CONSEQUENCES OF CLIMATE CHANGE FOR PLANT BIODIVERSITY IN HIGH MOUNTAIN ECOSYSTEMS

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CONSEQUENCES OF CLIMATE CHANGE FOR PLANT BIODIVERSITY IN HIGH MOUNTAIN ECOSYSTEMS

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Climate Vulnerability Assessment of the *Espeletia* Complex on Páramo Sky Islands in the Northern Andes

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Some of the largest impacts of climate change are expected in the environmentally heterogeneous and species rich high mountain ecosystems. Among those, the Neotropical alpine grassland above the tree line (c. 2,800 m), known as Páramo, is the fastest evolving biodiversity hotspot on earth, and one of the most threatened. Yet, predicting climate responses of typically slow-growing, long-lived plant linages in this unique high mountain ecosystem remains challenging. Here we coupled climate sensitivity modeling and adaptive potential inferences to efficiently assess climate vulnerability of *Espeletia*, Páramo's most iconic, predominant and rapidly evolving plant complex. In order to estimate climate sensitivity, we first modeled the distribution of 28 *Espeletia* taxa under a niche conservatism scenario using altitude and five current (1970–2000) and future (2050 RCP 8.5) bioclimatic variables across 36 different Páramo complexes in the northern Andes (49% of the world's Páramo area). As an alternative to range shifts via migration, we also computed the adaptive capacity of these Páramo complexes by considering three enhancing factors of the biodiversity's adaptive potential as well as three environmental limiting factors of the populations' plastic response. These predictors showed that diverse Páramos in the Eastern Cordillera were more vulnerable likely because the counteracting effects of the adaptive potential ($r = -0.93 \pm 0.01$) were not sufficient to buffer higher distribution losses ($r = 0.39 \pm 0.01$). Agriculture ($r = -0.48 \pm 0.01$), mining ($r = -0.36 \pm 0.01$), and rural population density ($r = -0.23 \pm 0.01$) also weakened the adaptive capacity. These results speak for a limited persistence via migration in the short-term responses of *Espeletia* to climate change, even though the past population dynamics in concert with glacial cycling is indicative of a predominant role of range shifts. Furthermore, changing climate, together with a general inability to adapt, may eventually constrain the rapid diversification in the *Espeletia* complex. Our integrative modeling illustrates how future climate may impact plant populations in a mega diverse and highly threatened ecosystem such as the Páramo, and encourages carrying out similar estimates in diverse plant complexes across other high mountain and island-like ecosystems.

Keywords: Neotropical alpine region, biodiversity hotspots, species distribution modeling, niche conservatism, range shifts, adaptation, Espeletiinae Cuatrec. (Asteraceae)

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INTRODUCTION

How tropical alpine biodiversity hotspots are affected and will react to climate change is among the most pressing questions in current biological research. Most studies on responses to changing climate assume populations migration to higher altitudes so that organisms would track their ecological niche (Lenoir et al., 2008; Steinbauer et al., 2018), and in some cases even to lower altitudes due to competitive release (Lenoir et al., 2010). Yet, when migration potential is restricted (Jump and Penuelas, 2005; Lees et al., 2020), the only way left for organisms to persist is by adjusting to the new environmental conditions, a traditionally neglected alternative in niche modeling. Adjustment via phenotypic plasticity is presumably important in long-lived taxa because plastic responses can happen within the lifetime of an individual (Nicotra et al., 2010). However, plasticity may be hampered when populations face novel anthropic conditions not found in their evolutionary history (Fox et al., 2019; Kelly, 2019), e.g., agriculture, mining and population density. Organisms can also adjust to climate change through adaptation (Waldvogel et al., 2020). Still, populations may not have enough adaptive potential (*e.g.* limited diversity) to keep up with the pace of climate change (Hoffmann and Sgro, 2011). Therefore, assessing biodiversity hotspots' vulnerability to climate change not only requires tracing ecological niche gaps under future scenarios – i.e., sensitivity (Berry et al., 2003), but also needs to explicitly account for the adaptive potential (Razgour et al., 2019) and plasticity to anthropic pressures. These improvements may make predictions more congruent with empirical data, even in potential climate refugia (Sweet et al., 2019).

One of the fastest evolving biodiversity hotspots is the Páramo (Madriñán et al., 2013), a highly threated (Vásquez et al., 2015; Pérez-Escobar et al., 2018) alpine ecosystem dominated by diverse grasslands above the treeline in the American tropics (at elevations of ca. 2,800–5,000 m). It is a main water supplier of wetland ecosystems and densely populated areas in the northern Andes (Cuesta et al., 2019a; Llambí et al., 2020). Despite it has a relatively small surface area (35,000 km^2), it contains over 3,000 plant species, several of which are endemic (Luteyn, 1999; Hughes and Atchison, 2015) and emerged as unique adaptations to extreme environments that evolved during the Andean uplift in the last five million years (Antonelli et al., 2009; Madriñán et al., 2013; Mutke et al., 2014). Unparalleled diversification rates across plant groups at these tropical "sky islands" (Sklenáø et al., 2014) has further been attributed to colonization by pre-adapted linages (Muellner-Riehl, 2019), and Pleistocene glacial cycling (Nevado et al., 2018; Perrigo et al., 2019; Rahbek et al., 2019) in the last 2.4 Myr that led to repeated periods of connectivity and spatial isolation (i.e., "species pump hypothesis"). Population fluctuations in concert with past glacial cycling indicate a general inability to adapt and a major role of range shifts (Martín-Bravo et al., 2010; Ronikier, 2011; Hazzi et al., 2018; Muellner-Riehl, 2019). Yet, it remains to be explored whether the rapidly evolving Páramo can keep pace with the quick rate of climate change and human expansion.

The most iconic, abundant and diverse group in the Páramo is the *Espeletia* complex (Monasterio and Sarmiento, 1991; Madriñán et al., 2013; Mavárez, 2019b). It originated in the Venezuelan Andes (Pouchon et al., 2018) from where it colonized southwards the Colombian Eastern Cordillera and the Ecuadorian Andes (Mavárez, 2019b), followed by a northwards migration into the Colombian Central and Western Cordilleras (Cuatrecasas, 2013). *Espeletia* taxa (ca. 120) are ecologically abundant across the Páramo landscape (Luteyn, 1999), occurring in highly heterogeneous habitats. *Espeletia* populations are adapted to grow in the wet depressions of high valleys, in the dry exposed slopes or even within the forests at the tree line, therefore experiencing a wide range of climatic conditions within elevations and localities (Peyre et al., 2018). This environmental heterogeneity may have important implications for the reaction of *Espeletia* to changing climatic conditions. For example, habitat variation may provide suitable locations for migrants within only a few meters of their current locations (Scherrer and Körner, 2011). However, populations adapted to a narrow range of conditions may respond poorly to future threats. In other words, environmental heterogeneity effects on the responses to climate change may be mixed, and sometime contradictory, especially for a long living tropical alpine plant with limited seed dispersal, such as *Espeletia*. Hence, a much-needed research is to balance climate sensitivity under the current ecological niche heterogeneity with the adaptive capacity of *Espeletia* to assess the climate vulnerability of this plant complex.

In order to bridge this gap, our goals in this study were to (1) quantify climate sensitivity in the *Espeletia* complex by comparing current and future (2050 RCP 8.5) distributions under a niche conservatism scenario (i.e., range shift via migration), (2) compute the adaptive capacity by incorporating enhancing factors of the biodiversity's adaptive potential (i.e., diversity, protected areas, and forest area in the buffer zone of the Páramo) as well as limiting factors of the plastic response (i.e., agricultural area, mining, and population density), and (3) estimate climate vulnerability of *Espeletia* by simultaneously coupling its climate sensitivity with its adaptive capacity. This work will help establishing how plant populations may respond to environmental change in a mega diverse tropical alpine region, where the largest impacts of climate pressures are expected (Körner, 2003; Rumpf et al., 2018).

MATERIALS AND METHODS

Study Area

The study area comprised the Colombian Andean Páramo biogeographical province (2,732–5,212 m, **Figure 1**), including the high-elevation northern Andes and the Sierra Nevada de Santa Marta, but excluding areas with similar altitudes in the Cordillera de Merida and Central American mountains (**Figure 2**). It was delimited in the north by the Sierra Nevada of Santa Marta (11°N), and in the south by the Colombia-Ecuador border (1 N). It spanned 36 Páramo complexes, as in Morales et al. (2007) and Londoño et al. (2014), denoting 49% of the world's Páramos. The surveyed Páramo zones were covered

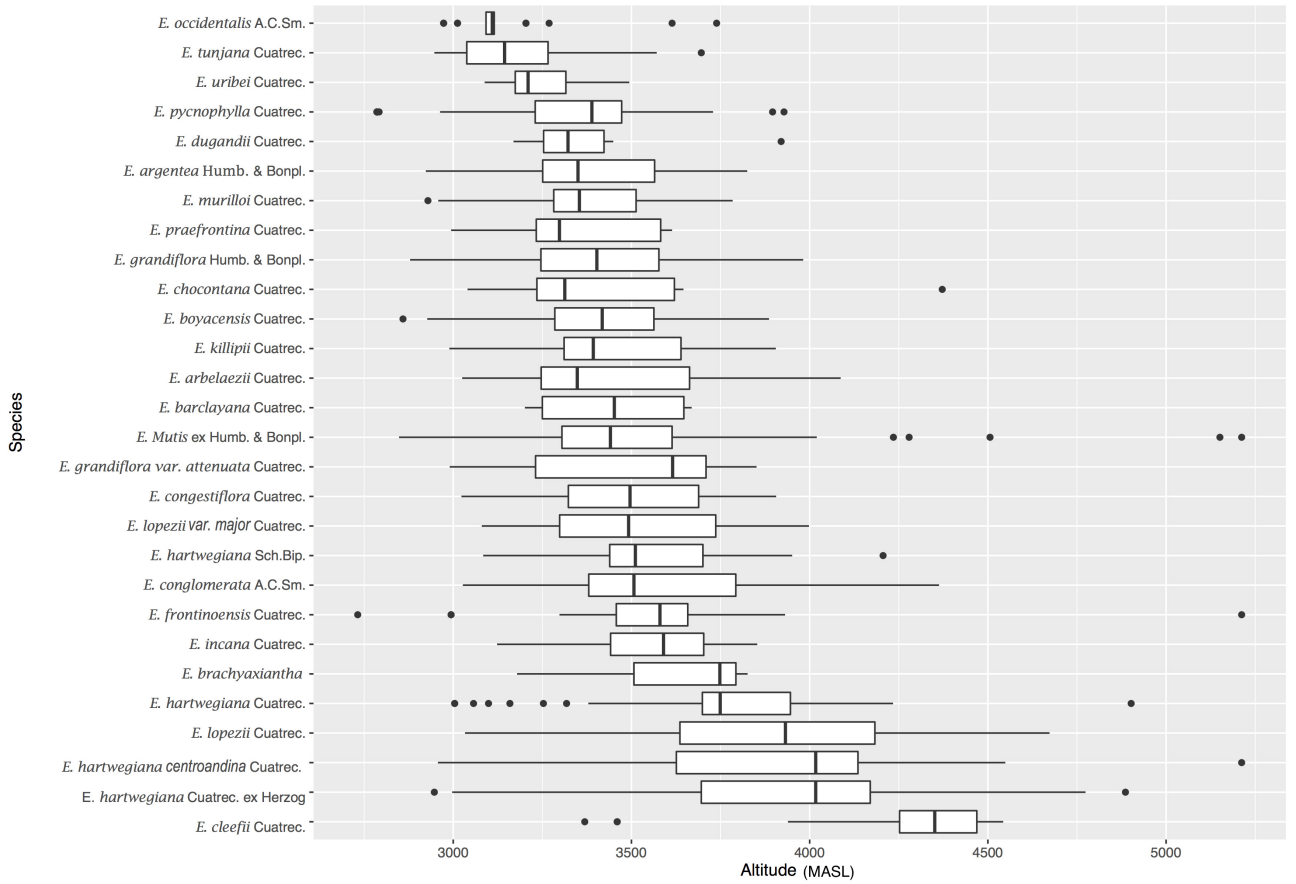


FIGURE 1 | Altitudinal profile (m asl) of 28 *Espeletia* taxa across Páramo sky islands in the Colombian Andes. A total of 1,432 occurrences, originally gathered as georeferenced specimen data from GBIF, were retained after cleaning for georeferencing consistency (i.e., non-redundant records located within the study area) and number of records per species (to avoid distributions with less than 10 points, following Van Proosdij et al. (2016) for narrow-ranged species). *Espeletia* taxonomy is in accordance with Cuatrecasas (2013).

in a 33% by protected areas[1], and surrounded in a 30% by forest patches[2]. Meanwhile, agriculture and mining, respectively, disturbed 6 and 7%[3] of these Páramos and their buffer belt (2 km around the tree line). The handling of these data sources is detailed below as part of the computation of the "Adaptive Capacity at the Study Area."

Climate Sensitivity in the *Espeletia* Complex

Climate sensitivity in the *Espeletia* complex was modeled by comparing current and future (2050 RCP 8.5) distributions under a niche conservatism scenario (i.e., range shift via migration). Occurrences of *Espeletia* samples for the 36 Colombian Páramo complexes were downloaded as georeferenced specimen data from providers served by GBIF[4]. From an initial set of 15,466 specimen records downloaded, a total of 1,432 (**Supplementary Table S1**), comprising 28 different *Espeletia* taxa according to Cuatrecasas (2013) – **Supplementary Table S2**, were retained after filtering for georeferencing consistency (i.e., non-redundant records within the study area) and number of records. In order to avoid bias due to distributions with low number of input points (Wisz et al., 2008), specially for presumably rare taxa (Fois et al., 2018), only putative species with at least 10 original records were retained (three taxa with more than 100 records, **Supplementary Figure S1A**). This threshold follows Van Proosdij et al. (2016) recommendation for narrow-ranged species, that is those with a moderate prevalence class (i.e., 27 taxa were present in less than seven different Páramo complexes, and only one taxon was present in 20 Páramos, **Supplementary Figure S1B**). This latter trend is expected in *Espeletia* species, for which distance (Diazgranados and Barber, 2017; Padilla-González et al., 2017) and ecology (Cortés et al., 2018a) are the major speciation drivers.

Historical climate data was extracted from the WorldClim[5] database, at a 30 s resolution, using the georeferencing of each record. All 19 bioclimatic variables were downloaded in the

[1]http://www.parquesnacionales.gov.co
[2]http://www.siac.gov.co
[3]https://www.igac.gov.co
[4]https://www.gbif.org
[5]http://www.worldclim.org

baseline period 1970–2000. The same variables projected to 2050, under the IPCC RCP 8.5 scenario, were gathered using CCAFS's downscaled delta method (Navarro-Racines et al., 2020). This projection averages 33 different global climatic models at a 1 km^2 resolution. A Digital Elevation Model (DEM) variable was further included. Prioritization of environmental variables (Zeng et al., 2016) is crucial to enhance model complexity (Warren and Seifert, 2011) for climate sensitivity assessments. By improving variable selection (Cobos et al., 2019), collinearity (Feng et al., 2019) and sampling bias (Syfert et al., 2013) can be minimized. Collinearity in particular refers to a strong correlation between two or more predictor variables, and may cause instability in parameter estimation (Dormann et al., 2013). A measure to diagnose collinearity is the Variance Inflation Factor (VIF), the square of the multiple correlation coefficient resulting from regressing a predictor variable against all other predictor variables (Chatterjee and Hadi, 2006). Hence, we performed variable selection by computing the VIF score using the *usdm* (Uncertainty Analysis for Species Distribution Models) package (Naimi et al., 2014) in R *v*.3.3.1 (R Core Team), and drew Pairwise Pearson correlations (*r*) among all variables by means of the same software (**Supplementary Figure S2**). Five bioclimatic variables and DEM did not exhibit collinearity (Naimi et al., 2014) at VIF $<$ 10 (**Supplementary Table S3**), with absolute *r*-values below 0.7 from 0.05 to 0.65, and therefore were retained hereinafter.

The six non-collinear variables fed Maxent model *v*.3.4.1. (Phillips et al., 2017), a "machine learning" outline that uses maximum entropy and Bayesian inference to estimate occurrence probability distributions. Ten split-sample models were run, after checking for stabilization in the ROC curves (Spiers et al., 2018), with an average of 10 k pseudo-absences for each of the 28 putative taxa. Each replicated run type was set to "cross-validate" with 500 iterations (Buffum et al., 2015). Presence $\geq$ 95% was binary reclassified (Liu et al., 2005) and the average across repetitions was recorded in each case. This procedure was performed for the historical dataset (1970–2000). To predict distribution changes of *Espeletia*, we projected trained models on current presence localities and present background climate to 2050 under the RCP 8.5 scenario through Maxent's "projection layer" tool. Current model validation considered AUC, an aggregate measure of performance across all possible classification thresholds. Minimum average testing AUC was above 0.98 (**Supplementary Table S4**). As AUC alone (**Supplementary Table S5**) may be deceiving (Lobo et al., 2008; West et al., 2016), the AIC and BIC criteria were also computed and checked for consistency (**Supplementary Table S6**).

All 56 distribution maps were converted to binary data, using a threshold of the 20th percentile, as suggested by the Maxent *v*.3.4.1 software (Phillips et al., 2017). These maps were then averaged to generate spatial information of current and future presence probabilities across all 28 taxa. Finally, the current distribution map was subtracted to the one obtained under a climate change scenario in order to estimate climate sensitivity in the *Espeletia* complex. Values close to zero in the sensitivity index meant few changes in the forecasted distribution of *Espeletia* due to climatic variation. On the other hand, negative and positive values corresponded to areas where the presence probabilities were, respectively, diminished or boosted by climate change.

Adaptive Capacity at the Study Area

We computed the adaptive capacity as the scaled additive effects of six main variables that expressed the capacity of the overall *Espeletia* biodiversity to respond without migration to the effects of climate change. Enhancing factors of the biodiversity's adaptive potential were diversity, protected areas, and forest area around the Páramo. On the contrary, limiting factors of the plastic response were agriculture, mining, and population density.

Diversity may buffer the effects of climate change by enriching complex interactions, such as adaptive inter-specific introgression (Marques et al., 2019) and hybrid speciation (Coyne and Orr, 2004; Abbott et al., 2013; Payseur and Rieseberg, 2016). Hybridization may allow for local adaptation in transitional environments and bi-directional transfer of adaptive variation through introgression (Isabel et al., 2020). *Espeletia* in particular exhibits weak species boundaries, rampant gene-flow and reticulate evolution (Cortés et al., 2018a; Pouchon et al., 2018), leading to a high incidence of natural hybrids (Rauscher, 2002; Cuatrecasas, 2013). In order to account for the enhancing role of diversity on the adaptive potential, the number of *Espeletia* taxa was totalized in a 1 km grid using the occurrence data downloaded from GBIF (see footnote), as described in the previous section, and scaled from 0 to 1.

Another enhancing factor of the biodiversity's adaptive potential is protected areas, where human intervention does not represent a direct threat, allowing biodiversity to be conserved while naturally coping with climate change. Protected areas selected for this analysis spanned 1,050 natural areas, according to the System of National Natural Parks (see footnote), and included 43 National Natural Parks, 663 Civil Society Reserves, 54 Regional Natural Parks, and 57 National Protected Forest Reserves. These categories were, respectively, rated as 1, 0.3, 0.1, and 0.1, following CIAT (2017). Higher ratings were assigned to areas that presumably provide greater protection for biodiversity due to their low anthropogenic pressure and extent.

Forest patches around the Páramo complexes (Henao-Díaz, 2019) may also contribute buffering climate change effects by facilitating (Bueno and Llambí, 2015) migration and gene-flow, which could increase the frequency of existing genetic variants adapted to particular conditions (Bridle and Vines, 2007). Additionally, forests provide conditions that may favor thermal and water regulation, decreasing the magnitude of climate change itself. Therefore, forest area in the buffer zone of the Páramo (2 km around the tree line) was gathered from the IDEAM (see footnote), and converted to binary points (1 = presence) in a 1 km grid.

On the other hand, major limiting factors of the plastic response are agriculture (Avellaneda-Torres et al., 2020), mining (Pérez-Escobar et al., 2018) and population density within the study area and in its buffer zone (Vásquez et al., 2015). In order to account for these threats, agricultural and mining areas were downloaded from the Geographic Institute Agustín Codazzi (see footnote) for the period 2010–2012, and transformed to binary data (0 = presence) in a 1 km grid within the study area and in its

buffer zone (2 km around the tree line). The population density indicator was computed as the inverse value of the normalized rural population density, scaled from 0 to 1, so that 1 meant absence of human settlements and 0 was the population density at the most populated rural zones in the study area. The population information was gathered from data projected to 2015 by the National Administrative Department of Statistics[6].

Climate Vulnerability Index for *Espeletia*

In order to estimate climate vulnerability at the overall *Espeletia* complex, we simultaneously coupled its climate sensitivity with its adaptive capacity. Negative values of the sensitivity index meant areas where the presence probabilities of *Espeletia* were diminished due to climate change. Meanwhile, lower adaptive capacity scores spoke for a prominent role of limiting factors of the plastic response (i.e., agriculture, mining, and population density) as compared to enhancing drivers of the biodiversity's adaptive potential (i.e., diversity, protected areas, and forest area around the Páramo). Therefore, the climate vulnerability score was computed as the complement value of the additive contributions of the sensitivity and the adaptive capacity indices. The climate vulnerability score was scaled from 0 to 1 for comparative purposes. Pairwise Pearson correlations (r) among all estimated scores were computed, and boxplots and ridgeline plots were drawn for the three indices in all 36 Páramo complexes. Computing and plotting were done in R *v*.3.3.1 (R Core Team).

[6]https://www.dane.gov.co

RESULTS

Climate Sensitivity of *Espeletia* Varied Widely Across Páramo Complexes

Climate sensitivity was estimated across all 36 Colombian Páramo complexes by comparing current (baseline period 1970–2000, **Figure 2A**) and future (2050 RCP 8.5, **Figure 2B**) average

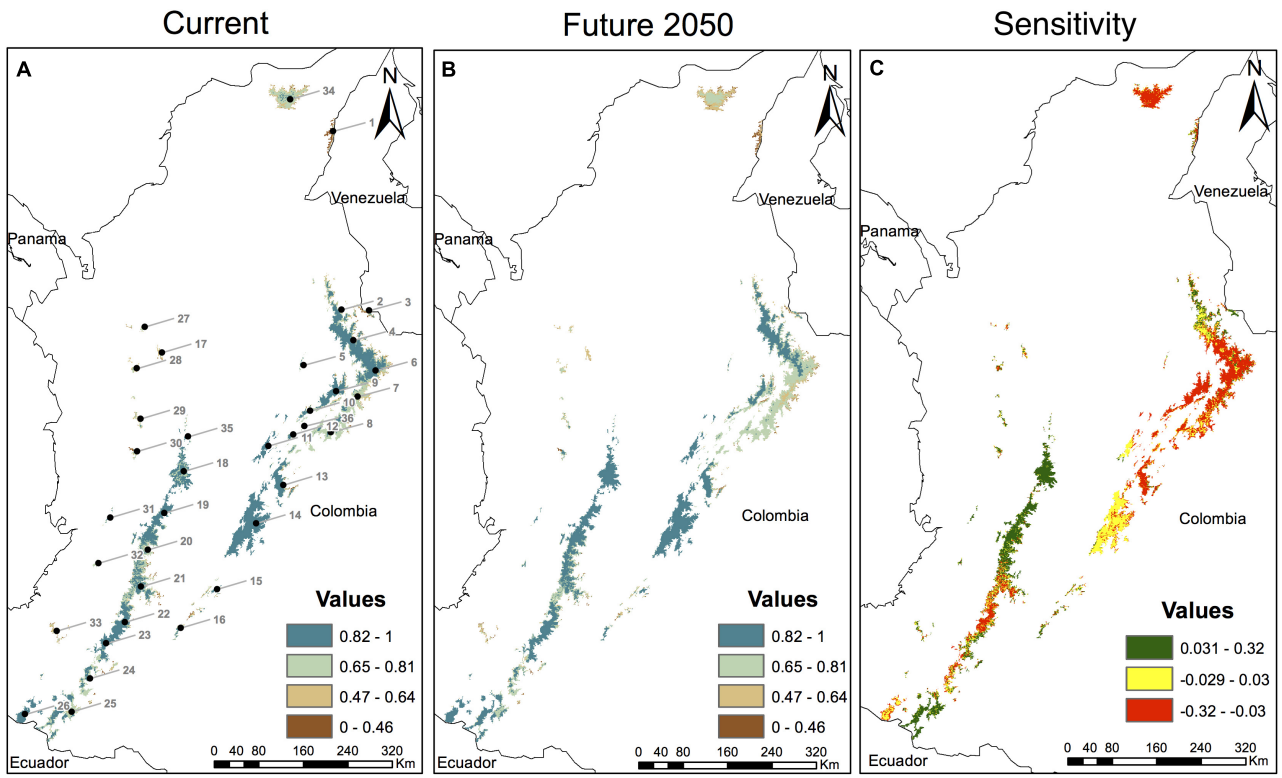


FIGURE 2 | Climate sensitivity estimates of *Espeletia* across 36 Páramo complexes in the Colombian Andes. Climate sensitivity was modeled by comparing **(A)** current (baseline period 1970–2000) and **(B)** future (2050 RCP 8.5) average distribution probabilities across 28 taxa, as obtained with Maxent *v*.3.4.1 (Phillips et al., 2017) under a niche conservatism scenario (i.e., range shift via migration). Values close to zero in the sensitivity index **(C)** indicate few changes in the forecasted distribution of *Espeletia* due to climate change, while negative and positive values correspond to range losses and gains, respectively, based on the averaged presence probabilities. Five non-collinear WorldClim bioclimatic variables (**Supplementary Table S3**) were considered at a 30 s (1 km^2) spatial resolution for the current and future scenarios, besides a Digital Elevation Model (DEM). *Espeletia* taxonomy follows Cuatrecasas (2013). Páramo complexes are numbered in **(A)** according to Morales et al. (2007) and Londoño et al. (2014), specifically: (1) Perijá, (2) Jurisdicciones-Santurbán, (3) Tamá, (4) Almorzadero, (5) Yariguíes, (6) Cocuy, (7) Pisba, (8) Tota-Bijagual-Mamapacha, (9) Guantiva-Rusia, (10) Iguaque-Merchán, (11) Guerrero, (12) Rabanal y río Bogotá, (13) Chingaza, (14) Cruz Verde-Sumapaz, (15) Los Picachos, (16) Miraflores, (17) Belmira, (18) Nevados, (19) Chilí-Barragán, (20) Las Hermosas, (21) Nevado del Huila-Moras, (22) Guanacas-Puracé-Coconuco, (23) Sotará, (24) Doña Juana-Chimayoy, (25) La Cocha-Patascoy, (26) Chiles-Cumbal, (27) Paramillo, (28) Frontino-Urrao, (29) Citará, (30) Tatamá, (31) Duende, (32) Farallones de Cali, (33) Cerro Plateado, (34) Santa Marta, (35) Sonsón, and (36) Altiplano Cundiboyacense.

distribution probabilities of 28 different *Espeletia* taxa (nine had average sensitivity scores below zero). Values surrounding zero in the sensitivity index (−0.029 to 0.030, 42% of the study area, **Figure 2C**) stood for few changes in the forecasted distribution of *Espeletia* due to climate change, and were predominant in the north of the Eastern Cordillera, the Cruz Verde-Sumapaz Páramo complex south of the Bogotá montane savanna (the southwestern wetland complex of the larger Eastern Cordillera's Andean highland plateau), and the small sky islands of the Western Cordillera.

Given a niche conservatism scenario, negative estimates of the climate sensitivity (≤−0.030, 32% of the study area, **Figure 2C**) predicted range losses based on the averaged presence probabilities. Páramo areas more likely to experience range losses were those in the Sierra Nevada de Santa Marta, and the Central Cordillera South of Las Hermosas (i.e., Nevado del Huila-Moras, and Guanacas-Puracé-Coconuco), as well as those in the northern corner of the Eastern Cordillera (i.e., Sierra Nevada del Cocuy, Pisba, and Tota-Bijagual-Mamapacha), where diverse summits surrounded the topographically intricate Chichamocha inter-Andean canyon. On the other hand, range shifts via migration enriched presence probabilities in other localities (positive estimates of the climate sensitivity ≥ 0.031, 27% of the study area, **Figure 2C**). Range gains were only

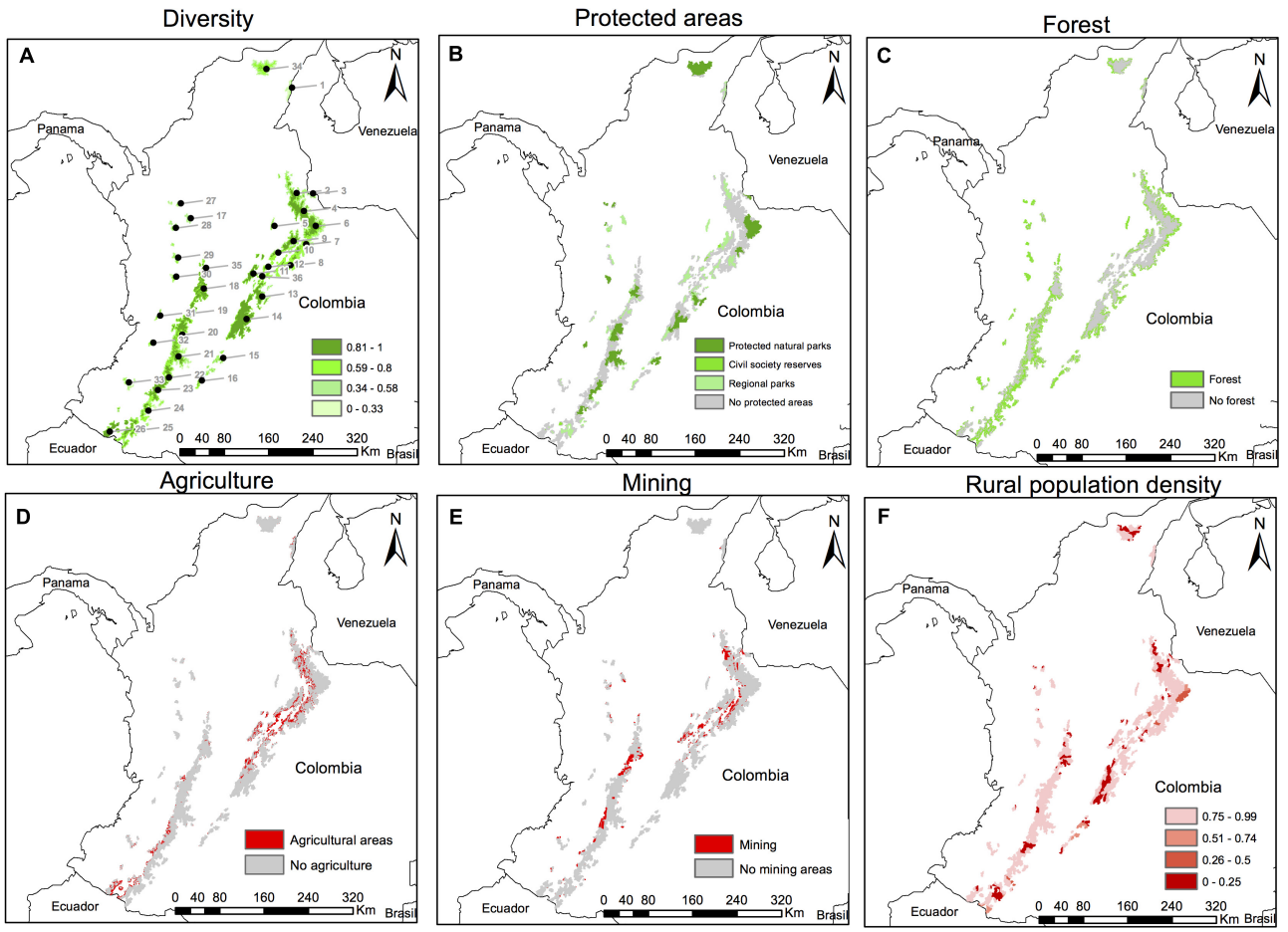


FIGURE 3 | Components of the adaptive capacity across 36 Páramo complexes in the Colombian Andes. Enhancing **(A–C)** and limiting **(D–F)** factors of the adaptive capacity are shown in green and red, respectively. Enhancing factors of the biodiversity's adaptive potential were **(A)** diversity, **(B)** protected areas, and **(C)** forest area around the Páramo, while limiting factors of the plastic response were **(D)** agriculture, **(E)** mining and **(F)** population density. Diversity **(A)**, which is the number of *Espeletia* taxa totalized in a 1 km grid based on the occurrence data downloaded from GBIF, and the inverse value of the normalized rural population density **(F)** were scaled from 0 to 1. A total of 1,050 protected areas **(B)** were rated as 1, 0.3, 0.1, and 0.1 for National Natural Parks, Civil Society Reserves, Regional Natural Parks and National Protected Forest Reserves, respectively, following (CIAT, 2017). Forest patches **(C)** around the Páramo complexes (2 km around the tree line, 1 km grid) were recorded as presence points (1 = presence), while agriculture **(D)** and mining **(E)** for the period 2010–2012 were marked as null data points (0 = presence) within the study area and in its buffer zone (2 km around the tree line, 1 km grid). Páramo complexes numbers in **(A)** follow Morales et al. (2007) and Londoño et al. (2014): (1) Perijá, (2) Jurisdicciones-Santurbán, (3) Tamá, (4) Almorzadero, (5) Yariguíes, (6) Cocuy, (7) Pisba, (8) Tota-Bijagual-Mamapacha, (9) Guantiva-Rusia, (10) Iguaque-Merchán, (11) Guerrero, (12) Rabanal y río Bogotá, (13) Chingaza, (14) Cruz Verde-Sumapaz, (15) Los Picachos, (16) Miraflores, (17) Belmira, (18) Nevados, (19) Chilí-Barragán, (20) Las Hermosas, (21) Nevado del Huila-Moras, (22) Guanacas-Puracé-Coconuco, (23) Sotará, (24) Doña Juana-Chimayoy, (25) La Cocha-Patascoy, (26) Chiles-Cumbal, (27) Paramillo, (28) Frontino-Urrao, (29) Citará, (30) Tatamá, (31) Duende, (32) Farallones de Cali, (33) Cerro Plateado, (34) Santa Marta, (35) Sonsón, and (36) Altiplano Cundiboyacense.

predicted for Páramo areas in the Central Cordillera (Los Nevados, Chilí-Barragán, Las Hermosas, and La Cocha-Patascoy, **Supplementary Figure S3**) where the dominant taxon was *E. hartwegiana*, as well as in the summits of the northern tip of the Eastern Cordillera (Perijá).

Adaptive Capacity Was Jeopardized Across Most Páramo Complexes

The adaptive capacity across 36 Colombian Páramo complexes was computed using a scaled additive model of enhancing (**Figures 3A–C**) and limiting (**Figures 3D–F**) factors of the biodiversity's adaptive potential and the plastic response. The adaptive capacity index (**Figures 4B**, **5A**, **Supplementary Figure S3A**, and **Supplementary Table S7**) was higher (≥0.72, 34% of the study area) in species-rich Páramo areas (Pearson's $r = 0.19 \pm 0.01$, **Figure 3A** and **Supplementary Figure S4**), and matched extensive National Natural Parks ($r = 0.59 \pm 0.01$, **Figure 3B** and **Supplementary Figure S4**), like the Eastern Cordillera's Sierra Nevada del Cocuy, Chingaza and Sumapaz. The Páramo adaptive capacity index was also moderately superior (0.62–0.71, 29% of the study area, **Figure 4B**) in the most representative National Natural Parks in the Central Cordillera (Los Nevados and Nevado del Huila) as well as in the Sierra Nevada de Santa Marta. In turn, the western slopes of the Páramo complexes at the Eastern and Central Cordilleras exhibited lower adaptive capacity values (≤0.61, 37% of the study area), and were enclosed by less forest patches (30% on average, **Figure 3C**, $r = 0.39 \pm 0.01$, **Supplementary Figure S4**).

However, positive effects due to protected areas and forest coverage on the adaptive potential were offset by limiting factors of the plastic response, primarily agricultural (6% of the study area and its buffer zone, **Figure 3D**, $r = -0.47 \pm 0.01$, **Supplementary Figure S4**) and mining (7% of the study area and its buffer zone, **Figure 3E**, $r = -0.41 \pm 0.01$, **Supplementary Figure S4**) pressures, mainly in the western slopes of the Páramo areas at the Eastern and Central Cordilleras. This let to the lowest adaptive potential estimates (≤0.52, 20% of the study area, **Figure 4B**) at the western slopes of the Páramo complexes in the Cauca province, in the Central Cordillera, and the Boyacá and Santander provinces (i.e., Almorzadero and Altiplano Cundiboyacense Páramo complexes), in the Eastern Cordillera. Rural population (**Figure 3F**) hampered the adaptive potential

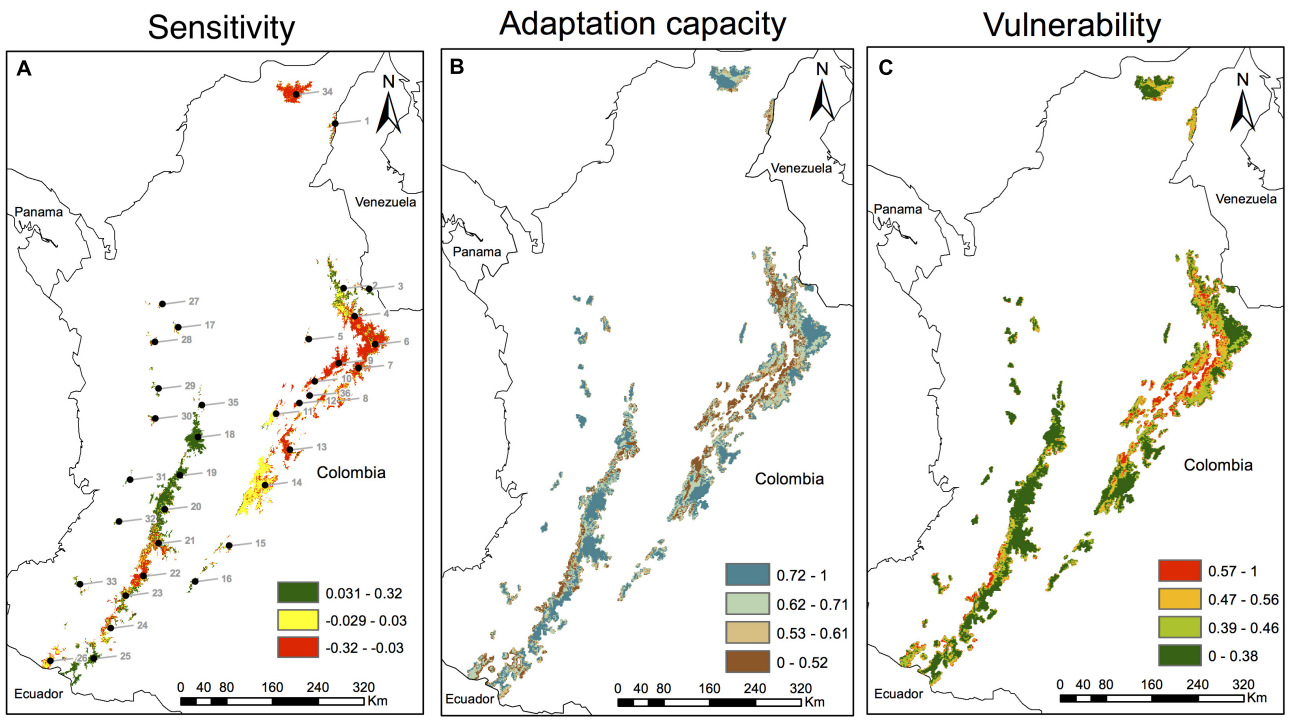


FIGURE 4 | Climate sensitivity, adaptive capacity and climate vulnerability in the *Espeletia* complex across 36 Páramo sky island complexes in the Colombian Andes. Climate sensitivity **(A)** is already depicted in **Figure 2C** and is brought here for comparative purposes. Adaptive capacity **(B)** was computed as the scaled additive effects of all six variables shown in **Figure 3**. Climate vulnerability **(C)** was calculated as the complement value of the additive contributions of the sensitivity and the adaptive capacity indices, scaled from 0 to 1. Negative values in the sensitivity index **(A)** suggest range losses in the presence probabilities of *Espeletia* due to climate change. Low values in the adaptive capacity score **(B)** indicate a major role of limiting factors of the plastic response (i.e., agriculture, mining, and population density, **Figures 3D–F**) as compared to enhancing drivers of the biodiversity's adaptive potential (i.e., diversity, protected areas, and forest area around the Páramo, **Figures 3A–C**). Páramo complexes are numbered in **(A)** following Morales et al. (2007) and Londoño et al. (2014), that is: (1) Perijá, (2) Jurisdicciones-Santurbán, (3) Tamá, (4) Almorzadero, (5) Yariguíes, (6) Cocuy, (7) Pisba, (8) Tota-Bijagual-Mamapacha, (9) Guantiva-Rusia, (10) Iguaque-Merchán, (11) Guerrero, (12) Rabanal y río Bogotá, (13) Chingaza, (14) Cruz Verde-Sumapaz, (15) Los Picachos, (16) Miraflores, (17) Belmira, (18) Nevados, (19) Chilí-Barragán, (20) Las Hermosas, (21) Nevado del Huila-Moras, (22) Guanacas-Puracé-Coconuco, (23) Sotará, (24) Doña Juana-Chimayoy, (25) La Cocha-Patascoy, (26) Chiles-Cumbal, (27) Paramillo, (28) Frontino-Urrao, (29) Citará, (30) Tatamá, (31) Duende, (32) Farallones de Cali, (33) Cerro Plateado, (34) Santa Marta, (35) Sonsón, and (36) Altiplano Cundiboyacense.

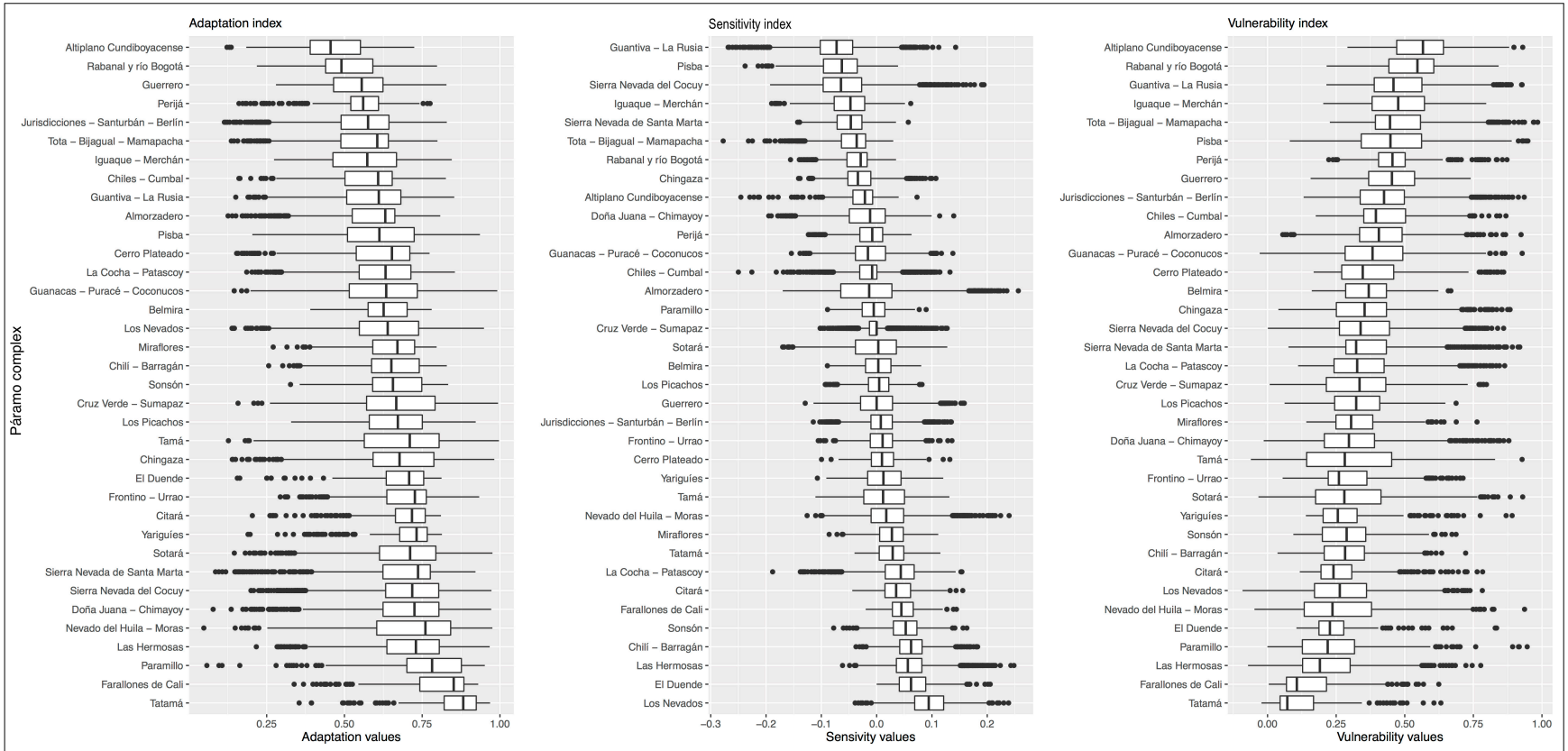


FIGURE 5 | Boxplots for the adaptive capacity **(A)**, climate sensitivity **(B)**, and vulnerability **(C)** across 36 Páramo complexes in the Colombian Andes. For comparative purposes, Páramo complexes are sorted in **(A)** from those with a low adaptive potential to anthropic pressures according to the enhancing and limiting factors depicted in **Figure 3**, in **(B)** from those likely to experience a range loss (distributions below zero) to those predicted to exhibit a range gain (distributions above zero) by 2050 (under RCP 8.5), while in **(C)** Páramo complexes at the top are the most vulnerable. Boxplots summarize grid estimates depicted in **Figure 4**.

in all three Cordilleras and Páramo areas ($r = -0.28 \pm 0.01$, **Supplementary Figure S4**).

Diverse Páramos in the Eastern Cordillera Were More Vulnerable

The scaled complement of the additive contributions of the sensitivity (**Figures 2C**, **5B** and **Supplementary Figure S3B**) and the adaptive capacity (**Figure 4B**) indices were used to compute the climate vulnerability score (**Figures 4C**, **5C** and **Supplementary Figure S3C**). The most vulnerable (≥0.57, 11% of the study area, **Figure 4C**) Páramo areas coincided with diverse Páramos in the Eastern Cordillera (**Figure 3A**) because of more distribution losses ($r = 0.39 \pm 0.01$, **Supplementary Figure S4**). Agriculture ($r = 0.48 \pm 0.01$, **Supplementary Figure S4**), mining ($r = 0.36 \pm 0.01$, **Supplementary Figure S4**) and rural population density ($r = 0.23 \pm 0.01$, **Supplementary Figure S4**) also boosted their vulnerability, mainly on the western slopes in the middle of the Central Cordillera (i.e., Guanacas-Puracé-Coconuco), and the northeast corner of the Eastern Cordillera (i.e., Almorzadero, Pisba, and Altiplano Cundiboyacense). On the other hand, the less vulnerable (≤0.38, 57% of the study area, **Figure 4C**) Páramo areas were those with the highest adaptive capacity (≥ 0.72, **Figure 4B**, $r = -0.93 \pm 0.01$, **Supplementary Figure S4**) due to large National Natural Parks (Sumapaz, Chingaza, Sierra Nevada del Cocuy, **Figure 3B**), as well as those for which predicted range shifts were neutral (−0.029 to 0.030, **Figure 4A**, i.e., the Cruz Verde-Sumapaz Páramo complexes in the Eastern Cordillera) or positive (≥0.031, **Figure 5B**, Los Nevados, Chilí-Barragán, and Las Hermosas in Central Cordillera).

DISCUSSION

The climate sensitivity score of *Espeletia* to climate change indicated range losses (negative values) widespread across most Páramo complexes. This suggests limited persistence of the *Espeletia* spp. populations via migration in the majority of Páramos, with some exceptions in the Central Cordillera possibly due to its higher connectivity. An alternative for populations to persist despite changing environments would be adaptation *s.l.* However, positive effects on the adaptive potential, like those due to protected areas and forests, are likely to be offset by limiting factors of the plastic response, such as rising agriculture and mining in the Páramo areas and their surroundings. Overall, this suggests that diverse Páramo areas of the Eastern Cordillera are the most vulnerable to changing climate. Climate change pushing *Espeletia* toward mountain summits where there is nowhere else to go (Cuesta et al., 2019a), together with a general inability of those populations to adapt, might constrain the rapid diversification that has made the *Espeletia* complex so unique.

Range Losses in the *Espeletia* Complex Will Widespread Throughout Most Páramos

Widespread range losses in the distribution of *Espeletia* are expected by 2050, a pessimistic prediction in line with previous modeling efforts (Buytaert et al., 2011; Crandall et al., 2013; Mavárez et al., 2018; Helmer et al., 2019; Peyre et al., 2020; Young and Duchicela, 2020). This tendency is broadly defined as thermophilization, a progressive decline of cold mountain habitats and their biota (Gottfried et al., 2012). Large reductions in modeled area and important upward shifts in the distribution of 28 Espeletiinae are already predicted by 2070 at the Venezuelan Páramo (Mavárez et al., 2018). Despite our projection is carried out for a shorter time frame at another, wider, study area (Colombian Andes spanning the Eastern, Central and Western Cordilleras besides Sierra Nevada de Santa Marta), overall results point toward the same trend and are complementary. Similar predictions of climate sensitivity at the Páramo complexes of the Ecuadorian Cordilleras (1 sp.) were not considered as part of this study because of the difficulties in compiling and comparing data from different national repositories, specifically for some of the variables that compose the adaptive capacity score (i.e., agriculture and mining pressures). Yet, considering analogous approaches in the Ecuadorian Páramo (Crandall et al., 2013; Helmer et al., 2019; Peyre et al., 2020) is essential to gather a more comprehensive understating on the consequences of climate change at high Andean ecosystems north of the Huancabamba depression, a major biogeographical barrier for high-elevation plant taxa (Weigend, 2002).

There are two important exceptions for the widespread range losses in the distribution of *Espeletia* in the Colombian Andes. First, small and fragmented Páramo sky islands in the Western Cordillera exhibit neutral climate sensitivity, a counterintuitive projection that Mavárez et al. (2018) also pointed out for Espeletiinae in isolated Páramo areas at Cordillera de Mérida. This reiterative finding suggests that scarce topographical complexity (terrain ruggedness) in recently colonized small islands (Flantua et al., 2019) may have favored habitat generalists, while intricate local-scale environmental heterogeneity at larger Páramo complexes could have triggered an explosive radiation (Cortés et al., 2018a; Naciri and Linder, 2020) of habitat specialists, each with a narrow ecological niche for migration to occur (Cuesta et al., 2019b). Modern colonization and limited topographical complexity, together with higher connectivity, may also account for the second exception, which is that range gains are only predicted for Páramo areas in the Central Cordillera (Los Nevados, Chilí-Barragán, Las Hermosas, and La Cocha-Patascoy), where the dominant taxon is *E. hartwegiana*. In line with this, Páramo areas more likely to exhibit range losses are those in the topographically complex Eastern Cordillera's northeast corner, where diverse (Luteyn, 1999; Cuatrecasas, 2013; Peyre et al., 2015, 2019) summits surround the deep (1,100 m) Chichamocha inter-Andean canyon.

Range shifts presuppose niche conservatism, but also depend on the intrinsic dispersal ability of the taxa (Pelayo et al., 2019; Tovar et al., 2020). In organisms with limited seed dispersal such as *Espeletia*, other ways for migration are key (Cochrane et al., 2019). For instance, migration via sporadic pollen flow would not be as constrained as seed dispersal (Cuatrecasas, 2013). Alternately, hybrids with intermediate niche requirements (Rieseberg, 2003) may serve as bridges for migration in a plant complex with rampant natural introgression (Rauscher, 2002; Cortés et al., 2018a; Pouchon et al., 2018). Finally,

sporadic episodes of habitat connectivity (Flantua et al., 2019) in the lifespan of a long-lived organism such as *Espeletia* (Cuatrecasas, 2013) could provide a window for migration across modern barriers.

Key traits may relax the niche conservatism assumption by conferring the ability to colonize new habitats. For instance, underground plant-soil interactions (Goh et al., 2013; Sedlacek et al., 2014; Little et al., 2016) across different water levels (Geange et al., 2017) and cellular-anatomical physiological adaptations (Sandoval et al., 2019) may serve as pre-adaptations for a wider range expansion (Cuesta et al., 2017). Plastic traits may also broad the spectrum of novel climates (Arnold et al., 2019). Coupling this modeling with eco-physiological studies in *Espeletia* (Leon-Garcia and Lasso, 2019; Llambí and Rada, 2019) is hence a key research line to better track future range shifts in the Páramo.

Climate Change May Constrain the Rapid Diversification of the *Espeletia* Complex

The fact that diverse areas in the Eastern Cordillera are vulnerable to climate change is not only counterintuitive, but it also means that the long-term rapid diversification rate, striking in the *Espeletia* complex, could be jeopardized. Diversity is regarded as a buffer of the effects of climate change by enriching interactions (Cáceres et al., 2014), such as facilitation (Bueno and Llambí, 2015; Wheeler et al., 2015; Llambí et al., 2018; Mora et al., 2018; Venn et al., 2019), adaptive inter-specific introgression (Schilthuizen et al., 2004; Seehausen, 2004) and hybrid speciation (Payseur and Rieseberg, 2016), of which there is abundant evidence in *Espeletia* (Rauscher, 2002; Cuatrecasas, 2013; Cortés et al., 2018a; Pouchon et al., 2018). Yet, these positive effects are offset by distribution losses in sensitive Páramo localities, besides rising mining, agriculture and population density. These are Páramo's current major anthropogenic threats (Vásquez et al., 2015; Pérez-Escobar et al., 2018) primarily in the western slopes of the Eastern and Central Cordilleras.

Permeable species barriers due to pervasive hybridization make the species concept highly debatable within the *Espeletia* complex. Therefore we refrained from emphasizing single-species responses that are likely to be overwhelmed by meta-population dynamics. Pouchon et al. (2018) suggested that the entire subtribe Espeletiinae requires an in deep taxonomic revision (Mavárez, 2019b) because most of Cuatrecasas' (2013) species are thought to be paraphyletic. This trend is not unexpected since the available taxonomy greatly relies on potentially plastic morphological and reproductive traits (Mavárez, 2020) that are susceptible to be driven by environmental effects, besides the fact that it disregards rampant hybridization (Rauscher, 2002). Then, a more appropriate scenario for the young rapidly-evolving *Espeletia* complex with weak species boundaries would be that ecotypes in the same locality, despite depicting microhabitat divergence, are capable of sharing through gene flow and introgression adaptive genetic variants (Cortés et al., 2018a). Gene swapping because of porosities in the species bounds would imply that (1) the effective size of "rare" taxa with few records is higher in terms of standing adaptive variation, and (2) concerted climate responses is feasible across lineages within the same Páramo sky island. Inter-mountain exchange is also plausible, as reported in other tropical sky islands (Chala et al., 2020; Tusiime et al., 2020).

A possible way forward for populations to persist is the very same driver that is thought to contribute with the unbeatable diversification rate in the Páramo flora (Madriñán et al., 2013) – local-scale adaptive variation (Huang et al., 2020; Todesco et al., 2020). As part of this study we considered major enhancers and constrainers of the adaptive potential at a regional scale. However, populations may still harbor intrinsic genetic variation naturally selected to cope with environmental differences at the microhabitat level (Ramírez et al., 2014). In highly heterogeneous mountainous environments, local-scale environmental effects are known for shaping genetic diversity and generating morphological diversity (Hughes and Atchison, 2015), which may prove useful in the reaction to climate change within the same locality. There is already compiling evidence of ecological-driven divergence in the radiation of Andean *Espeletia* (Cortés et al., 2018a), besides allopatric differentiation and isolation by distance (Diazgranados and Barber, 2017; Padilla-González et al., 2017, 2018). Ecological parapatric speciation is a likely consequence of local patterns of environmental variation (Naciri and Linder, 2020) typically found at the Páramo ecosystem (Monasterio and Sarmiento, 1991). For instance, frost is more extreme in the wind-exposed high valley slopes than in the wind-sheltered depressions of those valleys or in the upper boundary of the cloud forest. Soil moisture content, higher in the well-irrigated high valley depressions and in the upper boundary of the cloud forest than in the drier slopes of the valleys, could also contribute to this divergence. It now remains to be explored whether this mosaic of environmental heterogeneity has enforced enough heritable polygenic (Barghi et al., 2020) phenotypic variation as a pre-adaptation (Nürk et al., 2018) to the new selective forces imposed by climate change.

One potential caveat of our results precisely concerns data availability at the micro-scale level. Bioclimatic data is reliably modeled throughout the Páramo range at a 1 km^2 spatial resolution, which unfortunately overlooks micro-environmental drivers of the local-scale genetic adaptation (De La Harpe et al., 2017; Leroy et al., 2020). This is of particular importance at mountain/alpine ecosystems (Ramírez et al., 2014; Cortés and Wheeler, 2018), where microhabitats may serve as refugia (Zellweger et al., 2020), like across the treeline due to active landform processes (Bueno and Llambí, 2015; Arzac et al., 2019; Gentili et al., 2020). Populations may rely on specific small-scale habitat attributes that are likely to be overlooked by SDMs (Sinclair et al., 2010), such as topographic, geomorphic, and edaphic features, as well as the distribution of other taxa – e.g., competitors and facilitators (Yackulc et al., 2015). Due to this, SDMs may exhibit substantial uncertainty (Buisson et al., 2010) and lack of validation (Botkin et al., 2007) at local scales. Therefore, niche models could be insensitive to micro-environmental processes, leading to an underestimation of the mechanisms governing populations' responses. Even if bioclimatic models aim representing the realized niche, they may then disregard the fundamental niche (Loehle and Leblanc, 1996). Despite statistical imputation-like approaches such as environmental downscaling have been considered to model

climate at a higher resolution for specific Páramo complexes (Mavárez et al., 2018), consistently applying such techniques across cordilleras seems unfeasible because accuracy would be constrained by the accessibility of fine-scale bioclimatic data from regional weather stations. These restraints are likely to be overcome soon by incorporating microclimate into SDMs (Lembrechts et al., 2019) through mechanistic algorithms (Lembrechts and Lenoir, 2020) physiological models (Cortés et al., 2013; López-Hernández and Cortés, 2019), hydrological equations (Rodríguez-Morales et al., 2019; Correa et al., 2020), *in situ* data logging, and remote sensing (Ramón-Reinozo et al., 2019; Zellweger et al., 2019).

Another potential caveat of our inferences relate with data availability across the entire Páramo area in the northern Andes. Some components of the adaptive capacity score (i.e., protected areas, agriculture, and mining pressures) are downloadable only through national repositories, making standardizations and comparisons across all Páramo complexes in different countries impractical. Yet, it is worth to clarify that climate sensitivity, adaptation capacity and vulnerability indices are not meant to be absolute scores but rather relative values to compare among Páramo complexes. In this sense, the power of our inferences to rank Páramo areas is unlikely limited by the pessimistic predictions of the future climate scenario RCP 8.5, making our vulnerability index robust. Furthermore, our whole integrative analytical pipeline is in line with the most up-to-date approaches to carry out climate change vulnerability assessment (Ramirez-Villegas et al., 2014; Valladares et al., 2014; Foden et al., 2018).

By assessing climate sensitivity and adaptive capacity across Páramo sky islands, we predicted climate change vulnerability in the rapidly evolving *Espeletia* complex, a syngameon (Cannon and Petit, 2020) likely shaped by incomplete ecological speciation (Nosil et al., 2009) and adaptive introgression (Fitzpatrick et al., 2015; Martin and Jiggins, 2017). However, in order to gather a more comprehensive view of climate change responses in the high mountain ecosystems of the northern Andes, other diverse and highly abundant plant genera in the Páramo (Hughes et al., 2013) should be considered under a similar analytical framework as the described here. Immediate candidates to follow up this approach are *Bartsia* (Uribe-Convers and Tank, 2015), *Chusquea* (Ely et al., 2019), *Diplostephium* (Vargas et al., 2017), *Hypericum* (Nürk et al., 2013), *Loricaria* (Kolar et al., 2016), *Lupinus* (Hughes and Eastwood, 2006; Vásquez et al., 2016; Contreras-Ortiz et al., 2018), *Oreobolus* (Chacón et al., 2006; Gómez-Gutiérrez et al., 2017), *Puya* (Jabaily and Sytsma, 2013), and *Senecio* (Duskova et al., 2017; Walter et al., 2020). These combined efforts would ultimately reveal whether the fastest evolving biodiversity hotspot on earth, and in general tropical high mountain ecosystems (Hedberg, 1964; Sklenáø et al., 2014; Chala et al., 2016), have a chance to persist under current environmental and anthropogenic threats.

PERSPECTIVES

Besides range shifts (Chen et al., 2011; Feeley et al., 2011; Donoghue and Edwards, 2014; Kolar et al., 2016; Freeman et al., 2020) and adaptive responses (Hoffmann and Sgro, 2011; Franks and Hoffmann, 2012; Donoghue, 2019; Ørsted et al., 2019), introgression, hybridization (Lafon-Placette et al., 2016), and polyploidization (Han et al., 2019; Mason and Wendel, 2020; Nieto Feliner et al., 2020) which have not been explicitly explored in this study, may also provide innovative (Donoghue and Sanderson, 2015) adaptive sources in plants (Abbott et al., 2013; Marques et al., 2019). The *Espeletia* complex is known for a high incidence of natural hybrids as told by phenotypic and genetic markers (Rauscher, 2002; Pouchon et al., 2018). Hence, assessing the role of reticulate evolution and adaptive introgression for the *Espeletia* complex to face a changing environment will require sampling of hybrids and their putative parental populations across the Páramo's current micro-ecological mosaic.

Even though hybrids could occupy intermediate niches, persistence of populations in alpine environments that face climate change is regarded as mostly mediated by local-scale (i.e., microhabitat) variation (Cortés et al., 2014; Cortés and Wheeler, 2018). For example, environmental heterogeneity may provide new suitable locations for migrants within only a few meters of their current locations (Scherrer and Körner, 2011), driving in this way plant responses to warming (Zellweger et al., 2020). More localized environmental data must then be gathered (Zellweger et al., 2019), since current climate repositories lack the resolution needed to describe the microhabitat heterogeneity. Topographic and soil properties, often overlooked by climatic models, are presumably key in defining Páramo's wet and drier microhabitats in the wind-sheltered high valley depressions, and in the more wind-exposed slopes. This local-scale environmental trend has diversity implications for other endemic plant species in the northern Andes (Cortés et al., 2012a,b; Blair et al., 2016). Besides high-resolution environmental data, eco-physiological (Llambí and Rada, 2019; Sandoval et al., 2019) and ecological adaptive traits (e.g., Bruelheide et al., 2018; Cortés and Blair, 2018) must be noted more systematically at local scales in natural surveys and field tests, like reciprocal transplant assays of ecotypes among microhabitats (as in Sedlacek et al., 2015), and space-by-time substitution trials across altitudes (Cuesta et al., 2017) and habitats (Wheeler et al., 2014, 2016). Ultimately, micro-scales keep genetic variance (Cortés et al., 2011, 2018b; Galeano et al., 2012; Kelleher et al., 2012; Blair et al., 2013, 2018; Cortés, 2013; Wu et al., 2020), morphological diversity, and adaptive trait variation (Sedlacek et al., 2016; Pacifici et al., 2017).

Conservation efforts may also buffer the impact of climate change on Páramo ecosystems by enhancing their adaptive capacity. Vast areas of Páramo land now safe from conflict are not being rapidly protected, making them susceptible to deforestation for cattle and agriculture (Avellaneda-Torres et al., 2020), and illegal logging (Vásquez et al., 2015) and mining (Pérez-Escobar et al., 2018). Thus, improving the current network of protected areas would help minimizing ongoing threats (Duchicela et al., 2019), especially in the highly diverse Páramo complexes of the Eastern Cordillera where putatively new species are still being discovered (Diazgranados and Sanchez, 2017; Mavárez, 2019a). Alongside adoption of mitigation policies, adaptation policies (Elsen et al., 2020) may help safeguarding Páramos. Finding more sustainable land uses by empowering local communities

and developing ecotourism are then as essential to relieve human impact on tropical-alpine plant diversity in the northern Andes.

Last but not least, extending the modeling approach implemented in this work to other key pressures (e.g., fire, Wu and Porinchu, 2019; Rivadeneira et al., 2020; Zomer and Ramsay, 2020) and plant groups (Luteyn, 1999) in the Páramo, as well as to other sky islands around the mountains of the world (Hoorn et al., 2018; Pausas et al., 2018; Nürk et al., 2019; Testolin et al., 2020), and more broadly to other island-like systems (Papadopoulou and Knowles, 2015; Lamichhaney et al., 2017; Cámara-Leret et al., 2020; Flantua et al., 2020), will help understanding climate change effects on unrelated taxa experiencing similar evolutionary processes (Condamine et al., 2018; Cortés et al., 2020; Nürk et al., 2020). Such systems offer natural experiments to assess the role of colonization and adaptation (Ding et al., 2020; McGee et al., 2020; Tito et al., 2020) in the past and ongoing responses to climate change, which undeniably will complement ecological predictive modeling.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material** and in the Dryad repository under https://doi.org/10.5061/dryad.gf1vhhmmp. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AC, JV, and SM conceived the study. JV compiled the data. JV and JM analyzed the data with contributions from AC and JL. AC, JV, and SM interpreted the data. AC wrote the manuscript with contributions from JV, and further edits made by the other co-authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.565708/full#supplementary-material

Supplementary Figure 1 | Frequency distributions of the number of records per taxon **(A)**, and the number of Páramo complexes **(B)** spanned by each of the 28 *Espeletia* taxa across Páramo sky islands in the Colombian Andes. Number of records, and number of Páramo complexes spanned for each taxon are drawn from **Supplementary Table S2**.

Supplementary Figure 2 | Pairwise Pearson correlation coefficients (*r*) among 19 WorldClim bioclimatic variables (in the baseline period 1970–2000), and Digital Elevation Model (DEM) at a 30 s (1 km^2) spatial resolution. Five bioclimatic variables and DEM did not exhibit collinearity problems (**Supplementary Table S3**) and were retained.

Supplementary Figure 3 | Ridgeline plots for the adaptive capacity **(A)**, climate sensitivity **(B)**, and vulnerability **(C)** scores across 36 Páramo complexes in the Colombian Andes. For comparative purposes, Páramo complexes are sorted in **(A)** from those with a low adaptive potential to anthropic pressures according to the enhancing and limiting factors depicted in **Figure 3**, in **(B)** from those likely to experience a range loss (distributions below zero) to those predicted to exhibit a range gain (distributions above zero) by 2050 (under RCP 8.5), while in **(C)** Páramo complexes at the top are the most vulnerable. Ridgeline plots summarize grid estimates shown in **Figure 4**.

Supplementary Figure 4 | Pairwise Pearson correlation coefficients (*r*) among climate sensitivity (S), adaptive capacity (AC), climate vulnerability (V) and the components of the adaptive capacity in the *Espeletia* complex across Páramo sky islands in the Colombian Andes. The adaptive capacity enhancing components were diversity (D), protected areas (PA) and forest area around the Páramo (F), while its limiting factors were agriculture (A), mining (M) and rural population density (RPD). The area of each of the 36 Páramo complexes is also considered in hectares (Ha). Correlation estimates and 95% confidence intervals are presented above the diagonal, while below the diagonal circles are colored and sized accordingly. Estimates are gathered from **Supplementary Table S7** and are negatively transformed for agriculture (A), mining (M) and rural population density (RPD) in order to aid interpretation.

Supplementary Table 1 | Occurrence data of 28 *Espeletia* taxa across Páramo sky islands in the Colombian Andes. Occurrences were gathered as georeferenced specimen data from GBIF. Given a starting set of 15,466 specimen records, a total of 1,432 were kept after cleaning for georeferencing consistency (i.e., non-redundant records located within the study area) and number of records per species (to avoid distributions with less than 10 points, as suggested by

Van Proosdij et al. (2016) for narrow-ranged species). *Espeletia* taxonomy follows Cuatrecasas (2013).

Supplementary Table 2 | Number of records for each of the 28 *Espeletia* taxa across Páramo sky islands in the Colombian Andes, and altitudinal range for each taxon. Number of records, number of Páramo complexes spanned, and minimum and maximum altitudes for each taxon are gathered from **Supplementary Table S1**.

Supplementary Table 3 | Variance Inflation Factor (VIF) for five non-collinear (VIF < 10) WorldClim bioclimatic variables considered for modeling at a 30 s (1 km^2) spatial resolution for the current and future scenarios, besides a Digital Elevation Model (DEM). Absolute *r*-values ranged from 0.05 (BIO18∼DEM) to 0.65 (BIO15∼BIO18).

Supplementary Table 4 | Current model validation based on average Area Under the ROC Curve (AUC) and porcentual contribution of the six non-collinear environmental variables for each of the 28 *Espeletia* taxa. AUC in the training and testing datasets are an aggregate measure of performance across all possible classification thresholds. Pseudo-absences (number of background points) are also averaged for each of the 28 putative taxa. Un-averaged statistics are kept in **Supplementary Table S5**. The six non-collinear variables included five WorldClim bioclimatic variables at a 30 s (1 m^2) spatial resolution. Environmental variables (**Supplementary Table S3**) are abbreviated as follows: (BIO2) Mean Diurnal Range, (BIO4) Temperature Seasonality, (BIO15) Precipitation Seasonality, (BIO18) Precipitation of Warmest Quarter, (BIO19) Precipitation of Coldest Quarter, and (DEM) Digital Elevation Model.

Supplementary Table 5 | Current model validation based on Area Under the ROC Curve (AUC) and relative contribution of the six non-collinear environmental variables for each of the 10 runs in the 28 *Espeletia* taxa. The following overall summary statistics are kept: optimum training subset, regularized training gains, un-regularized training gains, effective iterations, training AUC, optimum testing subset, test gains, test AUC, AUC standard deviation, pseudo-absences (number of background points), and entropy. The following relative contribution scores are kept for the six environmental variables: contribution, permutation importance, training gain, test gain and AUC without and with only each variable. The six non-collinear environmental variables (**Supplementary Table S3**) included five WorldClim BIO variables at a 30 s (1 km^2) spatial resolution, and are coded as: (BIO2) Mean Diurnal Range, (BIO4) Temperature Seasonality, (BIO15) Precipitation Seasonality, (BIO18) Precipitation of Warmest Quarter, (BIO19) Precipitation of Coldest Quarter, and (DEM) Digital Elevation Model.

Supplementary Table 6 | Current model validation based on Akaike (AIC) and Bayesian (BIC) Information Criteria per repetition of each of the 28 *Espeletia* taxa. Optimum training and testing subsets are summed under N_{TR+TS}.

Supplementary Table 7 | Climate sensitivity (S), adaptive capacity (AC), and climate vulnerability (V) in the *Espeletia* complex across Páramo sky islands in the Colombian Andes. The adaptive capacity enhancing components were diversity (D), protected areas (PA) and forest area around the Páramo (F), while its limiting factors were agriculture (A), mining (M) and rural population density (RPD). Diversity (D), which is the number of *Espeletia* taxa totalized in a 1 km grid based on the occurrence data downloaded from GBIF, and the inverse value of the normalized rural population density (RPD) were scaled from 0 to 1. A total of 1,050 protected areas (PA) were rated as 1, 0.3, 0.1, and 0.1 for National Natural Parks, Civil Society Reserves, Regional Natural Parks and National Protected Forest Reserves, respectively, following (CIAT, 2017). Forest patches (F) around the Páramo complexes (2 km around the tree line, 1 km grid) were recorded as presence points (1 = presence), while agriculture (A) and mining (M) for the period 2010–2012 were marked as null data points (0 = presence) within the study area and in its buffer zone (2 km around the tree line, 1 km grid). The area of each of the 36 Páramo complexes is also presented in hectares (Ha), as well as its name and ID number following **Figure 2A**, Morales et al. (2007) and Londoño et al. (2014).

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Plant Regeneration Above the Species Elevational Leading Edge: Trade-Off Between Seedling Recruitment and Plant Production

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Mountains and their biota are highly threatened by climate change. An important strategy that alpine plants use to escape this change consists in seed dispersal and the ability of seeds to germinate and establish in new sites at higher elevation. Little is known about the environmental factors that can affect the regeneration of plants above the elevational limit of growth. We present the first field evidence of recruitment success and plant performance in consequence of upward shift from the alpine to the nival life zone. Seeds of four alpine grassland species were sown at the current elevational limit of growth (site A) and 200 m upward, in a nival environment (site N) located in the Italian Alps. At site N part of the seeds were subjected to experimental manipulation of temperature (using an Open Top Chamber, OTC) or soil (using soil from site A). Recruitment success, soil surface temperature and water potential were monitored for five consecutive years. At the end of the experiment, vegetative growth and foliar traits were measured on individuals from all treatments. Mean annual soil surface temperature and length of the growing season at site A were *ca.* 2°C higher and *ca.* 44 days longer than at site N. Seedling emergence and seedling establishment generally were higher at N (with or without OTC) on local soil than at site A or at site N with soil originating from site A. Conversely, production was higher at site A and at site N with soil originating from site A. Recruitment success above the elevational leading edge was enhanced by coarser and nutrient-poor soil, which promoted seedling emergence and establishment but constrained plant growth. This trade-off between seedling recruitment and plant production underlines adaptive consequence and environmental filtering, both critical to forecast community assembly and responses of alpine species to climate warming.

Keywords: alpine plants, ecological niche, foliar traits, germination, global warming, soil

INTRODUCTION

Mountains and their biota are highly threatened by climate change and are experiencing among the highest rates of warming (Pepin et al., 2015). Assessments of the impacts of climate change on global mountain systems foresee temperature warming between 1.8 and 4°C over the European Alps for the period 2051–2080 (Zimmermann et al., 2013). Such temperature increase is associated with a decrease in depth and duration of snow cover in mountain habitats (Marty, 2013; Frei et al., 2018), leading to longer growing seasons and higher frequency of frost events in winter (Gerdol et al., 2013). In response to these changes there are documented upward shifts of tree species within forests (Lenoir et al., 2008), tree-line ecotones (Du et al., 2018) and alpine species above tree line (Parolo and Rossi, 2008; Pauli et al., 2012), which results in plant community thermophilisation and changes in species richness (Gottfried et al., 2012). Recent observations revealed that this trend has accelerated, especially during the last 20–30 years (Steinbauer et al., 2018).

An important strategy that plants use to escape unsuitable environmental conditions driven by climate warming consists of seed dispersal and the ability of seeds to germinate and establish in new sites at higher elevations (Parolo and Rossi, 2008; Vittoz et al., 2009). Regeneration from seeds is also crucial for species persistence, because inherent genetic diversity and phenotypic plasticity of seeds may help species to adapt to future climate conditions (Walck et al., 2011; Bernareggi et al., 2016). Consequently, an increasing number of studies have addressed the effects of warming on seed germination and seedling establishment (see Briceño et al., 2015 for review). However, little is known about the regeneration capacity of plant species above the elevational leading edge of their distribution range, where new generations of species are expected to move.

The steep environmental gradients occurring in alpine habitats provide an ideal context for evaluating shifts of the species ecological niche (*sensu* Hutchinson, 1957) or just part of it, i.e., the recruitment niche (*sensu* Grubb, 1977; Young et al., 2005). Glacier foreland habitats are of additional interest because they represent highly attractive sites for studying primary dynamic succession (Matthews, 1992), thereby for understanding how the abiotic environment prevents establishment or persistence of plant species in the absence of (or at least in the presence of low) biotic interactions (Kraft et al., 2015). Glacier forelands experienced a substantial increase in species richness during the twentieth century (Holzinger et al., 2008; Fickert and Grüninger, 2018). Pioneer species inhabiting these habitats show high germination potential (Schwienbacher et al., 2012; Mondoni et al., 2015) and ability to grow relatively rapidly on nitrogen (N)—poor soils because of their inherently high nutrient uptake rates and photosynthetic efficiency (Chapin, 1993; Vittoz et al., 2009). Temperature warming *per sé* cannot represent the main driver of the upwards shift of species distribution because multiple environmental changes are needed to elicit vegetation dynamics in pioneer soils (Matthews, 1992). For example, seedlings are highly sensitive to desiccation (Leck et al., 2008) so that soil moisture may represent an important factor limiting seedling establishment, especially in glacier forelands where the coarse grain of the soil particles and the low humus content result in low water holding capacity of the soil (Körner, 2003). Furthermore, successful establishment of seedlings depends on availability of nutrients, mostly N and phosphorus (P), that limit plant growth in many alpine and arctic ecosystems (Van Wijk et al., 2004; Lett and Dorrepaal, 2018). Recruitment mechanisms in alpine plants are well known (e.g., Chapin et al., 1994; Jumpponen et al., 1999; Erschbamer et al., 2001). However, there is limited understanding of the environmental filters constraining species upward migration. Furthermore, little is known on how critical life stages, especially seedling recruitment and subsequent adult plant growth, respond to changing environment. Predicting the effects of global warming requires an understanding of how species are affected by the environment, which is most easily understood by investigating variation along gradients (McGill et al., 2006).

We present the first field evidence of seedling recruitment and plant performance in response to upward shift from the alpine life zone to the glacier foreland life zone, henceforth referred to as "nival zone" because of similarities among vegetation types (see paragraph "Study Area" for details). Seeds of four alpine grassland species were sown at the current elevational limit of growth and 200 m upward. At the high-elevation site part of the seeds were subjected to experimental manipulation of temperature or soil features. We hypothesized that alpine grassland species germinate less and are unable to establish in nival environment due to low temperature, dry and nutrient-poor soil, all constraining plant growth. We further hypothesized that weakly increased temperature and/or soil maturity enhance both seedling emergence and establishment of alpine species in nival environments.

MATERIALS AND METHODS

Study Area

The study area (**Figure 1**) is located in the eastern Alps of Italy (Sondrio Province, Lombardy). The low-elevation site is located in the alpine vegetation belt (henceforth referred to as site A), at ca. 2,300 m (46°24′25.4″N, 10°12′24.7″ E), on stable slopes colonized by *Carex curvula* grasslands and small patches of *Empetrum hermaphroditum—Loiseleuria procumbens* dwarf heathlands. The high-elevation site is located in the nival vegetation belt (*sensu* Körner, 2003; henceforth referred to as site N), at *ca.* 2,500 m (46°24′12.6″N, 10°12′46.4″E), on the Dosdè glacier moraine. At the N site, vegetation consists of highly fragmented pioneer scree communities typical of the nival life zone (Reisigl and Keller, 1990), with *Saxifraga aizoides*, *S. oppositifolia*, *Cerastium pedunculatum*, *Oxyria digyna,* and *Geum reptans* as the most frequent species.

Climatic features were derived from Worldclim datasets (Hijmans et al., 2005) at a spatial resolution of 30 arc s (i.e., 1 km), using the coordinates of the species growing site (site A). This area has mean annual rainfall of *ca.* 1,082 mm, mean annual temperature of 0.61°C, with average

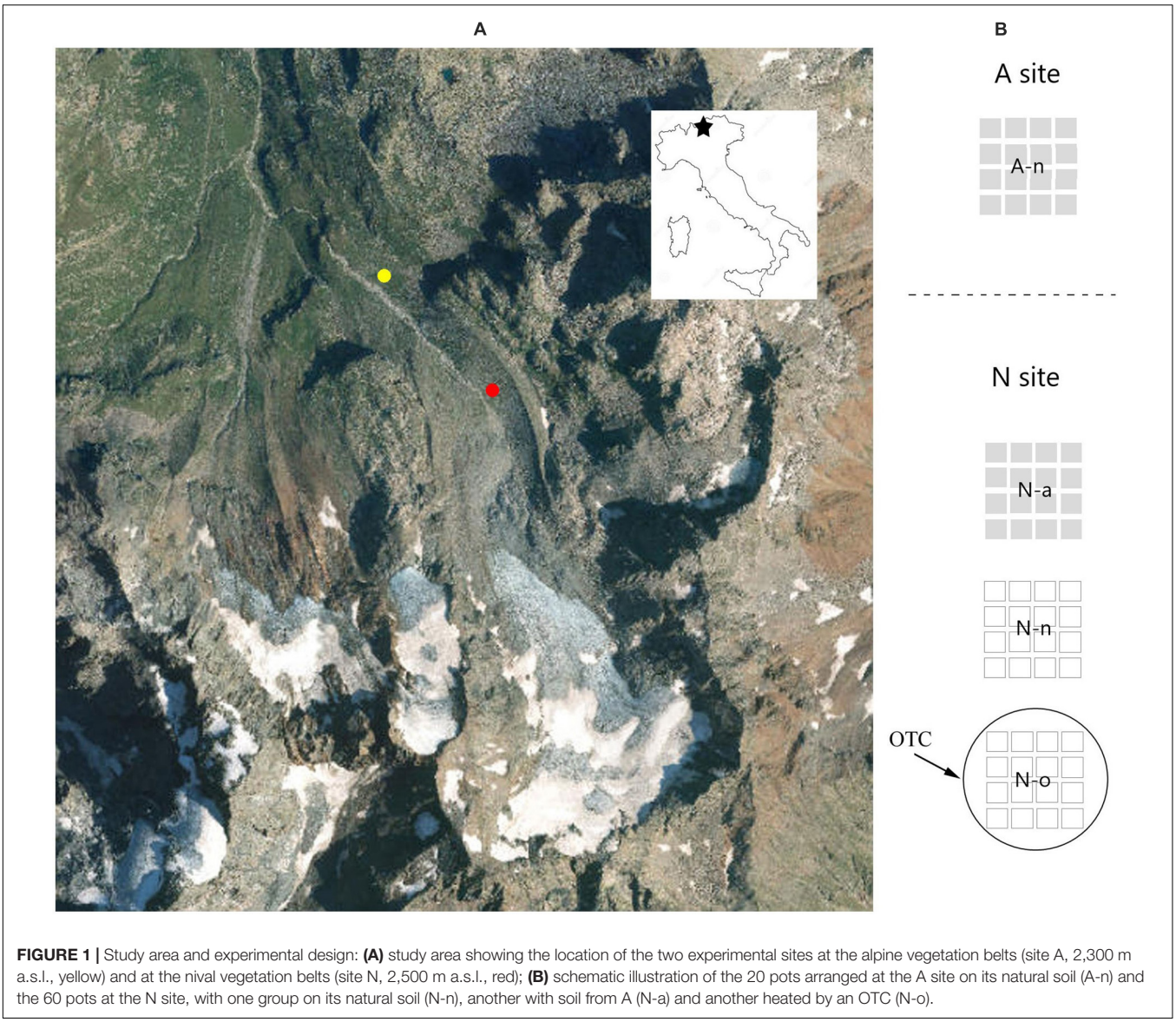


FIGURE 1 | Study area and experimental design: **(A)** study area showing the location of the two experimental sites at the alpine vegetation belts (site A, 2,300 m a.s.l., yellow) and at the nival vegetation belts (site N, 2,500 m a.s.l., red); **(B)** schematic illustration of the 20 pots arranged at the A site on its natural soil (A-n) and the 60 pots at the N site, with one group on its natural soil (N-n), another with soil from A (N-a) and another heated by an OTC (N-o).

minimum of −6.3°C in the coldest months (January-February) and average maximum of 8.45°C in the warmest month (July) at 2,300 m. Based on climatic data measured at the closest weather station (Livigno La Vallaccia, 46°28′40.9″N 10°12′22.0″E; 2,660 m above the sea level, *ca.* 7 km apart), during the last 18 years there was a weak but not significant increase in annual and summer temperature of *ca.* 0.3 and 0.8°C, respectively (**Supplementary Figure S1**). Bedrock geology in the whole area consists of siliceous acidic rocks (mostly gneiss).

Study Species and Experimental Set-Up

Seeds of four species belonging to the Asteraceae family [*Arnica montana* L., *Hieracium pilosella* L., *Homogyne alpina* (L.) Cass., and *Leontodon helveticus* Mérat] were collected on 9 September 2013, at the time of natural dispersal (Hay and Smith, 2003). Seeds were collected at site A, lying at approximately the local leading edge of the elevational distribution of the four species. These species were selected based on two criteria. First, they are overall abundant in the alpine vegetation belt. Second, they may represent potentially good colonizers of higher-elevation sites because of diaspore traits facilitating long-distance dispersal (Parolo and Rossi, 2008; Vittoz et al., 2009).

Immediately after collection, the seeds were exposed to environmental conditions occurring at both sites (A and N). At each site, experiments involved sowing four replicates of 100 seeds of the four species, each on a 10 × 10 cm square pot filled with natural soil at both sites, henceforth referred to as A-n (at site A) and N-n (at site N), respectively; soil was sieved removing any visible plant material from resident vegetation. The pots were adapted making large openings on the sides and removing the bottom to allow water circulation in the root zone. To investigate the effect of soil and temperature on recruitment success, the same experimental design was repeated two more times at site

N, either using soil originating from site A (henceforth referred to as N-a), or an Open Top Chamber (OTC; Marion et al., 1997) placed on the pots (henceforth referred to as N-o). The OTC followed the cone-design proposed by the International Tundra Experiment (ITEX), that is known to increase the mean daily air temperature by *ca.* 1.5°C, which approximately reflects the temperature difference between sites A and N (by assuming a vertical temperature lapse of *ca.* 0.6°C per 100 m elevation; Körner, 2003). Every year, the OTC was placed in the field immediately after snowmelt and removed at the end of the growing season. In each treatment, four more pots were left empty to serve as controls, so that a total of 80 pots were established, with 20 pots (4 species × 4 replicates × 1 treatment, plus 4 controls) at site A (i.e., treatment A-n) and 60 pots (4 species × 4 replicates × 3 treatments, plus 12 controls) at site N (i.e., treatments N-n, N-a, N-o).

Data loggers, recording soil temperature (Tiny Tag, Gemini, Chichester, United Kingdom) and soil water potential (SWP) at hourly intervals (MicroLog SP3, Environmental Measuring Systems, Brno, Czech Republic), were buried at a depth of 2 cm in each treatment (i.e., one data logger for measuring soil T and SWP in A-n, N-n, N-a, and N-o).

Periodic Measurements, Final Sampling, and Analyses

Prior to the start of the experiment, samples of soils were taken and subjected to granulometric analyses. The soils were sieved using five different meshes (10, 6, 3, 1, and 0.125 mm) and weighed. A 20 mg subsample of fine soil material (<0.125 mm) was used for analyzing total carbon (C) by a Shimadzu TOC-5000A (Shimadzu Corporation; Kyoto, Japan), connected with a solid sample module (Shimadzu SSM- 5000A). A 100 mg subsample of fine soil material was extracted in 3 ml of selenous H_2SO_4 at 420°C and analyzed for total N concentration by the salicylate method and total P concentration by the molybdenum blue method using a continuous flow autoanalyzer (FlowSys; Systea, Anagni, Italy). The whole soil material was used for setting up the square pots.

The experiment lasted 5 years (from September 2013 to July 2018). Seedling emergence (number of emerged seedlings/number of seeds sown) and seedling establishment (number of survived seedlings/number of seeds sown) were monitored at *ca.* 15-day intervals during the snow-free period for 2 years (from September 2013 to September 2015) and then at monthly intervals for the other 3 years (from September 2015 to July 2018).

At the end of the experiment (24 July 2018) all pots were harvested and the plants used for determining above ground production. The plant material was also used for measuring chlorophyll fluorescence (F_v/F_m), chlorophyll content, specific leaf area (SLA), leaf dry matter content (LDMC), foliar N and P concentrations and foliar N/P ratio, i.e., stomatal density (SD), stomatal length (SL) and C isotope discrimination ($\delta^{13}C$). F_v/F_m, chlorophyll content, SLA, LDMC, foliar N and P concentrations and foliar N/P ratio are functional traits controlling major plant functions, especially photosynthetic capacity (Pérez-Harguindeguy et al., 2013). On the other hand, SD and SL are related to drought stress (Sun et al., 2014) while $\delta^{13}C$ can be regarded as a proxy of water use efficiency (Cernusak et al., 2013). All of these variables eventually affect plant production and can hence be used as indicators of plant growth potential (Tonin et al., 2019). Below we provide details on the methods for each of these measurements.

Chlorophyll *a* fluorescence was determined on three mature healthy leaves by a portable fluorimeter (OS1-FL, Opti-Sciences, Hudson, NH, United States). A small leaf-clip adapter was placed on the central portion of each leaf for 1 min to achieve dark pre-adaptation before determining F_o. F_m was obtained after a saturating pulse of actinic light. The ratio F_v/F_m [where $F_v = (F_m - F_o)$] was used to assess the photochemical efficiency of photosystem II. Non-destructive assessment of chlorophyll content based on apparent leaf transmittance using leaf-clip-type sensors (CCM-200, Opti-Sciences, Hudson, NH, United States) was carried out on three other mature healthy leaves from different individuals. The mean values of chlorophyll fluorescence and chlorophyll content calculated across the three measurements were used as single values for each pot. Five to fifteen healthy leaves (depending on leaf size) taken from different individuals were used for determining SLA and LDMC. The leaves were fresh-weighed and scanned using the Scion Image version Alpha 4.0.3.2 (Scion Corporation, Frederick, MD, United States). Then, the scanned leaves were oven-dried at 40°C for 48 h and weighed for determining dry leaf mass and then calculating SLA. The ratio between dry mass and fresh mass was used for determining LDMC. The remainder of the aboveground plant material was oven-dried at 40°C for 48 h and subsequently weighed. The dry weight of this material plus that of the leaves used for determining chlorophyll fluorescence, chlorophyll content, SLA and LDMC represented aboveground production of a given species in a given pot. Three subsamples of dry leaves were used for further analyses.

- *Ca.* 50 mg of dry leaf tissue were powdered, extracted and analyzed for total N and total P concentrations as for the soils.

- Five to ten leaves (depending on leaf size) were used for stomatal measurements. As all four species are amphistomatic, stomatal measurements were carried out on both the abaxial and the adaxial sides of the leaves by the clear nail polish method (Hilu and Randall, 1984). All measurements were performed using a light microscope (Leica DMLS, Leica Biosystems, Nussloch, Germany), and an attached digital camera (DeltaPix, Måløv, Denmark), at 400× magnification. To estimate stomatal density (SD) the number of stomata was counted in five different fields of view for each epidermal impression. Stomatal length (SL) was measured using the software DeltaPix, v.3.2.x.

- *Ca.* 2 mg of dry leaf tissue were powdered and used for determining stable C isotope discrimination ($\delta^{13}C$) with an elemental analyzer (EA 1110, Carlo Erba, Milan, Italy) coupled online with an isotope ratio mass spectrometer (delta Plus XP, ThermoFinnigan, Bremen, Germany). Carbon isotope discrimination and expressed as in formula (1):

$$\delta^{13}C_{sample} = ({}^{13/12}R_{sample}/{}^{13/12}R_{V-PDB}) - 1 \qquad (1)$$

where V-PDB is the primary international standard *Belemnitella americana* from the Pee Dee Formation.

Statistical Analyses

Statistical analyses of the data were carried out with the objective to limit the effect of spatial autocorrelation of samples within sites. For this purpose, we ran partial regression analyses for all target variables on binary site explanatory variables and used the regression residuals for assessing effects of species and/or treatment on all target variables (Legendre, 1993).

The data on soil granulometry (i.e., the percentage of weight in the six particle size classes: 10, 6, 3, 1, 0.125 and < 0.125 mm) were analysed by a multivariate ANOVA with treatment as fixed factor. The data on soil chemistry, i.e., soil C concentration (soil C), soil N concentration (soil N), soil P concentration (soil P) and soil N/P ratio (soil N/P) were analysed each by a univariate ANOVA with the soil chemical variable as the response variable and treatment as the fixed factor. Significance of differences of the means among treatments were assessed using the Fishers' LSD *post hoc* test.

The data on total seedling emergence and establishment were analysed each by a generalized linear mixed model (GLMM) with binomial error and logit-link function. In the two GLMMs, seedling emergence and seedling establishment were the response variables, while species, treatment and their interaction were the fixed effects; replicates were considered as random effects. In order to avoid negative values in the GLMMs, the partial regression residuals were normalized according to formula (2):

$$X_{ni} = [(X_{oi} - X_{min})/(X_{max} - X_{min})] \times 100 \quad (2)$$

where X_{ni} is the normalized value of the i-th regression residual for the X-variable (i.e., seedling emergence or seedling establishment, respectively); X_{oi} is the original value of the i-th regression residual for the X-variable; X_{min} is the minimum value of all regression residuals for the X-variable and X_{max} is the maximum value of all regression residuals for the X-variable.

The relationship between seedling emergence and establishment was analysed by linear regressions.

The data on production and foliar traits, i.e., chlorophyll fluorescence (F_v/F_m), chlorophyll content, SLA, LDMC, foliar N concentration (leaf N), foliar P concentration (leaf P), foliar N/P ratio (leaf N/P), SD, SL and $\delta^{13}C$ were analysed each by a univariate factorial ANOVA with production or foliar traits as the response variable and species, treatment and their interaction as fixed factors. Significance of differences of the means among treatments were assessed by Fishers' LSD *post hoc* test. Relationships between production and foliar traits were analysed by Pearson's correlation coefficients.

Relationships among seedling emergence, seedling establishment, production, soil T, SWP, soil granulometry and soil chemistry were analysed by a multivariate statistical method (principal component analysis; PCA). The analysis was based on the correlation matrix among eight variables: percentage seedling emergence, percentage seedling establishment, production, mean soil T during the growing seasons 2014–2017, SWP during the growing seasons 2014–2017, percentage weight of coarse soil particles (i.e., 10 mm + 6 mm), soil N and soil P. The PCA provided centred standardized scores for the seven variables, after scaling to unit variance all variable vectors. Production was calculated as mean values of production of the four species in each of the four treatments. As production differed strongly among species because of intrinsic species-specific features, the data of production were normalized to maximum values prior to calculating the means. Normalisation was performed according to formula:

$$X_{ni} = (X_{oi}/X_{max}) \times 100 \quad (3)$$

where X_{ni} is the normalized value of production for the X-species at the i-pot; X_{oi} is the original value of production for the X-species at the i-pot; X_{max} is the maximum value of production for the X-species across all pots.

The statistical analyses were performed using the packages SPSS 21 (IBM SPSS Statistics, Armonk, New York, United States) and STATISTICA 6 (StatSoft Inc., Tulsa, OK, United States).

RESULTS

Environment: Climate and Soil

Mean annual soil temperature at site A (treatment A-n) was *ca.* 2°C higher than at site N (treatments N-n and N-a); the OTC (treatment N-o) increased the mean annual soil temperature by *ca.* 0.5°C at site N (**Table 1**). The growing season (considered as the snow-free period) was on average *ca.* 1.7°C warmer and *ca.* 44 day longer at A-n (approx. mid May–October) than at N-n and N-a (July–October), mostly because of earlier snowmelt in spring (**Supplementary Table S1**). In the absence of OTC, the plants at site N accumulated 642 degree-days less than at site A (**Supplementary Table S1**); during the growing season, the OTC strongly buffered the differences in soil temperature and slightly increased the accumulated heat at site N. The SWP was similar in the four treatments. The SWP always was high, i.e., barely below zero, and ranged from *ca.* −0.04 MPa during the growing season to *ca.* −0.30 MPa in winter. The mean annual SWP was *ca.* −0.20 MPa in all treatments (**Table 1** and **Supplementary Table S1**).

TABLE 1 | Mean annual soil temperature and mean annual soil water potential (SWP) in the four years at the alpine vegetation belt (site A) on its natural soil (A-n) and at the nival vegetation belt (N site) on its natural soil (N-n), on soil originating from site A (N-a), and under the OTC (N-o).

Year	Soil T (°C)				SWP (MPa)			
	A-n	N-n	N-a	N-o	A-n	N-n	N-a	N-o
2014	4.53	2.66	2.65	3.21	−0.03	−0.04	−0.05	−0.03
2015	4.76	2.65	2.67	3.02	−0.19	−0.16	−0.15	−0.17
2016	4.72	2.58	2.60	3.21	−0.30	−0.34	−0.37	−0.36
2017	5.48	3.54	3.54	4.16	−0.29	−0.32	−0.34	−0.34
Average	4.87	2.86	2.87	3.40	−0.20	−0.22	−0.23	−0.22

Data for 2013 and 2018 are not reported because incomplete (i.e., the monitoring started in September 2013 and ended in July 2018).

Soil granulometry differed significantly among treatments [*Wilks'*$\Lambda = 0.02$, $F_{(5,\ 15)} = 4.06$, $P = 0.002$]. The soils in A-n and N-a were poorer in coarse particles (size > 6 mm) compared to the soils in N-n and N-o (**Table 2**). The soils in A-n and N-a were richer in organic matter and hence presented higher C content [$F_{(3,\ 11)} = 85.40$, $P < 0.001$] compared to the soils in N-n and N-o (**Figure 2A**). The soils in A-n and N-a also had higher N content [$F_{(3,\ 11)} = 38.04$, $P < 0.001$; **Figure 2B**] whereas the soil P content [$F_{(3,\ 11)} = 0.53$, $P = 0.67$] did not differ among treatments (**Figure 2C**). The soil N/P ratio mirrored the trend of soil C and soil N contents with significantly [$F_{(3,\ 11)} = 63.46$, $P < 0.001$] higher values in A-n and N-a than in N-n and N-o (**Figure 2D**).

Seedling Recruitment

Seedling emergence was significantly different across species [$F_{(3,\ 46)} = 222.3$; $P < 0.001$] and treatments [$F_{(3,\ 46)} = 126.0$; $P < 0.001$] with significant species × treatment interaction [$F_{(9,\ 46)} = 29.6$; $P < 0.001$]. The overall mean emergence was 56%, with the four species being ranked as follows: *L. helveticus* (76%) = *H. alpina* (71%) > *A. montana* (43%) = *H. pilosella* (35%). In general, seedling emergence was highest in N-n and lowest in N-a for all species except for *H. alpina*, that presented lowest germination in A-n, and for *A. montana* for which germination was similar across treatments except in N-a (**Figures 3A–D**). Seedling emergence occurred in the growing seasons 2014 and 2015 and no further germination was observed in the growing seasons 2016–2018 (**Supplementary Figure S2**). Germination mostly occurred during the first month after snowmelt, i.e., in June at site A and in July at site N. Afterwards, there was only minor emergence (but see *A. montana* and *H. pilosella* in N-n and N-o; **Supplementary Figures S2A,C**).

Seedling establishment was significantly different across both species [$F_{(3,\ 46)} = 129.5$; $P < 0.001$] and treatments [$F_{(3,\ 46)} = 56.8$; $P < 0.001$], with strong species × treatment interaction [$F_{(9,\ 46)} = 18.9$; $P < 0.001$; **Figures 3A–D**]. Seedling establishment was overall lower than seedling emergence (21%), with following ranking: *L. helveticus* (39%) > *A. montana* (19%) = *H. pilosella* (16%) > *H. alpina* (8%). Seedling establishment was highest in N-n and N-o for *H. pilosella* and *L. helveticus* (**Figures 3C,D**). Seedling establishment was strongly depressed in N-o for *H. alpina* and was highest only in N-n for *A. montana* (**Figures 3A,B**). Similar to seedling emergence, seedling establishment was lowest in N-a for *A. montana*, *H. pilosella* and *A. helveticus* (**Figures 3A,C,D**). In contrast, seedling establishment in A-n was extremely low for *H. alpina* (**Figure 3B**).

Production and Foliar Traits

Production differed significantly among species and treatments (**Table 3**). Production of *A. montana*, *H. pilosella,* and *L. helveticus* was highest in A-n, intermediate in N-a and lowest in both N-n and N-o (**Figure 4A**). Conversely, production of *H. alpina* was similar in A-n, N-a, and N-n and could not be recorded in N-o because no seedlings of *H. alpina* survived in this treatment (**Figure 4A**). Such different production patterns resulted in a significant species × treatment interaction (**Table 3**). F_v/F_m was unaffected by species and treatments (**Table 3** and **Figure 4B**), whereas chlorophyll content did not differ among species but generally was highest in A-n and/or N-a (**Table 3** and **Figure 4C**). All of the other foliar traits differed significantly across species, which reflected intrinsic differences among species (**Tables 3**, **4** and **Figure 4**). Most foliar traits were strongly affected by treatment, with the exceptions of leaf P, SL, and foliar

TABLE 2 | Soil granulometry at the alpine vegetation belt (A site) on its natural soil (A-n) and at the nival vegetation belt (site N) on its natural soil (N-n), on soil originating from site A (N-a) and under the OTC (N-o).

Particle size	A-n	N-n	N-a	N-o
10 mm	0.5 ± 0.3	3.0 ± 2.0	0.7 ± 0.6	4.1 ± 0.5
6 mm	3.8 ± 1.0	7.9 ± 0.8	3.3 ± 0.4	8.6 ± 0.9
3 mm	14.6 ± 0.4	11.9 ± 0.4	8.3 ± 0.8	13.7 ± 0.8
1 mm	32.2 ± 1.6	28.3 ± 0.8	29.3 ± 0.8	28.3 ± 0.8
0.125 mm	23.7 ± 1.3	21.7 ± 0.5	27.1 ± 0.7	20.1 ± 0.1
< 0.125 mm	25.2 ± 1.4	27.1 ± 0.6	31.3 ± 2.0	25.2 ± 0.8

The data are weight percentages (mean ± 1 SE, n = 4) in six soil particle size classes.

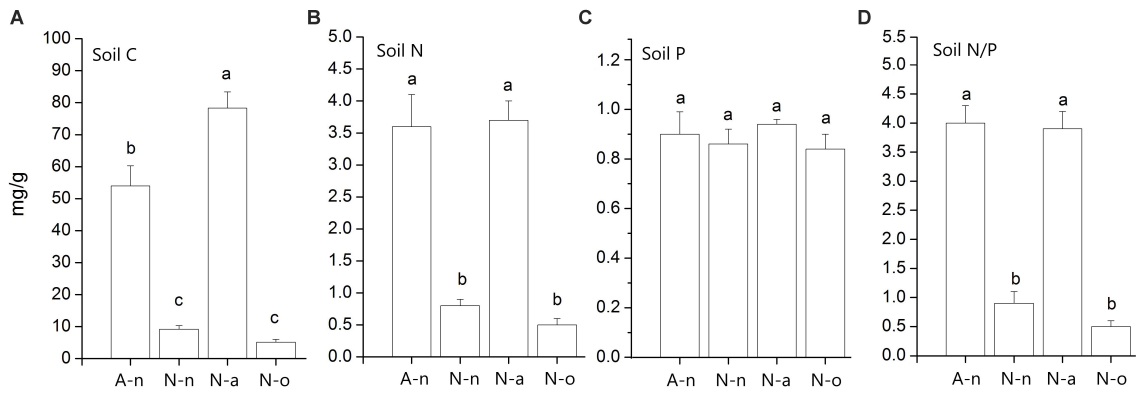


FIGURE 2 | Mean (± 1 SE; *n* = 4) values of soil C content **(A)**, soil N content **(B)**, soil P content **(C)** and soil N/P ratio **(D)** in the four treatments. Within each panel different letters represent significant ($P < 0.05$) differences.

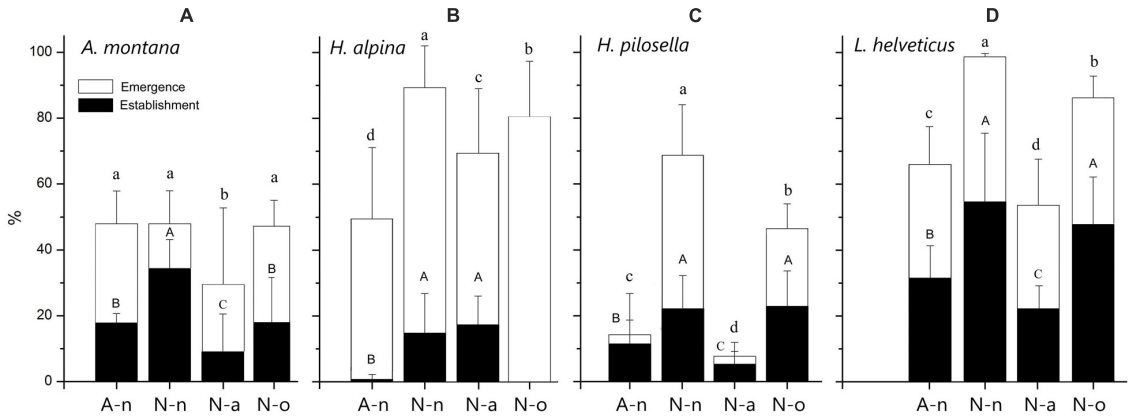


FIGURE 3 | Final seedling emergence and establishment percentage (± 95% CI) for all species at the alpine vegetation belt (site A) on its natural soil (A-n) and at the nival vegetation belt (site N) on its natural soil (N-n), on soil originating from site A (N-a) and under the OTC (N-o). Within each panel different letters represent significant ($P < 0.05$) differences of emergence (lower-case) and establishment (upper-case).

TABLE 3 | Results from univariate factorial analyses of variance (ANOVAs; *F*-values and associated d.o.f. for numerator and denominator in parenthesis) used to assess the effects of species identity, treatment and their interaction on production and foliar traits.

Variable	Species	Treatment	Species × treatment
Production	**7.73 (3,39)**	**3.28 (3,39)**	**6.74 (9,39)**
F_v/F_m	0.97 (3,40)	0.55 (3,40)	0.92 (9,40)
Chlorophyll	1.45 (3,40)	**4.09 (3,40)**	1.77 (9,40)
SLA	**83.73 (3,40)**	**2.99 (3,40)**	2.07 (9,40)
LDMC	**20.54 (3,40)**	**6.27 (3,40)**	1.43 (9,40)
Leaf N	**10.59 (3,35)**	**11.04 (3,35)**	1.34 (9,35)
Leaf P	**17.90 (3,35)**	1.09 (3,35)	1.37 (9,35)
Leaf N/P	**3.71 (3,35)**	**5.91 (3,35)**	1.57 (9,35)
SD	**19.04 (3,30)**	**10.12 (3,30)**	**3.03 (9,30)**
SL	**14.42 (3,30)**	0.13 (3,30)	1.16 (9,30)
$\delta^{13}C$	**13.85 (3,40)**	0.74 (3,40)	1.89 (9,40)

Bold characters represent significant values ($P < 0.05$).

$\delta^{13}C$ (**Tables 3**, **4**). The four species presented similar responses to the treatments in terms of SLA, LDMC, leaf N, and leaf N/P ratio, as shown by the lack of significant species × treatment interactions for these variables (**Table 3**). SLA generally was higher in A-n and N-a compared to N-n and N-o while the reverse was true for LDMC, although the differences were not always significant (**Figures 4D,E**). In *A. montana*, *H. pilosella,* and *L. helveticus* N concentrations always were ranked as follows A-n > N-a > N-n = N-o whereas the N/P ratio varied erratically across treatments. Few data on foliar nutrient concentrations were available for *H. alpina* because of scarcity of material for chemical analyses (**Figures 4F–H**). SD generally was higher at site N compared to site A but the response of SD to the treatments varied differently among species which resulted in a significant species × treatment interaction (**Tables 3**, **4**). Finally, a total of 6 individuals of *H. pilosella* and 9 of *L. helveticus* reached sexual maturity by the end of the monitoring, flowering only at site A (data not shown).

Relationships Among Seedling Recruitment, Production, Foliar Traits, and Environmental Factors

Across all species and treatments, seedling establishment was positively correlated with seedling emergence [$F_{(1, 60)} = 15.567$; $P < 0.001$]. Production was strongly positively correlated with chlorophyll content and leaf N (**Table 5**) and presented slightly lower positive correlations with leaf P and $\delta^{13}C$, and negative correlation with LDMC (**Table 5**). The results of PCA revealed a negative association between seedling recruitment and production as the seedling emergence and seedling establishment vectors were both located in the right-hand side of the diagram whereas the production vector had opposite orientation (**Figure 5**). The PCA diagram also revealed a close association between plant production and soil nutrient contents, i.e., soil N and to a lesser extent soil P. On the other hand, seedling emergence and seedling establishment were related to soil granulometry, i.e., the percentage of coarse size particles in the soil (**Figure 5**). Soil T and SWP were unrelated to both seedling recruitment and production because the vectors of soil T and SWP were oriented orthogonally to the vectors of seedling emergence, seedling establishment, and production (**Figure 5**).

DISCUSSION

In this study, we investigated seedling recruitment and plant production in response to upward shift from the alpine to the nival life zone. We hypothesized that alpine grassland species are unable to establish in nival environment due to low temperature, dry and nutrient-poor soil, but that weakly increased temperature and/or soil maturity can enhance both recruitment from seeds and production of alpine plants in nival environment.

Seedling Recruitment

Emergence and establishment of seedlings can be affected by both abiotic and biotic factors (see Larson and Funk, 2016

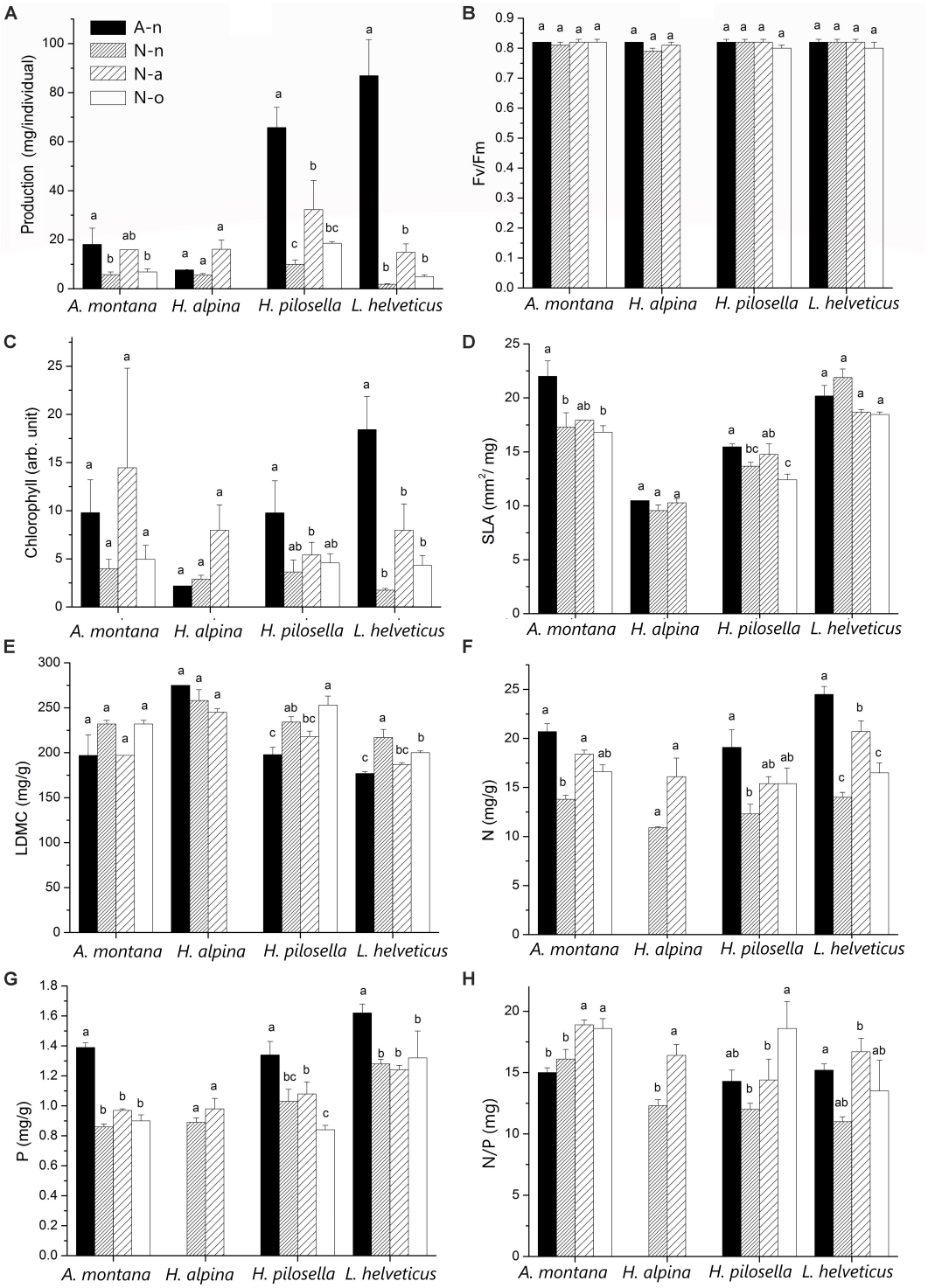


FIGURE 4 | Mean (± 1 SE; *n* varies among species and treatments in relation to availability of leaf material) values of **(A)** production, **(B)** chlorophyll fluorescence (F_v/F_m), **(C)** chlorophyll content, **(D)** specific leaf area (SLA), **(E)** leaf dry matter content (LDMC), **(F)** foliar N concentration, **(G)** foliar P concentration and **(H)** foliar N/P ratio for each species at the alpine vegetation belt (site A) on its natural soil (A-n) and at the nival vegetation belt (site N) on its natural soil (N-n), on soil originating from site A (N-a) and under the OTC (N-o). Within each panel, different letters represent significant ($P < 0.05$) differences.

TABLE 4 | Mean (± SE, *n* = 4) values of stomatal density (SD), stomatal length (SL) and foliar stable carbon isotope discrimination ($\delta^{13}C$) for the four species at the alpine vegetation belt (site A) on its natural soil (A-n) and at the nival vegetation belt (site N) on its natural soil (N-n), on soil originating from site A (N-a), and under the OTC (N-o).

Variable	Species	A-n	N-n	N-a	N-o
SD (mm^2)	*A montana*	197 ± 21^{b}	294 ± 25^{ab}	375 ± 2^{a}	401 ± 52^{a}
	H. alpina	–	294 ± 10^{b}	463 ± 16^{a}	–
	H. pilosella	387 ± 53^{b}	415 ± 38^{b}	700 ± 32^{a}	509 ± 43^{b}
	L. helveticus	334 ± 23^{a}	376 ± 27^{a}	403 ± 40^{a}	348 ± 31^{a}
SL (μm)	*A. montana*	39.6 ± 0.9	34.8 ± 0.4	35.4 ± 1.7	30.2 ± 4.8
	H. alpina	–	33.8 ± 7.0	40.1 ± 0.3	–
	H. pilosella	28.2 ± 1.0	28.0 ± 0.6	25.0 ± 1.5	23.9 ± 1.0
	L. helveticus	35.6 ± 1.6	39.7 ± 2.1	37.2 ± 1.6	40.7 ± 2.1
$\delta^{13}C$ (‰)	*A. montana*	−29.55 ± 0.53	−29.10 ± 0.07	−29.96 ± 0.07	−29.20 ± 0.74
	H. alpina	−28.38	−29.31 ± 0.56	−27.97 ± 0.68	–
	H. pilosella	−27.98 ± 0.27	−29.32 ± 0.52	−29.93 ± 0.44	−29.43 ± 0.45
	L. helveticus	-29.28 ± 0.18^{a}	-31.81 ± 0.06^{b}	-30.54 ± 0.26^{ab}	-30.91 ± 0.22^{b}

Whenever the main effect of treatment was significant, differences between mean values are indicated by letter. Within each row, the means followed by the same letter do not differ significantly (P < 0.05) based on Fishers' LSD post hoc tests.

TABLE 5 | Pearson's correlation coefficients between production and foliar traits calculated across all species in the four treatments (*n* = 42).

F_v/F_m	Chlorophyll	SLA	LDMC	Leaf N	Leaf P	Leaf N/P	SD	SL	$\delta^{13}C$
0.24	**0.62**	0.14	**−0.45**	**0.66**	**0.47**	0.15	0.05	−0.15	**0.38**

Bold characters represent significant (P < 0.05) correlations.

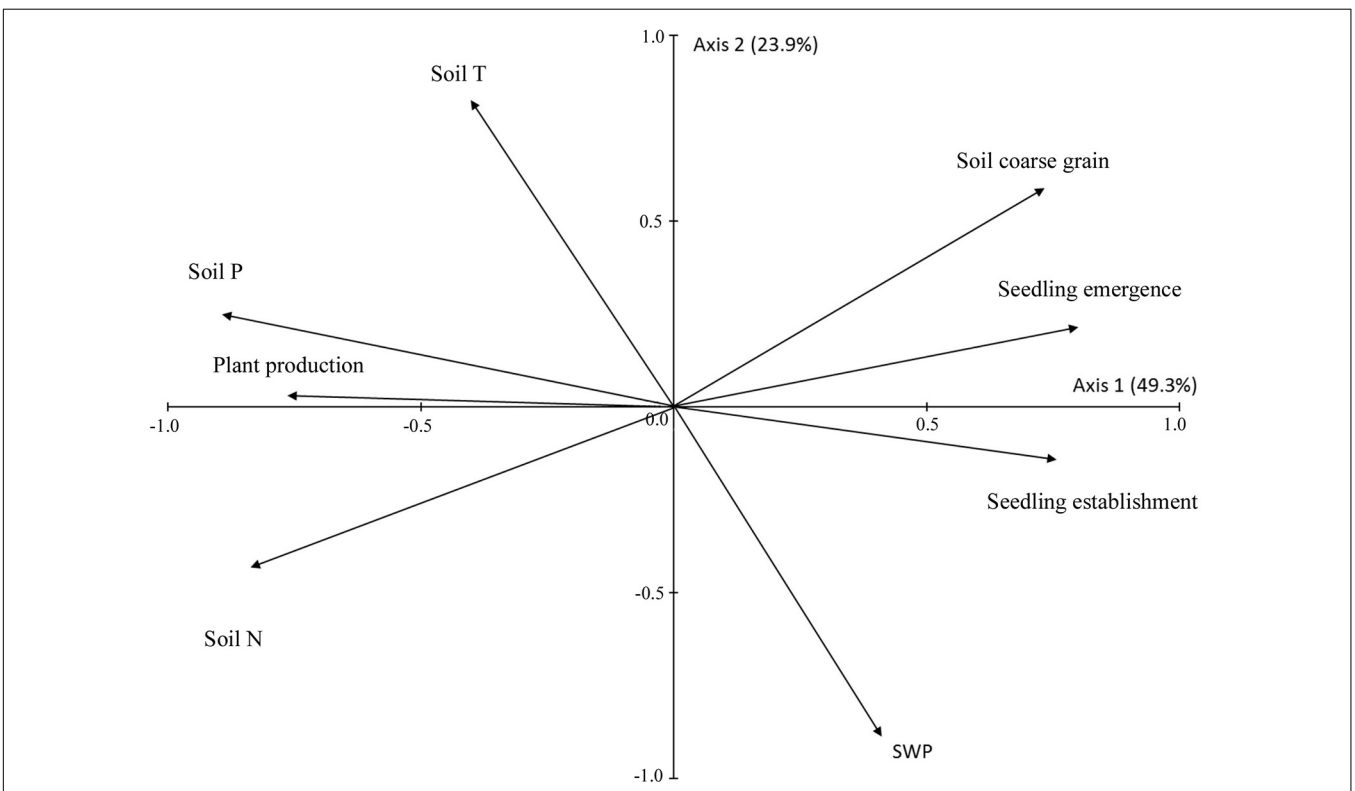


FIGURE 5 | Scores of seedling emergence, seedling establishment, plant production, soil chemistry variables and soil coarse grains (>6 mm) on the first two principal component (PCA) axes. Plant production is the mean production of all four species based on normalized values. The percentage of variance accounted for by each axis is in parenthesis.

for review). In recently deglaciated terrains seedling emergence and seedling establishment are principally controlled by abiotic filters (Jumpponen et al., 1999), especially water availability (Matthews, 1992) and temperature (Marcante et al., 2014). However, in our study the observed differences in recruitment success were generally unrelated either to SWP or temperature. Conversely, both recruitment traits for most species except *H. alpina* strongly differed between treatments experiencing similar temperature but different soil conditions. Contrary to our main hypothesis, seedling emergence and establishment were overall higher at site N, especially on coarse and nutrient-poor soil. This means that seed germination and seedling establishment were not hampered in cold, dry or nutrient-poor soils. Coarse soils are thought to facilitate seedling recruitment via multiple effects, such as enhancing moisture retention around seeds (Jumpponen et al., 1999), reducing the risk of predation (Enright and Lamont, 1989) and increasing the likelihood of radicle penetration (Sheldon, 1974). Soil C content and soil nutrient availability had minor effects on recruitment (Fraaije et al., 2015). Accordingly, recruitment traits showed strong relationships with soil granulometry and were unrelated to soil nutrient content.

Weaker recruitment success at site A compared to site N may either suggest that seeds do not germinate well in the field even under optimal conditions or that climate could be already too warm to elicit germination processes at the species growing site, but that it could have become warm enough for such process at higher elevation. Accordingly, during the last 18 years there was a subtle (but not significant) increase of temperature at the study area (**Supplementary Figure S1**). As a plant population migrates up a mountain in response to climate warming, the conditions for emergence and establishment at the leading edge become more favourable as the conditions at the trailing edge become less favourable (Mondoni et al., 2012). This threat might be strong especially for truly alpine species (i.e., those occurring only above the treeline), compared with those occurring also at lower elevations, which may show greater plasticity and, therefore, higher adaptability to both biotic and environmental changes. Warming has been found to decrease the emergence of several alpine species (Graae et al., 2008; Hoyle et al., 2013). Several mechanisms can play a role with this respect. For example, under a shorter winter season, seeds may remain partially dormant (Dong et al., 2010). In addition, advanced snowmelt (Frei et al., 2018) enhances frost penetration in the soil thereby hampering thermal protection of seedlings (Schaberg et al., 2008). Indeed, snow melted about 2 months earlier at site A although winter temperatures were on average similar across treatments. Another explanation of weaker recruitment is that temperature at site A exceeded the optimum for germination in some species (Cochrane et al., 2011). Contrary to our second hypothesis, warmer temperature did not improve seedling performance but even resulted in lower seedling emergence for three out of the four species. Lower emergence in N-o compared to N-n for *H. pilosella*, *L. helveticus,* and *H. alpina* indicates that warmer temperature could be responsible for weaker recruitment in site A. Importantly, our results can be generalized to emergence and establishment on bare ground, because our experimental set-up excluded possible indirect effects from resident vegetation.

Production and Foliar Traits

Plant growth in mountainous regions can be limited by temperature (Li et al., 2008), water availability (He et al., 2014) or nutrient availability (Boczulak et al., 2014). Temperature did affect plant growth because production was overall higher at site A. However, if plant growth at site N was primarily limited by cold temperature this would imply higher production in N-o compared to both N-n and N-a. This was not the case for any of the four species. Therefore, temperature did not appear to represent the major factor limiting plant growth across the four treatments. Plant growth in high-elevation habitats of the Alps generally does not experience drought limitation because precipitation increases and evapotranspiration decreases across elevational gradients (Van der Schrier et al., 2007). Nonetheless, we believe that water availability could be reduced by the coarse grain of the soil in N-n and N-o.In spite of marked differences in soil granulometry, SWP did not differ among treatments so that water availability, similar to temperature, was not responsible for the observed variation in plant production. Conversely, soil nutrient content was closely and positively related to production (**Figure 5**).

Relationships between production and environmental factors were consistently associated with variation patterns of foliar traits among treatments. Plants at the N site presented lower SLA, which supports the findings of previous studies demonstrating that plants in cold habitats generally exhibit low SLA (Midolo et al., 2019). If plant production was constrained by cold temperature, positive relationships would be expected between production and SLA because SLA is positively related to relative growth rate (Poorter and Remkes, 1990). Conversely, our study revealed no correlation between production and SLA (**Table 5**). Drought stress generally is associated with reduced size and density of stomata as a stomatal control of gas exchange. Low stomatal conductance improves water-use efficiency but meanwhile reduces net CO_2 exchange with consequent lowering of growth rates. If plant growth was constrained by water availability, production would be negatively correlated with SD, SL, and foliar $\delta^{13}C$. Indeed, drought stress generally is accompanied by low size and/or density of stomata, implying higher water-use efficiency and lower growth rates. The observed relationships of plant production with foliar traits did not point to water availability as a factor affecting plant growth because production was unrelated to both SD and SL and even exhibited positive correlation with foliar $\delta^{13}C$ for reasons that will be discussed below.

The positive correlations between production and leaf nutrient (especially N) content suggested that plant production was primarily controlled by foliar nutrient status, which partly supported our main hypothesis. In particular, high leaf N was associated with higher production in A-n and N-a. High foliar N content improves photosynthetic capacity (Hikosaka, 2004) because a large fraction of leaf N is allocated to the photosynthetic machinery and this fraction is largely constant (Evans, 1989).

Consistently, production exhibited positive correlation with chlorophyll content as well as with foliar $\delta^{13}C$ because C isotope discrimination is lower, i.e., the foliar $\delta^{13}C$ is less negative, when photosynthetic capacity is higher (Cernusak et al., 2013). Plants growing in more fertile habitats reduce resource investment in leaf structural elements (Hulshof et al., 2013), which is supported by negative correlation between production and LDMC. Higher leaf N content in A-n and N-a closely mirrored the soil N content expressed in terms of total soil N concentration. Production also showed positive correlation with leaf P content but higher leaf P content in A-n and N-a were not associated with consistent patterns of soil P content. We do not have a mechanistic explanation of this finding. However, previous studies showed that total soil P need not represent an index of P availability where most of the total soil P consists of recalcitrant organic or inorganic P forms (Gerdol et al., 2017). We speculate that soils richer in fine particle with higher C content enhanced microbial P mineralisation that is the main source of inorganic P supply to the plants in alpine soils (Gerdol et al., 2019). Whatever the mechanism accounting for soil P availability, adequate levels of P supply were important for preventing stoichiometric imbalance between N and P content in the leaves and consequent lowering of the growth rates (Gerdol et al., 2017). Consistently, production was unrelated to the leaf N/P ratio.

Trait Trade-Off and Niche Shift

A trait-based approach has been widely used to explain the distribution of species along environmental gradients and to predict shifts in species distributions (Laughlin, 2014). Selecting traits that can act as drivers of particular ecological processes is crucial to predict the performance of species in a changing environment (Funk et al., 2017). Various plant traits have been considered to explain the mechanisms governing succession in alpine environments. In particular, seed traits are considered as reliable indicators of colonisation potential (Schwienbacher et al., 2012) because strong colonizers of alpine summits possess wind-dispersed seeds (Holzinger et al., 2008) with high germination potential (Stöcklin and Bäumler, 1996). In this study seedling emergence and seedling establishment were positively correlated, underlining the importance of the earliest regenerative phases in determining species distributions (Grubb, 1977; Fraaije et al., 2015). However, species' responses to environmental conditions are better estimated considering different traits simultaneously (Kleyer and Minden, 2015). Indeed, our results showed that neither vegetative nor recruitment traits alone are as powerful as their combination to explain the processes by which species upward shift take place.

Upward shift of about 200 m from the range limit of alpine species had an opposite response in recruitment traits compared to vegetative traits, leading to a significant increase of recruitment success and a decrease of growth, respectively. This trade-off between recruitment traits and vegetative traits highlights that separate plant life stages differ in their ecological niche requirements. In particular, environmental conditions that allow a seed to germinate and establish (recruitment niche) differ from those needed for subsequent growth (adult niche). This is an example of shifts of the ontogenetic niche, i.e., the niche occupied by an organism during a specific life stage. Ontogenetic niche shifts have been often explored for animals but rarely for plants (Valdez et al., 2019 and references therein; but see Vitasse, 2013), for which it is assumed that life stages respond similarly to the environment. The ontogenetic niche shifts observed here indicate that alpine species have far greater potential to establish beyond rather than within their expressed niche, i.e., the niche where they are actually found, that is further limited by intra- and inter-specific competition not considered in this study. This may have important implications for community assembly and response to climate warming as such ontogenetic niche shifts enhance colonisation ability. With this respect, our results also show that alpine species did not reach the sexual maturity at the nival environment, indicating that their long-term persistence is not possible. Nevertheless, considering the exceptionally high rate of warming projected for alpine regions in this century (Hock et al., 2019), the possibility that alpine plants will establish in nival environments cannot be ruled out in a near future, at least for species with long-distance seed dispersal.

CONCLUSION

Our results show that adult niche represent a major bottleneck (filter) for alpine species to establish a viable population in nival environments. Hence, successional constraints in these habitats seem to be less related to colonisation abilities than to the ability to acquire (low) resources during subsequent growth. Furthermore, the trait trade-off highlighted here may differentiate ecological strategies for plant population maintenance and expansion. Seedling establishment was improved outside the adult niche, where there is low seed density and chance of growth and was constrained where environmental conditions support growth but there is higher risk of intraspecific competition.

Differential environmental filtering during regeneration and subsequent growth plays an important role in determining alpine plant population maintenance and expansion. In particular, soil granulometry and nutrients act as strong environmental filters improving recruitment in coarse, nutrient-poor soil and constraining it, but fostering growth on finer and nutrient-richer substrates. Thus, soil granulometry and nutrient form the major axes of an ecological framework for understanding species upward migration.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AM designed the study. AM, SO, TA, and GR carried out the periodic field work. AM, TA, GR, LB, and RG performed the final sampling. LB, AC, PI, RT, RM, and RG carried out the laboratory

work. AM and RG analysed the data and wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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frontiers in Ecology and Evolution

ORIGINAL RESEARCH
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Assessing the Role of Selected Osmolytes in Mediterranean High-Mountain Specialists

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Despite the constraining weight of summer drought over plant life which distinguishes Mediterranean high-mountains, and its anticipated exacerbation under the current climate crisis, there is still little knowledge of the underlying drought-endurance mechanisms in Mediterranean high-mountain species, such as osmolyte accumulation. To fill this gap, we studied the role of two of the most frequent osmoregulators in plants, proline and osmotically active carbohydrates (OAC), as pointers of the drought-stress response in seven high-mountain plant species representative of the high-mountain plant communities in Central Spain, along an elevation gradient. Overall, our results are consistent with the escalation of summer drought and suggest the involvement of osmolytes to sustain plant activity in these specialists during the growing season. Proline content showed a steadily increasing pattern in line with the seasonal aggravation of summer drought. The significant rise in mean proline in most species, coinciding with the periods with the greatest decline in soil water content, suggests the recurrent role of proline in the drought-stress response in the studied specialists. The lack of significant differences between elevations and the minimal seasonal variations in the OAC content suggest a fixed OAC content independent of functional type to sustain metabolic functions under summer drought. Moreover, these findings allow inferring the action of both OAC and proline as osmoregulators, allowing to support plant functions in these specialists under atypically dry conditions. Overall, our findings are consistent with proline as a major osmoprotectant strategy over OAC buildup in these specialists, which may be related to an adaptation strategy associated with the briefness of the growing season and the incidence of less favorable conditions in Mediterranean high-mountains.

Keywords: summer drought, proline, climate change, osmotically-active carbohydrates, Mediterranean high-mountains

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INTRODUCTION

The impact of drought on ecosystem functioning and services depends on both plant-related attributes, including the morphology and phenology of co-occurring species, and the interaction with fine-scale environmental factors that can exacerbate or mitigate climatic conditions (Körner, 2003; Kreyling et al., 2008). Mediterranean-type ecosystems are among those where drought exerts

a more critical impact, solely because the highest temperatures, registered during summertime, concur with the period of almost no precipitation (Ruiz-Labourdette et al., 2014; Piper et al., 2016). Mediterranean high-mountains are highly susceptible to the predicted aggravation of climate conditions, particularly during summertime (Bravo et al., 2008; Giorgi and Lionello, 2008). It is not known whether vegetation adapted to the current summer drought levels, will be able to survive the more severe droughts that are anticipated in the future. The incidence of drought in these high-mountain areas is almost counterintuitive since alpine habitats (i.e., temperate-zone areas above the tree line) are well-known for being subject to cold stress but seldom to water limitations (Körner, 2003). In contrast, Mediterranean high-mountain areas experience both summer stress (i.e., co-occurring rainfall shortfalls, high temperatures, and high irradiance) and, as in the case of other mountains, the occasional freezing events during the period in which temperatures are suitable for plant growth (Giménez-Benavides et al., 2007; Sanfuentes et al., 2012). Confirmation of the severe water deficit in these mountains during summer has been attained via direct measurements of topsoil water content (SWC), denoting an aggravation at lower elevations (<5% SWC in July; Giménez-Benavides et al., 2007; Gutiérrez-Girón and Gavilán, 2013; Piper et al., 2016). Summer drought has also been found to be a critical factor for phenology, reproduction, and distribution patterns in several high-mountain specialists in these summits (Giménez-Benavides et al., 2007; García-Camacho and Escudero, 2008; García-Fernández et al., 2013; Gutiérrez-Girón and Gavilán, 2013). The incidence of summer drought during the short active growing season has been determined as a critical ecological and evolutive challenge for plant life in Mediterranean high-mountains (Giménez-Benavides et al., 2011). Moreover, since summer drought is a primary constraint for plant performance and survival in these habitats (Gutiérrez-Girón and Gavilán, 2013), these specialists can be expected to deploy a wide variety of strategies and adaptations (e.g., biochemical) to overcome summer drought.

From a physiological perspective, plant responses to drought involve the synchronization of processes to alleviate cell hyperosmolarity and osmotic imbalance. Osmolyte accumulation is a widespread mechanism in plants targeting the avoidance of water loss and cell turgor (Hayat et al., 2012). However, it is worth stating that some of these metabolic changes are common to several stresses (i.e., salt, drought, and temperature stress), whereas others are specific to one type of stress (Krasensky and Jonak, 2012). Proline (hereafter "Pro") is an amino acid and one of the main osmolytes accumulated in plants in response to salinity, low temperatures, and drought stress (Hayat et al., 2012; Montesinos-Pereira et al., 2014; Per et al., 2017 and references therein; Roychoudhury et al., 2015). Thus, Pro buildup could be expected to occur in Mediterranean high-mountain plants in response to the seasonal aggravation of summer stress. Pro functions as a stabilizer of cell membranes, added to its alleged ROS scavenging capacity (Hayat et al., 2012). Another well-recognized response to drought and freezing stress in plants is the accumulation of osmotically-active carbohydrates (OAC; i.e., soluble sugars and fructans). OAC accumulation is acknowledged as a physiological response that causes sustained hydraulic function and osmoregulation maintenance, both essential mechanisms increasing plant survival under drought conditions (Gibson, 2005; Sala et al., 2012; O'Brien et al., 2014). For instance, the accumulation of fructans and raffinose-family-oligosaccharides enhances the stability of phospholipidic mono- and bilayers, as they directly insert between polar headgroups (Vereyken et al., 2003; Hincha et al., 2006; Valluru and Van den Ende, 2008). Sucrose buildup is closely related to decreasing membrane permeability, given its interaction with the phosphate in membrane-lipid headgroups to avoid cellular dehydration (Strauss and Hauser, 1986). Fructans have also been shown to play an essential role in the tolerance to drought, freezing and salt stress in plants. Numerous fructan-accumulating species are found in habitats characterized by periods of seasonal drought or frost, similar to those fructan-accumulating transgenic plants evidencing enhanced stress tolerance (Van den Ende, 2013 and references therein). Moreover, raffinose and sucrose enrichment in plastids suggests they play a role as cryoprotectants, as they promote photosystem stabilization against freezing damage and prevent electrolyte leakage in thylakoid membranes (Knaupp et al., 2011; Doltchinkova et al., 2013). Further compatible solutes have been identified as important contributors of osmotic adjustment in certain groups of plants under several abiotic stresses, e.g., glycine betaine (GB), polyols, trehalose (Krasensky and Jonak, 2012). Together with Pro, GB is acknowledged as one of the main osmolytes accumulated in plants during prolonged abiotic stresses, since it significantly contributes to increasing osmotic pressure, balance the vacuolar osmotic potential, and stabilizing the structural and functional integrity of protein complexes and membranes against ROS (Annunziata et al., 2019 and references therein; Krasensky and Jonak, 2012). However, GB primarily accumulates in young tissues, and with much higher levels in halophytes than in glycophytes (only found in few species from the Poaceae family; Annunziata et al., 2019). As for trehalose, it can function as an osmolyte and stabilize proteins and membranes at sufficient levels, though studies have only reported high trehalose levels in some desiccation-tolerant plants (Drennan et al., 1993; Paul et al., 2008). Meanwhile, the available studies show trehalose is present in trace amounts in most angiosperms, showing only relatively moderate increases in its levels under osmotic stress (Krasensky and Jonak, 2012 and references therein).

Dehydration stress in plant cells triggered by the incidence of drought or freezing reveals a close relationship between summer drought and freezing-stress responses in Mediterranean high-mountain specialists, as evidenced by their high endurance to extreme freezing events during their short growing season (Sierra-Almeida et al., 2009; Pescador et al., 2016; Sierra-Almeida et al., 2016). Although plants from Mediterranean high-mountains are distinguished for their resilience to limited water availability in the growing season (Larcher, 2000; Magaña Ugarte et al., 2019), the specific mechanisms underlying the summer-drought response in these specialists remain understudied. Understanding the extent of osmolyte accumulation (e.g., Pro and OAC) and its seasonal variation among species to withstand summer drought is a priority for Mediterranean high-mountain specialists and narrowly distributed species.

Osmolyte content varies according to the environmental seasonality experienced by plants, which results in higher osmolyte levels during stressful periods (Palacio et al., 2007; Meletiou-Christou and Rhizopoulou, 2017). These accumulation patterns may also vary as a consequence of microclimatic variations driven by elevation, exposition, or other local factors typical of high-mountain environments (Körner, 2003; Bansal and Germino, 2010; Li et al., 2016). Specifically, the present study aims to evaluate (1) the seasonal patterns of Pro and OAC accumulation in Mediterranean high-mountain specialists, and (2) whether this variation changes along an elevation gradient and in consecutive and climatically contrastingly years. More specifically, due to the incidence of summer drought on the short growing season in Mediterranean high-mountain habitats and its seasonal aggravation (Cavieres et al., 2000; Gutiérrez-Girón and Gavilán, 2013) we expect to find an increase in the accumulation of Pro and soluble sugars in these high-mountain specialists alongside the aggravation of summer drought. Similarly, we expect the osmolyte accumulation may differ depending on elevation. Since the drought-severity gradient increases as elevation decreases (Giménez-Benavides et al., 2007; Sierra-Almeida et al., 2009), populations from the lowest end of the elevation gradient could be subjected to most water stress. Thus, we would expect a local advantage to dry settings in the low-elevation populations due to better adaptation to more intense drought stress conditions compared to their high-elevation counterparts.

MATERIALS AND METHODS

Study Site and Selected Species

The study site was located in the Sierra de Guadarrama National Park (central Spain, 40° 44′ 14″ N, 3° 43′ 48″ W), an E-W running mountain range characterized by a Mediterranean-type climate with an intense dry season during summer, from June to September (less than 10% of total annual rainfall; Gutiérrez-Girón and Gavilán, 2013). Vegetation at the tree line (1,900–2,000 m.a.s.l.) is dominated by *Pinus sylvestris*, interspersed in a matrix of shrubby species (*Cytisus oromediterraneus* and *Juniperus communis* subsp. *alpina*) and perennial herbs, such as the dominant grass *Festuca curvifolia* Lag. *ex* Lange (Fernández-González, 1991). With increasing elevation (from 2,100–2,200 to 2,430 m), the *Cytisus-Juniperus* shrub community is replaced by patchy xerophytic pasture dominated by *Festuca curvifolia* (=*F. carpetana*; Fuente and Sánchez, 2014), cohabiting with several high-mountain specialists (Pescador et al., 2014). The study was set in the Bola del Mundo sector (40.785°N, 3.979°W) across the elevational gradient of this high-mountain grassland community on this summit (from 1,980 to 2,244 m). The sample collection took place during the 2017 and 2018 growing seasons (June–September), in two climatically contrasting years (**Figure 1** and **Supplementary Figure 1**). Average day-time temperatures in 2017 were 1.5°C higher in June and August 2017 than in the same months in 2018. Relatively cold temperatures were registered in the first half of the growing season in 2018, followed by extremely high mean daytime temperatures (>2°C than AEMET monthly reference values) in the second half (**Supplementary Figure 1** and **Supplementary Table 2**). Average SWC varied substantially through the growing season and between years, reaching considerably lower values at the end of the 2017 growing season, compared to the following year (**Figure 1**). Henceforth 2018 will be regarded as a "standard" year given the low number of days per month with zero rainfall and temperatures above mean monthly values, while 2017 will be classified as a dry year (**Supplementary Figure 1**).

Along the aforementioned elevation gradient, we selected four sites on NW and W facing slopes. One 20×20 m plot was established in each of the four sampling sites on homogeneous pasture areas. The most abundant species were identified in each plot and selected for this study, providing a complete representation of the phylogenetic diversity of this plant community (Pescador et al., 2014). The seven species selected included the chamaephytes *Armeria caespitosa* (Gómez Ortega) Boiss. in DC, and *Juniperus communis* subsp. *alpina* (Suter) Čelak (hereafter *J. alpina*); and the hemicryptophytes *Erysimum penyalarense* (Pau) Polatschek, *Festuca curvifolia* Lag. *ex* Lange, *Jurinea humilis* (Desf.) in DC, *Hieracium vahlii* Froelich. in DC, and *Senecio pyrenaicus* subsp. *carpetanus* (Willk.) Rivas-Mart. Further details on the growth form and family of each selected species are provided in **Supplementary Table 3**. All species are found in the two highest sampling sites (above 2,100 m), while only *E. penyalarense, J. alpina, F. curvifolia,* and *S. carpetanus* occur throughout the entire elevation gradient. The selected species encompassed from narrow-endemics, such as *E. penyalarense,* to widely distributed ones, e.g., *J. alpina*. All species included in this study are considered as high-mountain specialists (Clavel et al., 2011) as they appear almost exclusively in high-mountain grasslands above the tree line (Fernández-González, 1991; Jiménez-Alfaro et al., 2014).

Field Sampling

We sampled the available populations of the selected plant species in four sites located along a rough altitudinal gradient covering the presence of these oromediterranean pastures above the tree line. These four representative and well conserved remnants of these habitats where located close to the Bola del Mundo summit at the following elevations: 1,980, 2,052, 2,172, and 2,244 m asl. These four localities were sampled in two consecutive years, 2017 and 2018. For valid comparisons of osmolyte buildup between years and elevations, plants were sampled at the same developmental stages in both growing seasons. Available plants were sampled in these two years in June, July, August and September, covering the complete vegetative growth period in these mountains. The list of species per locality were at 1,980 m: *E. penyalarense, F. curvifolia, J. alpina,* and *S. carpetanus*; at 2,052 m: *E. penyalarense, F. curvifolia, J. alpina, J. humilis,* and *S. carpetanus*; at 2,172 and 2,244 m: *A. caespitosa, E. penyalarense, F. curvifolia, H. vahlii, J. alpina, J. humilis,* and *S. carpetanus.*

For each species, young, fully developed leaves were taken from randomly selected healthy, mature individuals to evaluate Pro and OAC concentrations. The number of individuals sampled per species varied depending on their natural abundance each year. Thus, a minimum of 10 individuals was sampled per

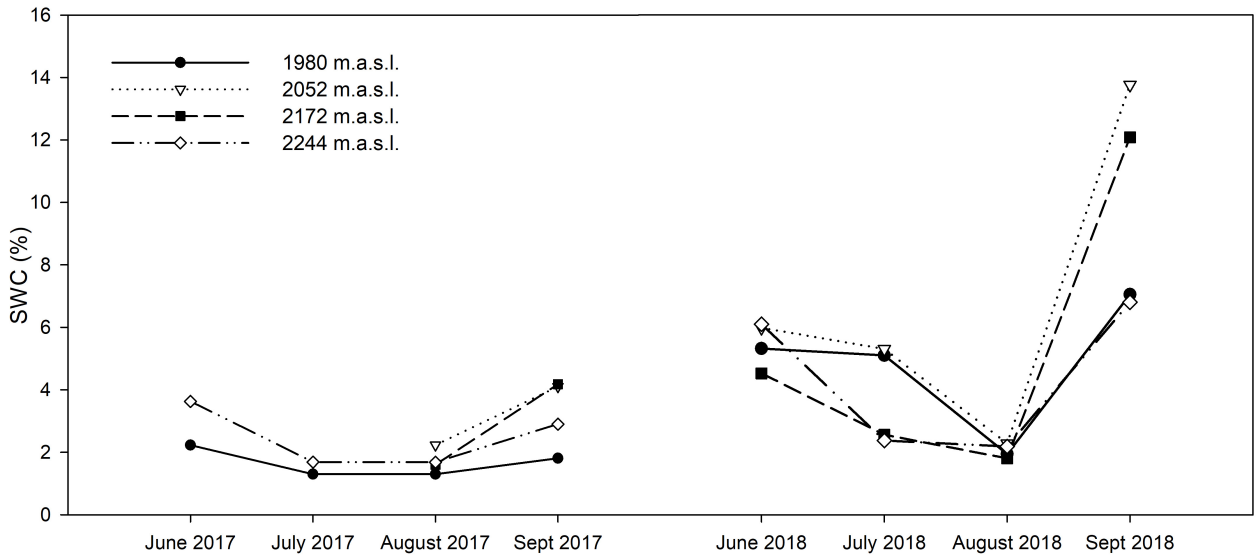


FIGURE 1 | Seasonal variation in SWC along the studied altitudinal gradient through the active growing season of vegetation in the study site. Data corresponds to in-site measurements on both years of study (2017, 2018).

plot when available, taking five leaves per individual. Mature green leaf samples were selected to guarantee less fluctuating osmolyte levels. Samples were stored immediately in liquid nitrogen to avoid degradation of metabolites during transfer to the lab. All samples for OAC analysis were collected in a brief period afore sunrise, to minimize the effect of diurnal OAC fluctuations. Fully developed green leaf samples collected from contrasting elevations (1,980 and 2,244 m) taken at the end of each growing season were used to study the effect of elevation on OAC accumulation in these species.

SWC was measured in all plots at the time of plant sampling on all dates in 2017 and 2018 at the highest and lowest elevations, and at mid-elevations at all dates in 2018 and only on the last two sampling events in 2017. SWC was measured using a hand-held, time-domain reflectometry sensor (FieldScout TDR 300; Spectrum Technologies, Plainfield, IL, United States), to a depth of 10 cm and taking a mean of ca. 5 measurements per plot in randomly located sites with plant cover. Climate records were obtained from the Puerto de Navacerrada meteorological station in Madrid, run by the Spanish Institute of Meteorology[1].

Proline Quantification

The proline content in the leaf tissue was determined using the ninhydrin-based colorimetric method (Abrahám et al., 2010), with minor modifications. Approximately 100 mg_{FW} of leaf samples were thoroughly ground in liquid nitrogen. Next, 3% sulfosalicylic acid was added to each grounded leaf sample, followed by the thorough grinding of plant material. Samples were centrifuged for 5 min at room temperature using a bench-top centrifuge at maximum speed (12,000 $\times g$). Afterward, 100 μL from the supernatant of each plant extract was added to the new tubes containing the reaction mixture (100 μL of 3% sulfosalicylic acid, 200 μL glacial acetic acid, and 200 μL acidic ninhydrin), and carefully mixed. Tubes were incubated at 96°C for 60 min, and the reaction was terminated on ice. Extraction was performed by adding 1 mL of toluene to each reaction tube. The tubes were then vortexed for 20 s and left to settle until the phase separation could be observed. The chromophore, organic part containing the toluene, was then used for measuring absorbance at 520 nm with a UV-Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan) in quartz cuvettes, using toluene as blank. Proline concentration was determined using a standard concentration curve and calculated on a dry weight basis ($\mu mol/g_{DW}$).

Quantification of Osmotically-Active Carbohydrates

In this research, the OAC refer to soluble sugars (fructose, glucose, sucrose, raffinose), and fructans (inulin). Samples were analyzed to determine the presence and concentrations of each OAC mentioned above, and total OAC content (OAC_{TOTAL}). Sample extraction and determination were performed following the protocol by Weiss and Alt (2017), with minor modifications.

The plant material was grinded into an ultrafine powder which was subsequently freeze-dried to dehydrate and adequately freeze the samples to avoid degradation. Approximately 50 mg of each freeze-dried sample was weighed, transferred into 12 mL Falcon tubes, and mixed with DIO water, and heated at 80°C for 30 min. Three consecutive centrifugation steps and dilution steps were performed to obtain a supernatant. Next the tubes were placed in a horizontal shaker, set at 30 revolutions/min for 1 h. After shaking, precisely 1.5 mL of the supernatant was filtered through an HPLC syringe filter (0.45 μm) into an HPLC vial. A volume of 20 μL per sample was injected. Soluble sugar concentrations were measured using a

[1]www.aemet.es

Carbo Sep CHO-682 Lead Carbohydrate HPLC-Column (300 × 7.8 mm), in a UHPLC Nexera System (Shimadzu Corp. Kyoto, Japan) in 50 min runs. HPLC water was used as the mobile phase. Samples were analyzed in a gradient mode. Sugar concentrations ($\mu g/mg_{DW}$) for fructose, glucose, inulin, raffinose, and sucrose were determined by comparison with standards, and OAC_{TOTAL} for each sample was calculated with the sum of all quantified carbohydrates.

Statistical Analysis

The effect of the temporal variation throughout the growing season (related to the progression of summer drought) and elevation on Pro content was assessed in seven Mediterranean high-mountain species in two consecutive and climatically contrasting years using Linear Mixed Models (LMMs); via restricted maximum likelihood (REML; Patterson and Thompson, 1971). Given that the data was collected repeatedly over time, the data analyses were performed as follows: (1) an overall analysis, with the additive effect of elevation and date of sampling (within a year) as fixed factors, and species as a random factor; and (2) a separate analysis for each species, including the effect of elevation and date of sampling (between years) as fixed factors, and individual as a random factor since they were submitted to several measurements along time. Mean Pro content per species and individual were modeled with Gaussian error distribution, using the function "lme" from package "nlme" (Pinheiro et al., 2018). The response variables were transformed (logarithmic) to achieve a normal distribution of the residuals when required. Full models were simplified by removing non-significant parameters to obtain more parsimonious models. Fixed factor significance was assessed via posteriori likelihood ratio tests.

Seasonal variations of OAC in leaves, total and by specific carbohydrates, were assessed per species at the beginning and end of each growing season (2017, 2018), and at the highest and lowest elevations using nested ANOVAs. Additional LMMs were implemented with the additive effect of elevation and sampling date (within a year) as fixed factors, and sample identity as the random factor to address and avoid temporal and pseudo-replication. The response variables were inulin, fructose, glucose, raffinose, sucrose, and OAC_{TOTAL}. Final model selection was achieved by deleting the non-significant interactions, followed by deleting those variables explaining the less variation.

All statistical analyses were performed with the R statistical package (R Core Team, 2018).

RESULTS

The climatic data retrieved from AEMET and the calculated ombrothermic indexes (Rivas-Martínez, 2007) indicated 2018 was a "standard" year given the low number of days per month with zero rainfall and air temperatures above mean monthly values, while 2017 will be classified as a dry year (**Supplementary Figure 1** and **Supplementary Table 2**). Moreover, the statistical analyses indicated that SWC was not strongly correlated with elevation in our gradient, while denoting a strong negative correlation with the date of sampling, i.e., the progress of the growing season (**Supplementary Table 4**).

Proline Content

Pro content varied significantly throughout the growing season in both years, whereas the effect of elevation depended on the species. In the overall analysis with species as a random factor, there was a significant accumulation in mean Pro as the growing season advanced ($p < 0.05$). Conversely, our model showed no significant mean changes in Pro along the elevation gradient ($p > 0.05$). Our analysis revealed a significant difference in the mean Pro between years, with a higher overall Pro accumulation during the atypically dry year 2017 than under the milder conditions of 2018. The most significant increase in mean Pro occurred between July and August in both years ($p < 0.001$), as were the lowest SWC measurements. Overall, there was a significant temporal increase in mean Pro per species during the second half of the growing season in both years (**Figures 2**, **3**). Seasonal mean Pro content per species was substantially higher in 2017 than in 2018 in *E. penyalarense* (295.5 vs. 268.3 $\mu mol/g_{DW}$), *J. humilis* (229.1 vs. 73.3 $\mu mol/g_{DW}$), *J. alpina* (89.4 vs. 71.5 $\mu mol/g_{DW}$), and *S. carpetanus* (357.4 vs. 290.9 $\mu mol/g_{DW}$), in contrast to the findings in *A. caespitosa* (78.1 vs. 111.4 $\mu mol/g_{DW}$), *F. curvifolia* (58.7 vs. 84.7 $\mu mol/g_{DW}$), and *H. vahlii* (102.4 vs. 141.0 $\mu mol/g_{DW}$). The seasonal accumulation pattern for mean Pro was recurring in *A. caespitosa* and *E. penyalarense*, while no clear accumulation pattern was observed in the remaining high-mountain specialists.

In chamaephytes, the Pro buildup showed significant changes throughout the growing season, in parallel with the seasonal decline of SWC (summer drought). In both years of study, Pro content in *A. caespitosa* significantly increased throughout the growing season ($p < 0.001$; **Figure 2**). Regarding *J. alpina*, Pro content only showed significant seasonal variation in plots located above the optimum elevation for *J. alpina* (∼2,000 m; $p < 0.001$) when the SWC suffered rapid declines.

In the case of hemicryptophytes, significant changes in Pro content with the aggravation of seasonal drought were almost unanimous in all models per species, while the effect of elevation diverged among them. In both years of study, Pro content increased significantly in *E. penyalarense* during the growing season and with elevation ($p = 0.021$ and 0.001, respectively), with a notable increase in the second half of the active growing season (**Figure 3**). Similarly, Pro content in *J. humilis* increased significantly during the growing season in 2017 ($p < 0.001$); while denoting no significant changes with elevation ($p = 0.35$). Furthermore, as SWC declined through the growing season, with a sharp decrease between July and August 2018, Pro buildup significantly increased throughout the growing season in *H. vahlii* ($p < 0.001$).

Seasonal Variation in OAC Content

Although OAC_{TOTAL} diverged between years in the studied species without significant differences between contrasting elevations (2,244 and 1,980 m), all species showed a general pattern whereby they maintained a substantial OAC_{TOTAL} during the course of the growing season (**Supplementary Table 1**).

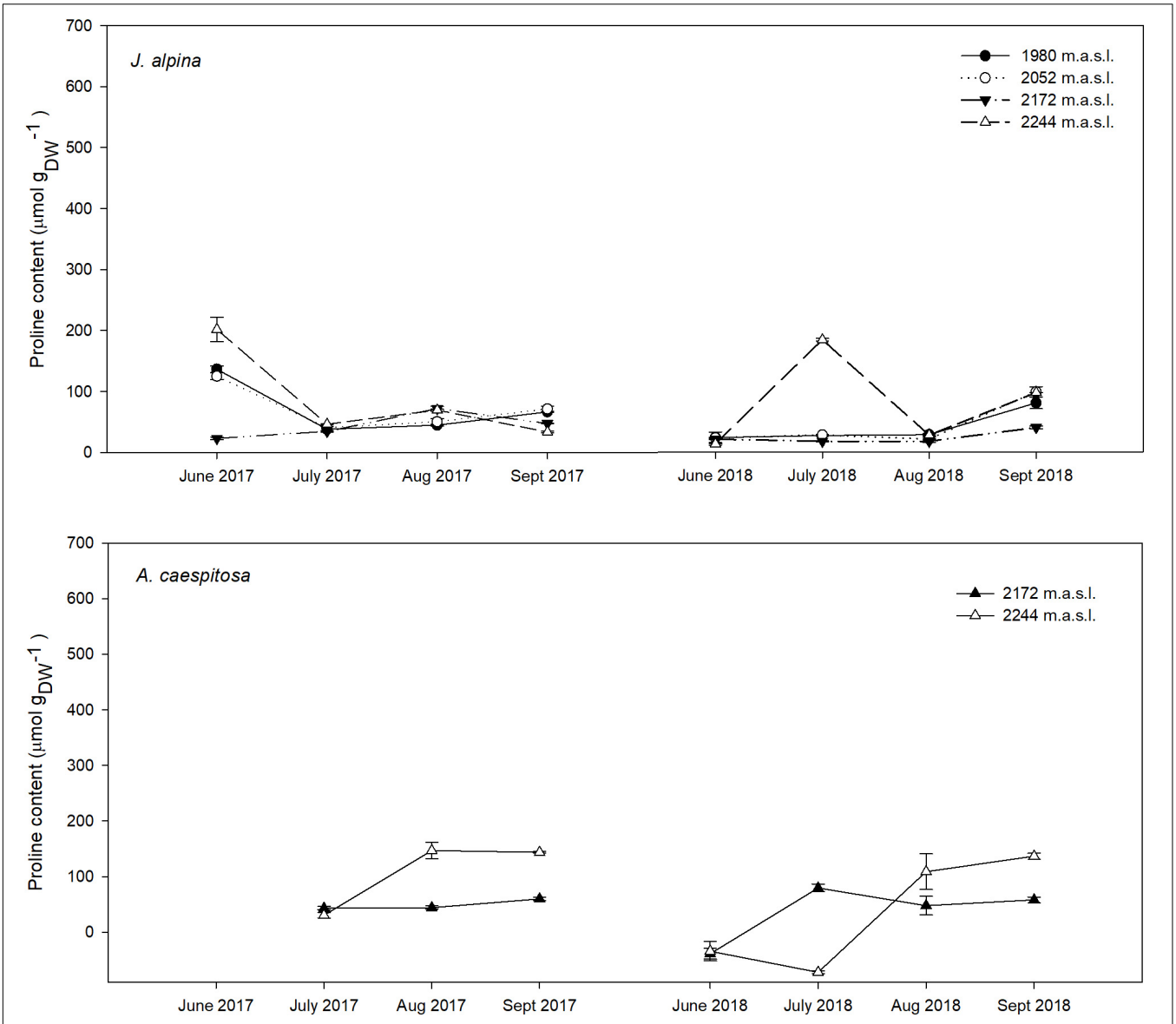


FIGURE 2 | Seasonal variation in Pro content in the studied chamaephytes (dwarf shrubs), *J. alpina,* and *A. caespitosa* at each sampling site and throughout their active growing season in 2017, 2018. For each sampling event, $n = 10$.

Overall, a significantly higher OAC_{TOTAL} was found during an atypically dry growing season (i.e., 2017) compared to a "standard" year (i.e., 2018; $p < 0.05$), without significant differences between elevations.

The analyses per species revealed that, in a "standard" year, OAC_{TOTAL} in *E. penyalarense* increased in line with the seasonal decrease in SWC. Additionally, a significant seasonal increase in inulin content was measured in this species (**Supplementary Table 1**). Comparably, OAC_{TOTAL} in *S. carpetanus* increased during the growing season in the lowest elevation, coupled with a significant seasonal increase in fructose and substantial rise in raffinose content. Inulin content maintained high seasonal levels in *S. carpetanus* without significant differences between elevations (**Supplementary Table 1**). Similarly, *J. alpina* maintained high inulin levels through the growing season in high-elevation individuals, contrasting with the substantial seasonal decline in those from the lowest elevation. The OAC_{TOTAL} decreased through the growing season in *F. curvifolia* at both elevations, though high inulin content was maintained all through the growing season without significant differences between contrasting elevations.

During the dry growing season of 2017, OAC content showed minor changes through the growing season and was exempt of significant differences between contrasting elevations. In 2017, *E. penyalarense* OAC_{TOTAL} increased substantially during the growing season, without significant differences between elevations, and a significant seasonal increase in inulin content (**Supplementary Table 1**). A significant seasonal increase in

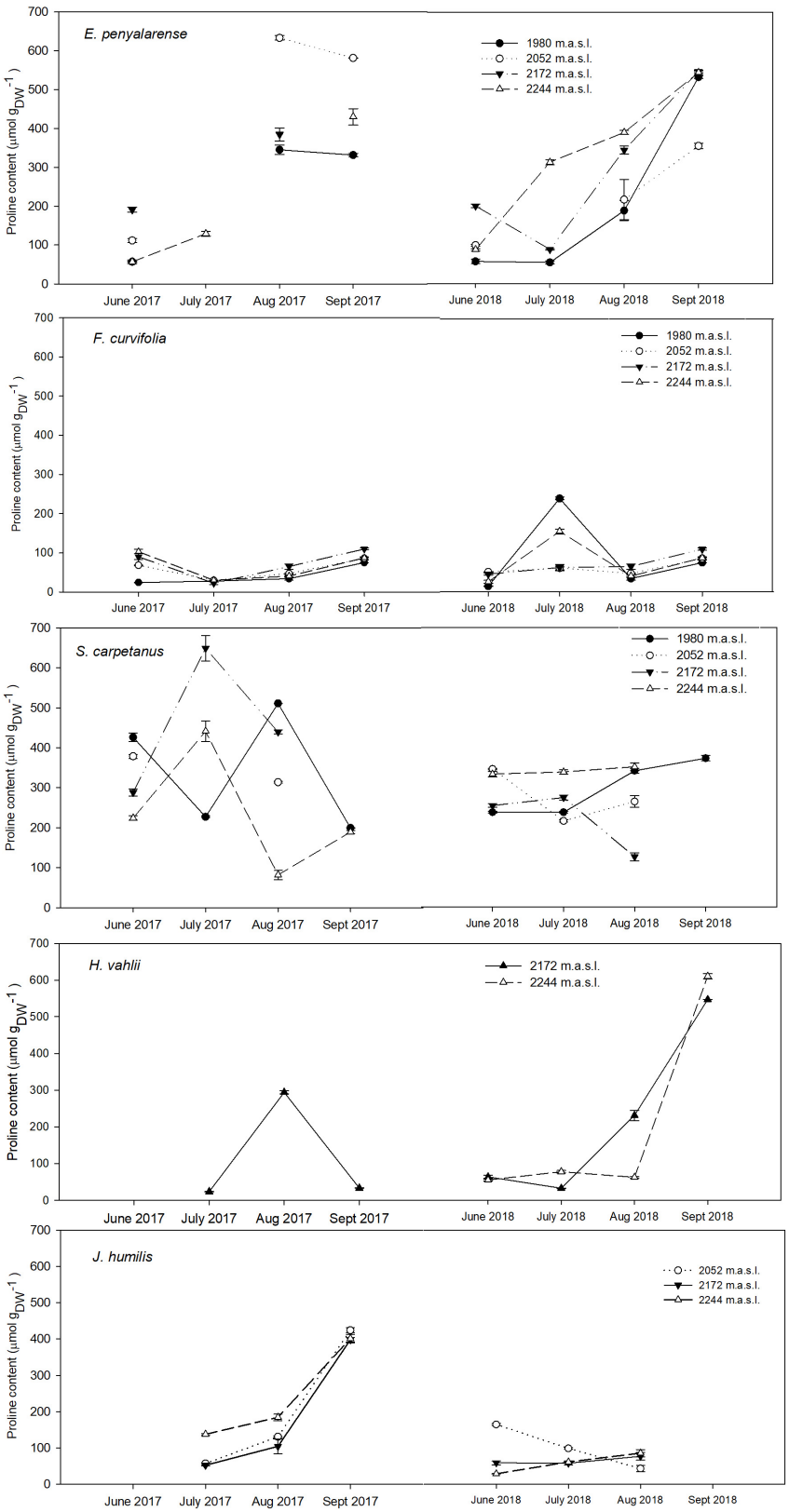


FIGURE 3 | Seasonal variation in Pro content in hemicryptophytes *E. penyalarense*, *F. curvifolia*, *S. carpetanus*, *H. vahlii,* and *J. humilis* in both years of study (2017, 2018) throughout the elevation gradient. For each sampling event, $n = 10$.

sucrose, fructose, and raffinose content was also observed in *E. penyalarense* at the highest elevation. In *F. curvifolia,* high inulin content was maintained throughout the growing season. Inulin and OAC_{TOTAL} significantly declined in *S. carpetanus* at both elevations (**Supplementary Table 1**), while raffinose content increased considerably only in the low-elevation individuals. As for *J. alpina*, a considerable reduction in inulin and significant raffinose buildup was found only in individuals from the highest elevation.

DISCUSSION

In the summits of Sierra de Guadarrama, snow-melting water is the primary source of soil moisture and it decreases progressively as summer advances (Giménez-Benavides et al., 2011; García-Fernández et al., 2013; Gutiérrez-Girón and Gavilán, 2013). Our results support our initial assumption, showing a significant seasonal accumulation in mean Pro in both years in parallel with the seasonal escalation of summer drought. Coupled with the significantly higher Pro accumulation during an atypically dry year (i.e., 2017) than under a "standard" year, our results showed that the most significant increase in mean Pro occurred during the period of maximum plant activity (July–August), and coincided with the most substantial decrease in SWC in the season. These findings suggest the involvement of Pro accumulation in sustaining plant activity during summer drought and reinforce the idea that summer drought is a major driver of plant life in these Mediterranean mountains (Giménez-Benavides et al., 2011).

Plants can increase their tolerance to several stresses (including heat, low temperatures and drought) in response to a gradual exposure to these constraints, a process known as acclimation (Krasensky and Jonak, 2012). In an osmotic-stress scenario, plants can foster a cytoplasmic osmotic adjustment through Pro and OAC accumulation, enabling the maintenance of cell turgor pressure and low leaf water potential to sustain photosynthetic rates, cell expansion, and growth (Chen and Jiang, 2010; Hayat et al., 2012). The significant seasonal increase in mean Pro content observed in consecutive and climatically contrasting years in *A. caespitosa* and *E. penyalarense* alongside the escalation of summer drought (**Figures 2**, **3**, respectively), suggests Pro accumulation as an important response mechanism in these specialists to maintain cell turgor when they are subject to conditions that can impose osmotic stress. In this sense, the different responses observed between climatically contrasting years in each species favor the idea that Pro accumulation in these Mediterranean high-mountain specialists may allude to an acclimation response related to the duration and severity of summer drought.

High-mountain plants have evolved and optimized their life strategies to survive the severe prevailing conditions in these habitats, eliciting different enduring mechanisms when the threshold for an environmental variable is exceeded (Bonhert and Sheveleva, 1998; Körner, 2003). In the Mediterranean high-mountains, the picture is more complicated since drought stress, together with freezing events, plays a critical adaptive role given its seasonal aggravation and the interannual variations it undergoes (Giménez-Benavides et al., 2007; **Figure 1** and **Supplementary Figure 1**). Responses to drought and low-temperature stress are clearly linked and widely found in high-mountain vegetation from temperate bioclimates (Magaña Ugarte et al., 2019). The incidence of freezing episodes during the growing season in Mediterranean mountains results in concurrent drought and freezing stresses, with both inflicting osmotic stress on plant cells and tissues (Valladares et al., 2004; Pescador et al., 2016). Exposure to summer drought during the growing season may heighten freezing resistance in these Mediterranean high-mountain specialists, as observed in their Andean counterparts (Sierra-Almeida et al., 2009; Sierra-Almeida et al., 2016). Increased freezing-drought resistance could be achieved through the accumulation of OAC and Pro; two physiological mechanisms related to the freezing-drought response in plants due to their role in sustaining cell growth and guarding cellular components against osmotic stress (Sierra-Almeida et al., 2016; Ahmad and Hameed, 2018). The high inulin and Pro levels found in *E. penyalarense* and *F. curvifolia* in our study suggest their role on the osmotic adjustment to protect against the osmotic stress likely imposed by summer drought. Moreover, our findings may provide an explanation for the remarkable freezing resistance described for these two species by Pescador et al. (2016), acting as osmoregulators to maintain cell-membrane integrity and delay ice crystal formation during the sporadic halting events occurring in the growing season (Körner, 2003; Valluru and Van den Ende, 2008). Although it could be argued that occasional freezing events during the growing season could be the primary driver for Pro accumulation, the available climate data indicates that freezing events were uncommon during the period where we detected the highest increase in Pro in most of these specialists (**Supplementary Figure 1**). Nevertheless, the nature of the study does not allow ruling out the influence of other environmental factors (e.g., temperature variation) on osmolyte accumulation, since both Pro and OAC play a multifunctional role in the plant stress defense (Krasensky and Jonak, 2012). The high Pro and OAC content found in some species toward the end of the growing season could be part of their cold acclimation process, triggered mainly by the low temperatures occurring more frequently late in the growing season. In view of that, and despite the absence of freezing events during our study period, the substantial Pro buildup observed at the end of the growing season in *E. penyalarense* and *A. caespitosa* at high elevations (>2,100 m) might imply an acclimation strategy to the annual climate variability in these mountains. This strategy could allow the maintenance of the aboveground portion of the plant through periods with a higher chance of freezing events and cold snaps, enabling a stretched resource-gaining period followed by the potential remobilization of osmoprotectants to meristems and roots to avoid freezing during winter. Thus, these findings suggest the role of Pro accumulation in the freezing-drought response in the studied Mediterranean high-mountain specialists.

Significant differences in the degree of responsiveness to changes in SWC levels were found among these specialists. Supporting our initial assumption, we observed a significant

increase in osmolyte contents alongside the seasonal escalation of summer drought and small variations in SWC in most of the studied hemicryptophytes (e.g., *E. penyalarense, H. vahlii, J. humilis*). Meanwhile, in the other species (e.g., *F. curvifolia, J. alpina, S. carpetanus*) significant osmolyte buildup was only observed when plants were subjected to severe climatic conditions or extremely reduced SWC. Despite osmolyte accumulation patterns differed among functional groups, these specialists can be classified in terms of their behavior during summer drought as either (1) resilient (i.e., most herbaceous hemicryptophytes), given their increased osmolyte accumulation consistent with summer drought; or (2) robust, comprising the chamaephytes *A. caespitosa* (dwarf cushion) and *J. alpina* (shrub), plus two hemicryptophytes: the dominant species *F. curvifolia,* and perennial herb *S. carpetanus*. The clear robustness to annual climate variations observed could be related to enhanced life strategies and functional trait differentiation in each biotype. For instance, robustness to climate extremes in chamaephytes is attributed to their atmosphere-decoupling capacity, which allows them to maintain a milder and relatively stable temperature and moisture conditions within their canopy, compared to their surroundings (Körner, 2003). In the case of *J. alpina,* these findings relate to the superior drought-tolerance reported for junipers, as they present an anisohydric regulation which allows high stomatal conductance and, thus, sustaining high photosynthetic uptake under dry conditions (McDowell et al., 2008; Zweifel et al., 2009). The suggested robustness in *S. carpetanus* may be linked to long-term feedbacks driven by long-standing environmental coarsening in this mountain range. These feedbacks may have caused the selection of functional traits that confer greater drought endurance, such as smaller leaf areas to reduce the ratio of hydraulic demand to supply (Magaña Ugarte et al., 2020). In *F. curvifolia*, the inferred robustness could be attributed to its strong competitiveness for resources and dominance in these xerophytic pastures (Pescador et al., 2014). The positive effects exerted by *F. curvifolia* and its "resource island," fostered by its dense root system and above-ground biomass, may become negative in the close proximity to the species and within its clumps, enhancing its competitiveness relative to the coexisting species in the community (Pescador et al., 2014).

The seasonal aggravation of drought could induce an increased osmolyte accumulation in plants as an adaptive response, particularly toward the dry-lower end of the elevational gradient (Sierra-Almeida et al., 2009). Moreover, water limitation is probably exacerbated in the thin, sandy-textured soils where *F. curvifolia* grasslands occur (Pescador et al., 2015). Contrary to our initial assumption, the absence of overall significant differences in osmolyte buildup between populations from contrasting elevations suggests the lack of enhanced endurance to summer drought in low-elevation populations relative to those from the high end. These summits are characterized by the combined effect of two opposed severity gradients operating along the elevation gradient: drought stress which decreases with elevation, and low-temperature stress which increases with elevation; which could neutralize the species net responses to changes in soil moisture availability (Pescador et al., 2015). The latter could provide an explanation of the weak response found in the studied species along the elevation gradient compared to the temporal gradient.

Fructans (e.g., inulin) and raffinose accumulation in vegetative tissues have been shown to contribute to osmotic adjustment in several species by acting as osmotic regulators or maintaining membrane integrity under stressful conditions (Taji et al., 2002; Valluru and Van den Ende, 2008; Krasensky and Jonak, 2012). Resembling the high drought-induced raffinose accumulation found in transgenic *A. thaliana* (Taji et al., 2002), the seasonal raffinose buildup found solely in low-elevation individuals of *E. penyalarense* and *S. carpetanus* in 2017 (**Supplementary Table 1**) could be a protective mechanism to delay or prevent the negative impact of more severe drought stress at the lowest elevation, compared to higher elevations (Sierra-Almeida et al., 2009). The exposure to constraining conditions over the course of several days could have resulted in high accumulation of raffinose and Pro (Krasensky and Jonak, 2012). Additionally, the maintenance of high inulin levels throughout the growing season in all species at both ends of the elevation gradient is consistent with the findings by Amiard et al. (2003); Vereyken et al. (2003), and Hincha et al. (2006), advocating for its pivotal role in protecting against drought stress via membrane stabilization. Furthermore, the minor reduction observed in the contents of soluble sugars and inulin in these specialists could suggest a requirement for a fixed level in the OAC, unconstrained by functional type, to sustain metabolic functions under summer drought in this elevation gradient (Martínez-Vilalta et al., 2016). In all the studied species, the OAC levels remained relatively constant and high throughout the season, similar to the low seasonal variability of soluble sugars and their slight increase in the summer months reported by Martínez-Vilalta et al. (2016) in a series of Mediterranean lowland species. The latter suggests a widespread OAC pattern in the Mediterranean flora.

The capacity of individual species to adjust to water availability likely drives their presence in a community. Our findings provide suggestive evidence of how summer drought could impose certain limits in these species. For instance, the substantial increase in Pro content in *J. humilis,* only during a dry year (i.e., 2017), compared with the lower Pro buildup recorded the following year under milder, wetter conditions (**Figure 3**). Relatedly, *J. humilis* showed an extended growing season during the atypically dry conditions of 2017 (personal observation), unlike in the milder growing season of 2018 (**Supplementary Figure 2**). Exposure to a longer growing season in 2017, coupled with the intensification of topsoil desiccation due to a substantially earlier snowmelt date and dry winter, could have prevented the efficient use of the limited water supply (Giménez-Benavides et al., 2007). Moreover, a limited rooting volume explored could have exposed *J. humilis* to more negative soil water potentials than other species with larger, denser root systems (e.g., *F. curvifolia, J. alpina*; McDowell et al., 2008) ultimately constraining water. Another example is the observed species occurrence (withdrawal of the aboveground portion and random re-sprouting) and high osmolyte accumulation in

E. penyalarense when subject to very low SWC. The observed onset of partial biomass recovery and leaf regrowth following extreme environmental conditions could be attributed to the availability of stored reserves, suggesting the role of the high pre-disturbance OAC_{TOTAL} in *E. penyalarense*'s recovery (Van der Heyden and Stock, 1996; Wyka, 1999; Morvan-Bertrand et al., 2001). In contrast, the low mean Pro generally maintained in *J. alpina* at all elevations and as the summer drought intensified, coupled with the seldom seasonal changes in OAC_{TOTAL}, resembles the notably unaltered behavior observed in other junipers undergoing severe drought (Zweifel et al., 2009; Herrero and Zamora, 2014). This high drought-tolerance can be partly explained by their ability to sustain very low-water potentials due to their cavitation−resistant xylem, low leaf/root area ratio, large water potential gradient from soil to leaf, and their relatively short stature (McDowell et al., 2008). The observed upholding of OAC_{TOTAL} levels is in keeping with the inferred survival strategy of hydraulic adjustment for junipers, by means of leaf area reductions to maintain hydraulic function and non-structural carbohydrate pools above critical levels (Limousin et al., 2013). Similarly, we observed the maintenance of low mean Pro content in *F. curvifolia* in climatically differing years (**Figure 3**). This could relate to the behavior of the roots of *F. curvifolia* capturing soil resources and its dense aboveground biomass plausibly trapping both nutrients and water from overflow and/or precipitation (Pescador et al., 2015). Nevertheless, the action of other osmoregulators is not discarded in this graminoid, particularly that of GB. The latter as GB is one of the main osmolytes accumulated in Poaceae to counter osmotic stress (Annunziata et al., 2019). Overall, these findings suggest enhanced hardiness to summer drought in these two species, compared to the other specialists studied. Moreover, the observed capacity of *F. curvifolia* and *J. alpina* to restore low Pro levels following the incidence of extreme climatic episodes in June 2017 (a combination of severe drought and heat) underlines their hardiness to extreme climatic events during their growing season. Nevertheless, further research is needed to assess the tolerance of these species to drought stress and determine whether osmolyte accumulation would suffice to avoid detrimental effects on plant fitness and survival in these specialists, given the predicted exacerbation of summer stress in the region.

Our approach, while not allowing for outlining single drought-stress effects, provides support of some of the roles played by the studied osmolytes on plant functions such as osmoregulation. For instance, it is worth noting that the highest increase in mean Pro in some of these species (e.g., *E. penyalarense, S. carpetanus, H. vahlii*) coincided with the periods with a sharp decline in SWC (July–August; **Figure 1**), and with their period of reproductive activity (Fernández-González, 1991). Our findings are therefore consistent with suggesting OAC and Pro act simultaneously as osmoregulators in the studied species, with Pro appearing as the primary osmoprotectant strategy, out of the ones studied, in these Mediterranean high-mountain specialists. The proclivity for Pro accumulation observed in these high-mountain plants in preference to OAC buildup could be related to an adaptation strategy associated with the briefness of the growing season and the incidence of less favorable conditions in these habitats. During less favorable conditions, plants could be seeking to optimize photosynthate investment into tissue growth, reproduction, and storage rather than osmoregulation, allowing this task to be generally undertaken by Pro, as observed in *A. thaliana* under severe water deficit (Hummel et al., 2010). The high Pro accumulation could be further associated with its redox potential and role as a stabilizer in other metabolic processes following stress (Montesinos-Pereira et al., 2014; Ahmad and Hameed, 2018), contributing to counteract the simultaneous effect of various abiotic stressors shaping plant life in high-mountain environments (Körner, 2003).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

RM and RG performed the material preparation and data collection. RM, AE, and RG performed the data analysis. RM wrote the manuscript with all authors commenting and proofreading the current and previous versions of the manuscript. AE and RG performed editorial guidance and supervision. All authors read and approved the final manuscript version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.576122/full#supplementary-material

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Changes in Community Composition Induced by Experimental Warming in an Alpine Meadow: Beyond Plant Functional Type

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Climate warming exerts profound effects on plant community composition. However, responses to climate warming are often reported at the community and functional type levels, but not at the species level. To test whether warming-induced changes are consistent among community, functional type, and species levels, we examined the warming-induced changes at different levels in an alpine meadow from 2015 to 2018. The warming was achieved by deploying six (open top) chambers [including three non-warmed chambers and three warmed chambers; 15 × 15 × 2.5 m (height) for each] that resulted in a small increase in mean annual temperature (0.3–0.5°C, varying with years) with a higher increase during the non-growing season (0.4–0.6°C) than in the growing season (0.03–0.47°C). The results show that warming increased plant aboveground biomass but did not change species richness, or Shannon diversity and evenness at the community level. At the functional type level, warming increased the relative abundance of grasses from 3 to 16%, but decreased the relative abundance of forbs from 89 to 79%; relative abundances of sedges and legumes were unchanged. However, for a given functional type, warming could result in contrasting effects on the relative abundance among species, e.g., the abundances of the forb species *Geranium pylzowianum*, *Potentilla anserine*, *Euphrasia pectinate*, and the sedge species *Carex atrofusca* increased in the warmed (compared to the non-warmed) chambers. More importantly, the difference in species identity between warmed and non-warmed chambers revealed warming-induced species loss. Specifically, four forb species were lost in both types of chambers, one additional forb species (*Angelica apaensis*) was lost in the non-warmed chambers, and two additional species (one forb species *Saussurea stella* and one sedge species *Blysmus sinocompressus*) were lost in the warmed chambers. Consequently, changes at the species level could not be deduced from the results at the community or functional type levels. These data indicate that species-level responses to climate changes must be more intensively studied. This work also highlights the importance of examining species identity (and not only species number) to study changes of community composition in response to climate warming.

Keywords: community composition, species diversity, functional group, species identity, alpine meadow

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INTRODUCTION

Global warming has exerted a profound influence on plant species diversity at the community level (Arft et al., 1999; Klein et al., 2004; Elmendorf et al., 2012; Pauli et al., 2012; Steinbauer et al., 2018), and will continue to influence ecosystem processes, functioning, and ecosystem services to human society (Hector and Bagchi, 2007; Naeem et al., 2010; Hooper et al., 2012; Tilman et al., 2014; Isbell et al., 2017; Liu et al., 2018). For example, plant species loss may significantly reduce primary productivity and plant litter decomposition rates (Hector and Bagchi, 2007; Hooper et al., 2012). Consequently, examining the responses of plant communities to warming is critical to accurately predict future changes in ecosystem properties.

Prior studies have revealed significant changes in species richness and relative abundance of plant communities in response to warming particularly in alpine and arctic ecosystems (Grabherr et al., 1994; Klein et al., 2004; Walker et al., 2006; Elmendorf et al., 2012). For example, experimental warming is reported to increase primary productivity and the relative abundance of grasses at the expense of the sedges and forbs in alpine meadows (Ganjurjav et al., 2016; Liu et al., 2018). A rapid loss of plant species has also been recorded in alpine meadows (Klein et al., 2004; Wang et al., 2012; Zhang et al., 2015, 2017). Analogous results have been obtained in arctic regions (Chapin et al., 1995; Walker et al., 2006). However, most of these reports have focused on changes in plant productivity or species diversity at the community level (Klein et al., 2004), or on changes in relative abundance at the plant functional type level (Chapin et al., 1996; Ganjurjav et al., 2016; Liu et al., 2018). Moreover, most of these studies have reported the number of species lost but not the identity of the species that were lost.

As a consequence, the effect of global warming on species diversity at the species level is incompletely understood. The paucity of information at the species level is likely to result in misleading conclusions for three reasons. First, it fosters the assumption that changes at the species level are consistent with changes at the plant functional type level. This assumption is most probably incorrect because it is unlikely that all of the species within a specific functional type will change in abundance in the same way. For example, it is possible that one species will increase its relative abundance even if the functional type to which it belongs decreases in overall abundance. Likewise, it is also possible that some species will increase in abundance while others will decrease, such that the abundance of the functional type to which they belong remains unchanged (Klanderud and Totland, 2005). Second, the assumption that changes at the species level are consistent with changes at the plant functional type level leads to the speculation that species loss occurs only in the plant functional types that decrease in relative abundance. Theoretically, this speculation is likely to be true only if the species decreasing in abundance are more likely to be lost, a speculation that thus far has not been tested. Third, in the context of global warming, it leads to the speculation that species loss is the result of warming-induced changes in species richness. This would be untrue if warming-induced changes in species identity differ among treatments. Specifically, if only species richness is considered, species loss could be masked by species invasion. Consequently, overlooking information at the species level can limit our ability to accurately predict future ecosystem functioning, particularly when the selection effect (i.e., species identity effect) of species diversity on ecosystem functioning dominates the biodiversity—ecosystem functioning relationship.

Here, we present data concerning changes in community species diversity, the relative abundance for four plant functional types (i.e., legumes, grasses, sedges, and forbs) and each plant species, and species identity in an alpine meadow in eastern Tibetan Plateau, which is considered among the most sensitive ecosystems to climate change (Piao et al., 2012; Zhang et al., 2017). We use the data to test the null hypothesis that warming-induced changes are consistent among the community level (as indicated by aboveground plant biomass, species richness, species diversity, and evenness), the functional type level, and the species level. To test this hypothesis, we examined (1) whether changes in relative abundance are consistent among different plant functional types, (2) whether changes in relative abundance are consistent among different plant species within specific plant functional types, and (3) whether species loss occurs in the functional types whose relative abundances decreased by warming.

MATERIALS AND METHODS

Study Site

This study was conducted in an alpine meadow in Hongyuan County, Sichuan province, China (32°48′ N, 102°33′ E), in the eastern part of Tibetan Plateau. The elevation is ∼3500 m above sea level. The climate is characterized by a short Spring and Autumn, cool mild Summer, and a long, cold Winter. The annual mean temperature is 1.7°C, with the minimum and maximum monthly mean temperature is −9.3 and 11.1°C in January and July, respectively. The annual mean precipitation is ∼756 mm (but fluctuates significantly among years, mostly ranging between 450 and 900 mm), over 80% of which occurs during May to September (Cao et al., 2018).

The meadow has been intensively grazed (e.g., yak *Bos grunniens*, Hequ horse *Equus caballus*, and Tibetan sheep *Ovis aries*) for decades. Local residents currently use the meadow as either summer pasture (grazing in the growing season from May to September only) or winter pasture (grazing in the non-growing season from October to April only). Our experiments were performed in a winter pasture. The vegetative cover of the meadow is more than 90%, and the average plant height is ∼30 cm. The vegetation is dominated by an assemblage of forbs (e.g., *Saussurea nigrescens*, *Polygonum viviparum*, *Potentilla anserine*, and *Anemone trullifolia* var. *linearis*), sedges (e.g., *Kobresia setchwanensis* and *Carex* spp.), and grasses (e.g., *Deschampsia caespitosa*, *Festuca ovina*, and *Elymus nutans*). Along with a diversity of plant species (Xiang et al., 2009), many arthropods, such as pollinators (Hu et al., 2019), herbivores (Xi et al., 2013), and dung decomposers (Wu et al., 2011) co-exist in the meadow.

Experimental Design

During October 2014, six 15 (length) × 15 (width) × 2.5 m (height) open top chambers (OTCs) were randomly deployed in a fenced, flat area of about 1.0 ha. Each OTC was large enough to include the local species pool, microhabitat variation in our study site. The sides of all OTCs were covered with thin (<0.1 mm) steel screen with a mesh size of 0.2 × 0.2 mm (**Supplementary Figure 1A**). Three of the chambers were additionally covered with 8 mm thick transparent tempered glass. Moreover, the roof of these three chambers was discontinuously covered by 250 0.3 m (width) transparent glass strips with an angle of 45°, with a 0.6 m space between strips (**Supplementary Figure 1A**), which was judged sufficient to prevent any strong airflow stack effect in the large warmed OTCs (Wilson and Tamura, 1968). Although one-third of the roof area of these chambers was covered by glass strips, these chambers are referred to as OTCs (**Supplementary Figures 1A,B**). All the six chambers were placed 1 m into the soil, and steel screens (with a mesh size of 0.6 × 0.6 mm) were placed 1 m into the soil along their sides to prevent rodents from entering (**Supplementary Figures 1A,B**). The three OTCs with transparent tempered glass are designated as "warmed chambers" and the other three (with only steel screens) as "non-warmed chambers". It should be noted that the non-warmed chambers were constructed and installed to remove the confounding effect of the physical setting of OTCs (if any) on plant reproduction. It was observed (Xiaoli Hu, personal observation) that the steel sheets prevented arthropods entering in both the warmed and non-warmed chambers, except for a few high-flying species such as honeybees (*Apis mellifera* and *Apis cerana*) that are abundant because of apiculture in the study site (Mu et al., 2014). Consistently, plant pollination was generally not limited, as indicated by the indistinguishable difference (judged on the basis of a generalized linear model with binomial errors, $P = 0.9$) in seed set rate (sound seed number/total seed number) of the self-incompatible species *Saussurea nigrescens* among warmed chambers (0.65, $n = 15$, sd = 0.18), non-warmed chambers (0.63, $n = 21$, sd = 0.21), and field sites outside chambers (0.68, $n = 12$, sd = 0.17). In addition, all six chambers were subject to horse grazing in the non-growing season (early October) in each study year from 2015 to 2018, making the study meadow a winter pasture (**Supplementary Figure 2**).

Measurements for more than 4-years using HOBO PRO (Onset Computer Corporation, USA) for air temperature and humidity and Watchdog2000 (Spectrum Technologies, Inc., USA) for soil temperature and moisture indicated that the mean annual temperature was 0.3–0.5°C higher (at 30 cm above ground surface) and 0.2–0.5°C higher (at a 5 cm soil depth) in the warmed chambers compared to the non-warmed chambers (see Table S1 and Figure S2 of Hu et al., 2020). During the non-growing season, the mean temperature was 0.4–0.6°C higher (at 30 cm above ground surface) and 0.8–1.1°C higher (at 5 cm soil depth) in the warmed compared to the non-warmed chambers, whereas temperature increases were 0.03–0.47°C and −0.2–0.8°C, respectively during the growing season (see Table S1 in Hu et al., 2020). The vapor pressure deficit, which was calculated based on air temperature and humidity measurements, was 2.6–3.7% higher in the warmed than in the non-warmed chambers during the growing seasons of 2018 and 2019. Moreover, soil moisture was 2–3% (v/v) higher at a 5 cm soil depth in the non-warmed compared to the warmed chambers (see Table S1 in Hu et al., 2020). In addition, transparency, which was calculated as inside-chamber light intensity/outside chamber light intensity (measured using light meters; TES-1336A, Taiwan, China) in the middle July of 2018, was slightly lower in the warmed chambers (94.4%, $n = 45$) compared to the non-warmed chambers (97.9%, $n = 45$) (Hu et al., 2020).

Vegetation Sampling

Each chamber was subdivided into nine 5 × 5 m subplots. Species abundance was determined during early-August each year using a quadrat sampling method (Pauli et al., 2015). Specifically, for each sampling (each year), we randomly placed five 100 × 100 cm quadrats in five of the nine subplots. Subsequently, we divided each quadrat into 10 × 10 cm grids and recorded species presence and abundance following the protocols described by Pauli et al. (2015). In order to determine whether species loss occurred during the experiment, we extensively surveyed the entire plant community within each chamber, recording the presence of all plant species. Species that did not emerge for two consecutive years were designated as "lost."

In the middle of August of each study year when most plant species completed reproduction, all aboveground plant parts were clipped to the ground surface and harvested in 16 1 × 1 m quadrats that were randomly deployed in each chamber. The harvests were carefully sorted into four different functional types (i.e., legumes, grasses, sedges, and forbs), and then dried for 72 h at 75°C and weighed.

Data Analysis

At the community level, we determined species diversity for each quadrat by calculating Shannon diversity (i.e., Hill number 1) [i.e., $D = \exp(-\sum_{i=1}^{S} p_i \ln p_i)$] and Shannon evenness (Hill ratio) (i.e., $E = D/S$), where p_i is the relative abundance (species-specific abundance/total abundance per quadrat) of the i^{th} species out of S species) (Chao et al., 2014). Generalized linear mixed models (GLMMs) were used to determine the effect of warming on aboveground plant biomass, species richness, Shannon diversity, and Shannon evenness (with Poisson errors for species richness, beta errors for Shannon evenness, and Gaussian errors for aboveground biomass and Shannon diversity). In each model, "treatment" (warmed vs. non-warmed) was set as the fixed factor, and "year" and "chamber" as random factors. GLMMs were performed using the package "lme4" (Bates et al., 2015) and "R2jags" (Su and Masanao, 2020).

At the functional type level, the differences in the relative abundance and relative biomass (i.e., plant functional type-specific aboveground plant biomass/total biomass) were determined between the warmed and non-warmed chambers, using GLMMs (with a binomial error structure) with "treatment" as the fixed factor and "year" and "chamber" as random factors. At the species level, the warming effect on relative species

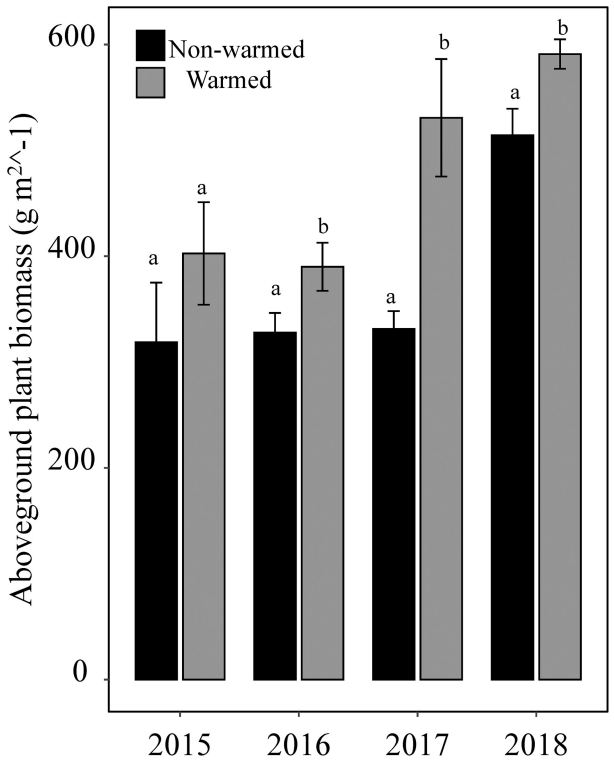


FIGURE 1 | Aboveground biomass in both non-warmed and warmed chambers from 2015 to 2018. Different letters indicate a significant difference ($P < 0.05$) between warmed and non-warmed chambers for each experimental year.

abundance was determined using GLMMs (with a binomial error structure) with "treatment" as the fixed factor and "year" and "chamber" as random factors. Only those species occurring in both warmed and non-warmed chambers were considered.

To further examine changes in the plant community composition during the experiment from 2015 to 2018, we conducted a two-way permutational analysis of variances (PERMANOVA) on the Bray-Curtis dissimilarity matrix including the relative abundance for all species [with log(n + 1) transformation]. The PERMANOVA was run with 999 permutations using the "adonis2" function in the R package vegan v. 2.5–4 (Oksanen et al., 2014). The dissimilarity in species compositions between warmed and non-warmed chambers was also computed using non-metric multidimensional scaling (NMDS) with Bray-Curtis distance and the "metaMDS" function in the R package vegan v. 2.5–4 (Oksanen et al., 2014).

All analyses were performed using R 3.5.3 (R Core Team, 2019).

RESULTS

Changes at the Community Level

Experimental warming significantly increased aboveground plant biomass starting in 2016 (**Figure 1**). However, the difference in species richness, Shannon diversity (as indicated by

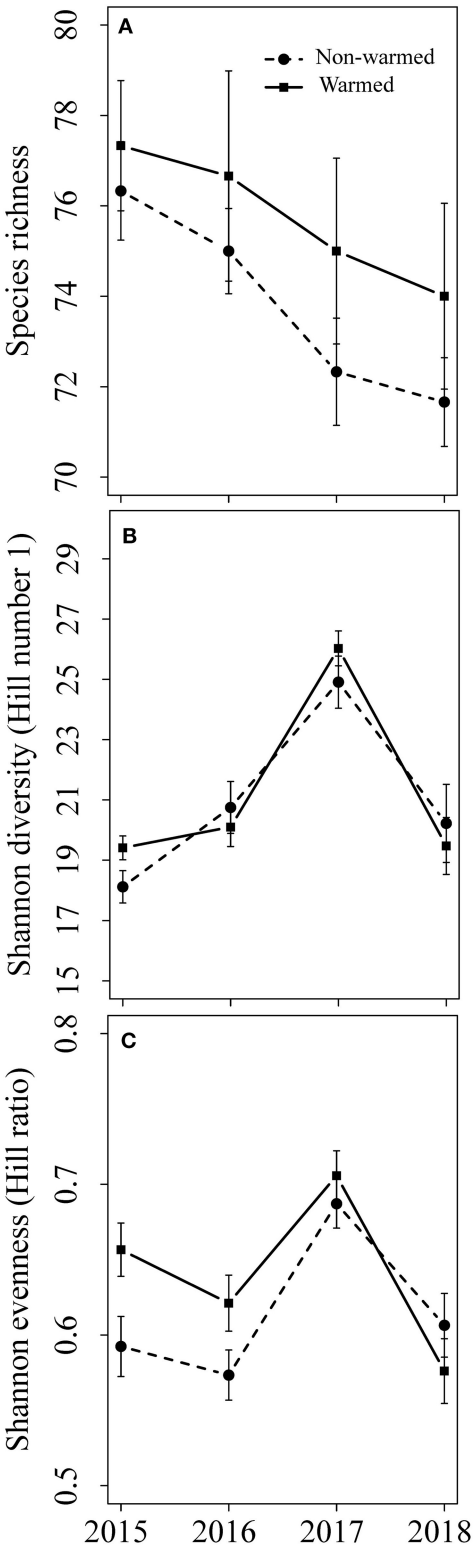


FIGURE 2 | Plant species diversity in both non-warmed and warmed chambers from 2015 to 2018. **(A)** Species richness; **(B)** Shannon diversity (Hill number 1); **(C)** Shannon evenness (Hill ratio). No significant difference in each variable was found between non-warmed and warmed chambers for each study year.

TABLE 1 | Summary of two-way PERMANOVAs (*F* and *P*-values) on Bray-Curtis community composition from 2015 to 2018.

Source of Variance	DF	*SS*	*F*	*P*
Treatment	1	1.439	13.322	**0.001**
Year	1	5.934	54.929	**0.001**
Treatment*Year	1	0.5084	4.706	**0.002**
Residual	104	11.235		

P-values in bold when <0.05.

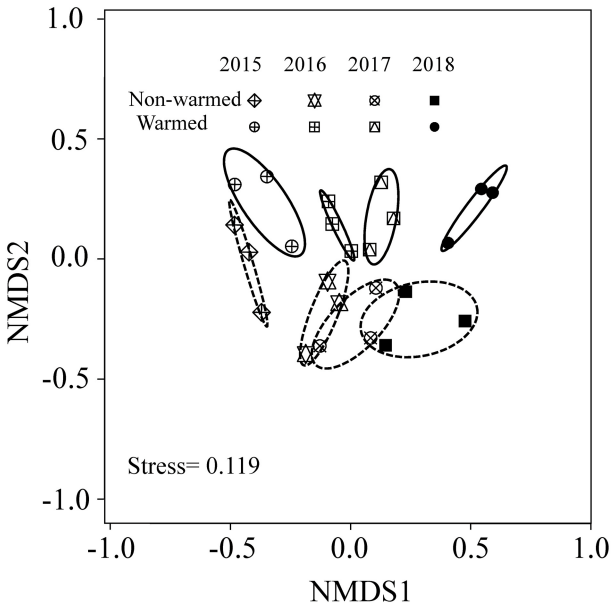


FIGURE 3 | Results of non-metric multidimensional scaling (NMDS) showing community dissimilarity between non-warmed and warmed chambers and years (2015–2018). Dissimilarity (Bray-Curtis) was estimated using the relative abundance of each species.

the Hill number 1) and Shannon evenness between the warmed and non-warmed chambers were statistically not significant (**Figure 2**). Moreover, the PERMANOVA and NMDS analysis showed that the overall warming-induced change in species composition was significantly different between 2015 and 2018 (**Table 1**; **Figure 3**).

Changes at the Functional Type Level

Warming-induced changes in relative abundance differed significantly among the four plant functional groups (**Table 2**). Warming increased the relative abundance of grasses from 3 to 16%, but decreased the relative abundance of forbs from 89 to 79% in 2018. In contrast, the relative abundance in the sedge and the legume functional groups did not significantly change (**Table 2**; **Figure 4A**). The relative biomass at the functional type level changed in a consistent way with respect to relative abundance (**Table 2**; **Figure 4B**).

Changes at the Species Level

Species belonging to the same plant functional type showed dissimilar responses in relative abundance to warming (**Table 2**; **Figure 5**). Specifically, in the forb group, warming significantly decreased the relative abundance of *Saussurea nigrescens* (**Table 2**; **Figure 5B**), but significantly increased the abundance of *Geranium pylzowianum*, *Potentilla anserine*, and *Euphrasia pectinate* (**Table 2**; **Figures 5C,F,I**) by 2018. Similarly, for the grasses, the relative abundance of *Festuca ovina* significantly increased, but remained unchanged for *Koeleria macrantha* (**Table 2**; **Figures 5K,L**). In the case of sedges, relative abundance decreased overall. However, *Carex atrofusca* significantly increased relative abundance as a consequence of warming (**Table 2**; **Figure 5N**)

Over the study years, four forb species (i.e., *Tibetia himalaica*, *Carum carvi*, *Microula sikkimensis*, and *Sanguisorba filiformis*) were lost in both the non-warmed and warmed chambers. Two species (i.e., the perennial sedge *Blysmus sinocompressus* and the perennial forb species *Saussurea stella*) were lost in the warmed chambers, and the forb species *Angelica apaensis* was lost in the non-warmed chambers.

DISCUSSION

Our results indicate that a warmed alpine meadow community has a higher primary productivity (as indicated by aboveground plant biomass), and warming-induced changes in the relative abundance differ significantly among plant functional types. These responses are consistent with previous studies addressing the effect of experimental warming on grassland communities (Arft et al., 1999; Wang et al., 2012; Zhang et al., 2015; Ganjurjav et al., 2016). Importantly, data show that species within the same plant functional type can respond differently to warming. These observations indicate that reporting changes at the community or functional type level is insufficient to predict changes at the species level. In addition, species loss occurred in both types of chambers and different species were lost in different chamber types, such that reporting a change in species richness is insufficient for describing the change at the plant community level. Thus, reporting the identity of lost species and understanding how they are lost are important when attempting to predict what species are more likely to go locally extinct under warming climatic conditions, a finding that has significance regarding conservation biology.

Although the warming-induced increase in aboveground plant biomass is consistent with previous warming studies in similar alpine meadows (Ganjurjav et al., 2016; Liu et al., 2018), changes in species diversity are less pronounced in this as opposed to other studies. For example, Chapin et al. (1995) showed that experimental warming reduced species richness by 30–50% in a tussock tundra; Klein et al. (2004) reported that warming over a period of 4 years resulted in a 26–36% decrease in species richness in Tibetan alpine meadow; and Sternberg et al. (1999) reported that manipulated warming

TABLE 2 | Summary of the GLMMs showing the warming effect on the relative abundance and biomass of 16 species and four functional groups.

	Name	Sum Sq	Estimates	*Z* value	*P* (>|*Z*|)
Relative abundance	*Gentiana abaensis*	12.29	−0.31	−3.5	**0.0005**
	Saussurea nigrescens	26.83	−0.27	−5.16	<**0.001**
	Geranium pylzowianum	92.03	0.33	9.58	<**0.0001**
	Polygonum viviparum	287.8	−0.72	−16.97	<**0.0001**
	Anemone rivularis	0.13	−0.027	−0.36	0.721
	Potentilla anserina	75.13	0.49	8.69	<**0.0001**
	Potentilla discolor	13.68	−0.39	−3.7	**0.0002**
	Anemone trullifolia var. *linearis*	9.29	−0.19	−3.054	**0.0022**
	Euphrasia pectinata	100.22	0.55	10.028	<**0.0001**
	Anaphalis flavescens	15.62	0.52	3.94	<**0.0001**
	Festuca ovina	65.94	0.6	8.07	<**0.0001**
	Koeleria macrantha	2.69	0.19	1.63	0.103
	Kobresia setschwanensis	8.34	0.31	2.9	**0.0038**
	Carex atrofusca	12.04	−0.29	−3.36	**0.0008**
	Oxytropis kansuensis	0.54	−0.088	−0.74	0.462
	Lathyrus quinquenervius	9.03	0.29	3.01	**0.0026**
	legume	10.27	0.289	3.21	**0.0013**
	grass	154.99	0.89	12.45	<**0.0001**
	sedge	6.2	−0.211	−2.48	**0.013**
	forbs	75.82	−0.44	−8.71	<**0.0001**
Relative biomass	legume	3.28	−0.217	−1.81	0.071
	grass	618.73	1.24	24.87	<**0.0001**
	sedge	51.91	−0.37	−7.21	<**0.0001**
	forbs	376.93	−0.82	−19.41	<**0.0001**

P-values in bold when <0.05.

decreased species richness by 10% in a calcareous grassland. In our study, warming did not significantly change species richness, Shannon diversity, or Shannon evenness over a 4-year period. This result contrasts with those of previous warming experiments conducted in alpine and arctic regions (Klein et al., 2004; Wang et al., 2012; Zhang et al., 2017). There are three possible explanations for why the warming-induced decrease in species richness is much smaller in our study system. First, the temperature increase in our experiment is smaller than that in other studies. Greater temperature increases often induce more pronounced changes in plant species diversity (Wang et al., 2019). Second, our experimental chambers are much larger than those used in previous studies. Large chambers might buffer the changes in species richness occurring at smaller and more homogeneous scales, i.e., larger chambers are likely to be more heterogeneous in their microclimate and micro-soil conditions compared to smaller ones (Vellend et al., 2013; Suggitt et al., 2018). Third, in the present study, the experimental control was non-warmed chambers compared to dispersal-unlimited field sites used in many other studies (e.g., Klein et al., 2004; Zhang et al., 2017). These chambers (including the warmed ones) were taller than those used in other warming studies (Klein et al., 2004, 0.4 m; Zhang et al., 2017, 0.4 m; Ganjurjav et al., 2016, 0.45 m), which could have significantly reduced or even prevented seed dispersal. Thus, seed dispersal would be equally confined in this study, whereas it was more confined in the warmed chambers than open sites in the other studies.

The difference in relative abundance among functional types reported here is also consistent with similar studies in our study region. Ganjurjav et al. (2016) and Liu et al. (2018) both reported a community shift from one dominated by forbs to one dominated by grasses in response to warming, whereas other studies indicated that sedge and legume species remain relatively unchanged in response to warming (Klanderud and Totland, 2005; Ganjurjav et al., 2016; Liu et al., 2018). These observations suggest that plant growth is normally limited by the low temperatures in alpine meadows and that increased temperatures likely increase aboveground plant biomass and plant height, which collectively increases interspecific competition for light. Tall grasses can competitively shade and exclude short forb and sedge species that tend to be rosette-like and short. Moreover, although soil moisture was on average high in our meadow study site, warming reduced soil moisture to a low level (<20% v/v) during the dry season (usually in August). This reduction in soil moisture might have limited the growth of sedge species and some forb species that are adapted to moist soil conditions (e.g., *B. sinocompressus* and *S. stella*; see also Klanderud, 2008; Little et al., 2015; Cao et al., 2017), but favored the growth of drought-tolerant species such as *D. caespitosa* and *Festuca* spp. (this study; see also Klanderud, 2008; Little et al., 2015).

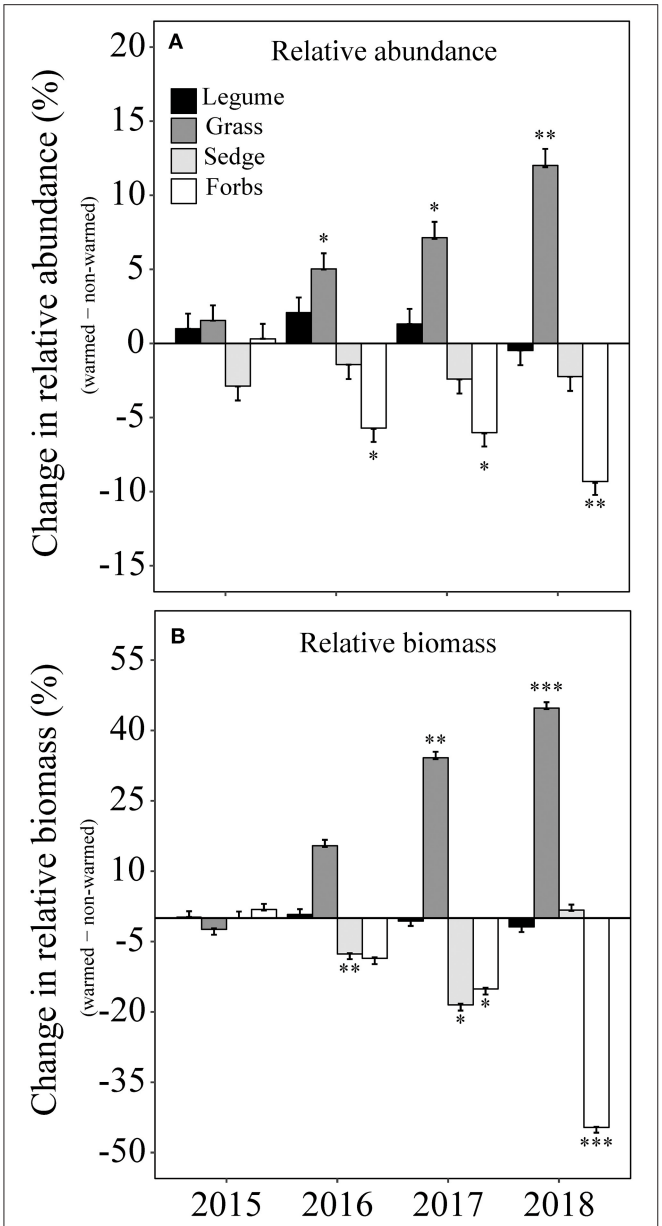


FIGURE 4 | Difference in relative abundance **(A)** and relative biomass **(B)** between non-warmed and warmed chambers for each functional type (i.e., Legume, Grass, Sedge, and Forbs) from 2015 to 2018. Positive and negative values indicate an increase or a decrease in the warmed chambers. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Importantly, individual species differed in their responses to warming both in direction and amplitude even for those within the same plant functional type. As noted, contrasting responses to warming have been observed in all plant functional types. This observation is attributable to differences in plant traits even those within the same functional type. For example, most forb species decreased in relative abundance probably because of competitive exclusion by tall grass species. Yet, three forbs (i.e., *G. pylzowianum*, *P. anserine*, and *E. pectinate*) increased substantially in their relative abundance in the warmed chambers. The abundance increase in *G. pylzowianum* and *P. anserine* is consistent with previous studies (Klanderud, 2008; Li et al., 2011), and can be attributed to their belowground storage organ (tubers) and the habit of climbing growing, which allows individuals to vegetatively overgrow other plants, thereby avoiding shade compared to plants growing in non-warmed chambers. The increase in the abundance of *E. pectinate* is also consistent with previous findings (Nyléhn and Totland, 1999; Klanderud, 2008). The underlying mechanism for its success is that this species is an annual root hemi-parasitic forb that can absorb water and nutrients from monocot species. Consequently, it increases in abundance in tandem with the abundance of grass species. It could be argued that species with different traits should not be placed within the same functional group. However, assigning species to too many different functional groups presents additional problems. Nevertheless, it is clear that subtle differences among the species assigned to any functional species group can result in substantive differences in response to warming, whether induced experimentally or experienced under natural field conditions.

Although differences in species richness were statistically indistinguishable between warmed and non-warmed treatments, species loss was demonstrably manifest as indicated by the fact that both warmed and non-warmed chambers experienced species loss and that different species were lost in the two different types of chambers. A number of factors may contribute to this observation. For example, in both warmed and non-warmed chambers, the loss of the forb species *Carum carvi* and *Microula sikkimensis* might have been the result of reduced disturbance and increased plant density during the experiment, because these species usually grow in ruderal or bare areas. The loss of the forbs *Tibetia himalaica* and *Sanguisorba filiformis* might be due to the shading of tall grasses because these two species are short and rare. The loss of the forb species *Saussurea stella* and the sedge species *Blysmus sinocompressus* in warmed chambers may perhaps be the result of decreasing soil moisture and their competitive exclusion by tall plants. Both of these species are short and often grow in high-moisture microhabitats (Xiaoli Hu, Personal Observation; Flora of China, http://www.iplant.cn/). Finally, the loss of the forb species *Angelica apaensis* in non-warmed chambers may reflect its demographic stochasticity because this species is extremely rare.

In summary, the results presented here show that a small increase in mean annual temperature and a winter-biased higher temperature during the non-growing season induces significant changes in an alpine meadow plant community, as evidenced by shifts in the relative abundance and biomass of different plant functional types. The results also indicate that changes in species relative abundance and biomass are not necessarily consistent with those of plant functional types, indicating that the data from plant functional types can obscure important information about the differential responses of individual species to climate change. Perhaps more important, whether species loss occurs cannot be deduced from changes in species richness. Collectively, species-level information is necessary to accurately predict plant

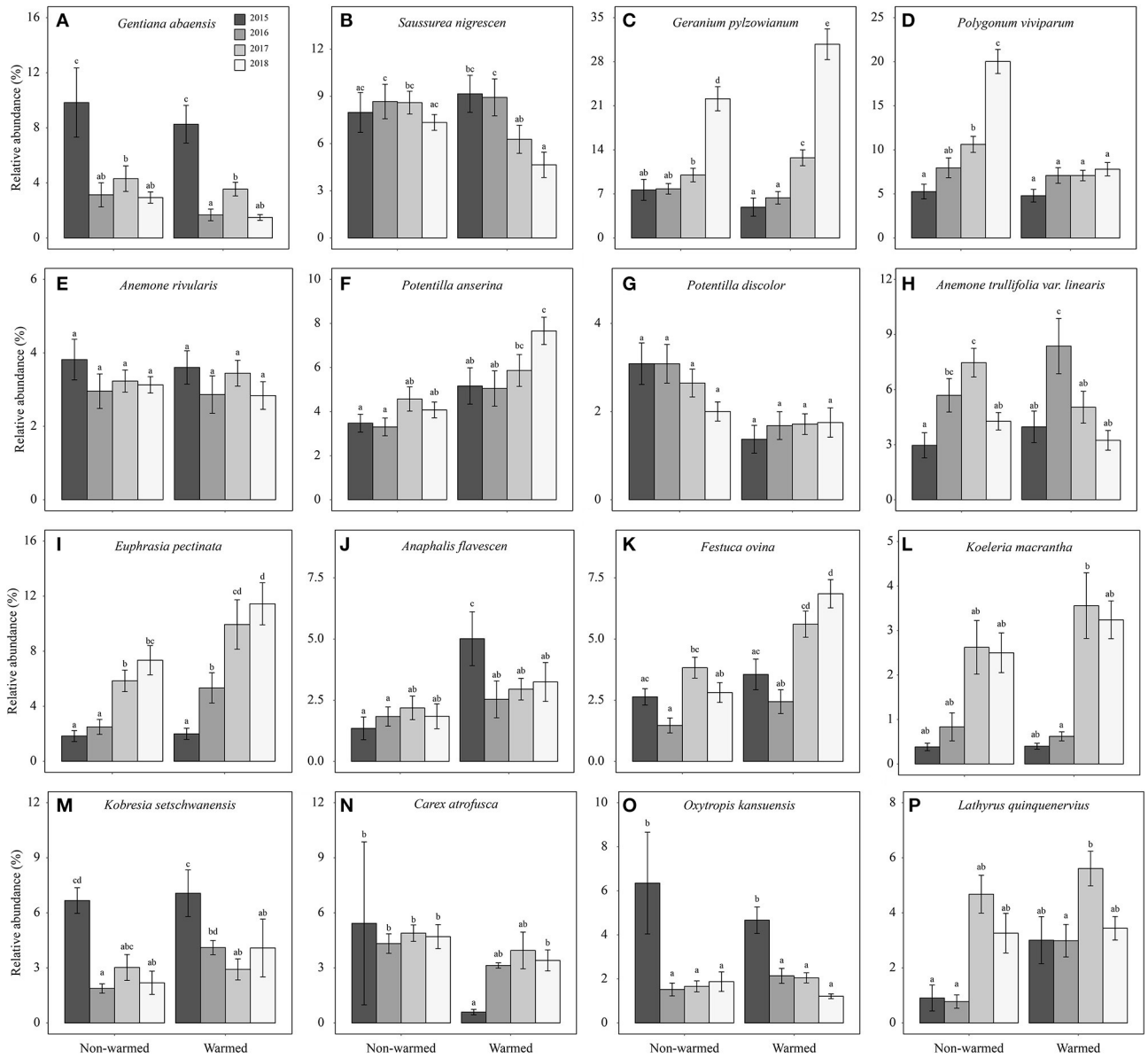


FIGURE 5 | Relative abundance of ten forbs (*Gentiana abaensis*, *Saussurea nigrescens*, *Geranium pylzowianum, Polygonum viviparum*, *Anemone rivularis, Potentilla anserine*, *Potentilla discolor*, *Anemone trullifolia* var. *linearis*, *Euphrasia pectinate,* and *Anaphalis flavescens*, **A–J**), two grasses (*Festuca ovina* and *Koeleria macrantha,* **K,L**), two sedges (*Kobresia setchwanensis* and *Carex atrofusca,* **M,N**), and two legumes (*Oxytropis kansuensis* and *Lathyrus quinquenervius,* **O,P**) in both non-warmed and warmed chambers from 2015 to 2018. Error bars = 1 standard error of mean. Different letters indicate a significant difference ($P < 0.05$) between warmed and non-warmed chambers.

community responses to global change. Future studies should consider the behavior of individual species when predicting changes in ecosystem functioning or service, or making policy decisions concerning biodiversity protection and conservation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SS started the project and designed research, and drafted the manuscript. XH, WZ, and XL performed research. SS, XH, and KN analyzed data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.569422/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Ecology and Evolution

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The Consequences of Glacier Retreat Are Uneven Between Plant Species

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Glaciers are retreating worldwide, exposing new terrain to colonization by plants. Recently-deglaciated terrains have been a subject of ecological studies for a long time, as they represent a unique natural model system for examining the effects of global warming associated with glacier retreat on biodiversity and the spatio-temporal dynamic of communities. However, we still have a limited understanding of how physical and biotic factors interactively influence species persistence and community dynamics after glacier retreat and glacier extinction. Using hierarchical joint species distribution models, we integrated data on plant species occurrence at fine spatial scale, spatio-temporal context, environmental conditions, leaf traits, and species-to-species associations in plant communities spanning 0 to c 5,000 years on average after glacier retreat. Our results show that plant diversity initially increases with glacier retreat, but ultimately decreases after glacier extinction. The 22% of plant species non-linearly respond to glacier retreat and will locally disappear with glacier extinction. At the local scale, soil carbon enrichment and reduction of physical (topographic) disturbance positively contribute to distribution patterns in 66% of the species, indicating a strong impact of community-level environmental conditions. Furthermore, positive and negative associations among species play a relevant role (up to 34% of variance) in driving the spatio-temporal dynamic of plant communities. Global warming prompts a shift from facilitation to competition: positive associations prevail among pioneer species, whereas negative associations are relatively more common among late species. This pattern suggests a role of facilitation for enhancing plant diversity in recently ice-free terrains and of competition for decreasing species persistence in late stages. Associated to that, species persisting the most show more "conservative" traits than species of concern. In summary, although plant diversity initially increases with glacier retreat, more than a fifth of plant species are substantially declining and will disappear with glacier extinction. Even for the "winners," the "victory" is not to be taken for granted due to the negative impact of rising competition. Integrating survey data with hierarchical and network models can help to forecast biodiversity change and anticipate cascading effects of glacier retreat on mountain ecosystems. These effects include the reduction of ecosystem services and benefits to humans, including food production from the pioneer species *Artemisia genipi*.

Keywords: biodiversity change, community dynamic, competition, facilitation, glacier forelands, global warming, hierarchical modeling, plant networks

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1. INTRODUCTION

Glaciers are retreating, shrinking, and disappearing worldwide due to global warming (Marzeion et al., 2014; Zemp et al., 2015; Roe et al., 2017). The retreat of glaciers affects landscape configuration, water availability, and natural hazards, impacting downstream ecosystems and society (Fell et al., 2017; Cauvy-Fraunié and Dangles, 2019). Therefore, plants inhabiting proglacial environments are highly sensitive to global warming and inherent glacier retreat (Erschbamer, 2007; Dullinger et al., 2012). Understanding how plant communities are responding to ongoing glacier recession is crucial for projecting biodiversity dynamics and planning sustainable ecosystem management (Walker and del Moral, 2003; Erschbamer and Caccianiga, 2016).

As glaciers retreat, new terrains are exposed to colonization by living organisms. The further away and downstream from the current glacier front, the longer ago the terrain was exposed by glacier retreat, the older the community. Thus, in general, community age increases with increasing the distance from the retreating glacier. With the support of geochronological records, one can reconstruct the past positions of glaciers, thus date the terrain and assign an age to the community (Matthews, 1992). During the last 10,000 years, the Little Ice Age (LIA, ca 1350–1850 CE) was the main episode of re-advance of glaciers in the European Alps (Maslin et al., 2019). Despite the rate of glacier retreat varies regionally, including two short recent periods of glacier advances in the 1920s and 1980s, glaciers are retreating at historically unprecedented rates globally (Zemp et al., 2015). For these reasons, the areas in front of retreating glaciers (hereafter, glacier forelands) are unique natural systems and prominent models for studying how populations change, communities assemble, and ecosystems develop over spacetime in response to global warming (Whittaker, 1993; Chapin et al., 1994; Caccianiga et al., 2006).

Various theories have been proposed to describe the orderly process of ecosystem development involving changes in species composition and communities over time, i.e., succession. The classic Connell and Slatyer models account for facilitation, tolerance, or inhibition between initial colonizers and later ones (Connell and Slatyer, 1977). In the facilitation model, initial colonizers ameliorate environmental constraints on plant colonization, growth and reproduction, facilitating the establishment of later colonizers. In the tolerance model, differential life history traits and the differential ability of species to tolerate initial environmental conditions determine the arrival and growth of species, with later colonizers growing in spite of the presence of initial ones. In the third one, initial colonizers inhibit the growth and establishment of later species. The hierarchical framework proposed by Pickett et al. (1987b) expanded on the importance of initial conditions (site availability) and on the concept of differential species availability and performance (e.g., dispersal, growth rate, life history strategies, environmental stress) to explain mechanisms of succession. The geoecological model (Matthews, 1992; Whittaker, 1993) postulates that the environment exerts more control over community development than organisms in the initial stages, while habitat modification by organisms and species interactions become more important in later development phases. Similarly, complex combinations of negative and positive biotic interactions structure plant communities (Chapin et al., 1994). In particular, facilitation is most common in harsh environments (initial stages), while competition becomes more important in more favorable environments (later stages) (Bertness and Callaway, 1994; Callaway and Lawrence, 1997). Finally, Grime's c-s-r theory (Grime, 2001) built on differences among plants in their competitive ability, tolerance to physical stress, and dependence on colonization over resource and disturbance gradients. It describes a pathway defined by control of disturbance initially that changes toward control of resources by competition and then tolerance to biotic stress (Caccianiga et al., 2006). Recent syntheses (Meiners et al., 2015; Erschbamer and Caccianiga, 2016; Pulsford et al., 2016) highlight that mechanisms underlying changes in biodiversity are often a complex interaction of species traits, physical environment, stochasticity, and effects of neighboring plants. However, the role of ecological networks for shaping biodiversity, driving species distribution and contributing to species response to global warming remains overlooked. Since biodiversity is more than a list of species (Bascompte and Jordano, 2014), understanding the contribution of ecological networks to species' response to global warming is of paramount importance for forecasting biodiversity, developing scenarios, and mitigating species loss.

There is evidence that biodiversity increases with glacier retreat (Raffl et al., 2006; Cauvy-Fraunié and Dangles, 2019), but this holds true only as long as glaciers are still present (Stibal et al., 2020). In fact, we are facing the extinction of glaciers around the globe (Dyurgerov, 2005; Zemp et al., 2015), especially in tropical mountain ranges and mediterranean mountains (Apennine, Sierra Nevada of California), while many glaciers will disappear within the next decade in the Alps, temperate Andes, Caucasus and Himalaya (Huss et al., 2017). Glacier forelands comprise unique habitats that host distinctive organisms and communities. Increasing evidence indicates high turnover rates in mountain environments (Steinbauer et al., 2018), where increase in species richness of lower-altitude plants is followed by decrease of populations and range contraction of high-altitude species (Thuiller et al., 2005; Pauli et al., 2007; Dullinger et al., 2012). Accordingly, it is possible that not all the species would have the possibility to migrate and colonize to suitable habitats nearby (Körner, 2005), so high-altitude plants associated to glacier environments will not persist locally following glacier extinction. However, the consequences of glacier retreat and extinction for plant species inhabiting glacier forelands as well as whether and how ecological networks mediate such impact remain poorly understood and quantified.

Here, we hypothesize that glacier extinction will severely reduce a set of plant species, ultimately causing biodiversity loss. Furthermore, we propose that facilitation will support persistence of initial colonizers while competition will exacerbate extinction risk. We answer the following questions: (i) How do plant species and communities respond to glacier retreat? (ii) Which and in what proportion will species persist or disappear after glacier extinction? (iii) How do plant networks change over spacetime and how they mediate species' response to glacier retreat?

2. MATERIALS AND METHODS

2.1. Data Collection

We integrated published datasets on plant species distribution and leaf traits with unpublished, original data on environmental conditions. Published datasets on plant community surveys were compiled including identity and abundance of plant species at local spatial scale (meters) at the following sites: (i) Vedretta d'Amola glacier (Adamello-Presanella Alps, Italy, 46°13'16" N, 10°40'41" E), characterized by a debris-covered glacier colonized by plants, glacier tongue position dated to 1994 and 1925, and LIA moraine (Losapio et al., 2016); (ii) Western Trobio glacier foreland (Orobic Alps, Italy, 46°03'36" N, 9°57'36" E), characterized by moraines dated to 1985, 1920, 1900, and LIA (Tampucci et al., 2015); (iii) Rutor glacier foreland (Graian Alps, Italy 45°38'0" N, 7°1'0" E), characterized by moraines dated to 1933, 1873, and LIA (Caccianiga and Andreis, 2004), and (iv) Vedretta di Cedec glacier foreland (Ortler Alps, Italy, 46°27'N, 10°35' E), characterized by three main moraines dated to 1986, 1965, and LIA (Gobbi et al., 2010). Each site comprised grasslands that are adjacent to each glacier foreland and occur in terrains beyond LIA moraines. These communities are dated to the Late Glacial period, from 200 to 10,000 years BP. Given this landscape configuration, we consider: (i) communities occurring in glacier forelands being representative of a scenario of glacier retreat, with glaciers still present and affecting ecosystems locally, and (ii) communities beyond LIA moraines (outside glacier forelands, the "potential natural vegetation", Caccianiga and Andreis, 2004) being the representative of a scenario of glacier extinction, which are not directly affected by Anthropocene glacier dynamic.

Surveys were replicated three to seven times in 25–100 m^2 plots in each community of different age along a transect starting from the glacier front. Leaf traits including Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC) were downloaded from TRY database (Kattge et al., 2020) and complemented with our own original measurements. SLA and LDMC were measured following standard protocols (Pérez-Harguindeguy et al., 2013). For each species, we sampled from 5 to 15 fully expanded leaves selected randomly from the outer canopy of different adult healthy plants within the same population. Leaf material was stored at 4°C overnight to obtain full turgidity for determination of fresh weight (LFW) and area (LA). LA was determined using a digital leaf area meter. Leaf dry weight (LDW) was then determined following drying for 24 h at 105°C. SLA was calculated as the ratio between LA and LDW. LDMC was calculated as the ratio between LDW and LFW.

In order to harmonize data across different studies for the further analysis of plant diversity dynamic, we considered species occurrence (presence/absence) in each study plot (sample). We then pooled data across sites into a single community matrix Y

$$\mathbf{Y}: \begin{bmatrix} y_{1,1} & y_{1,2} & \cdots & y_{1,j} \\ y_{2,1} & y_{2,2} & \cdots & y_{2,j} \\ \vdots & \vdots & \ddots & \vdots \\ y_{i,1} & y_{i,2} & \cdots & y_{i,j} \end{bmatrix}, \quad (1)$$

where plant species j are arranged in columns, plots i in rows, and the entries y_{ij} represent the species occurrence in the plot, with y_{ij} equals to 0 or 1 in case of absence or presence of the species, respectively.

Community age x of each plot i was estimated as the average years since glacier retreat between two moraines as $x_i = \sqrt{x_s - \frac{(x_{old}+x_{young})}{2}}$, where x_s is the year of sampling, x_{old} and x_{young} is the geochronological information (age) of older and younger moraines adjacent to the community, respectively. For instance, communities located between the glacier position in 2013 and the moraine dated 1994 were considered having an average age of 10 years. In the same way, communities surveyed in 2014 between two moraines deposited in 1985 and in 1920 have an estimated average age of 62 years, and plots beyond the LIA moraines have an estimated average age of 5,000 years. This approach allows us quantifying biodiversity patterns and dynamics with greater resolution than before by overcoming the limitations of using categorical data. As any other mean estimate, however, it has the limitation of focusing only on average trends excluding variation around such mean. In fact, notice that there is substantial geochronological uncertainty surrounding the age of communities beyond LIA moraines, since their age can span a range of 170–10,000 years.

In light of this potential shortcoming, we then explored the implications of such uncertainty and the robustness of our results against this assumption. We adopted a second method that relies on relative differences in elevation along the glacier foreland transect. In particular, for the seek of among-site comparison and synthesis, we standardized elevation v for each plot i at each site k as $v_i = 1 - \frac{v_i - \min(v_k)}{\max(v_k) - \min(v_k)}$, where the maximum and minimum values of elevation along the glacier retreat transect were taken for each site.

Then, we proceeded with measuring the following soil parameters in the Vedretta d'Amola glacier and Western Trobio glacier: (i) soil organic matter (SOM); (ii) gravel content; and (iii) pH. Soil samples were taken at the surface for physical and chemical analysis. A sample of about 1 kg was taken at every plot for particle size distribution analysis; a sample of about 200 g was taken for soil pH (in 1:2.5 soil:water), calcium carbonate content (Dietrich–Fruhling calcimeter) and organic matter content (Walkley–Black method) (Sleutel et al., 2007).

Spatio-temporal context and soil conditions data were organized into a matrix X

$$\mathbf{X}: \begin{bmatrix} x_{1,1} & x_{1,2} & \cdots & x_{1,m} \\ x_{2,1} & x_{2,2} & \cdots & x_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ x_{i,1} & x_{i,2} & \cdots & x_{i,m} \end{bmatrix}, \quad (2)$$

where m environmental variables (i.e., community age x_i, standardized glacier retreat v_i, and soil conditions) are arranged in columns, plots i in rows. The integrated datesets for the Vedretta d'Amola glacier foreland and Western Trobio glacier foreland comprised 51 plants species and two soil variables (SOM and gravel) over 54 plots and 54 plant species and three soil variables (SOM, gravel, and pH) over 30 plots, respectively.

2.2. Hierarchical Modeling

We analyzed plant diversity and community dynamics using the Hierarchical Modeling of Species Communities (HMSC) (Ovaskainen et al., 2017; Ovaskainen and Abrego, 2020; Tikhonov et al., 2020). This Bayesian framework accounts for the influence of environmental conditions, species' response traits, random variation in species occurrence, and species-to-species associations for modeling the joint distribution of species. In particular, we combined plant species occurrence data (Y matrix with plant species in columns and plots in rows) with the spatio-temporal context (X matrix defining sites and community age in columns for each plot in rows) and trait data (matrix with SLA and LDMC for each species) accounting for the potential effects of environmental conditions (SOM, gravel, and pH) and biotic interactions (positive and negative species associations) (**Figure 1**).

The occurrence y of each species j in each plot i was modeled as $y_{ij} \sim B(L_{ij})$, which follows a binomial distribution B, where L_{ij} is a linear predictor composed of fixed and random parts. Fixed effects F are modeled as the quadratic regression $L_{ij}^{F} \propto \sum_{i=1}^{S} x_i \beta_j$, where x_i indicates community age at plot i, and β_j the parameters of the second degree polynomial measuring species response j to glacier retreat dynamic across all S plant species. In addition, we ran the same model with standardized elevation v_i in place of x_i. The parameters β_j are estimated following a multivariate normal distribution and are influenced by species-specific traits (SLA and LDMC). Sites were included as random term. This model was fitted to the integrated dataset (Y matrix) comprising 117 plant species and two leaf traits over 170 plots at four glacier-foreland sites.

In a second model, environmental conditions (SOM, gravel, and pH) were included as fixed effects, in addition to community age, and spatial correlation among plots within each site was included as random term. This allowed us inferring signals of "biotic interactions" by identifying species pairs that co-occur more or less often than expected by chance, after controlling for the response of species to environmental conditions (Ovaskainen et al., 2016). This way, associations between species are reflected by significant co-occurrences that cannot be attributed to the responses of species to the environment (Ovaskainen and Abrego, 2020).

HMSC uses a latent variable approach to estimate species associations, which has much fewer parameters to be estimated than other approaches because the number of latent factors is much smaller than the number of species (Ovaskainen et al., 2017; Tikhonov et al., 2020). At the community level, this approach generates patterns of associations between species embedded in a variance–covariance matrix **R**. Matrix entries R_{j1j2} measure the degree to which species j_1 and j_2 co-occur more or less often than expected by chance net of the influences of environmental conditions. Thus, latent variables can represent the outcome of ecological interactions, with factor loading representing the response of species j_1 to that latent interaction with species j_2 (Ovaskainen et al., 2016). Two separate models were fitted for the Vedretta d'Amola site and Western Trobio site.

Parameters are estimated using Gibbs MCMC with a flat prior and 2,000 samples from the posterior distribution across four chains (thin = 2). The MCMC scheme converged as chains reached a stationary distribution. We evaluated model fit in terms of explanatory power. Analyses were conducted using the "Hmsc" package (Tikhonov et al., 2020) in R version 4.0.2 (R Core Team, 2020).

2.3. Network Analysis

To explore changes in facilitation and competition among plant species across glacier forelands, we adopted a multilink network approach (Losapio et al., 2019). Associations between species significantly different from zero (95% CI) R_{j1j2}^{*} were then retained to build species association networks. Species association networks were composed of plant species and

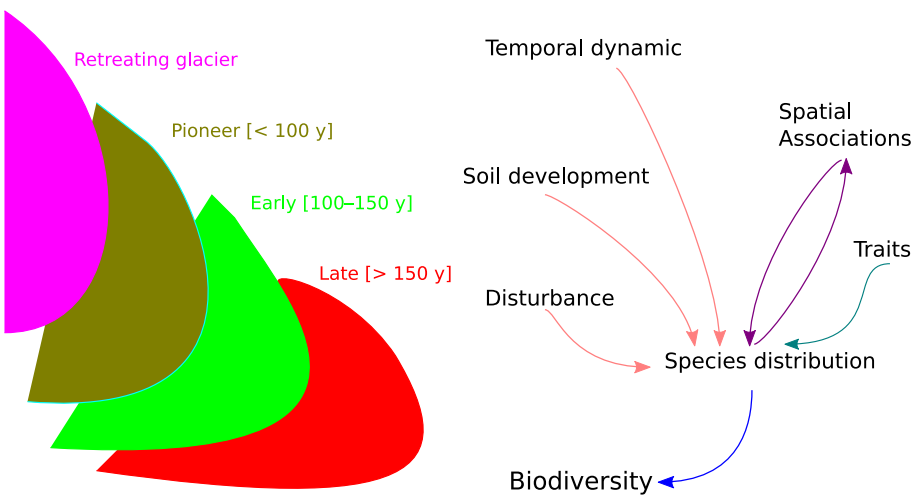


FIGURE 1 | On the left, schematic representation of glacier retreat dynamic, with "pioneer" (dark green), "early" (light green), and "late" (red) stages of ecosystem development. On the right, framework of the hierarchical joint species distribution modeling for forecasting biodiversity change.

associations between species taking the value of 1 or −1 if significant R^*_{j1j2} were higher or lower than 0, respectively.

Finally, to identify and classify species' response to glacier retreat, we implemented a species distribution network approach (Burns and Zotz, 2010; Losapio et al., 2019; Marini et al., 2019). We first transformed the plant community survey data into a bipartite network of species distribution over the landscape. In such network, plant species and community age of each plot are linked by species occurrence y_{ij}, representing the two parts of the network. Then, a fast greedy algorithm that optimizes a modularity score (Clauset et al., 2004) was used to analyze community structure and identify modules of plant species that are distributed across community ages. Network modules are dense subnetworks characterized by high occurrence frequency of a group of species within the same community age and low frequency or no occurrence between other species groups at different community age. This allowed us unveiling large-scale patterns of species response to glacier retreat. Two separate networks were built for the Vedretta d'Amola site and the Western Trobio site.

2.4. Statistics

First, we explored parameter estimates for β_j (species responses to glacier retreat), reporting mean and 95% posterior probability, as well as we generated predictions of plant diversity at the community level over glacier forelands as a function of terrain age, from zero (debris-covered glaciers) to 5,000 years.

Second, we estimated the probability of species persistence p_j after glacier extinction, at 5,000-years communities. Then, we related this probability to community type obtained from modularity analysis of species distribution networks. We used linear mixed-effects models including persistence probability as response, community type as predictor, and species identity nested within site as random effects.

Third, we quantified: (i) the frequency of positive and negative associations per species across network modules, and (ii) the density of positive and negative species associations per plot across the glacier retreat gradient in Vedretta d'Amola and Western Trobio sites. For the former, we used zero-inflated generalized linear models including association frequency as response (zero-inflated poisson distribution), association type (positive or negative), community type, and their statistical interaction as predictor. For the latter, linear models were fitted to association density in response to community age (continuous, square-root transformed), community type, and their statistical interaction. Two separate models were fitted for the two sites.

Finally, we analyzed the statistical association between traits and species distribution. We used a linear model for SLA and LDMC including community type (pioneer, early, late) as predictor.

Model output was evaluated in terms of both variance explained and parameter estimates. Analyses were conducted in R version 4.0.2 (R Core Team, 2020), using the packages "lme4" (Bates et al., 2015), "car" (Fox and Weisser, 2019), and "glmmTMB" (Brooks et al., 2019).

3. RESULTS

Glacier retreat affects the 51% of plants in a species-specific way (**Supplementary Table 1**), among which 29% of species will expand while 22% will disappear with glacier extinction. Examples of species that will flourish the most are: *Minuartia verna, Veronica fruticans, Achillea moschata, Trifolium pallescens, Draba aizoides, Poa alpina, Trisetum spicatum, Gentiana nivalis, Myosotis alpestris, Carex curvula, Antennaria dioica, Leontodon helveticus, Potentilla aurea, Senecio incanus*. Species of main concern for local extinction are: *Saxifraga bryoides, Artemisia genipi, Cardamine resedifolia, Leucanthemopsis alpina, Gnaphalium supinum, Sedum alpestre, Minuartia sedoides, Sempervivum arachnoideum, Hieracium staticifolium, H. glanduliferum*. Species that are ubiquitously distributed and do not show significant response to glacier retreat and environmental conditions are: *Anthoxanthum alpinum, Gentiana kochiana, G. punctata, Ligusticum mutellina, Pedicularis kerneri, Phyteuma hemisphaericum*. Functional traits contribute to explain the 4% of variation in species distribution.

Overall, plant diversity dynamic following glacier retreat is characterized by a hump-shaped trend (**Figure 2A**). In particular, our predictions show that diversity will increase immediately after glacier retreat with increasing community age x_i ($\beta = 0.017$). After saturating, plant diversity will decline toward poorer communities in the late stages of ecosystem development ($\beta = 0.020$). Results are qualitatively similar when using standardized differences in elevation v_i (**Figure 2B**).

We then considered plant community dynamics in the two sites (Amola and Trobio glacier forelands) with high resolution, local-scale data on environmental conditions. Glacier retreat, soil organic matter, gravel and species associations explain the 66–92%, 10–16%, 14–17%, 1–24% (95% CI) of the variance observed in species distribution along the Amola glacier foreland, respectively. In the Trobio glacier foreland, glacier retreat, soil organic matter, pH, and species associations explain the 61–94%, 1–11%, 1–12%, 1–34% (95% CI) of the variance observed in species distribution, respectively.

In both sites, species distribution networks were structured in three main modules (communities), which were composed by the 29, 21, and 50% of the species pool in the Amola glacier foreland and by the 42, 18, and 40% of the species pool in the Trobio glacier foreland. Results indicate that these communities are characterized by three distinct time periods of glacier retreat: "pioneer" (less than 100 years), "early" (between 100 and 150 years), and "late" (older than 150 years). We found that community type significantly predicted the persistence probability of species ($\chi^2 = 117.2$, $P < 0.001$, **Figure 3**). In particular, pioneer species were only 11.7 ± 8.0% likely to persist after glacier extinction, while early and late species were marginally ($P = 0.079$) and significantly ($P < 0.001$) more likely to persist, having a persistence probability of 21.3 ± 8.4% and 56.1 ± 7.8%, respectively. Variance among species and between sites was low ($\sigma = 0.038, \sigma = 0.011$).

Having shown that species differed in their persistence, we examined the distribution of network-level associations along the two glacier forelands. The Amola network comprised 150

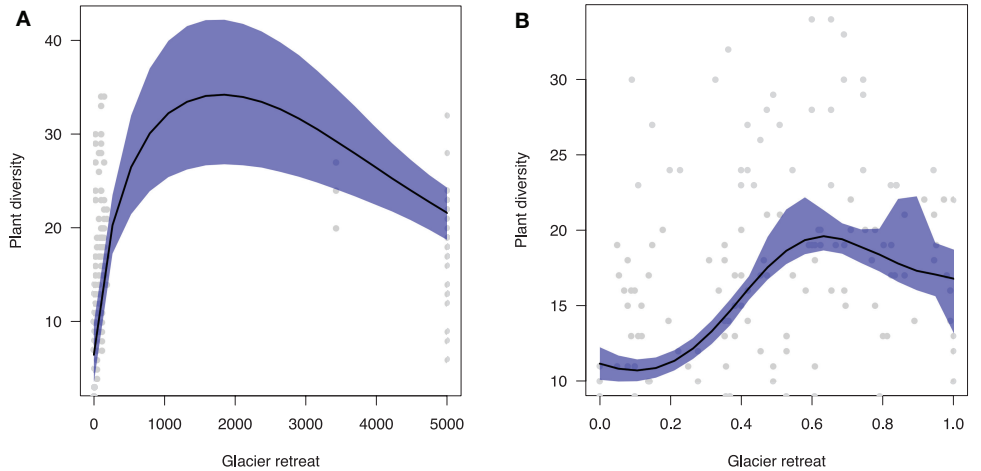


FIGURE 2 | Predicted effect of glacier retreat (*x*-axis) on plant diversity (*y*-axis). **(A)** Community age was estimated as the mean between two consecutive moraines. Notice that there is substantial geochronological uncertainty surrounding the age of communities beyond LIA moraines, since their age spans a range of 170–10,000 years (here, on average 5,000 years). **(B)** Community age was estimated as standardized differences in elevation along the glacier foreland transect.

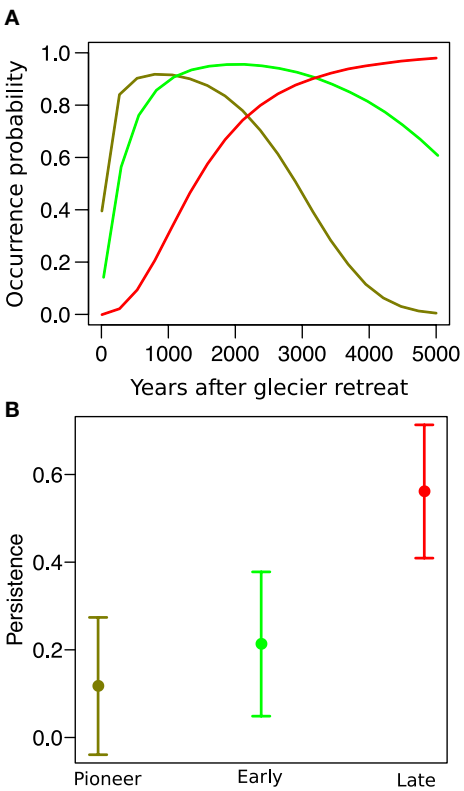


FIGURE 3 | (A) Distribution of three "model" species along the glacier foreland: the pioneer *Artemisia genipi* (dark green), the early *Luzula alpino-pilosa* (light green), and the late *Carex curvula* (red). **(B)** Persistence probability of species after glacier extinction across pioneer, early, and late species.

associations among 51 species (connectance = 0.06), among which 78 positive associations and 72 negative. Pioneer, early, and late species differ in their frequency of positive and negative associations ($\chi^2 = 5.2$, $P = 0.075$). In particular, late species were significantly more negatively associated to other species than early species ($\beta = 0.79 \pm 0.38$, $P = 0.037$; **Figure 4A**). Furthermore, the density of positive and negative associations depended on glacier retreat dynamic ($\chi^2 = 12.8$, $P < 0.001$), as negative associations became relatively denser than positive with increasing years after deglaciation ($\beta = 0.0007 \pm 0.0004$, $P = 0.011$; **Figure 4B**). The Trobio network comprised 90 associations among 54 species (connectance = 0.03), among which 54 positive associations and 36 negative. The frequency of positive and negative associations was significantly different ($\chi^2 = 5.9$, $P = 0.014$) and varied among plant communities ($\chi^2 = 22.8$, $P < 0.001$, **Figure 4C**). In fact, positive associations were significantly more prevalent than negative ones overall ($\beta = 1.13 \pm 0.29$, $P < 0.001$). Yet, negative associations were significantly more frequent among early and late species than pioneer ones ($\beta = 4.03 \pm 1.19$, $P < 0.001$, and $\beta = 4.48 \pm 1.24$, $P < 0.001$, respectively). Moreover, the density of associations marginally decreased with glacier retreat ($\beta = -0.002 \pm 0.001$, $P = 0.088$; **Figure 4D**).

Finally, we found that community type can predict plant traits ($P = 0.056$ for SLA and $P < 0.001$ for LDMC). In particular, pioneer and early species have different trait values from late species, having significantly higher SLA ($\beta = 3.34 \pm 1.60$, $P = 0.039$, **Figure 5A**) and significantly lower LDMC ($\beta = 7.62 \pm 1.90$, $P < 0.001$, **Figure 5B**).

4. DISCUSSION

We examined the poorly understood consequences of glacier retreat for biodiversity over a pool of 117 plant species in different alpine glacier forelands. Although glacier retreat triggers and favors the development of communities in the short term, plant diversity will decrease with glacier extinction in the long term: about one fourth of plant species would disappear locally once

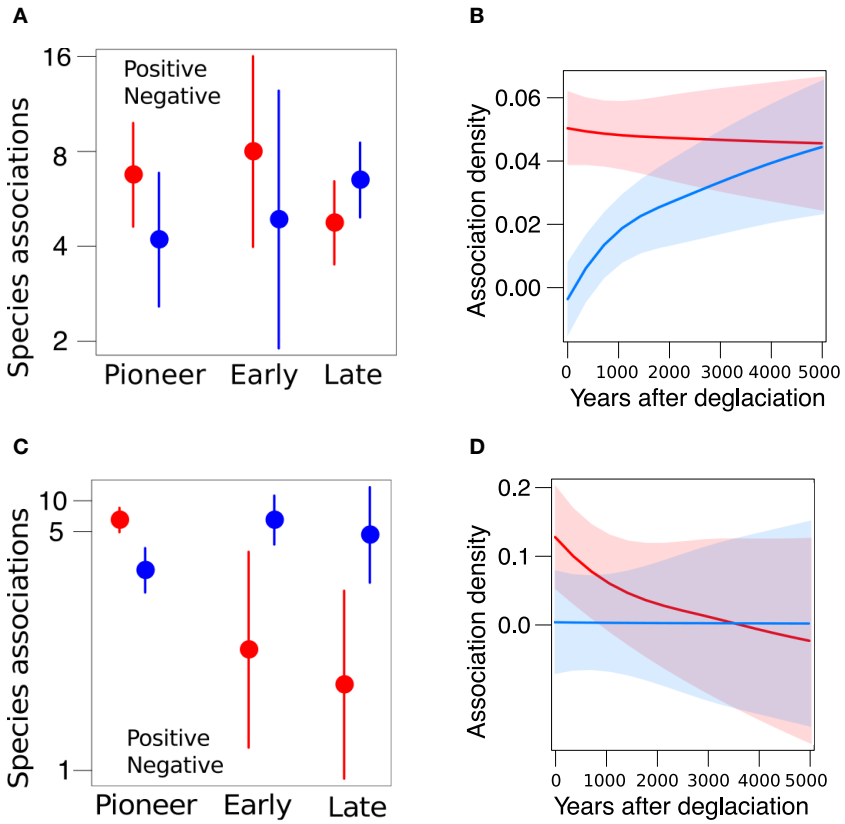


FIGURE 4 | Distribution of positive (red) and negative (blue) associations across species **(A,C)** and along the glacier foreland **(B,D)** in the Amola site (above) and Trobio site (below). Reported marginal means and 95% CI.

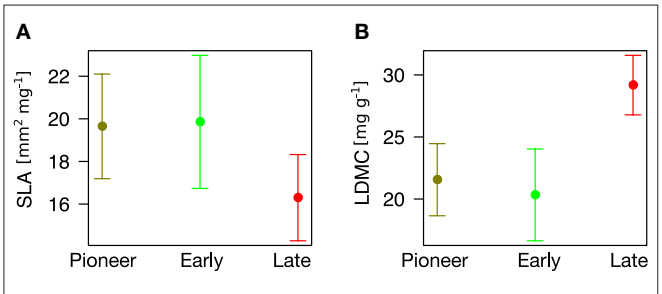


FIGURE 5 | Association between community type (*x*-axis) and plant traits (*y*-axis). **(A)** Specific Leaf Area. **(B)** Leaf Dry Matter Content.

glaciers vane. Soil organic matter and gravel further increase and decrease local diversity by enhancing and diminishing species ranges, respectively. Besides changes in species richness, we also observed changes in the way species are associated—and potentially interact—with each other. Positive associations tend to become less prevalent than negative ones with glacier retreat, potentially decreasing species persistence probability after glacier extinction. Species persisting the most with glacier extinction show more "conservative" leaf traits than species of concern. Taken together, these patterns reveal the species-specific impact of global warming on biodiversity is mediated by complex ecological interactions, highlighting the need of understanding them in a systemic and context-dependent way. Biodiversity conservation and ecosystem management require focusing on both species of concern and their networks of relationships with other species and the environment to support and ensure plant persistence after glacier extinction.

4.1. Plant Diversity Dynamic

Our results indicate that climate warming favors plant diversity as long as glaciers can withstand rising temperature. Model predictions show that plant diversity rapidly increases for c one thousand years after glacier retreat. Late species like *Carex curvula*, *Leontodon helveticus*, *Potentilla aurea*, and *Trifolium pallescens* are gaining from higher temperatures and their populations will likely flourish also when glaciers disappear. However, climate warming decreases species persistence once glaciers vane, ultimately prompting local extinctions. After a period of saturation and stasis, plant diversity will start declining substantially, losing half of the species across plant communities at the end of the dynamic and facing more than 20% of extinction from the species pool. In fact, not the whole ecosystem will gain species since pioneer and early plants like *Artemisia genipi*, *Saxifraga bryoides*, *Cardamine resedifolia*, and *Minuartia sedoides*

decline already 100 years after glacier retreat. With a probability of persisting lower than 0.2, these species will disappear locally in the absence of glaciers.

Such hump-shaped curve and idiosyncrasy reflect that the effects of climate warming produce "winners" and "losers" (Grabherr et al., 1994; Erschbamer, 2007). The likely reason is that the response of plants to increasing temperature varied among species and communities (Körner, 2005). Indeed, our results are consistent with experimental studies (Erschbamer, 2007), surveys (Walther et al., 2005; Pauli et al., 2007; Steinbauer et al., 2018), and species distribution modeling (Thuiller et al., 2005; Engler et al., 2011; Dullinger et al., 2012) indicating that although species richness increases with increasing temperature in high-altitude environments, there is also range contraction, replacement and biodiversity loss. Furthermore, they support the "replacement model" of succession (Pickett et al., 1987a; Van Andel et al., 1993; Walker and del Moral, 2003; Cutler et al., 2008), according to which changes in species richness and community composition occur as a result of changes in competitive "winners." Yet, it is worth noting that the persistence probability of late species after glacier extinction is on average 0.6. That is, the "victory" for the "winners" is not to be taken for granted. It is possible that competition among late plants decreases species persistence and therein biodiversity after glacier extinction.

Since ecosystem development following glacier retreat involves also changes in pollination networks (Losapio et al., 2015, 2016), arthropod community (Hodkinson et al., 2001; Kaufmann, 2001; Gobbi et al., 2010), food-webs (Raso et al., 2014; Sint et al., 2019), and soil microbial communities (Sigler et al., 2002; Jumpponen, 2003; Schütte et al., 2009), it is likely that the consequences of changing plant diversity will extend beyond plant communities. For instance, species of concern like *Saxifraga bryoides* and *Leucanthemopsis alpina* play a key role in the assembly and structure of ecological networks, supporting pollinators, herbivores, predators, detritivores, and parasitoids (Losapio et al., 2015). Thus, the local extinction of these plants would not only put other species at risk of extinction but would also severely affect the functioning of alpine ecosystems. The loss of biodiversity is going to reduce ecosystem services and benefits to humans, like food production from the pioneer, species of concern *Artemisia genipi*.

4.2. Plant Association Networks and Traits

After accounting for the response of species to glacier retreat and the effects of environmental conditions and random variation in species occurrence, we found a strong signal of biotic interactions in species distributions. In fact, species-to-species associations contribute to explain a significant proportion of variance, up to 34%, observed in the way plant species tended to be more or less associated to each other than expected by all other factors. Positive and negative species associations characterize plant networks, suggesting that both facilitation and competition may contribute to driving species distribution along glacier forelands and creating the hump-shaped diversity pattern over spacetime.

In particular, facilitation prevails among initial colonizers in pioneer communities, whereas competition was more prevalent in late communities. Overall, competition becomes relatively more important than facilitation with increasing community age. Particularly, this trend results from two distinct and non-mutually exclusive processes: in one case (Vedretta d'Amola site), the intensity of facilitation stays the same while competition becomes more prevalent, whereas in another case (Western Trobio site), the intensity of competition remains constant and it is the decrease in facilitation that changes through time. Taken together, these results provide evidence in support of both the facilitation model of community structure (Connell and Slatyer, 1977; Van Andel et al., 1993; Bertness and Callaway, 1994) as well as the geoecological model of succession (Matthews, 1992; Whittaker, 1993). Since the deglaciation gradient is also a gradient of decreasing harsher conditions, these results also support the shift from competition to facilitation in harsher environments (Callaway and Lawrence, 1997).

Notably, pioneer and early species have significantly different trait values as compared to late species, having higher SLA and lower LDMC. The remarkable differences we found locate the least and most persisting species at two opposite sides of the "leaf economic spectrum" of plant traits (Wright et al., 2004; Díaz et al., 2016). One one hand, pioneer and early species, adapted to physical disturbance ("ruderal" *sensu* Grime, 2001), show more "acquisitive" strategies (high SLA) that facilitate their colonization and development in recent ice-free areas (Caccianiga et al., 2006; Gobbi et al., 2010). On the other hand, late species pose more "conservative" strategies (low SLA and high LDMC, "stress tolerant" *sensu* Grime, 2001) that can allow them dominating over other plants (Caccianiga et al., 2006; Gobbi et al., 2010) and persisting after glacier extinction. Everything considered, it emerges that heavier "conservative" leaves may allow late species excluding pioneer and early plants and dominating once glaciers disappear.

Our findings have threefold implications. First, they suggest that both competition and facilitation are acting together within the same ecosystem. Therefore, studying mechanisms of species coexistence and succession should not be limited to competition only (Callaway and Lawrence, 1997; Losapio et al., 2019), especially in mountain ecosystems. The modeling, data-driven approach we adopted is useful for establishing correlative patterns between species. These inferred associations are putative interactions, thus are helpful for generating testable hypotheses concerning biotic patterns and for developing a deepen understanding of the role of species interactions and geophysical forces in dynamic environments. Addressing interactions with other organisms, such as pollinators, herbivores, seed predators, and soil microbes will also broaden our knowledge of community-structuring processes and ecological succession.

Second, global warming is changing environmental conditions, shifting the relative importance of facilitation and competition, and shuffling species and communities over spacetime. In the long run, the consequences of habitat loss that follows glacier extinction are negatively impacting biodiversity both directly and indirectly. Directly, due to the lack of available habitats: glacier extinction will decrease the number and size of areas in proglacial environments, which will progressively disappear without glaciers. Since the species—area relationship

is non-linear, a small reduction in glaciers may cause a much bigger species loss, reducing the occurrence of pioneer species. Indirectly, global warming exacerbates the impact of habitat loss via prompting competitive interactions and favoring fewer, dominant plants. Facilitation among pioneer plants may support species persistence in recently ice-free terrains. Indeed, it is well-established that plants can have beneficial, one-way or two-way, effects on the microenvironment and other plants, supporting community development (Connell and Slatyer, 1977; Bertness and Callaway, 1994; Chapin et al., 1994; Walker and del Moral, 2003). Nevertheless, glacier retreat is creating environmental conditions that disfavor "losers" pioneer species via creating an environment that favors competition and supports the "winners" late colonizers. Notice that the increase in competition with global warming would contribute to the local decline of late species too, beside the local extinction of pioneer plants. As experimental evidence supports that the fitness of high-altitude species is reduced due to competition with lower-altitude plants (Alexander et al., 2015), the same process would operate for the negative effects of competition between pioneer and late species.

Third, we highlight that there is no single factor, model or mechanism accounting neither for the response of species to global warming nor for the development of communities. Not only the effects of glacier retreat are uneven between species, which respond in different ways to global warming in general (Körner, 2005) and glacier retreat in particular (Raffl et al., 2006; Erschbamer, 2007), but also multiple factors interact with each other and a significant amount of uncertainty is associated with the future of glaciers and the development of communities. This includes the potential future (and past) actions of *Homo sapiens*, which have been overlooked in our estimates here. Interesting enough, almost half of the species pool shows inconsistent trends or weak responses to glacier retreat and environmental conditions, but random variation along the glacier foreland. Random events of colonization and dispersal as well as stochasticity associated with births and deaths may play a remarkable yet underestimated role in supporting plant persistence and driving successional change in community composition (Hubbell, 2001).

As we adopted a conservative scenario of glacier extinction ranging from 180 to 10,000 years (5,000 years on average), our predictions of species trends may be faster since it is likely that some of the glaciers examined here may disappear within the next decades. Although we used only two leaf traits with coarse average resolution, remarkable differences emerged between communities, providing meaningful insights into potential processes underlying species distribution patterns. Moreover, our estimates of plant diversity dynamic aid in overcoming the challenges associated with geochronology and unpredictable events, are important for furthering our understanding of biodiversity change, and could help in directing limited resources to critical species and communities. Although our predictions have a relatively high margin of uncertainty, making it difficult to estimate with accuracy and precision when exactly an extinction may happen, there is confidence on the overall pattern that indicates local species loss and extinction cascades following glacier extinction.

In conclusion, increasing the value of community survey data may help better understanding, conserving, managing and restoring ecosystems with scarce (economic) resources. To do so, addressing which and how species will interact with each other while being impacted by global warming is of paramount importance. Here, we have shown that plant diversity increases shortly after deglaciation, but declines in the long run following glacier extinction. This is particularly the case since pioneers and early colonizers are the most vulnerable to global warming and would not persist after glacier extinction. Despite facilitation can support plant establishment in recently-deglaciated terrains, the increase in competition over spacetime exacerbates habitat loss. Forecasting the fate of biodiversity change requires integrating hierarchical processes accounting for variation in the geophysical environment, biotic interactions and random events as well as embracing the challenge of including evolutionary changes, socio-economic drivers and feedbacks into a robust predictive science. Such framework shall be useful for guiding monitoring schemes and sustainable ecosystem management actions.

DATA AVAILABILITY STATEMENT

All raw original data used in this study are published on Stanford Digital Repository, publicly available at https://purl.stanford.edu/hp416gs2665.

AUTHOR CONTRIBUTIONS

GL conceived the study. MG and MC designed and coordinated data collection. GL, BC, CM, DT, and MC collected data. GL organized the dataset, performed statistical analyses and wrote the first draft of the manuscript. All authors contributed to the discussion and interpretation of data, revised the manuscript, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Living at the Edge: Increasing Stress for Plants 2–13 Years After the Retreat of a Tropical Glacier

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Rapid warming is a major threat for the alpine biodiversity but, at the same time, accelerated glacial retreat constitutes an opportunity for taxa and communities to escape range contraction or extinction. We explored the first steps of plant primary succession after accelerated glacial retreat under the assumption that the first few years are critical for the success of plant establishment. To this end, we examined plant succession along a very short post-glacial chronosequence in the tropical Andes of Ecuador (2–13 years after glacial retreat). We recorded the location of all plant individuals within an area of 4,200 m^2 divided into plots of 1 m^2. This sampling made it possible to measure the responses of the microenvironment, plant diversity and plants traits to time since the glacial retreat. It also made it possible to produce species-area curves and to estimate positive interactions between species. Decreases in soil temperature, soil moisture, and soil macronutrients revealed increasing abiotic stress for plants between two and 13 years after glacial retreat. This increasing stress seemingly explained the lack of positive correlation between plant diversity and time since the glacial retreat. It might explain the decreasing performance of plants at both the population (lower plant height) and the community levels (lower species richness and lower accumulation of species per area). Meanwhile, infrequent spatial associations among plants indicated a facilitation deficit and animal-dispersed plants were almost absent. Although the presence of 21 species on such a small sampled area seven years after glacial retreat could look like a colonization success in the first place, the increasing abiotic stress may partly erase this success, reducing species richness to 13 species after 13 years and increasing the frequency of patches without vegetation. This fine-grain distribution study sheds new light on nature's responses to the effects of climate change in cold biomes, suggesting that faster glacial retreat would not necessarily result in accelerated plant colonization. Results are exploratory and require site replications for generalization.

Keywords: abiotic stress, dispersal limitation, facilitation, glacial retreat, chronosequence, primary succession, tropical andes, plant height

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INTRODUCTION

The spatial distribution of plant communities along dated chronosequences after glacial retreat since the period of the little ice age (LIA) have been extensively used to characterize plant primary succession in temperate-to-arctic environments (Matthews, 1992; Walker and Del Moral, 2003; Cauvy-Fraunié and Dangles, 2019). Although this type of primary succession depends on a high number of local, regional and latitudinal parameters (D'Amico et al., 2017; Prach and Walker, 2020) as well as stochastic effects (Marteinsdóttir et al., 2013), structured patterns of plant diversity and plant interactions have been consistently correlated with time since the glacial retreat, including increased soil development and trophic interactions (Losapio et al., 2015; Cauvy-Fraunié and Dangles, 2019), increasing non-trophic interactions between plants (facilitation and competition: Fastie, 1995; Zimmer et al., 2018) and increasing plant cover and richness, as observed from field observations (Matthews, 1992; Schumann et al., 2016) and from satellite imagery analyses (Fischer et al., 2019). This goes along with the assumption that the temporal extent (and also the spatial extent) of a habitat will be correlated with the diversity of the species composing it (Tilman, 1994).

Under the effects of accelerated warming for half a century, the pace of glacier retreat has dramatically increased across the globe (Zemp et al., 2019). Large areas become rapidly available for plant primary succession. These landscapes constitute remarkable opportunities for the alpine biodiversity, especially plants, to enlarge their leading-edge distributions upward whereas their trailing-edge distributions at lower elevation mostly retract because of the combined effects of thermophilization and increased interspecific competition with up-migrating lowland species (Heckmann et al., 2015; Lenoir and Svenning, 2015). At the same time, however, these new habitats are characterized by a very short temporal extent, which is expected to impact negatively their biodiversity (Tilman, 1994). It remains unclear, however, to what extent the recent accelerated warming may affect the general patterns of plant primary succession previously observed at the scale of the LIA (Dullinger et al., 2012). Indeed, most studies on plant primary succession after glacial retreat have been carried out along chronosequences longer than 50 years, and often including the little ice age (Cauvy-Fraunié and Dangles, 2019). We expect that the recent accelerated warming and consecutive faster glacial retreat modifies greatly the organization of novel plant communities at the very first years of primary succession.

The few quantitative studies focusing on the early arrival of plants after glacial retreat since the acceleration of warming, in the 70s, reveal a number of singularities that have not necessarily been observed in longer chronosequences. This includes the lack of significant trends in plant composition and in the value of intraspecific functional traits along with time since the glacial retreat (Sitzia et al., 2017: chronosequences 15–66 years), an overwhelming majority of wind-dispersed species and a deficit in facilitative interactions between plants (Zimmer et al., 2018: 0–38 years; Rosero et al., 2021: 0–61 years) and an accelerated pace of primary succession in comparison with measures made before warming acceleration (Cannone et al., 2008: 1–25 years; Fickert et al., 2017: 1–35 years). Therefore, the accelerated pace of warming and glacial retreat is a game-changer for plants succession as it affords shorter time to perform upward migration (Klanderud and Totland, 2007). Shorter time may hinder site accessibility to seeds, especially those not dispersed by wind (Makoto and Wilson, 2019) and is expected to increase abiotic constraints on plant development as the soils do not have enough time to develop (Fastie, 1995; Sattin et al., 2009). Also, it may reduce the probability of positive interactions among species in stressful habitats where positive interactions have proven to be a central mechanism in the organization of plant communities (*stress-gradient hypothesis*; Bertness and Callaway, 1994; Anthelme et al., 2014). Consequently, to document the plant strategies designed to overcome the constraints limiting the first steps of primary succession is crucial to predict what types of alpine communities may develop in the next decades, and what species would face the highest extinction risks (Dullinger et al., 2012; Cauvy-Fraunié and Dangles, 2019).

Focusing on the study of plant primary succession after glacial retreat in the alpine, cold tropics is pertinent because they exhibit specific environmental features, with possibly unexpected impacts on successional processes. First, levels of biodiversity are globally higher due to specific climates, biogeography and isolation (Sklenár et al., 2014; Anthelme and Peyre, 2020) and plants exhibit narrower thermal niches breadth compared to higher latitudes (Cuesta et al., 2020). Second, warming is usually thought to be more pronounced (Pepin et al., 2015; Wang et al., 2016), which translates into faster glacial retreat (Vuille et al., 2018). Inverted precipitations gradients, a common feature in the alpine tropics at the origin of tropical alpine deserts (Leuschner, 2000) may increase stress for early colonists. A supplementary stress for the first colonizers may be the absence of a seasonal snowpack in the alpine tropics (Vuille et al., 2018) which exposes plants to low temperature at night all year round (e.g., Rada et al., 2001). Finally, during the dry period the absence of snowmelt generates higher soil aridity than in temperate-arctic environments (Vuille et al., 2018). For all these reasons, and despite the high stochasticity previously observed in plant distribution patterns along proglacial chronosequences (Marteinsdóttir et al., 2013; Buma et al., 2019), we expect to observe non-stochastic trends in plant distribution over the first years following glacial retreat in the alpine tropics.

Here we examined the first steps of plant primary succession, at a fine grain, in the foreland of a rapidly retreating glacier in the tropical high Andes, with the aim to reveal trends in plant distribution patterns. We used a 2–13 years chronosequence sequenced into four dated glacial retreat strips (Mount Antisana, Ecuador). In a 4,200 m^2 area, we measured plant traits at the community level and at the individual level and we compared them with data on the abiotic environment measured at each strip. We tested the following hypotheses: (1) the general trend of increasing plant diversity and productivity during early primary succession may be altered because of increasing stress a few years only after glacial retreat -for this purpose, we tested to what extent changes in soil macronutrients, temperature and soil humidity along the chronosequence modified plant

metrics, plant aggregation patterns and species-area relationships (Palmer, 2007; Storch et al., 2007)- and (2) facilitation among species are infrequent early after glacial retreat and successful colonizers are mainly dispersed by wind, two effects that may slow primary succession.

METHODS

Study Site

Our study site was located at 4870 m a.s.l. at the forefront of the glacier 15-alpha, on the western side of Antisana volcano (5,752 m), in the Eastern cordillera of the Ecuadorian Andes (0°29′06″S, 78°08′31″W; **Figure 1A**). The site lies in the "superpáramo" vegetation belt, where plant cover is discontinuous and plant communities are dominated by cushion-forming species [*Azorella aretioides* (Kunth) Willd. ex DC., *Xenophyllum rigidum* (Kunth) V.A.Funk], shrubs (*Chuquiraga jussieui* J.F.Gmel.) and tussock grasses [*Calamagrostis intermedia* (J.Presl) Steud.; Anthelme et al., 2012].

The glacier 15-alpha displayed a maximum extension during the Little-Ice Age, and reduced since with a severe acceleration of this reduction in the last 50 years (Jomelli et al., 2009). Inter-annual observations between 1997 and 2008 on glacial retreat using topographical measurements and differential GPS provided data for drawing a precise chronological map of recent glacial retreat (**Figure 1B**). An ORE meteorological station stands at only a few decametres of the sampling site. Data showed an annual rainfall amount of 907 mm in 2007 with a pronounced humid period between March and August (714 mm) and a monthly maximum of 276 mm in June. Between 2005 and 2007 annual mean temperature was 1.15°C and relative air humidity was 79.46%, without significant variations among months (Maisincho et al., 2009).

Study Design

Our study site consisted of a 2–13 years proglacial chronosequence divided into four strips delimited by precisely-dated and marked lateral moraines of the glacier (**Figure 1C**). We selected a site on the lateral border of the glacier in order to reduce the effects of meltwater flows into our plots. The area considered in each strip was calculated from **Figure 1C** with the open access software ImageJ (http://imagej.nih.gov/ij/): strip 2–5 years (moraines 2008–2005; 984 m^2); strip 5–7 years (2005–2003; 1,382 m^2); strip 7–9 years (2003–2001; 902 m^2); strip 9–13 years (2001–1997; 932 m^2). Between moraines, the mineral granulometry at soil surface was equally dominated by small rocks up to 10 cm in diameter and sandy material. Rocks over 1 m in diameter were almost absent. Slope gradient followed the lines drawn by moraines and was about 10–15% (clinometer Suunto Tandem, Vantaa, Finland). As a result, both topography and granulometry between moraines were relatively similar within each strip, reducing the potential effect of local heterogeneity between strips on plant distribution patterns. Moraines were relatively small (between 0.1 and 2 m high) and distributed in all the glacial retreat strips (**Figure 1C**; **Supplementary Material 1**). Slopes on moraines were steeper, but with a similar granulometry as in the rest of the sampling (**Supplementary Material 1**). We took them into account in our sampling as natural elements of the environment. Nevertheless, they were excluded from analyses related with species-area accumulation and from abiotic data analyses in order to focus on the effect of time since the glacial retreat on these variables.

Abiotic Data

We define abiotic stress as the effects of external constraints on the limitation of plant dry biomass (Grime, 1977). Soil samples were collected 30 plots, each of 1 m^2) for which we recorded the coordinates (**Figure 1**). Selection was random except they had similar slope and granulometry (coarse sand) at each of the four deglaciated strips. The aim was to study variation in soil moisture and soil macronutrient content along the chronosequence. Soil moisture was calculated as the difference between fresh and oven dried mass (48h, 70°C; Balance TU-OI FA-2104, Mhand, Fuzhou, China). In each strip, 10 of these soil samples were sent to the Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP, Quito, Ecuador) for analyses of soil macronutrients (NH4, P, K, Ca, and Mg; modified Olsen methodology; Sanchez, 1976).

At each soil sample location (30 in each strip) we measured soil temperature at 5 cm depth, considering that temperature stress at this depth will be especially harmful for plants (Matthews, 1992). We made a series of 30 measurements between 9:00 and 10:00 AM during a cloudy day, using probe thermometer (Fluke 574CF, Everett, USA). In parallel, we recorded temperature each 15 min over 3 months (March-June 2010), using 10 TidbiT v2 Temp data loggers (two or three loggers at each strip) from which we extracted daily minimum temperature as a proxy of stress for plants. The location of the loggers was different from that of the previous temperature measurements but was on similar slope and granulometry. These two types of temperature measurements allowed us to take into account both the temporal variability and the spatial variability of temperatures among the four strips.

Vegetation Data

Vegetation Sampling

A 60 x 70 m^2 rectangular grid was superimposed on the study site across the four glacial retreat strips (**Figure 1C**). Unlike scattered plots, the grid method allowed us to spatialize collected information on plants and to document plant diversity and plant spatial associations patterns at various spatial scales, from 1 to 100 m^2. This grid was composed by 42 squares, each one divided into 100 plots of 1 m^2 (**Figure 1D**).

Plant Metrics

In each plot of 1 m^2 ($N = 4{,}200$) all individuals of vascular plant species ($N = 5{,}574$) were recorded with XY coordinates (1–60 and 1–70 m, respectively) and identified in 2010. They were subject to various types of measurements (see below). Mosses and lichens were rare in the site and were not taken into account in the analysis. Two vascular species might be hybrids of two other Asteraceae species: *Senecio nivalis* (Kunth) Cuatrec. x *Lasiocephalus ovatus* Schltdl. (three individuals), and *Werneria nubigena* Kunth x *X. rigidum* (one individual); they

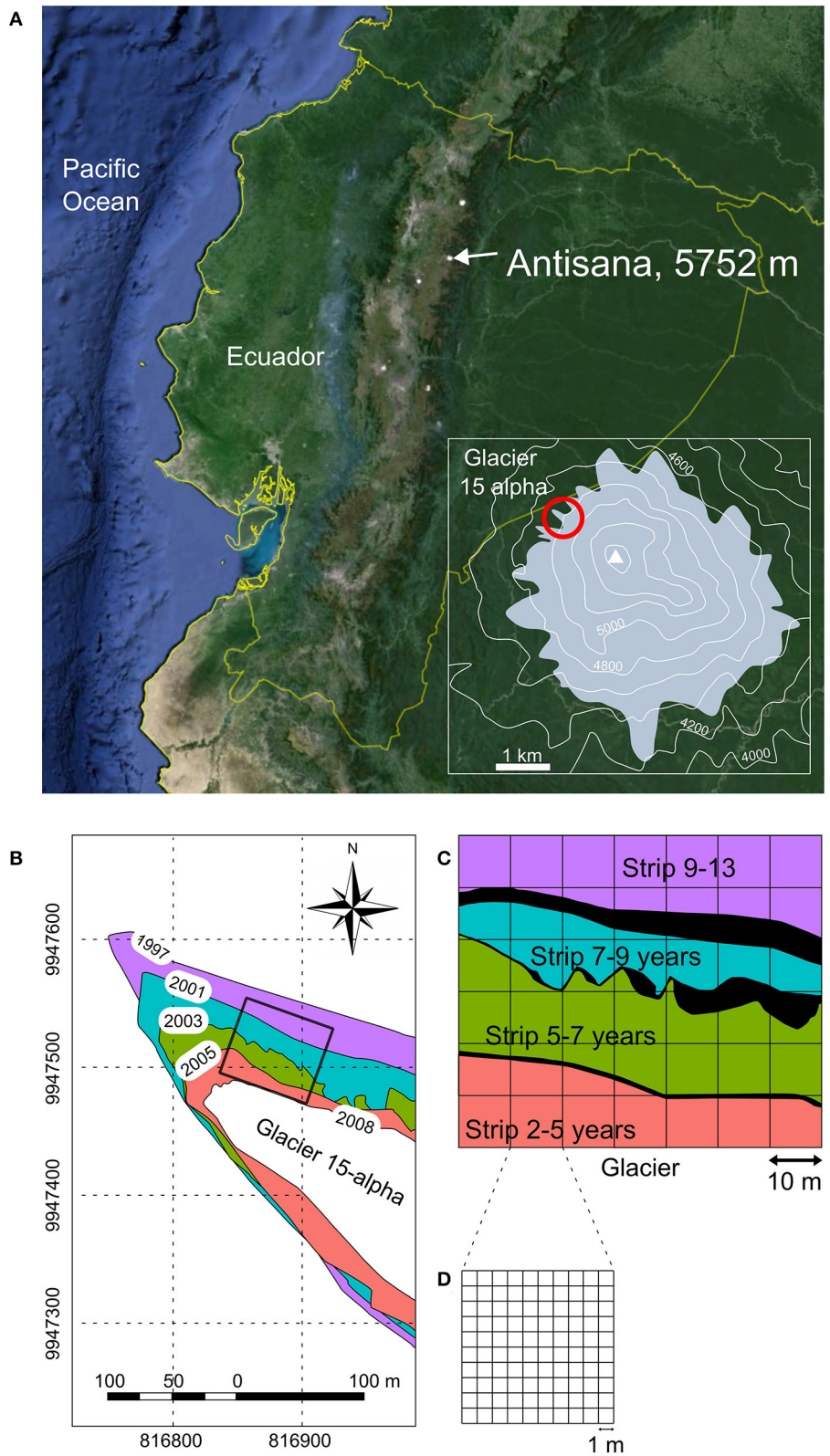


FIGURE 1 | Study area, study site and sampling design. **(A)** Volcano Antisana in the Ecuadorian Andes (extracted from Google map) and location of glacier 15-alpha within the Antisana ice-cap. **(B)** Map of the 15-alpha glacier between 1997 and 2008. Datum: WGMS 84; unit: UTM. **(C)** Sampling design: black areas represent the three lateral moraines of years 2001, 2003, and 2005, respectively; these areas were removed the species-area analyses because providing undesirable heterogeneity in the topography and granulometry. **(D)** Distribution of plots (1 m^2).

were coded H1 and H2, respectively. Two species did not display flowering material and were identified at the family level only (Asteraceae: A1 and A2; two and eight individuals, respectively). Eleven juvenile individuals belonging to two unidentified morphospecies were classified as Z1 and Z2. Plants were segregated into mature individuals and seedlings; the latter category not being taken into account when measuring plant traits in order to avoid ontogenic effects. Seedlings were defined as individuals without sexual reproductive attributes (flowers, fruits, or seeds) and with a cover at soil surface not exceeding 1 cm^2.

Plant community changes were characterized based on plant diversity, species richness, plant cover and plant functional traits along the chronosequence. Plant cover provided information on general primary succession process. It was calculated inside each 1 m^2 plot by summing the surface of all individuals ($N = 5{,}574$), as the product between plant area (extrapolated by multiplying length and width of each individual; cm) and its relative density (visually estimated as the % of vegetal material within the area). In addition, we measured plant height (height of the highest leaf, cm), the proportion of flowering individuals, the number of ramets per individuals (proxy of vegetative reproduction) and plant necromass (visual estimation of dead tissues, %, following Caccianiga et al., 2006) to characterize the responses of the plant community to the increasing time after glacial retreat. These traits were measured for the 1,539 mature individuals. Each of these traits were studied at the community level by averaging the values of all individuals, and at the population level for the three most abundant species: *Agrostis foliata* Hook.f., *Cerastium floccosum* Benth. and *Senecio nivalis* (Kunth) Cuatrec.

Spatial Associations Plant-Plant-Rock

We estimated the frequency of interspecific interactions among plants by observing the proportion of pairs of individuals spatially associated at each strip. We assumed associations to be a good proxy of positive interactions among plants because in alpine environments competition between plants is generally low (Cavieres et al., 2014). For this purpose, at each plot, we recorded each pair of spatially associated species. We assumed that spatial root interactions did not exceed aerial interactions among plants because the age of the target individuals did not exceed 13 years in our sampling, not giving sufficient time for plants to develop large root systems, as expected in mature alpine plants (Körner, 2003). Therefore, plants were considered spatially associated as soon as their aerial canopy overlapped. This method avoided the destruction of individuals to extract the roots. Nevertheless, we acknowledge that it may result in a slight underestimation of positive interactions. We also measured the proportion of individuals protected by an abiotic refuge, potentially providing protection from wind, moister conditions or temperature buffering to plants ("spatially associated with a nurse rock"; Scherrer and Körner, 2011; Moeslund et al., 2013). We considered plants as protected by a rock as soon as the rock exceeded the plant's height and the distance between plant and rock was not superior to the plant height.

Data Analysis

Chronosequence

Changes in plant parameters and abiotic parameters along the chronosequence (four strips) were first analyzed with simple linear regressions, plotting each variable against the distance to the glacier. The distance was the coordinate Y in our sampling as the sampling grid was parallel to the glacier border (**Figure 1C**). Thus, the distance to the glacier was used as a proxy of age of deglaciated terrains, which increased in the same direction as the distance. As an exception, data on T_{min} were assigned to each strip without having XY coordinates. Therefore, we used the variable "strip" to provide a regression, by averaging the age of each strip. In the specific case of nurse plants and nurse rocks (modalities: protected or not protected) we used logistic regressions. Second, comparisons between means of each variable in each strip were provided with one-way ANOVA and *post-hoc* Tukey tests, or Kruskall-Wallis tests as soon as data did not follow normal distribution. This helped providing simple values along the chronosequence.

Correlations between two microclimatic variables (Tsoil 9–10 AM and soil moisture), three diversity variables (species richness, abundance of individuals and relative plant cover) and the time since glacier retreat were possible on the subsample of 120 plots used for the microclimatic measurements (see above). For this purpose, we conducted a principal component analysis on these data and we used "strip" as a supplementary variable to visualize the belonging of plots to each strip on the biplot graph. PCA was designed with ade4 1.7-16 (Thioulouse et al., 2018) and factoextra 1.0.7 (Kassambara and Mundt, 2017) in R.

Spatial Analyses

We plotted all plant individuals as a marked point pattern at a one-meter resolution, with species identity as a mark [see Goreaud (2000), for a detailed presentation of the use of point pattern analysis in plant community ecology]. First, this spatial design allowed inferring species-area relationships (1) by calculating the frequency of patches without vegetation (hereafter termed "empty patches") at various spatial scales, and (2) by providing species-area curves. Empty patches permitted exploring the frequency of suitable sites for plant establishment (hereafter referred to as safe sites; Harper et al., 1961). Along the chronosequence, we calculated the number of patches without vegetation at the four deglaciated strips, taking into account different sizes of patches (1, 4, 49, and 100 m^2). For each strip, we calculated the proportion of empty patches (as a measure of environmental heterogeneity) for different patch sizes: 1, 4, 49, and 100 m^2, respectively. Moraine habitats, which were poorly colonized and under the influence of distinctive topographical parameters, were excluded from the analysis by making masks (see the R spatstat reference manual for details, http://cran.rproject.org/web/packages/spatstat/spatstat.pdf). For each patch size, we ran 100 simulations randomly located in each strip using a complete spatial randomness model, which is a completely random point set obtained from a homogeneous Poisson process. Using the same scale variation (1, 4, 49, and 100 m^2), we drew species-area curves for each glacial retreat strip. We used the slope of each curve to generate a Preston model, which was the

most appropriate for such type IIa curves [type IIa: plot arrayed in a contiguous grid, see Scheiner (2003) for a description of the different species-area curve types]. The equation can be written as follows:

$$S = cA^z$$

with S the number of species, A the patch area, and c and z constants. The z parameter (slope of the curve on log-log axis) has been widely used by ecologists to make comparisons among different habitats and communities (Storch et al., 2007). These spatial analyses were performed using the SpatStat package of R (Baddeley and Turner, 2004).

RESULTS

Increasing Abiotic Stress Along the Chronosequence

Soil temperature between 9:00 and 10:00 AM, daily minimum soil temperature along a three-month period and soil moisture all decreased significantly with increasing distance to the glacier (linear regressions: $R^2 = 0.41$, $R^2 = 0.22$, and $R^2 = 0.19$, respectively; **Figure 2**). Soil temperature 9–10 AM changed significantly along the chronosequence, decreasing from 5.88°C ± 0.13 in the strip 2–5 years after glacial retreat to 4.54°C ± 0.11 in the strip 9–13 (*post-hoc* Tukey tests $P < 0.05$; **Supplementary Material 2**). Minimum soil temperatures followed the same trend, decreasing from 1.48°C ± 0.08 in the strip 2–5 years to 0.39°C ± 0.07 in the strip 9–13 ($P < 0.05$). Soil moisture was also significantly variable between strips, shifting from 12% ± 0.34 in strip 2–5 to 8.8% ± 0.37 in strip 9–13 ($P < 0.05$; **Supplementary Material 2**). For soil macronutrients, the concentration of Ca, K, and Mg decreased significantly along the chronosequence (linear regressions: $R^2 = 0.43$, $R^2 = 0.50$, and $R^2 = 0.08$, respectively; **Figure 2**). As for microclimatic data, these nutrient values were significantly variables between strips, with higher values at the start of the chronosequence than at its end ($P < 0.05$).

Plant Diversity and Composition

A total of 23 vascular plant species and 5,574 individuals were recorded in our sampling area (**Table 1**). Overall, three species dominated the community: *Agrostis foliata* (66% of observed

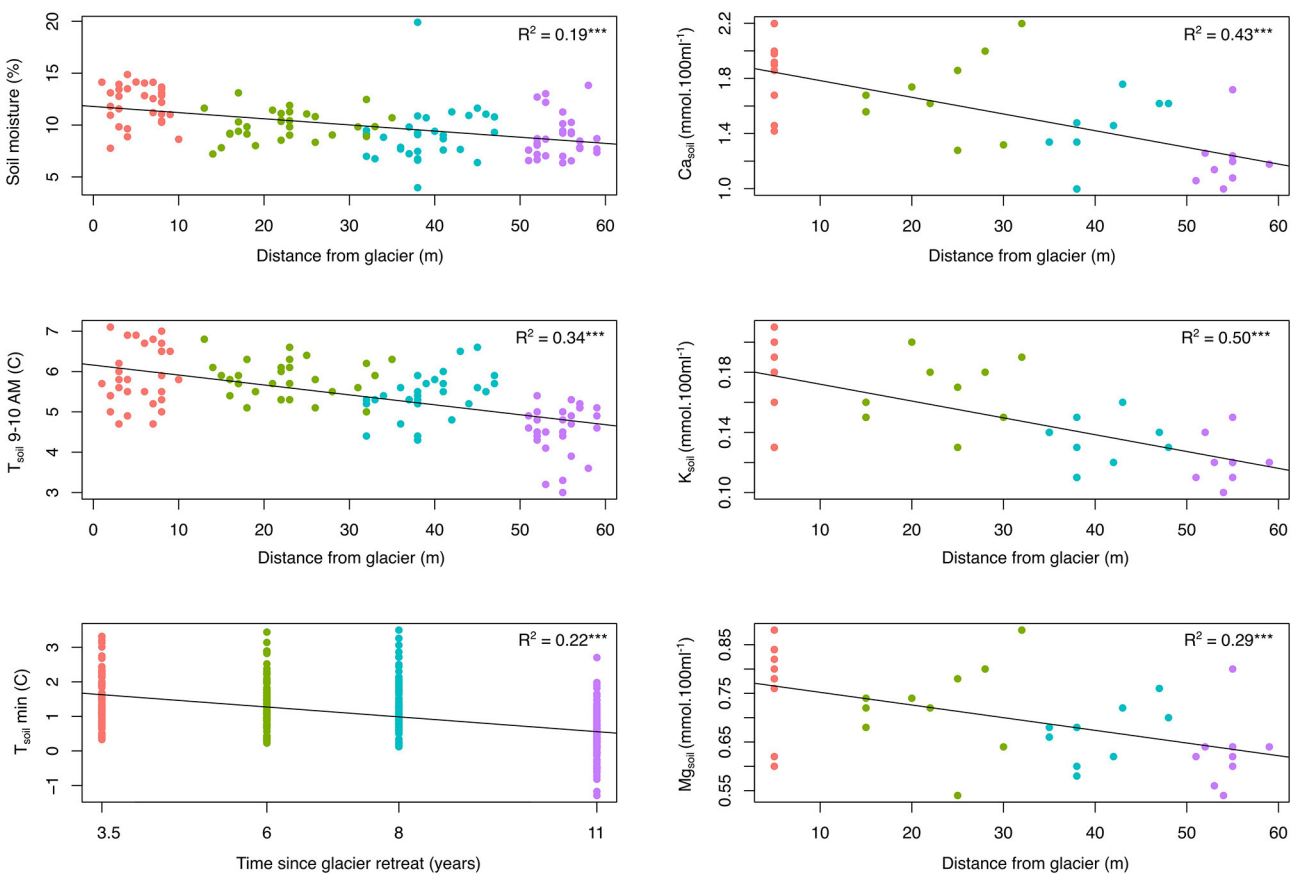


FIGURE 2 | Effects of the time since the glacial retreat on the microclimate and the relative content of three soil macronutrients (linear regressions; ***R^2 significant at $P < 0.001$). Time since the glacial retreat estimated through increasing distance from the glacier, except for T_{soil} min (mean age of each strip). Different colors represent the belonging of plots to the four dated strips along the chronosequence. See also **Supplementary Material 2** for comparisons among strips and data on other soil nutrients.

TABLE 1 | Species composition, dispersal mode, growth form, and number observed along each of the four strips of the proglacial chronosequence.

Species	Family	Dispersal mode	Growth form	2–5 years	5–7 years	7–9 years	9–13 years	Total
A1	Asteraceae	A	Rosette	1	0	1	0	2
A2	Asteraceae	A	Rosette	1	4	3	0	8
Agrostis foliata Hook.f.	Poaceae	A	Tussock grass	373	922	1146	1224	3665
Baccharis caespitosa (Ruiz and Pav.) Pers.	Asteraceae	A	Crawling shrub	3	5	6	0	14
Calamagrostis fibrovaginata Laegaard	Poaceae	A	Tussock grass	1	4	6	2	13
Calamagrostis intermedia (J.Presl) Steud.	Poaceae	A	Tussock grass	0	1	0	0	1
Cerastium floccosum Benth.	Caryophyllaceae	AZ	Rosette	70	474	412	513	1,469
Chuquiraga jussieui J.F.Gmel.	Asteraceae	A	Erect shrub	0	1	0	1	2
Ephedra americana Humb. and Bonpl. ex Willd.	Ephedraceae	Z	Crawling shrub	1	2	2	1	6
Erigeron ecuadoriensis Hieron.	Asteraceae	A	Rosette	0	2	0	0	2
H1 (*L. ovatus* X *S. nivalis*)	Asteraceae	A	Rosette	4	4	8	3	19
H2 (*W. nubigena* X *X. rigidum*)	Asteraceae	A	Cushion/Rosette	0	1	0	0	1
Hypochaeris sessiliflora aggr.	Asteraceae	A	Rosette	0	2	1	0	3
Lasiocephalus ovatus Schltdl.	Asteraceae	A	Stem rosette	4	9	3	1	17
Belloa kunthiana (DC.) Anderb. and Freire	Asteraceae	A	Rosette	0	1	2	0	3
Plantago sericea Ruiz and Pav.	Plantaginaceae	AZ	Rosette	0	1	0	0	1
Senecio nivalis (Kunth) Cuatrec.	Asteraceae	A	Rosette	9	36	143	90	278
Taraxacum officinale L.	Asteraceae	A	Rosette	0	0	1	1	2
Werneria nubigena Kunth	Asteraceae	A	Rosette	13	17	8	3	41
Werneria pumila Kunth	Asteraceae	A	Cushion/Rosette	0	2	2	1	5
Xenophyllum rigidum (Kunth) V.A.Funk	Asteraceae	A	Cushion	0	4	6	1	11
Z1	-	?	?	1	2	0	0	3
Z2	-	?	?	3	2	3	0	8
Species richness (strip scale)				13	21	17	12	23

A1, A2, Z1, Z2: (partially) unidentified morphospecies. Dispersal modes, A: anemochory; Z: zoochory.

individuals), *Cerastium floccosum* (26%), and *Senecio nivalis* (5%). Rosette was the overwhelming dominant growth form before tussock grasses whereas cushion, shrub and stem rosette growth forms were represented by a limited number of species and individuals, especially in the older glacial retreat strip. Almost all species observed in the sampling were dispersed by wind, with the exception of *Ephedra americana* Humb. & Bonpl. ex Willd., dispersed only by animals. Relative plant cover at the community level in 1 m^2 plots ranged between 0.08 and 4.10% and was significantly variable among strips (Kruskall-Wallis: $P < 0.001$; **Supplementary Material 3**).

The two first axes of the PCA extracted from a subsample of 120 plots combining microclimatic and diversity data gathered 69.5% of the plot ordination (**Figure 3**). Axis 1 of the biplot confirmed that soil moisture and soil temperature were negatively correlated with time since glacier retreat (here estimated as the distance of plots from the glacier). The variability of plant diversity (species richness, abundance, cover) is expressed on axis 2, but not on axis 1 of the PCA, which means that these indices were not linearly correlated with time since the glacial retreat.

Species richness at the strip scale reached 13 species in strip 2–5, peaked in strip 5–7 with 21 species observed, and then decreased to 12 species in strip 9–13. Nine species that were present in the younger strips were absent in strip 9–13, among which *Baccharis caespitosa* (Ruiz & Pav.) Pers., *C. intermedia*, *Hypochaeris sessiliflora* aggr. and *Belloa kunthiana* (DC.) Anderb. & Freire. On the contrary, no species were exclusively observed in strip 9-13 (**Table 1**).

Species-Area Relationship

Species richness increased with increasing area in each of the four strips of glacial retreat. However, the accumulation of species across area was reduced by half along the chronosequence [Preston coefficients of species-area curves (Z): 0.66 in strip 2–5 and 0.33 in strip 9–13; Spearman rank test $P < 0.001$; **Figure 4**]. This was the result of a higher frequency of large patches without vegetation in the older deglaciated strips (patches of 49 m^2: 11% ± 0 in strip 2–5 and 28% ± 0 in strip 9–13; patches of 100 m^2: 4% ± 0 in strip 2–5 and 23% ± 0 in strip 9–13; **Supplementary Material 4**).

Individual Traits

A total of 1,539 mature individuals were observed in the sampling and were subject to individual trait measurements. Plant height at the community level decreased significantly along the chronosequence (linear regression: $R^2 = 0.03$; $P < 0.001$; **Figure 5**). It was significantly different between strip 2–5 and strip 9–13 (6.0 cm ± 0.3 and 4.5 cm ± 0.1, respectively; **Supplementary Material 5**). A similar significant trend was

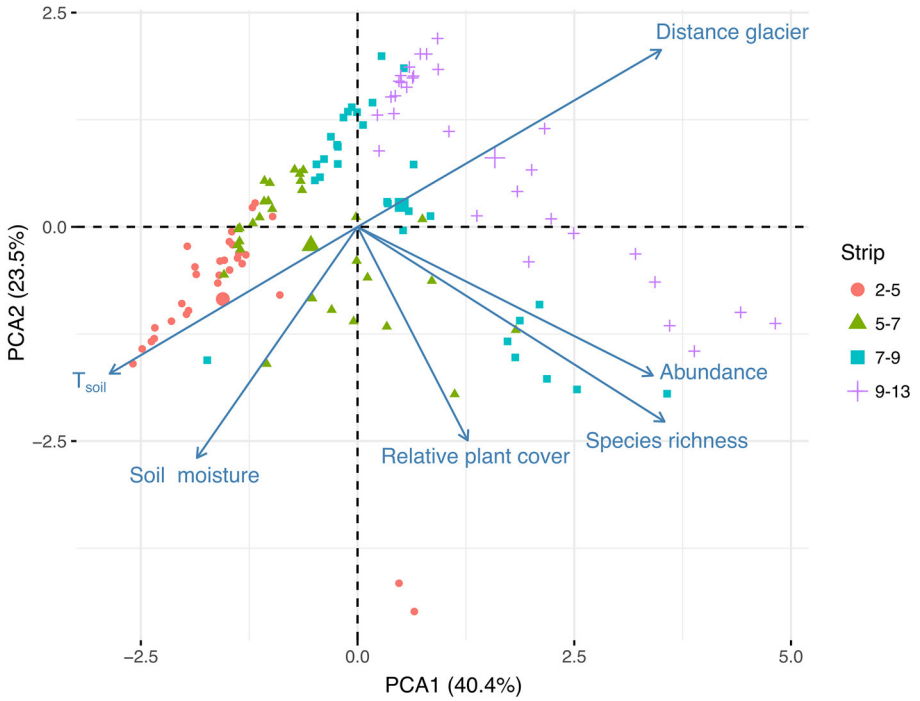


FIGURE 3 | PCA biplot combining diversity variables with microclimatic variables and the distance from the glacier on 120 plots. Each plot represented with a symbol assigned to one glacial retreat strip. Larger symbols represent the mean location of plots in each strip.

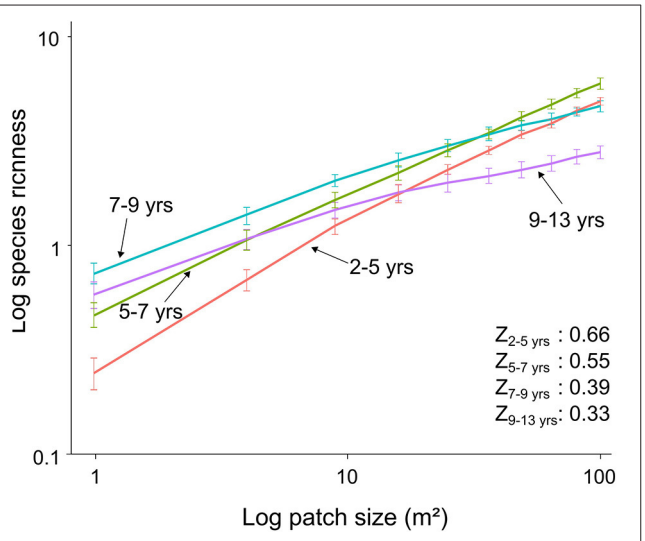


FIGURE 4 | Species-area curves at each glacial retreat strip along the chronosequence. Z values (see Preston equation in the section Methods) displayed for each strip. Symbols represent mean values ± SD based on 100 random simulations.

observed for *Agrostis foliata* and *Cerastium. floccosum* ($R^2 = 0.07$ and $R^2 = 0.05$, respectively; $P < 0.001$) and, to a lesser extent, for *S. nivalis* ($R^2 = 0.03$; $P < 0.05$). The relative necromass in plants increased significantly along the chronosequence at the community level (linear regression: $R^2 = 0.09$; $P < 0.001$). This trend was also observed in *A. foliata* and *C. floccosum* ($R^2 = 0.09$ and $R^2 = 0.11$, respectively; $P < 0.001$) but not in *S. nivalis* for which relative necromass remained stable at ∼20% at each strip of the chronosequence ($R^2 = 0.01$, not significant). The proportion of flowering individuals increased at the community level and for *A. foliata* and *C. floccosum* (Logistic regressions: $P < 0.001$) whereas it remained stable -and lower than for other species- for *S. nivalis*. The number of ramets per individual did not change at the community level along the chronosequence (linear regression: $R^2 = 0.00$) and it remained low and stable across all strips (1.04 ± 0.01). This pattern was also observed in *A. foliata* and *C. floccosum* whereas *S. nivalis* developed more ramets than the rest of the community (1.55 ± 0.10; **Supplementary Material 5**).

Spatial Associations

In the whole study site, almost half of the individuals were spatially associated with nurse rocks (722 out of 1,539), and this number significantly decreased along the chronosequence (Logistic regression: $z = -3.32$; $P < 0.001$; **Figure 6**). In contrast, only 68 individuals out of 1,539 were spatially associated with other plants. Still, the frequency of spatial associations among plants increased significantly along the chronosequence (Logistic regression: $z = 6.17$; $P < 0.001$), shifting from 0% of the individuals in strip 2–5 to 5.4% ± 1.0 in strip 9–13.

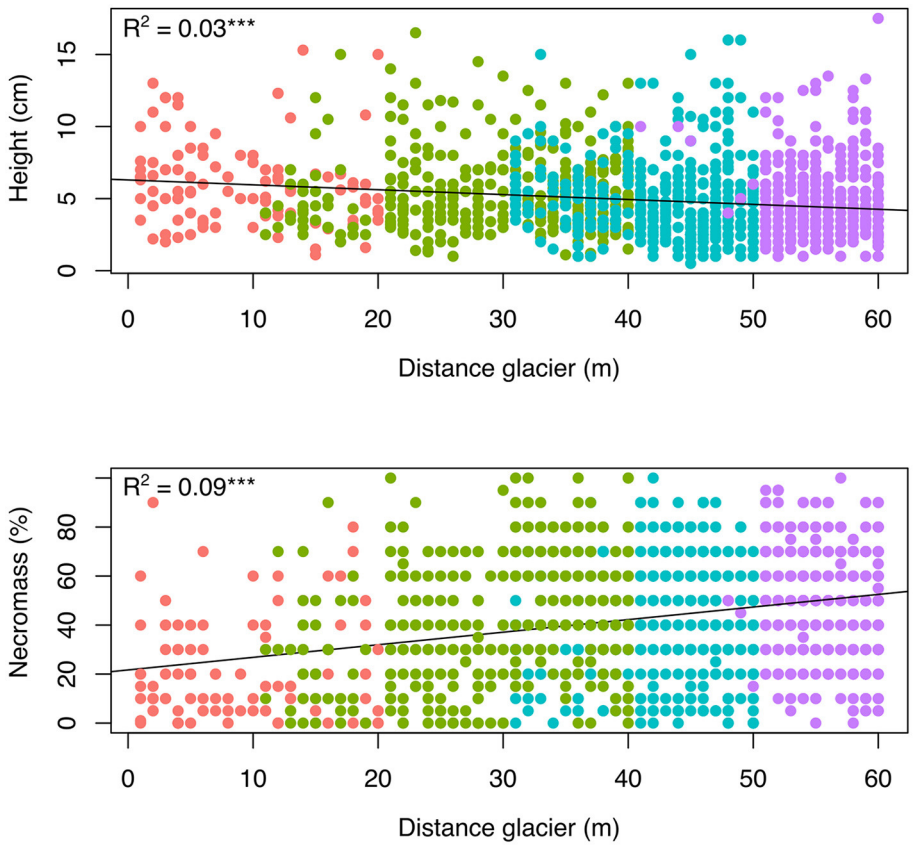


FIGURE 5 | Effect of time after glacial retreat on the plant height and the relative necromass of individuals along the chronosequence, at the community level (linear regressions; ***R^2 significant at $P < 0.001$). Time effect estimated through the distance from the glacier. Different colors represent the belonging of plots to the four dated strips along the chronosequence. See also **Supplementary Material 5** for comparisons among strips and data on complementary traits.

DISCUSSION

Revealing the drivers of plant distribution and plant individual traits during the first steps primary succession after glacial retreat has been proven challenging because of high stochasticity, complex environmental gradients, small-scale topography, specific substrates slowing succession and, possibly, unsuitable sampling with high environmental heterogeneity among plots or insufficiently precise time delimitations between glacial retreat strips (Marteinsdóttir et al., 2013; D'Amico et al., 2017; Sitzia et al., 2017; Buma et al., 2019). The finesse of our grid-sampling (location of each of the 5,574 plant individuals at a one-meter resolution) is uncommon but not new as a number of other studies had a similar approach (e.g., Wiegand et al., 2012). However, combining this approach with a proglacial chronosequence distributed over such a short period of time is original. This allowed revealing increasing abiotic stress and a shift toward greater stress-tolerance by plants on a time scale of only 11 years. Although requiring further site replications, this result is a significant step toward a better understanding of the first steps in primary plant succession after glacial retreat.

Plant Stress Increases During the First Steps of Succession

During primary succession, at the scale of the little ice age (250–350 years), it is commonly accepted that plants are gradually less constrained by the environment, as materialized by soil enrichment and stabilization, increase in both trophic and non-trophic interactions, increase in diversity and increase in local temperature (Cauvy-Fraunié and Dangles, 2019). However, during the first steps of glacial retreat, our results revealed that environmental constraints significantly increased between two and 13 years of glacial retreat; with lower soil temperature, soil moisture and soil macronutrient contents. This trend is in line with fragments of existing literature reporting (1) relative abundance of allochthonous nutrients just after glacial retreat as released by the glacier itself (Frenot et al., 1998; Caccianiga et al., 2006) and (2) a reduction in the allochthonous nutrient concentration in a few years only after glacial retreat, as being consumed by microbes (Sattin et al., 2009). At the same time enrichment in organic matter by plants themselves is likely negligible given the extremely low vegetation cover on the glacier foreland (**Supplementary Material 3**; Frenot et al., 1998). Decreasing humidity along the chronosequence may

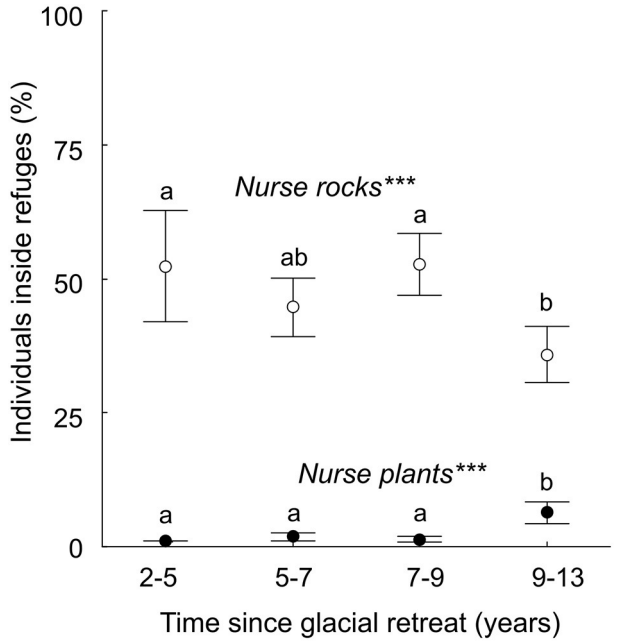


FIGURE 6 | Frequency of plant individuals developing under the shelter of abiotic refuges (nurse rocks) or heterospecific plants (nurse plants) at the community level, along the chronosequence. Mean values and Error bars: 95% CI are showed for each glacial retreat strip; different letters between strips indicate significant variations (*post-hoc* Tukey tests). The significance and the sign of the two trends were tested with logistic regressions: ***significant at $P < 0.001$.

be the result of increasing distance with melting ice (Stöcklin and Bäumler, 1996; Frenot et al., 1998). In the specific case of the alpine tropics, the absence of seasonal snowpack, which provides regularly water at soil surface (Vuille et al., 2018), and decreasing precipitation at higher elevation (Leuschner, 2000) may exacerbate this trend as the melting glacier becomes the only source of water availability. Reduced humidity has itself a negative impact on temperature amplitude and soil nutrients thus explaining possibly the pronounced abiotic gradients that we observed along such a short chronosequence. The reduced local environmental variations (slope, granulometry) between the plots and the strips of our sampling contribute to increase the reliability of the observed changes. It is necessary, however, to confirm them in future studies by considering other sites at various latitudes to gain representativeness.

In parallel, increasing stress for plants was observable between two and 13 years after glacial retreat, in several ways. First, this has been observed by the fact that plant communities reduce their vegetative height, a classic response to the effects of alpine stress (Körner, 2003). Second, the plant richness at the scale of the strip was also lower at the end of the chronosequence, with especially the absence of nine species previously recorded in the succession, this trend being possibly attributable to increasing stress for plants since the negative correlation between species richness and abiotic stress is commonly pattern in the alpine zone (e.g., Kammer and Möhl, 2002). Third, the fact that the most efficient clonal species in the sampling, *Senecio nivalis* (high abundance, high number of ramets and low investment in sexual reproduction) has not suffered an increase in necromass whereas the plant community does along the chronosequence goes with higher abiotic stress because clonal plants are generally more resistant to the alpine abiotic constraints (Stöcklin and Bäumler, 1996; Caccianiga et al., 2006). Fourth, the spatial distribution of plants at various spatial scales also evidenced diversity erosion along the chronosequence as both the reduction in the accumulation of species with increasing area (Preston curves) and in the proportion of safe sites (as evidenced by higher frequency of large empty patches) decreased with increasing time since the glacial retreat.

Focusing on the correlations between diversity, time since the glacial retreat and abiotic variables showed that diversity indices (abundance, species richness, plant cover) were not correlated linearly with time since they were variable along axis 2 of the PCA whereas time since the glacial retreat was variable along axis 1 (see **Figure 3**). This result contrasts with the positive correlation between plant diversity and time that is commonly observed during primary succession (Matthews, 1992; Zimmer et al., 2018; Cauvy-Fraunié and Dangles, 2019). An interpretation to be further tested is that the absence of linear relationship is the result of the antagonistic effects of time and the soil microclimate on diversity, which are opposite along axis 1 of the PCA. Such an antagonistic gradient may generate a unimodal relationship between time and diversity, which is not observable on the PCA biplot (based on linear correlations) but becomes visible when looking at the species richness at strip scale (see above). It could contribute to explain the lack of linear increase in diversity during early primary succession, as observed in some studies (e.g., Marteinsdóttir et al., 2013).

All in all, these results shed new light on recent studies observing a shift from relatively opportunistic to more stress-tolerant plant communities during the first steps of succession after glacial retreat. Indeed, three studies suggested that a shift from species with relative rapid growth rate (ruderals *sensu* Grime, 1977) to more stress-tolerant species occurred over a few years after glacial retreat (Caccianiga et al., 2006; Erschbamer and Caccianiga, 2016; Zimmer et al., 2018). By focusing on the first steps of colonization after glacial retreat (2–13 years), and by providing precise measurements on the abiotic environment (temperature, nutrients, soil moisture) our data provide that this trend can be explained with increasing abiotic stress. A decrease from 12 to 9% of the soil moisture between 2–5 years and 9–13 years, even statistically significant, might seem trivial to justify higher negative effects on plants. However, it represents a reduction by 25%, which, associated with decreases in temperature and nutrients, may be sufficient to exclude individuals of species that do not develop pronounced stress-tolerant traits. Such abiotic changes in longer chronosequences were not detected despite shifts from ruderal to stress-tolerant species (Zimmer et al., 2018, four chronosequences). This suggests (1) that increasing stress is observable only with a fine grain chronosequence, (2) that the consequences of this increasing stress after a few years can have an impact on plant traits on a longer term (see Zimmer et al., 2018) and (3) that

more evidence on this increasing stress is needed in other sites and regions.

Deficit in Facilitation and Dispersal Filtering

The first steps of plant primary succession after glacial retreat are generally assumed to rely strongly on biotic interactions (Cauvy-Fraunié and Dangles, 2019). This is especially true for non-trophic, positive interactions among plants (facilitation: Crocker and Major, 1955; Erschbamer et al., 2008). Indeed, there is a strong relationship between abiotic stress and the intensity of facilitation (stress-gradient hypothesis; Bertness and Callaway, 1994), which is particularly well-documented in alpine regions (Cavieres et al., 2014), including in the alpine tropics (Anthelme and Dangles, 2012), and is sustained by habitat amelioration provided by the nurse/facilitator. The high frequency of plants associated with volcanic rocks in our sampling indicated that plants in recently deglaciated terrains do require habitat amelioration (temperature, soil moisture, macronutrients, protection from wind; Erschbamer et al., 2001; Scherrer and Körner, 2011). This result is in line with another work in the Ecuadorian superpáramo evidencing lava rocks as much better refuges for plants than sandy areas soon after glacial retreat (Suárez et al., 2015). Contrastingly, facilitation by nurse plants, which are able to provide better habitat ameliorations than rocks (Filazzola and Lortie, 2014), was particularly infrequent in our sampling (1.7% of heterospecific plants spatially associated). This contrast between strong facilitation by rocks and poor facilitation by plants reveals a severe deficit in nurse plants, a pattern that was also observed in the dry tropical high Andes (Zimmer et al., 2018). Our results support this hypothesis with the fact that giant cushion-forming species, probably the most efficient nurse life form in the (tropical) alpine regions (Reid et al., 2010; Cavieres et al., 2014) are absent in our sampling site although they are present in older, adjacent deglaciated terrains (Anthelme et al., 2012).

In combination with facilitation deficit, the difficulties encountered by plants not dispersed by the wind to colonize the recently deglaciated site may inhibit the first steps of primary succession (Makoto and Wilson, 2019). In a context of unprecedented velocity of temperature warming, the absence of keystone, cushion-forming species dispersed by animals during the first steps of primary succession, such as *A. aretioides* in our sampling or *D. muscoides* and *O. andina* in the southern tropical high Andes (Zimmer et al., 2018), could influence the composition and functioning of novel alpine ecosystems on the longer terms, just as local species pool does (Schumann et al., 2016). The disproportionate abundance of wind-dispersed species in our sampled area was observable through the overwhelming majority of species being wind-dispersed (see also Erschbamer et al., 2008; Zimmer et al., 2018; Franzén et al., 2019 in other recently deglaciated terrains) whereas "only" 39% of the species in the tropical alpine regions of the Andes are expected to be dispersed by wind (Tovar et al., 2020).

The deficit in facilitative interactions among plants combined with strong dispersal filtering in novel alpine plant communities will likely slow primary succession in the context of rapid climate change/rapid glacial retreat. As confronted to an increasing abiotic stress during the few years after glacial retreat, plants may suffer even more from the absence of facilitation (stress-gradient hypothesis, Bertness and Callaway, 1994; Anthelme et al., 2014). This supports the already well-documented hypothesis that the collapse of positive interactions among organisms indebted to rapid climate change is an important driver of species vulnerability (Tilman, 1994; Gilman et al., 2010; Bellard et al., 2012). Nevertheless, the type of substrate, especially the presence of lava rocks, might also be an important driver of the plant colonization success, which deserves to be further tested (Suárez et al., 2015).

CONCLUSION

Under the effects of an unprecedented velocity of warming, exploring the first steps of primary succession following glacial retreat is key to build scenarios on the organization and dynamic of novel alpine plant communities. The increasing abiotic stress for plants we found between two and 13 years of glacial retreat seems to alter the commonly observed trend that plant diversity increases along with time early after glacial retreat. It reveals that some of the first colonizing species, brought by wind, could be present in vain as being seemingly unable to withstand the harsher environment farther from the glacier and disappear. This interpretation is consistent with the view that, at a time when global changes are extremely rapid, it is greatly difficult for plants to find successful trade-offs between high dispersal capacities and specific adaptations to the environmental constraints of the new habitats, leading to impoverished biodiversity (Tilman, 1994), resulting in a dispersal lag combined with an establishment lag (Alexander et al., 2018). The deficit of nurse plants that we observed in our sampling makes the colonization process even more difficult for the plant communities. These results are obviously exploratory and require further site replications to test if these results are generalizable to the alpine tropics, where the absence of snowpack (Vuille et al., 2018) and reduced precipitation is expected to increase stress for plants in comparison with alpine environments outside the tropics (Leuschner, 2000).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FA, OD, SC-F, and BF designed the study. SC-F and FA collected the field data. SC-F, FA, and OD analyzed the results. BC produced the glacial retreat map of glacier 15 alpha. All authors contributed to the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.584872/full#supplementary-material

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Homeostatic Response to Three Years of Experimental Warming Suggests High Intrinsic Natural Resistance in the Páramos to Warming in the Short Term

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Páramos, tropical alpine ecosystems, host one of the world's most diverse alpine floras, account for the largest water reservoirs in the Andes, and some of the largest soil carbon pools worldwide. It is of global importance to understand the future of this extremely carbon-rich ecosystem in a warmer world and its role on global climate feedbacks. This study presents the result of the first *in situ* warming experiment in two Colombian páramos using Open-Top Chambers. We evaluated the response to warming of several ecosystem carbon balance-related processes, including decomposition, soil respiration, photosynthesis, plant productivity, and vegetation structure after 3 years of warming. We found that OTCs are an efficient warming method in the páramo, increasing mean air temperature by 1.7°C and mean daytime temperature by 3.4°C. The maximum air temperature differences between OTC and control was 23.1°C. Soil temperature increased only by 0.1°C. After 3 years of warming using 20 OTC (10 per páramo) in a randomized block design, we found no evidence that warming increased CO_2 emissions from soil respiration, nor did it increase decomposition rate, photosynthesis or productivity in the two páramos studied. However, total C and N in the soil and vegetation structure are slowly changing as result of warming and changes are site dependent. In Sumapaz, shrubs, and graminoids cover increased in response to warming while in Matarredonda we observed an increase in lichen cover. Whether this change in vegetation might influence the carbon sequestration potential of the páramo needs to be further evaluated. Our results suggest that páramos ecosystems can resist an increase in temperature with no significant alteration of ecosystem carbon balance related processes in the short term. However, the long-term effect of warming could depend on the vegetation changes and how these changes alter the microbial soil composition and soil processes. The differential response among páramos suggest

that the response to warming could be highly dependent on the initial conditions and therefore we urgently need more warming experiments in páramos to understand how specific site characteristics will affect their response to warming and their role in global climate feedbacks.

Keywords: carbon emission, climate change, litter decomposition, IPPEX, OTC, tropical alpine ecosystems, carbon sinks, soil respiration

INTRODUCTION

The Earth is warming at an accelerated rate. The temperature is projected to increase 1.0–4.8°C by the end of this century (IPCC, 2014), with mountains experiencing a higher rise in temperature than the low-lands (Bradley et al., 2004, 2006). A recent compilation from 302 European mountain sites and 145 years of observations by Steinbauer et al. (2018) clearly shows accelerated warming and associated vegetation changes in mountain summits with unpredictable consequences to the ecosystem function and services they provide. In the tropics, projected to experience large changes in climate (Williams et al., 2007), the data void from mountain sites precludes us from understanding how the climate and vegetation have been changing (Feeley, 2015). The Andean highlands are already getting warmer (Vuille et al., 2015) and are projected to warm by 3–5°C by the end of this century (Urrutia and Vuille, 2009; Buytaert et al., 2011). There is building evidence that some tree species are already migrating upward and shrinking their distribution range (Pauli et al., 1996; Feeley et al., 2011; Duque et al., 2015; Báez et al., 2016). However, since not all species move at the same pace (Root et al., 2003; Walther et al., 2005), we could expect to see new plant communities form with yet unforeseen consequences to the whole region's biological diversity and ecosystem functioning (Báez et al., 2016). Whether the summits of the tropics are suffering floristic changes induced by the climate has yet to be detected through initiatives like GLORIA (The Global Observation Research Initiative in Alpine Environments) (Pauli et al., 2001), whose Andean branch has recently started to monitor floristic and climatic changes in the high tropical Andes (Cuesta et al., 2017). One piece of evidence that suggests vegetation is also shifting in the mountain summits of the tropics comes from the work by Cuesta et al. (2019) that documented important landscape transformations in the Andean highlands, including upward shifts of species and the formation of new communities in deglaciated forefronts (see also Zimmer et al., 2018). The consequences of these changes on species distribution, carbon storage, and ecosystem services across Andean alpine ecosystems are a pressing question that needs to be addressed.

Páramo ecosystems can be found at the top of several isolated mountains in the Northern Andean regions, Costa Rica, and Panamá and are considered biodiversity hotspots (Myers et al., 2000), important centers of speciation (Madriñán et al., 2013), crucial for local water supply, agriculture and hydropower (Buytaert et al., 2011), and important carbon sinks in the tropics (Hofstede, 1999; Hribljan et al., 2017). Páramos are usually found at elevations that range between 2800 and 4700 m above sea level and cover approximately an area of 41,700 km^2 (Kappelle, 2005; Llambí and Cuesta, 2014; Sklenář et al., 2014). Páramos are characterized by low-temperature conditions during night time, sometimes below zero, and cool to warm conditions during the day. Some páramos are relatively dry, but many can be described as high-elevation wetlands, given that some areas are waterlogged throughout part of the year. These environmental conditions, e.g., low temperatures and waterlogged soils, have led to low decomposition rates and a large buildup of organic matter through centuries (Podwojewski et al., 2002; Poulenard et al., 2003). Consequently, páramos are one of the most important carbon sinks in the tropics, storing up to six-fold more carbon per area than tropical forests (Hofstede, 1999), and globally they contain some of the largest stocks of soil organic carbon (Curiel Yuste et al., 2017). However, climatic factors that have favored soil organic carbon accumulation are likely to change by the end of this century, potentially shifting páramos from sinks to greenhouse gas emissions sources. Even though there is plenty of evidence that warming and changes in precipitation are already affecting biodiversity, decomposition rates, and soil carbon turnover in other biomes such as forests, mesic grasslands, and tundra (Cregger et al., 2014; Chen et al., 2015; Xue et al., 2016), tropical alpine regions are only beginning to receive greater attention (Buytaert et al., 2011). Páramo ecosystems could play a disproportionate role in driving climate feedbacks resulting from increased CO_2 release from their large soil carbon stores (Buytaert et al., 2011). Therefore, understanding how warming will impact carbon flux-related processes is of global importance.

Two recent publications highlight the potentially compounded aboveground and belowground challenges that would reduce soil carbon stocks. In the tropics, large carbon reservoirs could emit much carbon from soil respiration into the atmosphere with warming (Nottingham et al., 2020). Furthermore, there is evidence that accelerated growth in response to warming results in shorter tree lifespans, suggesting that increases in forest carbon stocks may be short-lived (Brienen et al., 2020). Results from a warming experiment in the tropical lowland forest of Panamá indicate that warming could increase the release of CO_2 from the soil by as much as 55% and thereby become a potent source of CO_2 in a warmer world (Nottingham et al., 2020). Likewise, there is a robust body of literature on the response of many carbon fluxes-related processes to changing temperatures that shows temperature increases soil respiration (Raich and Schlesinger, 1992; Bond-Lamberty and Thomson, 2010), litter decomposition (Hobbie, 1996; Cornelissen et al., 2001; Aerts, 2006), N mineralization and nitrification (MacDonald et al., 1995; Dalias et al., 2002; but see Emmett et al., 2004), plant nutrient uptake, and plant

productivity and fine root dynamics (Bassirirad, 2000; Gill and Jackson, 2000). However, it is still unclear how whole ecosystems will respond to warming and which ecosystems will be more affected. The general notion is that high-latitude and high-elevation ecosystems will be more affected by warming than equatorial and low-elevation ecosystems (Pepin et al., 2015; Wang et al., 2016) and the ecosystem responses would depend on the initial conditions of the carbon and nitrogen stocks, the soil water content, the size, and longevity of plants and their turnover rate (Shaver et al., 2000).

The recognition of the necessity to understand ecosystem responses to global warming has fueled a series of temperature-manipulation experiments worldwide that have been mainly concentrated in high-latitude sites. Two flagship examples are: (1) the Network of Ecosystem Warming Studies, which aims to evaluate ecosystem-level effects of rising temperature using a variety of warming methods in the tundra, low tundra, grassland, and forests at high-latitudes and elevation and (2) the ITEX (International Tundra Experiment) a network that has been warming circumpolar and high-elevation ecosystems using a common design of open-top chambers (Marion, 1997). Data from these warming experiments have been synthesized in various meta-analysis (e.g., Arft et al., 1999; Rustad et al., 2001; Aerts, 2006; Elmendorf et al., 2012; Lu et al., 2013), and have shown that warming can significantly alter soil and plant processes and ecosystem carbon cycling and storage (Melillo et al., 2002; McGuire et al., 2009; Craine et al., 2010; Conant et al., 2011; Schaefer et al., 2011). The meta-analysis of high-latitude ecosystems by Rustad et al. (2001) shows that warming can increase soil respiration by 20%, N mineralization rate by 46%, and plant productivity by 19%. A later meta-analysis by Lu et al. (2013) showed that although warming can stimulate soil respiration and carbon emission processes, it can also stimulate plant-derived carbon influxes like gross ecosystem photosynthesis by 15.7%, net primary productivity (NPP) by 4.4%, and plant C pools from above- and belowground parts by around 7.0%. Those carbon influxes offset the increases in warming-induced carbon efflux, indicating that climate change, specifically warming, may not always trigger strong positive carbon-climate feedback from terrestrial ecosystems. None of those large networks and meta-analysis includes tropical sites, and *in situ* warming experiments are urgently needed in the tropics to have a better picture of carbon-climate feedbacks from all terrestrial ecosystems (Cavaleri et al., 2015).

In the tropics, responses could be quite different from what has been observed in higher latitudes (Wood et al., 2019). The continuous growing season and higher temperature of the tropics are ideal for decomposers (Schlesinger and Andrews, 2000). Although temperatures are not higher in tropical alpine environments than in temperate alpine environments, they experience strong temperature amplitude all year round because of the absence of snow (e.g., Vuille et al., 2018). Some of the greatest soil respiration rates are found in the tropics, where tropical forests can contribute around 30% of the total global soil-derived CO_2 (Raich et al., 2002). However, because of the continuous growing season in moist and wet tropics, any negative effect of warming on decomposition and soil respiration could be offset if warming also increases plant productivity and carbon influxes all year around (Nemani et al., 2003). Therefore, the direction and magnitude of feedbacks could be different in the tropics. Two warming experiments were recently set in the tropical lowlands in Puerto Rico (Kimball et al., 2018; Kennard et al., 2020) and Panamá (Nottingham et al., 2020) and are starting to provide interesting data to understand how different the tropics may respond to warming. There are few *in situ* warming experiments running in Ecuador and Colombia in the tropical cold highlands, but no data have yet been published. However, translocation experiments set up along an elevation gradient in the Peruvian Andes (Salinas et al., 2011; Zimmermann and Bird, 2012; Nottingham et al., 2015) have provided some evidence of the temperature sensibility of soil organic matter decomposition in these high-elevation grasslands, which are drier than the páramos. Our previous work with páramos' incubated soils also points to a strong soil microbial responses to artificial warming (Curiel Yuste et al., 2017). These findings suggest that the response of heterotrophic respiration to warming in tropical mountain ecosystems could be stronger than predicted. More studies are needed to fill the unexplored gap in our understanding of carbon dynamics in páramos, where large quantities of soil organic carbon are present (Hofstede, 1999; Curiel Yuste et al., 2017; Hribljan et al., 2017).

In this study, we evaluated how warming affected several processes related to carbon emission and sequestration. We followed ITEX protocol using hexagonal open-top chambers (OTC), a standardized passive warming methodology used at sites that are difficult to access and have no access to power (Marion, 1997). We evaluated two key components of the global carbon budget; decomposition rate and annual CO_2 emissions from soil respiration, a process known to be one of the main sources of CO_2 to the atmosphere. We also evaluated carbon influx processes like photosynthesis and plant productivity (below and aboveground); and soil properties that could eventually lead to changes in those processes like soil water content, soil pH, carbon, phosphorous, and nitrogen pools. Additionally, we studied the warming effect on the vegetation structure to evaluate whether warming could alter the carbon sequestration potential trough vegetation changes, as has been reported in other cold biomes in the artic where woody shrub dominance has increased (Tape et al., 2006), and with it the carbon sequestration potential of the ecosystems. We hypothesize that in these high-elevation páramo ecosystems limited by temperature and nutrients (Hofstede, 1995), the increasing temperature would increase rates of litter decomposition, rates of soil respiration, net N mineralization, resulting in greater soil nutrient availability, and consequently plant growth and greater biomass production and net primary productivity (NPP). Moreover, we hypothesize that warming will favor shrubs over other growth forms since this group dominates warmer sites in the sub-páramo (Buytaert et al., 2011), has a physiological tolerator behavior in other páramos (Rada et al., 2019), and climate-driven shrub expansion has been amply documented across tundra and temperate alpine ecosystems (McDougall, 2003; Tape et al., 2006; Anthelme et al., 2007; Brandt et al., 2013; Zhou et al., 2020). How much and

in what directions will warming alter these different processes will be key to understanding the future role of the páramos as carbon reservoirs.

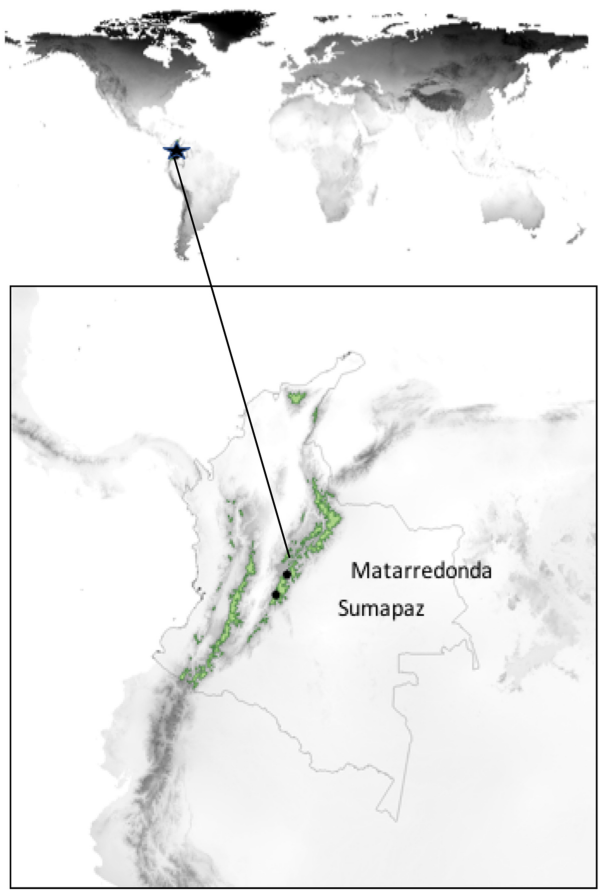
MATERIALS AND METHODS

Study Site

The warming experiments were conducted at two sites in the North-eastern Andean range in Colombia (**Figure 1**). Both páramos are part of the Cruz Verde-Sumapaz complex and are surrounded by a matrix of forest, roads, and agriculture. One site is Matarredonda, located 3300 m above sea level, with annual precipitation of 1178 mm and a mean annual temperature of 8.8°C. The minimum temperature we have registered in Matarredonda is −2.0°C and the maximum is 35.0°C. The experimental site in Matarredonda is 1 km away from the road and 2.5 km from the closest agriculture field. The second site is in Sumapaz, near the military base "Batallón de Alta Montaña N° 1" at 3400 m above sea level with annual precipitation of 1469 mm and a mean annual temperature of 7.6°C. The minimum temperature we have registered in Sumapaz is −4.5°C and the maximum is 39.0°C. In Sumapaz there are no asphalted roads close to the experimental site, only gravel roads located >2 km from the OTC. The distance between both páramo sites is approximately 96 km (in a straight line), but 7 h driving. In both sites, the vegetation is dominated by tussock grasses, especially *Calamagostri effusa*, intermixed with forbs, stem, and basal rosettes, including the páramo's endemic genus *Espeletia*, and sclerophyllous shrubs, with bryophytes and lichens commonly found in the prostrate vegetation strata. The soil type for both sites is Bh3-3b (FAO, 2009) with quaternary volcanic ashes and outflows, Paleozoic metamorphic and intrusive igneous rocks. In Matarredonda the soil has been classified as typic Hapludands while in Sumapaz as typic Dystrocryepts and typic Haplocryands (IGAC, 2000). A list of the most common species at the study sites are presented in **Supplementary Table 1**.

Experimental Warming

Climatic warming was simulated using fiberglass hexagonal open-top chambers (OTC) following the ITEX (International Tundra Experiment) design (**Figure 1**). OTC act both as windshields and solar traps increasing ambient air and soil temperature (Marion, 1997). OTCs were constructed using six pieces of 0.040-inch-thick fiberglass Sun-Lite HP (Solar



Characteristics	Matarredonda	Sumapaz
Lat/Long	4°33′00.1″ N 73°59′32.1″ W	3°49′36.3″ N 74°24′47.1″ W
Elevation range (m)	3280-3400	3324-3612
MAT (°C)	8.8	7.6
MAP (mm)	1178	1469
Tmax (°C)	14.3 (35.0)	12.7 (39.0)
Tmin (°C)	3.3 (-2.0)	2.7 (-4.5)
Soil type	Bh3-3b	Bh3-3b

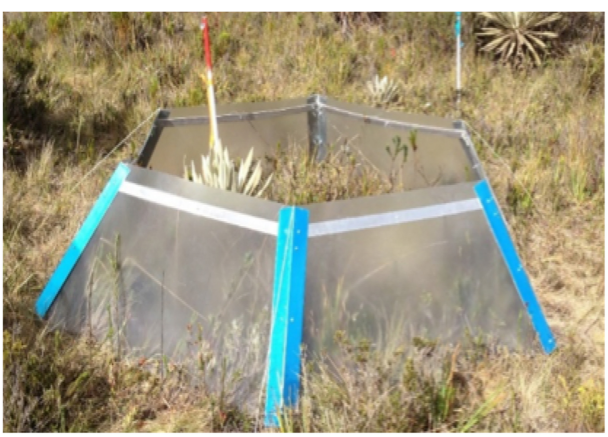


FIGURE 1 | Location, characteristics of the study sites and OTC design. Páramos of Colombia are depicted in green. Climatic variables were downloaded from http://www.worldclim.org/bioclim and the maximum and minimum temperatures registered by our temperature sensors during 2017–2019 are shown in parenthesis.

Components Corporation, Manchester, NH, United States) attached at 60° angles, with a side-to-side diameter of 2.08 m and 60 cm height, covering an area of 3.87 m^2 and attached to the ground by UV-resistant wires. This warming method is the preferred warming method on remote places and high-mountain tops without access to electrical power and has been successfully used in more than 20 experiments by the ITEX group.

Before establishing the plots, we searched the páramo for flat sites (with less than 15° of inclination) with similar vegetation of less than 60 cm in height. We marked two similar sites within 1–2 m distance to assign them the treatment (OTC and non-manipulated plot as its control) within the same block following a complete randomized block design. The distance between adjacent blocks was about 4–8 m. We included in the plots the most common species of the páramo and at least one individual of *Espeletia*, an iconic dominant genus of the páramo. A preliminary analysis considering the species' identity and its percentage of coverage showed that the plots assigned to each treatment within a block had similar vegetation at the beginning of the experiment (**Supplementary Figure 2** and **Supplementary Table 1**). The average slope in all plots in Sumapaz was 10°, and the average aspect 125.9°. In Matarredonda, the average slope was 6.8°, and the average aspect of 166.6°. Slopes and aspects were estimated using GRASS GIS software and the digital elevation model at 30 m spatial resolution from the shuttle radar topography mission (Farr et al., 2007). The OTC installation was completed in June 2016.

Environmental Monitoring

To quantify whether the OTCs are modifying the environment, we monitored ambient air temperature, air humidity, soil temperature, and soil water potential in and out the OTC using an automatic recording systems in Matarredonda that consisted of an EM-50 datalogger (Decagon Devices, Inc., Pullman, WA, United States) connected to two VP-4 humidity and temperature sensors with a radiation shield (Decagon Devices, Inc., Pullman, WA, United States) and two MPS-6 soil temperature and water potential sensors (Decagon Devices, Inc., Pullman, WA, United States). The ambient air sensor was set at 30 cm above the soil surface, which is the height of many of the plants in the OTC, and the soil sensor at 10 cm belowground. Data were recorded every 30 min from December 2017 to December 2019. This monitoring was done in only one OTC and its control paired plot in Matarredonda. Simultaneous records of temperature, relative humidity, and atmospheric pressure were used to calculate air vapor pressure deficit (VPD) using the equation as in Ficklin and Novick (2017). VPD is a direct measure of the atmospheric drying power and is defined as the difference between the water vapor in the air at saturation and the actual amount of water vapor.

To confirm that all OTC are functioning as warming devices, we measured the leaf temperature of *Espeletia* species in all 40 plots. We took thermographic images with a Fluke® Ti32 infrared thermal camera (Fluke, Avery, WA, United States) from healthy mature leaves of two common endemic rosettes species from the páramo, *Espeletia grandiflora* (10 pairs of plots) in Sumapaz, *Espeletia argentea* (six pairs of plots), and *Espeletia grandiflora* (four pairs of plots) in Matarredonda. Photos were taken at midday on a sunny day between September and October 2016, 4 months after establishing the experiment. Using the software SmartView 3.15^{TM} the temperature of the leaf was extracted from the thermal image of the whole leaf.

Effect of Warming in Processes Related to CO_2 Emissions

Decomposition Rate

To evaluate whether warming could alter decomposition processes and lead to an increase in carbon losses from the páramo, we used the litterbag method proposed by Powers et al. (2009) where litter mass loss of one gram of substrate was followed for 2 years. We used two substrates; bay leaves, *Laurus nobilis* (Lauraceae) a standard substrate that has been used on litter decomposition experiments across the tropics (Powers et al., 2009), and "frailejón" leaves, *Espeletia grandiflora* (Asteraceae) an endemic páramo species belonging to one of the predominant genera in the South American páramo. *E. grandiflora* retain their dead leaves around the stem and they were cut from the plant avoiding collecting eaten or diseased leaves. Litterbags (15 cm × 10 cm) of 1 mm nylon mesh were filled with 1.0 ± 0.1 g of air-dried UV sterilized substrate. In July 2016, 1 month after establishing the OTC chambers, 12 bags were deployed randomly (six from each species) on each plot. Bags were anchored to the surface of the soil using metallic wire. One bag per species per plot was collected at 1, 3, 6, 12, 18, and 24 months after deployment. The litter samples were then cleaned up, dried (55°C, 72 h), and weighed. To compare our values with the pantropical study on decomposition rate of Powers et al. (2009), we calculated the annual decomposition rate (k) following their protocol. That is, k was calculated as the slope of the linear regression of the natural logarithm of percent mass remaining versus time, forcing the Y-intercepts through 100% mass remaining at time 0. k values are presented as absolute values for ease of interpretation, where high k indicate steeper slopes and therefore higher decomposition rates. The response variable decay rate (k) was log-transformed before the analysis to meet assumptions of homogeneity of variance.

Soil Respiration

To evaluate whether warming could increase carbon losses from páramos' soil, we measured soil CO_2 fluxes using an infrared gas analyzer (EGM-5, PP Systems, Amesbury, MA, United States), connected to its soil respiration chamber (SRC-2) and soil temperature probe (STP-2). Two PVC collars (11 cm diameter × 8 cm height) were installed in each plot in February 2017 on bare soil at a depth of 4 cm, after carefully scraping away litter. Fluxes were measured by attaching the respiration chamber to the soil collar every 2–3 months in the late morning early afternoon, between 10:00 AM and 2:00 PM, starting February 2017 until June 2019. Before every measurement, vegetation encroached in the collar was removed. The soil respiration values that we obtained represent the combined respiration of soil micro- and macro-organisms and plant roots. Air and soil temperatures were measured at the same time as the CO_2 efflux. Measurements of CO_2 built up within the system were performed during 60 s and respiration was calculated from the slope of the increase in CO_2 concentration as a function of time. The volume

of the PVC collar was added to the soil chamber's volume to correct the final flux estimate.

Physical, Chemical and Biological Properties of the Soil

To evaluate whether warming changes soil properties like water content, bulk density, pH, total N, P, and C, we randomly collected, 36 months after establishing the experiment, two undisturbed soil cores at 20 cm depth per plot for a total of 40 cores per study site. Samples were then sieved to remove debris, roots, and soil particles >2 mm. Gravimetric water content was estimated from a subsample by weighting the sample before and after being oven-dried for 24 h at 105°C. For pH determination, 3 g of sieved soil were dissolved in 15 ml of distilled water for 30 min before reading was taken. The remaining soil sample was sent for total carbon and nitrogen (elemental analyzer), organic carbon (Walkley Black method and colorimetric), ammonia and nitrate (volumetric KCl 2M extraction), and phosphorous analysis (Bray II method) to the Soil and Water Laboratory from the Agricultural Sciences Faculty in Universidad Nacional de Colombia. Bulk density was calculated from the dry soil weight, divided by the core volume. We calculated soil organic carbon (SOC) stocks in the first 20 cm depth of soil by multiplying soil bulk density, soil depth, and % of organic carbon.

Effect of Warming on Processes Related to CO_2 Capture

Photosynthesis

To evaluate whether warming can potentially change the carbon influxes through carbon assimilation by the vegetation, we measured maximum net photosynthetic rate (A_{max}) and dark respiration (R_d, a measure of the leaf level autotrophic respiration) with a portable infrared gas analyzer Li-COR 6400XT (Li-Cor Inc., Lincoln, NE, United States). We selected in both páramos one species of the *Espeletia* genus, which is the most iconic, abundant and diverse genus in the páramo (Monasterio and Sarmiento, 1991; Madriñán et al., 2013; see **Supplementary Table 1**) and considered one of the most vulnerable to climatic change (Buytaert et al., 2011; Peyre et al., 2020; Valencia et al., 2020). Additionally, we selected two other abundant forbs species, that were commonly found in most of the OTCs and their paired control plots that are also endemic to the páramo. Measurements were taken 1 year after the establishment of the OTC in *Espeletia grandiflora* Humb. & Bonpl. (n = 8) and *Orthrosanthus chimboracensis* (Kunth) Baker. (n = 8) in Sumapaz and in *Espeletia argentea* Humb. & Bonpl. (n = 7), and *Valeriana pilosa* Ruiz & Pav. (n = 7) in Matarredonda. Then again, after 2 years for *E. grandiflora* in Sumapaz. Measurements were taken in upper mature fully expanded healthy leaf, setting the IRGA at: 420 μmol mol^{-1} reference CO_2, and a constant flow of 500 μmol s^{-1} at ambient humidity. For A_{max} leaves were exposed to 2000 μmol photons m^{-2} s^{-1} of light until stability was reached, and light curves were constructed with seven light points: 2000, 1500, 1000, 500, 250, 100, and 0 μmol photons m^{-2} s^{-1} of light. Values of A_{max} and R_d values were obtained from fitted curves after testing the best model (Lobo et al., 2013).

Net Ecosystem Exchange

Net ecosystem (CO_2) exchange (NEE) was measured using the chamber technique in the third year of warming in two spots inside the OTC and in two spots in the control plots. Measurements were taken in low stature vegetation that could be fit inside the chamber, on spots with similar species and vegetation cover on each paired plot. Dominant vegetation included juveniles of *Espeletia*, prostrated shrubs like *Pernettya prostrata*, small upright shrubs like *Diplostephium* and *Arcytophyllum*, some mosses, sedges, and grasses in the genus *Carex* and *Calamagrostis*, and forbs like *Valeriana*, *Orthrosanthus*, *Nertera*, and *Castratella*. Species were selected based on high abundance, though species that grow larger than the chamber diameter were excluded (**Supplementary Table 1**).

The changes in CO_2 concentration were measured using the EGM-5 Portable CO_2 infrared gas analyzer and its transparent canopy assimilation chamber CPY-5 (PP Systems International, Inc., MA, United States). The chamber is made of polycarbonate and includes a photosynthetically active radiation sensor, an air temperature sensor, an air mixing fan and a manifold system that ensures uniform circulation within the chamber. The chamber is 145 mm in height × 146 mm diameter and covers a surface area of 167 cm^2. At each spot, we took measurements in light and in darkness. In light, we measured net ecosystem production (NEE), which is the carbon sequestration by photosynthesis minus the carbon release from plant and soil microbial respiration. The dark measurement was taken after aeration of the chamber by covering it with a dark cloth that reduces the incoming light to zero; under this condition we measured the ecosystem (plant plus soil) respiration (Reco). The total uptake of carbon through photosynthesis or gross ecosystem production (GEP) was then calculated as GEP = NEE + Reco. Measurements were taken every second for 75 s. Initially, we worked on longer time series, but we had large variations in CO_2 concentration at the end, suggesting that we started to see the effect of lateral diffusion. After several trials, 75 s was finally estimated to be the best time series to obtain a reliable concentration time series to fit a linear equation, and estimate the flux from the slope. In this study, positive values indicate an uptake of CO_2 by the ecosystem from the atmosphere and negative values represent release of CO_2 from the ecosystem to the atmosphere.

Above- and Below-Ground Biomass

Belowground productivity was estimated at the community level using the ingrowth core technique (Li et al., 2013), that is, intact soil cores were removed from the ground and replaced with an equivalent volume of root-free soil enclosed in a mesh cylinder. In August 2016, two soil cores (5 cm diameter × 20 cm deep) were randomly extracted from each plot for a total of 40 soil cores (20 in OTC and 20 in control plots) per study site. The cored holes were then filled with the polyethylene net cylinders (5 cm diameter × 15 cm depth; mesh size 3.5 mm) previously filled with sterilized root-free soil. Given the low productivity of the páramos, ingrowth cores were left in the sites for a whole year and retrieved afterward. Cores were brought back to the laboratory, and fine roots were manually removed and rinsed with water to remove soil particles. Cleaned roots were then oven dried at

70°C until constant weight. Estimates of fine root productivity are presented as mass (g) of fine roots produced in one square meter in 1 year.

To evaluate whether warming is affecting aboveground productivity, we harvested all the vegetation present in 1 m × 10 cm transect inside the OTCs and in their paired control plots after 3 years of warming. The transect was randomly assigned and established in one of the four outer borders of the central 1 m × 1 m permanent vegetation plot and away from the soil respiration PVC collars and other manipulations. All the vegetation in the transect was harvested, including any tall vegetation and branches within the transect, even if it was rooting outside the transect. The harvest was divided into vascular, non-vascular plant biomass and necromass. We consider as necromass all tissue and leaves that were 100% senesced and dry. When a leaf or tissue was not entirely dried, we split the tissue in two, the green tissue was added to the biomass value and the dry tissue to the necromass value. Additionally, we extracted all the roots present in the first 15 cm of the soil surface from two soil core extracted from each OTC and each control plot in the third year of warming. Soil cores were 15 cm long by 5 cm width.

Vegetation Changes

To evaluate whether the carbon sequestration potential of the páramo vegetation changes were due to changes in the dominance of growth forms, for example from herbaceous to woody species, we monitored changes in the plant community composition in the OTC and control plots. We sampled each plot's vegetation using a 1 m × 1 m PVC frame with a grid with 100 squares (10 cm × 10 cm each square). Every square in the grid was identified with a combination of numbers (horizontally) and letters (vertically). To be able to relocate the grid on the same area every year, the grid was secured with four adjustable legs attached to four PVC tubes permanently anchored to the ground. The top center of the grid was always directed to the North and the 1 m × 1 m PVC frame occupied the center of the OTC, away from the walls. A stick of 3 mm in diameter was lowered through the upper left corner of each square until it reached the soil or lower lying vegetation and we registered each contact of the stick (a hit) with living and standing dead vegetation across all strata. Hits were recorded to the species level whenever possible or to genus level. Data were analyzed at the level of growth forms and all species were classified to one of the following forms: rosettes, shrubs, graminoids (grasses, sedges, and rushes), forbs, cushion plants, ferns, seedless vascular plants (ferns and club mosses), non-vascular plants (mosses, hornworts, and liverworts), and lichens. We also included bare soil cover and necromass (hits with standing dead vegetation) as additional categories. Vegetation was sampled upon the establishment of the OTC (in 2016) to obtain the base line vegetation cover to compare with, and then 3 years after establishing the experiment (June 2019). We obtained the cover of the major growth forms by dividing the number of hits for each species per plot by the total number of hits recorded for that plot each year. To evaluate how warming may have changed the vegetation, we calculated the change in cover as the differences in the cover found in 2019 from the cover found at the beginning of the experiment in 2016.

Statistical Analysis

For the analysis of all variables we checked for normality and homogeneity of variances using Shapiro–Wilk and Levene tests, respectively. Data were transformed before the analysis when needed. Most data were evaluated by a two-way ANOVA for a randomized block design with treatment (OTC and control) as main fixed factor and block as the second factor to control for the confounding effect of any spatial differences. Analyses were done separately for each páramo. We also evaluated the time effect on CO_2 fluxes by two-way repeated measurement ANOVA for the soil respiration data. For each date, we evaluated if respiration differed between the OTCs and the control plots by a Holm–Sidak test. When normal distribution was not reached after data transformation, non-parametric statistics were applied (Mann–Whitney *U*-test). All analyses were performed using JMP software (JMP 9.01. SAS Institute Inc.) and SigmaPlot V.12.0 (Systat Software Inc.).

To explore general patterns on the effect of warming on all variables on both páramos we computed the effect size (d) from the means and standard deviations of the variable analyzed in OTC and control plots. The effect size was calculated as the difference between the two means (OTC and control), divided by the pooled estimate of standard deviation. The effect size was then corrected using a factor provided by Hedges and Olkin (1985) and the confidence interval for an effect size was also calculated as explained by Hedges and Olkin (1985). When 95% CI of d do not overlap with zero, the warming treatment has a significant effect; positive values indicate a positive effect of warming and negative values a negative effect of warming.

RESULTS

Environmental Monitoring

The mean differences between the air temperature registered in the OTC with respect to the control plots was 1.7°C, the maximum air temperature differences between OTC and control was 23.1°C and the minimum air temperature differences was −1.0°C, evidencing a night-time cooling within the chambers (**Figure 2** and **Supplementary Figure 3**) that reduced the daily mean differences. When we analyzed only daily temperatures (temperature registered between 6:00 AM and 6:00 PM) the differences in air temperature between OTCs and control was 3.4°C. Soil temperature registered at 10 cm depth was more resistant to change, with only a mean increase in the OTC with respect to the controls of 0.1°C with maximum differences of 1.6°C. The soil was more resistant to abrupt temperature changes, while the lowest air temperature registered during the study was −3°C, the lowest soil temperature registered was 8.4°C. OTCs are also slightly drying the soil, but only during the dry season with the lowest soil water potential values registered at the end of April. OTC also reduced air humidity resulting in higher values of VPD in the OTC in comparison to the control plots all year round but specially in the dry season when highest VPD values paralleled with lowest soil water potential values (**Figure 2**). Changes in the air temperature by the OTC also resulted in changes in the mean leaf temperature of the two species analyzed,

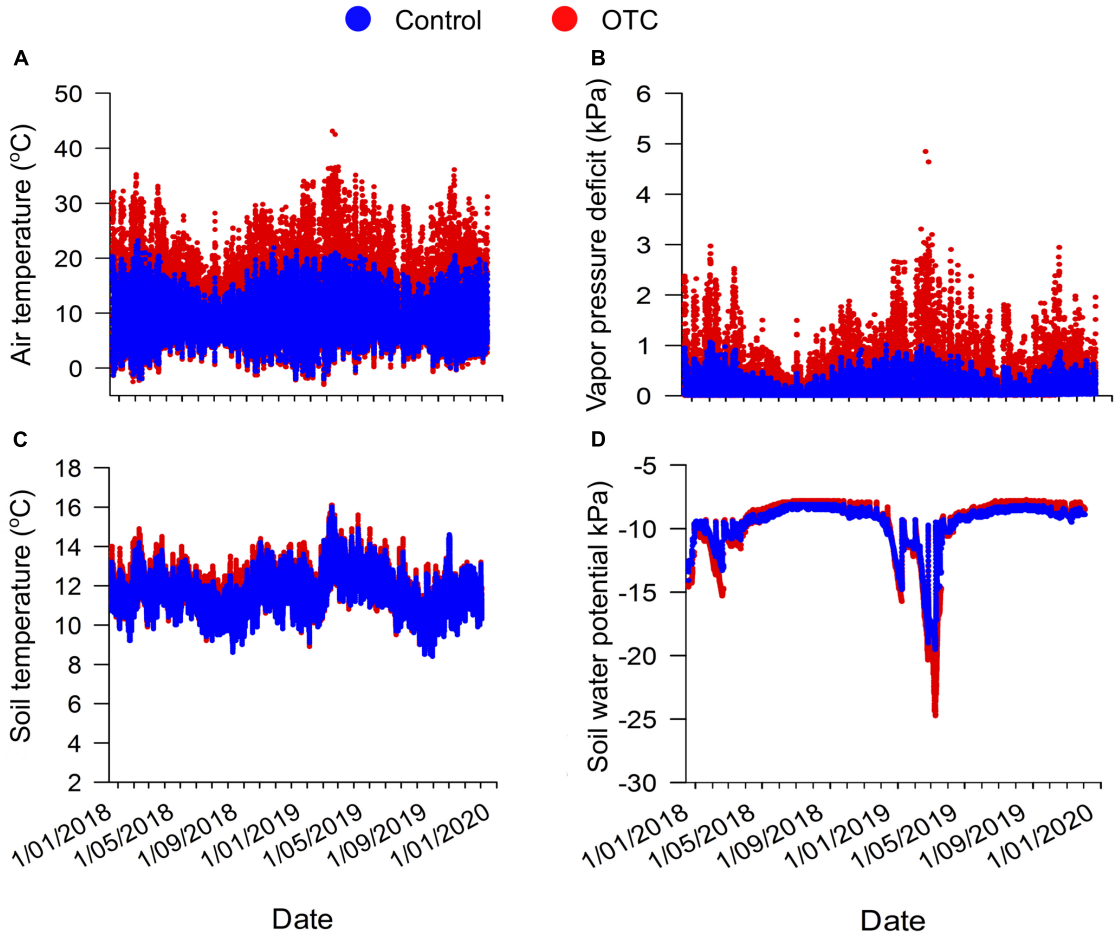


FIGURE 2 | Climatic variables collected inside the OTC (red) and outside the OTC (blue). Air temperature **(A)**, air vapor pressure deficit **(B)**, soil temperature **(C)**, and soil water potential **(D)** were collected every 30 min starting on December 19, 2017 until December 3, 2019 in Matarredonda.

which had warmer leaves by 3–4°C inside the OTC compared to the control plot (**Supplementary Figure 1**). The mean leaf temperature of *Espeletia grandiflora* in Sumapaz within the OTC was 29.5 ± 1.8°C, and outside the OTC was 25.6 ± 1.9°C ($t = 2.81$; $df = 9$; $P = 0.01$). The mean leaf temperature of *Espeletia argentea* in Matarredonda within the OTC was 23.1 ± 1.4°C, and outside the OTC was 19.1 ± 1.1°C ($t = 3.77$; $df = 5$; $P = 0.006$). The mean leaf temperature of *Espeletia grandiflora* in Matarredonda within the OTC was 23.6 ± 2.0°C, and outside the OTC was 18.7 ± 0.3°C ($t = 2.79$; $df = 3$; $P = 0.03$).

Effect of Warming in Processes Related to CO_2 Emissions

Decomposition Rate

Contrary to our initial expectation, the decomposition rates were similar or even lower in warmer plots than in control plots in both study sites (**Table 1** and **Figure 3**). In Sumapaz, the mean decay rate for *Espeletia's* substrate inside the OTC was lower than in the control plots ($F = 13.4$; $df = 1$; $P = 0.005$). Similarly, the decay rate for Laurel's substrate was reduced inside the OTC ($F = 15.2$; $df = 1$; $P = 0.004$; **Table 1**). In Sumapaz, only 44% of the original material has been decomposed after 2 years in the OTC and 46–53% in the control plots (**Figure 3**). In Matarredonda the mean decay for *Espeletia's* substrate was similar between treatments ($F = 1.93$; $df = 1$; $P = 0.19$; **Table 1**), while the decay rate for Laurel's substrate was lower in the OTC ($F = 6.48$; $df = 1$; $P = 0.03$; **Table 1**). With this low decomposition rate, in Matarredonda, only 46–49% of the original material has been decomposed after 2 years in the OTC and 48–53% in the control plots (**Figure 3**).

Soil Respiration

The regression between each of the measurements of soil respiration and its associated soil temperature shows that soil respiration increased with soil temperature in Matarredonda (temperature effect: $t = 3.23$; $P = 0.001$) and Sumapaz (temperature effect: $t = 1.92$; $P = 0.05$) but the slope of this relationship was no different between OTC and control plots in Matarredonda ($t = 1.28$; $P = 0.20$; **Figure 4**) and Sumapaz ($t = -0.83$; $P = 0.40$; **Figure 4**). Therefore, we did not find evidence that warming affects soil CO_2 fluxes at any time of

TABLE 1 | Soil related properties and processes in control and warmed (OTC) plots.

Site	Parameters	OTC	Control	*p-value*
Matarredonda				
	Organic C (%)	15.5 ± 0.81	15.9 ± 0.75	0.57
	Total C (%)	17.8 ± 1.01	18.1 ± 0.88	0.72
	Total N (%)	0.86 ± 0.05	0.89 ± 0.04	0.55
	SOC (tons C/ha)	149.4 + 6.25	141.4 + 3.82	0.21
	NH4 (mg/kg)	63.2 + 4.29	68.1 ± 3.96	0.31
	NO3 (mg/kg)	42.6 ± 4.99	35.9 ± 2.63	0.29
	P (mg/kg)	7.9 ± 3.41	5.9 ± 1.12	0.62
	Decay rate *E. grandiflora* (year^{-1})	0.35 ± 0.04	0.39 ± 0.03	0.19
	Decay rate *L. nobilis* (year^{-1})	0.40 ± 0.02	0.46 ± 0.01	**0.03**
	Root productivity (g/m^2/year)	9.10 ± 1.03	7.04 ± 0.61	**0.07**
	Bulk density (g/cm^3)	0.51 ± 0.03	0.46 ± 0.02	**0.03**
	Water content (g/g)	0.57 ± 0.01	0.59 ± 0.02	0.27
	pH	4.43 ± 0.06	4.35 ± 0.06	0.16
Sumapaz				
	Organic C (%)	8.3 ± 0.29	7.9 ± 0.34	0.28
	Total C (%)	12.0 ± 0.44	10.6 ± 0.35	**0.01**
	Total N (%)	0.66 ± 0.02	0.59 ± 0.02	**0.01**
	SOC (tons C/ha)	102.1 + 4.29	102.0 + 7.05	0.98
	NH4 (mg/kg)	102.0 ± 10.9	84.8 ± 7.09	0.22
	NO3 (mg/kg)	39.4 ± 4.03	47.6 ± 5.99	0.15
	P (mg/kg)	48.8 ± 5.9	42.6 ± 4.9	0.37
	Decay rate *E. grandiflora* (year^{-1})	0.30 ± 0.02	0.38 ± 0.04	**0.005**
	Decay rate *L. nobilis* (year^{-1})	0.35 ± 0.02	0.45 ± 0.01	**0.004**
	Root productivity (g/m^2/year)	13.63 ± 1.87	10.95 ± 1.79	**0.08**
	Bulk density (g/cm^3)	0.62 ± 0.03	0.64 ± 0.03	0.74
	Water content (g/g)	0.46 ± 0.01	0.46 ± 0.01	0.64
	pH	4.45 ± 0.06	4.37 ± 0.06	0.21

Physical and chemical properties come from 2 soils samples per plot collected in the first 20 cm of soil after 3 years of warming. Root productivity comes from 20 ingrowth soil cores, two per plot on each site on the second year of warming. Decay rates come from litter bags experiments during the first 2 years of the experiment. Numbers presented correspond to the mean ± standard error. P-values in bold when <0.1.

the year at both study sites (**Figure 4**). When we analyzed the seasonal pattern through a repeated ANOVA we found that in Sumapaz soil CO_2 fluxes did not vary in response to warming (Treatment effect: $F = 0.67$; $df = 1$: $P = 0.43$), but varied seasonally (Date effect: $F = 10.36$; $df = 4$; $P < 0.001$; **Figure 3**), with the lower fluxes measured during the rainy season on June 2017 and June 2018. However, the seasonal effects on soil CO_2 fluxes were not modified by warming (Treatment × date effect: $F = 0.74$; $df = 4$; $P = 0.57$). In Matarredonda a similar result was found where soil CO_2 fluxes did not vary because of warming (Treatment effect: $F = 0.09$; $df = 1$: $P = 0.77$), but varied seasonally (Date effect: $F = 11.58$; $df = 4$; $P < 0.001$), with the lower fluxes also measured during the rainy season June 2017. Like in Sumapaz, we did not observe that warming modified seasonal CO_2 fluxes (Treatment × date effect: $F = 1.48$; $df = 4$; $P = 0.19$).

Physical, Chemical and Biological Properties of the Soil

Most soil physical and chemical properties have not changed as result of warming in Matarredonda, only bulk density has increased in the warmed plots (**Table 1**). However, in Sumapaz we saw some changes in the N and C pools resulting from warming; total carbon and total nitrogen are higher in the OTC (**Table 1**). Belowground productivity has not been affected by warming either, with only a marginally significant increase in the OTCs' root production in both sites (**Table 1**). After 3 years or warming most soil related-processes and properties such as water content, organic carbon, SOC, pH, NH_4, NO_3, and soil respiration have remained the same, and show that both páramos can maintain their homeostasis with no evident short-term negative effect of warming (**Table 1**). If anything, warming (and the drying effect of the OTC) has slowed down decomposition rate and was slightly increasing root productivity.

Effect of Warming in Processes Related to CO_2 Capture

Photosynthesis

Maximum net photosynthetic rate (A_{max}) and respiration in the dark (R_d), were not affected by the warming treatment in *Espeletia grandiflora, Espeletia argentea*, *Orthrosanthus chimboracensis*, and *Valeriana pilosa* (**Table 2**), suggesting that they are not modifying their photosynthetic machinery because of warming or being inhibited by the higher temperatures. Whether carbon fluxes related to photosynthesis and aboveground autotrophic respiration may change in future warming scenarios, may ultimately depend on the net assimilation rate at the community level.

Net Ecosystem Exchange

The CO_2 fluxes measured at the small scale of the EGM chamber indicate that warming was not increasing the net carbon release from the ecosystem (**Figure 5**). Values of Gross primary production (GPP) or total uptake of carbon through photosynthesis after 3 years of warming were similar in the OTC and the control plots in Matarredonda ($F = 0.02$; $df = 1$; $p = 0.89$) and Sumapaz ($F = 0.08$; $df = 1$; $P = 0.79$). Values of total carbon dioxide release from autotrophic and heterotrophic respiration (Reco) were indistinguishable between OTC and controls plots in Matarredonda: ($X^2 = 1.42$; $df = 1$; $P = 0.23$) but in Sumapaz carbon dioxide release was slightly lower in the OTC ($X^2 = 3.94$; $df = 1$; $P = 0.05$), where less carbon was being released from respiration in the OTC in comparison to the control plots. The net ecosystem exchange (NEE) or final carbon dioxide effluxes from the ecosystem were not different between the OTC and control plots in Matarredonda ($F = 1.35$; $df = 1$; $P = 0.25$) nor in Sumapaz ($F = 0.59$; $df = 1$; $P = 0.48$).

Above- and Below-Ground Biomass

After 3 years of warming we did not detect any effect of warming on the production of belowground biomass (Sumapaz: $F = 0.59$;

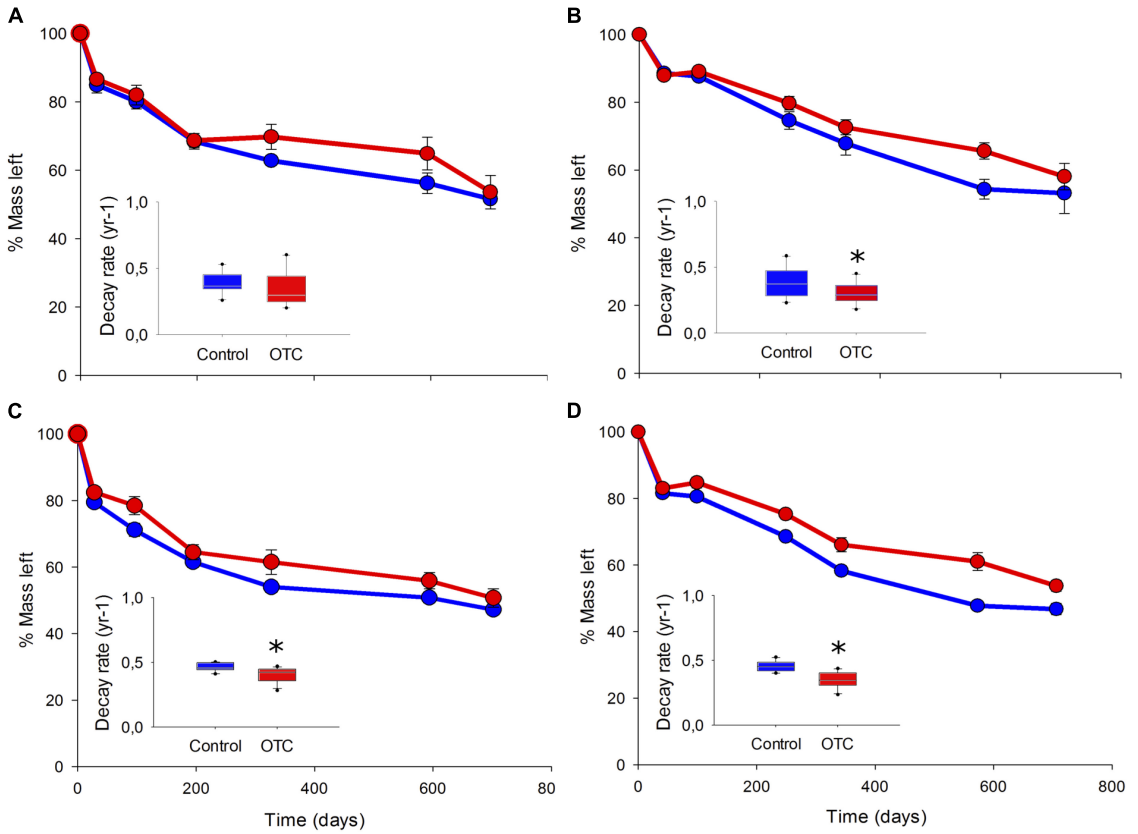


FIGURE 3 | Percentage of litter mass left at the end of 2 years of incubation in the field (outer plot) and the decay rate *k* (inner plot) for *Espeletia grandiflora* in Matarredonda **(A)** and Sumapaz **(B)** and for *Laurus nobilis* in Matarredonda **(C)** and Sumapaz **(D)**. Points are mean value and their error bars the standard error. Asterisk denote statistically significant differences between OTC and control plots at $P < 0.05$.

$df = 1$; $P = 0.45$, and Matarredonda: $F = 0.53$; $df = 1$; $P = 0.47$), and aboveground biomass; neither from vascular (Sumapaz: $X^2 = 2.29$; $df = 1$; $P = 0.13$, and Matarredonda: $F = 0.39$; $df = 1$; $P = 0.55$) nor from non-vascular plants (Sumapaz: $X^2 = 0.09$; $df = 1$; $P = 0.76$, and Matarredonda: $F = 0.03$; $df = 1$; $P = 0.88$). However, the standing necromass has increased slightly in the OTC in comparison to the control plots in Sumapaz ($X^2 = 5.1$; $df = 1$; $P = 0.02$) but not in Matarredonda ($F = 1.5$; $df = 1$; $P = 0.25$; **Figure 6**).

Vegetation Structure Changes (Carbon Sequestration Potential)

After 3 years of warming, we observed some natural changes in the plant community, some related to warming but most of them occurring regardless of the warming treatment, and interestingly those changes were páramo dependent (**Figure 7**). We only observed changes related to warming on the dominance of shrubs, graminoids, and lichens and they were completely different in the two páramos (**Figure 7**). In Matarredonda only lichens have increased as result of warming ($X^2 = 5.14$; $df = 1$; $P = 0.02$), increasing their cover by 2% in the OTC while in the control plots only by 0.36%. In Sumapaz, on the other hand, shrubs have increased in dominance in both the OTC (23%) and the control plots (19%) ($F = 3.9$; $df = 1$; $P = 0.08$); while warming is reducing graminoids losses, although they have decreased in both the OTC (0.9%) and the control plots (6%) the reduction was smaller in the OTC ($X^2 = 5.14$; $df = 1$; $P = 0.02$). For other groups, we did not find evidence that warming was changing their cover; forbs, rosettes, cushion plants, ferns, and club mosses (seedless vascular) and mosses and liverwort (non-vascular plants) have changed in similar ways in all treatments. In Sumapaz, we also observed that the bare soil cover has decreased and the standing necromass is increasing in similar ways in both treatments (Treatment effect: $F = 0.09$; $df = 1$; $P = 0.76$), while in Matarredonda we registered the opposite pattern, a reduction in necromass and increase of bare soil cover in all plots, regardless of treatment ($F = 1.21$; $df = 1$; $P = 0.29$).

Effect Size

Values of effect size indicate no experimental effect of warming on most measured variables (**Figure 8**). The largest effect size was observed for the decay rate of *Laurus nobilis* in Sumapaz

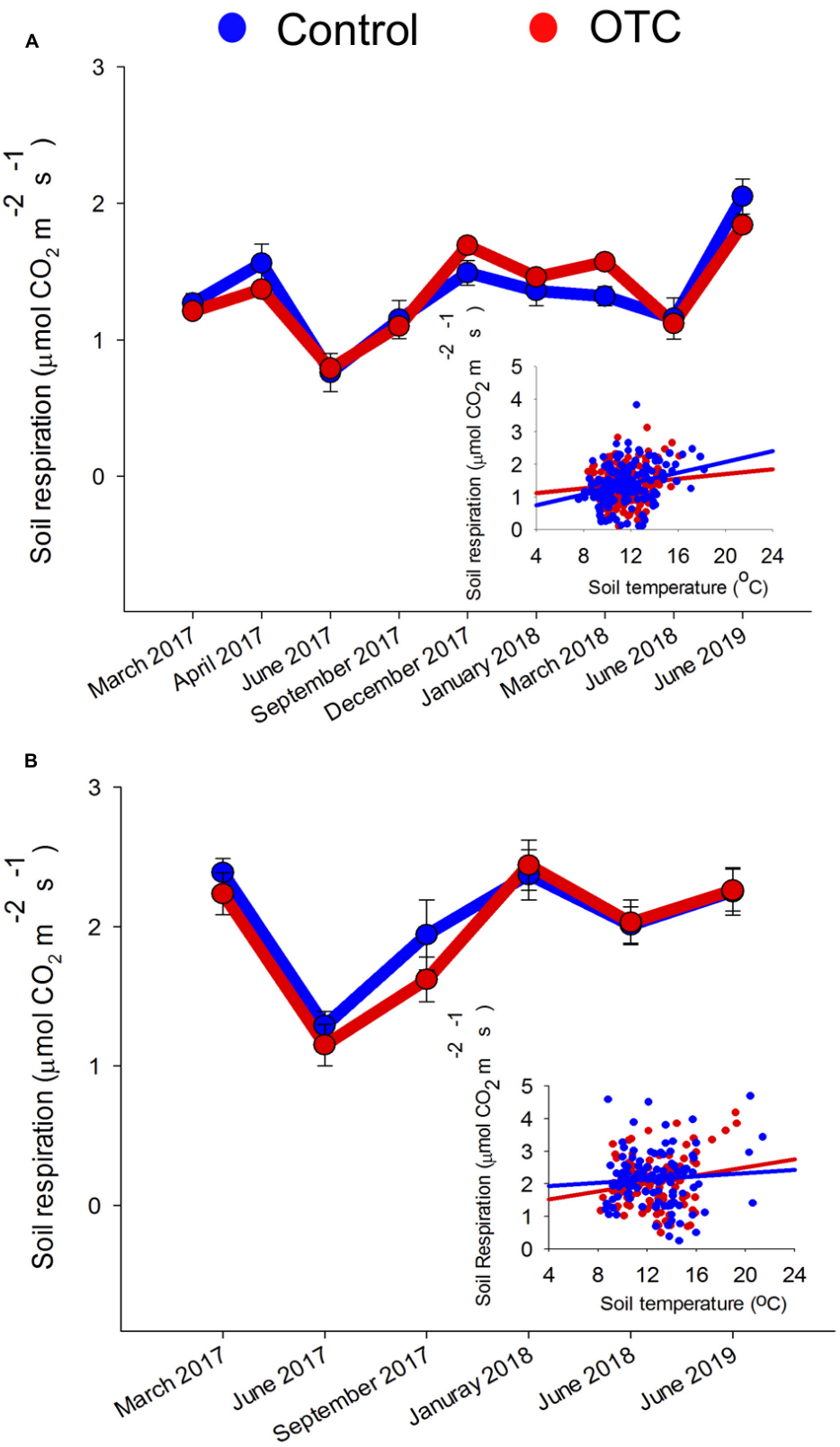


FIGURE 4 | Average CO_2 emissions from soil respiration measured inside the OTC and outside the OTC (Control) at Matarredonda **(A)** and Sumapaz **(B)**. Mean values (points) and standard errors (error bars) are given for each time and warming treatment. The inner plot shows the relationship between each soil respiration measurement and its associated soil temperature and the slope of the regression in the OTC and control plots.

TABLE 2 | Maximum net photosynthetic rate (A_{max}; μmol CO_2 m^{-2} s^{-1}) and dark respiration (R_d; μmol CO_2 m^{-2} s^{-1}), measured in leaves that developed in and out the OTC after 1 year of experimental warming.

Site	Species (years being warmed)	Parameters	OTC	Control	*p value* (n)
Matarredonda	*E. argentea* (1 year)				
		Amax	15.4 ± 1.4	15.8 ± 0.8	0.86 (7)
		Rd	−1.8 ± 0.3	−1.7 ± 0.3	0.37 (7)
	V. pilosa (1 year)				
		Amax	9.2 ± 0.8	11.3 ± 1.1	0.18 (7)
		Rd	−1.5 ± 0.4	−1.1 ± 0.3	0.27 (7)
Sumapaz	*E. grandiflora* (1 year)				
		Amax	10.1 ± 1.6	10.8 ± 0.8	0.76 (8)
		Rd	−1.6 ± 0.3	−1.9 ± 0.3	0.21 (8)
	E. grandiflora (2 years)				
		Amax	11.4 ± 1.1	10.6 ± 0.8	0.41 (9)
		Rd	−1.9 ± 0.5	−2.0 ± 0.3	0.51 (9)
	O. chimboracensis (1 year)				
		Amax	4.3 ± 0.8	5.0 ± 1.0	0.60 (8)
		Rd	−1.2 ± 0.4	−1.6 ± 0.4	0.24 (8)

For E. grandiflora values were taken again after 2 years of warming. Number in parenthesis after P-values indicate the number of blocks were measurement were taken. Numbers presented correspond to the mean ± standard error.

and Matarredonda, −1.96 and −1.27, respectively, and for the decay rate of *Espeletia grandiflora* in Sumapaz, −0.83. In this case, warming was significantly slowing down litter decomposition rate (**Figure 8**). Another large effect size observed was for the soil total N and C in Sumapaz (d = 0.80 and d = 0.76, respectively) where warming was significantly increasing the availability of C and N. Other large and medium effect size but whose confidence intervals were overlapping with 0 that may indicate a developing trend, are a change in graminoids cover observed in Sumapaz (d = 0.81) indicating that warming was slightly increasing graminoids cover, while in Matarredonda warming has the opposite effect (d = −0.64) of decreasing graminoids cover. Medium effect sizes were observed for the change in lichen cover (d = 0.59) and root productivity (d = 0.54) in Matarredonda only.

DISCUSSION

Results from 3 years of *in situ* experimental warming using open-top chambers (OTC) at two páramo sites in Colombia shows that warming has not so far significantly increased soil respiration, litter decomposition, aboveground and belowground biomass, leaf-level photosynthesis, net ecosystem exchange or gross primary productivity, indicating that the páramo has some resilience to warming at least in the short-term. The few changes observed in response to warming were páramo dependent. In Sumapaz, warming increased shrub, and graminoid cover, necromass, and total C and N in the soil while barren soil cover was reduced in the warmed plots. In Matarredonda, warming has resulted in an increase in lichen cover, a slightly higher fine-root productivity, and a small increase in soil bulk density with no other changes. With this result we conclude that the tropical high-elevation ecosystems of the Andes show an homeostatic response to warming at least in the short term and are less sensitive to warming as expected initially given their low temperatures (Lloyd and Taylor, 1994; Carey et al., 2016). However, changes observed in the vegetation suggest that changes could be slow, and that warming could affect those ecosystem processes indirectly and in the long run through changes in the vegetation and soil community. Site-specific differences underscore that the sensitivity of carbon-related processes to global drivers like warming depend on the background climate and ecosystem conditions. Earth system models should be evaluated on those underrepresented regions for an adequate forecasting of future terrestrial carbon-climate feedback (Song et al., 2019).

Application of OTC to Simulate Future Warming Scenarios in the Páramo

Since global climate change was recognized as a major threat, scientists have tried to determine how much carbon can different ecosystems soak up from the atmosphere and how much of the carbon that already resides in the vegetation and soil could be lost as temperatures increases. One realistic way to address these questions is through *in situ* warming experiments that include the vegetation, the soil community and their interactions. Here we show that hexagonal OTCs constructed following ITEX protocol are a suitable method to mimic future warming scenarios and to explore questions related to páramos' functioning in a future warmer world, especially giving the inaccessibility of some of these high-elevation sites with no access to power. Most of the experience using OTC come from temperate ecosystems, including tundra, and temperate alpine systems (Arft et al., 1999; Rustad et al., 2001; Aerts, 2006; Lu et al., 2013). Our knowledge of how warming will affect the cold tropics, where the climatic conditions are dramatically different without marked seasons and where vegetation can grow all year round, is scarce.

A series of warming experiments in the high alpine ecosystems of the tropics in Colombia, Ecuador, and Costa Rica are

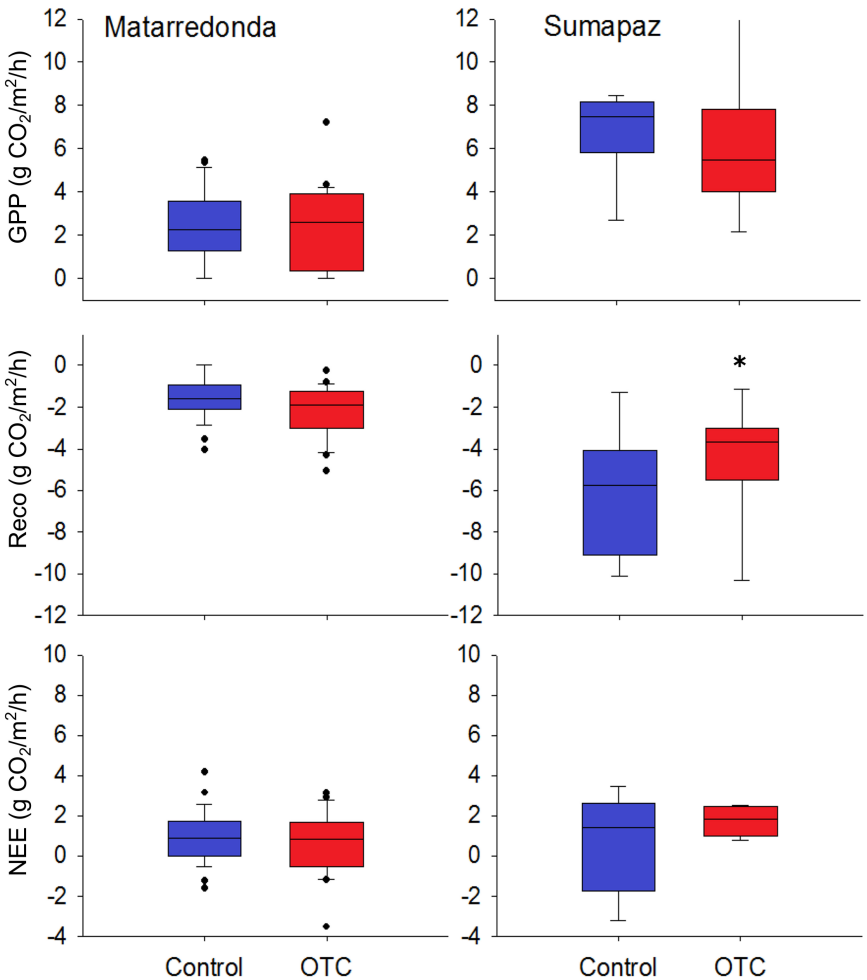


FIGURE 5 | Gross primary production (GPP), autotrophic and heterotrophic respiration (Reco), and net ecosystem exchange (NEE) measured inside the OTC and in the control plots after 3 years of warming. All box plots show the median, 25/75th percentiles within the box, 10/90th percentiles shown within the whiskers and the outlier. Asterisk denote statistically significant differences between OTC and control plots at $P < 0.05$.

running under the IPPEX network (International Network of Páramo and Puna experimental warming sites) with the goal of integrating and fostering research in ecosystem level effects of rising temperatures in the páramo; and in this special issue we present the first reports of two of these *in situ* warming experiments. We show that OTC is also an efficient warming method in the páramo capable of increasing mean air temperature by 1.7°C and soil temperature by 0.1°C, rising the air temperatures within the range predicted in the future warming scenarios (IPCC, 2014). This environmental data comes from 2-years monitoring but from only one site/block, limiting our ability to assess spatial variation in the ability of OTCs to increase temperature. However, this increase by 1.7°C, is within the general range (1.2–3.0°C) reported in many tundra and high-latitudes mountain warming experiments using OTCs (Marion, 1997; Hollister and Webber, 2000; Alatalo et al., 2017) which suggest that it is unlikely that all OTCs are not functioning within those ranges. Moreover, leaf temperature data that comes from all plots and sites consistently shows that OTC are increasing leaf temperature by at least 3°C. On the other hand, changes in soil temperature were smaller but consistent, in line with what is expected with air warming faster than the soil, and with vegetation and soils buffering abrupt temperature variations it is expected that changes in belowground processes will lag changes in aboveground processes (Pregitzer et al., 2000; Radville et al., 2016). All temperature enhancing systems, active or passive, have potential unexpected ecological effects such as: altering temperature extremes, light, moisture, gas concentration, wind, and animal visitants, so they invariably will alter other factors besides just temperature. However, they have provided insightful information on how warming might alter the vegetation and other ecosystem processes and have been used for more than 20 years. Here, by using OTC with the hexagonal more open design, we reduced some of those unwanted effects, for example the large opening in the top allows for direct solar radiation to the plants, allow for some wind flow and excess heat to dissipate through the top by free convection and reduces any significant difference in CO_2

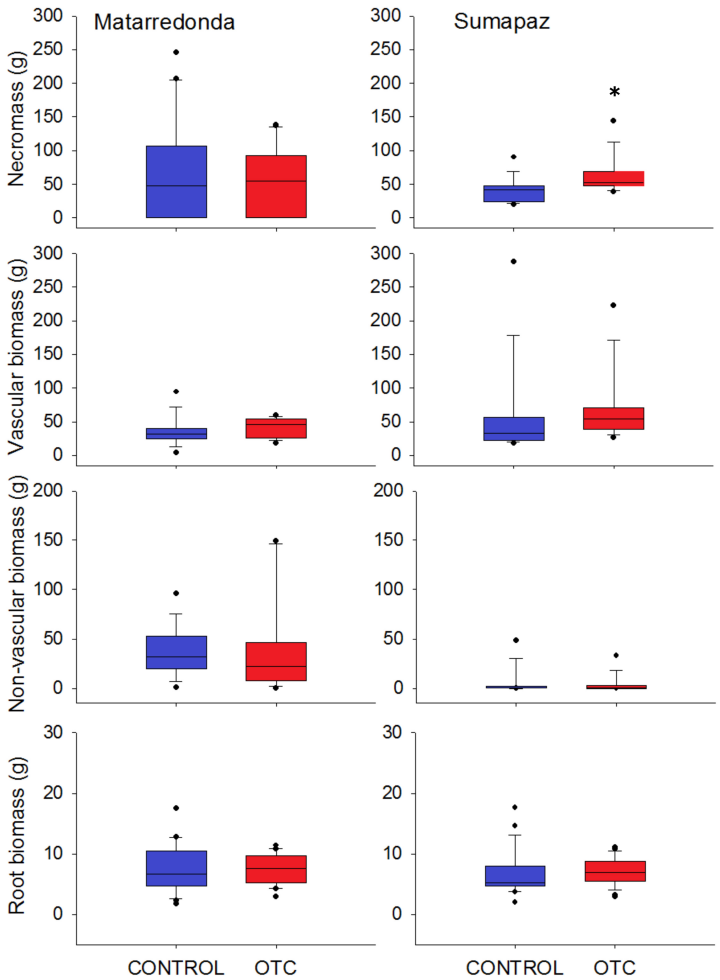


FIGURE 6 | Aboveground biomass separated by vegetation type [vascular, non-vascular plants, and necromass found in a 1 m × 10 cm strip on each plot (n = 10) and below ground biomass collected from 2 soil cores of 15 cm × 5 cm extracted from each plot (n = 20)]. All box plots show the median, 25/75th percentiles within the box, 10/90th percentiles shown within the whiskers and the outlier. Asterisk denote statistically significant differences between OTC and control plots at $P < 0.05$.

concentrations between controls and OTC chambers (Marion, 1997). Minimum air temperatures are sometimes lower in the OTC than in the control plots, but differences are small, and these events are infrequent. The large opening in the roof and the fact that the base is not buried in the ground allows air flow also through the base and the free entry of both flying and crawling insects. We have seen larvae, caterpillars, bees, and bumblebees inside the OTC, which tells us that the walls are not blocking the arrival of animals. Outside of these limitations, we believe that OTC can give us valuable information about how the páramo will respond to rising temperatures in places where it is difficult to get power to use other active heating methods.

Physical, Chemical and Biological Properties of the Soil

Páramos are carbon sinks, storing larger carbon quantities per hectare than tropical forests in their soil organic layer, which can extend from 1 m depth to 9.4 m in some peatlands (Hofstede et al., 2014; Hribljan et al., 2017). How much soil organic carbon (SOC) is stored depends on the climatic conditions and the land use history of the páramo (Gutiérrez et al., 2020) and in Colombia, SOC values reported range between 22 and 338 tons per hectare in the first 30 cm of the soil profile (Gutiérrez et al., 2020). Estimates from Sumapaz indicates values of SOC of 188.2 ± 41.2 ton/ha in the first 25 cm of the soil profile (Montes-Pulido et al., 2017) and values between 119 and 397 tons per hectare (t/ha) in the first 40 cm has been reported in other páramos (Castañeda-Martín and Montes-Pulido, 2017). Given the different sampling depth it is challenging to compare where our data stands in compared to other páramos but our values between 102.1 and 149.4 ton/ha from the first 20 cm of the soil, are within ranges reported for others carbon-rich Colombian and Ecuadorian páramos. Here we do not see evidence of a warming-induced reduction on SOC nor changes in most soil-related processes and properties such as water content, pH, NH_4 and NO_3 and soil respiration. However, in

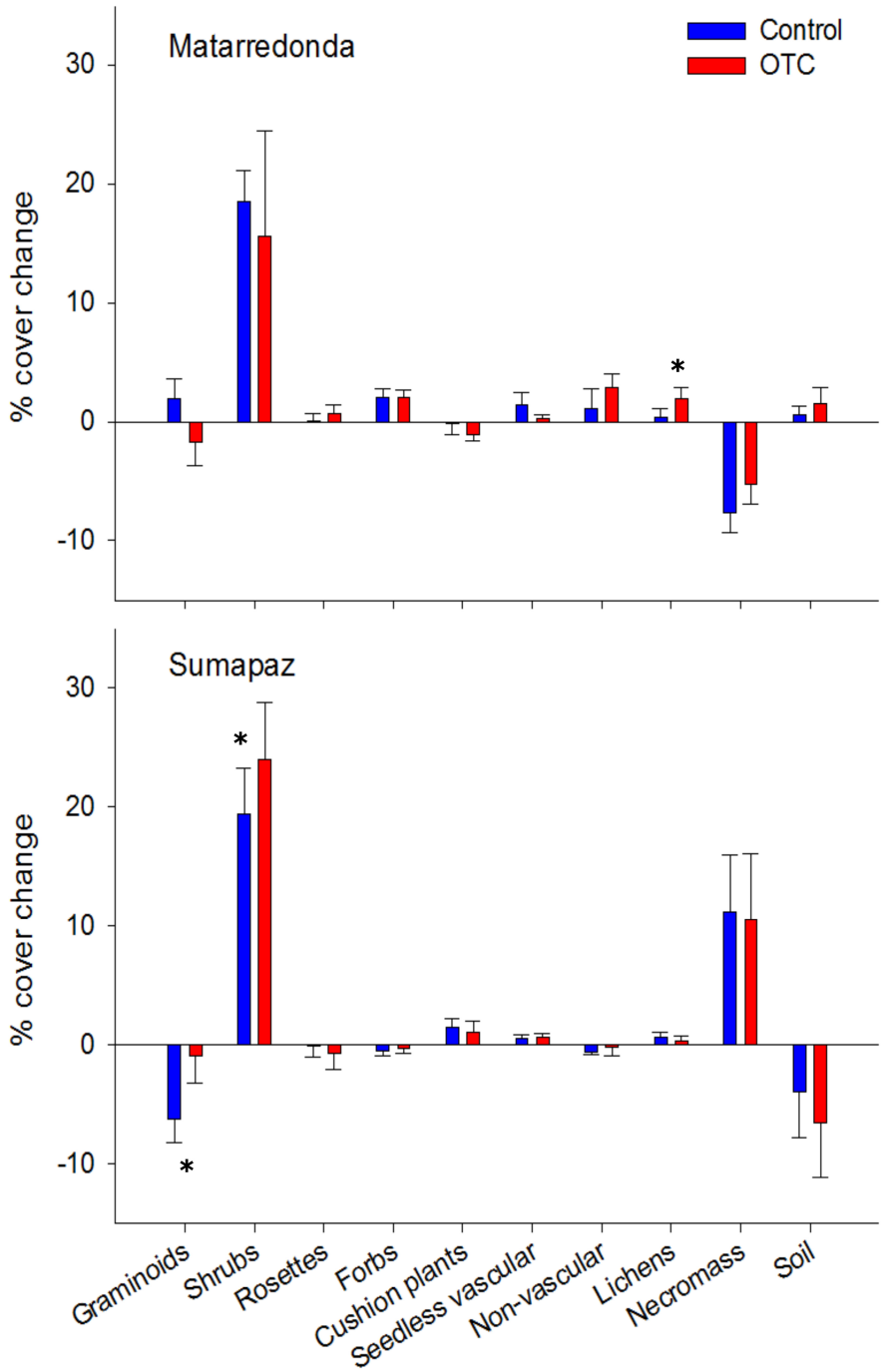


FIGURE 7 | Changes in vegetation cover of the main growth forms in the páramo and of the bare soil and necromass cover after 3 years of warming. We present the percentage cover change that has occurred during 3 years of warming. Positive values denote an increase in cover since the start of the experiment and negative values a reduction in cover. Errors bars represent the standard error. Asterisk denote statistically significant differences between OTC and control plots at $P < 0.05$.

Sumapaz we see an increase in total carbon and total nitrogen in the OTC that suggest an increase in N mineralization rate is taking place in response to warming as reported in other cold biomes (Rustad et al., 2001). Although we have not measured N mineralization rate *per se*, we do have instantaneous measurements of ammonium and nitrate and total N that suggest that mineralization may have increased as result of warming. If warming increases the mineralization rate, it could potentially increase plant productivity. A similar response was observed in a warming experiment in Alaska, where a 3-year lag between the initiation of treatment and ecosystem response was observed, probably related to changes in mineralization and

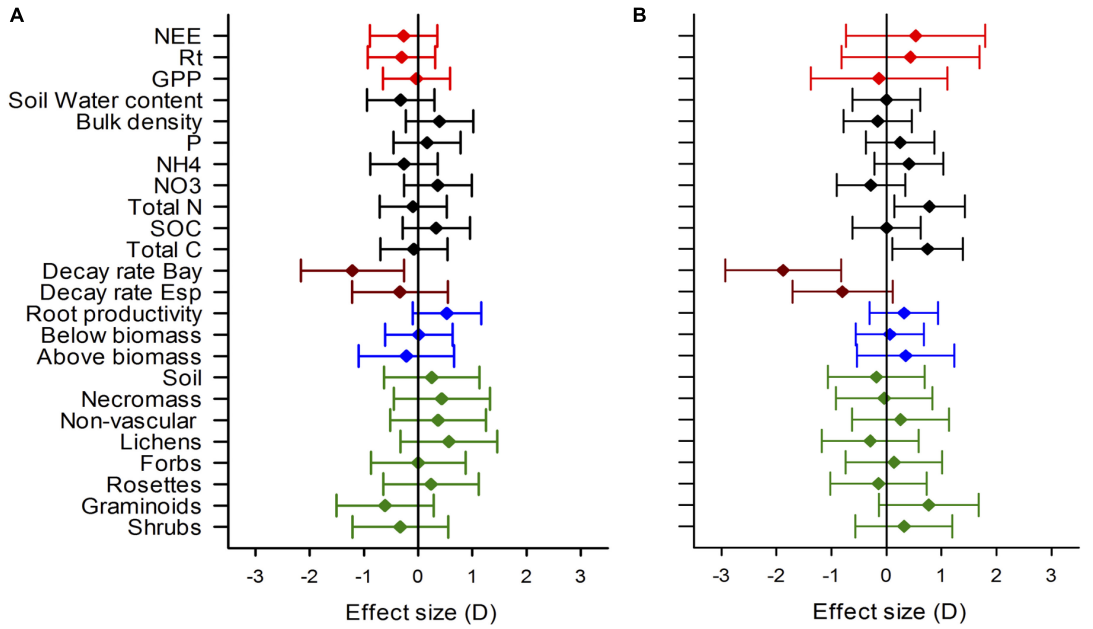


FIGURE 8 | Effects size of experimental warming on CO_2 fluxes (red symbols), soil related properties (black symbols), decomposition rate (brown symbols), productivity and biomass (blue symbols), and on vegetation cover (green symbols) in Matarredonda **(A)** and Sumapaz **(B)**. Diamond symbols show the warming effect size and the error lines the 95% CI. When 95% CI do not overlap with zero the warming treatment has a significant effect, positive values indicate a positive effect of warming and negative values a negative effect of warming. The effect size for most variables are for measurements taken in June 2019 after 3 years of the establishments of the experiment, except for decomposition and root productivity that were taken during the first 2 years of warming. Decay rate "Bay" refers to the estimate for *Laurus nobilis* and decay rate "Esp" for the estimates for *Espeletia grandiflora*.

an increase in nutrient availability (Chapin et al., 1995). If the trend holds and N increases trough time, any possible future negative effect of warming in decomposition and soil respiration could be offset if warming increases productivity, which in the unseasonal tropics with a continuous growing season, could be quite high. Moreover, the differential response among páramo sites indicate that páramo response to warming would depend on the site-specific conditions of the páramo; C and N pools and turnover rate, soil water content and precipitation regime, the disturbance history, the chemical composition of plants, the longevity, and population turnover rates of dominant species (Shaver et al., 2000).

Effect of Warming in Processes Related to CO_2 Emissions

Decomposition Rate

Plant litter decomposition is a key component of the global carbon budget (Couteaux et al., 1995; Aerts, 1997; Robinson, 2002) contributing approximately 70% to the total annual carbon flux (Raich and Schlesinger, 1992) and acting as critical feedback to climate change by simultaneously influencing CO_2 flux from the soil and productivity. Thus, any changes in factors that control litter decomposition rates may have significant repercussions for the global carbon budget. Given the sensitivity of decomposition to temperature, especially in cold biomes, it has been hypothesized that global warming will lead to increased litter decomposition rates, both through direct temperature effects and through indirect effects on litter quality and soil organisms. Here we did not find evidence that warming will increase litter decomposition and the loss of carbon from this process in the páramo. Evidence from different warming experiments in other cold biomes is still inconclusive. The analysis by Aerts (2006) of 34 warming experiments showed that warming resulted in slightly increased decomposition rates only when heating lamps were used, but in OTC decomposition rates was consistently reduced as we show here. OTC walls might be reducing UV light and therefore reduce decomposition. Alternatively, OTC may lead to stronger drought than heating lamps, and decomposition is moisture-limited, as suggested by Aerts (2006). This last possibility is further supported by the meta-analysis by Rustad et al. (2001), which shows that soil moisture was significantly lower in the heated plots than in the control plot across 14 experimental warming sites. If this is the case, global warming will increase litter decomposition rates only if there is sufficient soil moisture. For the Northern part of the high tropical Andes, temperature and precipitation are expected to change in the future (Urrutia and Vuille, 2009; Buytaert et al., 2011), and we could expect an increase in decomposition with the concomitant higher contribution to the total annual carbon fluxes (Salinas et al., 2011; Gutiérrez-Salazar and Medrano-Vizcaíno, 2019) in those future scenarios. However, in the current conditions, páramos' decomposition rate values are extremely low compared to what has been reported for other tropical ecosystems (Cusack et al., 2009; Powers et al., 2009). The decay rate for *Espeletia*'s substrate was between 0.30 and 0.39 year^{-1},

and for the bay leaf was between 0.35 and 0.46 year^{-1}. With this low decomposition rate, 1 g of litter has lost <50% of its mass after 2 years in the field, which strongly contrasts with the values reported for other tropical forests where values of decay rates ranged from 0.47 to 15.10 year^{-1} resulting in 95% of mass lost in just 1 year (Powers et al., 2009). The low decomposition rate in the páramo is not only related to the cold temperature of the páramo but also to the conditions of the soil, the clay-loamy soil texture, the high acidity that also induces a high content of aluminum, all factors that inhibit the degradation of the organic matter by the soil community (Gonzalez-Prieto et al., 1996; Bottner et al., 2006; Hofstede et al., 2014) so the effect of warming could be more intricate and deserves future evaluation.

Soil Respiration

Soil respiration is one of the main sources of CO_2 loss to the atmosphere, representing 40–90% of the whole CO_2 production in forest ecosystems (Schlesinger and Andrews, 2000), and warming is hypothesized to increase rates of soil respiration, potentially stimulating further intensification in global temperatures. Although warming has been shown to stimulate soil respiration in the tundra and other northern cold ecosystems (Melillo et al., 2002; McGuire et al., 2009; Conant et al., 2011; Chen et al., 2015) and in a tropical forest (Nottingham et al., 2020) we have not found evidence that the same is happening in the páramo. Our result contrasts with what should be expected in colder climates, which are supposed to be more responsive to increased ambient temperature than warmer climates (Lloyd and Taylor, 1994; Carey et al., 2016), but in agreement with the hypothesis that predicts that warming should cause only modest carbon loss from tropical soils relative to those at higher latitudes (Davidson and Janssens, 2006; Carey et al., 2016). This is not the first report of a neutral response to warming. Some studies have shown even negative responses to warming, often attributed to moisture limitation (Suseela et al., 2012), to the depletion of labile C pools (Melillo et al., 2002; Kirschbaum, 2004; Hartley et al., 2007) or shifts in the microbial composition and their physiological response (Luo et al., 2001; Crowther and Bradford, 2013).

While soil respiration in incubated soil from the páramo suggested a high sensitivity of CO2 emissions to changes in soil temperature (Curiel Yuste et al., 2017), our findings from this *in situ* long-term warming experiment indicates that processes are more complicated once the local vegetation and soil community become part of the story. It is clear that the vegetation is buffering changes in soil temperature, and the soil system *per se* is also buffering against air temperature fluctuations. Consequently, the soil system seems not to react to the 0.1°C net increase in temperature brought on by the OTC. The soil metabolism might have undergone a process of acclimation to the small increase in temperature (Allison et al., 2010; Wei et al., 2014) under OTC's that reflect a homeostatic behavior of the system. Another possibility is that this apparent acclimation reflects depletion of the readily decomposable substrate under warming (Kirschbaum, 2004), but our soil organic carbon data suggest otherwise. We have no evidence that the microbial community has changed during the first year of the experiment, where the bacterial, archaeal, and fungal communities measured through amplicon sequencing of the 16S rRNA and ITS1 rRNA gene regions remains similar in the OTC and control plots (Gallery and Lasso, 2017). However, given that páramo plants have shown surprisingly high heat tolerance (Leon-Garcia and Lasso, 2019), microbes may also be adapted to deal with the dramatic daily variation in temperature typical of the páramo, where temperatures can range from −2 to 30°C in one single day. Consequently, we do see a response to seasonal temperature fluctuation, but we do not see a strong response to the OTC's warming. Research on the soil microbial communities' thermal adaptation is still needed for the tropics, especially for the cold tropics (Bradford et al., 2008; Curiel Yuste et al., 2010; Wood et al., 2019).

Effect of Warming in Processes Related to CO_2 Capture and Net Ecosystem Exchange

The net effects of future warming on carbon fluxes in the páramo would depend on whether warming could boost up photosynthesis and primary productivity and offset any warming-induced CO_2 release thorough soil autotrophic and heterotrophic respiration. We did not found evidence that the higher temperatures are inhibiting species or that warming is modifying their photosynthetic machinery, all species had similar leaf level maximum photosynthetic rate and respiration in the OTC and the control plots. Consistent with this observation, we also saw that warming is not strongly affecting the production of below- and above-ground biomass, and we only see a trend in warming-induced fine-root production, which is a key component of carbon sequestration in soil (Matamala et al., 2003), accounting for 50–90% of primary production (Ruess et al., 2003; Steinaker and Wilson, 2008). So, warming could increase carbon sequestration through root production. Likewise, total uptake of carbon at the community level through photosynthesis (GPP) and total carbon dioxide release from autotrophic and heterotrophic respiration were indistinguishable between OTC and control plots in Matarredonda, while in Sumapaz we found that less carbon is being released from autotrophic and heterotrophic respiration in the OTC in comparison to the control plots. In both sites the net ecosystem exchange (NEE) or final carbon dioxide effluxes from the ecosystem were not different between the OTC and control plots. Our values of NEE range between 1.14 and 0.87 g CO_2 m^2/h and are slightly larger than the values reported for an undisturbed páramo in Coca National Park in Northern Ecuador with NEE = 0.69 g CO_2 m^2/h, but slightly smaller than values found in a grazed páramo in Antisana Ecuador with NEE = 1.25 g CO_2 m^2/h (Sánchez et al., 2017). Based on these data, which does not include night time respiration, gross primary productivity still exceeded respiration by around 17–30% in our sites and by 43–51% in Ecuador (Sánchez et al., 2017) and suggest that those páramos are still working as carbon sinks, absorbing more carbon than liberating it. However, the only study using eddy covariance technique following diurnal and nocturnal exchanges in the páramos, found that the páramo at Zhurucay in Northern

Ecuador behaved as a source of CO_2 most of the time and only 34% of the time was acting as a weak carbon sink; respiration was exceeding gross primary productivity by 6–11% (Carrillo-Rojas et al., 2018). Until more eddy covariance towers have been installed in other páramos we would not know to what extent páramos have lost their potential for carbon sequestration or if these results are specific to that Ecuadorian páramo.

Vegetation Structure Changes (Carbon Sequestration Potential)

Vegetation responses to warming were completely different between the two páramo sites, in Sumapaz, shrubs, and graminoids increased in response to warming while, in Matarredonda, we observed an increase in lichen cover and a slight increase (not significant) in moss cover. While we need to wait to see the long-term effect of warming in the páramo, these first 3 years of experimental warming indicate that warming has the potential to change the structure and function of the plant communities by increasing the dominance of shrubs, graminoids, and lichens. These changes could have several implications. For one, an increase in the dominance of woody shrubs has the potential to alter the C-sequestration of the páramo vegetation over the long-term if páramo's woody shrubs have greater C-sequestration capacity than other growth forms as found in the tundra (Shaver et al., 1998), and these changes could have in turn a positive feedback on the climate (Shaver and Jonasson, 1999; Shaver et al., 2000; Walker et al., 2006). This response is congruent with observations for the tundra where warming increased shrub cover (Sturm et al., 2001; Walker et al., 2006; Molau, 2010). The increase in woody shrubs in one of the páramo sites, together with the accumulation of standing dead necromass in the OTC, a response also registered in some warming experiment in the tundra (Hollister et al., 2005), implies a larger residence time for carbon in the vegetation. In Matarredonda, however, lichens have increased because of warming, if this trend is confirmed, it may imply a reduction in the complexity of the vegetation and on the carbon storage potential. The increase in lichens contrasts with the results of warming experiments in the tundra where warming resulted in a decreased cover of mosses and lichens (Graglia et al., 2001; Hollister et al., 2005; Jónsdóttir et al., 2005; Walker et al., 2006). Lichen and mosses in the páramo may not be particularly sensitive to warming and N_2 fixation may be maintained even in a warmer climate, where both groups might account for up to 50% of total N inputs as shown in the tundra cold biomes (Rousk and Michelsen, 2017). The greater stability of the vegetation observed in Matarredonda in response to warming could be connected to the larger presence of mosses and *Sphagnum* in this site, a group that buffered and stabilized the vegetation response to warming also in the Arctic tundra (Hollister et al., 2005; Klanderud, 2008; Keuper et al., 2011). Longer warming times, extended monitoring and more warming sites would be needed for a good prediction of long-term changes in the páramo vegetation in response to warming as it has been shown with a 20 years-long vegetation monitoring in a warming experiment in the tundra (Elmendorf et al., 2012). To fully understand how the vegetation changes connects with the carbon sequestration potential of the páramo we need more data on the carbon content and carbon sequestration potential of different growth forms in the páramo.

CONCLUSION

Understanding the sensitivity of páramo vegetation to climate warming is critical to forecasting future biodiversity and vegetation feedbacks to climate. Our results suggest that the carbon sequestration potential of the páramo will not be reduced by warming in the short term since none of the processes that could increase carbon release, neither soil or plant respiration nor the rate of decomposition are increasing in the OTC. However, such results must be interpreted cautiously because the large differences among sites suggest that not all páramos are going to respond in the same way. Moreover, there could be a tipping point after which the changes become irreversible and we don't know what this tipping point is going to be for the páramos. Here we are manipulating a single factor, temperature, and we are not accounting for all the complex interactions between environmental factors that limit growth and decomposition in the páramo, which are likely also constrained by water and/or nutrients. Long-term studies in different páramos will be crucial for resolving how all these factors interact with warming and will affect the vegetation and ecosystem services.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EL, JC, and RG conceived the ideas and designed methodology. EL, RG, JC, PM-A, CG-L, MC, IL-G, AA-P, and LA collected the data. EL and JC analyzed the data. EL led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.615006/full#supplementary-material

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Tolerance of Warmer Temperatures Does Not Confer Resilience to Heatwaves in an Alpine Herb

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Climate change is generating both sustained trends in average temperatures and higher frequency and intensity of extreme events. This poses a serious threat to biodiversity, especially in vulnerable environments, like alpine systems. Phenotypic plasticity is considered to be an adaptive mechanism to cope with climate change *in situ*, yet studies of the plastic responses of alpine plants to high temperature stress are scarce. Future weather extremes will occur against a background of warmer temperatures, but we do not know whether acclimation to warmer average temperatures confers tolerance to extreme heatwaves. Nor do we know whether populations on an elevational gradient differ in their tolerance or plasticity in response to warming and heatwave events. We investigated the responses of a suite of functional traits of an endemic Australian alpine herb, *Wahlenbergia ceracea*, to combinations of predicted future (warmer) temperatures and (relative) heatwaves. We also tested whether responses differed between high- vs. low-elevation populations. When grown under warmer temperatures, *W. ceracea* plants showed signs of acclimation by means of higher thermal tolerance (T_{crit}, T_{50}, and T_{max}). They also invested more in flower production, despite showing a concurrent reduction in photosynthetic efficiency (F_v/F_m) and suppression of seed production. Heatwaves reduced both photosynthetic efficiency and longevity. However, we found no evidence that acclimation to warmer temperatures conferred tolerance of the photosynthetic machinery to heatwaves. Instead, when exposed to heatwaves following warmer growth temperatures, plants had lower photosynthetic efficiency and underwent a severe reduction in seed production. High- and low-elevation populations and families exhibited limited genetic variation in trait means and plasticity in response to temperature. We conclude that *W. ceracea* shows some capacity to acclimate to warming conditions but there is no evidence that tolerance of warmer temperatures confers any resilience to heatwaves.

Keywords: phenotypic plasticity, climate change, heatwave, alpine, elevation, adaptation, thermal tolerance, fitness

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INTRODUCTION

Global climate change is threatening biodiversity and increasing extinction risk across diverse taxa and natural systems (Williams et al., 2008). Climate change is manifested as both gradual rises in average temperatures and increased frequency of extreme weather events such as heatwaves (rare and intense climatic conditions; Harris et al., 2018). A heatwave is an extreme weather event in

which air temperatures are substantially elevated above the mean for several consecutive days; these events are predicted to continue to increase in frequency, intensity (+3°C) and duration into the future (Cowan et al., 2014). Extreme events in concert with the sustained increase of mean temperature will likely affect species abundance and persistence, as well as interactions, and thus may affect community and ecosystem structure, function and food webs (Grimm et al., 2013; Wernberg et al., 2013; Kortsch et al., 2015; Harris et al., 2018).

Extreme events may exert stronger selection pressures on natural populations of plants and animals than more gradual, sustained changes in mean climatic parameters (Reyer et al., 2013; Kingsolver and Buckley, 2017). Heatwaves are associated with decreased biomass production and survival in plants (French et al., 2017; 2019). Previous studies have shown that plants can withstand heatwave events (Hoover et al., 2014; Poirier et al., 2012), but are negatively affected when heatwaves are concurrent with other stressors such as drought (De Boeck et al., 2016). Although heatwaves in future will occur in conjunction with warmer mean temperatures, to the best of our knowledge, the interaction between gradual warming and extreme heatwaves has only been investigated in two studies. Aspinwall et al. (2019) found that *Eucalyptus* species were more susceptible to heatwaves if grown under warmer temperatures, while Drake et al. (2018) found no differences in tolerance of heatwaves when *Eucalyptus parramattensis* was grown in warmer environments. Thus, to date, relatively little is known about the combined impact of exposure to both rising mean temperature and extreme events, or the extent to which exposure to the former might confer some form of resilience to the latter.

The effect of climate change is also expected to be stronger in vulnerable environments, such as alpine ecosystems (i.e., the portion of a mountain above the treeline), where mean air temperatures have increased twice as fast as lowland environments during the last century (Gobiet et al., 2014). Species endemic to alpine environments are further restricted by physical limitations on dispersal (Lara-Romero et al., 2014; Morgan and Venn, 2017), such that high elevation species may be increasingly confined to ever higher mountain peaks. These species are predicted to be progressively replaced at lower elevations by invasive or more competitive species from warmer sites as temperatures rise, and in some places species composition changes have already occurred (Pauli et al., 2012; French et al., 2017; Steinbauer et al., 2018). Further, to our knowledge, no study has looked at the interactions between warmer temperatures and heatwaves in alpine plants.

Within alpine environments, elevation is a key driver of climatic differences. While microclimatic conditions can vary at the same elevations because they are modulated by interactions of topographic and abiotic factors (Körner, 2003), climatic trends can be identified along elevation gradients. For example, solar radiation under a clear sky increases with elevation, while air temperature, atmospheric pressure, mineralization processes in the soil, and plant evapotranspiration all decrease (Körner, 2003, 2007). Snow cover lasts for longer at higher elevations, resulting in shorter growing seasons (spring and summer; Körner, 2003). It is therefore plausible that intraspecific phenotypic differentiation and adaptation occur between relatively high and relatively low elevation populations of alpine species (from now on, high- and low-elevation populations). Indeed, intraspecific trait differentiation along altitudinal gradients has been found in several plant species in morphology, phenology and growth (Vitasse et al., 2014; Nicotra et al., 2015; Halbritter et al., 2018). However, it is still not clear whether high- and low-elevation populations, adapted to their local climate, may also respond differently to the ongoing climate warming (Frei et al., 2014a; Vitasse et al., 2014; Hernández-Fuentes et al., 2015; Nicotra et al., 2015) or to weather extremes such as heatwaves.

Despite lower air temperatures at high elevations, alpine plants are frequently exposed to heat stress. Air and leaf temperatures are often decoupled: leaf temperatures can be up to 20°C above ambient temperatures on bright and warm days (Salisbury and Spomer, 1964). Higher solar radiation at high elevation exacerbates the decoupling because of passive warming by radiative heat, especially for metabolically active leaves (Körner, 2003). The leaf-air temperature decoupling is considered an adaptation to low air temperatures, since some typical alpine plant growth forms (prostrate and cushion) work as heat traps (Salisbury and Spomer, 1964; Körner, 2003; Buchner et al., 2015), but it may also expose plants to overheating (Buchner et al., 2015). Despite the frequency of heat stress events in alpine environments and the alarming threat imposed by climate change (both of which are accelerating), we currently know little about the ecophysiological responses of alpine plants to heat stress (Geange et al., 2021). To some extent, intraspecific variation in functional traits and plasticity over elevation gradients may alleviate the impact of climate change by providing standing genetic variation upon which selection can operate. Therefore, understanding how local adaptation influences plant responses to climate change is important to accurately predict resilience of alpine plants and the fate of alpine environments.

Phenotypic plasticity, defined as the capacity of individuals (or genotypes) to produce different phenotypes under different environments (Bradshaw, 1965), is commonly assumed to be an adaptive mechanism to cope with changing environmental conditions and persist *in situ*. Plastic changes have been documented for several plant functional traits under warming conditions (e.g., Atkin et al., 2006; Nicotra et al., 2008; Cochrane et al., 2015). Flowering time is frequently observed to shift to earlier in spring for most species under warmer temperatures (Frei et al., 2014a,b; Gugger et al., 2015). When water is not limiting, many plant species may benefit from mildly higher average growing temperature in terms of increasing above-ground biomass, which is constrained at low temperatures (De Frenne et al., 2011; Frei et al., 2014b; Rodríguez et al., 2015). The effect of warmer temperatures on plant reproduction (fitness) is highly species-specific, where some species increase flower production with higher temperatures (Frei et al., 2014a), others decrease (Scheepens and Stöcklin, 2013), or are unaffected (Choi et al., 2019). Seed production is less commonly used as fitness proxy in response to thermal stress, but studies on agricultural plants demonstrate that warming or heat stress during flowering strongly reduces seed production and individual seed weight (Young et al., 2004; Hampton et al., 2013; Alsajri et al., 2020),

which can negatively affect seed viability and germination (Ledgard and Cath, 1983; Veselá et al., 2020; Ge et al., 2020).

The physiological status of plants, as well as the efficiency and rate of photosynthesis, can be measured using chlorophyll fluorescence that takes advantage of the light re-emitted as fluorescence to estimate the amount of light used in photosynthetic reactions, given that the two processes are antagonistic (Maxwell and Johnson, 2000). The basal chlorophyll fluorescence (F_0, measured in the absence of photosynthetic light) measured while leaves are gradually heated generates temperature-fluorescence curves (T-F_0 curves) to investigate thermal tolerance of plants. The critical temperature (T_{crit}), corresponding to the temperature where a spike in F_0 occurs, and T_{50} and T_{max}, respectively the temperature at 50 and 100% relative F_0, are thermal metrics associated with damage to the photosynthetic machinery and represent upper thermal limits of plant tolerance (Neuner and Pramsohler, 2006; Ducruet et al., 2007; Ilík et al., 2007). Thermal safety margins (the difference between T_{crit} and maximum temperatures during treatments) can then be used to infer species' vulnerability to climate change (Leon-Garcia and Lasso, 2019) because they identify temperature ranges that can significantly reduce species performance.

Plastic increases in T_{crit} are associated with acclimation to higher temperatures (higher thermal tolerance) in many plant species (Downton et al., 1984; Weng and Lai, 2005; Buchner et al., 2017). In some cases, increases in T_{crit} and T_{max} can be induced after just a few minutes under high temperatures (Havaux, 1993), potentially giving resilience to short term fluctuations. Such plasticity is not universal; indeed, while for many plant species from different environments T_{crit} can rise by up to 9°C under warmer temperatures (Downton et al., 1984; Buchner et al., 2017), for others changes in T_{crit} are not observed, probably meaning that the plant is already operating close to its thermal limits in its natural environment (Krause et al., 2010, 2013). To our knowledge, no study has investigated the effect of a simulated heatwave on T-F_0 parameters and it is still not clear whether the potential acclimation and plasticity in T_{crit} or T_{max} that some species demonstrate under warmer growth temperatures will enable them to cope better with heatwaves, especially in ecosystems that are most vulnerable to climate warming.

In this study, we examined plastic responses to climate warming and extreme heatwaves of an Australian endemic alpine herb, *Wahlenbergia ceracea*, from an elevation gradient. In Australia, the mean temperature has risen by 0.5°C since 1970 and is projected to increase up to 5°C by 2,100 (Harris et al., 2018). We grew plants in conditions mimicking historical and future (projected for 2,100) temperatures of the Australian Alps (Harris et al., 2016). During this period, half the plants in each treatment were also exposed to a heatwave (+5°C above their temperature treatment for 5 days). We measured a range of functional traits pertaining to the biology and ecology of the species, and used these measures to: (1) characterize plastic responses of *W. ceracea* families in response to warming and/or extreme heatwaves; (2) test for differences between high- and low-elevation populations in their plastic responses to temperature and heatwaves; and (3) evaluate whether there is variation in the extent of plastic responses between families (intraspecific variation in plasticity).

MATERIALS AND METHODS

Study Species

Wahlenbergia ceracea Lothian (Campanulaceae; 'waxy bluebell') (**Figure 1**) is a short-lived perennial/biennial herb that grows in moist sites on high elevations in alpine and sub-alpine environments (Nicotra et al., 2015). This species is an Australian endemic that grows over a wide elevation gradient (1,550–2,100 m; Atlas of Living Australia, 2020) in Australian alpine regions. This species is hermaphroditic and facultatively autogamous, however, rates of autogamy in the wild are unknown. *W. ceracea* can be 10–60 cm tall and usually grows among shrubs and grasses in grassy herbfields above the treeline or in inverted treeline valleys and floodplains. From a previous investigation by Nicotra et al. (2015), *W. ceracea* plants grown at relatively warm temperatures (30/20°C day/night) emerge earlier, grow taller, and produce more leaves and capsules than individuals grown at relatively cool temperatures (20/10°C day/night). Individuals from lower elevations also express higher levels of plasticity compared to higher elevation conspecifics (Nicotra et al., 2015).

Parental (F0) Seed Collection and Crossing to Generate F1

Seeds of *W. ceracea* were collected directly from capsules of plants in the field during two spring collection trips in mid-March 2015 and mid-April 2016, to form a base (F0) generation. The natural elevational range of the species was divided in two to select seeds from relatively low (1,590–1,765 m a.s.l.) to high (1,837–2,095 m a.s.l.) elevation populations, from Charlotte's Pass up to Mount Kosciuszko (NSW, Australia), from plants that were growing at least 50 m apart. Individuals from these two elevation ranges have small, but significant genetic differentiation (Nicotra et al., 2015).

To minimize maternal effects and to ensure that each individual resulted from an outcross rather than self-cross of the mother plant, we generated an F1 generation by crossing F0 individuals. The F0 seeds (five seeds per pot from 100 plants, then reduced to one plant per pot) were grown from 20th July 2016 in soil in the glasshouse under controlled conditions of 20/15°C day/night, under natural photoperiod and then lights as natural daylight decreased. To maintain differences associated with elevation, we crossed F0 plants that on average differed in elevation by 21 m (minimum difference = 0 m, maximum difference = 48 m) but were not less than 50 m distance apart (altitudes where parental F0 seeds were collected and spatial distance of each parental pairs are presented in **Supplementary Table 1**). Parents of each family were on average 2.33 km apart. There were 65 F0 crosses that produced F1 seeds, and 52 of them were successful with at least 500 F1 seeds produced. Below, we refer to each cross as a family. From these, we chose the 15 families from the highest elevation crosses, and 15 from the lowest elevation crosses; these F1 families constitute full siblings.

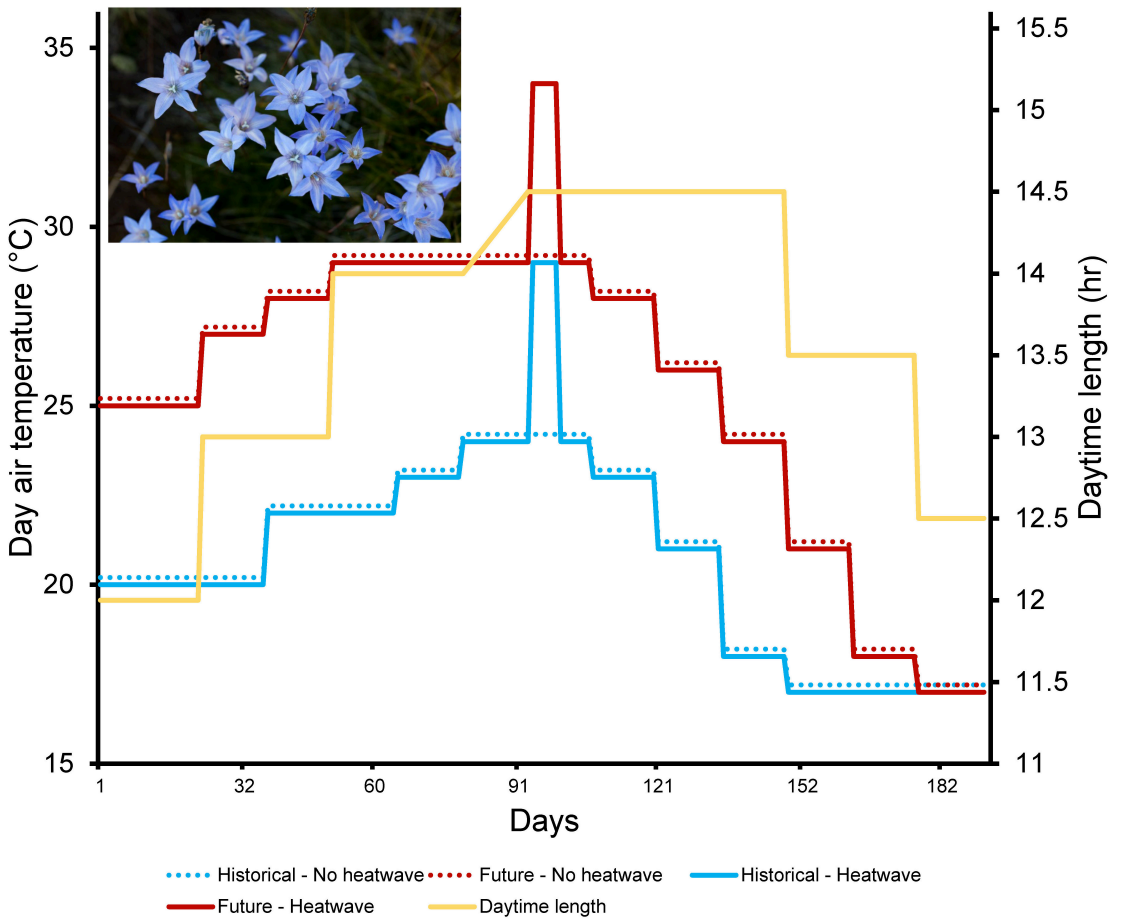


FIGURE 1 | Day temperatures and photoperiod across the duration of the experiment. Toward the middle of summer individuals were exposed to a 5-day heatwave treatment (solid lines), while control individuals remained under constant conditions of a historical and future scenario (dotted lines). Light blue = historical climate scenario; red = future climate scenario; yellow = photoperiod. To ease the view of the lines, which would be otherwise overlapping, 0.2°C were added to the groups "Historical – No heatwave" and "Future – No heatwave" only for the purposes of drawing the graph. Inset: *Wahlenbergia ceracea*. Credit: T Hayashi, February 2015, Kosciuszko National Park.

Plant Materials, Growth Conditions and Temperature Treatments

We sowed seeds of each family in seed raising mix in 50 mm diameter pots (three seeds per pot) that were then placed in two growth chambers (Phoenix room; Phoenix Research, Envirotec Pty. Ltd.; SA, Australia) at 25/15°C day/night to encourage early growth (12/12 h day/night length; photosynthetically active photon flux density (PPFD) = 330 μmol s^{-1} m^{-2}; RH = 70 ± 10%). Four to six weeks after germination, when the diameter of the rosette (tip-to-tip between the largest two leaves) was at least 15 mm, we selected 12 seedlings from each family and transplanted them into larger pots (80 mm × 80 mm). For each family, seedlings of similar size were paired and then randomly allocated: one of each pair to each of two temperature treatments, which was repeated until all 12 seedlings in a family had been allocated to the two temperature treatments (2 elevation × 15 families × 2 temperatures × 6 replicate individuals = 360 plants). Within each chamber, we randomly assigned plants to one of three blocks, corresponding to different positions in the chamber. Each block contained two trays with one replicate of each family (five rows × six columns in each tray). Plants were given 7 days to recover from transplant shock before the historical and future climate scenario conditions were applied: one growth chamber with the 'historical' climate, and one growth chamber with the 'future' climate. Day 1 of the experiment is taken as the day on which the temperature treatments were applied.

Treatment temperatures (**Supplementary Table 2** and **Figure 1**) were based on projections made using the Coupled Model Intercomparison Project Phase 6 (CMIP6) using the worst-case climate scenario (Representative Concentration Pathways, RCP8.5; Harris et al., 2014). The *historical scenario* treatment corresponds to the base period in the projection (1960–1970), while the *future scenario* treatment corresponds to the projected period (2090–2100). We call the baseline period 'historical' because it reflects the recent climate to which natural systems are adapted to according to the World Meteorological Organization (WMO), although some changes in temperature have clearly happened since then

(Harris et al., 2014). We averaged the base and projected maximum and minimum 10-year temperatures in 15-day intervals. We then corrected day temperatures to account for the difference between air and leaf temperature (see Salisbury and Spomer, 1964 and **Supplementary Material**) thus generating inside the chambers leaf temperatures experienced in the field. Temperature conditions in the growth chambers were then changed fortnightly, based on the averaged maximum (day) and minimum (night) historical base and future projected temperatures. Projected conditions resulted in higher temperatures (summer maximum day temperature: historical scenario = 24°C; future scenario = 29°C) and in a longer growing season (summer peak temperatures lasted 30 days under historical and 60 days under future scenario). Other conditions were held constant for both chambers (relative humidity = 80%; PPFD = 330 μmol s^{-1} m^{-2}). The focus of this experiment was plastic responses to temperature in isolation, therefore plants were watered to saturation daily, twice per day during the seedling stage and the warmest weeks, under all treatments to ensure they were not water stressed. The experiment started with temperatures mimicking late spring (November) and finished with temperatures mimicking early autumn (early April). To induce senescence in the plants, we also changed day length in concert with each temperature change (**Supplementary Table 2** and **Figure 1**), according to the relative period of the year. We note that since the growth chambers could not cope with large differences between day and night temperatures (which are typical of the Australian climate) for the future scenario, the night temperature was held at 20°C during the warmest weeks (**Supplementary Table 2** and **Figure 1**). Selected night temperatures (**Supplementary Table 2**) depended on the chambers capacity to reach those temperatures and maintain temperature excursion between night and day.

In the middle of the experimental summer, we exposed half the plants in each temperature treatment to a heatwave. One tray per block from each growth chamber was transferred to one of two 'heatwave chambers' (TPG-2400-TH; Thermoline Australia Pty Ltd.; NSW, Australia), which was set to +5°C above the growth chamber's temperature (29/17°C day/night for historical climate scenario, and 34/25°C for the future climate; **Figure 1**); other conditions were identical to the growth chambers. Trays were moved to the heatwave chamber sequentially starting day 88 and ending day 94 of the experiment, and then remained in the heatwave chambers for 5 days (heatwave: 'hw'), after which they were moved back to their original growth chambers; the last group of plants therefore left the heatwave chambers on the 98th day of experiment. All non-heatwave ('no hw') plants remained in their original growth chambers throughout this time (**Figure 1**). From day 100 onward, plants continued in the future vs. historical climate conditions, and the experiment was ended at day 191 (from when the temperature treatments were first applied).

Traits Measured

We measured a range of phenotypic traits reflecting different aspects of the phenology, physiology, growth and reproduction of *W. ceracea*. Measures were made on a total of 321 plants (out of the 360, see above) because 39 individuals died prior to exposure to the heatwaves.

Thermal Physiology

Chlorophyll content was measured by means of a SPAD-meter (SPAD-502; Konica Minolta, Inc., Tokyo, Japan) on the same day that the plant produced its first flower (maturity), therefore before the heatwave treatment. Measurements were taken as the average value of three spot measurements on the youngest fully-expanded green leaf from the main stem.

After the 5-day heatwave treatment, we removed one leaf per plant from both heatwave and non-heatwave plants to measure the potential quantum efficiency of photosystem II (F_v/F_m) and the temperature dependence of basal chlorophyll fluorescence. Harvested leaves were placed on a Peltier plate (CP-121HT; TE Technology, Inc., Traverse City, MI, United States) set to a constant ambient temperature (21 $\pm$ 1°C) and leaves were dark-adapted for 30 min.

After dark adaptation, we measured minimum chlorophyll fluorescence (F_0) (i.e., fluorescence in absence of photosynthetic light) and maximum fluorescence (F_m) (i.e., fluorescence after delivering to a saturating pulse for a short duration). Chlorophyll fluorescence was measured by means of a chlorophyll fluorescence imaging system (MAXI-Imaging-PAM; Heinz Walz GmbH, Effeltrich, Germany). We used the initial F_0 and F_m parameters to calculate F_v/F_m as the ratio of $(F_m - F_0)/F_m$ ($n = 276$). Unfortunately, the F_v/F_m measurements for 43 individuals were not recorded due to software errors.

After measuring F_v/F_m, we raised the temperature of the Peltier plate from 21 to 60°C at a heating rate of 60°C h^{-1} (Schreiber and Berry, 1977; Arnold et al., 2020). We recorded F_0 values every 10 s. The temperature of the leaf samples was measured using two type-T thermocouples that were attached to the underside of two randomly selected leaves. We logged the thermocouple data using a dual-channel data logger (EL-GFX-DTC; Lascar Electronics Ltd., Salisbury, United Kingdom). The average temperature of these two thermocouples was then used for all subsequent temperature calculations.

We visualized the temperature-fluorescence (T-F_0) curves and extracted values for critical hot temperature (T_{crit}), the temperature at 50% fluorescence (T_{50}) and the temperature at maximum fluorescence (T_{max}) values using the R Environment for Statistical Computing v3.5.2 (R Development Core Team, 2019) (**Supplementary Figure 1**). T_{crit} (n = 319) corresponds to the inflection point in the curve, i.e., the temperature at which an abrupt increase in F_0 is observed. We extracted T_{crit} as the intersection between the two straight lines tangential to the flat and steep part of the curve, using a break-point regression analysis conducted with the 'segmented' R package (Muggeo, 2017). For those curves that reached a maximum F_0 and then declined (n = 300) we were able to extract T_{max} and T_{50}. We included in the analysis the 19 individuals that did not reach a maximum F_0 considering them as having T_{max} = 61°C and calculating their T_{50} (see **Supplementary Material**). Removing those 19 individuals from the analysis did not change the pattern of the results.

We measured *thermal safety margins* for each individual as the difference between their T_{crit} and the maximum temperature they experienced under the respective treatment (historical/no heatwave = 24°C; future/no heatwave = 29°C; historical/heatwave = 29°C; future/heatwave = 34°C).

Additional information about the Peltier plate and chlorophyll fluorescence imaging system and data extraction can be found in the **Supplementary Materials** of this paper and in Arnold et al. (2020).

Phenology, Longevity and Biomass

Flowering onset was recorded as the day since the imposition of historical and future scenarios treatments on which the first flower opened (n = 295 plants). Flowering phenology was recorded every day during the first 56 days, then as flowering rate declined, plants were checked every 10 days for the next 35 days. Sampling for flowering onset was ended at the beginning of the heatwave treatment. Of the 26 individuals (8.1%) for which we do not have phenological data, eight (2.5% of the total) did not flower, while 18 plants (5.6% of the total) flowered after the heatwave imposition and were included in the analysis as flowering on the 98th day (corresponding to 1 week after the last sampling event that happened on the 92nd day). Individuals recorded as flowering on day 98 were evenly spread among climate scenarios, however, 14 out of 18 were from low elevations. 12 families had at least one such individual; family F1.118 had three unsampled individuals and family F1.125 had four.

After the heatwave we recorded the day of death of all the individuals that died before the end of the experiment. *Longevity* following the heatwaves was monitored every 3–4 days throughout the experiment. The exception to this was the first 3 weeks after the heatwave period, for which detailed mortality dates were not recorded. For the 49 plants that died in this 3 weeks period, we assigned them an estimated death date of 11 days (Exclusion of these plants from the analysis did not change the conclusions). Longevity was defined as days since the end of the heatwave (n = 321).

Of the total 321 plants that were alive at the time of the heatwave treatment, 161 plants died prior to the end of the experiment. Of these, most plants died after the changing of photoperiod, which was progressively reduced starting from the 149th day (**Supplementary Table 2** and **Figure 1**). Death of these plants was therefore mostly due to either the effect of our temperature treatments or to senescence. For the remaining 160 plants, at the end of the experiment (at 191 days since the imposition of climate treatments), we harvested the above-ground organs of all the plants that were still alive, placed them in bags and dried them at 70°C for at least 72 h, and then weighed the above-ground *biomass*. Therefore, we only measured the above-ground biomass on those individuals that survived until the end of the experiment.

Reproduction

We recorded the *total number of flowers* that each plant produced during the experiment and collected all seed capsules when these were mature. Seed capsules were stored in seed envelopes and stored in a desiccator for at least 36 weeks.

At the end of the experiment, we measured the total seed mass of the 236 plants that produced seed. We weighed three replicates of 50 seeds, calculated the average mass of 50 seeds and then obtained the *average individual seed weight* by dividing it by 50 (n = 233 plants). We estimated *the total number of seeds produced* (*total seed production*, n = 318) by one of three ways: (i) for the individuals with estimates of average individual seed weight, as the ratio between total seed mass and average individual seed weight; (ii) for seven individuals that produced fewer than 50 seeds, seeds were counted directly and the average individual seed weight was obtained as the ratio between total seed mass and total seed production; and (iii) for the 85 individuals that did not produce any seeds we assigned values of zero (results of the analysis without this last set of individuals are presented in **Supplementary Table 3**). Three individuals had unrealistically large estimates of the total seed production and were removed from the analysis. We also analyzed the *probability of producing any seeds*, assigning zero to individuals that did not produce seeds (85 individuals) and 1 to those that did (236 individuals).

Statistical Analysis

All traits were analyzed using linear mixed effect regression models fit by restricted maximum likelihood using the *lmer* function in 'lme4' R package (Bates et al., 2015) in R v3.5.2. Flowering onset and total seed weight were natural log-transformed before the analysis to obtain an approximately normal distribution, while F_v/F_m was multiplied by 10. The fixed effects were elevation ('high' or 'low'), climate scenario ('historical' or 'future'), heatwave ('no heatwave' or 'heatwave'), the interactions between these factors and block (3 blocks), whereas random effects were 'family' (30 families) and 'position' within the block (30 positions). We fitted position as a random effect because plants that were at the edges of blocks experienced less shading and lower competition for light than plants in the middle, whereas plants in the middle of blocks may have received on average more water because of pooling on the benches. There was evidence for significant variance in the effect of position for longevity and biomass, and it explained a small amount of variance for the other traits. Therefore, we do not think position has affected the pattern of results. We removed random effects from the model structure when explaining zero variance: 'family' for F_v/F_m and T_{max} and 'position' for seed production and individual seed weight.

We tested for all interactions, but as three-way interactions were never significant, we examined only two-way interactions (climate scenario × heatwave, elevation × climate scenario, elevation × heatwave); this is referred to as the 'full random-intercept model' from now on. Given that unconditioned main effects are incorrectly predicted when interactions are included in the model (because interactions contain the main effects they are composed of and because regression coefficients for one factor are estimated considering the other factors to be zero) we used models without any interactions between the main effects. To define whether there was statistical support for an effect of factors and interactions on traits, we evaluated P-values from each model, using the *summary* function of the 'lmerTest' R package (Kuznetsova et al., 2017).

We tested for variance in the intercept of plastic responses between families by means of a likelihood ratio test between the full random-intercept model and a model without the random effect of 'family.' We then fitted a model with additional random factors of the interaction between family and either climate scenario and heatwave treatment, a 'full random-slopes model' (specified as 'climate scenario| family' and/or 'heatwave| family'). We tested for significant variation in the slopes of plastic responses of families to climate scenario and heatwave by means of a likelihood ratio test between the full random intercept model and full random slope models. The interaction between family and heatwave was never significant so we do not present results for it.

Date to first flower and chlorophyll content were recorded before the heatwave treatment, therefore the model for these trait did not include the fixed effects of heatwave treatment. All traits were fitted with *lmer* assuming a normal error distribution, with the exception of the number of flowers, total seed production and the probability of producing seeds. Of these, number of flowers and total seed production were analyzed using a generalized mixed effect model with a negative binomial distribution (*glmer.nb* function in 'lme4'). The probability of producing seeds was analyzed using generalized mixed effects models (*glmer* function in 'lme4') with a binomial distribution. Longevity was analyzed using a proportional hazards Cox mixed effects model (*coxme* function in 'coxme' R package; Therneau, 2020). Again, the structure of fixed and random effects was identical to that for the other phenotypic traits.

Results for the traits seed mass, chlorophyll content and total seed production with lmer as well as details of the models are presented in **Supplementary Tables 3, 4**, respectively, and **Supplementary Figure 2**.

RESULTS

Thermal Physiology

Both warmer average growth temperatures and exposure to heatwaves altered thermal physiology in *W. ceracea*, however, there was no evidence of differences between high- versus low-elevation families in these responses.

The photosynthetic efficiency (F_v/F_m) of *W. ceracea* was reduced by both growth under future temperatures and heatwaves (**Table 1** and **Figures 2A,B**). Plants grown under future conditions showed a more extreme reduction in F_v/F_m when also exposed to a heatwave (**Figure 2B**) than did the plants grown under historical conditions (**Table 1** and **Figure 2A**), however the effect of the interaction between climate treatment and heatwave was marginal (P-value = 0.025).

Despite the impairment of F_v/F_m, growth under future, warmer temperatures induced an increase in thermal tolerance limits of leaves (**Table 1** and **Figures 2C–H**). T_{crit} was on average 0.7°C higher under the future scenario (46.3 ± 0.1°C SE) than the historical (45.6 ± 0.1°C SE), and this difference is maintained in the other two parameters derived from the T-F_0 curves, the temperature at 50% fluorescence (T_{50}), and the temperature at maximum fluorescence (T_{max}) (**Table 1** and **Figures 2C–H**).

In contrast to the effects of growth conditions, exposure to a transient heatwave led to a reduction in both T_{50} and T_{max}, but not in T_{crit}, regardless of growth temperatures (**Table 1** and **Figures 2C–H**). This trend was visible under both climate scenarios, but it was more pronounced under future temperatures where T_{crit} was not affected by the heatwave (no hw = 46.2 ± 0.19°C SE vs. hw = 46.4 ± 0.20°C SE; **Figure 2D**), while under a historical scenario the heatwave consistently reduced thermal tolerance (no hw = 45.8 ± 0.18°C SE vs. hw = 45.4 ± 0.18°C SE; **Figure 2C**).

We did not find any evidence that the acclimation to future, warmer temperatures conferred resilience to heatwaves, as the effect of the interaction between heatwave and climate scenario on thermal tolerance metrics was negligible (**Table 1**). There was also no evidence of any effect of elevation (either as a main effect, or in an interaction) for any of the thermal physiology traits (**Table 1**).

Phenology, Longevity and Biomass

Families of *W. ceracea* from higher elevations flowered 9.6 ± 2.2 days SE earlier than families from lower elevations (**Table 2** and **Figure 3A**). However, growth under a future, warmer scenario had little effect on flowering time, with plants flowering on average 1.0 ± 2.3 SE days earlier (**Table 2** and **Figure 3A**).

Under all treatment conditions, families from lower elevations produced more biomass than high-elevation families (**Table 2** and **Figures 3B,C**). According to the main effects model, biomass of the individuals that survived until the end of the experiment was not affected by growth in a future, warmer scenario nor by exposure to heatwaves (**Table 2** and **Figures 3B,C**). However, it seems that plants in historical conditions lost biomass, while those under future conditions accumulated biomass after a heatwave, although the effect of the interaction term between climate scenario and heatwave in our model is marginal (P-value = 0.028)(**Table 2** and **Figures 3B,C**).

Before the heatwave exposure, mortality rates were low: 39 (out of the starting 360 plants, i.e., 10.3%) individuals (mostly from the future scenario treatment) died before heatwaves, presumably for reasons related to the conditions inside chambers, shading from neighboring larger plants, or poor initial health. We analyzed longevity from the end of the heatwaves to the end of the experiment. Toward the end of the experiment, as temperatures were lowered and day-length decreased, plants began to die. Longevity did not differ for high and low elevation plants (**Table 2**). The effect of climate scenario and of the interaction between climate scenario and heatwave on longevity were also negligible (**Table 2**). In absence of heatwaves, longevity remained high until the end of the experiment under both temperature conditions (**Figure 4**). However, when plants were exposed to heatwaves there was a substantial reduction in survival under both climate scenarios (**Table 2** and **Figure 4**). Survival to the end of the experiment was 52.6 ± 4.1% SE for plants exposed to the heatwaves and 70.9 ± 3.6% SE for

TABLE 1 | Effects of temperature treatments on thermal physiology traits: results from linear random intercept models.

	F_v/F_m * 10			T_{crit} (°C)			T_{50} (°C)			T_{max} (°C)		
Main effects model:	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**
Intercept	8.015	0.04	NA	45.876	0.228	NA	48.58	0.263	NA	52.882	0.346	NA
Elevation (low)	−0.028	0.034	0.415	−0.291	0.196	0.148	−0.291	0.225	0.205	−0.352	0.291	0.227
Climate (future)	**−0.147**	**0.034**	**<0.001**	**0.703**	**0.182**	**<0.001**	**0.716**	**0.219**	**0.001**	0.635	0.29	0.03
Heatwave	**−0.264**	**0.034**	**<0.001**	−0.183	0.181	0.314	**−0.837**	**0.218**	**<0.001**	**−1.901**	**0.289**	**<0.001**
Block (B)	0.035	0.045	0.444	−0.141	0.224	0.531	0.189	0.269	0.484	0.646	0.358	0.072
Block (C)	0.052	0.039	0.186	0.071	0.222	0.748	0.32	0.266	0.229	0.614	0.354	0.084
	Marginal R^2: 0.221			*Marginal R^2: 0.057*			*Marginal R^2: 0.083*			*Marginal R^2: 0.142*		
	Conditional R^2: 0.233			*Conditional R^2:0.101*			*Conditional R^2: 0.111*			*Conditional R^2: 0.148*		
Full model:	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**
Fixed effects:												
Intercept	7.984	0.048	NA	45.869	0.271	NA	48.665	0.318	NA	53.1	0.418	NA
Elevation (low)	−0.038	0.057	0.503	0.011	0.315	0.959	−0.135	0.37	0.716	−0.312	0.486	0.522
Climate (future)	−0.046	0.056	0.415	0.551	0.306	0.072	0.414	0.364	0.256	0.074	0.489	0.879
Heatwave	**−0.23**	**0.056**	**<0.001**	−0.31	0.303	0.306	**−1.046**	**0.359**	**0.004**	**−2.268**	**0.481**	**<0.001**
Block (B)	0.038	0.045	0.396	−0.14	0.223	0.531	0.183	0.265	0.492	0.634	0.357	0.077
Block (C)	0.052	0.038	0.172	0.074	0.22	0.737	0.322	0.262	0.22	0.608	0.352	0.086
e(l)*c(f)	−0.063	0.067	0.352	−0.312	0.361	0.389	−0.098	0.43	0.82	0.138	0.578	0.811
c(f)*hw	−0.151	0.067	0.025	0.613	0.361	0.091	0.711	0.429	0.099	1.014	0.577	0.08
e(l)*hw	0.081	0.068	0.237	−0.325	0.369	0.38	−0.24	0.436	0.583	−0.208	0.58	0.72
	Marginal R^2: 0.242			*Marginal R^2: 0.070*			*Marginal R^2: 0.092*			*Marginal R^2: 0.151*		
	Conditional R^2: 0.258			*Conditional R^2:0.112*			*Conditional R^2: 0.120*			*Conditional R^2: 0.156*		
Random effects:	**var**		**Pr(ML)**	**var**		**Pr(ML)**	**var**		**Pr(ML)**	**var**		**Pr(ML)**
Family	NA		NA	0.0334		0.6405	0.0352		0.7169	NA		NA
Position	0.0016		1.0000	0.0894		0.2351	0.0799		0.4409	0.044		0.7983
Residual	0.0759			2.5692			3.6363			6.5914		

*Main effects model has no interactions; full model has all 2-way interactions. 3-way interactions were never significant and results for them are not presented. The intercept corresponds to high elevation, historical climate and no heatwave, and other parameter values are relative to this. Significant results, as for P-value < 0.01 from the lmerTest package in R, are presented in bold. e(l) = elevation (low); c(f) = climate (future); hw = heatwave; est. = estimated effect; Pr(ML) = P-value of the likelihood ratio test between the models with and without the random effect. Random effects parameters are the variance components associated with that random effect. * = multiplication.*

plants under non-heatwave growth conditions (**Table 2** and **Figure 4**).

Reproductive Traits

Wahlenbergia ceracea from high and low elevation origins and experiencing the two climate scenarios differed in their investment in reproduction and reproductive outcomes. Individuals belonging to families from high elevations overall produced more flowers (**Table 3** and **Figures 5A,B**). Under a historical climate, they also produced nearly three times more seeds than low-elevation individuals (**Table 3** and **Figure 5C**), however, that advantage was nullified under a future climate scenario (**Figure 5D**). Overall the individual seed weight was reduced under a future climate (**Table 3** and **Figures 5E,F**). We found a weak interaction effect between elevation and climate scenario on individual seed weight: under historical conditions high-elevation plants produced heavier seeds than low-elevation ones, however, when grown under a future scenario, high-elevation individuals produced lighter seeds, while low elevation individuals were not affected (**Table 3** and **Figures 5E,F**). High elevation individuals had higher probabilities of producing seeds under a historical climate than individuals from lower elevations, but they underwent a steep reduction in this probability when grown under a future climate (**Table 3** and **Figures 5G,H**).

Growth under the future climate scenario induced a higher flower production on average (**Table 3** and **Figures 5A,B**), however, this did not translate into higher fitness, as the total seed production was lower and individuals had lower probabilities of producing any seeds (**Table 3** and **Figures 5C,D,G,H**). Exposure to a heatwave in itself did not affect flower or seed production (**Table 3**).

Interestingly, despite the lack of a main heatwave effect on either flower production or the individual seed weight, individuals exposed to a heatwave in the future climate scenario halved the number of seeds produced (future scenario: no hw = 713 ± 196 SE vs. hw = 382 ± 111 SE; **Supplementary Table 3**, **Table 3**, and **Figures 5C,D**). Plants grown under historical conditions always produced more seeds, even when exposed to heatwaves (**Figures 5C,D**).

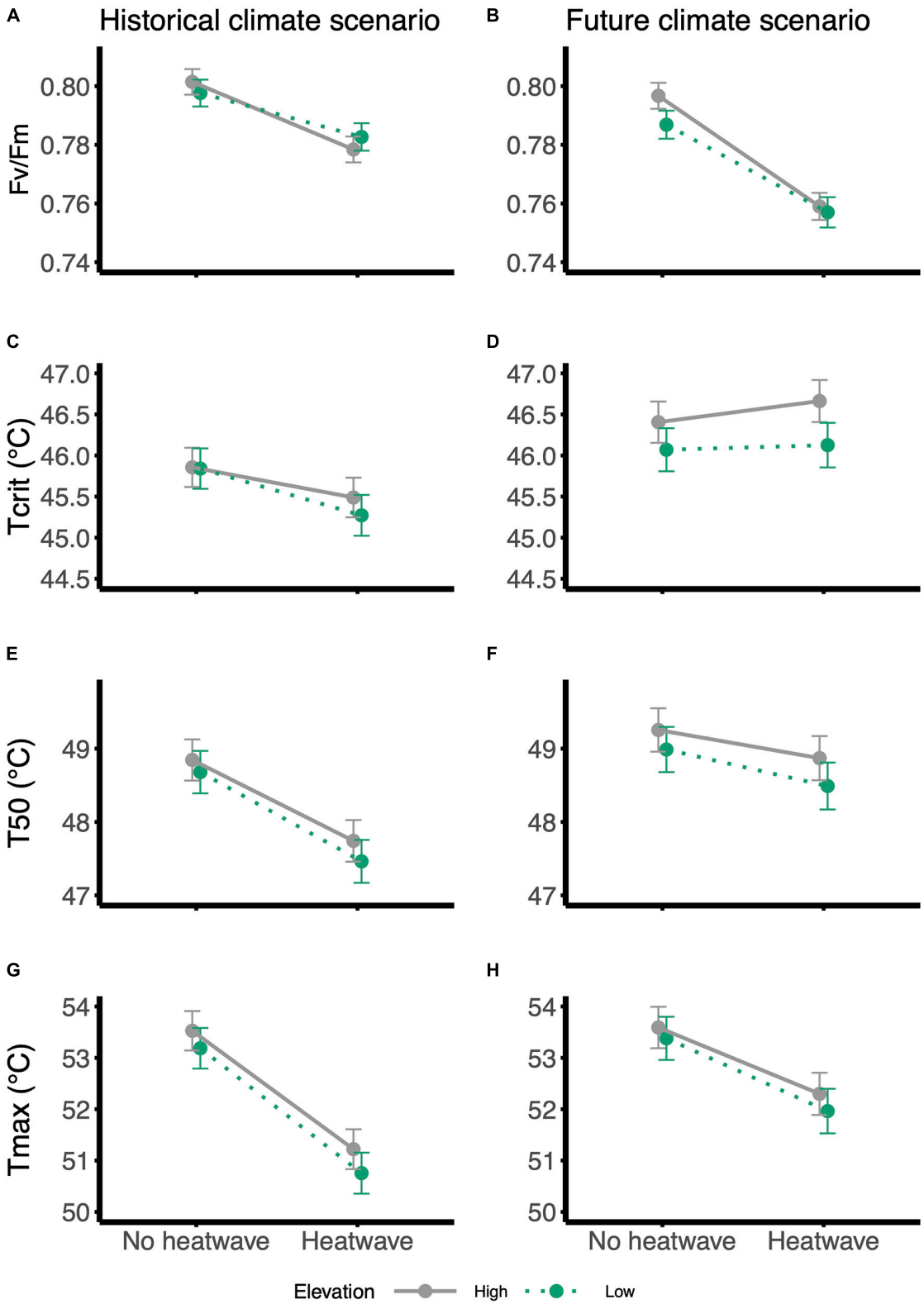


FIGURE 2 | Reaction norms for thermal physiology traits in response to the heatwave treatment: **(A,B)** F_v/F_m, **(C,D)** T_{crit}, **(E,F)** T_{50} and **(G,H)** T_{max}. Panels on the left: historical climate scenario; panels on the right: future climate scenario. Gray and solid lines = high-elevation families; green and dotted lines = low-elevation families. Values were estimated from the models using the *emmeans* function in 'emmeans' package, in R; error bars are standard errors.

TABLE 2 | Effects of temperature treatments on phenology, biomass and longevity: results from linear random intercept models (flowering onset), from linear random slope models (biomass) and from Cox proportional hazards model (longevity).

	Flowering onset (ln)			Biomass (g)			Longevity		
Main effects model:	**Est.**	**(*se*)**	***P*-value**	**Est.**	**(*se*)**	***P*-value**	**Est.**	**(*se*)**	***P*-value**
Intercept	3.594	0.064	NA	16.87	1.536	NA	NA	NA	NA
Elevation (low)	**0.252**	**0.08**	**0.004**	3.18	1.376	0.028	1.063	1.186	0.72
Climate (future)	−0.027	0.035	0.448	0.322	1.346	0.813	1.14	1.18	0.43
Heatwave	NA	NA	NA	−1.7	1.083	0.119	**1.585**	**1.179**	**0.005**
Block (B)	**−0.152**	**0.044**	**0.001**	−2.534	1.363	0.065	1.105	1.221	0.62
Block (C)	−0.046	0.043	0.287	−1.639	1.323	0.218	0.984	1.226	0.94
	Marginal R^2: 0.102			*Marginal R^2: 0.068*			*Marginal R^2: 0.038*		
	Conditional R^2: 0.387			*Conditional R^2: 0.434*			*Conditional R^2: 0.342*		
Full model:	**Est.**	**(*se*)**	***P*-value**	**Est.**	**(*se*)**	***P*-value**	**Est.**	**(*se*)**	***P*-value**
Fixed effects:									
Intercept	3.604	0.066	NA	18.845	1.732	NA	NA	NA	NA
Elevation (low)	0.233	0.086	0.01	0.587	1.942	0.764	1.21	1.334	0.51
Climate (future)	−0.046	0.048	0.345	−2.422	2.001	0.234	0.949	1.339	0.86
Heatwave	NA	NA	NA	**−5.364**	**1.694**	**0.002**	1.336	1.323	0.3
Block (B)	**−0.153**	**0.044**	**0.001**	−2.422	1.323	0.07	1.096	1.221	0.65
Block (C)	−0.047	0.043	0.284	−1.724	1.277	0.18	0.981	1.226	0.93
e(l)*c(f)	0.04	0.071	0.567	2.181	2.666	0.42	0.828	1.386	0.56
c(f)*hw	NA	NA	NA	4.748	2.139	0.028	1.658	1.393	0.13
e(l)*hw	NA	NA	NA	3.822	2.234	0.089	0.902	1.413	0.77
	Marginal R^2: 0.103			*Marginal R^2: 0.100*			**Marginal R^2: 0.050*		
	Conditional R^2: 0.389			*Conditional R^2: 0.487*			*Conditional R^2: 0.353*		
Random effects:	**var**		**Pr(ML)**	**var**		**Pr(ML)**	**var**		**Pr(ML)**
Family	**0.0388**		**<<0.001**	4.009		0.0862	0.0003		0.9016
Position	NA		NA	**15.541**		**0.0001**	**0.3593**		**<<0.001**
Climate| family(slope)	NA		NA	20.019		0.0436	NA		NA
Residual	0.0956			35.806					

Main effects model has no interactions; full model has all 2-way interactions. 3-way interactions were never significant and results for them are not presented. Estimates and standard error for flowering onset are in the logarithmic scale. Estimates from the Cox model correspond to the hazard ratio, so positive parameter estimates indicate increased mortality. NA = factor not included in the model for that trait or result not provided by the model. The intercept corresponds to high elevation, historical climate and no heatwave, and other parameter values are relative to this. Significant results, as for P-value < 0.01 from the lmerTest package in R, are presented in bold. e(l) = elevation (low); c(f) = climate (future); hw = heatwave; est. = estimated effect; Pr(ML) = P-value of the likelihood ratio test between the models with and without the random effect. Random effects parameters are the variance components associated with that random effect. The slope variation term (random effect climate| family, i.e., variation in plastic responses to climate treatment between families) is significant and therefore included in the model only for biomass. Given that a standardized method to calculate marginal R^2 is not available for Cox proportional hazards mixed effect models, we approximated it calculating the R^2 coefficient proposed by O'Quigley et al. (2005) on a model without random effects, i.e., on a Cox proportional hazards model with the same fixed effect structure.

Variation Between Families in Intercepts and Slopes of Reaction Norms

Finally, there was evidence of variation among the 30 full-sibling families for flower production and flowering onset (**Tables 2**, **3**) but not for thermal tolerance traits or longevity. Among the reproductive traits, total seed production did not vary among families, and we found only small variation between families in the individual seed weight. The proportion of the total variance accounted for by family ranged from 0.9% for T_{50} to 28.9% for flowering onset. The slopes of the family-level reaction norms (i.e., magnitude of the plastic response) varied for biomass production in response to growth conditions, but not in response to heatwaves (**Table 2**).

Thermal Safety Margins

Finally, as summary statistics, we calculated thermal safety margins for each individual as the difference between T_{crit} and maximum temperatures during treatments. Because we were interested in the responses to a relative heatwave, i.e., 5°C rise in temperature relative to historical and future temperature conditions, heatwaves reached different maximum temperatures for historical (heatwave = 29/17°C day/night) and future climate scenarios (heatwave = 34/25°C day/night). In our experiment this also resulted in different thermal safety margins. We demonstrated that, despite acclimation of T_{crit} to warmer temperatures, thermal safety margins were reduced by both future temperatures and heatwaves, but not their interaction (historical

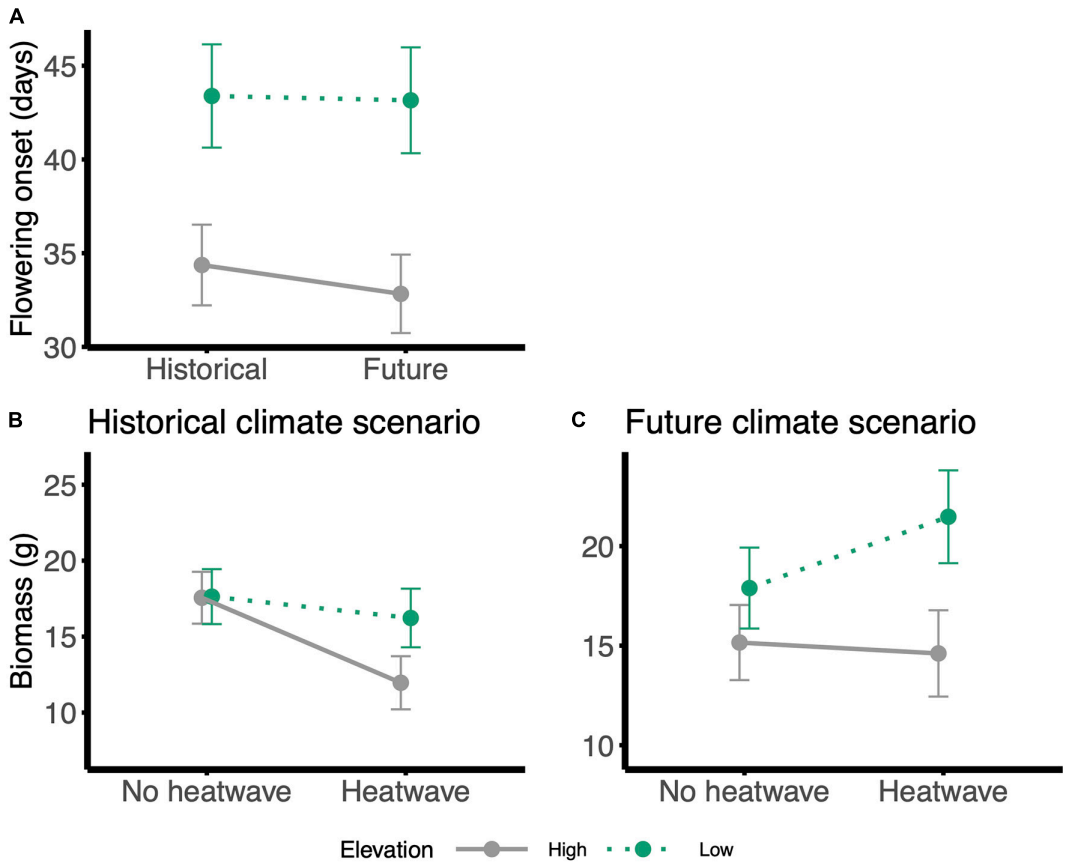


FIGURE 3 | Reaction norms for phenology and biomass traits: **(A)** flowering onset in response to the climate scenario, **(B,C)** biomass in response to the heatwave treatment, panel on the left: historical scenario; panel on the right: future climate scenario. Gray and solid lines = high-elevation families; green and dotted lines = low-elevation families. Values were estimated from the models using the *emmeans* function in 'emmeans' package, in R; error bars are standard errors.

climate/no heatwave = 21.9 ± 0.2°C SE; future climate/no heatwave = 17.3 ± 0.2°C SE; historical/heatwave = 16.4 ± 0.1°C SE; future/heatwave = 12.4 ± 0.2°C SE).

DISCUSSION

In this study, we grew progeny of families from high and low elevations of an Australian alpine plant, *W. ceracea*, under climate scenarios to test whether acclimation to a warmer climate would confer tolerance of heatwaves and to find evidence of intraspecific variation in plastic responses over an elevational gradient. Growth under warmer temperatures induced higher photosynthetic thermal tolerance in *W. ceracea*, but also affected growth and phenology in complex ways and caused a significant reduction in overall fitness, while heatwaves caused higher mortality rates. We found that the interactive effect of exposure to both warmer temperatures and a heatwave impaired seed production and plant fitness. There was no evidence in any trait that acclimation to, and hence tolerance of, higher temperatures in a future scenario conferred any resilience to heatwaves. Finally, the demonstration of elevation effects and differences in plasticity among families in some traits have implications for selective processes acting upon genotypes and plastic responses that can buffer the negative effects of climate change. Below, we discuss the responses that led to the strong reduction in fitness, and consider these in the context of the impacts of rapid climate change on alpine species.

Plastic Responses to Warming, Heatwaves and Their Interaction

Thermal Physiology

Photosynthetic thermal tolerance can indicate the potential of a species to tolerate and acclimate to warming (Yamori et al., 2014; Zhou et al., 2018). Despite being adapted to cold environments, alpine plants generally show broad thermal tolerance, with upper thermal limits well above maximum air temperatures, and capacity to acclimate very quickly to warming (Atkin et al., 2006; Larcher et al., 2010; Leon-Garcia and Lasso, 2019).

In our experiment, growth under a warmer scenario induced a small (∼1°C) but significant acclimation of photosynthetic thermal tolerance in *W. ceracea*, at the cost of a small reduction in photosynthetic efficiency. Many plant species, including those in alpine environments, have wide plasticity in T_{crit}, which can increase by up to 9°C under warming (Downton et al., 1984;

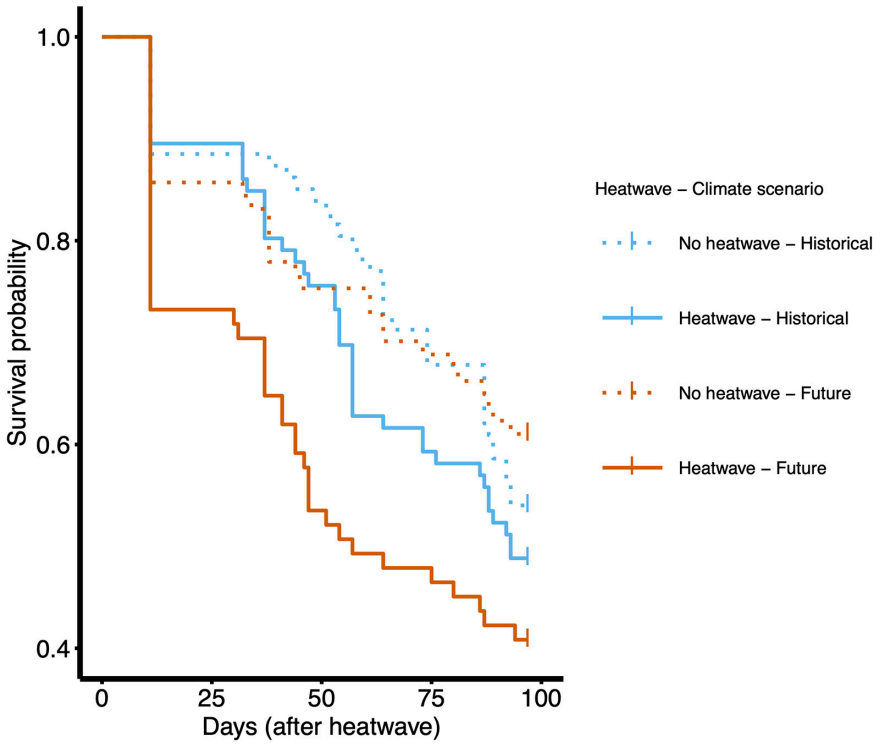


FIGURE 4 | Kaplan–Meier survival graph by longevity, analyzed as days from the end of the heatwave treatment to the end of the experiment, for the 321 plants alive at this point. The graph shows results for the interaction between factors 'Heatwave' and 'Climate scenario.' Kaplan–Meier curves were obtained using the *survfit* function in 'survival' package (random effects in Cox mixed effect model explain little of the variance, therefore results can be assumed to be comparable to a model without them). Curves were plotted using the *ggsurvplot* function in 'survminer' package. Dotted lines = no heatwave; solid lines = heatwave. Blue lines = historical climate scenario; red lines = future climate scenario. The effect of elevation was not significant and is not presented in this graph.

Weng and Lai, 2005; Buchner et al., 2017), but there are also examples of species that do not show measurable plasticity in T_{crit} (Krause et al., 2010, 2013). That plasticity in thermal tolerance in *W. ceracea* is only ∼1°C suggests that *W. ceracea* leaves might be experiencing occasional heat stress events in their natural environment close to their thermal limit (46–46.5°C). Indeed, despite air temperatures being low in alpine environments, under clear skies leaf surfaces exposed to intense sun radiation can very rapidly overheat during moments of wind stillness, reaching up to 50–55°C (Körner, 2003; Vogel, 2009; Larcher et al., 2010). Alternatively, during the experiment plants may have cooled their leaves through evapotranspiration given that they were not water stressed and may have only needed a small acclimation of the photosynthetic machinery.

Photosynthetic efficiency, F_v/F_m, has been widely used as an indicator of plant health and stress levels. In our experiment, the future scenario caused a reduction in F_v/F_m, but not so much to indicate that plants were chronically stressed or photosynthetically compromised. Reduced photosynthetic efficiency concurrent with an increase in thermal tolerance was also found by Buchner et al. (2017) and may represent a protective mechanism to reduce the excitation state of the photosystem II under stress through thermal dissipation (Krause, 1988; Buchner et al., 2017), thereby facilitating higher thermal tolerance.

In contrast with other alpine species that show physiological acclimation (De Boeck et al., 2016), thermal tolerance in *W. ceracea* was strongly affected by heatwaves. The number of studies that have looked at the effect of heatwaves on thermal tolerance is still limited, but similar results to ours were found by Aspinwall et al. (2019) in four *Eucalyptus* species that showed lower F_v/F_m and tissue damage after a 5-day heatwave, but also increased thermal tolerance measured by gas exchange. T_{crit}, T_{50}, and T_{max} relate to three different points of T-F_0 curves: while T_{crit} reflects the thermostability of the photosystem II, the temperature threshold where damage begins to occur, T_{max} represents its complete disruption (Kouřil et al., 2001; Neuner and Pramsohler, 2006; Ducruet et al., 2007; Ilík et al., 2007). We found that heatwaves did not affect T_{crit} in *W. ceracea*, however, the effect of heatwaves became stronger moving along the T-F_0 curve (**Supplementary Figure 1**) from T_{crit} to T_{50} and then to T_{max}, i.e., heatwaves did affect the speed of the disruptive processes [ΔT ($T_{crit} - T_{max}$); Kouřil et al., 2001] and the photosystem II was completely disrupted 8.3 ± 0.2°C SE above T_{crit} (compared to 9.9 ± 0.2°C SE in the absence of heatwaves), resulting in lower thermal tolerance. Our results clearly show that the small photosynthetic acclimation of T_{crit}, T_{50}, and T_{max} (at the cost of F_v/F_m) to a future, warmer scenario did not confer tolerance of higher temperatures during heat stress in *W. ceracea*, despite the effect of heatwaves being stronger under a historical scenario.

TABLE 3 | Effects of temperature treatments on fitness traits: results from generalized random intercept models [number of flowers, probability of producing any seeds and seed production (n)] and from linear random intercept models (individual seed weight).

	Number of Flowers			Number of seeds			Individual seed weight			Probability of producing seeds		
Main effects model:	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**
Intercept	3.361	0.142	NA	7.739	0.353	NA	23.128	1.341	NA	2.251	0.428	NA
Elevation (low)	−0.344	0.143	0.016	−0.54	0.272	0.047	0.245	1.383	0.861	−0.553	0.346	0.111
Climate (future)	**0.225**	**0.087**	**0.01**	**−1.194**	**0.261**	**<0.0001**	**−3.28**	**1.058**	**0.002**	**−2.122**	**0.334**	**<0.0001**
Heatwave	−0.146	0.086	0.09	−0.127	0.261	0.628	1.218	0.996	0.223	0.233	0.294	0.428
Block (B)	0.028	0.106	0.789	0.187	0.317	0.556	0.398	1.243	0.749	0.424	0.365	0.245
Block (C)	0.004	0.108	0.97	0.116	0.32	0.718	0.491	1.256	0.696	0.366	0.356	0.304
	Marginal R^2: 0.079			*Marginal R^2: 0.192*			*Marginal R^2: 0.043*			*Marginal R^2: 0.243*		
	Conditional R^2: 0.377			*Conditional R^2: 0.214*			*Conditional R^2: 0.143*			*Conditional R^2: 0.353*		
Full model:	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**	**Est.**	**(se)**	***P*-value**
Fixed effects:												
Intercept	3.342	0.158	NA	7.668	0.389	NA	23.357	1.463	NA	2.632	0.624	NA
Elevation (low)	−0.4	0.187	0.033	−1.016	0.439	0.021	−1.526	1.821	0.404	−1.243	0.669	0.063
Climate (future)	0.361	0.143	0.012	−0.985	0.43	0.022	−4.002	1.758	0.024	**−2.86**	**0.628**	**<0.0001**
Heatwave	−0.171	0.147	0.244	0.173	0.419	0.679	2.214	1.49	0.139	1.03	0.734	0.16
Block (B)	0.032	0.106	0.765	0.17	0.316	0.59	0.294	1.225	0.811	0.418	0.367	0.254
Block (C)	0.007	0.107	0.95	0.108	0.316	0.734	0.62	1.239	0.617	0.372	0.36	0.301
e(l)*c(f)	−0.109	0.171	0.524	0.566	0.532	0.288	4.872	2.085	0.02	1.529	0.715	0.032
c(f)*hw	−0.17	0.169	0.317	−0.953	0.501	0.057	−3.054	2.08	0.144	−0.475	0.673	0.48
e(l)*hw	0.209	0.186	0.262	0.341	0.506	0.5	0.202	1.974	0.144	−0.839	0.651	0.198
	Marginal R^2: 0.070			*Marginal R^2: 0.220*			*Marginal R^2: 0.072*			*Marginal R^2: 0.328*		
	Conditional R^2: 0.361			*Conditional R^2: 0.259*			*Conditional R^2: 0.164*			*Conditional R^2: 0.426*		
Random effects:	**var.(int.)**		**Pr(ML)**	**var.(int.)**		**Pr(ML)**	**var.(int.)**		**Pr(ML)**	**var.(int.)**		**Pr(ML)**
Family	**0.0846**		**0.0002**	0.092		0.4369	6.114		0.0202	0.1793		0.0566
Position	**0.1243**		**<<0.001**	NA		NA	NA		NA	0.3845		0.0746
Residual							55.367					

Main effects model has no interactions; full model has all 2-way interactions. 3-way interactions were never significant and results for them are not presented. NA = factor not included in the model for that trait or result not provided by the model. The intercept corresponds to high elevation, historical climate and no heatwave, and other parameter values are relative to this. Significant results, as for P-value < 0.01 from the lmerTest package in R, are presented in bold. e(l) = elevation (low); c(f) = climate (future); hw = heatwave; est. = estimated effect; Pr(ML) = P-value of the likelihood ratio test between the models with and without the random effect. Random effects parameters are the variance components associated with that random effect.

The present study focused on investigating temperature in isolation, however, it should be noted that heatwaves are often accompanied by drought. Although photosynthetic rates in *W. ceracea* are not reduced by moderate water limitation (Geange et al., 2017), drought can have strong negative effects on plants' photosynthetic physiology, which are amplified when drought is concurrent to heatwaves (De Boeck et al., 2016). Therefore, we would predict *W. ceracea* to be more negatively affected by the combination of warming and heatwaves when precipitation also declines in the future (annual precipitation is projected to decrease by 0–20% by the year 2,100 in the Australian Alps region and snow cover will be limited to only the highest peaks, Harris et al., 2016). Heatwaves are also predicted to increase in frequency in the future due to climate change, such that plants will face repeated heatwave events. Such exposure to repeated heatwaves may in effect prime plants, conferring acclimation and resilience to future events (French et al., 2019). Research on plant responses to heatwaves and to extreme events is still scarce and future studies are warranted to tackle the different properties of such events.

Phenology and Reproductive Allocation

Contrary to many other species, we did not find evidence that *W. ceracea* advanced flowering onset (reproductive maturity) under warmer conditions. Temperature cues are usually used by plants to tune their reproductive phenology with the start of the growing season, but other factors, such as photoperiod, can also influence phenology (Wang et al., 2020). In previous work on *W. ceracea,* Nicotra et al. (2015) found advances in phenology in response to warming. However, in that study plants were grown under natural light, and the difference between cool and warm temperatures was 10°C (cool = 20/11°C; warm = 30/19°C day/night, as compared to our 5°C difference between historical and future scenarios). When we consider only individuals that flowered within the first 56 days in our experiment, we do find an effect of warmer temperatures in advancing maturity, therefore the lengthening of the photoperiod from the 51st day

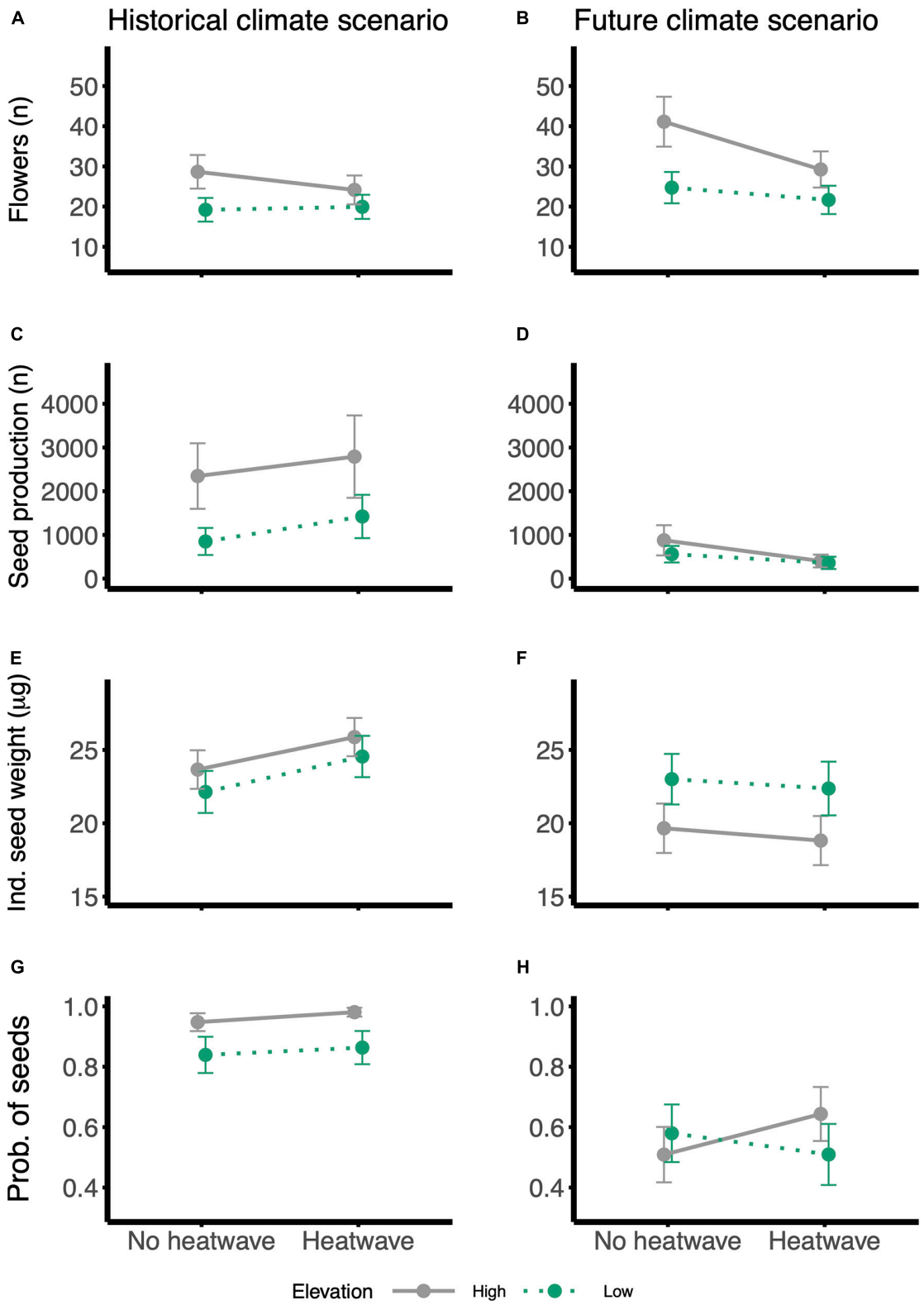


FIGURE 5 | Reaction norms for fitness traits in response to the heatwave treatment: **(A,B)** number of flowers, **(C,D)** total seed production, **(E,F)** individual seed weight and **(G,H)** probability of producing any seeds. Panels on the left: historical climate scenario; panels on the right: future climate scenario. Gray and solid lines = high-elevation families; green and dotted lines = low-elevation families. Values were estimated from the models using the *emmeans* function in 'emmeans' package, in R; error bars are standard errors. Ind., individual; prob., probability.

(**Supplementary Table 2** and **Figure 1** to mimic lengthening days in summer) or less frequent sampling from the 56th day may have confounded our results.

Though the shift in phenology was not statistically significant, *W. ceracea* did show significantly increased investment in flower production under warmed conditions. There are mixed reports of the effect of high temperatures on flower production. In Frei et al. (2014a), the number of inflorescences increases under warmer conditions. However, in other cases, flower production is not affected by warm temperatures (Choi et al., 2019) or can even be downregulated, as in the alpine plant *Campanula tyrsoides* (Scheepens and Stöcklin, 2013). Given that low temperatures usually limit and slow down growth in plants, *W. ceracea* and other alpine plants may take advantage of warmer temperatures to produce more flowers. Enhanced production of flowers may also be a response to compensate for the higher flower abortion and reduced flower longevity that can happen under warmer temperatures (Young et al., 2004; Pacheco et al., 2016).

Lifetime Fitness

Wahlenbergia ceracea is described as a short-lived perennial, most likely exhibiting a biennial strategy and a single reproductive event. Thus, survival to the end of the growing season is a good indicator of potential to produce seed. In our experiment, survival and total seed production were positively correlated (conditional R^2 = 0.37; **Supplementary Table 5** and **Supplementary Figure 3**), where individuals that died early produced fewer seeds.

Growth under future temperatures induced a strong inhibition of reproduction in *W. ceracea*, with a three-fold drop in seed production, and a reduction in average seed weight (**Table 3**, **Supplementary Table 3**, and **Figures 5C–F**). Moderate warming can affect development and longevity of flowers (Young et al., 2004; Pacheco et al., 2016), and heat stress during seed development and maturation inside capsules can reduce seed weight, germination and vigor and increase the number of shriveled and abnormal seeds (Shinohara et al., 2006; Hampton et al., 2013; Nakagawa et al., 2020). In general, germination and seed weight are positively correlated (Veselá et al., 2020; Ge et al., 2020), suggesting lower fitness in *W. ceracea* under future temperatures, but negative correlations were also found in a different alpine plant (Bu et al., 2007). The change in seed weight may indicate a parental effect, where parental plants alter investment to the endosperm and/or seed coat (Lacey et al., 1997), influence dormancy levels (Fenner, 1991) and/or diversify the timing of germination of their offspring to multiple seasons (Lampei et al., 2017; Satyanti et al., 2019). The reduction of the average seed weight could result in reduced viability or alter germination strategies; this will be investigated in a forthcoming experiment.

Plants exposed to a heatwave in a future scenario produced 72% fewer seeds relative to the mean seed production of plants in the rest of the treatments (**Figures 5C,D**). A major driver of that substantial reduction in fitness in our experiment was the higher mortality rates observed following heatwaves (**Figure 4**). Individuals that survived exposure to heatwaves in a future climate had greater biomass (**Figures 3B,C**), and it is therefore likely that those plants diverted investment to growth and preservation of organs, while stalling reproduction and limiting thermal tolerance.

Differences in Responses Due to Elevation of Origin

Intraspecific variation in traits and plasticity along elevation gradients has been invoked as a source of genetic variation that could buffer effects of climate change on alpine species (Henn et al., 2018; Heilmeier, 2019). Standing genetic variation is the raw material on which selection can act upon, assuming that at least part of the variation is heritable. Moreover, gene flow and/or migration of genotypes (or ecotypes) adapted to lower warmer sites may facilitate the spread of advantageous alleles to higher elevations (Anderson and Gezon, 2015; Moran et al., 2016).

Differences in Trait Means Between High- and Low-Elevation Populations

In a previous study on the same species, Nicotra et al. (2015) found variation in morphological and other growth-related traits across elevation as well as small, but significant, genetic differentiation between individuals from the two elevations, indicating some degree of adaptation to local conditions. In the present study, *W. ceracea* showed variation among the same populations in phenology, growth and fitness, but not in photosynthetic or thermal physiology. A lack of genetic or plastic variation in thermal physiology may indicate that both populations have historically experienced similar levels of temperature stress.

High-elevation populations of *W. ceracea* flowered earlier and increased flower production and consequently both seed mass and production (**Tables 2**, **3**, **Supplementary Table 3**, **Figures 3A**, **5A–D**, and **Supplementary Figures 2A,B**). At high elevations, the snow cover lasts for longer because lower air temperatures delay snow melt in spring and advance snowfall in autumn (Körner, 2003; Slattery, 2015). It follows that at high elevations, plants have to hasten growth until maturity and produce more flowers in order to take advantage of a shorter growing and reproductive season (Stinson, 2004; Ensing and Eckert, 2019). Of the plants that survived until the end of the experiment, those from low-elevation populations accumulated more biomass on average, indicating greater investment in vegetative growth, while the higher investment in reproduction and faster growth to maturity was associated with lower vegetative growth in high-elevation populations. This result is in line with Halbritter et al. (2018), who also found a clear trend of reductions in biomass with increasing elevations: in both cases, reduced stature may be a local adaptation to cooler air temperatures and stronger wind at high elevations (Körner, 2003).

Interestingly, under historical conditions, high-elevation individuals produced more seeds. Similarly, high-elevation individuals in Nicotra et al. (2015) produced heavier capsules and a higher total capsule mass, despite producing fewer capsules in total. High- and low-elevation populations of *W. ceracea* may have different optima such that historical temperatures in our experimental settings were optimal for high-, but sub-optimal

for low-elevation individuals that inhabit a warmer environment and may be experiencing warmer temperatures than the ones in our baseline (1960–1970). However, high-elevation individuals produced a higher total capsules mass even under 30°C in Nicotra et al. (2015). Therefore, we hypothesize that the higher investment in seed production may be a further response to a shorter growing season in high-elevation individuals of *W. ceracea*.

Variation in Plasticity Along an Elevation Gradient

Despite these significant elevation effects, we only found evidence of variation in plastic responses to warmer temperatures along an elevation gradient (i.e., an elevation × climate scenario interaction) in the individual seed weight and the probability of producing seeds (**Table 3**). High-elevation plants underwent a steeper reduction in the probability of producing seeds and produced much lighter seeds under future temperatures (**Figures 5E–H**), suggesting lower seed viability or active responses (parental effects) to influence germination timing of their offspring. In contrast, Nicotra et al. (2015) found greater evidence for variation in plasticity with elevation in the same study species (but using field collected seed): low-elevation individuals showed higher plasticity in growth and leaf-related traits. Therefore, our results indicate that there is no genetic variation across elevations in plastic responses when seed are generated under common conditions. This result is in accordance with Geange et al. (2017), which found limited variation in plastic responses of high- and low-elevation populations of *W. ceracea* to water limitation. Nevertheless, *W. ceracea* may still present variation in plasticity along elevation gradients for other traits and/or in response to other climatic and habitat factors.

In addition, we only used individuals from populations along the main range portion of Kosciuszko National Park (NSW, Australia) and we cannot exclude the possibility that other populations of the species may show variation in plasticity along elevation gradients. Although we selected families from the highest and lowest elevation collections of the species within the park, *W. ceracea* occupies a continuous elevation gradient in Kosciuszko National park. Individuals from mid-elevations (1,755–1,830 m a.s.l) showed similar responses to high-elevation individuals (Nicotra et al., 2015) and gene flow among populations is likely to occur. However, gene flow could reduce variation in both plasticity and trait means between elevation classes. A lack of variation in plasticity, but not in trait means, suggests that traits can be under selection, but the genetic architecture that underlies plastic responses is suitable for both environments.

Variation in Plasticity Among Families

Despite phenotypic plasticity being widely assumed to be an adaptive response to heterogeneous environments and climate change, the vast majority of studies that investigated responses to heat have ignored any associations with fitness-related traits, making it difficult to argue for the adaptive value of plasticity (Arnold et al., 2019). For selection to act upon plasticity, there has to be intraspecific variation in the extent of plasticity (i.e., in the slope of reaction norms). In this study, we did not find any evidence of variation in plasticity between families except for biomass, but we did find significant variation in trait means of fitness-related traits and flowering onset. Our results suggest that some families have higher fitness than others, but without variation in plasticity, it is not possible for to selection to act on plasticity.

Implications for Persistence of *W. ceracea* Under Climate Change

The use of climate model projections to implement realistic historical and future climatic conditions under controlled environments has been widely adopted in ecological research and is providing informative results on species responses and persistence under climate change (Harris et al., 2014). In our experiment, the implementation of realistic night temperatures was limited by technical reasons because growth chambers could not cope with the large temperature shift of the Australian alpine climate. However, previous studies have shown that night warming can promote acclimation to warm days, increasing photosynthetic capacity, and root and total biomass of plants (Turnbull et al., 2002; Su et al., 2020); therefore, we do not expect that this experimental limitation would have affected our overall pattern of results.

Although thermal safety margins in *W. ceracea* remained wide even under our treatments, they may be more easily exceeded in a future, warmer climate due to the leaf-air temperature decoupling. Under future climate scenarios and heatwaves, survival and fitness were severely hampered. We suggest that processes other than the photosystem II might well be disrupted under warmer conditions. If more individuals die and far fewer seeds are produced in future, *W. ceracea* will likely experience a reduction in population size, which could result in a contraction of its range. This may in turn lead to an upward movement of species from lower, warmer elevations and to a replacement of *W. ceracea* in its lower range limit. Seeds of *W. ceracea* are most likely dispersed by gravity and wind, limiting the dispersal capacity of the species (Morgan and Venn, 2017). Given that heatwaves affect survival in *W. ceracea* even under historical conditions, this successional process might already be occurring, as it is in the European Alps (Steinbauer et al., 2018).

CONCLUSION

Here we have demonstrated that the alpine herb *W. ceracea* can acclimate photosynthesis to moderate warming, resulting in higher T_{crit}, T_{50}, and T_{max}, and that thermal tolerance exceeds projected future temperatures and heatwaves. However, thermal safety margins will potentially be exceeded more often in the future as the narrow and daytime rapid overheating due to solar radiation become more frequent and intense. Warmer growth temperatures stimulated flower production, but also hampered seed development. Exposure to heatwaves reduced thermal tolerance and strongly reduced longevity of plants. We sought to determine whether growth under future, warmer temperatures would ameliorate or worsen the effects of heatwaves. We found that reduced longevity after heatwaves caused a further

reduction in seed production under a future climate scenario where many individuals did not produce seed. We did not find evidence that individuals acclimated to future temperatures had greater thermal tolerance after heatwave exposure compared to individuals grown under historical conditions.

We were able to add flower phenology and biomass to the list of morphological and growth traits that show genetic variation in *W. ceracea* along an elevational gradient. However, despite genetic variation, we did not find evidence that high- and low-elevation populations responded differently to climate change. We found little evidence of intraspecific variation in plasticity, suggesting that responses are consistent among families due to gene flow and/or climatic similarity along the elevation gradient.

We interpret our results as indicating increased chances for *W. ceracea* to be replaced on its lower range limits by lower elevation species and be constricted to the higher end of its range, but there is potential that the species might have the capacity to adapt to novel climatic conditions through selection on standing genetic variation. Nevertheless, future studies should consider adding replicates of the elevation gradient across a broader geographic range to test the generality of our results for *W. ceracea* and/or other Australian alpine plants. Finally, our study shows that not all alpine species are resilient to heatwaves. We suggest that future studies aimed at understanding the fate of plants under climate change should also consider how warmer growth temperatures will affect responses to heatwaves, drought, and their three-way interaction, and how growth temperatures affect plant responses to heatwaves of different intensities, frequencies and duration.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RN designed and conducted experiments, conducted statistical analyses, and wrote the manuscript. AN and LK developed ideas behind the experiments. All authors contributed to experimental design. LK and PA contributed to the statistical analyses. PA developed the chlorophyll fluorescence thermal tolerance methods and analyses. All authors contributed substantially to manuscript revisions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.615119/full#supplementary-material

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Impacts of Global Change on the Spatial Dynamics of Treeline in Venezuelan Andes

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The treeline in the Andes is considered an essential ecotone between the Montane forest and Páramo. This treeline in the Venezuelan Andes corresponds with a transitional ecosystem defined as the Páramo forest. In this work, we identify and analyze the impact of climate warming and land transformation as agents altering the Páramo forest ecosystem's spatial dynamics along the Venezuelan Andes' altitudinal gradient. We carry out multitemporal studies of 57 years of the land transformation at different landscapes of the Cordillera de Mérida and made a detailed analysis to understand the replacement of the ecosystems potential distribution. We found that the main ecosystem transition is from Páramo to the Páramo forest and from Páramo to the Montane forest. Based on the difference between the current lower Páramo limit and the Forest upper limit for 1952, the treeline border's displacement is 72.7 m in the 57 years of study, representing ∼12.8 m per decade. These changes are mainly driven by climate warming and are carried out through an ecological process of densification of the woody composition instead of the shrubland structure. We found that Páramo forest ecosystems practically have been replaced by the Pastures and fallow vegetation, and the Crops. We present a synthesis of the transition and displacement of the different ecosystems and vegetation types in the treeline zone. The impact of climate warming and deforestation on the Páramo forest as a representative ecosystem of the treeline shows us that this study is necessary for an integrated global change adaptation plan.

Keywords: climate warming, treeline, land use, woody densification, páramo forest ecosystem, landscape transformation

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INTRODUCTION

Global change impacts biodiversity by different drivers acting at the global and regional scales (Sage, 2020). Two of these drivers are climate warming and the alteration of precipitation operating on a worldwide scale. The land transformation works at a regional scale but with consequences globally (Sage, 2020). In the Andes mountains, forest loss is the main change observed at lower elevations, caused by increased pastures and croplands. In contrast, a shrub encroachment into highland grasslands, and an increase in woody vegetation, is probably due to a climate change, explicitly

the increment of temperature, between other explanations (Aide et al., 2019). In the Venezuelan Andes case, where shrubs and rosettes dominate the Páramo ecosystem, this encroachment is facilitated by a progressive shrub density change. Some isolated forest patches occurring up to 4,000 m are *Polylepis* spp. communities, with adaptative traits associated with these environments (Azócar et al., 2007). In general, species can respond to the climate warming through four responses: acclimate, adapt, shift their geographic distributions (migrate or dispersion), or eventual extinction (Holt, 1990; Huntley, 1992; Guisan et al., 1995; Bazzaz, 1996; Feeley et al., 2012; Vale and Araújo, 2018; Vinicius et al., 2018). In mountain landscapes, species can respond to climate change, with a vertical migration in a short distance (e.g., 500 m to counteract an increase of 3°C) (Peñuelas et al., 2002); the acceleration in the rate of increase in plant species richness (Steinbauer et al., 2018), with the local difference concerning to conditions as topography (Pauli and Halloy, 2019), and diversity of different spatial patterns for treeline dynamics (Bader et al., 2020). Most of these researches are focused on temperate alpine regions, but few on subtropical-tropical alpine regions (Pauli and Halloy, 2019).

How can forest migration occur in the Venezuelan Andes? Most of the works about the upward of treeline are for temperate or boreal systems, and little information is available about the impact of climate change on this migration in the tropical mountain (Fadrique et al., 2018). Tropical Andes have unique characteristics and very different from alpine zones. The Cordillera de Mérida in the Venezuelan Andes is a high and narrow mountain range located in the tropical belt with an extensive climatic variability (Andressen, 2007; Silva, 2010). Three particular determinants express this climatic variability (Andressen and Ponte, 1973; Monasterio and Reyes, 1980; Sarmiento, 1986; Vivas, 1992; Andressen, 2007; Silva, 2010; Llambí and Rada, 2019; Chacón-Moreno and Suárez del Moral, 2020): (a) the thermic gradient, (b) the configuration of the Cordillera de Mérida in two long mountain ranges and two long customary intermontane valleys, and (c) the latitudinal location of the Cordillera de Mérida, which confers it to be between two different climatic patterns in terms of the distribution of rainfall. These factors and the tropical localization are the central responsible higher diversity of ecosystems present in the region (Chacón-Moreno et al., 2013). As a result of this climate variability, vegetational belts are clearly defined along the altitudinal gradient (Monasterio, 1980a; Ataroff and Sarmiento, 2004; Costa et al., 2007; Josse et al., 2009; Chacón-Moreno et al., 2013; Llambí and Rada, 2019; Chacón-Moreno and Suárez del Moral, 2020).

One clear vegetation belt limit is the treeline, separating the Montane forest from the Páramo at the high Andes. In the high tropical mountains, the low temperatures, together with the steep slopes, the high radiation, water stress, and the considerable cloudiness, are the main adverse characteristics for the establishment of the arboreal vegetation, thus giving rise to what is known as the altitude limit of the forest or treeline (Tranquillini, 1979; Körner, 1998; Camarero and Gutiérrez, 2004; Cavieres and Piper, 2004; Körner and Paulsen, 2004; Bader et al., 2007; Wesche et al., 2008). Other environmental factors such as snow duration and elevation are also considered to influence the size and vulnerability of shrubs to freezing (Wheeler et al., 2014) and carbon limitation, although carbon was likely not limiting across the entire gradient (Wheeler et al., 2016). This limit, far from being a well-defined transition, is a gradual transition zone that is abrupt when observed on a small scale (Körner and Paulsen, 2004).

This treeline has been moving during the Andes geological history. The Andes ecosystems diversity has been changed dramatically in the Pleistocene and Holocene. Several palynological studies in the Andes support and indicate the forward and backward dynamics of the Andean vegetation belts due to climatic instability during the Pleistocene and Holocene, complemented by the high average net diversification rates of Páramo plant lineages (Van Der Hammen, 1974; Weingarten et al., 1990; Schubert and Vivas, 1993; Graf, 1996; Mahaney et al., 1997; Lachniet and Vazquéz-Selem, 2005; Rull et al., 2005; Stansell et al., 2005, 2007; Ni et al., 2006; Madriñán et al., 2013; Valencia et al., 2020). Specifically, higher temperatures brought about the advance of the Andean forest line over the Páramo, and lower temperatures caused the passage of the Páramo to lower altitudes. This process has been defined as the "flickering connectivity system" and works as an engine for ecological change and biodiversity in the Andean mountains (Flantua et al., 2019).

Between these two larges and contrasting ecosystems: Forest and Páramo exist an ecotone, considered an essential ecosystem that we defined as the Páramo forest. This ecosystem is a more open and smaller type of forest with three differentiable strata: tree canopy, bush, and intricated shrub strata (Arzac, 2008; Arzac et al., 2011), with the coexistence of floristic elements typical of higher elevations such as the *Libanothamnus* sp. and floristic aspects of lower altitudes characteristic of cloud forest or dry forest depending on the wet or dry slope, respectively, also finding the presence of elements typical of the transition between one ecosystem and another. This forest was called and defined by Monasterio (1980a) as a pre-páramo forest or páramo forest (Bosque Preparamero, Bosque Paramero). Like any transition between ecosystems, it is not abrupt. Still, gradual, presenting fluctuations in the altitude at which that transition zone is located, characterized by offering Páramo intrusions into the forest and vice versa as tongues of vegetation (Ramírez et al., 2009; Llambí et al., 2014; Llambí, 2015). The Páramo forest is considered a High-elevation Andean ecosystem, defined as an Under páramo ecosystem and Upper treeline forest, find between 3,000 and 3,500 m, and comprised of grasslands intermixed with isolated thickets of shrubs and dwarf trees (Arroyo and Cavieres, 2013; Malizia et al., 2020). For the treeline zone in the Cordillera de Los Andes in Venezuela, some studies describing species and growth form abundance patterns along the Páramo forest have been carried out (Ramírez et al., 2009; Arzac et al., 2011, 2019; Rodríguez et al., 2011; Llambí and Rada, 2019), and some others analyze the adaptive responses to environmental conditions to understand the restrictions to the establishment above the Páramo forest (Dulhoste, 2010; Puentes, 2010; Cáceres and Rada, 2011). Bader et al. (2007) made a comparative study of the spatial pattern for a wide range of tropical treelines, were found

an abrupt change between the Páramo areas and the Mountain forest, that calls "fringed," which usually consists of tall shrubs, tall grass, or trees with canopies down to ground level. Llambí (2015) presents a complete review of the vegetation structure, plant diversity, and dynamics of the Páramo forest ecosystem in the Venezuelan Andes.

Studies of tropical deforestation have traditionally focused on montane cloud forests but have disregarded the Páramo ecosystem; however, cattle grazing might alter a treeline (Bader et al., 2007). The Páramo forest ecosystem is one of the most fragile. Its disappearance could be due to the agricultural frontier's advance, so only small remnants of what was once dense forest remain. There are no quantitative measurement studies of the treeline's migration rate over the Páramo in the Venezuelan Andes. In this work, we identify and analyze the impact of climate change (climate warming and rainfall pattern distribution) and land transformation as primary change agents altering the cover and distribution of the Páramo forest ecosystem along an altitudinal gradient of the Venezuelan Andes.

Suppose that the temperature increases with elevation by climate change effects, and the rainfall distribution pattern is modified. In that case, we expect that the treeline's spatial dynamics could be affected in its distribution and structure. Forest ecosystems could migrate upward about the warming and precipitation changes. As responses mechanisms to migrate, it could be associate with changes in the density of woody vegetation.

In the actual High Andes' agricultural landscape, the transition between Mountain forest and Páramo ecosystems is interrupted with crops derived from the colonial time. We assume that the main agriculture uses were established over the Páramo forest ecosystem. Comparing the Páramo forest ecosystem's potential distribution with the actual distribution, we will understand the impact of land transformation.

We use multitemporal studies of the last five decades to determine the landscape transformation of the Cordillera de Mérida in the treeline area. We made a detailed analysis to understand the replacement of the ecosystems potential distribution.

MATERIALS AND METHODS

Study Area

The Cordillera de Mérida, as a representative area of the Venezuelan Andes, is a mountain range located between 7° 30′ and 10° 10′ north latitude and 69° 20′ and 72° 50′ west longitude (Schubert and Vivas, 1993; **Figure 1**), whose uplift began toward the end of the Eocene until reaching an elevation similar to the present one during the late Pliocene, and its current height at the end of the Pleistocene just before the Quaternary. We present three studies about landscape transformation and ecosystem distribution modeling for the Cordillera de Mérida. In **Figure 1**, we show the study areas in the context of the Venezuelan Andes Ecoregion (Chacón-Moreno et al., 2013). For each area, we consider a different landscape ecology approach. The first study is the Capaz river watershed, focused on the treeline zone, and where we detail the main transformation results and rate of change of the Páramo forest in almost six decades. The second area corresponds to the National Park Sierra Nevada's treeline zone, where we develop a study of the densification of the woody vegetation, comparing aerial photography from 1952 to 1998. In the third study, the Alto Chama area, we analyze and compare the ecosystem's potential distribution concerning the actual ecosystem cover, mostly focused on the high Andean mountain.

Capaz Study Case: Páramo Forest Migration

We made a detailed multitemporal landscape transformation analysis, considering four consuetudinary periods from 1952 to 2009 (57 years). This analysis was based on the interpretation of aerial photography and satellite imagery in the treeline area of Capaz watershed (**Figure 1**, area 1). The Capaz river basin extends from 100 to 4,200 m of altitude approximately in the Páramo of La Atravesada.

Ecosystems Maps

Following the methodology described by Pernía (1989), we interpreted the next aerial photographs covering the study area: (a) Mission A34 from 1952, scale 1: 40,000; (b) Mission 010380, the year 1973–1974, scale 1: 50,000; and (c) Mission 010493, the year 1997–1998, scale 1: 65,000. The interpretations were georeferenced, and we create ecological unit maps (Years: 1952, 1972, and 1998) following the approaches of Ataroff and Sarmiento (2004) and Chacón-Moreno (2004). The maps were processed using ILWIS Geographical Information System (Nijmeijer et al., 2001). Further description of the enterally work is presented in Rodríguez (2005) and Rodríguez-Morales et al. (2009). Based on the methods and approach of Chacón-Moreno (2007) and Chacón-Moreno et al. (2013), we processed and classified a Spot 5 image of January 2009 (Spot scene ID: 5 651-333 09/01/11 15:06:26 2 J; four spectral bands: Blue, Green, Red, and NIR; processing level 2A; pixel size 20 m) and a Landsat 7 image of June 2001 (Landsat scene ID: p006r54_7t20010616_z19, using five spectral bands: Blue, Green, Red, NIR, and SWIR 1; processing level 2A; pixel size 30 m), to obtain ecosystems maps of the Capaz study area for the years 2001 and 2009. We verify the Capaz ecosystem maps in eight field trips to the whole study area, with GPS and direct observation. The five maps previously elaborated were processed and analyzed to correct the georeference and coordinate system, to prepare maps in image raster format of 10 m pixel size, and create a detailed area at the treeline zone, where all the maps have the maximum coincidence (**Figure 2**). Georeference errors were calculated for each data processing.

Transitional Ecosystem Map

The five maps were crossed one by one in sequential order to obtain an ecosystem transitional matrix and map. The analysis was simplified, considering the unit of transition's predominance and size and the transition ecosystem's existence at the beginning of the study. From the transitional matrix, we elaborate on the main change trajectories for the ecosystems and replacement systems. We create the ecosystem transition model, including

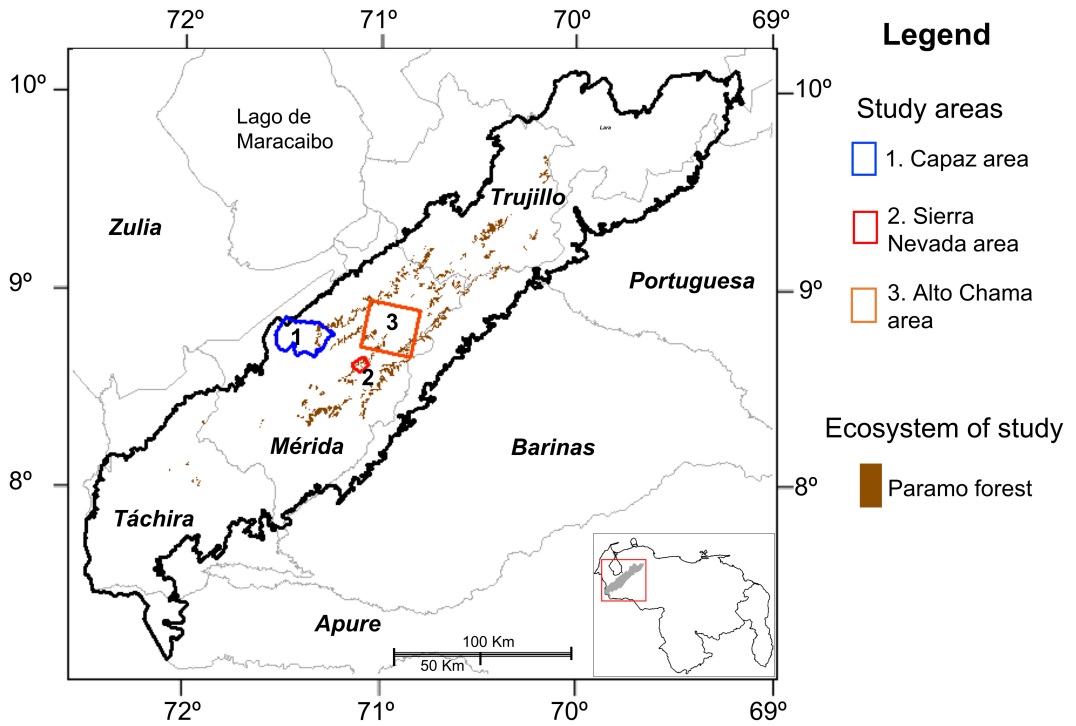


FIGURE 1 | Map of the Venezuelan Andes Ecoregion (Cordillera de Mérida) into the national context. State political divisions and names are presented. The three study areas are shown as well as the main ecosystem of study in this work.

a time sequence and percentages values of the rate change and accumulated area for each period.

Vertical Ecosystems Rate Displacement

We select on the map ten lines for the different transitional sequences of change (**Figure 2**). We create a profile diagram of the ecosystems sequence in line with the horizontal and vertical distances for each transect. The altitude data was recorded from a Digital Elevation Model (DEM) of the study area (Chacón-Moreno and Suárez del Moral, 2020). The range-limit average for the Páramo, Páramo forest, and Forest, for 2009 and 1952, was calculated. Based on the profile diagrams, we make measurements of the vertical and horizontal displacement to transition to Forest from the Páramo and the Páramo forest and create an index of change between ecosystems' horizontal and vertical displacement. We calculate the displacement dividend index as the vertical displacement between the horizontal displacement, multiplied by 100 m, and divided between the change period's years. We calculate the index's average for the transition from Páramo to Forest, and Páramo forest to Forest.

Sierra Nevada National Park Study Case: Páramo Forest Densification

The second study is a temporal comparison of Forest and Páramo forest densification between 1952 and 1998 on the treeline zone into the Sierra Nevada National Park, without human intervention. The study area has 44,169 ha and is located within the National Park (UTM coordinates 942984-951037 N and 266982-276012 E), and corresponds to the La Aguada sector with an altitude range from 2,500 to 4,000 m (**Figure 1**). The Sierra Nevada National Park is a broad legal protected area of the Andes, founded in 1952. From this date, the study area does not have any human intervention, and some small agriculture areas from 1952 have recovery in a successional process.

Vegetation Maps

The three main ecosystems: Mountain forest, Páramo Forest, and Páramo, were classified and described in detail based on the vegetation types of each one. Therefore, vegetation types are the principal spatial unit considered in this study case. We interpreted aerial photographs from 1952 to 1998 to obtain vegetation maps with detailed forest density in the transitional zone (treeline) following the methods described in Santaella (2007). A sequence of aerial photographs of the mission A-34, scale 1:40,000, the year 1952, and mission 010493 scale 1:65,000, the year 1998, were used. Aerial photographs were georeferenced using 92 geographic points, of which 40 were taken from topographic maps, and 52 points got hold of in the field. We create a digital elevation model (DEM) based on a map of level curves and the geographical points taken in the field. This DEM was used to correct the preliminary georeferencing of both images and created ortho-photomaps with an error of 8.7 m. We interpreted the ortho-photomaps of both dates and define and characterize the vegetation units based on the differentiation of the main photo-elements (tone, texture, and geometry of the photos) (Pernía, 1989). We carried out field trips

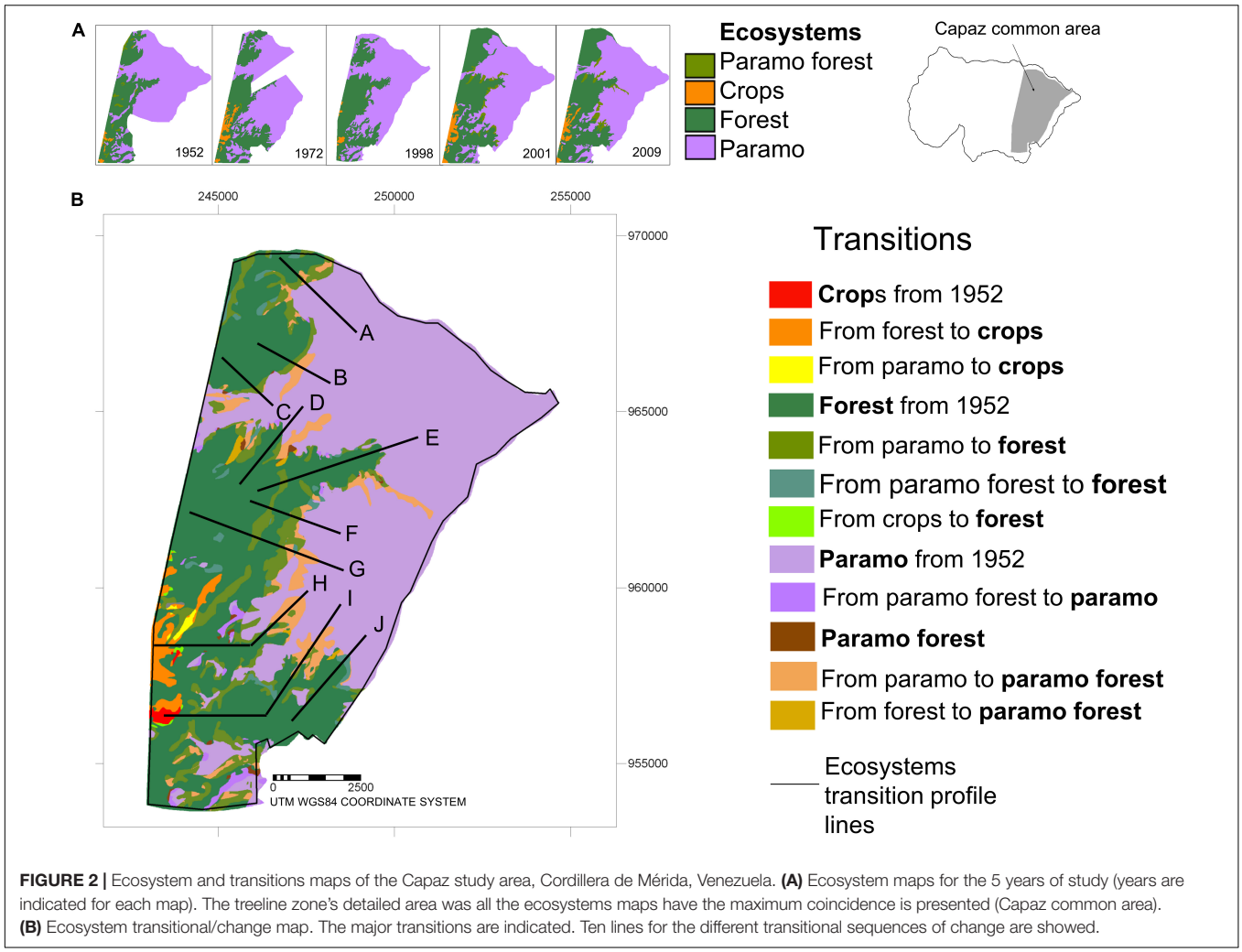


FIGURE 2 | Ecosystem and transitions maps of the Capaz study area, Cordillera de Mérida, Venezuela. **(A)** Ecosystem maps for the 5 years of study (years are indicated for each map). The treeline zone's detailed area was all the ecosystems maps have the maximum coincidence is presented (Capaz common area). **(B)** Ecosystem transitional/change map. The major transitions are indicated. Ten lines for the different transitional sequences of change are showed.

to the whole study area to check and confirm the vegetation units' descriptions previously identified in the aerial images. Two vegetation maps were obtained, showing the areas corresponding to each vegetation unit in both periods 1952–1998.

Matrix of Change

We compare, between both periods, the areas and percentages of each vegetation unit for the study area, as well as the spatial changes that occurred concerning the original surfaces (1952) of each vegetation unit and the area comprehensive study. An overlap was made with the vegetation units corresponding to both periods to analyze the map generated with the vegetation units replaced between 1952 and 1998.

Alto Chama Study Case: Páramo Forest Land Use Impact

The third study evaluated Andean ecosystems' potential distribution in the Chama River's upper basin (Alto Chama) in the Venezuelan Andes. We focused on the human impact on the Páramo forest ecosystem. The study area responds to the totality of a Miranda satellite image (**Figure 1**). It was taken in 2014 for the Venezuelan Andes and occupied approximately 77,792.4 ha. The altitude range between 2,500 and 4,200 m. The study area covers the municipalities Justo Briceño, Miranda, Cardenal Quintero, Rangel, Santos Marquina, Libertador, and Caracciolo Parra y Olmedo.

Ecosystems Map

We elaborate on an ecosystem map of the area following the approach and methods described in Josse et al. (2009); Chacón-Moreno et al. (2013), and Paredes (2018). The preliminary map of ecosystems was made using a supervised digital classification (Chacón-Moreno et al., 2013) based on a Miranda satellite image (Four bands: red, blue, green, and infrared) spatial resolution of 10 m, dated January 22, 2014. The image's possible ecosystems were defined by analyzing the combination of bands and colors and expert knowledge to determine the possible types of vegetation (coverage). The spectral signatures of the different ecosystems identified in the satellite image were created, starting from at least 30-pixel samples for each one. The spectral signatures were evaluated and statistically verified. A supervised parametric classification was generated using the maximum

probability algorithm. Subsequently, the classified image was filtered and homogenized (Paredes, 2018). The map uses the ecosystem class definition derived from Josse et al. (2009) and Chacón-Moreno et al. (2013). The preliminary map of the ecosystems was checked in fieldwork, using corroboration of the information and establishing control points for greater precision. In this study case, we made a differentiation between Upper montane páramo shrublands and rosettes, and High-Andean páramo rosettes and cushion vegetation, but in the two previous study cases were included in the Páramo ecosystem or Páramo vegetation class. That is because the 10 m image resolution gives us the possibility to define in detail these ecosystems.

Ecosystems Potential Map

We created a map of ecosystems potential distribution for the study area based on the climate envelope model following the methods described in Chacón-Moreno (2007); Suárez del Moral and Chacón-Moreno (2011), and Paredes (2018). From the ecosystem map, the spatial coordinate points of the presence of each ecosystem were extracted as sampling points to correlate with the environmental variables (calibration phase): Temperature (T), Precipitation (Pp), and Ombrothermal Index (Io), which were spatialized for the Andes (Chacón-Moreno and Suárez del Moral, 2020). The spatial information was crossed to generate a data or attribute table that shows the information of these three elements in each of the selected ecosystems. Box plots were constructed to define the ranges of distribution of ecosystems concerning each variable (Envelope method). The box plot represents the minimum and maximum data values on a rectangle (box), aligned horizontally or vertically. Each of the axes corresponds to an environmental variable on which the hypothesis of its correlation with ecosystems' distribution is hypothesized. From these graphs, the predictive equation of the model is determined. We elaborate on the map of the potential distribution of ecosystems based on the maps of environmental variables and the equations of the predictive models of the distribution of ecosystems (climate envelope model). We replace the environmental variables described in the equations with the environmental maps using logical operators of geographic information system (Chacón-Moreno et al., 2007). The ecological definitions and description of the statistical and mathematical procedures are presented in Ter Braak and Prentice (1988), Jongman et al. (1995), Ter Braak and Looman (1995), and Chacón-Moreno et al. (2013). We create an error matrix to compare the quality and sensitivity of the model, using a new data set of points (20 per ecosystem) in the same way as those obtained in the calibration phase (Congalton et al., 1983; Congalton, 1991; Janssen and van del Wel, 1994; Jenness and Wynne, 2007). We elaborate on a comparison map and matrix of change of the ecosystems potential distribution concerning the actual ecosystems distribution.

RESULTS

The landscape ecology approach used in the research allowed us to obtain maps of vegetation and ecosystems, with different time scales, to evidence the impacts of global change in the treeline's spatial dynamics in the Venezuelan Andes. The results address the processes of treeline migrations through change and transformation in a longtime sequence, and to know and understand the densification process as a driver of the treeline upward, all these under a climate change framework. Finally, we present the impacts of land use on the Páramo forest.

Capaz Study Case: Páramo Forest Migration

We present a sequence of ecosystem distribution maps for the Capaz study area's five dates (**Figure 2A**). The area corresponds with the five maps' coincidence zone at the treeline zone, ranging altitude gradient from 2,500 m (at the west side) to more than 4,000 m (at the east side). Maps from 1952 to 1972 have areas where data (aerial photographs) is missing for that zone. Following the sequence, we can observe that the Forest area is increasing over the Páramo area, and the Páramo forest is the smallest strip between the two large mentioned ecosystems.

We show a summary map of the landscape changes along the 57 years from 1952 (**Figure 2B**), where remark two main landscape transformation processes. The first one corresponds with the migration or displacement of the Forest ecosystem along the altitudinal gradient (from west to east), where areas than were Páramo ecosystem at the beginning of the study period (1952) change to Páramo forest or Forest ecosystems. Areas observed in the contact o transitional zone between "Páramo from 1952" and "Forest from 1952" in the map are showed as "from Páramo to Páramo forest" and "from Páramo to Forest." It is clear that the sequence of change from Páramo to Forest is gradual and includes the pass for the Páramo forest ecosystem. The second landscape transformation is replacing the Forest ecosystem with a Crops system, observed on the map's southwest side. Even some small Páramo areas change to the Crops system. Few and small areas back Forest ecosystem from Crop.

The crossing of the maps of **Figure 2A** results in a matrix of change. We present, in a simplified way, the main differences from ecosystems and replacement systems established in 1952 to the transition ecosystems along the period (57 years) (**Table 1**). This table complements the map (**Figure 2B**), where each ecosystem or replacement system increases or decreases the area. The area for crops is very few; however, the more significant transition comes from the Forest at the lowest altitude. The Forest ecosystem's principal transformation comes from converting Páramo to Forest with more than 5% of the area in the first 45 years. The shift from the Páramo forest to Forest in each period is less than 0.6%. Páramo ecosystem increases the area very little, just less than 0.5% in two periods, and less than this quantity in other periods; mainly derived from Forest. Forest downward migration representing less than10% of the total rate of change in each period. Páramo forest is the ecosystem that increases, mainly from Páramo, in the last two periods. Pastures are a new ecosystem in the ended period.

We summarize the total changes in the landscape transformation for the whole period (**Table 2**). The Crops system increases its area in a small percentage related to the

TABLE 1 | Ecosystems transitions at the Capaz area, Cordillera de Mérida.

Ecosystems/Replacement systems	Derived from	Period	Area (ha)	Area (%)
Crops	Settled down	1952	29.7	0.29
	Forest	1952–1972	90.5	0.87
		1972–1997	5.3	0.05
		1997–2001	35.0	0.34
		2001–2009	38.2	0.37
	Páramo	1952–1972	18.1	0.17
Forest	Settled down	1952	3, 539.5	34.04
	Crops	1972–1997	3.1	0.03
		1997–2001	1.9	0.02
		2001–2009	6.3	0.06
	Páramo	1952–1972	388.6	3.74
		1972–1997	279.8	2.69
		1997–2001	98.6	0.95
		2001–2009	110.5	1.06
	Páramo Forest	1952–1972	41.7	0.40
		1972–1997	18.7	0.18
		1997–2001	2.9	0.03
		2001–2009	60.3	0.58
Páramo	settled down	1952	5, 015.1	48.24
	Forest	1952–1972	38.2	0.37
		1972–1997	1.8	0.02
		1997–2001	47.4	0.46
		2001–2009	1.2	0.01
	Páramo Forest	2001–2009	2.7	0.03
Páramo Forest	Settled down	1952	28.8	0.28
	Forest	1997–2001	27.1	0.26
	Páramo	1972–1997	19.1	0.18
		1997–2001	243.6	2.34
		2001–2009	199.5	1.92
Pastures	Settled down	1952	–	0.00
	Forest	2001–2009	3.8	0.04
Total			10, 397.0	100.00

It is derived from map crossing and ecosystem change matrix. The main ecosystems and its change and origin are indicated for each period. The total area and % of the area for each transition is shown.

entire study area, with a transformation rate of 3.1 ha per year. Forest is the ecosystem that more gain superficies, with more than 1,000 ha (9.7%) and a positive change rate of 12.7 ha per year. On the other hand, the Páramo ecosystem reduces its superficies by 12.1%, with a negative rate of 22.2 ha per year.

We elaborate on a model of the principal transitional states for the Capaz study area's ecosystems and trajectories of change for the different periods (**Figure 3**), based on the landscape change represented in the map and transition results (**Figure 2** and **Tables 1**, **2**). The magnitude and % rate of transformation is indicated. We observe that the many ecosystem transitions are from Páramo to Páramo forest and Forest. In the last two periods, the change from Páramo to Páramo Forest is larger than the shift from Páramo to Forest. In this transition model, we present the initial and final Forest area (total area in ha and %). The Forest ecosystem increases from 34 to 41% (7%), mainly from the transition from the Páramo. The transition from the Páramo forest to Forest is essential in the last period, with a change of 0.58%. The decreasing or losses in the Forest area are small; only the difference from Forest to Crops in the first period is essential (0.87%).

We show the profile diagrams of the main ecosystems sequence and the horizontal and altitude distance for the ten lines indicated in **Figure 2** (**Figure 4**). All the profiles have the same scale in altitude and horizontal axles, except for line J. We clearly can observe that all transects show a transitional sequence of ecosystems along the altitudinal gradient. In seven of the lines, the transition to Forest from Páramo is directly, and in three of the lines, this transition occurs through the Páramo forest. There is no transition in two lines (Line D and Line E); it means that the contact between Forest and Páramo reflects neutral changes in these two of ten lines (20%). We observe the occurrence of Crops at the lowest altitude of the transects. All the lines present a different slope; however, lines A, D, E, G, H, I, and J show a slope of around ∼20%. Lines B and F show a higher slope with a change of ∼30%. The average for the current lower limit for the Páramo ecosystem is 3352 ± 68 m ($\alpha = 0.1$), and the upper limit for the Forest registered for 1952 was 3280 ± 61 m ($\alpha = 0.1$).

TABLE 2 | Total area and % gain and loss for each ecosystem at the Capaz study area during the whole period (57 years from 1952 to 2009).

Ecosystems/Replacement systems	Units	Initial area	Gain	Loss	Total end of the period	Rate of change (ha y^{-1})
Crops	ha	40.9	187.0	11.3	216.7	3.1
	%	0.4	1.8	0.1	2.1	
Forest	ha	3,828.0	1,012.4	288.4	4,551.9	12.7
	%	36.8	9.7	2.8	43.8	
Páramo	ha	6,373.0	91.4	1,357.8	5,106.5	−22.2
	%	61.3	0.9	13.1	49.1	
Páramo forest	ha	155.1	489.3	126.3	518.0	6.4
	%	1.5	4.7	1.2	5.0	
Pastures	ha	0.0	3.8	0.0	3.8	0.1
	%		0.0	0.0	0.0	

A rate of change is calculated for each ecosystem.

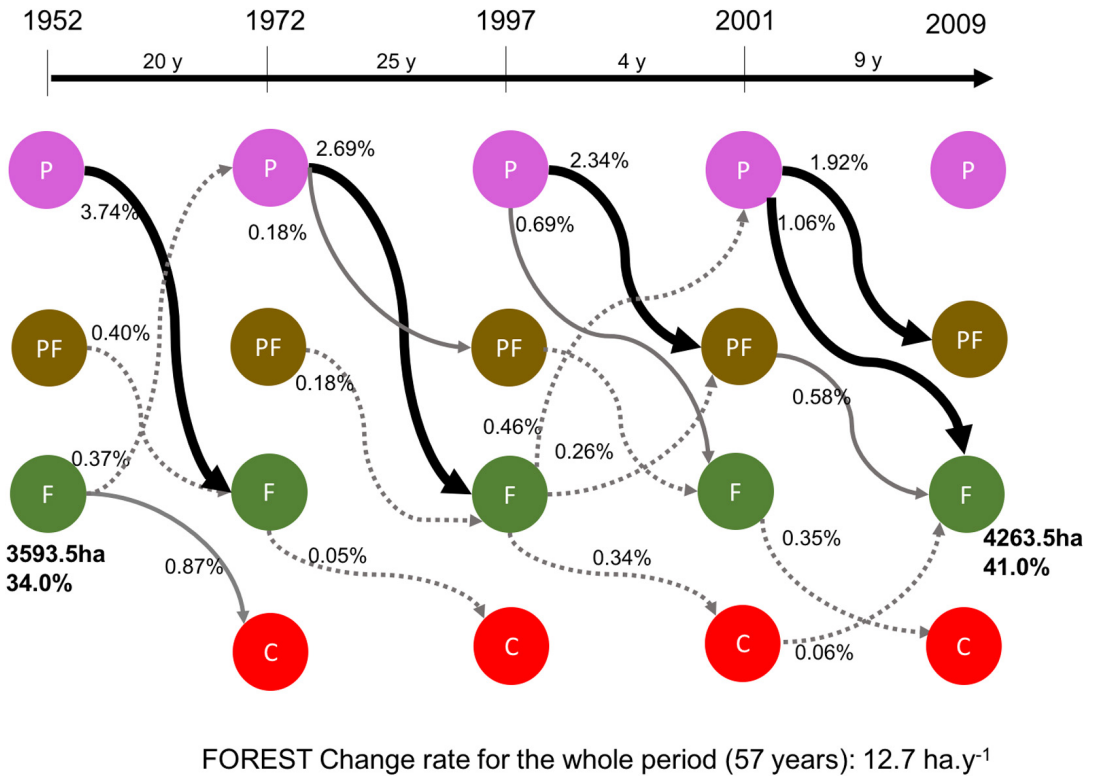


FIGURE 3 | Model of main ecosystems transition of the Capaz study area (P: Páramo, F: Forest, PF: Páramo forest, and C: Crops) and trajectories of change (arrows) of the different period of study for ecosystem and replacement systems (thick continuous black arrow % change >1, continuous thin gray arrow % change <1 and >0.5, thin dotted gray arrow % change <0.5 and >0.05, % <0.05 is not indicated). Accumulated % of change is indicated for each arrow and the total and % area for forest ecosystem at the beginning and the end of the whole period.

We found that the displacement of the treeline border, based on the difference between the current lower Páramo limit and the Forest upper limit for 1952, is 72.7 m in the 57 years of study, representing ∼12.8 m per decade.

The local features as slope and exposition and the microhabitat characteristics condition Forest's vertical displacement (Sedlacek et al., 2015, 2016). Therefore, we calculate the Forest and Páramo forest ecosystems' vertical displacement rate per 100 m of horizontal advance. The lines B and C have the transition in lower altitude related to the gradient, then these lines were not considered for calculations. We evaluate only 13 shifts (Five to Forest from Páramo; two to Forest from Páramo forest, and six to Páramo forest from Páramo). We found that the Forest ecosystem's vertical displacement derived from the Páramo ecosystem is 22.71 ± 9.75 m per decade per every 100 m of horizontal distance. For Páramo forest ecosystem derived from the Páramo ecosystem is 19.38 ± 8.91 m per decade per every 100 m of horizontal distance. For Forest ecosystem derived from the Páramo forest ecosystem is 31.81 ± 25.07 m per decade per every 100 m of horizontal distance. The transitions differences are

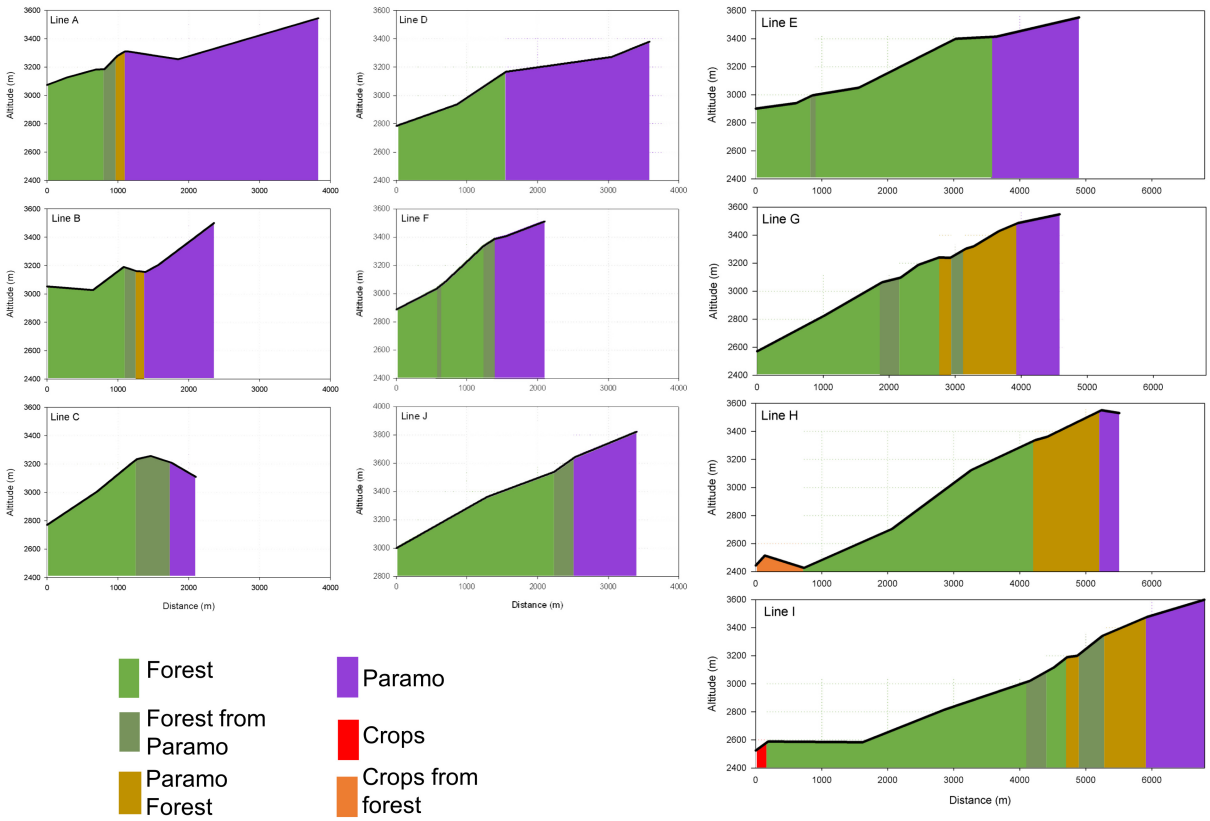


FIGURE 4 | Profile diagrams of the main ecosystems sequence along with the horizontal distance and altitude for the Capaz study area. Letter identification corresponds with the ten lines indicated in **Figure 2**.

not significant; consequently, we calculate the average for all 13 transitions evaluated. The vertical displacement is 22.57 ± 6.42 m per decade per every 100 m of horizontal distance.

Sierra Nevada National Park Study Case: Páramo Forest Densification

We present the vegetation maps for the Sierra Nevada National Park study area of 1952 and 1998 (**Figure 5**). We identified seven vegetation types in natural conditions, representing the three significant ecosystems in the study area. For the Páramo ecosystem, the vegetation types specified were Páramo vegetation and *Polylepis* forest (Josse et al., 2009; Chacón-Moreno et al., 2013). For the montane forest ecosystem, we define the Mountain cloud forest vegetation type. For the Páramo forest ecosystem, we differentiated four vegetation types, based on density and life form predominant: Low and dense Pre-páramo shrubland, Low and sparse Pre-páramo shrubland, High and dense Páramo forest, and High and sparse Páramo forest. In **Table 3**, we present a brief description of the vegetation types. Additional, seven secondary or replacement systems were recognized, associated with the main vegetation types: Low and secondary Pre-páramo shrubland, Low and secondary Pre-páramo shrubland in regeneration, Secondary Páramo, Secondary mountain cloud forest, High and secondary Páramo forest, High and secondary Páramo forest in regeneration, and Crops or fallow vegetation. These units correspond with areas used or replaced by human activities before the National Park establishment in 1952. The secondary vegetation has some degree of deterioration due to natural or anthropic causes. Abandoned areas do not have any management type; thus, it is in an early succession stage.

In both vegetation maps, 1952 and 1998, we observe the vegetation types' distribution along the altitudinal gradient (From northwest to southeast). We see the vegetation's variation, from the Forest to the Páramo, through the Páramo forest vegetation types. This variation goes from north to south, locating the cloud forest vegetation from northeast to the northwest of the study area. As one progresses further in a southeast and southwest direction, the vegetation types of dense and sparse high Páramo forest appear. Even more toward the south, the dense and sparse low Pre-páramo shrubland vegetation appears. Continuing in this direction emerges the Páramo vegetation, which is the predominant within the area. Together with the Páramo vegetation, the *Polylepis* forest comes into view.

In a comparison between the two maps, we observe a variation in the low and dense Pre-páramo shrubland vegetation type in the northwest of the study area, which has changed to high and dense Páramo forest. To the northwest of the study area, we can observe an evident displacement in the high and dense Páramo forest's altitude distribution limits, from 3,280 m in 1952

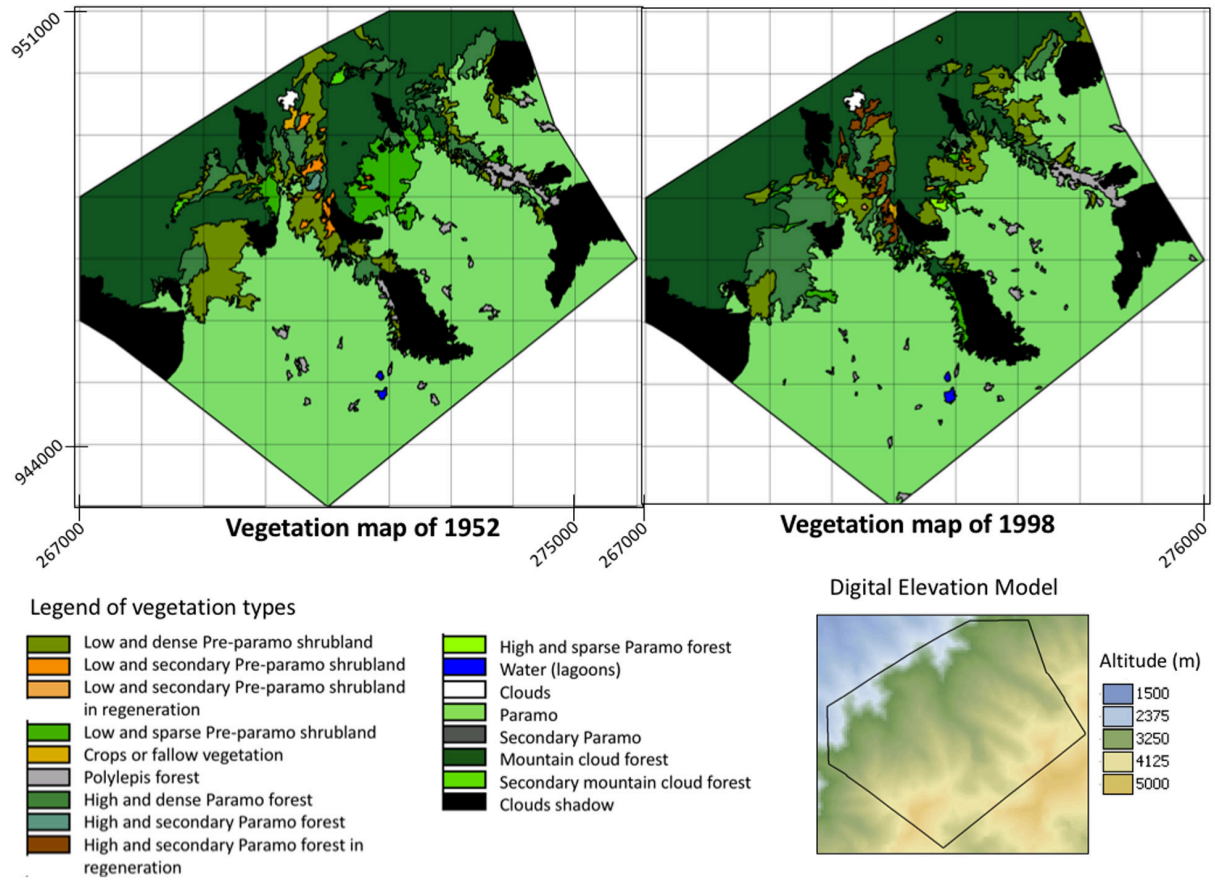


FIGURE 5 | Vegetation maps of Sierra Nevada National Park study area, for 1952 and 1998, Cordillera de Mérida, Venezuela. Legend of vegetation types is presented. The Digital Elevation Model (DEM) of the study area is shown, as well, the elevation gradient. Coordinates in the UTM system are indicated.

to 3,480 m in 1998. In the same way, we can notice a similar change toward the north and northwest. In a northeast direction, we can see how the dense Pre-páramo shrubland vegetation type is advancing on the Páramo. We observe the change in the south of the area from Low and Dense Pre-páramo shrubland and High and Dense Páramo forest vegetation types toward the *Polylepis* forest. Based on the results from **Table 4**, we found that the Páramo vegetation presented a variation of 0.5% concerning the area that this unit occupied in 1952, which is not a representative change in the total study area (0.2%). The cloud forest presented an increase of 5.3%, which implies an advance of this vegetation over other types, and at the level of the entire study area, it represents an increase of more than 1%. The secondary cloud forest size decreased by 90% compared to 1952, although the total area proportion is very low (0.2%). The Páramo forest vegetation presented an essential increase in its size, with more than 50% compared to 1952, and that represents 2.2% of the study area. While the shrub's vegetation types showed a decrease in their area for Low and dense Pre-páramo shrubland, Low and secondary Pre-páramo shrubland, and Low and sparse Pre-páramo shrubland of 23, 85, and 68%, respectively, related to presented in 1952. In Low and sparse Pre-páramo shrubland, this represents more than 2% of the study's total surface area. Secondary vegetation decreases the size in favor of the vegetation types in regeneration.

Related to the Páramo vegetation, this changed to the forest vegetation in 48 ha. Another 146 ha of this unit was occupied by the high and dense Páramo forest, which indicates another advance in successional stages and colonization of shrub species that could reach Forest's climax state. Besides, 398 ha of Low and dense Pre-páramo shrubland occupy the previous Páramo vegetation.

Alto Chama Study Case: Páramo Forest Land Use Impact

We present the ecosystems map for the Alto Chama study area (**Figure 6A**). We recognize and define seven ecosystems: Upper montane páramo shrublands and rosettes, High-Andean páramo rosettes and cushion vegetation, Páramo forest, Montane cloud forest, *Polylepis* forest, Evergreen dry Páramo forest, and Pre-páramo shrubland. These ecosystems' classification considers rosette diversity, which results from an adaptation process to this tropical high-altitude landscape (Madriñán et al., 2013; Cortés et al., 2018; Pouchon et al., 2018). Also, three replacement systems: Pine plantation, Pastures

TABLE 3 | Description of vegetation types for the Sierra Nevada National Park area, based on the vegetation maps for 1952 and 1998, Cordillera de Mérida, Venezuela.

Major ecosystem	Vegetation type	Brief description
Páramo	Páramo	Natural formation with a predominance of shrub and rosette life forms. As well as cushion plants and herbaceous cover. There is no continuity of the bush canopy, and the plants do not exceed three meters in height
	Polylepis forest	Forest composed practically only of populations of the species *Polylepis sericea*. *P. sericea* is the only tree species in Venezuela that grows between 3,400 and 4,600 m of altitude.
Páramo forest	Low and sparse Pre-páramo shrubland	Bush plant life form is dominant. Heights are less than 5 m and strongly branched from the base. The coverage of canopy is 25–50%. That is because there are spaces between plants or patches. There are some herbaceous plants. Little presence of rosettes compared to Páramo vegetation.
	Low and dense Pre-páramo shrubland	Bush plant life form is dominant. Heights are less than 5 m and strongly branched from the base. The coverage of canopy is greater than 75%. They are difficult to access because of the closed shrub canopy.
	High and sparse Páramo forest	Clear dominance of tree species. At least 5 m high. The coverage of the canopy is 25–50%. That is because there are spaces between the plants or patches.
	High and dense Páramo forest	Clear dominance of tree species. At least 5 m high. The coverage of the canopy is greater than 75%.
Montane forest	Montane cloud forest	Vegetation with a continuous distribution of trees and a variety of arboreal stratification. Mixed composition with a great variety of epiphytic plants. This forest is characterized by being evergreen. The trees have a canopy between 10 and 30 m high.

TABLE 4 | Vegetation types change in the Sierra Nevada National Park between 1952 and 1998.

Vegetation type	Area in 1952 (ha)	Area in 1998 (ha)	Change (ha)	Change (%)	Total change (%)
High and dense Páramo forest	1,887	2,857	970	51.4	2.2
High and secondary Páramo forest	62	14	−48	−77.4	−0.1
High and secondary Páramo forest in regeneration	0	393	393	100.0	0.9
High and sparse Páramo forest	0	80	80	100.0	0.2
Low and dense Pre-páramo shrubland	3,115	2,395	−720	−23.1	−1.6
Low and secondary Pre-páramo shrubland	246	35	−211	−85.8	−0.5
Low and secondary Pre-páramo shrubland in regeneration	0	8	8	100.0	0.0
Low and sparse Pre-páramo shrubland	1,370	431	−939	−68.5	−2.1
Crops or fallow vegetation	55	63	8	14.5	0.0
Polylepis forest	598	513	−85	−14.2	−0.2
Páramo	21,520	21,628	108	0.5	0.2
Secondary Páramo	0	0	0	0.0	0.0
Mountain cloud forest	9,910	10,433	523	5.3	1.2
Secondary mountain cloud forest	96	9	−87	−90.6	−0.2
Clouds. clouds shadow and lagoons	5,299	5,299	0	0.0	0.0

The total area for each vegetation type in each year is indicated. The % of change is related to the vegetation type and the change concerning the total study area.

and fallow vegetation, and Crops. Paredes (2018) presents a further description of the ecosystems and the replacement systems. There is a high correlation between the distribution of ecosystems and the primary environmental variables. For all cases, the first is the relationship with the temperature (altitudinal gradient, **Figure 6C**), which determines in the first instance the development of each ecosystem; precipitation is another determining factor in the location of ecosystems. In the narrow area between the two National Parks, it is noticeable that Pastures and fallow vegetation, and Crops (replacement systems) occupy this space in the middle of the map following the Chama River course.

We show the map of ecosystems potential distribution of the Alto Chama study area (**Figure 6B**). The potential ecosystem follows similar distribution to the observed for the ecosystems map; however, it shows a significant difference, especially about the extension of the ecosystems related to the ecotone between Upper montane páramo shrublands and rosettes and Montane cloud forest. We observe that the gradient extremes' ecosystems: High-Andean páramo rosettes and cushion vegetation, and Montane cloud forest, present a high similitude with the ecosystems map. To the left side of the Chama River (Southwest area), the forest and woody ecosystems offer more extension on the Upper montane páramo shrublands and rosettes ecosystem. It is essential to emphasize the large extension of the shrublands and páramo forest ecosystem, where today, the Crops and Pastures and fallow vegetation replacement systems are present.

Comparing both maps (**Figures 6A,B**), we found for the Upper montane páramo shrublands and rosettes, that Crops occupy the potential distribution in 7.18%. It can be considered

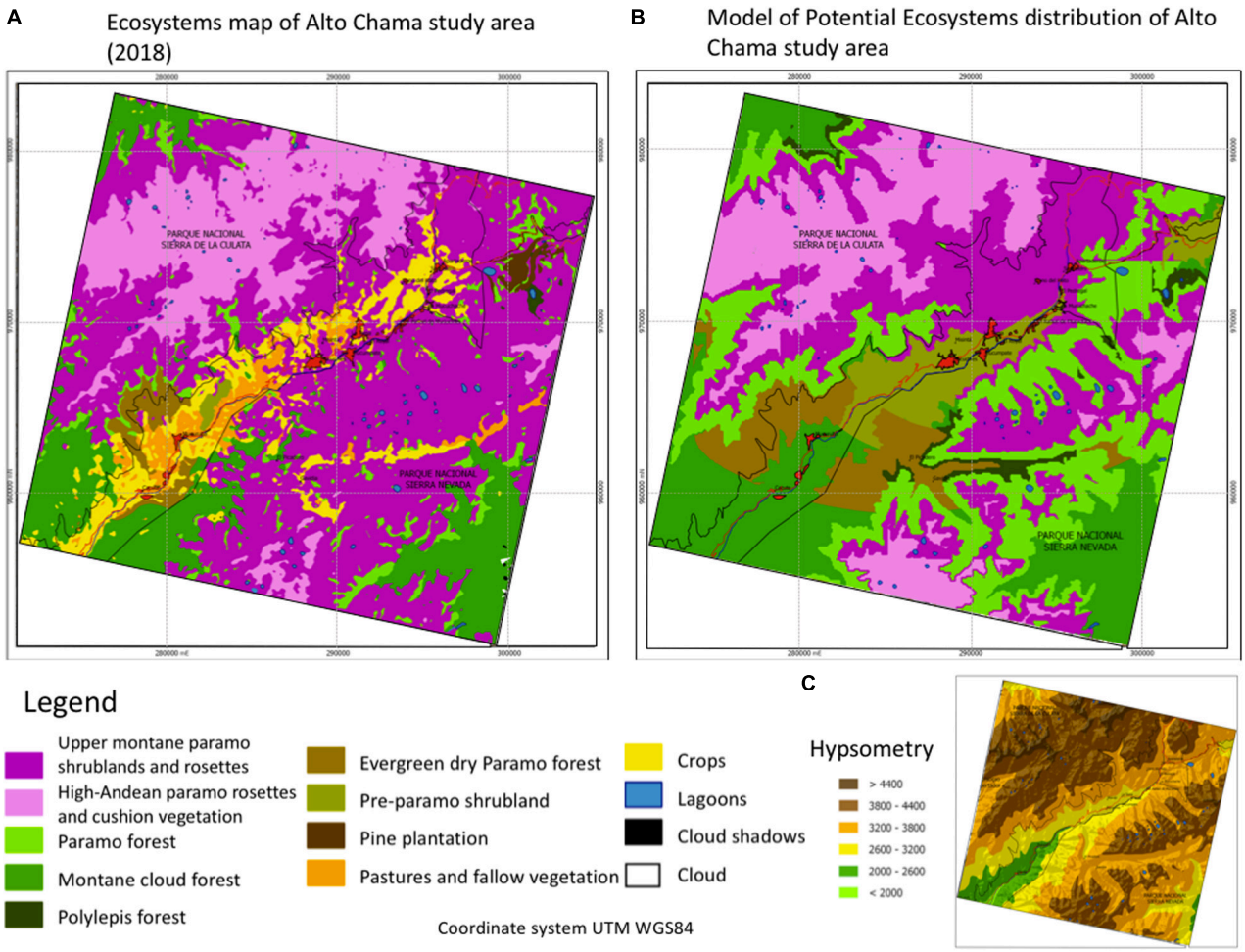


FIGURE 6 | (A) Ecosystems map, **(B)** Map of ecosystems potential distribution, and **(C)** Hypsometry map, for the Alto Chama study area, Cordillera de Mérida, Venezuela. The ecosystem types legend and of the study area are showed. Coordinates in the UTM system are indicated.

moderate change due to the rapidity with which this activity is occurring. Páramo forest is the ecosystem that presents a significant shift from potential to actual distribution. Currently, only 14.63% coincides with the potential total, showing an evident difference in its coverage, which is being occupied, mainly, by the Upper montane páramo shrublands and rosettes (72.53% of the potential area). The Montane cloud forest maintains 62.85% of the total potential; however, the main change is for replacement systems of Crops (13.20%) and Pastures and fallow vegetation (5.74%). Both replacement system sums a total substitution of 18.94% over forest cover. The Evergreen dry Páramo forest ecosystem maintains 13.50% of its potential coverage. Still, the rest of this ecosystem's potential area is mainly occupied by the Montane cloud forest (35.06%) and by the Upper montane páramo shrublands and rosettes (24.15%). The Pre-páramo shrubland ecosystem only conserves 1.58% of the total potential. The main changes of this ecosystem are to Upper montane páramo shrublands and rosettes (46.20%), to the Crops (16.47%), and Pastures and fallow vegetation (17.01%).

DISCUSSION

In this work, we have related and analyzed different study areas to give a comprehensive response to global change, limited by two of the significant impacts: climate change and deforestation. The first, showing a change in the increase in temperature, but without a clear tendency to rainfall distribution, with future continuity and uncertain consequences on vegetation migration. The other, deforestation, which shows a possible complete disappearance of the Páramo forest ecosystem by human action. Using the landscape ecology approach, we have been able to integrate and detail the processes of change and future transformations in the line of contact between the montane forest and the Páramo (treeline) in the Venezuelan Andes.

Páramo Forest Transition and Displacement

The temporal window of more than five decades and the possibility of combination aerial photographs and satellite images

under the landscape ecological approach for the Capaz area permit a detailed analysis of the woody vegetation movement along the altitudinal gradient. The displacement of the Montane cloud forest and Páramo forest ecosystems over the Páramo ecosystem is evident. Also, the localization of the study areas in protecting zones (National Parks) indicates that these changes have not direct human influence from almost the beginning of the study period.

Our results confirm that the treeline is a zone where the transition between the Montane cloud forest to Páramo is abrupt (Körner and Paulsen, 2004). However, the Páramo forest's spatial dynamics contribute to establishing a gradual change at a large spatial scale, with a diversity of spatial patterns (Bader et al., 2020). The difference (**Figure 2B**) involves the Páramo forest ecosystem presence in different vegetation structures and compositions. This sequence of transition state makes possible the advance of the forest to more elevated positions.

The rate of change in forest growth is less than the speed of loss of Páramo, and this is due in no small part to the development of the transition stages that include the Páramo forest (**Table 2**). This change rate further evidence the diversity of vegetation types associated with the Páramo forest (**Figure 5** and **Table 3**).

During the five decades of study, the woody component's movement showed in both Capaz and Sierra Nevada areas that the successional process is key in this displacement. The forest's increase is best evidenced near the river's margin, where humidity and possibly temperature conditions are more favorable for the forest movement to higher altitudes. We determine that the densification process occurs when shrubland areas in 1952 change to dense Páramo forest, and this dense Páramo forest of 1952 change to the montane cloud forest in 1998. In these successional stages, we defined four different vegetation types (Low and dense Pre-páramo shrubland, Low and sparse Pre-páramo shrubland, High and dense Páramo forest, and High and sparse Páramo forest), which can be assemblage in a transitional model (**Figure 7**). Works describing forest distribution patterns at the treeline show that tree density decreases with altitude due to reduced forest cover (Ramírez et al., 2009; Schwarzkopf et al., 2010; Llambí et al., 2014). These authors suggest that the forest tongues and islands function as nuclei of advancing woody pioneers of the forest toward the Páramo. Arzac et al. (2011) detected clear patterns of change in the abundance of life forms regarding altitude and aspect. Trees and ferns decreased in abundance abruptly with the increase in altitude and distance to the continuous forests. Besides, this can lead to shrubification that generates a change in ecosystems' structure and functioning. A large-scale increase in shrub cover will change ecosystems' structure and alter the flows of energy, regional climate, the exchange of water, carbon, and nutrients between the soil and the atmosphere, and ecological interactions between species. Increases in shrub are classified into three categories: (a) infill, an increase in shrub cover through the growth of currently existing shrubs; (b) increased growth potential; and (c) advanced shrub line, or colonization of areas beyond the previous distribution boundary (Myers-Smith et al., 2011).

Our results show that there is a matching between the forest upward shift and densification processes, and probably these processes are related to the highest plants diversity in the Andes mountain (Llambí et al., 2014; Llambí, 2015), and the number of plant life forms present in the treeline (Arzac et al., 2011, 2019). A similar observation is found for alpine treeline ecotone's spatial dynamics in the French Pyrenean (mainly of *Pinus uncinata*) (Feuillet et al., 2020), where an upward shift of the tree line and significant tree densification was observed. Still, they found a mismatch between these two ecological processes in 40% of the studied sites. They propose some hypotheses based on their results, mainly associated with the environmental factors (slope, soil, altitude) and the anthropogenic disturbances in the past. Bader et al. (2007) observed that the temperature fluctuations were much more extensive for the tropical alpine treeline. The average temperature was higher in alpine vegetation than in forests, and most treelines were abrupt. They explain that abruptness could result from positive feedback processes mediated by the microclimate differences between forest and Páramo. Recently, Bader et al. (2020) propose a framework for describing alpine treeline ecotones, where a diversity of spatial patterns contain information about the processes that control treeline dynamics. Therefore, our results show a spatial way that could drive the treeline dynamics. Some hypotheses have proposed to try to explain the treeline limit: – factors such as stress and mechanical damage could be affecting reproductive capacity, growth, and the acquisition of carbon (Körner, 1998); – low temperatures limit cell division as well as tissue differentiation during growth (Körner, 1998; Jobbágy and Jackson, 2000); – the temperature is the main factor associated with this disruption of tree vegetation (Körner, 1998; Jobbágy and Jackson, 2000; Hoch and Körner, 2003; Körner and Paulsen, 2004); – the high radiation leads to high evapotranspiration rates that are not necessarily compensated by the availability of water in the soil (Mayr et al., 2003; Smith et al., 2003). Accordingly, based on our results, we can say that climatic warming plus other indirect causes associated with this are principal factors driving forest migration. These associated factors revert those that controlling the treeline limit.

The newly colonized areas by the forest displacement and densities changes modify the microclimatic factors in the understory. These new microclimatic conditions permit to improve the physical and environmental features for a subsequent occupation of species that previously did not occupy these spaces (Sedlacek et al., 2016; Gentili et al., 2020; Zellweger et al., 2020). This increase in the density of woody vegetation increases the air's relative humidity by modifying radiation exposure in the air and soil. Similarly, the interception capacity of horizontal precipitation (fog) is rising since the contact surface increases, possibly allowing species with a higher water requirement to colonize these spaces (Correa et al., 2020). The soil organic matter may also increase since the types of vegetation such as the shrubland, Páramo forest, and the Montane cloud forest produce a more significant amount of necro-mass and organic matter (Little et al., 2016). This decomposition process increases the availability of nutrients and allows species with a higher nutrient requirement to colonize Páramo spaces that could not occupy previously because of nutrient stress. Open areas give opportunities and conditions for tree regeneration through

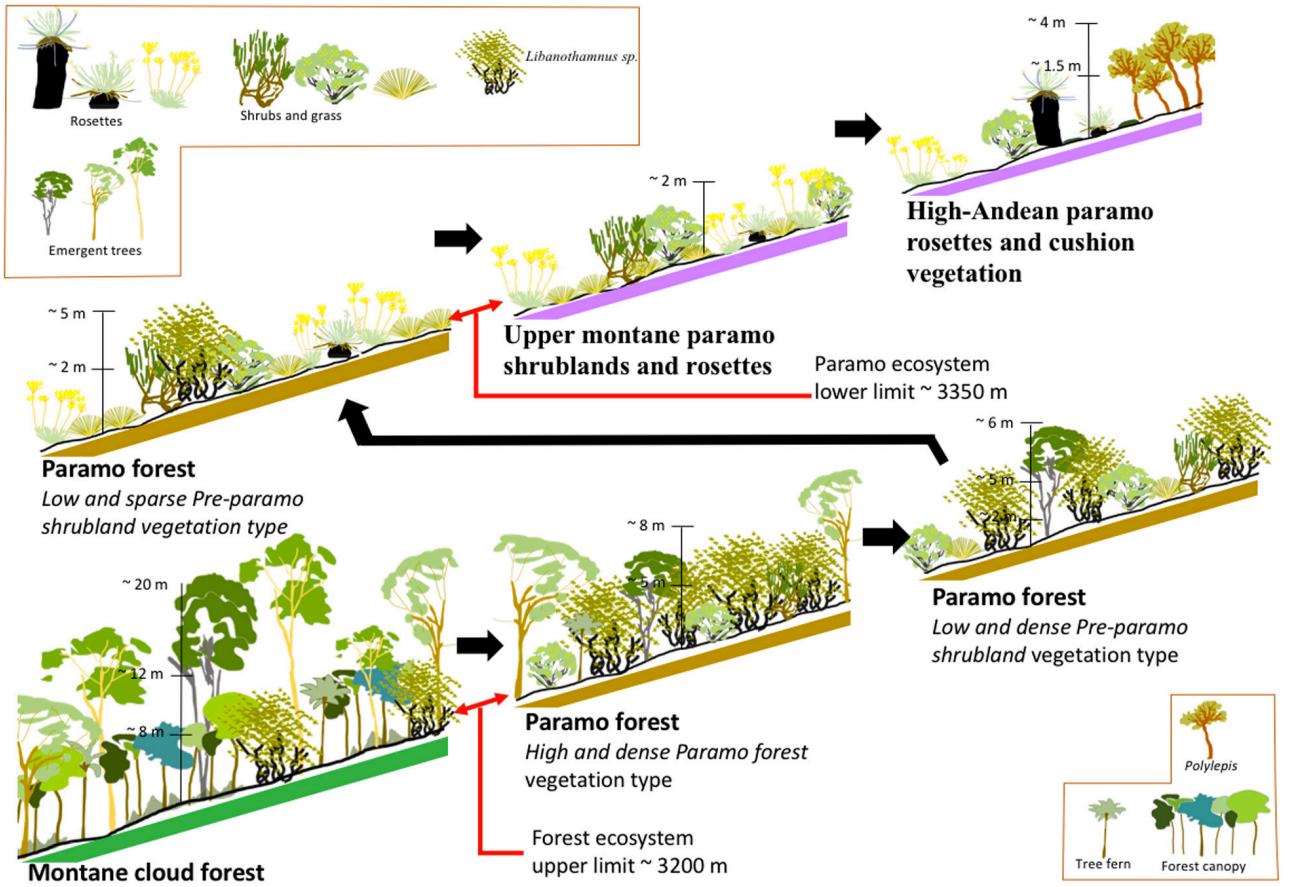


FIGURE 7 | Synthesis of the transition and displacement of the different ecosystems and vegetation types for the treeline zone in Cordillera de Mérida, Venezuela. It presents six profiles representing the four main ecosystems and three vegetation types for the Páramo forest ecosystem. Each profile shows the vegetation structure-based on the primary plant life forms, height of the canopy, and representative plants. Slope ~ 30% is indicated.

adaptation traits. Cierjacks et al. (2008) found that important regeneration traits in *Polylepis* species in the tropical tree line of Ecuador might contribute to the formation of the upper tree line.

Profiles lines (**Figure 4**) are no similar. That gives us a vision of heterogeneity; it means that the displacement of woody vegetation is not regular and linear; instead, topography, slope, and exposition make a difference in the area. Slopes of 20–30% in the Capaz area are considerably higher than the average for Sierra de la Culata with ~11%. This higher slope could harm the tree establishment; however, the montane forest's upward migration still occurs.

We elaborate the transition and displacement synthesis scheme of the different ecosystems and vegetation types, using and combining the further studies' results (**Figure 7**). It contains six profiles representing the four main ecosystems and three vegetation types for the Páramo forest ecosystem. Each profile shows the vegetation structure based on the principal plant life forms and the canopy and representative plants' height. Also, each profile is drawing with a slope of about 30%. The change from one profile to another is gradual; however, we can observe the difference in the composition of the life forms and the spaces open when the tree form is replaced by shrubs and grasses, increasing the presence. Montane cloud forest has a predominance of tree forms, with many emergent trees. The altitude ~ 3,200 m is the upper limit of the Montane cloud forest and the Páramo forest ecosystem's starting area, represented by three previously described vegetation types, similar to the border defined for Josse et al. (2009) for these ecosystems. The sequence in these three profiles shows how the tree life form decreases in predominance instead of *Libanothamnus* sp. and other shrub species. At the third Páramo forest profile, the woody plant density decreases, and some Páramo characteristic species appear. At the end of the highest Páramo Forest ecosystem profile, the altitude is ~3,350 m of altitude; it is mean that the wide belt for the Páramo forest is about 150 m vertically. The last two profiles in this sequence characterize the structure and predominance of plant life form for Páramo. Rosettes and shrubs represent the Upper montane páramo as a predominant life form, and the High-Anden páramo, with tall rosettes (*Coespeletia timotensis*), cushion vegetation, and *Polylepis* forest. Llambí (2015) define and describe similar structure pattern, and make a distinction about the Páramo forest with three different vegetation structure: (a) continuous Páramo forest; (b) the languages and islands of forest and their edges of contact

with the Páramo; and (c) the Upper montane páramo, which is represented by rosettes, grasses, and shrubs at different altitudes.

We observe that the treeline downward migration occurs with rates of change less than 10%, and ∼20% of the areas in the treeline area do not present changes, which it means is a neutral change. These results are coincident to the review about species migration of Lenoir et al. (2010). They founded that ∼65% of the species had shifted their mid-range positions upslope, 10% have not changed, and 25% had shifted downslope. Lenoir et al. (2010) suggest that downward range shifts of species primarily occur by a release from competition independently of climate warming.

Páramo Forest and Climate Change

One of the global change anthropogenic drivers that impact the biosphere is climate warming, which can alter the competitive dynamics, especially following the relaxation of lethal cold that suppresses cold-intolerant species as in the tree line disturb the timing of migration (Sage, 2020). Climate warming for the decade 2006–2015 has been of 0.87 (0.75–0.99) °C average about the period 1850–1900, with an increasing estimated anthropogenic global warming at 0.2°C per decade from the period 2006 to 2015 (IPCC, 2018). Minimum temperatures in Venezuela have increased by 0.37°C per decade, and precipitation has decreased from 1940 to 2002 (MARN, 2005). For the six decades of the study period (1950–2010), the temperature based on the global climate warming data (GCW) has increase 0.45°C (∼0.08°C per decade) (IPCC, 2013). However, considering the Coordinated Modeling Intercomparison Project Phase 5 (CMIP5) output for Venezuela, climatic warming (VCW) has a rate of 0.06°C per decade (IPCC, 2013). For the Mérida-airport meteorological station (the only station with a large number of records in the study area), the temperature data shows an increment between 1951 and 1990 (40 years) of 0.18°C per decade (MCW), which is higher compared with Venezuelan (VCW) and global data (GCW). Still, this data presents a higher interannual variability. Quesada-Román et al. (2020) indicate that climate-vegetation relationships in alpine systems play a fundamental role in the growth of dominant shrub species in Central America's Páramo ecosystems due to precipitation changes, temperature, and El Niño-Southern Oscillation (ENSO). The primary source of climate variability in the northern Andes is the ENSO phenomenon, characterized by hot (ENSO) and cold phases (La Niña or ANTI/ENSO). Both phases affect the temporal and spatial distribution of precipitation in a large part of South America. They have been the cause of extreme droughts and extraordinary rainfall in different geographical regions of the planet. In the northern Andes, hot phase events are associated with below-normal precipitation levels, while the opposite occurs during cold phases (Herzog et al., 2010).

If we considering the relation temperature – altitude for the montane areas in Venezuela of −0.564°C for 100 m of elevation (Chacón-Moreno and Suárez del Moral, 2020) and the climatic warming of 0.08°C per decade; it represents an altitudinal displacement of temperature during the period between 1950 and 2010 of 14 m per decade. However, if we use the Mérida-airport rate of warming of 0.18°C per decade, the displacement will be 32 m per decade. Our results show an increment of forest migration of about 13 m per decade, which has good concordance with the expected change due to the GCW data. Nevertheless, comparing with the MCW rate, forest migration has less than half of the possible displacement. The inter-annual variation of rainfall during the period study (Mérida-airport meteorological station) is more considerable than predicting climate change scenarios. The three study areas present a similar precipitation pattern, ranging between 1,800 and 2,100 mm per year at the treeline zone, and similar bioclimate (Pluvial Hyperhumid and Pluvial Ultrahyperhumid) (Chacón-Moreno and Suárez del Moral, 2020). Therefore, our results for the three areas are comparable for these parameters.

For our data derived from all 13 evaluated transitions, the value is more realistic because it considered the possibility of advance in 100 horizontal meters. It takes into account the slope (20–30%) and other local environmental conditions. The range of vertical displacement found of 16.15–28.99 m per decade per every 100 m of horizontal distance is slightly higher than the potential displacement considering the GCW rate. However, it is less than the possible displacement for the MCW rate.

If we considered an intermediate potential range of climatic warming and the correspondence potential vertical displacement between the GCW and MCW ∼23 m per decade, our results from the transect's detailed study are very similar: 22.6 m per decade. It means that the forest ecosystems migrate a little faster than climatic warming, only considering that the forest can horizontally displace 100 m. Nevertheless, if we considered only the whole forest ecosystems vertical displacement (14 m per decade), the rate of forest migration is lower than the potential of warming. This rate is realistic because we include the successional processes for forest ecosystems, explained in **Figure 7**.

Morecroft and Keith (2016) review and summarize the observed changes in treeline by climate change. They proposed that regardless of the migration mechanism, natural tree lines are determined primarily by the climate's warming. The IPCC (2014, 2018) remark, based on field observations over the past century in numerous locations in boreal, temperate, and tropical ecosystems, that biomes shifts, that this movement is due by the replacement at a place of one suite of species by another. This effect is usually of biomes moving upward in elevation and to higher latitudes.

Several studies present results about the treeline migration in the temperate zones, especially in Alpine areas, with evidence of changes in plants' altitudinal distribution (Walther, 2003; Walther et al., 2005). Peñuelas and Boada (2003), based on historical records, vegetation maps, and aerial photographs, found a progressive replacement of cold-temperate ecosystems by Mediterranean ecosystems; this upward shift of vegetation belts has upward by ∼70 m, with a rising annual temperature of 1.2–1.4°C during 50 years. However, the tree line shifts are subject to time lags in their response to environmental change because of their long generation time (Morecroft and Keith, 2016). Lenoir et al. (2008) found a significant upward shift in species optimum, averaging 29 m per decade. From plant surveys in 1954–1958 and 2003–2005, in the alpine to nival ecosystems in Italy, Parolo and Graziano (2008) register a median migration rate of 23.9 m per decade. This migration rate appears to be related to an estimated shift of the permafrost limit by +240 m during the last 50 years; they considered the climate warming a primary cause of the

observed upward migration of high mountain plants. In a study of the treeline dynamics in response to climate warming in the Mediterranean region of Cehennemdere, Turkey, Arekhi et al. (2018) found the geographical expansion of the treeline, with a migration rate of 20–40 m each decade, and the treeline altitude increased to 2,442 m from 2,335 m. In a study based on the supervised classification of historical (1953) and current (2015) aerial photographs on the spatial dynamics of the ecotone of alpine trees in the French Pyrenees, Feuillet et al. (2020) observed an upward displacement of the tree line by $\sim$ 45 m on average, with at an overall rate of 0.73 m year^{-1}. Furthermore, during the study period, the minimum mean air temperatures increased by 0.6°C, and the maximum mean air temperatures increased very slightly by 0.14°C.

Telwala et al. (2013) surveyed historical (1849–1850) and the recent (2007–2010) data on temperature and endemic species in elevational ranges at the alpine Sikkim Himalaya region and recorded a mean upward displacement rate of 27.53 $\pm$ 22.04 m per decade, which make to the montane floras, in particular, highly sensitive to climate change.

There are few studies about the Andes' forest migrations in response to climate change. Lutz et al. (2013) measured Peruvian Puna's treeline migration (Manu National Park) between 1963 and 2005. They found a rate of the treeline migration in protected areas of 2.4 m per decade, which is almost ten times lower than our results, showing a grassland-forest ecotone remarkable stable over the 42 years study period. Fadrique et al. (2018) use a community temperature index (CTI) to characterize communities and test the species migration prediction. The thermophilization, a progressive decline of cold mountain habitats and their biota (Gottfried et al., 2012) as the transition from cloud forest to open alpine grasslands (Páramo) at high elevations, show the thermophilization rates across elevations are affected by ecotones' presence. This study indicates that climate warming is causing rapid shifts in many tropical trees (Fadrique et al., 2018). In another study focused on the treeline of Cordillera de Mérida, Arzac et al. (2019) evaluate the response of plant life form to environmental conditions above the upper montane forest in the Venezuelan Andes and model their potential distribution under warming scenarios. They found that tree cover will sharply increase with increasing elevation, while shrub and caulescent rosette distribution will differ depending on their traits.

These studies about treeline in alpine temperate regions show similar migration patterns, and our results for tropical alpine treeline are not much different from this. However, it is essential to remark that the tropical treeline's spatial migration patterns could differ. The mechanism of densification of woody vegetation could explain the vegetation adaptation to the impact of global warming. The switch between the woody and non-woody component of the treeline is abrupt for most of the contact edges between the Páramo forest and the Páramo, and the rapid climate warming leads to a fast densification process. Therefore, we face a possible disappearance or narrowing of the Páramo forest ecosystem that serves as an ecotone between the Mountain forest and the Páramo.

Venezuela will expect an increase of 5°C and a decrease in rainfall on scenarios of greenhouse gas emissions and intermediate climate models (MPPEA, 2017). ACFIMAN (2018) indicates a possible increase in Venezuela's temperature in an unfavorable scenario (RCP8.5) of a maximum of 5°C and a decrease of 23.5% precipitation. The country is currently not prepared to mitigate or face any climate variability because the country's vulnerability to climate change is too high. Based on integrated climate models within the framework of the fifth phase of the Coordinated Modeling Intercomparison Project Phase 5: CMIP5 (IPCC, 2013), it elaborates on the possible climate change scenarios for the Venezuelan Andes. These scenarios show that for a moderately optimistic scenario (RCP 4.5), we could have, by the end of the century, temperatures 2–3°C above the average of the reference period (1986–2005) and decreased nearby rainfall to 0.1 mm day^{-1}.

Suárez del Moral and Chacón-Moreno (2011) determine at a regional scale the distribution model for Páramo forest in climate change scenarios based on the UKTR models developed by the United Kingdom Meteorological Office (UKMO) and the CCC from the Canadian Centre for Climate Modelling and Analysis (CCCMA). The climatic change CCCMA scenario found that it predicts more significant impacts on the Páramo forest distribution, with more considerable fragmentation in the forest continuum around the mountain range. The UKTR scenario, on the other hand, presents fewer impacts on the distribution of the forest and predicts displacement toward higher elevations without loss of forest toward the upper limit; in comparison with the CCCMA model, which predicts forest displacement toward lower elevations with the disappearance of areas toward the upper limit.

Páramo Forest Deforestation

The deforestation processes in the world are a well-documented problem; however, it has focused on lowland areas, where the forest's extensions are significant (Verburg et al., 2015; Aide et al., 2019). Andes forest (woody component) has been analyzed by many works at the regional and local levels, focusing on the montane forest ecosystem. Aide et al. (2019) evaluated the change in the distribution of woody vegetation in the tropical Andes of South America for 2001–2014. They found that the extensive loss of forest occurred in areas with less than 1,500 m of altitude. However, it increased the forest vegetation more than losses when the altitude was superior to 2,000 m. Also, Pérez-Escobar et al. (2018) report that mining is a potential threat to the Páramo forest, in some cases disproportionately excluded from Colombia's protected areas; this activity involves the involuntary displacement of human settlements and large-scale fragmentation and loss of habitat, thus threatening the extinction of endemic flora and fauna.

As we see in our results, we have two different processes of landscape change by deforestation. The first one corresponds with the agricultural advance over the montane cloud forest found in the Capaz study area to elevation under 2,500 m. The demographic growth, which translates into increased social pressure, is the more critical deforestation factor. Comparing the transformation rates of mountain forests reported in the bibliography with those calculated, our results with loss of forests in the basin between 1 and 1.3%, have very similar speeds to other countries as Vietnam 0.63%, Mexico 0.7%, and Bolivia

1.3%, and similar to the national average of deforestation (1.2%) (Rodríguez-Morales et al., 2009).

The second deforestation process is associated with treeline ecosystems. We found that the Evergreen dry páramo forest, the Pre-páramo shrubland, and the Páramo forest ecosystems, practically have been replaced by the Pastures and fallow vegetation, and the Crops (**Figure 6B**). This deforestation or landscape change is very few documented, probably, because the range and cover of these páramo forest ecosystems are very narrow. On the other hand, there are no multitemporal comparison studies due to these ecosystems' land use from ancient times. Monasterio (1980b) describes that for the climatic zone where we find the potential distribution of treeline ecosystems between 2,800 and 3,800 m of altitude, land use was based on potato and wheat crops from pre-hispanic and colonial times, respectively. The wheat was expanded in many areas with similar conditions of the potential treeline (mainly climate conditions) during the 16th and 17th centuries in the Cordillera de Mérida. Isolated areas remain current with the same peasant agricultural practices for wheat (De Robert and Monasterio, 1993, 1995; Monasterio, 2002). Since the middle of the 20th century, these areas have been used mainly for potato and vegetable crops, including irrigation and high mechanical technology (Velázquez, 2001). This modern technology allows the increase of agricultural activities with greater intensity.

In some cases, it gives the possibility of advancing the farming frontier on new lands for a human disturbance. Vásquez et al. (2015) show that the effect of human disturbances on plant species composition was determined by accessibility and significantly affects plant diversity by agricultural advance. However, the creation of the National Parks Sierra Nevada in 1952 and Sierra de La Culata in 1989 has slowed these agricultural frontier advances at higher altitudes.

Based on the potential distribution of ecosystems, our results show a historical transformation process of the Páramo forest, masked by not having recent spatial data on these forests' distribution. Also, it allows us to infer from a transformation process of the Andean landscape by understanding the distribution of crops such as wheat throughout the region.

Study Limitations and Future Recommendations

This work's primary limitations and methodological caveats are related to the combination of aerial photographs from the different missions and scales and satellite images, which makes the homogeneity of criteria very difficult, and some spatial data do not match. The three studies considered in this work have differences related to the vegetation and ecosystem definition; then, the homogenization and standardization of descriptions and names were hard work. The few financial resources to do fieldwork, reduce the number of collecting data, and the possibility to detail the environmental and social variables took into the field. The lack of meteorological stations with long data records and many stations' dismantling in the 21st century prevent a better interpretation of Andes' climatic data. Venezuela's current and terrible economic, social, and political situation makes difficult the fieldwork and data collecting, and the research in the country universities. However, we integrated these results in a coherent approach.

This work makes it possible to visualize an ecosystem's transformation that often goes unnoticed at the global, subcontinental, or even national scale due to its extension in the spatial context. Hence, it is necessary to implement collaborative international projects to monitor the landscape transformation at a regional and local level.

New trends and tools have to be incorporated into the research to understand the forest migration affected by climate change. The first is to consider the natural variation of abiotic and biotic factors along mountain elevation gradients. In this case, Tito et al. (2020) propose to take advantage of natural gradients to help us gain a complete understanding of the potential impacts of climate change on species, communities, and biodiversity. Second, to Include modern ecological modeling (Fulton et al., 2019), which is a crucial tool to understand vegetation and ecosystems' migration in climate change scenarios. Models of plant species distribution for the Páramo ecosystem have been made (Peyre et al., 2019, 2020), which need complete and adequate databases. Therefore, monitoring vegetation (Peyre et al., 2015; Cuesta et al., 2017) and landscape transformation studies are significant in implementing those models. Third, the use of new remote sensing classification techniques to know the distribution of species at a large scale, based on drones and hyperspectral images (Garzón-López and Lasso, 2020). The potential of this technology will help research in the tropical high-altitude ecosystems, to improve treeline prediction, expand existing databases (Peyre et al., 2015), make changes of scale and models (Fulton et al., 2019) improve, monitor, and detect threats to for these ecosystems. A fourth aspect to consider is the microscale studies, which allow us to understand the colonization process of plant species in confined spaces where the microclimatic characteristics are highly variable. Studies such as those carried out by Cortés et al. (2014); Ramírez et al. (2015), Sedlacek et al. (2016); Hupp et al. (2017), Gentili et al. (2020); Llambí et al. (2020), Zellweger et al. (2020), and Llambí et al., submitted allow the analysis of migration processes of mountain forest and High-Andean vegetation at microscales.

The impact of climate warming and deforestation on the Páramo forest as a representative ecosystem of the treeline shows us that this study is necessary for an integrated global change adaptation approach. This approach has to include nature-based solutions at regional and local scales to conserve the biodiversity of the High Mountain Ecosystems of the Andes region.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EC-M study conception, design, critical revision, and drafting of the manuscript. EC-M, MR-M, PS, DP, and AA acquisition

of data, analysis, and interpretation of the data. All authors contributed to the article and approved the submitted version.

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Climate Change Affects Vegetation Differently on Siliceous and Calcareous Summits of the European Alps

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The alpine life zone is expected to undergo major changes with ongoing climate change. While an increase of plant species richness on mountain summits has generally been found, competitive displacement may result in the long term. Here, we explore how species richness and surface cover types (vascular plants, litter, bare ground, scree and rock) changed over time on different bedrocks on summits of the European Alps. We focus on how species richness and turnover (new and lost species) depended on the density of existing vegetation, namely vascular plant cover. We analyzed permanent plots (1 m × 1 m) in each cardinal direction on 24 summits (24 × 4 × 4), with always four summits distributed along elevation gradients in each of six regions (three siliceous, three calcareous) across the European Alps. Mean summer temperatures derived from downscaled climate data increased synchronously over the past 30 years in all six regions. During the investigated 14 years, vascular plant cover decreased on siliceous bedrock, coupled with an increase in litter, and it marginally increased on higher calcareous summits. Species richness showed a unimodal relationship with vascular plant cover. Richness increased over time on siliceous bedrock but slightly decreased on calcareous bedrock due to losses in plots with high plant cover. Our analyses suggest contrasting and complex processes on siliceous versus calcareous summits in the European Alps. The unimodal richness-cover relationship and species losses at high plant cover suggest competition as a driver for vegetation change on alpine summits.

Keywords: bedrock, elevation gradient, GLORIA, microscale, surface cover type, species richness, species turnover, vascular plant cover

INTRODUCTION

Alpine plants are highly sensitive to global warming (Kammer et al., 2007; Grytnes et al., 2014; Rixen and Wipf, 2017; Winkler et al., 2019). With rising temperatures, species from lower elevations have been expanding their ranges upward (Rumpf et al., 2018), leading to an accelerated increase of species richness on mountain summits (Steinbauer et al., 2018) and a "thermophilization," i.e., a directional shift in community composition in favor of warm-affinity species, of mountain plant communities (Vittoz et al., 2008; Erschbamer et al., 2011; Gottfried et al., 2012; Pauli et al., 2012; Wipf et al., 2013; Unterluggauer et al., 2016). Warmer temperatures and a longer growing season have been enhancing plant productivity and causing a greening trend at high elevation during the last decades (Carlson et al., 2017; Rogora et al., 2018; Filippa et al., 2019). Since competitive species with a higher growth rate are particularly benefiting from warming (Farrer et al., 2015; Winkler et al., 2019) it is expected that slow-growing, cold-adapted species are threatened by competitive displacement (Alexander et al., 2015) and/or physiological constraints (Larcher et al., 1998; Marcante et al., 2012, 2014; Lamprecht et al., 2018) over time. Species distribution models predict drastic losses of such cold-adapted species by the end of the 21st century (Dirnböck et al., 2011; Engler et al., 2011; Dullinger et al., 2012). Recent studies in the Central Austrian Alps have already shown losses and decrease in cover of cold-adapted species from permanent plots (Lamprecht et al., 2018; Steinbauer et al., 2020). However, species distribution models might overestimate losses, as extinctions are still rarely encountered in revisitation studies in the European Alps (Kulonen et al., 2018).

The highly heterogeneous relief of alpine terrain (Nagy and Grabherr, 2009) forms a mosaic of closely located distinct climatic "micro-refugia," which might locally reduce or postpone extinction risks of alpine species (Scherrer and Körner, 2011; Graae et al., 2018). While, on the mesoscale of a whole summit, suitable microhabitats for new and resident species could be available side by side for a long time, especially when taking into account the contrasting temperature regimes between aspects (Winkler et al., 2016), open space for low-competitive alpine plants might get overgrown on the microscale. Here, the availability and distribution of surface cover types such as vascular plants, litter, bare ground, scree or rock might be relevant for species establishment. Open microsites such as rock crevices and scree could function as seed traps, providing new colonization areas (Graae et al., 2011). Kulonen et al. (2018) found that successfully colonizing high-alpine summit plants were most abundant on scree. In contrast, high-alpine species with a prevalence on organo-mineral substrate showed decreasing frequencies over time, suggesting competitive interactions in alpine microhabitats with favorable growing conditions (Kulonen et al., 2018).

The difference in species composition between siliceous versus calcareous bedrock types in the European Alps is an essential driver of the high plant diversity in the alpine region (Grabherr and Mucina, 1993; Wohlgemuth, 2002; Virtanen et al., 2003) and has been the focus of numerous early studies in plant ecology (Gigon, 1971 and references therein). The two bedrocks form contrasting landscape morphologies, typically with large rock walls and extensive scree slopes in calcareous regions, and dense grasslands up to high elevation in siliceous regions (Reisigl and Keller, 1994). Gigon (1971, 1987) and Kinzel (1983) found that most of the competitive species from siliceous grassland communities were inhibited by an excess of calcium ions, and by iron and phosphorus deficiency when they were grown on a calcareous substrate. Calcareous grassland species, on the other hand, were inhibited by an increased solubility of toxic aluminium ions and heavy metals due to a low pH, and – above all – by root competition of calcifuge species when they were grown on a siliceous substrate. These processes point toward a higher level of competition within siliceous plant communities and a higher level of specialization to abiotic conditions in species from calcareous substrates. Additionally, the different erosion potential (i.e., stronger weathering of calcareous bedrock due to carbonate dissolution) and different soil forming processes (Veit, 2002) most likely lead to more open and diverse surface cover types at calcareous sites, thus slowing down vegetation development, preventing the dominance of competitive species, and allowing a higher species richness (Wohlgemuth, 2002; Virtanen et al., 2003) and species turnover.

Whether vegetation in siliceous or calcareous regions will be more strongly affected by climate change is still an open, but highly relevant question in terms of conservation. Dense and competitive communities in siliceous regions might hamper new colonizers from low elevation and thus remain relatively stable, whereas open microsites on calcareous bedrock might facilitate the installation of new species and thus enhance a vegetation change. In the present study, we investigated divergences of ongoing vegetation changes between bedrock types, specifically focusing on the relationship between species richness/species turnover and vascular plant cover on calcareous and siliceous summits. On the microscale, a unimodal relationship of species richness and vascular plant cover might indicate increasing competition in dense vegetation (Grytnes, 2000). We analyzed monitoring data from 1 m × 1 m permanent plots, covering a 14-year period from three siliceous and three calcareous GLORIA (Global Observation Research Initiative in Alpine Environments[1]) regions across the European Alps (total of 24 summits). We assumed that warming effects on vegetation vary in magnitude (i) between siliceous and calcareous mountains, and (ii) along a gradient from low to high vascular plant cover. Specifically, we address the following three topics and inherent hypotheses:

(1) Surface cover types: (1a) Cover of vascular plants and litter is higher on siliceous bedrock, whereas on calcareous bedrock the cover of bare ground, scree, and rock is higher and, as a consequence, more open and competition free microsites should be present here. (1b) Over time, vascular plant and litter cover increases at the expense of bare ground, scree and rock, however more strongly on siliceous bedrock.

[1]www.gloria.ac.at

(2) Species richness: (2a) Species richness shows a unimodal relationship with increasing vascular plant cover and highest values at intermediate plant cover. (2b) Species richness increases over time, however at a slower pace on siliceous bedrock.
(3) New species and lost species: (3a) With an increasing vascular plant cover the number of lost species increases more than the number of new species. (3b) The number of lost species exceeds the number of new species at sites with high vascular plant cover, more so, on siliceous than on calcareous bedrock.

MATERIALS AND METHODS

Study Regions and Study Design

Our dataset comprised 24 summits from the GLORIA initiative across the European Alps, in three regions on siliceous and calcareous bedrock, respectively (**Table 1** and **Supplementary Figure 1**). Each region contained permanent plots on four different summits situated along an elevation gradient from the treeline to the subnival ecotone (treeline, lower alpine, upper alpine, subnival). The summits were mostly located in protected areas and were chosen to avoid pressure from human land use (i.e., tourism or intense grazing by livestock or anthropogenically strongly raised numbers of wild ungulates; Pauli et al., 2015; **Supplementary Table 1**). Species composition was surveyed in each permanent plot at least twice between 2001 and 2017 following the methodology in Pauli et al. (2015). To standardize elevation across different regions, we used the elevation of a summit relative to the position of the regional treeline (defined at the first survey as the highest elevation where groups of trees taller than 3 m occur), instead of absolute elevation (**Table 1**). On each summit, a quadrat cluster of 3 m × 3 m was established 5 m below the peak in each of the four cardinal directions. In each of the four 1 × 1 m corner plots of each quadrat cluster (in total 345 plots), percent cover was estimated for all occurring vascular plant species and for four different surface types, namely vascular plants (living), litter (dead plant material), bare ground (earthy or sandy surface), scree (unsealed rocks and debris material larger than sand fraction), and solid rock (outcrops and large boulders). As an attempt to minimize phenological variations, the first survey and resurvey were done in peak season. For the analyses, the scree and rock surface cover types were aggregated into one common substrate type "scree & rock," due to some potential ambiguity in the classification (larger boulders were sometimes classified as rocks and sometimes as scree) and because scree included a larger range of sizes (cm to m), with various potential for seedling establishment. Cover changes of cryptogams were excluded from the analyses due to their higher variability in cover estimation (Vittoz et al., 2010) and ambiguity in the classification. Due to inaccessible terrain on some summits, not all aspects could be equally documented (**Table 1**). After the baseline surveys from 2001 to 2003, monitoring was repeated approximately every seven years. To estimate long-term trends, we compared the first and the last survey (called resurvey hereafter) to gain the longest possible time interval. Time intervals varied from 12 to 14 years (**Table 1**).

Data Analyses

Climate

Annual Chelsa climate data[2] combined with data from CRU (Climate research unit[3], Harris et al., 2014) were downscaled to a resolution of 100 m (see **Supplementary Material** for the detailed downscaling process). To investigate different temperature trends between regions or summits over the last 30 years the mean summer temperature (June, July, August) was regressed on year, allowing different trends for each region or summit. Serial autocorrelation was considered with Newey West standard errors (Newey and West, 1987).

Surface Cover Types

The dependence of the percentage of the surface, covered by the four surface cover types (see study design), on (1) bedrock (siliceous, calcareous), (2) aspect (south, east, west, north), (3) elevation (distance to treeline), and (4) first survey/resurvey was investigated. Separately for each surface cover type, a mixed beta regression (with logit link) was used. A beta regression is appropriate for dependent variables that are percentages varying between zero and one (Ferrari and Cribari-Neto, 2004; Simas et al., 2010). The percentage values were transformed using the formula $\frac{y^*(n-1)+0.5}{n}$ (Smithson and Verkuilen, 2006), where y is the percentage value of the surface cover type (divided by 100), and n is the number of observations. The variables "bedrock," "aspect," "distance to treeline", and "first survey/resurvey" were used as fixed effects. In addition, summits nested in regions, i.e., distance to treeline were considered as random intercepts. All two-way interaction effects were included in the model. Thus, the final model looked like:

$$
\begin{aligned}
&g\left(E\left[y^{type}\right]\right)\\
&= (\beta_0 + U_0) + \beta_1\ bedrock\ + \beta_2\ aspect\\
&+ \beta_3\ distance_to_treeline + \beta_4\ bedrock\ \#\ distance_to_treeline\\
&+\beta_5\ first_survey/resurvey + \beta_6\ first_survey/resurvey\ \#\ bedrock\\
&+ \beta_7\ first_survey/resurvey\ \#\ distance_to_treeline + \varepsilon,
\end{aligned}
$$

where $g(\cdot)$ is an appropriate link function (logit link) and $E[\cdot]$ the expectation; y^{type} is the transformed ratio of the specific surface cover type following the beta distribution; the intercept consists of the overall intercept β_0 together with the random intercept (U_0) considering the summit nested in the region; the βs capture all fixed effects and two way interaction effects; # denotes an interaction effect between the corresponding variables; ε is the remainder noise.

To visualize the trends in surface cover types, we predicted mean cover values and their 95% confidence intervals for a gradient of distance to treeline from the smallest to the highest value per bedrock type in steps of 10 m.

[2]http://chelsa-climate.org/
[3]https://crudata.uea.ac.uk/cru/data/hrg/

TABLE 1 | Geographical distribution (GLORIA region, country: AT = Austria, CH = Switzerland, IT = Italy; mountain range), bedrock (Si = siliceous, Ca = calcareous), summit designation (combined of the region's three-digit code and the summit number in ascending elevation), number of 1 m × 1 m permanent plots, year of first survey/resurvey, elevation (in m), and relative position to the treeline of the 24 inventoried summits (Dist. treeline = elevation above the treeline, in m).

GLORIA region (country)	Mountain range	Bedrock	Summit	Plots (n)	First survey	Resurvey	Elevation (m a.s.l.)	Dist. Treeline (m)
Texelgruppe (IT)	Central Alps	Si	TEX-1	15	2003	2017	2180	30
Texelgruppe (IT)	Central Alps	Si	TEX-2	13	2003	2017	2619	469
Texelgruppe (IT)	Central Alps	Si	TEX-3	11	2003	2017	3074	774
Texelgruppe (IT)	Central Alps	Si	TEX-4	11	2003	2017	3287	987
Swiss National Park (CH)	Central Alps	Si	SN2-1	16	2003	2015	2424	24
Swiss National Park (CH)	Central Alps	Si	SN2-2	16	2003	2015	2519	119
Swiss National Park (CH)	Central Alps	Si	SN2-3	16	2003	2015	2797	397
Swiss National Park (CH)	Central Alps	Si	SN2-4	16	2003	2015	3104	704
Alps of Valais-Entremont (CH)	Western Alps	Si	VAL-1	16	2001	2015	2360	110
Alps of Valais-Entremont (CH)	Western Alps	Si	VAL-2	16	2001	2015	2550	300
Alps of Valais-Entremont (CH)	Western Alps	Si	VAL-3	8	2001	2015	2989	739
Alps of Valais-Entremont (CH)	Western Alps	Si	VAL-4	12	2001	2015	3212	962
Swiss National Park (CH)	Central Alps	Ca	SN1-1	16	2002	2015	2438	38
Swiss National Park (CH)	Central Alps	Ca	SN1-2	16	2002	2015	2542	142
Swiss National Park (CH)	Central Alps	Ca	SN1-3	16	2002	2015	2836	436
Swiss National Park (CH)	Central Alps	Ca	SN1-4	16	2002	2015	3092	692
Hochschwab (AT)	Eastern Alps	Ca	HSW-1	16	2001	2015	1910	60
Hochschwab (AT)	Eastern Alps	Ca	HSW-2	16	2001	2015	2065	215
Hochschwab (AT)	Eastern Alps	Ca	HSW-3	16	2001	2015	2214	364
Hochschwab (AT)	Eastern Alps	Ca	HSW-4	16	2001	2015	2255	405
Dolomites (IT)	Southern Alps	Ca	ADO-1	16	2001	2015	2199	99
Dolomites (IT)	Southern Alps	Ca	ADO-2	14	2001	2015	2463	363
Dolomites (IT)	Southern Alps	Ca	ADO-3	15	2001	2015	2757	657
Dolomites (IT)	Southern Alps	Ca	ADO-4	15	2001	2015	2893	793

Species Richness

With the number of vascular plant species present in the 1 m × 1 m plot, trends in plant species richness were analyzed. The relationship of species richness with vascular plant cover, bedrock, aspect, and first survey/resurvey was analyzed using a general linear mixed model (GLMM) with a negative binomial distribution appropriate for count data and overdispersion. Vascular plant cover was included both as linear and quadratic term to be able to model a unimodal relationship with decreases at higher values of vascular plant cover. The relationship was allowed to be different with respect to bedrock. Random intercept and slopes per region were taken into account, as this quadratic relationship differed between regions. The model was structured thus:

$$
\begin{aligned}
&g\left(E\left[y\right]\right)\\
&= (\beta_0 + U_0) + \beta_1 bedrock + \beta_2 aspect\\
&+ (\beta_3 + U_3)\, vascular_plants + (\beta_4 + U_4)\, vascular_plants^2\\
&+ \beta_5 bedrock \,\#\, vascular_plants + \beta_6 bedrock \,\#\, vascular_plants^2\\
&+ \beta_7 first_survey/resurvey + \beta_8 bedrock \,\#\, first_survey/resurvey\\
&+ \varepsilon,
\end{aligned}
$$

where $g(\cdot)$ is the appropriate link function (log link) and $E[\cdot]$ the expectation; y indicates species richness following a negative binomial distribution; the intercept consists of the overall intercept β_0 together with the random intercept variable for the region (U_0); random slopes are denoted with the corresponding random variables U_3 and U_4; the symbol # denotes an interaction effect between the corresponding variables; ε indicates the remainder noise.

The visualization was realized as described for surface cover types; species richness and 95% confidence intervals were predicted dependent on the continuous predictor variable "vascular plant cover" in steps of 1%.

Number of New and Lost Species

To further explore changes in richness patterns, the number of new species gained, and the number of species lost between the first survey and the resurvey were computed per plot. New species were defined as the species present in a plot in the resurvey, but not present in the first survey. Lost species were defined as species absent from a plot at the time of resurvey, but present in the first survey. New and lost species of every plot were checked and, in case of suspected determination problems (e.g., *Alchemilla, Hieracium)*, they were merged to species aggregates to avoid pseudo-turnover. No exotic species were on the summits. The relationship between the number of new or lost species and vascular plant cover, bedrock, and aspect was analyzed using GLMMs with a negative binomial distribution to account for overdispersion. A random intercept and slope per region was taken into account. Vascular plant cover values of the first survey were used (models using the values of the vascular plant cover of

the resurvey gave qualitatively the same results). The model was:

$$g\left(E\left[y\right]\right) = (\beta_0 + U_0) + \beta_1 bedrock + \beta_2 aspect + (\beta_3 + U_3)\, vascular_plants + \beta_4 bedrock \,\#\, vascular_plants + \varepsilon,$$

where $g(\cdot)$ is the appropriate link function (log link) and $E[\cdot]$ the expectation; y indicates number of new or the number of lost species, following the negative binomial distribution; the intercept consists of the overall intercept β_0 together with the random intercept variable for the region (U_0); a random slope was used (U_3) for vascular plant cover (vascular_plants) to allow for regional differences; between bedrock and vascular plants an interaction effect was used (denoted by #); ε indicates the remainder noise.

The number of new or lost species was predicted dependent on the predictor variable "vascular plant cover" with a step size of 1%.

For the categorical variables "bedrock," "aspect," and "first survey/resurvey," the first levels (i.e., siliceous, aspect S and first survey) were used as the reference level. For the readers' convenience to easily obtain the appropriate P-values additionally, the models with calcareous bedrock as the reference level were computed. The results are provided in the **Supplementary Tables 3, 4, 6**. A significance level of 5% was used. Assumptions and residual diagnostics were checked for all models.

All analyses were performed by using R 4.0.2 (R Development Core Team, 2015) and the R packages *glmmTMB* (Brooks et al., 2017) for models and *performance* (Lüdecke et al., 2020) for calculating marginal and conditional effects. For figures we employed *ggplot2* (Wickham, 2016). Moving averages (10 years) of summer temperatures were visualized by using the *smooth* function within the latter package.

RESULTS

Climate

Mean summer temperatures increased in all regions by 0.05 ± 0.01°C per year (estimate ± serial correlation robust standard error) over the period from 1986 to 2015 (**Figure 1** and **Supplementary Table 2**, Year: $p < 0.001$). This finding results in an estimated temperature increase of 0.7°C over the 14 years from the first survey to the resurvey. According to the downscaled climate data, trends in mean summer temperatures did not significantly differ neither between regions nor summits.

Surface Cover Types

Neither the mean vascular plant cover nor the mean scree & rock cover differed significantly between siliceous and calcareous bedrock (**Figures 2A,D** and **Table 2**, Calcareous: $p = 0.677$, $p = 0.138$ respectively). Bare ground was significantly larger in calcareous regions (4.2 ± 0.6% mean ± standard error, see **Table 3** for all mean values) than in siliceous regions (3.3 ± 0.5%; **Table 2**, Calcareous: $p = 0.05$). Generally, the proportion of litter and bare ground was low (**Figures 2B,C**) and poorly explained by the fixed effects of the model (**Table 2**, Litter cover: Marginal $R^2 = 0.256$; Bare ground: Marginal $R^2 = 0.125$). Surface cover types were affected by aspect, with vascular plant cover being higher at the southern and eastern plots compared to the western and northern ones (**Table 2**, Aspect W: $p < 0.001$, Aspect N: $p < 0.001$). Additionally, litter cover and bare ground cover were higher at southern and eastern aspects compared to western and northern ones (**Table 2**, Aspect W: $p = 0.002$, $p < 0.001$, Aspect N: $p = 0.005$, $p = 0.001$ respectively). The cover of scree & rock was higher at the western and northern aspects (Aspect W: $p < 0.001$, Aspect N: $p < 0.001$).

Vascular plant cover decreased with increasing distance to the treeline over both bedrock types (**Table 2**, Dist. treeline: $p < 0.001$). The proportion of scree & rock increased with increasing elevation (**Figure 2D** and **Table 2**, Dist. Treeline: $p < 0.001$). Litter decreased with the distance to treeline (**Figure 2B** and **Table 2**, Dist. treeline: $p < 0.001$). Bare ground decreased with the distance to treeline only on calcareous bedrock (**Supplementary Table 2**, Calcareous # Dist. treeline: $p = 0.006$).

Differences in vascular plant cover between the first survey and the resurvey depended on bedrock types (**Table 2**, Resurvey # Calcareous: $p = 0.040$). In siliceous regions, mean vascular plant cover decreased over time from 42.2 ± 2.6% in the first survey to 39.3 ± 2.4% in the resurvey (**Table 2**, Resurvey: $p = 0.010$). In contrast, in calcareous regions, mean cover was 38.6 ± 2.6% in the first survey and this did not change significantly in the resurvey (**Supplementary Table 3**, Resurvey: $p = 0.535$). The interaction effect of first survey/resurvey and distance to treeline was marginally significant for both bedrocks (**Table 2**, Resurvey # Dist. treeline: $p = 0.057$), resulting in a stronger decrease of vascular plants cover on lower siliceous summits and a slight increase of vascular plant cover on higher calcareous summits (**Figure 2A**). The decrease of the vascular plant cover from the first survey to the resurvey in siliceous areas was coupled with a significant increase in litter (**Table 2**, Resurvey: $p < 0.001$) from 4.0 ± 0.5% to 6.1 ± 0.8% (**Figure 2B**). For bare ground and scree & rock, no significant changes over time were detected (**Table 2**, Resurvey: $p = 0.780$, $p = 0.906$, respectively; **Figures 2C,D**).

Species Richness

Mean species richness per 1 m × 1 m plot was lower in the siliceous regions (13.6 ± 0.8) compared to the calcareous regions (15.0 ± 0.8, **Table 4**, Calcareous: $p = 0.033$), while the differences were higher in plots with a low vascular plant cover (**Figure 3**). The southern and eastern aspects were more species-rich than the western (**Table 4**, Aspect W: $p = 0.032$) and the northern aspects (**Table 4**, Aspect N: $p < 0.001$).

We found significant effects of the linear and the quadratic term of the vascular plant cover on species richness on both bedrock types (**Table 4** and **Supplementary Table 4**, Vascular plant cover: $p < 0.001$, Vascular plant cover^2: $p < 0.001$, $p = 0.012$ respectively), confirming a unimodal relationship between species richness and vascular plant cover (**Figure 3**). Species richness was highest at a vascular plant cover of 67% in siliceous regions and at 63% in calcareous regions (**Figure 3**), but

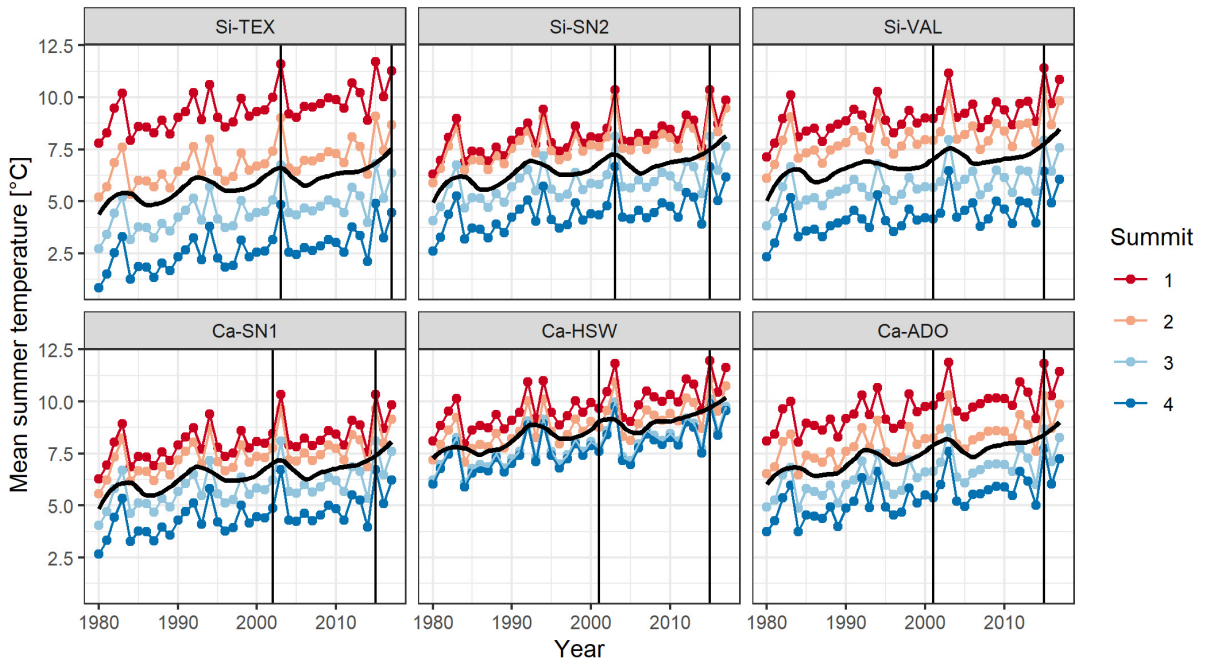


FIGURE 1 | Trend in mean summer temperatures on each four summits (1-4, in ascending order) in the six regions (**Table 1**). Shown are averages of summer temperature (monthly means June, July, August) calculated with downscaled Chelsa satellite data (resolution 100 m) combined with CRU V4.03. Black trend lines represent a 10-year moving average. Vertical lines indicate the year of the first survey and the resurvey.

the difference between bedrock types was not significant (**Table 4**, Vascular plant cover # Calcareous: $p = 0.107$, Vascular plant cover^2 # Calcareous: $p = 0.295$). Considering the single regions, the relationships between species richness and vascular plant cover varied considerably for calcareous bedrock from unimodal to nearly linear (**Supplementary Figure 2**), as indicated by the relatively low marginal R^2 of 0.640 (fixed effects, **Table 4**).

Changes in species richness over time differed significantly between siliceous and calcareous bedrock (**Table 4**, Resurvey # Calcareous: $p = 0.006$). In siliceous regions, mean species richness increased slightly but statistically significantly over time from 13.6 ± 0.8 to 14.6 ± 0.8 per plot (**Table 4**, Resurvey: $p = 0.013$, **Figure 3**), whereas in calcareous regions, it slightly decreased from 15.0 ± 0.8 to 14.3 ± 0.8, though not significantly (**Supplementary Table 4**, Resurvey: $p = 0.173$). In siliceous regions, the change ranged from −5 to 9 species and in calcareous regions from −17 to 10 species (**Supplementary Table 5**). On calcareous bedrock, the range of species richness varied among aspects from a maximum decrease per plot on eastern and western aspects (−17 and −11 species, respectively) to a maximum increase on the southern aspects (+10 species, **Supplementary Table 5**).

Number of New and Lost Species

On siliceous bedrock, the number of lost species (1.9 ± 0.2) was lower than on calcareous bedrock (2.8 ± 0.2; **Table 5**, Calcareous: $p = 0.012$). In general, the number of new species was higher on the southern aspects in comparison to the western aspects (**Table 5**, Aspect W: $p = 0.002$). Species losses were highest on southern aspects; however, significant differences were found only for the northern aspect (**Table 5**, Aspect N: $p = 0.008$).

In the siliceous regions, the number of both new and lost species increased with increasing vascular plant cover (**Table 5**, Vascular plant cover: $p < 0.001$). In total, the number of new species (3.0 ± 0.2) exceeded that of lost species (1.9 ± 0.2; **Figure 4**). In contrast, in calcareous regions, only the number of lost species increased with vascular plant cover (**Supplementary Table 5**, Vascular plant cover: $p = 0.001$), exceeding the number of new species in plots with more than 25% vascular plant cover (**Figure 4**). However, the relationship varied considerably among the three calcareous regions: the numbers of new and lost species were overall low in Ca-SN1, high for lost species in Ca-HSW, and higher for lost species than new species when vascular plant exceeded 55% in Ca-ADO (**Supplementary Figure 3**).

DISCUSSION

Temperature Increase

Our analyses of the downscaled climate data confirmed the drastic increase of summer temperature in all six studied mountain regions over the last three decades as reported from other studies (Marty and Meister, 2012; Gobiet et al., 2014; Pepin et al., 2015). As the GLORIA summits were chosen to avoid pressure from human land use (see section "Materials and Methods"), the observed changes from the first survey to the resurvey can be ascribed almost completely to the direct and indirect effects of climate change. Further, Pauli et al. (2012)

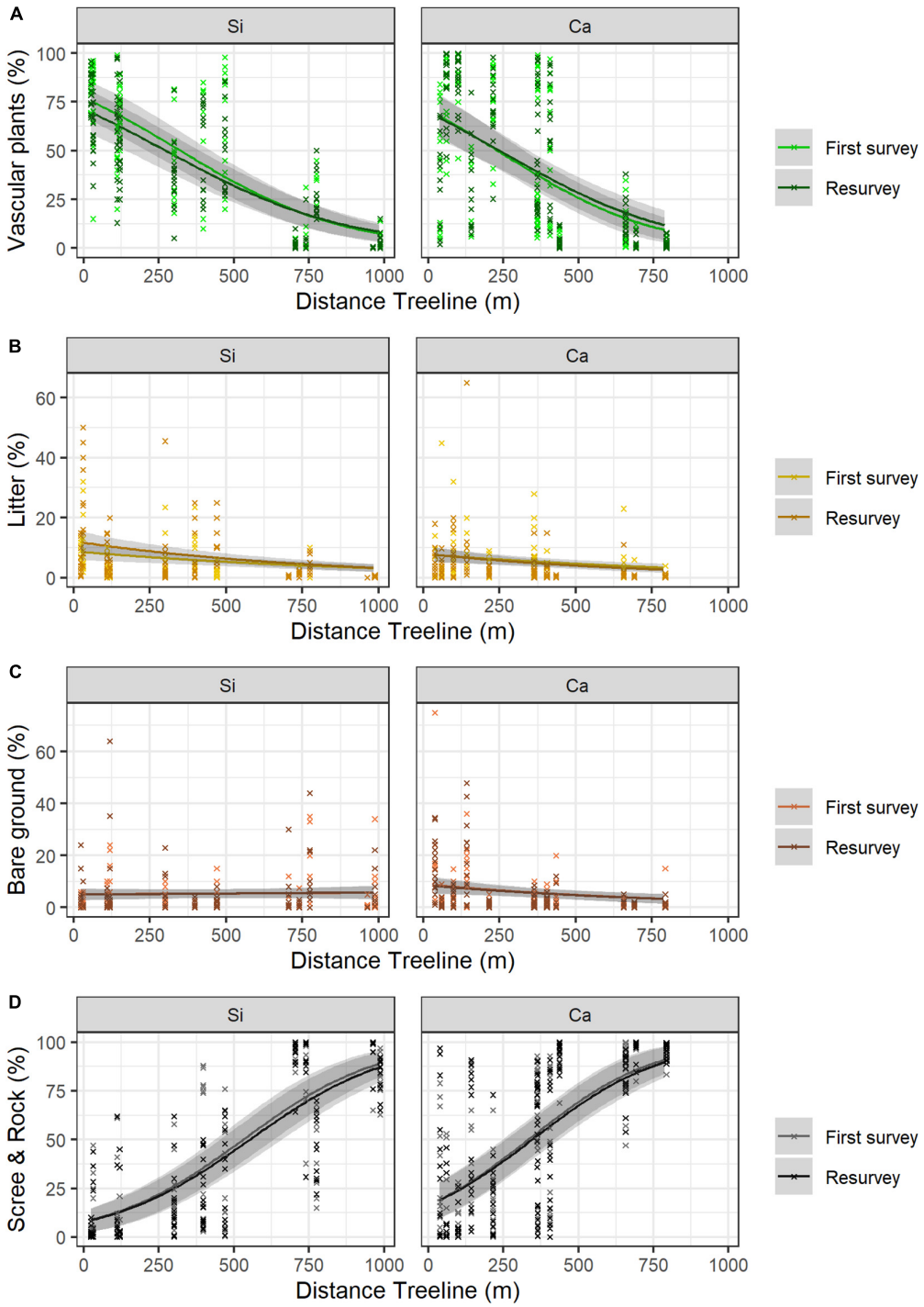


FIGURE 2 | Proportion of surface cover types **(A)** vascular plants **(B)** litter **(C)** bare ground and **(D)** scree & rock along the elevation gradient and changes over time (first survey vs resurvey) in siliceous (Si) versus calcareous regions (Ca). The lines show the predicted cover values with 95% confidence intervals (in grey) according to GLMMs. Mean values and standard errors were calculated across aspects and random regions. Crosses represent data. Values and significance levels of the effects are given in **Table 2**.

and Steinbauer et al. (2018) found that increased temperature is the main driver of the observed species increase on mountain summits across Europe. Gottfried et al. (2012) found a strong correlation between the ongoing "thermophilization" of alpine plant communities and the increase of the mean daily minimum temperature of June.

TABLE 2 | Effects of bedrock, aspect, distance to treeline, first survey/resurvey, and selected interactions on the cover of vascular plants, litter, bare ground, and scree & rock.

	Vascular plant cover			Litter cover			Bare ground cover			Scree & Rock cover		
	est.	SE	*P*-value	est.	SE	*P*-value	est.	SE	*P*-value	est.	SE	*P*-value
Intercept (siliceous, aspect S, first survey)	1.640	0.434	**<0.001**	−2.291	0.191	**<0.001**	−2.881	0.230	**<0.001**	−3.101	0.406	**<0.001**
Calcareous	−0.253	0.608	0.677	−0.090	0.262	0.731	0.621	0.317	**0.050**	0.832	0.561	0.138
Aspect E	−0.006	0.085	0.947	−0.136	0.070	**0.050**	−0.088	0.072	0.219	0.166	0.095	0.081
Aspect W	−0.738	0.090	**<0.001**	−0.228	0.072	**0.002**	−0.434	0.079	**<0.001**	0.885	0.101	**<0.001**
Aspect N	−0.545	0.088	**<0.001**	−0.199	0.071	**0.005**	−0.238	0.074	**0.001**	0.598	0.098	**<0.001**
Dist. treeline	−0.004	0.000	**<0.001**	−0.001	0.000	**<0.001**	1E-04	3E-04	0.715	0.005	0.001	**<0.001**
Calcareous # Dist. treeline	−0.001	0.001	0.418	−5E-05	4E-04	0.894	−2E-03	0.001	**0.006**	0.001	0.001	0.535
Resurvey	−0.321	0.125	**0.010**	0.376	0.095	**<0.001**	−0.031	0.113	0.780	−0.018	0.148	0.906
Resurvey # Calcareous	0.252	0.123	**0.040**	−0.351	0.101	**<0.001**	−0.018	0.108	0.870	0.003	0.138	0.982
Resurvey # Dist. treeline	4E-04	2E-04	0.057	−3E-04	2E-04	**0.067**	6E-05	2E-04	0.753	−2E-04	3E-04	0.509
Marginal R^2	0.694			0.256			0.125			0.824		

Estimates (est.), standard errors (SE) and P-values for fixed effects (P-values below 0.05 are in bold) are shown. For the categorical variables "bedrock," "aspect", and "first survey/resurvey," the first levels (i.e., siliceous, aspect S, and first survey) were treated as the reference level. Results with calcareous as reference level are available within the ***Supplementary Table S3****. Marginal R^2 is the coefficient of determination of fixed effects.*
Model is GLMM (betaregression), estimated by glmmTMB.

TABLE 3 | Table of means and standard errors (SE) for all analyzed response variables: surface cover types ("vascular plant cover," "litter cover," "bare ground cover"), "species richness," number of "lost species", and "new species" for the two bedrock types during the first survey/resurvey, and according to the aspects.

	Vascular plants [%]		Litter [%]		Bare ground [%]		Scree & rock [%]		Species richness		Lost species		New species	
Category	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Siliceous first survey	42.4	2.6	4.0	0.5	3.3	0.5	39.5	3.1	13.6	0.8				
Siliceous resurvey	39.3	2.4	6.1	0.8	3.7	0.6	37.6	3.0	14.6	0.8	1.9	0.2	3.0	0.2
Calcareous first survey	38.6	2.6	3.7	0.4	4.2	0.6	52.9	2.9	15.0	0.8				
Calcareous resurvey	40.0	2.6	3.0	0.4	3.9	0.6	51.8	2.9	14.3	0.8	2.8	0.2	2.4	0.2
Aspect South	45.9	2.6	5.2	0.7	5.1	0.7	38.5	3.0	16.9	0.8	3.0	0.3	3.2	0.3
Aspect East	44.3	2.7	4.2	0.5	4.2	0.6	42.9	2.9	15.7	0.8	2.7	0.3	2.7	0.3
Aspect West	34.7	2.6	3.7	0.6	2.6	0.6	51.7	3.1	12.9	0.7	2.2	0.3	2.0	0.2
Aspect North	34.8	2.3	3.6	0.4	3.2	0.5	50.7	2.8	12.1	0.7	1.7	0.2	2.6	0.2

The sum of surface cover types is not supposed to reach 100% as the proportion of cryptogams was excluded from analyses (see section "Materials and Methods").

Bedrock Specific Surface Cover Characteristics and Changes Over Time

Overall, we found small differences in surface cover between siliceous and calcareous bedrock. Therefore, the hypothesis (1a) that vascular plant cover and litter should prevail on siliceous bedrock, whereas bare ground, scree, and rock should dominate on calcareous bedrock could only be partially confirmed. Independently of bedrock, mean vascular plant cover decreased strongly along the elevation gradient and thus, is strongly related to the adiabatic temperature gradient (Bürli et al., 2021). Also, the proportion of scree and rock was independent of bedrock types. Nevertheless, the low bare ground cover on lower siliceous summits might indicate a lack of appropriate microhabitats for potential germination (Graae et al., 2011) in contrast to calcareous summits, where also at lower elevations small amounts of bare ground were present.

Although vascular plant cover strongly decreased with decreasing temperature along the elevation gradient, changes over time were smaller than expected from the considerable warming of 0.7°C of the mean summer temperature over the monitoring period. Using a space-for-time substitution approach, such warming would correspond to a 120 m upward shift in elevation necessary to conserve the past climatic conditions. In contrast to previous observations of vascular plant cover increases due to improved growing conditions and a longer growing season (Rogora et al., 2018), our hypothesis (1b) of an increase in vascular plant cover, more pronounced within siliceous regions, was not supported by our data. Vascular plant cover increased slightly, yet not significantly, only on the higher calcareous summits. On the lower siliceous summits mean vascular plant cover decreased by 3%, coupled with an increase of mean litter cover.

These observed decreases in plant cover on siliceous bedrock go in line with findings at Mt Schrankogel, the largest permanent plot site in the alpine-nival ecotone of the Alps on siliceous bedrock, where vascular plant cover also decreased over the last 20 years (Lamprecht et al., 2018). There, the decrease was mainly caused by a cover decrease of the cold-adapted species

TABLE 4 | Effects of bedrock, aspect, vascular plant cover (linear and quadratic), first survey/resurvey, and chosen interactions on species richness.

Species richness	est.	SE	*P*-value
Intercept (siliceous, aspect S, first survey)	0.877	0.336	**0.009**
Calcareous	1.008	0.472	**0.033**
Aspect E	−0.035	0.046	0.452
Aspect W	−0.106	0.050	**0.032**
Aspect N	−0.224	0.047	**<0.001**
Vascular plant cover	6.932	1.241	**<0.001**
Vascular plant cover^2	−5.142	1.274	**<0.001**
Vascular plant cover # Calcareous	−2.831	1.758	0.107
Vascular plant cover^2 # Calcareous	1.900	1.814	0.295
Resurvey	0.124	0.050	**0.013**
Resurvey # Calcareous	−0.186	0.068	**0.006**
Marginal R^2	0.640		

Estimates (est.), standard errors (SE) and P-values for fixed effects (P-values below 0.05 are in bold) are shown. For the categorical variables "bedrock," "aspect", and "first survey/resurvey," the first levels (i.e., siliceous, aspect S, and first survey) were treated as the reference level. Results with a calcareous reference level are available within the ***Supplementary Table 4****. Marginal R^2 is the coefficient of determination of fixed effects.*
Model is GLMM (negative binomial), estimated by glmmTMB.

(Steinbauer et al., 2020), while new species from lower elevations were not able to fill the open gaps. Vascular plant cover changes, therefore, may not necessarily be connected to the worldwide observed increase of species richness on summits (Dullinger et al., 2007; Pauli et al., 2012; Steinbauer et al., 2018). This is plausible as the soil development and establishment of vegetation can take centuries to millennia (Theurillat et al., 1998; Kulonen et al., 2018) and are hampered by the steep slopes and strong erosion through gravitational processes on alpine summits. At larger spatial scales, 'greening' of the European Alps was evidenced as an increase of the normalized difference vegetation index (NDVI) derived from satellite data on a spatial resolution of 30–125 m (Carlson et al., 2017; Filippa et al., 2019), which is, however, probably not comparable with the vascular plant cover changes in our plots.

Nevertheless, the slight increase of vascular plant cover on the higher calcareous summits points to a slow densification of the existing vegetation patches, either following a filling or a moving process (Grabherr et al., 1995; Gottfried et al., 1999). This increase was probably too small to be paralleled in a detectible decrease of surface cover types like bare ground or scree and rock. Additionally, it is possible that vascular plant species occupied microsites primarily covered by cryptogams, which were not considered in our analyses.

Furthermore, we found contrasting cover changes of litter between siliceous and calcareous regions. The increased proportion of litter on the lower siliceous summits and replacement of living vascular plants might be the first sign toward a more pronounced decreasing vascular plant cover in the long-term. Long-lived tussocks such as *Carex curvula* (Grabherr, 1989) produce a high quantity of old leaves and leaf sheaths, which decompose over a long timescale. More frequent drought events might have enhanced litter accumulation on lower siliceous summits during the last summers (Haslinger et al., 2016). The slow-decaying dead plant material can prevent colonization by new species and can negatively affect seed germination and seedling establishment (Xiong and Nilsson, 1999; Graae et al., 2011). However, effects of litter on seed germination and establishment depend on the quality and density of the dead plant material. Faster decomposing material of forbs might even be of advantage for seedlings under conditions of recurrent drought when increasing the water storing capacity or ameliorating the microclimate.

Competition Within Communities With High Vascular Plant Cover and Bedrock Specific Trends in Species Richness

The unimodal relationship between species richness and vascular plant cover, with a peak around 65% cover (**Figure 3**; siliceous 67%, calcareous 63%) confirms hypothesis 2a, in line with other results describing a decrease of species richness due to increased competition for resources such as light (Grime, 1979; Rosenzweig, 1995; Grytnes, 2000; Pierce, 2014). Besides competition for light, it is likely that root competition takes place in dense alpine grasslands (Grabherr, 1989). Naud et al. (2019) showed a unimodal relationship of species richness per 1 m × 1 m plot with elevation whereby species diversity was limited by competition at low elevation and by a smaller species pool at high elevation (Moser et al., 2005). However, at the mesoscale, there is a decrease in the richness of vascular plants along the elevation gradient above the treeline (Theurillat et al., 2003).

The unimodal relationship between species richness and vascular plant cover was found in both bedrock types. However, the decrease in richness at plant cover above 65% was more pronounced in siliceous communities. All three siliceous regions showed a remarkably similar decrease in richness at plots with high vascular plant cover, pointing toward a common species richness pattern at siliceous summits in the Alps above the treeline sharing similar communities and geological characteristics (Pfiffner, 2015). Contrastingly, patterns for the calcareous regions were rather heterogeneous, and relatively high species richness could also be found at high vascular plant cover (**Supplementary Figure 2**). An explanation for the high heterogeneity could be that the regions were situated in different parts of the Alps (**Supplementary Figure 1**) with different types of limestone and varying proportions of calcium, magnesium carbonate or clay, which further impacts erosion and soil formation: (1) SN1 within the geological complex calcareous part of the Swiss Central Alps consisting of heterogenous limestone formations (Wetterstein, Dolomite) high summits and a limitation of vegetation development (no high vascular plant cover, no high species richness); (2) HSW at the eastern edge of the Northern Limestone Alps with low maximum elevations, a relatively pure calcium carbonate limestone (Wetterstein, Gutensteiner Dolomite), and a nearly monotonic increasing species richness with vascular plant cover; and finally (3) ADO within the Southern Alps dominated by

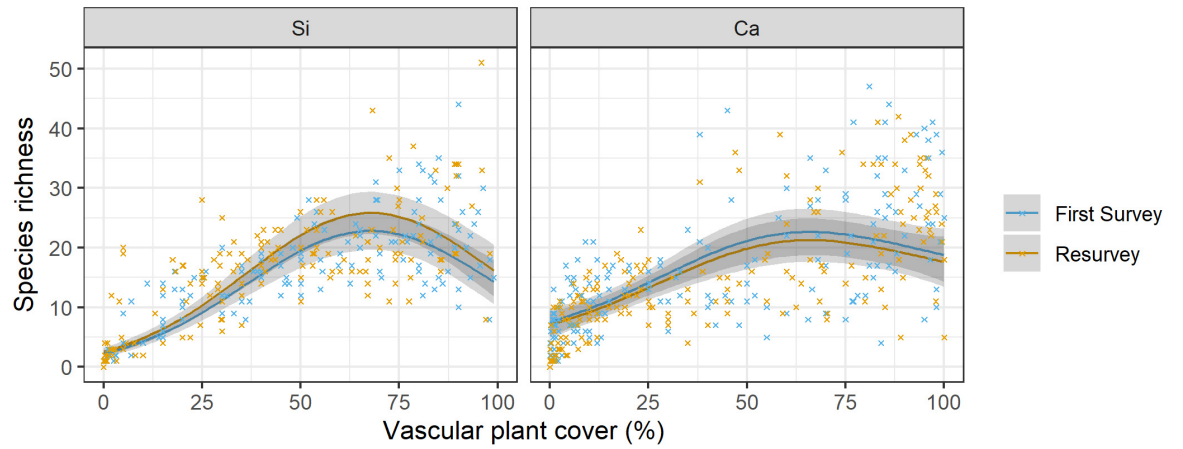


FIGURE 3 | Relationship of species richness with vascular plant cover per 1 m × 1 m plot over time (first survey vs resurvey) on siliceous and calcareous bedrock. The lines show the predicted mean species richness and the 95% confidence interval (in grey) from a negative binomial GLMM. Mean values and standard errors were calculated across aspects and random regions. Crosses represent data. Coefficients and significance levels of effects are given in **Table 4**.

Latemar limestone and Hauptdolomite showing a unimodal relationship with a high species richness maximum at high vascular plant cover.

High levels of competition in well-developed alpine grassland communities has been shown experimentally (Olsen and Klanderud, 2014) and via community analyses (Grabherr, 1989). Dense communities may be able to prevent the establishment of new species because of the presence of many long-lived, clonal species forming dense mats (Steinger et al., 1996; Theurillat and Guisan, 2001; but see discussion on species turnover below). Particularly impressive examples are *Carex curvula* communities with nearly no change over 32 yearsy (Windmaißer and Reisch, 2013) and communities at the Niwot Ridge, United States with minimal changes over 40 years (Naud et al., 2019). In the same way, the species-rich calcareous grassland communities with *Carex sempervirens* are known to be very competitive and stable (Grabherr, 1989; Gritsch et al., 2016; Matteodo et al., 2016). In comparison to the siliceous communities reaching a maximum species diversity at 65% vascular plant cover, single calcareous communities within our study reached their diversity maximum at up to 100% vascular plant cover. This goes in line with Gigon's (1971) investigations, showing that more species can coexist at calcareous sites because dominance of competitive species is not as strong as in siliceous communities. Additionally, in the European Alps the calcareous species pool is richer than the siliceous species pool, as a result of the outer calcareous regions being less impacted by glaciations than the central, siliceous regions (Wohlgemuth, 2002; Aeschimann et al., 2012). This might enable higher species richness in calcareous plots. Our results also confirmed higher species richness on calcareous bedrock, although the mean difference of just one species was relatively small and decreased over time.

Our results showed that changes in species richness at the scale of 1 m × 1 m plots were rather small over 14 years of observation. The mean increase of species richness by one species found in siliceous plots is comparable to the changes at the alpine-nival ecotone in the siliceous Austrian Central Alps (Mt Schrankogel), where Lamprecht et al. (2018) reported a slightly higher increase of two species over 20 years. Contrastingly, changes of species richness were not significant in calcareous regions, which argues against our hypothesis 2b, suggesting a more substantial increase of species richness within calcareous regions. Probably due to the increasing numbers of lost species, species richness increase is already limited in several plots. While species richness at whole summit areas is still rapidly increasing (Matteodo et al., 2013; Unterluggauer et al., 2016; Steinbauer et al., 2018), first threats of a loss in species richness might be observed at the finer scale of 1 m × 1 m plots.

TABLE 5 | Effects of bedrock, aspects, vascular plant cover, and the interaction between vascular plant cover and bedrock on the number of new/lost species.

	New species			Lost Species		
	est.	SE	*P*-value	est.	SE	*P*-value
Intercept (siliceous, aspect S)	0.312	0.304	0.305	−0.935	0.437	**0.033**
Calcareous	0.446	0.413	0.280	1.465	0.583	**0.012**
Aspect E	−0.162	0.114	0.156	−0.024	0.123	0.845
Aspect W	−0.383	0.126	**0.002**	−0.252	0.135	0.062
Aspect N	−0.073	0.115	0.527	−0.355	0.134	**0.008**
Vascular plant cover	0.015	0.004	**<0.001**	0.028	0.004	**<0.001**
Vascular plant cover # Calcareous	−0.011	0.006	0.085	−0.016	0.006	**0.004**
Marginal R^2	0.215			0.518		

Estimates (est.), standard errors (SE) and P-values for fixed effects (P-values below 0.05 are in bold) are shown. For the categorical variables "bedrock", and "aspect," the first levels (i.e., siliceous and aspect S) were treated as the reference level. Results with a calcareous reference level are available within the ***Supplementary Table 6****. Marginal R^2 is the coefficient of determination of fixed effects. Model is GLMM (negative binomial), estimated by glmmTMB.*

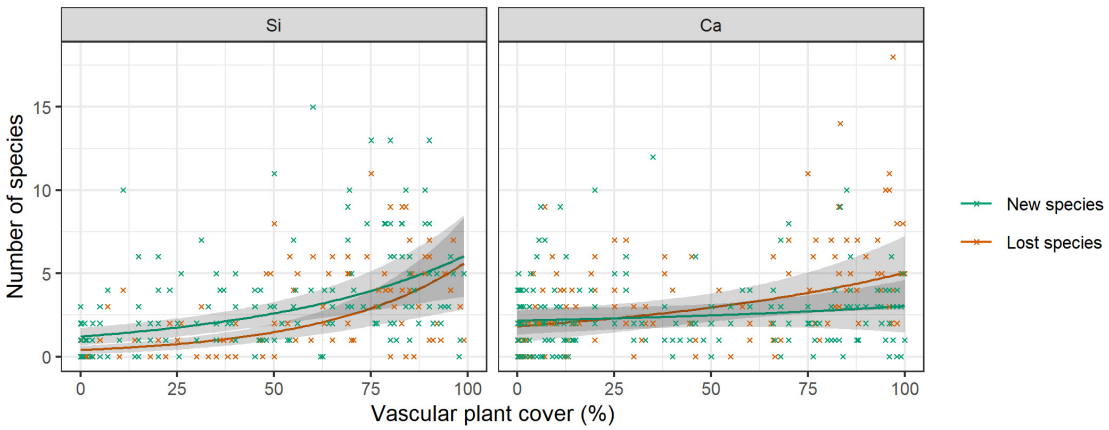


FIGURE 4 | Number of new (green) species and lost (red) species per 1 m × 1 m plot in relation to the vascular plant cover on siliceous and calcareous bedrock. The lines show the predicted mean number of lost or new species ±95% confidence intervals (in grey) from a negative binomial GLMM. Mean values and standard errors were calculated across aspects and random regions. Crosses represent data. Values and significance levels of the effects are given in **Table 5**.

High Species Losses in Communities With a High Vascular Plant Cover

The number of new species and lost species provided additional insight to detect vegetation changes over time. So far only native species have been found on the studied summits and colonization involved only species that have been present in the region for a long time. With increasing vascular plant cover, the number of lost species increased, which confirms our hypothesis 3a. However, contrary to our expectations, the number of lost species exceeded the number of new species at the highest vascular plant cover in calcareous regions only (hypothesis 3b). This might be the first sign of diversity loss in some sites with higher vascular plant cover. Erschbamer et al. (2011) and Unterluggauer et al. (2016) for example, reported an ongoing encroachment of treeline species and grasses on the lowest summit in the calcareous region ADO. They suggested that treeline species might outcompete alpine species in the future. Eventually, due to the increased water permeability and lower water contents of soils on calcareous bedrock (Gigon, 1971), the effect of drought may be more severe in calcareous regions and lead to a higher number of lost species. Indeed, several summers (2003, 2015) within the monitoring period were dry and hot (Haslinger et al., 2016). According to predictions, the probability of increased frequency and severity of summer drought events will increase in some parts of the Alps (Gobiet et al., 2014; Haslinger et al., 2016; Spinoni et al., 2018). Together with high temperatures, which increase evapotranspiration, this might pose a serious problem for Alpine plants. However, the low resolution of the downscaled climate data and the low frequency of monitoring cycles within our study make it difficult to capture the direct effect of drought on the 1 m × 1 m plot level.

In siliceous regions, well-developed communities with a high vascular plant cover were expected to be competitive and stable. Overall, however, there was a high species turnover on siliceous bedrock, and the numbers of new and lost species both increased with increasing vascular plant cover. It seems therefore quite probable that due to the special conditions taking place on summits, a higher turnover is possible in comparison to the well-developed grasslands on homogenous slopes or flatter areas in the alpine belt (Windmaißer and Reisch, 2013; Gritsch et al., 2016; Naud et al., 2019) at least at the micro-scale of 1 m × 1 m plots. However, the observed species turnover does not mean a conversion into a new community. Most of the new species belong to the local species pool around or below the summits (Unterluggauer et al., 2016). Nevertheless, several studies have already reported a homogenization and thermophilization of the summit communities (Vittoz et al., 2008; Erschbamer et al., 2011; Gottfried et al., 2012; Pauli et al., 2012; Wipf et al., 2013; Matteodo et al., 2016; Unterluggauer et al., 2016; Lamprecht et al., 2018).

Overall, the models used in our study to predict the number of new and lost species had a comparably low marginal R^2 with considerable differences between regions (**Supplementary Figure 3**). This is not surprising as colonizations and disappearances are rare events that are influenced by several other factors besides temperature and vascular plant cover (Graae et al., 2011), making them more prone to stochastic events occurring between plots and regions.

Additional Factors Influencing Surface Cover, Species Richness and Turnover

In their pan European GLORIA analysis, Winkler et al. (2016) clearly showed that aspects matter for species diversity on summits in temperate mountains, enabling a higher species richness on eastern and southern exposures due to warmer temperatures and better-developed soils. Therefore, it appeared straightforward to include the effect of aspect in the models in order not to omit a variable and to introduce a bias. The results of all our models confirmed the strong effect of the aspect. Winkler et al. (2016) showed that new species mostly invade the warmer southern and eastern sides. The variation between aspects might also be the reason for the large range of species richness change over time observed in the present study. Probably the higher

species turnover on calcareous bedrock resulted from a higher instability of vegetation on certain aspects.

Besides the discussed factors influencing vegetation changes, grazing might be a variable that is difficult to take into account correctly. Since summits were initially chosen in areas with no or the least possible domestic grazing (**Supplementary Table 1**), we expect grazing effects to be small, although no quantitative data exist in this way for wild grazers. Therefore, we cannot exclude that some variation in the grazing activity may have caused some local heterogeneity or could have temporarily masked the effects of warming. For instance, the general trend of a continuous decrease of pasturing by livestock in the alpine zone of the European Alps over the last decades could have an impact on vegetation succession (Tasser and Tappeiner, 2002; Czortek et al., 2018; Frate et al., 2018). Conversely, there is a long-term trend of increasing wild ungulates (Apollonio et al., 2010). According to Steinbauer et al. (2018) land-use change may account for local variation in species richness trends, but it is unlikely at a larger scale. In a joint analysis with Mediterranean GLORIA regions, Lamprecht et al. (2019) analyzed the impact of land-use in three of our regions (ADO, HSW, VAL). Although land-use varied locally, structural equation models could not find a significant effect on species richness trends.

Observer errors, in particular visual cover estimation and wrong species identifications, are a common issue in resurveys. The relative coefficient of variation of cover estimation may reach up to 60% (Vittoz et al., 2010; Futschik et al., 2020). Wrong identification of species, leading to pseudo-turnover and pseudo-variation in species richness, may occur in 10 to 30% of the cases, according to Morrison (2016) and Futschik et al. (2020). However, Futschik et al. (2020) showed that changes in species turnover and species cover were significantly larger than pseudo-changes due to observer errors even after seven years within the tested GLORIA regions Hochschwab, Mt Schrankogel, and High Tatra. Additionally, to avoid pseudo-turnover, we checked all the new and lost species for possible misidentification. Remaining mistakes are likely to be random, and hence our extensive dataset allows us to find trends beyond potential observer errors.

Besides observer errors, several phenological variations caused by water availability, warm or cold season, and inherent population fluctuations between years in the reproductive allocations might still be apparent within our dataset and superimpose a long-term effect of climate change. As an attempt to minimize phenological variations, the first survey and resurvey were done in peak season.

CONCLUSION

The present study provides evidence that vegetation attributes such as species richness and species turnover, and the distribution of surface cover types (vascular plants, litter, bare ground, scree & rock) generally differ between calcareous and siliceous summits and show contrasting changes over 14 years of climate warming on the micro-scale of 1 m × 1 m. Surface cover types showed minor general differences between bedrock types but changed differently over time: on siliceous bedrock, vascular plant cover decreased, coupled with an increase in litter, and it marginally increased on the highest calcareous summits. Instead of the expected increase, vascular plant cover might thus even decrease at the expense of litter accumulation quite probably due to drought. Counterintuitively, this effect is more pronounced at the moment on siliceous bedrock; however, similar effects might occur in calcareous regions.

We could confirm a unimodal relationship of species richness and vascular plant cover indicating competition in communities with a high vascular plant cover. Species richness decreased more strongly beyond 65% plant cover within siliceous regions. Nevertheless, overall species richness increased over time, and the number of new species exceeds the number of lost species. Contrastingly and contrary to expected, in calcareous regions, the exceeding number of lost species might be the first sign of a negative trend in species richness with drought possibly being an increasing problem for summit vegetation. Continued monitoring will be necessary to confirm the observed trends.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LN did the analyses and prepared the manuscript. JWa supervised the analyses. MM, PU, LN, SW, CR, J-PT, AL, KS, and PV contributed GLORIA data. DM, AG, and JWe prepared the climate data and did the downscaling. BE and SW supervised all steps from analysis design to manuscript preparation. All authors contributed to the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.642309/full#supplementary-material

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Evaluating Assisted Gene Flow in Marginal Populations of a High Mountain Species

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Many species cannot either migrate or adapt at the rate of temperature increases due to climate warming. Therefore, they need active conservation strategies to avoid extinction. Facilitated adaptation actions, such assisted gene flow, aim at the increase of the evolutionary resilience of species affected by global change. In elevational gradients, marginal populations at the lower elevation edges are experiencing earlier snowmelt and higher temperatures, which force them to adapt to the new conditions by modifying their phenology. In this context, advancing the onset of flowering and seed germination times are crucial to ensure reproductive success and increase seedling survival prior to summer drought. Assisted gene flow may bring adaptive alleles and increase genetic diversity that can help throughout ontogeny. The main aim of this work is to assess the effects that different gene flow treatments could have on the desired trait changes in marginal populations. Accordingly, we established a common garden experiment in which we assayed four different gene flow treatments between *Silene ciliata* Pourr. (Caryophyllaceae) populations located in similar and different elevation edges, belonging to the same and different mountains. As a control treatment, within-population crosses of low elevation edge populations were performed. The resulting seeds were sown and the germination and flowering onset dates of the resulting plants recorded, as well as the seedling survival. Gene flow between populations falling on the same mountain and same elevation and gene flow from high-elevation populations from a different mountain to low-elevation populations advanced seed germination time with respect to control crosses. No significant effects of gene flow on seedling survival were found. All the gene flow treatments delayed the onset of flowering with respect to control crosses and this effect was more pronounced in among-mountain gene flows. The results of this study highlight two important issues that should be thoroughly studied before attempting to apply assisted gene flow in practical conservation situations. Firstly, among-populations gene flow can trigger different responses in crucial traits throughout the ontogeny of plant species. Secondly, the population provenance of gene flow is determinant and plays a significant role on the effects of gene flow.

Keywords: *Silene*, facilitated adaptation, gene flow, marginal populations, outbreeding, climate change, flowering phenology

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INTRODUCTION

Global warming is changing environmental conditions of habitats where species used to live. This climate change is forcing species to move to higher latitudes or elevations looking for adequate environments to survive (Hughes, 2000; Parmesan and Yohe, 2002; Walther et al., 2002). Another response implemented by species to deal with climate change is the adaptation to the new environmental conditions through phenotypic plasticity or evolutionary adaptation (Hoffmann and Sgró, 2011). However, some species cannot either migrate or adapt at the needed rate to cope with the effects of increasing temperatures (Hoffmann and Sgró, 2011). This situation is especially striking in alpine plant species which are particularly threatened due to limitations of dispersal, habitat availability and requirements of microclimate conditions. In this context, the rise of temperatures is a real threat to mountain species, which cannot migrate higher than the mountain summits (Nogués-Bravo et al., 2007; Colwell et al., 2008). Moreover, populations of high mountain species are frequently isolated from other populations, due to natural geographic barriers. This natural fragmentation may reduce the ability of species to adapt to stressing conditions, among other things, due to the lower populations' sizes, greater inbreeding loads and genetic drift, and reduced gene flow between populations (Ouborg et al., 2006; Anderson et al., 2011). Thus, species that cannot migrate or adapt to the new conditions may need active conservation strategies to escape from the extinction vortex (Aitken and Whitlock, 2013; Hällfors et al., 2014).

Multiple conservation strategies have been suggested in the last decade to mitigate the effects of global warming. Among them, facilitated adaptation actions aim to increase the evolutionary resilience of the species affected by global change (e.g., Jones and Monaco, 2009; Shoo et al., 2013; van Oppen et al., 2015). Two approaches proposed for developing facilitated adaptation actions are the traditional Assisted Migration and the novel Assisted Gene Flow. Assisted Migration, the translocation of populations to a new suitable habitat, is nowadays the most frequently used approach but still under debate (Ricciardi and Simberloff, 2009; Hällfors et al., 2014). This action may entail some ecological risks (e.g., disease transmission, competitive exclusion, pollination and dispersal failure, etc.) for the assisted population and the new hosting habitat (Loss et al., 2011; Weeks et al., 2011; Williams and Dumroese, 2013). Assisted Gene Flow, the movement of gametes from populations adapted to the changed environment to populations threatened by new conditions, has recently emerged as a less hazardous alternative to manage plant populations or species in the verge of extinction (Aitken and Whitlock, 2013; Whiteley et al., 2015). In this context, gene flow has been proposed to overcome the maladaptation to climate warming in trees (Aitken and Bemmels, 2016) and proved to be a valuable tool for the genetic rescue of small populations suffering inbreeding depression (Frankham, 2015). However, gene flow effects on the adaptive potential of recipient populations are closely related to the specific environmental conditions of the original and recipient populations. In this sense, Morente-López et al. (2020a) showed that gene flow between marginal populations that share similar environmental conditions increase genetic diversity and provide adaptive alleles. Other studies point to a reduction of fitness in marginal populations due to the incorporation of maladapted alleles from optimal populations (Dawson et al., 2010; Fedorka et al., 2012). On the contrary, Bontrager and Angert (2019) reported that gene flow from a warmer optimal population provided better adaptive response in a cooler marginal population in a temperature increase scenario.

In essence, the potential pros and cons of assisted gene flow have yet to be ground-proved in natural populations under contrasting situations to gain insight on the applicability of this approach. Also, it is important to measure the effects of gene flow on different traits throughout the ontogeny and assess whether the same gene flow can produce contrasting fitness effects on different life stages (Angert et al., 2008; Morente-López et al., 2020a). This is crucial because the outcome of a conservation action would depend on the overall response of the different fitness components measured.

Adjusting plant phenology through assisted gene flow actions can be an alternative conservation strategy for alpine plant populations that face the consequences of climate warming. The long snow cover duration results in a short period for alpine plants to grow and reproduce before the early frosts of autumn (Stinson, 2004; Molau et al., 2005; Sedlacek et al., 2015). This period is even shorter in the Mediterranean Alpine ecosystems where the soil moisture availability is lower due to high temperatures and reduced rainfall in spring and summer (Giménez-Benavides et al., 2007a, 2018). Under seasonal variation, the germination timing is crucial for the subsequent seedling survival (Baskin and Baskin, 1998; Shimono and Kudo, 2003). Moreover, germination and seedling survival are highly affected by summer drought conditions in the Mediterranean mountain species (Cochrane et al., 2014; Giménez-Benavides et al., 2018). An early germination makes possible a growth of seedling roots deep enough to extract soil water and survive the summer drought. Alpine plants are expected to reduce the prefloration interval (number of days from emergence date to full flowering) to take advantage of the moist soil duration in an increasing temperatures scenario (Stinson, 2004; Hülber et al., 2010). However, earlier flowering may cause flower damages in a context of snow cover reduction due to higher temperatures and the subsequent higher risk of late spring frosts.

Silene ciliata Pourr. (Caryophyllaceae) is an alpine plant whose southernmost locations in Europe are in central Spain. Within each mountain, populations are spread along local altitudinal ranges (from ∼1,800 to 2,500 m). Lower elevation populations are experimenting earlier snowmelt and higher temperatures than the upper populations, and are better adapted to drought stress than populations of the summit (Giménez-Benavides et al., 2007a, 2018; García-Fernández et al., 2013; Morente-López et al., 2020b). However, even if plants are adapted to survive under warmer conditions, they need to adjust their reproductive phenology to new conditions through phenotypic plasticity or local adaptation (Giménez-Benavides et al., 2011b, 2018). In fact, low elevation populations of *S. ciliata* have showed a declining trend in the number of large reproductive

individuals and smaller plants have lower flowering probability due to summer drought (Giménez-Benavides et al., 2011a). *S. ciliata* high-elevation populations bloom earlier than low-elevation populations in *ex situ* common garden experiments because of their lower requirement of flowering cumulative temperature (°C days) (Morente-López et al., 2020b). Based on this result, gene flow from high elevation populations could be expected to advance the flowering time in low elevation populations. If so, early bloom may entail *in situ* an increase of the flowering period and hence reproductive performance (Giménez-Benavides et al., 2011b).

On the other hand, previous works have reported high mortality rates during early life stages of the plant (Giménez-Benavides et al., 2007a; García-Fernández et al., 2012a; Lara-Romero et al., 2014, 2016). These findings highlight that not only adaptations on the flowering onset are necessary, but also on the timing of germination and on seedling survival rates. Gene flow from high elevation population to low elevation population may have undesirable effects on germination and survival of the seedlings. The increase of genetic diversity due to the gene flow among populations may rise plant fitness or, on the contrary, the gene flow between isolated populations can produce a decrease in fitness due to maladaptation and outbreeding depression (Frankham et al., 2011; Aitken and Whitlock, 2013).

The main aim of this paper is to evaluate the effect of assisted gene flow coming from different origins on the adaptive potential of *S. ciliata* populations inhabiting the lower elevation edges, in response to changing environmental conditions. The distribution of the species in the study area is fragmented in three mountain ranges and comprises a steep environmental gradient. This implies that the genetic differentiation between mountains is higher than the genetic differentiation within mountains, even though populations inside the same mountain at different elevations experienced contrasted environmental conditions (Morente-López et al., 2018). This scenario allows testing the potential contrasting effects of gene flow between populations that are subject to more frequent natural gene flow between them (within mountains) versus gene flow between populations that have been isolated from each other for a longer period (among mountains). We hypothesize that the effect of the assisted gene flow will depend on the origin, and thus, on the particular environmental and historical conditions that populations experienced. We predict that gene flow from high elevation populations will induce an earlier flowering onset in lower elevation populations. However, this gene flow from high to low elevation population may bring poorly adapted genes to the more severe temperature conditions for the germination and survival stages. On the other hand, we also predict that gene flow within mountains will generate higher seedling survival rate and shorter time until germination and flowering onset in the progeny than gene flow between mountains, due to the shared evolutionary history in the former. For this purpose, we studied in a common garden the effects of the different gene flow treatments on the flowering onset, germination time and seedling survival in *S. ciliata* plants. Specifically, we aim to answer the following questions: (i) Can gene flow between populations advance flowering onset? (ii) What are the consequences of between population gene flow on germination and seedling survival? (iii) Do the pros overtake the cons in any of the gene flow treatments? To our knowledge, this is the first study to test whether assisted gene flow can be used to improve the viability of the marginal (low elevation) populations of an alpine plant species in response to global warming, considering different critical life stages in the same study such as seed germination, seedling survival and flowering phenology.

MATERIALS AND METHODS

Study Species and Populations

Silene ciliata is a long perennial cushion plant which inhabits a marked environmental gradient in Mediterranean alpine habitats. This species occurs in grasslands above the tree line in the Mediterranean mountain ranges of Southern Europe, from the Sistema Central in the center of the Iberian Peninsula to the Massif Central in France, the Apennines in Italy and the Balkan Peninsula (Sanz-Elorza et al., 2003; Escudero et al., 2004; Kyrkou et al., 2015) and has a late flowering period from the end of June until mid-September. Germination of *S. ciliata* seeds requires a previous exposure to cold temperatures, that normally takes place right after snowmelt to make the best use of soil moisture (Giménez-Benavides et al., 2005, 2007b). Summer drought is the most lethal factor for seedling survival (Giménez-Benavides et al., 2007b). Flowering onset initiates after snowmelt, with lower elevation populations flowering earlier in the field but later under *ex situ* common garden conditions than higher elevation populations (Giménez-Benavides et al., 2011b; Morente-López et al., 2020b). Thus, higher elevation populations may have the capacity to advance flowering onset. Similar genetic differentiation patterns concerning other functional traits associated, not to elevation but, to latitudinal gradients have been observed in other *Silene* species (e.g., Abeli et al., 2015; Mondoni et al., 2018). *S. ciliata* is a self-compatible species. Xenogamic and geitonogamic hand pollination treatments induce both fruit and seed set. However, the presence of a pronounced protandry limits passive autogamy (Giménez-Benavides et al., 2007b).

Study populations were chosen from the three main mountain ranges, Guadarrama, Béjar and Gredos of the Sistema Central mountain region, located in the center of the Iberian Peninsula (**Figure 1A**). These populations are considered to be relict because of their isolation from the rest of the populations of the species located in other mountain systems such as the Pyrenees, the Massif Central, the Apennines and the Balkan Peninsula (Tutin et al., 1964). The shortest distance between mountain ranges is approximately 52 km between Béjar and Gredos, whereas the greatest distance is 150 km between Guadarrama and Béjar. Within each mountain, plants from three populations were selected: two from the lowest edge and one from the highest edge of the altitudinal range. Low elevation populations are located between 1,850 and 2,000 m a.s.l., while high elevation populations are near to the mountain summits (2,350–2,400 m a.s.l.) (**Table 1**).

In spite of the short distance existing between high and low elevation populations within each mountain, plants inhabiting

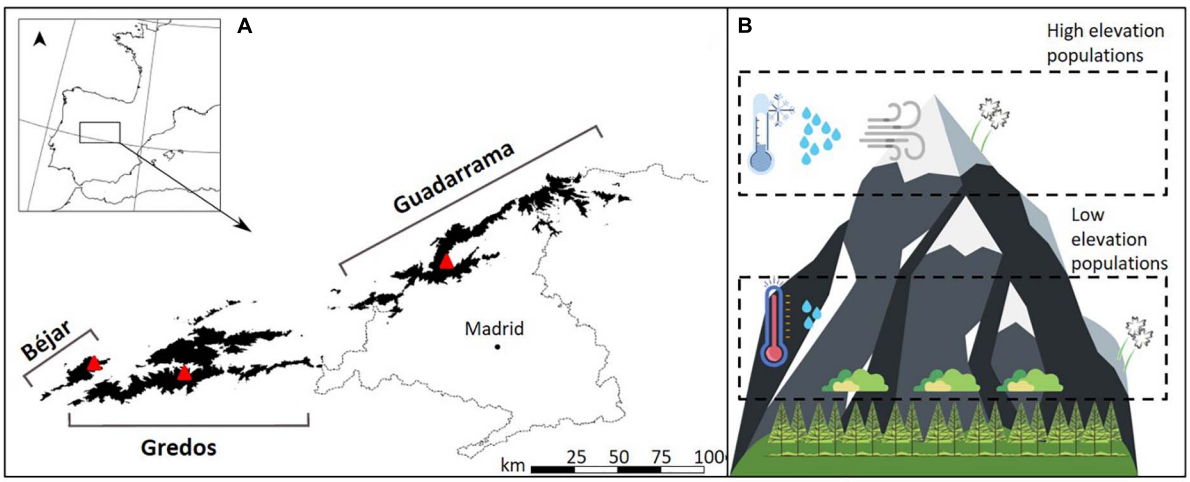


FIGURE 1 | Location and conditions experienced by *Silene ciliata* populations at the Sistema Central mountain region in the Central Spain. **(A)** Guadarrama, Béjar and Gredos constitute the three main mountain ranges. Each red triangle denotes the three sample sites (two low elevation populations and one high elevation population) within each mountain range. Black shades denote the areas above 1,500 m a.s.l. **(B)** Populations occur along an elevation gradient from the low edge at the timberline to the high edge at the mountain peaks. Environmental conditions are warmer, drier and less windy at the low elevation populations.

TABLE 1 | Location and elevation classification of the populations.

Mountain range	Population	Pop. ID	m a.s.l.	Elevation class	Latitude	Longitude
Guadarrama	Najarra baja	NAJ	1,850	Low	40°49′23.46″N	3°49′52.53″W
	Morrena Peñalara	MOR	1,980	Low	40°50′11.82″N	3°57′0.91″W
	Pico Peñalara	PEN	2,400	High	40°51′2.11″N	3°57′24.02″W
Gredos	El Sestil	SES	1,900	Low	40°16′24.45″N	5°14′54.93″W
	Los Campanarios	CAM	2,000	Low	40°15′42.63″N	5°12′55.74″W
	Altos del Morezón	ZON	2,380	High	40°14′57.5″N	5°16′8.3″W
Béjar	Las Cimeras	RUI	2,000	Low	40°21′7.03″N	5°40′59.71″W
	Pico El Águila	AGI	1,950	Low	40°21′12.36″N	5°41′46.52″W
	Canchal Negro	NEG	2,360	High	40°20′19.97″N	5°41′22.27″W

these two sites experience contrasting biotic and abiotic conditions (**Figure 1B**). Low elevation populations are located next to the timberline (*Pinus sylvestris*) in an interspersed shrub-pasture matrix (*Cytisus oromediterraneus, Juniperus communis,* and *Festuca curvifolia*). High elevation populations share the habitat only with pastures of *F. curvifolia* and *Nardus stricta*. Low elevation sites are 2.9°C warmer during the reproductive season than high elevation populations, have higher minimum temperatures, less wind and the snow cover remains for a significantly shorter period of time (Giménez-Benavides et al., 2007a,b; Morente-López et al., 2020b). These environmental differences have been documented to create selective pressures acting on genetically based phenotypic differentiation and adaptive patterns in populations at different elevations (Morente-López et al., 2020a,b).

Plant Collection

At the end of the summer of 2013 we collected a minimum of 29 randomly selected plants from each of the nine populations (**Figure 2A** and **Table 2**). Collected plants were divided in a variable number of cuttings (depending on the size of the plant) to obtain a greater number of clonal individuals and potted in 0.5 L plastic pots with commercial potting substrate (**Table 2**). They were then kept in a common garden at the Universidad Rey Juan Carlos CULTIVE facility laboratory greenhouse (690 m a.s.l.; 40°20′02″ N/3° 52′59″ W) to let them root and grow. The plants resulting from the cuttings conformed the parental generation.

Gene Flow Treatments

In the flowering season of 2014 we performed crosses within and between populations, and within and between mountains to generate five different gene flow treatments as follows (**Figure 2A**):

I. Control, within-population crosses in the low elevation populations (AGI, CAM, and MOR). We considered this gene flow treatment as the reference to compare the rest of the treatments, since it represents no change in the genetic composition of the populations.
II. HLWM, crosses using pollen from high elevation populations on plants from low elevation populations

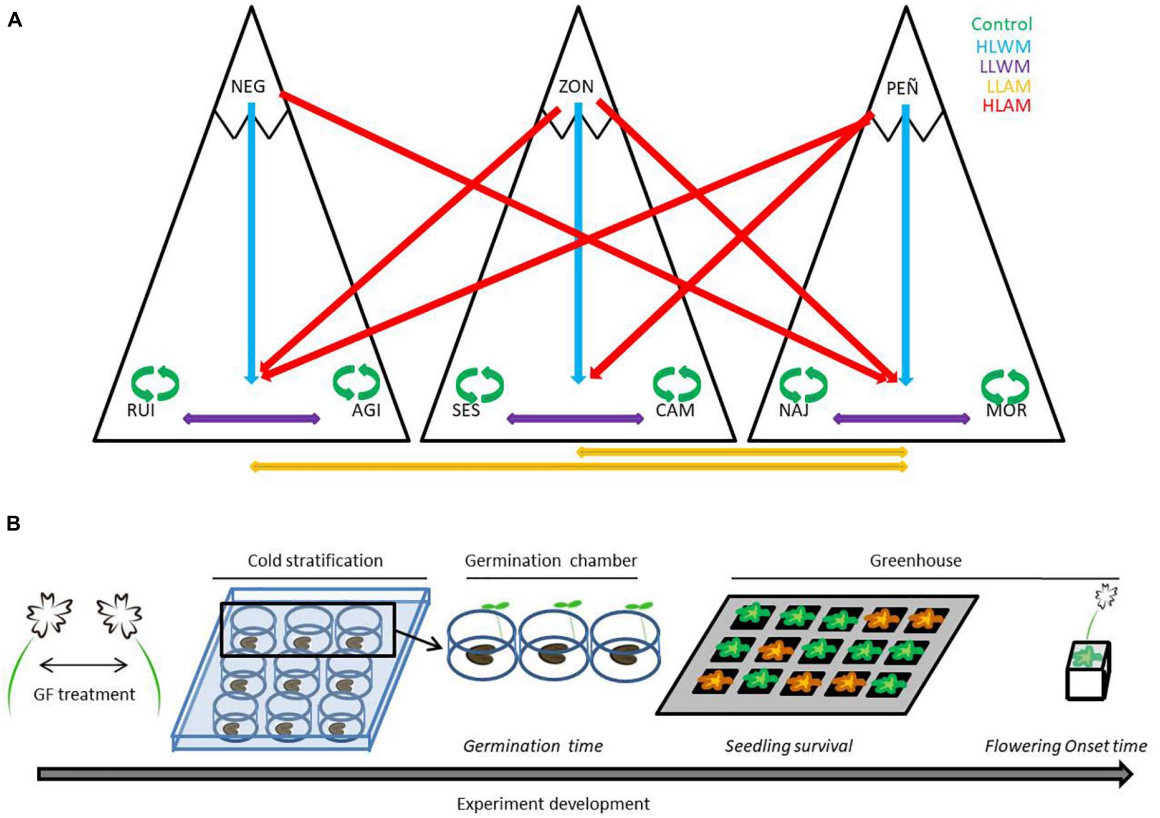


FIGURE 2 | Experimental methodology followed in the study. **(A)** Gene flow within and between populations from the three mountain ranges considered in this study (Béjar, Gredos, and Guadarrama). The arrow marks the direction of the pollen flow between populations. Green arrows denote the within population treatment (Control). Purple and orange arrows denote flow from low to low elevation population, within (LLWM) and among mountains (LLAM), respectively. Blue and red arrows denote flow from high to low elevation population, within (HLWM) and among mountains (HLAM), respectively. Blue, red and orange gene flows were implemented in the two low elevation populations of each mountain. Gene flow from NEG to low elevation populations of Gredos mountain range could not be performed. **(B)** Workflow from seed germination to flowering onset data collection. Seeds from the five gene flow treatments were deposited in multiwell plates, kept under cold stratification and then incubated in the germination chamber. Germinated seeds were first transferred to small pots where seedling survival was recorded. Four months later, plants were transferred to larger pots and flowering onset was recorded in due time.

within the same mountain. We expected that this gene flow treatment would induce earlier flowering. Moreover, it would increase genetic diversity without outbreeding depression due to gene flow from a geographically close but environmentally differentiated population from the same mountain. Seedling survival might vary depending on the outcome of potential maladaptive allele combinations vs. reduction of inbreeding depression.

III. LLWM, crosses within mountain using pollen from a low elevation population on plants from another low elevation population. We expected that this gene flow treatment would not change flowering date (gene flow from low population). Nevertheless, it would increase genetic diversity without outbreeding depression from a naturally connected and environmentally similar population from the same mountain, reducing inbreeding depression and, thereby, increasing seedling survival.

IV. LLAM, crosses using pollen from a low elevation population on plants from another low elevation population from a different mountain. We considered that this gene flow treatment would not change flowering date (gene flow from low population). Nevertheless, this gene flow would increase genetic diversity from naturally isolated (different mountain range) but environmentally similar populations. Seedling survival might vary depending on the outcome of potential outbreeding depression vs. reduction of inbreeding depression.

V. HLAM, crosses using pollen from high elevation populations on plants from low elevation populations from a different mountain range. We considered that this gene flow treatment would induce earlier flowering (gene flow from high population). Moreover, it would increase genetic diversity from naturally isolated (different mountain) and environmentally differentiated populations. In spite of a reduction of inbreeding depression, seedling survival could decrease as a result of potential outbreeding depression and the arrival of maladaptive allele combinations.

TABLE 2 | Number of mother plants (collected in the field) and number of clonal plants (generated from cuttings) of each low elevation population. Number of seeds, seedlings and flowered plants used to measure the germination time, seedling survival and flowering onset time, respectively. The low elevation population is the plant population that received the pollen of the gene flow treatments.

Low elevation population	No. of mother plants	No. of clonal plants	Treatment	No. of seeds	No. of seedlings	No. of flowering plants
AGI	46	79	Control	103	68	3
			HLWM	119	87	27
			LLWM	149	88	NA
			LLAM	149	101	14
			HLAM	197	142	31
RUI	31	125	Control	105	72	NA
			HLWM	125	95	NA
			LLWM	149	100	NA
			LLAM	86	64	NA
			HLAM	137	99	NA
MOR	29	80	Control	271	175	36
			HLWM	167	104	29
			LLWM	269	161	NA
			LLAM	227	153	41
			HLAM	223	139	42
NAJ	43	74	Control	215	143	NA
			HLWM	272	183	NA
			LLWM	287	181	NA
			LLAM	205	126	NA
			HLAM	188	145	NA
CAM	46	83	Control	211	137	6
			HLWM	236	157	26
			LLWM	244	180	NA
			LLAM	257	170	35
			HLAM	202	161	39
SES	33	103	Control	241	134	NA
			HLWM	243	174	NA
			LLWM	253	170	NA
			LLAM	176	124	NA
			HLAM	315	196	NA
			Total	6,021	4,029	329

Treatments were: Control, low elevation within-population crosses; HLWM, High low elevation within mountain crosses; LLWM, Low low elevation within mountain crosses; LLAM, Low low elevation among mountain crosses; HLAM, High low elevation among mountain crosses. NA denotes populations and treatments where flowering onset was not measured.

The experimental gene flow design was not reciprocal as it was focused on studying the effects of assisted gene flow on the marginal populations occurring at the low elevation distribution edge. Thus, the recipients of gene flow treatment were always the marginal populations as in previous study assessing similar questions (see Morente-López et al., 2020a).

Cultivation and Data Collection

In 2014, the fruits obtained from the above-mentioned crosses were collected and the seeds were counted and cleaned. 11201 seeds were subjected to a cold and wet stratification treatment to ensure a high percentage of germination (Giménez-Benavides et al., 2007a; **Figure 2B**). The number of obtained seeds per cross varied from 8 to 24, with the 89.5% of the crosses having at least 24 seeds. Seeds were placed separately in multiwell plates with Whatman filter paper wetted with distilled water. Dishes were stored in a refrigerator in the dark at 1°C for 3 months (following Giménez-Benavides et al., 2005; **Figure 2B**). After the stratification process, multiwell plates with the seeds were placed in a growth chamber under a 16-h light/8 h-dark regime at 20°C (Selecta Hotcold GL, Barcelona, Spain) (**Figure 2B**). From April 2015, germination was checked every 2 days. Seeds were considered to be germinated when they had developed a 2 mm radicle. Germination time was obtained counting the days from the start of the incubation to the germination day. Seedlings were transferred to 4 × 4 cm pots with a commercial potting substrate enriched with NPK fertilizer and kept, for 4 months, in an *ex situ* common garden at the greenhouse (**Figure 2B**). The pots with the seedlings were randomly placed on the greenhouse bench. The survival of the obtained seedlings was recorded in 2015 just before they were transferred to 5 L pots with the same type of substrate to maximize the plant growth and obtain reproductive individuals (**Figure 2B**). Plants remained in this common garden when flowering onset was recorded. From 1 January to 30 August 2017, the date of flowering onset of the surviving plants was registered and flowering time calculated by

counting the days between the first of January and the first flower bloom. After the first flower appeared, the maximum diameter of all individuals was measured.

Growth conditions in the greenhouse were warmer than those found in the natural populations. In the greenhouse mean temperature from May to September (the growth season) was 23.8°C whereas in nature it is about 15°C (Giménez-Benavides et al., 2007a). As a consequence of this difference, plants flowered earlier in the greenhouse compared to natural sites (Morente-López et al., 2020b). Furthermore, plants grown in the greenhouse were watered daily, whereas natural populations experience a significant summer drought (Giménez-Benavides et al., 2007a).

Statistical Analysis

In each mountain range, the flowering onset date was recorded on one of the low elevation populations. The results of the LLWM gene flow treatment were not used in the flowering onset analysis due to the low number of plants available for this treatment when the experiment was performed (**Figure 2**). To test possible differences on the days until first bloom (onset) among gene flow treatments, Kaplan-Meier survival curve comparisons based on Cox proportional hazards regression models were used. GLMMs were applied to assess differences among treatments in germination time and seedling survival. A *Poisson* error distribution and a binomial distribution were used for germination time and seedling survival, respectively. In the three models, gene flow treatment was included as a fixed factor and the maternal plant population as a random factor. Plant size was included as a co-variable in the flowering onset survival curves models. All statistical analyses were performed in R. For the Kaplan-Meier survival curve analysis, we used the function *coxme* from package *coxme* (version 2.2-7, Therneau, 2018) which allows the analysis of mixed effects Cox models. For the GLMMs, the function *glmer* from the lme4 package (Bates et al., 2015) was applied, and, for the *post hoc* analysis, the function *glht* from multcomp package (version 1.4-8, Hothorn et al., 2008).

RESULTS

Germination and Seedling Survival

The time until germination was recorded for 6021 germinated seeds. Survival rate was noted for 4029 seedlings (**Table 2**). Significant differences on germination time were found among gene flow treatments ($X^2_4 = 127.39$, $P < 0.001$). LLWM and HLAM had shorter germination time than Control, HLWM and LLAM (**Figure 3**). LLAM seeds were the latest to germinate (**Figure 3**). The R^2 of this model was 13.4% and the differences among treatments were, on average, approximately 1 day. There were no differences among treatments on seedling survival ($X^2_4 = 3.57$, $P < 0.47$, $R^2 = 0.003$). HLAM had the highest seedling survival and LLWM the lowest, with just a 2.4% difference between them (84.5, 84, 82.73, 83.47, and 85.83% in Control, HLWM, LLWM, LLAM, and HLAM, respectively).

Flowering Onset

The flowering onset was different between gene flow treatments in the survival curve comparison ($X^2_3 = 39.04$, $P < 0.001$). Plants from within population crosses (Control) flowered earlier than plants from between population crosses regardless of the pollen provenance (HLWM, LLAM, and HLAM) (**Figure 4**). Plants from crosses between mountains (LLAM and HLAM) flowered later than plants from crosses within the same mountain (Control and HLWM).

DISCUSSION

The results of this study highlight that gene flow affects differently several crucial life history traits on *S. ciliata*, and that these effects depend on gene flow origin. Therefore, when considering the use of assisted gene flow, it is crucial to evaluate the various effects of gene flow treatments on the different traits along the ontogeny. Then, the pros and cons of different types of gene flow should be pondered to determine which type of gene flow will provide the greatest benefits, if any, for the survival of the endangered population.

One of the main effects of gene flow regardless of its origin is its capacity to reduce inbreeding depression. The mixed mating system of the species allows the build-up of inbreeding depression in small-sized marginal populations. Previous studies suggest that inbreeding depression plays an important role in the fitness of early life stages of *S. ciliata* (García-Fernández et al., 2012a).

Assisted Gene Flow Effects on Germination and Seedling Survival

LLWM (within mountain low-low elevation population crosses) and HLAM (between high and low elevation localities among mountains) significantly decreased seed germination time with respect to within-population crosses. In the natural populations of *S. ciliata*, germination occurs right after snowmelt; thus, plants that germinate earlier have more time to grow before the arrival of the summer drought (Giménez-Benavides et al., 2007b). LLWM and HLAM decreased the seed germination time in almost six percent with respect to within-population crosses. On the contrary, LLAM (gene flow among low elevation populations among mountains) increased the seed germination time. These results seemingly reflect an interaction between elevation and mountain origin of the pollen donor population (**Figure 3**). At short distances (within mountain), the gene flow that causes an advance of germination comes from the low population. However, when pollen comes from far distances (among mountains) it was high elevation gene flow that shortened the germination time. This interaction could be explained by two different mechanisms playing a role at once: genetic diversity increase and acquisition of adaptive alleles. As the HLAM gene flow came from a contrasting environment from the target low elevation population, the reduction on germination time may be related with the gain in genetic diversity and the decrease of inbreeding depression in

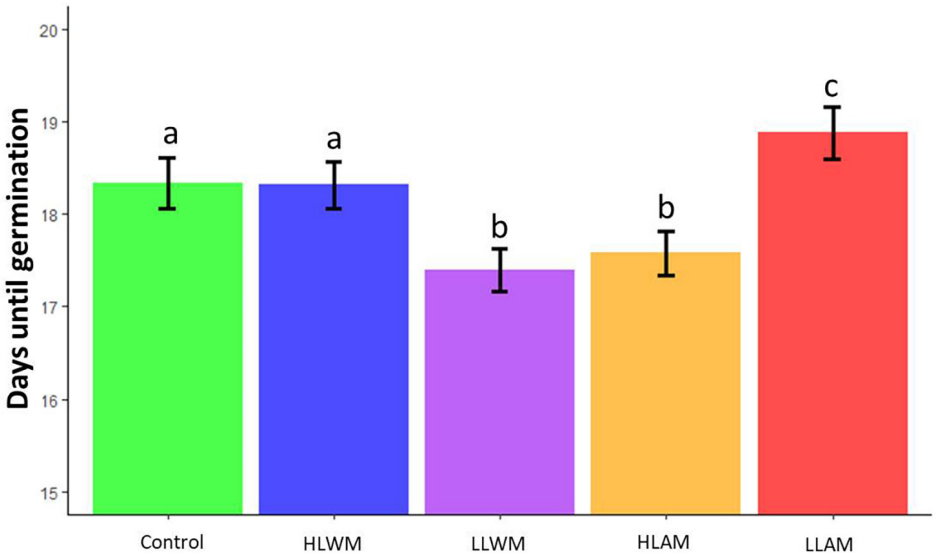


FIGURE 3 | Number of days until seed germination on different gene flow treatments. Bars denote mean ± SE. Different letters denote significant differences among treatments. Treatments were: Control, low elevation within-population crosses; HLWM, High low elevation within mountain crosses; LLWM, Low low elevation within mountain crosses; LLAM, Low low elevation among mountain crosses; HLAM, High low elevation among mountain crosses.

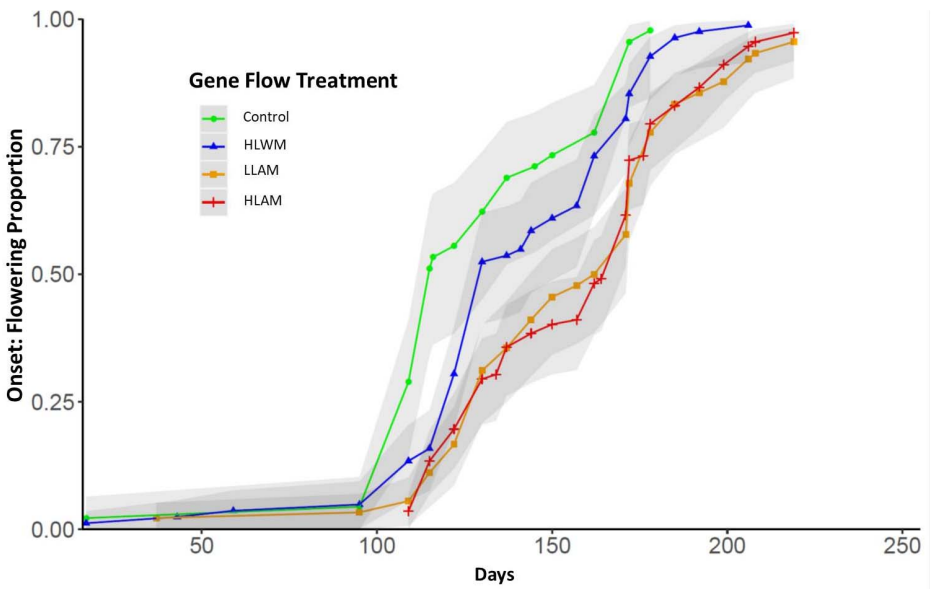


FIGURE 4 | Flowering time curves of each gene flow treatment. LLWM plants could not be included in this part of the experiment. The curves denote the accumulative increase of plants that flowered for the first time.

the target populations (Bontrager and Angert, 2019; Morente-López et al., 2020a). Even individuals with maladapted alleles may have a better performance in the first life stages if they benefit from an inbreeding reduction, which involves less genetic load and more vigorous seeds (Stanton and Galen, 1997; Sexton et al., 2009). The reduction in germination time by LLWM gene flow may be explained by the combined effect of genetic variation increase (gene flow from other population) and by the acquisition of adaptive alleles developed under similar environmental conditions in low elevation areas, that promotes faster germination (Sexton et al., 2011; Morente-López et al., 2020a). However, in HLWM gene flow, the net effect is null because the effect of genetic variation increase would be compensated by the effect of the incoming of maladapted alleles developed under contrasted environmental conditions.

The effects of LLAM on germination time cannot be interpreted on simple additive terms under the previous premises, and show an interaction pattern (**Figure 3**). A possible

explanation is that the small size of low elevation populations may have originated significant genetic drift. This fact together with the absence of natural gene flow between mountains may have generated very different genotype complexes involved in germination that, when put together, cause a delay in germination (Frankham et al., 2017). The lack of effect of the different gene flow treatments on seedling survival could be explained by the growing conditions of the *ex situ* common garden. In nature, selective pressures such as summer drought negatively affect seedling survival (Giménez-Benavides et al., 2007a). In this way, in a parallel experiment performed in the natural populations, seedlings from LLWM gene flow treatment had greater survival rates than Control and HLWM (Morente-López et al., 2020a). In the common garden used in this study, seedlings did not experience the stressors that occur in natural populations (e.g., lack of soil moisture) and thus did not show differences in survival rate between treatments.

Assisted Gene Flow Effects on Flowering Phenology

Flowering time is a crucial step in the reproduction of *S. ciliata* (Giménez-Benavides et al., 2007b). A common garden experiment carried out with this species showed that high elevation populations bloom earlier (at lower temperatures) than low elevation populations and that this difference is genetically based (Morente-López et al., 2020b). Therefore, we expected that gene flow from high elevation populations would induce an advance in the onset of flowering time of the low elevation populations. For instance, genetic modification of the flowering time owed to natural gene flow in the context of climate change has been suggested in populations of *Beta vulgaris* (van Dijk and Hautekèete, 2014). Against expectations, gene flow from high elevation populations delayed flowering onset with regard to the control treatment, regardless of whether the gene flow originated from the same mountain or a different mountain (HLWM and HLAM, respectively). Furthermore, flowering onset delay was higher when gene flow originated from other mountains (LLAM and HLAM), than when it came from the same mountain (HLWM).

It is important trying to understand why the flowering onset did not change as expected following the gene flow treatments. The delays obtained in flowering onset as a result of the different gene flow treatments could be considered an expression of outbreeding depression. Outbreeding depression is a fitness reduction originated after crossing populations with somewhat distant phylogenetic lineages and/or occurring in different environments (Edmands, 2007; Frankham et al., 2011). One of the main causes of outbreeding depression is the breakup of the gene networks involved in the target trait after recombination (Aitken and Whitlock, 2013). Coadapted gene complexes may provide a phenotype than confers fitness in specific environments but interpopulation hybridization may reduce the fitness due to a disruption of coadapted genes on the gene pathways (Templeton et al., 1986; Edmands, 1999; Rawson and Burton, 2002; Ellison and Burton, 2008). Taking into account that the timing of flowering is polygenic and controlled by several gene networks (Liu et al., 2009; Amasino, 2010; Wellmer and Riechmann, 2010), the fact that the early flowering onset trait from high elevation populations was not transferred through the pollen treatment to the recipient low elevation populations suggests that the polygenic genetic control of flowering onset is not additive and that epistatic interactions may be controlling the outcome in *S. ciliata* (Fenster and Galloway, 2000; Montalvo and Ellstrand, 2001). In a similar way, the flowering onset delay caused by gene flow from other mountains may also derive from the interactions generated from the mixing of genetic complexes with different evolutionary histories. Intensification of the epistatic modification has been observed with increasing distance (Fenster and Galloway, 2000). Genetic differences between *S. ciliata* populations are higher between than within mountains, suggesting a more probable natural gene flow within mountains and gene isolation between mountain (García-Fernández et al., 2012b; Morente-López et al., 2018). Hence, the effects of epistatic interactions would be more pronounced between the populations that are more isolated and thus more genetically differentiated (i.e., LLAM) than between the populations that are naturally connected by gene flow (i.e., LLWM) (Frankham et al., 2011). Nevertheless, in most scenarios, the outbreeding depression owed to epistatic interactions is expected to be temporary and could recover in a few generations (Aitken and Whitlock, 2013). Other potential causes of outbreeding, such as, chromosomal incompatibilities and introduction of locally maladapted alleles, are discarded by the fact that our studied populations of *S. ciliata* have the same karyotype and that gene flow from low elevation populations LLAM (i.e., same environment as the recipient populations) originated the plants that were the latest to flower (Frankham et al., 2011; García-Fernández et al., 2012b; unpublished data for Gredos and Béjar mountains; Aitken and Whitlock, 2013).

One limitation of this study regarding the evaluation of gene flow effects on flowering phenology, but also on germination time and seedling survival, is the fact that the environmental conditions of the common garden experiment were different from those occurring in the low elevation edge marginal populations. Specifically, the absence of water stress in the common garden experiment does not allow to evaluate the effects of the possible rise of outbreeding depression and maladaptation that might take place under the natural environmental conditions.

CONCLUDING REMARKS

This is the first study in an alpine plant that tests the importance of gene flow provenance for implementing effective facilitated adaptation conservation strategies considering different life history traits. The effects of gene flow on the offspring should be assessed in a sufficient number of traits to ensure a positive balance of pros and cons throughout the ontogeny of the species, as it is important to evaluate the effects along the different stages of the life cycle (Aronne, 2017). In this way, it should cover not only the survival rates of the individuals but also

their reproductive success. The results of the present work support the idea that the effects of assisted gene flow are not easily predictable and that it can cause unexpected responses in the traits aimed to be modified and also in others. Since the main functional traits of a plant are under polygenic control (e.g., Holdsworth et al., 2008; Ó'Maoiléidigh et al., 2014), the phenotypic modification of a population to improve its fitness after a change in the environmental conditions appears to be not as straightforward as we initially thought. Thus, the use of conservation strategies based on assisted gene flow should be considered with caution. Consequently, the importance of evaluating these effects prior to any conservation action in nature should be highlighted. Plants of threatened alpine populations facing a climate warming scenario will need seed germination in shorter times after snowmelt assuring in this way more time to the seedling for growing before summer drought and autumn frosts arrive. This will also help the following year to advance the onset of flowering in the next spring, right after snowmelt, to maximize seed production. Further ongoing studies on this species, which are currently testing the effects of distinct gene flow treatments on different traits in *in situ* common gardens near to the endangered populations should provide additional insight on the pros and cons of this potential conservation approach.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed for this study can be found in Figshare repository (10.6084/m9.figshare.13061693).

AUTHOR CONTRIBUTIONS

JM-L, SP-B, AG-F, CL-R, and JI designed this study. JM-L, SP-B, AG-F, CL-R, MLRT, and JI collected the plants from nine populations and performed the laboratory work at CULTIVE facility. SP-B and JM-L analyzed the data. SP-B wrote the manuscript with the help of JM-L, MLRT, CL-R, AG-F, ET, and JI. All authors reviewed the manuscript and approved the final manuscript.

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Climate Change Can Drive a Significant Loss of Suitable Habitat for *Polylepis quadrijuga*, a Treeline Species in the Sky Islands of the Northern Andes

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It is predicted that climate change will strongly affect plant distributions in high elevation "sky islands" of tropical Andes. *Polylepis* forests are a dominant element of the treeline throughout the Andes Cordillera in South America. However, little is known about the climatic factors underlying the current distribution of *Polylepis* trees and the possible effect of global climate change. The species *Polylepis quadrijuga* is endemic to the Colombian Eastern Cordillera, where it plays a fundamental ecological role in high-altitude páramo-forest ecotones. We sought to evaluate the potential distribution of *P. quadrijuga* under future climate change scenarios using ensemble modeling approaches. We conducted a comprehensive assessment of future climatic projections deriving from 12 different general circulation models (GCMs), four Representative Concentration Pathways (R) emissions scenarios, and two different time frames (2041–2060 and 2061–2080). Additionally, based on the future projections, we evaluate the effectiveness of the National System of Protected Natural Areas of Colombia (SINAP) and Páramo Complexes of Colombia (PCC) in protecting *P. quadrijuga* woodlands. Here, we compiled a comprehensive set of observations of *P. quadrijuga* and study them in connection with climatic and topographic variables to identify environmental predictors of the species distribution, possible habitat differentiation throughout the geographic distribution of the species, and predict the effect of different climate change scenarios on the future distribution of *P. quadrijuga*. Our results predict a dramatic loss of suitable habitat due to climate change on this key tropical Andean treeline species. The ensemble Habitat Suitability Modeling (HSM) shows differences in suitable scores among north and south regions of the species distribution consistent with differences in topographic features throughout the available habitat of *P. quadrijuga*. Future projections of the HSM predicted the Páramo complex "Sumapaz-Cruz Verde" as a major area for

the long-term conservation of *P. quadrijuga* because it provides a wide range of suitable habitats for the different evaluated climate change scenarios. We provide the first set of priority areas to perform both *in situ* and *ex situ* conservation efforts based on suitable habitat projections.

Keywords: climate change, citizen science data, habitat suitability modeling, high-Andean forests, Páramo, street view imagery

INTRODUCTION

The Andean region is a South American biodiversity hotspot, home to an impressively high diversity and endemism (Myers et al., 2000; Antonelli and Sanmartín, 2011). Regrettably, tropical Andean mountain species are classified as highly vulnerable to ongoing climate change, which may lead to strong shifts in distribution ranges, populations decline, and local extinction (Mittermeier et al., 2011; Mountain Research Initiative EDW Working Group, 2015). Although future distribution predictions for tropical Andean species are often challenging and carry a high uncertainty, due to the complex topography and climate of the region, they often point toward an extreme sensitivity to climate change (Lawler et al., 2009; Tovar et al., 2013; Tejedor Garavito et al., 2015; Peyre et al., 2020). Specifically, it is hypothesized that the main factors impacting future species distribution in the Andes will be the increase of temperature, changes in water fluxes, and the upward shift of the lower snowfall limit (Vuille et al., 2008, 2015; Herzog et al., 2011). Furthermore, several studies have already predicted an increase in threats and local extinction risks by the second half of the century in the Andes region (Ramirez-Villegas et al., 2014; Peyre et al., 2020; Valencia et al., 2020).

Polylepis forests are unique montane ecosystems, naturally occurring at high elevations in the Andes (Hoch and Körner, 2005; Navarro et al., 2005). These forests are generally present as sparse patches separated by vast areas of *Páramo* or *puna* grasslands (Fjeldså, 2002; Jameson and Ramsay, 2007) dominated mainly by *Polylepis* spp., that are endemic to South American mountain ranges, and tend to occur along the upper treeline (Simpson, 1979, 1986; Fjeldså et al., 1996). *Polylepis* treeline forests are the source of essential cultural, economic, and ecosystem services, providing pivotal ecological functions such as absorbing moisture from the clouds and releasing water in springs and rivers while being optimal habitats for other threatened and endemic species (Kessler, 2006; Donald et al., 2010). These forests require special conservation efforts (Purcell and Brelsford, 2004; Pinos, 2020) as they are threatened by deforestation, fires, farming, timber extraction, and land-use change (Fjeldså et al., 1996; Etter and Villa, 2000; Kessler, 2002; Hensen et al., 2011).

Several studies already support the importance of environmental variables such as elevation, precipitation, and mean annual temperature in shaping the current distribution patterns of different *Polylepis* species (Zutta et al., 2012; Boza Espinoza et al., 2019). Constraints related to water availability and soil water content during the dry season, hence precipitation and low-temperature extremes define the central eco-physiologic adaptations of *Polylepis* species (Velez et al., 1998; Morales et al., 2004; Ramos et al., 2013). Moreover, the decline of *Polylepis* diversity and abundance is one of the hypothesized effects of global or regional climate change patterns on Andean ecosystems (Gareca et al., 2010; Anderson et al., 2011). In particular, recent studies predicted severe reductions of suitable habitat for *Polylepis tarapacana, P. incana*, and *P. reticulata* before the end of the twenty-first century (Ramirez-Villegas et al., 2014; Cuyckens et al., 2016). It is known that closed forest stands can maintain their own microclimate and that their irregular cohort establishment depends on the occurrence of favorable (wet) years (Rada et al., 2011). The possible importance of moisture conditions for *Polylepis* spp. has already been reported for northern Chile (Rundel et al., 2003). Also, it has been postulated that any increase in temperature will further restrict *Polylepis australis* distribution in central Argentinian Andes to the uppermost elevations (Marcora et al., 2008), therefore underlining a high sensitivity to high temperatures for the genus. Therefore, understanding the consequences of climate change on *Polylepis* woodlands, as one of the most sensitive Andean biomes (Kessler et al., 2014), should be a priority for conservation biology in the Andean region, as necessary means to guide conservation policies and management strategies in the coming decades (Gosling et al., 2009; Sousa-Silva et al., 2014; Cuyckens et al., 2016). Also, it has to be taken into account that plausible niche specialization processes taking place in the different regions of the species distribution may lead to a reduced model ability to predict suitable areas under the assumption of niche equilibrium (Guisan and Thuiller, 2005; Parmesan, 2006; Hargreaves and Eckert, 2014).

In the specific case of the Colombian Andes, it is known that *Polylepis quadrijuga* Bitter was one of the first native species to be exploited (Rodríguez et al., 2018) and that continuous expansion of agricultural and livestock frontiers has led to increased fragmentation and isolation of *P. quadrijuga* woodlands. Thus, it is important to expand the understanding of the main ecological drivers of *P. quadrijuga* distribution, and to estimate the extent of its current distribution, while evaluating the effects of ongoing climate change on the species. Here, we selected this treeline species as a case study to address the influence of climate change on the distribution of key highland species in the northern Andean region. We apply Habitat Suitability Modeling (HSM) to infer the climate-change vulnerability of *P. quadrijuga*, one of the recorded species of *Polylepis* in Colombia (Boza Espinoza et al., 2019). Moreover, we specifically aim to (i) identify the key climate variables shaping the current geographical distribution of *P. quadrijuga*, (ii) estimate present and future suitable areas and, (iii) identify climatically stable areas as potential sites for the implementation of protection efforts. Additionally, we evaluate to what extent the current protected areas network and Páramo

complexes of Colombia enclose the predicted future suitable areas under climate change for *P. quadrijuga,* and if these can be sufficient for the *in situ* conservation of this species. Finally, we discuss possible conservation strategies and effective management actions that can ensure the persistence of this Colombian iconic Andean treeline species, addressing the specific protected areas that could host suitable habitat for *P. quadrijuga* under current and future climate change conditions.

MATERIALS AND METHODS

Study Species and Area

Polylepis quadrijuga (Rosaceae), locally known as "*colorado*" or "*sietecueros*" is an evergreen tree of up to 10 m height with compound leaves and a twisted trunk with continuously peeling reddish bark (Simpson, 1986). This species is easily recognized, its taxonomy resolved, and its distribution does not overlap with other *Polylepis* species (Kessler and Schmidt-Lebuhn, 2006; Segovia-Salcedo et al., 2018). *P. quadrijuga* is wind-pollinated and the seeds are dispersed by the wind during the dry season (Simpson, 1979; van Schaik et al., 1993; Schmidt-Lebuhn et al., 2007). Particularly, *P. quadrijuga* is endemic to the Colombian Eastern Cordillera slopes and rocky areas that are usually immersed within the Páramo grassland matrix (Simpson, 1986; Schmidt-Lebuhn et al., 2006; Rangel-Ch and Arellano-P, 2010; Pérez-Escobar et al., 2018; Peyre et al., 2018). Also, *P. quadrijuga* occurs often above the timberline and forms dense stands at elevations as high as 4,000 m, usually along streams (Simpson, 1979).

The Northern Andes present a high physiographic and topographic complexity, are considered one of the most diverse areas in the world (Myers et al., 2000; Jenkins et al., 2013; Jung et al., 2020), and are characterized by a very moist climate, low thermal seasonality, and marked diurnal temperature variations (Buytaert et al., 2006). Regionally, the climate of the Colombian Eastern Cordillera ranges from humid on the eastern flank to sub-humid or slightly dry on the western flank. The precipitation regime is bimodal, with pronounced dry and wet seasons, and annual rainfall ranging from 600 to 1,300 mm/y (Etter and Villa, 2000).

Polylepis species possess physiological adaptations to low temperatures and physiological drought that allow them to grow under colder conditions than the global mean for high elevation treeline species (Fjeldså et al., 1996; Kessler et al., 2014). Key physiological traits such as high foliar concentration of flavonoids and seasonal phenology could play an important role in the adaptation of this species to the severe environmental conditions of the Andean highlands (Velez et al., 1998). Despite these appealing features, *Polylepis* has been little studied. Moreover, as a result of the long history of deforestation in the high Andean forests of Colombia, the remaining patches of *Polylepis* forests are spread out over the open modified landscape (Etter and Villa, 2000; Rangel-Ch and Arellano-P, 2010), and several studies highlight that the relict patches of *Polylepis* forests in Colombia should be considered as a priority for conservation programs.

Species Occurrence Data

We compiled occurrence data for *P. quadrijuga* from three different sources: (i) the Global Biodiversity Information Facility (GBIF), initially 104 georeferenced records, where we also checked the nomenclature for synonyms (Borja-Acosta, 2017, 2019; Raz and Agudelo, 2019; García et al., 2020; Grant and Niezgoda, 2020; Marín and Moreno, 2020; Rodríguez Bolaños, 2020; Lozano Bernal, 2021; Magill et al., 2021); (ii) curated citizen science projects observations (iNaturalist, 49 records), which included geographical coordinates and a photographic record, allowing an accurate identification check (Ueda, 2021); and (iii) direct virtual exploration inside the main Páramo complexes of the Eastern Cordillera, using the Google Street View Imagery tool (Google Maps, 23 records). We surveyed all the rural roads with available street view imagery, crossing the Páramo districts "Boyacá," "Cundinamarca," and "Santanderes" (Sarmiento et al., 2013). Through direct inspection of hundreds of 3D georeferenced panoramas, we certified the occurrence of *P. quadrijuga* when we observed multi-trunk trees or shrubs showing the combination of vegetative characters as the distinctive reddish bark peeling in thin papery layers and compound leaves with grayish-green color[1]. No other group of plants could be mistakable with *Polylepis* in the Páramo landscape (Simpson, 1979), and *P. quadrijuga* is the only species of the genus recorded from the Eastern Cordillera of Colombia (Rangel-Ch and Arellano-P, 2010; Fajardo-Gutiérrez et al., 2018). Therefore we considered this methodological approach adapted from Hardion et al. (2016) to be adequate in this study.

Only records with global positioning system (GPS) coordinates and detailed localities were used, and then visually inspected for obvious geocoding errors. We included all the occurrences covering the entire known geographic distribution of the species (**Figure 1** and **Supplementary Table 1**) except the ones belonging to the Páramo of Frontino, located in the Western Cordillera of Colombia in the Antioquia department, which were removed, as these are now believed to form a separate species, and are currently under taxonomic reevaluation (Boza and Kessler, pers. comm.). We removed records with uncertain locations and retained occurrences with a minimum distance of 1 km from each other to avoid spatial pseudo-replication, yielding a final set of 99 records: 37 from GBIF (41%), 40 from iNaturalist (40%) and 22 from Street View Imagery (22%) (**Supplementary Table 1**). We also evaluated potential geographical sampling bias in our species occurrence dataset due to differences in accessibility. For that, we assessed the biasing effect of roads on the sampling intensity using the Bayesian approach implemented in the R package *sampbias* (Zizka et al., 2020). We calculated the bias effect on the three different sources of observed records (GBIF, iNaturalist, and Google Street View Imagery) and ten simulated datasets of same size as our complete dataset under complete random sampling and other 10 highly biased simulated datasets limiting the random sampling to maximum 1 km from roadways within the species distribution range (both geographical and altitudinal). The biasing effects show that differences in the observations among the three data

[1]https://goo.gl/maps/AFXnvdGFjrtxPgkJ6

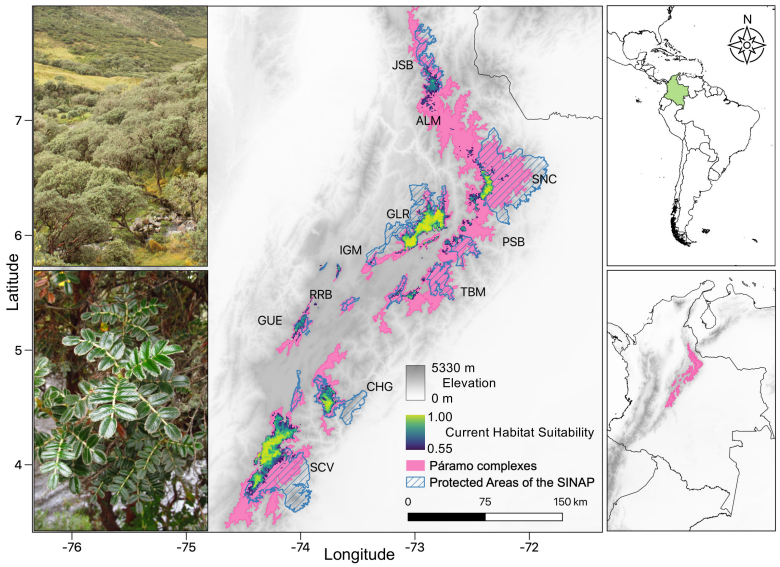


FIGURE 1 | Habitat and distribution of *Polylepis quadrijuga* woodlands in the Colombian Eastern Cordillera. Left panel: habitat and architecture of the *P. quadrijuga* (top) and morphology of the plant *in situ* (bottom; Photos by F. Fajardo). Central panel: Projection of the *P. quadrijuga* ensemble Habitat Suitability Model to current climatic conditions (showing suitability values > 0.554). Continuous color scale represents the habitat suitability scores. The pink shading denotes the spatial extent of the Páramo complexes along the Colombian Eastern Cordillera *sensu* IAvH (2012). The protected areas of the Colombian National System of Protected Areas (SINAP) that overlap with Páramo regions are represented by the diagonal blue-lined areas. Right panel: broad geographical context of the main map in South America (top) and Colombia political boundaries (bottom) including the Andes Cordilleras (gray) and Eastern Cordillera's Paramo complex areas (pink).

sources and the simulated full random sampling are much smaller compared to the random sampling around the roads (**Supplementary Figure 1**).

Environmental Data Sets and Abiotic Predictor Variables

We gathered a total of 23 biologically relevant climatic variables: 19 bioclimatic variables derived from monthly temperature and precipitation climatology for the years 1979–2013 available at 30 arc sec resolution (1 km at the equator) were retrieved from the CHELSA dataset (Karger et al., 2017)[2]; Slope and Aspect variables derived from a SRTM Digital Elevation Model using the raster package (Hijmans, 2015); and two topographic variables (Terrain roughness index and Topographic wetness index) at same resolution to the bioclimatic variables were obtained from the ENVIREM data set (Title and Bemmels, 2018). The bioclimatic variables have shown a high importance for defining the environmental niche of species of *Polylepis* (Zutta et al., 2012; Boza Espinoza et al., 2019) and are directly affected by climate change. The CHELSA climatic dataset has been proven to provide better results for topographically complex montane regions (Bobrowski and Schickhoff, 2017). Moreover, the topography-related variables that we incorporated, are secondary geographic predictors that are used to characterize spatial topographic diversity, terrain complexity and soil moisture patterns (Lin et al., 2006), and are very sensitive to local differences in elevation, being able to capture the presence of steep gradients and high spatial heterogeneity (Różycka et al., 2017).

[2]http://chelsa-climate.org/bioclim/

We cropped and aligned the explanatory environmental layers to an area encompassing 0°–12.5° N and 79.5°–69.1° W, using the raster package (Hijmans, 2015) in R. This geographic extent includes the full known extant range of *P. quadrijuga*. In order to avoid multicollinearity-related noise, we performed variable selection using the function *corselect* of the R package fuzzySim (Barbosa, 2015). This function calculates Pairwise Pearson correlations among all explanatory variables and then, among each pair of highly correlated variables (correlation coefficient > 0.75), it retains the variable with the highest individual response in a preliminary binomial Generalized Linear model. The final explanatory data sets included 11 variables, all showing variation inflation factors below 10. Correlation values among the selected variables were calculated and plotted with the function *ecospat.cor.plot* of the package ecospat (Di Cola et al., 2017; **Supplementary Figure 2**).

Habitat Suitability Models

To perform the HSM we implemented an ensemble modeling framework (Araujo and New, 2007) using the BIOMOD package (Thuiller et al., 2009) in R version 4.0.3 (R Core Team, 2020). This approach combines models obtained by several algorithms to account for the uncertainty associated with each modeling technique (Thuiller et al., 2009, 2020). We selected seven modeling algorithms: (1) generalized linear models—GLM (McCullagh and Nelder, 1983); (2) generalized additive models—GAM (Hastie and Tibshirani, 1990); (3) generalized boosting models—GBM (Death, 2007); (4) classification tree analysis—CTA (Breiman et al., 1993); (5) multiple adaptive regression splines—MARS (Leathwick et al., 2005); (6) random

forests—RF (Breiman, 2001); (7) artificial neural networks—ANN (Ripley, 1996); and (8) maximum entropy—MAXENT; (Phillips et al., 2006). We performed individual parameter tuning for each algorithm using the *BIOMOD_tuning* function.

Following Barve et al. (2011), we sampled the pseudo-absences within the accessible area for the species. We outlined the area including the Colombian Eastern Cordillera above 1,500 m of elevation and outside a 5 km buffer around the presence records (**Supplementary Figure 3**). Within this area, we randomly selected 10,000 pseudo-absences. Prevalence was maintained at 0.5, allowing the sum of presence-weights to be equal to the sum of pseudo-absence weights in the model calibration process (Barbet-Massin et al., 2012). We ran ten cross-validation replicates where presence records were split into sets of 75% for training and 25% for testing the models. For each cross-validation replicate we performed five runs with different pseudo-absence sets, therefore completing a total of 400 models. To assess the predictive performance of the models, we used both the threshold-independent area under the receiver operating characteristic curve statistic—AUC (Phillips et al., 2006) and the threshold-dependent true skill statistic—TSS (Allouche et al., 2006). Consensus models were obtained using a TSS-weighted average method to account for the predictive power of each model. Models with low predictive power (TSS < 0.7) were discarded.

As an additional characterization of the multi-dimensional occupied environmental space of *P. quadrijuga*, we extracted selected predictor values in correspondence of our species occurrences and performed a principal components analysis (PCA), with the function *prcomp* in R (R Core Team, 2020).

Evaluation of Future Range and Elevation Shifts and Representation in the Protected Areas System

We assessed the possible impacts of climate change on the distribution of *P. quadrijuga* through the projection of the HSMs to two time periods (2041–2060 and 2061–2080). For future projection we retrieved the projected bioclimatic variables from the CHELSA dataset, according to four greenhouse gas concentration scenarios, including low (RCP 2.6), moderate (RCP 4.5), high (RCP 6.0), and strong (RCP 8.5) increases in global radiative forcing (IPCC, 2013). These scenarios express the intensity of climatic changes by representative concentration pathways, using predicted values for radiative forcing for 2100, in comparison to the preindustrial era (Harris et al., 2014). The RCP2.6 scenario assumes CO_2 concentrations of 450 ppm in 2100, that global mean temperatures will increase 0.2–1.8°C and has no strict reference in previous (Fourth Report) IPCC guidelines. RCP4.5 assumes 650 ppm CO_2 and 1.0–2.6°C in 2100 and refers to the previous guideline scenario B1. RCP6.0 assumes 850 ppm CO_2 and 1.3–3.2°C in 2100 and refers to the previous guideline scenario B2. RCP8.5 assumes 1350 ppm CO_2 and 2.6–4.8°C in 2100 and refers to the A1F1 scenario of previous IPCC guidelines (Harris et al., 2014).

In addition, to consider the inherent uncertainty derived from each different general circulation model, we performed a comprehensive assessment including twelve different CMIP5 General Circulation Models (GCMs) [CESM1(CAM5), CSIRO-Mk3-6-0, FIO-ESM, GFDL-ESM2G, GFDL-ESM2M, GISS-E2-H, IPSL-CM5A-MR, MIROC-ESM-CHEM, MIROC5, MRI-CGCM3, NorESM1-M, and CCSM4]. For the resulting future climate projections, we generated consensus distribution maps for each time period and RCP scenarios, outlining the suitable areas predicted by at least three binary-transformed ensemble projections for the different GCMs. Future bioclimatic variables for all the GCMs, RCPs and time periods were retrieved from the CHELSA database. The topographic variables were kept invariant for all future projections.

We intersected the current and future HSMs projections to estimate the sensitivity to climate change at the species level, and assess the distribution range stability for all future RCPs scenarios and time periods. To this aim, we calculated the percentage of predicted area that remains stable, as well as range expansions or contractions in relation to the current projected area. Values of less than 100% indicate a decrease in environmental suitability over time (i.e., a high vulnerability of the species to climate change), whereas values greater than 100% indicate an increased extent of suitable habitat (i.e., a low vulnerability of the species to climate change).

In order to identify the specific Protected Areas (PAs) that could be relevant for the protection of *P. quadrijuga* in the future, we measured the overlapping area between the different projections of suitable habitat and the areas of 10 out of 36 Páramo complexes of Colombia (PCC), belonging to the Páramo districts "Boyacá," "Cundinamarca," and "Santanderes" (Sarmiento et al., 2013). Colombia has defined 17 Páramo districts and 36 Páramo complexes, by grouping natural areas that are divided by geographical features or human transformed landscapes that constitute special management and protection zones (Sarmiento et al., 2013). We also calculated the overlap between the suitable habitat projections and 36 protected areas of the National System of Protected Areas of Colombia (SINAP, 2020), including five National Natural Parks[3]. We used the PCC and the SINAP maps and calculated the portion of the suitable area for *P. quadrijuga* within these protected areas under the current conditions and future scenarios (RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5), for the periods 2041–2060 and 2061–2080.

RESULTS

Model Performance and Current Suitable Area Distribution

The projection of the ensemble-HSM to current climate conditions predicted a suitable area of 4444.9 km^2, retrieving the highest and spatially continuous values of suitability throughout the southern range of the species distribution (SCV and CHG **Table 1** and **Figure 1**), and less suitable and

[3]https://runap.parquesnacionales.gov.co

TABLE 1 | Number of occurrences of *P. quadrijuga*, and resulting suitable areas for each Páramo Complex of Colombia (PCC; Sarmiento et al., 2013).

Páramo district	Páramo complex	N° of records within the PCC	Suitable area within the PCC (km^2)	(%) of the Páramo complex area	Mean (sd) suitability value	Maximum suitability value
Cundinamarca	Sumapaz Cruz Verde (SCV)	42	1328.4	39.8%	0.858 (± 0.13)	1.000
Boyacá	Guantivá La Rusia (GLR)	35	940.9	78.6%	0.883 (± 0.12)	1.000
Boyacá	Sierra Nevada del Cocuy (SNC)	17	562.3	20.7%	0.787 (± 0.13)	0.996
Cundinamarca	Chingaza (CHG)	11	322.9	29.4%	0.834 (± 0.12)	0.997
Santanderes	Jurisdicciones Santurbán Berlín (JSB)	4	317.1	22.2%	0.687 (± 0.08)	0.932
Cundinamarca	Guerrero (GUE)	2	158.3	37.4%	0.707 (± 0.07)	0.878
Boyacá	Tota Bijagual Mamapacha (TBM)	2	201.6	13.3%	0.728 (± 0.09)	0.973
Boyacá	Pisba (PSB)	1	250.8	23.6%	0.674 (± 0.07)	0.913
Santanderes	Almorzadero (ALM)	0	63.6	4.1%	0.624 (± 0.04)	0.798
Boyacá	Iguaque Merchán (IGM)	0	22.9	8.1%	0.683 (± 0.07)	0.839

more fragmented areas in the northern range. The predicted potential current distribution is consistent with the known distribution of this species. Ten PCCs along the Colombian Eastern Cordillera contained suitable areas for *P. quadrijuga* (**Table 1** and **Figure 1**). Less than a half of the predicted suitable area (46.1%, 1813.4 km^2) was covered by the protected areas network of the SINAP, but most of the suitable areas (95.8%, 3765.3 km^2) were located inside of the PCC boundaries, with only 4.2% was located outside of the PCC protection (163.4 km^2) (**Figure 1** and **Table 1**). The algorithms returning higher model performance metrics were GBM, GLM, Maxent, and RF and, the HSM returned overall high model evaluation metrics, with average AUC = 0.96 ± 0.04 and TSS = 0.9 ± 0.07 (**Supplementary Figure 4**), underscoring a good model performance in discriminating the species' fundamental climatic niche.

Importance of Environmental Predictors

The PCA identified several important climatic variables that highlight differences in niche breadth through the species distribution. The analysis revealed differences in niche occupancy with a strong geographic signal represented by the different Páramos complexes (**Figures 1**, **2**). The first axis of PCA (35% of the total variation) synthesized a distribution gradient between the eastern and western flanks of the Eastern Cordillera, with the variables Mean Diurnal Range and Precipitation of Warmest Quarter showing the highest loadings, as well as Temperature Seasonality and Topographic wetness index, but with an opposite direction (**Figure 2**). The second axis (ca. 21% of the variation) with Precipitation of Driest Quarter showing the highest loading, highlighted a north-south gradient, with the exception of the localities from the SCV Páramo that had a wide niche breadth along this axis (**Figure 2**).

We found that Mean Temperature of Warmest Quarter (Bio 10) was the variable with consistently higher individual influence (mean = 77% ± 11.3) for all modeling techniques applied. Isothermality (Bio 3, mean = 51% ± 19.1) and Precipitation of Driest Quarter (Bio 17, mean = 45% ± 25.4) had also relatively high importance values (**Supplementary Figure 5**). Topographic variables appeared to be less informative in predicting the species occurrences but showed more relevance for differentiating the different geographical groups within the species (**Figure 2** and **Supplementary Figure 5**).

Predicted Suitable Distribution of *P. quadrijuga* Under Climate Change

The potential geographic range projected for *P. quadrijuga* under different climate change scenarios presented strong reductions in suitable areas (**Figure 3**), and also in areas encompassed by PAs (**Table 2**). Our results predicted a substantial decline of the suitable habitat under all climate change scenarios by 2040s, the fragmentation of currently suitable areas, and the loss of most of the suitable habitat patches in the north of the Eastern Cordillera (Páramo districts "Boyacá" and "Santanderes"). In fact, the predicted future distribution of the suitable habitat for *P. quadrijuga* shows that only the population of SCV, would be able to persist under all the four different RCP scenarios (**Figure 3**), while for all the other areas, a strong reduction and fragmentation (or even disappearance) of suitable areas is predicted. Moreover, the HSMs predicted a potential loss of suitable area especially in the northern part of the Eastern Cordillera, even for the most optimistic scenario (RCP 2.6 for 2041–2060; **Figure 3**).

For all RCPs and for both the evaluated time periods, a decrease of more than 80% of the suitable habitat for *P. quadrijuga* is evident, and underscores a general scenario of dramatic range reduction and loss. The effect of global climate change on the potential distribution of *P. quadrijuga* is particularly drastic under the most pessimistic scenario (RCP 8.5), leaving only small relictual patches of the current suitable area in the northern species range (SNC and PSB Páramo complexes; **Figure 1**), for the 2061–2080 period. Interestingly, for the 2041–2060 period, the HSM predicted a higher loss of suitable area under the RCP 4.5 scenario than under all the other RCPs, followed by the RCP 8.5 (**Figure 3**). The potential loss of climatically suitable areas ranges from 76.4% (RCP 2.6—2041–2060) to 98.5% (RCP 4.5 2041–2060), even for the more climatically stable regions (i.e., SCV Páramo complex; **Figure 1**).

Additionally, the ensemble projection of the HSMs to future climate conditions predicted a fast contraction of the altitudinal range around medium-high elevation values, together with

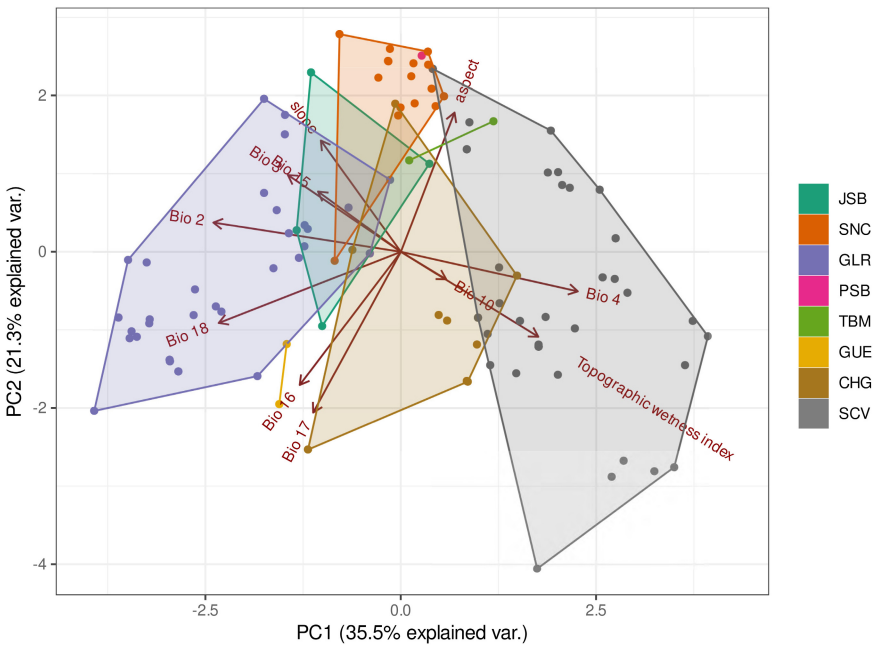


FIGURE 2 | Scatter plot of the principal components analysis (PCA) based on environmental space values occupied by *Polylepis quadrijuga* from Colombian Eastern Cordillera. Each different color dots represent the whole set of occurrences and the polygons highlight the occurrences superimposed to each Páramo complex. Niche area corresponds to the area of the hull relative to the largest polygon (*P. quadrijuga* = 1).

more fragmentation of suitable areas and even local population extinction in several of the northernmost localities (**Figures 3**, **4**).

Distribution of *P. quadrijuga* in the Protected Areas Network

Within the predicted current potential distribution of *P. quadrijuga* (4444.9 km^2), we identified about 2036.9 km^2 (45.8%) overlaps SINAP network areas. Moreover, 4168.8 km^2 (93.8% of the current suitable area) overlays Páramo complexes areas (**Table 1**). From the 99 presence records, 41 occur inside the protected areas of the SINAP. The Natural Parks Sumapaz and Pan de Azúcar el Consuelo are the protected areas with the highest number of records (16 and 11 occurrence points, respectively; **Supplementary Table 2**). Among the National Natural Parks (PNN) we found species records in the PNN Chingaza, PNN Sierra Nevada del Cocuy, and PNN Sumapaz. With regard to Regional Natural Parks (PNR), *P. quadrijuga* occurrences were recorded inside the PNR Pan de Azúcar el Consuelo, PNR Mutiscua Pamplona, and PNR Páramo de Santurbán.

The future projections of *P. quadrijuga* predicted a strong decline of protected areas covering current highly to moderately suitable habitats. The most dramatic shift of suitable areas was observed for the period 2041–2060, compared to the current distribution, affecting the northernmost portion of the species distribution. Although the species is of high conservation interest, the known occurrences located inside protected areas in Colombia are limited to only 41% of the reported occurrences (**Supplementary Table 2**).

DISCUSSION

This study reinforces the growing evidence of significant range shifts and drastic reduction of high Andean plant species driven by global climate change. The predicted potential future distribution of the Colombian Eastern Cordillera highland endemic *Polylepis quadrijuga*, highlights a strong decline in its suitable habitat. Thus, the implementation of management and conservation strategies becomes necessary as a basis for ensuring *P. quadrijuga* persistence and the preservation of the entire ecosystems that support this species. To this aim, our study identifies the areas of highest vulnerability for *P. quadrijuga* under future climatic conditions and provides useful information about stable suitable areas, serving as a baseline for decision-makers to implement effective conservation and management strategies to preserve *Polylepis* forest in Colombia. Lastly, we underline the importance of Protected Natural Areas in conservation studies and planning in the higher Andes, and their relevance for mountain species whose distribution is often naturally fragmented and more susceptible to habitat loss.

Current Suitable Habitat and Climatic Predictors for the Distribution of *P. quadrijuga*

We present the most detailed model to date regarding the potential distribution of *P. quadrijuga*, which shows four main areas with high habitat suitability values (**Figure 1**). Compared to a previous assessment (Fajardo-Gutiérrez et al., 2018), we included an updated dataset of herbarium records and verified

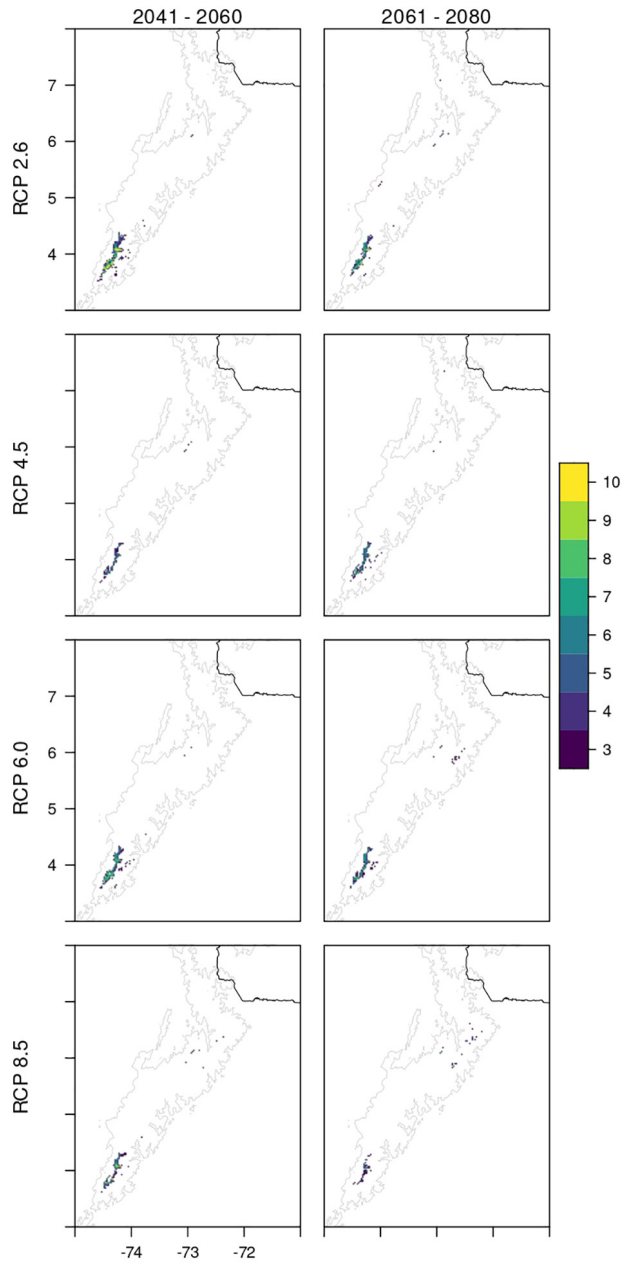


FIGURE 3 | Projection of the Habitat Suitability Model (HMS) of *P. quadrijuga* to future climate conditions derived from twelve general circulation models (GCMs) for the periods (2041–2060 and 2061–2080) under four Representative Concentration Pathway (26, 45, 60, and 85). The discrete color scale represents the number of different projections derived from the 12GCMs that support the presence of the species for future climate scenarios.

records from citizen science projects and street imagery sources, enhancing the model ability to characterize the species niche (Wisz et al., 2008). In addition, we applied a multiple algorithm ensemble modeling approach using a climatic dataset suited for montane regions, overall increasing the robustness in the identification of the climatic predictors of *P. quadrijuga* distribution and the spatial prediction of suitable areas.

The HSM predicts large suitable areas for *P. quadrijuga* in the western slopes of the Eastern Cordillera of Colombia, specifically in the SCV and CHG Páramo complexes, located in the southern range of the species, but lower and more fragmented suitable areas in the northern Páramos (JSB and ALM; **Figure 1**). In fact, our occurrences dataset shows a northward decrease in records, which may suggest a sampling bias problem (**Table 1**). However, we interpret this observed pattern of decreasing suitability toward the north as a natural response to the change of available niches driven by three interrelated factors: slope, topographic complexity, and soil water content. SCV and CHG Páramos host larger and topographically less complex valleys, allowing the establishment of bigger populations of *P. quadrijuga*. At higher latitudes, the suitable climatic belt for the species presents a more complex topography, with steep slopes and lower water availability. This restricts the species establishment to smaller forests on selected-facing slopes that provide a sheltering effect and enable the species to avoid long-term freezing, which limits tree growth, as well as seedling dehydration and photoinhibition caused by strong solar radiation (Toivonen et al., 2018).

Interestingly, the PCA grouped the populations occurring in the Páramos complexes of the larger Eastern Cordillera highland plateau (i.e., CHG and SCV) along similar values of the variables Topographic wetness index, Bio 4 and Bio 6, and separated them from the populations of the northeastern corner of the Eastern Cordillera (i.e., GLR, JSB, PSB, SNC), that were instead more related with variables such as Slope and Bio 3 (PC1, **Figure 2**), showing a temperature and topographic gradient. The PC2 highlighted a precipitation gradient spanning from the CHG Páramo (with high values of Bio 14, Bio 16, and Bio 19), to PSB, JSB, and SNC, that showed low values of those variables, and that in other words classify as atmospherically dry Páramos. Two slightly different trends were observed regarding the environmental niche of *P. quadrijuga*, one for the 35 records found in GLR, which forms part of the Boyacá Páramo district, and another for the 42 records of the SCV Páramo complex in the Cundinamarca district (**Table 1** and **Figure 2**), where the variables Bio 2 and Bio 4 play an important explicative role in the PC1. The GLR Páramo complex had a higher temperature diurnal range (Bio 2) compared to SCV, and SCV had a higher temperature seasonality (Bio 4) compared to GLR (**Figure 2**).

An alternative explanation of the lower amount of occurrences of *P. quadrijuga* in the northernmost part of the species distribution (Boyacá and Santanderes Páramo districts) is that these areas, where environmental conditions are suitable to host high andean forest and Páramo communities, had undergone severe deforestation through the past decades, thus resulting in the impoverishment of the local flora. In fact, 22–47% of the land belonging to the ALM, GLR, GUE, IGM, JSB, and PSB Páramo complexes is highly transformed, and as of 2011, these hosted complessively 168 km^2 of legal mining titles (Sarmiento et al., 2013). Oppositely, SCV and CHG Páramo complexes are also protected areas of the SINAP, hosting only 10 and 6% of transformed area, respectively, and 3556.4 km^2 of natural ecosystems, mostly Páramos and high andean forests (Sarmiento et al., 2013). Also, the northern east corner of the Eastern

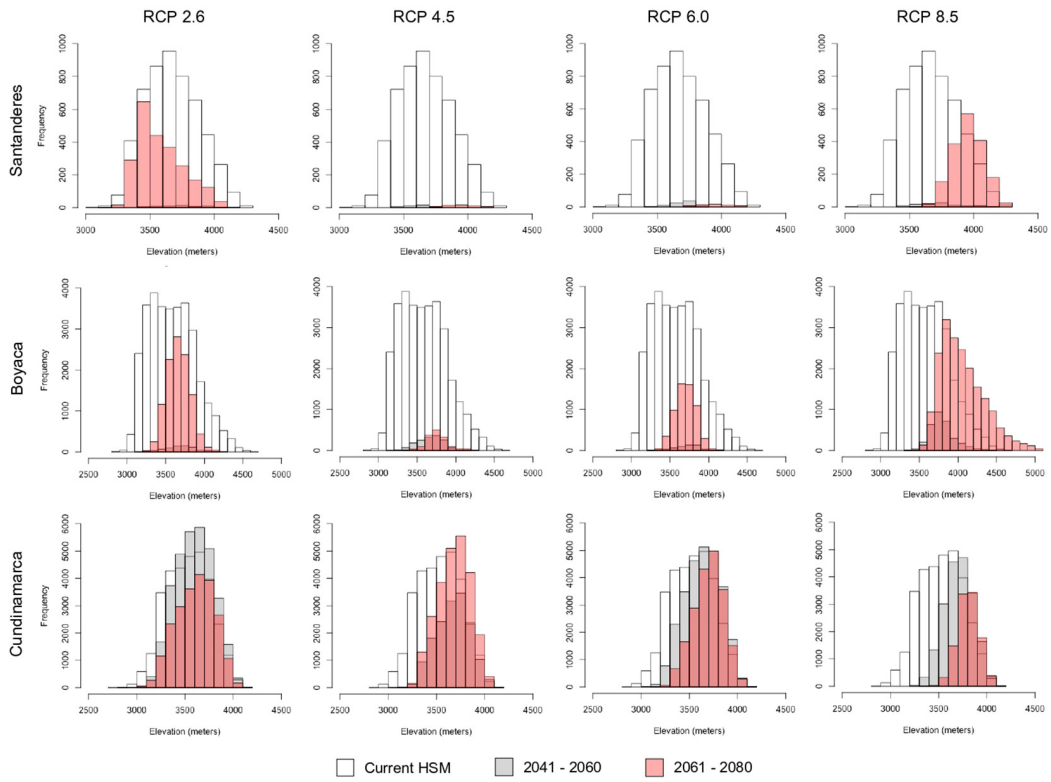


FIGURE 4 | Altitudinal range distribution of the projected suitable regions under current and future climate scenarios of *P. quadrijuga* for each of the three Páramo complex districts (**Table 1**). Santanderes (ALM, JSB), Boyacá (TBM, RRB, PSB, GLR, SNC, IGM), and Cundinamarca (SCV, SHG, GUE).

TABLE 2 | Change of suitable area for the projections of the ensemble Habitat Suitability Model of *Polylepis quadrijuga* to four climatic change scenarios (RCP) and two time periods.

Scenario	2041–2060			2061–2080		
	Stable	Loss	Gain	Stable	Loss	Gain
RCP 2.6	15.28 (12.94)	84.72 (12.94)	0.47 (0.34)	16.86 (14.57)	83.14 (14.57)	1.42 (1.9)
RCP 4.5	10.43 (12)	89.57 (12)	0.91 (2.01)	10.9 (10.96)	89.1 (10.96)	0.51 (0.67)
RCP 6.0	12.64 (11.69)	87.36 (11.69)	0.31 (0.39)	9.72 (7.93)	90.28 (7.93)	0.38 (0.52)
RCP 8.5	11.1 (8.77)	88.9 (8.77)	0.52 (0.81)	8.25 (7.09)	91.75 (7.09)	1.11 (1.7)

Values indicate the mean (sd) proportion of the stable, loss, or gain area over the projections from the 12 Global Circulation Models compared to the current conditions. Stable suitable habitats (Stable), predicted loss of suitable habitat (Loss) and predicted gained suitable habitat (Gain).

Cordillera displays a remarkably high topographic complexity, due to the presence of numerous high peaks surrounding the deep Chicamocha inter-Andean canyon (Peyre et al., 2019; Valencia et al., 2020). As a consequence, we found that these Páramo complexes (SNC, GLR, ALM, and PSB) host smaller suitable areas for *P. quadrijuga*, as already pointed out for the *Espeletia* complex (Valencia et al., 2020).

Polylepis trees are exposed to the harsh climatic conditions that characterize highland areas. Constraints related to water availability, hence precipitation, soil water content during the dry season, and low-temperature extremes define the main eco-physiologic adaptations of *Polylepis* species (Velez et al., 1998; Morales et al., 2004; Ramos et al., 2013). Consistently, our results showed that temperature-related variables targeting extreme diurnal/annual ranges are among the better climatic predictors of *P. quadrijuga* occurrence (**Supplementary Figure 5**). These results are in agreement with previously published studies focusing on other *Polylepis* species showing that relatively warm diurnal and freezing night temperatures are expected to limit the distribution in *Polylepis* species (Purcell and Brelsford, 2004; Toivonen et al., 2014; Cuyckens et al., 2016; Zutta and Rundel, 2017). Field observations reports support that *P. quadrijuga* reproduces sexually inside the forest fragments, while in edge zones, clonal reproduction becomes the primary natural propagation strategy. This change relates to the scarce success of seedling establishment in ecotonal zones, where the open vegetation does not protect nor create favorable conditions for seedling survival.

Predicted Shifts and Putative Future Climatically Stable Areas for *P. quadrijuga*

According to our HSM future predictions, climate change can reduce the suitable area for *P. quadrijuga* by up to 80% for the most optimistic scenario (**Figure 3** and **Table 2**). Notably, according to our HSMs, we expect *P. quadrijuga* to become severely threatened in the medium-term (2041–2060) and locally extinct in large part of its range under the pessimistic scenarios RCP 6.0 and 8.5 in the long-term (2061–2080). This issue is a concerning situation considering that high elevation tree species are often unable to colonize new habitats in higher (hence colder) areas (Feeley et al., 2011; Duque et al., 2015; Fadrique et al., 2018).

Several studies predict a higher sensitivity to climate change of highland species and mainly relate it to upward distribution shifts along the altitudinal ranges, thus leading to a contraction of the available suitable area (Thuiller et al., 2005; Morueta-Holme et al., 2015). In the case of *P. quadrijuga,* our results predict significant reductions in the projected area and an altitudinal range shrinkage to the uppermost edge as a possible consequence of climate change (**Figures 3**, **4**). Our projections show that only 11–15% of the suitable habitat of *P. quadrijuga* will be able to persist under the different 2041–2060 RCP scenarios and that only 8–17% will persist under the 2061–2080 RCP scenarios. These results are compatible with the projection of a severe reduction of *P. tarapacana* in the South American Altiplano (Cuyckens et al., 2016) and with a climate vulnerability assessment of the *Espeletia* complex, which shows that several Páramos of the Eastern Cordillera are highly vulnerable to a changing climate (Valencia et al., 2020). Overall, our results agree with the prediction that ongoing climate change could compromise the long−term persistence of the genus *Polylepis* (Cuyckens et al., 2016; Pinos, 2020).

Warming temperatures and changes in precipitation patterns are causing many high Andean biomes to retreat upslope and suffer changes in their composition as higher-altitude environments undergo faster temperature changes than lower-altitude environments (Costion et al., 2015; Mountain Research Initiative EDW Working Group, 2015). In particular, for some species that will need to track the isotherms throughout the Tropical Andes, a general upslope migration of 600 m is predicted to happen by the year 2100 (Anderson et al., 2011). However, forests' range shift dynamics in response to climate change are a complex process, and a possible Andean treeline upward displacement to areas occupied by Páramos can be limited by many factors, including high radiation levels, freezing events, and human disturbances (Rehm and Feeley, 2015a,b).

Even if *P. quadrijuga* forests' extent will not decrease, their distribution is unlikely to increase at higher elevations. For example, some *Polylepis* woodlands from the Cordillera de Vilcanota (Perú) can be highly stable (Jameson and Ramsay, 2007; Rehm and Feeley, 2013, 2015b). However, since our result predicted an altitudinal range shrink under climate change conditions, the species reproduction needs to be considered because treeline stands may show slow and species-specific responses (Camarero and Gutiérrez, 2004; Dullinger et al., 2004). Interestingly, Toivonen et al. (2018) found that the topographic heterogeneity of the high-Andes could help *P. subsericans* forests to persist regionally, facilitating these treeline species by providing dispersal and establishment microsites, achievable by local relocation.

Importance of Protected Areas Network to the Conservation of *P. quadrijuga*

We observed that PAs covered almost 75% of all remaining *P. quadrijuga* forests in Colombia. However, in all the examined PAs, the total suitable area for this species tends to be reduced under future climate change scenarios, especially toward the northern Páramos complex (**Figure 3**). In this context, our results serve as a baseline to adequately protect *Polylepis* woodlands, while highlighting relevant areas to expand the current PAs network to include suitable areas for *P. quadrijuga* (e.g., SCV, GLR, SNC, and GUE; **Figure 1**). The future HSMs projections define some important climatically stable areas where *P. quadrijuga* could be able to persist. However, these stable areas are located principally inside the SCV and CHG Páramos, where the model predicts a lower decrease in suitable areas. Nonetheless, remaining *Polylepis* patches might also undergo further fragmentation and occupy sub-optimal conditions at the periphery of their future ranges. A severe concern underlines the areas located at the northeast edge of the Eastern Cordillera, where a dramatic decrease or even a complete loss of suitable habitat is predicted (**Table 2**).

Our study reiterated the value and importance of an adequate representation and protection of biological entities in the Páramo complexes network in Colombia. According to our predictions, the SCV area supports stable conditions within the intervened local landscape despite the regional biotic and abiotic change. Interestingly, Zutta and Rundel (2017) predicted refugial areas along the Andes for many *Polylepis*-associated species indicating that temporally stable areas will be crucial for conservation during anthropogenic-induced climate change, and that their protection and management may reflect the persistence of *Polylepis* woodlands in the future. Correspondingly, our findings suggest that conservation efforts targeting *P. quadrijuga* should be directed first toward the large National Natural Parks (Sumapaz, Chingaza, Sierra Nevada del Cocuy; **Supplementary Table 2**).

According to our future projections, the current PAs network will protect ∼36–48% of the extent of the predicted *P. quadrijuga* suitable area. Additionally, the few areas that could maintain part of its future suitable habitat (CE-CM-SCV) are currently strongly threatened by human activities. For example, only 142,112 of the 333,420 ha of the *Sumapaz* Páramo are currently protected as part of the Sumapaz Natural National Park (IAvH, 2012), and fracking projects currently threaten the area. Thus, for *P. quadrijuga,* habitat protection and connectivity restoration should become a central objective of conservation strategies. In this sense, management plans of already established protected areas such as SCV and CHG Páramos, which our study reports as climatically suitable, should receive special attention to preserve *Polylepis* woodlands. We recommend additional protection efforts in the

north of the species distribution, where areas under protection are currently scarce.

Here we pinpoint that several areas within SCV, CHG, and SNC Páramos could be considered as stable suitable areas for *P. quadrijuga*, since our models predicted their persistence under some of the evaluated future time frames and for most of the evaluated RCPs. Interestingly, these Páramos coincide with identified high diversity and Pleistocene refuge areas (Cleef, 2008; Zutta and Rundel, 2017). These Páramos were already identified as future persistence areas for different highland plant species (Ramirez-Villegas et al., 2014; Valencia et al., 2020). Thus, we assume that our results and their conservation implications can be of high relevance for protecting the highland Andean biodiversity in the future. However, it is very worrying that Páramo ecosystems have long been at risk due to agriculture, mining and that now they are susceptible to climate change. For example, for the CHG complex, it was found that increasing temperatures and changing precipitation regimes will reduce the 39–52% of the currently suitable area for the Páramo ecosystem during the dry season, and 13–34% during the wet season (Cresso et al., 2020).

We suggest that five of the PAs that showed at least a minimal area of persistence in some of the future scenarios and time frames, should be initially targeted for management: "Parque Nacional Natural Chingaza," "Parque Nacional Natural Sumapaz," "Parque Nacional Natural Sierra Nevada del Cocuy," "Parque Nacional Natural Pisba," "Pan de Azúcar—El Consuelo," and "Páramo de Guantivá y La Rusia" regional parks. Prioritizing and expanding small protected areas in the correspondent mountain ranges and PCCs will be essential for protecting this species *in situ* and *ex situ,* and pivotal to safeguard it from further habitat loss. Interestingly, our model projections to future scenarios highlighted several areas of climatic stability or even suitable habitat expansion in many protected areas that currently host no records of *P. quadrijuga* (e.g., Pisba and Páramo de las Oseras; **Supplementary Table 2**), or small private reserves (e.g., La Reserva, Villarica; **Supplementary Table 2**). These can be effective for *ex situ* conservation measures for the scattered mature *P. quadrijuga* individuals and seedlings, and can be particularly relevant to carry out seed germination or seedling transplant experiments in the field.

Implications and Recommendations for the Conservation of *Polylepis* Woodlands Under Climate Change

Currently, few sustainable management strategies have been proposed for *Polylepis* woodlands, and where implemented, they do not match the widespread management practices in Colombian rural areas (Gareca et al., 2010; Vásquez et al., 2014; Boza Espinoza et al., 2019). Since many *Polylepis* woodlands are located in densely populated areas and are under considerable pressure from human disturbance, it is urgent to identify hotspots of vulnerability and resilience and define management interventions. We also emphasize the need to maximize protection efforts targeted around treeline ecotones biodiversity in the Andes, as these are essential providers of ecosystem services and home to a diverse array of unique wildlife (Fjeldså, 1993; Arnal et al., 2014; Sevillano-Ríos et al., 2018).

We consider *P. quadrijuga* as an appropriate umbrella species to monitor the upward shift of treeline Andean ecosystems. However, it is essential to gather and consider more information about its interactions with other species for the selection of appropriate sites for protection. For example, high habitat specialization with strong confinement inside *Polylepis* forests was observed for some specialist bird species (Fjeldså, 1993; Cahill and Matthysen, 2007; Gareca et al., 2010; Meneses and Herrera, 2013). In agreement with Sevillano-Ríos and Rodewald (2017), we suggest protecting large remnants at lower elevations and maintaining all relictual patches of *Polylepis* forests irrespective of size become two key strategies constituting the cornerstones of future conservation efforts. Also, protected areas in the higher Andes must incorporate the study of potential species distributions and species responses to local climate changes.

Although it is known that the presence of a species inside PAs offers no guarantees for effective species long-term conservation (Hoffmann et al., 2019), protection efforts are the most successful tools to maximize landscape connectivity of *Polylepis* forest. Simultaneously, identifying areas of historical and potential future refugia or climatically stable areas is important to direct the focus of conservation efforts (Keppel et al., 2015). Moreover, studies predicting species' future distribution considering the idiosyncratic species responses (i.e., phenotypic acclimation, rapid evolution, ecophysiological studies of selected species, and local adaptation patterns) are necessary to set the methodological baseline for the management of *Polylepis* woodlands inside PAs. Some examples include Ramos et al. (2013), showing that the physiological performance of *P. quadrijuga* was significantly affected by landscape fragmentation, and Velez et al. (1998) supporting that *P. quadrijuga* trees develop a high protection strategy against UV-B radiation, which tends to increase with altitude. However, more detailed studies are still needed to clarify relevant aspects of species biology and deliver comprehensive eco−evolutionary data in the context of global climate change.

Toivonen et al. (2018) suggested that a better understanding of the environmental niche preferences of *Polylepis* species is crucial to identifying potential climatic resilience areas for the *Polylepis* forests. Accordingly, we show that HSM could be crucial for possible conservation strategies to address changing environmental conditions. However, the incorporation of species−specific dispersal constraints and the influence of local topographic and edaphic factors are essential additions to projections of plant species distribution models in the future (Rull and Vegas-Vilarrúbia, 2017). Moreover, since treeline species responses to climate changes are species-specific, climate, land-use, fragmentation, and soil degradation are all critical factors determining whether an upwards migration is possible. For this reason, intensive fieldwork might be relevant to monitor plant altitudinal migration and could increase the knowledge of the potential responses of threatened treeline species in the higher Andes.

Under climate change conditions, plants face various barriers to their dispersal that may hamper gene-flow and recruitment,

leading to extinction. Although *Polylepis* species are wind-pollinated, palynological and population genetics analyses show that pollen dispersal is limited to relatively short distances (Schmidt-Lebuhn et al., 2007), underlining that forest fragmentation can lead to an increase of genetic drift, inbreeding, and a consequent loss of adaptive capacity in local populations. On average, for the northern Andean region, is expected an increase in annual precipitation by values as high as 300 mm/year (Buytaert et al., 2011). This issue is crucial for biological processes such as *Polylepis* species dispersion. Also, the dispersion ability of tropical treeline species could be limited by the complex topography of the Andes and the harsh microclimatic conditions near the soil surface, which may be one of the reasons limiting seedlings recruitment (Wesche et al., 2008). We still have much to learn about the genetic mechanisms behind climate change adaptations of high-Andean treeline species, and the recognition of regional patterns of genetic diversity of *Polylepis* populations could offer the opportunity not only to preserve ecosystems with high biological diversity but also to understand and protect evolutionary processes from climate change (i.e., Schierenbeck, 2017).

In this work, we incorporated data deriving from citizen science projects to increase the number of occurrences of *P. quadrijuga*. We observed that integrating data from alternative databases could substantially increase sample size and could open up new opportunities to identify declining populations earlier to evaluate the effectiveness of policy interventions. We remark that citizen science has the potential to generate valuable biological observations suitable for species distribution modeling (Tiago et al., 2017; Roy-Dufresne et al., 2019; Petrovan et al., 2020) and ecological restoration projects (Edwards et al., 2018; Callaghan et al., 2019). Citizen science projects also provide the users with informal learning experiences that can contribute to the knowledge on ecological traits, seed collection, plant germination, and propagation of species of the higher Andes, which are often neglected in the scientific literature. However, our results should be interpreted with caution, given that the distribution of utilized species records is heavily biased toward the PAs and the central part of Colombian Eastern Cordillera. Probably, due also to the fact that patches of *Polylepis* are challenging to identify using current air photographs and satellite images (Aucca and Ramsay, 2005), most of our occurrences of *P. quadrijuga* are located in PAs situated and along roads, whereas large areas in the northeast and southeast of the Eastern Cordillera remain poorly sampled or not sampled at all.

Our model is subjected to limitations, since we did not account for physiological processes or any information about the species' ecology and natural history (e.g., interspecific competition, migration rate), and recent human activity and disturbance (e.g., land use change, fires). Moreover, we could not incorporate data on microclimatic conditions into the modeling, due to the lack of available high resolution data for our study area. Nevertheless, an improved understanding of *P. quadrijuga* distribution and the possible future response to climate change represents a precondition for more precise modeling efforts of potential range shifts of treeline species in the Andes. Our results provide a better comprehension of the role of environmental constraints in shaping treeline species potential ranges. Specifically, we highlight a "Change in the climatically suitable area" and a "Change in average climatic suitability in already occupied cells" for *P. quadrijuga*, which have been defined as two quantifying components of risk for woody species under climate change (Ohlemüller et al., 2006). For this reason, based on our results, we suggest implementing an evaluation criterion to categorize the conservation status of *P. quadrijuga*, targeting a key treeline forming species on the northern Andes, a region that is still being heavily transformed and fragmented by agricultural and mining activities. Immediate implementation of *in situ* conservation programs could be crucial to ensure the persistence of the species. Monitoring the upward distributional shift would help us to detect the predicted decline in *Polylepis* populations over time. Lastly, the creation of a germplasm bank from specimens from all the different localities would be fundamental.

CONCLUSION

According to our future projections, the threat status of *P. quadrijuga* is alarming and needs attention due to the predicted considerable reduction in climatically suitable habitat for the species. The detailed information provided here could represent a valuable tool to guide the future establishment of new and efficient conservation efforts. Moreover, our findings can help restore *Polylepis* forests and preserve them through *in situ* and *ex situ* strategies, planned according to the predicted future stable climatic areas. Medium- and long-term conservation strategies for *P. quadrijuga* woodlands should be focused on actions to prevent deforestation of the extant patches and to implement ecological restoration measures in the Cruz Verde—Sumapaz and Páramo complex, which is the only predicted future suitable area for six out of eight of the future scenarios employed here. Our results can be used to define high-priority areas in the Andean region for conservation and management plans for *Polylepis* forest and can offer a baseline for similar analyses of other endangered and threatened treeline species in the region. Finally, we reinforce the need to strengthen management strategies inside those protected areas that comprise the remaining patches of *P. quadrijuga* forest in Colombia.

DATA AVAILABILITY STATEMENT

The original occurrence data used in the study is included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GS-A and LC-V designed the study and conducted the formal analysis. FF-G and MC performed data curation. GS-A, FF-G, and MC prepared the figures, tables, and **Supplementary Data**. LC-V and MC wrote the first draft of the manuscript. All authors interpreted the results, contributed substantially to revisions and worked together to define the structure and content of the manuscript, and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

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Effects of Climate Change vs. Grazing Exclusion on Species Diversity Over 18 Years Along an Elevation Gradient in the European Alps

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Climate change was already shown to increase species numbers in high elevations. In contrast, grazing might interfere with climate change effects. To disentangle both the effects remains a major challenge of alpine ecology. The present study investigated both the effects on species diversity along an elevation gradient in the Austrian Central Alps. We aimed to answer the following questions: How do species diversity and frequency of subalpine–alpine–subnival plant communities change in grazed sites with time? Do competitive plant species increase in the communities? How does grazing exclusion affect species diversity, functional groups, and strategy types? Are environmental changes (temperature, sunshine duration, precipitation) responsible for diversity changes or does grazing override climate effects? The study was carried out for 18 years along an elevation gradient from 1,958 to 2,778 m a.s.l. at Obergurgl (Tyrol, Austria), including six different plant communities. A total of 11 grazing exclusions were established. At each community, the frequency of the species was counted in 1 m^2 plots yearly or at least every 3–4 years. Environmental data were obtained from the weather station Obergurgl. Changes of the community parameters and the species composition were analyzed by partial redundancy analyses and mixed-effect models. Species diversity increased with time at all grazed sites, but this increase was suppressed under grazing exclusion. Grazing exclusion effects became pronounced after 5 years. The most consistent result was the increase of bryophytes throughout. At the subalpine grassland, tall-growing species expanded in the exclosures; at the upper alpine *Carex curvula* grassland, snow bed species decreased with grazing exclusion. Among the environmental factors, sunshine duration of the previous year's autumn quartal was found to be the essential variable for the changes. We concluded that diversity increases in grazed communities of the Austrian Central Alps can be attributed to climate change. An indication of slightly reduced and altered weather effects under grazing exclusion was found.

Keywords: diversity, environmental factors, exclosures, long term monitoring, plant community

INTRODUCTION

In view of the ongoing climate warming (Gobiet et al., 2014), biodiversity changes in high elevations are one of the central topics in today's ecological research (Winkler et al., 2019). A severe biodiversity loss is expected worldwide (Un Report, 2019) with major impacts also in high elevations, although probably with some time lags in the alpine zone (Dullinger et al., 2012; Alexander et al., 2018). Data comparing new and old relevés or data from long-term monitoring projects revealed increases in alpine plant species diversity (Grabherr et al., 1994; Holzinger et al., 2008; Pauli et al., 2012; Wipf et al., 2013) with striking accelerations during the last decades (Steinbauer et al., 2018). However, these studies focused on diversity changes of upper summit areas. Plant communities in the alpine zone were less investigated (e.g., Vittoz et al., 2009; Gritsch et al., 2016; Matteodo et al., 2016). Responses to climate change were found to be weak or even missing (Windmaißer and Reisch, 2013) or they were rather heterogeneous (Gritsch et al., 2016). Only snow bed communities seem to be an exception, showing significant changes with time (Matteodo et al., 2016; Liberati et al., 2019).

Especially in the Austrian Central Alps, a long tradition of grazing prevails in high elevations (Vorren et al., 1993; Bortenschlager, 2000). More or less all landscapes from the subalpine to the subnival zone are grazed—even today—by sheep at higher elevations and cattle/horses at the subalpine zone. Cessation of grazing may cause severe changes in community composition (Tasser and Tappeiner, 2002; Dullinger et al., 2003) as shown recently in the Tatra Mts (Czortek et al., 2018), although also accessibility of the sites has to be considered. Mayer and Erschbamer (2017) reported that alpine plant communities exhibit an individualistic reaction to grazing exclusion, depending on the kind of grazers. Abandonment of livestock grazing might decrease short-statured plants and promote competitive species (Vittoz et al., 2009) such as trees (Gehrig-Fasel et al., 2007; Vittoz et al., 2008; Wieser et al., 2019). Generally, the literature shows rather inconsistent results on the effects of grazing in high elevations (review in Pardo et al., 2015), the slow reaction of alpine species to grazing exclusion causing interpretation problems and aggravating predictions (Evju et al., 2009; Pardo et al., 2015).

All in all, alpine ecology has to deal with two major drivers governing diversity and abundance of species. On the one hand, the ongoing warming with mean temperature increases of 1.2°C during the last 55 years in the Austrian Central Alps (Fischer, 2010) might continuously enhance species numbers. On the other hand, permanent grazing activity might reduce species diversity (Song et al., 2020); however, it may also interact with climate change in the way that community changes are decelerated or avoided (Speed et al., 2012; Vowles et al., 2017; Wang et al., 2019) or enhanced during warm and dry periods (Pardo et al., 2015). In the Southern Scandes, Norway, alpine grasslands were found to shift upward by 3 m over 8 years of grazing exclusion (Speed et al., 2012). Such striking effects were never seen in the Alps. However, machine-based models predicted that alpine calcareous pastures with *Carex firma* will be reduced by future climate while *Nardus stricta* and *Carex curvula* pastures will gain distribution area in the Italian mountains (Dibari et al., 2020).

With our study, we aimed to assess ongoing climate change effects in grazed and ungrazed plant communities along an elevation gradient from the subalpine to the alpine and subnival zone in the Austrian Central Alps. We selected seven widespread plant communities and followed the changes in species number, frequency, and composition over time (up to 18 years of monitoring). We expected significant changes in species diversity depending on community type. In order to evaluate the impact of climate change on vegetation structure (Pardo et al., 2015), we classified the species according to functional groups in graminoids, herbs, dwarf shrubs, mosses, and lichens.

Climate change scenarios outline a migration of lowland species toward higher elevations (Pauli et al., 2007, 2012; Lamprecht et al., 2018). Alpine species are known as strictly adapted to the harsh conditions (Körner, 2003), having a limited plasticity compared to species from lower elevations (Gugger et al., 2015). Due to their higher growth rate, competitive species are particularly benefiting from warming (Farrer et al., 2015; Winkler et al., 2019) and the low-statured alpine species are threatened by competitive displacement (Alexander et al., 2015, 2018). Finally, new communities could evolve (Alexander et al., 2015). We expected that such tendencies should be observable with our long-term investigations. We grouped the species by means of the C-S-R strategy concept of Grime (1979), differentiating between competitive, stress-tolerant, and ruderal species. By this, we will be able to answer the question if competitive species tend to increase in the studied communities. However, not only may competitive species increase due to invasions from lower elevations (direct climate change effect, Farrer et al., 2015) but also predominant resident species may inhibit companion species due to their increased growth intensity and competitive ability, provoking "indirect effects" of climate change (Farrer et al., 2015). Consequently, an increase of the competitive species group might be attributed to invasion of new competitive species or by enhanced growth and competition of predominant resident species. In addition to functional attributes, we used also ecological indicator values (Landolt et al., 2010) to detect if increases in temperature values and nitrogen values occurred with time. Calculating indicator values for each studied plant community, we expected to gain information on shifts of community composition and thermophilization tendencies (Pauli et al., 2007, 2012; Gottfried et al., 2012).

Moreover, using regional weather data (temperature, sunshine duration, precipitation) for the whole study period, we aimed to analyze the environmental effects on grazed and ungrazed sites. By this, we tried to disentangle climate and grazing effects and to evaluate if grazing had the power to modify climate-induced changes in diversity (Speed et al., 2013).

Altogether, our objectives were (i) to study changes and environmental effects in species diversity, functional groups, strategy types, and ecological indicator values of all grazed and ungrazed communities; (ii) to investigate the dynamics of species composition; and (iii) to highlight grazing effects on alpine species. The overall goal of this paper was to unravel

environmental vs. grazing effects. The following hypotheses were tested:

H1 Species diversity and frequency of all functional groups (graminoids, herbs, dwarf shrubs, mosses, lichens) will be enhanced with time at all grazed sites.

H2 Competitive strategists and species with high Landolt temperature values (i.e., species from lower elevations or species with wide distribution ranges) as well as species with high nitrogen values will increase at all grazed sites.

H3 Diversity will decrease in grazing exclusion plots.

H4 Changes in temperature, sunshine duration, and precipitation are responsible for diversity changes. Grazing will not override environmental effects.

MATERIALS AND METHODS

Study Sites

The investigated sites (**Table 1** and **Supplementary Material 1**) are located along an elevation gradient, from the subalpine to subnival zone at Obergurgl in the inner Ötztal (Tyrol, Austria). The study sites belong to the Long-Term Ecosystem Research Network (LTER) within the Long-Term Socio-Ecological Research (LTSER) platform Tyrolean Alps[1] and LTER-Austria[2]. The subalpine zone (1,960–2,200 m a.s.l.) is grazed by cattle during summer; the alpine and subnival zone (2,300–2,900 m a.s.l.) by sheep; and the area of the sites S03–S05, S06, and S13 by sheep, Haflinger horses, and partly also by goats. Details on number of grazers and stocking rates are given in Mayer et al. (2009).

Field Work

Permanent plots of 1 m^2 were marked at S01–S04, S07–S09, S12, and S14–S16 in 2000; at S06 in 2001; S13 in 2003; and at S05, S10, and S11 in 2005 (**Table 1**). Within fenced exclosures (**Table 1**), three ungrazed × 1 m^2 plots were established per exclosure site together with three × 1 m^2 plots as controls outside the fence. At the grazed sites without exclosures (**Table 1**), six × 1 m^2 plots were installed. The sites were monitored regularly (except 2007 when monitoring was restricted to S05, S10, and S11) till 2018 (**Table 1**) by means of a frequency frame, divided into 100 subplots of 1 dm^2. Within each subplot, presence/absence of the species and the functional groups (graminoids, herbs, dwarf shrubs, mosses, lichens) were recorded. The total frequency per 1 m^2 was given as percentage of the 100 subplots.

The nomenclature of vascular plant species follows Fischer et al. (2008); bryophytes (with exception of *Polytrichum* species, which were identified at the genus level) and lichens were treated as functional groups. Some lichens were identified at the species level (*Cetraria islandica*, *Peltigera aphthosa*) and integrated in the species analyses. Vascular species not present at the first monitoring but present in 2018 were defined as newcomers. The frequency sum of each species/functional group per plot was calculated for each sampling year.

Statistical Analyses

Statistical analyses and graphs were done using PRIMER 7 with the PERMANOVA + add-on (Anderson et al., 2008; Clarke and Gorley, 2015), CANOCO 5.12 (Ter Braak and Šmilauer, 2018), and R 3.6 (R Core Team, 2019) with packages lme4, lmerTest, effects, ggplot2, and grid.

The plant communities were grouped by means of a principal coordinate ordination (PCO, PRIMER) with Bray–Curtis similarity of square-root-transformed species data (**Table 1** and **Supplementary Material 2**). Seven communities were distinguished: siliceous subalpine *Agrostis–Festuca* grassland (one exclosure, one control, S01), siliceous lower alpine *Nardus stricta* grassland (three exclosures, three controls, S02–S04), siliceous upper alpine *Carex curvula* grassland (three exclosures, three controls, S07–S09), siliceous lower alpine peat bog (three exclosures, three controls, S05, S10, S11), calcareous upper alpine *Kobresia* grassland (one exclosure, one control, located at the Rotmoos glacier foreland, S06; one grazed subnival site, no exclosure, S14), siliceous lower alpine *Festuca nigrescens* grassland (one grazed site, no exclosure, S13), and siliceous subalpine dwarf shrub communities (S12, S15, S16, all of them grazed, no exclosure, **Table 1**). Dominant species per site are shown in **Table 1** and more comprehensively in **Supplementary Material 3**. In total, 33 × 1 m^2 plots were monitored inside the exclosures and 33 × 1 m^2 outside the exclosures (controls). These plots were analyzed to detect exclosure effects. To detect climate change effects, a total of 63 × 1 m^2 grazed plots were analyzed.

For the functional groups, frequency sums (graminoids, herbs, dwarf shrubs, mosses, and lichens) and frequency fractions were calculated. C-S-R strategies were extracted from Flora Indicativa (Landolt et al., 2010) and converted to numeric indicators (values 1/3, 2/3, 1 according to the number of letters per strategy type; for instance, CCS was converted to 2/3 value C). For further calculations, weighted sums and fractions of total sum were used. Landolt indicator values temperature, light, nitrogen, and soil reaction were checked in Flora Indicativa (Landolt et al., 2010) and calculated as weighted means of species frequencies (omitting species without indicator values).

The temporal changes of all grazed communities (**Table 1**) and their species composition were analyzed by partial redundancy analyses (pRDA on correlations, CANOCO) testing for linear trends (year as metric variable) and for additional non-linear fluctuations around this trend (year as categorical variable). To isolate these effects, and to account for repeated measures, the large differences between plots were partialed out. Species frequencies were square-root transformed and species with < 5 presences excluded. All significance tests were done with 4,999 Monte-Carlo permutations (giving minimal p-values of 0.0002).

Univariate analyses of the diversity parameters [total frequency N, species number S, Pielou evenness J, and Shannon diversity H' ($\log_2$)] and all species (bryophytes were regarded as species, though being a functional group; the functional group containing bryophytes and *Polytrichum* spp. was called "mosses") were done for a screening of the same effects (year as metric

[1]http://www.lter-austria.at/ta-tyrolean-alps

[2]https://www.lter-austria.at/

TABLE 1 | Overview on the sites and their elevation (m above sea level, a.s.l.), description of the plant communities and the dominant species, number of grazed and ungrazed plots per community, size of the fence in m, and sampling years.

Site	Ma.s.l.	Description	Dominant species	Plot no.	Fence size (m)	2000	2001	2002	2003	2004	2005	2006	2007	2008	2011	2014	2018
S01	1,958	Subalpine ***Agrostis-Festuca*** grassland	*Agrostis capillaris* *Festuca rubra*	3 grazed 3 ungrazed	12 × 12	+			+	+	+	+		+	+	+	+
S02	2,292	Lower alpine ***Nardus stricta*** grassland	*Nardus stricta* *Potentilla aurea*	3 grazed 3 ungrazed	6 × 6	+	+	+	+	+	+	+		+	+	+	+
S03	2,310	Lower alpine ***Nardus stricta*** grassland	*Nardus stricta* *Scorzoneroides helvetica*	3 grazed 3 ungrazed	12 × 12	+	+	+	+	+	+	+		+	+	+	+
S04	2,320	Lower alpine ***Nardus stricta*** grassland	*Nardus stricta* *Geum montanum*	3 grazed 3 ungrazed	6 × 6	+	+	+	+	+	+	+		+	+	+	+
S07	2,576	Upper alpine ***Carex curvula*** grassland	*Carex curvula* *Gnaphalium supinum*	3 grazed 3 ungrazed	6 × 6	+	+	+	+	+	+	+		+	+	+	+
S08	2,592	Upper alpine ***Carex curvula*** grassland	*Carex curvula* *Avenula versicolor*	3 grazed 3 ungrazed	12 × 12	+	+	+	+	+	+	+		+	+	+	+
S09	2,590	Upper alpine ***Carex curvula*** grassland	*Carex curvula* *Geum montanum*	3 grazed 3 ungrazed	6 × 6	+	+	+	+	+	+	+		+	+	+	+
S05	2,265	Lower alpine **peat bog**	*Carex nigra* *Eriophorum angustifolium*	3 grazed 3 ungrazed	12 × 12						+	+	+		+	+	+
S10	2,270	Lower alpine **peat bog**	*Nardus stricta* *Carex nigra*	3 grazed 3 ungrazed	12 × 12						+	+	+		+	+	+
S11	2,261	Lower alpine **peat bog**	*Trichophorum cespitosum* *Carex rostrata*	3 grazed 3 ungrazed	12 × 12						+	+	+		+	+	+
S06	2,289	Upper alpine ***Kobresia*** grassland	*Kobresia myosuroides* *Poa alpina*	3 grazed 3 ungrazed	12 × 12		+		+	+	+	+		+	+	+	+
S14	2,778	Upper alpine ***Kobresia*** grassland	*Festuca pumila* *Kobresia myosuroides*	6 grazed		+			+	+				+	+	+	+
S13	2,292	***Festuca nigrescens*** grassland	*Festuca nigrescens* *Nardus stricta*	6 grazed					+	+				+	+	+	+
S12	2,040	Subalpine **dwarf shrub** (tall)	*Rhododendron ferrugineum* *Vaccinium* spp.	6 grazed		+			+	+				+	+	+	+
S15	2,250	Subalpine **dwarf shrub** (prostrate)	*Loiseleuria procumbens* *lichens*	6 grazed		+			+	+				+	+	+	+
S16P	2,334	Potentially **dwarf shrub** (ski piste)	*Festuca nigrescens,* *Achillea millefolium* agg.	3 grazed		+			+	+				+	+	+	+
S16H	2,335	**Dwarf shrub** (close to the ski piste)	*Vaccinium* spp. *Potentilla aurea*	3 grazed		+			+	+				+	+	+	+

For further explanation, see text. Community names are given in bold.

variable and year as categorical variable) by linear mixed-effect models with plot as random factor (done in R). Significances were Bonferroni-corrected for the number of species tested. The strength of time trends of all community parameters (diversity, functional group, C-S-R strategy, Landolt indicator values) was shown graphically, using the loadings of axis 1 from pRDA.

Exclosure effects on species composition were tested by PERMANOVA (also with plot as random factor, 4,999 permutations) using Bray–Curtis distances of square-root-transformed species frequencies. Effects should appear as differences in the changes over time between the fenced and grazed plots, therefore, the interaction "exclosure × years (categorical)" was the target effect for this analysis. Also for the exclosure effects, univariate analyses were performed for diversity parameters and single species, again using mixed-effect models but now with the focus on the interaction "exclosure × years."

Data from the weather station Obergurgl (1,938 m a.s.l.) were obtained by © ZAMG (Christian Posch 18/02/2020), spanning the years 1999–2018. Quartal means of air temperature in 200 cm height, sunshine duration, and precipitation sums were calculated for months 1–3 (Q1 winter), 4–6 (Q2 spring), 7–9 (Q3 summer), and 10–12 (Q4 autumn). Two quartals of the previous year (*_Q3p, *_Q4p) were included to allow analysis of delayed weather effects. The weather data were used as explanatory variables for the community parameters of all samples in a pRDA and tested for simple and conditional effects (using CANOCO's summarize-effects option).

In all analyses, the repeated measures of plots were taken into account, as covariates in partial analyses or as random factor in mixed-effect models.

RESULTS

Changes in Grazed Communities Over Time

All grazed communities showed more or less pronounced changes over time. Variation partitioning (**Table 2**) revealed that across all sites a common linear trend of change accounted for 27.3% of the variation (Yr, range for communities 17.4–35.5%) and another 13.2% were due to synchronous fluctuations between years (Yrc, range for communities 7.9–42.3%). These fractions refer to the variation remaining after accounting for the very large plot (and site) differences (86.2% of the total variation, range for communities 26.3–81.4%). The time effects were highly significant throughout ($p < 0.001$, except effect of Yrc in the peat bog). The diversity parameters species number S, frequency N, Pielou evenness J, and Shannon index H′ increased at all grazed communities (**Figure 1**). The increases of S and H′ were lowest at the peat bog and highest at the subalpine *Agrostis–Festuca* grassland. Also J increased with time with exception of the *Festuca nigrescens* grassland. Lowest changes per site occurred at S15 and S11. Details on diversity changes per site are reported in **Supplementary Material 4**.

Among the functional groups, clear linear increases resulted in all grazed communities for mosses (**Figure 1**). All other functional groups exhibited divergent increasing/decreasing patterns especially when considering the relative values. Small changes occurred in dwarf shrubs and in lichens (**Figure 1**). The competitive strategists decreased mainly at the *Carex curvula* and *Kobresia* grassland while stress tolerators decreased at the *Agrostis–Festuca* and *Nardus stricta* grassland (**Figure 1**). Temperature values decreased at the *Agrostis–Festuca* and *Festuca nigrescens* grassland, light values at the *Nardus stricta* grassland, the peat bog, and the *Festuca nigrescens* grassland. Nitrogen and soil reaction showed minor changes with exception of the *Agrostis–Festuca* grassland where these values remarkably increased (**Figure 1**).

Changes at the grazed sites did not follow a straight trajectory even if the main change occurred along axis 1 of the pRDA (**Figure 2**). There were remarkable fluctuations between the years 2000 and 2003, and also 2005 and 2008. The most relevant species/species groups for the changes were bryophytes, *Polytrichum* spp., *Phyteuma hemisphaericum*, *Pinus cembra* (newcomer), *Luzula multiflora*, *Pseudorchis albida*, and one species which disappeared during the second half of the study period (*Diphasiastrum alpinum*, **Figure 2**).

Bryophyte frequencies linearly increased throughout the years ($p < 0.001$, 17 plots, **Table 3**) with steepest increases at S01, S10–S12, and S16P (**Supplementary Material 5**). A broad variety of herbs (*Phyteuma hemisphaericum*, *Scorzoneroides helvetica*, *Homogyne alpina*, *Geum montanum*, *Campanula scheuchzeri*, *Leucanthemopsis alpina*, *Veronica alpina*) and graminoids (*Nardus stricta*, *Anthoxanthum odoratum* agg., *Carex curvula*, *Poa alpina*, *Festuca halleri*, *F. nigrescens*) had significant linear increases together with one lichen (*Cetraria islandica*, **Table 3**). Only one species (*Salix herbacea*, **Table 3**) showed significant decreases over time.

Grazing Exclusion Effects

Across all sites (without the peat bog because of later installation of the exclosure fences), diversity was significantly lower within the fences than in the controls outside (species number S and Shannon index H′: $p < 0.001$, mixed effect models), and the effect started to appear after 5 years of exclosure (**Figure 3**).

Regarding functional groups, only at the *Agrostis–Festuca* grassland was the exclusion effect strong with responses of both graminoids ($p < 0.001$) and herbs ($p = 0.012$). At the *Nardus stricta* grassland, there was a decrease in graminoids ($p = 0.018$) and at the *Carex curvula* grassland in herbs ($p < 0.001$), while none of the functional groups were significantly affected by grazing exclusion at the peat bog and the *Kobresia* grassland.

Grazing exclusion effects on species composition were not consistent across the plant communities. PERMANOVA results revealed that 8 out of 11 sites were significantly affected by grazing exclusion (**Table 4**). Two of the three sites at the *Nardus stricta* grassland (S03, S04) and the *Carex curvula* grassland (S07, S09), respectively, showed significant exclusion effects. Also at the peat bog only two sites had significant exclosure effects (S05, S10) while on the wettest site S11 weakest changes occurred ($p = 0.07$). Exclosure plots diverged also at the *Agrostis–Festuca* grassland (S01) and the *Kobresia* grassland (S06) from the control plots ($p = 0.0002$, **Table 4**). Within the exclosures, different species were significantly affected by grazing exclusion; some

TABLE 2 | Results of the pRDA (of all diversity parameters, total frequency, C-S-R strategies, and Landolt indicator values): changes over time for all grazed sites and grazed communities separately.

	All sites	*Nardus stricta*	*Carex curvula*	Peat bog	Dwarf shrub	*Kobresia*	*Agrostis-Festuca*	*Festuca nigrescens*
Number of sites	17	3	3	3	4	2	1	1
Samples	510	99	99	54	126	69	27	36
EV pRDA1	0.0527	0.1148	0.1834	0.0343	0.0782	0.2069	0.3545	0.1956
Correlation Yr-pRDA1	0.6876	0.6483	0.7446	0.8392	0.794	0.5802	0.8255	0.7158
Variation partitioning (of partial variation after accounting for plot differences)								
Linear trend (Yr)	27.3%	17.4%	35.5%	17.4%	35.0%	20.7%	35.4%	26.1%
Non-linearity (additional by Yrc)	13.2%	15.4%	24.1%	11.4%	7.9%	42.3%	31.2%	29.4%
Variation partitioning (of total variation)								
Plot	86.2%	52.3%	66.1%	81.4%	80.5%	56.4%	26.3%	54.7%
Linear trend (Yr)	3.8%	8.3%	12.0%	3.2%	6.8%	9.0%	26.1%	11.8%
Non-linearity (additional by Yrc)	1.8%	7.3%	8.2%	2.1%	1.5%	18.4%	23.0%	13.3%
Unexplained	8.2%	32.0%	13.7%	13.3%	11.1%	16.1%	24.6%	20.2%
Significances of explanatory variables								
p (Yr)	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
p (Yrc | Yr) additional non-linearity	0.0002	0.0006	0.0002	0.0516	0.0004	0.0002	0.0008	0.0010

Test of linear time changes (variable Yr) and superimposed non-linearity (variable Yrc), after partialing out plot differences. Variation partitioning is given with respect to total variation and to partial variation (after accounting for plot differences).

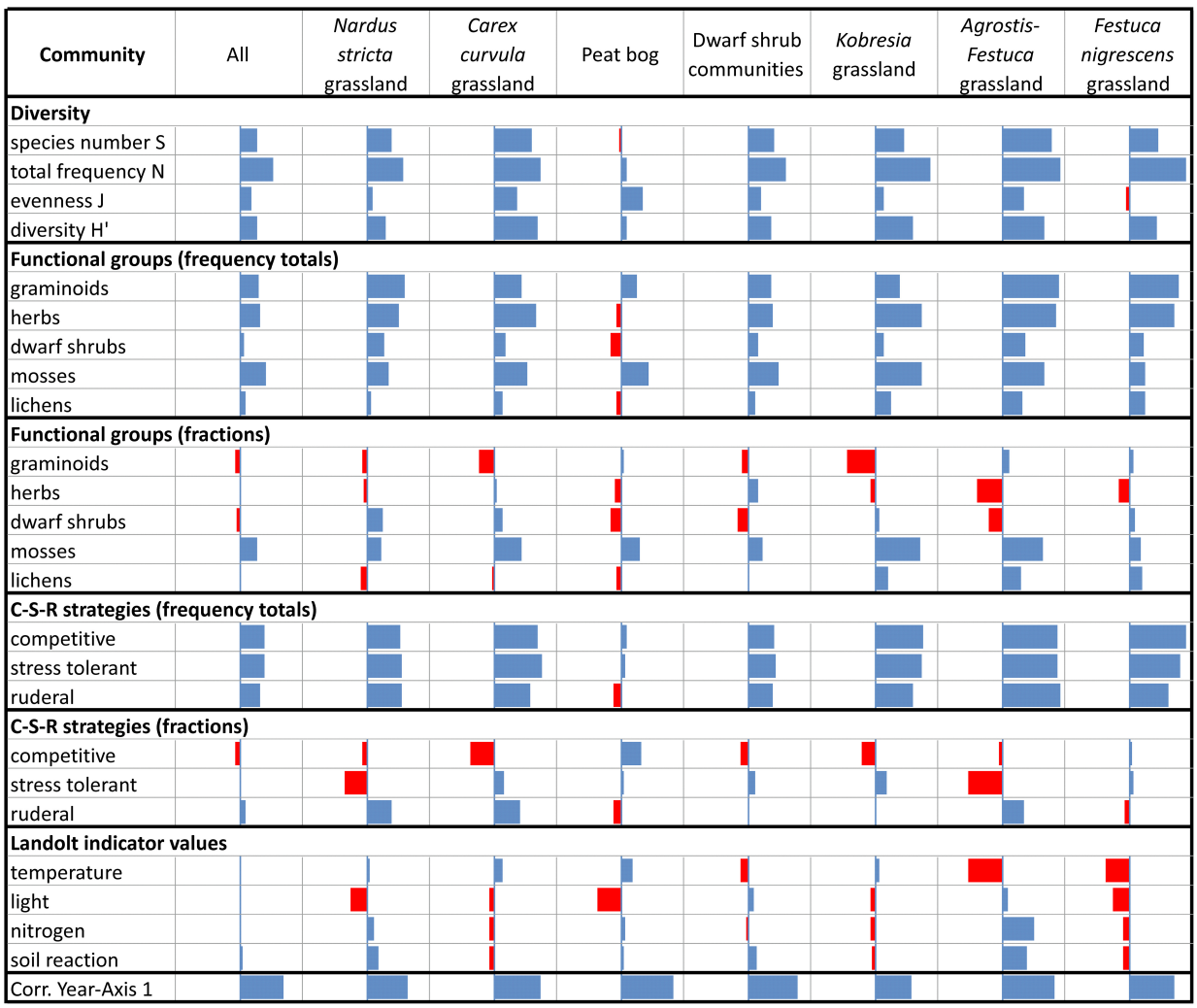


FIGURE 1 | Strength of time trend of community parameters (grazed sites): loadings on axis 1 (correlations) from pRDA representing the linear increase or decrease over time (range of bars −1 to +1). Bottom line shows correlation of explanatory variable Yr with pRDA axis 1. Results shown for the seven communities and for all communities together.

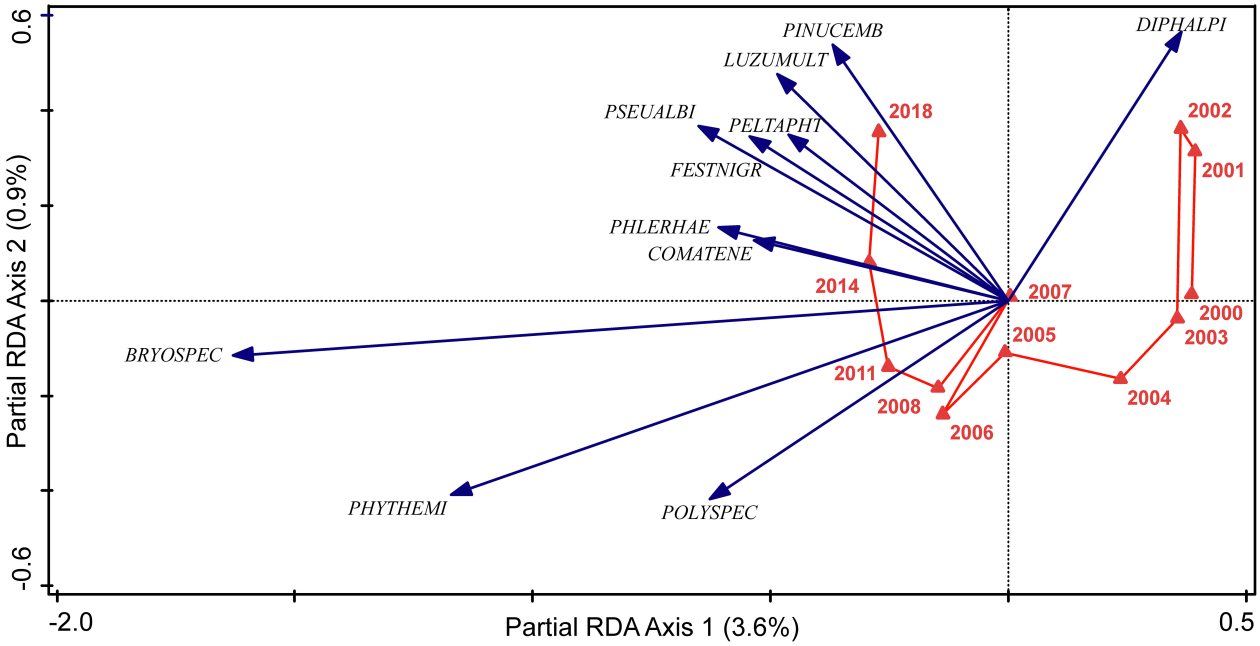


FIGURE 2 | Changes of the grazed sites from 2000 to 2018. The most relevant species (fit > 2%) are plotted as vectors (POLYSPEC = *Polytrichum* species, PHYTHEMI = *Phyteuma hemisphaericum*, BRYOSPEC = brophyte species, COMATENE = *Comastoma tenellum*, PHLERHAE = *Phleum rhaeticum*, FESTNIGR = *Festuca nigrescens*, PSEUALBI = *Pseudorchis albida*, PELTAPHT = *Peltigera aphthosa*, LUZUMULT = *Luzula multiflora*, PINUCEMB = *Pinus cembra*, DIPHALPI = *Diphasiastrum alpinum*). Partial RDA with plot as covariate, Monte-Carlo p = 0.0002, explained variation of axes indicated.

enhanced their frequency, and some decreased considerably with respect to the controls. At the *Nardus stricta* grassland, within the exclosures bryophytes increased after 10 years and *Geum montanum* from the beginning; *Anthoxanthum odoratum* agg. first increased more than in the controls but decreased in the end (**Figure 4**). At the *Carex curvula* grassland, snow bed species (*Sibbaldia procumbens*, *Gnaphalium supinum*) decreased in the exclosures while the dominating *Carex curvula* was not affected (the species showed parallel changes of exclosures and controls, **Figure 4**). At the peat bog, only *Carex rostrata* increased in the exclosures. At the *Agrostis–Festuca* grassland, increasing species were *Geranium sylvaticum* (in the last years) and *Potentilla aurea* (only temporarily over 10 years), whereas some graminoids (*Nardus stricta*, *Poa alpina*) and other herbs decreased (*Trifolium pratense* ssp. *nivale*, *Galium anisophyllon*, *Leontodon hispidus*) (not all species shown in **Figure 4**). At the *Kobresia* grassland, the dominant *Kobresia myosuroides* further increased, *Minuartia gerardii* increased temporarily during the first 10 years, and *Sedum atratum* reacted unclearly, because it was initially present only in the exclosure plots where it rapidly decreased toward the controls.

TABLE 3 | Selection of species with significant increase/decrease (only *Salix herbacea*) over time (linear trend), number of grazed plots with presences, slope for Yr ± S.E., and Bonferroni-corrected *p*-values (*p*).

Species	No. plots	Slope Yr ± S.E.	*p*
Bryophyta	17	2.07 ± 0.14	<0.000001
Cetraria islandica	13	0.87 ± 0.11	<0.000001
Potentilla aurea	13	0.75 ± 0.10	<0.000001
Phyteuma hemisphaericum	12	0.61 ± 0.07	<0.000001
Nardus stricta	11	0.57 ± 0.11	0.000038
Polytrichum spp.	11	1.09 ± 0.15	<0.000001
Scorzoneroides helvetica	11	1.30 ± 0.11	<0.000001
Anthoxanthum odoratum agg.	10	1.53 ± 0.17	<0.000001
Carex curvula	10	0.36 ± 0.06	0.000004
Homogyne alpina	10	1.91 ± 0.14	<0.000001
Geum montanum	9	0.61 ± 0.09	<0.000001
Poa alpina	9	0.69 ± 0.15	0.000717
Campanula scheuchzeri	8	1.18 ± 0.16	<0.000001
Festuca halleri	8	1.08 ± 0.18	<0.000001
Festuca nigrescens	8	1.76 ± 0.21	<0.000001
Leucanthemopsis alpina	8	0.61 ± 0.12	0.000076
Salix herbacea	8	−0.71 ± 0.19	0.032456
Veronica alpina	8	0.42 ± 0.10	0.002640

Environmental Effects

Increases of temperature at Obergurgl from 1999 till 2018 ranged from 0.03 to 0.08°C per year for the different quartals. The weather effects on the community parameters proved to be highly significant and mostly consistent across the communities (pRDA with site as covariate, $p = 0.0002$). Interestingly, the sunshine duration of the previous autumn quartal ($p = 0.003$) had by far the highest explanatory power (11.8% explained variation) and defined the first pRDA axis, which was highly correlated with total frequency, bryophytes, competitive and stress-tolerant species, and to a lesser degree diversity (**Figure 5**;

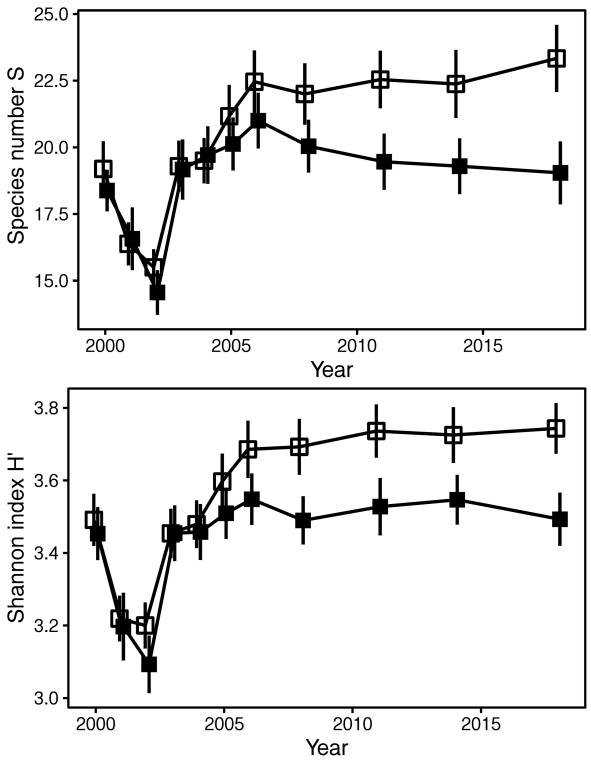


FIGURE 3 | Grazing effects on diversity [species number S and Shannon index H′ (log_2)]: time courses for exclosure and control plots diverging after 5 years. Means ± S.E. for exclosure plots (filled squares) and grazed control plots (open squares). Sites with later installation of fences excluded (peat bog: fences in 2005).

TABLE 4 | Exclosure effects (exclosure × year interaction) on species composition of the communities and single sites.

Community	Site	df	SS	MS	Pseudo-F	p
Nardus stricta grassland	All	10	1928	192.8	2.04	**0.0030**
	S02	10	738.2	73.82	1.18	0.2116
	S03	10	1493.8	149.38	1.58	**0.0066**
	S04	10	1584.9	158.49	1.79	**0.0008**
Carex curvula grassland	All	10	1315.4	131.54	1.01	0.475
	S07	10	1684.1	168.41	3.57	**0.0002**
	S08	10	418.6	41.86	1.78	0.0808
	S09	10	1818.4	181.84	2.55	**0.0002**
Peat bog	All	5	326.4	65.28	0.51	0.8498
	S05	5	833.4	166.67	6.76	**0.0002**
	S10	5	560.9	112.19	3.02	**0.0026**
	S11	5	222.8	44.57	1.62	0.0726
Agrostis-Festuca grassland	S01	8	4048.9	506.11	4.02	**0.0002**
Kobresia-grassland	S06	8	1579.7	197.46	3.98	**0.0002**

PERMANOVA results on Bray–Curtis distances of square-root transformed species frequencies. Fixed factors = exclosure, year; random factor = plot (and community for analyses All). Significant p-values in bold.

see also **Supplementary Material 6**). Summer precipitation of the previous year and spring/summer temperatures were also positively correlated with this axis. Precipitation and temperature of the previous autumn quartal (p = 0.003) were negatively correlated. For the exclosure sites, we also tested if the weather response was affected by grazing exclusion. Indeed, there was a small but significant (p = 0.0002) effect, with a slightly attenuated (and altered) response to sunshine duration of the previous autumn quartal in the fenced plots (**Supplementary Material 7**).

DISCUSSION

Diversity Changes in Grazed Communities

The most consistent result was that species diversity (species number, Shannon index) and species frequencies increased significantly with time at all grazed communities. Thus, hypothesis 1 can be confirmed. Primarily, bryophytes increased significantly in the course of 18 years (however, they increased also in the exclosures, see section "Discussion" below). This is in line with long-term observations in the alpine vegetation of Dovrefjell, Norway (Vanneste et al., 2017). The benefit for bryophytes from grazing was already highlighted by several other authors (Väre et al., 1996; Austrheim and Eriksson, 2001; Takala et al., 2014), showing that grazers promote certain bryophytes by creation of gaps.

Lichens—as a functional group—were not affected throughout the years in our study area, maybe due to a rather low importance in most of the investigated communities. This holds also for dwarf shrubs. At arctic sites, lichen richness was found to decline due to increased shading by dwarf shrubs and shrubs (Elmendorf et al., 2012; Vanneste et al., 2017) and litter increase (Cornelissen et al., 2001) as a consequence of climate warming.

The overall increase in species numbers in the grazed plots is in line with the accelerated species increase at high elevations in Europe (Steinbauer et al., 2018), as an effect of the ongoing climate warming. The lowest changes in diversity throughout the years occurred at S15 (prostrate dwarf shrub) and S11 (wettest site of the peat bog). At the prostrate dwarf shrub, obviously hardly any microsites for seedling recruitment (Graae et al., 2011) are present and sheep grazing also seems to have no influence on this *Loiseleuria procumbens* lichen heath, the dense carpet obviously preventing invasions of other species. This might be an example of "community-level resistance," defined by Hudson and Henry (2010). Similarly, also the calcareous *Kobresia* grasslands showed low diversity changes. This result is comparable to the low effects in subalpine calcareous grasslands in the Apennines found by Frate et al. (2018). At the peat bog site S11, the canopy of very few species remained stable throughout time. Grazers almost avoid this site due to the high water level and unpalatable species.

No Increase of Competitive Species

Hypothesis 2 suggested that competitive strategists and species with high temperature and/or nitrogen values should increase at the grazed sites due to climate change effects. Although the absolute frequency number of all the three C-S-R strategy types increased at all grazed communities, the relative changes did not really support our hypothesis. The fraction of competitors slightly decreased at most of the grazed communities (except the

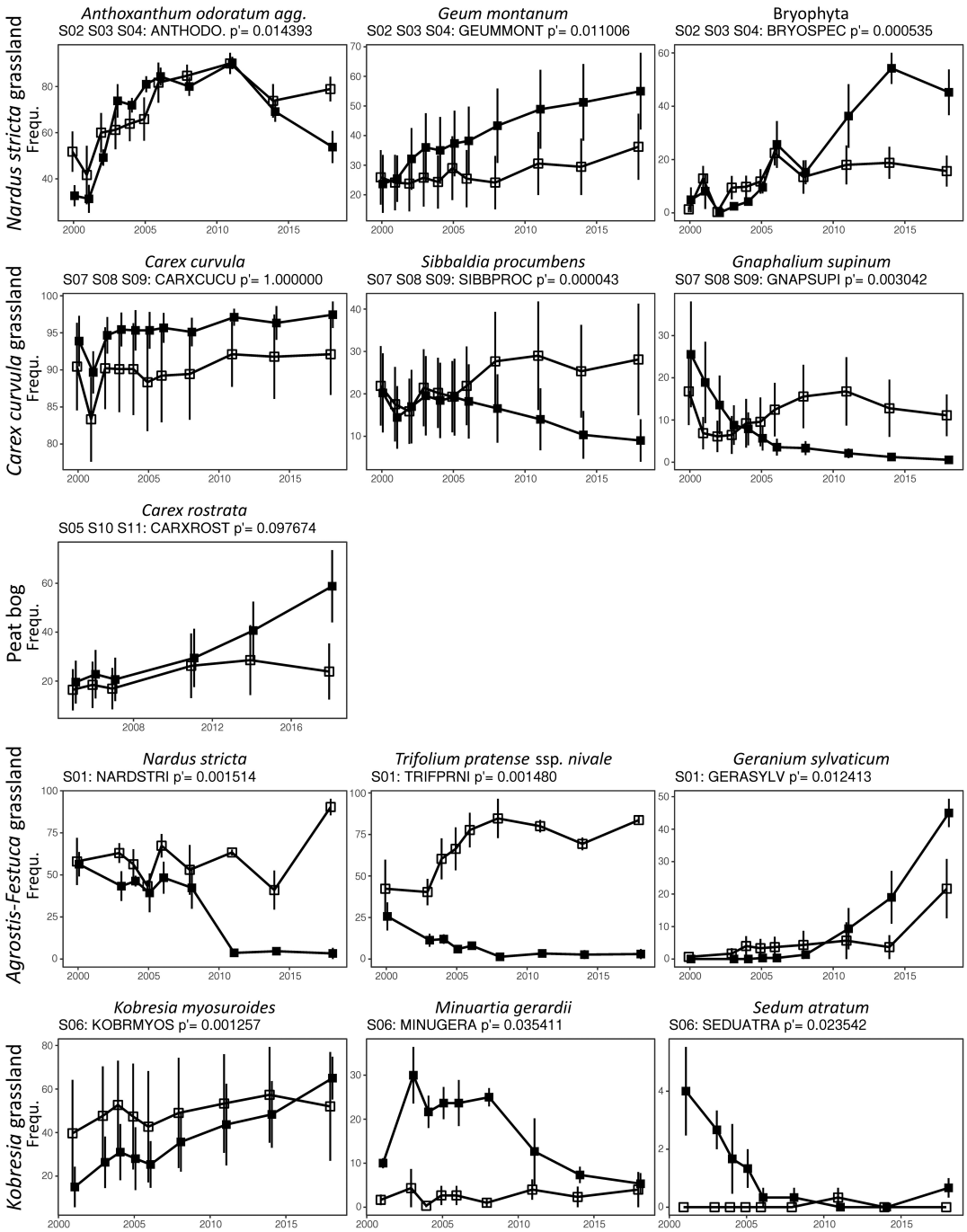


FIGURE 4 | Grazing effects on species frequency (tested by mixed-effect models, Bonferroni-corrected $p < 0.05$ for the exclosure × year interaction) for the different communities. Means ± S.E. for exclosure plots (filled squares) and grazed control plots (open squares).

peat bog, **Figure 1**) or did not change at all. The climate change scenarios suggest an increase of competitive, tall-growing species. This was not the case on our grazed communities. Similar to the competitors, the stress tolerators showed no uniform response in our study; only at the *Agrostis–Festuca* and the *Nardus stricta* grassland was this group reduced. In contrast, ruderals increased slightly, though not significantly. Our finding may be a clear indication of rather stable species composition and vegetation structure, probably due to long-term evolution of grazing in the area, grazing lasting already more than 6,000 years (Vorren et al., 1993; Bortenschlager, 2000).

For the Landolt temperature values, an increase was predicted due to the ongoing climate warming. However, also here rather divergent results were obtained. The findings suggest that hardly

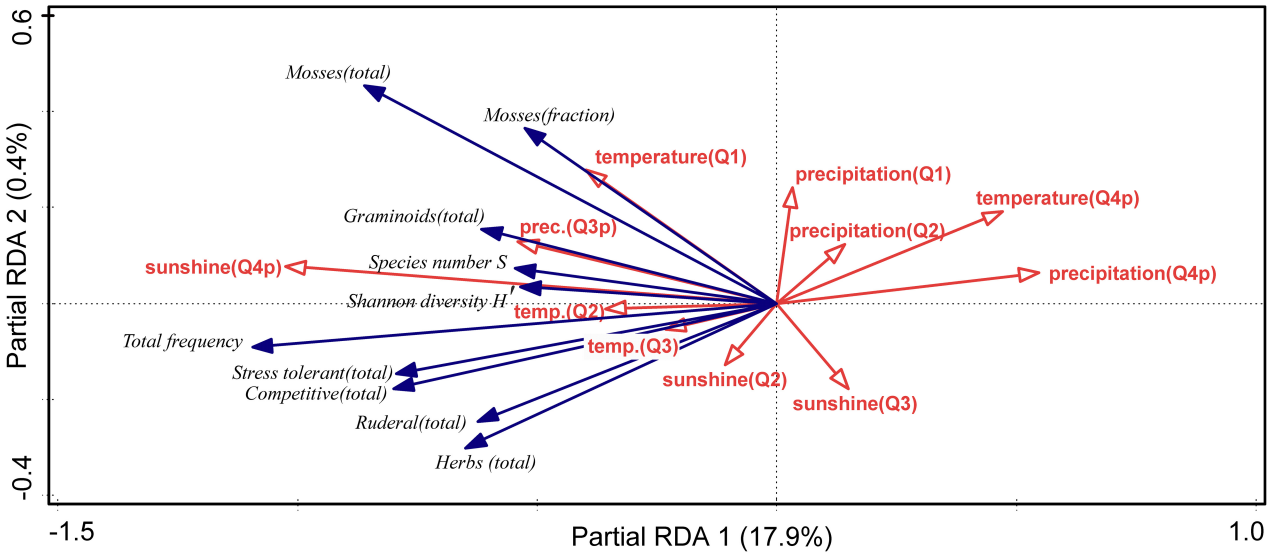


FIGURE 5 | pRDA showing the environmental effects on diversity (S, H′), total frequency, functional groups, and strategy types of the grazed sites (total frequencies and fractions indicated for the latter). Explanatory weather variables (sunshine duration, temperature, precipitation) shown with quartals in brackets (Q1 Jan–Mar, Q2 Apr–Jun, Q3 Jul–Sep, Q3p Jul–Sep previous year, Q4p Oct–Dec previous year). Partial RDA (site as covariate) on all community parameters (variables with fit > 5% shown), explained variation of axes indicated, $p = 0.0002$.

any species from lower elevations (having higher temperature values) invaded the grazed areas of the lower and upper alpine grasslands. The reaction of the light values seems to follow a decreasing trend (except at the dwarf shrub communities and *Kobresia* grasslands). This might be explained by the increasing cover and canopy height in the plots (Mayer and Erschbamer, 2014, 2017), promoting shade-tolerant species. Remarkable was the increasing amount of nitrogen-demanding species at the subalpine *Agrostis–Festuca* grassland and the changes in soil reaction values. Here, the grazed control plots were fertilized during the first years of the monitoring period, without our knowledge, by the owner of the cattle (Mayer et al., 2009). This might explain the surprising increases of nitrogen and soil reaction values. Unfortunately, we have no data on atmospheric N-deposition and amount of fertilizers spread. On the other hand, Evangelista et al. (2016) also found an increase in N-demanding species in the Apennines at 2,400–2,790 m a.s.l. after four decades.

Decreasing Diversity in Grazing Exclusions

With hypothesis 3, we predicted a decreasing diversity in grazing exclusion plots. While this can clearly be confirmed (i.e., species number S and Shannon index H′) when analyzed across the communities, the effect did not consistently occur at all sites. However, at 8 sites out of 11 there was a strong response of the entire species community (**Table 4**). This is by far a higher response compared to the results of the long-term study by Pardo et al. (2015) in the Pyrenees. There, two decades of grazing exclusion did not result either in diversity changes or in changes of the community structure. The authors explained the missing response mainly by (i) the absence of dwarf shrubs and trees in the surrounding, (ii) the predominance of long-lived alpine species, and (iii) possibly depletion of the species pool due to intensive grazing throughout the last centuries.

Considering the functional groups at our study sites, the communities showed a rather heterogeneous behavior: at the *Nardus stricta* grassland, mostly graminoids were negatively affected, at the *Carex curvula* grasslands mostly herbs, and at the *Agrostis–Festuca* grassland both. Contrasting responses of functional groups were highlighted also by Austrheim et al. (2008) in southern Norway, and the authors suggested that individual plant life histories should be known to evaluate the results. *Nardus stricta* is more or less avoided by grazers and may largely extend in extensively grazed areas at the treeline and in the lower alpine zone (e.g., Schaminée and Meertens, 1992). This resulted also by modeling future conditions (Dibari et al., 2020). In our sites, *Nardus stricta* significantly increased in most of the grazed plots, but decreased in the subalpine *Agrostis–Festuca* exclosure as well as at its typical grassland exclosures.

Graminoids were not affected in the *Carex curvula* grassland. *Carex curvula* is known to be highly persistent (Grabherr, 1989), lacking moderate grazing effects (Schneiter, 1977; Cernusca, 1989; Erschbamer et al., 2003). Here, the most striking indicator species for the grazing exclusion effects were snow bed species (*Sibbaldia procumbens*, *Gnaphalium supinum*). This might be an alarming signal, as snow bed species are also among the highest threatened species due to climate warming (Matteodo et al., 2016; Liberati et al., 2019). Also in the arctic tundra, herbivore removal caused increasing losses of 'short-statured' species (Kaarlejärvi et al., 2017).

At the subalpine *Agrostis–Festuca* grassland, *Geranium sylvaticum* became dominant throughout the years in the

exclosure. The species is known as tall herb, selectively grazed by sheep (Evju et al., 2006). The developing dominance of tall-growing herbs following grazing exclusion parallels mowing cessation effects (Bohner et al., 2020), i.e., land-use abandonment in general provokes a remarkable decrease in species richness and Shannon index. This conclusion is not new: several authors highlighted the role of grazing (or mowing) in preserving grasslands and enhancing species diversity (Olff and Ritchie, 1998; Adler et al., 2001; Austrheim and Eriksson, 2001; Dupré and Diekmann, 2001; Pavlu et al., 2005; Sebastià et al., 2008; Mayer and Erschbamer, 2017 and citations therein; Wang et al., 2017; Perotti et al., 2018). With the selection of tall-growing species and their biomass accumulation, microsites for seedling recruitment may become rare on such sites (Graae et al., 2011).

The increase of bryophytes also within the exclosure plots may be attributed to enhanced shading effects by a remarkably increased canopy height due to grazing exclusion (Mayer and Erschbamer, 2017). Generally, bryophytes benefit from grazing (as mentioned above); however, canopy structure changes due to grazing exclusion led to similar frequency increases as in the grazed plots.

Environmental Factors Govern Diversity Changes

Hypothesis 4 postulated that environmental factors drive diversity changes. At our study site, a steady increase in temperature was observed (Fischer, 2010; Kuhn et al., 2013). Generally, temperature was identified as the major climatic factor, driving vegetation changes in high elevations (Körner, 2003; Gottfried et al., 2012). Indeed, temperatures of the spring and summer quartals were positively correlated with total frequency, species number, and Shannon index, while precipitation of the winter and spring quartals as well as precipitation and temperature of the autumn quartals of the previous year were negatively correlated. Additionally, water deficit in warmer summers might increase, and together with elevated N deposition, these factors might be responsible for shifts of grassland communities (Wüst-Galley et al., 2020). Sunshine during winter (quartal 4) was the weather variable with the strongest steady increase, making it the dominating predictor in our study. We had expected more influence of the variables temperature and precipitation, but this could not be confirmed.

Interaction of Climate Warming and Grazing Effects

According to Speed et al. (2011, 2012), grazing exclusion may provoke upward shifts of species, especially advances of the treeline. Such signals were hardly detected at our study area. Only one tree sapling—*Pinus cembra*—newly appeared at one plot (**Figure 2**), although not within an exclosure. The distribution range of this tree is highly depending on the bird *Nucifraga caryocatactes* (spotted nutcracker) and its caching activity (Neuschulz et al., 2017). The nutcrackers hardly perform seed caching above the actual treeline due to deep snow (Mattes, 1982) or the lack of landmarks at high elevations (Neuschulz et al., 2017). Our results, showing missing shrub and tree invasions within exclosures are comparable to land-use changes in the Eastern Alps: formerly mown grasslands exhibited no invasion of shrubs and trees even after 60 years of abandonment (Bohner et al., 2020). Missing effects despite climate warming and grazing such as in our study area are known also from the LTER site Niwot Ridge (Scharnagl et al., 2019). In contrast, experiments (Alexander et al., 2015) demonstrated that lowland competitors might outperform alpine plants. At our study site, no montane or colline-centered species were recorded throughout the 18 years of observation. All newcomers and species with increasing frequency belonged to the treeline and alpine species pool.

Long-term processes caused by climate warming and/or grazing exclusion may interact and are difficult to disentangle. Major changes due to climate warming can be weakened or completely overruled by high grazing intensity (Speed et al., 2012, 2013; Jäschke et al., 2018). In contrast, continuous low-intensity grazing may retard or even inhibit community changes (Speed et al., 2013; Wang et al., 2019). Pardo et al. (2015) recorded low impacts of grazing exclusion on species richness but highlighted synergistic effects with extreme weather events such as dry and warm periods. Our study was not conclusive in this respect, but we found an indication of slightly reduced and altered weather effects under grazing exclusion. Our sites generally exhibited a relatively low grazing intensity, as the grazers forage large areas with exception of the subalpine *Agrostis–Festuca* grassland, where six cattle per 1 ha graze from the beginning of July to the end of August each year, provoking a higher grazing pressure (Mayer et al., 2009; Mayer and Erschbamer, 2017).

The problem of disentangling climate change and grazing exclusion effects is an aspect which certainly has to be analyzed in more detail. For a more stringent interpretation, data of the local conditions per individual site would be helpful and a much higher sampling intensity would be necessary.

Shortcomings of the Study

Long-term monitoring studies are expected to give comprehensive answers to research questions such as effects of climate or land-use changes (Evangelista et al., 2016; Frate et al., 2018; Scharnagl et al., 2019). However, even in long-term studies we have to deal with directional and non-directional changes over time as well as plot variability (Spasojevic et al., 2013), exacerbating a clear interpretation. Initial differences between exclosure and control plots often aggravated the interpretation, the effects being very small in comparison to general changes over time and to site differences.

Moreover, we are aware of several limitations of the study design. First, replications of exclosures at the subalpine *Agrostis–Festuca* grassland and the upper alpine *Kobresia* grassland as well as at the grazed *Festuca nigrescens* grassland are missing due to local circumstances. Second, due to technical and financial problems, monitoring was not equal for all sites and years. Third, the uneven number of replicated permanent plots in the grazed and exclosure/control sites, respectively, might limit the strength of our results for species numbers.

CONCLUSION

Diversity increases in grazed subalpine–alpine–subnival plant communities in the Central Austrian Alps were attributed to the ongoing climate change. During the 18 years of investigation, the communities were not invaded by species from low elevations; the newcomers mainly belonged to the treeline and alpine species pool. The investigated sites were not affected by the increase of competitive species with exception of the subalpine *Agrostis–Festuca* exclosure. Here, grazing exclusion lead toward a tall-forb community. The missing increase of competitive species in all other sites may be attributed to the typical vegetation structure of alpine communities where already highly competitive species predominate as residents, such as *Kobresia myosuroides* in the subnival grasslands, *Carex curvula* in the upper alpine grassland, *Nardus stricta* in the lower alpine grassland, *Vaccinium* spp. in the tall dwarf shrub, or *Loiseleuria procumbens* in the prostrate dwarf shrub. In the *Carex curvula* grasslands, snow bed species decreased within the exclosures. Cessation of grazing might multiply the threat of this species group as it was found to be also endangered by climate warming.

An indication of slightly reduced and altered weather effects under grazing exclusion was found. The interaction of climate change and grazing is not easy to disentangle. However, this topic definitely should be considered for predictions of community shifts in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s. The raw data are included in the GRASSPLOT database (https://edgg.org/databases/Grassplot).

AUTHOR CONTRIBUTIONS

RK and BE designed the study. RM set up the exclosures and carried out the field work. NS provided equipment and set up the exclosures during the last years. RK analyzed the data. BE wrote the drafts of the manuscript. All authors contributed to the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.640103/full#supplementary-material

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Spatial Scale Dependence of Ecological Factors That Regulate Functional and Phylogenetic Assembly in a Mediterranean High Mountain Grassland

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Understanding how functional and phylogenetic patterns vary among scales and along ecological gradients within a given species pool is critical for inferring community assembly processes. However, we lack a clear understanding of these patterns in stressful habitats such as Mediterranean high mountains where ongoing global warming is expected to affect species fitness and species interactions, and subsequently species turnover. In this study, we investigated 39 grasslands with the same type of plant community and very little species turnover across an elevation gradient above the treeline at Sierra de Guadarrama National Park in central Spain. In particular, we assessed functional and phylogenetic patterns, including functional heterogeneity, using a multi-scale approach (cells, subplots, and plots) and determined the relevance of key ecological factors (i.e., elevation, potential solar radiation, pH, soil organic carbon, species richness, and functional heterogeneity) that affect functional and phylogenetic patterns at each spatial scale. Overall, at the plot scale, coexisting species tended to be more functionally and phylogenetically similar. By contrast, at the subplot and cell scales, species tended to be more functionally different but phylogenetically similar. Functional heterogeneity at the cell scale was comparable to the variation across plots along the gradient. The relevance of ecological factors that regulate diversity patterns varied among spatial scales. An increase in elevation resulted in functional clustering at larger scales and phylogenetic overdispersion at a smaller scale. The soil pH and organic carbon levels exhibited complex functional patterns, especially at small spatial scales, where an increase in pH led to clustering patterns for the traits related to the leaf economic spectrum (i.e., foliar thickness, specific leaf area, and leaf dry matter content). Our findings confirm the presence of primary environmental filters (coldness and summer drought at our study sites) that constrain the regional species pool, suggesting the presence of additional assembly mechanisms that act

at the smallest scale (e.g., micro-environmental gradients and/or species interactions). Functional and phylogenetic relatedness should be determined using a multi-scale approach to help interpret community assembly processes and understand the initial community responses to environmental changes, including ongoing global warming.

Keywords: clustering, community assembly, elevation gradient, functional traits, overdispersion, phylogenetic distance, species diversity

INTRODUCTION

Coexistence theory considers that community assembly is a process regulated by multiple mechanisms, both stochastic and deterministic, which act at different spatial scales and that are subject to different ecological factors (Palmer, 1994; Chesson, 2000). Understanding how these assembly mechanisms and their drivers operate simultaneously at different spatial scales and along environmental gradients is critical for forecasting community responses to environmental changes, including the major issue of ongoing global warming. Approaches based on functional traits, which are valuable indicators of resource use strategies and fitness, can provide critical information about how ecological factors affect assembly because only species with the appropriate set of traits can be part of the local (or realized) plant community (Cornelissen et al., 2003; Violle et al., 2007). In addition, phylogeny seems to be involved in assembly processes because it summarizes the evolutionary history of the local species pool (Losos, 1996; Webb et al., 2002; Cavender-Bares et al., 2009; Kraft and Ackerly, 2010; Mouquet et al., 2012; Gerhold et al., 2015), and it is often used as a proxy of current functional information as it is assumed that evolutionary diversification has generated predictable trait diversification (Flynn et al., 2011). Thus, comparisons of the functional (and/or phylogenetic) diversity of species assemblages and those expected from null assemblages have been performed routinely during the last two decades to identify the assembly processes that structure plant communities at different spatial scales (Díaz et al., 1998; Cavender-Bares et al., 2006; Kraft and Ackerly, 2010; Chalmandrier et al., 2017; López-Angulo et al., 2021).

Among the so-called deterministic processes, environmental filtering and limiting similarity are two major ecological forces that drive community assembly. It is known that these mechanisms act at different spatial scales on the regional species pool (Levin, 1992; Swenson et al., 2006; Emerson and Gillespie, 2008; Götzenberger et al., 2012) but their importance in specific plant communities has traditionally been studied at a single spatial scale under similar environmental conditions (but see Kraft and Ackerly, 2010; Messier et al., 2010; de Bello et al., 2013b; Carboni et al., 2014; Chalmandrier et al., 2017) and/or in systems with high species turnover. Environmental filtering, which is considered to act at relatively large scales, is expected to lead to a community where species are more functionally similar or closely related (i.e., functional and phylogenetic clustering; Weiher and Keddy, 1995; Leibold, 1998; Webb, 2000) because filters will select species that can thrive under the local conditions (but see Gerhold et al., 2015). By contrast, limiting similarity (*sensu* MacArthur and Levins, 1967), which is related to the existence of niche differences where plant-to-plant competitive interactions prevail (i.e., smaller scales), predicts that competitiveness among species with similar requirements leads to niche segregation where the coexisting species are functionally and/or phylogenetically different (Mason and Wilson, 2006; Kraft et al., 2008), thereby implying trait and phylogenetic overdispersion for exploiting the diversity of niches at small scales. However, alternative combinations have also been described. For example, strong environmental heterogeneity may potentially lead to a functional overdispersion pattern at relatively small spatial scales because different niches are available (Mayfield and Levine, 2010; de Bello et al., 2013b), which would determine intense turnover. In addition, trait clustering may be linked to biotic competitive processes at small spatial scales when the dominance of closely related species excludes weaker competitors (Chesson, 2000; Mayfield and Levine, 2010).

Evaluating patterns of site-to-site functional variation, i.e., a type of β-diversity referred to as functional heterogeneity in the following, among spatial scales may be necessary for understanding the importance of mechanisms involved at both local and regional scales in community assembly (Seabloom et al., 2005; Siefert et al., 2013; Yang et al., 2015). Thus, high functional heterogeneity would be expected when comparing the functional variation between sites or assemblages (at large spatial scales) simply because geographic and environmental distances often imply strong species turnover (i.e., different community types) or shifts in their functional traits (Myers et al., 2013). However, functional heterogeneity could be stabilized at the regional scale when a strong environmental filter acts and selects species with a particular combination of associated functional traits (Körner, 2003). In addition, functional heterogeneity could be increased further at local scales (within sites) by small-scale environmental variations such as those induced by soil heterogeneity (Freestone and Inouye, 2006) or species interactions (Pescador et al., 2014).

Mountains exhibit steeper environmental gradients over short geographic distances and at various scales (Körner, 2003). Thus, mountains are excellent systems for investigating how assembly processes operate at different spatial scales and the relevance of ecological factors for structuring plant communities (McCain and Colwell, 2011). Previous trait- and phylogenetic-based studies in high mountain communities suggest that environmental filtering increases along the elevation by affecting functional and phylogenetic patterns (de Bello et al., 2013a; Pistón et al., 2016; Xu et al., 2017; Le Bagousse-Pinguet et al., 2018). In addition, these patterns can be shaped by local factors in alpine communities, such as the potential solar

radiation, soil pH, or nutrient heterogeneity (Cadotte et al., 2011; Bernard-Verdier et al., 2012; Luo et al., 2016). However, functional and phylogenetic patterns have generally been assessed across large environmental gradients, which imply high species turnover with major changes in the habitat type, and thus it is difficult to understand assembly mechanisms at different scales.

In the present study, we investigated functional and phylogenetic patterns as well as their ecological predictors by considering a nested spatial design in a single plant community type located along a 500-m elevation gradient in a Mediterranean high mountain area. This community is characterized by strong environmental filtering acting along the whole study gradient, which excludes species that are vulnerable to cold (Pescador et al., 2016), and a severe summer drought, especially at lower elevations (Giménez-Benavides et al., 2007; Olano et al., 2013). These conditions limit the species pool to specialists that are well adapted to such conditions, thereby resulting in the same type of plant community with low species turnover (Pescador et al., 2015). To the best of our knowledge, a low turnover scenario has not been considered in previous studies for assessing functional and phylogenetic patterns, but it probably represents the best system for investigating how a habitat-specific species pool is affected by ongoing global warming before a change in turnover. Thus, we hypothesized that an environmental adjustment would prevail in this community with environmental filtering acting at large scales and across the elevation gradient, which would define the realized assemblages through functional and phylogenetic clustering, especially at higher elevations. By contrast, micro-environmental heterogeneity and species interactions would prevail at smaller scales, thereby leading to functional and phylogenetic divergence, which should be correlated with the soil characteristics at these scales. As a consequence, the functional heterogeneity should be relatively similar across the spatial scales studied because the primary environmental filters should stabilize the large functional heterogeneity between sites (regional scale), whereas micro-environmental heterogeneity and species interactions could force the opposite. Thus, in the present study, we addressed the following three questions: (i) Are the functional and phylogenetic clustering–overdispersion patterns within assemblages similar across spatial scales? (ii) Does the functional heterogeneity between assemblages vary among spatial scales? (iii) How do functional and phylogenetic patterns vary along the elevation gradient and with other key ecological factors (e.g., potential solar radiation, soil pH or nutrient heterogeneity, and species richness) at each spatial scale?

MATERIALS AND METHODS

Study Site and Field Sampling

This study was conducted above the treeline at Sierra de Guadarrama National Park located northwest of Madrid in Spain (40°46′39″ to 40°51′8″ N; 3°49′44″ to 4°4′59″ W; 1,940 to 2,419 m a.s.l.). The climate is Mediterranean, with a mean annual temperature and precipitation of 6.4°C and 1,350 mm, respectively, and a drought from May to October (less than 10% of the annual precipitation), which is more intense at lower elevations (Giménez-Benavides et al., 2007; Olano et al., 2013).

The treeline is located between 1,900 and 2,000 m a.s.l., and it is dominated by Scots pine (*Pinus sylvestris* L.) interspersed in a shrubby matrix of *Cytisus oromediterraneus* Rivas Mart. et al. and *Juniperus communis* L. subsp. *alpina* (Suter) Čelak. Above this elevation, the dominant shrubby matrix extends up to 2,100–2,200 m a.s.l., with dry and cryophilic grassland dominated by *Festuca curvifolia* Lag. *ex* Lange in less steep locations. In the highest areas (from 2,200 to 2,419 m a.s.l.), grassland represents the dominant vegetation and it is structured in a mosaic of patches dominated by *F. curvifolia* and some creeping and cushion chamaephytes clumped in a bare ground matrix (Pescador et al., 2014).

Thirty-nine sites covering the whole elevation range of *F. curvifolia* grasslands were selected in the summer of 2011 (**Supplementary Figure 1A**). At each site, we established a sampling plot of 20 m × 20 m and surveyed the vegetation at three different spatial scales: cell, subplot, and plot (**Supplementary Figure 1B**). First, a square measuring 2.4 m × 2.4 m was haphazardly located in the center of the plot and divided into 64 cells of 30 cm × 30 cm, which represented the cell scale (smallest scale). Second, five subplots of 2.4 m × 2.4 m (including the grid of cells) were established in the center and four corners of the plot as the intermediate scale. The coverage was visually estimated for every plant species, soil, and rock at the three spatial scales. At the plot scale, all plant species and their percentage cover were estimated as the average cover per species in the five subplots after also considering the presence and cover of the plant species not found in the cells or subplots but present in the plot (for more details, see López-Angulo et al., 2019).

Each plot was also characterized based on a set of environmental variables. In particular, we measured the elevation and orientation using a GPS system (Garmin Colorado-300) and the slope with a clinometer (Silva Clinomaster CM-360-%, LA). Orientation and slope values were employed to estimate the potential solar radiation according to Gandullo's method (Gandullo, 1974; López-Angulo et al., 2018). Finally, 15 soil samples (diameter = 5 cm, depth = 10 cm) were randomly collected from each plot (**Supplementary Figure 1B**) under vegetated patches of the dominant grass (usually *F. curvifolia*; five samples), shrub canopies (*C. oromediterraneus* and *J. communis* subsp. *alpina*; five samples), and on bare ground (five samples). The soil samples were sieved (2-mm mesh) and air dried for 1 month, before the pH and soil organic carbon (SOC, %) were determined at the NutriLab-URJC laboratory. The pH was determined using a pH meter GLP 21 (Crison, Barcelona, Spain) and SOC was determined by colorimetry after oxidation with $K_2Cr_2O_2$ and H_2SO_4.

Plant Functional Traits

During the summer of 2011 and 2012, five plant functional traits were measured in a total of 56 species (**Supplementary Table 1**). These species represented more than 83% of the species in the community study and approximately 99% of the species

cover. At least 10 individuals per species were selected and five functional traits were measured according to standardized protocols (Cornelissen et al., 2003): plant height (Hmax; distance between the ground and top of photosynthetic tissues), foliar thickness (FT), specific leaf area (SLA; ratio of leaf fresh area relative to dry weight), leaf dry matter content (LDMC; ratio of leaf dry weight relative to fresh weight), and seed mass (for more details, see Pescador et al., 2015).

DNA Isolation, Sequencing, and Phylogenetic Analysis

We collected fresh young leaves from three individuals per species and they were stored under dry conditions in silica gel for 1 month. Up to 20 mg of each dry leaf sample was disrupted and homogenized with a TissueLyser LT (Qiagen, Valencia, CA, United States) using glass beads. Total genomic DNA was extracted with a DNeasy Plant Mini-Kit (Qiagen, Valencia, CA, United States) according to the manufacturer's instructions. Standard polymerase chain reactions (PCRs) were conducted to amplify two barcoding loci (i.e., *rbcL* and *matK*). In the PCR amplification mixture, 2 μl of DNA was added to 23 μl of reaction mixture comprising 2.5 μl of Taq buffer 2 mM with $MgCl_2$, 1 μl of dNTP Mix (0.4 mM), 1.25 μl of reverse and forward primers, and 1.25 U Taq DNA Polymerase (Biotools, Madrid, Spain). PCR was performed with an S1000 Thermal Cycler (Bio-Rad, United States) using the specific PCR conditions given in **Supplementary Table 2**. The PCR products were cleaned up using ExoSap-IT® (USB Corporation, Cleveland, OH, United States) and submitted to Macrogen Inc. (Seoul, South Korea) for sequencing in the forward and reverse directions. In total, 288 sequences [three individuals per species × (56 species successfully sequenced with *rbcL* + 40 species successfully sequenced with *matK*)] were edited with Sequencher 4.1.4 (Genes Code Corporation), and the consensus sequences in each species were determined from the forward and reverse sequences. The *rbcL* and *matK* markers were aligned independently using Mafft online-version 7 (Katoh and Standley, 2013) following the G-INS-I strategy recommended for < 200 sequences with global homology. MacClade 4.06 (Maddison and Maddison, 2003) was used to concatenate primer configurations (*rbcL* + *matK*) and to create a combined matrix with missing data when the marker could not be sequenced.

The *rbcL* + *matK* combined matrix was used to reconstruct the phylogeny topology and the divergence times for our community using Bayesian inference and the Markov chain Monte Carlo (MCMC) approach in the BEAST 1.6.1 program (Drummond and Rambaut, 2007). We ran three MCMC chains with 50 million generations and sampled one tree and their parameters every 10,000 generations (partition). We estimate the rooted, time-measured phylogeny inferred using an uncorrelated relaxed molecular clock, which assumes that the substitution rates associated with each branch are drawn independently from a single lognormal distribution (Drummond et al., 2006). The substitution model used for each partition was GTR + Γ. The substitution model and estimated frequencies were unlinked among partitions. To calibrate the tree temporally, we constrained the divergence times for the minimum crown age at seven nodes based on the fossil record (**Supplementary Table 3**). Each constrained node was drawn following a lognormal or exponential prior with different parameters (for more details, see **Supplementary Table 3**). The Tracer 1.6 program (Rambaut et al., 2014) was used to evaluate the effective sample size for parameters, and we verified that three runs converged on the posterior distribution and reached stationarity. We combined the independent MCMC parameters and sample trees using the LogCombiner 1.6.1 program. Finally, all trees were summarized in a single maximum clade credibility tree (**Supplementary Figure 2**) after removing 2,500 trees as the burn-in and calculating the clade posterior probabilities and 95% honest posterior density intervals for node-specific parameters using TreeAnnotator 1.6.1.

Community Structure Metrics

We calculated the mean pairwise distance (MPD_{obs}; see de Bello et al., 2016) between the coexisting species observed in each cell, subplot, or plot to assess the functional and phylogenetic clustering–overdispersion patterns at different spatial scales. The functional and phylogenetic distance between each pair of coexisting species were calculated based on Gower's dissimilarity matrix (Pavoine et al., 2009) using the information for the plant functional traits and the cophenetic correlation for the phylogenetic approach. Each MPD_{obs} value was expressed relative to the MPD simulated (MPD_{exp}) based on a null model to calculate a standardized effect size (SES; Gotelli and McCabe, 2002) for each scale, trait, all traits together, and phylogeny. Choosing an appropriate null model is a critical step when testing the effects of different mechanisms (Perronne et al., 2017), so we designed two "realistic" null models where the species names in each simulated assemblage were the names of the species in the plot to which the cell/subplot belonged (i.e., local pool, NM1), or the species names in all plots (i.e., regional pool, NM2). Thus, at the cell and subplot scales, the species that generated the null assemblage could come from the local or regional pool, whereas only the regional pool was considered at the plot scale. In addition, irrespective of the null model used, the number of species and total cover in each cell, subplot, or plot were kept fixed to control for differences in resource availability among scales and species (Gotelli, 2000; Le Bagousse-Pinguet et al., 2018). The likelihood that one species appeared in null assemblages was directly related to its abundance at the plot or regional scale, and the cover for each species in the null distribution was proportional to its abundance at the plot or regional scale. Based on these constraints, we generated 1,000 null assemblages for each cell (64 cells/plot × 39 plots), subplot (5 subplots/plot × 39 plots), and plot (39 plots), where the MPD_{exp} values were estimated using multi-trait, individual trait, and phylogenetic information, and each null model (i.e., NM1 and NM2). Comparing each MPD_{obs} to the corresponding 1,000 random

MPD_{exp} values allowed us to calculate the SES of the MPD as follows:

$$SES - MPD = {}^{(MPD_{obs} - \overline{MPD_{exp}})}/_{sd\ (MPD_{exp})},$$

where MPD_{obs} is the MPD obtained from the data observed at the pertinent working scale and using the functional or phylogenetic distance, *MPDexp* is the mean value for the 1,000 null MPD distributions, and *sd* (MPD_{exp}) is the standard deviation value for the 1,000 null MPD distributions. It should be noted that this index is equivalent to the inverse of the net relatedness index used in many phylogenetic approaches (Webb et al., 2002), and positive values indicate that the co-occurring species are more functional or phylogenetically distant than in the null assemblages (overdispersion pattern), whereas negative values denote that species are more closely related (clustering pattern). In total, we calculated SES–MPD values for 64 × 39 cells, 5 × 39 subplots, and 39 plots by considering each plant functional trait individually (i.e., Hmax, FT, SLA, LDMC, and seed mass), all traits together, and phylogenetic distances. At the cell and subplot scales, the null assemblage was built by considering the local (NM1) or regional (NM2) species pool. In each situation, the average SES–MPD value per plot was considered for each of the 64 cells and five subplots.

We estimated the coefficient of variation of the community weighted mean (CV-CWM; ratio of the standard deviation to the mean for average traits weighted by species abundances) for the realized assemblages at each spatial scale (i.e., cell, subplot, and plot) to characterize the functional heterogeneity at three spatial scales. We considered the information for 64 cells per plot to estimate the functional heterogeneity within each subplot (i.e., CV-CWM_w) because the summed area of the 64 cells was equivalent to the subplot area. We calculated the between-subplot heterogeneity by considering the five subplots per plot (i.e., CV-CWM_b). Finally, a unique CV was obtained for the 39 plots at the regional scale (i.e., CV-CWM_r). The latter variation component can be considered as the total variation between plots, and thus of the plant community in study area. All of these functional heterogeneity components were estimated by considering all traits together (in a multi-trait approach) and each trait independently (i.e., Hmax, FT, SLA, LDMC, and seed mass).

Statistical Analyses

We performed *t*-tests using the SES–MPD values at each spatial scale to determine whether the functional and phylogenetic diversities differed significantly from expectations. In particular, SES–MPD values significantly higher than 0 indicated overdispersion among assemblage species, whereas SES–MPD values lower than zero denoted clustering patterns. We performed paired *t*-test to evaluate the differences between functional heterogeneity within (CV-CWM_w) and between subplots (CV-CWM_b). We established linear models to assess the response of ecological factors on each SES–MPD following a multi-model inference (MMI) over all possible combinations of the factors and with AIC_c. A total of 35 independent linear models were drawn as a result of considering SES–MPD values for all traits together, each individual trait, and phylogeny at each of the three spatial scales and two null models. We used non-correlated ecological factors (i.e., elevation, potential solar radiation, pH, SOC, and species richness). We also considered the functional heterogeneity as an additional predictor factor. Within each independent model, all possible combinations of ecological factors were considered, and the resulting "partial models" were ranked according to the AIC_c value with respect to the "partial model" with the minimum AIC_c ($\Delta AIC_c = AIC_i - AIC_{min}$). "Partial models" with $\Delta AIC_c < 2$ were considered to be indistinguishable (Burnham and Anderson, 2002) and an average explanatory model was defined according to this threshold. The average estimate, significance, and relative importance of each predictor (w+) were estimated for each model. The relative importance of a predictor was evaluated by summing the w_i values for the partial models that contained the predictor of interest. Finally, the phylogenetic signal was calculated for each functional trait with Blomberg's *K* index to capture the effect of trait evolution and to assess the existence of phylogenetic niche conservatism in our system. Basically, Blomberg's *K* represents the ratio of the observed phylogenetically correct mean-square error divided by the mean-square error using a variance matrix derived from the phylogeny standardized by the expectation under a Brownian motion model (Blomberg et al., 2003). The significance of *K* (*p*-value) was calculated by comparing it to the null distribution with 9,999 replicates. Thus, $K < 1$ indicates that closely related species resemble each other less than expected under the Brownian motion model of trait evolution (no phylogenetic signal), whereas $K > 1$ suggests that closely related species are more similar than predicted by the model (stronger phylogenetic signal).

All statistical analyses were conducted with R 3.6.0 (R Core Team, 2019) and the following functions: dbFD and gowdis functions implemented in the FD package (Laliberté and Legendre, 2010; Laliberté and Shipley, 2011), dredge and importance functions in the MuMIn package (Kamil, 2013), melodic function (de Bello et al., 2016), and phylosignal function in the picante package (Kembel et al., 2010).

RESULTS

Overall, we identified significant deviations in the functional and phylogenetic clustering–overdispersion patterns depending on the spatial scale (i.e., cell, subplot, or plot scale) and null model (i.e., species consideration from the plot pool, NM1, or from the regional pool, NM2; **Figure 1** and **Supplementary Table 4**). At the plot scale, the trait clustering pattern (SES–MPD values < 0) was most relevant for all single-trait and multi-trait approaches. At the subplot and cell spatial scales, significant trait overdispersion (SES–MPD values > 0) had considerable importance for most traits irrespective of whether we considered the plot (NM1) or regional pool species (NM2). By contrast, Hmax had a significant clustering pattern at both spatial scales and when considering both null models (**Figure 1B**). The community had a weak phylogenetic clustering pattern at the plot scale (species at plot scales were more closely related than

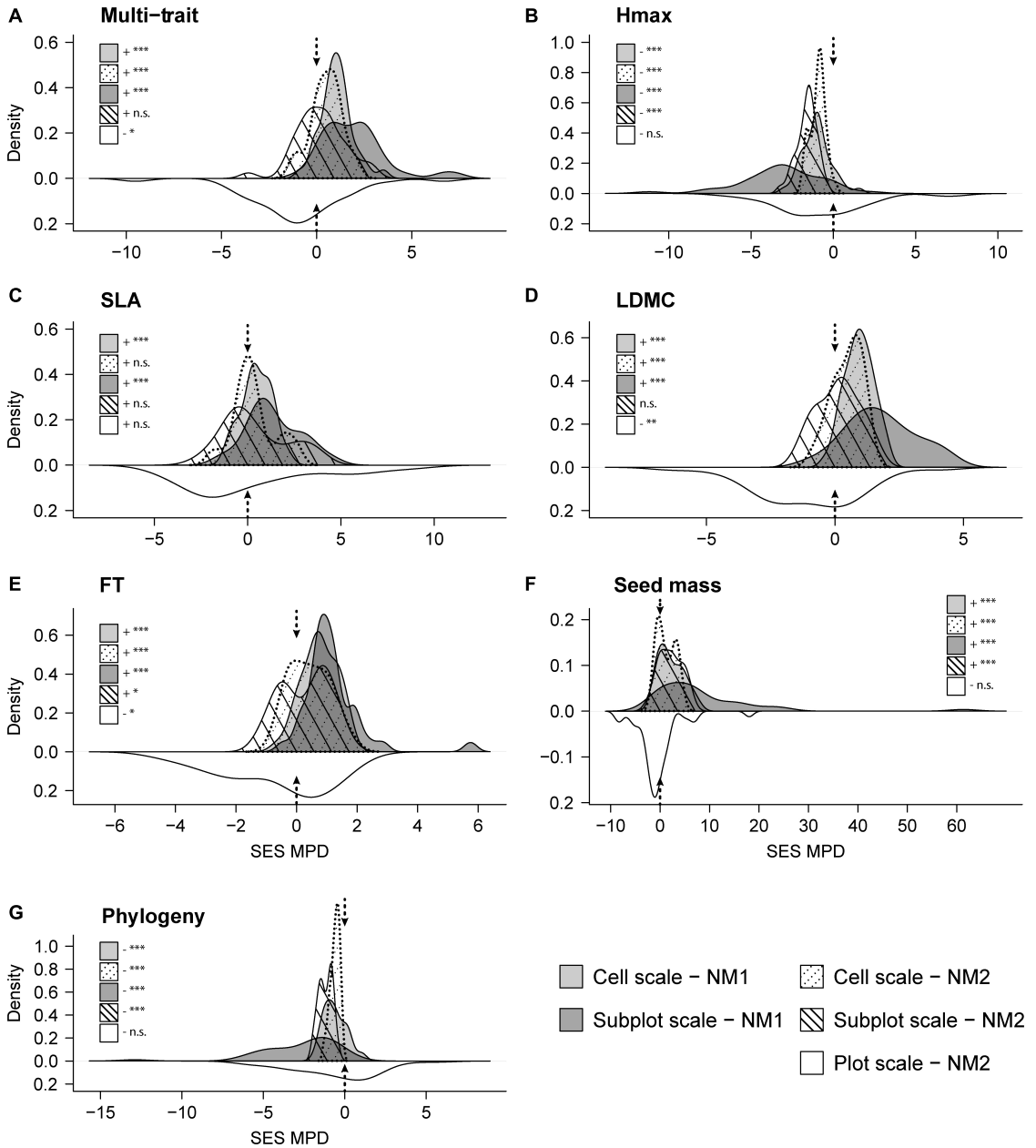


FIGURE 1 | Density plots of standardized effect size of mean pairwise distance (SES–MPD) for **(A)** multi-trait, **(B–F)** each individual plant functional trait and **(G)** phylogeny. Three different operating spatial scales (cell, subplot, or plot scale) and two null models (simulated assemblages using plot pool, NM1, and regional pool, NM2) are included in each plot. Negative values indicate that co-occurring species within the assemblages are more related than expected by chance (i.e., clustering), whereas positive values denote distances greater than expected by chance (i.e., overdispersion). Black-dashed arrows represent the SES threshold of 0. Each panel shows the results of one-sample *t*-tests conducted to determine whether SES–MPD values for each approach, null model, and scale were positive (+) or negative (–), and significantly different from 0 as: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Full scores for the one-sample *t*-tests are presented in **Supplementary Table 4**. Hmax, plant height; SLA, specific leaf area; LDMC, leaf dry matter content; FT, foliar thickness.

expected from the null model), and significant clustering was found at the subplot and cell scales when both null models were used (**Figure 1G**).

The functional heterogeneity across spatial scales exhibited similar patterns for the multi-trait approach and based on each plant functional trait (**Figure 2** and **Supplementary Table 5**). On average, the functional heterogeneity was usually greater at the regional scale (i.e., CV-CWM$_r$) than those within and between subplots (i.e., CV-CWM$_w$ and CV-CWM$_b$, respectively), except for traits related to the leaf economic spectrum (i.e., FT, SLA, and LDMC), where the values of CV-CWM$_w$ were similar or even higher than those of CV-CWM$_r$. Paired *t*-tests between the CVs at the plot level (i.e., CV-CWM$_w$ and CV-CWM$_b$) indicated lower variability between subplots than within subplots

in most cases, except for Hmax (**Figure 2B**) and FT (**Figure 2C**), where both components were not significantly different (*p*-values according to paired *t*-test = 0.50 and 0.09, respectively; **Supplementary Table 5**).

The effects of ecological factors on the SES–MPD values depended on the spatial scale and the null model considered (**Figure 3**). In particular, clustering patterns (negative relationships) were detected for multi-trait, SLA, and seed mass as the elevation increased at the plot scale and under NM2. By contrast, a phylogenetic overdispersion pattern (positive relationship) was more obvious at the smallest spatial scale and under NM2 as the elevation increased. The potential solar radiation effect led to clustering patterns for multi-trait and seed mass for co-existing species at the subplot scale. Among the soil factors, an increase in SOC resulted in the overdispersion of SLA at different scales but a clustering pattern of FT at the plot and subplot scales and under NM2. Higher soil pH levels resulted in overdispersion patterns for Hmax and seed mass at the subplot and cell scale, respectively. By contrast, clustering patterns were found for FT, SLA, and LDMC when the soil pH levels increased at the subplot scale and under NM1. Increased species richness was related to clustering patterns for multi-trait, Hmax, FT, and phylogeny at the three spatial scales. Finally, overdispersion patterns for Hmax and FT at the cell scale were directly dependent on the functional heterogeneity characterized as the CV of the corresponding CWM.

DISCUSSION

Our results indicate the existence of three assembly mechanisms operating at different spatial scales in the same plant community type on a Mediterranean high mountain. First, we established the scale dependence of the mechanisms involved in the assembly process ranging from functional clustering at regional spatial scales to functional overdispersion at the smallest scale (Mason et al., 2011; de Bello et al., 2013b; Carboni et al., 2014), and phylogenetic clustering irrespective of the spatial scale. Second, we found that the functional heterogeneity at the smallest scale represented a source of variation, which was as important as the heterogeneity detected along the whole elevation gradient, especially for the traits related to the leaf economic spectrum. Third, we showed that different ecological factors (i.e., elevation, potential solar radiation, SOC, pH, species richness, and functional heterogeneity) regulated the functional and phylogenetic patterns among co-occurring species and ultimately determined the community assembly. Overall, the results obtained in our study confirmed the importance of considering approaches based on functional traits and phylogenetic information across spatial scales in plant coexistence assessments (Kraft and Ackerly, 2010; Perronne et al., 2014).

Diversity Patterns Across Spatial Scales

By assessing the SES–MPD according to two null models (simulated community assemblages using plot pool, NM1, and regional pool, NM2) and at three spatial scales (cell, subplot, or plot scale), we detected contrasting clustering–overdispersion patterns in the functional and phylogenetic diversity (Freschet et al., 2011; Bernard-Verdier et al., 2012; de Bello et al., 2013b). We found that an increase in the spatial granularity (i.e., plot scale of 20 m × 20 m) implied a clustering pattern for most plant functional traits, thereby supporting the idea that the effects of environmental filters are more conspicuous at larger scales (Weiher and Keddy, 1995). By contrast, when the spatial granularity was reduced (i.e., subplot and cell scales), functional overdispersion was the most frequent pattern for all functional traits, except for Hmax. Similarly, some previous studies found an overdispersion pattern at small spatial scales (Stubbs and Wilson, 2004; de Bello et al., 2013b), and our results support the limiting similarity hypothesis for most functional traits at these scales. Hmax was the only functional trait that exhibited a clustering pattern at these scales (**Figure 1B**), and thus the heights of species in a subplot or cell tended to be similar. In high mountain areas, cold is a strong abiotic filter and greater height may be considered a limitation for the survival of species (Körner, 2003). Consequently, low stature appears to be the most suitable strategy for this type of environment. Furthermore, Mayfield and Levine (2010) suggested the existence of similarity patterns in Hmax linked to competitive ability. The phylogenetic relationship assessment detected clustering patterns at three study scales, thereby indicating that the species were more closely related than the null expectation. This pattern is consistent with the prevalence of environmental filtering indicated for most of our functional traits and the idea that phylogenetic relatedness suggests functional proximity (Webb, 2000; Cavender-Bares et al., 2006; Kraft and Ackerly, 2010). In fact, environmental filtering could be identified with phylogeny as a proxy of functional differences among species at larger scales, but this pattern might have been caused by competitive exclusion leading to clustering after the elimination of the weakest competitors (Mayfield and Levine, 2010). It should be noted that this pattern was more obvious with NM1, which implies that the species pool selected passed a specific environmental filter, and thus the patterns detected could probably be attributed to the incidence of biotic factors (species competition for the same resources) rather than environmental heterogeneity (Stubbs and Wilson, 2004).

Functional Heterogeneity Across Spatial Scales

Overall, functional heterogeneity at the smallest scale (CV-CWM$_w$) for the traits related to the leaf economic spectrum showed a variation similar to or even higher than that observed at the regional scale (CV-CWM$_r$). These findings suggest the existence of (a) a primary abiotic filter at the regional scale determining low functional turnover throughout the distribution area and minor differences between plots (i.e., low CV-CWM$_r$); (b) the existence of local environmental heterogeneity acting within each plot to yield functional heterogeneity (i.e., plots with relatively similar CV-CWM$_b$ values rather than CV-CWM$_r$ values); and (c) different and significant determinist processes at the smallest scale, which would also have been conditioning the species assembly (i.e., similar or higher CV-CWM$_w$ values

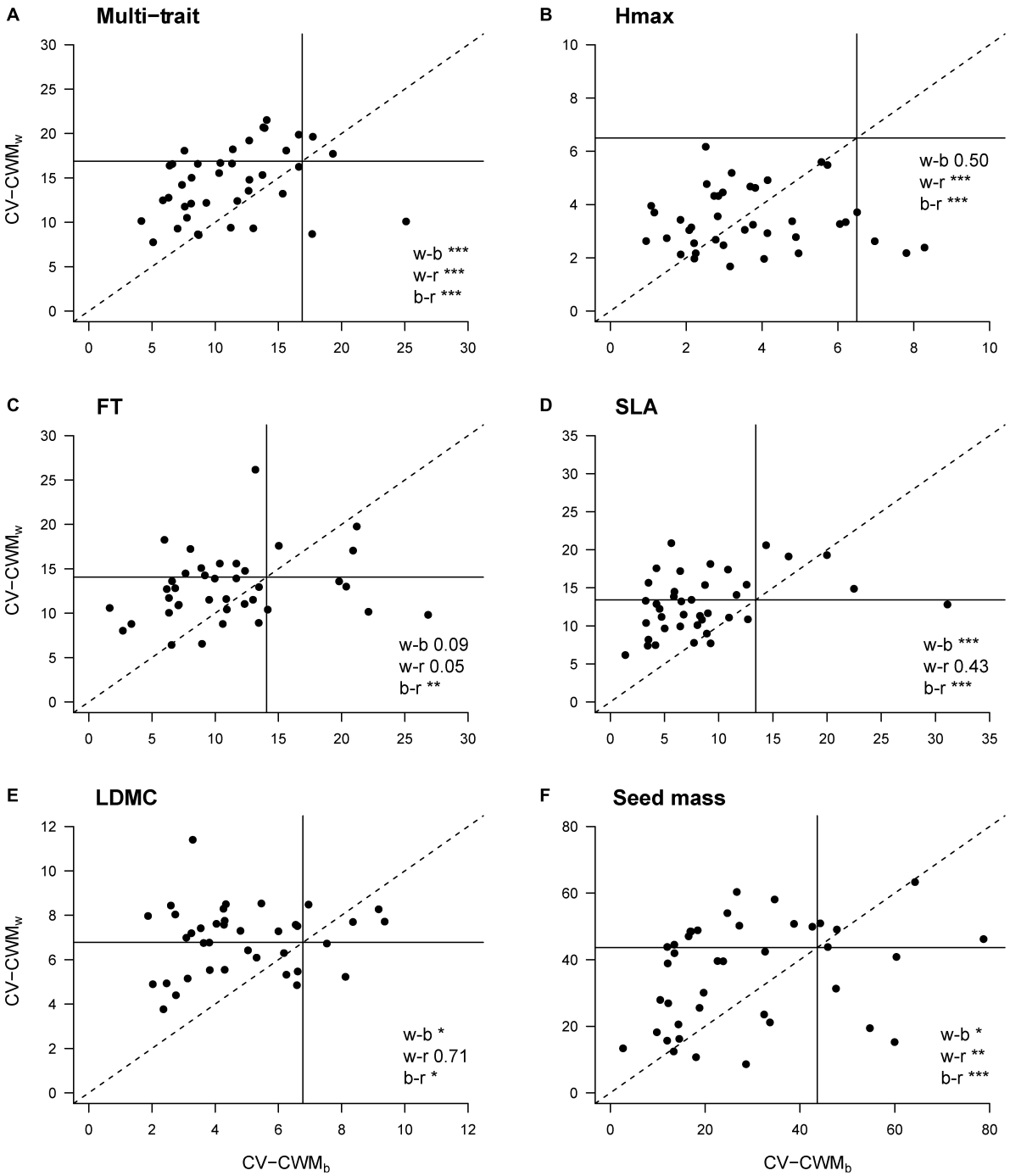


FIGURE 2 | Relationships among coefficient of variation (CV) of community weighted mean (CWM) at the three spatial scales assessed [within subplots (CV-CWM$_w$; *y*-axis in each panel), between subplots (CV-CWM$_b$; *x*-axis in each panel), and between plots (CV-CWM$_r$; solid lines in each panel)] and considering **(A)** multi-trait approach and **(B–F)** each individual plant functional trait. Dashed lines represent a 1:1 relationship. In each panel, *p*-values obtained from paired *t*-tests for within subplots (w), between subplots (b), and between plots (r) are denoted as: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. Full results for these paired *t*-tests are presented in **Supplementary Table 5**. Hmax, plant height; FT, foliar thickness; SLA, specific leaf area; LDMC, leaf dry matter content.

to those between plots). As noted above, cold and summer drought are primary filters in Mediterranean high mountains that promote the existence of a similar and well-adapted species pool among sites (i.e., low composition and functional turnover along elevation; Helmus and Ives, 2012). This relatively limited pool of species (no more than 60 species along a 500-m elevation gradient

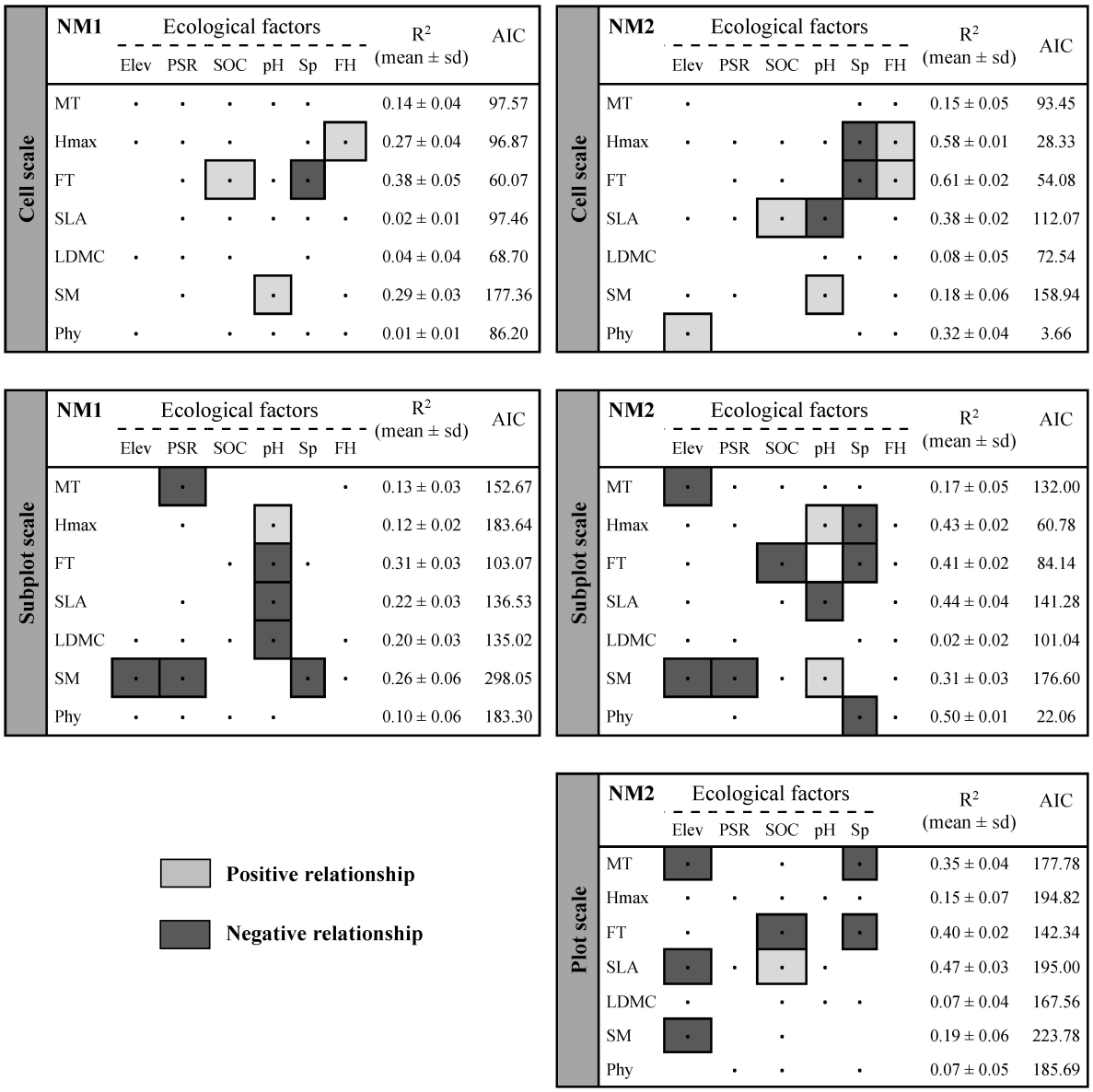


FIGURE 3 | Linear averaged models showing the responses of non-correlated ecological factors on each standardized effect size of mean pairwise distance (SES–MPD) for multi-trait, individual functional traits, and phylogenetic approaches. SES–MPD values were considered at three spatial scales (i.e., cell, subplot, or plot scale) and under two null models (i.e., simulated assemblages using plot pool, NM1, and regional pool, NM2). In each case, all possible predictor combinations were tested and "partial models" were ranked according to Akaike's information criterion (AIC_c). Averaged models were drawn from the best partial models based on $\Delta AIC_c < 2$ ($\Delta AIC_c = AIC_i - AIC_{min}$). Predictors included in each averaged model are denoted by a dot (.) and positive or negative significant relationships (according to the average-slope value) are highlighted in light gray or dark gray, respectively. R^2 (mean ± standard deviation) and AIC_c values are shown in each case for the best partial models. Full results for the averaged linear models are shown in **Supplementary Table 6**. MT, multi-trait; Hmax, plant height; FT, foliar thickness; SLA, specific leaf area; LDMC, leaf dry matter content; SM, seed mass; Phy, phylogeny; Elev, elevation (m a.s.l.); PSR, potential solar radiation; SOC, soil organic carbon (%); pH, soil pH; Sp, number of species per plot; FH, functional heterogeneity (CV-CWM).

in Sierra de Guadarrama National Park) may be subject to the effects of other sources of environmental heterogeneity (potential solar radiation, light competition, soil characteristics, and shrub encroachment) or simply to stochasticity. These sources could constrain the species assemblage in each plot and lead to functional heterogeneity between plots (i.e., CV-CWM_r), which is also limited by the primary filters. Nevertheless, the ecological variables that lead to other sources of heterogeneity are relatively similar between the subplots in each plot (same elevation, potential solar radiation, or soil characteristics), thereby resulting in redundant species assemblages between subplots with similar functional heterogeneity (i.e., CV-CWM_b). By contrast, at small spatial scales, the species in a plot are subject to additional assembly mechanisms based on micro-environmental

gradients (Hutchings et al., 2003; Burton et al., 2011) or species interactions (Pescador et al., 2014), which may increase the functional heterogeneity at the cell scale. These additional assembly mechanisms lead to a scenario where the deterministic processes that operate at small scales are as important for generating functional variability as those observed at larger scales. In general, if the functional heterogeneity at the smallest scale is in the range of that at the regional scale, then any model that attempts to explore the species distribution and community composition based on the environmental conditions and species functional traits (see Shipley et al., 2006) should consider at least these sources of spatial variability.

Relevance of Ecological Factors on the Diversity Patterns

Linear averaged models showed that assembly processes were influenced by ecological factors that depended on the scale, functional traits, and null model considered (Mason et al., 2011; Spasojevic and Suding, 2012; de Bello et al., 2013a; Price et al., 2014; Reitalu et al., 2014). Species in plots located at the highest elevations exhibited a clustering pattern according to the multi-trait approach as well as for the SLA and seed mass traits. However, when the sampling granularity decreased to the cell level, phylogenetic overdispersion increased with the elevation but with no effects on functional responses. These results suggest that species at the plot level use a similar functional strategy to cope with the harsh conditions on the summits (Weiher and Keddy, 1995; Stubbs and Wilson, 2004; Schwilk and Ackerly, 2005; Chalmandrier et al., 2017). However, a phylogenetic overdispersion pattern is needed at the smallest scale to reduce competition with direct neighbors for the same local resources (Cavender-Bares et al., 2004, 2006; Pescador et al., 2014). Phylogenetic patterns have been investigated previously along elevation gradients with contrasting findings (Bryant et al., 2008; Culmsee and Leuschner, 2013; González-Caro et al., 2014; Li et al., 2014; Qian et al., 2014). In particular, similarly to our results, Bryant et al. (2008) found that plant communities tended to exhibit phylogenetic overdispersion at higher elevations, probably due to the prevalence and intensification of facilitation with elevation, whereas competition was more important at lower elevations. Furthermore, high levels of stress can lead to more open and discontinuous vegetation cover providing more gaps for colonization, and thus higher phylogenetic diversity (Reitalu et al., 2014). Based on the conceptual framework of functional and phylogenetic patterns (Webb et al., 2002; Cavender-Bares et al., 2004; Kraft and Ackerly, 2010), our findings imply that important traits for habitat specialization in harsh conditions probably exhibit convergent evolution (i.e., a low phylogenetic signal; **Supplementary Table 7**).

The absence of responses in terms of the functional and phylogenetic diversity to potential solar radiation at the plot and cell scales suggest that difference in the total annual radiation between north- and south-facing slopes was less important as a filter (Méndez-Toribio et al., 2017). However, multi-trait and seed clustering was detected at the subplot scale when the potential solar radiation values were higher. Thus, at this scale, co-occurring species must employ similar physiological and regenerative strategies to cope with water stress in plots with high potential solar radiation, especially in the Mediterranean-type climate. Irrespective of the spatial scale considered, the multiple effects of soil factors on functional patterns suggest high heterogeneity in the soil characteristics at small scales (Jackson and Caldwell, 1993; Hutchings et al., 2003; Gazol et al., 2013), especially given the strong effects of soil pH (Chytrý et al., 2003) and SOC in grasslands. Remarkably, we found clustering patterns for the traits related to the leaf economic spectrum and overdispersion patterns for Hmax and seed mass at higher pH values, and these patterns have not been described previously to the best of our knowledge. Furthermore, the prevalence of clustering as the species richness increases was found previously using functional (Carboni et al., 2014; Niu et al., 2014) and phylogenetic approaches (Reitalu et al., 2014). Thus, if the environment favors the co-occurrence of a great number of species, they will tend to be functionally and phylogenetically more redundant and similar (Cadotte et al., 2011), possibly because the regional species pool is unable to provide new candidates with different functional strategies to cope with harsh conditions (Yachi and Loreau, 2007; Pakeman, 2011). As a consequence, this predictor together with elevation had hardly any effect on the ecological clustering–overdispersion patterns when NM1 was used (i.e., the species pool was represented by the species present in the plot considered), probably because the species in null assemblages were from a species pool subject to the same elevation and species richness as the realized assemblage. Finally, the overdispersion patterns for the Hmax and FT traits were modulated by an increase in the functional heterogeneity at the smallest scale (CV-CWM$_w$), and thus site-to-site functional variation should be considered as a relevant factor when assessing community assemblies.

Conclusion

Our multi-scale approach applied to a single plant community type with a low species turnover highlights the importance of evaluating functional and phylogenetic patterns at different spatial scales to understand the current community assembly. We showed that complex functional and phylogenetic patterns emerge depending on the spatial scale considered. Environmental filtering was found at larger spatial scales where species coexist under particular conditions, whereas functional overdispersion was present at small scales, thereby suggesting secondary niche differentiation after the effects of primary filters such as cold and summer drought. Phylogenetically based analysis indicated a pattern consistent with the functional analysis at larger scales, but this congruence was absent at the subplot and cell scales where the phylogenetic pattern remained clustered. Our results also demonstrated the importance of functional heterogeneity at the smallest scale in our plant community, thereby confirming the presence of primary environmental filters and suggesting the existence of additional assembly mechanisms based on micro-environmental gradients

and species interactions at the smallest scale. Finally, our results show that functional and phylogenetic diversity patterns differ in terms of their responses to ecological factors across spatial scales, which highlights the need to develop adequate tools for predicting the response of vegetation to global warming (Reu et al., 2011) or the distributions of species based on the relationships between environmental conditions and species functional traits (Shipley et al., 2006). Given the shifts in the functional and phylogenetic responses along the elevation and among spatial scales, both sources of diversity should be considered by employing a multi-scale approach to help understand the future of alpine biodiversity under ongoing warming.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, MW927222–MW927321. Data sets on vegetation co-occurence patterns, environmental variables, and functional traits for each plant species can be found in López-Angulo et al. (2019).

AUTHOR CONTRIBUTIONS

DP, AE, and FV conceived and designed the study. DP and JL-A conducted the fieldwork. DP and FB performed the statistical analysis. DP wrote the manuscript. All authors contributed to the discussion and interpretation of data, revised the manuscript, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.622148/full#supplementary-material

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Plant Phenology Dynamics and Pollination Networks in Summits of the High Tropical Andes: A Baseline for Monitoring Climate Change Impacts

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Analyzing plant phenology and plant–animal interaction networks can provide sensitive mechanistic indicators to understand the response of alpine plant communities to climate change. However, monitoring data to analyze these processes is scarce in alpine ecosystems, particularly in the highland tropics. The Andean páramos constitute the coldest biodiversity hotspot on Earth, and their species and ecosystems are among the most exposed and vulnerable to the effects of climate change. Here, we analyze for the first time baseline data for monitoring plant phenological dynamics and plant–pollinator networks along an elevation gradient between 4,200 and 4,600 m asl in three mountain summits of the Venezuelan Andes, which are part of the GLORIA monitoring network. We estimated the presence and density of plants with flowers in all the summits and in permanent plots, every month for 1 year. Additionally, we identified pollinators. We calculated a phenological overlap index between species. We summarized the plant–pollinator interactions as a bipartite matrix and represented a quantitative plant–pollinator network, calculating structural properties (grade, connectance, nestedness, and specialization). We also evaluated whether the overall network structure was influenced by differences in sampling effort, changes in species composition between summits, and phenology of the plant species. Finally, we characterized the pollination syndrome of all species. Flowering showed a marked seasonality, with a peak toward the end of the wet season. The overall phenological overlap index was low (0.32), suggesting little synchrony in flowering among species. Species richness of both plants and pollinators decreased along the elevation gradient. Flies, bumblebees, and hummingbirds were the most frequent pollinators in the network, while entomophily and anemophily were the prevailing pollination syndromes. The interaction network in all summits showed high connectance values, significant specialization (H2), and low nestedness. We did not find a significant effect of sampling effort, summit plant species

composition, or plant phenology on network structure. Our results indicate that these high tropical alpine plant communities and their plant-pollination networks could be particularly vulnerable to the loss of species in climate change scenarios, given their low species richness and functional redundancy coupled with a high degree of specialization and endemism.

Keywords: plant reproduction, alpine ecosystems, GLORIA, animal-plant interactions, vegetation dynamics

INTRODUCTION

Climate change is accelerating and is having important impacts on species range shifts, which are particularly well documented in cold biomes, where there is increasing evidence that alpine species distributions are shifting upwards (Lenoir and Svenning, 2015; Steinbauer et al., 2019). Long-term research within the framework of monitoring networks such as the Global Observation Research Initiative in Alpine Environment (GLORIA) has shown that global warming is affecting community structure (Steinbauer et al., 2019). For example, in European mountains, climate warming and changes in precipitation are having contrasting effects on the summit floras, increasing species richness in boreal-temperate summits but decreasing richness in Mediterranean sites, probably because of increased drought severity in the European south (Pauli et al., 2012; Lamprecht et al., 2021). Monitoring within the GLORIA–Andes network in northwestern Argentina has shown an increase in the abundance of small herbs and disperse grasses (Carilla et al., 2017). However, in the context of monitoring long-term vegetation dynamics in alpine ecosystems, much less is known about the impacts of climate change on plant reproduction (but see Tovar et al., 2020 in the case of plant dispersal strategies), including phenological responses, changes in plant-pollination, and the possible relation between these two processes.

In plants and crops from the temperate zone, evidence has shown that increases in temperature in recent years, can have a significant effect on phenological patterns, including the time for the beginning or the amplitude of the flowering or fruiting period, as well as significant disruptions of pollination interactions (Harrison, 2000; Chapman et al., 2005; Inouye, 2008). In diverse and/or generalist systems, the wide availability of different pollinators can provide a buffer against alterations in phenological patterns (Anderson et al., 2012). Moreover, in systems with a high degree of functional redundancy, pollinator species with strong preferences for certain floral traits have less need to synchronize their dynamics with the phenology of particular plant species (Benadi et al., 2014). However, in systems with less functional redundancy and/or with many specialists, there can be a greater susceptibility to species losses and changes in phenology, resulting in adverse effects on the stability of plant–pollinator networks (Bartomeus et al., 2011, 2013).

In alpine systems, pollinator's diversity is generally low compared with lowland diversity (Ramirez and Brito, 1992). In fact, in mountain systems, the ratio of pollinator/plant abundance decreases with elevation (i.e., pollinators become increasingly scarce for plants), while generalist and specialized systems coexist, although generalist systems tend to dominate (Medan et al., 2002; Ramos-Jiliberto et al., 2010; Trøjelsgaard and Olesen, 2013). Species displacements from lower to higher elevations, changes in species abundance, or species extinctions linked with climate change could all cause alterations in mutualistic plant–pollinator systems. Hence, there is an urgent need for studies on this topic that encompass the wide spatial and temporal variability (within and between years) characteristic of alpine areas around the world and which can provide a baseline for future comparison of plant phenological dynamics and plant–pollinator interactions in global change scenarios (Forrest, 2014; Inouye, 2020).

In this context, the study of ecological interactions and complex networks should offer key insights to analyze primary ecological properties like the interdependence of the components of the network (Bascompte et al., 2006). For example, the analysis of connectance allows one to assess the degree of association between species; metrics such as the degree of nesting can help to evaluate the robustness of the system to species loss, while specialization indexes can provide a measure of the degree of species interdependence (Jordano, 1987; Bascompte et al., 2006; Blüthgen et al., 2006, 2007; Burgos et al., 2007; Memmott et al., 2007; Tylianakis et al., 2007; Almeida-Neto and Ulrich, 2011; Devoto et al., 2012; Dormann and Strauss, 2014). Moreover, the probability of alternative matrices of interactions can be computed to evaluate variables that could influence network structure on the basis of important ecological attributes that are thought to have explanatory value (e.g., species abundance patters and flowering phenology), which can then be compared to null models (Vázquez et al., 2009; Vázquez, 2010).

The available studies of plant–pollinator networks in temperate alpine ecosystems have generally reported a high degree of nestedness (Dupont et al., 2003) and low connectance values (Olesen and Jordano, 2002; Santamaría et al., 2014). However, the study of plant reproductive ecology and plant–animal interactions has received substantially less attention in the case of the tropical mountain ecosystems, with the few available studies concentrating on the páramos of the tropical Andes (Aguirre et al., 2011; Llambí and Rada, 2019). The páramos are diverse high tropical mountain ecosystems distributed between 3,000 and 4,800 m along the Northern Andes (Monasterio, 1980b; Josse et al., 2011). The high specialization of the flora to the conditions of the cold tropics suggests that these species are particularly vulnerable to global warming (Anthelme et al., 2014). In fact, recent continental scale research in long-term monitoring summits of the GLORIA–Andes network has shown that vegetation in páramo summits of the northern Andes is particularly vulnerable to habitat loss in climate change scenarios

because of a high abundance of endemic species with narrow thermal niches (Cuesta et al., 2020).

The few available studies on the reproductive ecology and pollination in the high tropical Andes reinforce the idea that these systems could be sensitive to the loss of species due to global changes, given that they are relatively poor in pollinator species and show high endemism (Ramos-Jiliberto et al., 2010; González and Loiselle, 2016; Pelayo et al., 2019). Analyses on plant phenology and reproductive aspects in the páramos have focused on very emblematic species such as the giant rosettes (*Espeletia* spp.), as well as some exploratory studies on small herbs and cushions (Berry, 1986; Berry and Calvo, 1994; Fagua and Bonilla, 2005). Thus, we know that giant rosettes species are entomophilous and anemophilous, while in the case of herbs and cushions, self-fertilization seems to predominate.

One of the few available studies on plant phenology and pollination at a community scale in the alpine tropics was carried out in the Venezuelan páramos between 3,000 and 4,200 m (Pelayo et al., 2019). This study showed that flowering occurs gradually from the beginning to the end of the rainy season (from May to November), with a low overlap of flowering between species. Few species exhibited constant flowering throughout the year, and few species bloomed during the dry season (from December to April), which tends to be particularly harsh in the Venezuelan páramos (compared to other páramos in the Northern Andes). A high proportion of the studied species were pollinated by animals, including hummingbirds and bumblebees. Moreover, the structure of plant–pollinator networks showed significant specialization; low values of connectance, asymmetry, and nestedness; and high values of functional complementarity (Pelayo et al., 2019). However, this study did not fully cover the superpáramo or subnival belt (above 4,200 m asl), an ecological belt where more extreme climatic and edaphic conditions could be linked to a decrease in richness of plants and pollinators. In a more humid superpáramo in the Sierra Nevada de Mérida (4,155–4,220 m), Manrique et al. (2019) reported a network with significant specialization, which was also influenced by phenotypic and phenological adjustments.

In this context, the presence of three permanent monitoring summits of the GLORIA–Andes network in the Venezuelan superpáramos (between 4,200 and 4,600 m asl, see Cuesta et al., 2017; Gámez et al., 2020) offers a unique opportunity to (a) establish a baseline to analyze the potential impacts of climate change on the reproductive behavior of high elevation species in tropical mountain ecosystems; (b) develop a protocol for recording the phenology of annual flowering in the different summits and orientations; and (c) document the dynamics of the interactions between plants with flowers and their pollinators. Hence, the objective of this study was to analyze changes in the flowering phenology and the structure of the plant–pollinator network in three summits along an elevation gradient in a high belt of Venezuelan páramos.

Based on previous results from páramos at lower elevations (Pelayo et al., 2019), we evaluated the following predictions: (1) the phenological behavior of the plant community will be strongly influenced by the marked rainfall seasonality of the Venezuelan páramo, and the phenological overlap index could increase with elevation as the climatic filtering for flowering becomes more severe (i.e., more severe seasonal thermal and hydric stress at higher elevations); (2) we expect a higher incidence of pollinator visits in periods when flower availability is higher; (3) we expect a reduction in the species richness of both plants and pollinators and a shift in the relative importance of the different plant growth forms (increase in the predominance of small flowering herbs) and pollinator taxa (reduction in the importance of birds and bumblebees vs. flies); (4) these networks are expected to show high levels of specialization but low levels of connectance and nestedness compared to other systems at lower elevations; and (5) the network structure should be influenced by plant phenology and the patterns of change in plant species composition between summits. The assessment of these predictions should provide a more detailed and mechanistic understanding of the vulnerability to climate change of these unique high elevation tropical communities.

MATERIALS AND METHODS

Study Area

This study was carried out in the Piedras Blancas páramo, in the northeast region of the Sierra de La Culata National Park, Venezuela. The region is influenced by the dry climate of the high Chama river basin, with a total annual precipitation of 860 mm (Pico El Águila weather station, 4,118 m asl). Less than 5% of this precipitation falls during the dry season, extending from December to March (Sarmiento, 1986). Mean monthly temperatures vary less than 2–3°C, and the growing season extends all year. However, there are large daily temperature oscillations, that increase in amplitude during the dry season, when air temperatures can reach 25°C during the day and drop at night below –5°C (Monasterio, 1986; Azócar and Rada, 2006).

The subnival belt (locally known as high Andean páramo or superpáramo) extends between 4,200 and 4,800 m asl. Within this belt, two types of vegetation can be distinguished: (a) the desert páramo, dominated by scattered giant rosettes (genus *Espeletia*) together with a lower stratum dominated by cushion plants, acaulescent rosettes, low shrubs, tussock grasses, and herbs (4,200–4,500 m asl); and (b) the periglacial desert, where plant cover is normally less than 10% and small grasses and herbs are dominant (typically above 4,500 m, Monasterio, 1980a).

The sampling sites were located in the three highest monitoring summits of the GLORIA–Andes network in Venezuela (4,207, 4,402, and 4,604 m asl) in La Culata-Piedras Blancas site (VE-CPB, Cuesta et al., 2017; Gámez et al., 2020). This site was installed in 2012 and a first resampling campaign was implemented in 2017. The total richness of vascular plants in these summits is 71 species, distributed in 53 genera, and 24 families (the most common being Asteraceae and Poaceae). Vegetation cover and total species richness in these summits decrease from 59% and 59 spp. at 4,207 m to 9.1% and 14 species at 4,604 m asl (**Table 1**).

In the lowest summit (PI2), vegetation corresponds to a shrubland-rosette páramo; the upper stratum is dominated by the sclerophyllous shrub *Hypericum laricifolium* and the stem

TABLE 1 | Characteristics of the selected summits for monitoring the flowering phenology and plant–pollinators networks within the framework of the GLORIA–Andes network in Venezuela.

Summit	Total species richness *	Average plant cover**	Dominant species ***	Vegetation belt
PI2	59	59.1%	*Hypericum laricifolium* (20%) *Aciachne acicularis* (8%) *Espeletia timotensis* (7%) *Rumex acetosella* (4%) *Luzula racemosa* (4%)	Subnival/shrub rosette transition from páramo to superpáramo
PB4	40	43.4%	*Rumex acetosella* (21%) *Castilleja fissifolia* (4%) *Espeletia timotensis* (4%) *Agrostis tolucensis* (3%) *Conyza uliginosa* var *uliginosa* (2%)	Subnival/rosette superpáramo. Upper stratum dominated by giant rosettes
MO6	14	9.1%	*Pentacalia imbricatifolia* (4%) *Festuca fragilis* (3%) *Castilleja fissifolia* (0.8%) *Draba chionophila* (0.4%) *Lasiocephalus longipenicillatus* (0.3%)	Nival/Superpáramo, periglacial desert dominated by small shrubs, grasses and herbs, sparse vegetation

Total site richness: 71 species, 53 genera, and 24 families.*
**Total number of vascular plant species.*
***Average cover in the 16 1 × 1 m permanent plots per summit.*
****Average percent cover in the 16 1 × 1 permanent plots per summit.*

rosette *Espeletia schultzii*, while the lower stratum is dominated by the graminoids *A. acicularis* and *L. racemosa* and the exotic herb *R. acetosella*. In the intermediate summit at 4,402 m (PB4), vegetation corresponds to a giant rosette superpáramo; the giant stem rosette *Espeletia timotensis* is dominant in the upper stratum, while the lower stratum is dominated by herbs (*R. acetosella*, *Castilleja fissifolia*) and grasses (e.g., *Agrostis tolucensis*). Finally, the 4,604 summit (MO6) corresponds to a periglacial desert, with a single stratum dominated by small shrubs (*Pentacalia imbricatifolia*), grasses (*Festuca fragilis*), small herbs (*C. fissifolia* and *Lasiocephalus longipenicillatus*), and basal rosettes (*D. chionophila*) (Gámez et al., 2020).

Flowering Phenology

The quantitative analysis of plant phenology focused on the 3 × 3 m permanent plots (n = 4) located in the four cardinal orientations of each summit, established following the standard GLORIA network protocol (Pauli et al., 2015). During 1 year (December 2017–November 2018), we carried out 1-day visits between the last 2 weeks of each month to each summit, in order to count the number of individuals of vascular plants with flowers in the total area of each 3 × 3 m plot. We decided not to consider grasses, because this group is anemophilyc and relatively abundant. Hence, this allowed optimizing the time used for observing plants with flowers that could possibly receive visits from pollinators.

A complementary qualitative sampling was also implemented, registering the presence/absence of plants with flowers of each species in the whole summit area sections located in each cardinal orientation between the highest summit point and the 10-m-elevation contour line (see Pauli et al., 2015). Based on the qualitative information of presence/absence of plants with flowers for the whole summit each month, we represented the phenological patterns in graphs and calculated the overlap index (Primack, 1985) to analyze the degree of synchrony of flowering among species. This index ranges from 0 to 1, with 0 corresponding to a complete lack of synchrony and 1 to total synchrony. For this analysis, we integrated the information from the four orientations of each summit, and analyzed each summit separately or all summits together for a general synthesis of the three summits in the superpáramo belt. We also analyzed changes between the three summits in the proportion of species that exhibited different pollination syndromes, based on the morphology of their flowers, following the classification proposed by Faegri and van der Pijl (1979).

Plant–Pollinator Network

During each monthly visit to each of the summits, we recorded all the interactions observed between flowers and pollinators, while collecting the phenological data. For this, we used direct observation and binoculars (10 × 42), when necessary (a single expert carried out the visitation observations in all cases). In each case, the number of visits made by the pollinator was recorded, but only if it contacted the reproductive structures of the flowers. The unknown insects were collected for later identification at the species level, in the case of bumblebees and at the morphospecies and family level in the rest. They were deposited in the Entomological Collection at Universidad de Los Andes (Mérida, Venezuela). Bird pollinators were identified directly in the field by comparison with the Bird Guide of Venezuela (Hilty, 2003). The observation time varied between 2 and 6 h for each visit, depending on the weather conditions, as we were not able to register interactions during precipitation events (animal activity normally ceases). Simultaneous with direct observations, we placed 2 *GOPRO White 3* cameras next to the plants with more abundant flowering during each visit (Pelayo, 2017). These normally focused on one or two individuals within the field of vision of the camera. The cameras took high-resolution pictures every 0.5 s. In total, during the year, we made a total sampling effort of 92 h of direct observations and

107 h of camera recordings and visually analyzed a total of 385,200 images. A limitation of the use of these cameras is that in many cases, the small insects are not observed in detail in the photographs, so it is very important and advisable to become familiar with the floral visitors in the field, for the subsequent analysis of the photographs.

Rarefaction curves and the Chao 2 richness index were calculated for the observed pollinators using EstimateS 9.1.0 (Colwell, 2013) to evaluate the completeness of our sampling effort. In the case of plants (excluding grasses) we observed 55 species with flowers across the year of observation, corresponding to 87.3% of the 63 species that we know to be present based on the full plant inventory in these summits from 2012 to 2017 (Gámez et al., 2020). Some of the few missing species (e.g., *E. timotensis*) are in fact known to flower every 2 or 3 years, and they are pollinized by wind, so these would not affect our analysis of the pollination network (Berry, 1986; Berry and Calvo, 1994).

We calculated the correlation (Spearman) between the number of monthly visits with (a) the total number of plants with flowers in all the 3 × 3 quadrants; and (b) number of species with flowers each month (for the whole summit).

With the information collected each month, we built matrixes for each summit (plant species in the rows and the pollinators in the columns), summarizing the total number of visits received by the flowers of each plant species by each type of pollinator during the sampling year. Based on these quantitative matrixes, we developed a plant–pollinator network for each summit and for the three summits together, and calculated their structural properties by means of the following metrics: (i) degree of a species (Bascompte et al., 2006), (ii) connectance (Jordano, 1987), (iii) weighted nestedness metric based on overlap and decreasing fill (NODF; Almeida-Neto and Ulrich, 2011; based on the quantitative interaction weights), and (iv) specialization index (H2; Blüthgen et al., 2006; based on quantitative interaction weights). Calculations of network metrics were conducted with the bipartite package (Dormann et al., 2008) in R 2.15.2 (R Core Team, 2016). Significance of weighted NODF and H2 was tested by comparing empirical values vs. a null model (10,000 repetitions of similar dimension network) in R 2.15.2.

The structure of the general plant–pollinator network was contrasted with alternative models based on attributes of the plant species (Vázquez et al., 2009), including phenology (qualitative and quantitative) and spatial distribution (presence–absence of each species in each of the three summits). We also evaluated if network structure could be explained by sampling effort (focal and video observation time per species). In some cases, combined models were also performed to explore the joint relationship of two variables, for example, phenology and plant distribution, focal and video hours. We carried out this analysis for the whole network based on the data for the three summits combined, because network sizes were too small to allow meaningful tests based on data from each individual summit taken separately. We did not implement models including variables related to fauna, since we only registered their visitation rates. Finally, a null model was created in which all interactions were assigned the same probability of occurrence [$p = 1/(P * A)$] (where P and A were equal to the number of plant and animal species, respectively). Akaike's information criterion (AIC) was calculated for each of these modeled matrices, which allowed to select the most plausible model (lowest AIC), to explain the observed matrix. Given a set of candidate models for the data, the preferred model is the one with the minimum value in the AIC and therefore $p = 1$, where $p = \exp[(\text{AICmin} - \text{AICj})/2]$ and AICmin represents the lowest AIC of the four models explored (Burnham and Anderson, 2002). This analysis was carried out using the "mlik" function of the "ecolnet" package (Vázquez, 2010) of R 2.15.2.

RESULTS

Flowering Phenology

In the three studied summits, we collected information on the phenology of flowering of 55 species that produced flowers during the study year, belonging to 19 families (excluding grasses). The families Asteraceae and Rosaceae presented the highest number of species with 26 spp. and 5 spp., respectively, followed by Brassicaceae, Caryophyllaceae, and Orobanchaceae, with three species each. Forty-seven species have characteristics related to entomophily, 2 to anemophily, 3 to ornithophily, and 3 exhibit mixed characteristics between entomophily and ornithophily (**Figure 1**).

Most species flowered during the wet season (**Figure 2**), and tended to show limited overlap with each other during their flowering periods. However, a few species such as *C. fissifolia*, *Draba pulvinata*, *H. laricifolium, Lachemilla polylepis*, *L. longipenicillatus*, *Conyza uliginosa* var *uliginosa*, and *Valeriana*

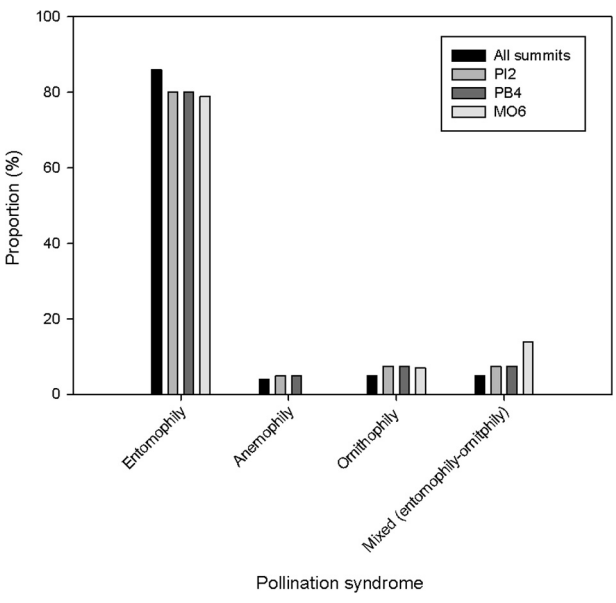


FIGURE 1 | Proportion (%) of plant species that belong to each pollination syndrome in the three summits (PI2, PB4, and MO6) of the GLORIA–Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela.

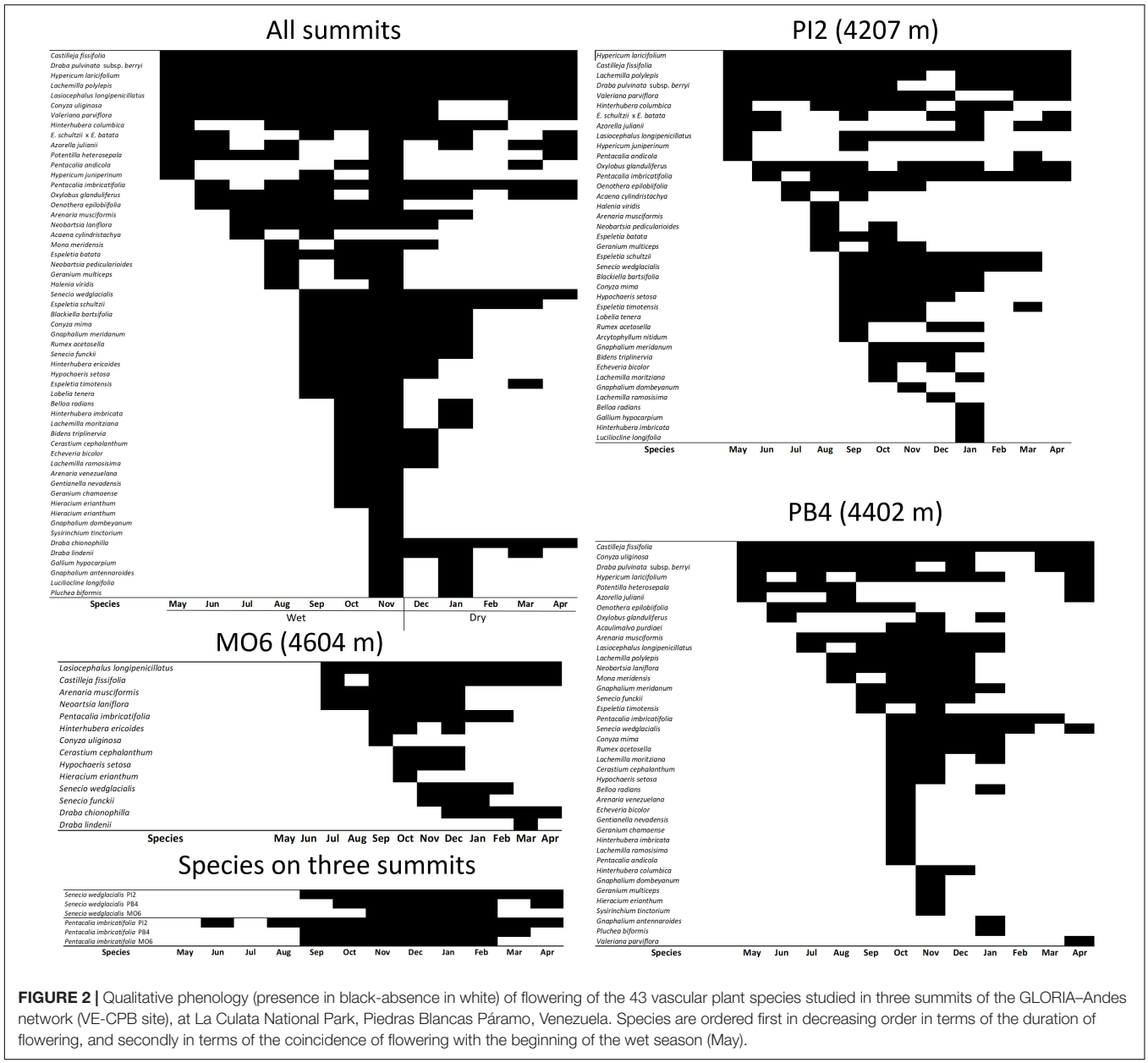


FIGURE 2 | Qualitative phenology (presence in black-absence in white) of flowering of the 43 vascular plant species studied in three summits of the GLORIA–Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela. Species are ordered first in decreasing order in terms of the duration of flowering, and secondly in terms of the coincidence of flowering with the beginning of the wet season (May).

TABLE 2 | Phenological overlap index between plants and structural properties of pollination networks in superpáramo summits (la Culata-Piedras Blancas site, GLORIA–Andes, Venezuela).

Indices and network metrics	All summits	PI2 (4,207 m)	PB4 (4,402 m)	MO6 (4,604 m)
Phenological overlap	0.32	0.24	0.32	0.36
No. of plants	26	14	15	4
No. of pollinators	13	10	9	8
Chao2 pollinators	13.45 (13–21)	10.91 (10–20)	11.75 (9–32)	7.69 (7–15)
Degree plants	2–7	1–7	1–5	1–2
Degree pollinators	1–11	1–6	1–9	1–4
Connectance	0.17	0.20	0.20	0.34
H2	0.72**	0.82**	0.78**	0.69*
Weighted NODF	20.01**	19.63**	22.39*	14.71**

*Statistical significance compared to the null model: **$p < 0.001$; *$p \leq 0.05$; ns (not significant) $p > 0.05$. Chao 2 estimates for pollinator richness is presented with the 95% interval in parenthesis.*

parviflora showed continuous flowering. There were also some species that were able to produce flowers during the dry season, some of them with periods of explosive blooming limited to a single month per year. The rest of the species showed a long bloom period. These general patterns were characteristic of the two lower summits (PI2 and PB4). However, in the highest summit (MO6), flowering began in the middle of the rainy season and lasted until the dry season (**Figure 2**). The overall phenological overlap index between plant species was low (0.32), suggesting little synchrony in flowering between species. This index increased with elevation (**Table 2**), suggesting that flowering could become more synchronic in the highest summit. For example, *Senecio wedglacialis* and *P. imbricatifolia,* which were abundant species present in the three summits, showed a tendency to bloom later in the year with increasing elevation, while their flowering period become shorter at higher altitudes (**Figure 2**).

We found that the species with the highest density of plants with flowers changed with summit elevation (**Figure 3**). In PI2, the dominant species were the shrub *H. laricifolium* and the herbs *Conyza mima* and *Oenothera epilobifolia*. In PB4, the herbs *C. fissifolia*, *C. mima*, and *Gnaphalium meridanum* were dominant. Finally, in MO6, the herbs *P. imbricatifolia*, *Bartsia laniflora*, and *L. longipenicillatus* dominated. Regarding the effect of orientation within the summits, in the lowest summit (PI2), the highest number of individuals with flowers were recorded in the south and east orientations. In PB4 and MO6 summits, the highest density of plants with flowers was recorded in the north and west orientations (**Figure 4**).

Plant–Pollinator Networks

Of the 55 species that produced flowers during the year, 26 were included in the general pollination network (**Figure 5**), while the rest were not visited during our observations. We registered a total of 13 species of pollinators. This was within the 95% confidence interval of the Chao 2 richness index for pollinators (13–21 species), suggesting that the pollinator sampling was nearly complete (**Table 2**). The guild of floral visitors observed covered taxa as diverse as hummingbirds (2 species), flower piercers (1), flies (5), bumblebees (2), bees (1), wasp (1), and butterflies (1). With 47.68% of all of the interactions observed in the three summits (total frequency), flies were the most frequent floral visitors. Two bumblebees (*Bombus rohweri* and *B. rubicundus*) accounted for 35.31%; bees and the wasp, 9.80%; hummingbirds, 5.67%; flower piercer, 1.25%; and butterflies, 0.29% (**Figure 5**).

In terms of species, the largest number of visit records corresponded, in order of importance, to *B. rohweri* (Apidae, 33.3%), Bibionidae 1 (Diptera, 23.8%), Bombyliidae (Diptera, 17.83%), Colletidae (Hymenoptera, 9.65%), *Oxypogon lindenii* (Trochilidae, 3.7%), and Muscidae (Diptera, 3.61), while the rest of morphospecies together contributed to less than 8.1% of the visits (**Figure 5**). The general interaction network was dominated by *B. rohweri* and Bibionidae 1 with 11 and 9 connections (grade K), respectively; the remaining pollinators had between 7 and 1 connections. Regarding the plant species, *E. schultzii* and *C. fissifolia* were the most connected with seven and five connections each. The richness of plants and pollinators decreased with elevation. In all cases, the pollinator sampling was within the 95% confidence interval of the Chao 2 richness index (10–20 in PI2, 9–32 in PB4, and 7–15 in MO6), suggesting that the pollinator sampling was nearly complete (**Table 2**).

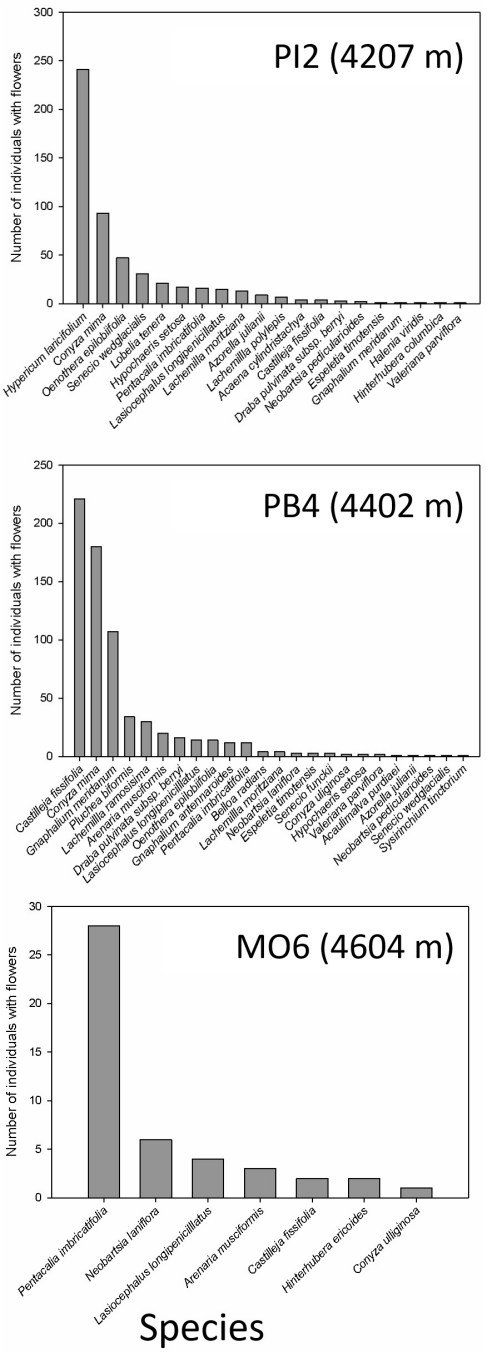


FIGURE 3 | Total number of recorded individuals with flowers per species in the four 3 × 3 m permanent plots (36 m^2) during 1 year, in each summit of the GLORIA–Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela.

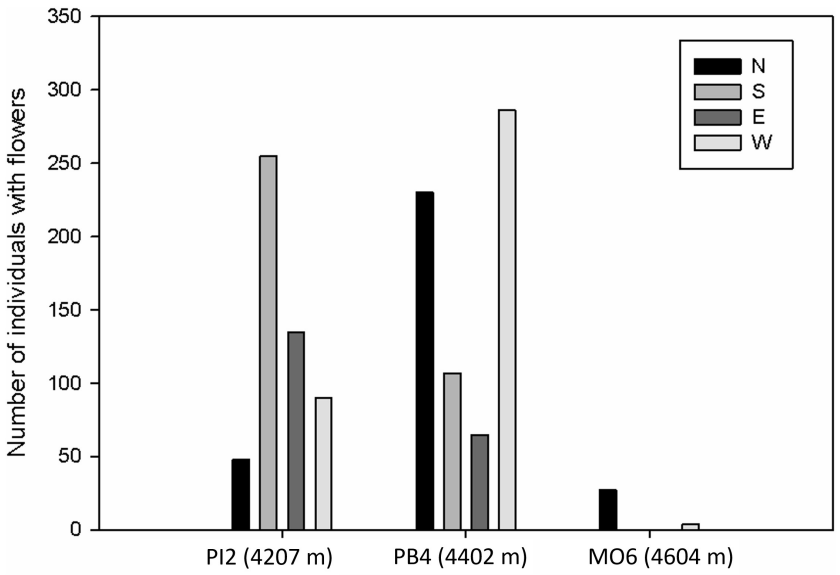


FIGURE 4 | Number of individuals with flowers in each cardinal orientation in the summits of the GLORIA–Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela.

We registered 17.75% of all possible interactions. The interaction network showed a high and significant degree of specialization (H2), a high value of connectance compared to other systems, and a low value of nestedness, both at a general level and when analyzed separately in each summit (**Table 2**; Jordano et al., 2009). The two lowest summits (PI2 and PB4) had very similar plant and pollinator richness values and similar values for the degree of pollinators and plants. The two lowest summits also showed similar values of connectance, specialization, and nestedness. At the highest summit (MO6), species richness was lower for plants and pollinators, and the value for all the indices decreased, although this network was still highly specialized. The null model, which assumed the same probability of interaction between all plants and pollinators, presented the lowest AIC, compared with models in which interaction structure was related with plant phenology, plant species distribution between summits, or the sampling effort for each species of plant based on both camera recordings and direct observations (**Table 3**).

In the three summits, a marked seasonality in the total number of visits of pollinators was observed. In the lowest summit (PI2), visits concentrated between October and May, with a peak in the total number of visits in November. At the intermediate summit (PB4), visits occur between July and December, with a peak in the last 3 months. Finally, in the highest summit (MO6), visits occur from December to April, with a marked peak in December (**Figure 6**). The highest visitation frequencies seemed to coincide with the months when there were more plant individuals and plant species with flowers. However, the correlation (Spearman rank) between the monthly pollinator visits and the number of plants with flowers ranged between 0.10 and 0.51 for our three summits, while for the species richness of plants with flowers, the correlation ranged between 0.18 and 0.64 (with $p > 0.05$ in all cases, except for this last one in PB4; see details in **Supplementary Table 1**).

DISCUSSION

Our knowledge of the effects of global warming on mutualistic interactions is still limited (Visser and Both, 2005; Walther et al., 2005) and few empirical studies exist (Hegland et al., 2009). Long-term studies that encompass the temporal variability characteristic of alpine areas, and studies that provide a baseline for future comparison should provide particularly valuable contributions, particularly on tropical alpine systems, which comprise about 10% of global alpine areas but remain particularly understudied (Inouye, 2020).

The behavior of flowering phenology in the superpáramo summits studied was similar to the only previous report available at a whole-community scale in páramos at lower elevations (Pelayo et al., 2019), while it also agrees with the behavior of other species analyzed individually in Colombia and Venezuela (Berry and Calvo, 1994; Velez et al., 1998; Gutiérrez-Zamora et al., 2004; Fagua and Bonilla, 2005). Along the elevation gradient studied, the species with continuous flowering and with the highest number of individuals with flowers coincide with the species of non-graminoid plants that show the highest general abundance in these summits (Cuesta et al., 2017).

Common species in our study region with continuous flowering, such as *C. fissifolia*, *D. pulvinata*, and *H. laricifolium*, could be important to the maintenance of the assemblage of pollinators. These species have very wide altitudinal distributions in the páramos (Briceño and Morillo, 2002) and are considered as key elements in the ecology of these ecosystems. *C. fissifolia* is a hemiparasite (Steyermark, 1957) that provides floral resources

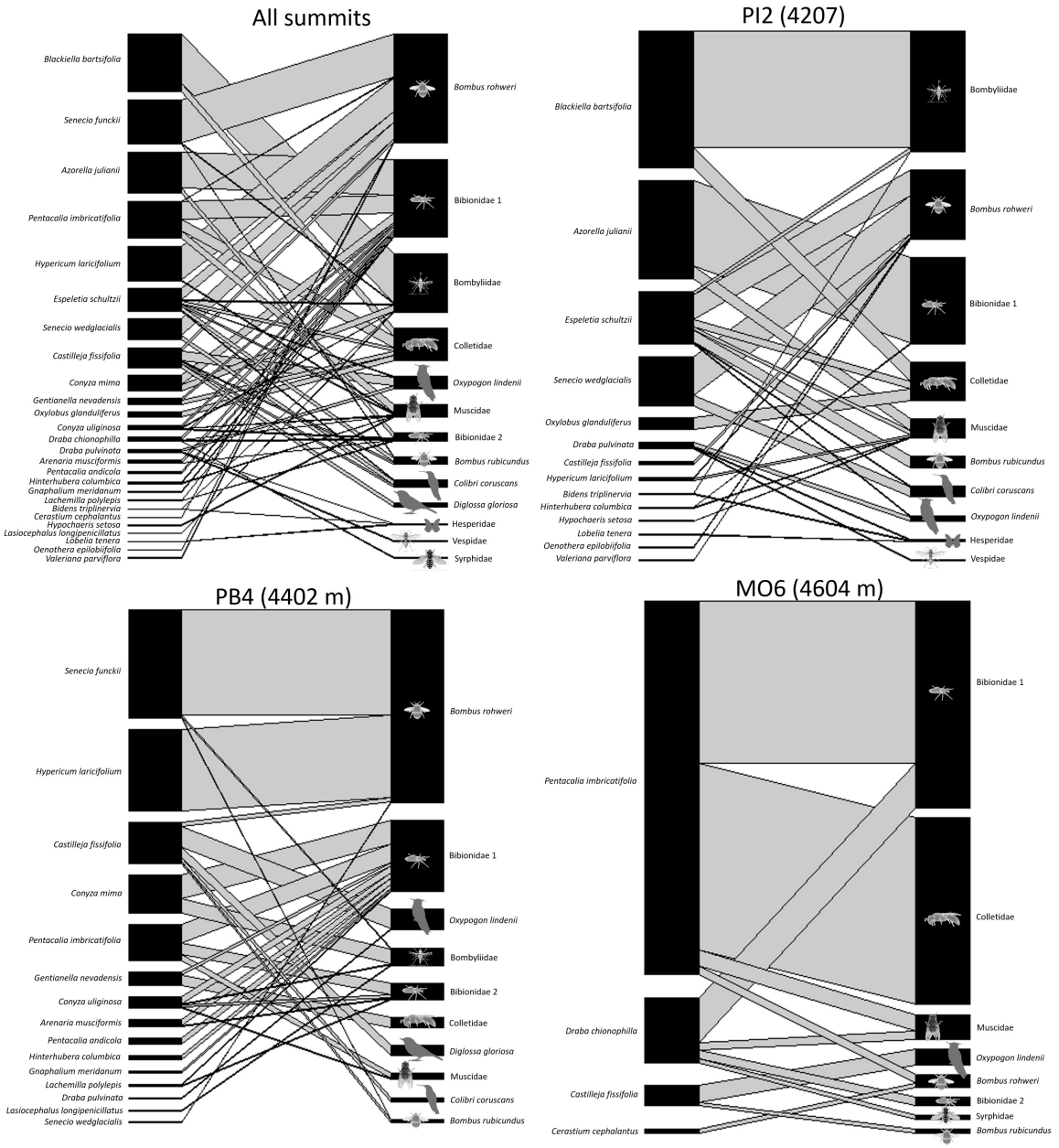


FIGURE 5 | Plant–pollinator networks of the GLORIA–Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela. The thickness of the links represents the total intensity of the interaction (visitation frequency). The size of the rectangles represents the relative importance of the species in the network (in terms of the number and intensity of its interactions with other species).

for endemic pollinators throughout the year both in superpáramo and in páramo ecosystems (Pelayo et al., 2019, 2020). Moreover, *H. laricifolium*, which is an important ecosystem engineer in the superpáramo (with a positive effect on microhabitat conditions and the richness/abundance of other plants), showed high connectance values with pollinators (Cáceres et al., 2014; Ramírez et al., 2015; Manrique et al., 2019). *D. pulvinata* is an endemic species, pollinated by few species including *O. lindenii*, an endemic hummingbird from Mérida. Hence, it would be important to analyze in more detail the interdependence between this endemic pair of plant and pollinator species, both of which act as high elevation specialists that could be particularly vulnerable in climate change scenarios (Cuesta et al., 2020). Finally, *E. schultzii,* the more connected species in the network, is endemic from Venezuelan páramos and one of the more abundant and widely distributed species in the Cordillera de Mérida (Briceño and Morillo, 2002).

As indicated by our first prediction, the phenological behavior of the plant community seemed to be influenced by the marked rainfall seasonality of the Venezuelan páramo, with a decrease in

TABLE 3 | Processes related to the network structure through Akaike's information criterion (AIC) models; they are ordered from smallest to largest.

Model	AIC
Random	7,703.25
Focal hours	7,846.21
Qualitative phenology (presence–absence of species per month)	7,846.49
Spatial distribution (presence–absence of plant species by summit)	7,885.17
Total effort (focal + video)	8,140.57
Qualitative phenology * Spatial distribution	8,219.59
Quantitative phenology	10,042.27
Quantitative phenology * Spatial distribution	10,343.99

the number of species flowering toward the end of the dry season and beginning of the wet season (February–May). However, there was a low value of the phenological overlap index across species in the three summits, which could be interpreted as a mechanism to minimize competition between plant species in conditions of low availability of pollinators and low visitation frequencies (Waser, 1983). In addition, as suggested by our first prediction, the index seemed to increase with elevation, suggesting that flowering could become more synchronic as conditions become harshest. However, further studies of inter-annual variability in plant phenology and its relationship with climatic variability would be required to evaluate the generality of these initial conclusions. In addition, it could be important to increase the sampling frequency within the year to be able to better estimate the phenological overlap index (although we did record flowering in 87.3% of the species with flowers we know to be present in these summits).

Interestingly, total pollinator visitation frequency showed a marked seasonality (generally concentrating between October and December), which seemed to coincide with the months with the maximum richness or the total number of plants flowering in each of the summits (prediction 2). However, we observed no significant correlations between these two variables and visitation frequencies. This could be partly linked with the fact that flowering is much more widely spread during the year than pollinator visitation frequencies (see **Figure 6**), which is reflected in the low phenological overlap index observed.

The decline in pollinator richness and visitation frequencies with increasing elevation has also been reported at lower altitudes within the páramo belt (Pelayo et al., 2019) and at high elevations in extra-tropical regions (e.g., Arroyo et al., 1982; Totland, 2001). Additionally, the total density of plants with

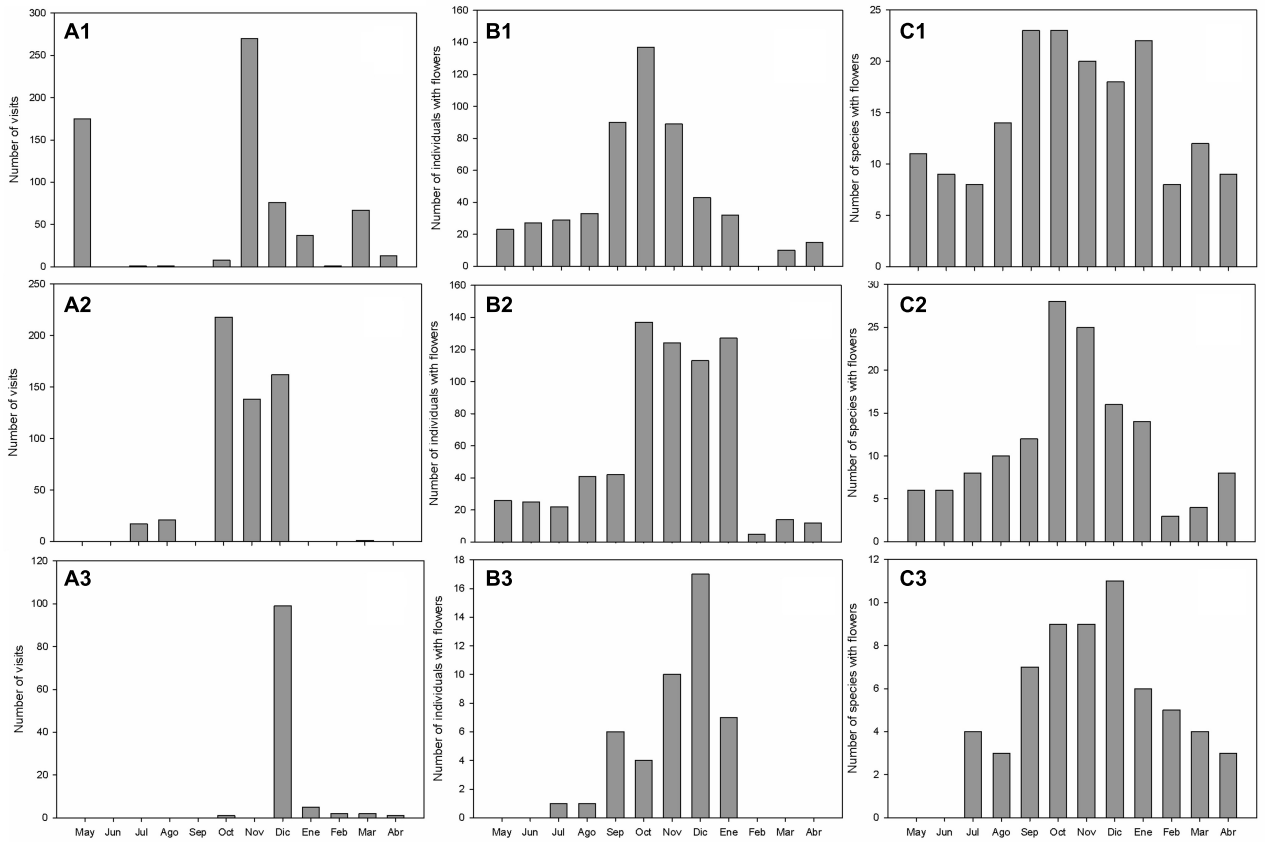


FIGURE 6 | Dynamics of the total number of visits of pollinators (**A1** = PI2, **A2** = PB4, and **A3** = MO6), total number of individuals with flowers in all the 3 × 3 quadrants (**B1** = PI2, **B2** = PB4, and **B3** = MO6), and number of species with flowers (**C1** = PI2, **C2** = PB4, **C3** = MO6) in summits of the GLORIA-Andes network (VE-CPB site), at La Culata National Park, Piedras Blancas Páramo, Venezuela.

flowers decreased with elevation, although we found patches with relatively high density in some of the orientations, S and E in the lowest summit and N and W in the two highest summits. Additionally, an analysis of total plant cover in the three summits (data unpublished) reveals that the West and North orientation (generally more humid) always show the highest total cover when compared with South and East orientations (facing in intermountain dry enclave). Consequently, these differences could be due to microclimatic conditions associated with environmental heterogeneity, as has been described in other alpine ecosystems (Scherrer and Körner, 2011; Young et al., 2016). Differences in rock cover and the presence of rocky outcrops (i.e., act as thermal refuges, Rada et al., 2009), differential incidence of wind (which tends to be stronger in East and South faces, especially in the two highest summits), and the varying influence of humid vs. dry slope orientations could all contribute to generate these complex and patchy distribution of plants and flowers among the different summit orientations (with a possibly stronger influence of rock cover in the lower summit where there is less climatic contrast between orientations because of its geographic position inside a more humid watershed).

It should be noted that more than half of the plants with flowers registered in the summits had visits from pollinators, mainly from insects, indicating that, in these superpáramo ecosystems, entomophily is the predominant reproductive mechanism (prediction 3). This has also been documented in another superpáramo community in the Sierra Nevada in Mérida (Manrique et al., 2019). This contrasts with the results of previous studies in the Andean páramo at lower elevations, where pollination by hummingbirds were more important (Pelayo et al., 2019).

As expected in this ecosystem (e.g., Cuesta et al., 2017), we observed a decrease in both plants and pollinators' richness along the elevation gradient. Moreover, there was a change in the composition and relative importance of both plants and animals (prediction 3). The increase with elevation in the relative importance of Diptera as pollinators observed here has also been reported in other studies along elevation gradients in tropical and temperate alpine regions (Arroyo et al., 1982; Manrique et al., 2019; Inouye, 2020). Interestingly, high elevation specialist plants such as *D. chionophila* showed a strong dependence on these flies, many of which we have only recorded in the two highest summits. However, hummingbirds and bumblebees with high metabolic rates and energy demands remained as important pollinators species, albeit they showed higher visitation frequencies and relative importance in the two lowest summits. In the case of the high elevation specialist hummingbirds *O. lindenii*, we have seen them foraging in these summits even during snowfalls, demonstrating their remarkable ability to adapt to these extreme environments (**Figure 7**).

Our networks exhibited values of connectance similar to those found for alpine and temperate pollination networks



FIGURE 7 | *Oxypogon lindenii* (Trochilidae) visiting flowers of *Castilleja fissifolia* (Orobanchaceae) during a snowfall event at 4,400 m in the Venezuelan superpáramo.

(Olesen and Jordano, 2002; Santamaría et al., 2014) and a low nestedness, as has been reported in other páramos (prediction 4, Manrique et al., 2019; Pelayo et al., 2019). However, the high elevation networks studied here could be more vulnerable, as they showed the lower richness of plants and pollinators and higher values of specialization (prediction 4); they are also more exposed to fast warming and exhibit smaller populations (with less habitat in terms of area and climatic suitability, see Cuesta et al., 2020). Both a low nestedness and a high degree of specialization agree with what is expected for networks with a low richness of plant and animals (Bascompte et al., 2003).

In the superpáramo, self-reproducing and apomictic plants (see Berry, 1986; Samaniego et al., 2018) coexist with others with specialist pollination systems such as *D. chionophila* (which were visited by few species of flies) and with some generalists' species such as *E. schultzii* and *S. wedglacialis*, which were visited by a wider range of insects and birds. However, these generalist species showed in our superpáramo summits a less rich pollinator assembly than previously reported in other páramos (Pelayo et al., 2015, 2019; Manrique et al., 2019). Hence, at higher elevations, even the more generalist plant species show a relatively poor assembly of pollinators (i.e., low functional redundancy for pollinators).

We found no relationship between plant phenological responses and the overall network structure in the studied summits (prediction 5). This could be partially due to the small size of these networks, which complicates the construction of robust models and detection of significant departures from randomness (see Dormann et al., 2014), preventing us for carrying out separate analysis of these relationships for each summit. In addition, it would be important to explore other plant and fauna attributes that could help to explain network structure, such as floral morphology, quality of rewards, phylogenetic relationships, or the patterns of change in the abundance pollinators (Fort et al., 2016; Manrique et al., 2019).

Even though the number of observed pollinators in the three studied summits was within the estimated values based on the Chao 2 estimator, the generally low visitation frequencies observed in the high páramos suggest that a higher sampling effort each month would be important to obtain a more exhaustive characterization of all the plant–pollinator interactions that could occur. However, given the remoteness of many sampling summits within the context of the GLORIA–Andes network, sampling effort could face important limitations for long-term monitoring purposes that need to be kept in mind.

Plant–pollinators systems with a high degree of specialization and with low functional redundancy like the ones studied here have been considered to be more sensitive to climate change (Gilman et al., 2010; Benadi et al., 2014; Miller-Struttmann et al., 2015; Valiente-Banuet et al., 2015). Moreover, comparative research across the GLORIA–Andes summits in South America has shown that the high elevation summits in the Northern páramo exhibit particularly high levels of endemism and are dominated by species with narrow thermal niches that are more prone to loss of niche space in future warming conditions (Cuesta et al., 2020). Together, all of these observations indicate that vegetation in superpáramo areas and their plant-pollination networks could be particularly vulnerable to species and functional diversity losses driven by climate change.

Longer-term monitoring of multiple pressures on pollinators and pollinator-dependent plants will help elucidate the mechanisms underpinning observed vegetation shifts, and inform the design and assessment of mitigation and adaptation options (Settele et al., 2016). Our preliminary analyses of plant community dynamics between the establishment of the baseline (2012) and the first resampling (2017) in the permanent plots of the GLORIA–Andes network showed an increase in species richness due to new arrivals of small herbs from the lower elevations, in agreement with reports by Carilla et al. (2017) in the northern Argentinian Andes. All of these new species were involved in our plant and pollinator networks (e.g., *C. uliginosa* var *uliginosa*, *Oxylobus glanduliferus*, *Senecio funckii,* and *Lucilliocline longifolia*), showing in some cases very high visitation frequencies (e.g., *S. funckii*). These preliminary observations reinforce the idea that the GLORIA–Andes summits can function as an excellent natural laboratory for evaluating the long-term effects of climate change on interactions between plants and pollinators. However, a more detailed analysis of these patterns is required.

CONCLUSION

Most of the flowering plant species (excluding graminoids) recorded in the three summits received visits from insects and, to a lesser extent, from hummingbirds. These plants showed little synchrony in flowering among species, but synchrony increased with elevation. Flies and bumblebees were the most important pollinators in areas above 4,200 m asl with flies increasing in relative importance in the highest summit. The interaction networks showed a high and significant specialization (H2) and connectance but a low degree of nestedness. The baseline information collected here in the context of the GLORIA–Andes monitoring program should provide key information to evaluate changes in the reproductive behavior of plants, which could be more sensitive than species composition and abundance to climate change impacts. Moreover, this information could help understand the differential response of plant species in the High tropical Andes to climatic drivers along elevation gradients, proving a more process-oriented understanding of the climatic risks faced by these unique high elevation plant communities.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

RP and LL conceived, designed the study, and wrote the first draft of the manuscript. RP, LL, JT, and LG collected

field data. LG carried out all taxonomic identifications. LR manages the GLORIA-Venezuela database. RP, LL, and YB performed statistical analyses. All authors contributed to the discussion and interpretation of data, revised and complemented the manuscript, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Snow Cover and Snow Persistence Changes in the Mocho-Choshuenco Volcano (Southern Chile) Derived From 35 Years of Landsat Satellite Images

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Mountain regions have experienced above-average warming in the 20th century and this trend is likely to continue. These accelerated temperature changes in alpine areas are causing reduced snowfall and changes in the timing of snowfall and melt. Snow is a critical component of alpine areas - it drives hibernation of animals, determines the length of the growing season for plants and the soil microbial composition. Thus, changes in snow patterns in mountain areas can have serious ecological consequences. Here we use 35 years of Landsat satellite images to study snow changes in the Mocho-Choshuenco Volcano in the Southern Andes of Chile. Landsat images have 30 m pixel resolution and a revisit period of 16 days. We calculated the total snow area in cloud-free Landsat scenes and the snow frequency per pixel, here called "snow persistence" for different periods and seasons. Permanent snow cover in summer was stable over a period of 30 years and decreased below 20 km^2 from 2014 onward at middle elevations (1,530–2,000 m a.s.l.). This is confirmed by negative changes in snow persistence detected at the pixel level, concentrated in this altitudinal belt in summer and also in autumn. In winter and spring, negative changes in snow persistence are concentrated at lower elevations (1,200–1,530 m a.s.l.). Considering the snow persistence of the 1984–1990 period as a reference, the last period (2015–2019) is experiencing a −5.75 km^2 reduction of permanent snow area (snow persistence > 95%) in summer, −8.75 km^2 in autumn, −42.40 km^2 in winter, and −18.23 km^2 in spring. While permanent snow at the high elevational belt (>2,000 m a.s.l.) has not changed through the years, snow that used to be permanent in the middle elevational belt has become seasonal. In this study, we use a probabilistic snow persistence approach for identifying areas of snow reduction and potential changes in alpine vegetation. This

approach permits a more efficient use of remote sensing data, increasing by three times the amount of usable scenes by including images with spatial gaps. Furthermore, we explore some ecological questions regarding alpine ecosystems that this method may help address in a global warming scenario.

Keywords: Andes (South America), remote sensing, global warming, alpine plants, npphen

INTRODUCTION

In addition to increasing average temperatures globally, climate change is also disrupting and altering seasonal patterns that drive ecosystem function (IPCC, 2021). There is growing evidence that the rate of warming is amplified at higher elevations, such that high-mountain environments experience more rapid changes in temperature than environments at lower elevations (Beniston et al., 1997). Indeed, mountain regions have been identified as having experienced above-average warming in the 20th century, a trend likely to continue (Beniston et al., 1997; Liu and Chen, 2000). These accelerated changes in alpine areas means that ecosystems are being impacted by both earlier snowmelt and reductions in snowfall. Seasonal snow makes up the largest portion of the cryosphere with up to 31% of the earth's surface being covered by seasonal snow (Vaughan et al., 2013). However, globally snow cover has decreased by 10% compared with snow coverage in the late 1960's (Walther et al., 2002) and since 1972 snow cover duration in the Northern hemisphere has been declining at a rate of 5 days/decade, attributable to earlier spring snowmelt (Choi et al., 2010). A recent global assessment of mountain regions reported that around 78% of the world's mountain areas have experienced a snow cover decline between the 2000 and 2018 study period (Notarnicola, 2020).

Snow is a critical component of alpine areas. It drives hibernation of animals and determines the length of the growing season for plants. In addition, snow provides an insulating layer for plants and animals, protecting them from temperature extremes during winter (Callaghan et al., 2011; Pauli et al., 2013). Warming in alpine areas will not only reduce snowfall but also advance the timing of snowmelt. Ecologically, this timing is particularly important for the plants and animals that rely on the insulation provided by the snow to cope with freezing temperatures (Wipf et al., 2009; Callaghan et al., 2011; Pauli et al., 2013; Wheeler et al., 2014). Early snowmelt has been found to dramatically change soil microbial composition (Zinger et al., 2009), expose plants to freezing events they cannot tolerate (Bokhorst et al., 2009; Gerdol et al., 2013) and advance plant reproduction and growth (Lambert et al., 2010; Livensperger et al., 2016). Hence, a change in the time of the snowmelt, as well as a reduction in snowfall, can have significant consequences for high elevation ecosystems. Furthermore, snow cover and duration also influences lowland ecosystems, as the decrease in snow fall can decrease freshwater for downstream ecosystems. Snow water equivalent, a parameter that takes into account snow depth and density, has a direct influence on the hydrologic cycle and water supply in snowmelt-dominated regions (Barnett et al., 2005; Gan et al., 2013). Shrinkage of spring snow water equivalents over the last few decades has been reported for the Arctic region (Brown et al., 2010), East Europe (Bednorz, 2004), and the Northern Hemisphere (Brown, 2000; Brown and Robinson, 2011).

In spite of the crucial role played by snow in alpine areas, there are gaps in the knowledge as to how alpine snow is responding to a changing climate. It is still unclear whether all alpine areas will respond in a similar fashion or if differences exist between hemispheres, climate types, and environments. A search on the "Web of Science" with the keywords: snow, snow duration, mountains, climate change, and warming shows 60 studies ranging from 1999 till 2020 that specifically investigated how snow regimes were changing in alpine areas. Of these 60 studies, 39 were done in the Northern hemisphere, including 21 studies undertaken in Europe. In the Southern hemisphere, 21 studies were undertaken and 15 of these were based in South America (two of these studies also included North American sites), with 11 studies along the Andes Cordillera, particularly the Northern and Central Andes. Fourteen of these 21 Southern hemisphere studies used satellite images to quantify snow cover. Most of the South American studies describe glacier dynamics (Bodin et al., 2010; Durán-Alarcón et al., 2015), measure snow effects on albedo (Lhermitte et al., 2014) or validate methodologies to describe snow patterns (Soncini-Sessa and Volta, 2005). Surprisingly, only four studies focused particularly on the effect of climate change on snow accumulation (Masiokas et al., 2012; Migliavacca et al., 2015; López-Moreno et al., 2017; Notarnicola, 2020). The Andes Cordillera maintains both permanent and seasonal snowpack (Masiokas et al., 2020), with a seasonal snowpack coverage of approximately 61,000 km^2 Saavedra et al., 2017). The seasonal snowmelt from the Andes provides the majority of the water required for human consumption, agriculture, industrial use, electricity generation, and aquifer recharge (Masiokas et al., 2006, 2012, 2020). Therefore, understanding how the snowpack duration in these mountains will be impacted by a changing climate is extremely important.

Given this, studies of the effects of climate change on snow cover and duration in the Andes are starting to emerge. Existing studies have mainly used data on snowfall and accumulation extracted from the closest weather stations. Thus, this kind of data does not consider spatial variation in snowfall or duration of snow, which is known to vary over a short spatial scale in the mountains. To overcome the spatial and temporal constraints imposed by the use of weather station data, remote sensing appears as a cost-efficient approach to measure and monitor snow cover systematically over large areas. The main limitations are the availability of cloud free images, especially when we want to study seasonal patterns. Besides measuring snow cover in cloud-free images, in this study, we used a per-pixel approach to take advantage of incomplete images due to cloud cover and other

causes of data loss (e.g., the SLC instrument failure of the Landsat 7 ETM+ sensor). A similar approach was used by Macander et al. (2015) to study snow persistence in Alaska, although the classification algorithm they proposed returns maps of snow-free dates rather than snow persistence. Here we use Landsat satellite images from 1984 till 2019, including cloud free and incomplete scenes, to study the spatio-temporal changes in both snow cover and snow persistence in the Mocho-Choshuenco Volcano in the Southern Andes of Chile. Besides snow cover per date, traditionally studied from available cloud-free remote sensing data, here we propose to calculate a pixel based snow frequency measure per season (e.g., the number of times that a pixel is covered by snow considering the available satellite records), from now on called "snow persistence." This provides robust snow persistence maps per season and per different time periods. Pixels showing snow persistence of 100% during the four seasons will show the areas with permanent snow cover. Changes in snow persistence, for example from one decade to the next one, can show a reduction of the area with permanent snow (less pixels with 100% snow persistence) or highlight the areas where the permanent snow changed to seasonal snow (e.g., 50 or 75% snow persistence) or ephemeral snow (e.g., <25%). In the present study, we derived two remote sensing metrics (snow cover area and snow persistence) to address the following questions regarding temporal variations in snow cover: Is the total area covered by snow changing through the years? What is the seasonality of the total snow cover in the volcano? and questions looking at the spatial and temporal variation in snow persistence: Are there temporal differences in snow seasonality at different elevational belts? What are the spatial patterns of snow persistence in the different seasons? Is there a reduction of snow persistence in the different seasons through the years? And finally, which elevational belts are changing the most in regard to snow persistence? We expect the total area covered by snow in this volcano to be reduced through the years as seen in other alpine areas around the world and we expect mid and low elevation belts to have a more pronounced reduction of snow persistence particularly in Spring and Autumn, indicating early snowmelt and late snowfall as has been predicted for alpine areas.

MATERIALS AND METHODS

Study Area

We studied snow cover and its persistence through time in the Mocho-Choshuenco volcano complex, located in Los Ríos Region in the South of Chile (**Figure 1**). The Mocho-Choshuenco is an active stratovolcano compound located in Los Ríos Region, inside the Southern Chile Volcanic Zone (Rawson et al., 2015, 2016). The volcanic compound covers an area of 250 km^2, an approximate volume of 100 km^3 with a summit of 2,422 m a.s.l. (Moreno and Lara, 2007). Currently, the compound is 7th in the specific risk ranking developed by National Service of Geology and Mining of Chile. Most of the study area belongs to the Mocho-Choshuenco National Reserve under the administration of the Chilean National Forest Service CONAF. We selected the area above the treeline (above

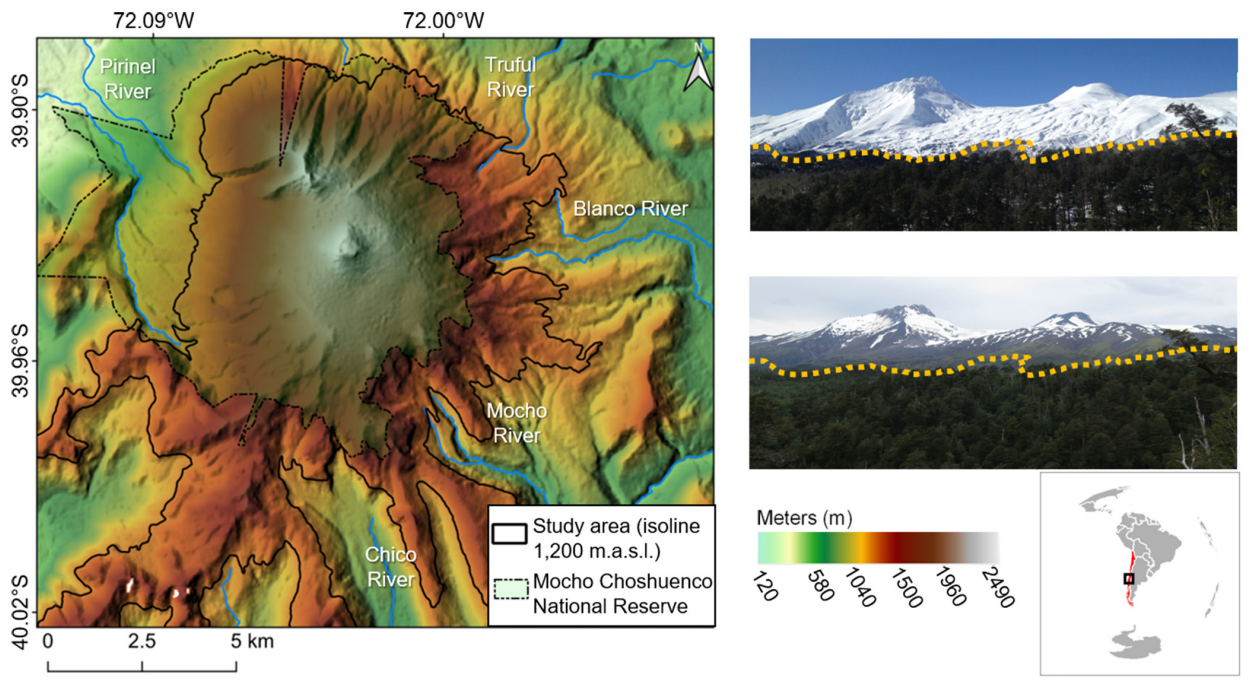


FIGURE 1 | Study area comprising the alpine area above the tree-line of the Mocho Choshuenco volcano in Southern Chile, with a total area of 137 km^2. The tree line (1,200 m.a.s.l.) is indicated in the pictures by dotted yellow lines.

1,200 m.a.s.l.) equivalent to the alpine area to analyse the snow duration. The alpine area in this volcano is dominated by shrubs such as *Adesmia longipes*, *Escallonia alpina*, *Gaultheria poepigii*, *Ovidea andina*, and *Senecio chionophilus* and grasses such as *Anthoxanthun juncifolium*, *Festuca thermarum*, and *Poa obvallata*. Toward higher elevations small perennial herbs like *Nassauvia revoluta* and *Nassauvia lagascae* sparsely inhabit the scoria (Teillier et al., 2012).

Landsat Images Availability and Definition of Periods of Analysis

All available Landsat images, including Landsat 5, 7, and 8, were accessed and analyzed in the cloud using the Google Earth Engine capabilities (Gorelick et al., 2017). The specific code of this and other sections of the manuscript are available in this link.[1] Specifically, we used the atmospherically corrected product Landsat Surface Reflectance Collection 1 Tier 1 corresponding to the highest radiometric and precision terrain quality. All Landsat scenes have 30 m pixel resolution. A total of 678 scenes from Path 232/233 and Row 88 were used, from which 23.5% of the scenes were completely cloud free and 76.5% were incomplete (with data gaps). Incomplete images are the result of partial cloud cover or sensor data loss, e.g., failure of the SLC instrument on Landsat 7 (**Supplementary Material** and **Supplementary Figure 1**). Cloud-free images were used for calculating total snow cover area per date of the study area and both cloud-free and incomplete images were used for snow persistence calculations which are applied on a pixel basis. Before 1998, Landsat data was scarce with a temporal gap of 7 years between 1990 and 1997 (**Supplementary Material** and **Supplementary Figure 1A**). From 1998 on, the dataset provides images consistently over time with 25–50 images per year, considering the revisit time of 16 days that each satellite has, but also that the different satellites overlapped in time, e.g., Landsat 5 and 7 between 1999 and 2013 and Landsat 7 and 8 between 2013 and 2020. Following the meteorological conditions of the South Hemisphere, there is a marked seasonality in data availability with a minimum of data during winter, particularly in June, due to increased cloud cover. In order to have sufficient data points for snow frequency calculations (snow persistence maps), we grouped the Landsat data by season: summer (Dec–Jan–Feb), autumn (Mar–Apr–May), winter (Jun–Jul–Aug), and spring (Sep–Oct–Nov) and by five periods of time: P1 (1984–1990), P2 (1998–2003), P3 (2004–2009), P4 (2010–2014), and P5 (2015–2019).

Total Snow Area Estimation and Validation Using the NDSI

Snow detection was automatically performed using the Normalized Difference Snow Index (NDSI) proposed by Dozier (1984) and the spectral bands of Landsat images, specifically the short-wave infrared (SWIR) and green (G) bands (**Figure 2**). This snow index has been widely used for snow detection in different regions of the world (Burns and Nolin, 2014; Zhang et al., 2019; Masiokas et al., 2020). The formula of the NDSI is given as follows:

$$\mathrm{NDSI} = \frac{\mathrm{SWIR} - \mathrm{G}}{\mathrm{SWIR} + \mathrm{G}}$$

From all original Landsat images (**Figures 2A–D**), where the snow is already recognizable by simple visual inspection in different seasons (A: summer, D: winter), the NDSI images were retrieved (**Figures 2B,E**). To classify pixels as "snow" or "not-snow" several NDSI thresholds have been proposed (**Figures 2C,F**). In the literature, thresholds of 0.4 (or similar) have been used or recommended (Hall and Riggs, 2007; Sankey et al., 2015; Masiokas et al., 2020) but this threshold has shown large standard deviations across different areas (Härer et al., 2018). In this study, we carried out a calibration exercise to define a NDSI threshold for the Mocho-Choshuenco volcano and later a validation of the snow mapping exercise. To achieve this, we set two transects (**Figure 2**) in a gradient from permanent snow (top of the volcano) to ephemeral snow (downward the volcano). In the west–east transect we defined four calibration points (C1–C4) and in the east-west transect four validation points (V1–V4). Thus, we have 636 data points for NDSI threshold definition and 636 data points for an independent validation of the defined NDSI threshold. For each calibration point and for each of the 159 cloud-free Landsat images (**Supplementary Material** and **Supplementary Figure 1**), we visually inspected whether the corresponding pixel was "snow" or "not-snow" using a false color composition (SWIR-NIR-Red) of the original Landsat images (**Figures 2B,E**) supported by available Google Earth high spatial resolution images, especially in the transition between snow and not snow. With this database we were able to study the dynamic range of the NDSI in a gradient of permanent snow to ephemeral snow and across the seasons and years. By constructing boxplots of NDSI for pixels with "snow" and "not snow" of the four calibration points, we identified the optimal threshold to detect snow. Finally, we constructed a confusion matrix and calculated the overall accuracy of the defined NDSI threshold using the validation dataset. By using this NDSI threshold, binary snow and not-snow maps were constructed from which the total snow area per date was calculated (**Figures 2C,F**). Additionally, we constructed a confusion matrix of the snow flag included with the Landsat Surface Reflectance product level 2.0 to compare the results obtained from NDSI thresholding and the default Landsat snow flag. Visualizations of the annual cycle of total snow area for the entire volcano and also at three different elevational belts (1,200–1,530, 1,530–2,000, and >2,000 m a.s.l.) were constructed using kernel density estimations of the snow area – time space. For this purpose, we used the PhenKplot function from the "npphen" R function (Chávez et al., 2017).

Snow Persistence Modeling and Maps

As indicated in previous sections, cloud free satellite images are scarce at high latitudes and in mountainous areas, limiting remote sensing based assessments of snow cover dynamics. For example, in our study site, only 23% of the 678 Landsat images were cloud-free. To make use of incomplete satellite images (e.g., the 77% of our Landsat dataset) we use a complementary approach to total snow cover area calculation (Section "Total

[1] https://github.com/JoseLastra/Frontiers_paper

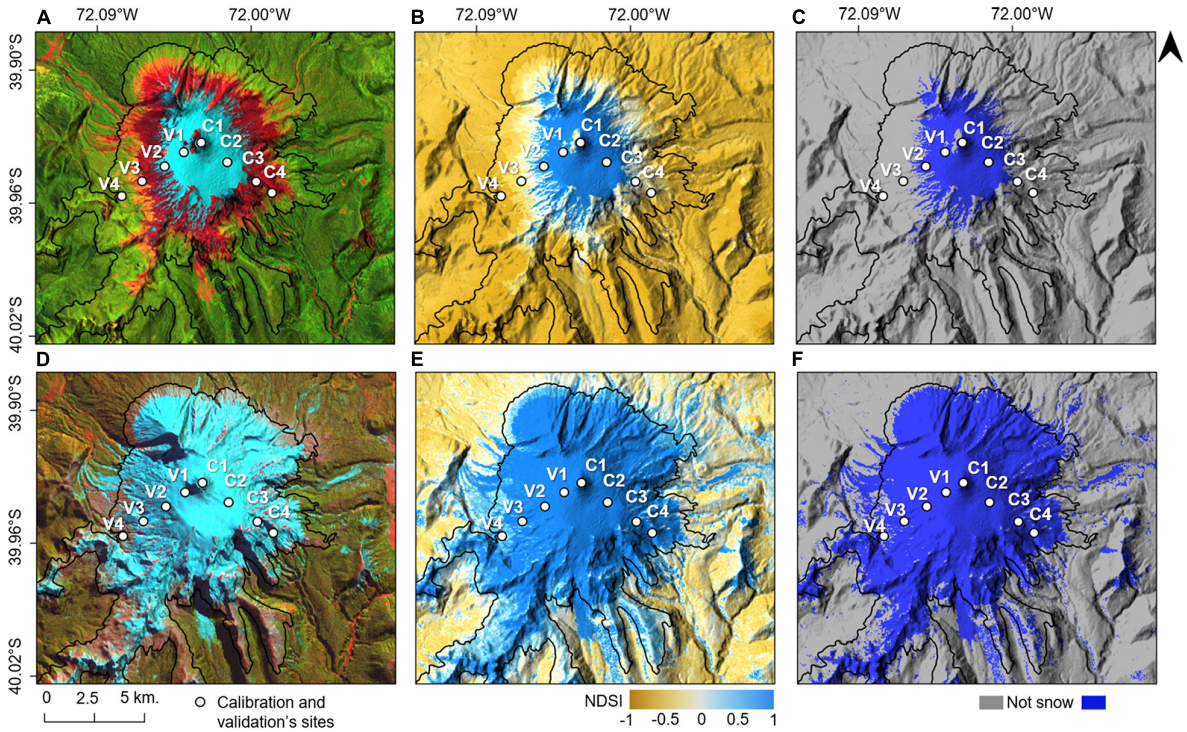


FIGURE 2 | Snow detection approach using Landsat images. **(A,D)** correspond to a false color composition (SWIR-NIR-Red) of Landsat images, **(B,E)** are the Normalized Difference Snow Index (NDSI) images calculated from Landsat, and **(C,F)** are binary "snow" and "not-snow" images calculated using a fixed threshold. Upper panels **(A–C)** correspond to a Landsat image acquired in summer (23-01-2019) and **(D–F)** in winter (24-08-2006).

Snow Area Estimation and Validation Using the NDSI") which we call "snow persistence". Snow persistence is the probability of the presence of snow for a given pixel at a given day of the year (DOY) or through a time interval. Frequencies are calculated per pixel and per DOY based on all available Landsat data (cloud free and incomplete images) and can also be calculated for different periods of time (e.g., the five periods defined in this study, see **Supplementary Material** and **Supplementary Figure 1**). Based on the "snow"/"not-snow" binary images (**Figures 2C,F**) we extract for each pixel the time series of snow presence per date. In order to have more binary observations for the fitting process, the data can be aggregated by month or season. Then we fit a binomial generalized linear model to the "snow"/"not-snow" data per month or season.

Different from the study of Macander et al. (2015), where the classification algorithm returns the snow-free date per pixel, in this study, the response variable corresponds to the probability that a given pixel is covered by snow at a given date or time interval. Here we calculated snow persistence per season for the whole Landsat time series and also for the five periods of analysis. Pixels with snow persistence of 100% in summer, winter, autumn, and spring can be considered as permanent snow. Changes in snow persistence through time can demonstrate the potential impacts of climate change: e.g., areas where snow persistence in summer was 100% and now is 80% indicates permanent snow is becoming seasonal or ephemeral. Since this is calculated at a pixel basis, there is no need for complete cloud-free images and thus all valid pixels of all available satellite images can be used. We developed a function in R Core Team. (2020) to calculate snow persistence maps for the entire study area and also at three different elevational belts (1,200–1,530, 1,530–2,000, and >2,000 m a.s.l.) using the capabilities of the "raster" package (Hijmans, 2019) to handle large Landsat raster-stacks and also R capabilities to take advantage of multi-core processing. To evaluate the quality of the binomial GLM models, we calculate the Area Under the ROC Curve (AUC-ROC) per pixel. AUC values close to 0.5 means the classifier is no better than a random classifier, and values close to 1 means the classifier has a high quality performance. We finally constructed AUC-ROC maps per period, showing the spatial distribution of the model performances in terms of AUC-ROC grouped in four categories suggested by Hosmer et al. (2013) which are <0.7 (poor), 0.7–0.8 (acceptable), 0.8–0.9 (excellent), and 0.9–1.0 (outstanding).

RESULTS

Total Snow Cover Area and Seasonality

The boxplots obtained from the calibration exercise showed that a threshold of NDSI of 0.5 maximizes classification accuracy (**Supplementary Material** and **Supplementary Figure 2**). Using this threshold to classify snow and not-snow for the

validation dataset, we achieved an overall accuracy of 98.27% (**Supplementary Material** and **Supplementary Table 1**). The NDSI > 0.5 threshold showed higher overall accuracy than the default Landsat snow flag (98.27% against 90.88%) and lower omission error for the snow class (2.8% against 15%) (**Supplementary Material** and **Supplementary Tables 1, 2**). Hereafter, all calculations were performed using this threshold.

The total snow cover area of our study site during the 1986–2019 time frame is presented in **Figure 3**. This is the longest time series available in remote sensing records at this spatial scale (30 m). As expected, there is a strong seasonality in total snow cover with a total area reaching between 80 and 105 km^2 in winter and a minimum and consistent area of about 18–20 km^2 in summer. In **Figure 3B**, we show a kernel density estimation of the total snow area – DOY space using all annual cycles of snow area. This way, the long-term snow seasonality of the Mocho-Choshuenco volcano is deployed. The dark-red line shows the most likely snow area at different DOY's along to its frequency distribution (colored area). From this figure, we observed that around DOY 150 (end of May) the snow starts to fall and there is a sharp increase in the area covered by snow, reaching its maximum by the end of the winter (August, about DOY 240). A wide range of total snow is observed at the peak of the winter season demonstrated by the broad frequency distribution around the most probable value of about 100 km^2. In spring, the melting process begins and therefore this period has the most variation in snow cover through time. By the DOY 300 (end of October) the melting process ends and the total snow area goes down to about 40–30 km^2, and keeps going down to the basal summer snow cover of 20 km^2. This minimum area covered by snow in summer was relatively stable over 25 years until 2014 when it decreased and remained below 20 km^2 until 2019 (basal red line in **Figure 3A**). Besides this decrease in the summer cover area, there is no other clear evidence of total snow area changes through time.

When we decomposed the total snow area time series into three different elevational belts (**Figure 4**), we observed clear seasonal variation in the area covered by snow at the low and middle elevations, but not at high elevation (>2,000 m a.s.l.). In the middle elevation, the snow area in winter remains more stable (about 40 km^2) and for longer (about 170 days) compared with low elevation (40–55 km^2 lasting about 100 days). From this Figure, we can see that the decrease in total snow area in the Mocho-Choshuenco volcano, starting in 2014, takes place mostly at middle elevations (**Figure 4C**).

Snow Persistence Spatio-Temporal Patterns

The AUC-ROC maps calculated for the five different periods (**Supplementary Material** and **Supplementary Figure 3**), show that the pixel based models used for snow persistence mapping present high efficiency for practically the entire study area. **Supplementary Table 3** in the **Supplementary Material**, summarizes the area at different levels of model efficiency, showing that for the five periods less than 0.5% of the study area presented poor efficiency (AUC-ROC < 0.7). Furthermore, less than 5% of the study area presented "acceptable" efficiency for all periods while the categories "excellent" and "outstanding" were the most represented. Overall, these results suggest a good performance of the snow persistence modeling and mapping for all periods.

The snow persistence maps calculated with 29 years of Landsat data (678 satellite images) show distinctive spatial features for the four different seasons (**Supplementary Material** and **Supplementary Figure 4**). The area with highly persistent snow (i.e., snow persistence > 75%) is much larger in winter and spring than in summer and autumn. Consequently, the low elevational belt is snow-free in summer and autumn, while in the middle elevational belt, some areas remain with highly persistent snow,

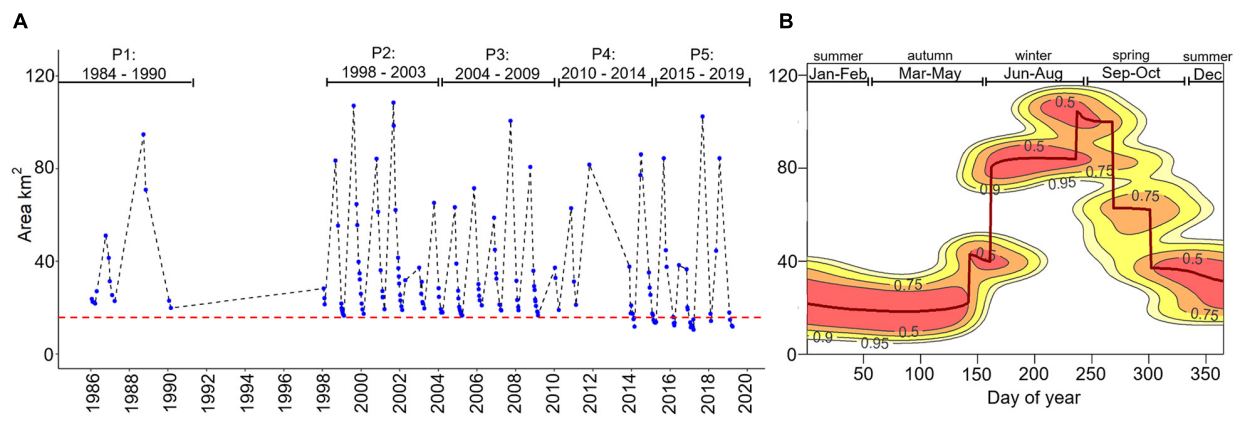


FIGURE 3 | Total snow cover time series 1986–2019 **(A)** and seasonality **(B)** of the Mocho Choshuenco volcano based on 159 cloud-free Landsat images. Seasonality of total snow cover area was calculated using Gaussian kernel density estimations of the snow cover along the year based on all historical records. Red color in panel **(B)** indicates high frequency of a given snow cover at different days of the year and yellow color lower frequencies of snow cover areas. Summer is very stable and the area covered by snow is about 20 km^2. The end of winter and the start of spring are the periods with larger snow cover, approximately 100 km^2. Then the snow cover decreases gradually in spring till the basal 20 km^2 of snow cover in summer. Note that from 2014 onward the lowest snow cover area is lower than the basal 20 km^2 (red dotted line).

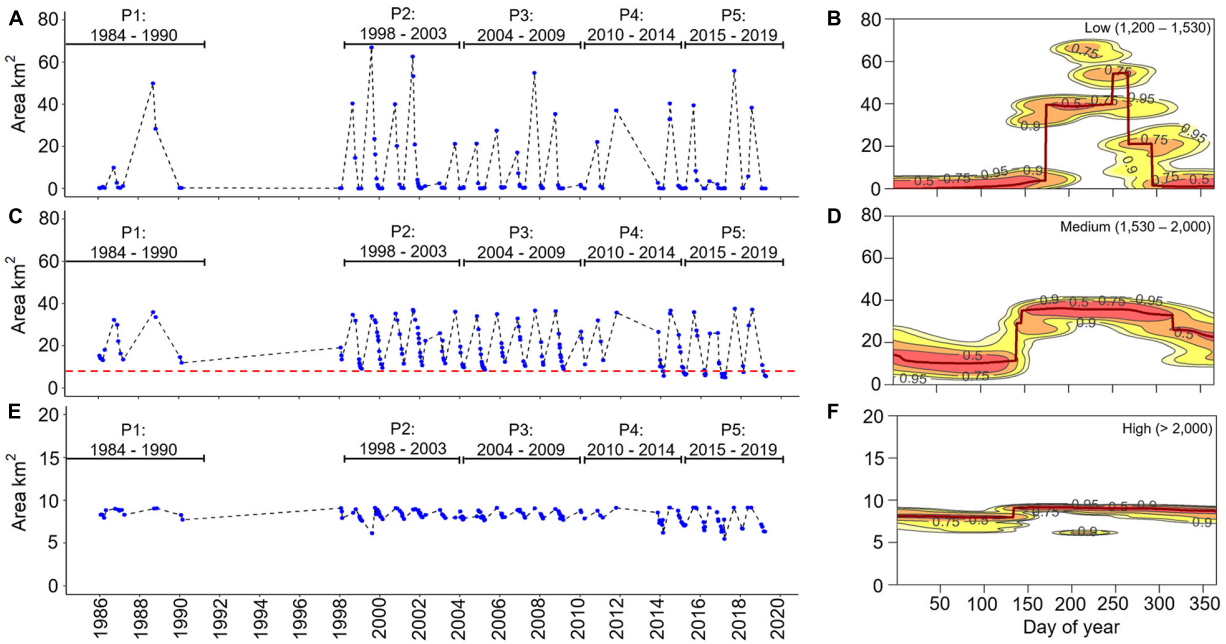


FIGURE 4 | Total snow cover time series 1986–2019 **(A,C,E)** and seasonality **(B,D,F)** of the Mocho Choshuenco volcano at three different elevation belts where L, Low (1,200–1,530 m. a.s.l.); M, Medium (1,530–2,000 m. a.s.l.); and H, High (>2,000 m. a.s.l.). Note that from 2014 onward the lowest snow cover area is lower than the basal area only for the middle elevational belt (red dotted line).

especially along the East–South slope of the volcano. During winter and spring this middle elevational belt is permanently covered by snow. Finally, the high elevational ring is covered by snow practically all year long, besides some specific areas with high slope near the crater and on the northern slope, which are snow-free during summer and autumn.

The total area with different snow persistence ranges observed for the five different periods at three elevational belts is presented in **Figure 5** while the changes per period considering all elevational belts together is summarized in the **Supplementary Material** and **Supplementary Figure 5**. Pixels with snow persistence > 95% in summer can be considered as permanent snow and when setting the first period (with 20 km^2) as a baseline, our results show that permanent snow area has decreased by 17% in period 2, then recovered 4% in period 3 with an accumulated decrease of 13%, then decreased again by 20% in period 4 to finally reach its minimum extent in the last period with 5.75 km^2 (28% reduction). Temporal changes in autumn and spring are variable. In winter we observed a sustained reduction of the area with snow persistence > 95% in periods 2 (24%), 3 (56%), 4 (25%), and 5 (42%) with respect to period 1 (**Figure 5**, **Supplementary Material**, and **SupplementaryFigure 5**).

When subtracting the snow persistence maps of the different periods from the map of period 1 (**Figure 6**), we can identify specific locations with a gain (light blue to blue colors) or a reduction (yellow to red colors) in snow persistence. Changes in snow persistence through the periods are seen for summer and autumn at the middle elevation belt (**Figure 6**). In spring and winter, the lower elevational belt is decreasing in snow persistence especially after 2010. The affected area in the middle elevation belt is smaller and less drastic than in autumn. Extreme changes in the persistence of snow are seen for low elevations in winter.

DISCUSSION

In this study, we have investigated the spatial and seasonal variation of snow cover and snow persistence in the Mocho-Choshuenco volcano in Southern Chile using a long record (35 years) of Landsat Satellite images. Snow cover in mountain areas has been traditionally assessed using remote sensing data and the NDSI from global (Notarnicola, 2020) to regional scale, e.g., the South American Andes (Masiokas et al., 2020), using MODIS products (250 m resolution), and to local scale (Burns and Nolin, 2014) using Landsat data (30 m resolution). Data loss is huge, since all images with a partial coverage of the study area have to be neglected. MODIS 8-day composites cope partially with this problem, but still for the 8 day temporal window the scene has to be complete in order to be used. In this manuscript, in addition to the traditional snow cover calculation, we introduced a different metric: seasonal snow persistence calculated at a pixel basis, allowing us to take full advantage of Landsat images both complete cloud-free (159 scenes) and incomplete scenes (519). Using this approach we can construct robust snow persistence change maps for different periods and across seasons with 326% more data than the traditional approach. The AUC-ROC maps for the five different periods showed a good performance of the snow persistence models for almost the entire study area with less than 0.5% of the study area presenting a "poor" performance (AUC-ROC < 0.7).

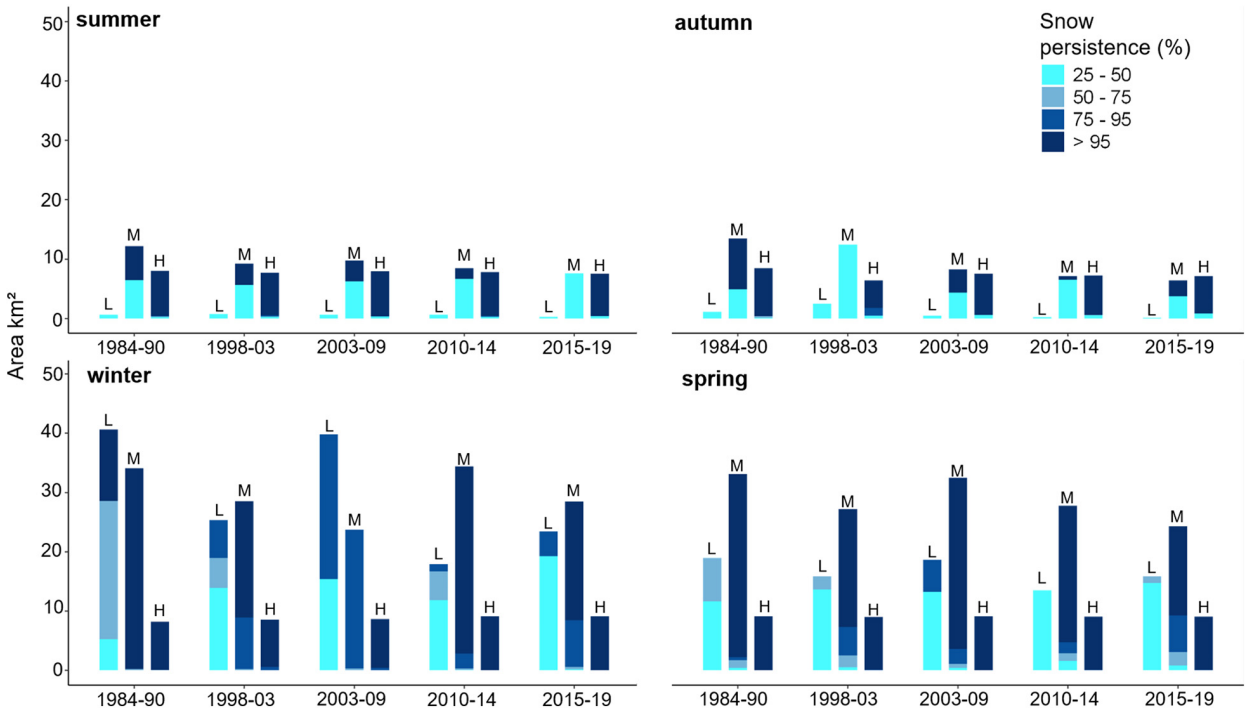


FIGURE 5 | Area changes in snow persistence for different seasons and periods. Bars represent the different elevation belts where L, Low (1,200–1,530 m. a.s.l.); M, Medium (1,530–2,000 m. a.s.l.); and H, High (>2,000 m. a.s.l.).

We have found a clear seasonal variation in snow cover and persistence for the whole area above the treeline, as well as across different elevational belts, with the low elevation belt being the most variable in terms of snow cover and persistence. Comparing across periods, the areas of high snow persistence (75–95 and >95%) are the most variable across periods. Indeed, a clear reduction of the area of high snow persistence is seen in summer (28% reduction in the period 2015–2019 with respect to the base period 1984–1990). Evidence of reduction of snow persistence was also found when comparing periods for the middle and low elevational belt. The middle elevational belt is decreasing in snow persistence in summer and autumn while a decrease in snow persistence in winter and spring is seen in the lowest elevational belt. In addition, we found that the minimum snow cover area is decreasing through the years, particularly in the middle elevation belt. Here we compare our findings with the ones found in other mountain areas. We explore the ecological relevance of our findings and highlight the advantages and constraints of this methodology. Finally we discuss the potential ecological questions that can be addressed with this method.

Contrasting Snow Seasonality Between Elevational Belts in the Mocho-Choshuenco Volcano

The kernel density estimation applied to the snow area along the year allows us to measure the long-term snow seasonality for the whole volcano and its temporal variation, given by the frequency distribution along the most expected snow area along the year. This non-parametric approach has been proposed and used to study vegetation phenology and disturbances (Chávez et al., 2019a,b,c; Decuyper et al., 2020) and it is a useful approximation to assess the seasonality of snow cover. The seasonality of the area covered by snow varies strikingly between elevational belts. The snow lasts approximately 70 days more in the middle elevation belt compared to the low elevation belt, this is a big difference in snow duration over short elevational scale, that accentuates the environmental differences between elevations. Mountains are usually viewed and studied in terms of the air temperature decreasing across the elevational gradient. However, snow decouples plants from air temperature and thus paradoxically, plants at lower elevations are exposed to colder temperatures than higher elevation ones. Considering the effects of climate change on the seasonality of snow duration is crucial to understanding how climate change will impact alpine vegetation.

Snow Cover and Persistence Reduction Through the Years in Mocho-Choshuenco Volcano

We have found evidence of snow cover and persistence reduction in the Mocho-Choshuenco volcano. Firstly, using all the snow free scenes, a decrease in the minimum area covered by snow can be seen since 2014 onward. This was also evident when studying the seasonality of the snow cover by elevational belts, with the middle elevational belt showing the decrease in minimum snow cover. The maps of difference in snow persistence per season also show a striking variation in the area of snow persistence.

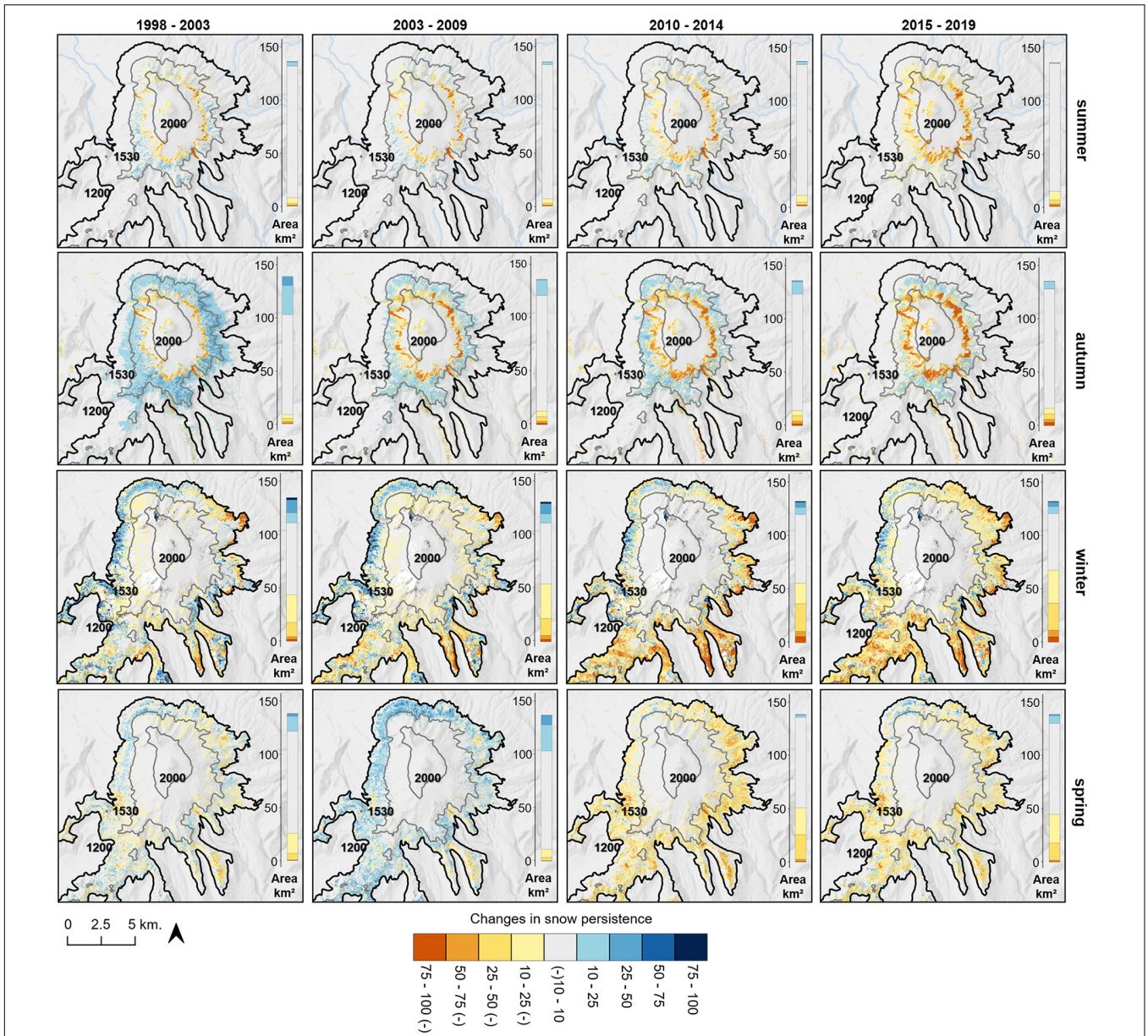


FIGURE 6 | Changes in snow persistence for different periods (columns) and seasons (rows). Reference period (P1: 1984–1990).

The middle elevational belt is decreasing in snow persistence in summer and autumn while a decrease in snow persistence in winter and spring is seen in the lowest elevational belt. The highest elevational belt is not experiencing any changes in snow persistence with the years. A range of studies have identified that the most rapid and consistent losses of snow, in terms of depth and duration, are mid-elevation areas (e.g., sub-alpine zone) and those with Mediterranean/maritime climates, where mean air temperatures are close to freezing (Brown and Mote, 2009; Steger et al., 2013). In the Tibetan Plateau, areas below 3,500 m a.s.l. experienced shorter snow cover duration in a 15 years study using MODIS (Wang et al., 2017). A review of the status of the European cryosphere found a general negative trend for snow depth and snow water equivalents below 2,000 m a.s.l. and no trend at higher elevations in the Alps (Beniston et al., 2018). For Western United States, Mote et al. (2018) found that 33% of the monitored sites showed a significant decline in snowpack, these changes occur mainly in spring and at milder elevations. Recently, a study looking at snow cover in mountains around the globe, found that 78% of the global mountains are undergoing a decline in snow cover duration of up to 43 days and a decrease of snow cover area up to 13%. In this study, the area of the Andes mountains considered (between 42°S and 29°S) exhibited the most drastic change in snow cover, with a decrease of snow cover duration of −26.6 days between 2,500 and 4,000 m a.s.l. (Notarnicola, 2020), similar results were found

by Saavedra et al. (2018) and Malmros et al. (2018). Our results show reductions on snow persistence in every season. Indeed, besides spring, the area with snow persistence > 75% is the lowest in the last of the five periods, and excluding winter, the area with snow persistence > 95% is also the lowest in the last period. The effects of a reduction in snow persistence on alpine vegetation at different elevations and for different seasons is of critical relevance if we are to make predictions of the effects of climate change on alpine ecosystems.

Climate predictions for the Andes are extremely complex, due to the sharp vertical rise of the Andes and the length of the Cordillera covering several climatic regions and the influences of additional climate systems including El Niño patterns (Kohler and Maselli, 2009). Therefore, studies on changes in snow persistence here need to encompass several sites across this mountain chain and, for example, findings on the Southern Andes cannot be extrapolated to what might occur in the tropical, Mediterranean or Patagonian Andes. Studies covering most of the climatic regions of the mountain range are needed to understand the local effects of climate change or climate systems on snow persistence in the Andes.

Potential Ecological Effects of Reduction of Snow Persistence

There is clear evidence that snow duration is changing in mountains worldwide, however, the question of how plants will respond to changes in snow conditions is still unclear and most of the studies looking at the ecological responses to variation in seasonal snow cover are done in the northern hemisphere (Slatyer et al., 2021). Advanced snowmelt could potentially increase plant fitness by prolonging the growth period and hence increasing resource allocation to seed production (Galen and Stanton, 1993). However, there is increasing evidence that early snowmelt also increases the risk of frost damage in adult life stages. We already know that naturally occurring warm spells and early snowmelt increase the mortality of flower buds due to frost damage (Molau, 1993; Inouye, 2008; Lambert et al., 2010). Further, experimental snow reduction and advancing snowmelt have been linked to reductions in biomass and fruit production, again resulting from frost damage (Gerdol et al., 2013). The effects of reduced snow persistence will depend on the season and elevation where this reduction occurs, with perhaps more detrimental effects for winter and for mid and high elevations areas where plants are acclimated to late snowmelt. Therefore, methods that allow us to look at the spatial and temporal variation in snow persistence are extremely relevant.

Snow duration determines species composition in the mountains, therefore, changes in snow conditions could promote changes in species composition and modify the dominant plant life-form (e.g., Hagedorn et al., 2014; Elliott and Petruccelli, 2018), and even impact on other trophic levels (Roland et al., 2021). In addition, a potential slow upward colonization of shrubs over areas currently occupied by perennial herbs might occur as a consequence of early snow melt in these areas. A second potential effect could be observed in the distribution of the species along the elevational gradient. In several alpine environments it has been observed a significant change in the optimal elevation of the plant species distribution. Similar changes have been observed in Western Europe (Lenoir et al., 2008). In these cases, elevational changes in the maximum value for some fitness metrics (e.g., seed production, seed germination, and growth, etc.) could be expected. The effects of early snow melt on plant fitness in alpine areas requires urgent assessment to provide key information for the conservation of these environments. Using this methodology we have found reduction of snow duration at a temporal and spatial scale in the Mocho-Choshuenco volcano. This method could be used to study the influence of snow duration on plant reproduction, phenology, seed germination, species persistence, and stress tolerance in environments where snow is a major feature. In addition, this methodology can be used to find priority areas for conservation in the mountains, such as areas where a strong pattern of snow reduction is seen or areas where plant species exist without significant potential for adaptation.

Advantages and Disadvantages of Seasonal Snow Persistence Calculations

We have already highlighted that our approach to the study of snow persistence optimizes the use of remote sensing data, including scenes with spatio-temporal gaps due to cloud cover or sensor malfunction. It can be applied to different remote sensing data such as MODIS snow products (250 m), Landsat (30 m), or Sentinel 2AB data (20 m). In this case, we were able to use three times more Landsat data than a traditional cloud-free based assessment. This allowed us to provide a robust description of snow spatio-temporal patterns and changes even for the cloudy Seasons. The main limitation of this approach is that it does not allow monitoring of snow cover on a short-term temporal basis, since several observations are needed to calculate a robust snow persistence. For example, it is difficult to calculate snow persistence on a monthly basis instead of seasonal as we did here, especially during cloudy seasons. Additionally, we advise to use this methodology for areas above the treeline (i.e., alpine areas), given that taller vegetation below the treeline will likely interfere with the snow visualization. In this study, we took careful consideration of this limitation when defining our study area: above the treeline (1,200 m.a.s.l.). Snow duration studies in the field are extremely laborious and of short span. We hope this methodology helps to understand how climate change is affecting snow cover and persistence patterns worldwide, especially for cloudy areas or seasons with patchy satellite data, and the ecological consequences of it.

DATA AVAILABILITY STATEMENT

The data and scripts supporting the conclusions of this article are made available by the authors in this link https://github.com/JoseLastra/Frontiers_paper, without undue reservation.

AUTHOR CONTRIBUTIONS

RC and VB conceived the research article. RC, SE, and JL developed the R code for the remote sensing analyses. RC, VB,

SE, and DH-P prepared and edited the manuscript. JL and RC conceived and prepared the figures. All authors have revised and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.643850/full#supplementary-material

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Microclimatic Warming Leads to a Decrease in Species and Growth Form Diversity: Insights From a Tropical Alpine Grassland

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Due to warming, changes in microclimatic temperatures have shifted plant community structure and dynamics in tundra and alpine regions. The directionality and magnitude of these changes are less known for tropical alpine ecosystems. To understand the likely trajectory of these shifts in the Andes, we conducted a warming experiment in the northern Andes—using open-top chambers (OTC). In this study, we ask (1) how do OTCs affect air and soil temperatures in microclimates of tropical alpine regions, year-round and during the dry season? (2) What are the effects of 7 years of warming on (a) the aboveground biomass (AGB) and (b) the plant taxonomic and growth form diversity? We installed five monitoring blocks in 2012 at ca. 4,200 m asl with 20 OTCs and 50 control plots randomly distributed within each block. We measured AGB, plant community diversity, and growth form diversity between 2014 and 2019. After 7 years of warming, we found significant increases in mean monthly (+0.24°C), daily (+0.16°C), and night air temperatures (+0.33°C) inside the OTCs, and the OTCs intensified microclimatic conditions during the dry season. Additionally, OTCs attenuated extreme temperatures—particularly in the soil—and the number of freezing events. AGB significantly increased in OTCs, and by 2019, it was 27% higher in OTCs than in control. These changes were driven mainly by a progressive increment of tussock grasses such as *Calamagrostis intermedia*, typical of lower elevations. The increase of tussocks led to a significant decrease in species diversity and evenness inside OTCs, but not in species richness after accounting by sampling time. Furthermore, cushions and herbs decreased inside OTCs. Our results show that experimental warming using OTCs in equatorial regions leads to decreased daily thermal amplitude and night temperatures rather than the level of increase in mean temperatures observed in temperate regions.

The increase of tussocks and decrease in diversity of species and growth forms due to prolonged modifications in microclimatic temperature might be a step toward shrub-dominated ecosystems. Further research on this topic would help understand shifts in growth form dominance and the direction and rate of change of the system.

Keywords: climatic conditions, field experiments, mountain ecosystems, temperature, functional trait, Andes, biomass

INTRODUCTION

In tropical alpine ecosystems, macroclimatic conditions and large-scale climatological phenomena play an important but secondary role in limiting plant life compared to microclimatic conditions (Sklenář et al., 2015). Microclimates are subtly affected by the topography, substrate, and aspect of the landscape and are also modified by plants (Anthelme et al., 2014; Opedal et al., 2015). While regional climate warming predictions for tropical alpine regions forecast an increase in temperature between 1 and 5°C with mean values of 2 and 3.5°C (Vuille et al., 2018), at a small scale, these projected increases in temperatures may differ (Cuesta et al., 2019). In tropical alpine ecosystems, the interaction between microclimatic change and its effect on vegetation is not well understood. One potential strategy is that plants might alter important functional characteristics to cope with this change (Körner, 2003) or become less dominant or even locally extinct. Therefore, it is vital to examine how changes in microclimatic temperatures affect plant diversity and productivity to further assess the consequences of climate warming on tropical alpine ecosystems.

Temperature, humidity and seasonality gradients due to elevation and latitude (Weberbauer, 1945; Troll, 1959; Sarmiento, 1986; Cuesta et al., 2017, 2020) define different ecological niches in tropical alpine systems. To fill this mosaic of niches, tropical alpine plants developed various morphological and physiological adaptations (e.g., Cleef, 1978; Smith and Young, 1987; Ramsay, 2001; Ramsay and Oxley, 2001). For example, plant community composition and growth forms change gradually as elevation increases as a coping response to harsh environmental conditions particularly, cold temperatures, where facilitation mechanisms prevail over competition (Sklenář and Ramsay, 2001; Anthelme et al., 2012).

To empirically examine the effects of climate change on mountain ecosystems, experimental studies shorten the space-time scale (i.e., warming experiments and transplant experiments) and link global warming with the dynamic changes of plant communities and their effect on ecosystem functions (Tito et al., 2020). Among the benefits that space for time experimentation provides is the potential to compare with studies that look at changes over a prolonged timeframe. Passive warming experiments use various methodologies and structures to simulate the temperatures and conditions predicted for the future. While similar studies have used open-side chambers (Pancotto et al., 2020), International Tundra Experiment (ITEX) corners, and circular chambers (Rustad et al., 2001), the method that has been successful in comparable systems is the open-top chamber (OTC) designed by ITEX (Marion et al., 1997). This OTC has a hexagonal shape made of polycarbonate sides hinged together to have a smaller area at the top of the structure than at the bottom. It allows airflow above, below and between the corners of the chamber yet retains warmth when shortwave radiation enters and delays the escape of longwave radiation, as would occur in a greenhouse, passively and minimally increasing the area's temperature.

Currently, passive warming studies have been reported mainly for the tundra and Northern hemisphere alpine ecosystems and two in southern South America (Rustad et al., 2001; Sierra-Almeida and Cavieres, 2010; Pancotto et al., 2020; Fazlioglu and Wan, 2021). These studies have focused on understanding vegetation community responses to climate warming, including changes in biomass, plant phenology, freezing resistance mechanisms, species distribution shifts, increased dominance of certain species, and changes in the *alpha* and *beta* diversity of the ecosystem (Klein et al., 2004; Elmendorf et al., 2012; Liu et al., 2018; Yang et al., 2018). Studies that have used OTCs have reported temperature increases in mean air temperatures of 1.7°C for tropical alpine ecosystems (Lasso et al., 2021) and increases in daily soil temperatures of up to 0.4°C (Klein et al., 2004, 2007; Pancotto et al., 2020), as well as changes in the number of freezing events and their intensities (Sierra-Almeida and Cavieres, 2010). Presently, only one study has reported the effect of experimental warming on plant communities of tropical alpine ecosystems (Lasso et al., 2021), but no study has reported the long-term (over three years) effects of experimental warming in these systems.

The observed impacts of passive warming experiments on tundra/alpine vegetation are shifts in the abundance of dominant species, dominant growth forms, and changes in aboveground biomass (AGB). Studies that successfully undertook experimental warming (Klein et al., 2004; Yang et al., 2018) attributed the change in species composition to an increase in competition between current dominant species and species that migrated from lower elevations, which resulted in a decrease in species richness. For example, Klein et al. (2004) reported that warming decreased species richness by five to 14 species, depending on habitat and grazing history, in one year and by nine to 15 species in another year. Regarding growth forms, Lasso et al. (2021) reported shifts in growth form dominance, attributed to an increase in graminoids and shrubs, observed after three years of warming in the tropical alpine region of Colombia. Therefore, although it seemed that the upward migration of new species would increase species richness (Steinbauer et al., 2018, 2020), these experimental studies suggest that increase

may be temporary. Moreover, the effect of climate warming could be the loss of species and growth forms characteristic of tundra/alpine ecosystems. Regarding AGB, studies have reported decreases (by a maximum of 23%; Fu et al., 2013), increases (by 72 g/m^2 in 2000 and 102 g/m^2 in 2001 in one site; Klein et al., 2007), or no effect on AGB or aboveground productivity due to experimental warming (Liu et al., 2018). Given these results, it is important to assess the direction and magnitude of the effect of climate warming on tropical alpine vegetation.

The tropical region of the northern Andes, also known as paramo, is a highly diverse alpine ecosystem (Sklenář et al., 2014), where the potential impact of warming on biodiversity is not well known (Buytaert et al., 2011). It has three main belt-units defined by the physiognomy and structure of the vegetation: (1) the subpáramo or shrub *páramo*, (2) grass *páramo*, and (3) superpáramo. These belt units are equivalent to the subalpine, alpine, and subnival belts. Along the elevational gradient, shrubs (e.g., *Pentacalia*, and *Diplostephium*) and tussock grasses (e.g., *Calamagrostis*, *Festuca*) decrease in dominance in favor of cushion plants (e.g., *Azorella*), acaulescent rosettes (e.g., *Werneria*), sclerophyllous shrubs (e.g., *Loricaria*), and short-stem tussock grasses (e.g., *Poa*) (Cleef, 1981; Ramsay and Oxley, 1997; Sklenář and Balslev, 2005, 2007).

In this study, we analyze for the first time the effects of a prolonged passive warming experiment (7 years) on plant communities in the *páramo* habitats of Ecuador. By implementing a warming experiment using OTCs installed in the Yanacocha Reserve in 2012, we seek to answer the following questions: (1) How do OTCs affect air and soil temperatures in the microclimate of tropical alpine regions year-round and during the dry season? Furthermore, how are these temperatures affected in the dry season? (2) What are the effects of 7 years of continuous passive experimental warming on (a) the AGB and (b) species diversity, and (c) growth form diversity of tropical alpine ecosystems?

In terms of the effects of the OTCs on microclimate temperatures, given the results reported from multiple studies (Klein et al., 2004, 2007; Sierra-Almeida and Cavieres, 2010), we expected daily mean air temperature to increase a minimum of 0.4°C and a smaller increase in soil temperature to a maximum of 0.4°C. We expected these differences to be particularly noticeable in the dry season. Regarding biodiversity, we expected to find a decrease in species diversity and evenness due to a dominance of certain species characteristic of lower elevations. Given the gradual change in species and growth form diversity as elevation increases, we expected changes in growth form diversity. For example, we expected an increase in graminoid and shrub species because their growth form might be more resilient to changes in microclimatic conditions or, in the case of shrubs, higher temperatures result in favorable conditions for them (Harte and Shaw, 1995; Cruz-Maldonado et al., 2021; Lasso et al., 2021). While there has not been a clear trend for AGB under warming conditions, we expected an increase in AGB due to the previously mentioned increase in shrubs and graminoids that may contribute more biomass per square meter than other growth forms.

MATERIALS AND METHODS

Study Area

The Yanacocha Reserve, located about 15 km northwest of Quito, the capital of Ecuador (**Figure 1A**), was created by the Jocotoco Foundation, a World Land Trust Partner spanning 1,200 ha (960 ha of montane evergreen forest and 240 ha of *páramo*). The study area, embedded in the eastern flank of the Pichincha Volcanic Complex, is located in the western mountain range of the Andes in Ecuador. The western mountain range is found in Ecuador's north and central regions and is geologically more recent than the eastern mountain range (Hughes and Pilatasig, 2002). It is influenced climatically by air masses that originate in the Pacific Ocean (Vuille et al., 2000). Precipitation varies seasonally—there is more precipitation between January and May and October to December, whereas June through September is the dry season (Navarro-Serrano et al., 2020). The average annual precipitation is 1,500 mm year^{-1} (Rugel, 2019) and less than 250 mm for the dry season (Sklenář et al., 2008). Therefore, a decrease in monthly rainfall during the dry season, coupled with increased UV radiation due to cloud-free skies leads to augmented thermal convection, resulting in higher maximum and minimum daily temperatures. Soils within the Yanacocha Reserve study area are Andosols, typical of the high Andes (WRB, 2015) with well-defined layers of volcanic ash intercalated with the organic layers. The topsoil layer in both forest and *páramo* varies from 15 to 18 cm in depth containing more than 7% of soil organic matter (Calderón-Loor et al., 2020).

The *páramo* vegetation in the Pichincha Volcanic Complex, dominated by shrubs like *Monnina crassifolia* (Bonpl.) Kunth and *Brachyotum ledifolium (Desr.) Triana*, herbs like *Hieracium frigidum* Wedd. and *Lachemilla orbiculata* (Ruiz & Pav.) Rydb., and tussock grasses like *Calamagrostis intermedia* (J. Presl) Steud., starts at ca. 3,900 m asl. At 4,200 m asl—the location of the experimental plots—the dominant growth-forms shift to mainly tussock grasses with interspersed sclerophyllous shrubs [including *Chuquiraga jussieui* J.F. Gmel. and *Loricaria thuyoides* (Lam.) Sch. Bip.], and prostrate herbs, acaulescent rosettes and cushions (including *Cerastium floccosum* Benth., *Werneria nubigena* Kunth, and *Plantago rigida* Kunth). Our study area lies in the transition zone between grass *páramo* and superpáramo.

Experimental Design

In 2012, the Yanacocha Reserve became the location of a long-term ecological research site to monitor vegetation changes at different elevations and under different scenarios (Teunissen van Manen et al., 2019, 2020; Calderón-Loor et al., 2020; Llerena-Zambrano et al., 2021). For example, the Yanacocha Reserve houses two of the GLORIA monitoring summits for the Pichincha Volcanic Complex (Cuesta et al., 2020; Tovar et al., 2020). In July of 2012, a passive warming experiment was established. It started only with control plots using a hierarchical block design and later, after the installation of the OTCs in 2012 (**Figure 1B**), the monitoring of the experimental plots started in 2013.

Five monitoring blocks—installed at 4,200 m asl (−0.13518 Lat, −78.57458 Long)—contained ten units of 6 m × 6 m

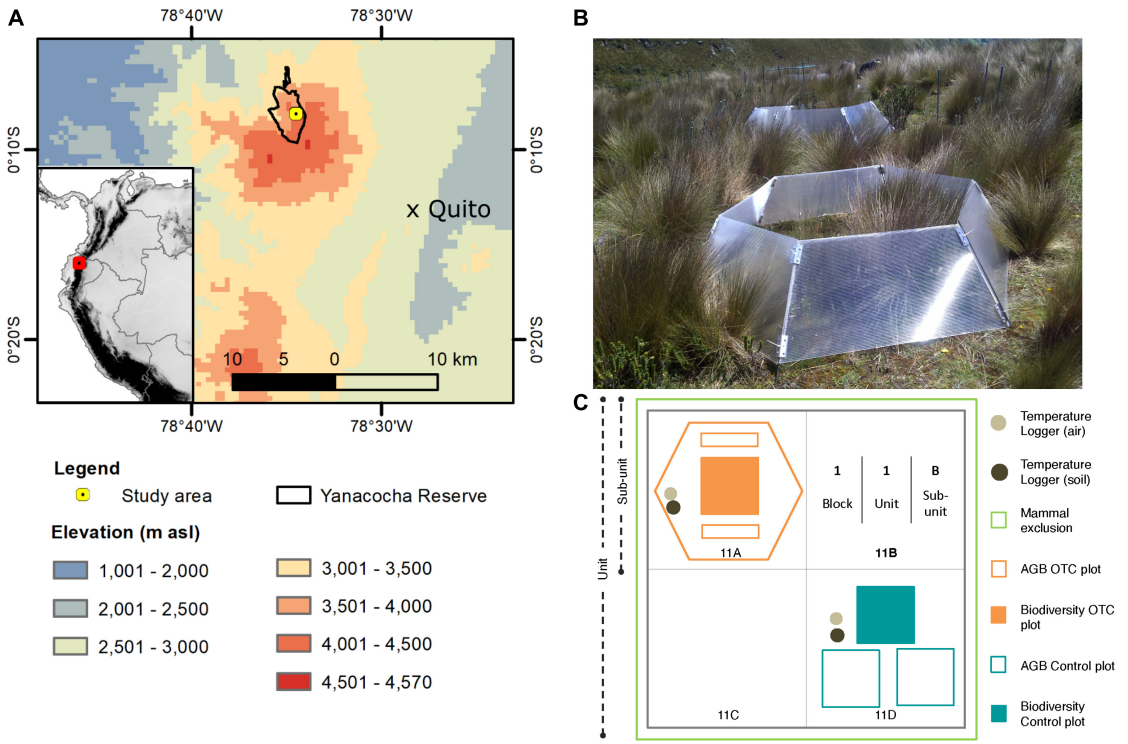


FIGURE 1 | Location and set-up of study site. **(A)** Map of location with respect to the city of Quito. **(B)** Image of an open-top chamber in 2013. The total area of the OTC covers the space of one sub-unit. **(C)** Diagram of unit set-up. Each unit is subdivided into four sub-units, where one sub-unit contains biodiversity and biomass control plots, and one sub-unit contains an OTC. The sub-units are named by the number of the block, followed by the number of the unit and lastly, the letter of the sub-unit. This figure is based on a freely available Digital Elevation Model produced by hydrosheds: https://www.hydrosheds.org/downloads.

each, with four sub-units of 3 m × 3 m for every unit. Two sub-units contained the control and experimental plots: (1) one biodiversity control plot of 1 m × 1 m and two control AGB plots of 1 m × 1 m each; and (2) one experimental biodiversity plot of 1 m × 1 m and two 1 m × 0.1 m experimental AGB plots (**Figure 1C**). Furthermore, OTCs were placed randomly in four units per block, covering the biodiversity and AGB experimental plots. Therefore, four units per block had an OTC sub-unit, making a total of 20 OTC sub-units and 50 control sub-units among all five blocks (Báez et al., 2013). The blocks were placed at a minimum distance of 5 m from each other to reduce spatial autocorrelation. Through incidence-based species accumulation and rarefaction curves—using the functions specaccum and rarecurve both from the Vegan package in R (Oksanen et al., 2020)—the saturation of the curves showed an appropriate sampling effort (**Supplementary Figure 1**).

To confirm that temperature caused the effects observed in the OTCs and that any change in biomass or diversity would not be attributed to a possible reduction in herbivory—caused by the physical blocking of the OTC walls (Marion et al., 1997)—six herbivore exclusion units were installed among the five blocks in 2012. The exclusions consisted of 1-meter-tall fences buried 30 cm below ground and covered the area of a whole unit within a block. Before installing the exclusion, we cleared all small mammals: genera *Thomasomys*, *Cryptotis*, *Sylvilagus*, and *Microryzomys*. To prepare the area, we trapped and then released them outside of the exclusions.

Field Methods

Control and OTC biodiversity was monitored once a year between 2014 and 2019 (**Table 1**) using permanent the 1 m × 1 m plots, sub-divided in 10 cm × 10 cm squares to determine species cover (percent) and composition. In addition, we collected control AGB samples from one of the two designated AGB plots located within one of the sub-units, following Calderón-Loor et al. (2020), once per year between 2012 and 2019 (**Table 1** and **Figure 1C**)—through two strips of 10 cm × 1 m at the top and the bottom of the plot. In the OTC sub-units, we collected AGB from the outer limit of the biodiversity plot, where 1 m × 10 cm strips were harvested outward instead of inward, as was the case for the control plots (**Figure 1C**). AGB samples were tagged, bagged, and placed inside plastic coolers to preserve the integrity of the collected samples before laboratory processing. In sum, the monitoring included assessing the effect of temperature on the following variables over time: AGB, which refers explicitly to live plus dead plant material collected above ground level, and vegetation composition.

Thermochron ibuttons® (DS 1922L-F5#) were installed in the soil (at 10 cm in depth) and at 10 cm above the ground to measure soil and air temperature, respectively, in 18 plots (9 for both

TABLE 1 | Summary of plots per treatment per year and sampling time periods.

	Control		OTC		Sampling times per variable						
	Plots (N) [Exclusion plots]	Year of set-up	Plots (N) [Exclusion plots]	Year of set-up	Years	2012 (0)	2013 (1)	2014 (2)	2015/2016 (3)	2017 (4)	2019 (5)
Biodiversity	20 [6]	2012	20 [0]	2012	2014–2019			x	x	x	x
AGB	24 [6]	2012	20 [0]	2012	2012–2019	x	x	x	x	x	x
Temperature/ Logger plots	9 [0]	2012	9 [0]	2012	2012–2018	2x	2x	2x	2x	2x	2x

Temperature data was retrieved or downloaded twice every year. Once at the same time as biodiversity and biomass data collection and a separate time during the year.

control and OTCs), making a total of 36 loggers, within blocks 1 through 3. The loggers recorded the temperature every hour. We also placed Hygrochron® temperature and humidity data loggers (DS 1923-F5#) when installing the blocks, but they failed after the second year of sampling. Therefore, for this study, we focused on temperature data only.

Laboratory and Herbarium Methods

We first weighed the AGB samples in the field with a manual scale to obtain fresh weight. Then we transferred the samples to a laboratory where we sorted them between above ground necromass and live mass. We distinguished between necromass and live mass based on the color and rigidity of the plants—yellow/brown leaves for necromass and rigid green leaves for live mass (Calderón et al., 2013). Samples were then dried at 60°C for 72 h in an oven until they reached a stable weight. Once the samples were dried, we weighed them again separately. This study's statistical analysis used the sum of the dry aboveground necromass and live mass.

At the QCA Herbarium, we processed and deposited vouchers of plants identified in the field and confirmed the plant identifications (**Supplementary Table 1**). Identification of plant vouchers were done in the QCA Herbarium where samples were also deposited. We identified 51 out of 70 morphospecies to species level. The rest of the 19 morphospecies were left at their most substantiated level and grouped into family groups. For example, the "Apiaceae group" housed individuals that look like species within the *Oreomyrrhis* and *Niphogeton* genera but were not identified up to species. The "Asteraceae group" included individuals that look like species from the *Aphanactis, Gamochaeta,* and *Achyrocline* genera. The "Poaceae group" included most species from the Poaceae family except *C. intermedia* and *Calamagrostis fibrovaginata* Lægaard, grouped in their own *Calamagrostis* group (**Supplementary Table 1**).

Trait Data

We obtained the growth form data for each species and morphospecies from the GLORIA-Andes database (Muriel et al., unpublished data), which uses a revised version of the Ramsay and Oxley classification (Ramsay and Oxley, 1997). We used the following growth form categories: cushion, herb, rosette, shrub, and tussocks.

Statistical Analysis

Temperature Analysis

Temperature data was downloaded from all loggers twice a year from June 2012 to 2019 to obtain a complete yearly log of hourly temperatures. However, due to a malfunctioning in some of the loggers, gaps in the annual sequence occurred in 2015, 2016, and 2018. Therefore, we bootstrapped the dataset, with the boot function in the boot package in R, to resample the years 2012, 2013, 2014, 2017, and 2018—using 1,000 bootstrap replicates—to minimize the impact of gaps in the dataset (Canty and Ripley, 2021). With this resampled data, we calculated summary statistics—including freezing events and intensities and the mean, minimum and maximum temperatures for air and soil, for every day of the year, of the dry season and night temperatures for OTC and control loggers—as well as the 95% confidence intervals (Crowley, 1992; Dixon, 1993).

We estimated statistics for the dry season using temperature data from June to September. Night temperatures were estimated using the values between 6 PM and 6 AM. Finally, we calculated freezing times by counting the number of times in 24 h the temperature was below zero and the intensity of the freezing period by counting the consecutive hours the temperature was below zero in 24 h (Sierra-Almeida and Cavieres, 2010).

We assessed the difference between control and OTC plots for every month of the year with *T*-tests on the temperature data (not bootstrapped) and Wilcoxon two-sample test for freezing lengths (Sokal and Rohlf, 2012)—using the stat v3.3 package in base R (R Core Team, 2021). Afterward, we used a linear mixed-effects model to assess the effect of the OTC on temperature, where the treatment interacts with the position of the logger (either air or soil), and the random effects are the hour of the day and the logger number. We used the lme4 package in R (Bates et al., 2014). We ran this model for the total temperature dataset, dry season temperatures, and night temperatures separately, using the bootstrapped data.

Estimation of Diversity Metrics

We used three different metrics to estimate taxonomic diversity: species richness (count), Pielou's Evenness ($J = H'/ln(S)$), and Hill's Diversity Index (N1; $N1 = \hat{e}\left(-\sum_{i=1}^{s} p_1 log\, p_1\right)$) following Chao et al. (2014). We also calculated evenness and N1 for growth form coverage data.

Assessing the Effect of Warming

Aboveground biomass was estimated as the sum of dry aboveground necromass and live mass in kilograms per square meter and then tested for normality using Q-Q plots in R. Since we did not obtain normality from these data, the dataset was log-transformed for our analysis. No transformations were done for any of the other variables since they all met the normality assumption.

Like with temperature, we compared all diversity metrics and AGB between control and OTC plots through two methods. First, to show differences when we do not account for time and other factors, we used the Wilcoxon two-sample test to compare warmed and control plots. Then, we used generalized linear mixed-effects models and general linear mixed-effects models to analyze the effects of the warming treatment and time (year) on these response variables using the glmer and lmer functions from the lme4 package in R (Bates et al., 2014). For AGB we tested the following three models: (a) biomass from control plots against OTC plots, (b) biomass from exclusion plots against control plots, and (c) biomass from exclusion plots against OTC plots. Finally, we used a Poisson distribution for the species richness model.

RESULTS

Microclimate

Compared to controls, daily mean air temperature increased in OTCs by 0.16°C (**Table 2**). During the dry season, the OTCs had the most increase in air temperature for the daily average maximum by 2.33°C (**Table 2**). At night, the mean temperature increased for OTCs compared to controls by 0.33°C, and the highest increase was in the average maximum temperature by 1.31°C (**Table 2**). On the contrary, the OTCs significantly decreased the daily mean soil temperature by −0.08°C (**Table 2**). The highest increase in soil temperature was for the daily average minimum by 0.32°C (**Table 2**).

The OTCs significantly increased the mean monthly air temperature by an average of 0.24°C except in September and October, at the end of the dry season (**Figure 2A**). In contrast, the monthly mean and maximum soil temperature in OTCs were lower than controls from July to October and April to December, respectively (**Figure 2B**). The OTCs attenuated soil monthly minimums and maximums in most of the year except for the height of the wet season in February and March (**Figure 2B**).

There were significantly fewer freezing events and of less intensity in OTCs than in control in soil and air during the dry season, but OTCs and control had a similar number of freezing events in the air during the whole year (**Supplementary Tables 3**, **4**). Temperature loggers in the soil recorded zero freezing events during the dry season (daily and nightly; **Supplementary Table 3**).

Biomass

Over the entire study period, mean AGB was significantly higher within OTCs (mean = 1.11 kg/m^2; sd = ± 0.13) than in control plots (mean = 0.93 kg/m^2; sd = ± 0.13) by 9% (W = 9,423; p = 0.029; **Figure 3A**; **Supplementary Table 5**). The biggest difference in AGB within the same year between OTC and control was in 2019 with 27.9% (p = 0.007). However, AGB decreased over time in both OTCs and control plots as shown by the negative values of the mixed-effects model coefficients (**Figure 3B** and **Supplementary Table 5**). Exclusion plots (mean = 1.24 kg/m^2; sd = ± 0.16) did not show significant differences with OTC plots (mean = 1.11 kg/m^2; sd = ± 0.16) or control plots (mean = 0.93 kg/m^2; sd = ± 0.16; **Figure 3B** and **Supplementary Table 6**).

Biodiversity

Species diversity (Hill index) in OTCs (mean = 3.66; sd = ± 1.9) was significantly lower than in control over the entire study period (mean = 4.47; sd = ± 1.9; W = 3,499; p = 0.041; **Figure 4A**). However, this difference is not significant when accounting by sampling time in the mixed-effects model (t = −1.85; p = 0.065). In the mixed-effects model the years 2016, 2017, and 2019 had lower diversity values than 2015 (**Figure 4B** and **Supplementary Tables 7**, **8**).

Evenness in the OTCs (mean = 0.46; sd = ± 0.17) was significantly lower than in control plots (mean = 0.54; sd = ± 0.16) over the entire study period (W = 3,434; p = 0.0088; **Figure 4A**). This pattern was further confirmed when time was held as a fixed factor in the mixed model, and evenness for 2016, 2017, and 2019 was lower than in 2015 (**Supplementary Table 8** and **Figure 4B**).

There was no significant difference in species richness between control plots and OTCs (**Figure 4A**). Only in 2019, species richness was significantly lower in OTCs (mean = 9.6; sd = ± 2.4) than in control (mean = 12.19; sd = ± 2.7) as was suggested by the Wilcoxon test (p = 0.015) and visualized by the mixed-effects model (**Supplementary Table 7** and **Figure 4B**). Nevertheless, by 2019, five out of the 57 recorded species [e.g., *Astragalus geminiflorus* Bonpl., *Azorella pedunculata* (Spreng.) Mathias & Constance] were no longer present inside the OTCs, and six more demonstrated a progressive reduction in the warmed plots (**Supplementary Table 1**). On the other hand, only two species appeared in the OTC plots as the warming experiment progressed without being present in the control plots, *Bartsia laticrenata* Benth. and *G. acostae* Cuatrec.

Growth Forms

Growth form diversity was significantly lower (p = 0.0026) in the OTC plots (mean = 2.36; sd = ± 0.7) than in control plots (mean = 2.73; sd = ± 0.71) but decreased in both treatments over time (**Figure 5A**). This difference holds after accounting by sampling time in the mixed-effects model (**Figure 5B** and **Supplementary Table 9**). Growth form evenness was also significantly lower (p = 0.003) in OTC plots (mean = 0.56; sd = ± 0.2) than in control plots (mean = 0.66; sd = ± 0.17) over the entire study period and decreased over time in both (**Figure 5B**). However, after accounting by time in the mixed-effects model, evenness in OTCs was not significantly different from control, and all times had lower evenness compared to 2014 (**Figure 5B** and **Supplementary Table 9**).

Percent cover of cushion growth form was significantly lower in OTCs than in control plots, while tussock cover was

TABLE 2 | Summary of statistics for temperature in control and OTC plots for air and soil loggers.

	Control				OTC				Significance	
	Temperature	**SE**	**conf.low**	**conf.high**	**Temperature**	**SE**	**conf.low**	**conf.high**	***p*.value**	***p*.signif**
Air loggers										
Dry season										
Max	25.73	0.24	25.25	26.18	28.06	0.33	27.48	28.72	0.00	****
Mean	7.31	0.07	7.17	7.45	7.44	0.07	7.31	7.59	0.20	ns
Min	−0.11	0.08	−0.26	0.05	−0.20	0.06	−0.32	−0.07	0.40	ns
Dry season night										
Max	9.33	0.17	8.99	9.67	11.70	0.34	11.07	12.40	0.00	****
Mean	2.45	0.07	2.32	2.59	2.74	0.06	2.63	2.86	0.00	**
Min	−0.10	0.08	−0.24	0.06	−0.19	0.06	−0.31	−0.06	0.38	ns
Daily										
Max	23.36	0.15	23.08	23.65	23.91	0.18	23.54	24.28	0.02	*
Mean	6.70	0.04	6.63	6.77	6.86	0.04	6.78	6.94	0.00	**
Min	−0.17	0.05	−0.26	−0.08	−0.03	0.05	−0.13	0.06	0.04	*
Night										
Max	8.39	0.08	8.22	8.56	9.70	0.14	9.43	9.98	0.00	****
Mean	2.66	0.04	2.59	2.73	2.99	0.04	2.91	3.06	0.00	****
Min	−0.14	0.05	−0.23	−0.05	−0.01	0.05	−0.10	0.09	0.05	ns
Soil loggers										
Dry season										
Max	14.75	0.23	14.31	15.20	12.49	0.11	12.26	12.71	<2e-16	****
Mean	7.88	0.05	7.79	7.97	7.60	0.03	7.55	7.66	0.00	****
Min	4.63	0.08	4.47	4.80	4.91	0.04	4.83	4.98	0.00	**
Dry season night										
Max	11.29	0.09	11.12	11.47	10.42	0.07	10.29	10.56	0.00	****
Mean	7.47	0.05	7.38	7.56	7.10	0.03	7.04	7.16	0.00	****
Min	4.80	0.09	4.63	4.97	5.05	0.04	4.97	5.12	0.01	**
Daily										
Max	13.11	0.11	12.90	13.35	11.56	0.08	11.42	11.72	<2e-16	****
Mean	7.44	0.03	7.39	7.49	7.36	0.02	7.32	7.40	0.02	*
Min	4.46	0.05	4.36	4.55	4.78	0.03	4.71	4.84	0.00	****
Night										
Max	10.22	0.05	10.13	10.31	9.72	0.04	9.65	9.80	0.00	****
Mean	7.09	0.02	7.04	7.14	6.99	0.02	6.96	7.04	0.00	**
Min	4.61	0.05	4.51	4.71	4.89	0.03	4.82	4.96	0.00	****

$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; ns: $p > 0.05$; not statistically significant.

significantly higher over the entire study period (**Figure 6A** and **Supplementary Table 10**). Over time, cushion species cover decreased in the OTCs but increased in control plots (**Figure 6B** and **Supplementary Table 10**). Meanwhile, percent cover of tussock species (e.g., *Calamagrostis* group) increased over time in OTCs by 16.5% but kept similar cover values in control plots (**Figure 6B** and **Supplementary Table 10**).

DISCUSSION

The Role of Temperature in Microclimatic Conditions

In our study, the OTCs changed the microthermal conditions, potentially leading to shifts in AGB, plant community composition, and growth form diversity. Generally, air temperature and soil temperatures had different responses to the OTCs, where air temperature on average increased and soil temperature on average decreased. Specifically, the thermal conditions changed by first, a general increase in daily and monthly air temperature with the highest increase in means occurring for night temperatures. Second, the dry season accentuated the differences in microclimatic conditions between control and OTC plots, for both air and soil temperatures, where soil temperature in OTCs was mainly lower than control, and air temperature in OTCs had its highest increase in temperature compared to controls. Third, extreme temperatures were attenuated, including reducing freezing events.

Daily (+0.16°C) and monthly (+0.24°C) mean air temperature increased but in a lower amount than what we hypothesized and less than observed in tundra and subtropical grassland environments. For subtropical grasslands, Buhrmann et al. (2016) reported an increase of 1.6 to 2.4°C in air temperature during the day in OTCs compared to controls

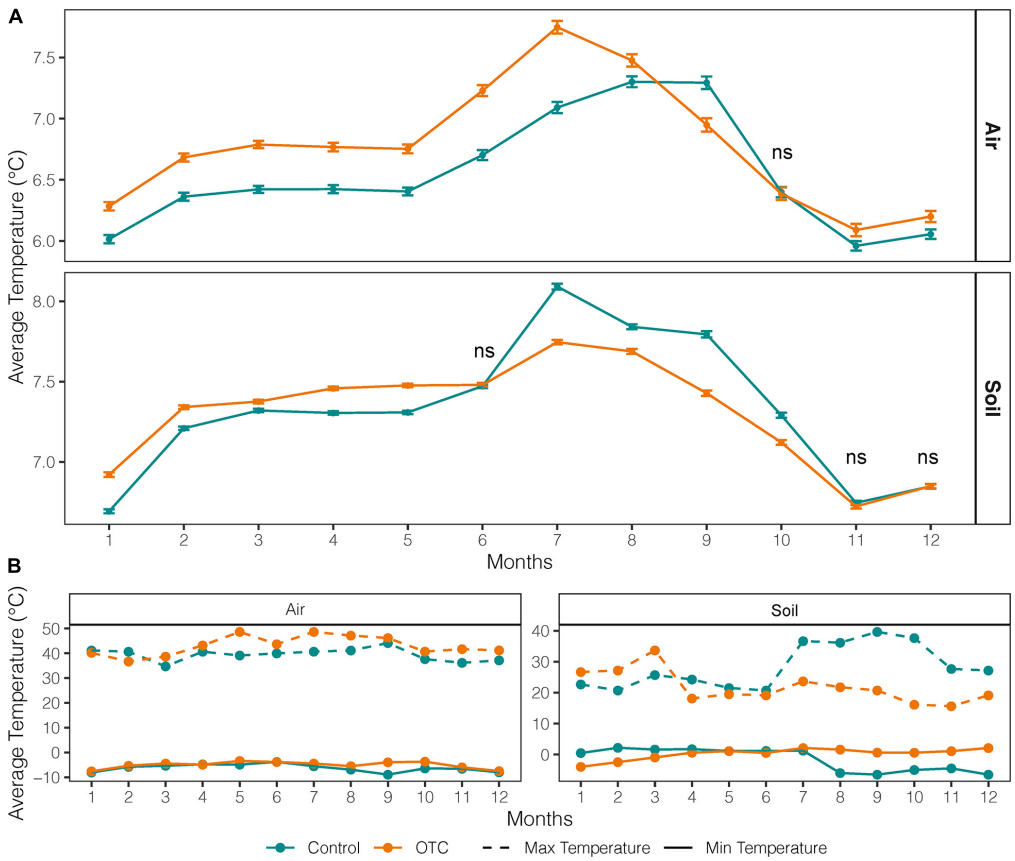


FIGURE 2 | Averaged monthly mean, minimum and maximum air and soil temperature inside and outside the OTC (plots under warming and control plots, respectively). Data covers the period 2012–2018. **(A)** Average monthly temperature from air and soil loggers in control and OTC plots. Bars represent standard error. **(B)** Average monthly minimum and maximums in air and soil from air and soil loggers in control and OTC plots.

and 0.0 to 0.6°C during the night across all four seasons. In our study, night temperatures in OTCs were significantly different from control and showed higher mean air temperatures by an average of +0.33°C ($p < 0.001$), which falls within the range of change in temperate regions. Our results show that in equatorial regions, larger changes occur for night temperatures than from daily temperatures, different from temperate and subtropical alpine areas. Additionally, lower values of temperature change than Lasso et al. (2021) in the Colombian *páramos* may result from differences in OTC design, such as the type of fiberglass used in their warming experiment.

Our study found smaller differences in the daily mean soil temperature between OTCs and control than in the mean air temperature. This result was similar to the results reported by Pancotto et al. (2020) in southern Patagonia. However, a meta-analysis of 32 experimental sites across four temperate biomes: tundra, low tundra, grassland, and forest found a broader range, of 0.3 to 6.0°C, for mean soil warming temperature increases (Rustad et al., 2001). The studies analyzed in that meta-analysis used different warming methods, not only OTCs, which, along with latitudinal differences, may contribute to the differences from our results. For example, in subtropical grasslands, the absolute maximum temperature in OTCs increased by 0.9 to 1.8°C while the absolute minimum was increased by 0.3 to 0.8°C (Buhrmann et al., 2016). Meanwhile, we obtained a mean maximum temperature increase of 0.55°C and an increase in mean minimum temperature of 0.4°C.

The OTCs stabilized soil temperatures by keeping the monthly minimums and maximums consistent throughout the year with maximum temperatures lower than those in control plots. This reduction in the soil thermal range may reduce plants' ability to tolerate extreme events under normal conditions (Sierra-Almeida and Cavieres, 2010). Further, soil temperature attenuation also reduces the frequency and intensity of freezing events (**Supplementary Table 3**). In this study, the mean monthly soil minimum temperature for OTCs was above freezing. This attenuation could be caused by greater evapotranspiration during the day, which could generate a buffer that reduces heat transfer through convection during the night (Sierra-Almeida and Cavieres, 2010). Consequently, the thermal amplitude experienced by plants is reduced, which would alter their physiological response to extreme conditions (Sklenář et al., 2016).

The dry season analyses revealed an important pattern in the temperature trends observed in the OTCs, including the intensification of temperature and shifts in trends around the

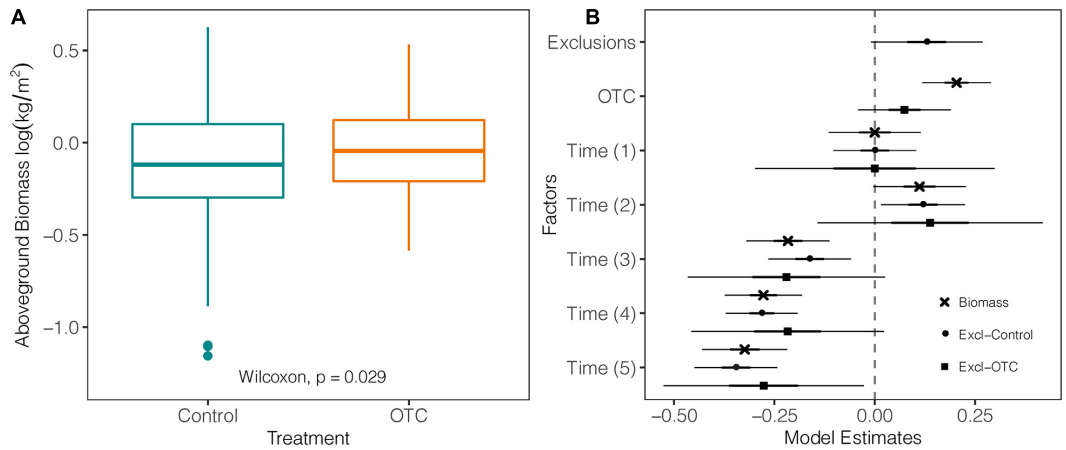


FIGURE 3 | Impact of warming on biomass. **(A)** Difference in above ground biomass measured in plant communities under natural and warming conditions over the five sampled years between 2012 and 2019 [Time (0) = 2012 used as a reference against the other sampling years, Time (1) = 2013, Time (2) = 2014, Time (3) = 2015/2016, Time (4) = 2017, and Time (5) = 2019]. **(B)** Coefficients of three mixed-effects models testing log-transformed biomass from control against the effects of exclusions, warming (OTC), and the effects of exclusion against warming (OTC). The vertical line represents the null value (where fixed effects are not significant when their CI cross this line). Fixed effects to the right of the vertical line have a significant positive relation with the response variable, and a negative one if they are on the left.

start and end of the dry season. In our results, at the beginning of the dry season (June), air temperatures in OTCs intensified, obtaining their maximum difference from controls in July by 0.7°C. During June and July, the OTC trapped higher amounts of UV radiation, causing this peak in temperature (Marion and Pidgeon, 1992). Additionally, monthly maximum air temperature in OTCs only fell below the control plots in January and February, during the wet season, where days have higher humidity, cloud cover, and lower radiation (Vuille et al., 2003; Letts and Mulligan, 2005). Studies in tundra found that passive warming treatments increased mean growing season air temperature by 1 to 3°C, a warming range predicted and observed for tundra regions (Walker et al., 2006). Therefore, variations in monthly precipitation and UV radiation over a year-long period are important in describing OTC temperature trends.

Relationship Between Biomass, Biodiversity, and Growth Forms

Over the study period, OTCs showed higher values of AGB but lower values of species and growth form diversity and evenness than control plots. This pattern seems to be linked to an increase in the vegetation cover of tussocks and a decrease of cushion and herb species in the OTCs, leading to a less diverse community. While AGB reduced in both control and OTCs over the study period, AGB was highest in exclusion plots followed by OTCs, and the lowest means of AGB were found in controls.

By the end of our 7-year experiment, OTCs had 27% more AGB than control plots, as we hypothesized, and which is consistent with other experiments in the alpine meadows of the Hedguan mountains (Yang et al., 2018) and Patagonia (Pancotto et al., 2020). However, other studies have reported no warming impact or decreases in AGB. For example, Fu et al. (2013) found that AGB significantly decreased with experimental warming at 4,313 m asl. This study used different methods than ours and had a much shorter experimental time of 1 year, potentially explaining the difference from our results. Although OTCs generally had more AGB than controls, we observed an overall decrease of AGB for both controls and OTCs. This contrasts with results reported by Calderón-Loor et al. (2020) in the same *páramos* of the Yanacocha Reserve for the period 2012–2014 in which AGB increased, despite showing similar values of AGB to ours for 2014. Finally, the higher amount of AGB in exclusion plots than OTCs shows that the OTCs did not entirely exclude herbivory by small mammals, as was also observed by Barrio et al. (2021). However, herbivory intensity is dependent on site and OTC design. In our study, given the visual evidence of herbivory in OTCs, more AGB in OTCs may be more likely related to the changes in thermal conditions rather than the potential exclusion effect of herbivores by the OTCs. However, further research on herbivory intensity related to OTCs in tropical alpine regions would help clarify this topic.

The higher AGB in OTCs (by 9%) in our study area is mainly related to increased vegetation cover of tussocks (e.g., *C. intermedia*), which increased by 16.5% by the end of the experiment and it is a pattern that has been observed in other studies as well. For example, in subtropical alpine grasslands, annual graminoid biomass production significantly increased by nearly 20% in OTCs (Buhrmann et al., 2016). Similarly, Na et al. (2011) found an increase in AGB inside OTCs after 2 years of warming in the Tibetan Plateau, where sedges were responsible for 90% of the total biomass gain. Lastly, in the *páramos* of Colombia, graminoid and shrub coverage increased inside OTCs in one out of the two study sites (Lasso et al., 2021).

In our study, an increase in tussocks in OTCs could be due to three reasons: (1) tussocks have specific adaptations that allow them to become dominant, (2) the drier environments created by OTCs favor tussocks, and (3) at this point, tussocks

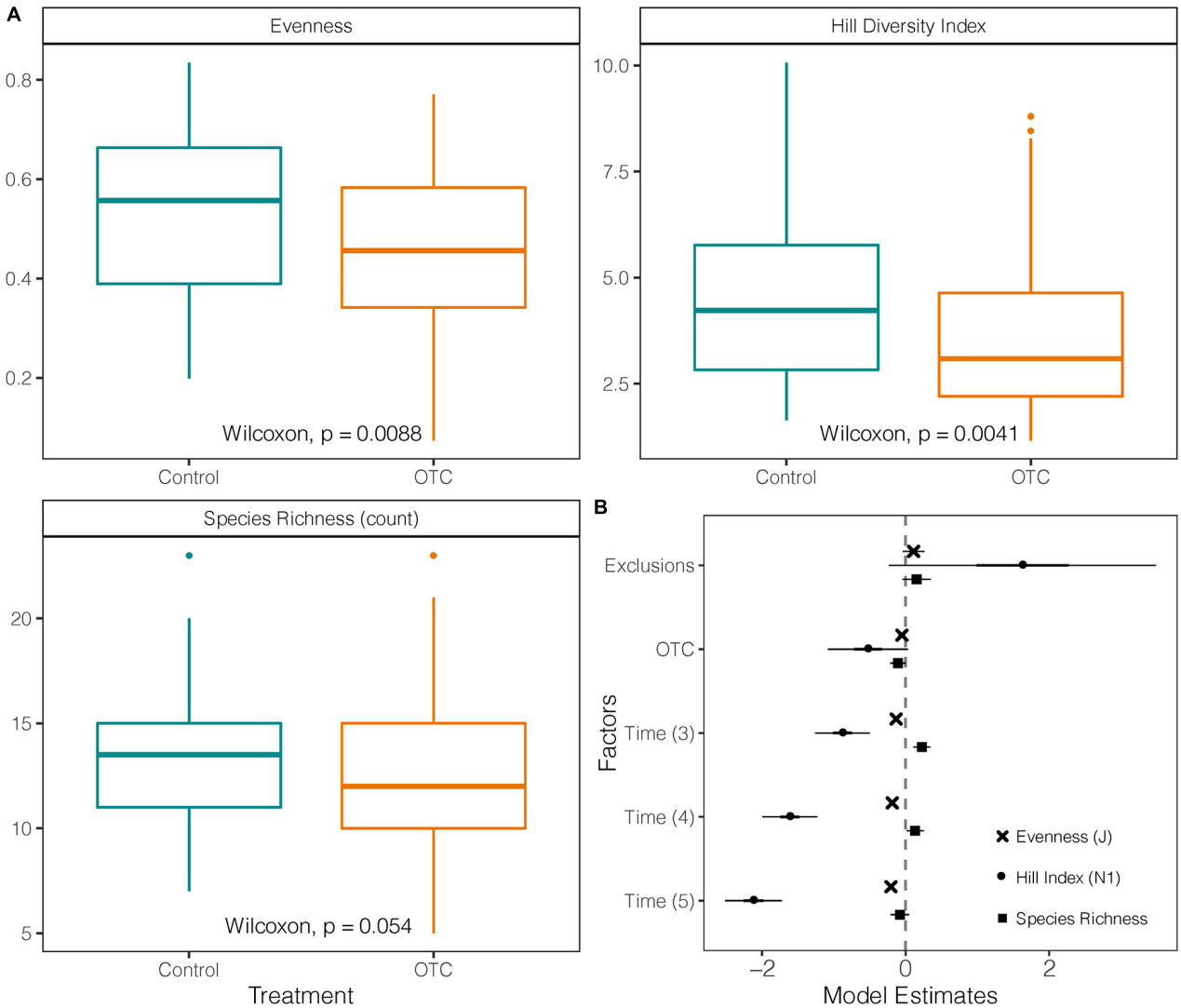


FIGURE 4 | Impact of warming on taxonomic diversity. **(A)** Species richness, evenness and Hill Diversity Index (N1) measured in plant communities under warming (OTC) and control conditions (outside OTC) in 4 sampling times between 2014 and 2019. **(B)** Coefficients of three mixed-effects models testing Hill Diversity Index, Evenness and Species Richness against the effects of exclusions, warming (OTC) and sampling year. The vertical line represents the null value (where fixed effect are not significant when their CI cross this line). Fixed effects to the right of the vertical line have a significant positive relation with the response variable, and a negative one if they are on the left [Time (2) = 2014 used as a reference against the other sampling years, Time (3) = 2015/2016, Time (4) = 2017, and Time (5) = 2019].

are favored but may be replaced later by shrubs and woody plants. First, tussock grasses have broad thermal niches (Cuesta et al., 2020) and are highly tolerant to freezing (Márquez et al., 2006; Rada et al., 2019). Second, they compete by being taller, shading out shade-intolerant species, which leads to local/plot extinctions (Wong, 1991), dispersing by wind (Tovar et al., 2020), and by being photosynthetically efficient (i.e., reduced leaf area index and higher leaf dry matter content (Leegood, 2002; Cuesta, unpublished data). Next, a secondary effect of the OTCs is that they create drier environments (e.g., Lasso et al., 2021), and tussocks are resistant to cold and resistant to drought (Monteiro et al., 2011; Rada et al., 2019). Therefore, tussock grasses seem suited to take advantage of conditions created by increased temperatures coupled with increased water limitation.

Lastly, we hypothesized that both graminoid and shrub coverage would increase but only the increase in graminoid cover was significant. In their monitoring studies, Porro et al. (2019) and Hamid et al. (2020) found that the vegetation cover of shrubs and graminoids increased with warmer temperatures. However, in our study, we found that shrubs as a group did not increase, but that individual species, such as *C. jussieui*, showed an increase in cover. The magnitude of this trend was small and may depend on more prolonged warming exposure periods and higher warming temperatures. Previous studies on the tropical upper forest line suggest that woody plants cannot grow adequately at soil temperatures below 5 or 6°C (Hoch and Körner, 2003; Körner and Paulsen, 2004; Hoch and Korner, 2005; Wiley and Helliker, 2012). Therefore, in the long term, as the world continues to

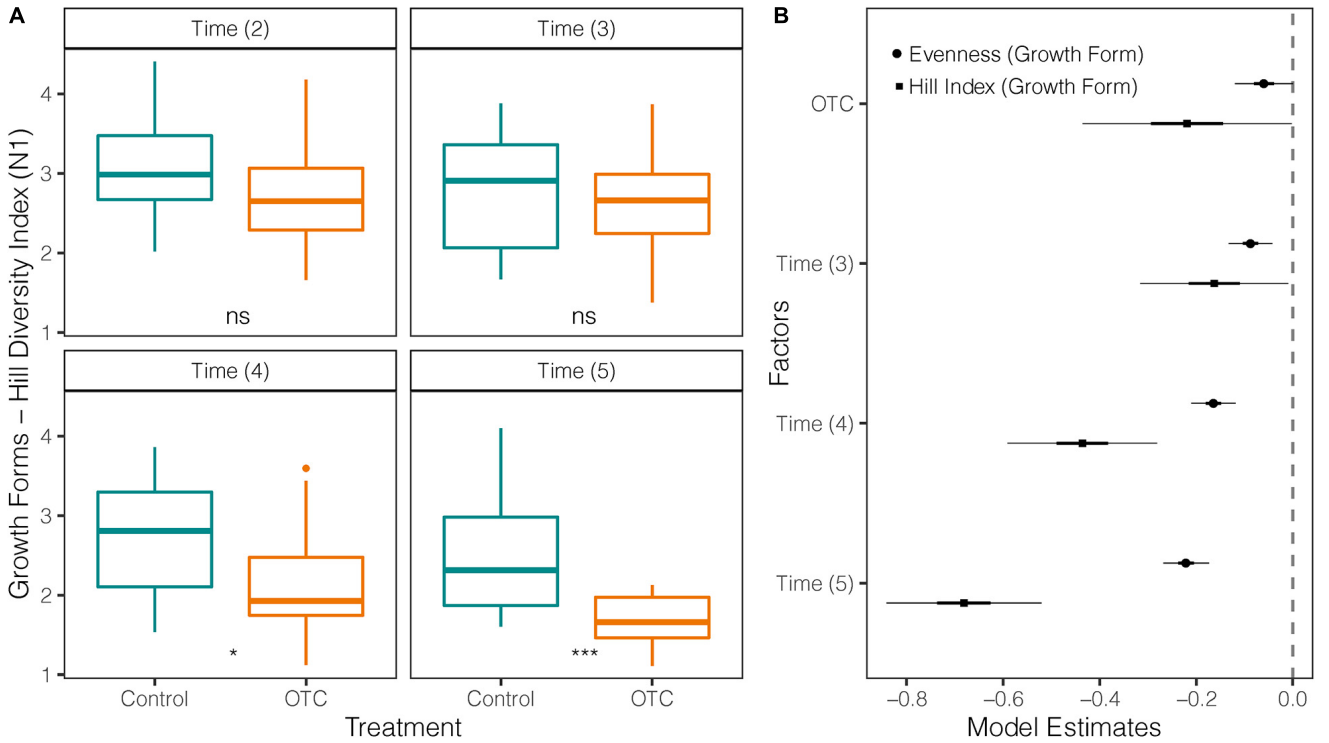


FIGURE 5 | Impact of warming on the diversity of growth forms in 4 sampling times between 2014 and 2019. Time (2) = 2014 used as a reference against the other sampling times, Time (3) = 2015/2016, Time (4) = 2017, and Time (5) = 2019. **(A)** Comparison of Hill's diversity index between plant communities under warming (OTC) and control conditions (outside OTC) over time. **(B)** Coefficients of two mixed-effects models testing Hill Diversity Index and Evenness of growth forms against the effects of warming (OTC) and sampling year. The vertical line represents the null value (where fixed effect are not significant when their CI cross this line). Fixed effects to the right of the vertical line have a significant positive relation with the response variable, and a negative one if they are on the left. $^{*}p < 0.05$ and $^{***}p < 0.001$.

get warmer in the future, shrubs [e.g., *Hypericum laricifolium* Juss., *Hesperomeles ferruginea* (Pers.) Benth.] and trees (e.g., *Polylepis pauta* Hieron., *Gynoxys acostae*) might outcompete tussock grasses, and there is evidence for this in the paleo records (Flantua et al., 2019). Here our hypothesis was partially met, so given our results, tussock dominance could be a temporary state on the way to a forested landscape.

The increase in percent coverage of tussock species and some shrubs (e.g., *C. intermedia, C. jussieui*) in OTCs is related to the downward trend observed for diversity and evenness of species and growth form, as we hypothesized. This downward trend is consistent with observations made in a 14-year monitoring study in the Apennines (Porro et al., 2019). Furthermore, Walker et al. (2006) found that warming increased the cover of deciduous shrubs and graminoids and decreased species diversity and evenness. While our results for species evenness and diversity and growth form diversity were significant, species richness was not, yet the downward trend for species richness was similar to that found in previous studies in alpine/tundra regions (Klein et al., 2004; Yang et al., 2018). For example, after the fourth sampling time, five out of the 57 recorded species (e.g., *A. geminiflorus*, *A. pedunculata*) were no longer present inside the OTCs, and six more evidenced a progressive reduction in the warmed plots (mainly herbs and cushions). This result was contrary to that of Steinbauer et al. (2018), who found increased species richness in nine regions across Europe over 145 years, mostly related to temperature change. However, after 20 years of monitoring, Steinbauer et al. (2020) showed that previously recorded species richness increases in the Alps were slowing down due to the disappearance of species, specifically cold-adapted species. In our study, decreasing species richness was more in line with this trend. Yet, the slower rate of decrease compared to other OTC studies may be due to the OTCs raising the daily mean temperature less than the increased mean temperature in those studies.

Besides the increase in tussock percent cover, we also observed decreased cushion (e.g., *Plantago sericea* Ruiz & Pav.), herb (e.g., *Geranium humboldtii* Willd. ex Spreng.), and rosette cover in the OTCs. Cushion plants are stress-tolerant, which allows them to be a dominant growth form at the highest elevations, along with rosettes, which decreased in both control and OTC plots in our study (Sklenář et al., 2010; Anthelme et al., 2017; Rada et al., 2019). However, when subjected to increased temperatures, Pancotto et al. (2020) found that their study subject *Astelia pumila* (G. Forst.) R. Br., a cushion plant, decreased their photosynthetic efficiency. Thus, the increase in temperature could be a potential explanation for the decrease in cushion plant coverage in our site. The reduction in cushion plant cover, together with the overall decrease of rosettes, which in tropical alpine ecosystems are characteristic of high elevations

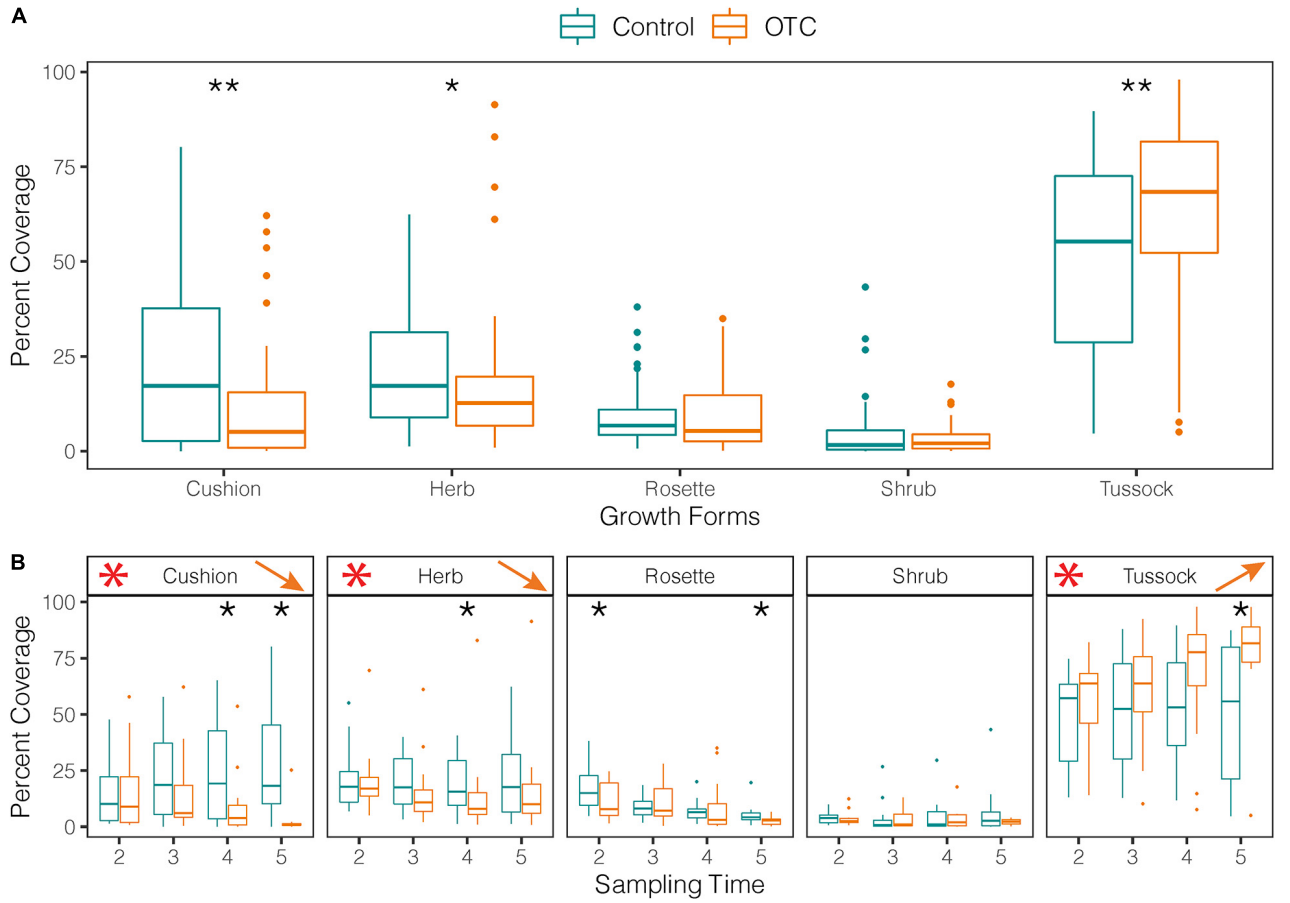


FIGURE 6 | Impact of warming on categories of growth forms. **(A)** Vegetation cover per category of growth form measured in plant communities under warming (OTC) and control conditions (outside OTC) in 4 sampling times between 2014 and 2019. Black stars represent significant differences between OTC and control. **(B)** Vegetation cover by sampling time for each category of growth form. Black stars show significant differences between OTC and control for a given time. Red stars show results found in **(A)** for comparison and arrows the direction of change over time inside the OTCs (Time 2 = 2014, Time 3 = 2015/2016, Time 4 = 2017, and Time 5 = 2019).

(Sklenář and Balslev, 2007; Rada et al., 2019), in OTC and control plots over time, may suggest that a macro-scale process may be already occurring in the Yanacocha Reserve. One potential explanation for this pattern is that rosettes are already migrating upward. On the Antisana volcano, about 63 km southeast of our study site, Moret et al. (2019) compared Humboldt's collections to a resurvey of the same collection route. This study reported upward shifts for three rosettes [*W. nubigena*, *Valeriana alypifolia* Kunth, and *Senecio nivalis* (Kunth) Cuatrec.] and one herb [*Phlegmariurus crassus* (Humb. & Bonpl. ex Willd.) B. Øllg.] where *S. nivalis* expanded upslope 216 to 266 m in 215 years.

Time is an essential variable in warming experiments. OTCs accelerate the rate to visualize change compared to prolonged comparative studies (Porro et al., 2019; Steinbauer et al., 2020; Anthelme et al., 2021), and our study found significant changes after 7 years of sampling. However, studies in non-tropical ecosystems found differences in a much shorter timeframe; for example, studies in tundra found changes in plant communities after two growing seasons (Walker et al., 2006). The distinct seasonality found in tundra and sub-tropical ecosystems, contrasting from the tropics, may make the tropics more resilient to temperature changes and may reveal the effects of climate warming over more time. Furthermore, those studies reported much higher temperature changes than what we obtained in our OTCs, which could also be contributing to the longer response times.

CONCLUSION AND FUTURE RESEARCH

Given the rapid rate of global temperature rise, it is crucial to understand its effect on ecosystems that support millions of people and are home to a diverse and unique group of life forms. In this study, the first long-term OTC study in a tropical alpine ecosystem, we experimentally increased the temperature of vegetation plots to assess the effects of warming on AGB and taxonomic and growth form diversity. While we hypothesized that air temperature would increase like other studies in similar regions, we obtained a smaller increase than expected. However, OTCs did modify the microclimatic conditions that plants were

experiencing. Two limitations of this study in characterizing the microclimatic conditions and their relationship to tropical alpine vegetation are the lack of humidity data together with photosynthetically active radiation and the use of one study site. Having multiple experimental sites would allow for more robustness and the feasibility of upscaling our results into a regional context.

Shifts in thermal conditions caused by the OTCs altered AGB, taxonomic, and growth form diversity, confirming the importance of small-scale temperature on shaping plant community composition and vegetation physiognomy in alpine ecosystems. Within OTCs, we found a trend toward the dominance of one or a few species and growth forms, specifically tussock grasses, leading to a decrease in species and growth form diversity and evenness, specifically tussock grasses. The dominance of tussocks may be a transitional phase; in the long term, as warming continues to increase, shrubs may outcompete the tussocks.

We obtained two unexpected results from this study: (1) a decrease in total biomass in both control and OTCs, and (2) a decrease in rosette coverage in both OTCs and control plots. Further research is needed to elucidate the overall decrease in AGB in the Yanacocha Reserve under control and experimental conditions. Measuring necromass decomposition rates together with soil properties (moisture, respiration, and *N* mineralization rates), belowground biomass, and soil organic carbon will help improve our understanding of the mechanisms driving AGB changes over time. Additionally, AGB contributions by growth forms are needed to understand shifts in growth form dominance and their links to resource acquisition traits.

Unlike in shrub *páramo* or Andean forests, demographic patterns in the grass *páramos*—such as species mortality and recruitment rates—have not been studied yet. Furthermore, because many species have clonal reproduction, it is challenging to determine individuals and perform population dynamics studies in tropical alpine systems. In our study, analyses on population dynamics would further the results reported here by providing information on immigration from lower elevations and possible extinction processes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

FC, PM, and SD conceived the experiment. SD, FC, PM, and CT built the general structure of the datasets. SD, FC, and CT analyzed the data and wrote the manuscript. SD, FC, PM, RJ, ES, and EP collected field data. All co-authors commented on and/or approved the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.673655/full#supplementary-material

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Vegetation Assembly, Adaptive Strategies and Positive Interactions During Primary Succession in the Forefield of the Last Venezuelan Glacier

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Glaciers are receding at unprecedented rates in the alpine tropics, opening-up new areas for ecosystem assembly. However, little is known about the patterns/mechanisms of primary succession during the last stages of glacier retreat in tropical mountains. Our aim was to analyze soil development and vegetation assembly during primary succession, and the role of changing adaptive strategies and facilitative interactions on these processes at the forefront of the last Venezuelan glacier (Humboldt Peak, 4,940 m asl). We established a chronosequence of four sites where the glacier retreated between 1910 and 2009. We compared soil organic matter (SOM), nutrients and temperatures inside vs. outside biological soil crusts (BSCs) at each site, estimated the cover of lichen, bryophyte and vascular plant species present, and analyzed changes in their growth-form abundance and species/functional turnover. We also evaluated local spatial associations between lichens/bryophytes and the dominant ruderal vascular plant (the grass *Poa petrosa*). We found a progressive increase in SOM during the first century of succession, while BSCs only had a positive buffering effect on superficial soil temperatures. Early seral stages were dominated by lichens and bryophytes, while vascular plant cover remained low during the first six decades, and was almost exclusively represented by wind dispersed/pollinated grasses. There was a general increase in species richness along the chronosequence, but it declined in late succession for lichens. Lichen and bryophyte communities exhibited a higher species turnover than vascular plants, resulting in the loss of some pioneer specialists as succession progressed. Lichen and bryophyte species were positively associated with safe-sites for the colonization of the dominant ruderal grass, suggesting a possible facilitation effect. Our results indicate that lichens and bryophytes play a key role

as pioneers in these high tropical alpine environments. The limited initial colonization of vascular plants and the progressive accumulation of species and growth-forms (i.e., direct succession) could be linked to a combination of severe environmental filtering during early seral stages and limitations for zoochoric seed dispersal and entomophilic/ornithophilic pollination. This could potentially result in a slow successional response of these ecosystems to accelerated glacier loss and climate change.

Keywords: Andes, bryophytes, climate change, facilitation, lichens, pollination, seed dispersal, soil development

INTRODUCTION

The process of plant community structuring during succession can be interpreted as being the result of several interacting filters which determine: (a) the species that can reach a site (biogeographic/dispersal filters); (b) the species that can tolerate conditions at the site (adaptive/functional filters); and (c) the species that are able to coexist at the site as a result of both positive and negative biotic interactions (Lortie et al., 2004). Some generalizations have been proposed on the changing importance of these processes for vegetation dynamics, including the key role played by effective seed dispersal, local micro-site availability and autogenic habitat amelioration *via* facilitation during the first stages of primary succession (Walker and Chapin, 1987; del Moral and Wood, 1993; Jones and del Moral, 2009). However, an integrated understanding of how these and other drivers operate across diverse taxa, succession types and biomes is still being developed (Walker and del Moral, 2003; Cauvy-Fraunié and Dangles, 2019; Pratch and Walker, 2020).

A significant contribution to the study of primary succession under climate change has come from research in glacier forefields, particularly following the worldwide tendency for glaciers to retreat after the Little Ice Age (LIA, Matthews, 1992; Fastie, 1995; Cannone et al., 2008). Glacier retreat rates have accelerated in the last five decades (Zemp et al., 2019), while there is increasing evidence that alpine species distributions are shifting upward (Lenoir and Svenning, 2015). This offers a unique opportunity to study climate change impacts in high mountain environments and opens up important questions regarding the development of novel ecosystems and the ability of high elevation specialists to become established and to maintain viable populations in newly exposed areas (Cannone et al., 2008; Zimmer et al., 2018; Cuesta et al., 2019; Anthelme et al., 2021).

Moreover, we are starting to witness the complete extinction of glaciers in many mountain regions, including Mediterranean and tropical alpine ecosystems, where most glaciers are likely to disappear during this century (Rabatel et al., 2013; Huss et al., 2017; Zemp et al., 2019). Alpine areas where glaciers will soon go extinct constitute particularly limiting environments because of steep slopes and dispersal barriers linked with island-like conditions near mountain peaks, high incident radiation but low partial pressures of CO_2 and O_2, incipient soil development and reduced water supply from glacier melt water (Zimmer et al., 2018; Cauvy-Fraunié and Dangles, 2019). In tropical alpine regions additional limiting factors include reduced plant protection from seasonal snow and particularly fast rates of high-elevation warming and glacier retreat (Rabatel et al., 2013; Wang et al., 2016; Vuille et al., 2018). However, studies of the patterns and mechanisms of primary succession in glacier forefields are scarce in the alpine tropics (see Spence, 1989; Mizuno, 2005; Mizuno and Fujita, 2014; Suárez et al., 2015; Zimmer et al., 2018; Anthelme et al., 2021; Rosero et al., 2021), especially under the harsh conditions that occur in mountains where they are about to become extinct.

The tropical Andes concentrate more than 95% of all tropical glaciers (Veettil et al., 2017). In the few available chronosequence studies of glacier forefields in the region, analyses of spatial association patterns and plant dispersal strategies have suggested that vegetation development could be slowed down by strong limitations in zoochoric seed dispersal and the effectiveness of facilitation mediated by nurse-plants (Zimmer et al., 2018; Anthelme et al., 2021). These studies have focused on the first two to six decades of succession, but how these findings apply for longer successional periods and to areas where remaining glaciers are less extensive or about to go completely extinct, remains largely unexplored. In fact, several essential aspects for understanding primary succession remain little understood in the alpine tropics, including the effect of biological soil crusts (BSCs) on soil development. Moreover, the available studies have focused on vascular plants, while community composition/dynamics and the role as pioneers of lichens and bryophytes have received limited attention [but see Sklenář et al. (2010) for a volcanic sere], despite being key structuring components of Arctic, Antarctic and alpine ecosystems, particularly during early succession (Longton, 1992; Bardgett and Walker, 2004; Cutler et al., 2008; Favero-Longo et al., 2012; Rosero et al., 2021).

In this context, a key unexplored aspect to understand vegetation assembly is to compare successional patterns of change in α and β diversity between lichens, bryophytes and vascular plants. Previous studies in glacial forefields have generally found an increase in vascular plant species richness (α diversity), at least for sites deglaciated after the LIA (Zimmer et al., 2018; Pratch and Walker, 2020). This has been associated with a progressive accumulation of plant species. Hence, in terms of the two main components of β diversity (nestedness and species turnover, see Baselga, 2010), during early primary succession vascular plants tend to show a predominance of nestedness (i.e., species accumulation) over temporal turnover (i.e., species replacement), a pattern called *direct succession* (Svoboda and Henry, 1987; Walker and del Moral, 2003; Pratch and Walker, 2020). However, incorporating bryophytes and lichens in these analyses could result in different patterns, as they could exhibit a different successional response to vascular plants. A higher species turnover could be expected in this case,

as competitive displacement between lichens, bryophytes and vascular plants could become increasingly more important as vegetation develops. This could also result in a decrease of species richness for lichens/bryophytes in more advanced seral stages.

Another key aspect relates to a comparative analysis of changes in functional / growth-form strategies along succession. Previous research in the alpine tropics suggests that ruderal vascular plants tend to be composed of small grasses and non-graminoid herbs with a predominance of anemochoric seed dispersal strategies (Sklenář et al., 2010; Suárez et al., 2015; Zimmer et al., 2018; Anthelme et al., 2021), while a more diverse array of growth forms should be expected later in succession (Sarmiento et al., 2003). The hardy stress tolerant strategies of tropical alpine grasses from high elevation would also support the idea of them being the predominant growth-form under the limiting conditions expected during early succession (Márquez et al., 2006; Rada et al., 2019). In the case of bryophytes we would expect cushion and turfs to be the pioneer growth-forms during early seral stages, as they are well adapted to sun-exposed rocky surfaces and desiccation at high elevations (Vanderpoorten and Goffinet, 2009; Kürschner and Frey, 2012). Later in succession, we would expect the colonization/dominance of other growth forms such as bryophyte mats, better adapted to less exposed sites with more developed soils and higher humidity (Vanderpoorten and Goffinet, 2009). In the case of lichens, crustose growth forms are expected during early seral stages, as their bi-dimensional structure (closer to the substrate) has been found to be more resistant to desiccation produced by high radiation in rocky areas (Büdel and Scheidegger, 2008). In later stages we would expect the colonization/dominance of more complex growth forms like foliose, fruticose and dimorphic lichens (Sklenář et al., 2010; Bässler et al., 2015). Consequently, in terms of functional structure, strong environmental filtering during early succession could result in a progressive accumulation of growth-forms of lichens, bryophytes and vascular plants (i.e., a predominance of nestedness over growth-form turnover). Obviously, competitive displacement as succession progresses could also play a role, but it is more likely to modify the relative abundance of the different growth-forms than to result in the complete displacement of some functional groups.

Our general aim in this study was to analyze successional soil development and the assembly of lichen, bryophyte and vascular plant communities during the last stages of glacier retreat in the high tropical Andes, and to link them to potential underlying processes reflected in their changing functional strategies (i.e., growth-forms, dispersal and pollination syndromes) and local spatial interactions (i.e., facilitation/competition). A detailed historical reconstruction allowed establishing a chronosequence of four sites in the forefront of the last Venezuelan glacier at Humboldt's Peak (4,945 m asl), in areas where the glacier retreated between 1910 and 2009 (c. 100 years, Ramírez et al., 2020). Our specific objectives were: (1) to analyze the role of BSCs on soil development, documenting successional changes in soil organic matter, nutrients and superficial soil temperatures in areas inside vs. outside their influence along succession; (2) to quantify changes in the composition, richness and abundance of lichen, bryophyte and vascular plant species and compare the degree to which species and growth-form accumulation vs. turn-over (the two components of between-site β-diversity) characterize successional community assembly; (3) to analyze the importance of abiotic vs. animal mediated dispersal and pollination, quantifying changes in the relative abundance of vascular plants with different dispersal/pollination syndromes; and (4) to explore the role of facilitation mediated by pioneer lichens/bryophytes on early vascular plant colonization, analyzing local spatial association between them and favorable sites for the establishment of the dominant ruderal plant (the grass *Poa petrosa*).

Based on the previous research on primary succession in temperate and tropical alpine regions, and considering the expected limitations for ecosystem development in glacier forefields of vanishing glaciers, we evaluated the following predictions: (1) the presence of BSCs will be associated with an increase in soil organic matter and nutrients; (2) a progressive accumulation of species (nestedness), more than species turnover, should characterize the dynamics of vascular plants under these extreme conditions, resulting in a successional increase in the richness of species and growth forms (i.e., direct succession). However, these patterns should differ if we incorporate lichens and bryophyte species in the analysis, reflecting a possible increase in species turn-over and competitive exclusion between them and with vascular plants as vegetation develops; (3) limitations in zoochoric seed dispersal and pollination by birds and insects in these extreme environments should result in less diverse vascular plant pollination and dispersal syndromes during early succession; and (4) the presence of lichens and bryophytes will be positively associated with favorable microhabitats for the early establishment of ruderal plants suggesting a facilitation role.

MATERIALS AND METHODS

Study Area

The study was carried out in the North-West flank of the glacier forefront at Humboldt peak (8°33′0.8″ N, 71°59′50.56″ W), in the Sierra Nevada National Park, Venezuela (**Figure 1**). Venezuela will soon be the first Andean country to lose all of its glaciers. Ramírez et al. (2020) showed that glaciers in the Sierra Nevada de Mérida, where the highest peaks in the country are located, have lost 99% of their area since 1910. There is only one remaining ice mass at Humboldt Peak (4,942 m asl), which has lost 98.7 % of its surface since 1910, with an area for 2019 of only 0.0454 Km^2 (Ramírez et al., 2020). These glaciers have been retreating since the local expression of the LIA, with moraines at the terminus reaching c. 4,200 m elevation (Mahaney et al., 2000; Ramírez et al., 2020).

Average annual precipitation at the highest elevations in the Sierra Nevada de Mérida range from 1,000 to 1,200 mm, with high inter-annual variability (Andressen, 2007). Both precipitation and temperature decrease with elevation, from an

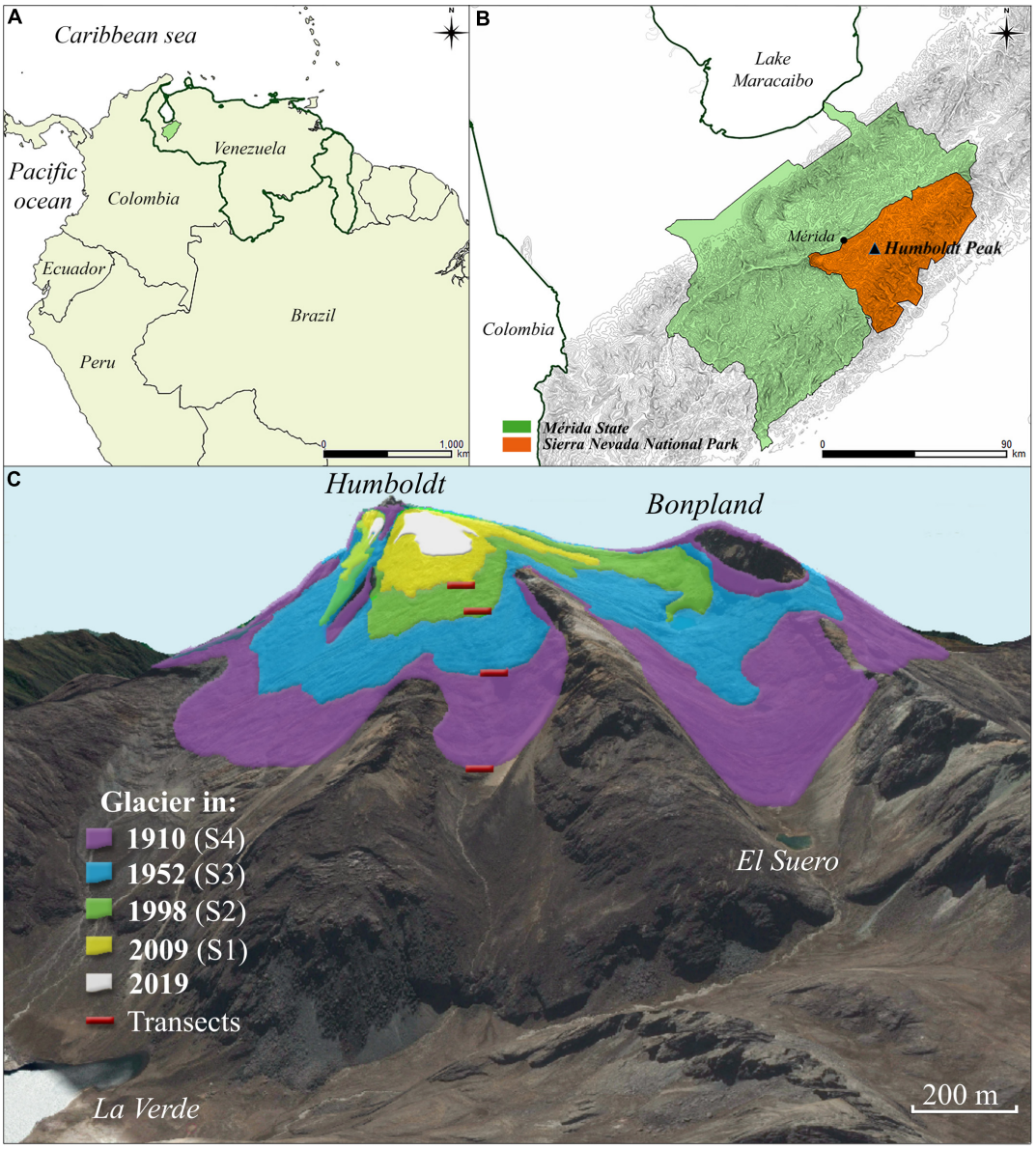


FIGURE 1 | Study region maps. **(A)** Study region in the northern South America; **(B)** Detail of Mérida State, the Sierra Nevada National Park and Humboldt Peak, Venezuela. **(C)** General view of the North West face of Humboldt and Bonpland Peaks indicating the location of our study transects (red lines) and glacier cover for different moments between 1910 and 2020 [based on an integrated historical reconstruction by Ramírez et al. (2020)]. Glacier cover polygons drawn over a Google Earth image.

average of 1,811 mm annual precipitation and 7.1°C mean air temperature at La Aguada station of the cable car system (3,446 m asl) to 1,173 mm and −0.4°C at Pico Espejo station (4,766 m asl, the closest station to Humboldt peak). Although there is little monthly variability in average temperature (≤3°C), precipitation is seasonal, with a dry period between December and March (see Andressen, 2007). Regarding the area's geology, the Iglesias group formation is present at the highest elevations, corresponding to Precambrian high-grade metamorphic rocks including banded gneiss, schist, and amphibolite, with frequent granitic dikes and quartz veins (Schubert, 1975).

Sampling Sites

Field work was organized in three 1-week expeditions that occurred between May 2019 and December 2019. For site selection we used a chronosequence approach, based on a detailed reconstruction of glacial retreat in Humboldt Peak (1:5,000 scale, Ramírez et al., 2020). The reconstruction integrated historical photographs with the analysis of maps from 1910 (Jahn, 1925), high-resolution aerial photographs for 1952 and 1998 and satellite images for 2009 (Spot V, 2.5 m). This allowed the selection of four study sites along a successional/elevation gradient in the NW face of Humboldt's peak (**Figure 1**): (a)

Site 1: 10 years since glacial retreat, corresponding to the glacier position in 2009 (4,670 m asl); (b) Site 2: 21 years of succession, corresponding to 1998 (4,637 m asl); (c) Site 3: 67 years, for the 1952 line (4,537 m asl); and d) Site 4: 109 years, for the 1910 line (4,365 m asl). Although site replication would have been desirable to allow more robust generalizations, selecting more study sites on other mountain faces, logistic limitations prevented it: this is a remote area, requiring two full days to reach base camp; we worked on the more accessible face of the peak (Northwest), as working on other faces can be considerably more dangerous because of the steepness of the terrain.

The glacier forefront area corresponds to a periglacial landscape dominated by steep slopes (45–70°) formed by hard bedrock polished by glacier action. In each study site, we established one 50 × 20 m study area (1,000 m^2) on sections with similar geomorphological and topographic characteristics, to allow a better comparison of vegetation and soil development between sites (**Figure 1**). All vegetation and soil sampling was carried out within each of these 1,000 m^2 areas (see details below and **Supplementary Figure 1**). Although it would have been desirable to nest the soil sampling points within the sampling plots used for studying vegetation cover (eight replicate plots, 5 × 5 m each), time/logistic constraints prevented this.

The 50 × 20 m study areas were placed parallel to the glacier front on slopes ranging between 10° and 30°, coinciding with rocky steps created by glacier erosion / scouring along rock foliation lines. The flatter local topography in the study sites allowed the accumulation of loose glacial till mixed with gelifraction materials (small angular rocks and scree), coming from both lateral slopes of the valley. We decided to work on these flatter rocky-steps as they allowed us to analyze areas less limiting for soil and vegetation development than the steep polished glacier bedrock walls, where plant colonization is almost completely absent.

Soil Analyses

Soil samples (0–3 cm depth) were collected in 12 random points along each study area, six in open soils and six directly under BSCs (see **Supplementary Figure 1**). Soils were transported to the laboratory, where they were dried and sieved using 4 and 0.5 mm mesh strainers to then determine pH (in water), total soil organic matter (SOM, ignition), total nitrogen (N_t, Kjeldahl), available phosphorus (P, Bray-Kurtz for acid soils), and exchangeable bases (calcium, magnesium, potassium and sodium, see details in Gilabert de Brito et al., 1990).

Soil properties were analyzed using a two-way Permutational Analysis of Variance (PERMANOVA+ for Primer 6.0 software, Anderson et al., 2008), defining the successional time as a fixed factor with four levels (10, 21, 67, and 109 years) and the presence/absence of BSCs as a fixed factor with two levels (inside vs. outside of BSCs) and each of the edaphic response variables taken separately (i.e., univariate tests). We used PERMANOVA instead of fully parametric ANOVA procedures, as our data was not normally distributed. In all PERMANOVA tests, the number of permutations was set to 9,999 and the probability of type I error was set at 95% (a = 0.05), although it was adjusted to 99% for *post hoc* tests (permutational t-tests).

At each study site, we also placed two thermometer data-loggers (Onset HOBO TidbiT v2), 2 cm below the soil surface, one inside and one outside of a BSC (always less than 2 m apart, **Supplementary Figure 1**). These recorded temperatures every hour for 6 months (between July 1st and December 10th 2019), allowing us to calculate for each situation average, minimum, and maximum superficial soil temperatures, average temperature amplitudes and the proportion of days with freezing superficial soil temperatures (see **Supplementary Table 1**). However, because of lack of replication (not enough temperature sensors for this), we were not able to statistically compare these results between sites/seral stages.

Patterns of Community Assembly and Diversity

To assess changes in community composition and species abundances, we first carried out a detailed inventory of total species richness in each site recording all the lichenized fungi, bryophyte and vascular plant species found within each 1,000 m^2 study area. Then, to quantify the abundance of each species, we visually estimated the cover of vascular plants in eight 5 × 5 m plots randomly placed within each study area (**Supplementary Figure 1**). We estimated lichen and bryophyte cover in three 0.5 × 0.5 m subplots placed along on the diagonals of each 5 × 5 m plot (and averaged results from the three sub-plots for subsequent analyses). Within each 5 × 5 m plot we also estimated the cover of rocks (≥ 50 cm in diameter), scree, loose plant necromass and bare soil/BSCs (with these superficial substrates summing 100% cover in each plot, without taking into account plant cover.

The visual estimation of cover for vascular plants, lichens and bryophytes was each done by a single person in all studied plots (i.e., the corresponding expert in the group). In the case of bryophytes and lichens, cover estimation was aided by placing a frame subdivided into one hundred 5 × 5 cm cells over the 50 × 50 cm sub-plot (with both experts working together to reach consensus). Confirmation consensus between the three experts was also reached for the cover of vascular plants in each plot. Admittedly, the fact that a different researcher estimated the cover of lichens, mosses and vascular plants could have introduced some unintended bias, but the observed differences in cover between groups are sufficiently large to make this small sampling bias unimportant.

We compared community composition and species abundance between 5 × 5 m plots with a Principal Coordinate Analysis (PCoA, Legendre and Legendre, 2003) based on a Bray Curtis similarity matrix (using Primer v6, Clarke and Gorley, 2006). Because of the high incidence of rare species, the original data was transformed with fourth root and then standardized by species for comparison.

Finally, we analyzed the degree to which the successional process involved species replacement (turnover) or species accumulation (nestedness) between sites, using a partitioned β-diversity analysis, following Baselga (2010). First, we separately established presence/absence matrices for lichens, bryophytes and vascular plants in each site using the full species inventory.

We then established a similar matrix for all species of photosynthetic organisms taken together. Then, for each type of organism taken separately and for the whole group of species, we calculated total multisite β-diversity using Sørensen's index (β_{SOR}), and decomposed it into two additive components: β-diversity resulting from turnover (using Simpson's index, β_{SIM}) and β-diversity resulting from nestedness (β_{NES}). The same analysis was then repeated using a presence-absence matrix of all the growth-forms of lichens, bryophytes and vascular plants for each of the four study sites (see below for details on the grouping of species into growth-forms). These indices were calculated with the "*beta.multi*" function of the *betapart 1.5.1* package (Baselga and Orme, 2012), using RStudio v. 1.1.463 (RStudio Team, 2016).

Changes in Functional Structure: Growth-Forms, Dispersal and Pollination Strategies

To assess successional changes in key aspects of the functional structure of lichen, bryophyte and vascular plant communities, we characterized the growth-form of each species. For lichens, we used the classical system including three growth forms (crustose, foliose and fruticose, see Büdel and Scheidegger, 2008). We defined dimorphic lichens as a mixed form with a primary thallus usually crustose or squamulose/foliose, and a secondary fruticose thallus. For bryophytes, we used Vanderpoorten and Goffinet's (2009) system, based on the direction of main shoots. The categories included "cushion" (radiating from a central point); "turfs" (erect habit of acrocarps); and "mats" (creeping habit of pleurocarps). Finally, for vascular plants, we adapted the framework proposed by Hedberg and Hedberg (1979), adding to the five growth forms defined by them (grasses, cushions, shrubs, acaulescent rosettes and caulescent rosettes), two important growth forms in many alpine ecosystems: non-graminoid herbs and ferns.

For vascular plants only, we also defined the pollination and dispersal syndrome of each species present, based on on-site observations of their floral visitors (75 h of onsite field observations) and the morphology of their flowers and diaspores, following classifications by Faegri and van der Pijl (1979) and Tovar et al. (2020), respectively. For pollination syndromes we also relied on previous detailed studies of observed plant-pollinator interactions in the high Venezuelan páramo (Pelayo et al., 2019, 2021). Hence, plants were classified as anemophilous, entomophilous or ornithophilous for pollination and as anemochoric, ballochoric, barochoric or zoochoric in terms of dispersal. Given their importance during early colonization, we sub-divided anemochoric species in two groups, those with minute/dust seeds (<3 mm, in our Poaceae species), and those with additional morphological adaptations for wind dispersal (i.e., Asteraceae with plumose, hairy or winged seeds). The total cover of each growth form or syndrome was calculated as the sum of the cover of each species included in a given category in each sampling plot. These were then averaged in each study site to calculate the relative cover of each growth form or dispersal/pollination syndrome in each site.

Spatial Association Between Lichens/Bryophytes and Ruderal Vascular Plants

The possible role of lichens and bryophytes on the establishment of the dominant early- succession vascular plant species (the ruderal grass *Poa petrosa)* was studied in Sites 2 and 3 (21 and 67 years), using their local spatial association as a proxy. We did not include Site 1 (10 years) because the density of *P. petrosa* was too low, while in the case of Site 4 (109 years) a much higher vegetation cover of many different plant species complicated establishing clear local spatial association patterns. Moreover, this ruderal grass was no longer among the most abundant vascular plant species at this site.

In sites 2 and 3, we compared the cover of all bryophyte and lichenized fungi species (visual estimation, always by the same two experts working together), within 10 circular micro-plots (30 cm diameter) centered on mature *P. petrosa* plants (≥5 cm height) and 10 paired micro-plots placed outside, centered on a randomly chosen point within 1–2 m from the target plant (for a total of 40 microplots, 20 in each study site). The small size of the micro-plots facilitated visual estimation.

We compared the total cover of lichens and bryophytes (taking them as a group and also for each species separately) using the average of the relative interaction index [$RII = (C_n - C_a)/(C_nC_a)$], where C_n is the cover of the organism type or species near *P. petrosa*, and C_a the cover of the same organism or species away from *P. petrosa* (Armas et al., 2014). RII values range from 1 to −1, where significant positive values (i.e., 95% CI does not include zero) would indicate a positive local association of lichens/bryophytes with favorable sites for the growth of the pioneer grass, and significant negative values would suggest negative, inhibitory effects.

RESULTS

Soil Development and Substrate Cover Changes

Soil organic matter (SOM), total soil nitrogen (N_t), calcium (Ca), magnesium (Mg) and available phosphorus (P) showed statistically significant differences between sites along succession ($p \leq 0.0001$), while the rest of the variables did not (**Table 1**). In the case of SOM and N_t there was a clear trend to increase from the youngest to the oldest sites along the chronosequence. However, Ca and Mg showed the opposite trend, with significantly higher concentrations in the two youngest sites in the case of Mg, and in Site 1 (10 years) for Ca. P was significantly higher in Site 2 (21 years) than in the rest of the sites. The presence of BSCs did not show a significant effect for any variable, and there was no significant interaction between time and BSC presence ($p \geq 0.05$ for the presence of BSC and the interaction term in all the two-way PERMANOVAs for each variable). However, BSCs consistently reduced superficial soil temperature amplitudes in all study sites. This was mainly the result of a consistent reduction in maximum temperature, but the proportion of days during the study period

TABLE 1 | Average (standard error) for soil properties (0–3 cm) studied within biocrusts (Bio) and in paired areas outside (Out) in four sites (S1 to S4) along a glacier chronosequence (years since glacier retreat) in the forefront of the Humboldt glacier, Venezuela.

Variable	S1 (10 years)		S2 (21 years)		S3 (67 years)		S4 (109 years)		*pseudoF (p)* Time
	Bio	Out	Bio	Out	Bio	Out	Bio	Out	
SOM(%)	0.49 **a**(0.05)	0.48 **a**(0.03)	0.75 **b**(0.12)	0.56 **b**(0.05)	1.08 **c**(0.07)	0.92 **c** (0.21)	1.51 **d**(0.07)	1.41 **d**(0.21)	**37.8(0.0001)**
Nt(%)	0.016 **a**(0.004)	0.011 **a**(0.002)	0.028 **b**(0.004)	0.027 **b**(0.004)	0.049 **c**(0.006)	0.035 **c**(0.004)	0.050 **c**(0.007)	0.049 **c**(0.013)	**13.5(0.0002)**
pH	5.03(0.19)	4.99(0.18)	4.52(0.11)	4.72(0.14)	4.94(0.08)	4.99(0.12)	4.75(0.11)	5.02(0.19)	2.66(0.0646)
Ca(meq/100 g)	3.50 **b** (0.62)	2.22 **b**(0.23)	1.18 **a**(0.40)	1.06 **a**(0.20)	0.92 **a**(0.26)	0.57 **a**(0.21)	0.40 **a** (0.06)	0.89 **a** (0.23)	**18.9(0.0001)**
Mg(meq/100 g)	10.41 **b**(1.65)	8.47 **b**(0.80)	13.02 **b**(2.88)	12.07 **b**(3.89)	2.25 **a**(0.65)	1.53 **a**(0.68)	2.50 **a**(0.92)	3.96 **a**(1.44)	**17.1(0.0001)**
Na(meq/100 g)	0.59(0.09)	0.42(0.03)	0.51(0.20)	0.44(0.09)	0.40(0.07)	0.42(0.07)	0.38(0.04)	0.47(0.04)	0.40(0.7886)
K(meq/100 g)	1.00(0.07)	0.82(0.17)	1.01(0.12)	0.84(0.15)	0.76(0.13)	0.67(0.14)	0.69(0.07)	0.80(0.11)	1.49(0.2344)
P(ppm)	0.79 **a**(0.18)	1.45 **a**(0.72)	3.69 **b**(0.81)	4.50 **b**(0.72)	0.87 **a** (0.35)	1.10 **a**(0.57)	0.94 **a** (0.13)	0.67 **a**(0.31)	**17.13(0.0001)**

Values between successional stages and local sampling situations (Bio vs. Out) were compared using a two-way PERMANOVA (α = 0.05).
The effect of successional time is indicated (pseudoF, p value).
No significant effects were observed for the local sampling situation or the interaction term for any of the studied variables ($p \geq 0.05$ for all variables).
Different letters indicate significant differences between successional stages based on a permutational t test.
Values in bold indicate statistically significant differences.

with freezing temperatures was also consistently lower within BSCs than outside (**Supplementary Table 1**).

Average soil/BSCs cover was lowest (1.8 % ± 1.3 SE) in Site 1 (10 years), while it ranged between 10.3% (± 3.2) and 8.7 % (± 1.2) in Sites 2–4. Rock cover varied between 60.6% (± 6.0) and 49.4% (± 6.5) in the three earlier successional sites and was lowest in Site 4 (28.1% ± 7.4). Average scree cover ranged between 50.0% (± 6.4) in Site 1 and 28.4 % (± 4.7) in Site 2, with intermediate values in the other two sites. Finally, plant necromass cover ranged between 0.0 and 1.0% in the three younger sites and was highest in Site 4 (12.1 % ± 2.2).

Patterns of Community Assembly and Diversity

Based on our complete inventory in each site (1,000 m^2 sampling areas), a total of 47 species of lichenized fungi were identified across the four seral stages (19 genera and 16 families). Of these, 25 species (53%) were new reports for Venezuela (**Supplementary Table 2**). For bryophytes, we identified a total of 55 species (27 genera and 16 families). Six were new records for Venezuela and 8 endemic to the Andean páramos (15%). Finally, 36 species of vascular plants were registered inside the four transects/sites (13 families and 30 genera). There were no new records for Venezuela, but 24 species were endemic to the Andean páramos (67%) and 8 endemic to Venezuela.

Total species richness increased monotonically along succession for bryophytes and vascular plants, but not for lichens, which showed a maximum in site 3 (67 years, **Figure 2**). Average richness was always lowest in Site 1 (10 years), and increased in the older sites, but leveled-off in Site 3 for lichens and bryophytes, while it continued to increase for vascular plants, with a maximum in Site 4 (109 years). Interestingly, bryophytes showed higher total and average richness in the two earlier sites than plants and lichens, but lichens showed much higher average cover in these initial stages. Average lichen cover peaked after 21 years (29.2%), and then declined, while bryophytes and vascular plants monotonically increased their cover along succession, from very low values in Site 1 (1.17 and 0.38% respectively) to a maximum in Site 4 (**Figure 2**).

The PCoA ordination analysis indicated very clear successional changes in species composition and cover (**Figure 3**). Sampling plots were organized in terms of successional time along the 1st axis (31.7% of total variance), 10 year plots occupying the left side and 67 / 109 year plots the right-hand side. The two late-succession sites were clearly separated along the 2nd axis (14.7% variance). The two earliest seral stages were dominated by lichens (e.g., *Rhizocarpon umbilicatum*). Bryophytes showed much lower cover in Site 1 (10 years), with *Molendoa peruviana* and *Racomitrium crispipilum* being among the dominant pioneers. In the next three sites *Racomitrium subsecundum* and *R. crispipilum* became the dominant moss species (see **Supplementary Table 2** for details). Only three vascular plant species were present in Site 1: the grass *Poa petrosa*, and two Asteraceae species. However, only *P. petrosa* was present within the 5 × 5 m sampling plots, showing a very low average cover (0.38%). Other species progressively colonized the next two sites, with grasses dominating Sites 2 and 3 (e.g., *Festuca fragilis*). Finally, in Site 4 (109 years) a more diverse community developed, dominated by the shrubs (e.g., *P. imbricatifolia)*, the grass *F. fragilis*, erect herbs (e.g., *Castilleja fissifolia*) and the giant caulescent rosette *Espeletia moritziana* (see details in **Supplementary Table 2**).

The analysis of multisite β-diversity revealed contrasting responses for plants, bryophytes and lichens. We found a highest total β-diversity and a highest contribution of nestedness in the case of vascular plants, indicating a predominance of species accumulation over turnover. The lowest total β-diversity and the highest contribution of temporal turnover was observed for bryophytes, while an intermediate situation was observed for lichens (see **Table 2** and **Supplementary Figure 2**). Interestingly, when the three groups of organisms were analyzed together, the species turnover component showed a slight predominance over the nestdness component (54.7 vs. 45.3%), reflecting the higher relative importance of species turnover shown by bryophytes and lichens compared to vascular plants.

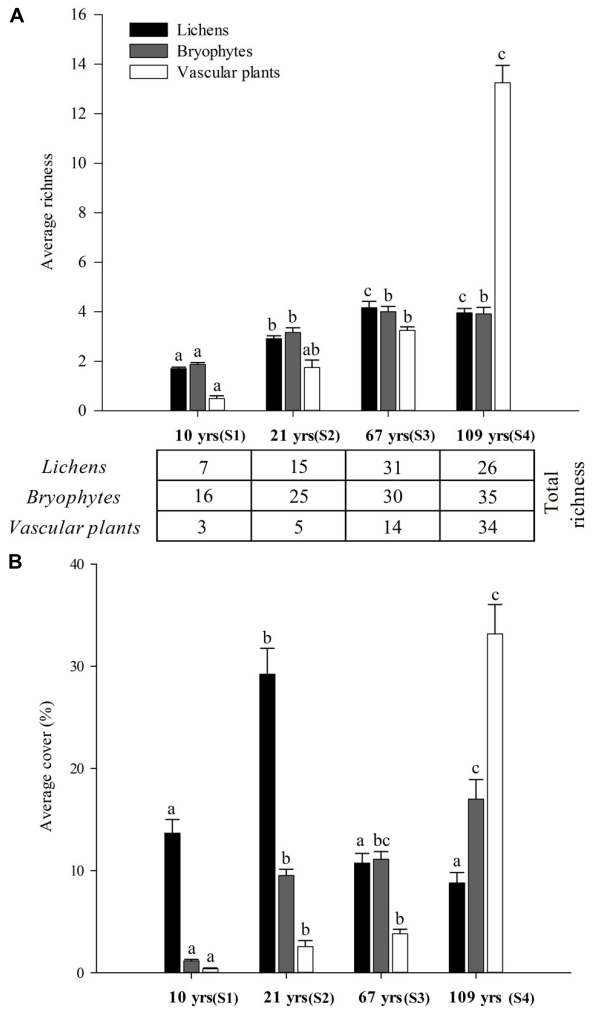


FIGURE 2 | Changes in total species richness and average richness **(A)** and cover **(B)** for lichens, bryophytes and vascular plants in four study sites (S1-S4) along a post-glacial chronosequence (10–109 years of succession) at Humboldt Peak, Venezuela. Different letters indicate significant differences across successional stages for each group of organism analyzed separately (i.e., differences between seral stages, permutational *t*-test, $\alpha = 0.01$).

Changes in Functional Strategies

In the case of lichenized fungi all sites were dominated by crustose species, while fruticose lichens were the sub-dominant growth form (**Figure 4**). Foliose lichens were present from the earliest stages and increased in relative cover, but remained below 10% in all sites, while dimorphic species were only present in the two oldest sites, with a maximum of 4.5% of relative cover in Site 4 (109 years). For bryophytes, the three growth-forms were present across the four seral stages, but turfs were clearly dominant during early seral stages, while in late succession mats dominated. Small cushions showed a low relative importance in all sites/seral stages. Finally, regarding vascular plants, growth-form richness increased across sites, grasses strongly dominating the three youngest sites with values between 100 and 85% of relative cover. Vegetation showed a much higher growth form richness in Site 4, with shrubs becoming dominant (37.5% of relative cover), followed by grasses and non-graminoid herbs. Caulescent and acaulescent rosettes, cushions and ferns were less abundant, with relative cover values below 6% in all cases (**Figure 4**).

When multisite β-diversity was analyzed in terms of the presence-absence of growth-forms of lichens, bryophytes and vascular plants taken together, its total value was lower than the value based on the presence/absence of individual species, and there was no observed growth-form turnover. Hence, the observed β-diversity between sites was entirely due to the progressive accumulation of growth forms along succession, with an observed increase in growth-form richness for lichens and vascular plants and no loss from early to late succession, so that the nestedness component comprised 100% of the overall β-diversity in this case.

Regarding plant dispersal and pollination strategies, anemochoric grasses with small seeds and anemophilous pollination dominated vegetation in the first three sites (**Figures 5A,B**). In contrast, plants with anemochoric seeds with appendages (e.g., plumes/hairs, Asteraceae) became dominant in Site 4 (109 years, with 55.2 % relative abundance), followed by dust seed grasses (27.8%), and a much smaller representation of ballochoric and barochoric species (**Figure 5A**). We found no species with zoochoric dispersal in any of the four study sites. Pollination syndromes also became more diverse in Site 4, with a dominance of insect pollinated species (entomophily), followed by anemophilous and ornithophilous species (**Figure 5B**).

Spatial Association Between Lichens/Bryophytes and Ruderal Plants

The relative interaction index (RII) indicated that bryophytes were significantly overrepresented in the neighborhood of the ruderal grass *Poa petrosa*, suggesting a positive association in both sites. In contrast, total lichen cover showed a neutral spatial relation, with the RII not being significantly different from zero (**Figure 6**). When RII was analyzed at the species level (see **Supplementary Figure 3**), two bryophyte species showed a significant positive association with the grass in Site 2 (21 years), *R. subsecundum* and *Bryum* cf. *chryseum (*among the most abundant species in this site, being particularly frequent within BSCs). Only one species showed a significant negative RII (in Site 2), the saxicolous lichen *Tremolecia atrata*. In Site 3 (67 years) four bryophytes showed a significant positive RII, including the abundant moss *C. flexuosus* var. *flexuosus* (the 2nd most common species within BSCs). In this site four lichenized fungi species also showed a positive association with the grass; three of them were dominant within BSCs and are known to have atmospheric N-fixing photobionts (e.g., *S. verruciferum*). No bryophyte or lichen showed significant negative spatial associations with the pioneer grass in S3.

DISCUSSION

Climate change is resulting in accelerated glacier retreat in the tropical Andes (Rabatel et al., 2013; Zemp et al., 2019), exposing new areas for ecosystem development at high elevations.

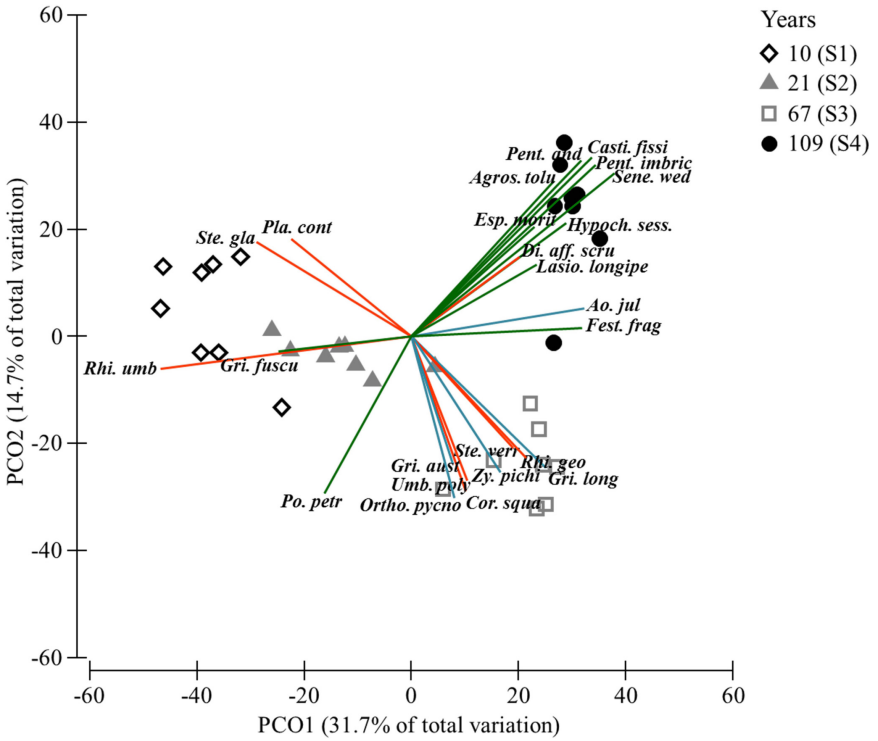


FIGURE 3 | Principal Coordinates Analysis based on a Bray–Curtis similarity matrix of the cover of all species present in 5 × 5 m plots (n = 32) in four study sites (S1-S4) along a post-glacial chronosequence at Humboldt Peak, Venezuela. The vectors correspond to the Spearman Rank correlation ($r_s \geq 0.5$) of the dominant species of lichenized fungi (red), bryophytes (blue) and vascular plants (green) with the first two principal axes (full species names in **Supplementary Table 2**).

However, little is known in the alpine tropics about the dynamics of primary succession under the extreme conditions that occur during the last stages of glacier retreat. Our results in the forefield of the last Venezuelan glacier show a strong dominance of lichens and then bryophytes during the first six decades of succession and a progressive accumulation of vascular plant species and growth forms characteristic of direct succession (Svoboda and Henry, 1987; Pratch and Walker, 2020). Moreover, they suggest that in the alpine tropics lichens and bryophytes could play an important role in modulating early vascular plant colonization, generating favorable micro-sites, particularly within BSCs. However, they also support the idea that animal mediated dispersal and pollination could be limited in these extreme conditions, with a complete absence of zoochoric (animal dispersed) vascular plant species during the first century of succession and a strong dominance of anemophily (wind pollinated) grasses in the first six decades (see also Zimmer et al., 2018). Below, we discuss in more detail the dynamics of soil and vegetation development, and some of the key processes that could help us understand the limitations faced by ecosystem development during the first 100 years of post-glacial succession in the highland tropics.

TABLE 2 | Analysis of multi-site β-diversity for lichens, bryophytes and vascular plants and for the whole vegetation analyzing all species of photosynthetic organisms together.

Organism	Total β-diversity	Nestedness	Temporal turnover
Lichens	0.61	0.32 (52.8%)	0.29 (47.2%)
Bryophytes	0.51	0.16 (31.3%)	0.35 (68.7%)
Vascular plants	0.71	0.59 (82.5%)	0.13 (17.5%)
All species	0.59	0.27 (45.3%)	0.33 (54.7%)
All growth-forms	0.30	0.30 (100%)	0.00 (0.0%)

We also present the analysis in terms of the β-diversity of growth-forms present (all growth-forms taken together).
In each case, total β-diversity between sites (Sorensen's index) is decomposed in its nestedness and turnover (Simpson's index) components.
The percent contribution of each component to total β-diversity is presented in parenthesis.

Soil Development

Against our expectations (prediction 1), BSCs did not show a significant effect on SOM or soil nutrients in any of our study sites. This does not agree with what has been documented in several other glacial seres, and the fact that N-fixing organisms are frequently associated with BSCs (Longton, 1992; Belnap and Lange, 2001; Walker and del Moral, 2003; Bardgett and Walker, 2004; Breen and Lévesque, 2006, 2008). Even so, BSCs appeared to reduce daily superficial soil temperature variability (see also Breen and Lévesque, 2008) and the number of days with below zero temperatures (72–21% lower frequency within BSCs in the three highest sites). This could be important in terms of microhabitat amelioration for colonizing plants, as it

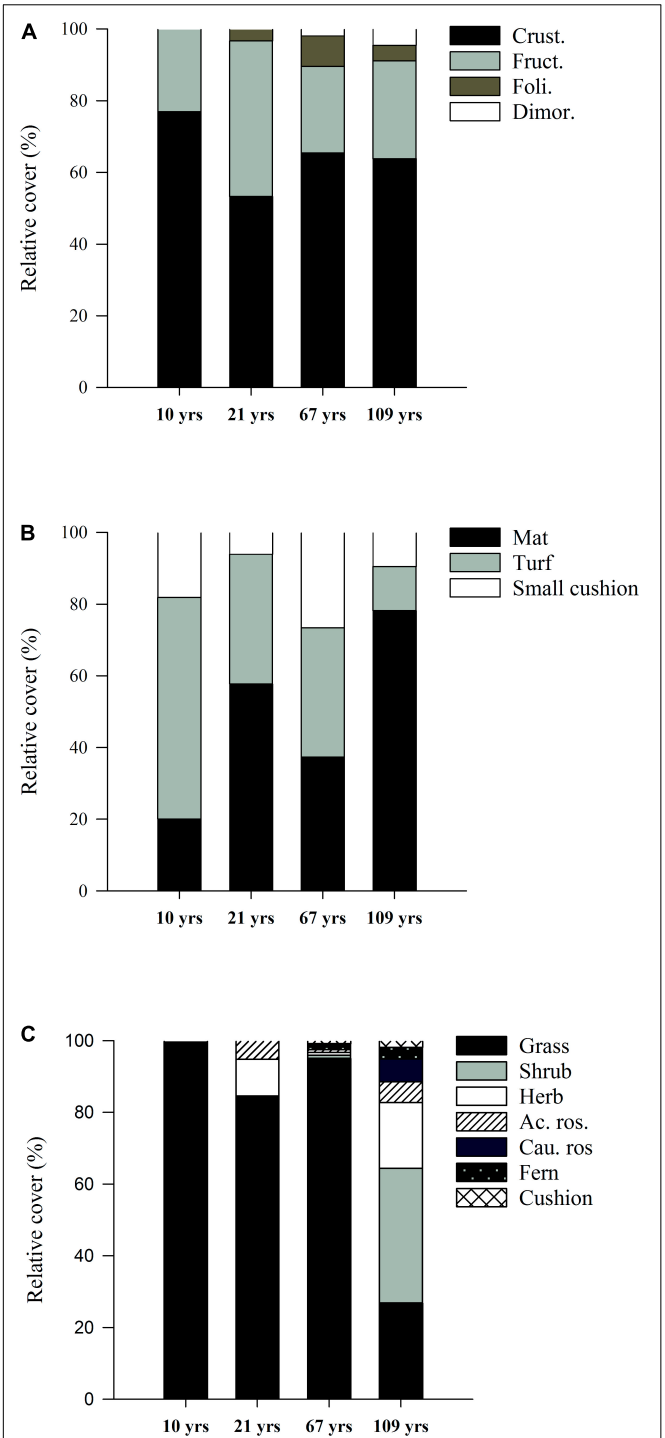


FIGURE 4 | Average relative cover (%) of the different growth forms of lichens **(A)**, bryophytes **(B)** and vascular plants **(C)** in four study sites (S1-S4) along a post-glacial chronosequence (10–109 years of succession) at Humboldt Peak, Venezuela.

could reduce soil instability and soil water limitations associated with daily freeze-thaw cycles (Pérez, 1995; Ramírez et al., 2015).

However, in agreement with what has been frequently reported for glacial primary succession (Matthews, 1992; Walker and del Moral, 2003; Pratch and Walker, 2020; Rosero et al., 2021), we found a clear successional increase in superficial SOM and N_t. Values were relatively high considering the limiting conditions for soil development in the study area (e.g., steep slopes with hard exposed metamorphic bedrock and low vegetation cover), although they remained below typical values reported in the superpáramo belt in Venezuela between 4,000 and 4,400 m asl (Malagón, 1982; Pérez, 1995). The accumulation of soil N_t could be partly due to the presence of several species of lichenized fungi which are known to involve nitrogen fixing cyanobacteria as primary (e.g., *Sticta*, *Peltigera*, and *Cora*) or secondary photobiont (e.g., species of *Stereocaulon* and *Placopsis*; Rikkinen, 2002; Lücking et al., 2013).

Interestingly, Ca and Mg showed higher values in the two earliest sites and then sharply declined. These initial high content of Ca and Mg could be linked with the presence of amphibolite rocks rich in these cations in the study area (Schubert, 1975). The successional decline of Ca and Mg could be associated with their accumulation in microbial or plant biomass and/or their loss *via* leaching (given the typically low cation exchange capacity of these sandy soils with low SOM; Malagón, 1982), as reported in other post-glacial succession studies in temperate and tropical sites (Walker and del Moral, 2003; D'Amico et al., 2014; Whelan and Bach, 2017; Anthelme et al., 2021).

Patterns of Vegetation Assembly

Lichens strongly dominated the two early sites (mostly saxicolous species) and shared dominance with bryophytes in Site 3 (67 years, mostly terricolous species present within BSCs). Vascular plants only became the dominant structural component of vegetation after one century of succession (see **Figures 2**, **3**). A similar pattern was reported by Sklenář et al. (2010) in volcanic sere in Cotopaxi, while Rosero et al. (2021) found a relative dominance of bryophytes over lichens and vascular plants during the first 65 years of post-glacial succession in a vanishing glacier in Carihuairazo (both in Ecuador). In agreement with our 2nd prediction, plant species richness increased during the first one hundred years of succession, which paralleled the observed increase in SOM and plant cover. This has been reported in most primary succession studies in glacier forefields, at least for sites deglaciated after the LIA (Zimmer et al., 2018; Pratch and Walker, 2020; but see Anthelme et al., 2021). However, in the case of lichens, total richness peaked after 67 years, but then declined (see also Sklenář et al., 2010).

The general increase in species richness can be linked with the documented predominance of species accumulation over turnover during the initial stages of post-glacial succession (Svoboda and Henry, 1987; Walker and del Moral, 2003; Pratch and Walker, 2020). This was the pattern observed for vascular plants: species accumulation with almost no species loss; of the 36 species recorded across the four sites, 34 species were present in Site 4, with only two high elevation *Draba* species missing (see **Supplementary Figure 1**). This was confirmed by decomposing multisite β-diversity in its

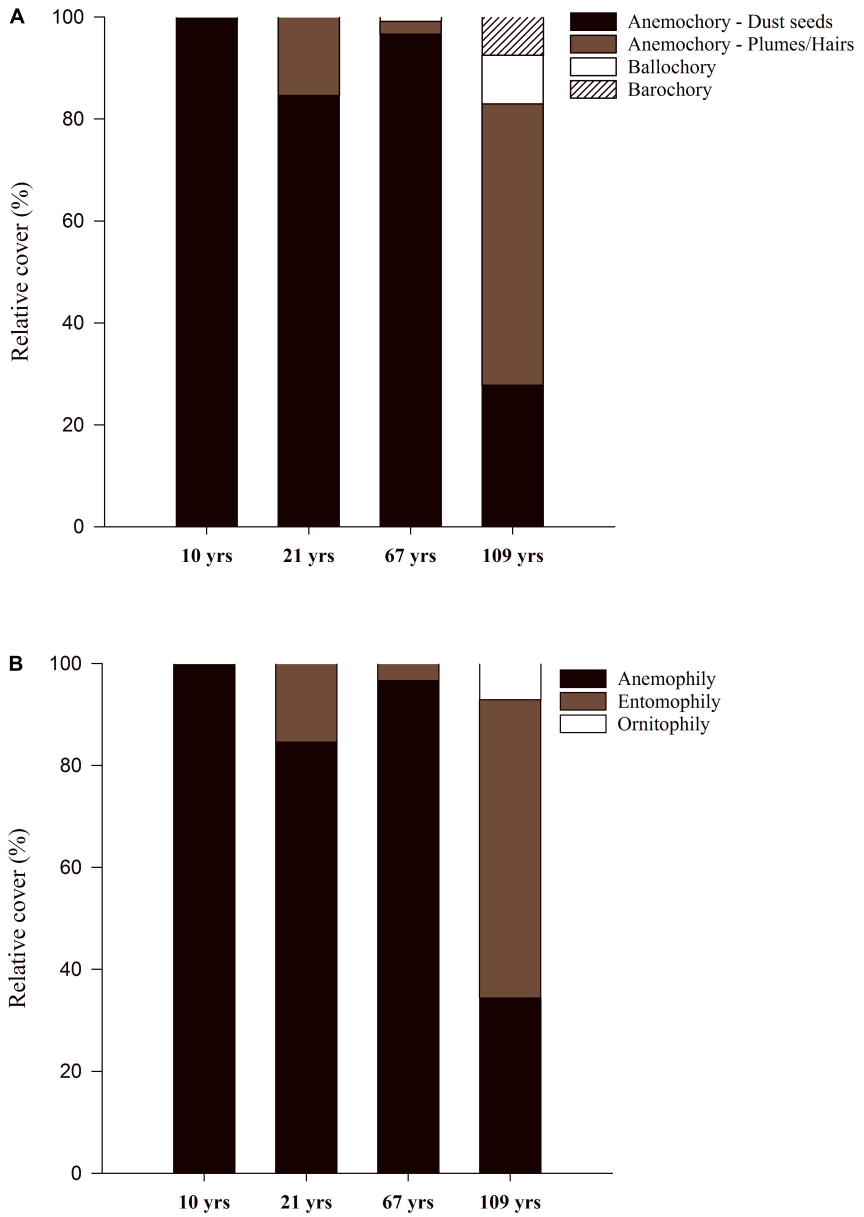


FIGURE 5 | Average relative cover (%) of vascular plant species with different dispersal **(A)** and pollination **(B)** syndromes in four study sites (S1-S4) along a post-glacial chronosequence (10–109 years of succession) at Humboldt Peak, Venezuela.

nestedness and turnover components, with a limited between-site turnover between sites, corresponding to only 17.5% of total β-diversity.

Interestingly, the response was very different when lichens and bryophytes were considered (as proposed in our 2nd prediction). Multisite β-diversity was lower for lichens and bryophytes, but species turnover accounted for a much higher proportion of it, representing 47% of total β-diversity for lichens and 69% for bryophytes. This also resulted in a higher turnover component when all three groups of organisms were analyzed together than when vascular plants were analyzed separately. Both a higher turnover for bryophytes and lichens, and the decline of total species richness during late succession for lichens, suggest that conditions can become less favorable as succession proceeds for some specialist early-succession cryptogamic species. This could be the result of increased competition (among them, and also with vascular plants), and/or a reduced availability of suitable micro-habitats (e.g., open rocky sites for pioneer saxicolous species). Hence, as glaciers completely disappear and vegetation develops, some specialist species (e.g., mosses like *Molendoa peruviana* and *Grimmia fuscolutea*) could be lost in the long-term from these periglacial environments (see review by Cauvy-Fraunié and Dangles, 2019).

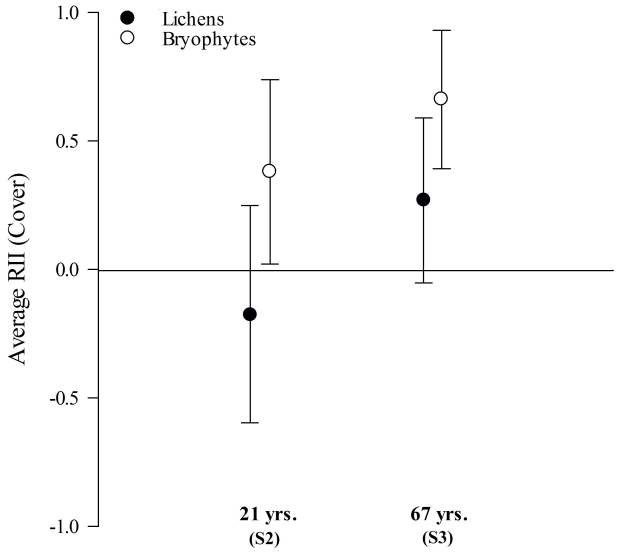


FIGURE 6 | Mean relative interaction index (RII) and 95% confidence interval for the total cover of lichens (black circles) and bryophytes (white circles) in paired sampled in rings (n = 40) centered on mature individuals of the pioneer grass *Poa petrosa* vs. random sites outside, in two study sites (S2-S3) along a post-glacial chronosequence (21 and 67 years) at Humboldt Peak, Venezuela.

Changes in Functional Strategies

In the case of lichens and vascular plants, there was a clear increase in growth-form richness, which was maximum after 109 years (hypothesis 2), resulting from a progressive accumulation of growth-forms. This was clearly reflected by decomposing β-diversity in its nestedness and turnover components for the presence-absence of growth-forms, with nestedness accounting for 100% of total β-diversity in this case. For lichens, only two growth forms were present in Site 1 (10 years), while four different growth forms were found after 109 years. However, all sites were dominated by crustose species, with fruticose lichens being sub-dominant. In contrast, Sklenář et al. (2010) found fruticose lichens to dominate their youngest lahar sites, while foliose lichens were more abundant in intermediate stages, possibly as a result of more developed soils and a lower cover of exposed bedrock in their older volcanic study region. In the case of bryophytes, we observed no overall change in the number of growth forms present, but turfs showed a higher relative abundance during early succession. This growth-form is typical in disturbed, sun-exposed areas with high light intensity and low humidity (Vanderpoorten and Goffinet, 2009). In contrast, mats increased their relative cover in the late succession site, possibly as a result of lower sun exposure associated with much higher vascular plant cover in this site.

For vascular plants, Site 4 showed a much higher functional diversity than the three earlier sites. Grasses constituted by far the most important early-succession growth form, strongly dominating the first three sites, while a few non-graminoid herbs, small shrubs and acaulescent rosettes (all Asteraceae) were able to colonize Sites 1 and 2, but showed extremely low cover values. Grasses, non-graminoid herbs and small shrubs from these two plant families (Poaceae and Asteraceae) have also been found to be among the most important ruderal species in available post-glacial succession studies in the tropical Andes (Suárez et al., 2015; Zimmer et al., 2018; Anthelme et al., 2021; Rosero et al., 2021).

The dominance of grasses during early succession in our high elevation sites could be associated with them including many of the most tolerant species to low/freezing temperatures and seasonal drought in the alpine tropics (Márquez et al., 2006; Rada et al., 2019). In Site 4 vegetation showed higher functional diversity, with seven different growth forms and a more even distribution of relative abundance. Here shrubs, grasses and non-graminoid herbs were the most abundant, while the cover of giant rosettes and cushion plants remained low compared to typical superpáramo sites at these elevations in the Venezuelan Andes (Cáceres et al., 2015; Cuesta et al., 2017; Hupp et al., 2017; Mora et al., 2019).

The strong dominance of ruderal grasses in the first six decades of succession was associated with the prevalence of anemochoric dispersal *via* small dust seeds and anemophilous pollination syndromes. Plants with specialized structures for seed dispersal (hairs/plumes), like those of many Asteraceae, became dominant in Site 4 (109 years). In this site, plants with entomophilous pollination syndromes became dominant, while ornithophilous species were also present. No zoochoric species were found in any of our study sites, although they can have higher relative importance in alpine communities at lower elevations across the tropical Andes (see Tovar et al., 2020). These results support our 3rd prediction regarding the low prevalence seed dispersal and pollination mediated by insects and birds in early post-glacial succession, which has been reported both in tropical and temperate alpine seres (Walker and del Moral, 2003; Jones and del Moral, 2009; Erschbamer and Mayer, 2011; Zimmer et al., 2018; Anthelme et al., 2021; Rosero et al., 2021).

Spatial Association Between Lichens/Bryophytes and Ruderal Plants

Our plant-centered analysis of favorable micro-sites for the colonization of the dominant ruderal grass (*Poa petrosa*), indicated a significant spatial association with the local cover of bryophytes, but not with lichens when analyzed as a group. Even so, when their cover was analyzed at the species level, we did find several bryophyte and lichenized fungi species that were significantly associated with *P. petrosa*, supporting our 4th prediction. Most of these species were dominant within BSCs, and three of the lichens are known to have atmospheric N-fixing photobionts (Rikkinen, 2002; Lücking et al., 2013). Hence, our results support the idea that in extreme tropical alpine areas, facilitation mediated by BSCs, more than by nurse plants, could be an important mechanism for the establishment of ruderal plants (see also Zimmer et al., 2018). In fact, growth forms reported to act as effective nurse plants in superpáramo ecosystems were absent or rare in our three early successional sites (i.e., compact shrubs, cushions and giant rosettes, see Cáceres et al., 2015; Hupp et al., 2017; Mora et al., 2019).

However, without further experimental evidence, we cannot fully rule out the alternative explanation of shared local habitat preferences between lichens/bryophytes and *P. petrosa*, although the paired sampling design used should have helped to reduce the likeliness of this alternative explanation.

CONCLUSION

The results presented here emphasize the key role played by lichens and bryophytes as pioneer photosynthetic organisms in these high tropical alpine ecosystems, and their potential for ameliorating local conditions within BSCs (buffering superficial soil temperature changes). Their local spatial association with the most abundant grass during early succession also supports the idea that they can facilitate the colonization of ruderal vascular plants (see review in Belnap and Lange, 2001). Moreover, while vascular plants exhibited a response characteristic of a direct succession, with a progressive accumulation of species and limited species relay (Walker and del Moral, 2003), there was a higher species turnover in the case of lichens and bryophytes, indicating that different mechanisms, including competitive exclusion, could drive their long-term successional response. This could imply the loss of some pioneer specialists from periglacial environments where a denser vegetation cover develops over time, such as saxicolous species characteristic of rocky open sites (Cauvy-Fraunié and Dangles, 2019).

More generally, our results indicate that under the extreme conditions associated with the last stages of glacier retreat in the northern Andes, ecosystem development can face important barriers, particularly during early seral stages (see also Zimmer et al., 2018; Anthelme et al., 2021). The limited initial colonization of vascular plants and the progressive accumulation of species and growth-forms during the first 100 years of succession (i.e., a nested pattern characteristic of direct succession) could be linked with a combination of: (a) strong dispersal limitations, with a complete absence of animal dispersed vascular plants; (b) severe environmental filtering, with ruderal vascular plants being strongly dominated by stress tolerant grasses; and (c) limitations in the effectiveness of positive biotic interactions, with a complete absence of facilitation mediated by nurse-plants and of insect/bird pollination during early succession. This could have important implications for understanding climate change impacts in high tropical alpine ecosystems, especially near mountain summits. In particular, these results suggests that the projected colonization and upward shifts in the distribution of high alpine communities and plant species linked with global warming (Lenoir and Svenning, 2015) could face important abiotic and biotic limitations in the forefields of vanishing glaciers, which need to be more explicitly incorporated into prospective/predictive models of vegetation dynamics (e.g., Tovar et al., 2013).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LL and AM conceived the project. LL, AM, MC, LG, RP, and CR designed the study. LL, AM, LG, MC, CR, RP, JT, and BH collected field data. NR, AM, and LL mapped the chronosequence. LG, MC, CR, and JH carried out all taxonomic identifications. LL and MC performed statistical analyses and wrote the first draft of the manuscript. All authors contributed to the discussion and interpretation of data, revised and complemented the manuscript and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.657755/full#supplementary-material

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