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Change in relative abundances, more so than species turnover, modulates β -diversity and network complexity in communities of eukaryotic microorganisms from glacial to periglacial environments

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The cryosphere is facing unprecedented changes due to global change, and one of its main consequences is on the connectivity of ice-snow and downstream habitats in the glacial and periglacial landscape. Here, we analyze the eukaryotic community composition and the ecological processes structuring eukaryotic communities in the glacial environment of Mount Tronador (North-Patagonia, Argentina). The study was carried out in Castaño Overo and Alerce glaciers, including three different compartments: glacial ice, snow, and ponds in the glacier forefield. All compartments showed low dissolved and particulate nutrient concentrations. Genomic DNA analyses revealed a high eukaryotic microorganism's diversity, and a high proportion of shared taxa (>35%), resulting in low presence-absence β -diversity. However, abundance-based β -diversity is higher among compartments, underscoring that changes in abundances are significant regardless of the large number of shared ASVs. Ice and snow showed a predominance of Fungi, while Chlorophyta increased its importance in ponds. Ciliophora and Dinoflagellates were almost exclusively present in ice and ponds, respectively. The co-occurrence network analysis showed a higher number of modules and modularity in ice and snow relative to ponds. The presence of modules with algae and fungi (Puccinmycotina and Chytridiomycotina) would reflect a functional association due to the nutritional dependence of fungi on the algae. More connectors between modules were detected in ice and snow (5 and 6) relative to ponds (only 1). Most of these connectors were fungi that could also indicate their dependence on more than one species association (module). Overall, our results showed that as ice and snow melt due to global change, eukaryotes will undergo community reorganization that will affect downstream environments.

KEYWORDS

eukaryotic communities, glacier, snow, glacial forefield, eukaryotic diversity, network analysis, North-Patagonian Andes

Introduction

Microeukaryotes in glacial and periglacial systems are a fascinating topic because these ecosystems, though extreme, host a variety of microbial life that has adapted to harsh conditions (Anesio et al., 2017). The eukaryotic organisms found in glaciers and ice-covered environments primarily include protists, fungi, and some algae, many of which are adapted to survive in low temperatures, nutrient-poor conditions, and high levels of ultraviolet (UV) radiation (Anesio and Laybourn-Parry, 2012).

In mountain regions, changes in near-surface temperature and in precipitation patterns lead to a decrease in snow accumulation, earlier spring melt, and prolonged summer ice melt (Fell et al., 2017). Consequently, these changes in the distribution, thickness, and permanence of ice sheets result in the retreat and thinning of many mountain glaciers (Zemp et al., 2015). For the year 2100, the estimated loss in glacier area is projected to range from 22 to 51%, depending on the climate scenario considered (Bosson et al., 2023). The retreat and imminent loss of glaciers have physical, chemical, and biological implications for the aquatic ecosystems receiving their meltwater (Vanderwall et al., 2024). In addition, the physical processes associated with glacial retreat and meltwater runoff erode the rock, modeling a new landscape that includes the formation of lakes and a still largely unexplored periglacial environment.

The periglacial environment is defined as that in which frost action and/or permafrost related processes dominate (French and Thorn, 2006). In addition, proglacial environments are located immediately in front of glaciers, ice caps, and ice sheets (Slaymaker, 2011). However, the criteria of ice-marginal location and intensive freeze-thaw and/or permafrost related processes are not exclusive of each other, and proglacial and periglacial environments commonly overlap (Slaymaker, 2011). Frozen water is an important erosive agent in these environments, as ice pressure can break the rock, allowing the formation of ponds (Martini et al., 2011). In particular, during the warm season, substantial melting of glaciers and snow contributes with water, nutrients, and microbial communities to downstream environments, where they play a crucial role in biogeochemical cycling (Anesio and Laybourn-Parry, 2012). These cold ecosystems are especially sensitive to climate change; thus, understanding the role of microbial life in these habitats is crucial in scenarios of glacier retreat and permafrost thawing (Margesin and Collins, 2019; Winkel et al., 2022).

Mountain peri/proglacial regions are changing rapidly due to loss of snow and ice, and this will produce important environmental changes, including physical parameters (temperature and light), chemical (nutrients), and biological (element stoichiometry, microorganisms' diversity) (Ren et al., 2019; Elser et al., 2020; Vanderwall et al., 2024). In addition to ice melting, snow melt can also influence lake nutrient inputs (Vincent et al., 2011) and snow microbiome (Krug et al., 2020; Yakimovich et al., 2020) can remain in the active fraction of downstream aquatic environments (Comte et al., 2018). Thus, these cold environments represent an

interesting spatial pattern of ice-snow-ponds of hydrological interaction, where microorganisms colonize the different water states.

Eukaryotic communities in glacial and peri/proglacial environments are dominated by heterotrophic microorganisms such as fungi and protozoa (flagellates and ciliates), and by autotrophs with algae such as Chlorophyceae, Zygnematomyceae, Dinophyceae, and Bacillariophyceae (Anesio and Laybourn-Parry, 2012). Microorganisms in glacial habitats exhibit metabolic adaptations such as the production of cold-active enzymes, antifreeze proteins, and exopolymeric substances that protect against low temperatures (Margesin and Miteva, 2011). These communities remain biogeochemically active, contributing to processes like carbon fixation, iron cycling, and methanogenesis, with implications at both local and global scales (Hood et al., 2009). Most studies on eukaryotic diversity in these cold environments have been carried out in the Arctic and Antarctic (Laybourn-Parry and Pearce, 2007; Cameron et al., 2015; Comte et al., 2018), in snow packs (Brown and Jumpponen, 2019; Vimercati et al., 2019), and in high altitude lakes (Triadó-Margarit and Casamayor, 2012; Kammerlander et al., 2015; Filker et al., 2016). Despite its ecological importance in nutrients and elements cycling (Anesio and Laybourn-Parry, 2012), our knowledge about eukaryotic communities from mountain glacial and periglacial environments is still scarce, and this is of particular importance since, as glaciers continue to recede, these communities will be gradually more vulnerable and lost (Vimercati et al., 2019). Studies of the connectivity and biogeography of the snow microbiome (Comte et al., 2018; Brown and Jumpponen, 2019) and biogeographic analyses of glacial lakes (Filker et al., 2016; Schiaffino et al., 2020) have indicated differences in community structure among regions, but little attention has been given to spatial patterns within connected hydrologic systems. Solid water environments such as glacial ice and snow packs can promote spatially structured microbial microhabitats and increase ecological complexity compared to the more homogeneous and diluted conditions typically found in meltwater ponds (Bachy et al., 2011; Anesio et al., 2017).

This study aims to analyze the eukaryotic communities of a glacial and periglacial environment of North Patagonian Andes. Glacial ice and snowpacks can sustain distinct microbial microhabitats (Bachy et al., 2011), whereas meltwater typically results in more diluted and mixed conditions. Based on these contrasting physicochemical settings, we hypothesize that the eukaryotic community varies among these hydrologically connected environments according to water state and physical heterogeneity. Specifically, we predict a more complex community structure in solid water (ice and snow) than in meltwater ponds.

To evaluate this, we analyzed eukaryotic community dissimilarities, diversity indices, diversity components, and performed a network analysis in the ice-snow-ponds hydrological system. The present study was carried out in Mount Tronador, the most important ice cap in the North Patagonian Andes, with

different glaciers with radial flow (Ruiz et al., 2015). The retreat of Tronador glaciers has been well documented over recent decades (Ruiz et al., 2017). Samples were collected from ice and snow of the Alerce and Castaño Overo glaciers, and from ponds located in the glacier forefield.

Materials and methods

Study site and sampling

Mount Tronador is an inactive stratovolcano located in the North Patagonian Andes (41.15°S, 71.88°W), and has a height of 3478 m (Figure 1). The mountain straddles the border between Chile and Argentina. The upper slopes of Mount Tronador host the accumulation zones of 13 major glaciers, 5 of which are located in Argentina (Zorzut et al., 2020). In the last decades, the equilibrium line altitude changed due to increasing warming trends (Ruiz et al., 2017; Ghilardi Truffa and Ruiz, 2024). Thus, its glaciers have exhibited a continuous retreat trend (Ruiz et al., 2017; Masiokas et al., 2020). This retreat leaves new landscapes, modeling the existing ones and generating new aquatic environments, such as lakes and periglacial ponds (Ruiz et al., 2017; Modenutti et al., 2018, this study).

The study was carried out in Alerce and Castaño Overo glaciers (Argentina), where samples of glacial ice and snow were obtained. In addition, ponds in the glacier forefield at circa 1900 m a.s.l. were also sampled (Figure 1A). Sampling was carried out in summer 2021–2022. An ice drill (IDDO Hand Auger Kit) was used to obtain the ice core samples. This equipment is specially designed for boring and extraction in remote, high mountain locations. We sampled two ice sites, one in Alerce and the other in Castaño Overo glaciers (Figure 1A). At each sampling site, two cores (90 cm long) were obtained, and the upper 5 cm were discarded to avoid contamination from the equipment head. The remaining core sections were carried to the laboratory in sterile bags, divided into two portions. Considering the different sections and replicates, we analyzed 8 ice samples.

The snow samples were collected in four sampling sites (2 samples per site) located on both mentioned glaciers (Figure 1A). At each site, an area of 1 m² of virgin snow cover was delimited, and the surface layer (10 cm) was collected in Nalgene® bottles with sterile gloves. Considering all sites, we analyzed 8 snow samples.

Finally, we obtained water samples from 3 ponds located in the glacier forefield (Figure 1A). These ponds resulted from proglacial processes that occurred around and within the margins of the former glacier and produced the formation of ponds scoured into bedrock (Figures 1B–D). Surface water samples (two samples per pond) were taken using sterile 1L Nalgene® containers. Thus, a total of 6 water samples were analyzed.

All samples were transported in thermally insulated containers until they arrived at the laboratory, where they were stored at -80°C until processing.

Laboratory procedures for chemical parameters

Sample processing consisted of filtering the water from the different sources (glacial ice, snow, and ponds). To determine the concentrations of total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), and dissolved organic carbon (DOC) we filtered onto pre-combusted GF/F filters. The TDP samples were digested with potassium persulfate at 125 °C and 1.5 atm, and then the concentration was determined using the ascorbic acid reduction method with molybdenum (APHA, 2005). DOC was measured in 50 mL of sample using a high-combustion analyzer (Shimadzu TOC-VCSH) and TDN was determined using the TNM-1 unit on the Shimadzu TOC-VCSH.

In addition, unfiltered water samples (ice, snow, and ponds) were used for total phosphorus (TP) and total suspended solids (TSS) concentration determinations. TP was determined following APHA (2005) as explained for TDP, except for the filtering. TSS was quantified by filtration of 200 mL of unfiltered water onto GF/F filters previously combusted and weighed. Then, filters were dried for at least 48 h at 60°C and then reweighed. Particulate C and particulate N were measured on precombusted GF/F filters (200 mL) and processed in a High Temperature Combustion elemental analyzer (Thermo Finnigan EA1112).

DNA extraction, and amplicon sequencing

Water samples (250–300 mL) from different sources (ice, snow, and ponds) were filtered through 0.22 µm pore-size nucleopore filters (PALL®) for DNA extraction. The filters were frozen at -80 °C until DNA extraction.

Genomic DNA was extracted using Qiagen DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. The quantity and purity of DNA samples were determined with a Take3 Micro-Volume Plate (BioTek® Instruments, Inc., Winooski, VT, USA) in a Synergy™ HTX Multi-Mode Microplate Reader. The extracted DNA was stored at -80°C for further processing. The extracted DNA was sent to Novogene Bioinformatics Technology Co. (Beijing, China) to be processed using Illumina, the sequencing platform and strategy used was NovaSeq 6000 PE250.

To determine the eukaryotic community diversity, we used the tagged amplicons of the V4 region of 18S rRNA eukaryal gene obtained with the primers 528F (5'-GCGGTAATTCAGCTC CAA-3') and 706R (5'-AATCCRAGAATTTACCTCT-3') 350bp (Cheung et al., 2010). These primers amplify a specific hypervariable region of the eukaryotic 18S ribosomal RNA (rRNA) gene, which is widely used as a genetic marker for eukaryotic microorganisms. The 18S gene contains conserved regions (for primer binding) flanking variable regions that provide taxonomic resolution (Pawlowski et al., 2012). Amplification, library preparation, sequencing and demultiplexing

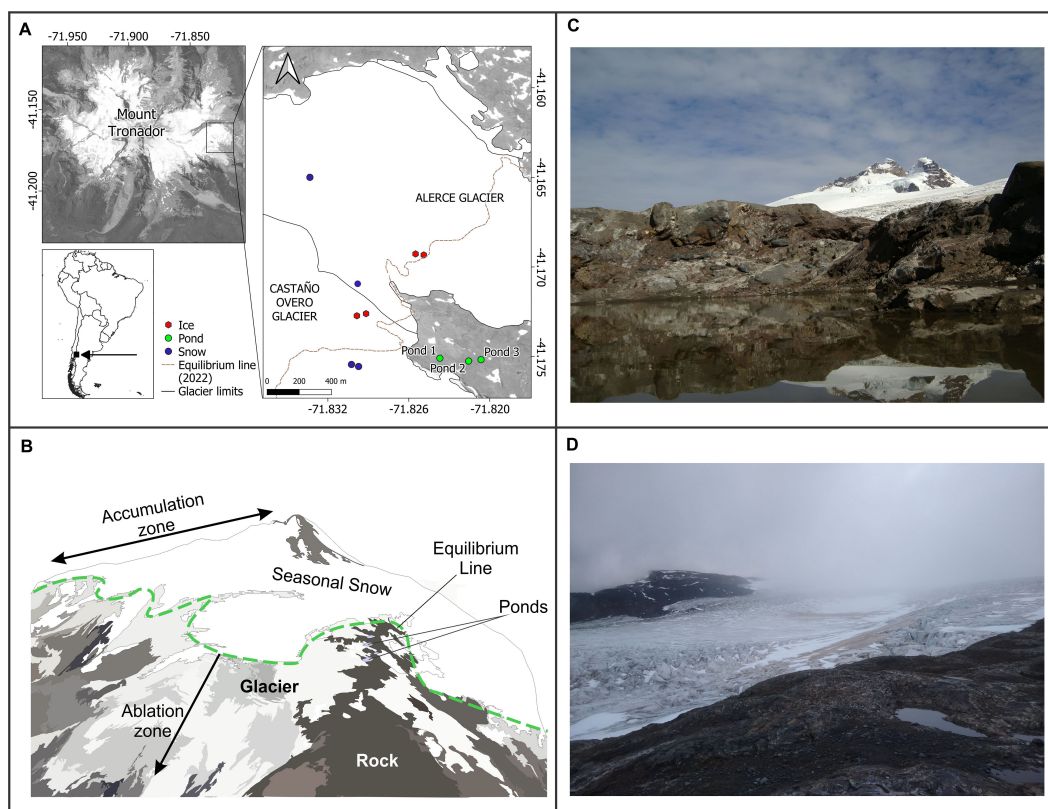


FIGURE 1

Map of the studied area. Sampling sites in the three glacial and periglacial environments (Glacial Ice, Snow and Ponds) are indicated with dots. (A) Map, (B) Scheme of the Tronador Mountain, (C) Photographs of Pond 2, and (D): Glacier Castaño Overa and Pond 1.

were conducted by Novogene Bioinformatics Technology Co. The PCR conditions informed by Novogene Bioinformatics Technology Co. were: an initial denaturation of 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C, and a final cycle of 5 min at 72°C.

Processing of illumina sequencing data

Amplicon sequences provided by Novogene Bioinformatics Technology Co. were processed using DADA2 v1.22.0. The entire sequencing project was analyzed with the same filtering parameters following Callahan et al. (2016). We used the filterAndTrim function from DADA2 with the following quality values: maxEE = c(2,2) and truncLen = c(220,200). The chimera sequences were excluded using the function removeBimeraDenovo. Sequences were identified as unique amplicon sequence variants (ASV) based on single nucleotide differences. The taxonomic classification was performed using the PR2 database (version 2.0.2) as a reference. A second table was generated by discarding ASVs with fewer than 20 reads. Multicellular organisms were also eliminated to avoid bias due to multiple copies of the 18S rRNA gene, when they were amplified from the same organism. This last table was normalized to a sampling depth using the rarefy function of the vegan package (Oksanen et al., 2013). To have a lower taxonomic level, the

taxonomic search for some ASVs was carried out on the National Center for Biotechnology Information (NCBI) website, introducing the sequence corresponding to each ASV in the tool “blastn.” The sequencing data have been deposited in NCBI BioProject PRJNA1270387 with the Biosample accessions SAMN48824426-47.

Data analysis

Nutrients were compared among systems (Glacial ice, snow, and ponds) with a one-way ANOVA or Kruskal-Wallis test when normality or homoscedasticity were not fulfilled.

We constructed rarefaction curves to analyze the depth of sequencing and evaluate whether the richness of ASVs of each sample was adequate (Supplementary Figure S1). The obtained rarefaction matrix of ASVs was based on an iterative process to bring all samples to the smallest number of readings. These ASVs were used as taxonomic identities (see *Processing of Illumina sequencing data*) for the calculations of diversity indices, and network analysis.

To test our hypothesis, we calculated diversity indices, community dissimilarities, and performed network analysis as follows. We calculated alpha diversity indices (Shannon-Wiener index), ASV richness, and Pielou's evenness index. Eukaryotic community dissimilarities were visualized using dendrograms and

non-metric multidimensional scaling (NMDS) analyses based on Bray-Curtis distance with the total ASVs in 22 samples, followed by an analysis of similarities (ANOSIM) to determine if there were significant differences among the groups generated by the NMDS analysis. All the above statistical analyses and calculations were performed in R (v. 4.4.3) (R Core Team, 2025) with vegan v. 2.6.1. Heatmaps were constructed using pheatmap v.1.0.12, while Venn diagrams were created using the VennDiagram package v.1.7.3.

Total eukaryotic β -diversity was calculated using the Sørensen dissimilarity index (β_{SOR}) and total Bray-Curtis dissimilarity index (β_{BC}), applied to presence-absence and abundance data, respectively. Then, we applied the Baselga β diversity partitioning (Baselga and Orme, 2012; Baselga and Chao, 2017) by calculating the contributions of turnover (β_{SIM} ; Simpson dissimilarity index) and nestedness (β_{NES}) to the presence/absence dataset. In addition, we analyzed the contributions of balanced-variation ($\beta_{BC,BAL}$) and abundance-gradient ($\beta_{BC,GRA}$) components to the abundance dataset. The dissimilarity indices of eukaryotic communities were calculated in R, using the function 'beta.pair' and 'beta.pair.abund' in the R package 'betapart' (v1.6). Total eukaryotic β -diversity and its partitioning were analyzed by comparing the systems: a) glacial ice-snow, b) snow-ponds, and c) glacial ice-ponds.

We generated null models of eukaryotic communities in the periglacial environments for both presence-absence and abundance data. In the former, we applied the functions 'nullmodel' and 'simulate' of the package 'vegan', with a non-sequential algorithm for binary matrices that preserves the site (row) frequencies (option 'r1'). In the abundance null models, we used the function 'taxo.null' of the package 'NST' (v3.1.10), with the abundance option 'region'. In both cases, we generated 1000 null models, we estimated the distance matrices (β -diversity) and then we calculated the average distance matrix for each kind of index. Then, we compared the β diversity of the observed datasets (glacial ice, snow, and ponds) with those of the corresponding null model with a t-test.

Network analysis was conducted for each environment (glacial ice, snow, and ponds) to determine the co-occurrence patterns among the eukaryotic taxa and to identify key ASVs or significant module members in eukaryotic communities (Guimera and Amaral, 2005). A module is a group of nodes (ASVs) with high interactions among them and low interactions with other nodes outside the module; while modularity measures how well a network separates into different modules (Deng et al., 2012). Modularity may indicate habitat heterogeneity and an increase in modularity seemed to reflect more intensive functional associations (Zhou et al., 2021). Analysis was performed using the Igraph package (v2.0.3) and MetaNet (v0.1.2) in R (v.4.4.3). A similarity matrix was constructed based on Pearson correlation coefficient between pairwise ASVs. The edge list was constructed based on the similarity matrix considering only absolute values > 0.8. The different roles of each node in the network were identified using the values that measure within-module connectivity (Z_i) and the values that measure among-module connectivity (P_i). Then, we develop Z_i - P_i diagrams, which can be used to study key species in a community (Zhou et al., 2011; Deng et al., 2012). Co-occurrence

graphs were constructed with Cytoscape v3.10.3 (Shannon et al., 2003).

Results

Chemical features of the glacial and periglacial environments

Dissolved nutrient concentrations (TDP, TDN and DOC) were significantly different among the glacial environments (Table 1). Glacial ice showed the highest DOC concentration and TDN: TDP ratio, while snow exhibited the highest TDN and TDP concentrations but the lowest TDN: TDP ratio. The ponds showed a very low DOC concentration (Table 1).

The particulate fraction showed a different pattern. C concentration was highest in snow and lowest in glacial ice, similar to particulate N and P, but in the latter, the lowest values were observed in the ponds (Table 2). Elemental ratios showed only significant differences in N:P, with the lowest values in glacial ice (Table 2).

Taxonomic composition of the eukaryotic community in glacial and periglacial environments

After the quality control and rarefaction, we obtained a total of 759550 reads in 1363 ASVs. The eukaryotic communities are dominated by Chlorophyta, fungi, Dinoflagellata, and Ciliophora with different relative abundances in the three systems (Figures 2A, B). Ice and snow showed a predominance of fungi, while in the ponds, Chlorophyta increased its importance. In addition, Ciliophora and Dinoflagellates were present almost exclusively in the ice and the ponds, respectively.

Comparing the three environments, the eukaryotic richness, Shannon diversity, and evenness did not show significant differences (richness: one-way ANOVA, $F_{2,19} = 0.233$, $P = 0.794$; Shannon diversity: one-way ANOVA $F_{2,19} = 1.234$, $P = 0.313$; Evenness: one-way ANOVA, $F_{2,19} = 1.761$, $P = 0.199$). However, in the three parameters, the greatest variation was observed in the

TABLE 1 Dissolved nutrients in the three glacial and periglacial environments (glacial ice, snow and ponds) and results of ANOVA and Kruskal Wallis *post-hoc* analysis.

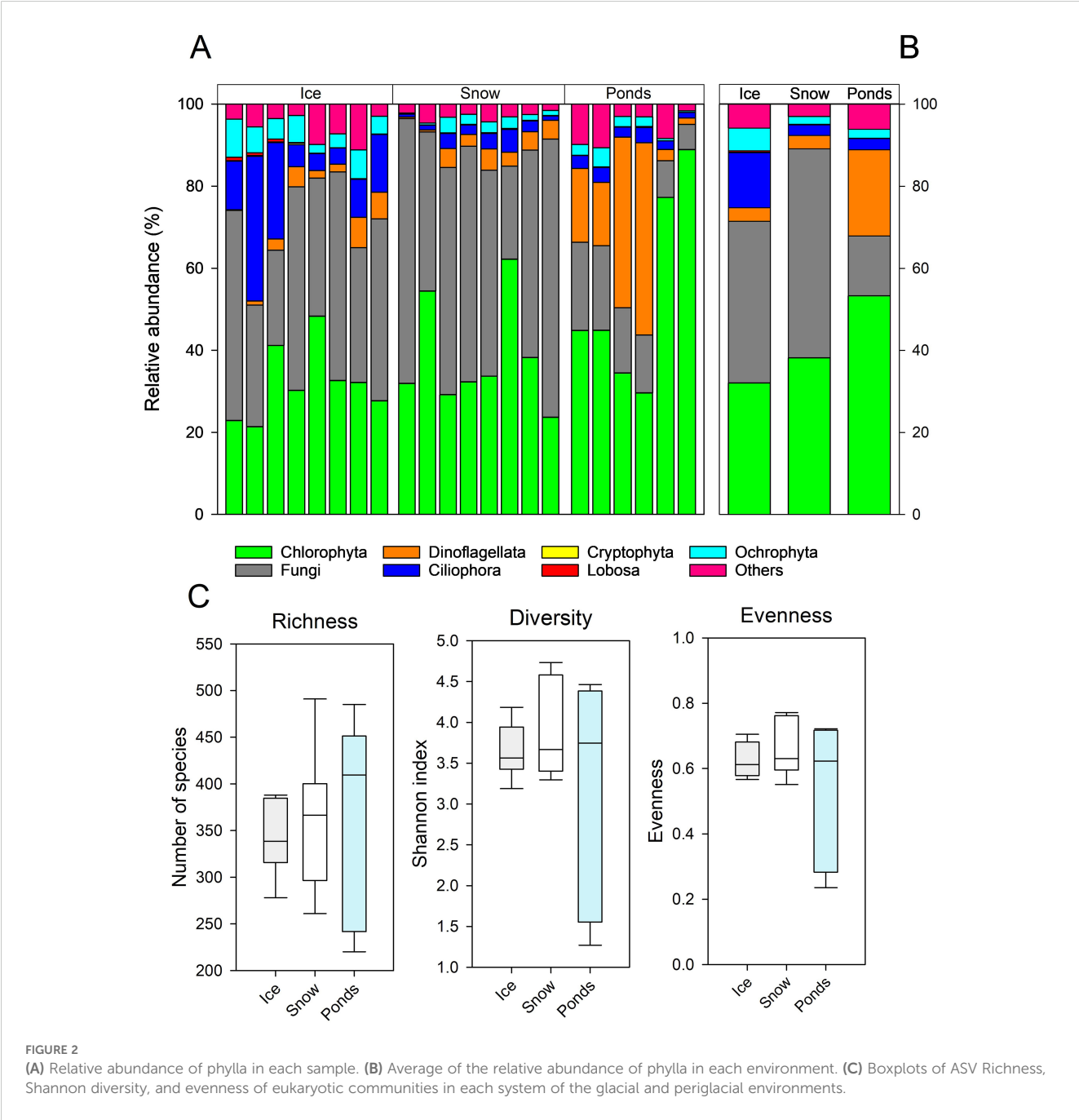
	DOC	TDN	TDP	TDN: TDP
Glacial Ice	19.3 ± 1.19	107.7 ± 17.25	3.3 ± 0.35	74.5 ± 12.11
Snow	14.1 ± 5.87	140.6 ± 28.66	12.9 ± 3.08	25.9 ± 2.85
Ponds	1.5 ± 0.61	120.6 ± 33.08	6.1 ± 1.41	43.3 ± 7.88
	* K-W	NS	** K-W	*** ANOVA

DOC: dissolved organic carbon; TDP: total dissolved phosphorus, TDN: total dissolved nitrogen; NS: non significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 2 Particulate carbon (C), phosphorus (P) and nitrogen (N) and elemental ratios in the three glacial and periglacial environments (glacial ice, snow and ponds) and results of Kruskal Wallis (K-W) *post-hoc* analysis.

	$\mu\text{g C L}^{-1}$	$\mu\text{g N L}^{-1}$	$\mu\text{g P L}^{-1}$	C:P	C:N	N:P
Glacial Ice	452.8 ± 88.5	39.5 ± 13.3	34.3 ± 10.6	93.8 ± 26.7	36.2 ± 8.1	3.0 ± 0.6
Snow	5210.0 ± 1237.2	469.3 ± 89.8	192.6 ± 87.4	128.6 ± 21.0	13.3 ± 1.2	11.8 ± 2.9
Ponds	1253.3 ± 616.6	118.3 ± 58.5	17.2 ± 6.6	176.7 ± 35.8	14.5 ± 3.0	13.8 ± 3.2
	*** K-W	*** K-W	*** K-W	NS	NS	*** K-W

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.



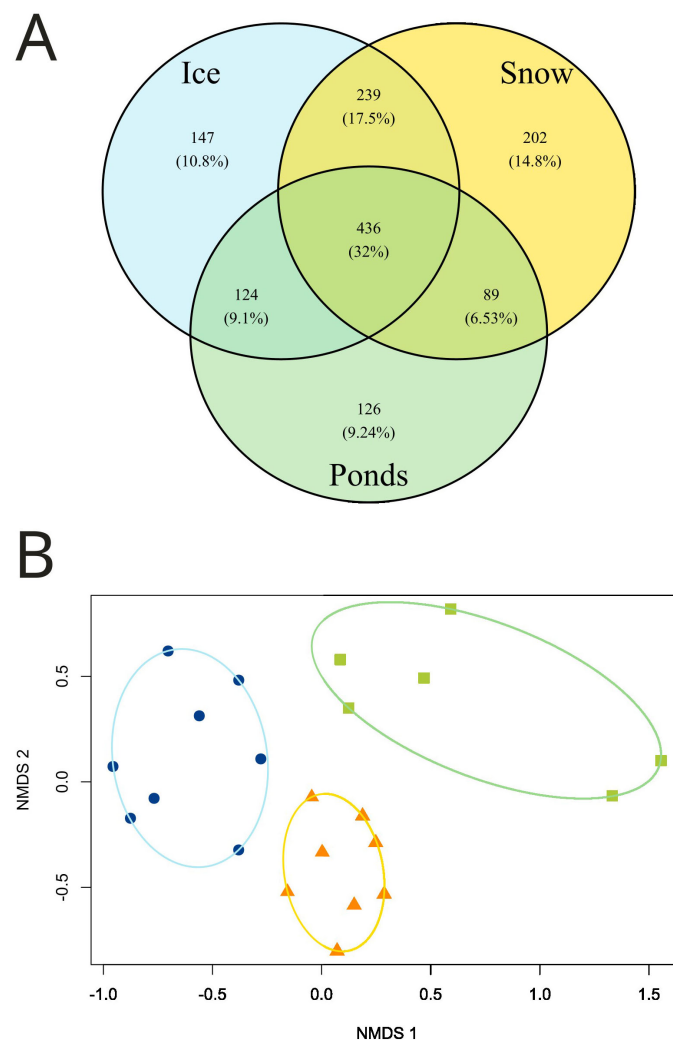


FIGURE 3
(A) Venn diagram of the ASVs present in the glacial and periglacial environments. **(B)** Results of the Nonmetric multidimensional analysis (NMDS) based on Bray–Curtis dissimilarity considering all ASVs present in all samples.

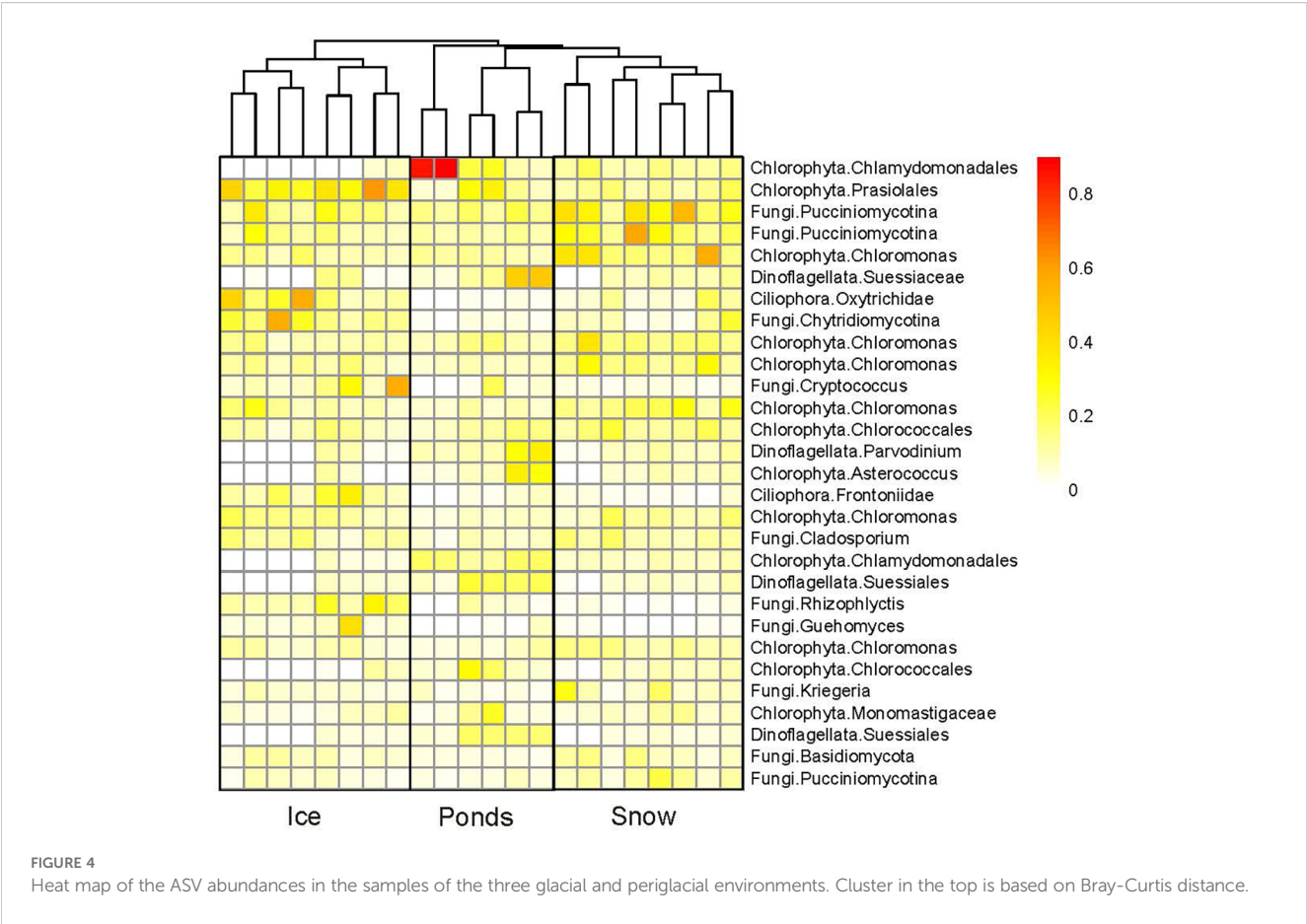
ponds (Figure 2C). Glacial ice and snow presented 946 ASVs and 966 ASVs, respectively, while ponds presented a lower value (775 ASVs). A total of 436 ASVs were shared by the three environments, representing 32% of the total ASVs. A similar percentage of ASVs (34%) were unique for each one of the three environments (Figure 3A).

However, when we analyze communities based on abundance of ASVs (Bray Curtis index), the NMDS ordination analysis showed that the three environments constitute separate groups (Figure 3B). This was confirmed by the ANOSIM analysis, which showed significant differences among the sampled environments (ANOSIM, global $R = 0.70$, $p < 0.001$).

A more deeply analysis of the sequences (heatmap and the cluster analysis in Figure 4) confirmed the separation of the eukaryotic communities in the three environments (ice, snow, and ponds). These partitions resulted from differences in the abundance of the dominant phylotypes: Chlorophyta, fungi, Ciliophora, and Dinoflagellates. The two most abundant ASVs

correspond to algae: Chlamydomonadales and Prasiolales, and contributed to the segregation of the ponds and ice, while the next two correspond to Pucciniomycotina (Fungi) that are mostly exclusive from snow samples (Figure 4). In addition, the following ASVs were also abundant in different environments: ASV5 *Chloromonas* in snow, ASV6 Suessiaceae (Dinoflagellates) in ponds, and ASV7 Oxytrichidae (Ciliophora) in ice.

The analysis of β -diversity indices enables comparison of communities across different environments, while the partitioning of these indices reveals whether changes in community composition are driven by species turnover or differences in relative abundances. The presence/absence dissimilarity analyses of the total β -diversity (β_{SOR}) and turnover (β_{SIM}) showed low values among sectors (all β_{SOR} and $\beta_{SIM} < 0.59$) (Table 3, Supplementary Figure S2). Accordingly, nestedness (β_{NES}) values were very low (< 0.08). On the contrary, values of β -diversity based on ASVs abundance were higher (all β_{BC} and $\beta_{BC,BAL} > 0.75$). In all cases, maximum values were observed when comparing glacial ice and ponds (Table 3).



When we compared the observed β -diversity values of our presence/absence dataset with those generated by the null models, we observed that the differences between glacial ice and snow, and snow and ponds are significant, with an increase in the turnover component (Table 3). On the contrary, in the comparisons based on the abundance dataset, all pairwise comparisons gave significant

results, indicating that although environments share ASVs, the changes in abundance differed more (Table 3, Supplementary Figure S2).

The network analysis was conducted to identify the eukaryotic organism co-occurrence patterns in the three glacial environments (Figure 5A). The glacial ice and snow exhibited higher modularity

TABLE 3 Observed values of β diversity and components among glacial and periglacial environments (glacial ice-snow and ponds) based on presence-absence data (Sorensen index) and abundance data (Bray Curtis index) and the statistical results of the comparison between observed and null models.

Index	Glacial Ice-Snow		Snow-Ponds		Glacial Ice-Ponds	
	Observed	Observed-Null Model	Observed	Observed-Null Model	Observed	Observed-Null Model
β_{SOR}	0.536 $\pm$ 0.006	P<0.001*	0.493 $\pm$ 0.010	P<0.001*	0.593 $\pm$ 0.013	P>0.05
β_{SIM}	0.491 $\pm$ 0.007	P<0.001*	0.408 $\pm$ 0.013	P<0.001*	0.527 $\pm$ 0.014	P>0.05
β_{NES}	0.045 $\pm$ 0.004	P=0.003*	0.088 $\pm$ 0.010	P<0.001*	0.065 $\pm$ 0.005	P>0.05
β_{BC}	0.721 $\pm$ 0.010	P<0.001*	0.762 $\pm$ 0.009	P<0.001*	0.793 $\pm$ 0.014	P<0.001*
$\beta_{\text{BC,BAL}}$	0.639 $\pm$ 0.016	P<0.001*	0.665 $\pm$ 0.020	P<0.001*	0.757 $\pm$ 0.015	P<0.001*
$\beta_{\text{BC,GRA}}$	0.045 $\pm$ 0.008	P<0.001*	0.097 $\pm$ 0.015	P<0.001*	0.035 $\pm$ 0.003	P<0.001*

For details of null models, see methods. Values are average $\pm$ standard error. β_{SOR} : total β diversity based on Sorensen index, β_{SIM} : turnover, β_{NES} : nestedness; β_{BC} : total β diversity based on Bray-Curtis dissimilarity index, $\beta_{\text{BC,BAL}}$: contributions of balanced-variation, $\beta_{\text{BC,GRA}}$: abundance-gradient component. * significant difference.

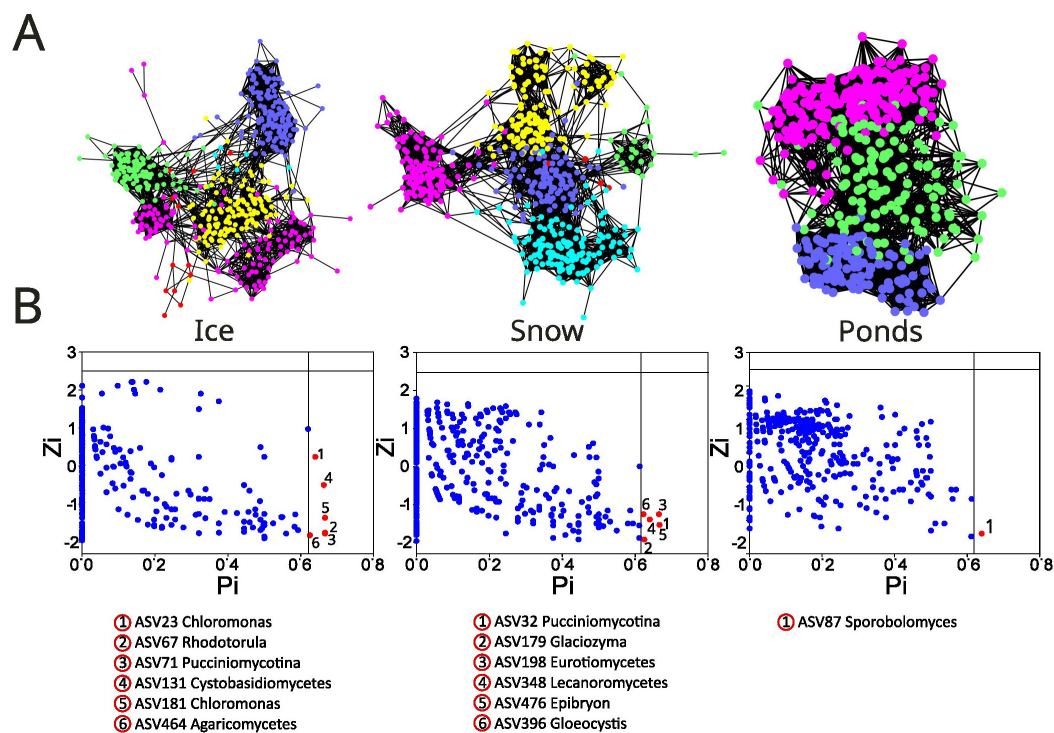


FIGURE 5

(A) Module structures of eukaryote co-occurrence networks in the three glacial and periglacial environments showing changes in modularity.

(B) Topological roles in the network according to the within-module connectivity, Zi, and among-module connectivity, Pi. Blue dots: Peripherals ($Z_i \leq 2.5$ and $P_i \leq 0.62$), and yellow dots: connectors ($Z_i \leq 2.5$ and $P_i > 0.62$). No nodes were categorized as modules and network hubs in any of the three glacial environments ($Z_i > 2.5$).

(0.74 in ice and 0.66 in snow) and module number (6 and 5, respectively). Modularity was lower in the ponds (0.52) and we also observed a low number (3) of modules. In the three systems, we identified modules with more than 100 ASVs. In the ponds, 45% of ASVs do not correspond to any module > 5 ASVs, and more than 25% of the total ASVs co-occurred in only one module. Algae and fungi co-occurred in most of the modules; however, there were modules composed mainly of algae. In particular, module 3 in ice and 2 in snow correspond to Chlamydomonadales, Chrysophyceae, Dinophyceae, among others, that were positively connected.

The topological roles of individual nodes (ASVs) in the network were classified according to the relationship within-module connectivity (Z_i) and among-module connectivity (P_i) (Figure 5B). We did not observe the presence of network hubs or module hubs in any of the three glacial environments. The presence of connectors ($P_i > 0.625$) was detected in the three environments, however, with different numbers. In the glacial ice and snow, the number of connectors was much higher (6) than in the ponds (1). In all cases, connectors are fungi (especially *Pucciniomycotina*) that include species that produce both yeast and hyphal states. In both the glacial ice and snow, there was one algal species (*Chloromonas* and *Gloeocystis*, respectively) acting as a connector. In the ponds, the only identified connector was *Sporobolomyces* (*Pucciniomycotina*, *Microbotryomycetes*).

Discussion

Dissolved nutrient concentrations are low, as is typical in many supraglacial environments (Ren et al., 2019). However, particulate elements are present at higher concentrations, suggesting that nutrients may exist in alternative forms such as decaying organic matter, particulate debris, and other materials (Antony et al., 2017). Additionally, the input of glacial clay affects both light penetration and nutrient availability in lakes and streams (Martyniuk et al., 2014; Rose et al., 2014). As glacial inputs decrease, an increase in phosphorus (P) limitation is expected (Elser et al., 2020; Martyniuk et al., 2022), or alternatively, nitrogen–phosphorus (N:P) co-limitation may occur (Ren et al., 2019). Ren et al. (2019) predicted a more rapid decline in nitrogen (N) availability compared to P as glacier retreat progresses, leading to a shift from P limitation to N–P co-limitation. In contrast, Elser et al. (2020) suggested that N availability would increase while P availability would decrease with diminishing glacial influence, resulting in a shift from N limitation to P limitation.

Studies have highlighted that certain aspects of phosphorus cycling in glacial environments have been largely overlooked, particularly the processes of P adsorption and re-adsorption onto mineral surfaces, which can lead to different outcomes in terms of elemental limitation (Stibal et al., 2008). In our study, the low

particulate N:P ratios suggest nitrogen (N) limitation; however, the dissolved fraction presents a contrasting picture. Dissolved N:P ratios were well above 15, indicating potential phosphorus (P) limitation. Indeed, in a previous study, Modenutti et al. (2018) also found that total phosphorus (TP) concentrations were high and strongly correlated with total suspended solids, whereas total dissolved phosphorus (TDP) showed no such relationship. Thus, the apparent discrepancy in nutrient limitation may stem from P adsorption onto various mineral surfaces, such as glacial clays. Supraglacial primary production (by algae in glacial ice and snow) promotes the accumulation of autochthonous organic C (Williamson et al., 2019; Vanderwall et al., 2024). During summer, with warm temperatures and high solar radiation, there is a near constant melt. Thus, any organic matter that was fixed on the supraglacial surface (ice and snow) and not consumed by local heterotrophs (in our study, mainly fungi and ciliates, but also bacteria) is exported to downstream environments (ponds in our study). In hydrologically connected habitats, the metabolic activity of supraglacial organisms may affect the environmental availability of nutrients and shape trophic relationships in downstream habitats.

The analyses of the eukaryotic communities showed a clear dominance of fungi, algae, and ciliophora, though with different relative abundances in the three systems. We did not observe an important number of ASVs losses in the hydrologically connected systems but a community reorganization with different relative abundances. This was particularly evident when comparing beta-diversity based on presence-absence (Sorensen index) and relative abundances (Bray-Curtis index). This means that changes in relative abundances dominate over species turnover in the reorganization of the communities across environments. Moreover, the maximum differences were observed when comparing ice and ponds, with the balanced-variation (changes in relative abundances) having a higher contribution, while the abundance-gradient is low. In addition, the β -diversity obtained from the null models differed from that of the observed ones. This implies that the relative abundances observed across environments were not due to random processes, but due to changes in the environmental conditions where the communities are established. As physical conditions of the systems changed (ice-snow-ponds), some species will be favored (environmental filtering) and this seemed to be the case of algae that increased their importance in the ponds. Several studies have discussed that non-motile algal cell stages rest beneath the snow during winter and rely on cellular motility to emerge, swimming upward during summer meltwater (Détain et al., 2025). Indeed, in the ponds that received meltwater from ice and snow, we observed an increase in the abundance of motile cells such as Dinoflagellates.

Microbial communities can be considered as a network of species connected by interspecific interactions (i.e. competition, predation, mutualism, and parasitism) (Barberan et al., 2012; Jia et al., 2021), that play substantial roles in promoting microbial diversity and ecosystem functioning (Ratzke et al., 2020). A module is a group of nodes (ASVs) with high interactions among them and low interactions with other nodes outside the module; while

modularity measures how well a network separates into different modules (Deng et al., 2012). The co-occurrence network analysis showed a decrease in modularity in the ponds in which only three modules (with five or more nodes) were identified. Modularity may indicate habitat heterogeneity (Olesen et al., 2007) and an increase in modularity seemed to reflect more intensive functional associations (Zhou et al., 2021). Thus, the increased modularity observed in ice and snow suggests greater heterogeneity, likely driven by higher physical complexity (Bachy et al., 2011; Détain et al., 2025). At the same time, the presence of modules (6 in ice and 5 in snow) composed of fungi and algae would reflect the increase in functional associations between these components.

Algae generate bioavailable dissolved organic matter profited by heterotrophic communities (Anesio et al., 2010), and their presence in snow and ice has been reported on all the continents (Lutz et al., 2016). The most frequent algae belong to the Chlamydomonadales (Chlorophyta) group, that includes species responsible for snow algal blooms (Brown and Tucker, 2020; Hoham and Remias, 2020). The interplay between carbon-fixing algae and carbon-respiring heterotrophic microorganisms ultimately controls the amount and composition of organic matter in ice and snow (Rossel et al., 2025).

Among the fungi we identified Pucciniomycotina psychrophilic yeasts (ASV3 and ASV4, Figure 4) that inhabit snowfields and glacial ecosystems worldwide, including glaciers in Greenland and Svalbard, the Alps, and Antarctica (Margesin and Collins, 2019; Perini et al., 2021). Different Pucciniomycotina species have a key role in snow and ice ecosystems, since they are capable of degrading organic molecules during algal blooms, connecting the carbon cycle in cold environments (Marechal et al., 2025). We also found members of the Chytridiomycotina subdivision (ASV 8, Figure 4) that were previously cited in the studied area (Duo Saito et al., 2018). Chytridiomycota have been described as abundant in alpine and Arctic snow, snowpacks of Colorado and Switzerland (Lutz et al., 2017), and in glacier meltwater and sediments in a fjord in Chilean Patagonia (Gutiérrez et al., 2015). Chytridiomycetes are nutritionally dependent on the algae enrichment of snow patches (Brown et al., 2018), and the presence of this group associated with cryophilic algae in our ice and snow environments suggests a tied dependence. Moreover, the nutritional dependence of fungi on algae (Rossel et al., 2025) would be related to the observed increase in functional associations in the modules of ice and snow.

In the context of network analysis, the presence of a connector refers to a node (ASV) that links two modules within a network (Zhou et al., 2021). These nodes play a role in connecting or facilitating interactions across different modules of organisms within the community. In our case, the presence of more connectors in ice and snow can also be related to the interaction between fungi and algae (Brown et al., 2018; Rossel et al., 2025). Most of these connectors are fungi that would indicate their dependence on more than one species association (module). On the one hand, fungi depend on algae for nutrition, and on the other hand, fungal and algal communities may be influenced by similar environmental drivers (Brown et al., 2018). In our case study, in all three systems, the connectors are fungi, and two algal genera

(*Chloromonas* and *Gloeocystis*, in ice and snow, respectively). The identification of fungi as the main connectors across all environments suggests their potential role as functional integrators within the eukaryotic community network, linking different modules and possibly mediating resource flows or ecological interactions between algae and other microbial groups (Brown et al., 2018). The few algae identified as connectors and the existence of modules composed mainly of algae would suggest that primary producers are more involved as module members.

The change in water state towards meltwater in the ponds produces a substantial reduction in the number of modules in the network. Indeed, in the ponds, we only observed three modules and one connector. The unique connector was *Sporobolomyces* (Pucciniomycotina), a common yeast previously found on glaciers in Patagonia (de Garcia et al., 2012). Studies have shown that these yeasts are cold-adapted and can produce extracellular enzymatic activities even at low temperatures (de Garcia et al., 2007). This capacity may allow this yeast to prevail as a connector in early aquatic ecosystems.

Currently, the ponds located at the glacier forefield of Castaño Overo and Alerce are still hydrologically connected to glaciers as the direct contact with glaciers has been lost. As glaciers retreat, direct contact is lost; however, some ponds continue to receive meltwater, maintaining the hydrological connectivity. Glaciers in the northern Patagonian Andes have shown a retreat in the last decades (Masiokas et al., 2020). Here, we showed that the organization of the eukaryotic community changes substantially as water melts, in particular in the relative abundance of taxa and in the community network organization. Based on the present data on glacial-periglacial environments, we did not find evidence of potential biodiversity loss. Although we cannot predict if these environments will definitely lose their glacial inputs, the reductions in ice masses in Patagonia will affect downstream aquatic environments, reducing the glacial influence in their biotic communities.

Data availability statement

The data presented in this study are publicly available. This data can be found here: <https://www.ncbi.nlm.nih.gov>, BioProject accession number PRJNA1270387 and BioSample accession numbers SAMN48824426-SAMN48824447.

Author contributions

BM: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. EV: Data curation, Formal analysis, Investigation, Writing – review & editing. MBN: Data curation, Investigation, Writing – review & editing. NM: Data curation, Investigation, Writing – review & editing. EB: Conceptualization, Formal analysis, Funding acquisition, Investigation, Validation, Writing – original draft, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2025.1670481/full#supplementary-material>

SUPPLEMENTARY FIGURE 1
Rarefaction curves.

SUPPLEMENTARY FIGURE 2
 β -diversity matrices.

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