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EDITED BY

Pengpeng Duan,
Chinese Academy of Sciences (CAS), China

REVIEWED BY

Justin Bastow,
Eastern Washington University, United States
Yangyang Jia,
Xinjiang University, China

*CORRESPONDENCE

Yanying Han,
✉ 1064336015@qq.com

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Effects of nitrogen addition on SOC in alpine grasslands of the Qinghai-Tibetan Plateau and adjacent mountain regions: a meta-analysis

Yanhui Ye, Zheng Wu, Shaobing Zhang, Lingchen Tong, Wenqiang Huang, Zhipan Cui and Yanying Han*

Xizang Agriculture & Animal Husbandry University, Environment College, Nyingchi, Xizang, China

Aims: Nitrogen (N) deposition has emerged as a major driver of ecological change in alpine grasslands of the Qinghai-Tibetan Plateau under global climate change. To predict the ecological consequences of increasing nitrogen deposition, nitrogen addition experiments have been widely employed as a key methodological approach to simulate this process. However, the effects of nitrogen addition—considering its rate, duration, and form—on carbon (C) dynamics in these ecosystems remain inconsistent across studies. Understanding these effects is critical for predicting global carbon stocks and guiding sustainable grassland management.

Methods: We conducted a meta-analysis of 57 peer-reviewed studies (794 observations) to quantify the response of alpine grassland C dynamics to N addition.

Results: N addition significantly increased plant-derived carbon inputs, increasing aboveground biomass by 42.7%, belowground biomass by 16.2%, and dissolved organic carbon (DOC) by 10.7%. The soil organic carbon (SOC) content increased by 3.6% overall. Conversely, soil respiration decreased by 5.1%, whereas the microbial respiration rate increased by 21.9%. The addition of nitrogen decreased the soil pH by 0.20 units and the soil C/N ratio by 1.7%. The soil ammonium (NH₄⁺) and nitrate (NO₃⁻) contents decreased by 20.1% and 52.1%, respectively. The microbial biomass nitrogen (MBN) increased by 14.5%, whereas the microbial biomass carbon (MBC) decreased by 2.8%. The soil fungal-to-bacterial ratio (F/B) decreased by 31.0%.

Conclusion: These results indicate that shifts in microbial community structure drive SOC dynamics in alpine grasslands. Short-term N addition (≤ 5 years; ≤ 30 kg N ha⁻¹ yr⁻¹) enhances SOC through increased plant biomass and microbial C sequestration. However, long-term additions promote C loss via soil acidification and a critical shift in the microbial community, notably a decreased fungal-to-bacterial ratio. To sustain alpine ecosystem function, N addition rates should not exceed 10 kg N ha⁻¹ yr⁻¹. Future research should prioritize interactions between N deposition status and soil acidification/microbial function in high-altitude regions.

KEYWORDS

alpine grassland, nitrogen addition, microorganisms, carbon dynamics, meta-analysis

1 Introduction

Since the mid-20th century, global anthropogenic emissions of reactive nitrogen (N) have increased at approximately 1.3% per year, profoundly influencing terrestrial carbon (C) cycles and soil organic carbon (SOC) dynamics (Galloway et al., 2004; Zheng et al., 2020). However, research findings on alpine grassland SOC responses to nitrogen addition remain inconsistent. Some studies indicate that N addition promotes plant productivity and increases SOC (Seabloom et al., 2021; Shen et al., 2020), whereas others report accelerated SOC decomposition (Luo et al., 2020) or negligible effects (Chen et al., 2021). These discrepancies may be modulated by factors such as soil type, nitrogen addition regimes, and environmental conditions (Li et al., 2023; Xu et al., 2021a).

Grasslands occupy ~40% of the global land surface (Buisson et al., 2022) and store approximately 34% of global terrestrial carbon stocks (Bai and Cotrufo, 2022). Alpine grasslands represent only ~3.5% of total grassland area yet constitute disproportionately large carbon reservoirs (Dinerstein et al., 2017; Wang et al., 2023a). The Qinghai-Tibet Plateau, located in southwestern China and being the world's largest plateau, experiences an atmospheric nitrogen deposition rate of 8.7–13.8 kg N ha⁻¹ yr⁻¹, which significantly influences biochemical processes and the global carbon cycle (Zhang et al., 2025; Lü and Tian, 2007). Despite this, consensus is lacking on whether N addition promotes or suppresses SOC accumulation. Enhanced microbial activity under N inputs may accelerate SOC mineralization (Li et al., 2021), while other studies report neutral or positive SOC responses (Chen et al., 2021; Zi et al., 2022).

Microbial responses to nitrogen inputs are temporally dynamic (Wang et al., 2019), and their role as mediators of SOC in alpine grasslands remains unresolved. Furthermore, interactions between geographic factors—soil properties, elevation, and climate—modulate these effects. Global syntheses reveal spatiotemporal heterogeneity in SOC responses: short-term additions (<3 years) often show minimal effects, while long-term experiments (>12 years) report SOC responses up to 4.3 times stronger than short-term treatments (Liu et al., 2016; Xu et al., 2021b; Tang et al., 2023). While N addition increases surface SOC by an average of 3.1% in global grasslands, alpine meadows/grasslands show no significant response (Liu et al., 2023). This divergence may arise from variation in nitrogen forms, addition rates, and their effects on carbon sequestration (Yin et al., 2022) or from contrasting microbially mediated dynamics. Long-term organic N inputs, for example, can indirectly enhance carbon sequestration by increasing MBC (Li et al., 2023). Clarifying these dynamics is essential to maintaining alpine grasslands' carbon sink function and assessing the impacts of climate change and nitrogen deposition on carbon-nitrogen cycling and feedbacks to global warming.

A mechanistic understanding of N-driven SOC dynamics in Qinghai-Tibet Plateau alpine grassland alpine grasslands is urgently needed. This study synthesizes data from the past 2 decades to evaluate how geographic factors (climate, elevation, grassland type) and nitrogen addition regimes (duration, rate, form) affect SOC responses. Using meta-analysis, we address the following questions:

1. How do carbon pools in Qinghai-Tibet Plateau alpine grassland ecosystems respond to different types, duration and gradients of N addition?
2. What are the key drivers of the effects of N addition on soil organic carbon in Qinghai-Tibet Plateau alpine grassland and the mechanism of multi-dimensional response to environmental factors?
3. What are the thresholds for defining the short-term facilitating and long-term inhibiting effects of N addition on soil organic carbon?

The aim is to promote scientific understanding of soil C dynamics and optimization of theoretical models, and to provide recommendations for alpine grassland management in the context of global nitrogen deposition.

2 Materials and methods

2.1 Data collection and organization

We systematically collected peer-reviewed studies from the Web of Science (WOS, <http://apps.webofknowledge.com>), published between 1 January 2003, and 31 December 2023. The search was conducted using combinations of the following keyword categories:

1. Ecosystem: “alpine grassland” OR “alpine meadow”;
2. Nitrogen input: “nitrogen addition”, “N addition”, “nitrogen deposition”, “N deposition”, “nitrogen input”, “N input”, “nitrogen fertilization”, “N fertilization”, “nitrogen application”, “N application”, “nitrogen enrichment”, “N enrichment”, “nitrogen amendment”, “N amendment”, “nitrogen elevation”, “N elevation”, “nutrient addition”;
3. Carbon outcome: “soil organic carbon”, “SOC”, “carbon sequestration”, “C stock”, “carbon storage”;
4. Geographical position: “Qinghai-Tibetan”, “Xizang”, “Qinghai Tibetan Plateau”, “Tibet”.

Searches were limited to the title, abstract, and keywords fields. The scope included the Tibetan Plateau and its surrounding areas (Figure 1, Supplementary Figure S1).

For each study, we extracted variables related to nitrogen addition and carbon dynamics in alpine grasslands, including:

Carbon input indicators: aboveground biomass, belowground biomass, and dissolved organic carbon (DOC); Soil physicochemical properties: soil moisture content, bulk density, pH, C/N ratio, nitrate (NO₃⁻), and ammonium (NH₄⁺) concentrations; Microbial indices: microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), fungal/bacterial PLFA ratio, Shannon-Wiener index; Carbon loss indicators: soil respiration rate and microbial respiration rate; Site characteristics: latitude, longitude, elevation, mean annual temperature (MAT), mean annual precipitation (MAP), grassland type, soil type, N addition rate (kg N·ha⁻¹·yr⁻¹), form and duration of N addition, and soil sampling depth (limited to 0–20 cm).

Suitable papers were screened based on the following criteria:

1. The experiments were conducted on natural grasslands located at elevations greater than 2,900 m or restored from abandoned fields for at least 30 years with field observations.
2. The ongoing experiments were conducted at the same locations and time scales with clearly documented variables related to soil carbon dynamics, excluding reviews, modeling studies, indoor studies, and additions of N contents of unknown organic fertilizers.
3. Each experiment reported three or more variables required for inclusion in the analysis;
4. When extracting variables, the type was mean.
5. When there were multiple identical measurements available for the same study, the mean value of the variable was calculated, and when multiple soil data were available for the report, only topsoil layers up to 0–20 cm were used, such as when the data were only available for the 0–10 cm and 10–20 cm soil layer data, then subgroups were used and processed.
6. When multiple N addition gradient levels existed in the same experiment, each gradient was considered a separate experiment and the relevant values were calculated independently.
7. When a two-factor experiment appeared in the study, only control and N addition group experimental data were taken. For example, when the two-factor experiment of nitrogen addition and litter addition has four treatments (control, litter addition alone, nitrogen addition alone, litter and nitrogen addition at the same time), only the observed values of the control and nitrogen addition alone are selected.
8. For experiments with missing environmental factors such as elevation, mean annual temperature, and precipitation, the missing data were supplemented by downloading them from the WorldClim website (<http://www.worldclim.org/>).

On the basis of the above selection criteria, we were able to use 57 publications on nitrogen addition (including nitrogen deposition and fertilization) to alpine grasslands, as shown in the reference list in the Supplementary Material, which included 794 observations at 52 sites. In our dataset, we used WebPlotDigitizer v4.4 (Burda, O'Connor, Webber, Redmond and Perdue, 2017) to extract values (if relevant data were presented in numerical form).

2.2 Meta-analysis method

A meta-analysis was used to investigate the response of SOC and its related indicators after the addition of nitrogen (Hedges et al., 1999). The response ratio (RR) of each variable was used to indicate the effect size of nitrogen addition (Xu et al., 2021a; Zhang et al., 2018; Yang et al., 2023), which was calculated as follows Equations 1–6:

$$RR = \ln\left(\frac{\bar{X}_t}{\bar{X}_c}\right) = \ln(\bar{X}_t) - \ln(\bar{X}_c) \quad (1)$$

Where, \bar{X}_t and \bar{X}_c are the mean values of the added N group and control group. variables, respectively. By fitting a Gaussian normal function (Zhou, Zhou, Zhang, Lu, Luo, Liu and Li, 2014; Wei, Zhang, Zhang, Qin, Zhang, Sun and Huang, 2022), the response

ratios of all individual variables related to soil carbon pools obeyed a normal distribution. The within-study variance (v) of each response ratio was estimated as follows:

$$V_{RR} = \frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} \quad (2)$$

Where S_t and S_c are standard deviations (SD) and n_t and n_c are sample sizes for N addition and control, respectively. The random effects model was calculated by applying the restricted maximum likelihood (REML) method for the weighted response ratios and confidence intervals:

$$w_i^* = 1/(V_{RR} + \tau^2) \quad (3)$$

In this equation, w_i^* : weights of individual studies, V_{RR} : within-study variance, τ^2 : between-study variance.

Cumulative effect values were calculated as follows:

$$\bar{y} = \frac{\sum_{i=1}^k w_i^* RR}{\sum_{i=1}^k w_i^*} w_i^* = 1 / (V_{RR} + \tau^2) \quad (4)$$

The 95% confidence interval (CI) of the cumulative effect value is calculated as follows:

$$CI = \bar{y} \pm 1.96 \times SE = \bar{y} \pm 1.96 \times \sqrt{\frac{1}{\sum_{i=1}^k w_i^*}} \quad (5)$$

The 95% confidence intervals were calculated using OpenMEE software (Wallace et al., 2017). In this meta-analysis, we used a restricted maximum likelihood (REML) random effects model to calculate the ratio of the ratio of the effects of N on each response variable, which was based on the recommendations of (Veroniki et al., 2016). These effects are considered significant if the 95% confidence interval does not cross the zero point (Koricheva et al., 2013).

The CI were converted to percentage changes as follows:

$$\text{Percentage change (\%)} = (\exp(\bar{RR}) - 1) \times 100\% \quad (6)$$

Percentage changes are more intuitive and easier to interpret than weighted effect sizes (RR), however, backward transformations may introduce bias into response ratios due to the convexity of logarithmic transformations.

To maximize the sample size, some studies with missing SD were included, and in other cases, where neither SD nor SE was given, a conservative approach was adopted whereby they were assigned the maximum variance (v) observed among studies with complete data for the respective variable. This approach minimizes the weight of studies with missing precision data in the overall analysis. In cases where neither SD nor SE was reported, the SD was imputed by multiplying the reported mean by the overall mean coefficient of variation ($CV = SD/\text{Mean}$) calculated from the complete dataset, following the methodology of Weir et al. (Weir et al., 2018). When I^2 was greater than 50%, i.e., when between-study differences due to heterogeneity could not be ignored, a random effects model was used; otherwise, a fixed effects model was used (Sedgwick, 2012). The effect of added nitrogen on the variable of interest was considered statistically significant if the CI did not coincide with zero.

The overall heterogeneity (Q_t) of effect values for each subgroup (SOC, PH, C/N, MBC, MBN, F/B, microbial respiration rate, soil respiration rate, above-ground biomass, below-ground biomass, ammoniacal nitrogen, nitrate nitrogen, Shannon-Weiner index, species richness) and the influence of explanatory variables on the effect values (Q_m) (Supplementary Table S2) were examined by the Q-test method (Viechtbauer, 2010), when the overall heterogeneity of the effect values is strong (and $P < 0.05$) or when the I^2 coefficient is $>60\%$, it means that the effect values of each nitrogen application trial are significantly influenced by other relevant factors (Benitez-Lopez et al., 2017; Zhao et al., 2020), the introduction of the explanatory variables introduces the sub-type variable Nitrogen treatment Duration of N addition, Rate of N addition, Form of N addition, Grassland type, Mean annual temperature (MAT), Mean annual precipitation (MAP) were used to analyze the data in subgroups (Higgins et al., 2003), and we conducted meta-regressions using the REML mixed-effects model in the OpenMEE software package to explore the effects of each categorical group on the variables.

2.3 Statistical and modeling analysis

2.3.1 Heterogeneity and publication bias test

We assessed publication bias using Egger's regression test, with $p < 0.05$ indicating potential bias (Sterne et al., 2011). Publication bias was present for some variables. The Rosenthal fail-safe number was used to determine the extent of the effect of publication bias, and when it was greater than $5n + 10$ (n , sample size of the variable), publication bias was not considered to affect the results of the meta-analysis (Supplementary Table S3). Linear mixed-effects models were developed using the 'lme4' package (Bates et al., 2015) to explore the relationships between grassland type, climate, nitrogen addition status, pH response ratios (RR (pH)) and soil carbon related variables. A random forest analysis was conducted using the "randomForest" software package (Liaw and Wiener, 2002) to obtain relative importance rankings of factors affecting carbon dynamics. Piecewise Structural Equation Modeling (Piecewise SEM) (Lefcheck 2016) is a nested mixed-effects model with "study" as a random factor to explore how climatic conditions, duration and rate of nitrogen additions, soil acidity (pH), and carbon dynamics variables influence changes in SOC under nitrogen additions.

2.3.2 Explanatory variables and model analysis

In this study, subgroup analyses were conducted based on grassland type and the duration, rate, and form of N addition, respectively. Meadow ecosystem type:

Meadow soil type (Alpine grassland/meadow): alpine meadow soil/subalpine meadow soil, subalpine meadow soil, meadow soil, artificial grassland.

Cambisol soil type (Cambisols): Cambisol, Gelic Cambisol, Mat-Grylic Cambisol.

Calcareous/arid soils (Calcereous): Cryic Aridisol, Kastanozem, Chernozem, Cambisol, Alpine chernozem.

Special soil types (Special): red loam, ice-accumulated humus, Cambrian soil, chalky clay, chalky soil, gravelly sandy loam, Ombrotrophic blanket bog.

Nitrogen addition rates were categorized into three levels: low ($\leq 60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), medium ($60\text{--}120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and high ($>120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). The experimental duration of nitrogen addition was categorized as short-term (≤ 5 years), and medium-to long-term (5–10 years). In our collection of N addition experiments, N fertilizers included NH_4NO_3 , urea, NaNO_3 or KNO_3 , NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$, and mixed additions (NH_4NO_3 , urea), the first two of which accounted for nearly 91.5% of the total dataset of observations of SOC content in topsoil (108) using NH_4NO_3 or urea as the form of N addition. Thus, the forms of N addition were categorized as urea and NH_4NO_3 .

In all structural equation modeling (SEM) analyses, variables were standardized by dividing each by its standard deviation to maintain comparable scales across datasets. Model fit was evaluated using Fisher's C statistic (Shipley, 2009). All statistical analyses were performed in R version 4.4.3 and AMOS.

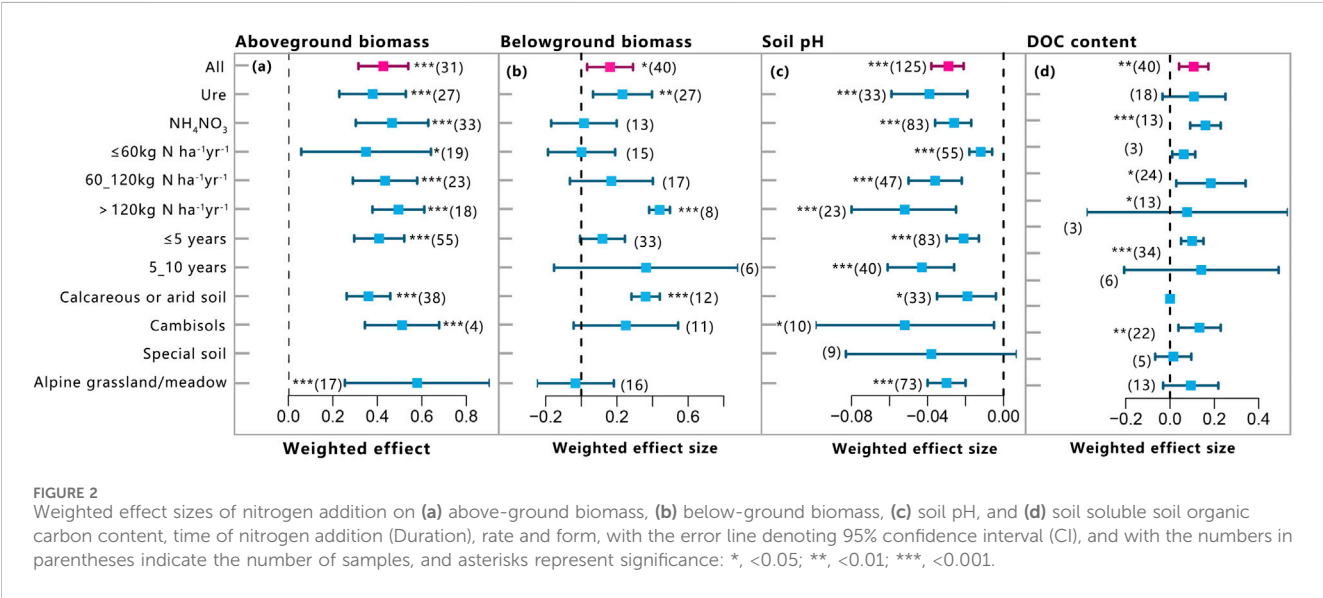
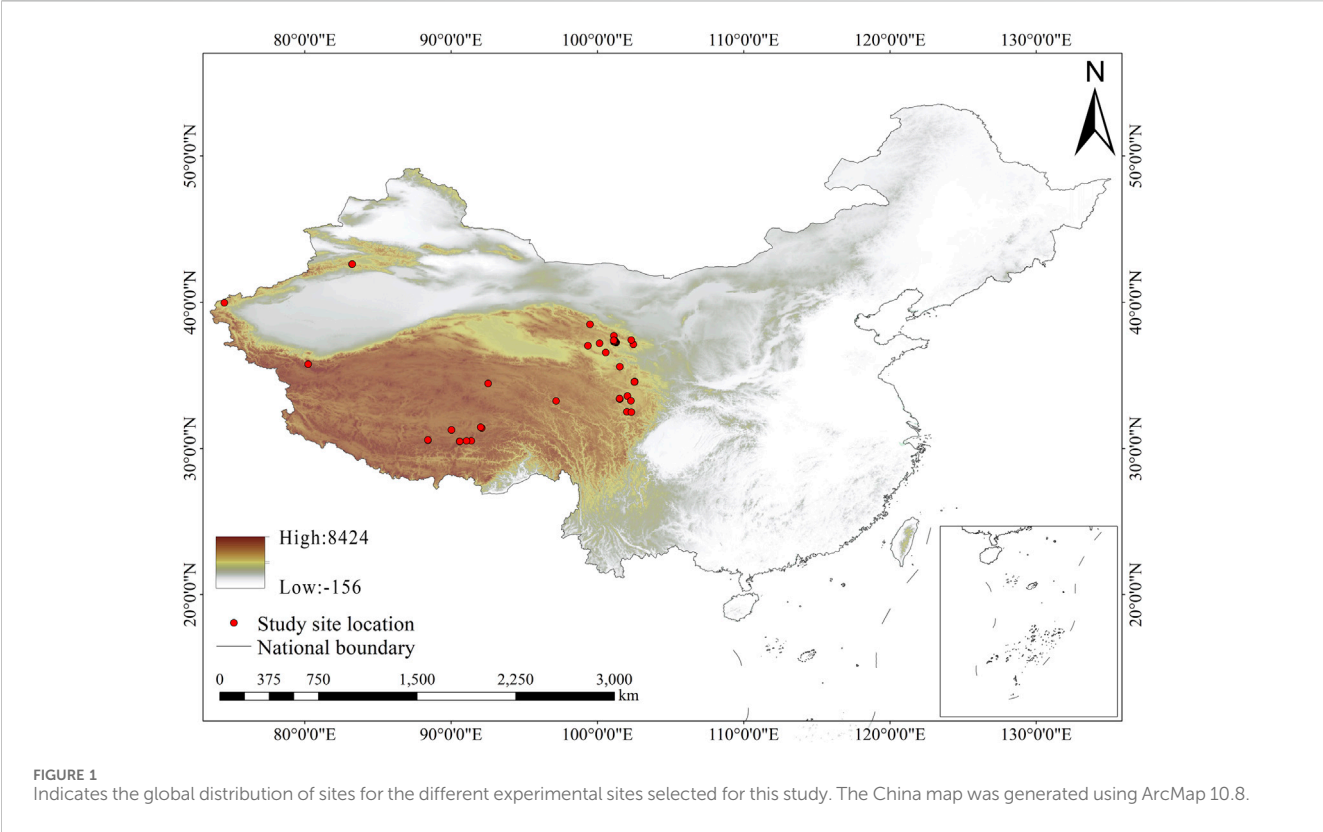
3 Results

3.1 Overall response of alpine grassland C dynamics to N addition

Nitrogen (N) addition had a significant overall effect on carbon (C) dynamics in alpine grasslands across all studies. (Figures 2–6). Meta-analysis demonstrated an overall 3.6% increase in SOC content under nitrogen addition ($P < 0.05$), with calcareous/arid soils showing more pronounced responses than Cambisols (Figure 3a). The increase in SOC was significantly greater under short-term N addition (≤ 5 years; +4.1%) than under long-term addition (5–10 years; +3.1%) ($P < 0.01$; Figure 3a), the relationship between SOC and N addition rate was nonlinear. Under both short-term and medium to long-term nitrogen addition, SOC increased with rising nitrogen addition rates at lower doses, but showed a significant decline after reaching its peak (Figure 3b). Enhanced SOC resulted primarily from increased plant-derived carbon inputs. Nitrogen addition elevated aboveground biomass by 42.7% and belowground biomass by 16.2% (Figures 2a,b). These increases correlated positively with experimental duration and nitrogen addition rate, though grassland types exhibited varying responses: alpine meadows/grasslands showed 57.9% AGB increase accompanied by 3.3% BGB decrease. Concurrently, the concentration of DOC, a key component of the soil active carbon pool, also increased significantly by 10.7% (Figure 2d). However, N addition led to significant decreases in species richness and Shannon-Weiner indices (Figures 4a,b). Contrasting with carbon input enhancements, soil carbon efflux through respiration by 5.1% ($P = 0.131$) (Figure 5c).

3.2 Microbial and soil chemophysical responses to nitrogen addition

In addition to altering soil carbon dynamics, nitrogen addition significantly modified soil chemical properties and microbial communities. It resulted in an overall decrease in soil pH by 0.20 units (Figure 2c) and the soil C/N ratio by 1.7%, and



induced a substantial restructuring of the microbial community. This restructuring was characterized by a 31.0% decrease in the fungal to bacterial ratio ($P < 0.001$; Figure 6a). Under short-term N addition (≤ 5 years), both MBC and MBN increased significantly by 23.2% and 25%, respectively; however, they exhibited a declining trends under long-term N addition (Figure 4c). Concurrently, the microbial respiration rate increased significantly by 21.9% ($P < 0.001$; Figure 6b).

3.3 Response of soil C dynamics to environmental factors

Random forest modeling identified mean annual precipitation (MAP), mean annual temperature (MAT), and altitude as primary predictors of SOC dynamics among tested variables (Figures 7, 8). Climate and elevation factors dominated carbon response patterns in these ecosystems. MAP emerged as the principal regulator for

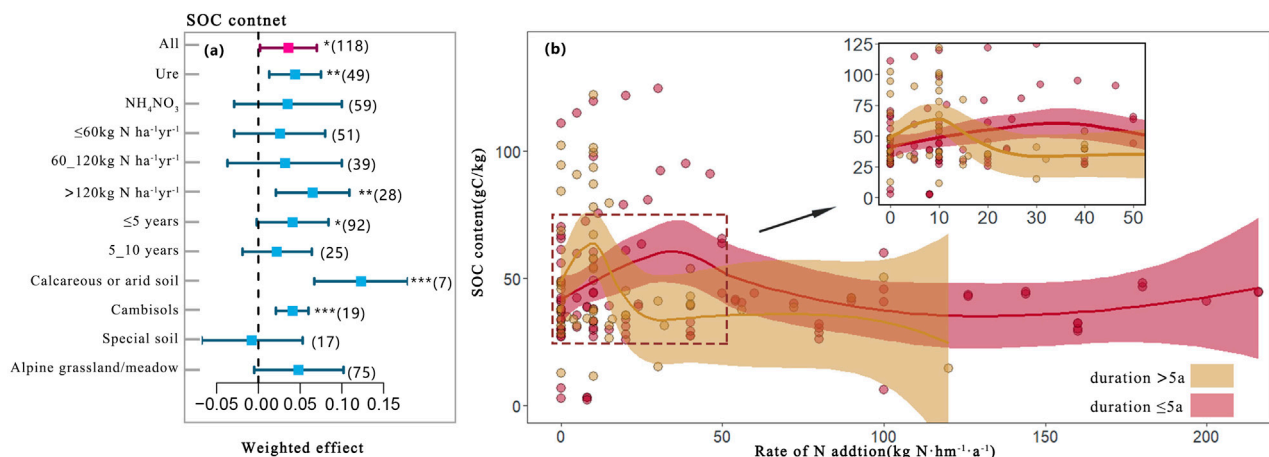


FIGURE 3

Weighted effect sizes of nitrogen addition on (a) soil organic carbon content. (b) represents the linear fit of soil organic carbon content in relation to the time of N addition and the amount of N added, the width of the cross-section denoting 95% confidence interval (CI).

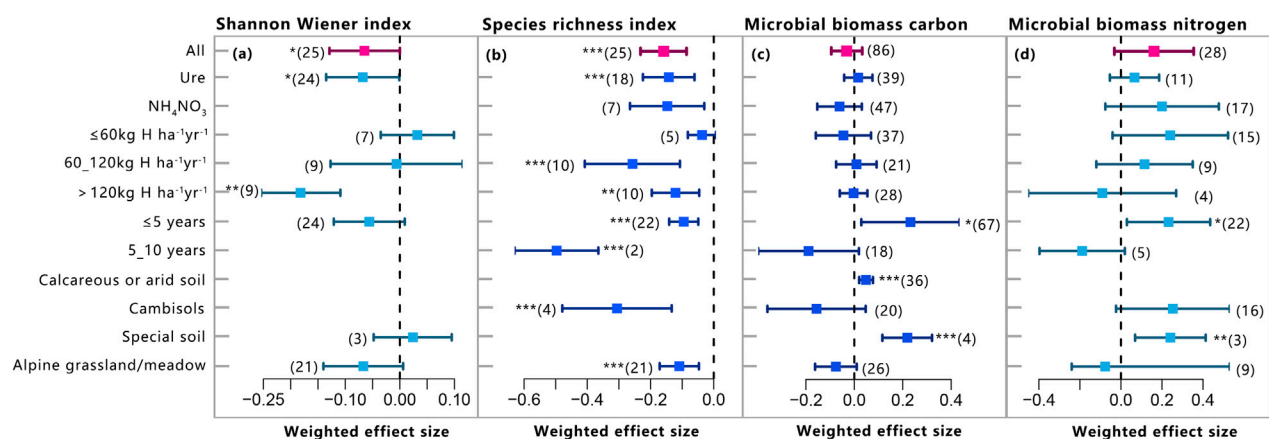


FIGURE 4

Weighted effect sizes of nitrogen addition on (a) Shannon-Wiener index, (b) species richness index, (c) microbial biomass carbon, and (d) microbial biomass nitrogen, time of nitrogen addition (Duration), rate, and form, with the error line denoting 95% confidence interval (CI), and with the numbers in parentheses indicate the number of samples, and asterisks represent significance: *, <0.05; **, <0.01; ***, <0.001.

multiple variables, exhibiting strongest influence on aboveground biomass, soil pH, microbial biomass nitrogen, dissolved organic carbon, and species richness index (Figures 7a,e,f; Figures 8b,d). MAT served as the main governing factor for SOC and microbial biomass carbon. Altitude represented the most critical factor for belowground biomass, fungal-to-bacterial ratio, microbial respiration rate, and dissolved organic carbon (Figures 7, 8).

Carbon-related variables demonstrated differential environmental sensitivity: aboveground biomass showed greater sensitivity to MAT, while belowground biomass and pH responded strongly to MAT, MAP, and altitude (Figures 7, 8). The relative effect values (RR) of AGB, BGB, and pH did not differ significantly among soil types (Figures 2a,c), but the effect on MBC was negative in alpine meadow/grassland differing significantly from other soil types (Figure 2d).

3.4 Key drivers of SOC dynamics

Structural equation modeling elucidated Indirect pathways of nitrogen addition effects on SOC (Figure 9). Soil pH, aboveground biomass, belowground biomass, and microbial biomass carbon exerted significant direct effects regardless of nitrogen addition status. A strong negative pathway connected soil pH and SOC (−34%, $P < 0.01$), while positive associations emerged with aboveground biomass, belowground biomass, and microbial biomass carbon (Figure 9). Furthermore, soil types and duration indirectly influenced SOC through the regulation of soil pH ($P < 0.01$), while also being affected by climatic factors (Figure 9). BGB shows a strong positive correlation with nitrogen addition time in SEM equation analysis (+40%, $P < 0.05$) and is positively influenced by nitrogen addition type (+24.4%, $P < 0.1$). It is also driven by

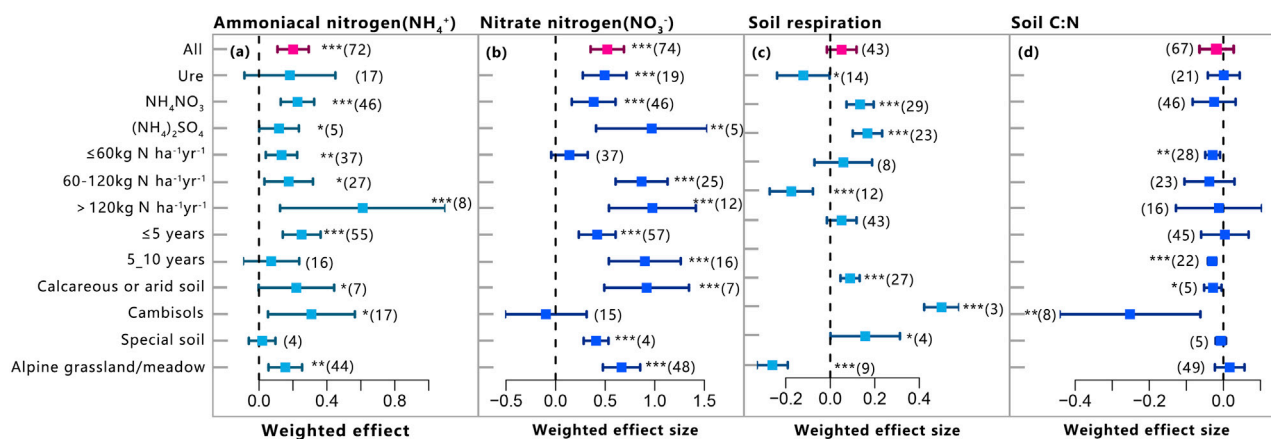


FIGURE 5

Weighted effect sizes of nitrogen addition on (a) soil ammoniacal nitrogen, (b) soil nitrate nitrogen, (c) soil respiration rate, and (d) soil carbon to nitrogen ratio, time of nitrogen addition (Duration), rate, and form, with the error line denoting 95% confidence interval (CI), and with the numbers in parentheses indicate the number of samples, and asterisks represent significance: *, <0.05; **, <0.01; ***, <0.001.

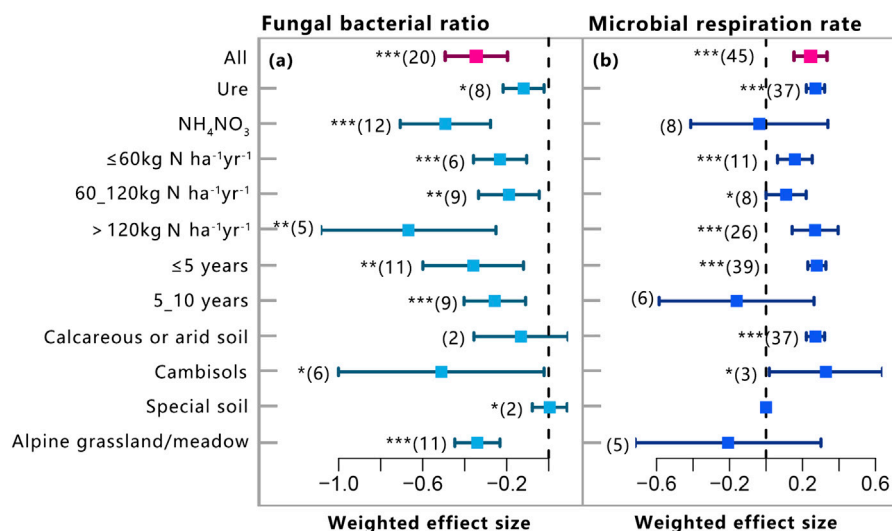


FIGURE 6

Weighted effect sizes of nitrogen addition on (a) fungal to bacterial ratio, (b) soil microbial respiration rate, time of nitrogen addition (Duration), rate, and form, with the error line denoting 95% confidence interval (CI), and with the numbers in parentheses indicate the number of samples, and asterisks represent significance: *, <0.05; **, <0.01; ***, <0.001.

environmental factors such as altitude, annual average temperature, and annual average precipitation (Figure 7b), which significantly increase BGB accumulation through direct and indirect effects. In contrast, AGB in alpine grasslands was more climate-dependent and less sensitive to elevation (Figures 7a, 9). Overall, BGB was more sensitive to elevation and MAP than AGB (Figures 7a,b). MBC is significantly influenced by climate, altitude, and soil type ($P < 0.05$), and has a significant positive correlation with SOC (+50%, $P < 0.01$). Nitrate nitrogen (NO_3^-) dynamics were mainly regulated by N addition rate and precipitation (MAP), with the direct effect of N input being more significant (Figure 7d). Climate factors (MAT and MAP) represented the most important overarching drivers of

surface SOC, exceeding contributions from grassland type, elevation, and nitrogen addition status.

4 Discussion

4.1 Response and threshold effect of SOC to nitrogen addition in alpine grassland

This study demonstrates that SOC in Qinghai-Tibet Plateau alpine grasslands responds positively and significantly to N addition (Figure 3a), aligning with global evidence that N input enhances

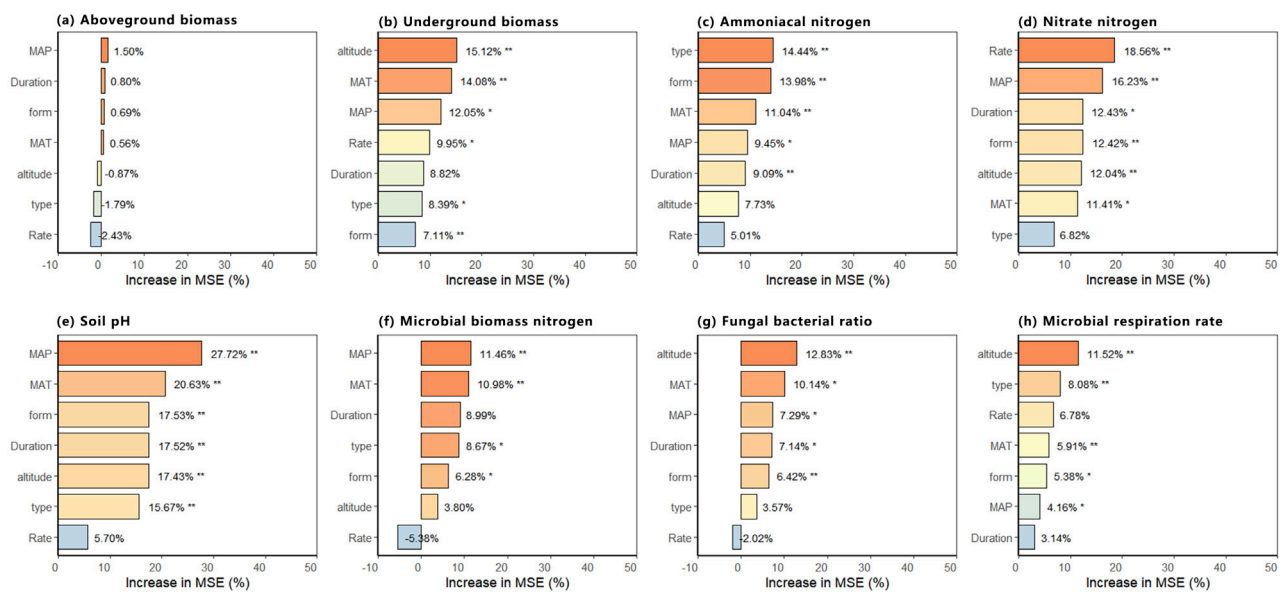


FIGURE 7

Analysis of factors affecting carbon dynamics based on a random forest model, with relative importance ranking of soil type (type), mean annual temperature (MAT), mean annual precipitation (MAP), and nitrogen addition duration (Duration), rate, and type (Form), as expressed by the mean squared error (MSE, %) of (a) above-ground biomass; (b) below-ground biomass; (c) ammoniacal nitrogen; (d) nitrate nitrogen; (e) soil pH; (f) microbial biomass nitrogen; (g) fungal-bacterial ratio; and (h) microbial respiration rate, with asterisks denoting significance: *, <0.05; **, <0.01; ***, <0.001.

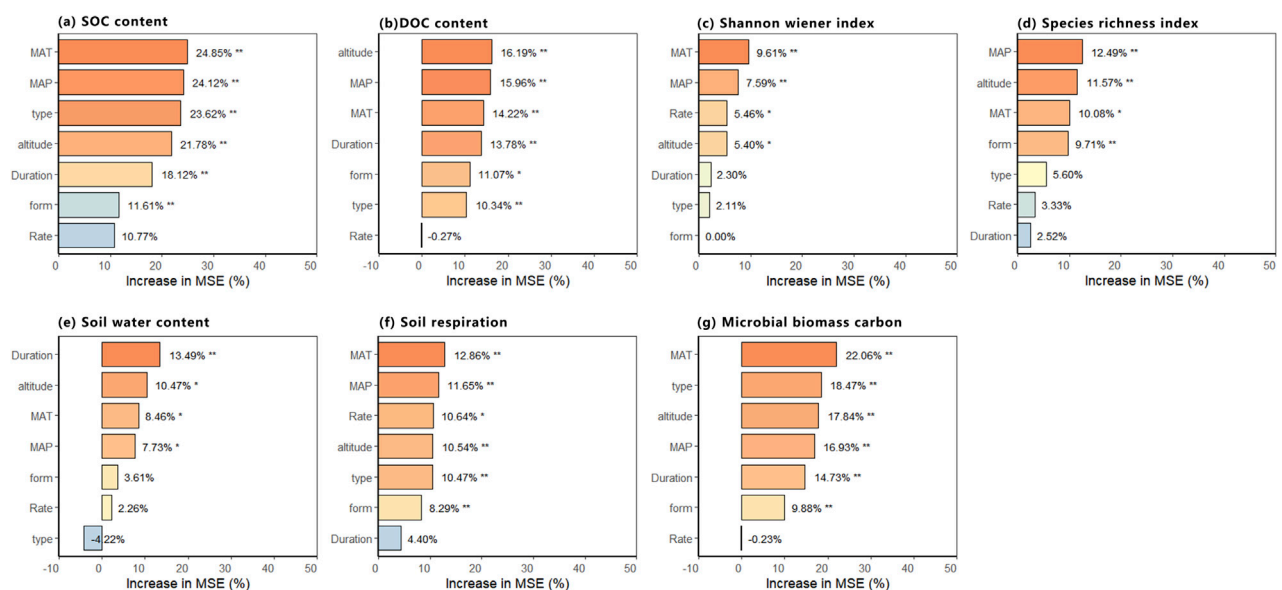


FIGURE 8

Analysis of factors affecting carbon dynamics based on the random forest model, with relative importance ranking of soil type (type), mean annual temperature (MAT), mean annual precipitation (MAP), and nitrogen addition duration (Duration), rate, and type (Form), as expressed in the mean squared error (MSE, %): (a) soil organic carbon content; (b) soil soluble organic carbon content; (c) Shannon-wiener index; (d) species richness index; (e) soil water content; (f) soil respiration rate; and (g) microbial biomass carbon, with asterisks representing significance: *, <0.05; **, <0.01; ***, <0.001.

carbon sequestration in grassland ecosystems (Liu et al., 2023). Owing to the inherent sensitivity of alpine ecosystems, N addition tends to exert stronger effects on the soil environment in these regions (Li et al., 2020), N addition significantly increased

aboveground biomass and soluble organic carbon, mainly due to the increase of NH_4^+ and NO_3^- content in the soil by N addition (Figure 5a; Figure 5b) which stimulated the growth of plants (Perring et al., 2008) and later indirectly increased soil C storage

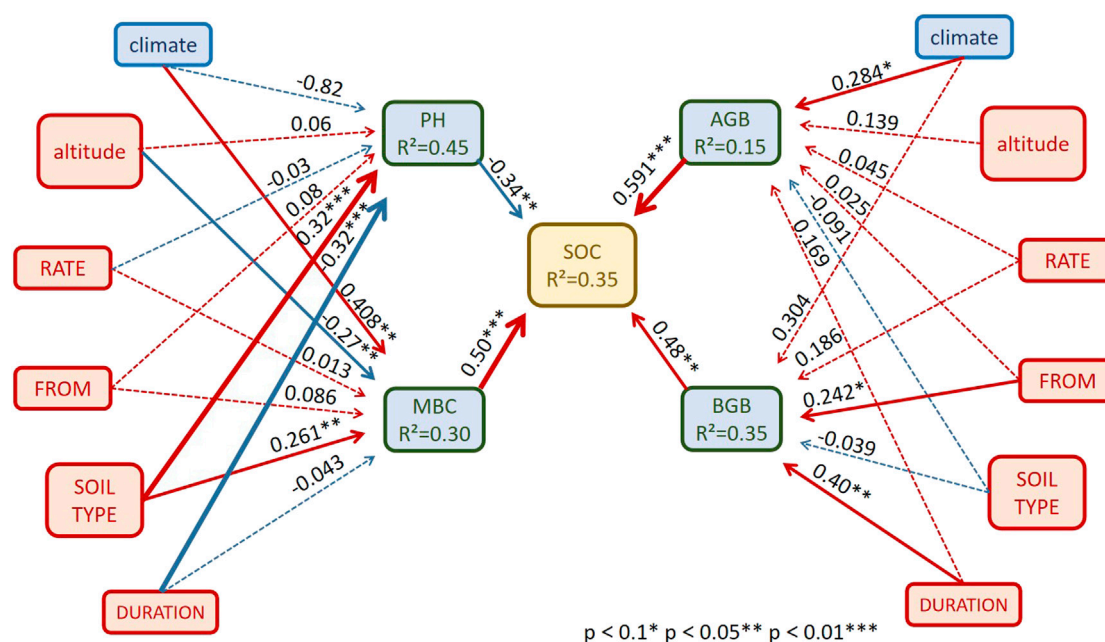


FIGURE 9

Structural equation modeling (SEM) of the main factors of N addition on soil C dynamics. It mainly includes the direct and indirect effects of climate (Climate), altitude, soil type, and nitrogen (N) addition status (duration and rate of N addition) on pH, above-ground biomass (AGB), below-ground biomass (BGB), and microbial biomass carbon (MBC), as well as their effects on the soil organic carbon content (SOC) and their effects on soil organic carbon content (SOC). Climate includes temperature (MAT) and precipitation (MAP). Blue and red arrows indicate negative and positive effects, respectively. Solid and dashed lines indicate significant and insignificant pathways, respectively. R² values associated with variables indicate the proportion of variation explained by correlation with other variables. Asterisks represent significance: *, <0.05; **, <0.01; ***, <0.001.

through litter input (Xu et al., 2021a). This may also be attributed to shifts in microbial community composition, improved plant nutrient uptake efficiency, or suppression of soil pathogens under N enrichment (Wang et al., 2023a). Secondly, N addition reduced the species richness index and Shannon-Weiner indices, which was mainly due to N selection by aboveground plants and altered soil pH (Midolo et al., 2019). Because plant-derived carbon contributes to the labile carbon pool, enhanced aboveground growth under N input indirectly increases SOC input, especially in alpine systems with rapid turnover of organic matter (Lu et al., 2023).

Regarding soil carbon loss, N addition altered microbial community structure, reducing the fungal-to-bacterial ratio and increasing microbial respiration rates (Figures 6a,b). This indicates a microbial shift toward bacterial dominance—characterized by higher metabolic activity—which enhances SOC decomposition (Creamer et al., 2015). Secondly, soil respiration showed a nonlinear response of increasing and then decreasing with the gradient of nitrogen addition (Figure 5c), which may be due to the change of soil pH, the same as the results of previous studies (Xing et al., 2022).

Further analysis showed significant differences in the response of different grassland soil types to N addition. For example, there was a significant difference in SOC changes between Special soil and soil types such as alpine grassland/meadow (Figure 3a), which may be closely related to the effectiveness of soil organic matter content and nitrogen formations (Li et al., 2021; Zou et al., 2021; Yu et al., 2023). Alpine meadow soils, which are richer in organic matter, may enhance nitrogen availability and plant carbon use efficiency (Zhao

et al., 2023), promoting deeper carbon storage through belowground biomass allocation (Figure 2b) (Dolezal et al., 2019). This is consistent with root-prioritized carbon allocation strategies commonly observed in alpine ecosystems, where elevation and mean MAT significantly influence BGB accumulation (Figure 7b). In terms of timing and gradient, alpine grasslands showed a unique response to N addition gradient and duration: the short-term (≤ 5 years) effect was most pronounced for middle dose (≤ 30 kgN-hm⁻¹-a⁻¹), but long-term high-dose additions led to a decline in SOC due to soil acidification (Figure 3; Figure 2c; Figure 9). This result is consistent with the study of Wang et al. (2023b). In the linear fitting plot we found that low doses (≤ 10 kgN-hm⁻¹-a⁻¹) of long-term inputs (> 5 years) optimized C sequestration (Figure 3), providing a threshold reference for nitrogen management in alpine grasslands.

4.2 Mechanisms underlying the microbial regulation of SOC

The response of SOC to N addition in Qinghai-Tibet Plateau alpine grasslands exhibits a trade-off between short-term accumulation and long-term depletion. This duality is largely driven by microbial community responses, which regulate soil nitrogen availability and subsequently affect carbon cycling processes (Zhou et al., 2017). Under N enrichment, the microbial community shifted toward bacterial dominance, with fungal abundance suppressed—evident in a 31% decrease in the fungal-

to-bacterial PLFA ratio (Figure 6a). This pattern aligns with prior findings indicating that ammonium-based N inputs selectively reduce fungal biomass (Zi et al., 2022). The rapid bacterial response to nitrogen (2.7% negative MBC vs. 15% positive MBN) (Figures 4c,d), a bacterial-dominated preference for nitrogen utilization, led to an increase in microbial metabolic carbon demand, resulting in a “carbon-nitrogen imbalance”, with short-term nitrogen addition accelerates carbon turnover and increases microbial respiration rate by 21.9% (Figure 6b), which was partially consistent with the mechanism of “short-term nitrogen addition promotes carbon input and long-term inhibition of microbial decomposition” proposed by Dong et al. (2022). Our findings further suggest that microbial responses are particularly sensitive to N addition under low-temperature alpine conditions, amplifying short-term respiration responses (Zhang et al., 2020). This carbon and nitrogen imbalance was intensified at low temperatures, further explaining the contradiction between the short-term positive response of SOC to nitrogen addition and the attenuation of the long-term effect in alpine grassland.

Over the long term, high N input caused soil acidification, which offset SOC gains by reducing microbial activity and C-use efficiency (Jiang et al., 2019). Soil pH declined significantly (Figure 2c), and this acidification was strongly driven by MAP (27.7%) and MAT (20.6%) (Figure 7e). Reduced pH suppressed microbial activity, resulting in decreased MBC and enhanced soil respiration, consistent with studies linking acidification to C loss (Figure 4c; Figure 5c) (Ye et al., 2022; Dharanishanthi et al., 2021), regulating SOC stability, reflecting the inhibitory effect of low temperature interacting with nitrogen dose on microbial processes in alpine environments. This is consistent with Chen’s study on the effect of soil acidification on SOC stability (Chen et al., 2020), validating the mediating role of soil acidification in the carbon cycle of alpine grasslands. Compared with the growing season dynamics of microbial biomass in Canadian tundra and northwestern Russian soils (Pold, et al., 2021; Sun et al., 2022), the immediate microbial response to nitrogen in alpine grasslands is more susceptible to low-temperature limitation. In addition, low temperatures and altitudinal in alpine grasslands may inhibit microbial respiration (Figures 6b, 7h) and reduce SOC decomposition under long-term nitrogen addition, a “low-temperature protection” effect that echoes the carbon pool stabilization mechanism in high-latitude ecosystems in the Northern Hemisphere (Harris et al., 2022), explaining the positive response of SOC to nitrogen addition in alpine grasslands at low temperatures (Pold, et al., 2021; Sun et al., 2022). The positive response of alpine grassland SOC to N addition was enhanced at low temperatures. The interaction between microbial communities and the soil environment together constitute a core mediating pathway for SOC dynamics.

In summary, microbial communities mediate SOC responses through dual pathways of respiration and nitrogen assimilation. Nitrogen addition drives SOC accumulation through “carbon catabolism-fixation”. This is consistent with the mechanism proposed by Zhang et al. for the regulation of carbon dynamics by microbial processes (Zhang et al., 2020), which shows a unique response in alpine environments due to low temperatures and changes in microbial community structure (bacterial dominance) (Pushkareva et al., 2024). Structural changes in microbial communities (decline in fungal-bacterial ratio) affect carbon

metabolism, which in turn affects carbon cycling through plant root-microbial interactions (Kuzayakov and Xu, 2013), deepening the mechanistic analysis of carbon and nitrogen coupling in their alpine ecosystems.

4.3 Mechanisms of carbon dynamics in response to environmental factors under nitrogen inputs

Our meta-analysis showed that N addition significantly influenced soil carbon dynamics in Qinghai-Tibet Plateau alpine grasslands, with mean MAT, elevation, and MAP emerging as the most influential environmental drivers (Figures 7–9). These factors shape the soil environment and indirectly mediate SOC, DOC, and MBC responses (Figure 8). Under the same altitude and temperature environment, elevated precipitation is more favorable for plant growth and enhances soil microbial activity (Diao et al., 2022), thus stimulating soil respiration (Yang et al., 2023). Second, climate and topography also dominated biomass allocation, with belowground biomass promoting microbial carbon sequestration through MAP- and MAT-mediated accumulation (Figure 7b). MAP’s contribution of 16.93% to MBC (Figure 7g) is consistent with Kazanski et al.’s conclusion that plant productivity contributes to SOC accumulation (Kazanski et al., 2019), and emphasizes the belowground carbon input’s centrality (Figure 7b), reflecting the fact that plant allocation strategies for resources in alpine environments favor the below-ground fraction to adapt to low temperatures and nutrient limitation. Nitrogen input and moisture conditions regulated soil N forms, and nitrate N was mainly affected by N addition rate (Rate) and MAP (Figure 7d), which echoed the conclusion of the significant effect of medium- and high-dose N additions on carbon fluxes (Yue et al., 2016), and further explained the significant elevation of NO_3^- in alpine grassland (Figure 5b). The interaction between N addition and precipitation determines N speciation, which affects plant uptake and microbial processing, forming a nitrogen–moisture–carbon feedback network.

Soil type also played a critical role, especially in regulating NH_4^+ and pH (Figures 7c,e), both of which shaped microbial responses and thus influenced SOC dynamics (Figure 7f). This is in general agreement with Chen et al.’s study on the effect of soil type in regulating nitrogen (Chen et al., 2020). Differences in the physicochemical properties of different soil types (Figures 3a, 5a,b) led to the heterogeneity of nitrogen effects, further validating the key role of soil type in mediating regulation.

4.4 Uncertainty and its implications for future research

The mechanism of microbial community regulation on SOC stability has received increasing attention, but the dataset of this study did not fully cover the details of microbial response to N addition, especially the dynamic effects of different N addition patterns on microbial functional genes (e.g., *nirS*, *amoA*). Existing data mainly focus on the mid-to long-term changes in carbon dynamics in alpine soils from 0 to 10 years, but lack long-term changes of more than 10 years, which may underestimate the

risk of carbon and nitrogen imbalance caused by soil acidification induced by N addition. In the future, we need to incorporate more than 10 years of soil dynamics and microbial genetic changes to analyze the interaction mechanism between different microbial communities and SOC, so as to provide a precise scientific basis for nitrogen management and carbon neutrality in alpine ecosystems around the world.

5 Conclusion

This meta-analysis quantitatively assessed the effects of nitrogen addition on SOC dynamics in Qinghai-Tibet Plateau alpine grassland ecosystems. Our findings reveal a nonlinear and context-dependent relationship between N addition and SOC, driven by both application rate and duration, as well as climatic and edaphic heterogeneity.

Regardless of the nitrogen form applied, nitrogen addition significantly increased carbon input-related indicators, including AGB, BGB, and DOC. It also elevated the soil concentrations of NH_4^+ and NO_3^- . However, prolonged nitrogen addition significantly reduced soil pH and species richness. Short-term N addition (≤ 5 years) at moderate doses ($\leq 30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) effectively promoted SOC accumulation by stimulating plant growth and microbial carbon sequestration. In contrast, long-term and high-dose N addition led to significant soil acidification and a fundamental shift in the soil microbial community structure, notably a 31.0% decrease in the fungal-to-bacterial ratio. Climate and elevation as the main environmental factors determined the spatial heterogeneity of SOC. In alpine grassland ecosystems, the long-term N addition rate should be kept below $10 \text{ kg N} \cdot \text{h}^{-1} \cdot \text{y}^{-1}$ to maintain its ecological balance. More rational carbon management strategies should be developed at this stage to support soil management in alpine grassland ecosystems and the achievement of global carbon neutrality goals.

Author contributions

YY: Data curation, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing. ZW: Conceptualization, Data curation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing. SZ: Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review and editing. LT: Data curation, Investigation, Project administration, Software, Writing – review and editing. WH: Data curation, Methodology, Validation, Writing – review and editing. ZC: Formal Analysis, Investigation, Writing – review and editing. YH: Funding acquisition, Project administration, Resources, Supervision, Writing – review and editing.

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Supplementary material

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