Alpine meadow could be better restored before moderately degradation using inorganic nitrogen on the Tibetan Plateau

Jianbo Sun[12*], Chimin Lai[3*], Fei Peng[14], Junzhou[5], Mengting Hu[6], Xingzhi Xu[12], Xiaowei Gou[7], Huakun Zhou[8], Carly Stevens[9]

[1] Key Laboratory of Ecological Safety and Sustainable Development in Arid Lands, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

[2] University of Chinese Academy of Sciences

[3] School of Geography and Environment, Jiangxi Normal University, Nanchang, China

[4] Beiluhe Observation and Research Station on Frozen Soil Engineering and Environment in Qinghai-Tibet Plateau

[5] Key Laboratory of Mountain Surface Processes and Ecological Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu, China

[6] Anhui Normal University, Wuhu, China, 241002

[7] Wildfire Research Center, National Institute of Natural Hazards, Beijing 100085, China

[8] Institute of Tibetan Biology Research, Chinese Academy of Sciences, Xining, China

[9] Lancaster Environment Centre, Lancaster University, Lancaster, UK

*Equally contribute to the paper

Correspondence to Fei Peng, pengfei@lzb.ac.cn

Abstract

Nitrogen (N) fertilization is considered as a powerful alternative approach to restore degraded alpine meadows. However, whether form of available N matters and their efficiency along a degradation gradient remains largely unexplored. A four-year N addition experiment with different available N forms (NH_4^+-N, NO_3^--N and Glycine) was carried out on the undegraded, moderately (MD) and severely degraded (SD) alpine meadows on the Tibetan Plateau. Plant aboveground productivity was greatly increased in undegraded and MD alpine meadows but belowground productivity was enhanced in SD alpine meadow, especially in the two inorganic N treatments. When inorganic N was added, plants accumulated more N compared to the control in undegraded alpine meadow. Plants accumulated more N in the MD alpine meadow than in undegraded alpine meadow in all forms of N addition treatments as the plant N uptake rate was almost doubled in MD alpine meadow when N was added. In SD alpine meadow, most of the added N moved to the microbial N pool with the largest increase observed in the NO_3^--N treatment. Leaching remained almost unchanged in NH_4^+-N and Glycine in undegraded and MD alpine meadows but increased in SD alpine meadow, especially in NO_3^--N treatment. However, leaching was low, accounting for less than 5% of the added N even in the SD alpine meadow. Our results indicate 1) the added N were mostly up taken and retained in plants or microorganisms regardless of N forms, 2) inorganic N is more efficient in increasing plant productivity for MD but not for SD alpine meadow, 3) fertilization using inorganic N to restore the degraded alpine meadow is efficient for MD but not for SD alpine meadows.

Keywords: Nitrogen addition, degradation, nitrogen partitioning, alpine meadow, Tibetan plateau

Introduction

Alpine meadows on the Qinghai-Tibetan Plateau (QTP), are of great importance for biodiversity conservation, soil and water conservation, carbon sequestration and supporting the livelihood of local people (Liu et al., 2022; Wang et al., 2022) but 70 % of it is severely degraded (Bardgett et al., 2021). Although there have been restoration efforts (Dong et al., 2024), sustainable restoration still requires further understanding of ecological processes and mechanisms involving alpine meadows' degradation (Bardgett et al., 2021). In nitrogen (N) limited ecosystems such as these (Du et al., 2020), manipulative N addition experiments have revealed an increase in plant biomass, litter decomposition rate, and soil microbial activity, thereby increasing soil organic carbon (SOC) and possibly being conducive to restoring the degraded ecosystem (Liu et al., 2019; Tang et al., 2023; Tian et al., 2019). Although N from atmospheric deposition has increased twofold in last two decades on the QTP (Chen et al., 2023), it is still much lower than the plant N requirements (Fu & Shen, 2016). Thus, N fertilizer application is practiced in many restoration actions on the QTP (Gu et al., 2023).

The form in which the N is applied (ammonia nitrogen: NH_4^+-N, nitrate nitrogen: NO_3^--N, and smaller molecular organic nitrogen like urea and amino acids) matters for two reasons: firstly, plant preferences for specific available N forms may determine the efficiency of them for plant growth performance in a short term (Xu et al., 2012); secondly, differences in mobility and associations with soil minerals differ between N forms, together with stoichiometry of plants, soils and microorganisms affecting the capability of them to retain the added N in the system and consequently long-term potential for restoration (Yang et al., 2022; Yang et al., 2021). All added N will be fiercely competed for by plants and microorganisms and is likely to be fully retained in the ecosystem regardless of the form in a N limited ecosystem (Choudhary et al., 2016). Plants can assimilate both inorganic N and smaller molecular compounds of organic N but they generally prefer inorganic N (Ashton et al., 2010; Harrison et al., 2007; Liu et al., 2017). Recent studies have indicated that plants in undegraded alpine meadows on the QTP mainly assimilate NH_4^+-N most effectively ((Lai et al., 2024; Lai et al., 2022). Any form of added N may first enter into the microbial N pool due to their competitive advantage resulting from their large volume to surface area ratio (Zhang et al., 2024) but more NH_4^+-N will be immobilized in plant organs or microbes because of its lower energy requirement for being synthesized into protein in contrast to NO_3^--N (L'Espérance et al., 2024). As they assimilate additional N, soil microorganisms would need to decompose more soil organic matter in order to maintain a stoichiometric homeostasis (Li et al., 2021; Zhu et al., 2021), thereby increasing the net mineralization rate and providing N for plants. In undegraded alpine meadows, NH_4^+-N is the most abundant form of available N in the soil. Even when NO_3^- -N or organic forms or N such as glycine are added, NH_4^+-N is still the most abundant available form (Lai et al., 2024; Lai et al., 2022).

The fate of different forms of additional N, whether it is transformed into NO_3^--N, quickly up taken by plants, lost by leaching, or immobilization or mobilization by microbes, depends on the soil environment. With degradation of alpine meadows, changes in the soil environment such as an increase in soil pH, improvement in aeration, and reduction in soil moisture (Peng et al, 2016, 2018) favoring different microbial processes. The added NH_4^+-N and smaller molecular N may be transferred into oxidized forms like NO_3^--N and N_2O (Che et al., 2019; Liu et al., 2016). In conditions that result in more NO_3^--N in soil, the high mobility of NO_3^- anion may lead to a higher plant N uptake rates and thus retention of a large proportion of added N in plants (K. E. Chen et al., 2020); or on the other hand, less N may be retained in the ecosystem because of the high inclination of NO_3^- anion to be leached (Di & Cameron, 2002). Although the soil conditions in degraded alpine meadows facilitate nitrification and make NO_3^--N the most abundant available N in the soil, degradation also promotes the loss of N via leaching due to a lack of clay particles to adsorb NO_3^--N. This means that degraded soils are even more N limited. In severely N limited ecosystems such as arctic tundra, soil microbes retain 39 and 70% of the additional N in mineral and organic layers, acting as an effective buffer for leaching (Choudhary et al., 2016).

How the forms of N that are added impacts partitioning among active N pools in plants, soil and microorganisms along degradation gradients remain unexplored. We conducted a four-year field experiment with NH_4^+-N, NO_3^- -N and Glycine addition in undegraded (IN), moderately (MD) and severely degraded (SD) alpine meadows in the inland of QTP to investigate the fate of N after addition, and the efficiency of different N forms in restoring the degraded alpine meadow. We hypothesized that 1）NH_4^+-N addition will result in the highest plant N uptake rate, the largest increase in aboveground net primary productivity (ANPP), and the greatest retention of added N within the plant N pool compared to NO_3^--N or glycine in undegraded meadows. 2）Microbial biomass nitrogen (MBN) will increase disproportionately relative to plant N uptake in degraded (meadows, especially for NO_3^--N and glycine additions, leading to a higher microbial C:N ratio. 3）Inorganic N fertilization (particularly NH_4^+-N) will be most effective at increasing overall ecosystem N retention and plant productivity (ANPP & BNPP) in moderately degraded (MD) meadows, but will have diminished efficacy in SD meadows where microbial immobilization dominate N fate.

Materials and methods

Site description

Alpine meadows which were undegraded, moderately degraded (MD) and severely degraded (SD) were set up near Beiluhe Permafrost Engineering and Environment

Comprehensive Observation and Research Station (34° 49' N, 92° 55' E) in a permafrost region in the hinterland of the QTP (Fig. S1). The meadows were classified according to (Xue et al., 2009). In the classification framework, plant coverage, plant community composition, and the aeolian activities are considered. The study site is in an alluvial and diluvial high plain with relatively flat terrain. The altitude in the study site is 4620–4640 m. Annual average temperature and precipitation was -3.8 ℃ and 290 mm (2009–2024). More than 90% of the precipitation occurs in May-September when plants grow, and the freezing period is from October to April. The permafrost in the study area is 30–70 m deep, and the depth of active layer is 1.5–3.5 m (Li et al., 2021a). The plant community of the undegraded alpine meadow in the study area is dominated by sedges, namely *Kobresia humilis* and *Kobresia pygmaea*. With degradation, forb species like *Leontopodium nanum* and *Saussurea pulchra* typically increase (Li et al., 2024).

N fertilization experiment

An experimental area covering the undegraded, MD and SD meadows (100 × 100 m) was set up near the research station. Twelve 2 × 2 m plots were randomly established in each alpine meadow, and the spacing between each pair of plots was about 5 m.

Starting in 2019 N fertilizer was added as NH_4Cl, KNO_3 and Glycine with an equivalent N amount of 3.1 g m^{-2} yr^{-1} in July each year, 20 times the average N deposition in the eastern QTP (Liu et al., 2015). All N forms were first dissolved in water and sprayed manually. The unfertilized control plot received the same amount of water. All the N addition and control treatments had three replicates in each meadow.

Plant community, productivity and C, N concentration

A 30 × 30 cm quadrat was set in each plot to study plant community characteristics. All vegetation was clipped at ground level to determine the above-ground net primary productivity (ANPP) in mid-August 2020, 2021, and 2023. The below-ground net primary productivity (BNPP) was determined using the in-growth method. In September 2019, a PVC collar with 13 cm in diameter and 30 cm in depth was inserted into the center of each plot. The PVC was refilled with local soil after removing root debris with 2 mm sieve. In September 2020, 2021, and 2023, roots in each PCV were collected and washed. The oven-dried roots was considered BNPP (Lai et al., 2021). Following collection, above-ground and root biomass were dried, ground, and analyzed for C and N. The foliar and root C and N concentrations were measured with an elemental analyzer (Elementar Vario EL, Hanau, Germany).

Soil sampling and measurement of N concentration

Soil samples were taken in each plot using the five-point sampling method. Soil cores (5 cm in diameter) were collected at 0–10, 10–20, 20–30 cm depths, and sub-samples of the same depth were mixed to form a composite sample. After the visible roots and other debris were picked out, each composite soil sample was sifted through a 2 mm sieve and then divided into two sub-samples. One was stored in a refrigerator at 4 ℃ for the measurement of NH_4^+-N, NO_3^--N, soil microbial biomass carbon (MBC) and nitrogen (MBN) and water content, and the other part is naturally air-dried for the determination of soil pH, SOC and total nitrogen (TN).

Soil MBC and MBN were determined using the chloroform fumigation-potassium sulfate extraction method (Brookes et al., 1985).Soil pH was tested using a acidimeter

(FE28K, Mettler Toledo, Shanghai).The NH_4^+-N, NO_3^--N in soil were determined by flow autoanalyzer (SEAL Analytical AutoAnalyzer 3, Northern Ireland, UK). Soil inorganic N was the sum of NH_4^+-N and NO_3^--N.

Nitrogen partitioning among soil, plant and soil microorganisms

A randomized block design was adopted, with three identical plots for each treatment. All measurements were conducted during two major field surveys: the initial sampling in June 2022 and the final sampling in September 2023. This period covered the important growing season while taking into account practical limitations such as the impact of the COVID-19 pandemic and worker availability.

Plant aboveground parts were collected at the peak of plant growth (mid-August). ANPP and BNPP were multiplied by the N concentrations of leaves and roots respectively, and then added to obtain the plant N pool. By comparing the increase in the plant N pool between the fertilized plots and the unfertilized plots during the two sampling dates, the amount of additional N accumulated by the plants due to N addition measures was determined.

The determination of soil and microbial N content involved collecting soil cores up to 30 centimeters deep, which were divided into three depth layers (0 - 10 cm, 10 - 20 cm, 20 - 30 cm). For each layer, we calculated the N storage, taking into account the soil bulk density. Three types of soil N were measured: MBN, inorganic nitrogen, and DON. The calculation of the additional N stored in the microorganisms and the N retained in the soil was the same as that for plant N: by comparing the changes in these N reserves between the fertilized plots and the control plots during the period from June 2022 to September

2023.

From June 2022 to September 2023, in situ anion-cation exchange resin bags were buried in PVC pipes with a diameter of 5 centimeters, placed at depths of 10 centimeters and 20 centimeters respectively. To assess the N loss due to leaching. These resins were extracted with 1 M KCl solution and the concentrations of NH_4^+-N and NO_3^--N in the resins were detected by a flow automatic analyzer. The concentration of inorganic N (the sum of NH_4^+-N and NO_3^--N concentrations, mg kg^{-1}) at each depth (10 cm and 20 cm) was divided by the area of the PVC tube. The inorganic N concentrations were then converted into unit area N leaching (Karhu et al., 2021).

^{15}N labeling experiments were conducted to explain the N partitioning between plant and soil microorganisms. The ^{15}N labeling experiment was conducted in August 2021. We used three types of ^{15}N-labeled chemicals: $^{15}NH_4^+$ (98 atom% ^{15}N), $^{15}NO_3^-$ (99 atom% ^{15}N), and ^{15}N-Glycine (98atom% ^{15}N). The specific labeling methods, sampling and calculations refer to our previous article (Lai et al., (Lai et al., 2024; Lai et al., 2022).

Statistical analysis

Analyses of observational and experimental data were performed using SPSS 27.0 (SPSS, Chicago, IL, USA) software. Separate one-way analyses of variance (ANOVA) were conducted for each degradation level (undegraded/IN, moderately degraded/MD, severely degraded/SD) to assess the impact of nitrogen addition treatment (Control, NH_4^+-N, NO_3^--N, Glycine) on individual response variables (e.g., pH, water content, ANPP, BNPP, plant C and N concentration, soil available N, N absorption rates, N pools, leaching). Where the ANOVA indicated a significant treatment effect (*p* < 0.05), Tukey's Honestly

Significant Difference (HSD) post-hoc test was used to identify significant differences between individual treatment means within that degradation level. Homogeneity of variances was verified using Levene's test and normality of residuals was assessed using the Shapiro-Wilk test; data met ANOVA assumptions. Structural equation modeling (SEM) was employed to investigate the specific mechanisms and pathways through which N addition affects ecosystem productivity. The model was developed based on established ecological theory and estimated using the *lavaan* package in R 4.5.1. N addition was specified as an exogenous variable directly affecting key soil properties (including soil pH, soil moisture, soil available N, and soil C/N) and plant C/N, which were hypothesized to subsequently influence ANPP and BNPP(Sun et al., 2020; Zhang et al., 2026). After N addition (especially NH_4^+), the nitrification process ($NH_4^+ > NO_3^-$) releases H^+ ions, which is the main cause of soil acidification (Wang et al., 2023). The effect of N addition on soil moisture was based on the fact that N addition could promote plant growth (especially aboveground parts), thereby increasing transpiration water loss, which may lead to a decrease in soil moisture(Liang et al., 2020). The effect of N addition on soil C/N was based on the ecological processes that exogenous N input dilutes the relative concentration of soil organic matter, usually resulting in a decrease in the soil C/N (Li et al., 2023; Wang et al., 2020; Xu et al., 2022). When N is abundant, microorganisms accelerate the decomposition of organic matter (priming effect), which may also release CO_2 and indirectly affect the C/N(Qin et al., 2024; Widdig et al., 2020). Correlations among mediating variables were permitted in the model specification. All figures present the data as mean ± standard error (SE). The significance level was set at $p = 0.05$.

Results

Changes in ANPP and BNPP after the N addition

In the control treatment, NPP (the sum of ANPP and BNPP) decreased with the severity of alpine meadow degradation. The responses of ANPP and BNPP varied annually, with degradation severity and different forms of added reactive N (Fig.1). In 2020 and 2021, ANPP was significantly increased by all N additions, with a relatively higher increase under inorganic N addition both in undegraded and MD alpine meadows (Figs.1a and b). In 2020, the addition of NH_4^+-N and NO_3^--N increased ANPP by 29.6% and 37.3% in undegraded, and by 57.3% and 61.5% in MD alpine meadow, respectively. In 2021, the addition of NH_4^+-N and NO_3^--N increased ANPP by 34.6% and 8.0% in undegraded, and by 81.2 % and 92.3 % in MD alpine meadow, respectively. But in SD alpine meadow, ANPP showed no change under any of the N addition treatments in 2021 (Fig.1b). In 2023, ANPP only increased in NH_4^+-N and Glycine treatments in MD alpine meadow but enhanced in all three N additions in SD alpine meadow (Fig.1c). BNPP showed no change or a slight decrease in all the three alpine meadows in 2020 and 2021, but it dramatically increased in the undegraded and MD alpine meadows under all N addition treatments in 2023 (Fig.1).

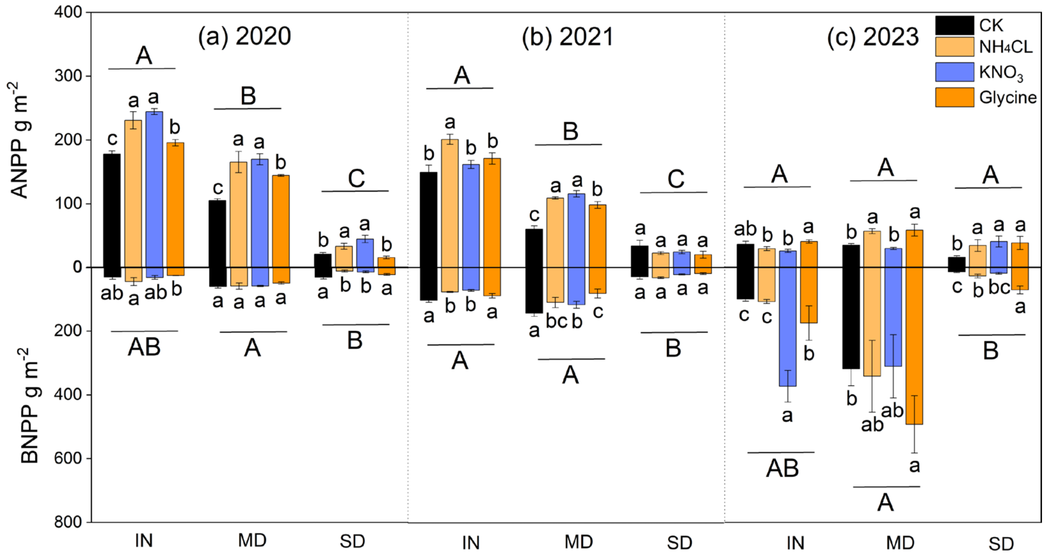

Fig. 1 The effect of different forms of available nitrogen (N) addition on ANPP and BNPP in undegraded, moderately degraded and severely degraded alpine meadows during 2020-2023. IN: undegraded; MD: moderately degraded; SD: severely degraded alpine meadows, respectively; CK: the control. Values are means ± SE (n=3)

Soil physical-chemical properties

The average pH value at the 0–30cm soil in three alpine meadows was above 7 regardless of treatment (Table 1). It decreased in the undegraded meadow but increased in MD and SD alpine meadows with the N addition. TN increased in all the N addition treatments in undegraded and MD alpine meadows, with the largest increase in NH_4^+-N

Table 1 The effects of N addition (NH_4Cl, KNO_3 and Glycine treatment) on soil pH, water content and inorganic N in alpine meadows at different degradation stages

	IN				MD				SD			
	CK	NH_4Cl	KNO_3	Glycine	CK	NH_4Cl	KNO_3	Glycine	CK	NH_4Cl	KNO_3	Glycine
pH	8.16 (0.00)^{a}	8.06 (0.02)^{b}	8.12 (0.01)^{ab}	8.13 (0.02)^{ab}	8.34 (0.01)^{b}	8.38 (0.01)^{b}	8.56 (0.00)^{a}	8.58 (0.01)^{a}	8.85 (0.01)^{a}	8.79 (0.02)^{a}	8.82 (0.00)^{a}	8.84 (0.01)^{a}
TN (g kg^{-1})	0.77 (0.03)^{c}	1.17 (0.06)^{a}	0.97 (0.05)^{b}	0.93 (0.02)^{b}	0.28 (0.01)^{b}	0.40 (0.02)^{a}	0.32 (0.01)^{b}	0.31 (0.01)^{b}	0.48 (0.05)^{a}	0.46 (0.03)^{a}	0.41 (0.03)^{a}	0.50 (0.07)^{a}
Soil C:N	15.93 (0.21)^{a}	16.04 (0.08)^{a}	15.38 (0.10)^{c}	15.56 (0.02)^{b}	12.11 (0.27)^{b}	13.13 (0.14)^{a}	12.34 (0.11)^{b}	12.28 (0.17)^{b}	7.59 (0.20)^{c}	8.55 (0.10)^{b}	8.34 (0.11)^{b}	9.44 (0.37)^{a}
Water content (%)	14.60 (0.18)^{c}	18.62 (0.31)^{b}	17.95 (0.55)^{b}	15.54 (0.44)^{a}	7.60 (0.14)^{c}	9.01 (0.17)^{a}	8.35 (0.04)^{b}	8.58 (0.24)^{ab}	10.81 (0.06)^{b}	10.93 (0.22)^{b}	11.66 (0.31)^{a}	10.07 (0.49)^{c}
Inorganic N (mg kg^{-1})	38.08 (0.68)^{b}	43.16 (0.47)^{a}	43.06 (0.62)^{a}	41.98 (1.34)^{a}	20.74 (0.71)^{b}	34.08 (3.49)^{a}	39.13 (1.68)^{a}	36.17 (1.92)^{a}	14.28 (0.29)^{c}	22.54 (1.88)^{b}	24.38 (1.02)^{b}	28.25 (0.90)^{a}

and no difference between NO_3^--N and glycine treatments (Table S1). The soil C:N was significantly increased in MD and SD but showed no significant response in undegraded alpine meadows in all N addition treatments. On average, the relative increase in C:N was larger in SD than in MD alpine meadows (Table S1).The soil inorganic N significantly increased in all meadows. On average, the increase of inorganic N was much higher in MD and SD than in undegraded alpine meadows (Table S1).

Changes in leaf and microbial carbon and nitrogen content and stoichiometry

The community leaf C content showed no significant response to any N addition treatments (Fig. 2a) in undegraded alpine meadow. It slightly increased in the Glycine (5.4%) and NH_4^+-N (4.2%) application in MD and SD alpine meadows, respectively. The community leaf N content was only slightly enhanced in Glycine and NH_4^+-N treatments in undegraded and MD alpine meadows and it was largely promoted in SD alpine meadow (Fig. 2c). Leaf C:N generally reduced under all the N addition treatments in the three alpine meadows, except the 9.2% increase of Glycine treatment in the MD alpine meadow (Fig. 2e).

The MBC was only increased significantly (by 8.2%) in the NO_3^--N addition but MBN increased by 94.7% and 116.2% in NH_4^+-N and NO_3^--N addition in undegraded alpine meadow (Fig.2b). The NO_3^--N and Glycine addition reduced both the MBC and MBN in MD alpine meadow (Figs. 2b and d). By contrast, NH_4^+-N addition increased them although was only statistically significant for MBC (Fig. 2b). The addition of inorganic N largely reduced the MBC and MBN, whereas the Glycine had opposite effect on MBC and MBN in SD alpine meadow (Figs. 2b and d). The microbial C:N responses to N addition greatly varied with different N forms and degradation status. It declined in all three N addition treatments in undegraded alpine meadow but increased in MD alpine meadow (Table S1). In undegraded alpine meadow the greatest reduction of microbial C:N occurred in NO_3^--N treatment (58.7%) while the greatest increase in MD alpine meadow was in NH_4^+-N treatment (161.7%) (Fig. 2f).

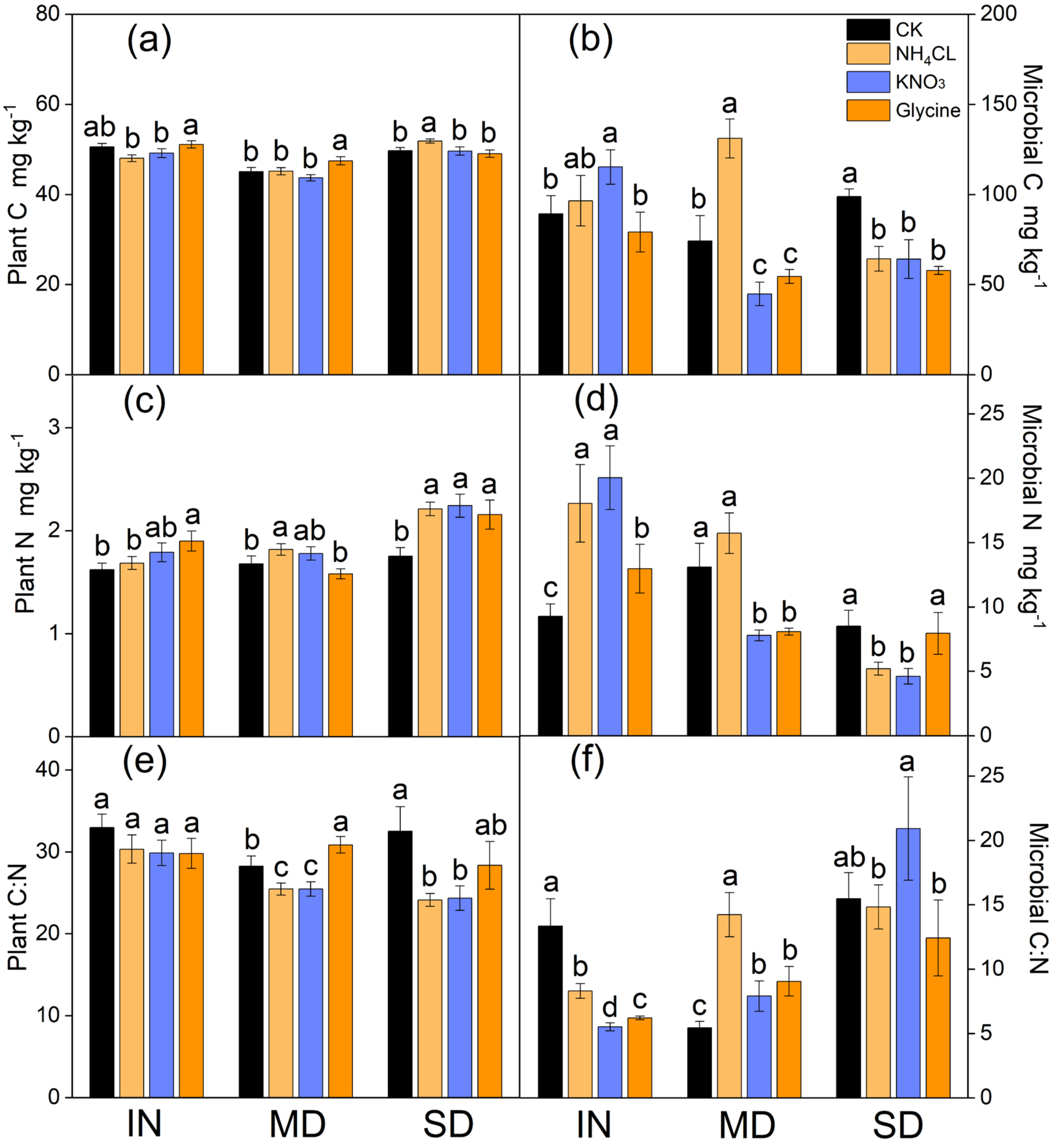

Fig. 2 The effect of different forms of available N addition on leaf C (a), soil microbial C (b), plant N (c), microbial N (d), plant C:N ratio (e) and microbial C:N ratio (f) in alpine meadow at different degradation stages. IN: undegraded; MD: moderately degraded; SD: severely degraded alpine meadows; CK: the controls. Values are means ± SE (n=3)

Changes in various N pools after different available N forms addition

The plant N pool increased under all N treatments (Fig. 3a). The increase was higher in inorganic N treatments than that of Glycine treatment (Fig. 3a). NH_4^+-N increased the plant N pool by 35.2%, 80.3% and 33.6% in undegraded, MD and SD alpine meadows, respectively. NO_3^--N treatment increased them by 52.4%, 77.7% and 65.5%, respectively (Fig. 3a). The plant N uptake rate was only increased in the NH_4^+-N treatment (84.8%) in undegraded alpine meadow, but it was increased in all N addition treatments in MD and SD alpine meadows. The increase in plant N uptake rate was not significantly different among the three N additions in either MD or SD alpine meadows (Fig. S2a).

Accumulation of soil inorganic N after one year was much lower in N addition treatments than control with 75.7%, 85.3% and 50.4% less N accumulated in NH_4^+-N, NO_3^--N and Glycine treatments relative to the control in undegraded meadow (Fig. 3b). In MD and SD alpine meadows, the soil inorganic N was decreased in N addition treatments contrast to the accumulation of it in control. In undegraded alpine meadow, the microbial N pool was decreased in the inorganic N additions but increased in the Glycine treatment (Fig. 3c). By contrast, microbial N pool only increased in Glycine treatment in MD and increased in all N addition treatments in SD alpine meadow. The microbial N uptake rate only slightly increased in Glycine treatment in MD (13.8%) and decreased in SD alpine meadows (56.0%) (Fig. S2).

In the undegraded meadow, leaching was only increased in the NO_3^--N treatment. But it increased in the Glycine treatment in MD and in both NO_3^--N and Glycine treatment in SD alpine meadows (Fig. 3d).

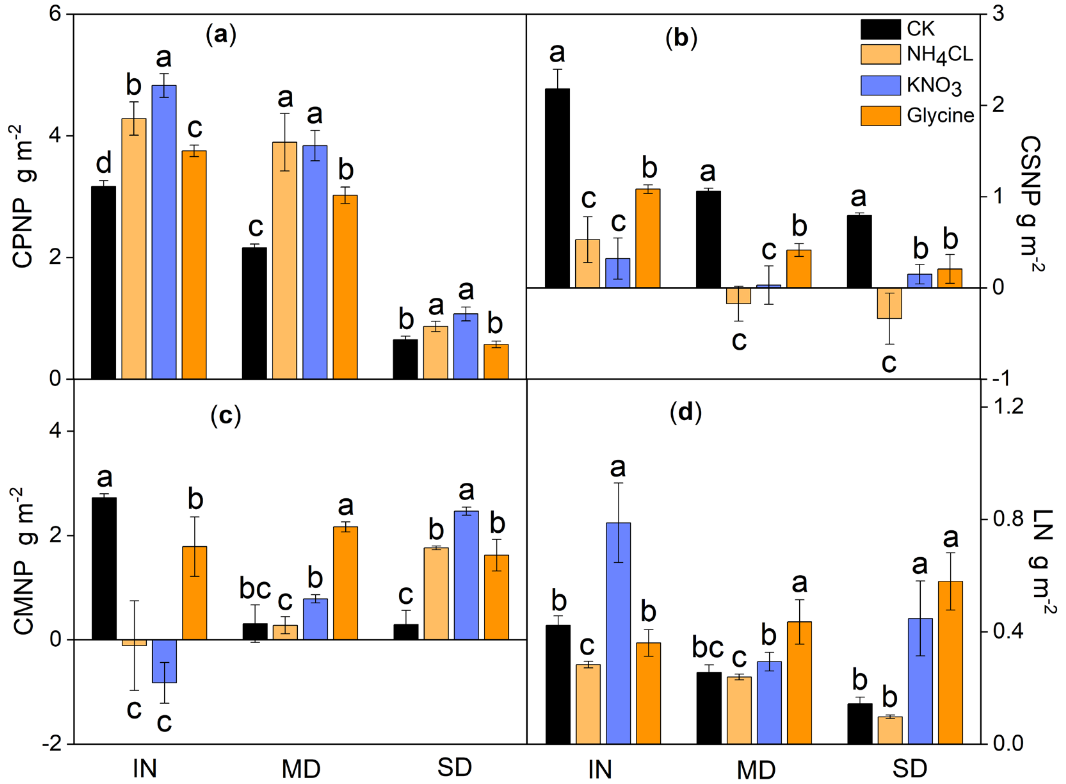

Fig. 3 The effect of different forms of available N addition on plant N pool changes in aboveground and belowground plants in the growing season of 2021 (a), soil available N pool between June 2021 and 2022 (b), microbial N pool (c) and LN (potential N loss due to leaching at the same period) (d) in alpine meadow at different degradation stages. See Figure 1 for abbreviations. Values are means ± SE (n=3)

Partitioning of N into different active N pools

In undegraded alpine meadow, plants retained 1.11 and 1.66 g N m^{-2} of added NH_4^+-N and NO_3^-—N, respectively. The soil inorganic N and microbial N pool shrank, and leaching showed minimal change (Fig. 4a, b, c). The sum of the four N pools showed a net loss in NH_4^+-N but a minor gain in NO_3^--N and Glycine treatments.

The N retained in plants was greater in any N treatment in MD compared to undegraded alpine meadows. Like the undegraded, the soil inorganic N pool declined in all N addition treatments in MD, yet microbial N pool increased, especially in the Glycine treatment. This contrasted with the reduction observed in the undegraded alpine meadow where the four N pools showed a net gain after N addition, with the greatest gain in NO_3^--

N treatment (Figs. 4d, e, f).

The soil inorganic N pool also declined in the SD alpine meadow with N addition but the microbial N pool was greatly enhanced in all N treatments compared to the undegraded and MD alpine meadows. The N addition resulted in a net gain in SD but all of which was lower than in the MD alpine meadow (Figs. 4 g, h, i).

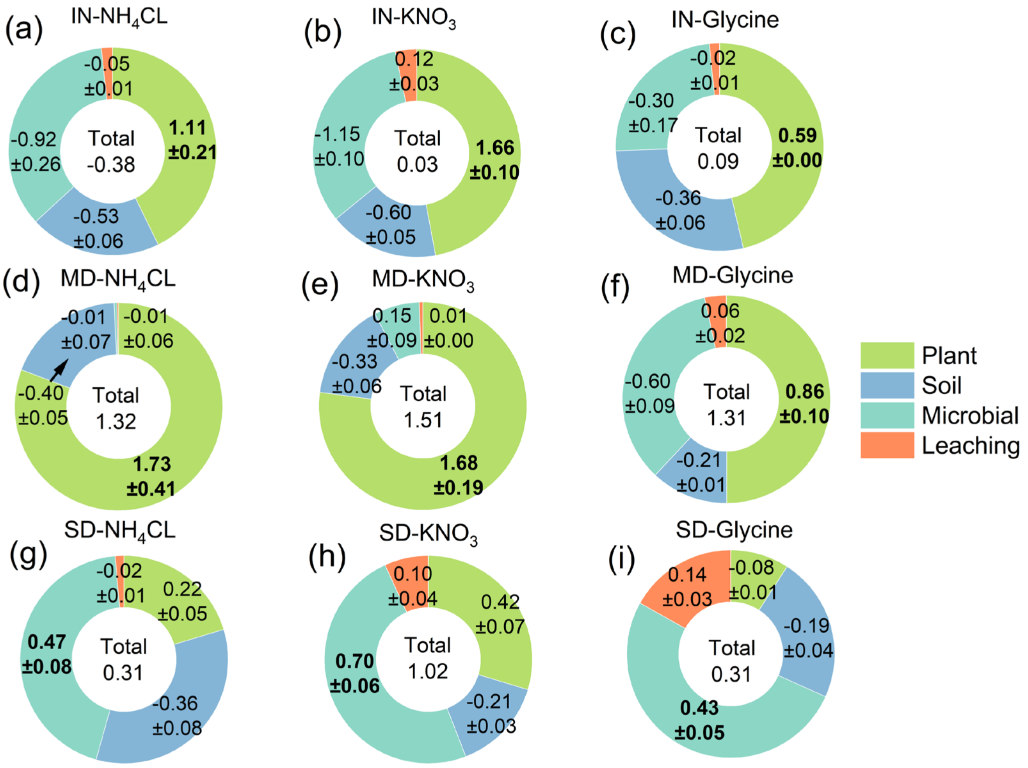

Fig. 4 The partitioning of added N (mg m^{-2}) into active N pools and the net balance among them in responding to addition of different available N forms relative to the control (simple mass balance method). Although leaching is a flux not a N pool, the accumulated N in the anion-cation resin could be treated as potential loss N during a period. IN: undegraded; MD: moderately degraded; SD: severely degraded alpine meadows, respectively. Values are means ± SE (n=3)

Discussion

Impact of different forms N addition on NPP of alpine meadows with different degradation statuses

The observation of the largest increase in ANPP in NH_4^+-N treatment in the undegraded alpine meadow in 2021 (Fig. 1) supported our first hypothesis, which is in agreement with recent findings that plants use the most abundant available N in grassland ecosystem (Liu et al., 2024). The significant increase in plant N uptake rate in NH_4^+-N also could contribute to the largest increase of ANPP in undegraded alpine meadow (Fig. S2). But this phenomenon was not consistent throughout the three years. There was no difference in N addition induced change in ANPP between the N addition treatments in 2020 but in 2021 ANPP increase was much higher in NH_4^+-N than in other two forms of N addition. This implies that the impact of different forms of N addition on ANPP may take time to happen. In 2023, the measurement of ANPP and BNPP was conducted close to the senescence period, which may account for the lack of treatment response. Further observations are needed to confirm the impact of different N forms on ANPP.

Plant, microbial and soil inorganic N and C:N change with different forms N addition

Ecosystem retention of N depends largely on the size of plant and soil C pools and their C:N stoichiometric ratio (Lovett & Goodale, 2011; S. Yang et al., 2022). In an N limited ecosystem with high plant and soil C:N, plants and microbes will fiercely compete for the added N (Zhang et al., 2023). Under these circumstances, soil microbes will quickly absorb N to induce a priming effect on soil organic matter decomposition (Chen et al., 2020; Riggs et al., 2015), thereby improving plant N uptake and alleviating N limitation and declining the plant C:N (Lu et al., 2010). In our study, the higher microbial C:N in the control treatment of the undegraded relative to MD alpine meadows (Fig. 2f) suggests a relatively severe N limitation, thus a greater increase of microbial N and decrease in microbial C:N when N was added (Figs. 2d and f). The significant increase in plant and microbial N contents but no change in C contents (Figs. 2a-d) could contribute to the decline in their C:N in almost all treatments at the three alpine meadows. Microbial C:N generally declined more than plant C:N with added N (Figs. 2e, f) as MBN increased more than leaf N content (Figs. 2c, d), which might be due to superiority of microorganisms in competing for available N (Ouyang et al., 2016). Although the plant N uptake rate was greatly improved in MD (Fig. S2a) and the plant N pool increase was much larger in undegraded and MD than in SD alpine meadows (Figs. 3, 4), the largest increase in plant leaf N content increase occurred in SD (Fig. 2c), which is probably due to growth dilution of assimilated N with the much larger increase in plant productivity in undegraded and MD alpine meadows (Fig. 1).

N rention in active N pools with different forms of additional N in alpine meadows with different degradation statuses

In our study, the difference in N rention among different N forms addition in each alpine meadow is much lower than the difference among degradation statuses (Fig. 3 and 4). The addition of N will significantly increase the available N content in the soil. The available N in the soil will have a significant negative effect on plant C/N and soil C/N, thereby affecting the nANPP and BNPP, promoting plant growth, and leading to the accumulation of N in plants, litter, and soil (Fig. S3). In both the undegraded and MD alpine meadows, no matter what form of N was added, plants retained most of the added N, with the highest retention in inorganic treatments (Fig. 4). Although microbes are superior to plants in absorbing available N in the short term (Kuzyakov & Xu, 2013), in the long-term, plants will get the extra N when microbes mineralize organic N after its N limition is eased (Kuzyakov & Xu,

2013) with an increase in MBN and decline in C:N (Figs. 2d,f). In addition, the increased plant N uptake also could contribute to a higher retention of N in plants (Fig. S2). Although plants in arctic tundra typically prefer to ultilize N in the form of amino acids, our previous studies revealed that dominant species mainly uptake the most avaible form of N (Liu et al., 2024). In our study area that is inorganic N (Lai et al., 2022), which suports the greater increase of plant N uptake rate in the inorganic N addtion than the Glycine addition (Fig. S2).

The increase in plant N could be counteracted by the reduction in soil inorganic N pool . The sum of the changes in the active N pools in the undegraded alpine meadow are close to zero (Fig. 4a,b,c) suggesting an internal closed cycing of the added N and no N loss in terms of gaserous or leaching, which is also supported by a near zero ^{15}N in plants and soil in undegraded alpine meadow (Li et al., 2021b). The net loss of added N in NH_4^+-N treatment in the undegraded alpine meadow could be attributed to the larger increase in plant productivity (Fig. 1) as dominant plants of undegraded apline meadow prefer NH_4^+-N (Lai et al., 2022). The larger increase in plant N will transfer the active N into orgnaic N pools which is evidenced by the greater increase of TN in the undegraded alpine meadow (Table 1). The higher net N gain in MD compared to the undegarded alpine meadow could be the result of a lower reduction in soil inorgnaic N pool and a higher increase in plant N pool (Fig. 3 and 4). The larger rention of N in plants in MD alpine meadow could be the result of the increase in plant N uptake rate and relatively low microbial N uptake rate (Fig. S2). When alpine meadows was degrade, easily decomposable plant litter with lower C:N increases (Lai et al., 2021). These changes would acclelerate the soil orangic matter decompostion and replenish the soil inorganic N pool, therefore result in less reduction of soil inorganic N in MD alpine meadow (Fig. 3 and 4). The smaller increase in TN in MD relative to the undegarded alpine meadow (Table 1) support this.

In SD alpine meadow, microbes retained most of the added N relative to plants (Fig. 4g,h,i) which partly support our second hypothesis as plants retained most the added in MD alpine meadow. The higher microbial C:N in SD than in undegraded and MD alpine meadows (Fig. 2f) indicates a relatively severe N limitation for microbes in SD alpine meadow. Thus, the added N would be mobilzied by microbes which is supported by the realtively greater increase of microbial N pool (Fig. 3c). Although microibal N rention is higher, the net N gain is lower than in MD alpine meadow as leaching is enhanced (Fig. 3 and 4). The changes in soil texture, in particular an increase in silt and sand with alpine meadow degradation (Peng et al., 2018) will increase leaching because of reduction in soil minerals and soil organic matter's adsoprition to added N. The negative charge of soil minerals and organic matter could result in a higher leaching in NO_3^--N and Glycine but not in NH_4^+-N treatment in SD alpine meadow (Fig. 3d).

Conclusion

Although leaching increased with alpine meadow degradation in NO_3^- -N and Glycine treatments, it only accounted less than 5% of added N. The added N was mostly incorporated into plant biomass in undegraded and MD alpine meadows while it was retaiend primarily in microorganisms in SD alpine meadows. Plant productivity and plant N uptake rate was higher in the MD alpine meadow with a relatively larger increase in the inorganic N addition treatment. Thus, to restore the degraded alpine meadow, N fertilization should be applied before the meadow reaches the stage of being moderatley degradaded and use of inorganic N, epseicaly the NH_4^+-N is more efficient.

Conflict of Interest: The authors declare that they have no conflict of interest.

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