Changing soil moisture and pH with alpine meadow degradation determines nitrogen preference of dominant species

Abstract

1. Our previous results revealed dominant plant species of alpine meadows under various degradation stages showed differential preference to the three available N forms (ammonia, nitrate, and amino acids). However, the perseverance of the N-uptake preference of the species in different soil conditions and its affecting factors remains unknown, which determines efficacy of nutrients addition in the restoration of degraded alpine meadows.

2. An indoor pot experiment was conducted to investigate the plasticity and determinants of different plant species' N-uptake preference using ^{15}N-labeled inorganic N ($^{15}NH_4^+$ and $^{15}NO_3^-$) and one of dual-labeled (^{13}C-^{15}N) amino acid (glycine). In the experiment, dominant species of alpine meadow under specific degradation status were planted in soils of alpine meadows with three different degradation status.

3. Different with the changing preference of dominant species in the field, results of this study showed that all species preferred to uptake nitrate in all soils, except the *Kobresia humilis*, *Carex moorcroftii*, and *Aster flaccidus* planted in the soil of severely degraded alpine meadow (SD-soil), which took up more ammonia. The relative abundance of different available N forms directly affects the N-uptake preferences of all species. Either soil moisture or pH was controlled, the partial correlations between percentage uptake and availability of various nitrogen forms changed. Differences in soil moisture and pH among the three alpine meadows determines the N uptake preference of the nine species through their impacts on the relative abundance of different available N forms.

4. *Synthesis*. The forms of available N added into soil will change with N cycling processes regulated by soil moisture and pH, thereby affecting the plant N uptake. Our results imply 1) during the restoration of degraded alpine meadow the use of appropriate N form that favors the target plant species should well match the soil environment, 2) adjusting the soil moisture and pH, and consequently affecting the different abundance of various available

N forms therefore drive the plant community restoration may be better than directly adding different available N to degraded alpine meadow.

Keywords: Alpine meadow, grassland degradation, ^{15}N tracer, N acquisition strategies, Organic and inorganic N

1. Introduction

Alpine meadows on the Qinghai-Tibetan plateau (QTP) are home to and the livelihood source of 5 million people belonging to ethnic minorities. However, even though a few local and national restoration projects have been implemented, alpine meadows on the QTP are still regarded as severely degradation (Harris, 2010; Miehe et al., 2019). Degradation greatly harms the provision of ecosystem services such as carbon storage and the livelihood of local farmers (Wen et al., 2013). Fertilization is the most common and effective measure used to restore the productivity of degraded alpine meadows (He et al., 2020; Dong et al., 2020). Nitrogen (N) is an essential macronutrient that often limits net primary productivity in terrestrial ecosystems (Vitousek & Howarth, 1991; Lebauer & Treseder, 2008; Moreau et al., 2019), especially in cold ecosystems where N mineralization is slow due to low temperatures (McKane et al., 2002; Song et al., 2007; Zhang et al., 2021). Thus, N fertilizer application has been widely used to restore the alpine meadows. However, several studies have shown that plants have a preference with respect to the uptake of different forms of available N (NH_4^+, NO_3^- and low-molecular-weight organic N) (Wang & Macko, 2011; Wang et al., 2012; Lai et al., 2023). Given this fact, the effectiveness of N fertilizer application in the restoration of degraded alpine meadows may depend on the use of the most appropriate form of N.

Most plants obviously prefer to absorb and utilize inorganic N in soil (Harrison et al., 2007; Ashton et al., 2010; Liu et al., 2017; Yi et al., 2023). However, plants may differ in their capacity to utilize different forms of inorganic N. Some plants prefer to absorb NO_3^- in soil, while others prefer to utilize NH_4^+ (Zhang et al., 2018; Hong et al., 2019; Liu et al., 2022). For example, an *in-situ* ^{15}N-labeling experiment found that alpine meadow species *Kobresia humilis* and *Kobresia pygmaea* preferred to take up NH_4^+ while *Stipa aliena* and *Saussurea pulchra* take up more NO_3^- (Xu et al., 2004; Wang et al., 2012; Lai et al., 2023).

However, most previous studies have typically focused on the dominant species' N utilization preference in specific environments, leaving the response of plant N utilization preference to environmental fluctuations largely unexamined. Moreover, the proportion of different N forms that plants absorb can depend on many interacting factors, making it hard to isolate the factors influencing plant N utilization preference in previous *in-situ* experiments.

Plants have evolved nutritional adaptations to different forms of N, thus can often be found on soils enriched in the particular N source to which they are most adapted. Indeed, some plants appear to be so well adapted to a specific N source that they appear to prefer it under a wide range of conditions (Britto & Kronzucker, 2013). For example, seedlings of *Picea glauca* and *Pinus radiata* showed greater growth and N uptake with NH_4^+ than with NO_3^-, regardless of changes in soil environment (McFee & Stone, 1968). Other studies have indicated plant preferences for one inorganic N source over another for various plant species/genotypes (von Wirén et al., 1997; Britto & Kronzucker, 2013). Therefore, closely phylogenetically related species may exhibit similar plant N-uptake preferences (Li et al., 2022). Some studies have also shown that plant N-uptake strategies may be related to plant functional traits (Moreau et al., 2019). For example, plants promote the uptake proportion of inorganic N by increasing specific root length and specific root surface area (Hong et al., 2017). These studies suggested that plants may adapt to environmental conditions and complete their life history through long-term evolutionary optimal N-uptake strategies.

Nevertheless, another view states that plant N-uptake strategies are flexible and plastic, adjusting quickly in a short-term under the influence of environment. The relative amounts of N in soils is identified as the main factor determining which forms of N are used by plants and plant N-uptake strategies may change with the concentration of available N (Chapin et al., 2002; Andersen & Turner et al., 2013; Song et al., 2015). Soil moisture, and pH influence the forms of N in soil and, therefore, the forms of N uptake by plants (Britto & Kronzucker, 2013). For example, plants may switch N sources from NH_4^+ to NO_3^- along an environmental gradient from wetter to drier conditions (Houlton et al., 2007; Mansson et al., 2014; Wen et al., 2021) due to intensified nitrification and a higher NO_3^- availability in

relatively dry environments. By comparison, a shift in the main source of plant N from NH_4^+ to NO_3^- was reported when the soil changed from acidic to alkaline (von Wirén et al., 1997; Hawkins & Robbins, 2010). In order to determine the most appropriate approach for restoration of degraded meadows, the perseverance of N use preference of dominant species along degradation gradients and the factors that affect it should be examined.

Degraded alpine meadows provide a natural platform for assessing the changes in plant N acquisition strategies along an environmental gradient. Soil moisture decreases and pH increases with grassland degradation (Peng et al., 2018). The total available soil N also decreases, and the concentration of different forms of N may change with grassland degradation (Che et al., 2017; Lai et al., 2023). Grassland degradation can also change the plant community composition, and plant properties (Lai et al., 2021). Our previous studies have shown that the dominant species at non-degraded alpine meadow prefer to absorb NH_4^+ whereas the dominant species at moderately degraded alpine meadow prefer to absorb NO_3^- (Lai et al., 2023). Although the dominant species at severely degraded prefer to absorb inorganic N, the proportion of organic N used increased (Lai et al., 2023). Whether the N use preference of plants persists under changing environmental conditions, and what are the main mechanisms of plant N uptake preference in degraded alpine meadows remains unknown. To solve this problem, we conducted a greenhouse pot experiment to grow the dominant species from each degradation stage in soils of various degradation stages, and a short-term ^{15}N labeling experiment to examine the plant uptake of different available forms of N when the plant reaches maturity.

2. Materials and Methods

Study site and species selection

Collection of “field-conditioned” soils was done at the non-degraded (Intact), moderately degraded (MD), and severely degraded (SD) areas of an alpine meadow in the central QTP (92°56'E, 34°49'N), near the source of the Yangtze River, with a mean elevation of 4635 m above sea level. The detailed information about environmental background of the field area and identification of land degradation stages can be seen in our previous study (Lai et al., 2023). We selected three dominant species at each degradation stage based on our previous vegetation survey data (Lai et al., 2021, 2023).

Kobresia humilis Sergievskaya (Cyperaceae), *Kobresia pygmaea* C. B. Clarke (Cyperaceae), and *Poa pratensis* L. (Poaceae) were the dominant species at the non-degraded grassland. *Saussurea pulchra* Lipsch (Asteraceae), *Leontopodium nanum* (Hook. f. et Thoms.) Hand.-Mazz. (Asteraceae), *Potentilla acaulis* L. (Rosaceae) were the dominant species at the moderately degraded grassland. *Carex moorcroftii* Falc. ex Boott (Cyperaceae), *Allium sikkimense* Baker (Amaryllidaceae), and *Aster flaccidus* Bge. (Asteraceae) were the dominant species at the severely degraded grassland. For detailed information about the relative cover, height, frequency, and biomass of each dominant species please refer to Lai et al. (2023).

Pot experiment

In the greenhouse experiment, the nine species from three degradation stages in an alpine meadow on the QTP were used in this study. Seeds of all species were collected in August 2020 near the field experiment site. The greenhouse experiment was conducted at the Linze Experimental Station of Lanzhou University (103°05'E, 38°38'N; 1400 m a.s.l.) from June to September 2021. The seeds were initially germinated in a glass culture dish and placed in an incubator. The percentage seed germination was determined in trials for each species (*Poa pratensis* L., 95%; *Saussurea pulchra*, 50%; *Leontopodium nanum*, 90%; *Potentilla acaulis* L., 50%; *Allium sikkimense*, 80%; *Aster flaccidus*, 90%). Due to very low germination rates, underground buds of sedge species (*Kobresia humilis*, *Kobresia pygmaea*, and *Carex moorcroftii*) were transplanted from field plots. This approach was also used in a pot experiment about sedge species (Phoenix et al., 2020). Soils were collected across three plots (3 m × 3 m) within each degradation stage nearby the research station. As roots of all species were mainly concentrated at 0–20 cm soil layer (Lai et al., 2023), we collected soil at 0–20 cm depth and sieved to 2 mm to remove large roots and rocks. Soil of the same degradation stage was mixed. The field-collected and processed soil was cooled immediately to −4°C in refrigerated trucks and then transported to the experimental station.

To investigate the plant N-uptake preferences of each species under changing environments, we conducted the experiments with 9 dominant species in soils of three degraded alpine meadows, and 4-^{15}N-labeling treatments ($^{15}NH_4^+$,$^{15}NO_3^-$, [$^{13}C_2$,^{15}N]-

glycine, and control), 3 replicates for each ^{15}N-labeling treatments, with a total of 324 pots (3 (degradation stages)×9 (dominant species)×4 (^{15}N-labeling treatments)×3 (replicates)). Pots (15 × 15 × 17 cm deep) were filled with 2 kg prepared soil and placed at room temperature for 2 weeks. All seedlings emerging from the soil seed bank were removed in the 2 weeks. Five seedlings of same species (about 1 cm high) and underground buds of sedge species were planted in each pot. Seedlings that died within the first week after transplanting were removed and replanted. The environment in the greenhouse was set similar as much as possible to the field during the growing season. The air temperature in the greenhouse was 24℃ during the day (7:00 am to 22:00) and 16℃ at night (22:00 to 7:00 am), and the relative humidity was consistent at 65%. Throughout the study, soil moisture in pots was maintained at 65% of field capacity by adding distilled water every 1-3 days according to the water loss determined by weighing (Zhang et al., 2020). The pots were placed randomly and moved periodically in the greenhouse.

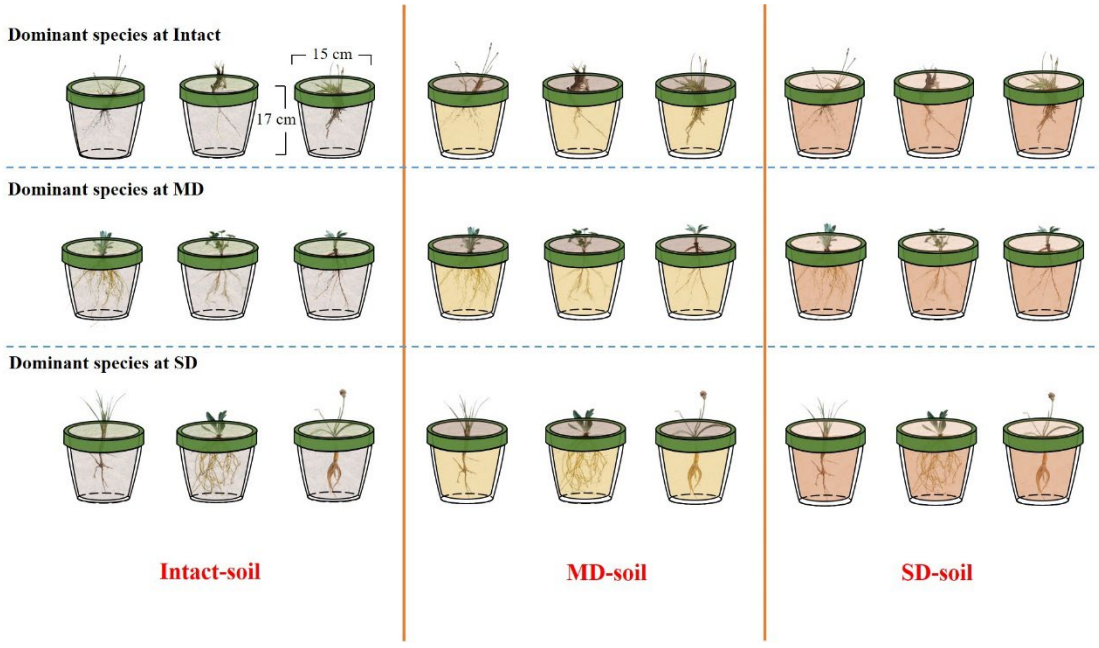

Fig. 1 Design of greenhouse pot experiment. Each row of pots represents one of the dominant species at the same degradation stage planted into soils of different degradation stages. Five seedlings of same species were planted in each pot. The figure above shows only one of the four ^{15}N-labeling treatments ($^{15}NH_4^+$,$^{15}NO_3^-$, [$^{13}C_2$,^{15}N]-glycine, and control), and the other three treatments were set as the same method. Intact, non-degraded alpine meadow; MD, moderately degraded alpine meadow; SD, severely degraded alpine

meadow

Isotope labelling and harvest

Short-term ^{15}N-labeling experiments were carried out on September 14th, 2021, after the seedlings in each pot had been growing for 12 weeks. We used three types of ^{15}N labels: $^{15}NH_4^+$ (98 atom% ^{15}N), $^{15}NO_3^-$ (99 atom% ^{15}N) and [$^{13}C_2$,^{15}N]-glycine (98 atom% ^{15}N) (Fig. 1). The ^{15}N concentration of the solutions with these labeled N sources was all 8 mM. The three ^{15}N-labeled treatments and a water control were injected one at a time at two soil depths (2.5 cm and 7.5 cm) in each pot. In total, 16 ml solution (1 ml per injection) was injected at the two soil depths at each of the eight injection sites around individuals of dominant species at each pot. There was no irrigation the day before labeling.

Six hours after the ^{15}N solution adding to the soil, plant and soil samples were collected from the pots (Xu et al., 2011). The soil was carefully separated from the plant roots. Harvested soil samples were immediately brought to the laboratory and stored at -4°C until measurements were made.

Sample analysis

Shoots and roots were rinsed first with tap water, and carefully separated, then roots were soaked in a 0.5 mM $CaCl_2$ solution for 30 min, and again with deionized water to clear the ^{15}N adsorbed onto the root surface (Xu et al., 2011). Roots were put in transparent plastic root disk, the root images were scanned with Epson scanner (10000XLPro, Canada) with a resolution of 300 dpi, then root characteristics were analyzed by Win Rhizo Pro (V2012b, Canada). The total root length and root diameter of each scanned roots were recorded, and specific root length ($cm \cdot g^{-1}$) was calculated. Root with diameter less than 2 mm was fine roots (Makkonen et al., 1999). Finally, shoots and roots were dried at 75°C for 48 h, weighed, and ground to a fine powder separately with a mortar and pestle. About 2 mg of plant material (shoots and roots separately) was weighed into tin capsules to analyze the C, N content, ^{13}C and ^{15}N atom% using a Vario EL cube interfaced with an IsoPrime100 isotope ratio mass spectrometer (Elementar Analysensysteme GmbH, Hanau, Germany).

The concentrations of exchangeable NH_4^+, and NO_3^- in soil were determined using a flow autoanalyzer (SEAL analytical AutoAnalyzer 3, Northern Ireland, United Kingdom)

after extraction with 1 M KCl. Soil water content was determined by the drying method, pH was measured using the acidimeter (FE28K, Mettler Toledo, Shanghai) with dry soil: water ratio of 1:2.5. The concentrations of soil dissolved organic N (DON) was determined using a TOC-TN analyzer (Elementar vario TOC select, Hanau, Germany) after extraction with 0.5 M K_2SO_4. The concentration of soil glycine was estimated considering the percentage of glycine to total dissolved organic N in alpine grasslands (Liu et al., 2022; Lai et al., 2023). The sum of three N fractions (exchangeable NH_4^+, NO_3^-, and dissolved organic N) in soil was estimated to indicate the available soil N under different degradation stages (Lai et al., 2023).

Calculation and statistics

^{15}N atom% excess (APE) was calculated as the atom% ^{15}N difference between labeled and control plants samples. ^{15}N amount in the shoot or root material (μg) was calculated by multiplying root or shoot N concentration (μmol N g^{-1} d.w.), the corresponding atom% (APE/100), biomass (B, g), and the relative molecular mass of ^{15}N (15), according to the following equation (Liu et al., 2020):

$$^{15}N\ (\mu g) = N_{concentration}\left(\frac{\mu mol}{g}\right) \times \frac{APE}{100} \times B\ (g) \times 15\left(\frac{g}{mol}\right) \quad (1)$$

Plant ^{15}N uptake rates ($P^{15}NUR$, μg ^{15}N g^{-1} h^{-1} d.w. root) was calculated by adding the root and shoot ^{15}N amount together, and then dividing by the labeling time (h), root biomass (g), as shown in the following equation:

$$P^{15}NUR\ \left(\mu g\ ^{15}N\ g^{-1}h^{-1}\right) = \frac{Root\ ^{15}N\ amount\ (\mu g) + Shoot\ ^{15}N\ amount\ (\mu g)}{Root\ biomass\ (g)\ Time\ (h)} \quad (2)$$

Plant N uptake rates (NUR, μg N g^{-1} h^{-1} d.w. root) were calculated by multiplying plant $P^{15}NUR$ by the concentration of native NH_4^+, NO_3^-, and N-glycine in soil (MN, μg N g^{-1}), and then divided the amount of ^{15}N labeling and ^{15}N labeling abundance, as follows (McKane et al., 2002):

$$NUR\left(\mu g\ N\ g^{-1}\ h^{-1}\right) = \frac{P^{15}NUR\left(\mu g\ g^{-1}h^{-1}\right) \times MN\left(\mu g\ g^{-1}\right)}{^{15}N_{added} \times\ ^{15}N\ labeling\ abundance} \quad (3)$$

Total N uptake was calculated as the sum of the uptake of exchangeable NH_4^+, NO_3^-, and N-glycine. Preferences for different N forms were calculated as the ratio of individual uptake to the total N uptake.

One-Way ANOVA was conducted to test the differences in plant N-uptake rate and

preference of different available forms of N, soil moisture, pH, concentration of different available N forms, total soil available N concentration and root characteristics at different degradation stages soils. All the data satisfied normal distributions and homogeneity of variance tests when performing One-Way ANOVA. The uptake of intact glycine molecules by plants was corrected by the linear correlation slope of the percentage excess of ^{13}C and ^{15}N of the labeled plants (Fig. S1). We used Pearson correlations to test the relationships between plant N-uptake preference and soil environment, soil available N and plant properties. We used the "phytools" package of R (R Development Core Team, 2016) to construct the phylogenetic tree and the "ape" package was used to calculate the phylogenetic trees distance.

Variation partitioning analysis (VPA) that partitioned the variance shared by all factors was then used to quantify the independent and interactive contribution of each group of factors. We firstly used the VPA to examine the contribution of soil environment, soil available N and plant properties to plant N-uptake preferences. Soil moisture and pH were grouped as soil environment, the different forms of N and total available N were classified as soil available N, and root traits and phylogenetic trees distance were grouped as plant properties. The variation partitioning analyses were conducted with the R package "vegan" v.3.2.4 (R Development Core Team, 2016). The "caret" package of R was used to standardize all the data before the variation partitioning analysis.

Then, as soil pH or moisture may affect the availability of various N forms therefore the plant nitrogen uptake preference, its effect on the N-uptake preference was determined by comparing the zero-order and partial correlation coefficients when one of them was controlled. The greater the difference between the zero-order and the partial correlation coefficients, the stronger the effect of the factor being controlled (Doetterl et al., 2015). These analyses were conducted using the packages "ggm" and "psych" of the R statistical software v.3.2.4 (R Development Core Team, 2016).

Structural equation modelling was further used to evaluate the direct and indirect relationships between the plant N-uptake preference and soil environment (soil moisture and pH), different forms of N concentration and total soil available N concentration. This approach can partition the direct and indirect effects that one variable may have on another

and is therefore useful for exploring complex relationships in natural ecosystems. Owing to strong multicollinearity among the factors of soil N forms, we calculated the variance inflation factor (VIF) to remove the linearly dependent indicators before structural equation modelling construction (Table S1). The fit of the final model was evaluated using the model χ2 test and the root mean-squared error of approximation. The structural equation modelling analyses were conducted using AMOS 26.0 (Amos Development Corporation, Chicago, IL, USA).

3. Results

Soil characteristics

The concentration of total soil available N, inorganic N, DON, and glycine-N decreased significantly with degradation (Fig. 2a-e). The concentration of NO_3^- was the highest in soils of all degradation stages. The concentration of NO_3^- was 18, 11 and 4 times higher than that of NH_4^+ in soil of non-degraded (Intact-soil), moderately degraded (MD-soil) and severely degraded alpine meadow (SD-soil), respectively (Fig. 2a). The ratio of NH_4^+ to NO_3^- significantly increased in SD-soil (Fig. 2b).

Soil moisture was significantly declined in degraded soil (Fig. S3a), but there was no significant difference between MD-soil and SD-soil. Soil pH was significantly increased in degraded soil (Fig. S3b). It was 8.2 ± 0.02, 8.7 ± 0.03 and 8.9 ± 0.02 at Intact, MD and SD -soil, respectively.

Species characteristics

There were no significant differences in average total root length, fine root length, thick root length, root surface area, specific root length and specific root area of any species when planted in soils of different degradation stages (Fig. S4a-f). Phylogenetic trees of the same genus have close distance to each other (Table S2). For example, there is a close genetic distance between *Carex moorcroftii, Kobresia humilis* and *Kobresia pygmaea* of *Cyperaceae* family (Table S2).

N-uptake of the nine species at different soils

Kobresia humilis, *Carex moorcroftii*, and *Aster flaccidus* had higher uptake percentage for NO_3^- when planted in Intact-soil and MD-soil (Fig. 3c, g, and i), but took up more NH_4^+ when planted in SD-soil (Fig. 3c, g, and i). Other species mainly absorbed NO_3^- when

planted in any of the three soils (Fig. 3a, b, d, e, f and h).

Factors affecting plant N-uptake

Soil environment and soil available N had significant effects on plant N-uptake (Fig. 4). Soil available N explained 17% variance of the nine species' plant N-uptake preference whereas soil environment explained 4% of the variance (Fig. 4), which also interactively affected plant N-uptake preference (Fig. 4). The percentage uptake of NH_4^+ (Fig. 5a and S5) and NO_3^- (Fig. 5b and S5) were closely correlated with the ratio of NH_4^+ to NO_3^-, NH_4^+ to total available N and NO_3^- to total available N. No significant correlations were observed between plant N-uptake preference with root functional traits and phylogenetic tree distances (Fig. S5). When either soil moisture or pH was controlled, the zero-order correlation coefficients decreased (Fig. 5). The structural equation modelling analysis revealed that the change in soil environments (soil moisture and pH) affected the availability of different forms of soil N and thus influenced the plant N-uptake preferences (Fig. 6a). The total standardized effects of the soil environment were higher than for available soil N (Fig. 6b).

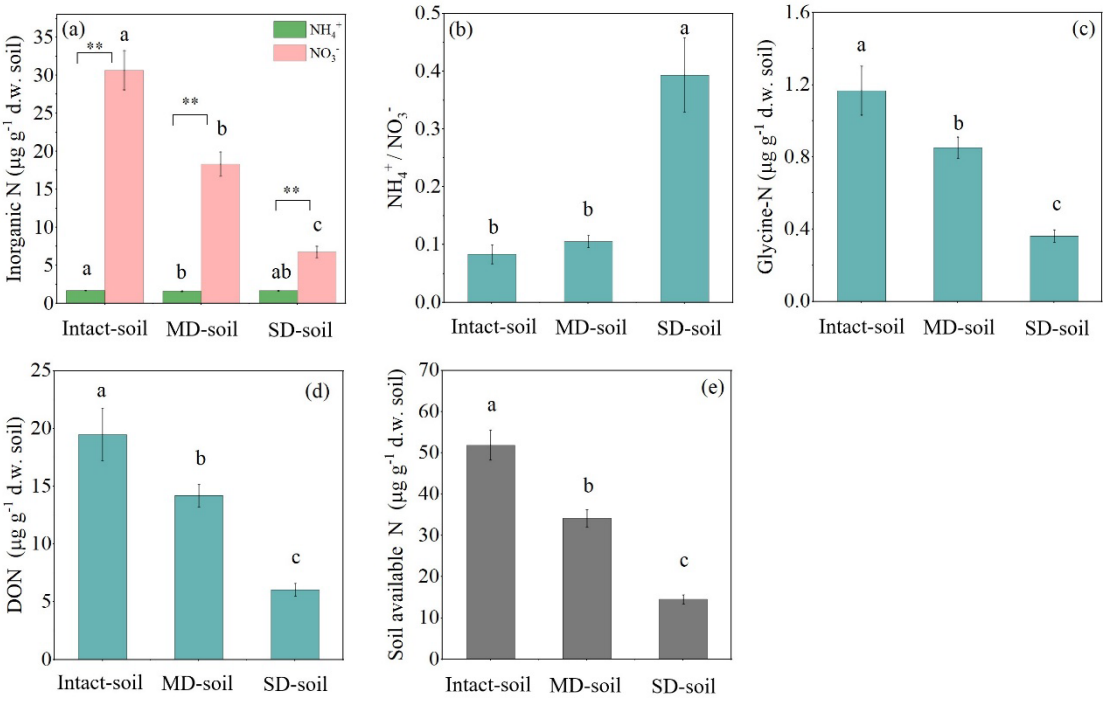

Fig. 2 Different forms of soil available N and total available soil N concentrations at different degradation stages soil. The values are means ± SE (n=27). Different lowercase letters above the columns indicate significant differences at $p < 0.05$ among different degradation stages soil

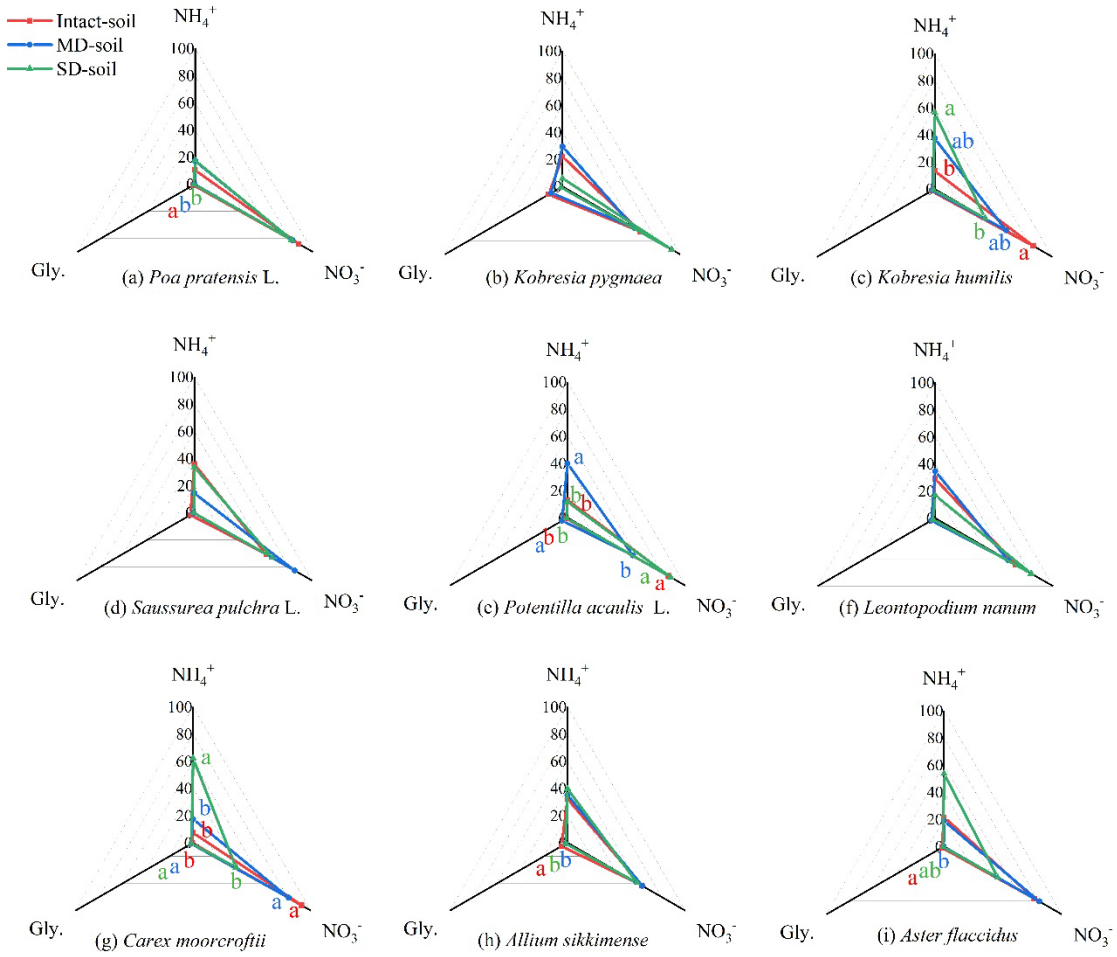

Fig. 3 The uptake preference of NH_4^+, NO_3^-, and glycine by dominant species at different degradation stages soil. The three-axis present the contribution of NH_4^+, NO_3^-, and glycine to total N uptake (%) by plants (total sum of three N forms in plants= 100%). The different colored lines represent the N uptake preference by all dominant species planted at different degradation stages soil. Different lowercase letters indicate the significant difference of plant uptake preference for the same form N in soil at different degradation stages soil (p<0.05)

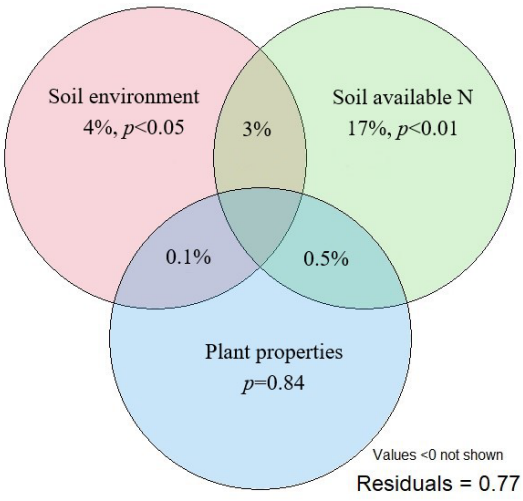

Fig. 4 Variation partitioning analyses (VPA) reveal the relative contribution of soil

environment, soil available N and plant properties to different form of N-uptake (%) by plant. Soil environment includes soil moisture and pH; Soil available N includes the concentration of different forms of N and total available N; Plant properties includes total root length, fine root length, thick root length, root surface area, specific root length, specific root area and phylogenetic trees distance

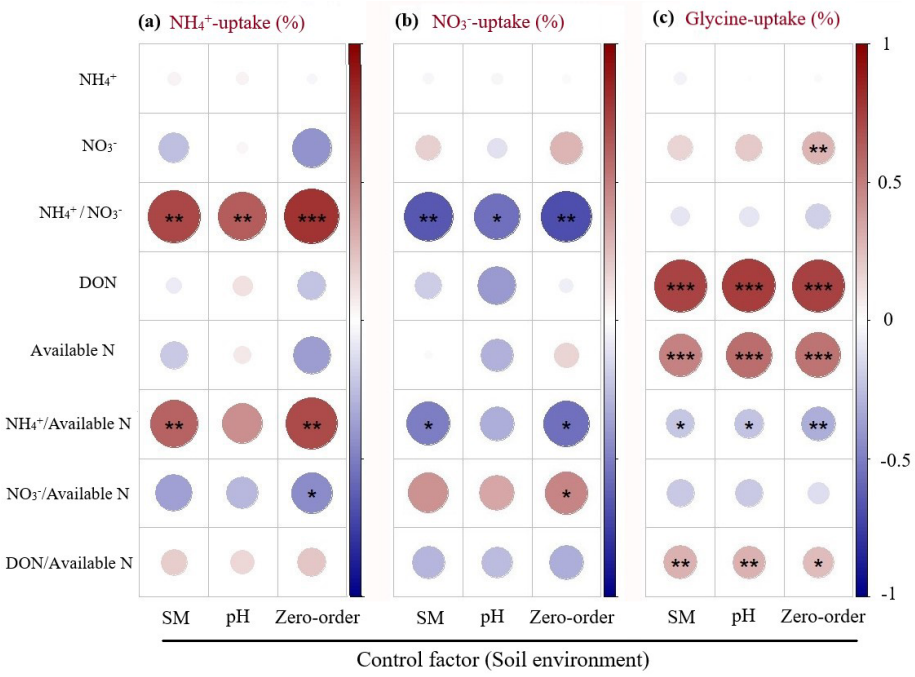

Fig. 5 Partial correlations between the N-uptake percentage of different available N forms and soil available N after controlling soil environment (soil moisture and pH). The x-axis shows the zero-order (without controlling any factors) and the factors being controlled. The y-axis shows the N-uptake percentage of NH_4^+ (a), NO_3^- (b), and Glycine (c) correlations with soil available N. The size and color of the circles indicate the strength and sign of the correlation. Differences in circle size and color between the zero-order and controlled factors indicate the level of dependency of the correlation between the different form of N-uptake (%) by plant and the examined factor on the controlled variable (no change in circle size and color between the controlled factor and zero-order = no dependency; a decrease/increase in circle size and color intensity = loss /gain of correlation).

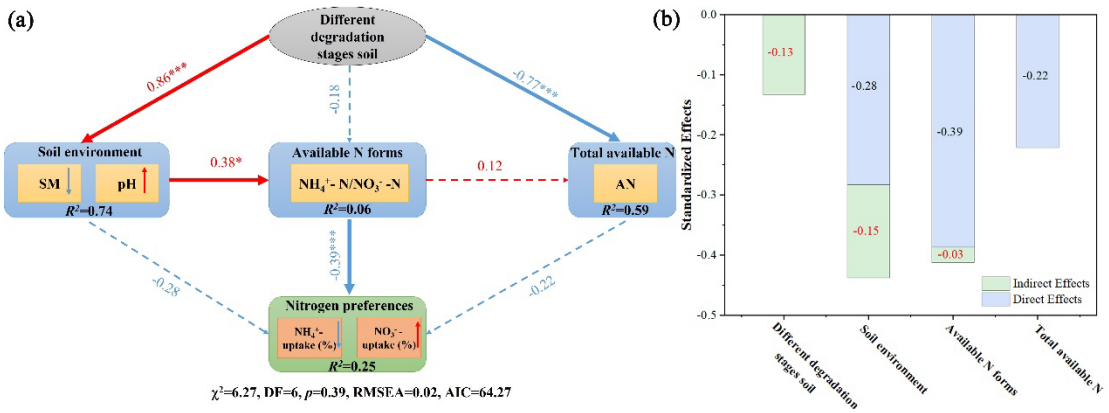

Fig. 6 Direct and indirect effects of soil environment, concentration of different forms of N

and total available N on the N-uptake preferences by plant (a), and direct and indirect path standardized effects (b). Solid and dashed arrows represent significant ($p < 0.05$) and non-significant ($p > 0.05$) paths. Blue and red lines represent negative and positive correlation. The numbers adjoining the arrows are standardized path coefficients. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

4. Discussion

4.1 Plant nitrogen uptake preferences in degraded alpine meadows

The dominant species in degraded alpine meadows examined here showed flexibility in using available N forms compared to field experiment. Houlton et al. (2007) found community-wide shifts in preferences for N sources that tracked N pools along a precipitation gradient, suggesting that tropical plants are flexible in their N uptake strategies. In boreal forest (Nordin et al., 2001) and temperate forest (Finzi & Berthrong, 2005; Liu et al., 2017; Zhou et al., 2019), plants preferred to uptake NH_4^+, the dominant available N forms in soil. In our pot experiment, NO_3^- was the most abundance form of N in soils of all degradation stages, and the NO_3^- concentration was 18 times, 11 times and 3.9 times higher than that of NH_4^+ in the three soils, respectively (Fig. 2a). However, in the field, NH_4^+ was the most abundance form of N in soil at non-degraded stage (Lai et al., 2023). The relatively higher concentration of NO_3^- in pot experiment may be due to the enhancement of nitrification because of low soil pH in pot experiment (0.5 unit lower than in the field). Six of the nine dominant species preferred to uptake NO_3^- at soils of all degradation stages, and the proportion of NO_3^- uptake by plants was more than 50% (Fig.3). Different with the results of pot experiment, *K. pygmaea* and *P. pratensis* in the field mainly absorbed NH_4^+ (Lai et al., 2023), which indicate the flexibility of the two species' nitrogen uptake preference. However, *K. humilis*, *C. moorcroftii*, and *A. flaccidus* planted in SD-soil took up more NH_4^+ than other forms (Fig. 3c, g, and i), which is mainly due to the increase of NH_4^+/NO_3^- in SD-soil supported by the positive correlation between NH_4^+ uptake percentage and the NH_4^+/NO_3^- (Fig. S5). The significant increase in the proportion of NH_4^+ to soil available N (Fig. 2b) and the correlation between it and uptake percentage of different N forms suggests that plant N use preferences may be influenced not only by the most abundant form of N in the soil but also by the proportion of different forms of available N. In a word, the difference in N uptake preference of the nine species in various soils (Fig. 3) and

corresponding changes in concentrations of available N forms (Fig.2) imply the apparent plasticity of dominant species' N uptake in different degraded alpine meadows, which are in line with previous studies that dominant species often use the most abundant N forms (Mckane et al., 2002; Andersen & Turner, 2013; Wen et al., 2021).

4.2 The main influencing mechanisms of plant nitrogen utilization strategies in degraded alpine meadows

Nitrogen uptake preferences of dominant plant species in alpine meadows of various degradation status is determined by soil environments (soil moisture and pH) and availability of different N forms rather than the root functional traits and phylogenetic trees distance (Fig. 4 and S5). Soil moisture is an important environmental factor that affects the transformation of N in soil, therefore, the forms of N uptake by plants (Britto & Kronzucker, 2013). In most cases, ammoxidation is an aerobic process (Zhalnina et al., 2012; Che et al., 2017). Thus decrease in soil moisture and improved soil aeration with alpine meadow degradation should promote the activities of soil nitrifiers (Che et al. 2017). The increase in soil nitrifiers might make NO_3^- the most abundant available N form, therefore plants are likely to uptake more NO_3^- (Houlton et al., 2007; Lai et al., 2023). Our partial correlation results show that after controlling soil moisture, the correlation coefficients between the percentage uptake of NH_4^+ (Fig. 5a), NO_3^- (Fig. 5b) and glycine (Fig. 5c) by dominant species with concentration of NO_3^- significantly decreased by 9%, 6% and 12%. This indicates that soil moisture regulates concentration of NO_3^- in soil and thus affects the plant N-uptake preference. Soil pH affects NH_4^+ and NO_3^- transport differentially, with more uptake of NO_3^- in alkaline conditions (Britto & Kronzucker, 2013). In addition, soil pH also significantly alters the diversity, abundance, and function of ammonia oxidation genes (AOB-*amoA* and AOA-*amoA*), determining the nitrification and changes the NO_3^- concentration in the soil (Gubry-Rangin et al., 2011). Nitrification is inhibited under acidic conditions (Watanabe et al., 1998) because acidic conditions in the rhizosphere inhibit the associated soil bacterial activity (Falkengren-Grerup, 1995), while nitrification capacity may be increased in alkaline soils. A study on alpine meadow has shown that a weak increase in pH after grassland degraded could result in a significant increase in the abundance of AOA-*amoA* genes (Che et al., 2017). The soils at different degradation stages in this study

are all weakly alkaline (Fig. S3b), which was conducive to nitrification and further increased NO_3^- concentration in the soil. Moreover, the correlation coefficients significantly decreased by 19%, 10% and 9% between the percentage uptake of NH_4^+ (Fig. 5a), NO_3^- (Fig. 5b) and glycine (Fig. 5c) with concentration of NO_3^- in soil after controlling soil pH. Thus, soil moisture and pH ultimately affect plant N-uptake preferences by influencing the concentration of different forms of N in soil (Fig. 6a).

Our study found that root traits and phylogenetic trees distance had no significant effect on plant N-uptake preference across degraded alpine meadow species (Fig. 4 and S5). Roots are vital for plants to acquire nutrients (Ma et al., 2018). Hong et al. (2017) found that the root biomass, volume, surface area and average diameter were negatively correlated with the N uptake rate, while the specific root length and the specific root area had significantly positive effects on the N uptake rate across ten alpine plant species. However, the plant root characteristics did not determine the N-uptake preference of our study species. The contrast between Hong's and our results suggest that root functional traits determine the uptake rate of various N forms but not the N uptake preference. Plant N-uptake preference may be an inherent property of plant evolution, and there was a possibility that consistent plant preference was genetically inherited (Wang & Macko, 2011; Daryanto et al., 2019). Wang et al. (2011) found that plant seeds retain the adaptation towards the N uptake preference of their parents, even when the abundances of NH_4^+ and NO_3^- changed. However, the phylogenetic trees distance did not show an effect on plant N-uptake preference in this study (Fig. 4 and S5), and plant progeny does not continue to exhibit the same N preference as the parent plants in the field (Lai et al., 2023). There may be differences in physiological traits and genotypes among different species. The insufficient phylogenetic trees between dominant species in our study also may mask the influence of evolutionary traits on the plant N uptake preference. More species and wider phylogenetic trees species should be further studied in the future.

4.3 Implications for restoration of degraded alpine meadows

Nitrogen fertilizer application can significantly improve the productivity of alpine meadows, and it is an effective means for restoration of degraded grassland in short-term. Our *in-suit* ^{15}N-labeling experiment have shown that dominant species at non-degraded

alpine meadow prefer to absorb NH_4^+; dominant species at moderately degraded prefer to absorb NO_3^- (Lai et al., 2023). Thus, applying NH_4^+-N fertilizer would be most efficient approach to recover the plant community. However, results of this pot experiment raise the possibility that restoration of degraded alpine meadow grassland productivity is not necessarily linked to the particular form of N fertilizer. The manipulation of soil moisture and pH of degraded alpine meadow to ensure the higher concentration of NH_4^+ in soil may restore the dominance of non-degraded alpine meadow species.

As the dominant species of non-degraded alpine meadow showed plasticity to uptake the most abundant N form available in soil, either NH_4^+-N or the NO_3^--N fertilizer could help to restore the degraded alpine meadow. However, the synthesis of NO_3^- into proteins consumes four times higher energy than NH_4^+ (Wang & Macko, 2011), which suggests that NH_4^+-N fertilizer may be more efficient in the restoration. But further restoration practices either by applying different form of N fertilizer or manipulating the soil environment and addition of N fertilizer should be tested in the field.

5. Conclusions

Our results shown that dominant species of degraded alpine meadows have evolved a uniformly plastic ability to switch among different N sources, and different species showed different levels of flexibility in degraded meadows. The change in soil moisture and pH alters the different forms of available N concentration, thereby influencing plant N-uptake preferences. From the perspective of grassland restoration management, our results revealed that plant N-uptake preferences were mainly regulated by the soil environments. To better understand the mechanism of plant N acquisition strategies, further studies should consider the effects of competing neighboring plants and soil microorganisms on the N acquisition strategies of degraded alpine meadow plants.

6. Author Contributions

7. Acknowledgements

8. Conflict of Interest Statement

References

Andersen, K. M., & Turner, B. L. (2013). Preferences or plasticity in nitrogen acquisition by understorey palms in a tropical montane forest. *Journal of Ecology*, 101(3), 819☼825.

Ashton, I. W., Miller, A. E., Bowman, W. D., & Suding, K. N. (2010). Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology*, 91, 3252– 3260.

Britto, D. T., & Kronzucker, H. J. (2002). NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, 159(6), 567☼584.

Britto D. T., & Kronzucker, H. J. (2013). Ecological significance and complexity of N-source preference in plants. *Annals of Botany*, 112(6), 957☼963.

Chapin, F. S., Matson, P. A., & Mooney, H. A. (2002). Terrestrial plant nutrient use. In Principles of Terrestrial Ecosystem Ecology, (pp. 176–196). New York, NY: Springer‐Verlag New York, Inc.

Che, R. X., Wang, F., Wang, W. J., Zhang, J., Zhao, X., Rui, Y. C., Xu, Z. H., Wang, Y. F., Hao, Y. B., & Cui, X. Y. (2017). Increase in ammonia-oxidizing microbe abundance during degradation of alpine meadows may lead to greater soil nitrogen loss. *Biogeochemistry*, 136(3), 341☼352.

Daryanto, S., Wang, L. X., Gilhooly III, W. P., & Jacinthe, P. A. (2019). Nitrogen preference across generations under changing ammonium nitrate ratios. *Journal of Plant Ecology*, 12(2), 235☼244.

Doetterl, S., Stevens, A., Six, J., Merckx, Roel., Oost, V. K., Pinto, M. C., Casanova-Katny, A., Muñoz, C., Boudin, M., Venegas, E. Z., & Boeckx, P. (2015). Soil carbon storage controlled by interactions between geochemistry and climate. *Nature Geoscience*, 8, 780☼783.

Dong, S. K., Shang, Z. H., Gao, J. X., & Boone, R. B. (2020). Enhancing sustainability of grassland ecosystems through ecological restoration and grazing management in an era of climate change on QinghaiTibetan Plateau. *Agriculture, Ecosystems & Environment*, 287, 106684.

Falkengren-Grerup, U. (1995). Interspecies differences in the preference of ammonium and nitrate in vascular plants. *Oecologia*, 102, 305☼311.

Finzi, A. C., & Berthrong, S. T. (2005). The uptake of amino acids by microbes and trees in three cold-temperate forests. Ecology, 86, 3345–3353.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B. C., James, P., Schloter, M., Griffiths, R. I., Prosser, J. I., & Nicol, G. W. (2011). Niche specialization of terrestrial archaeal ammonia oxidizers. *Proceedings of the National Academy of Sciences of the United States of America*, 108(52), 21206☼21211.

Harris, R. B. (2010). Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. *Journal of Arid Environments*, 74(1), 1☼12.

Harrison, K. A., Bio, R., Bardgett, R. D. (2007). Preferences for different nitrogen forms by coexisting plant species and soil microbes. *Ecology*, 88(4), 989–999.

Hawkins, B. J., & Robbins, S. (2010). pH affects ammonium, nitrate and proton fluxes in

503 the apical region of conifer and soybean roots. *Physiologia Plantarum*, 138(2),
504 238☼247.
505 He, J. S., Bu, H. Y., Hu, X. W., Feng, Y. H., Li, S. L., Zhu, J. X., Liu, G. H., Wang, Y. R., &
506 Nan, Z.B. (2020). Close-to-nature restoration of degraded alpine grasslands:
507 Theoretical basis and technical approach. *Chinese Science Bulletin*, 65(34), 3898☼
508 3908.
509 Hong, J. T., Ma, X. X., Yan, Y., Zhang, X. K., & Wang, X. D. (2017). Which root traits
510 determine nitrogen uptake by alpine plant species on the Tibetan Plateau?. *Plant*
511 *and Soil*, 424(1-2), 63☼72.
512 Hong, J. T., Qin, X.J., Ma, X.X., Xu, X., & Wang, X.D. (2019). Seasonal shifting in the
513 absorption pattern of alpine species for NO_3^- and NH_4^+ on the Tibetan Plateau.
514 *Biology and Fertility of Soils*, 55, 801–811.
515 Houlton, B. Z., Sigman, D. M., Schuur, E. A., & Hedin, L. O. (2007). A climate-driven switch
516 in plant nitrogen acquisition within tropical forest communities. *Proceedings of the*
517 *National Academy of Sciences of the United States of America*, 104(21), 8902–
518 8906.
519 Lai, C. M., Li, C. Y., Peng, F., Xue, X., You, Q. G., Zhang, W. J., & Ma, S. X. (2021). Plant
520 community change mediated heterotrophic respiration increase explains soil
521 organic carbon loss before moderate degradation of alpine meadow. *Land*
522 *Degradation & Development*, 32, 5322–5333.
523 Lai, C. M., Peng, F., Sun, J. B., Zhou, J., Li, C. Y., Xu, X. L., Chen, X. J., You, Q. G., Sun,
524 H. Y., Sun, J., Xue, X., & Lambers, H. (2023). Niche differentiation and higher
525 uptake of available nitrogen maintained the productivity of alpine meadow at early
526 degradation. *Biology and Fertility of Soils*, 59(1), 35–49.
527 Lebauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in
528 terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379.
529 Li, J., Zhao, Q. X., Li, W. L., He, J. Z., & Xu, X. L. (2022). Distinct kin strategies of the
530 legume soybean and the non‐legume balsam by accomplishing different nitrogen
531 acquisition and rhizosphere microbiome composition. *The Plant Journal*, 110, 103–
532 113.
533 Liu, M., Li, C. C., Xu, X. L., Wanek, W., Jiang, N., Wang, H. M., & Yang, X. D. (2017).
534 Organic and inorganic nitrogen uptake by 21 dominant tree species in temperate
535 and tropical forests. *Tree Physiology*, 37(11), 1515–1526.
536 Liu, M., Yu, C. L., Zhu, T. B., Xu, X. L., & Wang, Y. F. (2022). Restoration of degraded alpine
537 grasslands alters plant–microbial competition for nitrogen. *Biology and Fertility of*
538 *Soils*, 58, 803–814.
539 Ma, Z. Q., Guo, D. L., Xu, X. L., Lu, M. Z., Bardgett, R. D., Eissenstat, D. M., McCormack,
540 M. L., & Hedin, L. O. (2018). Evolutionary history resolves global organization of
541 root functional traits. *Nature*, 555(7694), 94–97.
542 Makkonen, K., & Helmisaari, H. S. (1999). Assessing fine-root biomass and production in
543 a Scots pine stand-comparison of soil core and root ingrowth core methods. *Plant*
544 *and Soil*, 210, 43–50.
545
546 Månsson, K. F., Olsson, M. O., Falkengren-Grerup, U., & Bengtsson, G. (2014). Soil

moisture variations affect short-term plant-microbial competition for ammonium, glycine, and glutamate. *Ecology and Evolution*, 4(7), 1061–1072.

McFee, W. W., Stone, E. L. (1968). Ammonium and nitrate as nitrogen sources for Pinus radiata and Picea glauca. *Soil Science Society of America Proceedings*, 32, 879–884.

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A., & Murray, G. (2002). Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, 415, 68–71.

Miehe, G., Schleuss, P. M., Seeber, E., Babel, W., Biermann, T., Braendle, M., Chen, F. H., Coners, H., Foken, T., Gerken, T., Graf, H. F., Guggenberger, G., Hafner, S., Holzapfel, M., Ingrisch, J., Kuzyakov, Y., Lai, Z. P., Lehnert, L., Leuschner, C., Li, X. G., Liu, J. Q., Liu, S. B., Ma, Y. M., Miehe, S., Mosbrugger, V., Noltie, H. J., Schmidt, J., Spielvogel, S., Unteregelsbacher, S., Wang, Y., Willinghofer, S., Xu, X. L., Yang, Y. P., Zhang, S. R., Opgenoorth, L., & Wesche, K. (2019). The Kobresia pygmaea ecosystem of the Tibetan highlands – origin, functioning and degradation of the world's largest pastoral alpine ecosystem: Kobresia pastures of Tibet. *Science of the Total Environment*, 648, 754–771.

Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, 33(4), 540–552.

Nordin, A., Högberg, P., & Näsholm, T. (2001). Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. *Oecologia*, 129(1), 125–132.

Peng, F., Xue, X., You, Q. G., Huang, C. H., Dong, S. Y., Liao, J., Duan, H. C., Tsunekawa, A., & Wang, T. (2018). Changes of soil properties regulate the soil organic carbon loss with grassland degradation on the Qinghai-Tibet Plateau. *Ecological Indicators*, 93, 572–580.

Phoenix, G. K., Johnson, D. A., Muddimer, S. P., Leake, J. R., & Cameron, D. D. (2020). Niche differentiation and plasticity in soil phosphorus acquisition among co-occurring plants. *Nature Plants*, 6(4), 349–354.

Schimann, H., Ponton, S., Hättenschwiler, S., Ferry, B., Lensi, R., Domenach, A. M., & Roggy, J. C. (2008). Differing nitrogen use strategies of two tropical rainforest late successional tree species in French Guiana: Evidence from ^{15}N natural abundance and microbial activities. *Soil Biology and Biochemistry*, 40(2), 487–494.

Song, M. H., Xu, X. L., Hu, Q. W., Tian, Y. Q., Ouyang, H., & Zhou, C. P. (2007). Interactions of plant species mediated plant competition for inorganic nitrogen with soil microorganisms in an alpine meadow. *Plant and Soil*, 297(1-2), 127–137.

Song, M. H., Zheng, L. L., Suding, K. N., Yin, T. F., & Yu, F. H. (2015). Plasticity in nitrogen form uptake and preference in response to long-term nitrogen fertilization. *Plant and Soil*, 394(1-2), 215–224.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: How can it occur?. *Biogeochemistry*, 13(2), 87–115.

von Wirén, N., Gazzarrini, S., & Frommer, W. B. (1997). Regulation of mineral nitrogen uptake in plants. *Plant and Soil*, 196, 191–199.

Wade, M. J. (1980). An experimental study of kin selection. *Evolution*, 34(5), 844–855.
Wang, L. X., & Macko, S. A. (2011). Constrained preferences in nitrogen uptake across plant species and environments. *Plant, Cell & Environment*, 34(3), 525–534.
Wang, W. Y., Ma, Y. C., Xu, J., Wang, H. C., Zhu, J. F., & Zhou, H. K. (2012). The uptake diversity of soil nitrogen nutrients by main plant species in *Kobresia humilis* alpine meadow on the Qinghai-Tibet Plateau. *Science China Earth Sciences*, 55(10), 1688–1695.
Watanabe, T., Osaki, M., & Tadano, T. (1998). Effects of nitrogen source and aluminum on growth of tropical tree seedlings adapted to low pH soils. Soil Science and Plant Nutrition, 44(4), 655–666.
Wen, L., Dong, S. K., Li, Y. Y., Wang, X. X., Li, X. Y., Shi, J. J., & Dong, Q. M. (2013). The impact of land degradation on the C Pools in alpine grasslands of the Qinghai-Tibet Plateau. *Plant and Soil*, 368(1–2), 329–340.
Wen, S. H., Tian, Y. Q., Ouyang, S. N., Song, M. H., Li, X. B., Zhang, Y., Gao, S., Xu, X. L., & Kuzyakov, Y. (2021). High frequency of extreme precipitation increases Stipa grandis biomass by altering plant and microbial nitrogen acquisition. *Biology and Fertility of Soils*, 58, 63–75.
Xu, X. L., Ouyang, H., Cao, G. M., Pei, Z. Y., & Zhou, C. P. (2004). Uptake of organic nitrogen by eight dominant plant species in Kobresia meadows. *Nutrient Cycling in Agroecosystems*, 69, 5–10.
Xu, X. L., Ouyang, H., Richter, A., Wanek, W., Cao, G. M., & Kuzyakov, Y. (2011). Spatio-temporal variations determine plant-microbe competition for inorganic nitrogen in an alpine meadow. Journal of Ecology, 99, 563–571.
Yi, R. J., Liu, Q. Y., Yang, F. T., Dai, X. Q., Meng, S. W., Fu, X. L., Li, S. G., Kou. L., & Wang, H. M. (2023). Complementary belowground strategies underlie species coexistence in an early successional forest. *New Phytologist*, 238, 612–623.
Zhalnina, K., de Quadros, P. D., Camargo, F. A. O., & Triplett, E. W. (2012). Drivers of archaeal ammonia-oxidizing communities in soil. *Frontiers in Microbiology*, 3, 210.
Zhang, R., Degen, A. A., Bai, Y. F., Zhang, T., Wang, X. M., Zhao, X. Y., & Shang, Z. H. (2020). The forb, *Ajania tenuifolia*, uses soil nitrogen efficiently, allowing it to be dominant over sedges and Graminae in extremely degraded grasslands: Implications for grassland restoration and development on the Tibetan Plateau. *Land Degradation & Development*, 31(10), 1265–1276.
Zhang, Y. W., Guo, Y. P., Tang, Z. Y., Feng, Y. B., Zhu, X. R., Xu, W. T., Bai, Y. F., Zhou, G. Y., Xie, Z. Q., & Fang, J. J. (2021). Patterns of nitrogen and phosphorus pools in terrestrial ecosystems in China. *Earth System Science Data*, 13, 5337–5351.
Zhang, Z. L., Li, N., Xiao, J., Zhao, C. Z., Zou, T. T., Li, D. D., Liu, Q., & Yin, H. J. (2018). Changes in plant nitrogen acquisition strategies during the restoration of spruce plantations on the eastern Tibetan Plateau, China. *Soil Biology and Biochemistry*, 119, 50–58.
Zhou, J., Li, X. L., Peng, F., Li, C. Y., Lai, C. M., You, Q. G., Xue, X., Wu, Y. H., Sun, H. Y., Chen, Y., Zhong, H. T., & Lambers, H. (2021). Mobilization of soil phosphate after 8 years of warming is linked to plant phosphorusacquisition strategies in an alpine meadow on the Qinghai-Tibetan Plateau. *Global Change Biology*, 27,1–14.

635 Zhou, M. X., Yan, G. Y., Xing, Y. J., Chen, F., Zhang, X., Wang, J. Y., Zhang, J. H., Dai, G.
636 H., Zheng, X. B., Sun, W. J., Wang, Q. G., & Liu, T. (2019). Nitrogen deposition and
637 decreased precipitation does not change total nitrogen uptake in a temperate forest.
638 *Science of the Total Environment*, 651, 32–41.
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Supplementary materials

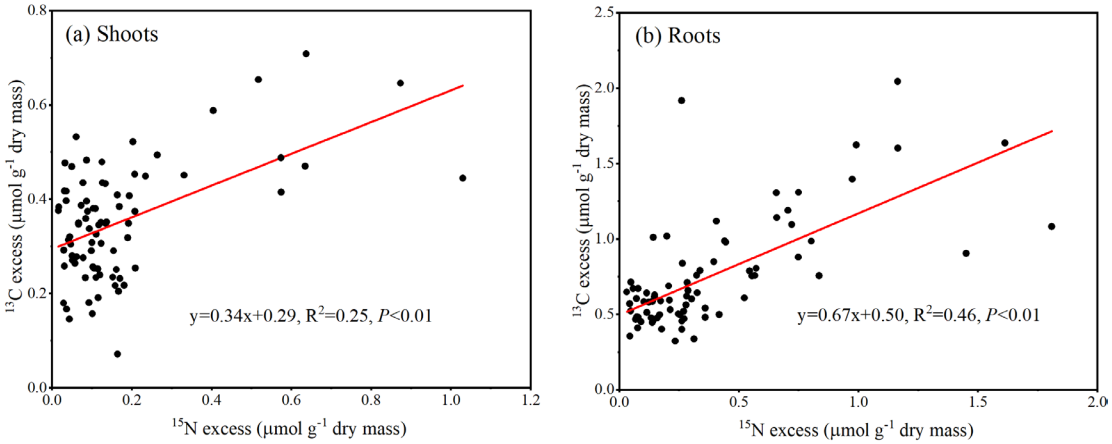

Fig. S1 The linear relationship of excess ^{13}C and excess ^{15}N in plant leaf (a) and root (b) of double-labeled glycine

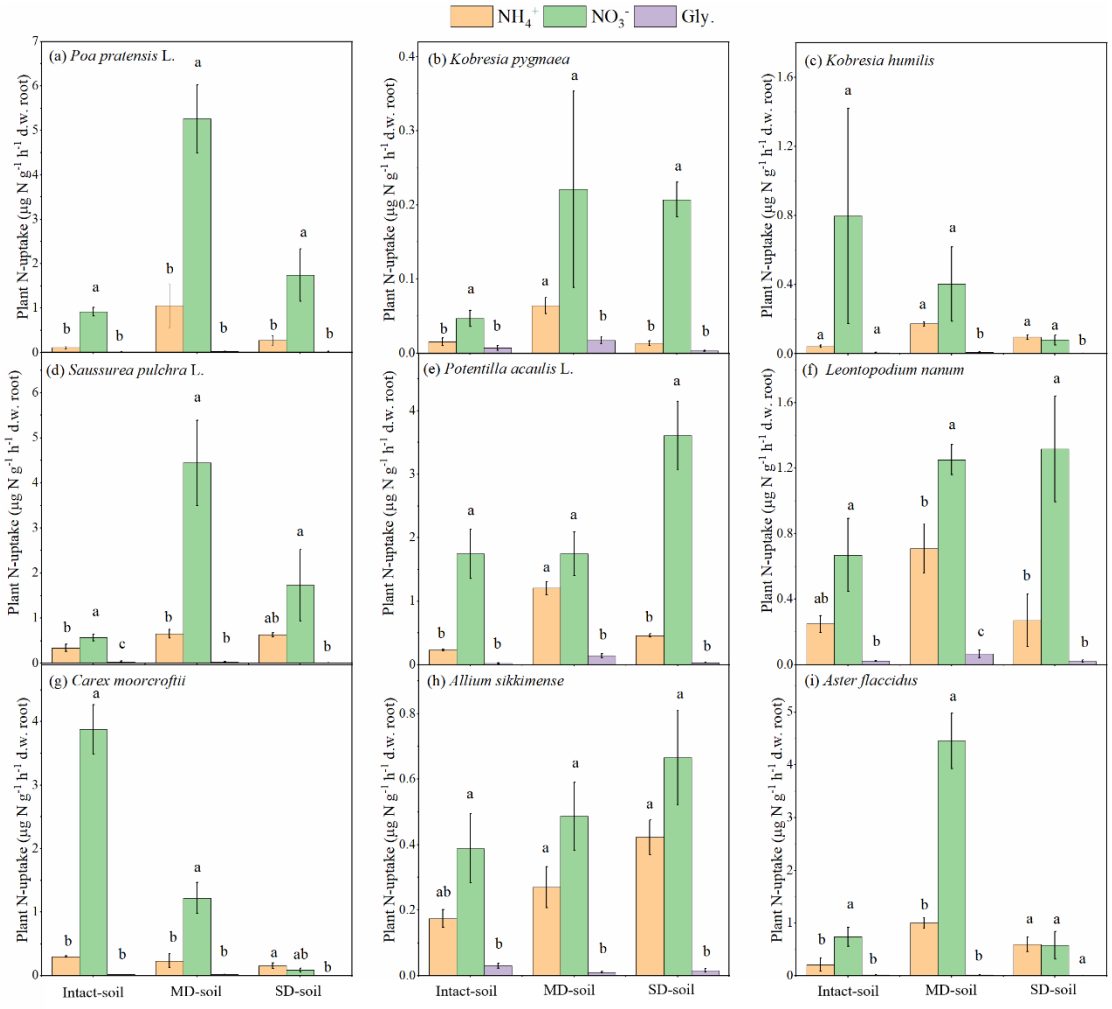

Fig. S2 The uptake rates of different forms N by dominant species at different degradation stages soil. Different lowercase letters indicated significant differences in the absorption rates of different forms of N by different dominant species in soil at different degradation stages (p<0.05).

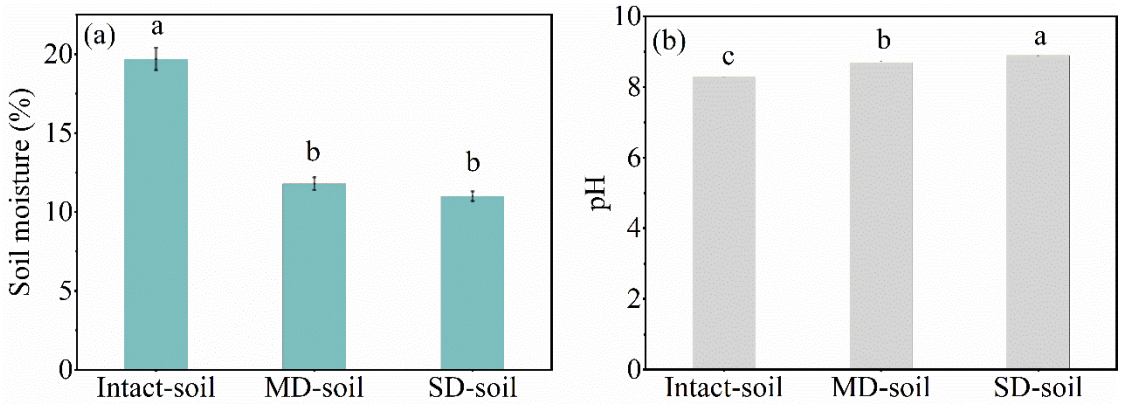

Fig. S3 Soil moisture (a) and pH (b) at different degradation stages soil. The values are means ± SE (n=27). Different lowercase letters above the columns indicate significant differences at $p < 0.05$ among different degradation stages soil.

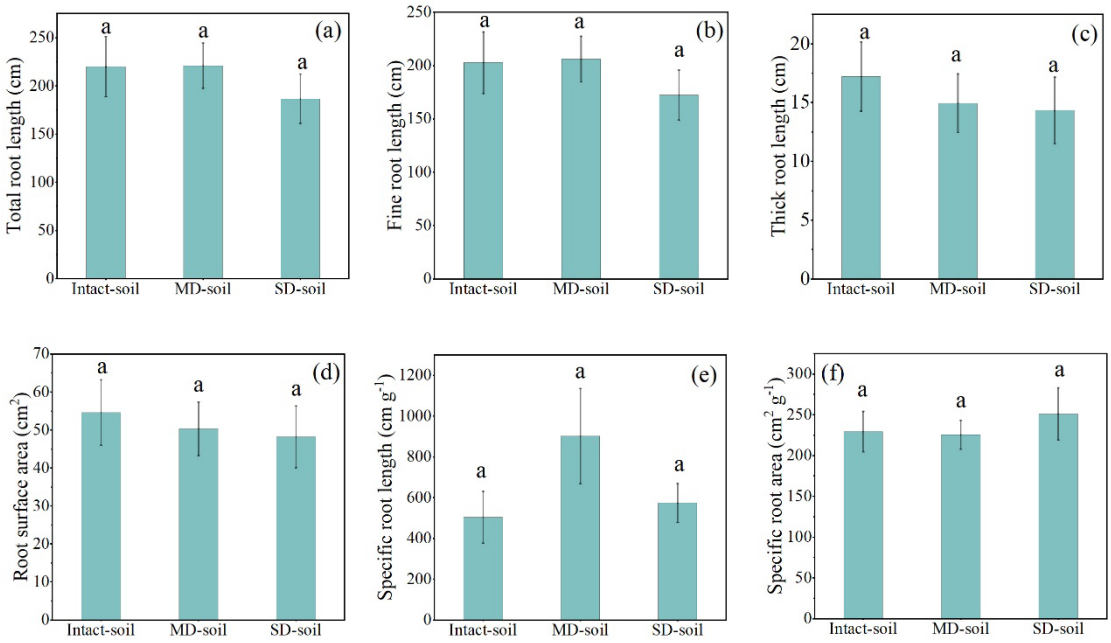

Fig. S4 Total root length (a), fine root length (b), thick root length (c), root surface area (d), specific root length (e), and specific root area (f) of all dominant species planted at different degradation stages soil. The values are means ± SE (n=27). Different lowercase letters above the columns indicate significant differences at $p < 0.05$ among different degradation stages soil.

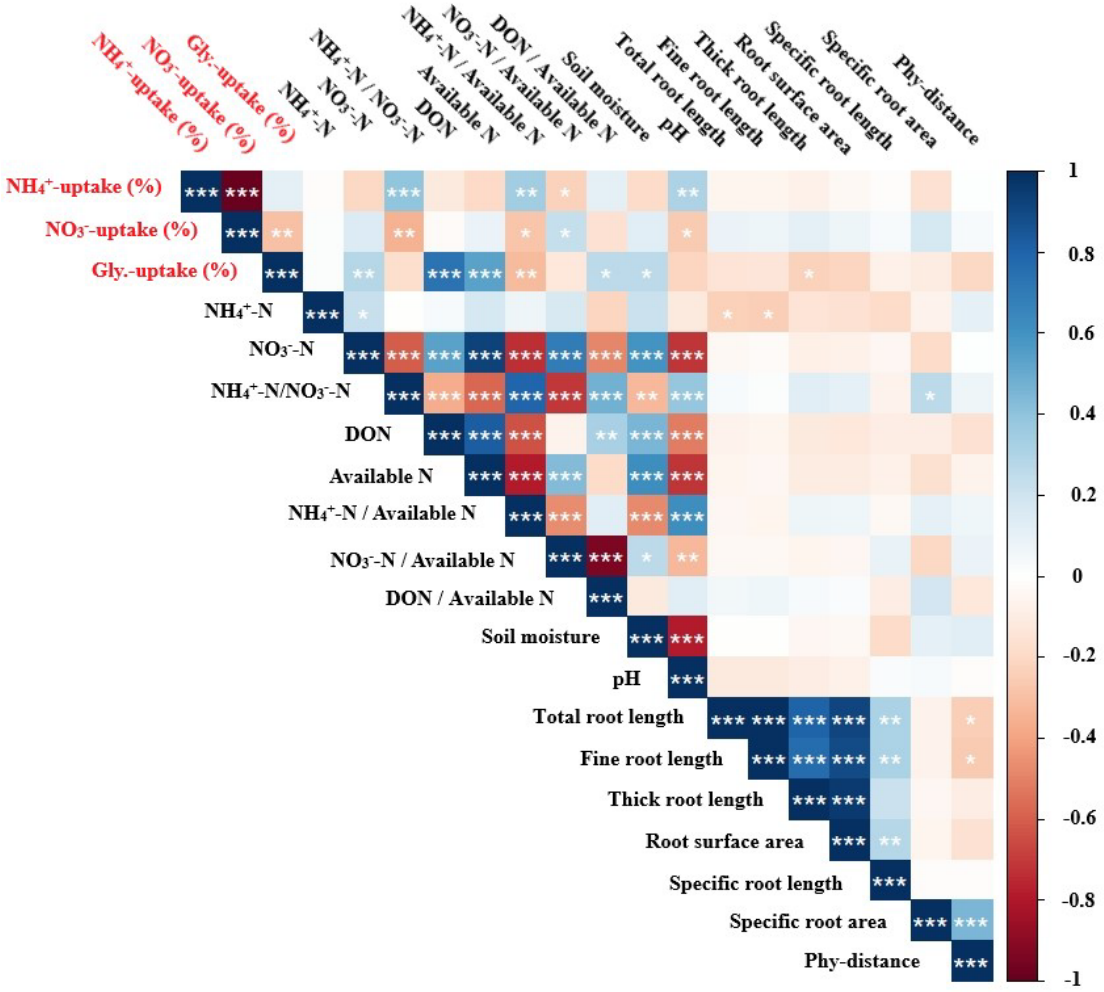

Fig. S5 Correlation between uptake preference of NH_4^+, NO_3^-, and glycine by dominant species and plant and soil parameters. *, ** and *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. DON: dissolved organic nitrogen; Phy-distance: phylogenetic trees distance.

Table S1 Multicollinearity was removed by variance inflation factor (VIF) before structural equation models.

Factors	VIF
NH_4^+-N	7.72
NO_3^--N	6.43
NH_4^+-N / NO_3^--N	4.20
DON	5.67
NH_4^+-N/AN	8.02
NO_3^--N/AN	9.58
DON/AN	Na

Delete values where VIF is greater than 5. AN: total available N

Table S2 The phylogenetic trees distance matrix of dominant species at different degradation stages

	Ln.	*Af.*	*Sp.*	*Pa.*	*As.*	*Pp.*	*Cm.*	*Kh.*	*Kp.*
Ln.	0								
Af.	45.98	0							
Sp.	68.56	68.56	0						
Pa.	247.47	247.47	247.47	0					
As.	271.52	271.52	271.52	271.52	0				
Pp.	271.52	271.52	271.52	271.52	229.20	0			
Cm	271.52	271.52	271.52	271.52	229.20	187.64	0		
Kh.	271.52	271.52	271.52	271.52	229.20	187.64	13.40	0	
Kp.	271.52	271.52	271.52	271.52	229.20	187.64	13.40	12.61	0

Ln.: *Leontopodium nanum*, *Af.*: *Aster flaccidus*, *Sp.*: *Saussurea pulchra* L., *Pa.*: *Potentilla acaulis* L., *As.*: *Allium sikkimense*, *Pp.*: *Poa pratensis* L., *Cm.*: *Carex moorcroftii*, *Kh.*: *Kobresia humilis*, *Kp.*: *Kobresia pygmaea*