Title：Plant uptake of available N from different layers varies among species in an alpine meadow of permafrost regions on the Qinghai-Tibetan Plateau

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Abstract

Background and aims: The uptake of permafrost thaw released nitrogen (N) could benefit plant growth and change vegetation community composition in a warming climate in cold regions. However, the capacity of co-existing species to take up different forms of available N beyond the root zone remains largely unknown in permafrost areas with a deep active layer.

Methods: *In situ* $^{15}NH_4Cl$, $K^{15}NO_3$ and $C_2H_5NO_2$ (glycine) labelling were conducted up to 70 cm depth for five species.

Results: Averaged across the five species, the summed ^{15}N recovery rate of the three tracers was 10.71 ± 10.69 %, 1.69 ± 2.51%, 1.54 ± 4.16% and 0.7 ± 2.23% at 0-15, 15-30, 30-50 and 50-70 cm, respectively. *Kobresia. humilis* had the largest N uptake diversity. The NO_3^--N recovered from 30-70 cm for *K. humilis* and *Saussurea. japonica* was much higher than other species, accounting for 23% and 13% of the total N recovered at 0-70 cm. Root surface area was positively related to the recovery rate of inorganic N at soil below 15 cm whereas a species' N requirement negatively to the N recovery at 0-15 cm. The relative cover of a species in a community was negatively related to a species' N requirement but showed no relationship with the N recovery rate or N uptake diversity. Plant community composition may not be affected by vertical N uptake patterns of co-existing species.

Conclusions: Species that can take up N from deep soil layers may gain competitive advantages, thereby altering the plant community structure in a warm climate in the future.

Keywords: nitrogen uptake, ^{15}N tracing, alpine meadow, permafrost, plant functional groups

Introduction

Plants in permafrost region are extremely nitrogen (N) limited (Kou et al. 2020) because of 1) low temperatures restricting microbial decomposition of soil organic matter therefore, resulting in low N availability and 2) confined space for plant roots to acquire available N in seasonally thawed active layer (Bjorkman et al., 2020; Iversen et al., 2015; Xu et al., 2011). Climate warming enhances soil N availability by accelerating mineralization and thawing frozen N pools (Natali et al. 2014; Wickland et al. 2018; Beermann et al. 2017; Hansen and Elberling 2023; Yun et al. 2023; Strauss et al. 2024) The increased N availability could improve plant productivity and modify species composition (Schuur et al. 2007; Elmendorf et al. 2012; Wild et al. 2018; Heijmans et al. 2022), thereby affecting carbon and energy balances, and large-scale feedback to climate warming (Schuur et al. 2015; Chen et al. 2022). Examining the capability of co-existing species to use N from deep soil can help to predict which species would gain a competitive advantage in a warm climate in cold regions.

Co-existing plant species in Arctic tundra and subarctic peatlands demonstrate spatial and chemical niche differentiation in taking up available N (Keuper et al., 2012, 2017; Hewitt et al. 2019; Pedersen et al. 2020; Lacroix et al. 2022). Deep-rooting graminoid species, such as *Arctagrostics latifolia* Griseb. and *Eriophorum vaginatum*, could make use of available N at the thaw front (as deep as 50 cm) while shallow-rooting shrubs, such as *Betula nana* L. and *Rhododendron tomentosum* Harmaja take up N only from the uppermost 30 cm organic layer (Keuper et al. 2012, 2017; Wang et al. 2018). Graminoids are therefore predicted to profit more than shrubs from permafrost thaw released N (Wang et al., 2018). Dominant species of alpine grasslands take up more nitrate N (NO_3^--N) due to its high mobility in upper organic layers whereas they primarily take up ammonium N (NH_4^+-N) in deep layers as alleviated competition between microbes and plants make it available to be taken up by plants (Hewitt et al., 2019; Pedersen et al., 2020; Salmon et al., 2018; Xu et al., 2011; Zhu et al., 2016). Previous studies have investigated plant N uptake via an injection of ^{15}N into the soil, often with a depth less than 50 cm, which is also the maximum depth of active layer that thaws at summertime and freezes at wintertime in high latitude permafrost areas. By contrast, active layer thickness is often deeper than 100 cm in alpine ecosystems on the Tibetan Plateau (Wu et al., 2010), which is far beyond the root reach. Thus, permafrost thaw released N is considered equally inaccessible for all plants in the alpine grassland on the Tibetan plateau and it will not affect the plant community(Mao et al., 2020). However, plants with shallow roots can still use N derived from deep soil via several mechanisms: 1) plant roots with mycorrhizal symbionts will capture deep-soil N and transport upward, which could become available for plants with shallow roots (Clemmensen et al. 2008; Ravn et al. 2017; Hewitt et al. 2019), 2) cryoturbation induced vertical soil mixing can move the available N at thaw front to upper layers (Klaminder et al. 2013) and make it available for shallow-rooted species. Species that could take up available N beyond root zone may have a large sourcing space for N, therefore gain advantage in the community. But plant uptake of soil available N deeper than the root zone is less examined in alpine grassland on the Tibetan Plateau.

Alpine grassland plant community on the Tibetan Plateau is dominated by two plant functional groups, graminoids and forbs (Miehe et al. 2019), which have contrasting root traits and root depth (Li et al., 2011). In N addition experiments, the aboveground biomass of graminoids was found to be enhanced more than forbs (You et al. 2017). According to the root spectrum and nutrient acquisition hypothesis (Eissenstat 2000; Scheifes et al. 2024), graminoids with fast and “do-it-yourself” strategies will invest more photosynthates in their short-lived fine roots, thereby have a high N uptake capacity at the root zone. In addition, the deep roots of graminoids help them to take up more N from the thaw front to further strengthen their advantage over forbs (Keuper et al. 2012, 2017; Wang et al. 2018; Pedersen et al. 2020). However, fine roots are also more prone to freezing-thawing damage at the thaw front (Iversen et al., 2015), possibly cancelling out the acquisitive root traits induced advantage in taking up permafrost thaw released N in deep soil. Forbs in this habitat are typically shallow rooted, they are more likely to develop mycorrhizal symbiosis because of their relatively large roots (Li et al., 2024). This expands the opportunities for forbs to utilize N redistributed by biological and physical processes beyond the root zone. However, the effect of root depth, acquisitive root traits and association with mycorrhizae on the plant uptake of permafrost thaw released deep soil N is largely unknown. Despite leaf and root spectrum based N acquisition strategies, plants will increase root branching, clustering and growing dense root hairs to enlarge root surface area for nutrient sorption and acquisition (Lambers et al., 2011). We, therefore, hypothesized that species with greater root surface area would show a higher uptake of N from deep soil layer.

Plants can take up both inorganic (NH_4^+-N and NO_3^--N) and organic N (smaller molecular amino acids) and show preference to specific forms (Mao et al., 2025). The preference for N form varies among species and is mediated by the proportions of different available N forms (Lai et al. 2024; Mao et al. 2025). In Arctic tundra, the uptake of amino acids could account for as much as 70% of the total plant N uptake (Raab et al. 1996; Näsholm et al. 2009), while plants dominantly take up the inorganic N in alpine meadows on the Tibetan Plateau (Lai et al. 2023, 2024). Ammonium is the most abundant soil available N in spring while the concentration of NO_3^--N would gradually increase in the growing season (Xie et al. 2020). The capacity to move with soil water, adsorption to soil mineral, energy consumption to assimilate are different among NH_4^+-N, NO_3^--N and amino acids (Nacry et al. 2013). Thus, plants may have different vertical uptake patterns for different N forms. However, previous studies investigated the variations of plant N uptake at different depths only applied ^{15}N-labelled NH_4^+-N.

We conducted a $^{15}NH_4Cl$, $K^{15}NO_3$ and glycine injection experiments in an alpine meadow on the Qinghai-Tibetan Plateau to 1) investigate the variations of graminoids' and forbs' uptake of different available N forms at different soil depths and 2) to reveal the impact of root depth, acquisitive root traits, association with mycorrhizal fungi and N requirement of a species on the N uptake variations.

Materials and Methods

Study site

The field experiment was carried out in an alpine grassland on Qinghai-Tibetan Plateau near the Beiluhe Permafrost Observation Station (92° 56' E, 34° 49' N, 4635 m a.s.l.), Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences. The mean annual temperature and annual precipitation were -3.8 °C and 290.9 mm (Lai et al., 2024, Li et al., 2024). It is a continuous permafrost area with maximum active layer of ~ 2 m and permafrost depth of 70-150 m (Wu et al., 2010). From early May, the land surface will gradually thaw. The active layer reaches the maximum thaw in late

September and then gradually freezes again.

The N uptake experiment was conducted on an undegraded alpine meadow. Sedge species, *Kobresia humilis* and *K. pygmaea* (Cyperaceae, sedge) dominates the plant community with a relative coverage more than 20%. Forbs species, *Leontopodium nanum* has a relative coverage of 12%, whereas *Aster flaccidus* and *Saussurea japonica* are the rare species with relative coverage less than 5% (Table S1). More than 85% of the roots of these species are in the top 30 cm (Xu et al., 2016). A small number of roots were visible at 50-70 cm for four of the five species, except *A. flaccidus* which root are only visible above 50 cm. The soil bulk density, pH, soil organic carbon and soil total nitrogen at 0-15 cm were 0.96 ± 0.05 g m^{-3}, 8.5 ± 0.01, 13.9 ± 0.3 g kg^{-1} and 0.92 ± 0.01 g kg^{-1} for the undegraded alpine meadows (Lai et al., 2024).

^{15}N labelling

The *in-situ* labelling was carried out in late August 2021 and 2023, respectively. Each time, the ^{15}N labelling was conducted for three species. $^{15}NH_4Cl$ (98% atom% ^{15}N), $K^{15}NO_3$ (99 atom% ^{15}N) and $^{13}C_2H_5{}^{15}NO_2$ (99 atom%, ^{15}N) were used. Three trenches (representing three replicates) of 12 m in length and 30 cm in width were dug to 100 cm deep or until water saturation occurred at depths of ~70 cm. The distance between trenches was 50-100 m. 1 ml of tracer or water (control) was horizontally injected at 2.5, 7.5, 12.5 cm (representing plant N uptake at 0-15 cm), 17.5, 22.5, 27.5 cm (at 15-30 cm), 35, 40, 45 cm (at 30-50 cm), 55, 60 and 65 cm (at 50-70 cm) three times to form a 20 x 20 cm grid around target species (Fig. S1). The distance between injections of different N forms was ~ 2 m apart within a trench. A total of 24 ml ^{15}N labelled compound was injected at each depth. The control was injected with the same amount of water. The ^{15}N concentration of solutions of the three tracers were 11 mM, 8 mM and 4 mM. In total, 3.88 mg, 2.82 and 1.41 mg N were injected for each individual plant at a specific depth according to the content of different available N forms (Lai et al, 2023, 2024).

Plant and soil sampling and measurement

Plant and soil samples were collected 24 hours after the injection. Soil samples were collected horizontally at 0-15, 15-30, 30-50 and 50-70 cm using an auger with a diameter of 7 cm for each individual plant labelled with specific compound. The auger was pushed horizontally 20 cm. For each species injected with one of the three labelled compounds or water, 9 (3 replicates × 3 soil depths) or 12 (3 replicates × 4 soil depths), soil samples were not collected at lower levels if the soil was saturated. In total, 171 soil samples were collected. The aboveground biomass of each plant individual was collected by cutting at ground level. All soil and plant samples were put in a cooler at 4 ^{o}C and transported into the lab in Lanzhou.

The soil samples were gently crumbled by hand to separate roots and bulk soil. Approximately 10 g of soil was dried in an oven at 105 ^{o}C for 48 hours to determine the soil moisture content. The root surface was first brushed to get the rhizosphere soil and then the roots were soaked in a 0.5 mM $CaCl_2$ for 30 min and rinsed with deionized water to clear the ^{15}N adsorbed on the root surface. The rhizosphere soil from each labelled compound of the same species at a specific depth were mixed to get to a composite sample for analysis. Aboveground part of plants was washed with distilled water to remove dust on the surface. Cleaned shoots and roots were then dried at 75 ^{o}C for 48 h, weighed, and ground to fine power. About 2 mg ground sample was put into tin capsules to determine C and N concentrations and stable isotopes using a vario EL cube interfaced with an IsoPrime 100 (Elementar Analysen systeme GmbH, Hanau, Germany). The N pool of an individual plant was calculated by multiplying N concentration and biomass of aboveground and belowground respectively, and then summed.

The rhizosphere soil was sieved through a 2 mm mesh and then extracted with 1 M KCl. Concentrations of exchangeable NH_4^+ and NO_3^- were determined using a flowautoanalyzer (SEAL Analytical AutoAnalyzer 3, Northern Ireland, UK). The soil was extracted by 0.5 mM K_2SO_4 at 25 ^{o}C and put in a vibrator with 250 rpm for 1 hour for dissolved N measurement by a TOC analyzer (Vario TOC Select, elementar, UK). Dissolved organic N was the difference between dissolved N and exchangeable inorganic N in the soil sample. Soil glycine was estimated by multiplying DON with 6% (the percentage of glycine to total dissolved organic N in alpine grasslands (Liu et al. 2022).

Maximum root depth and root traits

In our study, soil samples were collected up to 70 cm depth even though trenches were dug to 100 cm because deeper soils were water saturated. If no roots of the species were found in the soil sample, the maximum root depth was determined as the bottom of the layer above. Because of the great reduction in root biomass with soil depth, it is impossible to distinguish the root traits and get the whole root of a plant individual in deep layers. Thus, root traits of each species were determined by growing them in pots in a greenhouse. Briefly seeds of each species, or underground buds for sedges, were collected near the study site. Seeds or buds were grown in pot (15 cm in diameter and 20 cm in depth) with field soil collected at 5-20 cm near the study site. Pots were put in a greenhouse with environmental settings (relative humidity 60%, light intensity 800 μ mol m^{-2} s^{-1}, and 21 ^{o}C and 16 ^{o}C for 16 hours daytime and 8 hours nighttime) that resemble the natural conditions in summertime. Four pots were grown for each species and watered once a week. They were harvested after growing for 100 days, the duration of growing season in natural condition. Plants were gently pulled out and then washed in tap water. The entangled roots were separated carefully to get clear images during the scan. The separated roots were then scanned (EPSON, 10000XLPro, Canada) and analyzed for topology characteristics using Win RhizoProf (V2012b, Canada). Then roots of each pot were oven-dried and weighed. Specific root length (SRL, mm g^{-1}), root length density (RLD, m m^{-3}), root surface area (RSA, cm^2 g^{-1}), total root length (TRL, mm), fine root length (FR, mm), aboveground and belowground biomass (g per plant) of each plant individual was calculated referring to Lai et al., 2024. Roots with a diameter less than 2 mm were considered as fine roots.

During the ^{15}N labelling, root samples were also collected with an auger at 0-30 cm depth to measure the mycorrhizal infection rate of different species. The root mycorrhizal infection in deep layer was not determined because it is impossible to distinguish the root of a specific species and the sharp decline in large roots with depth. This was tested using the ink and vinegar staining method recommended by (Vierheilig et al. 1998). Nine live root segments of 1 cm in length of each species were first cleared in a 10% KOH solution at 90°C, followed by acidification in 2% HCl for 5 minutes. They were then stained in a 95% ink-vinegar solution at 60°C for 30 minutes and subsequently destained in deionized water for 12 hours. Mycorrhizal structures, including arbuscules, vesicles, and hyphae, were quantified under a microscope at 200× magnification following the method proposed by

(McGONIGLE et al. 1990). For each segment, 10 view fields in the microscope were cross-checked (Li et al., 2025). The number of arbuscules, vesicles, and hyphae were identified in each view field and recorded and then were divided by the total 10 view field to get the infection percentage of arbuscular mycorrhizal fungi for each root segment.

Calculation of plant N uptake and N recovery

^{15}N atom% excess (APE) was calculated as the atom% ^{15}N difference between labelled and control of aboveground and belowground plant tissue (Eq. 1).

$$APE = atom\%\ ^{15}\mathrm{N}_{labelled} - atom\%\ ^{15}\mathrm{N}_{control} \qquad \text{(Eq. 1)}$$

^{15}N amount in the shoot or root (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) in Eq. 2.

$$^{15}\mathrm{N}\ (\mu\mathrm{g}) = \mathrm{N}_{\mathrm{concentration}} \left(\frac{\mu\mathrm{mol}}{\mathrm{g}}\right) \times \frac{\mathrm{APE}}{100} \times \mathrm{B}\ (\mathrm{g}) \times 15 \left(\frac{\mathrm{g}}{\mathrm{mol}}\right) \qquad \text{(Eq. 2)}$$

Plant ^{15}N uptake rate (μg ^{15} N g^{-1} d.w. root) was calculated by adding the root and shoot ^{15}N amount together and then dividing by root biomass (g) in Eq. 3.

$$\mathrm{P}^{15}\mathrm{NUR}\ (\mu\mathrm{g}\ ^{15}\mathrm{N}\ \mathrm{g}^{-1}\mathrm{h}^{-1}) = \frac{\text{Root }^{15}\text{N amount }(\mu\text{g}) + \text{Shoot}^{15}\text{N amount }(\mu\text{g})}{\text{Root biomass (g) Time (h)}} \qquad \text{(Eq. 3)}$$

The plant ^{15}N recovery (*R*, %) was calculated by dividing the plant ^{15}N uptake in the ^{15}N labeling time by the amount of ^{15}N labeling in Eq. (5)

$$R(\%) = \frac{P^{15}NU}{^{15}N_{added}} \times 100 \qquad \text{(Eq.4)}$$

The plant ^{15}N uptake rate and recovery rate indicated the preference to and capacity to take up specific N forms. As the ^{15}N uptake rate and recovery were closely correlated, we only reported the recovery rate in the Results section. The N recovery rate of a plant at a specific depth was the sum of the recovery rate of all three N forms at the same depth. The recovery rate of specific N form in the whole soil profile was the sum of the recovery at all depths. The N recovery rate of the specific N form at the specific depth was divided by the summed N recovery rate of all three N forms at the whole soil profile to determine the vertical and chemical pattern of species' N uptake pattern.

N uptake preference and diversity

The ^{15}N recoveries of one tracer at different depths of the same soil profile was summed to get the whole soil profile ^{15}N recovery. Then, the recovery of all three tracers was summed up to obtain a total recovery of one species. The ratio of the recovery of a specific N form at a specific depth to the total recovery of a species was calculated to indicate the N uptake preference.

The N uptake diversity (Jesch et al. 2018) was calculated referring to Shannon-winer diversity index, which represented niche breadth of species' N uptake and was calculated as follows:

$$D_{uptake} = -\sum R_i \times \ln{(R_i)} \quad \text{(Eq. 5)}$$

Where D_{uptake} was the N uptake diversity of the species, R_i is the recovery rate of specific N form at a specific depth. The larger the index, the higher the diversity of a species to take up N from various sources at different depths.

N uptake partitioning overlap

The proportional similarity (PS) index was used to measure the amount of overlap between species pairs in using different sources of N (Jesch et al., 2018).

$$PS = 1 - 0.5\sum_{i=1}^{12}|p_{1i} - p_{2i}| \quad \text{(Eq. 6)}$$

Where p_i is the percentage uptake of a specific N forms at different soil depths for a species. Proportional similarity measures the intersecting area of the frequency distributions of N sources used by two different species. It ranges from 0 to 1 with 0 meaning overlap and 1 meaning complete overlap (Jesch et al., 2018). The PS was calculated for all species pairs.

Statistical analysis

The values of all indexes were tested for normal distribution and homogeneity of variance before ANOVA analysis. If the index did not meet the requirement, it was root square mean, or logarithm transformed. If it still did not meet the requirements of ANOVA, a Kruskal-Wallis non-parametric test was carried out to analyze the difference among species, soil depth and available N forms. Principal component analysis was conducted to understand the root traits difference and difference in N uptake from different sources among species in a reduced dimension. Stepwise linear regression was used to build the relationship between maximum root depth, root traits, species' N requirement and association with AM on the recovery of available N forms at 0-15, 15-30 and 30-70 cm. As three species did not have the recovery data for glycine at 50-70 cm, the regression analyses were conducted for pooled data at 30-50 and 50-70 cm. Zero order and partial correlation was determined using the Pearson correlation in SPSS. 24. Graphs were made using Origin 18.0.

Results

Available N at the soil profile

At all soil depths, the concentrations of NO_3^--N were higher than NH_4^+-N and DON, especially at 0-15 cm. NH_4^+-N was the similar at the four soil depths, while the NO_3^--N content was 40% lower at 0-15 cm compared to the other three depths. DON was the highest at 15-30 cm (Fig. 1).

Leaf N concentration, N requirement and ^{15}N of various species

The leaf N concentration was the highest in *S. japonica* (26.87 ± 2.65 mg N g^{-1} d.w) and *A. flaccidus* (22.63 ± 1.76 mg N g^{-1}). It was significantly lower in *K. humulis* relative to *S. japonica* ($P<0.01$). It was the lowest in *L. nanum* (18.01 ± 0.88 mg N g^{-1}). The N requirements of all species were significantly different, with the highest in *S.japonica* (6.44 ± 0.70 mg N per individual), followed by *A. fluccidus* (4.72 ± 0.32 mg), *K. humulis* (3.56 ± 0.26 mg), *L. nanum* (1.66 ± 0.20 mg) and *K. pgymaea* (0.53 ± 0.03 mg) (Fig. 2).

The natural leaf $\delta^{15}N$ of *K. humulis* and *L. nanum* was higher than 0, while it was lower than 0 for the other three species. The natural leaf $\delta^{15}N$ of forbs species were significantly lower than sedge species (Fig. 2).

Maximum root depth, root traits, mycorrhizal fungi and individual sizes

Roots were found up to 70 cm for four out of the five species. At 50-70 cm, only roots of *A. fluccidus* were not observed. Among the five species, *K. humilis* showed the greatest while *S. japonica* the smallest nutrient acquisition-related root traits, such as RLD, SRL, RSA, TRL and fine roots (Table 1). When compared between sedges and forbs, RLD, RSA, TRL and fine roots were greater in forbs than in sedges while SRL was larger in sedges (Fig. S2). All root traits of *S. japonica* were smaller than the other two forb species (Table 1). Examining root traits in a two-dimensional PCA space , *S. japonica* and *K. pygmaea* were clearly differentiated from other species (Fig. S3). The AM infection percentage was 20-37% for all species with no significant differences among them.

The belowground biomass of all species were 2-5 folds larger than aboveground biomass. The aboveground, belowground and total biomass of *K. humilis*, *S. japonica* and *A.flaccidus* were greater than that of *K. pgymaea* and *L. nanum* (Table 1).

Recovery rate, N uptake diversity and partitioning of different species

When data for all species is pooled together, the ^{15}N recovery of the three tracers was the highest at 0-15 cm (10.71 ± 10.69 %), then decreased at 15-30 cm (1.69 ± 2.51%), 30-50 cm (1.54 ± 4.16%) and 50-70 cm (0.7 ± 2.23%) for all five species ($P < 0.01$). The percentage of summed N recovery of the three forms at a specific depth to the 0-70 total recovery was 31.52 ± 3.81%, 4.58 ± 0.84%, 4.32 ± 1.12% and 4.34 ± 1.56% at 0-15, 15-30, 30-50 and 50-70 cm. The average ^{15}N recovery rate was lower below than above 30 cm regardless of species or tracers and highest at 0-15 cm (Fig. 3). Only *A. flaccidus* took up the similar amount of glycine at 0-15 and 15-30 cm (Fig. 3).

Across all five species, the recovery of NH_4^+-N and NO_3^--N showed no difference either for the summed 0-70cm recovery or at specific depths (Fig. 4). The average recovery rates across all soil depths and species were 4.56 ± 10.69%, 4.29 ± 8.12% and 0.33 ± 0.49% for the NH_4^+-N, NO_3^--N and glycine, respectively. The recovery rate of NO_3^--N was relatively higher for *K. humilis* and *S.japonica* at all depths than that of NH_4^+-N (Fig.3), whereas the other three species recovered more NH_4^+-N (Fig. 3).

The *K. humilis*' ^{15}N recovery was higher than that of *S. japonica* for inorganic N either at a specific depth or the summed 0-70 cm recovery (Fig. 5). Averaged across the three traces at all soil depths, the recovery rate of *K. humulis* was 8.25±11.88%, followed by *L. nanum* (6.65 ± 15.37%), *K. pgymaea* (2.79 ± 4.30%), *A. flaccidus* (2.10 ± 3.34%) and *S. japonica* (1.01 ± 1.93%). The ^{15}N recovery of NH_4^+-N or NO_3^--N showed no difference among species in either forbs or sedge groups. *S. japonica* and *A.flaccidus* had a relatively larger recovery of glycine than other species at all soil depths (Fig. 5).

The two sedges species mainly took up NH_4^+-N and NO_3^--N from 0-15 cm (Fig. 6), which accounted for 28% and 31% of the total N recovered at 0-70 cm depth of the three N forms for *K. humilis*, and 42% and 41% for *K. pgymaea*. Different with *K. pgymaea* that took up inorganic N mostly from surface layer, *K. humlilis* took up 12% and 11% NO_3^--N at 30-50 and 50-70 cm, respectively. Different with the other two forbs species that mainly took up NH_4^+-N at the 0-15 cm depth: 59.90 ± 14.4% and 57.53 ± 4.73 % for *L. nanum* and *A. flaccidus*, *S. japonica* primarily took up NO_3^--N at 0-15 cm (53% ± 6.50 %) and 30-50 cm (13.52 ± 0.85%).

The N uptake diversity (D_{uptake}, niche breadth in term of N uptake) of various forms at different depths of a species was the largest in *K. humilis* (1.13 ± 0.20), which is significantly higher than that of *K. pygmaea* (0.58 ± 0.08, P=0.04), *S. japonica* (0.43 ± 0.05, P=0.01) and *A.flaccidus* (0.51 ± 0.08,P=0.08) but showed no difference with *L. nanum* (0.74 ± 0.24, P=0.20).

The proportional similarity between *K. pygmaea* and the two forbs species, *L. nanum* and *A. flaccidus* was the largest, while it was small between *K.pygmaea* and *K.humilis* and *S.japorica* (Table 2). It was also larger between *L.nanun* and *A.flaccidus* than other species pairs (Table 2).

Impacts of root depth, root traits, AM and plant N requirement on N uptake variations

At 0-15 cm, maximum root depth and N requirement of a species explained 41% and 18% of the variation in NH_4^+-N recovery rate, and 22% and 31% of the variation in NO_3^--N recovery; the maximum root depth positively but the N requirement negatively related to recovery rate of inorganic N (Table 3). At other depths, the RSA was the most influential factor, positively affecting recovery rate of NH_4^+-N and NO_3^--N. It explained 65% and 59% of the variations in NH_4^+-N and NO_3^--N recovery at 15-30 cm and 31% and 44% at 30-70 cm. In addition to RSA, the maximum root depth also positively impacted the recovery rate of NH_4^+-N at 15-30 cm. Contrast to the 0-15 cm layer, N requirement positively related to the NO_3^--N recovery rate, which explained 22% of variations in NO_3^--N recovery at 30-70 cm (Table 3). The R^2 of the regression models were higher at shallow layers than the deep layers.

Relationship of abundance in a plant community with N requirement and N uptake

The D_{uptake} was positively correlated with total recovery at 0-70 depth (r = 0.87, n = 15, P<0.01). Only the N requirement of a species significantly negatively correlated with the relative abundance of the species in the plant community (r = -0.54, P = 0.038, n = 15, Fig.). When the total recovery rate was controlled, the correlation between N requirement and relative abundance of species was strengthened (r = -0.60, P = 0.022). Yet it was unchanged when the D_{uptake} was controlled ($r = -0.54$, $P = 0.047$).

Discussion

The vertical and chemical niche partitioning of N uptake

There was a higher plant ^{15}N recovery from 0-15 cm than other layers for all three available N forms in all species even though there was not much difference in total dissolved N among different soil layers (Figs.1, 3, 4B). This supports the prevailing hypothesis that vertical pattern of plant N uptake is affected by fine root biomass density (De Baets et al. 2007), as more than 85% of roots were distributed above 20 cm in the alpine grassland (Li et al., 2011). Acquisitive species at the fast end of the leaf economy spectrum are more likely to place their roots in the uppermost layers, thereby taking up resources primarily from shallow soil layers, whereas conservative species take up relatively more nutrients from deep layers (Herben et al., 2018, 2022). *K. humilis*, a conservative species with low specific leaf area and leaf N content (Fig.S2), taking up more N uptake from deep layers than acquisitive species, *S. japonica* and *A. flaccidus* (Figs. 3-6, Table 1), lends credence to this hypothesis. Nonetheless, more uptake of NO_3^--N by *S. japonica* from 30-50 cm than from 15-30 cm (Figs. 3-6), given similar NO_3^--N content in the two layers (Fig. 1), which contradicts the relationship between leaf spectrum and N acquisition.

Besides the traits, transport systems also impact the plant uptake of available N. The available N taken up by *K. humilis* and *S. japonica* from deep layers was primarily NO_3^- (Fig. 6). Root NO_3^- transport systems are consisted of different carrier proteins, with functional properties and specific regulatory patterns, including the induction by NO_3^-, feedback repression by N status, or stimulation by photosynthesis (O'Brien et al., 2016). Kinetics studies revealed a biphasic pattern of plant NO_3^- influx. It showed a saturable component in the low concentration (e.g., <0.2–0.5 mM) but a linear one at higher concentrations (Siddiqi et al., 1990). This suggested the existence of high-affinity (HATS) and low-affinity transport systems (LATS), respectively (Crawford & Glass, 1998) .As we have no physical data related to transporters for HATs and LATS, we focused on the regulation of N limitation and photosynthesis on the transportation of NO_3^- from soil solutions to plant cellular. Root NO_3^- influx was improved when plants suffer from N limitation while it was depressed with a high N provision (Lee, 1993). With this observation, all the plants should have a high NO_3^- influx from deep layers because of the low concentration of soil available N in deep layers. Yet, the high-low affinity transport systems predict a slow NO_3^-influx. Thus, it is hard to explain the higher uptake of deep layer NO_3^- by *S. japonica* from view of N limitation and high-low affinity transporters. The root NO_3^- uptake is also dependent upon photosynthesis. When more photosynthates was transported from shoot to root, more NO_3^- will be taken up by root (Delhon et al., 1996). Although there is no data of shoot to root transport, the larger root biomass of *K. humilis* and *S. japonica* (Table 1) may result in a higher uptake of NO_3^- from deep soil layer.

Maximum rooting depth is more important than root traits and the association with mycorrhizal fungi for studying N availability change on plant community in Arctic ecosystems (Keuper et al. 2012, 2017; Moreau et al. 2015; Zhu et al. 2016; Wang et al. 2018; Freschet et al. 2021). Deep rooting species can access existing and newly thawed deep soil N (Keuper et al., 2017; Wang et al., 2018; Hewitt et al., 2020; Pedersen et al., 2020). Although four species having roots as deep as 70 cm could took up N at 50-70 cm, only *K. humulis* and *S.japonica* recovered 29% and 15% of the $^{15}NO_3^-$-N at 30-70 cm whereas N recovery beyond root zone accounted less than 1% of the total N recovery for the other two species (Fig. 6). Thus, maximum depth may not be a reliable indicator of a species' capacity to take up N beyond root zone in alpine grassland. In deep layers, plants' uptake of N was more related to its N requirement (Table 3). Although the N requirement of *S. japonica* was the largest (Fig. 2), the percentage uptake of N from deep layer was less than *K. humilis* (Fig. 6). Both the relatively high N requirement and largest RSA of *K. humilis* (Table 1) contributed to the high recovery rate of ^{15}N from deep layer because RSA was positively related to the uptake of both NH_4^+-N and NO_3^--N at deep layer (Table 3). Our results suggest that despite root traits, a species' N requirement also affects a species' N uptake capacity from soil beyond root zone in alpine grassland.

In nutrient limited ecosystems, coexisting species could differentially take up different chemical N forms to avoid interspecific competition (Näsholm et al. 2009; Ashton et al. 2010). Although, the recovery of $^{15}NO_3^-$-N and $^{15}NH_4^+$-N was the similar either for the summed 0-70 cm or at a specific depth when pooled the five species together (Figs. 3-5), different species showed distinct preference (Fig. 6). The preference of *L. nanum* and *A. fluccidus*' for NH_4^+-N was offset by *K. humilis* and *S. japonica*'s preference to NO_3^--N (Fig. 6). The soil available N composition determines plant's N uptake preference across different ecosystems (Lai et al., 2024; Mao et al., 2025). But species in a plant community with the same available N composition still may show divergent preference because their difference in N acquisition traits mediates competition with microorganisms and the tradeoff between carbon investment and N acquisition (Xu et al. 2011; Hewitt et al. 2020, 2024). Species with more effective fine roots or establishing mycorrhizal fungi associations may take up more NH_4^+-N at surface layer (Miller and Cramer 2005; Kuzyakov and Xu 2013; Hewitt et al. 2024). Although all the species examined were associated with AM, the absence of AM but presence of root traits in regression models (Table 3) support Hewitt's findings that root traits accounted for a greater proportion of variation in permafrost N uptake than root associated fungi in cold regions (Hewitt et al., 2024). *K. pygmaea*, *L.nanum*, and *A. flaccidus'* preference to NH_4^+-N (Fig. 6) could be related to their relatively large SRL, SRA and fine roots (Table 1) even NO_3^--N was the most abundant available N at 0-15 cm (Fig. 1). However, *K. humilis and S. japonica* took up more NO_3^--N than NH_4^+-N at 0-15 cm (Fig. 6) even SRL, SRA and fine roots of *K. humilis and S. japonica* were clearly different (Table 1), probably due to the faster diffusion rate of NO_3^- (Miller and Carmer, 2005) and the higher abundance of it in topsoil layer (Fig. 1). The preference for specific available N forms was consistent for a species between root zone and beyond the root zone. The contrasting preference to different available N forms among species at 30-70 cm was positively related to the SRL ($r = 0.56$, $P = 0.027$, $n = 15$). Species that invest more carbon to grow fine roots deeper in the soil may develop roots low in tissue density but high in SRL and SRA (Hong et al., 2017; Hewitt et al., 2024), thereby enhancing the uptake of the most abundant available N at deep layer, like *K. humilis* and *S. japonica* in our study (Fig. 6).

Leaf natural $\delta^{15}N$ could indicate the uptake of N from deep layer (Wang et al., 2018) as the ^{15}N become enriched with soil depth (Li et al., 2021). Although both the *K. humilis* and *S. japonica* showed a relatively larger percentage of N recovered from deep layers (Fig 6), their $\delta^{15}N$ was significantly different (Fig. S2). The contrasting $\delta^{15}N$ of the two species could be due to their distinct ^{15}N recovery rate (Figs. S2,3). Our results suggest that the natural δ^{15}N is more reliable to indicate the N uptake rate rather than the partitioning of N uptake from deep layers.

Plant N uptake pattern and the relative abundance in plant community

Nutrient acquisition strategies mediate the species coexistence and community assembly (Tian et al. 2023). The relative abundance of a species in a community along a water table gradient is found to be positively correlated with ^{15}N recovery in a sedge dominated ecosystem in an alpine grassland (Gong et al. 2025). Species that can make full use of available N are superior competitors, which in turn appear to be associated with high abundance (Cao et al. 2021). According to the leaf economic spectrum, plants with conservative traits would show higher performance under low resource availability (Reich 2014; Díaz et al. 2016; Ficken and Wright 2019). In our study, the plant N requirement negatively related to the relative abundance of a species in a community but total ^{15}N recovery and the N uptake diversity did not. High N requirement species such as *K. humilis* could maintain a relatively high abundance given greater N recovery rate and N uptake diversity, otherwise, would result in a low abundance such as *S. japonica* (Fig.2, Table S1). The biomass of individual *S. japonica* plants was the largest among the five species (Table 1) but it showed the lowest ^{15}N recovery and N uptake rate (Figs. 3 ,5). The performance of species in the community often relates to its tolerance to limiting resources (Tilman, 1982). Species that can tolerate lower level of limiting resources will better compete with other species, which should have a low nutrient content in plant organs. A high leaf N content and N requirement of a species is accompanied by limited capacity to acquire N from soil. It may show a small relative coverage in the community. Species with low N uptake diversity and low N requirement but high total ^{15}N recovery could also maintain a relatively high abundance in the community such as *K. pygmaea* (Figs 3-6, Tables, 1,4). The dominant species with large N requirement and N uptake diversity showed a lower overlap with other species in taking up different sources of N (Table 2). The two dominant sedges primarily take up inorganic N (Figs. 3,6) but showed vertical niche partitioning with *K. pgymaea* taking up most of the inorganic N at 0-30 cm while *K. humulis* absorbing 23% of N in the form NO_3^--N beyond root zone. Species with intermediate coverage in the plant community showed a large overlap in taking N.

Due to climate warming or long-term fertilization, plants in some tundra ecosystems are increasingly rooting into organic soil layers (Björk et al., 2007; Wu et al., 2014), thus the plant community will benefit less from the permafrost released N. Yet in our study site, a climate manipulation experiment demonstrated that roots moved downward to absorb water in the middle layers because of soil drought in surface layer (Xu et al., 2016), therefore the whole plant community may benefit from access to N in deep soil. Although the 15N recovery rate is low (<2%), K. humilis and S. japonica may gain competitive advantages by utilizing deep-layer N, therefore altering plant community structure under sustained warming.

Reliability of the study for assessing N uptake patterns at a depth of 70cm

Even though small root fractions in deep soil (<5% of total root mass) could lead to disproportionate uptake of nutrients in deep soil (Blume-Werry et al. 2019; Pedersen et al. 2020), deep roots are least likely to occur in Arctic, boreal or cool-temperature regions (Iversen et al., 2015). On the Qinghai-Tibetan Plateau, very few roots are observed deeper than 70 cm in alpine grassland (Yun et al., 2023), thus we conducted the ^{15}N labelling at a maximum depth of 70 cm. Some species might develop roots deeper than 70 cm but the soil below 70 cm is often saturated in summer and autumn, which is unfavorable for root growth therefore constraint N uptake at deep layers (Finger et al., 2016; Albano et al., 2021). Subsurface runoff in water saturated conditions below 70 cm would take away the available N by lateral flow (Hansen and Elberling, 2023) and make it less accessible for plants. In addition to these, soil conditions beyond the root zone only allow root growth for several weeks in the later summer before the deep soil re-freezes, greatly limiting the potential for build-up of root biomass deeper than 70 cm (Lacroix et al., 2022). Across all species and the three available N forms, small percentage N recovered beyond root zone (5% in 30-70cm V.S. 31% at top surface layer, and less than 1% a 50-70 cm) support the reliability to examine the N uptake pattern with a maximum labelling depth of 70 cm for the alpine grassland.

Conclusion

Although some species can make use of available N beyond root zone, plants took up most of the N from surface layer of the soil. The vertical N uptake pattern follows the root distribution profile. The vertical and chemical partitioning showed no consistent patterns within plant functional groups. Each species develops a unique N uptake strategy to survive in the community. Species with large root surface area and high N demand tend to take up the most abundant available N from soil beyond root zone in alpine grassland. High N demand but limited capacity and N uptake diversity will result in a less presence of a species in plant community. Both the dominant and rare species that can take up N from deep layers may benefit more in a warming climate.

Acknowledgements: This study was financially supported by the National Key Research and Development Program of China (Grants No. 2022YFF0801902), the National Natural Science Foundation of China (Grants No. 42471074, W2412147), Western Light Project of Chinese Academy of Sciences (Grants No. xbzg-zdsys-202316) and the complete set of technologies for ecological restoration of sandy land along the Golmud section of the Qinghai Tibet Railway (QZ2022-Z01).

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