



In situ nitrogen uptake from inorganic and organic sources by the fine root systems of five alpine woody plant species

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Abstract

Alpine forests are characterized by cold conditions and poor nutrient availability. Knowledge of nitrogen (N) uptake by fine roots is necessary to understand the survival strategies of plants in these severe environments. We aimed to characterize species-specific inorganic and organic N uptake rates and their root traits in five dominant alpine woody plant species: the ectomycorrhizal (ECM) species *Pinus pumila* and *Alnus maximowiczii*, and the ericoid mycorrhizal (ERM) species *Empetrum nigrum* var. *japonicum*, *Rhododendron aureum*, and *Vaccinium vitis-idaea*. The inorganic and organic N uptake rates from the fine-root systems were measured directly using the solution-depletion method. Root traits, such as diameter, specific root length, root tissue density, and root N concentration, were also determined through morphological and chemical analyses of the incubated roots. Total N uptake rates were higher in ERM species, particularly in *Vaccinium vitis-idaea*. The proportion of inorganic N uptake was greater in ECM species than in ERM species, while the proportion of organic N uptake was generally higher in ERM species. Root diameter was the best predictor of N uptake rates, and specific root length and root tissue density were linked to preferences for inorganic and organic N, respectively. Thus, alpine woody plants altered N uptake and preference by modifying root traits, potentially promoting N niche acquisition in competition among species or mycorrhizal types, and enhancing plant survival in the alpine zone.

Keywords Ectomycorrhiza · Ericoid mycorrhiza · Nitrogen acquisition · Root morphology · Solution-depletion method · Tree line

Introduction

Alpine forests are typically characterized by severe environmental conditions, such as low temperatures, low nutrient availability, and stormy winds, which determine the upper limit of tree growth and distribution (Körner 2021). These climatic stresses can negatively affect plant nutrient availability, growth, reproduction, and ultimately population densities (Jump and Woodward 2003). Soil nutrients in alpine regions are generally deficient due to reduced soil microbial activity and slow decomposition and mineralization processes (Zhu and Zhuang 2013). Under these stresses, alpine

plants must acquire nutrients from limited soil resources through their roots, especially fine roots, which are defined as those with a diameter of less than 2 mm. Fine roots are responsible for nutrient and water uptake (McCormack et al. 2015; Freschet et al. 2021) and have high production and turnover rates (McCormack et al. 2015; Ding et al. 2020). Therefore, the field examination of fine roots is crucial for evaluating how alpine plants acquire nutrients in harsh alpine environments.

Generally, inorganic N, such as ammonium (NH_4^+) and nitrate (NO_3^-), serves as a major nutrient source for plants (Haynes et al. 1978). In soils, there is a physiological trade-off between the two N forms. NO_3^- is soluble in soil water and is highly mobile, but must be reduced using energy before incorporation into amino acids. NH_4^+ is more rapidly assimilated into amino acids but tends to adhere to soil particles and is less mobile (Stewart et al. 1992). Because N is one of the primary limiting factors for plant growth (Vitousek and Howarth 1991), it is important to examine which forms of N are absorbed by fine roots and in what

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quantities to understand the nutrient acquisition strategies of alpine plants.

Plants can absorb organic N as a significant resource (Jones et al. 2005; Näsholm et al. 2009), although they generally uptake inorganic N, such as NH_4^+ and NO_3^- . By directly taking up organic N, plants can acquire nutrients through the shortest pathway, bypassing processes such as mineralization or nitrification (Jones et al. 2005). Glycine, an amino acid, is generally used as an N substrate for measuring organic N uptake because of its small molecular weight (Wang et al. 2012), high fluidity in soil (Raab et al. 1999), and low contribution to microbial utilization (Lipson et al. 1999). Li et al. (2015) demonstrated that the uptake of the glycine by the fine roots of nine dominant tree species in a subtropical forest accounted for approximately 5–10% of total N uptake. Guo et al. (2021) also found that glycine uptake by the fine roots of spruce in alpine forests accounted for approximately 10–33% of the total uptake. In addition, the glycine uptake accounted for 6–20% of total N uptake in six Ecuadorian tropical montane forest species (Wittich et al. 2015), approximately 6% in nine dominant subtropical plantation species in southern China (Li et al. 2015), and 11–43% in seedlings of *Pinus koraiensis*, *Larix kaempferi*, *Quercus mongolica*, and *Juglans mandshurica* under greenhouse conditions (Zhu et al. 2019). A meta-analysis in natural ecosystems at the global scale found that amino acid uptake constituted 25.6% of total N uptake (Mao et al. 2025). Given this information, it is essential to perform field experiments on the uptake of both inorganic and organic N to understand nutrient acquisition strategies of alpine plants better.

Another important factor influencing root N uptake is interspecific variation. The preference for N uptake by fine roots varies significantly among species, even within the same environment. For example, Ito et al. (2023) reported a clear distinction in N uptake between NH_4^+ and NO_3^- in small root segments among four cool-temperate coniferous species. These differences among woody species are believed to be related to distinct traits of root functional morphology and chemistry (Comas et al. 2014; Yahara et al. 2019). For instance, absorptive roots are typically characterized by small diameter, high specific root length (SRL, the ratio of root length to mass), or low root tissue density (RTD, the ratio of root mass to volume) (Hodge 2004; Bergmann et al. 2020). These root traits reflect resource availability and indicate physiology, lifespan, and foraging strategies (Ostonen et al. 2011; Makita et al. 2011, 2015). Furthermore, inherent variations in root functional traits among tree species are characterized by their mycorrhizal types (Liese et al. 2017; Caplan et al. 2019). For instance, arbuscular mycorrhizal (AM) species exhibit acquisitive traits (e.g., high branching roots with a large surface

area), which enable rapid nutrient uptake but typically have shorter lifespans (Averill et al. 2019; Weigelt et al. 2021). In contrast, ectomycorrhizal (ECM) and ericoid mycorrhizal (ERM) species exhibit conservative traits, including thicker, less-branched roots with longer lifespans (Ward et al. 2022), which favor the conservation of resources and the efficient use of acquired nutrients. ECM and ERM species thrive in high-elevation and latitude regions where organic matter stocks are abundant and organic N is readily available. Notably, ERM species exhibit a high capacity for environmental acclimation, allowing for growth even in barren and acidic soils (Ward et al. 2022). Therefore, measuring inorganic and organic N uptake, as well as root traits of species in alpine forests, is crucial for determining how each species approaches nutrient acquisition.

Overall, we aimed to determine the nutrient acquisition of alpine woody plants by measuring inorganic and organic N uptake rates and root traits. We directly measured the uptake rates of NH_4^+ , NO_3^- , and glycine by the root systems of seedlings under field conditions using the depletion method. This net N uptake method quantifies N solution changes by soaking fine roots in a known N solution (Socci and Templer 2011; Ito et al. 2023). We also assessed the morphological traits of root systems—such as root diameter, SRL, and root tissue density (RTD) and their chemical traits (N concentration) using the same root systems to evaluate resource acquisition capacity. To conceptualize their fine-root traits for resource uptake, we referred to the root economic space proposed by Bergmann et al. (2020), which combines a fungal collaboration gradient (root diameter and SRL) and a conservation gradient (RTD and N concentration). We also considered differences in soil conditions, plant traits, and fungal partners between ECM and ERM plants (Ward et al. 2022). Based on this framework, we hypothesized that (i) N uptake rates vary among species and between mycorrhizal types, with ERM species showing higher organic N uptake and ECM species relying more on inorganic N; and (ii) variation in N uptake rates is linked to root functional traits along the root economic space: ERM species with finer, high-SRL roots and lower RTD would show higher uptake rates, indicating an acquisition for efficient resource foraging, while ECM species with thicker, low-SRL roots and higher RTD would have lower uptake rates, consistent with a conservation.

Materials and methods

Study site and species selection

This study was carried out at Nishikoma research forest (35°80'N, 137°83'E; 2,672 m above sea level), Shinshu

University, Nagano, Japan (Fig. 1a). This area is located beyond the tree line and is dominated by shrubs, including Siberian dwarf pine, crowberry and cowberry (Fig. 1b). The mean temperature and precipitation during the study period from July to September 2023 was 12.1 °C and 1,661 mm, respectively (Iwata 2025).

We selected seedlings of five common woody species in this forest: one gymnosperm species, *Pinus pumila* (Siberian dwarf pine), and four angiosperm species, *Alnus maximowiczii* (Montane alder), *Empetrum nigrum* (Black crowberry), *Rhododendron aureum* (yellow-flowered rhododendron), and *Vaccinium vitis-idaea* (Lingonberry). *Alnus maximowiczii* is a deciduous species; the others are evergreen. Seedlings, being highly sensitive to severe environmental conditions, represent a crucial stage in their growth for environmental acclimation. *P. pumila* and *A. maximowiczii* are associated with ECM fungi. *E. nigrum*, *R. aureum*, and *V. vitis-idaea* are associated with ERM fungi. ERM species also occur in the understory and forest edge of ECM species (Mitchell and Gibson 2006; Pölme et al. 2013; Koizumi

and Nara 2017). The roots of *Alnus maximowiczii* also host N-fixing fine roots, in addition to forming ECM associations, which bear nodules along the root surface.

Field experiment design and N uptake measurement

We established an area of approximately 100 m × 100 m spanning the ridge and the north and south slopes, where the target species are distributed. N uptake by the target woody plant species was measured on sunny days. The N uptake rates by plants per unit of fine root mass ($\mu\text{mol g}^{-1} \text{h}^{-1}$) were assessed using the in situ depletion method (Ito et al. 2023). First, we selected 18 locations within the study area where multiple target tree species were growing. At each location, we collected two seedlings from nearby: one for sampling organic N uptake and one for sampling inorganic N uptake. That is, inorganic and organic N uptake rates were measured using different seedlings, even within the same location. NH_4^+ and NO_3^- were measured for inorganic N, and

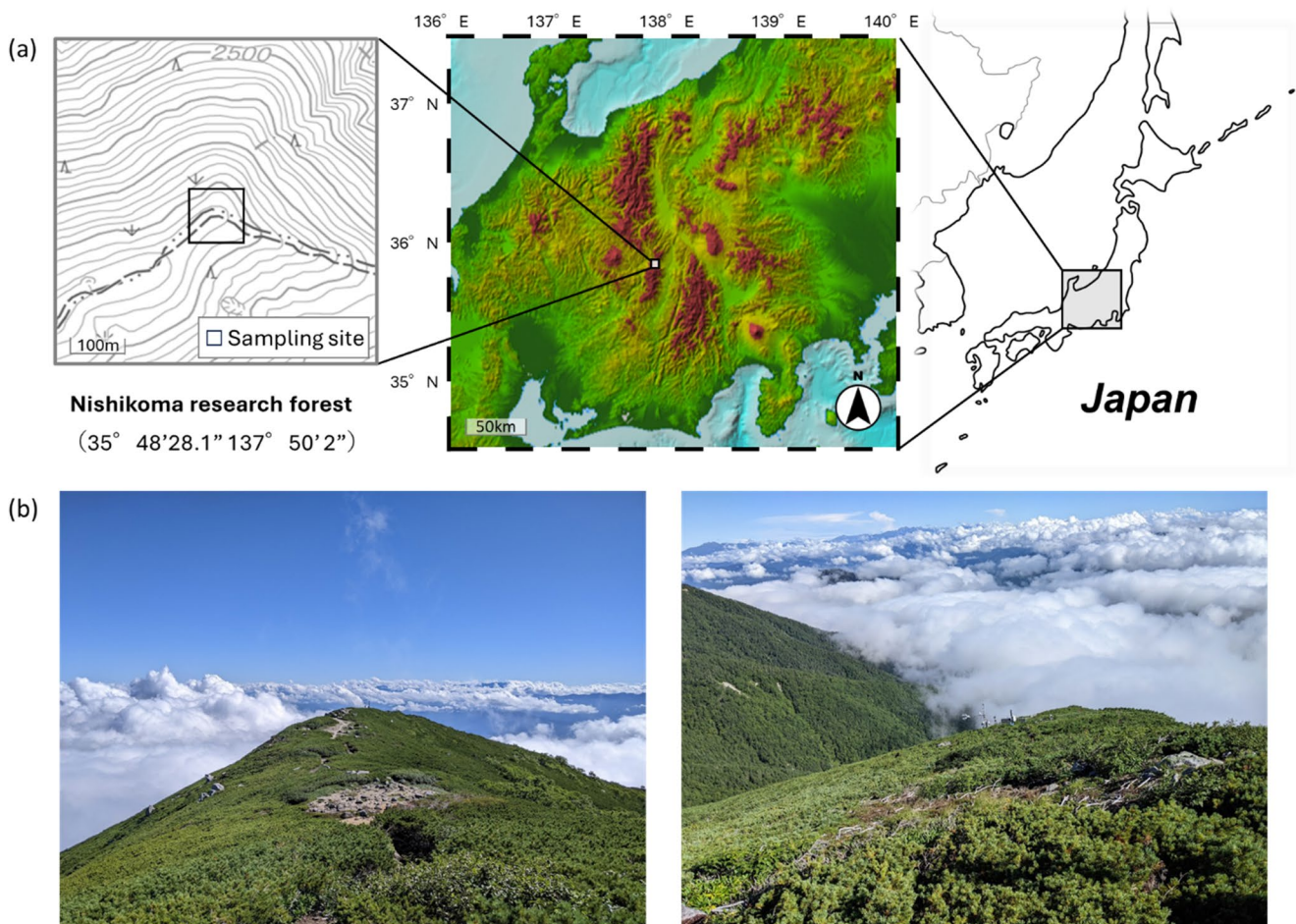


Fig. 1 **a** The topographic map and location of the study site, Nishikoma research forest. The squares on the topographic map represent the sampling sites for measurements of plant root nitrogen uptake.

Elevation data was provided by the Geospatial Information Authority of Japan. **b** Photographs of the ridge and slope at the study site

glycine for organic N. Eighteen replications were performed for each species for both inorganic and organic N uptake measurements. Seedlings less than 10 cm tall were carefully excavated to prevent damage to the roots. We focused on fine roots as they are the most active in nutrient acquisition (Nadelhoffer and Raich 1992). For our measurements, we selected seedlings with healthy and intact fine root systems. These roots were defined as roots without darkened, decayed areas, dry white tissues, and exhibiting abundant branching and an elastic structure (Ohashi et al. 2025). The soil was removed from the root systems by washing with deionized water. Subsequently, the roots were wiped with a paper towel. The entire root system was placed in a 5 mL syringe containing an N solution (200 $\mu\text{mol L}^{-1}$ NH_4Cl and KNO_3 and 200 $\mu\text{mol L}^{-1}$ glycine) and incubated for 90 min. Each syringe was positioned vertically in a sunlit location, sealed with parafilm, and covered with aluminum during incubation to minimize evaporation and contamination. After incubation, N solutions were filtered through a 0.45 μm syringe filter (Membrane Solutions Limited, Plano, TX, USA) and placed in a 5 mL tube. Control solutions were prepared and incubated under the same conditions in the field without root insertion. The incubated roots in the syringe were cut and stored in zip lock bags. Collected solutions and root samples were placed in an icebox and quickly transported to the laboratory.

Measurement of N concentration

NH_4^+ , NO_3^- , and glycine concentrations in the solution samples were determined by colorimetric analysis using microplate technology (Shand et al. 2008; Ito et al. 2023). NH_4^+ and NO_3^- in solutions were determined by color formation due to the Berthollet reaction and reaction of the vanadium oxidizing agent and Griess reagent, respectively. Glycine in solution was measured using the same procedure as for NO_3^- , following its oxidation to NO_3^- by the addition of a persulfate-oxidizing reagent and subsequent incubation (Isobe et al. 2011). The concentration of each N solution sample was measured using a Multiskan Sky microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). N uptake rate was calculated as follows:

$$N_{\text{uptake}} = (N_{\text{control}} - N_{\text{sample}}) \times \frac{V_{\text{solution}}}{M_{\text{root}} \times \text{Time}}$$

where N_{uptake} ($\mu\text{mol N g}^{-1} \text{ h}^{-1}$) indicates uptake rates of NH_4^+ , NO_3^- , and glycine by the root; M_{root} (g) is the dry weight of the incubated root; N_{control} ($\mu\text{mol L}^{-1}$) is the concentration of control N solution before measurement; N_{sample} ($\mu\text{mol L}^{-1}$) is the concentration of N solution sample after

incubation; V_{solution} (L) is the volume of N solution sample; and Time (h) is the incubation time.

Determinations of root morphological and chemical traits

The root samples were scanned in color at 600 dpi using a model GT-X980 double-lamp bed scanner (Epson, Nagano, Japan). The mean root diameter (mm), total length (cm), and total volume (cm^3) of the root sample were determined using WinRHIZO 2013 Pro (Regent Instruments, Quebec City, Quebec, Canada) root system analyzer software. The root samples were then dried at 50 °C for 48 h and weighed. The SRL (m g^{-1}) and RTD (g cm^{-3}) were calculated from the samples' total root volume, length, and dry mass. After morphological measurements, the dried root samples were ground with a mortar and pestle to measure the N concentration (%) using a FLASH 2000 elemental analyzer (Thermo Fisher Scientific, USA).

Soil sampling and measurement

Soil samples were collected at the N uptake measurement plot. The sampling points were evenly distributed across the site. A total of 12 soil sampling points were evenly distributed across the plot to reduce bias caused by spatial clustering, slope aspect, or elevation. Soil traits included pH, electrical conductivity (EC), concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, dissolved organic N (DON), and total dissolved N (TDN), and water content. Surface soil samples (0–10 cm) were collected from six locations in the plot using a shovel. The samples were taken to the laboratory and sieved through a 2 mm mesh to remove debris. $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and DON concentrations (mg kg^{-1}) were measured following the extraction of the soil solution with 0.5 M K_2SO_4 solution (soil; 8 g, 0.5 M K_2SO_4 ; 40 mL). During extraction, the soil samples were shaken for 12 h and filtered using 5 C filters (ADVANTEC, Tokyo, Japan). The $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations of the filtered and root culture solutions were measured using colorimetric analysis. The remaining soil samples were air-dried for two weeks, and the pH and EC were determined in a 1:5 mixture of dried soil and distilled water using a LAQUA D-210PC-S pH and EC meter (Horiba, Kyoto, Japan). Fresh soil was weighed and dried at 105 °C for over 24 h to measure water content. After determining the dry soil weight, the gravimetric soil water content (%) was calculated per unit mass of oven-dried soil.

Statistical analyses

The effect of species on both uptake rates and root traits was tested using a one-way analysis of variance. Tukey's

honestly significant difference (HSD) test was employed to identify differences in uptake rates among the species ($p < 0.05$). It is noted that the targeted-species seedlings for inorganic N uptake and organic N measurements were placed at a distance of less than 1 m at multiple coexisting locations, which we refer to as paired samples. In such cases, the paired measurement values would be considered as point-level uptake rate values for statistical comparison. Principal component analysis (PCA) was performed using the following parameters: uptake rate, root diameter, SRL, RTD, and N concentration. These root traits were included in the PCA because they are important when considering root economic space (Bergmann et al. 2020). All statistical analyses were performed using R version 4.2.2 software.

Results

Soil properties

The mean soil pH and EC ($\pm$ SE: standard error) in the study site was 5.99 (0.12) and 17.77 (4.19) $\mu\text{S cm}^{-1}$, respectively. The concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and DON ($\pm$ SE) were 4.24 (0.63), 0.03 (0.01), and 21.49 (3.01) mg N kg^{-1} , respectively. The soil water content per weight in the 0–10 cm layer was 48.8% (0.01). While our sampling design might minimize potential bias, microscale variation in nutrient concentrations, in particular, may still have existed. This variation reflects realistic soil conditions that roots encounter and could have subtly influenced nutrient uptake.

N uptake rates

The uptake rate of NH_4^+ , NO_3^- , and glycine by fine roots for five woody plant species was 2.01–4.49 $\mu\text{mol N g}^{-1} \text{h}^{-1}$, 0.06–1.71 $\mu\text{mol N g}^{-1} \text{h}^{-1}$, and 1.19–7.28 $\mu\text{mol N g}^{-1} \text{h}^{-1}$, respectively (Fig. 2, Table S1). The NH_4^+ uptake rate of *V. vitis-idea* was significantly higher than that of any other species (Fig. 2a; $p < 0.01$), as was the NO_3^- uptake rate (Fig. 2b; $p < 0.001$). There were no significant differences in inorganic N uptake rates of other species (Fig. 2a, b). The glycine uptake rates of *V. vitis-idea* and *E. nigrum* were significantly higher than those of any other species (Fig. 2c; $p < 0.001$). The total N uptake rates of ERM species were significantly higher than those of ECM species. (Fig. 3a; $p < 0.001$). In proportion to N uptake rates, ECM species absorbed more inorganic N (NH_4^+ and NO_3^-), whereas ERM species absorbed more glycine (Fig. 3b).

Root morphological and chemical traits

There were significant differences in the measured root morphological and chemical traits among the species (Table 1, S1). The values of mean root diameter, SRL, RTD, and root N concentration for each species were 0.27–0.65 mm, 8.60–25.04 m g^{-1} , 0.37–1.18 g cm^{-3} , and 0.62–1.41%, respectively. The root diameter was significantly larger in *P. pumila* and *A. maximowiczii* ($p < 0.001$). SRL was significantly higher in *A. maximowiczii*, *R. aureum*, and *V. vitis-idaea* than in the other two species ($p < 0.001$). RTD was significantly higher in *E. nigrum*, *R. aureum*, and *V. vitis-idaea* than in ECM species ($p < 0.001$). Root N concentration was significantly higher in *A. maximowiczii* than in the other four species. ECM species had roots with larger root diameters and smaller RTD, whereas ERM species had smaller root diameters and larger RTD.

Variation in N uptake and root traits among the five species

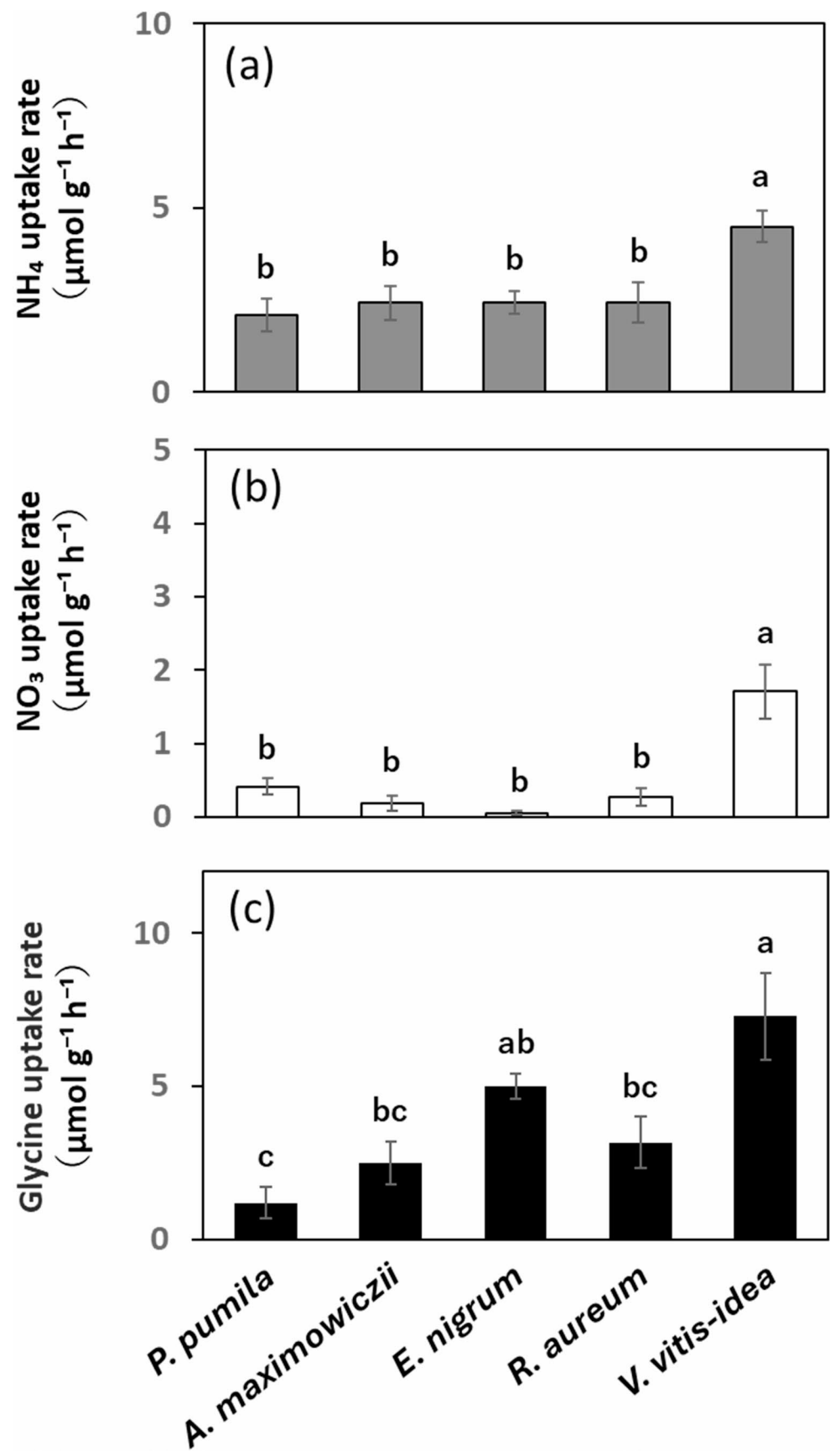
PCA of the uptake rates of NH_4^+ , NO_3^- , and glycine and root traits (mean root diameter, SRL, RTD, and root N concentration) of the five species showed that the first and second principal component (PC1 and PC2, respectively) accounted for 56.0% and 20.6% of variation, respectively (Fig. 4). NH_4^+ , NO_3^- , and glycine uptake rates correlated negatively with PC1. N uptake rates contributed slightly to PC2, with NH_4^+ and NO_3^- uptake rates contributing positively and the glycine uptake rate contributing negatively. PC1 differentiated species according to their mycorrhizal associations, with ECM species (*P. pulila* and *A. maximowiczii*) positioned towards the positive side of PC1 axis. In contrast, ERM species (*R. aureum* and *V. vitis-idaea*) were positioned towards the negative side. PC2 further distinguished species within each mycorrhizal type, with *V. vitis-idaea* (ERM) and *A. maximowiczii* (ECM) positioned on the positive side of the PC2 axis. In root traits, the mean root diameter aligned positively with PC1. SRL and root N concentration aligned positively with PC2. RTD was considered to influence both axes, with a positive and negative contribution to PC1 and PC2, respectively.

Discussion

NH_4^+ , NO_3^- , and Glycine uptake rates of alpine woody plants

The results provide new insights into N acquisition strategies of alpine species. Among inorganic N forms, the uptake rate of NH_4^+ was higher than that of NO_3^- , possibly because

Fig. 2 **a** NH_4^+ , **b** NO_3^- , and **c** glycine uptake rate of fine roots in the five species. Different letters above the bar indicate significant differences among the five species according to Tukey's HSD test ($P < 0.05$). Error bars indicate standard error



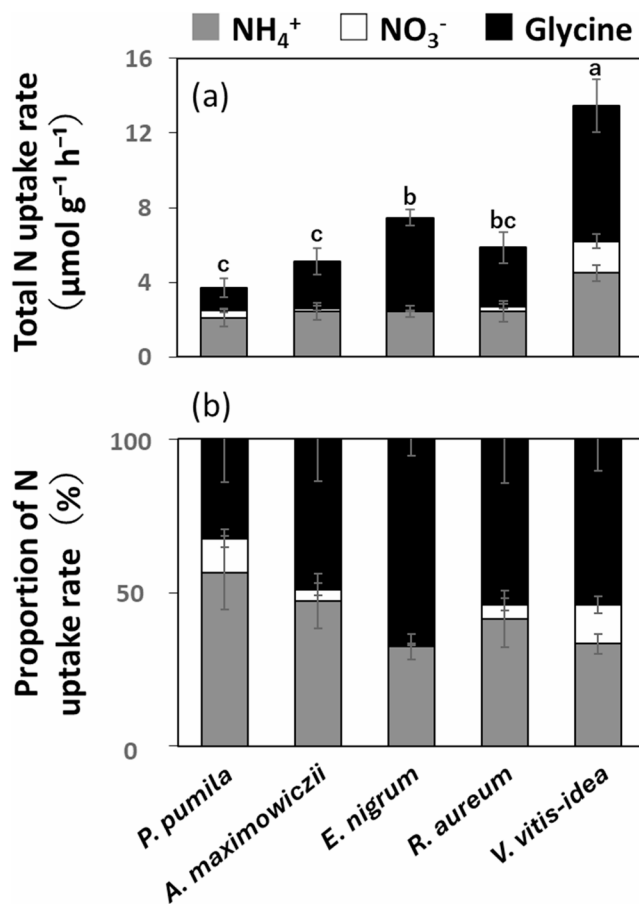


Fig. 3 **a** Total N uptake rate and **b** proportion of each N uptake rate for each N form of fine roots in the five species. Different letters above the bar indicate significant differences among the five species according to Tukey's HSD test ($P < 0.05$). Error bars indicate standard error

Table 1 Mean values of (a) morphological and (b) chemical traits of root samples used for N uptake measurement

Species		Morphological trait			Chemical trait
		Diameter (mm)	SRL (m g^{-1})	RTD (g cm^{-3})	Root N (%)
<i>Pinus pumila</i>	Mean	0.65 a	8.60 c	0.40 c	0.86 b
	SE	0.02	0.69	0.01	0.04
<i>Alnus maximowiczii</i>	Mean	0.50 b	21.63 ab	0.37 c	1.41 a
	SE	0.03	2.80	0.02	0.05
<i>Empetrum nigrum</i>	Mean	0.39 c	11.40 bc	0.76 b	0.62 c
	SE	0.01	1.06	0.03	0.01
<i>Rhododendron aureum</i>	Mean	0.33 cd	25.04 a	1.18 a	0.83 b
	SE	0.02	4.88	0.10	0.04
<i>Vaccinium vitis-idea</i>	Mean	0.27 d	20.80 ab	0.81 b	0.80 b
	SE	0.01	2.11	0.03	0.02

Different letters next to the values indicate significant differences among the five species according to Tukey's HSD test ($P < 0.05$). SRL stands for Specific Root Length, and RTD represents Root Tissue Density

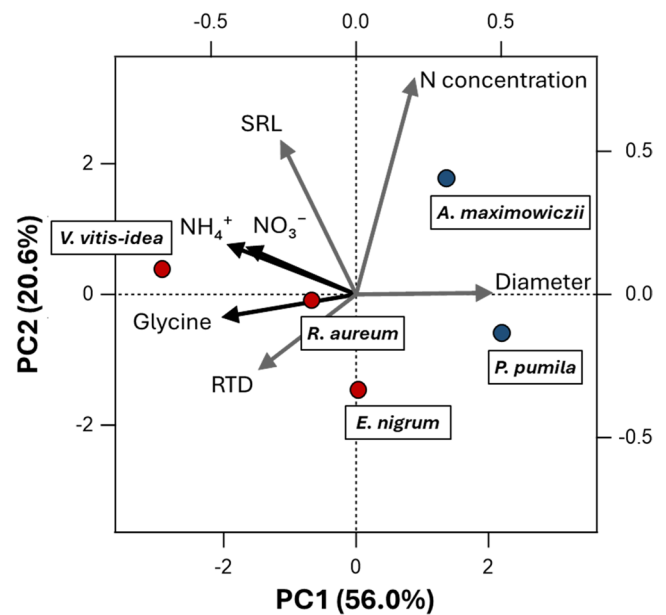


Fig. 4 Principal component analysis of NH_4^+ , NO_3^- , and glycine uptake rates and four root traits across the five species examined in this study. Black arrows indicate the uptake rates of NH_4^+ , NO_3^- , and glycine. Gray arrows indicated root traits, mean root diameter, specific root length (SRL), root tissue density (RTD), and root N concentration. Red and blue circle symbols indicate ECM and ERM species, respectively

NH_4^+ can be directly incorporated into amino acids without the energy-intensive reduction process required for NO_3^- assimilation. Notably, the proportion of glycine uptake in this study was generally higher than reported in previous research across various ecosystems. By comparison, the high glycine uptake observed in our alpine species aligns with findings from nutrient-limited taiga environments (Kielland et al. 2006) and suggests that alpine plants may be particularly acclimated to utilize organic N forms, such as glycine, more effectively than plants in lower-elevation forests. This enhanced capacity for organic N uptake in alpine species may be attributed to the low availability of inorganic N in alpine soils. According to national soil metadata in Japan (Urakawa et al. 2015), mean NH_4^+ and NO_3^- concentrations are 6.25 mg kg^{-1} and 4.85 mg kg^{-1} , respectively, indicating a relatively low supply of inorganic N (especially NO_3^-) in our study site. Organic N compounds, such as amino acids, can be used more efficiently because they do not require further assimilation after uptake (Persson et al. 2006). Thus, alpine woody plants may enhance their organic N acquisition to maximize efficiency under nutrient-limited conditions.

Our findings also support previous studies showing that plants have higher uptake capacity for abundant N type (Kielland et al. 2006; Wang et al. 2012; Andersen and Turner 2013). However, they contrast with the commonly reported pattern, in which dominant species preferentially

use the most abundant N form (McKane et al. 2002). At our alpine site, the dominant species *P. pumila* exhibited a preference for NH_4^+ , the second most abundant form, over the more abundant organic N. This pattern may reflect traits such as root morphology or mycorrhizal associations, but it is important to consider that three of the five species studied belong to Ericaceae, and no explicit phylogenetic contrast was incorporated. Therefore, the observed uptake differences may partly reflect phylogenetic constraints rather than purely species-specific acclimation. Nonetheless, these results highlight potential ecological differentiation in N acquisition among alpine species, providing insight into how different species exploit available N forms under nutrient-limited conditions.

Differences in N uptake among species

Uptake rates of NH_4^+ and NO_3^- were significantly higher in *V. vitis-idea*, with no differences observed among the other species (Fig. 2a,b). On the other hand, glycine uptake rates were elevated in ERM species, *E. nigrum* and *V. vitis-idea* (Fig. 2c). The roots of *V. vitis-idea* (ERM species) are characterized by a unidirectional distribution and longer soil exploration distances, while ECM species typically show shorter exploration distances and a more concentric root distribution pattern (Fruggens et al. 2023). By spreading their roots in N-rich areas, *V. vitis-idea* may enhance the uptake capacity of the explored roots. ERM species, such as *V. vitis-idea*, also contribute to increased soil acidity and microbial activity (Makarov et al. 2019). This suggests that *V. vitis-idea* may have a unique nutrient acquisition compared to the studied other species in the alpine zone.

The total N uptake rate tended to be higher in ERM species (Fig. 3a). This result suggested that their nutrient acquisition capacity is particularly robust under alpine conditions. This elevated uptake likely reflects their high ecological flexibility, enabling them to thrive in environments with poor nutrient availability. In terms of N form preference, ECM species favored inorganic N, while ERM species favored organic N (Fig. 3b). The results supported hypothesis (i). This difference in preferences may stem from the varying process of nutrient acquisition among mycorrhizal types. Both ECM and ERM species are characterized by hyphal extension on soil organic matter and can compete with microorganisms for decomposition products. However, ERM species appear to spread their hyphae more extensively than ECM species, making them more adept at utilizing organic N (Ward et al. 2022). Therefore, organic N is the primary N source for ERM species.

Relationship between N uptake rates and root traits

The results of the PCA showed that each N uptake rate, root diameter, and RTD contributed to PC1 (Fig. 4), while SRL, root N concentration, and RTD contributed to PC2. Compared to the root economic space proposed by Bergmann et al. (2020), multidimensionality, such as collaborative or conservative gradients, was not observed. Our results did not completely support hypothesis (ii). The reason is that the root traits of ECM species do not fit into the conventional theoretical framework (Valenzuela-Estrada et al. 2008). The PC1 axis represents the N uptake rate and is most strongly influenced by root diameter. Root diameter serves as an indicator of nutrient acquisition capacity, as thinner roots can acquire nutrients more easily due to their ability to penetrate the soil matrix and increase the contact area (McCormack et al. 2017; Ma et al. 2018; Freschet et al. 2021). ERM species, such as *V. vitis-idea*, develop very fine roots and have high nitrogen uptake rates. By producing the roots with high access potential and enhancing the uptake rate, these species are likely to achieve high total N acquisition. In ERM roots, the vessel diameter is small and comparable to the root diameter, which makes them less susceptible to damage from freezing and drying (Hargrave et al. 1994; Davis et al. 1999). These roots, with small root diameters, may enhance both nutrient acquisition and tolerance to freezing or drying stress. Although nutrient acquisition is positively correlated with SRL and root N concentration (Ryser 2006; Reich et al. 2008; Holdaway et al. 2011; McCormack et al. 2017), our results suggest that root diameter may be the most significant factor influencing N uptake rates in alpine zones. In our study site, we found that ERM species achieved higher total N uptake than ECM species, indicating that fine root morphology enhances efficient nutrient acquisition under nutrient-limited conditions. However, it is important to recognize that our study was conducted at a single high-elevation site, and environmental conditions at other elevations or in different alpine environments may vary. Nonetheless, our results clearly demonstrate that root morphological traits are connected to N uptake rates in alpine species and emphasize potential functional acclimation that could be vital for plant performance in stressful, nutrient-poor conditions.

It is noteworthy that inorganic and organic N uptake contributed slightly to the positive and negative sides of PC2, respectively. The PC2 axis represents N preference and is influenced by SRL, N concentration, and RTD. In other words, roots with higher SRL and N concentrations can absorb more inorganic N, while roots with higher RTD can absorb more organic N. Furthermore, the relationship between inorganic N uptake and root traits appears to vary among mycorrhizal types. To enhance inorganic N uptake rates, ERM species like *V. vitis-idea* produce roots with

higher SRL (indicating better soil nutrient exploration), whereas ECM species like *A. maximowiczii* develop roots with higher root N concentrations (reflecting increased root metabolic activity). The N uptake rate in cool-temperate forest conifers is positively correlated with SRL and N concentration (Ito et al. 2023); however, this relationship may be specific to inorganic N. Hong et al. (2018) demonstrated that root traits associated with nutrient acquisition, such as SRL, were significantly correlated with inorganic N uptake, but showed weaker correlations with glycine uptake rates. The relationship between organic N uptake and RTD is also of considerable interest. RTD, which can indicate physical defense and stress tolerance, has been reported to be higher in ERM species than in ECM species (Hewitt et al. 2024). The positive correlation between organic N uptake and RTD suggests that increasing RTD may serve as an adaptive trait in response to environmental conditions. RTD is positively associated with root stele diameter and root hydraulic conductivity (Kong et al. 2014; Masumoto et al. 2022) and relates to the transport function. Given the abundance of organic N in the soil, the efficient transport of organic N from the rhizosphere into the plant body may be more important than enhancing root exploration capacity. These findings represent the first detailed exploration of the relationship between N uptake and root traits in alpine zones for each form of N. This understanding may be crucial for comprehending the strategies species use to survive in demanding environments.

Overall, our study revealed that species-specific differences in N uptake arose from variations in root traits. ECM species exhibited lower N uptake than ERM species, likely due to their relatively thicker roots, which were less suitable for absorbing nutrients. Nevertheless, *A. maximowiczii* appears to enhance inorganic N uptake by increasing root N concentration. The high root N concentration in *A. maximowiczii* suggests a potential dependence on N fixation from the atmosphere, in addition to N uptake from the soil. Root N concentrations are particularly elevated in N-fixing plants (Yahara et al. 2019). ERM species also absorb more N by possessing thinner roots. The high N uptake in *V. vitis-idea* can be attributed to their smallest root diameters. *E. nigrum* seemed to absorb N at the second-highest rate among the ERM species. The similarity in nutrient acquisition between *E. nigrum* and *V. vitis-idea* may stem from their common growth form as creeping shrubs. In contrast, the distinctive individual size and root morphology of *R. aureum* appeared different from those of the other ERM species, potentially influencing the variation in N uptake. However, it is challenging to fully explain species-specific differences in N uptake in terms of root traits. For instance, mycorrhizal fungal symbiosis plays a significant role in N uptake. Hobbie and Hobbie (2006) reported that ECM and ERM species

derive 61–86% of their acquired N through associations with mycorrhizal fungi. Additionally, infection rates and the composition of mycorrhizal fungi have been shown to alter N uptake (Grelet et al. 2009; Qiao et al. 2016; Hewitt et al. 2024). Microtopographic variations, such as slope aspect and elevation, may also influence N uptake (Yu et al. 2024). To further investigate N uptake in alpine zones, it is imperative to examine the effects of mycorrhizal fungi function, soil N availability, and environmental conditions on N uptake in greater depth. In the future, integrated approaches, such as phylogeography and biochemistry, may facilitate a deeper understanding of species-specific nutrient acquisition and ecosystem functions in alpine environments.

Conclusion

In five alpine woody plant species, the in situ N uptake rate by fine roots was higher in ERM species than in ECM species. The higher N uptake in ERM species is attributed to their smaller root diameter, which enhances their ability to absorb nutrients. The N uptake rate of ECM species was more dependent on inorganic N, while that of ERM species relied more on organic N. This difference is thought to stem from N partitioning due to competition for resources among species or mycorrhizal types. Furthermore, inorganic N uptake was enhanced in ECM and ERM species through increased root N concentration and SRL, respectively. Higher RTD also facilitated the uptake of organic N. Thus, the results of this study suggest that alpine plants acquire nutrient niches by displaying different N preferences (inorganic N or organic N) under poor nutrient conditions. Differences in N uptake among species or mycorrhizal type are largely controlled by root traits, indicating that root morphology and chemistry are crucial for maintaining nutrient acquisition strategies in alpine zones.

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Data availability The datasets are available on request to the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

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