Different Relationships of Fine Root Traits With Root Ammonium and Nitrate Uptake Rates in Conifer Forests

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Research Article

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Abstract

Purpose: Nitrogen (N) uptake by fine roots of trees is important for understanding the root physiological function in forest ecosystems, but a direct investigation of *in situ* rate of ammonium and nitrate uptake is limited. Thus, we aimed to clarify the inorganic N uptake rates among tree species and to determine the factors controlling N uptake through relationships with fine root traits in cool temperate forests.

Methods: Using a solution depletion method for measuring N uptake, we observed the relationship of N uptake rate in the form of NH_4^+ and NO_3^- by an intact root system with root morphological traits, such as root diameter, specific root length (SRL), and root tissue density (RTD), and chemical traits, including root nitrogen (N) content.

Results: The coniferous roots in this study preferred NH_4^+ form more than NO_3^- form. Across species, there were significant relationships between NH_4^+ uptake and diameter, SRL, and RTD, while these were significant only for RTD in NO_3^- form. Relationships between N uptake rates and root morphological traits differed between NH_4^+ and NO_3^- .

Conclusions: We found that the relationship of inorganic N uptake with the morphological traits depends on the characteristics of the N form adsorbed through soil and tree N assimilation efficiency. An approach on the relationships of *in situ* N uptake with root traits will provide a breakthrough in our understanding of the root physiological function and the prediction of fundamental N acquisition strategies.

Introduction

Nitrogen (N) is one of the most essential and limiting factors for plant growth and development in temperate forest ecosystems (LeBauer and Treseder 2008; Vitousek and Howarth 1991). The existing N content in forest ecosystems is approximately $5,300 \text{ kg ha}^{-1}$, and the amount of N stored in the soil is 90% (Bormann et al. 1977). In soil, complex organic polymers in litter are decomposed and converted into bioavailable N-containing monomers (such as amino acids, amino sugars, and nucleic acids) that can be used by either plants or microorganisms (Schimel and Bennett 2004). The N content is mineralized by microorganisms, producing ammonium (NH_4^+), and transformed to nitrate (NO_3^-) by nitrification. Trees acquire almost all their N demand through root uptake. Generally, NH_4^+ and NO_3^- as inorganic N are the major N sources for trees (Haynes et al. 1978). The NH_4^+ and NO_3^- differ in their assimilation efficiency and solubility in soil (Lambers et al. 2019). While NO_3^- is readily available due to its high solubility in soil water, plants must use energy to reduce NO_3^- before incorporation into amino acids. Thus, it may be more efficient for plants to take up NH_4^+ because it can be immediately incorporated into amino acids. However, NH_4^+ is an immobile form of N, which is not always available for uptake because it is strongly adsorbed onto soil particles. Our crucial concern in this field is the form and amount of N that the tree roots acquire from the soil. The answer is important in interpreting the findings from the nutrient

acquisition strategy of trees. Inorganic acquisition by trees is one of the major N flows in ecosystems (Bormann et al. 1977). Therefore, N uptake by tree roots is important for understanding the N cycles in forest ecosystems.

Previous studies have directly measured the N uptake of fine roots (< 2 mm in diameter) and evaluated the effects of tree species (Gessler et al. 1998; Templer and Dawson 2004). For example, fine roots of American beech (*Fagus grandifolia* Ehrh.) seedlings took up NO_3^- more than NH_4^+ , while sugar maple (*Acer saccharum* Marsh.) preferred to take up NH_4^+ over NO_3^- (Templer and Dawson 2004). In warm and cool temperate forests, the uptake preference of chemical N forms varies among plant species (Gao et al. 2020). In temperate and tropical forests, all 21 tree species took up NH_4^+ more than NO_3^- , but the contribution of each N form was different (Liu et al. 2017). Because there is inadequate data on N preferences in tree species, we still do not know the background mechanisms of N uptake characteristics among tree species.

An approach based on root functional traits can be one of the most useful for understanding the physiological mechanisms of tree species (McCormack et al. 2017). For example, absorptive roots are generally characterized by a small diameter, high specific root length (SRL: ratio of root length to mass), or low root tissue density (RTD: ratio of root mass to volume), resulting in longer and thinner roots that are considered to have greater capacity for water and nutrient acquisition when compared to short and thick roots (Comas et al. 2002; Comas and Eissenstat 2004; Hodge 2004). These traits would reflect the availability of resources and indicate physiology, lifespan, and foraging strategy (Tjoelker et al. 2005; Ostonen et al. 2011; Adams et al. 2013; Makita et al. 2015, 2016), allowing comparisons across species (Comas and Eissenstat 2009; Liese et al. 2017; Yahara et al. 2019). In addition, fine roots with high SRL and low RTD have high root respiration rates (Makita et al. 2012) and exudation rates (Akatsuki and Makita 2020). In the present study, we aimed to focus on the relationship between root N uptake and the morphological traits of fine root systems, including SRL and RTD. We also aimed to determine the relationship with tissue N content. Here, we hypothesized that differences in root N uptake rates among tree species could be explained by morphological and chemical root traits. Determining these relationships would be helpful for understanding the generality of their impacts on root N uptake from the soil.

To achieve the direct field measurement of N uptake by fine roots, we used *in situ* depletion method for measuring N uptake in solution by roots (BassiriRad et al. 1999; Campbell et al. 2014; Socci and Templer 2011). This depletion method for net N uptake could quantify the change in N solution by soaking fine roots in a known N solution. It is suitable for evaluating fine roots with a thinner size, which have high physiological activity (Makita et al. 2009, 2015). This study addressed the following three questions:

- (i) Is there a difference in species of NH_4^+ and NO_3^- uptake rates by tree fine roots?
- (ii) How do morphological and chemical traits, including SRL, RTD, and root N content, relate the uptake rates of each N form?

(iii) Do the relationships between root NH_4^+ and NO_3^- uptake and root traits exhibit common patterns across species?

Material And Methods

Study site

The study was conducted in Terasawayama Station Research Forest (35° 53 N, 138° 02 E; 960–1,450 m a.s.l.) in Nagano, Japan. The annual temperature and precipitation are 9.8 °C and 1,469 mm, respectively. This is a cool-temperate forest, with an area of 228 ha for coniferous plantations.

The forest consists of the plantations in larch (*Larix kaempferi* (Lambert) Carrière) and red pine (*Pinus densiflora* Sieb. et Zucc.), Japanese cypress (*Chamaecyparis obtusa* Sieb. et Zucc.), and Japanese cedar (*Cryptomeria japonica* D. Don), which were selected as the target species. In Japan, these four tree species have been widely planted for timber products and cover approximately 70% of the plantation area in Japan (Forestry Agency 2018). *Larix kaempferi* is a deciduous conifer, and *Pinus densiflora* is an evergreen conifer that is associated with ectomycorrhizal (ECM) fungi. *Cryptomeria obtusa* and *C. japonica* are evergreen conifers that are associated with arbuscular mycorrhiza (AM) fungi. At this site, studies have been conducted on the root functional traits (Tanikawa et al. 2019; Yahara et al. 2019) and root exudation (Akatsuki and Makita 2020) in several woody species.

Field experiment of root N uptake measurement

We established a 30 × 30 m plot for each species at the site and selected eight mature trees per plot. The mean diameter at breast height of the mature trees was 38.5 cm. The root uptake of NH_4^+ and NO_3^- by the target tree species was measured on seven sunny days in August 2020. The N uptake measurement by the depletion method was conducted during daytime (9:00–17:00) because the physiological activity of the plants was presumed to be maximum during this period. The procedure for root N uptake was as follows: intact root systems of the target tree species were exposed at a depth of 10 cm from the top of the soil, including the organic layer, which was attached to the mother root. The root systems of the target tree species were distinguished by their morphological traits such as color, diameter, branching pattern, root tips, and mycorrhizal type (Yahara et al. 2019). Living root systems were distinguished from dead roots based on their color and elasticity. The soil was removed from the larger root system by carefully washing with deionized water. Larger root systems were re-buried until the N uptake measurements (generally 24 h later). The re-burial process was used for root exudate measurement (Phillips et al. 2008) and was intended to allow additional time for roots to recover from any potential injury or stress sustained during the excavation and rinsing process.

After the re-burial process, two intact root segments (< 2 mm diameter, approximately 10 cm length of the main axes) were selected for N uptake measurements. They were rinsed with de-ionized water, gently

wiped with a paper towel, and inserted into the tube (12 mL). Next, 10 mL of N mixed solution (containing $200 \mu\text{mol L}^{-1}$ NH_4Cl and KNO_3) was injected into the tube. The remaining root parts outside the tube were covered with wet kitchen paper to maintain root moisture. To shade the sample, the whole root system was wrapped with aluminum foil and covered with a shading sheet. The root segments soaked in the N solution were incubated for 90 min. After incubation, the root segments were collected from the N solutions and sampled. The N solution samples were immediately filtered using syringe filters ($0.45 \mu\text{m}$; Membrane Solutions Limited, Plano, TX, USA) and placed in centrifuge tubes. The root samples were stored in zipper storage bags. In total, we evaluated 64 samples of solutions and fine roots (four tree species $\times$ 16 replications). Both root and N solution samples were placed in an icebox and transported to the laboratory.

Measurement of N concentration in solutions

In the laboratory, the NH_4^+ and NO_3^- concentrations of the solution samples were measured using a microplate technique (Shand et al. 2008). The N solution samples were colored by the Berthelot reaction for NH_4^+ and by the vanadium oxidizing agent and Griess reagent for NO_3^- . The concentration of each N solution sample was measured using a microplate reader (Multiskan Sky, Thermo Fisher Scientific, Cambridge, MA, USA).

For the calculation of *in situ* N uptake, the difference in N solution concentration before (N_{control}) and after (N_{solution}) the measurement was multiplied by the solution volume (V_{solution}) and divided by the dry weight of the root sample (DW_{root}) and incubation time (T). The calculation of each N from the uptake rates by root (N_{uptake}) was calculated as follows:

$$N_{\text{uptake}} = (N_{\text{control}} - N_{\text{solution}}) \times \frac{V_{\text{solution}}}{DW_{\text{root}} \times T}$$

where N_{uptake} ($\mu\text{mol g}^{-1} \text{h}^{-1}$) is the uptake rate of NH_4^+ or NO_3^- by the fine roots; DW_{root} (g) is the dry weight of the root sample; N_{control} ($\mu\text{mol L}^{-1}$) is the NH_4^+ or NO_3^- concentration of the control N solution (before measurement), while N_{solution} ($\mu\text{mol L}^{-1}$) is the NH_4^+ or NO_3^- concentration of the N solution samples (after measurement); V_{solution} (L) is the volume of the N solution; and T (h) is the incubation time.

Root morphological and chemical analysis

The root samples were scanned in gray at 300 dpi using a double-lamp bed scanner (GT-X980; EPSON, Nagano, Japan). We analyzed the total root length, volume, and mean diameter using WinRHIZO Pro 2013a (Regent Instruments, Quebec, Canada). The total root length, volume, and dry mass were used to calculate the SRL (m g^{-1}) and RTD (g cm^{-3}) of the samples. After scanning, the root samples were dried

at 50°C for 48 h and weighed. The dried root samples were ground with a ball mill and analyzed for their tissue N content (%) using an NC analyzer (Flash EA 1112, Thermo Fisher Scientific, Cambridge, MA, USA).

Soil sampling and measurement

The soil samples were collected next to the location of root sampling. For the analysis of chemical characteristics, the soil samples were taken from the soil surface (0–10 cm) by a shovel and transported to the laboratory while cooled. In the laboratory, the samples were immediately sieved through a 2-mm mesh, 8 g of soil was measured, mixed with 40 mL of 2 M KCl solution, and shaken for 12 h. Next, the shaken soil samples were filtered using the ADVANTEC 5C filters (ADVANTEC, Tokyo, Japan). The NH_4^+ and NO_3^- concentrations of the filtered solutions were measured using the same process as the N solution measuring method, and the concentration of the samples was converted to unit soil weight. The other soil samples were air-dried for two weeks, and the pH and electrical conductivity (EC) were determined in a 1: 5 dried soil: distilled water mixture using a pH/EC meter (LAQUA D-210PC-S, Horiba, Kyoto, Japan). Then, we measured the soil C and N concentrations (%) using the same method that was used for root N measurement.

For the analysis of water content and bulk density, the soil samples were taken from the soil surface using a 100-cc core sampler. In the laboratory, fresh soil was weighed and dried at 70°C to a constant mass. After the dry soil weight was determined, the gravimetric soil water content (%) was calculated per unit mass of oven-dried soil. In addition, the dry soil samples were sieved through a 2-mm mesh for bulk density. Finally, the bulk density, which is the weight of dry soil divided by the total soil volume, was calculated.

Statistical analysis

The effects of the tree species on NH_4^+ and NO_3^- uptake rates or root traits were tested using the Brunner–Munzel test (Brunner and Munzel 2000) and multiple tests by Benjamini and Hochberg (1995). The relationships between N uptake rates and root traits (diameter, SRL, and RTD) in all pooled data were analyzed using regression analysis. Principal component analysis (PCA) was conducted using NH_4^+ and NO_3^- uptake rates, diameter, SRL, RTD, and root N as the parameters. All statistical analyses were performed using R version 3.6.2 software (R Development Core Team 2019).

Results

Soil characteristics

The means of the soil pH, EC, and CN ratio in each plot ranged from 4.58–5.97, 37.4–104.7 $\mu\text{S cm}^{-1}$, and 15.14–26.09, respectively (Table 1). The soil NH_4^+ and NO_3^- concentrations ranges were 9.12–11.69 mg

kg⁻¹ and 0.12–6.23 mg kg⁻¹, respectively. The soil NH₄⁺ concentration was higher than the NO₃⁻ concentration in all the plots. The soil water content and dry bulk density ranges were 14.9–35.0% and 332–509 g L⁻¹, respectively.

Table 1
The mean values of the soil characteristics in each plot of the target tree species.

	Plot under target species			
	<i>Larix kaempferi</i>	<i>Pinus densiflora</i>	<i>Chamaecyparis obtusa</i>	<i>Cryptomeria japonica</i>
pH	5.34	4.58	4.70	5.97
EC (μS cm ⁻¹)	45.4	45.7	37.4	104.7
CN ratio	15.14	26.09	16.22	17.36
NH ₄ -N (mgN kg ⁻¹)	9.12	11.69	10.83	10.66
NO ₃ ⁻ -N (mgN kg ⁻¹)	1.57	0.12	2.29	6.23
Water content (%)	14.9	16.6	34.6	35.0
Bulk density (g L ⁻¹)	509	392	425	332

Root uptake rates of NH₄⁺ and NO₃⁻

The uptake rates of NH₄⁺ and NO₃⁻ by fine roots in the four coniferous species were 2.37–14.55 μmol g⁻¹ h⁻¹ and -1.59–2.09 μmol g⁻¹ h⁻¹, respectively (Fig. 1). The fine roots in all the species absorbed N forms of more NH₄⁺ than NO₃⁻. Comparing the N uptake capacity among the species, the NH₄⁺ uptake rate of *L. kaempferi* was significantly higher than those of the other tree species (Fig. 1a). The NO₃⁻ uptake rates of *C. obtusa* and *C. japonica* were higher than those of *L. kaempferi* and *P. densiflora* (Fig. 1b).

Fine root morphology and chemistry

For the root morphological traits, the sample roots in the four coniferous species ranged from 0.26 to 0.69 mm for the mean root diameter, 8.72 to 34.10 m g⁻¹ for the SRL, and 0.21 to 0.70 g cm⁻³ for the RTD. For the root chemical trait, the root N content ranged from 0.60–1.71%. There were significant differences in the measured root traits among the species (Table 2; *P* < 0.05).

Table 2

The mean values of the fine root traits of root segments used for the N uptake experiment. Different letters indicate significant differences among the four species ($P < 0.05$; Brunner–Munzel and multiple test).

	<i>Larix kaempferi</i>		<i>Pinus densiflora</i>		<i>Chamaecyparis obtusa</i>		<i>Cryptomeria japonica</i>	
mean diameter (mm)	0.36	b	0.47	a	0.38	b	0.52	a
SRL (m g ⁻¹)	20.46	ab	14.91	c	21.72	a	16.69	bc
RTD (g cm ³)	0.52	a	0.41	b	0.42	b	0.30	c
tissue N content (%)	1.23	b	1.06	b	1.48	a	1.41	a

Relationships NH₄⁺ and NO₃⁻ uptake rates with fine root traits

The NH₄⁺ uptake rates significantly increased with decreasing mean diameter ($P < 0.001$) and with increasing SRL ($P < 0.001$) across species (Fig. 2a, b) and had a significantly positive correlation with the RTD ($P < 0.001$; Fig. 2c). On the other hand, the NO₃⁻ uptake rates had no relationship with the mean diameter and SRL (Fig. 2d, e), whereas they were negatively correlated with the RTD ($P < 0.05$; Fig. 2f).

Overall variation in NH₄⁺ and NO₃⁻ uptake rates and fine root traits

The principal component analyses of the NH₄⁺ and NO₃⁻ uptake rates and four fine root traits of four coniferous tree species showed that the first and second principal components (factors 1 and 2) accounted for 46.36% and 24.42% of the variation, respectively (Fig. 3). Factor 1 was negatively correlated with the NH₄⁺ uptake rates. The mean diameter, SRL, and RTD were related to factor 1, with low mean diameter, high SRL, and RTD with high NH₄⁺ uptake rates. Factor 2 was positively correlated with the NO₃⁻ uptake rates. Root N was related to factor 2, with high root N tending to have high NO₃⁻ uptake rates. Four coniferous tree species were distributed in each quadrant. *Larix kaempferi* tended to have a high NH₄⁺ uptake rate, SRL and RTD, low NO₃⁻ uptake rate, diameter, and tissue N content. *Pinus densiflora* tended to have a high diameter, low NH₄⁺ and NO₃⁻ uptake rate, SRL, RTD, and tissue N content. *Cryptomeria obtusa* tended to have high NH₄⁺ and NO₃⁻ uptake rates, SRL, RTD, and tissue N content and low diameter. *Cryptomeria japonica* tended to have a high NO₃⁻ uptake rate, tissue N content, diameter and low NH₄⁺ uptake rate, SRL, and RTD.

Discussion

We observed *in situ* inorganic N uptake rates by fine roots in four coniferous forest species, providing direct evidence for root strategies for nutrient acquisition. Using influx in the solution depletion

experiments, the NH_4^+ and NO_3^- uptake rates of this study ranged from 2.37 to 14.55 $\mu\text{mol g}^{-1} \text{h}^{-1}$ and -1.59 to 2.09 $\mu\text{mol g}^{-1} \text{h}^{-1}$, respectively (Fig. 1). These values overlap with those of other studies in boreal (Socci and Templer 2011), temperate (Liu et al. 2017), and tropical forest (Liu et al. 2017), and laboratories (Gruffman et al. 2014). For example, Liu et al. (2017) reported all 21 species in temperate and tropical forest took up NH_4^+ , with mean values of $12.8 \pm 1.22 \mu\text{g N g}^{-1} \text{h}^{-1}$ in the temperate forest and $6.77 \pm 0.43 \mu\text{g N g}^{-1} \text{h}^{-1}$ in the tropical forest. In contrast, the NO_3^- uptake rate of this study ranged from 0.13 to 1.60 $\mu\text{g N g}^{-1} \text{d.w. root h}^{-1}$ in the temperate forest and 0.58 to 2.17 $\mu\text{g N g}^{-1} \text{d.w. root h}^{-1}$ in the tropical forest. Socci and Templer (2011) reported the NH_4^+ uptake rates of sugar maple (*Acer saccharum*) and red spruce (*Picea rubens* Sarg.) ranged from 7.05 to 10.59 $\mu\text{mol N g}^{-1} \text{h}^{-1}$, and the NO_3^- uptake rate ranged from -36.38 to 2.49 $\text{g}^{-1} \text{h}^{-1}$ in the depletion method. In addition, when comparing the NH_4^+ and NO_3^- uptake rates in all the target tree species, the NH_4^+ uptake rates were over 30 times higher than those of the NO_3^- uptake rates (Fig. 1). This result is consistent with the findings of previous studies in boreal (Socci and Templer 2011), temperate (Liu et al. 2017), subtropical (Liu et al. 2020), and tropical forests (Liu et al. 2017). In this study, the fine roots of the coniferous species preferred to take up N in the form of NH_4^+ from the soil. Overall, we found a clear distinction in the specific N preference through N uptake between NH_4^+ and NO_3^- in a small root segment in a cool-temperate coniferous forest.

To understand how N uptake rates by tree fine roots differed between NH_4^+ and NO_3^- , their functional traits would be useful. Surprisingly, the relationships between N uptake rate and root morphological traits across species were different for NH_4^+ and NO_3^- (Fig. 2). The NH_4^+ uptake rate by the fine roots was correlated with negatively mean diameter and positively SRL (Fig. 2a, b). On the other hand, the NO_3^- uptake rate had no relationship with the mean diameter and SRL (Fig. 2d, e). In general, both root diameter and SRL are known parameters of the ability to absorb nutrients and water (McCormack et al. 2015). Smaller roots and higher SRL could allow the efficient exploitation of soil resources and aid in resource foraging (Bowsher et al. 2016), which would be highly beneficial for the absorption of N by plants (Holdaway et al. 2011; Ryser 2006). These results reveal different factors controlling N uptake through fine root morphological changes between N forms.

The relationship between N uptake and morphology in our study may suggest that different soil adsorptions between NH_4^+ and NO_3^- can affect the magnitude of diameter and branching of the roots. In soil, NH_4^+ is strongly adsorbed by soil particles because the surface of soil particles has a negative charge (Lambers et al. 2019). The roots must modify the length and diameter for high foraging ability and may be able to access NH_4^+ in soil, resulting in a higher NH_4^+ uptake rate. In contrast, NO_3^- has high solubility in soil water because soil particles electrically repel it (Lambers et al. 2019). In other words, NO_3^- is easily diffused to the root surface through water flow, realizing NO_3^- uptake without dependency on root morphological adaptation for NO_3^- . The roots can absorb the NO_3^- solution more easily in the

soil. Thus, the characteristics of soil adsorption in the field are reflected in the difference in the mobility of different N forms and, thus, are essential for improving the specific nutrient acquisition by fine roots.

Furthermore, the higher uptake rates of NH_4^+ by fine roots can be attributed to differences in the N assimilation process of plants (Lambers et al. 2019). Plants must use energy to reduce NO_3^- before being incorporated into amino acids. In contrast, NH_4^+ can be immediately incorporated into amino acids to reduce energy. Thus, it could be more efficient for plants to take up NH_4^+ than NO_3^- . Another factor could be that NH_4^+ in soil was more abundant than NO_3^- in all the target species of this study site (Table 1). Some studies have shown that plant species co-existing in a single community prefer the specific N form that is most abundant in the soil (Andresen et al. 2008; Wang et al. 2012). Thus, coniferous species in this study may acquire and utilize N forms that are abundant in the soil and facilitate efficient assimilation.

Interestingly, there were significant relationships between the RTD and N uptake rates of both NH_4^+ and NO_3^- but in different directions. The RTD was positively correlated with NH_4^+ and negatively correlated with NO_3^- (Fig. 2c, f). From this result, we imply that RTD is assumed to follow a trade-off between rapid growth rate/resource capture and high survival/resource conservation through NH_4^+ and NO_3^- uptake. In general, fine roots with high RTD have been associated with slow growth, low water permeability, low nutrient availability, while high resistance to external stress, and longer life (Weemstra et al. 2016). The RTD and N uptake relationships in our study may be helpful for interpreting N acquisition strategies with potential functional trade-offs. However, it is still unclear whether the relationship between root RTD and N uptake is reflected in the form of NH_4^+ and NO_3^- . Further studies are needed to explore the general mechanisms of N uptake function.

The principal component analysis showed that the trend of each N form uptake rate and root morphological and chemical traits differed by tree species (Fig. 3). Gao et al. (2020) also reported that based on the root chemistry and uptake abilities, different plant species were distinctly scattered in plant species. Trends in the N uptake rate by plant species may be caused by different N acquisition strategies. The factor 1 axis was negatively correlated with the NH_4^+ uptake rate, negatively correlated with the SRL and RTD, and positively correlated with the mean diameter. Therefore, the NH_4^+ uptake rate can be regulated by root morphological traits. The factor 2 axis was positively correlated with the NO_3^- uptake rate and positively correlated with the root tissue N content. Thus, the NO_3^- uptake rate may be related to the root tissue N. In a previous study, the relationships between root nutrient uptake and root traits have been discussed acquisitively or conservatively (Weemstra et al. 2016), but the fact may be more complex and the relationships can be changed by chemical forms of nutrients, such as NH_4^+ and NO_3^- . Further research on species-specific N uptake in the field is required to fully understand the relationships between root N uptake and their control over morphological processes in various species and environments.

Overall, we evaluated *in situ* NH_4^+ and NO_3^- uptake rates using the depletion method in coniferous forests. Our study showed that the roots of coniferous species preferred the NH_4^+ form of more than the NO_3^- (Fig. 1). In addition, the relationships between N uptake rates and root morphological traits differed between NH_4^+ and NO_3^- (Fig. 2). The coniferous species in this study might optimize their root morphological traits in response to the availability of NH_4^+ and NO_3^- . Until now, it is known that root traits are ideally related to nutrient uptake function (McCormack et al. 2015). However, we found that the relationship of nutrient uptake with morphological traits depends on the characteristics of the essential mineral elements. Finally, we propose that an approach to the relationship of N uptake with root traits will be helpful for identifying the reason why there are differences among species in their uptake of N (Fig. 3). We believe that advances in our understanding of the *in situ* N uptake of tree fine roots will provide a breakthrough in our understanding of the root physiological function and the prediction of the fundamental N acquisition strategy of trees.

Abbreviations

N, nitrogen; NH_4^+ , ammonium; NO_3^- , nitrate; SRL, specific root length; RTD, root tissue density

Declarations

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Author Contributions

N.M and T.I designed the study. N.M, T.I, T.M. and M.A. collected the data. N.M, T.I and A.T.O analyzed the data. All authors wrote the manuscript and approved the final manuscript.

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Data availability

Data are available on request from the corresponding author

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Figures

Figure 1

The boxplot of (a) ammonium uptake rate and (b) nitrate uptake rate of four conifer species. Different letters above the boxplots indicate significant differences among the four species ($P < 0.05$; Brunner–Munzel and multiple test).

Figure 2

The relationship between fine root NH_4^+ or NO_3^- uptake rate and (a) mean root diameter (mm), (b) specific root length (m g^{-1}), (c) root tissue density (g cm^{-3}) of *Larix kaempferi* (yellow squares), *Pinus densiflora* (red circles), *Chamaecyparis obtusa* (blue triangles), and *Cryptomeria japonica* (light blue diamonds). A black line across the figure indicates a significant correlation among all species ($P < 0.05$; regression analysis).

Figure 3

Principal component analysis of NH_4^+ and NO_3^- uptake rates (NH_4^+ , NO_3^- respectively) and root traits (D, mean diameter; SRL, specific root length; RTD, root tissue density; N, tissue N content) of four conifer species. Each plot indicates one root segment sample.