



Research paper

Root nitrogen uptake capacity of Chinese fir enhanced by warming and nitrogen addition

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Received March 1, 2022; accepted August 30, 2022; handling Editor David Whitehead

There is a knowledge gap in the effects of climate warming and nitrogen (N) deposition on root N absorption capacity, which limits our ability to predict how climate change alters the N cycling and its consequences for forest productivity especially in subtropical areas where soil N availability is already high. In order to explore the effects and mechanism of warming and the N deposition on root N absorption capacity of Chinese fir (*Cunninghamia lanceolata*), a subtropical arbuscular mycorrhizal conifer, the fine root $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$ uptake kinetics at a reference temperature of 20 °C were measured across different seasons in a factorial soil warming (ambient, +5 °C) × N addition (ambient, +40 kg N ha⁻¹ yr⁻¹) experiment. The results showed that (i) compared with the control, warming increased the maximal uptake rate of NH_4^+ ($V_{\text{max},20\text{ }^\circ\text{C-NH}_4^+}$) in summer, while N addition enhanced it in spring and summer; compared with non-warming treatments, warming treatments increased the uptake rate of NO_3^- at a reference concentration of 100 μmol ($V_{100,20\text{ }^\circ\text{C-NO}_3^-}$) in spring. (ii) The analysis of covariance showed that $V_{\text{max},20\text{ }^\circ\text{C-NH}_4^+}$ was positively correlated with root mycorrhizal colonization rate (MCR) and $V_{100,20\text{ }^\circ\text{C-NO}_3^-}$ was positively correlated with specific root respiration rate (SRR), whereas no N uptake kinetic parameter was correlated with specific root length, root N and non-structural carbon concentrations. Thus, our results demonstrate that warming-increased root NH_4^+ uptake might be related to warming-increased MCR, whereas warming-increased root NO_3^- uptake might be related to warming-increased SRR. We conclude that root NH_4^+ and NO_3^- uptake capacity of subtropical Chinese fir can be elevated under warming and N deposition, which could improve plantation productivity and mitigate N leaching loss and soil acidification.

Keywords: root nitrogen uptake kinetics, subtropics.

Introduction

Nitrogen (N) is a critical limiting factor for plant growth and productivity in most terrestrial ecosystems (Galloway et al. 2008, Joseph et al. 2021). The two leading global change factors, climate warming and atmospheric N deposition, increase soil N availability (Kou et al. 2018, Xiong et al. 2018). The plant productivity can be raised, if the increased soil available N is fully utilized by the plants. On the contrary, the increased accumulation of available soil N might lead to soil N leaching or

acidification (Hietz et al. 2011, Kou et al. 2018), especially in tropical and subtropical areas where soil N availability is already high (Zhang et al. 2011), consequently hindering plant growth (Simkin et al. 2016, Coskun et al. 2017). The plant root uptake is a critical component of the forest N cycle and plays an essential role in regulating plant N acquisition, storage and ecosystem N retention (Kronzucker et al. 2000, Sanders-DeMott et al. 2018). The effect of increased N availability induced by global warming and N deposition on plant productivity and N retention

in the ecosystem depends to a large extent on the N uptake capacity of plant roots (Bassirrad 2000).

In general, the Michaelis–Menten kinetics described the uptake at realistic soil concentrations; therefore, root uptake capacity can be expressed in maximal uptake capacity (V_{\max}) and the affinity ($1/K_m$, where K_m is the Michaelis–Menten constant). These root N uptake kinetics can be an efficient biological index to evaluate the N uptake capacity of ecosystems (Kronzucker et al. 1995a, 1995b, Joseph et al. 2021). However, several studies determined the rate of uptake based on only a single concentration representing the maximum velocity of uptake, V_{\max} (Gessler et al. 1998, Bassirrad, 2006, Jordan et al. 2014, Qiao et al. 2016, Sanders-DeMott et al. 2018). Although these parameters could provide an indication of whether the uptake capacity of the system has been altered in relative terms, these single data points do not provide a complete picture of kinetic behavior.

So far, there are large bodies of reports that demonstrate factors affecting root N uptake rates, such as available soil N, temperature, root non-structural carbohydrate (NSC), root N content and mycorrhizal colonization. However, only a few of them focus on the effects on root N uptake kinetics (Britto and Kronzucker 2002, Finzi et al. 2007, Garnett et al. 2009, Hodge and Storer 2014, Jordan et al. 2014, Mei et al. 2015, Griffiths and York 2020). The effect of N addition on the N uptake kinetics and the mechanisms underlying this variation remains unclear. For instance, field experiments in temperate areas have shown that the N addition reduced the maximal uptake rate (V_{\max}) of the N (Bassirrad et al. 1996, Rothstein et al. 2000), which was related to the feedback control of the N concentration between the root and soil (Rothstein et al. 2000, Britto et al. 2001, Kronzucker et al. 2003). An indoor experiment also demonstrated that the N addition had an inhibitory effect on the V_{\max} of the N uptake (Wu et al. 2020). However, Rothstein et al. (1996) reported that the N addition had no significant effect on the V_{\max} and half saturation constant (K_m) of *Acer saccharum* roots. In response to the N addition, the variation of root N absorption capacity may depend to a large extent on the plant's N demand (BassiriRad 2015, Joseph et al. 2021). In comparison, the warming effect on root N uptake kinetics is rarely studied (Bassirrad 2000, Rennenberg et al. 2009, Sanders-DeMott et al. 2018). Some studies have shown that rising temperature increases the N uptake rate (Chapin et al. 1986, Gessler et al. 1998, Bassirrad 2000, Socci and Templer 2011, Huang et al. 2012, Qiao et al. 2016, Jayawardena et al. 2021) until the optimal growth temperature is exceeded. Nevertheless, warming from the optimal to superoptimal temperature may decrease this rate (Bassirrad et al. 1993, Mainali et al. 2014, Giri et al. 2017, Sanders-DeMott et al. 2018). So far, however, field experiments directly examining warming effects on root N uptake kinetics are limited, except for Bassirrad et al. (1993). They reported that warming (10 °C of increase on average)

reduced the V_{\max} of NO_3^- uptake of *Agropyron desertorum* roots in temperate regions. Furthermore, the knowledge of the combined effect of warming and N deposition on root N uptake is lacking, although it has already been noted that soil warming might stimulate root N uptake rates particularly when N is available as NO_3^- , which also increased the most in N deposition in recent decades (Liu et al. 2013). The overall response of root N uptake capacity might not be entirely appreciated unless the interactive effects of these two global changes are considered.

On long timescales (days to years), plants may acclimate to warming and N deposition by adjusting phenotypically (Mou et al. 2013, Wang et al. 2013), such as tissue NSC, N concentrations and mycorrhizal fungi colonization at the scale of the individual root, which can affect fine root N uptake kinetics (Rothstein et al. 2000, Wu et al. 2020, Joseph et al. 2021). Notably, the previous studies demonstrated that mycorrhizal fungi could increase the root active absorbing area and strengthen the competitiveness of the host to uptake soil inorganic N (Kuzyakov and Xu 2013), which was evidenced by a recent study that mycorrhizal fungi can contribute to ~66% of total plant N absorption (Shi et al. 2016). Thus, it would be possible that mycorrhizal plants had a higher V_{\max} than non-mycorrhizal plants (Wu et al. 2020). However, a few studies examined the relationship between mycorrhizal colonization rate (MCR) and N uptake kinetics of fine roots. Furthermore, root morphology and respiration rate were found to significantly affect the ion absorption rate (Griffiths and York 2020), which could be related to the N uptake kinetics. For example, specific root length (SRL), which reflects the level of physiological function of fine roots and represents the benefit of nutrient absorption under specific carbon (C) invest of plant roots (Parts et al. 2019), is relevant to nutrient absorption capacity (Razaq et al. 2017). Plant root specific respiration rate (SRR) uses substrates involving proteins and amino acids for metabolism, which provides energy (ATP) for ion absorption (Jarvi and Burton 2018), and may also play a role in root N uptake kinetics. Our previous studies in the subtropical forests demonstrated that both warming and the N addition affect these above-mentioned root phenotypical factors (Xiong et al. 2018, 2020). However, how these above-mentioned root traits together affect root N uptake kinetics remains unknown.

Despite the substantial increase in both temperature and the N deposition in the subtropical and tropical regions (Cavaleri et al. 2015, Kanakidou et al. 2016), their effects on forest ecosystems (Xiong et al. 2020) are rarely studied. The effects of these factors may differ greatly from those in high-latitude ecosystems due to the relatively abundant N in many subtropical and tropical forests (Lu et al. 2021). As an essential timber and arbuscular mycorrhizal (AM) conifer species in subtropical China, Chinese fir (*Cunninghamia lanceolata* (Taxodiaceae))

accounts for 6% of the world's plantation forests (FAO 2006), and plays an important role in forest C sequestration (Piao et al. 2009). However, AM-dominated forests have been shown to be subject to more NO_3^- leaching in response to the N deposition than ectomycorrhizal forests (Midgley and Phillips 2014). Conifers are also less capable of taking up NO_3^- than angiosperms (BassiriRad et al. 1997, Kronzucker et al. 1997). Therefore, whether subtropical ecosystems dominated by AM conifers (such as Chinese fir) will be strongly affected by NO_3^- deposition has attracted attention. The present study aims to examine the effect of warming and the N deposition on the N uptake kinetics of Chinese fir roots, by using a factorial warming and the N addition experiment. To reveal the mechanisms, root N and NSC concentration, SRL, SRR and MCR are measured concurrently. Our previous studies in young Chinese fir plantations were informative. For example, the N addition resulted in the increase of both plant growth (Zhang et al. 2017) and root NSC concentration (Xiong et al. 2020); warming increased significantly the average tree height of Chinese fir (Xiong et al. 2018), which probably enhanced the demand of the N; in particular, warming also increased fine root MCR and enhanced or at least maintained SRR of Chinese fir (Xiong et al. 2018), which may contribute to improve the N uptake capacity. Therefore, we hypothesize that (i) the N addition and warming would increase fine root N uptake capacity of Chinese fir; and (ii) this root N uptake capacity increase is related to root phenotypical adjustments, such as increasing NSC concentration, SRL, SRR, MCR and decreasing N concentration separately or in combination with warming and the N addition.

Materials and methods

Study site description

The study area was located in the Chenda Observation Study Site of Fujian Sanming Forest Ecosystem National Observation and Research Station in Fujian Province, China ($26^{\circ}19' \text{ N}$, $117^{\circ}36' \text{ E}$), with a typical mid-subtropical monsoonal climate and a mean annual temperature of 19.1°C , mean annual precipitation of 1750 mm (primarily concentrated in March to August), a mean relative humidity of 81% and mean annual evaporation of 1585 mm. A previous study showed that the mean annual air temperature and precipitation during the study year were 18.8°C and 1198 mm, respectively, which were lower than the long-term average (Xiong et al. 2020). The study area is 300 m above sea level and the soil is mainly composed of red granite soil (State Soil Survey Service of China 1998).

Experimental design

A complete randomized design was established in August 2014, which was factorial warming (two levels, ambient and

ambient $+4^{\circ}\text{C}$) \times N addition (two levels, ambient and ambient $+40 \text{ kg N ha}^{-1} \text{ year}^{-1}$) experiment. The warming of 4°C was set to simulate the maximum projected increase in global surface temperature (3.7°C) by the end of this century (IPCC 2013). The N addition rate of $40 \text{ kg N ha}^{-1} \text{ year}^{-1}$ was adopted because it approximated the local ambient N deposition rate (average $36.3 \text{ kg N ha}^{-1} \text{ year}^{-1}$) (Zhang 2013). There were four treatments: control, warming, N addition and warming plus N addition, each with six replicates. A total of 24 plots were established, each with an area of $2.5 \text{ m} \times 2.5 \text{ m}$. The buffer width between plots was 2 m. This experiment was performed in a flat area, the area was not affected by the apparent hydrological impact of the neighboring forest. The soil heating cables (Nexans type TXLP, Oslo, Norway), with a diameter of 6.5 mm, were buried in the plot at a depth of 10 cm along an S-shaped route and spaced 20 cm apart between the two neighboring parallel lines to ensure the uniformity of temperature in October 2014. Cables were installed in the same way in non-heated plots to compensate for potential soil interference, but these cables were not energized. Our previous study showed that, compared with the unheated plots, the soil temperature in the 0–10 cm layer of the heated plots remained at $4.0 \pm 0.1^{\circ}\text{C}$ higher throughout the observation period, and that warming reduced the average gravimetric soil moisture in the 0–10 cm layer by 15.5% (Xiong et al. 2020).

The separate proportional integral differential controller next to the sample plot kept the soil temperature of the warmed plots constantly higher ($4.0 \pm 0.1^{\circ}\text{C}$) than that of the unheated plots. The thermistor temperature probe was used to measure the soil temperature, which was placed in the unheated plot set with the reference. This control method could respond quickly to the daily fluctuation of soil temperature. A soil temperature sensor (F109; Campbell Scientific Inc., Logan, UT, USA) and a moisture probe (CS616; Campbell Scientific Inc.) were installed between the cables in each plot to monitor the changes in soil temperature and moisture. A meteorological station 200 m northwest of the research site could record meteorological data (Xiong et al. 2020). Five 1-year-old Chinese fir seedlings with similar height ($53.0 \pm 2.2 \text{ cm}$) and ground diameter ($4.8 \pm 0.5 \text{ mm}$) were planted in each plot in January 2015. Each Chinese fir seedling was planted between two parallel cable lines. Our previous study showed that warming and the N addition increased the average height growth of Chinese fir in spring and summer (Xiong et al. 2020).

In the N addition plot, N was applied monthly by sprinklers filled with NH_4NO_3 solution ($\geq 99\%$), and the rate of N application was $40 \text{ kg N ha}^{-1} \text{ year}^{-1}$. The solution was mixed with 800 ml of deionized water and applied evenly to each treated plot. The plot without the N fertilizer was sprayed with deionized water of the same volume. The soil heating and the N addition treatments began in March 2016, and other plants germinating in the plots were removed.

Soil chemical analyses

In the spring (April), summer (July) and autumn (October) of 2017, five soil cores (2 cm diameter) of 0–10 cm soil layer were randomly collected from each plot. After picking out the roots and debris in the soil, some subsamples were dried to constant weight at 105 °C to measure the soil water content. The residual subsamples were passed through a 2-mm mesh sieve, and the content of soil inorganic N was determined by a continuous flow analyzer (Skalar san+++, The Netherlands). Our previous study has shown that the effects of warming and the N addition on soil inorganic N content vary with the seasons (Xiong et al. 2020).

Measurement of fine root traits

Fine root traits were measured three times in the spring, summer and autumn of 2017. Fine root SRL, NSC and the N concentration were measured from the total six plots, whereas root N uptake rate, MCR and SRR were measured from three randomly selected plots each time. In the present study, only data from the three randomly selected plots were included. The data on fine root SRL, NSC and the N concentration computed using the total six plots had been reported in our previous study (Xiong et al. 2020). In the present study, the corresponding data were calculated using the randomly selected three plots were provided.

For measuring root morphological and chemical traits, six soil cores were taken from the 0–10 cm soil layer from each plot using a soil corer with an inner diameter of 3.5 cm. The roots (diameter ≤ 1 mm) were carefully picked out from the soil sample, and fine roots were divided into living and dead roots according to color, texture and shape (Chen et al. 2013). For measuring root SRR, MCR and root N uptake capacity, an additional four to six soil cores per plot were taken from the three randomly selected plots per treatment to collect fresh root samples. Only living roots were used in this study. We collected a pool of roots at each sampling time and divided them up between different measurements.

When measuring root morphology, subsamples were scanned and analyzed by the Win-Rhizo software (Pro 2009B; Regents Instruments Inc., Quebec, Canada) to determine root diameter and length and dried to constant weight at 65 °C. The SRL was calculated by dividing root length by dry weight. The total C and the N concentrations of fine roots were measured by the C-N analyzer (Elementar Vario, MAX, Germany) using ground subsamples. The NSC (soluble sugar and starch) of fine roots were determined by the phenol-sulfuric acid method (Buysse and Merckx 1993). A 40 mg ground dry sample was added to 10 ml of 80% ethanol and extracted for 24 h determination of soluble sugar. The fine root residue of the extracted soluble sugar sample was oven-dried to constant weight at 60 °C, and 3 ml of 3% HCl was added and hydrolyzed in boiling water for 0.5 h and filtered for starch content determination.

The concentrations of glucose and starch in the supernatant and filtrate were determined by phenol-sulfuric acid and UV-Vis spectrophotometer (UV-2450; Shimadzu, Japan) at 490 nm.

Fine root respiration rate was measured within 2 h after being excised. The fresh fine roots were placed in physiological buffers (10 mmol MES buffer and 1 mmol CaSO₄) at 20 °C for 10 min, to maintain the cell membrane activity of fine roots, and then respiration rate of fine roots was measured by the Clark-style liquid-phase oxygen electrode system (Hansatech Instruments Ltd, UK) at 20 °C (Clark et al. 2010).

Fine root MCR was determined by the acid fuchsin staining method (Kormanik et al. 1980). Fresh roots were cut into root segments (200 segments) of ~ 1 cm in length, and 10% KOH solution was added to 90 °C water. After 60 min, 2% HCl solution was added for acidification, and finally, the fine root samples were placed in acid fuchsin lactic acid glycerin staining solution overnight at room temperature. The root segments were placed on a glass slide and observed under a high-power microscope. According to the weighted method of root MCR (Biermann and Linderman 1981), roots were divided into different groups with a degree of root infection of 0%, 10%, 20%, 100%. The MCR was calculated as follows:

$$C\% = \sum [(10 \times i)\% \times n] / N,$$

where C is the MCR, i is an integer from 0 to 10, n is the number of root segments with colonization rate $(10 \times i)\%$ and N is the total number of root segments.

The fine root N absorption rates of different forms were determined by the ¹⁵N labeling method (Rothstein et al. 2000). Fresh subsamples were used to assay for ¹⁵N uptake. The actual uptake assay was performed within 2 h of root excision to minimize potential carbohydrate-starved roots and possible inhibition of nutrient transport mechanisms. The soil particles on the surface of fine roots were washed away with CaCl₂ solution (0.5 mmol) to ensure the integrity of cell membrane. Fine roots were soaked in beakers containing 0.5 mmol CaCl₂ solution (20 °C), and then 10 aliquots of 0.1 g subsamples (~ 0.32 – 0.49 m of root length) were soaked in 10, 50, 100, 250 and 500 μ mol of ¹⁵NH₄Cl solution and of K¹⁵NO₃ solution (100 ml, 20 °C), for 15 min. Then these aliquots were washed with 5 mmol KCl and 0.5 mmol CaCl₂ solution three times to remove the ¹⁵N adsorbed on the fine root surface. The washed aliquots were dried until constant weight at 65 °C, and crushed and ground. Finally, the ¹⁵N concentration of fine roots was determined by an isotope ratio mass spectrometer (Finnigan MA T-253; Thermo Electron, San Jose, California, USA). The NH₄⁺ absorption rate was obtained by subtracting ¹⁵N concentration from the fine roots in the blank from the total ¹⁵N concentration in fine roots after soaking in ¹⁵NH₄Cl solution and then divided by the absorption time (NO₃⁻ absorption rate was obtained by the same method).

The kinetic parameters of Michaelis–Menten equation were calculated using the Lineweaver–Burke double reciprocal method (Eisenthal and Cornish-Bowden 1974, Kronzucker et al. 1995c, Kronzucker et al. 1996). The formula used is as follows:

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \times \frac{1}{C_s},$$

where V is the ion uptake rate ($\mu\text{mol g}^{-1} \text{h}^{-1}$), C_s is the concentration of a solute ($\mu\text{mol l}^{-1}$), V_{\max} is the maximal uptake rate ($\mu\text{mol g}^{-1} \text{h}^{-1}$) and K_m is the half saturation constant ($\mu\text{mol g}^{-1} \text{h}^{-1}$).

Statistical analysis

Nitrate uptake by higher plants typically shows dual-phase kinetics, with an initial saturation of a high affinity transporter followed by a linear response at higher concentrations (Pilbeam and Kirkby 1990). Excised Chinese fir roots exhibited an initial saturation at $\sim 100 \mu\text{M}$. Thus, the NO_3^- uptake rate of $100 \mu\text{M}$ ($V_{100-\text{NO}_3^-}$) was used as our index of NO_3^- uptake capacity index for all statistical analyses (Rothstein et al. 2000). Since the three selected plots were randomly selected from the six replicate plots at each season, the season factor (S) cannot be considered as a repeated-measure variable. Therefore, three-way analysis of variance (ANOVA) was used to examine the effect of warming, the N addition and season on fine root $V_{\max-\text{NH}_4^+}$, $K_{m-\text{NH}_4^+}$, $V_{100-\text{NO}_3^-}$, N concentration, NSC concentration, SRR, MCR and SRL (logarithmic transformation is carried out to conform to the normal distribution). The significance level was set as $P < 0.05$, except for the interaction term where $P < 0.10$ was considered significant, and $0.10 \leq P < 0.15$ was marginally significant (Stehman and Meredith 1995; Augustine and McNaughton 2007; Mueller et al. 2016; Wang et al. 2022). If the interaction terms, including season, were $P < 0.15$, ANOVA was conducted separately for each season. If the interactive effect of warming and the N was $P > 0.15$, the pairwise comparisons were conducted only for the main effect term that was significant ($P < 0.05$). If the interactive effect of warming and the N was $P < 0.15$, the multiple pairwise comparisons were then conducted among treatments (i.e., control, warming, the N addition and warming plus the N addition by season). Taking fine root N concentration, NSC concentration, specific root respiration rate, MCR and SRL as covariates, the analysis of covariance (ANCOVA) was used to examine how the effect of warming, the N addition and season on fine root $V_{\max-\text{NH}_4^+}$, $K_{m-\text{NH}_4^+}$ and $V_{100-\text{NO}_3^-}$ is mediated by fine root phenotypical adjustments. The fixed effects include season, warming and the N addition and their interactions and the main effects in the ANCOVA. A collinearity test showed that there was no collinearity among these covariates ($\text{VIF} < 3$). The best fitting model was selected by a sequential forward addition of the candidate covariates that most improved Akaike information criterion.

To graphically demonstrate the relationships between $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ and MCR from the fitted model (Table 2), we used the residuals from the fitted model plus the mean intercept coefficient and the MCR coefficient times MCR (Table S1 available as Supplementary data at *Tree Physiology Online*), to plot the fitted line, which mainly represents the relationships between uptake kinetics parameters and MCR after accounting for the effects of other fixed factors and covariates (Chen et al. 2016). Similarly, the relationship between $V_{100,20^\circ\text{C}-\text{NO}_3^-}$ and SRR from the fitted model (Table 2) was also illustrated graphically.

SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Diagrams were drawn using Origin 2019 software (Origin Lab, Northampton, MA, USA).

Results

Nitrogen absorption rate of fine roots under different treatments

The absorption of ammonium by the excised roots for the four treatments in different seasons followed the Michaelis–Menten kinetic equation ($R^2 > 0.940$ for all treatments in each season) (Table 1), and absorption rate of ammonium increased with the concentration of ammonium solution (Figure 1). In all three seasons, absorption rate of ammonium was faster than that of nitrate under the same solution concentration (Figure 1). Absorption of nitrate by the excised roots of four treatments in different seasons did not completely follow the Michaelis–Menten kinetic. Instead, it showed dual-phase kinetics (Figure 1): the absorption rate of nitrate showed an upward trend at $10\text{--}50 \mu\text{mol}$, but decreased at $50\text{--}100 \mu\text{mol}$, and showed initial saturation at $\sim 100 \mu\text{mol}$, then continued to increase (Figure 1).

Effects of warming and N addition on N uptake kinetics of fine roots

The interaction of season, warming and the N addition had a significant effect on fine root $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ ($P = 0.100$) (Figure 2a). In spring, the N addition treatments had significantly higher $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ than non-N addition treatments; in summer, $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ in all the treatments was significantly higher than that in the control, while there was no significant difference in $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ among treatments in autumn (Figure 2a). Compared with spring, $V_{\max,20^\circ\text{C}-\text{NH}_4^+}$ was higher in summer and autumn for most of the treatments (Figure 2a).

The interaction of season, warming and the N addition had a marginal significant effect on fine root $K_{m,20^\circ\text{C}-\text{NH}_4^+}$ ($P = 0.147$), but there was no significant difference among treatments in each season (Figure 2b). Compared with spring, $K_{m,20^\circ\text{C}-\text{NH}_4^+}$ was higher in summer and autumn for most treatments (Figure 2b).

The interaction of season and warming had significant effect on fine root $V_{100,20^\circ\text{C}-\text{NO}_3^-}$ ($P = 0.016$) (Figure 2c).

Table 1. R^2 values (mean \pm SD, $n = 3$) of Michaelis–Menten kinetic equation for root ammonium uptake under four treatments in different seasons.

Season	Treatment			
	Control	Warming	Nitrogen	Warming + nitrogen
Spring	0.975 \pm 0.009	0.947 \pm 0.007	0.988 \pm 0.004	0.985 \pm 0.001
Summer	0.993 \pm 0.003	0.994 \pm 0.003	0.996 \pm 0.002	0.985 \pm 0.016
Autumn	0.985 \pm 0.008	0.989 \pm 0.006	0.992 \pm 0.006	0.994 \pm 0.003

Table 2. Analysis of covariance on fine root N uptake kinetics across seasons in a factorial warming and N addition experiment.

Sources of variation	$\lg V_{100,20}^{\max} \text{ } ^\circ\text{C-NH}_4^+$		$\lg K^m_{,20} \text{ } ^\circ\text{C-NH}_4^+$		$\lg V_{100,20} \text{ } ^\circ\text{C-NO}_3^-$	
	F	P	F	P	F	P
S	2.529	0.105	0.286	0.755	4.077	0.034
W	0.304	0.588	3.206	0.089	0.007	0.934
N	7.647	0.012	1.254	0.276	3.806	0.066
S \times W	1.126	0.344	0.791	0.467	3.091	0.069
S \times N	1.093	0.354	0.864	0.437	1.223	0.316
W \times N	0.090	0.767	2.629	0.121	0.098	0.758
S \times W \times N	0.961	0.400	1.416	0.266	1.334	0.287
\lg RN	0.264	0.613	0.156	0.697	0.726	0.405
\lg SRL	0.612	0.443	2.760	0.112	0.000	0.988
\lg NSC	0.150	0.703	0.004	0.948	0.183	0.674
\lg MCR	4.675	0.043	1.928	0.180	2.967	0.101
\lg SRR	–	–	–	–	4.569	0.046

S: season; W: warming; N: N addition; RN: root N concentration; SRL: specific root length; NSC: root NSC concentration; MCR: mycorrhizal colonization rate; SRR: specific root respiration rate. The bold type indicates that the fixed effect was significant.

Compared with non-warming treatments, warming treatments increased $V_{100,20} \text{ } ^\circ\text{C-NO}_3^-$ by 47.4% in spring, but had no effect in the other two seasons (Figure 2c). Compared with that in spring, $V_{100,20} \text{ } ^\circ\text{C-NO}_3^-$ was lower in autumn for both warming and non-warming treatments and lower in summer for the warming treatments (Figure 2c).

Effects of warming and N addition on fine root mycorrhizal, physiological and morphological traits

Warming had a significant effect on fine root MCR ($P = 0.012$) (Figure 3a); compared with non-warming treatments, warming treatments significantly increased fine root MCR (17.6% vs 19.2%) (Figure 3a). The interaction between the N addition and season had a significant effect on fine root MCR ($P = 0.002$) (Figure 3a); compared with non-N addition treatments, the N addition treatments significantly reduced fine root MCR in summer (25.6% vs 19.5%), and autumn (16.0% vs 14.1%), and not at all in spring (17.4% vs 17.7%) (Figure 3a). The MCR was generally higher in summer than in other seasons.

The interaction of season, warming and the N addition had a significant effect on SRR ($P = 0.017$) (Figure 3b). Compared with control, SRR was significantly increased only in the warming treatment in spring (by 30.9%) but decreased in summer by 29.0%, 39.3% and 34.6%, and increased in autumn by 56.5%, 36.9% and 60.7%, respectively, in the warming, the N addition and warming + N treatment (Figure 3b). The SRR generally

increased from spring to autumn in all treatments, except for the control, where SRR was higher in summer than in other seasons.

The interaction of season, warming and the N addition had a significant effect on the N concentration ($P = 0.091$) (Figure 4a). There was, however, no significant difference among treatments for each season (Figure 4a). The N concentration was generally lower for all treatments in spring than in other seasons.

The N addition had a significant effect on NSC concentration ($P = 0.019$) (Figure 4b); compared with non-addition treatments, the N addition treatments increased NSC concentration by 8.8%, on average (Figure 4b). The interaction of warming and season had a significant effect on NSC concentration ($P = 0.042$) (Figure 4b); compared with non-warming treatments, warming treatments reduced NSC concentration by 12.5% in spring (Figure 4b). Root NSC concentration was lower in spring than in autumn for warming treatments and showed no significant seasonal difference for non-warming treatments.

The interaction of warming and the N addition had a significant effect on SRL ($P < 0.001$) (Figure 4c); there was, however, no significant difference between any treatment and the control (Figure S1 available as Supplementary data at *Tree Physiology* Online). The interaction between season and warming had a significant effect on SRL ($P = 0.075$) (Figure 4c). Compared with non-warming treatments, warming treatments increased SRL by 13.2% and 16.9% in spring and

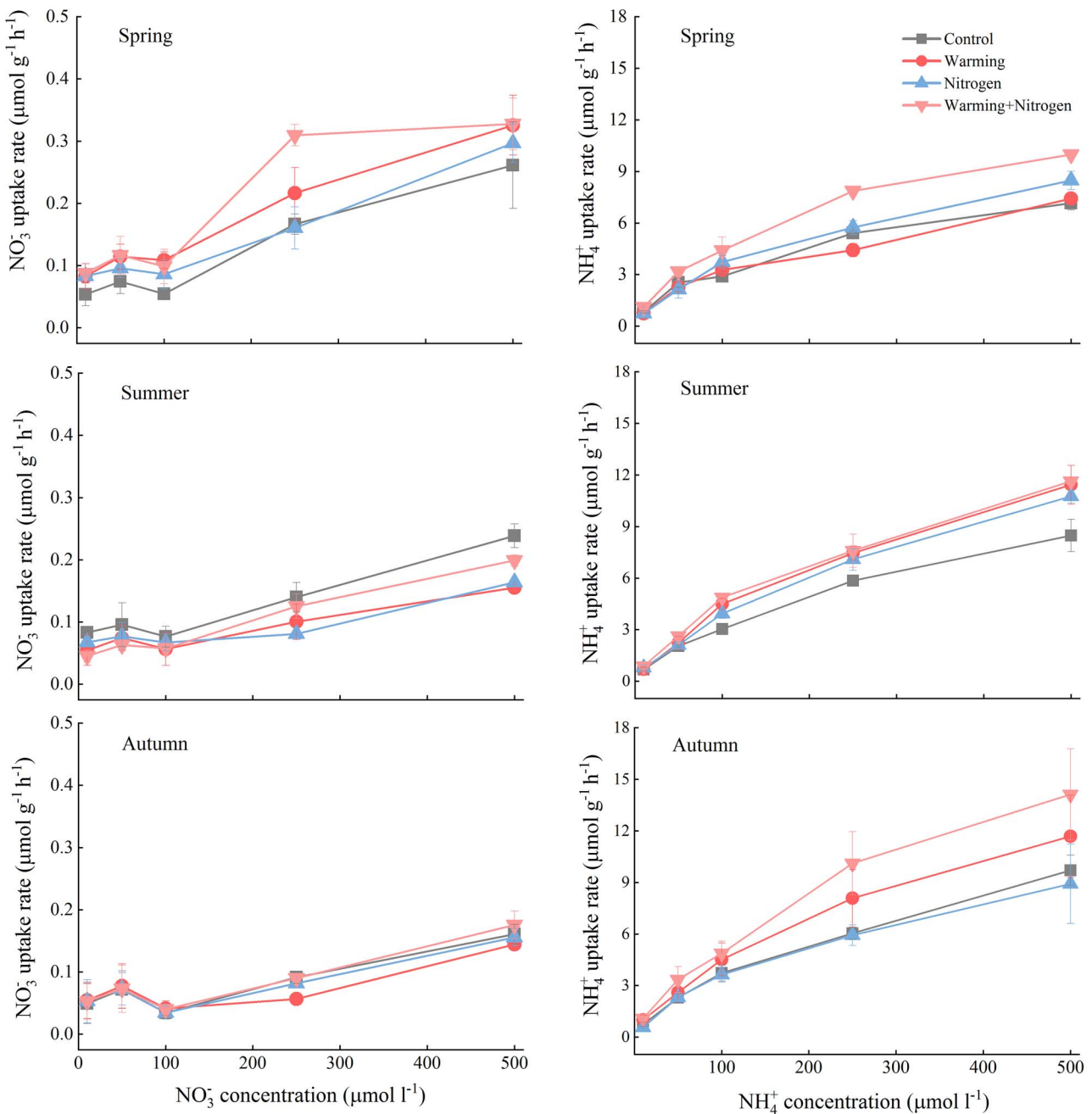


Figure 1. Kinetics curve of fine roots NO_3^- and NH_4^+ uptake across seasons in a factorial warming and N addition experiment (mean \pm standard deviation (SD), $n = 3$).

summer, respectively (Figure 4c). The interaction of season and the N addition also had a significant effect on SRL ($P = 0.036$) (Figure 4c). However, there was no significant difference between the N addition and non-N addition treatments for any season (Figure 4c).

Mechanism of the effect of warming and N addition on the N uptake kinetics of fine roots

Fine roots MCR had a significant effect on $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ (Table 2), and $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ increased with increasing MCR

(Figure 5a), when accounting for other factors and covariables. SRR had a significant effect on $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ (Table 2), and $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ showed an increasing trend with increasing SRR (Figure 5b) when other factors and covariables are accounted for. After considering covariates, warming had no significant effect on $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ (Table 2), which indicated that warming-increased $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ may be related mainly to warming-stimulated MCR. After considering covariates, the N addition still had a positive effect on $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ ($P < 0.05$, Table 2; the estimated fixed effect of the N addition was 0.110, Table S1 available as Supplementary data at *Tree Physiology*

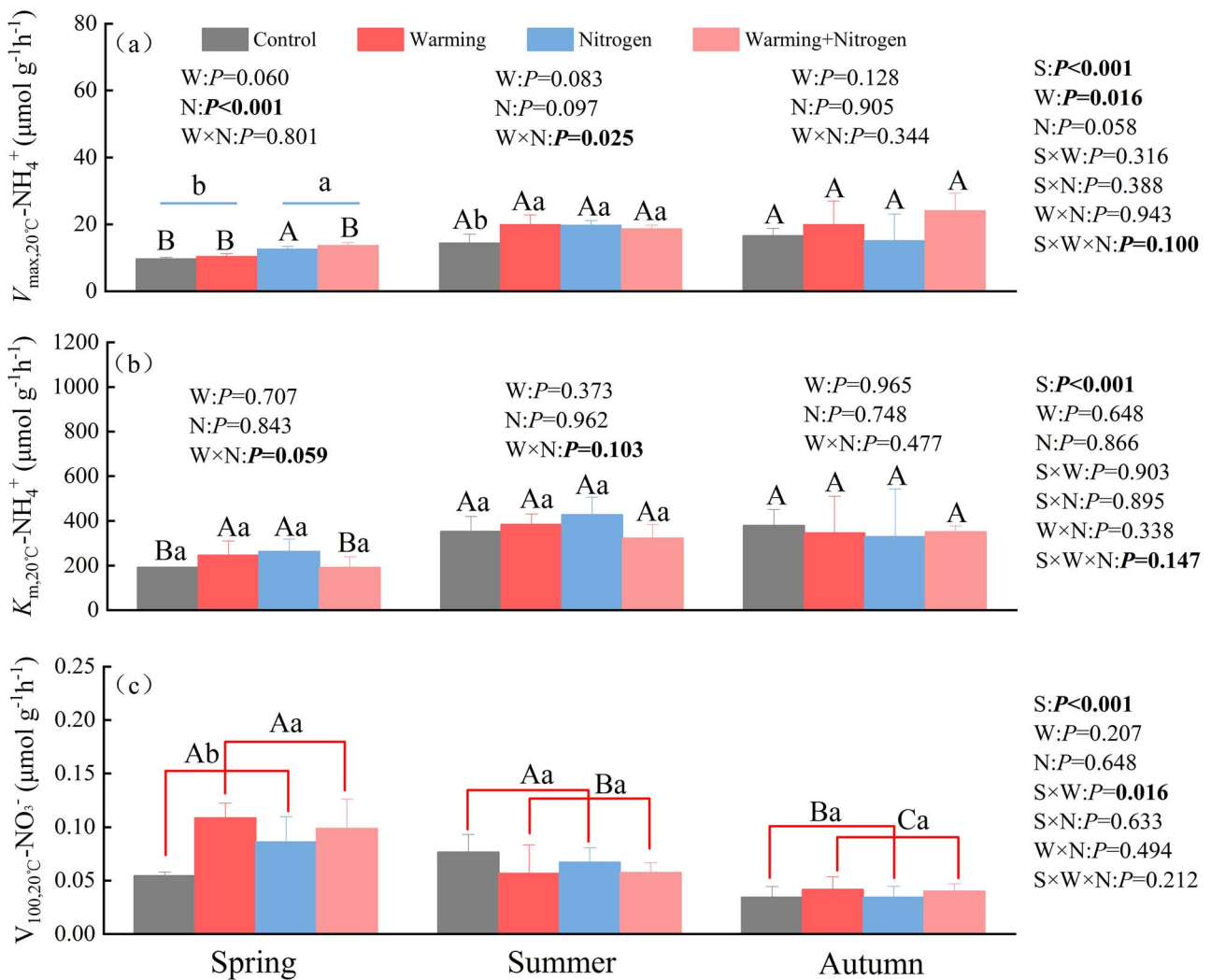


Figure 2. Variation of fine roots $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$, $K_{m,20^{\circ}\text{C}}\text{-NH}_4^+$ and $V_{100,20^{\circ}\text{C}}\text{-NO}_3^-$ at reference temperature of 20°C across seasons in a factorial warming and N addition experiment (mean \pm SD, $n = 3$). S: season, W: warming; N: N addition. Different lowercase letters and uppercase letters on the bars indicate significant differences between different treatments in the same season and different seasons in the same treatment, respectively. Different lowercase letters on the blue line (a) indicate significant differences between different N addition levels; different lowercase letters on the red line (b) indicate significant differences between different warming levels; and different uppercase letters on the red line (c) indicate significant differences between different seasons.

Online), which might be due to factors other than those considered in current study. This positive effect could override the possible negative effect of the N addition-induced decreased MCR (Figure 2a) on $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$, which together might lead to overall positive apparent effect of the N addition on $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$.

After considering covariates, the interaction of warming and the N addition had a marginal significant effect on $K_{m,20^{\circ}\text{C}}\text{-NH}_4^+$ (Table 2). However, the multiple pairwise comparisons indicated no significant difference among treatments (Table S2 available as Supplementary data at *Tree Physiology* Online). This further showed that both warming and the N addition can hardly affect $K_{m,20^{\circ}\text{C}}\text{-NH}_4^+$, whether or not the phenotypical adjustments in fine roots were accounted for.

After considering covariates, the interaction of season and warming still had a significant effect on $V_{100,20^{\circ}\text{C}}\text{-NO}_3^-$

(Table 2), but the multiple pairwise comparisons showed no significant difference between warming and non-warming treatments in any season (Table S3 available as Supplementary data at *Tree Physiology* Online), which indicated that warming-increased $V_{100,20^{\circ}\text{C}}\text{-NO}_3^-$ in spring (Figure 2c) might be related to warming-increased SRR.

Discussion

In the present study, compared with the control, warming increases the fine root $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$ in summer, whereas the N addition increases $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$ in spring and summer. Compared with non-warming treatments, warming treatments increase $V_{100,20^{\circ}\text{C}}\text{-NO}_3^-$ in spring, which thus supports our first hypothesis. Our results also indicate that an increase in $V_{\max,20^{\circ}\text{C}}\text{-NH}_4^+$ and $V_{100,20^{\circ}\text{C}}\text{-NO}_3^-$ is related to root phe-

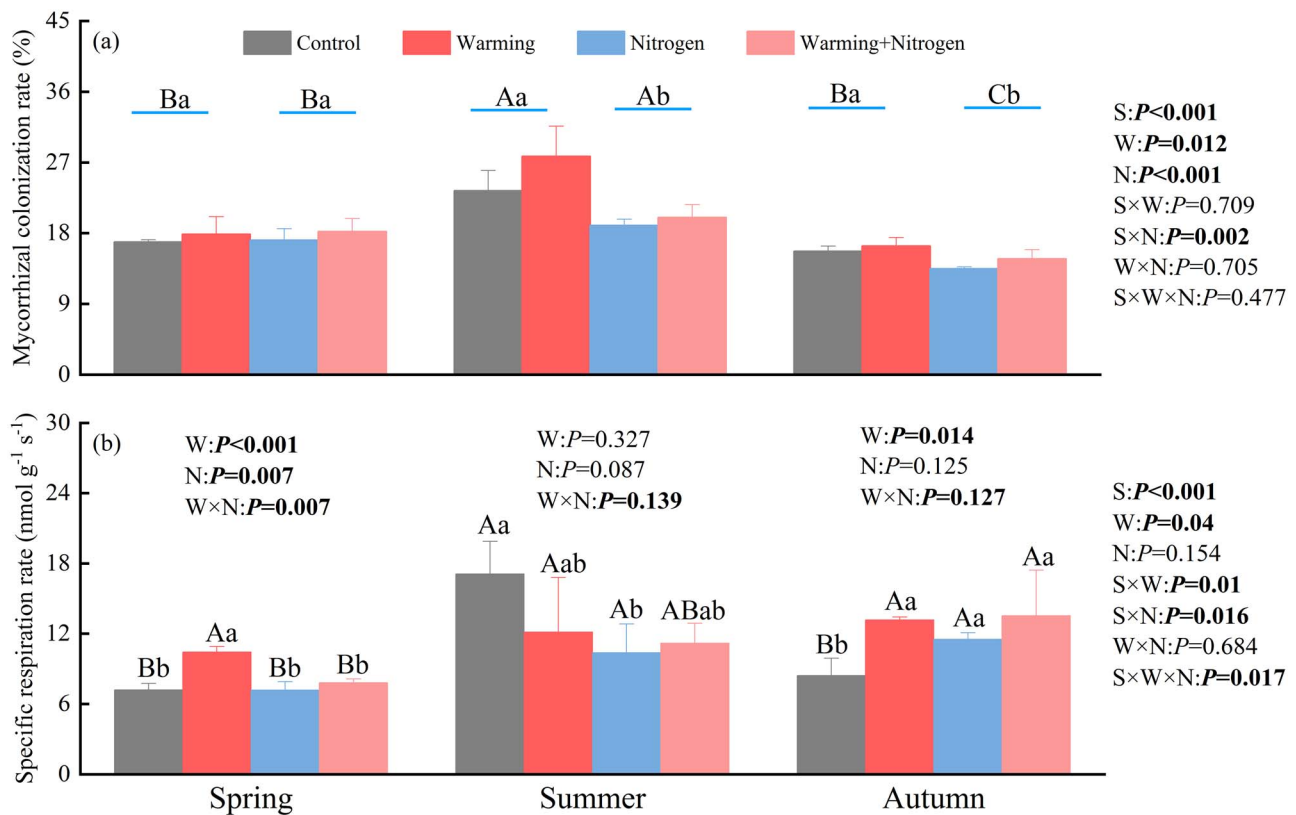


Figure 3. Variations of fine roots MCR (a) and SRR (b) across seasons in a factorial warming and N addition experiment (mean \pm SD, $n = 3$). S: season; W: warming; N: N addition. Different lowercase letters and uppercase letters on the bars indicate significant differences between different treatments in the same season and different seasons in the same treatment, respectively. Different lowercase and uppercase letters on the blue line (a) indicate significant differences between different N addition levels and between seasons.

notypical adjustments, especially to increasing MCR and SRR by warming. Hence, our second hypothesis was also partly supported.

Effects of warming and N addition on NH_4^+ uptake kinetics of fine roots

Our results indicated that warming increased the fine root $V_{\text{max},20^\circ\text{C}}\text{-NH}_4^+$ in summer in the control and was consistent with some previous studies showing that warmer soils increased NH_4^+ uptake rate (Gessler et al. 1998, Soggi and Templer 2011), which might, to some extent, reflect the potential increase of root N uptake capacity. Increased N uptake with warming is often attributed to enhanced soil N availability (Butler et al. 2012, Lim et al. 2019) resulting from accelerated N mineralization (Bai et al. 2013, Jarvi and Burton 2018). In contrast, some studies showed that warmer soils reduced NH_4^+ uptake rate (Lucash et al. 2005, Sanders-DeMott et al. 2018), which could be simply due to concomitant drought effect in warmer soils (Sorensen et al. 2018), decelerating the movement of ions (Joseph et al. 2021).

Our results showed that, compared with the control, the N addition significantly increased fine root $V_{\text{max},20^\circ\text{C}}\text{-NH}_4^+$ (Figure 2a) in spring and summer, which contrasts with previous

studies that root V_{max} of NH_4^+ uptake generally decreases in response to the N addition (Bassirad et al. 1996, Rothstein et al. 2000). For example, Rothstein et al. (2000) found that V_{max} of root NH_4^+ uptake of *Populus tremuloides* in Michigan was significantly lower at high N than at low N availability. Despite considerable variation among species, the effect of the N addition on root NH_4^+ uptake kinetics often exhibits a strong correlation with the growth rate of plant species (Ingestad and Kähr 1985, Schenk 1996). Generally, reduction of root NH_4^+ uptake occurs only after the plant's demand for the N is met (Bassirad 2000). Our previous study found that the N addition accelerated the tree growth in the same site of Chinese fir (Xiong et al. 2020), indicating a massive demand for the N. As a fast-growing species, the demand for the N in Chinese fir is high, particularly in its early growth stage, and the absorption capacity of NH_4^+ was not inhibited but was even enhanced by the N addition.

An interactive effect of warming and the N addition on $V_{\text{max},20^\circ\text{C}}\text{-NH}_4^+$ occurred in summer, and the combined warming + N addition treatment did not further increase $V_{\text{max},20^\circ\text{C}}\text{-NH}_4^+$ compared with warming and the N addition alone treatments (Figure 2a). This could be explained by no further increase in tree growth in the combined treatment (Xiong

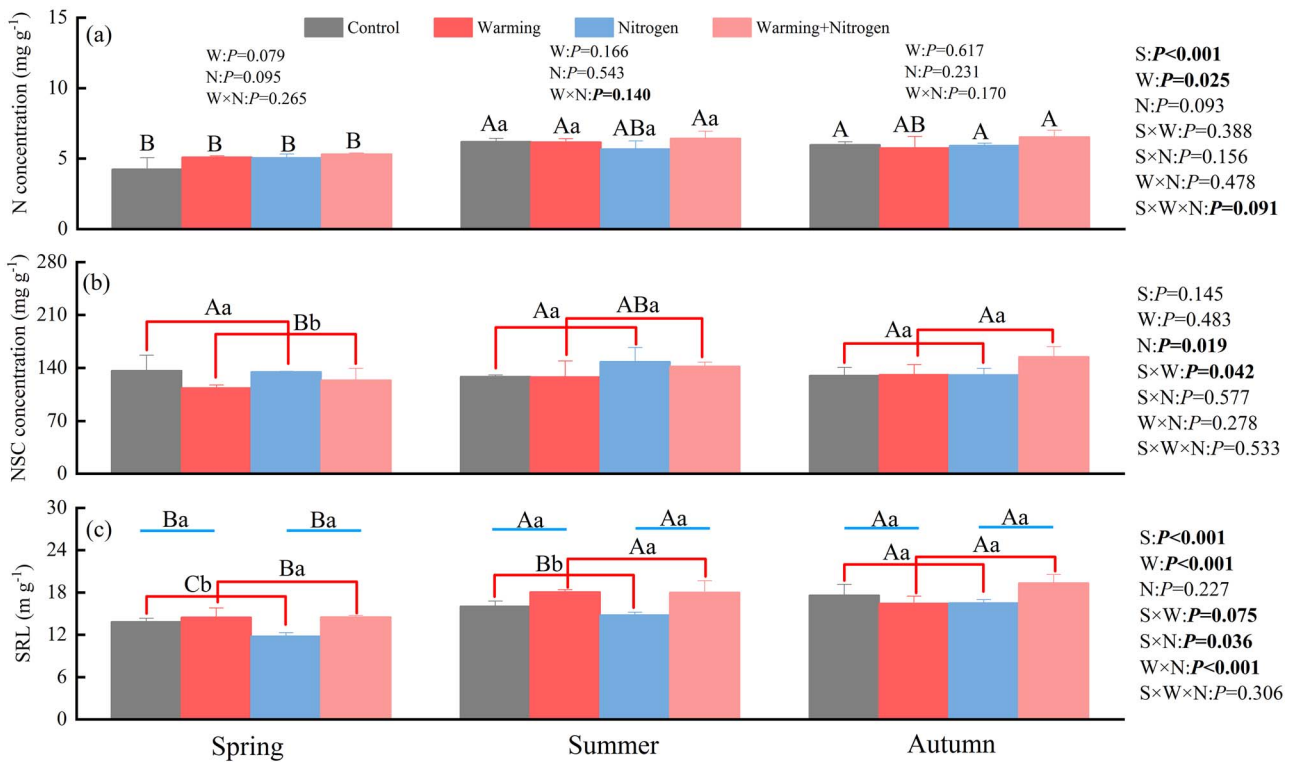


Figure 4. Variation of fine root N concentration (a), SRL (b) and NSC concentration (c) across seasons in a factorial warming and N addition experiment (mean \pm SD, $n = 3$). S: season; W: warming; N: N addition. Different lowercase letters and uppercase letters on the bars indicate significant differences between different treatments in the same season and different seasons in the same treatment, respectively. Different lowercase letters on the red and blue lines (b and c) indicate significant differences between different warming levels and between different N addition levels, respectively; different uppercase letters on the red and blue lines (b and c) both indicate significant differences between different seasons.

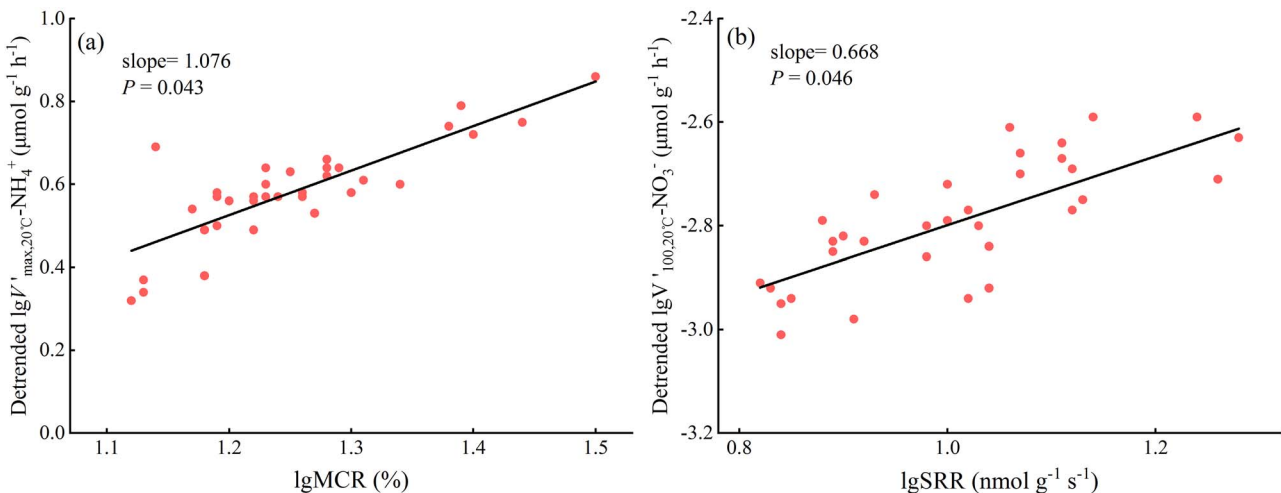


Figure 5. Trends of log10-transformed $V_{\max,20\text{ }^{\circ}\text{C-NH}_4^+}$ associated with mycorrhizal colonization rate (MCR) (a), and of $V_{100,20\text{ }^{\circ}\text{C-NO}_3^-}$ associated with specific root respiration rate (SRR) (b), when other factors and covariables in Table 2 were accounted for. The y-axis is detrended for the effects of other factors and covariables in Table 2.

et al. 2020) and hence no extra N demand, as compared with the warming and the N addition alone treatments.

We found a positive correlation between MCR and $V_{\max,20\text{ }^{\circ}\text{C-NH}_4^+}$, similar to Bassirirad et al. (2001), who reported a positive effect of AM fungi (AMF) on the specific root uptake rate of NH_4^+ . Indeed, the plant metabolic system is markedly affected

by the AMF, even though the extramatrical hyphae are removed during root processing. For example, inoculation with AMF increased glutamine synthase activity (N assimilation enzyme) in roots (Azcón and Tobar 1998) and AMF hyphae (Smith et al. 1985) by improving the phosphorus status required for the enzymatic reaction (Harrison et al. 2002). GintAMT1 and

GintAMT2, two high-affinity NH_4^+ transporters, were expressed both in the intramatrical and extramatrical hyphae of AMF, and the transcription level of GintAMT2 in the intramatrical hyphae is even higher than that in the extramatrical hyphae (Pérez-Tienda et al. 2011).

The MCR was stimulated by soil warming in the present study, which was consistent with other studies (Zavalloni et al. 2012, Mohan et al. 2014, Bennett and Classen 2020), and might be explained by an enhanced nutrient demand of trees under warming or better growth of fungi at warmer temperatures. The finding that the N addition reduced MCR is consistent with Wheeler et al. (2017) and Sheldrake et al. (2018). It suggests that plants might reduce their C investment in AM fungi when nutrient availability increases (Johnson et al. 2010). Warming-enhanced root $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ in summer could be explained by the significant increase of warming-stimulated MCR during this season (Figure 3a). The N addition reduced MCR in summer and autumn (Figure 3), which might also have a negative effect on $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$, although such effect was overridden by the positive effect of the N addition through an unaccounted mechanism. This unaccounted mechanism leading to a positive effect of the N addition on $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$ might be the stimulated production of plasma membrane H^+ -ATP enzyme in response to the N addition (Tang et al. 2019), which can create a gradient of electrode potential to drive the influx of NH_4^+ (Hawkins and Robbins 2010, Luo et al. 2013).

Unlike the $V_{\max,20^\circ\text{C}}\text{-NH}_4^+$, we showed that the $K_{m,20^\circ\text{C}}\text{-NH}_4^+$ was not affected by warming or the N addition, which could be explained by the characterization of ion transporter properties. The V_{\max} positively relates to an increased transporter density, which differed significantly among species and is easily affected by environmental factors, whereas K_m might not be influenced (Griffiths and York 2020).

Effects of warming and N addition on NO_3^- uptake kinetics of fine roots

Our results showed that, compared with non-warming treatments, warming treatments increased fine root $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ in spring (Figure 2c), which contradicts Bassirirad et al. (1993), who reported that warming reduced the V_{\max} of NO_3^- uptake of *A. desertorum*, a common perennial grass in the Great Basin of Utah. However, the significant temperature increase (10 °C) in that experiment was far beyond the soil temperature range that *A. desertorum* experienced during the growing season, which might result in a negative warming effect on nitrate uptake capacity (Hungria and Kaschuk 2014, Mainali et al. 2014).

In the present study, $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ was significantly correlated with SRR (Figure 3b). As we know, the energy cost of NO_3^- uptake is higher than that of NH_4^+ (Boudsocq et al. 2012, Britto and Kronzucker 2013). Up to 20% of total plant respiration has been determined to be devoted to nitrate uptake (Veen 1980, Jarvi and Burton 2018). Our previous study

indicated that warming accelerated turnover of fine roots in Chinese fir plantation (Xiong et al. 2018), which might result in a younger fine root population with high metabolic activity and thus higher respiration rate, which are beneficial for fast resource acquisition (Jarvi and Burton 2020, Noh et al. 2020). Therefore, the observed increased fine root $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ with soil warming may be attributed to the increased SRR (Table 2; Figure 5). Although NSC was considered as the energy source of fine root N uptake (Bassirirad et al. 1996, 1997, Rothstein et al. 2000), Chinese fir fine root N uptake kinetics were found to be independent of root NSC in the current study (Table 2; Figure 4). The NSC storage in fine root tissues is generally small and the energy to maintain root activity mainly comes from the belowground allocation of recent photosynthate (Hartmann and Trumbore 2016, Joseph et al. 2021).

Boczulak et al. (2014) reported that the highest NO_3^- net uptake occurred at temperature between 16 °C and 20 °C. Similarly, we found that warming-stimulated $V_{100,20^\circ\text{C}}\text{-NO}_3^-$ occurred only in spring due to the optimum temperature range for NO_3^- uptake. Soil temperature in summer and autumn in our study site (Xiong et al. 2020) was mostly beyond this optimum temperature, which is why we did not observe an increased NO_3^- uptake in these seasons.

Limitations and implications

Nitrogen uptake kinetics measured on excised root are limited to a certain extent, because root excision might lead to a decrease in N uptake rate due to either direct effect of excision, such as a wounding response, or more indirect effects, such as reduction of water movement through the roots and limitations to the root carbohydrate supply (Bloom et al. 1992). However, excised root technique has been used extensively, either on physiology or whole-tree and ecosystem processes, in nutrient uptake studies (Hawkins et al. 2014, Sanders-DeMott et al. 2018, He et al. 2018, Chen et al. 2021), which can provide good estimate of inorganic N uptake rate as comparable to attached roots, if the excised root is measured within a few hours of excision (Bloom et al. 1992, Bassirirad et al. 1996, Rothstein et al. 2000). Hawkins et al. (2012) have shown that ion flux in roots remains stable for several hours after root excision. Furthermore, using excised roots is often necessary for field-grown plants and always valuable for in-depth analysis of ion influx across root zones and root classes (Griffiths and York 2020), as long as the excision and the measurement are conducted without any delay to avoid disruption to uptake processes (BassiriRad et al. 1999). In the current study, Nitrogen uptake assays were always performed within 2 h of root excision to minimize potential carbohydrate starvation of roots and possible inhibition of nutrient transport.

The rate of nutrient absorption by a root depends upon both nutrient supply to the root surface and active absorption by root cortical cells (Chapin 1980). N, phosphorus and potassium

are diluted in the soil solution, and, consequently, the bulk of these elements moves to the root surface by diffusion (Chapin 1980). Thus, nutrient diffusion (an important part of nutrient supply) is the rate-limiting step in absorption by plant roots (Chapin 1980). However, Chapin et al. (1986) also suggested that the importance of nutrient uptake kinetics depends on ion mobility. For mobile ions (such as nitrate and ammonium), there is a substantial selection for a high absorption capacity in sites where these ions may limit plant growth, because uptake may be more critical than diffusion as a rate-limiting step in absorption of mobile ions, and uptake kinetics become an essential component of root competition in soils (Chapin et al. 1986). In addition, as soil solution concentration increases, root absorption capacity and temperature become increasingly important relative to diffusion in controlling nutrient acquisition by plants (Chapin 1980).

This and our previous study in the same plot (Xiong et al. 2020) demonstrated that both warming and the N addition increased soil N availability and improved root N uptake capacity, and consequently increased tree height. Similarly, Melillo et al. (2011) and Grant (2014) in Harvard forest and Harrison et al. (2020) in Hubbard brook experimental forest also reported that warming increased soil N availability, plant N uptake and tree growth. Thus, warming accelerated N cycling and redistributed N from soil to trees, which may lead to a substantial increase in C storage in woody tissue. Our previous study found that warming had no significant effect on fine root biomass (Xiong et al. 2020), which is consistent with Jarvi and Burton (2020) but inconsistent with Zhou et al. (2011) and Grant (2014), who found that warming decreased fine root biomass in Harvard forest. The optimal partitioning theory indicates that plants optimize the capture of limited resources by allocating C to maximize growth rates (Tilman 1988, McConnaughay and Coleman 1999). As the climate warms, N becomes more available, trees do not have to allocate as much C to acquire N, consequently fine root biomass decreases (Melillo et al. 2011). However, Chinese fir is a fast-growing species, demanding plentiful nutrients even in the early growth stage (Ma et al. 2007, Chen et al. 2013), which may necessitate the maintenance of fine root biomass. In the meantime, warming-induced photosynthesis enhancement and growing season extension help sustain this needed belowground C allocation (Malhotra et al. 2020). This study also showed that root respiration decreased only in summer, but even increased in spring and autumn, indicating a partial acclimation under warming, which was consistent with previous studies (Jarvi and Burton 2018), and may lead to large increases in annual CO₂ emissions (Atkin et al. 2000). As a result, the increased tree growth under warming due to the improvement of soil N availability and root N uptake capacity may offset the warming increased soil C loss to some extent.

The trees have a distinct preference for different N forms and generally absorb fewer NO₃⁻ than NH₄⁺ (Boudsocq et al. 2012, Britto and Kronzucker 2013, Zou et al. 2020, Chen et al. 2021). As indicated above, conifers usually take up even fewer NO₃⁻ than angiosperm (BassiriRad et al. 1997, Kronzucker et al. 1997), which was also evidenced by the present study that documented much faster root absorption rates of NH₄⁺ than of NO₃⁻ in Chinese fir during the entire experimental period (Figure 1). Under the scenario of global warming and N deposition, soil N availability can be enhanced (Kou et al. 2018, Xiong et al. 2018, Lim et al. 2019). If root N absorption capacity is reduced, NO₃⁻ might accumulate in soil and losses of excessive NO₃⁻ (exceeding biological absorption capacity) may happen, which might decrease cation availability, and lead to soil acidification and eutrophication, and eventually forest decline (Simkin et al. 2016, Gessler et al. 2017). Our study showed that fine root N uptake capacity of Chinese fir did not decrease, but even increase, in response to warming and N addition, which could alleviate possible N leaching and improve forest productivity. As a fast-growing tree species, nutrient demand for the growth of Chinese fir is high even in the early growth stage (Ma et al. 2007, Chen et al. 2013), and increases consistently until maturation (Ma et al. 2007). Thus, increased N availability from warming and N deposition can meet nutrient demand and accelerate the growth of Chinese fir, which is evidenced by the present and previous studies (Zhang et al. 2017, 2018, Xiong et al. 2018, 2020). Increased N may lead to an imbalanced supply of N and phosphorus however, and limit plant productivity in the low phosphorus soils of subtropical forests in the longer term (Lu et al. 2021). Therefore, care must be taken in extrapolating our findings in a short-term experiment at seedling stage to a long-term warming and N deposition effect on root N uptake capacity in mature forests. To better characterize root uptake capacity, belowground allocation of recent photosynthate, closely related to plant nutrient absorption and assimilation, should be included in future studies on root N uptake kinetics. In addition, gene sequencing on the membrane-bound transport proteins encoded by plant genomes is essential to understanding how NH₄⁺ and NO₃⁻ are intercepted and shuttled across the membrane by individual transporters under climate change.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Acknowledgments

We thank the Fujian Sanming Forest Ecosystem National Observation and Research Station for analytical support. Thanks also

go to the anonymous reviewers and editors, whose comments and suggestions have helped us enormously to improve this manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (grant numbers 31830014, 31930071 and 32071743).

Conflict of interest

None declared.

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