Tree Physiology 37, 1515–1526 doi:10.1093/treephys/tpx046

# **Research paper**



# Organic and inorganic nitrogen uptake by 21 dominant tree species in temperate and tropical forests

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Received October 19, 2016; accepted April 14, 2017; published online May 8, 2017; handling Editor Torgny Näsholm

Evidence shows that many tree species can take up organic nitrogen (N) in the form of free amino acids from soils, but few studies have been conducted to compare organic and inorganic N uptake patterns in temperate and tropical tree species in relation to mycorrhizal status and successional state. We labeled intact tree roots by brief <sup>15</sup>N exposures using field hydroponic experiments in a temperate forest and a tropical forest in China. A total of 21 dominant tree species were investigated, 8 in the temperate forest and 13 in the tropical forest. All investigated tree species showed highest uptake rates for NH<sub>4</sub><sup>+</sup> (ammonium), followed by glycine and NO<sub>3</sub><sup>-</sup> (nitrate). Uptake of NH<sub>4</sub><sup>+</sup> by temperate trees averaged 12.8  $\mu$ g N g<sup>-1</sup> dry weight (d.w.) root h<sup>-1</sup>, while those by tropical trees averaged 6.8  $\mu$ g N g<sup>-1</sup> d.w. root h<sup>-1</sup>. Glycine uptake rates averaged 3.1  $\mu$ g N g<sup>-1</sup> d.w. root h<sup>-1</sup> for temperate trees and 2.4  $\mu$ g N g<sup>-1</sup> d.w. root h<sup>-1</sup> for tropical trees. NO<sub>3</sub><sup>-</sup> uptake was the lowest (averaging 0.8  $\mu$ g N g<sup>-1</sup> d.w. root h<sup>-1</sup> for temperate trees and 1.2  $\mu$ g N  $q^{-1}$  d.w. root  $h^{-1}$  for tropical trees). Uptake of NH<sub>4</sub><sup>+</sup> accounted for 76% of the total uptake of all three N forms in the temperate forest and 64% in the tropical forest. Temperate tree species had similar glycine uptake rates as tropical trees, with the contribution being slightly lower (20% in the temperate forest and 23% in the tropical forest). All tree species investigated in the temperate forest were ectomycorrhizal and all species but one in the tropical forest were arbuscular mycorrhizal (AM). Ectomycorrhizal trees showed significantly higher  $NH_4^+$  and lower  $NO_3^-$  uptake rates than AM trees. Mycorrhizal colonization rates significantly affected uptake rates and contributions of  $NO_3^-$  or  $NH_4^+$ , but depended on forest types. We conclude that tree species in both temperate and tropical forests preferred to take up NH4<sup>+</sup>, with organic N as the second most important N source. These findings suggest that temperate and tropical forests demonstrate similar N uptake patterns although they differ in physiology of trees and soil biogeochemical processes.

Keywords: AM fungi, ECM fungi, inorganic N, organic N, root nitrogen uptake, temperate forest, tropical forest.

# Introduction

Nitrogen (N) is considered as a major factor limiting plant growth and development in many terrestrial ecosystems (Elser et al. 2007). Traditional concepts assumed that plants can only use inorganic N ( $NH_4^+$  and  $NO_3^-$ ) released by microbial

mineralization of organic N and nitrifiers (Nadelhoffer et al. 1985, Schimel and Bennett 2004). However, increasing evidence suggests that various plant species can take up organic N directly in the form of intact amino acids from soils (Jones et al. 2005, Näsholm et al. 2009, Warren 2014). Most previous

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studies have been conducted in ecosystems that store a substantial amount of soil organic N. For example, alpine (Lipson et al. 2001, Xu et al. 2004, 2006, 2011) and boreal (Kielland 1994, Näsholm et al. 1998, Näsholm and Persson 2001, Kielland et al. 2006, Leduc and Rothstein 2010) ecosystems in cold climates are characterized by low mineralization rates of soil organic matter due to low temperature and high soil moisture favoring soil organic N build-up and relatively high organic versus low inorganic N availability. More recently, several studies have been conducted in temperate (Finzi and Berthrong 2005, Berthrong and Finzi 2006, Warren and Adams 2007, Warren 2009) and subtropical (Li et al. 2015) forests, showing that dominant tree species have the capacity to take up organic N from soils. However, this has not been well investigated for trees in tropical forests (Turnbull et al. 1996, Wanek et al. 2002) although they occupy a sizeable proportion of global forests.

Across the globe, soil N availability and its composition widely varies from boreal to temperate and tropical forests. It has been postulated that ammonification is dominating in temperate forests while nitrification is prevailing in tropical forests (Schimel and Bennett 2004). Accordingly, dominant plant species may have evolved distinct N uptake patterns to optimize their N acquisition across latitudinal gradients. This suggests that temperate trees may prefer NH<sub>4</sub><sup>+</sup> uptake whereas tropical trees may prefer to take up NO<sub>3</sub><sup>-</sup>. However, so far this hypothesis has not been tested by field experiments using the same methodology.

Additionally, loss of soil N can contribute to environmental problems and pollution, e.g.,  $N_2O$  is an important greenhouse gas and can cause damage to the  $O_3$  layer while leaching of  $NO_3^-$  and organic N can pollute drinking water sources and can cause eutrophication of aquatic ecosystems. Soil N losses are well correlated with plant N uptake patterns (Groenigen et al. 2015). For example, if plants take up more  $NO_3^-$ ,  $NO_3^-$  will have fewer chances to be lost by leaching or denitrification. This indicates that changes or differences in plant preferences for N forms (the N form predominantly taken up by plants) may differentially affect ecosystem N cycling and loss processes (Kraiser et al. 2011, Britto and Kronzucker 2013).

In mature old-growth forests, tree fall caused by natural mortality or storms and pest outbreaks causes opening of large (tree-fall) gaps, which are colonized by early successional species. Gap dynamics are therefore an important characteristic of old-growth forests, allowing the establishment and coexistence of early and late successional species in the same forest. Previous studies showed that pioneer species acquire more  $NO_3^-$  and less  $NH_4^+$  than late successional species (Britto and Kronzucker 2013), but this is seldom explored in the same forests. Additionally, several studies showed that ectomycorrhizal (ECM) plants have the ability to mobilize and take up organic N (Kielland 1994, Sokolovski et al. 2002, Smith and Read 2008), while arbuscular mycorrhiza (AM) can help to take up  $NO_3^-$  (Ho and Trappe 1975, Azcón and Tobar 1998, Azcón et al. 2001). To compare N uptake patterns between temperate and tropical forest trees, a temperate forest in Donglingshan and a tropical forest in Xishuangbanna were selected. Eight dominant tree species in the temperate forest and 13 dominant tree species in the tropical forest were chosen based on their mycorrhizal type and successional stage as these factors can strongly influence plant N uptake patterns (Marschner and Dell 1994, Turnbull et al. 1996, Bonfante and Anca 2009, Miransari 2011). As glycine is one of the dominant amino acids in most soils and often used in experiments of plant organic N uptake (Schiller et al. 1998, Miller and Bowman 2002, Finzi and Berthrong 2005, Berthrong and Finzi 2006, Forsum et al. 2008, Svennerstam et al. 2008, Brackin et al. 2015), here we also selected glycine to represent uptake of other amino acids for comparison with published studies. Using brief <sup>15</sup>N exposures of intact roots in field hydroponic experiments, we aimed to test the following three hypotheses: (i) temperate trees have higher uptake rates of organic N and  $NH_4^+$  than tropical ones, (ii) temperate trees prefer  $NH_4^+$  while tropical trees prefer NO<sub>3</sub><sup>-</sup>, accompanied by a change from ECM to AM tree species and (iii) early successional species show greater uptake for  $NO_3^{-}$  than  $NH_4^{+}$  compared with late successional species.

#### Materials and methods

# Study sites and tree species

The temperate forest site was located at Donglingshan Ecological Experiment Station (39°58'N, 115°26'E, 2303 m above sea level), in Beijing Municipality in northeastern China. The tropical forest site was located at Xishuangbanna Ecological Experiment Station (21°54'N, 101°46'E, 580 m above sea level), in the south of the Yunnan Province of China.

The Donglingshan site is characterized by a typical warm temperate seasonal climate. In the past 20 years, mean annual air temperature averaged 6.3 °C. Mean annual precipitation averaged 612 mm, and is mainly concentrated from June to August. Forests are dominated by temperate deciduous broad-leaved trees. Eight dominant tree species (i.e., *Pinus tabuliformis, Betula dahurica, Betula platyphylla, Populus davidiana, Juglans mandshurica, Quercus wutaishanica, Acer mono* and *Larix principis-rupprechtii*) were chosen. The soils are classified as brown soil, corresponding to Eutric cambisol (World Reference Base for Soil Resources 2006).

The Xishuangbanna site is characterized by a typical tropical seasonal climate. In the past 20 years, mean annual air temperature approximated 21.8 °C. Mean annual precipitation averaged 1493 mm. The forest is dominated by evergreen broad-leaved trees. Thirteen dominant tree species (i.e., *Cleidiocarpon cavaleriei, Ficus tinctoria, Macaranga denticulata, Sapindus mukorossi, Syzygium szemaoense, Anogeissus acuminata var. lanceolata, Parashorea chinensis, Pometia tomentosa, Chukrasia tabularis* var. *velutina, Tectona grandis, Flacourtia rukam, Lithocarpus balansae* and *Ficus altissima*) were chosen. The soils belong to lateritic red soils, developed from siliceous rocks (Wang et al. 1996, Cao et al. 2006, World Reference Base for Soil Resources 2006). Detailed information on the tree species is presented in Table 1.

# Field hydroponic experimental design

A mixture of NH<sub>4</sub><sup>+</sup>, glycine and NO<sub>3</sub><sup>-</sup> was employed to test tree root N uptake. Glycine was used as an organic N source (Lipson and Näsholm 2001). Equal concentrations of NH<sub>4</sub><sup>+</sup>, glycine and NO<sub>3</sub><sup>-</sup> (33 µmol N I<sup>-1</sup> for each N form) were used so that the roots could choose between N forms. There were four treatments with four replicates for each treatment and plant species. Each labeling solution contained only one of the three N forms <sup>15</sup>N labeled (99 atom% <sup>15</sup>N-glycine, 10 atom% <sup>15</sup>N-KNO<sub>3</sub> or 10 atom% (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), while the other two N forms were not labeled with <sup>15</sup>N. In addition, a solution containing all three N forms in an unlabeled form was used as a control. All solutions used contained 10 mg I<sup>-1</sup> of ampicillin to minimize microbial activity and avoid decomposition of the amino acid as well as 0.2 mmol CaCl<sub>2</sub> to maintain the function and integrity of roots (Warren and Adams 2007).

Labeling experiments were performed in September 2014. Four trees of similar size were randomly selected for each tree species and four pairs of fine roots in four directions (one pair for each direction) with diameter less than 2 mm (weight ranged from 0.05 to 0.20 g for one fine root; length about 15 cm for each root) were dug out diagonally along the stem base. Each pair of roots of the same direction was gently washed with deionized water and then the root pair immersed in the respective labeling solutions in 15 ml centrifuge tubes. After 2 h, fine roots were excised and washed with 50 mM KCl solution for 3 min and then gently rinsed with deionized water to remove the <sup>15</sup>N remaining on the surface of the fine roots. The pairs of roots were used as independent replicates (i.e., one of the four root pairs were labeled by only one <sup>15</sup>N activities and main a function for a min and then gently replicates (i.e., one of the four root pairs were labeled by only one

<sup>15</sup>N solution; one pair of roots for each tree therefore represents two replicates for a specific N form, with a total of four trees, i.e., eight replicates per N form). The fourth pair of roots treated with unlabeled N solution was used as natural stable isotope abundance control. At the same time, additional fine roots were collected, gently washed in deionized water and immediately fixed in Formalin-Aceto-Alcohol solution (90 ml 50% ethanol, 5 ml 100% glacial acetic acid, 5 ml 37% methanol) to investigate mycorrhizal type and mycorrhizal colonization rate (Guo et al. 2008). All labeled and unlabeled fine roots collected were dried at 70 °C for 48 h for dry mass determination. Dried roots were ground into a fine powder and weighed into tin capsules for measurements of N content and <sup>15</sup>N/<sup>14</sup>N ratios by continuous-flow isotope ratio mass spectrometry (IRMS). These measurements were performed with an elemental analyzer (EA 1110, CE Instruments, Milan, Italy) connected by a ConFlo II device to the IRMS (Finnigan MAT 253, Bremen, Germany). Mycorrhizal colonization rates were measured by the grid line intersect method (Giovannetti and Mosse 1980). Arbuscular mycorrhiza- colonized root samples were stained with

Table 1. Main properties (functional group, mycorrhizal type and successional stage) of 21 dominant tree species in a temperate forest at Donglingshan and a tropical forest at Xishuangbanna. ECM refers to ectomycorrhiza while AM indicates arbuscular mycorrhiza.

Tree species	ID	Family	Genus	Functional group	Mycorrhizal type	Successional stages
Temperate species						
Pinus tabuliformis Carr.	PTA	Pinaceae	Pinus	Coniferous	ECM	Early
Betula dahurica Pall.	BDA	Betulaceae	Betula	Broad-leaved	ECM	Early
Betula platyphylla Suk.	BPL	Betulaceae	Betula	Broad-leaved	ECM	Early
Populus davidiana Dode	PDA	Salicaceae	Populus	Broad-leaved	ECM	Early
Juglans mandshurica Maxim.	JMA	Juglandaceae	Juglans	Broad-leaved	ECM	Early
Quercus wutaishanica Mayr	QWU	Fagaceae	Quercus	Broad-leaved	ECM	Late
Acer mono Maxim.	AMO	Aceraceae	Acer	Broad-leaved	ECM	Late
Larix principis-rupprechtii Mayr	LPR	Pinaceae	Larix	Coniferous	ECM	Late
Tropical species						
Cleidiocarpon cavaleriei (H. Lév.) Airy-Shaw	CCA	Euphorbiaceae	Cleidiocarpon	Broad-leaved	AM	Early
Ficus tinctoria Forst. f.	FTI	Moraceae	Ficus	Broad-leaved	AM	Early
Macaranga denticulata (Bl.) Müll. Arg.	MDE	Euphorbiaceae	Macaranga	Broad-leaved	AM	Early
Sapindus mukorossi Gaertn.	SMU	Sapindaceae	Sapindus	Broad-leaved	AM	Early
Syzygium szemaoense Merr. et Perry	SSZ	Myrtaceae	Syzygium	Broad-leaved	AM	Early
Anogeissus acuminata var. lanceolata Wall. ex C. B. Clarke	AAC	Combretaceae	Anogeissus	Broad-leaved	AM	Early
Parashorea chinensis Hsie Wang	PCH	Dipterocarpaceae	Parashorea	Broad-leaved	AM	Late
Pometia tomentosa (Bl.) Teysm. et Binn.	PTO	Sapindaceae	Pometia	Broad-leaved	AM	Late
Chukrasia tabularis A. Juss. var. velutina King	CTA	Meliaceae	Chukrasia	Broad-leaved	AM	Late
Tectona grandis L.f.	TGR	Verbenaceae	Tectona	Broad-leaved	AM	Late
Flacourtia rukam Zoll. et Moritzi	FRU	Flacourtiaceae	Flacourtia	Broad-leaved	AM	Late
Lithocarpus balansae (Drake) A. Camus	LBA	Fagaceae	Lithocarpus	Broad-leaved	AM	Late
Ficus altissima Blume	FAL	Moraceae	Ficus	Broad-leaved	ECM	Late

acid fuchsin to have a better contrast between the fungal colonized and the non-mycorrhizal root part, while ECM-infected root samples were not stained as ECM roots can easily be distinguished from non-mycorrhizal roots by differences in color, thickness, texture and branching patterns (Vierheilig et al. 2005). A dissecting microscope was used to quantify intersections between grid lines and roots, which were designated as either colonized or nonmycorrhizal.

Soil samples in both temperate and tropical forests were collected from beneath each tree species individual at 0–10 cm at four positions, then soils were mixed, immediately brought to the laboratory, sieved to 2 mm and stored at 4 °C until measurements. Fresh soil was extracted with 0.05 M K<sub>2</sub>SO<sub>4</sub> and soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in extracts were measured on an auto analyzer (AA3, Bran-Luebbe, Hamburg, Germany). Soil glycine concentrations were measured by high-performance liquid chromatography (HPLC-MS/MS API3200 Q-TRAP, Foster City, CA, USA) on the water extracts after derivatization of amino acids (Näsholm et al. 1987). Soil characteristics are presented in Table 2.

#### Calculations and statistical analyses

In this study, we measured net uptake rates over 120 min which represent the difference of gross uptake (influx) minus efflux, and that efflux at low concentrations ( $\mu$ M range) constitutes between 10% and 30% of gross uptake (Macduff and Jackson 1992, Kronzucker et al. 1995, Min et al. 1999, Warren 2016). <sup>15</sup>N atom% excess (APE) was calculated as the difference in atom%<sup>15</sup>N between <sup>15</sup>N-labeled roots and unlabeled (control) roots. Net N uptake rates (NUR:  $\mu$ g N g<sup>-1</sup> d.w. root h<sup>-1</sup>) were calculated by multiplying root N content ( $\mu$ g N g<sup>-1</sup>) by the corresponding (APE/100), divided by labeling time in hours and (atom%<sup>15</sup>N/100) of the applied <sup>15</sup>N-labeled N form (10% for NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, 98% for glycine).

NUR (
$$\mu$$
g N g<sup>-1</sup>root d.w. h<sup>-1</sup>) =  $\frac{N \operatorname{content}\left(\frac{\mu g}{g}\right) \times \frac{APE}{100}}{\operatorname{time}(h) \times \frac{\operatorname{at} \%^{15} N \operatorname{tracer}}{100}}$ 

Uptake rates of each N form were normalized to the sum of uptake rates of the three forms to calculate its contribution to total N uptake. Arbuscular mycorrhizal colonization was calculated as the proportion of colonized root counts of total counts by the dissecting microscope. Ectomycorrhizal colonization was calculated as the ratio of root length that was mycorrhizal to total root length.

Differences in uptake rates of and contributions for three N forms among tree species, mycorrhizal types, forest types and

Table 2. Soil nitrate, ammonium, glycine and total free amino acid (TFAA) contents and mycorrhizal root colonization rates of 8 dominant tree species in a temperate forest (Donglingshan) and 13 dominant tree species in a tropical forest (Xishuangbanna). Values are presented as means  $\pm$  SE of four replicates. Mean values of each index for temperate and tropical forests are showed in bold.

Tree species	$NO_{3}^{-}-N \ (\mu g \ g^{-1})$	$NH_4^+-N \ (\mu g \ g^{-1})$	Gly-N ( $\mu$ g g <sup>-1</sup> )	TFAA ( $\mu g g^{-1}$ )	Mycorrhizal colonization rate (%)
					ECM/AM
Temperate species					
Pinus tabuliformis	8.10 ± 0.20	20.61 ± 1.42	0.12 ± 0.00	0.95 ± 0.02	20 ± 2.67
Betula dahurica	7.61 <u>+</u> 3.47	24.9 <u>+</u> 4.84	0.13 <u>+</u> 0.01	0.89 <u>+</u> 0.07	30 <u>+</u> 1.46
Betula platyphylla	14.95 <u>+</u> 4.02	13.29 <u>+</u> 4.72	0.11 <u>+</u> 0.01	1.05 <u>+</u> 0.07	31 <u>+</u> 5.41
Populus davidiana	6.52 <u>+</u> 0.31	20.76 ± 1.16	0.12 ± 0.00	0.94 ± 0.02	17 <u>+</u> 4.29
Juglans mandshurica	12.67 <u>+</u> 1.52	5.00 ± 0.40	$0.09 \pm 0.00$	1.17 ± 0.01	33 <u>+</u> 3.35
Quercus wutaishanica	5.67 ± 0.61	12.80 <u>+</u> 0.97	0.11 ± 0.00	1.06 ± 0.01	23 ± 6.04
Acer mono	9.73 ± 0.70	31.49 ± 1.61	0.14 ± 0.00	0.79 ± 0.02	26 <u>+</u> 6.77
Larix principis-rupprechtii	3.64 ± 0.89	7.72 <u>+</u> 2.63	0.10 ± 0.00	1.13 ± 0.04	40 ± 3.51
Mean value	8.61 <u>+</u> 0.94	17.07 <u>+</u> 1.92	0.11 ± 0.00	1.00 <u>+</u> 0.03	27 <u>+</u> 1.86
Tropical species					
Cleidiocarpon cavaleriei	2.17 ± 0.64	35.32 ± 10.88	0.14 ± 0.02	0.74 ± 0.15	52 ± 7.96
Ficus tinctoria	35.51 <u>+</u> 2.45	51.46 ± 6.55	0.17 ± 0.01	0.51 ± 0.09	38 ± 1.66
Macaranga denticulata	7.34 ± 3.46	43.44 <u>+</u> 7.08	0.16 ± 0.01	0.62 ± 0.10	57 <u>+</u> 4.73
Sapindus mukorossi	3.58 <u>+</u> 1.06	29.83 <u>+</u> 3.97	0.13 <u>+</u> 0.01	0.82 <u>+</u> 0.06	54 <u>+</u> 7.29
Syzygium szemaoense	1.92 ± 0.24	32.46 <u>+</u> 2.79	0.14 ± 0.00	0.78 ± 0.04	54 <u>+</u> 1.52
Anogeissus acuminata var. lanceolata	16.89 <u>+</u> 2.68	34.91 ± 4.61	0.14 ± 0.01	0.74 ± 0.07	54 <u>+</u> 2.38
Parashorea chinensis	11.40 <u>+</u> 0.90	61.15 <u>+</u> 7.44	0.19 <u>+</u> 0.01	0.37 <u>+</u> 0.10	27 <u>+</u> 0.49
Pometia tomentosa	4.62 ± 1.03	34.35 <u>+</u> 4.88	0.14 ± 0.01	0.75 ± 0.07	47 <u>+</u> 0.71
Chukrasia tabularis var. velutina	2.49 ± 0.65	32.35 ± 3.17	0.14 ± 0.01	0.78 ± 0.04	38 <u>+</u> 2.23
Tectona grandis	4.66 <u>+</u> 0.39	30.99 <u>+</u> 1.28	0.14 ± 0.00	0.80 <u>+</u> 0.02	46 <u>+</u> 5.34
Flacourtia rukam	13.39 ± 4.06	29.70 ± 3.94	0.13 ± 0.01	0.82 ± 0.06	35 <u>+</u> 2.65
Lithocarpus balansae	12.34 <u>+</u> 0.82	33.21 ± 6.10	0.14 ± 0.01	0.77 ± 0.09	35 <u>+</u> 5.11
Ficus altissima	3.83 <u>+</u> 1.55	45.02 <u>+</u> 5.31	0.16 <u>+</u> 0.01	0.60 <u>+</u> 0.07	23 <u>+</u> 4.30
Mean value	9.36 <u>+</u> 1.33	37.77 <u>+</u> 1.77	$0.15 \pm 0.00$	0.70 <u>±</u> 0.02	43 <u>+</u> 1.80

successional stages were analyzed by one-way analysis of variance (ANOVA). The effects of N form and forest type on N uptake rates were examined by two-way ANOVA. Correlations between mycorrhizal colonization rates and N uptake rates and contributions were analyzed by Pearson correlation. To better obey normal distribution, natural logarithmic transformation (In) of primary data were used for every ANOVA and correlation analyses. Statistical analysis was carried out by SPSS 18 (SPSS Inc., Chicago, IL, USA) and considered as significant at P < 0.05. Figures were plotted with SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). All differences were tested for significance at P = 0.05.

# Results

Soil N concentrations were tested across the two forest types. Soil NH<sub>4</sub><sup>+</sup> concentrations in the temperate forest (mean value  $17.1 \pm 3.19 \,\mu\text{g} \text{ N g}^{-1}$ ) were significantly lower than those in the tropical forest (mean value 38.0  $\pm$  2.65 µg N g<sup>-1</sup>, P < 0.001), while soil  $NO_3^-$  concentrations in the temperate forest (mean value 8.57  $\pm$  1.33 µg N g<sup>-1</sup>) were similar to that in the tropical forest (mean value 9.24  $\pm$  2.58 µg N g<sup>-1</sup>). Soil glycine concentrations in the temperate forest were on average 0.11  $\pm$ 0.001  $\mu$ g N g<sup>-1</sup>, being significantly lower than in the tropical forest (P < 0.05, Table 2). Soil glycine contributed 14.5% and 15.8% to total free amino acids (FAA) in the temperate forest and the tropical forest, respectively. Total extractable N (i.e., FAA +  $NO_3^- + NH_4^+$ ) averaged 26.68 ± 2.24 µg N g<sup>-1</sup> d.w. soil in the temperate forest and 47.84  $\pm$  2.50 µg N g<sup>-1</sup> d.w. soil in the tropical forest (P < 0.001). However, total FAA comprised only a small fraction of available N in soils (2-5%) of the sum of NH<sub>4</sub><sup>+</sup>,  $NO_3^-$  and FAA) in both forests, while  $NO_3^-$  constituted 34% and 17% and  $NH_4^+$  61% and 81% in temperate and tropical forests, respectively.

Among the three N forms, all 21 species took up  $NH_4^+$  at the highest rate, with mean values of 12.8  $\pm$  1.22 µg N g<sup>-1</sup> d.w. root h<sup>-1</sup> in the temperate forest, being significantly higher than in the tropical forest with 6.77  $\pm$  0.43  $\mu g$  N  $g^{-1}$  d.w. root  $h^{-1}$ (Figure 1, P < 0.001). In the temperate forest, L. principisrupprechtii showed the highest uptake rates for NH4<sup>+</sup> and P. tabuliformis and A. mono the lowest. In the tropical forest, M. denticulata and P. chinensis showed highest uptake rates for  $NH_4^+$ , with the lowest uptake rates for S. szemaoense. Glycine uptake rates of tree species in the temperate forest ranged from  $0.88 \pm 0.16$  to  $5.93 \pm 0.87 \,\mu g \,\text{N g}^{-1}$  d.w. root h<sup>-1</sup>, and were significantly higher than  $NO_3^-$  uptake rates (ranging from 0.13  $\pm$ 0.03 to 1.60  $\pm$  0.54 µg N g<sup>-1</sup> d.w. root h<sup>-1</sup>) (P < 0.001). In the tropical forest, glycine uptake ranged from 1.17  $\pm$  0.82 to 4.87  $\pm$  $0.60 \ \mu g \ N \ g^{-1} \ d.w.$  root  $h^{-1}$ , and was also significantly higher than uptake of  $NO_3^-$  (ranging from 0.58 ± 0.05 to 2.17 ±  $0.20 \ \mu g \ N \ g^{-1} \ d.w. \ root \ h^{-1}) \ (P < 0.001)$ . Among the temperate tree species, L. principis-rupprechtii had the highest uptake rates for glycine while P. tabuliformis showed the lowest. In the tropical forest, *P. chinensis* demonstrated the highest uptake for glycine and *C. cavaleriei* the lowest (Figure 1). Overall, temperate tree species showed higher uptake rates for  $NH_4^+$  (*P* < 0.001) and lower uptake rates for  $NO_3^-$  (*P* < 0.001), but similar ones for glycine (*P* = 0.347) compared with tropical tree species (Figure 1).

Overall, the uptake preference for the three N forms was in the order:  $NH_4^+ > glycine > NO_3^-$  (P < 0.001), both for temperate and tropical tree species (Figure 2). Uptake of  $NH_4^+$  accounted for 76% of total N uptake (sum of uptake of the three N forms) in the temperate forest and was significantly higher than that in the tropical forest (64%, Figure 2, P < 0.001). The contribution of glycine uptake for the temperate forest trees was about 20% of total uptake of all three N forms, being significantly lower than the 23% for the tropical forest (P = 0.038). Moreover, the contribution of NO<sub>3</sub><sup>-</sup> uptake was also significantly higher in tropical (13%) than in temperate forest had significantly higher NH<sub>4</sub><sup>+</sup> and lower glycine and NO<sub>3</sub><sup>-</sup> preferences than that in tropical forest.

There was no significant effect of forest type on N uptake rates when tested across all three N forms in a two-way ANOVA (P =0.715), but we found a significant interaction term between N form and forest type (Table 3). All tree species investigated in the temperate forest were ECM, while all trees in the tropical forest were AM except for one ECM species. The sampling design therefore did not allow us to statistically separate between mycorrhizal type and forest biome effects on root N uptake. Ectomycorrhizal trees showed significantly higher  $NH_4^+$  and lower  $NO_3^-$  uptake rates (P < 0.001), but similar glycine (P = 0.173) uptake rates than AM trees. The uptake of NO3<sup>-</sup> was significantly higher in AM than in ECM trees (P < 0.001) while that of NH<sub>4</sub><sup>+</sup> was lower (P <0.001). In the temperate forest, mycorrhizal colonization rates positively affected uptake rates of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, while they only negatively affected NH<sub>4</sub><sup>+</sup> in tropical forest. In total, mycorrhizal colonization rates positively affected rates (P = 0.001) and contributions for NO<sub>3</sub><sup>-</sup> uptake (P < 0.001) but did not significantly affect  $NH_4^+$  (P > 0.05) and glycine (P > 0.05), as shown in Table 4. Successional stage had no significant effect on the uptake rate of any N form ( $P_{(NO3^{-})} = 0.113$ ,  $P_{(NH4^{+})} = 0.125$ ,  $P_{(glycine)} =$ 0.491). Successional stage and its interaction with forest type was also not significant in a two-way ANOVA ( $P_{(NO3^{-})} = 0.330$ ,  $P_{(NH4^{+})} =$ 0.305,  $P_{(\text{glycine})} = 0.469$  for successional stage, Figure 3). Temperate tree species took up more  $NH_4^+$  and less  $NO_3^-$  than the tropical tree species in either early or late successional stages (one-way ANOVA,  $P_{(NO3^{-})} = 0.003$ ,  $P_{(NH4+)} = 0.016$ ,  $P_{(glycine)} =$ 0.254 for early succession,  $P_{(NO3^{-})} = 0.001$ ,  $P_{(NH4^{+})} = 0.012$ ,  $P_{(\text{qlycine})} = 0.731$  for late succession, Figure 3).

# Discussion

In this study, 21 dominant tree species (8 in the temperate forest and 13 in the tropical forest) were selected to examine their

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Figure 1. Uptake rates of  $NH_4^+$ , glycine and  $NO_3^-$  by 8 dominant tree species in a temperate forest (A) and 13 dominant tree species in a tropical forest (B). Values are presented as means  $\pm$  SE of eight replicates. Different letters above the bars indicate significant differences in uptake rates between N forms for each species (one-way ANOVA, *P* < 0.05). Three-letter species codes are given in Table 1.

uptake rates of and contributions for organic and inorganic N forms by  $^{15}N$  labeling of intact roots in field hydroponic experiments. We found that all targeted trees in both temperate and tropical forests showed a strong preference for  $\rm NH_4^+$  over glycine and  $\rm NO_3^-$  uptake although equimolar concentrations were simultaneously supplied for all three N forms. Such uptake patterns differ from a previous study in a temperate forest of Australia

(Warren 2009), but were to some extent similar to a study in a subtropical forest in China (Li et al. 2015). This indicates that there is plasticity in plant N uptake that is affected by various factors such as ecosystem type, soil development, plant identity, mycorrhizal type and colonization rate as well as successional stage.

Beyond the general preference for  $NH_4^+$ , all targeted trees showed higher uptake rates for glycine than for  $NO_3^-$ . This



Figure 2. The contribution of glycine,  $NO_3^-$  and  $NH_4^+$  to total N uptake by 8 dominant tree species in a temperate forest (A) and 13 tree species in a tropical forest (B). Values are presented as means  $\pm$  SE of eight replicates. Three-letter species codes are given in Table 1.

confirms that tree species have the capacity to take up organic N in the form of FAA from soils, though most previous studies focused on plant species from regions that are characterized by low annual temperature and high dissolved soil organic N content, e.g., alpine (Lipson et al. 2001, Xu et al. 2004, 2006) and boreal ecosystems (Näsholm et al. 1998, Näsholm and Persson 2001,

Leduc and Rothstein 2010). The reason for this is that the contribution of organic N to plant nutrition has been predicted to increase from tropical to arctic ecosystems due to increasing dominance of organic N in the plant-available N pool (Read 1991). Our study does not support this prediction, either in terms of the composition of available soil N or in patterns of N uptake. Though

Tree Physiology Online at http://www.treephys.oxfordjournals.org by Xishuangbanna Tropical Botanical Garden, CAS user on 15 December 2017 temperate forests showed a trend towards higher glycine uptake rates than tropical forest, the contribution of glycine to total N uptake was slightly lower than for tropical trees (20% for temperate trees versus 23% for tropical trees). However, this trend was not statistically significant and the data therefore contradict our first hypothesis. In addition, there are a variety of other FAA

Table 3. Two-way ANOVA testing the effects of type of forest (temperate and tropical forest) and N forms (glycine,  $NO_3^-$  and  $NH_4^+$ ) on N uptake rate by 21 tree species. *P* values for significant effects and interactions are reported.

Factors	Mean square	df	F	P values
Forest type	0.051	1	0.134	0.715
N form	104.487	2	272.037	<0.001
Forest type $\times$ N form	8.046	2	20.947	<0.001
Error	91.029	237	-	-

available in soils, and in this study, soil glycine constituted about 15% of total FAA in both forests. Moreover, total FAA constituted only a small fraction (2-5%) of the extractable labile N pool in soils. Such low FAA fraction is ascribed to its measurement on water extraction but  $NH_4^+$  and  $NO_3^-$  measured on 0.05 M K<sub>2</sub>SO<sub>4</sub> extracts. The relatively high uptake of glycine in both forests relative to the proportion of total FAA (and glycine) in the extractable N pool is striking and points to greater root uptake of total FAA than evident from soil extracts. Recently, Inselsbacher and Näsholm (2012) applying microdialysis approaches demonstrated that in boreal forests bulk soil extracts strongly underestimate N supply rates through diffusion to roots for total FAA. The contribution of total FAA to soil extracts was ~15% but diffusive fluxes provided ~80% in the form of total FAA to root imitates compared with soil inorganic N. But certainly, uptake rates of N forms measured at equimolar concentrations (at 100 µM) cannot

Table 4. Correlation analysis between mycorrhizal colonization rates and N uptake rates and contributions. *P* values for significant effects and interactions are reported at P < 0.05 (\*) or P < 0.01 (\*\*) level. Number in bold indicates that the effect was significantly different.

		N uptake rates			N uptake contributions		
		NO <sub>3</sub> -	Glycine	NH4 <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	Glycine	${\rm NH_4}^+$
Temperate forest	Pearson correlation	0.352*	0.344	0.568**	0.041	-0.224	0.244
	P values	0.049	0.054	0.001	0.824	0.217	0.179
Tropical forest	Pearson correlation	-0.060	-0.149	-0.383**	0.129	0.166	-0.274
	P values	0.683	0.307	0.007	0.378	0.255	0.057
Total	Pearson correlation	0.372**	-0.041	-0.154	0.428**	-0.076	-0.213
	P values	0.001	0.717	0.170	0.000	0.502	0.056



Figure 3. Uptake of glycine,  $NO_3^-$  and  $NH_4^+$  by trees of early and late successional stages in a temperate forest and a tropical forest. Different lowercase letters above the bars indicate significant differences between temperate and tropical forest tree species in either early or late successional stages (one-way ANOVA, P < 0.05). No significant differences were found for early and late successional species for any N form within either temperate or tropical forests.

be directly transferred to in situ uptake rates. Another uncertainty relates to using glycine uptake as a surrogate for total FAA uptake. Only few data are available to assess this issue, but recently Warren (2016) showed for <sup>13</sup>C-labeled metabolite mixtures administered to wheat roots that net uptake rates (NUR, recalculated) of individual amino acids were strongly related to their external concentration ( $R^2 = 0.83$ , NUR = concentration  $\times 0.064$  + 0.055), with the exception of Asp showing low uptake rates at high concentrations. To avoid this, we would in future studies recommend to apply FAA mixtures representative of their composition in soils instead of only glycine to study amino acid uptake and to test the hypothesis that high latitude tree species have a greater uptake of amino acids. Particularly, we have to mention that we used an equimolar solution of the three N forms in this study while the quantity and composition of N forms could be different from their native concentrations in soils. Additionally, NO<sub>3</sub><sup>-</sup> loading into the xylem and shoot export can be substantial, which might cause an underestimation of NO3<sup>-</sup> uptake rates in this study where plant parts distal to incubated fine roots were not investigated which would comprise an almost impossible task because of the large tree biomass and for sensitivity reasons. However, the fact that a substantial proportion of NO3<sup>-</sup> taken up by tree roots can be already assimilated into NH4<sup>+</sup> and amino acids in roots (Andrews 1986), and the low export of the latter assimilatory products, greatly reduces this uncertainty (Min et al. 1999). The extraction efficiency of  $NO_3^-$  is therefore greater than for  $NH_4^+$  and amino acids in soil water extractions, causing a shift from FAA and  $NH_4^+$ towards NO<sub>3</sub><sup>-</sup> in water extracts (Inselsbacher and Näsholm 2012, Li et al. 2012, Shaw et al. 2014). Different N uptake rates between temperate and tropical trees could be a strategy for them to adapt to their habitats in terms of N availability and length of growing season. In temperate regions, trees have shorter growing seasons with lower precipitation and temperature than in the tropical region. Although uptake of N was measured in the late growing season, temperate trees still demonstrated higher uptake rates to acquire N, which has to be accomplished in a shorter time period to meet their N demand for growth. In contrast, tropical forest trees have longer growing seasons and longer time periods to take up soil N. Temperate tree species may therefore need to up-regulate their N uptake capacity to obtain enough available N for growth during the shorter vegetation period. However, the mechanisms behind higher N uptake rates (mainly for  $NH_4^+$ ) in temperate versus tropical tree species still need further investigation, but the relation with differences in season length and mycorrhizal type may be important.

All investigated tree species in temperate and tropical forests showed a preference for  $NH_4^+$  uptake. Schimel and Bennett (2004) predicted that plant N preferences will be different across arctic to tropical ecosystems as dominant N forms shift because different N transformation processes dominate. They predicted that plants would prefer organic N in boreal forests and tundra, temperate plants would prefer  $NH_4^+$  whereas tropical plants would prefer  $NH_4^+$  whereas tropical plants would prefer  $NH_4^-$  whereas tropi

tropical tree species showed great preference to take up NH<sub>4</sub><sup>+</sup> (accounting for 76% in the temperate forest and 64% of total N uptake in the tropical forest). Our results further showed that  $NH_4^+$  preference was significantly greater in temperate than in tropical tree species while NO3<sup>-</sup> uptake was significantly higher in tropical than in temperate tree species, which partly confirms our second hypothesis. However, a main point to be made from our study is that temperate and tropical tree species behaved more similar than different in their N uptake preferences, which contradicts the prediction by Schimel and Bennett (2004). Studies of N uptake in tree species along N availability gradients showed that N uptake rates can change markedly depending on atmospheric N deposition and soil N availability (Högberg et al. 1998, Nordin et al. 2001). Under similar concentrations of N forms in soils (e.g.,  $NH_4^+$ :glycine:  $NO_3^-$  being 1:1:1), trees take up  $NH_4^+$  at the maximum rate. Considering there was more  $NH_4^+$  than  $NO_3^-$  and FAA extractable from the soils sampled around the tree species (Table 2) and the proportion of  $NH_4^+$ :  $NO_3^-$  was greater than 1:1 (2:1 temperate, 4:1 tropical forest), these trees may also show higher uptake rates for  $NH_4^+$  than for  $NO_3^-$  in their native environments. However, we have to admit that  $NO_3^-$  uptake rates might be underestimated due to fast xylem loading and transport of NO3<sup>-</sup> to aboveground tissues. Besides, more mobile NO3<sup>-</sup> than  $NH_4^+$  (Owen et al. 2001) and strong microbial competition for amino acids and  $NH_4^+$  with plants in the rhizosphere (Jansson 1971, Haynes and Goh 1978, Kuzyakov and Xu 2013) could also underestimate NO<sub>3</sub><sup>-</sup> uptake. The uncertainty above calls for further investigations of N uptake by plants and microbes over wider spatiotemporal scales to better understand plant N uptake patterns across sites, regions and biomes. The low uptake rates of NO3<sup>-</sup> in both temperate and tropical forests could be ascribed to the low availability of  $NO_3^-$  in these soils. The application of field hydroponic approaches excluded the effects of microbes, which can strongly compete with plants for N in the rhizosphere in the short term (Lipson and Näsholm 2001, Kuzyakov and Xu 2013). Considering the plasticity in plant N acquisition, more in situ labeling experiments should be conducted to explore controls on root N uptake in forests over large spatiotemporal scales in the future (Xu et al. 2011).

Plant preferences for N forms can trigger important ecological consequences mainly through affecting biogeochemical processes (Britto and Kronzucker 2013). First, high uptake rates of  $NH_4^+$  by roots may cause soil acidification in the rhizosphere and thereby further affect the nutrient status in these forests. This is because roots can secrete protons to take up  $NH_4^+$  and  $NH_4^+$  assimilation releases protons in the cytoplasm (Nye 1981, van Breemen et al. 1983). Second, the strong preference of plants for  $NH_4^+$  could decrease nitrification and  $NO_3^-$  production through providing less  $NH_4^+$  to nitrifiers. Consequently, such tree N preferences could reduce the risk of  $NO_3^-$  leaching and  $N_2O$  emissions (Britto and Kronzucker 2013). Third, approximately 80% of total cations and anions taken up by roots

are either  $NH_4^+$  or  $NO_3^-$  (Cui et al. 2016). A plant preference for  $NH_4^+$  could lead to excess uptake of cations and affect the balance between cation and anions in cells and thus cell metabolism (Marschner 1995, Torres-Olivar et al. 2014).

All targeted tree species were further classified into ECM versus AM trees and early versus late successional species (Table 1). Numerous studies have suggested that mycorrhizal plants can take up more N by roots than non-mycorrhizal ones (Sokolovski et al. 2002, Alexander 2007, Smith and Read 2008) and many studies have explored the differences in N uptake patterns between ECM and AM trees (George et al. 1995, Miransari 2011, Veresoglou et al. 2012). A previous study in a subtropical forest of China demonstrated that AM tree species have the ability to acquire  $NO_3^-$  faster than ECM tree species (Li et al. 2015). In the current study, ECM trees had higher uptake rates for NH<sub>4</sub><sup>+</sup>, lower ones for NO<sub>3</sub><sup>-</sup> and similar ones for glycine compared with AM trees.  $NO_3^-$  uptake rates were significantly higher in AM than in ECM trees while that for NH4<sup>+</sup> was lower. Particularly, in total mycorrhizal colonization rates significantly affected the uptake rates and the contributions for NO3<sup>-</sup>. Considering that AM trees have higher uptake rates and contributions for  $NO_3^-$ , it should be the AM fungi that enhance uptake of NO3<sup>-</sup>. Uptake and transport of NO<sub>3</sub><sup>-</sup> by AM plants can be affected by its concentration in soils and the age of AM symbiosis (Azcón et al. 2001). However, recent studies showed that AM external hyphae can mobilize soil NO3<sup>-</sup> and transfer it to plant root cells, increasing AM plants' inflow of N (Johansen et al. 1993, Azcón et al. 1996). Until now, it has been reported that AM fungi can decrease soil NO3<sup>-</sup> concentrations by uptake and  $NO_3^-$  assimilation, indicating that AM fungi have the gene set for this process (Ho and Trappe 1975, Kaldorf et al. 1994, 1998, Azcón and Tobar 1998), and our results supported this. Moreover, ECM trees take up NH<sub>4</sub><sup>+</sup> at higher rates than AM trees, which means that ECM fungi have a greater ability to promote NH<sub>4</sub><sup>+</sup> uptake than AM fungi. One reason for this may relate to ECM fungi excreting ectoenzymes for organic N decomposition and mineralization. However, so far there is no strong evidence to prove that this may enhance  $NH_4^+$  utilization. Several studies showed that ECM fungi can greatly improve the access to high-molecular weight organic N for the host plant compared with AM fungi and with non-mycorrhizal plants (Marschner and Dell 1994). We did not find significant differences in glycine uptake rates between ECM and AM tree species. Moreover, all tree species investigated in the temperate forest were ECM plants and those in the tropical forest, except one (F. altissima), were AM plants. The differences in the uptake of N forms between tropical and temperate trees therefore almost reflect the patterns found between ECM and AM trees, and testing for ECM versus AM effects independent of forest biome did not increase the statistical power but rather decreased it. Effects of mycorrhizal type independent of forest biome on N uptake rates should therefore be further explored, but could not be tested here.

Previous studies showed that late successional tree species acquire more NH4<sup>+</sup> and less NO3<sup>-</sup> than early successional species (Britto and Kronzucker 2013). Our results did not corroborate this as there was no significant difference in N uptake rates or contributions between early and late successional tree species in this study. In addition, successional stage and its interaction with forest type showed no significant effect of successional stage. The general predicted tendency of early successional tree species to have enhanced uptake rates and contributions for NO<sub>3</sub><sup>-</sup> is based on the high availability of this N form after tree fall or clear cutting due to enhanced mineralization and nitrification after disturbance (Binkley and Hart 1989, Pardo et al. 2002). We showed here that this is not necessarily based on inherent differences in uptake capacities between early and late successional tree species. In this study, we sampled tree species in relatively mature forests and classified them as early and late successional, based on their occurrence in secondary successional chronosequences reported by others. In mature oldgrowth forests, tree fall caused by natural mortality or storms and pest outbreaks causes opening of large (tree-fall) gaps, which are colonized by early successional species. Gap dynamics are an important characteristic of old-growth forests, allowing the establishment and coexistence of early and late successional species in the same forest. However, decades after disturbance when early successional species have grown to large size, soil biogeochemistry is expected to have reached an equilibrium, closely resembling undisturbed sites where, e.g., NO3<sup>-</sup> has declined to background levels.

In summary, based on one of the most extensive studies of tree N uptake, we conclude that in both temperate and tropical forest ecosystems, tree root uptake rates and preferences for soil N forms were in the following order:  $NH_4^+$  > glycine >  $NO_3^-$ . Uptake preference for  $NH_4^+$  was significantly higher in temperate trees than in tropical trees, but glycine and NO3uptake preferences for temperate trees were lower than those of tropical trees. Arbuscular mycorrhizal trees showed higher  $NO_3^-$  uptake and lower  $NH_4^+$  uptake rates than ECM trees. Mycorrhizal colonization rates can affect uptake rates and contributions of inorganic N, but the effect relies on forest types. These findings suggest the great complexity of uptake of N by trees in the field, constrained by various factors such as tree size, seasonal changes in uptake capacity, and the inherent difficulty of measuring available N concentrations, etc. Our results only provide a snapshot of N uptake by mature trees. In future studies, new approaches should be developed to monitor in situ uptake of organic and inorganic N to connect with biogeochemistry in forests.

# **Conflict of interest**

None declared.

Nitrogen uptake by temperate and tropical tree species 1525

# Funding

This study was supported by the National Natural Science Foundation of China (31470560 and 41601318) and Youth Innovation Research Team Project (LENOM2016Q0004).

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