



Niche partitioning in nitrogen uptake among subtropical tree species enhances biomass production



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HIGHLIGHTS

- Niche partitioning in N uptake forms among tree species increases with richness.
- Plant coexistence increased at higher species richness by decreasing N niche overlap.
- N uptake complementarity is reflected in above- and belowground functional traits.
- N uptake niche can explain for biomass productivity increasing with species richness.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrogen (N) is a main nutrient limiting plant growth in most terrestrial ecosystems, but so far it remains unknown which role plant N uptake plays for the positive relationship between species richness and productivity. An *in situ* ¹⁵N labeling experiment was carried out by planting four subtropical tree species (*i.e.*, *Koeleruteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*) in pots, at richness levels 1, 2 and 4 species per pot. Plant N uptake preference for inorganic N form of NO₃⁻ to NH₄⁺ and organic N form of glycine, as well as biomass and plant functional traits was evaluated under different tree species richness level. Overall, pot biomass productivity increased with tree species richness. Biomass of the most productive species, *K. bipinnata* increased, but not at the expense of a decreased growth of the other species. In mixtures, the species shifted their preference for the inorganic N form, from NO₃⁻ to NH₄⁺ or vice versa. The uptake preference for glycine remained stable along the species richness gradient. Plant N uptake was well correlated with numerous functional traits, both aboveground, such as height and shoot diameter, and belowground, such as root diameter and root length. We conclude that increased ecosystem biomass production with tree species richness could be largely explained by niche partitioning in N uptake among tree species. Our findings highlight that niche partitioning for N uptake should be a possible important mechanism maintaining species diversity and ecosystem production in subtropical forests.

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1. Introduction

The relationship between plant diversity and ecosystem functioning has become one of the key topics in ecological studies (Reich et al., 2001; Zak et al., 2003). Positive biodiversity effects on ecosystem productivity have been demonstrated by various studies (von Felten et al., 2009; Cardinale, 2011). Because of the global importance of forests for ecosystem services (Mori et al., 2017), corresponding studies demonstrated positive effects of tree richness on carbon storage (Hulvey et al., 2013), stand volume (Huang et al., 2018) and biomass productivity (Liang et al., 2016; Mori, 2018; Tatsumi, 2020). Niche partitioning, brought about by a complementary resource use of the coexisting plant species (Ashton et al., 2010), has been suggested to be the prevailing mechanism to explain these positive relationships (Loreau and Hector, 2001; Zhu et al., 2015; Huang et al., 2018). Niche partitioning in resource use among coexisting plant species can enhance the acquisition for limiting resources such as soil nutrients (Liu et al., 2020; Phoenix et al., 2020). Moreover, niche partitioning can enhance resource use efficiency, and thus, lead to higher productivity (Harrison et al., 2001; Cardinale et al., 2007).

One of the most important soil resources for plants is nitrogen (N), which is made available to plants through microbial mineralization of organic matter (Moreau et al., 2019). In consequence, ecosystem productivity in various terrestrial ecosystems is tightly linked to microbial N mineralization and subsequent mineral N uptake by plants (Reich et al., 2006; Elser et al., 2007; LeBauer and Treseder, 2008). Except for some plants that can fix atmospheric N_2 through symbiosis with bacteria, most plant species in terrestrial ecosystems rely on N uptake from the soil solution by roots (Kiers et al., 2003). As a result, coexisting plant species frequently compete for available N from the soil solution (Henneron et al., 2020; Liu et al., 2020). The form of N taken up by the roots is either inorganic (i.e., mineral) or organic (Chapin et al., 1993; Näsholm et al., 1998; Liu et al., 2017). Among the two mineral N forms, NH_4^+ is leached less from soil than NO_3^- , because NH_4^+ can be adsorbed on the negative surface charges of the soil clay particles (Gurevitch et al., 2020). As increased N uptake by plants can decrease NO_3^- leaching or denitrification, plant N uptake patterns play an important role in soil N loss (Van Groenigen et al., 2015).

Nitrogen uptake by plants is primarily dependent on the concentrations of available N in the soil (Sauheitl et al., 2009), microbial activities (Kuzakov and Xu, 2013), plant functional traits (Leffler et al., 2013) and plant species identity (Zhang et al., 2015). However, under conditions of N limitation, neighboring plant species can alleviate competition for N in the soil solution through niche partitioning, by either preferring different chemical forms (Liu et al., 2020) or by varying the spatial (McKane et al., 2002) and temporal pattern of N uptake (Liu et al., 2016; Cui et al., 2017). This complementary utilization of soil available N is potentially an important mechanism underlying the observed species richness-productivity relationships (Cardinale, 2011; Lambers and Oliveira, 2019). This idea is supported by the observation that soil N retention is increased at high plant species richness levels (Lang et al., 2014; Furey and Tilman, 2021). So far, these findings have been explained by an increased niche partitioning through combining plant species that display species-specific N uptake patterns. However, no study has explored yet to which degree the same plant species plastically adjust their N uptake patterns to increase niche partitioning in mixtures.

We addressed the question of whether plants are plastic in their N uptake preference when diversity increases using subtropical tree species. Subtropical/tropical forests are thought to be characterized by high soil NO_3^- availability and uptake, because of the high N nitrification rates under high temperatures, resulting in high NO_3^- but low NH_4^+ and organic N uptake (Schimel and Bennett, 2004). To detect whether plastic niche partitioning in N uptake by trees is affected by species richness, we studied four subtropical tree species of *Koeleria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei* in a common garden experiment. The same species are part of the BEF-China tree diversity experiment in Jiangxi, China (Bruehlheide et al., 2014). To assess the uptake rates of different N uptake forms, *in situ* ^{15}N labeling of NO_3^- , NH_4^+ and glycine

was performed. Glycine was chosen to represent organic N, as glycine is one of the dominant amino acids in soil and generally used in experiments of plant N uptake (Näsholm et al., 1998; McKane et al., 2002; Liu et al., 2017). Plant biomass, soil available N and plant N uptake were measured and calculated, including plant functional traits of stems, leaves and roots, to explore how N niche partitioning contributes to biomass production over a tree species richness gradient.

Considering the positive effects of plant diversity in previous studies, we hypothesized that: i) the biomass of the planted tree community increases with tree species richness, which is also reflected in increased total plant N uptake, ii) the underlying mechanism is increased niche partitioning in N uptake between the tree species in the community, brought by both species-specific N uptake but also by a plastic adjustment of N uptake form, and (iii) the plastic adjustment in N uptake as response to community richness is also reflected in plant functional traits, in particular in below-ground traits.

2. Materials and methods

2.1. Study site, soil, and species preparation

This study was conducted in a greenhouse of the Jiangxi Key Laboratory of Plant Resources and Biodiversity, Jingdezhen University, China (29°22' N, 117°13'E, 43.4 m a.s.l.). This area is characterized by a typical subtropical seasonal climate, with a mean annual temperature of 17.5 °C and a mean annual precipitation of 18.5 mm.

We chose the local red earth, sandy soil and nutrient soil, then homogeneously mixed them with a proportion of 2:2:1. The red earth is the main local soil type belongs to orthic Acrisol category (World Reference Base for Soil Resources, 2014). Nutrient soil was the land surface soil rich in organic matter, red earth was sampled from 0–15 cm depth and sandy soil was sampled from the 15–30 cm depth from land surface. The mixed soil was then sieved by 4 mm mesh. The soil available mineral and organic N concentrations are shown in Fig. S1 and Tables S1, S2.

To examine the effects of tree species richness on N uptake patterns, we selected four local tree species of *K. bipinnata*, *L. glaber*, *Cy. myrsinaefolia* and *Ca. eyrei*. These tree species represent one quarter of the broken stick design of the first random extinction sequence of site A of the biodiversity-ecosystem functioning experiment BEF-China (Bruehlheide et al., 2014), which is located nearby in Xingangshan, Jiangxi Province. The seeds used in this experiment were collected in autumn 2018 and spring 2019 from the Gutianshan National Nature Reserve in Zhejiang Province, which was the same source as used in BEF-China. Seeds were then carefully simultaneously germinated, and seedlings raised in the greenhouse for about 6 months.

2.2. Experimental design

To investigate the effects of tree species richness on N uptake and the consequence for tree productivity, we conducted a three-factorial experiment, including tree species richness (1, 2 and 4), species identity (*K. bipinnata*, *L. glaber*, *Cy. myrsinaefolia* and *Ca. eyrei*), and three N forms (NH_4^+ , NO_3^- and glycine). In October 2019, four individual seedlings each were planted in pots at three richness levels. The four targeted tree species were planted in monocultures, in two different pairs that corresponded to the field design of BEF-China (pair 1: *K. bipinnata* and *L. glaber*; pair 2: *Cy. myrsinaefolia* and *Ca. eyrei*), and in a mixture of all four species. The pots had a diameter of 25 cm and a height of 16.5 cm. Each pot contained exactly 3.5 kg soil. The tree seedlings were irrigated once every two or three days after planting. No extra N fertilizer was applied to the pots during the growing period.

2.3. *In situ* ^{15}N labeling, sampling, and analysis

Short-term *in situ* ^{15}N labeling experiments were carried out on September 10th, 2020 after the tree seedlings had been growing in the

experiment for about one year. A mixture of NH_4^+ , NO_3^- , and glycine (1:1:1 $\text{NH}_4^+ : \text{NO}_3^- : \text{glycine}$) solution was provided to determine tree root N uptake. The labeling solution contained 35 mg N L^{-1} for each N form. In each of the three labeling solutions only one N form was labeled with either 99.14 atom% ($^{15}\text{NH}_4$) $_2\text{SO}_4$, 99.19 atom% $^{15}\text{N-KNO}_3$, or 99.04 atom% glycine. The same volume of distilled water served as a control treatment. Five replicates were set for each treatment/richness level and totally 140 pots were harvested. ^{15}N tracers were gently sprayed on the soil surface around the plants by syringes. There was no irrigation the day before labeling.

Twenty hours after the ^{15}N solution was added to the soil, plant and soil samples were collected from the pots. Soil and plants were taken from the pots and the soil was carefully separated from the plant roots. Harvested soil samples were immediately brought to the laboratory, sieved through a 2 mm-mesh and stored at 4 °C until measurements were made. Fresh soil was extracted with 0.05 M K_2SO_4 and soil NO_3^- and NH_4^+ in the extracts were measured using an AA3 auto analyzer (Bran-Luebbe, Hamburg, Germany). Plants were carefully separated by species and cut at the growing point to separate shoot and roots. Root samples were gently washed by distilled water to remove the attached soil particles and by 50 mM KCl for 3 min and then deionized water to remove the surface ^{15}N . Plant shoots and roots were dried at 60 °C for 72 h before measuring dry biomass. Dried shoots and roots were ground to a fine powder (MM2, Fa. Retsch, Haan, Germany) and weighed in tin capsules. Nitrogen content and $^{15}\text{N}/^{14}\text{N}$ ratios were determined using a continuous-flow isotope mass spectrometry (IRMS) system comprising an EA 1110 elemental analyzer (CE Instruments, Milan, Italy) connected by a ConFlo II device to the spectrometer (Sercon Ltd., Crewe, UK).

Tree functional traits were also measured in the different species richness levels. Leaves were counted and plant height was measured by a ruler from the soil surface. Shoot diameters were measured by vernier calipers at the 1/3 of the total length from the stem base. Leaf thickness was also measured by vernier calipers. After these measurements, the fresh leaf and root samples were immediately placed into Ziplock bags, put into an ice box and brought to the laboratory (Liu et al., 2019). Root samples were ringed under running water. Fresh leaves and roots were arranged on a flat surface without overlap and scanned on an Epson Expression desktop scanner (resolution 300 dpi). Leaf and root images were analyzed by ImageJ software (2.1.0/1.53c; Java 1.8.0_172 [64-bit]) to obtain leaf area, average root diameter and total root length.

2.4. Statistical analysis

^{15}N atom% excess (APE) was calculated by the difference of atom% ^{15}N between labeled and control plant samples. Shoot or root ^{15}N content (μg) was calculated by multiplying root or shoot N content ($\mu\text{mol N g}^{-1}$) by the corresponding ^{15}N atom% excess value (APE/100), biomass (g), and relative molecular mass of ^{15}N (i.e., 15 g mol^{-1}), as shown in the following equation:

$$^{15}\text{N content } (\mu\text{g}) = \text{N content } \left(\frac{\mu\text{mol}}{\text{g}} \right) \times \frac{\text{APE}}{100} \times \text{biomass (g)} \times 15 \left(\frac{\text{g}}{\text{mol}} \right) \quad (1)$$

Plant ^{15}N uptake rates (^{15}NUR : $\mu\text{g g}^{-1} \text{ d.w. root h}^{-1}$) of $^{15}\text{N-NH}_4^+$, $^{15}\text{N-NO}_3^-$, or $^{15}\text{N-glycine}$ were separately calculated by adding root and shoot ^{15}N content together and then divided by the labeling time (h), root biomass (g), as shown below:

$$^{15}\text{NUR } (\mu\text{g g}^{-1} \text{ h}^{-1}) = \frac{\text{root } ^{15}\text{N content } (\mu\text{g}) + \text{shoot } ^{15}\text{N content } (\mu\text{g})}{\text{time (h)} \times \text{root biomass (g)}} \quad (2)$$

Plant N uptake rates (NUR: $\mu\text{g g}^{-1} \text{ d.w. root h}^{-1}$) were calculated by multiplying ^{15}NUR by MN (the concentration of native NH_4^+ , NO_3^- or glycine in soil, $\mu\text{g g}^{-1}$), and then divided by $^{15}\text{N}_{\text{added}}$ (the concentration of added $^{15}\text{N-NH}_4^+$, $^{15}\text{N-NO}_3^-$ or $^{15}\text{N-glycine}$, $\mu\text{g g}^{-1}$) and ^{15}N labeling

abundance (99.14 atom% ($^{15}\text{NH}_4$) $_2\text{SO}_4$, 99.19 atom% $^{15}\text{N-KNO}_3$, or 99.04 atom% glycine) (McKane et al., 2002). The equation used was as follows:

$$\text{NUR } (\mu\text{g g}^{-1} \text{ h}^{-1}) = \frac{^{15}\text{NUR } (\mu\text{g g}^{-1} \text{ h}^{-1}) \times \text{MN } (\mu\text{g g}^{-1})}{^{15}\text{N}_{\text{added}} (\mu\text{g g}^{-1}) \times ^{15}\text{N labeling abundance}} \quad (3)$$

Plant total N uptake rates of each species were calculated by the sum of NH_4^+ , NO_3^- and glycine uptake rates. The uptake preference of each N form was calculated by the percentage of the targeted N form to the total N uptake rates. The root:shoot ratio was calculated by the ratio of root biomass to shoot biomass. Specific root length was calculated by the length of root per gram of root biomass. Multiple linear models were used to analyze i) the effects of $\log_2(\text{Richness})$, species and N form and all interactions of these factors on the soil available N concentrations, N uptake rates and N form preference; ii) the effects of $\log_2(\text{Richness})$ and species on total N uptake rates, biomass (shoot, root and total biomass per species), and plant functional traits; iii) the effect of $\log_2(\text{Richness})$ on biomass per pot (shoot, root and total biomass). Response variables were \log_e -transformed (N uptake) or 1/3 power-transformed (N uptake preference) to achieve normal distribution of residuals. Tukey *post hoc* tests and confidence intervals were calculated using the “emmeans” and “multcomp” package in R (R version: 3.6.1, R core team, Vienna). Principal components analysis (PCA) of plant, soil and N uptake traits was carried out by the package of “factoextra” in R. Correlation between plant and soil characteristics was analyzed by the package of “corrplot” in R.

3. Results

3.1. Soil available N concentrations as a function of tree species richness

Soil available N concentration was significantly affected by tree species richness and the interaction of N form and species (Table S1). With increasing tree species richness, the concentrations of NO_3^- and total free amino acids were significantly decreased in soil under *L. glaber* (Fig. S1, Table S2). Insignificant decrease trends were observed in soil NO_3^- concentrations for *Cy. myrsinaefolia* and *Ca. eyrei*, soil glycine concentrations for all four species, and total concentrations of soil free amino acids for *K. bipinnata*, *Cy. myrsinaefolia* and *Ca. eyrei*.

3.2. N uptake rates as a function of tree species richness

Plant N uptake rates were significantly affected by tree species richness, species, N form and the interactions among these factors (Table 1). The increase in N uptake rates with tree species richness was mainly driven by the species *K. bipinnata*, which showed the highest uptake rates and a positive slope, which, however, was not significantly different from zero (Table S3). With increasing tree species richness, *L. glaber* significantly decreased the uptake of NH_4^+ and glycine, *Cy. myrsinaefolia* decreased the uptake of NO_3^- , and *Ca. eyrei* decreased the uptake of NO_3^- and glycine (Table S3, Fig. 1). In consequence, when summing up NO_3^- , NH_4^+ and glycine, total N uptake did not show any general relationship to tree species richness, but displayed opposing trends for different species (Fig. 1, Table 2). Total N uptake by *K. bipinnata* increased (but not different from zero) with tree species richness, while it decreased in the other three species (significantly different from zero only in *L. glaber*, Fig. 1, Table S4).

3.3. Preference of N uptake as a function of tree species richness

The N uptake preference was significantly altered by the tree species richness (Table 3). The preference for glycine significantly declined for *Ca. eyrei* with tree species richness, while that for *K. bipinnata* and *L. glaber* also showed decrease trends and for *Cy. myrsinaefolia* showed increase trend (not significant when tested species-wise, Fig. 2). The uptake preference for NO_3^- increased for *K. bipinnata* and *L. glaber* (not significant

Table 1

Analysis of variance table of a linear model predicting nitrogen (N) uptake rates from $\log_2(\text{richness})$, species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*) and N form (NO_3^- , NH_4^+ , Glycine). The data of N uptake rates was \log_e -transformed to obtain a normal distribution of residuals. Significant results for P values are shown in bold fonts. Asterisks are added to P values at the levels of “***” for $P < 0.001$, “**” for $P < 0.01$, and “*” for $P < 0.05$.

Factors	df	Sum Sq	Mean Sq	F value	P value
$\log_2(\text{richness})$	1	5.62	5.619	17.661	<0.001***
species	3	111.78	37.261	117.118	<0.001***
N form	2	381.10	190.549	598.928	<0.001***
$\log_2(\text{richness})$: species	3	7.23	2.410	7.576	<0.001***
$\log_2(\text{richness})$: N form	2	2.75	1.376	4.325	0.0149*
species: N form	6	7.03	1.172	3.684	0.0019**
$\log_2(\text{richness})$: species: N form	6	5.12	0.853	2.681	0.0167*
Residuals	156	49.63	0.318		

when tested species-wise, Table S5), while that for *Cy. myrsinaefolia* and *Ca. eyrei* significantly decreased (Fig. 2). On the contrary, the uptake preference of NH_4^+ by *K. bipinnata* and *L. glaber* displayed a decreasing trend (albeit not significant when tested species-wise, Table S5), while the uptake preference by *Cy. myrsinaefolia* and *Ca. eyrei* increased (Fig. 2, significant only for *Ca. eyrei*, Table S5).

Table 2

Analysis of variance table of a linear model predicting total nitrogen (N) uptake rates from $\log_2(\text{richness})$ and species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*). The data of total N uptake rates was \log_e -transformed to obtain a normal distribution. Significant results for P values are shown in bold fonts. Asterisks are added to P values at the levels of “***” for $P < 0.001$, “**” for $P < 0.01$, and “*” for $P < 0.05$.

Factors	df	Sum Sq	Mean Sq	F value	P value
$\log_2(\text{richness})$	1	0.126	0.1265	0.5880	0.447
species	3	35.154	11.7180	54.4798	<0.001***
$\log_2(\text{richness})$: species	3	1.930	0.6432	2.9903	0.039*
Residuals	52	11.185	0.2151		

3.4. Biomass with tree species richness

At the individual level, tree species richness, species and their interaction significantly affected the shoot, root, and total biomass per plant (Table S6). The shoot and root biomass of the most productive species, i.e., *K. bipinnata*, significantly increased with increasing of tree species richness (Fig. S2a, b). In contrast, the shoot and root biomass of all other species showed a stable or slightly decreasing trend, which however was only significant for shoot biomass of *Cy. myrsinaefolia* (Table S7). Correspondingly, the total biomass of *K. bipinnata* increased with tree species richness while it remained more or less stable for the other three species (Fig. 3a). The root:shoot ratio of *K. bipinnata* significantly decreased with the increasing of tree

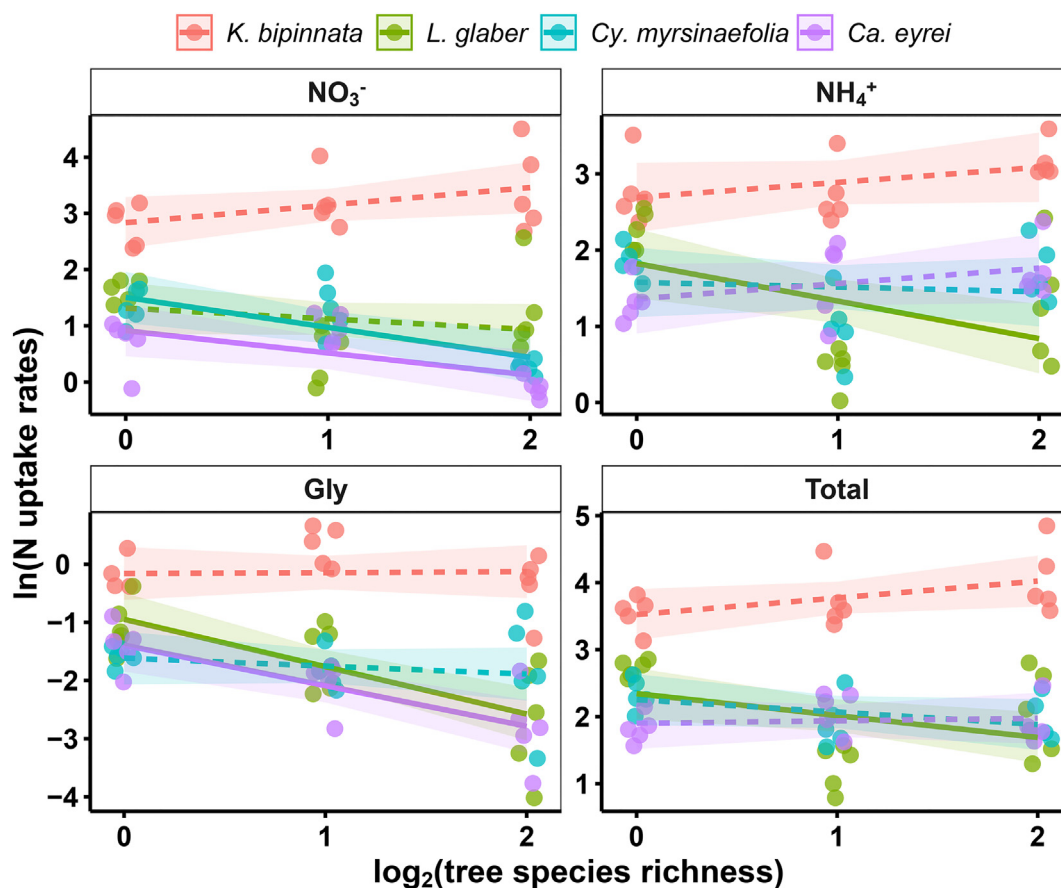


Fig. 1. Nitrogen (N) uptake rates of nitrate (NO_3^-), ammonium (NH_4^+), glycine (Gly) and total N (Total) by the targeted four species under different tree species richness levels (\log_2). The N uptake rates were \log_e -transformed to obtain a normal distribution of residuals. Regression lines and confidential intervals are presented. Solid lines indicate significant confidence intervals (i.e., significantly different from zero), while dotted lines indicate insignificant confidence intervals. Analysis of variance table of a linear model predicting N uptake rates from $\log_2(\text{richness})$, species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*), and N form (NO_3^- , NH_4^+ and Gly) is presented in Table 1 and the detailed trends and confidence intervals are listed in Table S3. Analysis of variance table of a linear model predicting the total N uptake rates from $\log_2(\text{richness})$ and species is presented in Table 2 and the detailed trends and confidence intervals are listed in Table S4.

Table 3

Analysis of variance table of a linear model predicting nitrogen (N) uptake preference from $\log_2(\text{richness})$, species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*) and N form (NO_3^- , NH_4^+ , Glycine). The data of N uptake preference was 1/3 power-transformed to obtain a normal distribution of residuals. Significant results for *P* values were shown in bold fonts. Asterisks were added to *P* values at the levels of “****” for $P < 0.001$, “***” for $P < 0.01$, and “**” for $P < 0.05$.

Factors	df	Sum Sq	Mean Sq	F value	<i>P</i> value
$\log_2(\text{richness})$	1	0.0279	0.0279	4.7727	0.0304*
species	3	0.0115	0.0038	0.6538	0.5817
N form	2	9.7558	4.8779	833.2498	<0.001***
$\log_2(\text{richness})$: species	3	0.0149	0.0050	0.8498	0.4686
$\log_2(\text{richness})$: N form	2	0.0319	0.0160	2.7268	0.0686
species: N form	6	0.4122	0.0687	11.7367	<0.001***
$\log_2(\text{richness})$: species: N form	6	0.1790	0.0298	5.0950	<0.001***
Residuals	156	0.9132	0.0059		

species richness (Fig. S2c). At the same time, the root:shoot ratio by other three species slightly increased (albeit not significant different from zero, Table S7).

At the community level, tree species richness significantly increased the shoot and root biomass per pot and decreased the corresponding root:shoot ratio (Fig. S3, Table S8). In consequence, there was also a significant increase in total biomass per pot with increasing tree species richness (Fig. 3b).

3.5. Plant functional traits with tree species richness

Tree species richness did not significantly affect typical aboveground traits such as height or leaf number, but significantly increased shoot diameter and root length while decreased leaf thickness (Table S9). At the species level, these patterns were significant for shoot diameter of *K. bipinnata* and *Ca. eyrei*, leaf thickness of *K. bipinnata* and root length of *K. bipinnata*, *L. glaber* and *Ca. eyrei* (Fig. S4, Table S10).

3.6. Correlations between plant and soil traits

Total biomass displayed significantly positive correlations to aboveground traits of height, shoot diameter, leaf number and leaf area, belowground traits of root diameter and root length (Fig. S5). Total biomass, aboveground traits, and belowground traits had significantly positive correlations to the uptake rates of NO_3^- , NH_4^+ , and glycine (Fig. 4). In contrast, the biomass variables were negatively related to root:shoot ratio, leaf thickness and soil available NO_3^- concentration (Figs. 4 and S5).

4. Discussion

To explore whether N niche partitioning leads to positive effects of tree species richness on biomass production reflected in plant above- and belowground functional traits, an *in situ* ^{15}N labeling experiment was carried out for four subtropical tree species at a richness gradient of 1, 2 and 4 after growing for about one year in pots. With increasing tree species richness, we found a significant increase in biomass productivity at the community level and in the complementary of the N uptake form. Total mesocosm biomass was positively related to N uptake rates and to above- and belowground functional traits (Fig. 4).

Biomass production significantly increased with increasing tree species richness, thus supporting our first hypothesis. It is remarkable that our simple pot experiment that ran for a bit more than one year was able to reproduce results from decades-old field experiments of BEF-China that uses the same species in the same combinations (Hulvey et al., 2013; Huang et al., 2018; Mori, 2018). Experiments with young plants in small pots or plots have the advantage that tree-tree interactions develop more quickly, but they seem to arrive at principally the same results at early stages of stand development (Niklaus et al., 2017). These positive relationships to tree

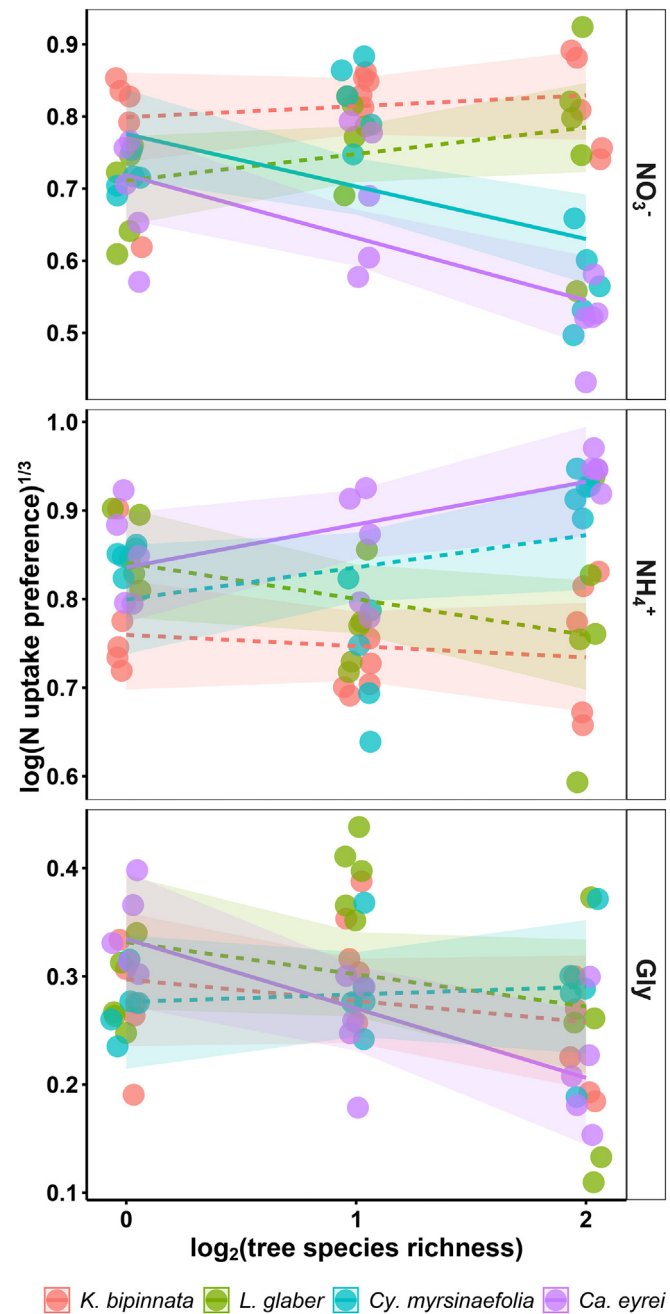


Fig. 2. Preference of nitrate (NO_3^-), ammonium (NH_4^+), or glycine (Gly) to the total nitrogen (N) uptake rates by the targeted four species under different tree species richness levels (\log_2). The N uptake preference were 1/3 power-transformed to obtain a normal distribution of residuals. Regression lines and confidential intervals are presented. Solid lines indicate significant confidential intervals, while dotted lines indicate insignificant confidential intervals. Analysis of variance table of a linear model predicting preference of N uptake rates from $\log_2(\text{richness})$, species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*), and N form (NO_3^- , NH_4^+ and Gly) is presented in Table 3 and the detailed trends and confidence intervals are listed in Table S5.

species richness applied to all compartments, *i.e.*, not only shoots but also roots, which usually cannot be analyzed in field experiments *e.g.* Huang et al. (2018). The biomass increase with increasing tree species richness was mainly brought about by the most productive species, *i.e.*, *K. bipinnata*, while biomass of the other three species remained almost stable. This indicates that the increased growth biomass of species did not occur at the expense of decreased growth of the other species. Phylogenetic distance effects can be used to explain this phenomenon, as *K. bipinnata*

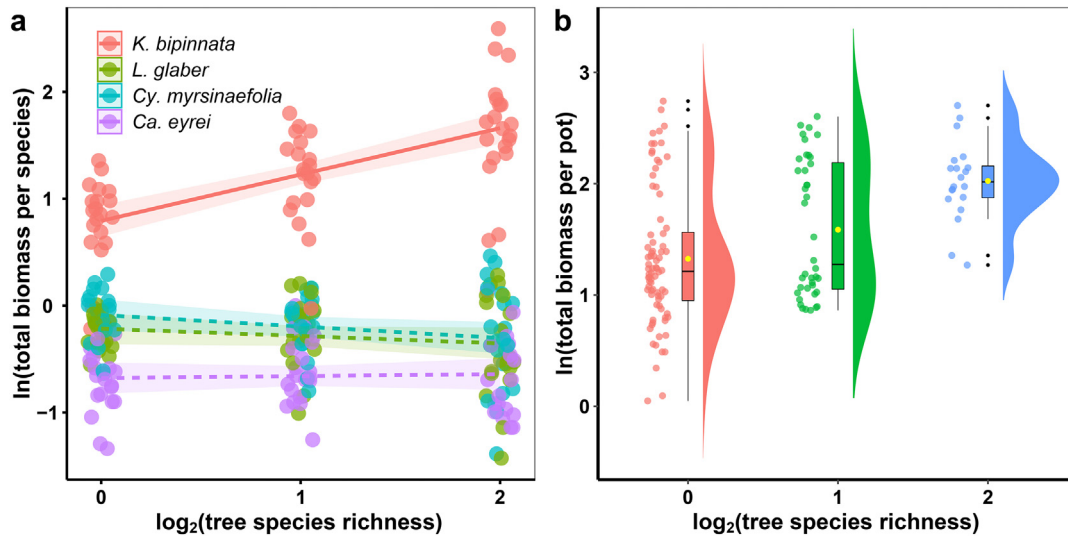


Fig. 3. Total biomass per species (a) and per pot (b) under different tree species richness levels (\log_2). The unit of biomass is g. The values for N uptake preference were \log_e -transformed to achieve normal distribution of residuals. a, solid regression lines indicate confidence intervals that were significantly different from zero, while dotted lines indicate insignificant slopes. Analysis of variance table of a linear model predicting biomass per species from \log_2 (richness) and species (*Koelreuteria bipinnata*, *Lithocarpus glaber*, *Cyclobalanopsis myrsinaefolia* and *Castanopsis eyrei*) on biomass is presented in Table S6 and the detailed trends and confidence intervals are listed in Table S7. b, violin and box pots of total biomass per pot under different tree species richness levels (\log_2). Total biomass per pot increased with the increasing of tree species richness, with detailed analysis results listed in Table S8.

belongs to the family of Sapindaceae while the other three species are members of the Fagaceae family. Thus, the stronger biomass growth of *K. bipinnata* could have been a family trait. Overall, increased biomass with increasing tree species richness was largely related to N uptake (Fig. 4), thus also confirming this part of our first hypothesis. *K. bipinnata* had a larger shoot diameter and height, but also longer roots (Fig. S4). These advantages may have helped *K. bipinnata* to acquire more resources,

such as space, light, water and soil available N (Weiner and Damgaard, 2006; DeMalach et al., 2016). Although the trend of total N uptake with tree species richness differed among tree species, total biomass and N uptake rates were generally positively correlated (Figs. 4, S5). Thus, plant N uptake can be considered responsible for the increasing of biomass affected by tree species richness.

We also find support for our second hypothesis of increased trends of N niche partitioning with increasing tree richness. Interestingly, this was only partly brought about by different preferences of the different species for different N forms. While the most productive species *K. bipinnata* clearly showed high rates of N uptake, the other species did not vary much from each other, which could reflect a phylogenetically conserved trait in the Fagaceae family. In contrast, most of the niche partitioning effect was caused by a chemical niche shift in the preferred N uptake form with increasing tree species richness. While *K. bipinnata* and *L. glaber* increased their preference for NO_3^- , *Cy. myrsinaefolia* and *Ca. eyrei* showed the opposite pattern and increasingly preferred NH_4^+ . Compared to NH_4^+ , NO_3^- is more mobile and is thus easier captured by roots (Gurevitch et al., 2020). Individuals of *K. bipinnata* and *L. glaber*, which were taller and had a larger leaf number, increased the preference trend for uptake of NO_3^- with increasing tree species richness. This confirms previous findings that comparably larger plants preferentially use the dominant form of NO_3^- when coexisting with other species (McKane et al., 2002). In contrast, with increasing of tree species richness, the smaller individuals of *Cy. myrsinaefolia* and *Ca. eyrei* adjusted their uptake preference from NO_3^- to NH_4^+ . Although NH_4^+ is much less mobile in the soil, its assimilation after uptake needs less energy compared to NO_3^- (Gurevitch et al., 2020). When plants grow well and have enough NAD(P)H to reduce NO_3^- , this form is the preferred N uptake form. By comparison, less vigorous plants prefer the “cheaper” N form of NH_4^+ . As net primary productivity is largely related to the N uptake of the ecosystem (Reich et al., 2006; Elser et al., 2007; LeBauer and Treseder, 2008), the higher biomass productivity could be largely related to the complementarity in the preferred N uptake forms among different species (McKane et al., 2002; Liu et al., 2020). The opposite trends of N uptake preferences by different species decrease the N uptake niche overlap in the ecosystem (von Felten et al., 2009). Decreasing niche overlap does not only increase ecosystem functioning but also allows coexistence of different plant species at high tree species richness (McKane et al., 2002; Liu et al., 2020; Phoenix et al., 2020). Compared to mineral N, the uptake preference

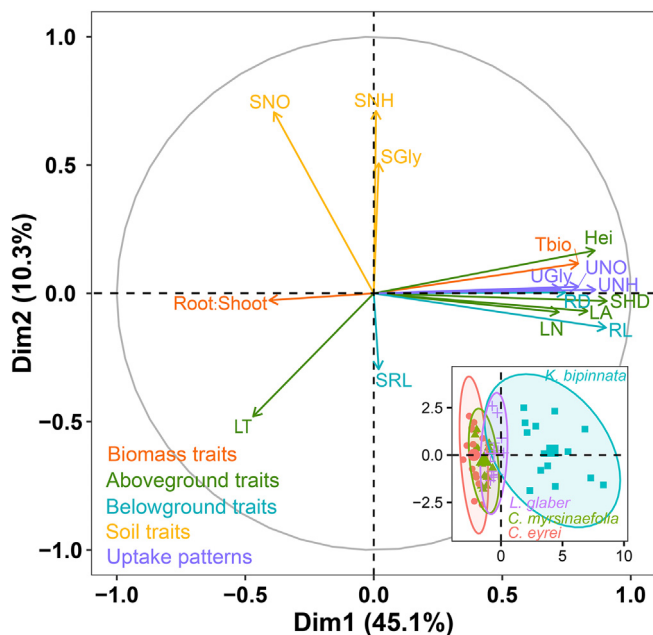


Fig. 4. Principal components analysis (PCA) plot of plant, soil and N uptake traits. Abbreviations in the figure are listed below: Tbio (total biomass), Root:Shoot (root to shoot biomass ratio), Hei (height), DBH (diameter at breast height), LN (leaf number), LT (leaf thickness), LA (leaf area), RD (root diameter), RL (root length), SRL (specific root length), SNO (soil NO_3^-), SHN (soil NH_4^+), SGly (soil glycine), UNO (uptake of NO_3^-), UNH (uptake of NH_4^+), Ugly (uptake of glycine). Different classifications of the indexes are distinguished by colors. Species identity are represented by points with circle at the right bottom of the figure.

for glycine declined with tree species richness for all targeted species, presumably because of a more active soil microbiome that competes with plant uptake (Moreau et al., 2019). It might well be that more active soil bacteria release lower amounts of amino acids, because a higher microbial activity in mixtures enhances mineralization and nitrification, thus increasing the provision of inorganic N and decreasing that of organic N (Malchair et al., 2010). However, this has to be studied in more details in future research.

The finding that different species shifted their niche occupations in N uptake is consistent with the distinct trait values (Kahmen et al., 2006; Cui et al., 2017). We observed a significant response of functional traits as response to increasing tree species richness, thus confirming our third hypothesis. Particularly, we found root length increased with tree richness (Fig. S4). Longer roots facilitate N uptake, and thus explain the increased uptake rates and biomass production with increasing species richness. Plant above- and belowground functional traits can have tight correlations to N uptake rates and total biomass (Weih et al., 2021). We found positive correlations between N uptake rates and numerous traits, both aboveground, such as height and shoot diameter, and belowground, such as root diameter and root length (Figs. 4, S5). Plants can be generally grouped into conservative and acquisitive types (Henneron et al., 2020). *K. bipinnata* belongs to typically acquisitive type, as it grows faster and takes up N more rapidly than other species. Particularly, with increasing of tree species richness, shoot diameter increased and leaf thickness decreased for *K. bipinnata* (Fig. S4). Thin leaves have been demonstrated to have high photosynthetic capacity and large amounts of N per unit mass (Loomis, 1997). Moreover, the larger root diameter of *K. bipinnata* can potentially benefit from an increased collaboration with mycorrhizal fungi, which increases the uptake surface and efficiency for N (McCormack et al., 2017). The larger root diameter also indicates an increasing of cortex:stele ratio, which can optimally balance the N uptake with transportation (Kong et al., 2017). This could also be an additional explanation of the high N uptake rates of *K. bipinnata* compared to the other species. From this perspective, our results demonstrate that the plant above- and belowground traits are integrated as predicted by Reich (2014).

5. Conclusion

Niche partitioning with respect to preferred N uptake forms among coexisting tree species increases with tree richness. By decreasing niche overlap among tree species, plant coexistence is increased at higher species richness. Nitrogen uptake niche partitioning is reflected in both above- and belowground plant functional traits, and in N-limited ecosystems, should be considered as a possible important explanation of the increasing of biomass productivity with increasing tree species richness.

CRedit authorship contribution statement

ML, XX, NMvD and HB developed the experimental idea; ML, XX, BY, NZ and ZM conducted the planting, sampling and laboratory analyses; ML and HB analyzed the data; ML, XX and HB led the writing of the manuscript and all authors contributed substantially to revisions.

Data availability

The data that support the findings of this study is available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.153716>.

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