

Contents lists available at ScienceDirect

### Journal of Plant Physiology



journal homepage: www.elsevier.com/locate/jplph

# Coordination of nitrogen uptake and assimilation favours the growth and competitiveness of moso bamboo over native tree species in high-NH<sub>4</sub><sup>+</sup> environments

Huijing Chen<sup>a,b,1</sup>, Xiaofeng Huang<sup>a,b,1</sup>, Weiming Shi<sup>c</sup>, Herbert J. Kronzucker<sup>d,e</sup>, Lihan Hou<sup>a,b</sup>, Haiyan Yang<sup>a,b</sup>, Qingni Song<sup>a,b</sup>, Jun Liu<sup>a,b</sup>, Jianmin Shi<sup>a,b</sup>, Qingpei Yang<sup>a,b</sup>, Na Zou<sup>a,b,\*</sup>

<sup>a</sup> College of Landscape and Art, Jiangxi Agricultural University, Nanchang, Jiangxi, 330045, China

<sup>b</sup> Jiangxi Provincial Key Laboratory for Bamboo Germplasm Resources and Utilization, Jiangxi Agricultural University, Nanchang, Jiangxi, 330045, China

<sup>c</sup> State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing, 210008, China

<sup>d</sup> Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

<sup>e</sup> School of BioSciences, The University of Melbourne, Parkville, Victoria, 3010, Australia

### ARTICLE INFO

Keywords: Moso bamboo Camellia oleifera Sapium sebiferum Machilus pauhoi Castanopsis fargesii Phenotypic plasticity Interspecific competition N assimilation <sup>15</sup>N Stable isotope

### ABSTRACT

Phenotypic plasticity and competitive strength are major mechanisms determining the success of invasive species and are influenced by abiotic factors. A rise in the ratio of ammonium  $(NH_4^+)$  to nitrate  $(NO_3^-)$  in soils is frequently associated with the invasion of bamboo into broad-leaved evergreen forests. However, the influence of soil nitrogen (N) chemistry on plant growth and interspecific competition in the context of invasion remains insufficiently studied. In the present work, differences in plasticity and interspecific competition between native tree species in broad-leaved evergreen forests and invasive bamboo in response to different N forms were investigated using seedlings grown in a controlled environment. We show that moso bamboo responded positively and strongly to increased soil  $NH_4^+/NO_3^-$  ratios, while the native tree species Sapium sebiferum, Camellia oleifera, and Machilus pauhoi responded negatively and exhibited limited plasticity. Native tree species growth was significantly inhibited in the presence of moso bamboo under high-NH<sub>4</sub><sup>+</sup> conditions, whereas native tree species were less affected by interspecific competition when  $NO_3^-$  was supplied as the sole N source. By contrast, moso bamboo growth was significantly inhibited, followed by seedling death, in both monoculture and in mixed culture with prolonged  $NO_3^-$  treatment. All species tested exhibited significantly higher rates of  ${}^{15}NH_4^+$  than  $^{15}$ NO<sub>3</sub><sup>-</sup> uptake, but the Michaelis constant (K<sub>m</sub>) for  $^{15}$ NH<sub>4</sub><sup>+</sup> uptake was lower in moso bamboo, indicating higher substrate affinity. Nitrate reductase (NR) and nitrite reductase (NiR) activities showed no inducible effects in moso bamboo compared to the induction response seen in the native tree species in response to  $NO_3^-$ . Activities of glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH) significantly increased with NH4<sup>+</sup> provision in roots of moso bamboo, contrasted by a less plastic response in the native tree species. Enhanced ammonification and reduced nitrification in soils is typically observed during bamboo invasion and appears to create a positive soil-plant feedback loop that, due to highly flexible and opportunistic  $NH_4^+$ acquisition pathways, favours bamboo fitness and invasion into native forests when  $NH_4^+$  is the dominant N form.

### 1. Introduction

Plant invasion successfully occurs when an alien species is capable of establishing self-sustaining populations outside its natural range (naturalization). Exotic plant invasion seriously threatens biodiversity and community structure of native ecosystems and has led to profound changes in ecosystem processes and function worldwide. Invasive plants tend to increase soil N pools and/or accelerate N transfer rates (Ehrenfeld, 2003; Liao et al., 2008). High rates of soil N mineralization and nitrification can lead to increases in soil ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate

https://doi.org/10.1016/j.jplph.2021.153508

Received 26 July 2021; Received in revised form 25 August 2021; Accepted 30 August 2021 Available online 7 September 2021 0176-1617/© 2021 Elsevier GmbH. All rights reserved.

<sup>\*</sup> Corresponding author. College of Landscape and Art, Jiangxi Agricultural University, Nanchang, Jiangxi, 330045, China.

E-mail address: nzou@jxau.edu.cn (N. Zou).

<sup>&</sup>lt;sup>1</sup> These authors contributed equally to this work.

(NO<sub>3</sub><sup>-</sup>) concentrations, dramatically enhancing the supply of plant-available N in invaded ecosystems (Liao et al., 2008). For example, it has been shown that plant-N, soil-NH4<sup>+</sup>, and soil-NO3<sup>-</sup> concentrations were 40, 30, and 17% higher, respectively, in invaded compared to native ecosystems (Liao et al., 2008; Min et al., 2021 a,b). N is one of the most frequently growth-limiting mineral nutrients for plants, limiting gross and net primary production in most ecosystems (LeBauer and Treseder, 2008), and increased N availability often favours invasive plants because of greater plasticity in the response to N and/or increased interspecific competitive ability compared to native species (Huangfu et al., 2016; Liu et al., 2018). Therefore, invading species can effectuate positive feedback loops, helping the species to become dominant and reducing plant diversity in the invaded communities (Ehrenfeld et al., 2003; Valliere and Allen, 2016). However, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> differ profoundly in their chemical properties, and plants differ in their capacity to use various forms of N. Altered concentrations and ratios of  $\mathrm{NH_4^+}$  to NO<sub>3</sub><sup>-</sup> in soils have been proposed to be a significant driving force producing shifts in plant communities, and a favourable response to one form of N over another may help to explain the dominance of many plants (Kronzucker et al., 1997, 2003; Britto et al., 2001; Britto and Kronzucker, 2013). Although rates of soil N mineralization and nitrification in soils are known to be altered during plant invasion, little attention has been paid to these N-chemistry-based feedback mechanisms in biological invasions (Huangfu et al., 2016; Zou et al., 2020 a).

Biomass production is typically a useful indicator of species-specific N-acquisition strategies, and the magnitude and direction of competitive interactions between species (Kronzucker et al., 1997; Rennenberg et al., 2009; Trinder et al., 2013). Furthermore, phenotypic plasticity, the fundamental property of a given organism to produce different phenotypic traits in response to different environments, plays an important role in biological invasions (Callaway et al., 2003; Matesanz et al., 2012). The capacity to alter biomass allocation patterns, leaf traits, and root morphology, and to adjust N uptake and metabolism according to plant demand are considered key aspects of individual adaptive plasticity (Glass et al., 1997, 2001; Ryser and Eek, 2000; Zhao et al., 2010; Huangfu et al., 2016). Plant root system plasticity in response to nutrient availability is a fundamentally important trait for optimizing nutrient resource interception and nutrient-absorptive area, and, ultimately, plant growth and fitness (Yu et al., 2014; Plett et al., 2020; Zhang et al., 2021). Divergence in N form preferences among species reflect differential capacities of plant roots to take up various forms of N, and this has been shown to significantly affect species' phenotypic responses and the interactions between native and exotic species (Kronzucker et al., 1997, 2003; Lavorel and Garnier, 2002; Bueno et al., 2019). The kinetic uptake parameters V<sub>max</sub> and K<sub>m</sub> are widely used to gauge and compare uptake capacity among species and provide insight into uptake mechanisms (Kronzucker et al., 1995a,b; Min et al., 2000; Kronzucker et al., 2010; Sun et al., 2016; Silverstein, 2019). In the assimilation process, NO<sub>3</sub><sup>-</sup> is first reduced to NO<sub>2</sub><sup>-</sup>, a step catalyzed by the enzyme NR, and then to  $NH_4^+$ , by NiR;  $NH_4^+$ , derived from such reduction of  $NO_3^-$  or, alternatively, absorbed directly by the roots, is then assimilated into glutamine and glutamate by GS and GOGAT, respectively (reviewed in Masclaux-Daubresse et al., 2010). Additionally, NADH-glutamate synthase can incorporate NH4<sup>+</sup> into glutamate in response to high levels of NH4<sup>+</sup> under stress conditions (Skopelitis et al., 2006). The participation of a variety of enzymes in the uptake and assimilation of different N forms permits specialization among species for the NH4<sup>+</sup> or NO3<sup>-</sup> sources of N at a variety of intersection points (Knoepp et al., 1993). Essentially, plant invasion is the process of exotic plant species displacing native species, and subsequently dominating invaded communities as an outcome of complicated competitive interactions (Funk and Vitousek, 2007; Parepa et al., 2013). Therefore, comparative studies of phenotypic plasticity, N acquisition strategies, and competitive interactions between native species and invasive plants under varied NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> ratios can elucidate mechanisms of invasion success and ecosystem impacts as a function of altered N chemical composition in soils.

Moso bamboo (Phyllostachys edulis (Carrière) J. Houzeau), a tree-like evergreen woody bamboo species in the family Poaceae, is known to be a problematic invading species in Asian forests (Lima et al., 2012; Liu et al., 2019). Tree species in secondary evergreen broad-leaved forests (EBF), are the most vulnerable and can be readily displaced by bamboo (Okutomi et al., 1996; Song et al., 2016). In addition to altering community structure and species diversity, unrestrained bamboo expansion also significantly affects the distribution patterns of N in plants and soils, and alters available soil N pools and rates of N cycling and N mineralization in soils (Fukushima et al., 2015; Song et al., 2016; Li et al., 2017). Decelerated nitrification and decreased soil  $NO_3^-$  content are generally contrasted by accelerated ammonification and elevated ratios of  $NH_4^+/NO_3^-$  in invaded ecosystems, compared with native ones (Song et al., 2013, 2016; Li et al., 2017, 2019). Therefore, this interaction provides an ideal system to test the roles of altered N chemical composition in soils in driving plant invasion. Our previous work showed that moso bamboo possesses superior growth, N uptake, and NH4<sup>+</sup> tolerance under elevated NH4<sup>+</sup> compared to the dominant native tree species Castanopsis fargesii Franch (Zou et al., 2020a), even though both species displayed preference for  $NH_4^+$  over  $NO_3^-$  as a source of N. However, whether other tree species in EBF respond in a similar manner to increased  $NH_4^+/NO_3^-$  ratios during bamboo invasion is not known. As well, how interspecific competition between bamboo and native tree species is affected by altered soil N chemical composition is still unclear. Responses to N form, in the context of invasion and differential phenotypic plasticity, must clearly be examined more closely at the mechanistic level.

To further assess the effect of elevated  $NH_4^+$  in favouring the expansion of bamboo, in contrast to dominant native tree species, such as Castanopsis fargesii we reported on previously (Zou et al., 2020a), this study aimed to compare growth traits and phenotypic plasticity between several native tree species and exotic bamboo in response to varied N chemistry, examine bamboo-tree competitive interactions, and analyse the underlying physiological mechanisms.  $^{\rm 15}{\rm N}\text{-tracer}$  analysis, and determination of the kinetics of N uptake, was used to assess NH4+- and NO<sub>3</sub><sup>-</sup>-uptake differences among the species. Furthermore, activities of the N-assimilation enzymes NR, NiR, GS, GOGAT, and GDH were analyzed under varying NH4<sup>+</sup> and NO3<sup>-</sup> supply to assess metabolism-based physiological adaptations. Seedlings, grown from seed, of Castanopsis fargesii Franch., Sapium sebiferum (L.) Roxb., Camellia oleifera Abel., and Machilus pauhoi Kanehira, the dominant and associated native tree species in EBF invaded by bamboo (Song et al., 2013, 2016), and Phyllostachis edulis (Carrière) J. Houzeau, the leading invasive bamboo species in these forests, were used in this study. The experiments were conducted in a greenhouse with varying concentrations (0.4 mM and 8 mM) and forms (NH<sub>4</sub><sup>+</sup>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>) of N in vermiculite culture, to advance our understanding of the role of altered soil N chemical composition and of interspecific competition under such conditions. We hypothesized that: (1) growth traits and phenotypic plasticity in response to N chemistry differ among species coexisting in native plant communities and moso bamboo; (2) altered soil N chemical composition affects interspecific competition between invasive bamboo and native trees; (3) differences in  $NH_4^+/NO_3^-$  uptake and the activity of N-assimilation enzymes underpin the species' phenotypic responses to different N forms.

### 2. Materials and methods

### 2.1. Plant materials and growth conditions

Seeds of *P. edulis* were collected in September from a single stand in GuanYang of Guangxi province, China, and stored at 4 °C. Seeds were soaked overnight, shelled, sterilized using 20% NaClO for 20 min, rinsed in sterile water five times, and then germinated in vermiculite in a greenhouse, located at Jiangxi Agricultural University (28° 41′ N, 115°

52′ E), with a temperature regime of approximately 25/18 °C  $\pm$  3 °C, 65–70% relative humidity, and a 14/10 h day/night photoperiod (Zou et al., 2020 a,b).

Seeds of *C. fargesii* were collected from the Xiayang state forest farm in Fujian province, *C. oleifera* was from Jiangxi Academy of Forestry, *S. sebiferum* and *M. pauhoi* were obtained from Jiangxi Agricultural University in Jiangxi province, China, and their seeds were stratified in sand, in 1:3 (V/V) medium in outdoor pits during winter, prior to germination in the greenhouse in the coming spring. Three-foliate seedlings of similar height for each species were selected for N treatments.

### 2.2. N treatments

The six N treatments consisted of three N forms  $(NO_3^-, NH_4^+)$  and  $NO_3^-$ , and  $NH_4^+$ ) at two N levels (0.4 mM and 8 mM). Different N forms and concentrations were supplied by KNO3, Ca(NO3)2.4H2O, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and K<sub>2</sub>SO<sub>4</sub> to maintain the same potassium and calcium concentration in the different N treatments, as displayed in Table 1. The nutrient solution in each treatment was adjusted to pH 4.0 with 0.1 M NaOH or 0.1 M HCl.  $C_2H_4N_4$  (7  $\mu$ M) was used as a nitrification inhibitor and added to all treatments to prevent nitrification (Zou et al., 2020 a,b). There were two seedlings of the same species per pot (diameter 150 mm, height 130 mm) and four pots in a tray as a replicate. There were three replicates with 24 seedlings of the same species per N treatment. These seedlings were grown in the greenhouse, under the conditions described above (at ambient conditions of temperature of max 28 °C/min 15 °C, 65-70% relative humidity and 14/10 h day/night photoperiod). Pots were rotated every week to avoid location effects. After two months, growth traits and root system architecture were analyzed.

### 2.3. Interspecific competitiveness experiments

In order to evaluate interspecific competitive responses under  $NH_4^+$ rich conditions, uniform seedlings of *P. edulis, C. oleifera,* and *S. sebiferum* were selected and transplanted to plastic pots (length 435 mm, width 200 mm, height 140 mm) with vermiculite. There were eight seedlings per pot with tray in the following three planting systems (Zou et al., 2020 a): bamboo monoculture (8 seedlings of *P. edulis* per pot with tray), broadleaved tree monoculture (8 seedlings of the same tree species per pot with tray), mixed culture of bamboo and trees species (6 seedlings of *P. edulis* + 2 seedlings of *C. oleifera* per pot with tray, and 6 seedlings of *P. edulis* + 2 seedlings of *S. sebiferum* per pot with tray), to mimic bamboo forest, bamboo-broadleaved tree mixed forest (with 3:1 ratio of *P. edulis* to broadleaved trees), and evergreen broadleaved forest, respectively. The seedlings were watered with deionized water three times a week until new roots were produced, then seedlings were treated with normal nutrient solution supplemented with 8 mM  $NH_4^+$  (Table 1) once a week, from September 2017 to August 2018.

The long-term growth response and interspecific competitiveness of *P. edulis* and the native tree species were further studied with different N forms. In monoculture, 4 seedlings of the same species of *P. edulis*, *C. fargesii* or *C. oleifera* were planted in separate pots (diameter 300 mm, height 280 mm), with four pots of the same species per tray as a replicate. In mixed-culture, a 3:1 ratio of 3 *P. edulis* seedlings and 1 *C. fargesii* seedling were planted in the same pot, with four such pots per tray as a replicate or 3 *P. edulis* seedlings and 1 *C. oleifera* seedling in the same pot with four such pots per tray as a replicate. There were three replicates for monoculture and six replicates for mixed-culture per treatment. When new roots were produced, the seedlings were treated with normal nutrient solution supplemented with 8 mM NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> (Table 1) once a week, from July 2019 to January 2020.

### 2.4. Growth analysis and root morphology

Plant height was measured with a ruler. Leaf number was determined for all of the unfolded leaves above the cotyledonary node. The chlorophyll content of the leaves was measured using a chlorophyll meter (SPAD-502, Minolta). Root morphology of root total length (RL), average diameter (AD), root surface area (RS), root volume (RV) and root tips, and leaf surface area were determined with an automatic scanning apparatus (EPSON color image scanner LA1600+, Toronto, Canada), equipped with WinRHIZO 2012 software (Regent Instruments, Quebec, Canada). When treatments were completed, all plants were harvested for morphology analysis and then separated into foliage, stems, and roots, dried at 105 °C for 30 min, and then to a constant weight at 70 °C for biomass determination.

### 2.5. Nitrogen uptake rate ( $^{15}NH_4^+$ and $^{15}NO_3^-$ )

Uniform three-foliate seedlings of *P. edulis*, *C. fargesii*, *C. oleifera*, and *S. sebiferum* were selected for determination of N-absorption kinetics. After two weeks of cultivation with deionized water until new roots were produced, the seedlings were treated in full nutrient solution containing 0.1 mM NH<sub>4</sub>NO<sub>3</sub>, and then the seedlings were transferred to solutions without N for two days prior to N uptake measurements, as described by Zhang et al. (2019). Then, the white roots were excised from the seedlings and treated with 100 ml nutrient solutions supplemented with 0, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0, and 2.0 mM <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> in the forms of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and Na<sup>15</sup>NO<sub>3</sub>, respectively, for 3 h under light. 0.2 g fresh weight was used for a bulked replicate, with three replicates per <sup>15</sup>NH<sub>4</sub><sup>+</sup> or <sup>15</sup>NO<sub>3</sub><sup>-</sup> treatment per species. Then, roots were removed from the treatment solutions, washed in 0.2 mM CaCl<sub>2</sub>, dried to a constant weight at 70 °C, and ground into powder. 0.2 mg of

Table 1
---------

Nutrient solution composition with	different N concentrations	[N] and NH <sub>4</sub> <sup>+</sup>	$^{\prime}/NO_{3}^{-}$ ratios (N form).
------------------------------------	----------------------------	--------------------------------------	---

[N]		0.4 mM		8 mM			
Compound	NO <sub>3</sub> <sup>-</sup>	$\mathrm{NH_4}^+\mathrm{+NO_3}^-$	$\mathrm{NH_4}^+$	$NO_3^-$	$\mathrm{NH_4}^+\mathrm{+NO_3}^-$	$\mathrm{NH_4}^+$	
KNO3	0.4	0.2	0	3	0	0	
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	0	0	0	2.5	2	0	
$(NH_4)_2SO_4$	0	0.1	0.2	0	2	4	
K <sub>2</sub> SO <sub>4</sub>	1.3	1.4	1.5	0	1.5	1.5	
CaCl <sub>2</sub> .2H <sub>2</sub> O	2.5	2.5	2.5	0	0.5	2.5	
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.25	0.25	0.25	0.25	0.25	0.25	
Na <sub>2</sub> HPO <sub>4</sub> .10H <sub>2</sub> O	0.6	0.6	0.6	0.6	0.6	0.6	
Fe-EDTA	0.01	0.01	0.01	0.01	0.01	0.01	
H <sub>3</sub> BO <sub>3</sub>	0.02	0.02	0.02	0.02	0.02	0.02	
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.002	0.002	0.002	0.002	0.002	0.002	
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.002	0.002	0.002	0.002	0.002	0.002	
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.002	0.002	0.002	0.002	0.002	0.002	
Na2MoO4.2H2O	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	

Concentrations are in mM.

dried powder was used for determination of total N and  $\delta^{15}$ N abundance, with a Flash 2000 HT elemental analyzer interfaced to an Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Inc., USA), according to Zou et al. (2020 a). The kinetic parameter of V<sub>max</sub> represents the maximum velocity asymptotically approached by the system at saturating substrate concentrations, and K<sub>m</sub> is the substrate concentration at which a velocity of 1/2 V<sub>max</sub> is reached, and these were derived from the Michaelis–Menten equation and also using Lineweaver-Burk, Hanes-Woolf and Eadie-Hofstee analysis, as described by Kronzucker et al. (1995d, 1996). The total <sup>15</sup>N amount per dry weight of root/3 h, yielding the amount of <sup>15</sup>N taken up per unit weight of root per unit time, was used in the formula (Zhang et al., 2019).

### 2.6. Assays of N-assimilation enzyme activities

Enzyme activities of NR, NiR, GS, GOGAT, and GDH were determined in fresh root, stem, and leaf tissue of the four species' seedlings in monoculture after two weeks of treatment with normal nutrient solution supplemented with 8 mM  $NH_4^+$  or  $NO_3^-$  in vermiculite as described above. When treatments were completed, seedlings were separated into foliage, stems, and roots, mixed respectively per replicate, and about 0.2 g such fresh weight was used for each assay as a replicate. There were three replicates of 24 seedlings per species per treatment. The activities of NR and NiR were determined using the NR and NIR assay Kits (NR-2-Y and NIR-2-G), respectively, and the activities of GS, GOGAT, and GDH were detected by using GS, GOGAT, and GDH detection Kits (GS-2-Y, GOGAT-2-Y, and GDH-2-Y), respectively, according to the manufacturer's instructions, as provided by Comin Biotechnology Co. Ltd, China. Enzyme activities of both NR and NiR ( $\mu$ mol g<sup>-1</sup> FW h<sup>-1</sup>) were defined as the amount of enzyme units catalyzing to yield 1 µmol NO2<sup>-</sup> per hour per gram fresh weight. Enzyme activity of GS  $(U.g^{-1})$  was defined as the amount of enzyme units in a per-mL reaction system to produce an absorption change by 0.01, at 540 nm, per minute and per gram fresh weight. 1 U enzyme activity of both GOGAT and GDH (nmol  $g^{-1}$  FW min<sup>-1</sup>) was defined as per gram protein to consume 1 nmol NADH per min.

### 2.7. Statistical analysis

The data were subjected to analysis of variance (ANOVA) with LSD (least significance difference) to identify differences using the statistical software program SPSS version 13.0 (SPSS, Chicago, IL, USA). Significant differences (P < 5%) between treatments are indicated by different letters. Sigma Plot 13.0 was used for the generation of graphs.

### 3. Results

### 3.1. Comparison of growth between native tree species and moso bamboo under different N treatments

Elevated N concentration significantly increased almost all morphological measures in the four species determined (Figs. 1 and 2, Table 2). Different N forms significantly increased biomass, most leaf and root morphological parameters in *P. edulis*, part of the growth characteristics in *S. sebiferum*, but only the SPAD value in *C. oleifera*, and the average diameter and root volume in *M. pauhoi* (Figs. 1 and 2, Fig. S1-S2, Table 2).

Tree species, especially *C. oleifera* and *S. sebiferum*, had higher biomass than *P. edulis* (Fig. 1 A, B). The highest biomass in *P. edulis* was achieved when  $NH_4^+$  was provided as the sole N source, at both N concentrations, while *S. sebiferum* had significantly higher biomass with  $NO_3^-$  at 0.4 mM and with mixed-N treatments at 8 mM compared to other treatments at the same N levels. *C. oleifera* and *M. pauhoi* seedlings displayed no significant difference in biomass accumulation at three



**Fig. 1.** Biomass accumulation and allocation in moso bamboo and native tree species in response to different N forms at 0.4 mM and 8 mM N. Seedlings were grown with different N treatments for two months, and the biomass of all plant parts was measured at the end of the experiment. Data are the means from three replicates of 24 seedlings, and error bars represent standard errors (SE). Different letters above the bar indicate statistical differences among treatments for each species, NSD means no significant different. *P*-values of the two-way ANOVAs of N concentration, N form, and their interaction are indicated. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.



Fig. 2. Root biomass and morphology of bamboo and native tree species in response to different N treatments. Root biomass (A, B) and root volume (C, D) were measured at the end of the experiment. Data are the means  $\pm$  SE, n = 24.

**Table 2** Two-way ANOVA (*P* values) for N concentrations ([N] = 0.4, 8 mM) and N forms ( $NH_4^+$ ,  $NH_4^+/NO_3^- = 1/1$ ,  $NO_3^-$ ) on the growth index of moso bamboo and tree species.

Growth index	P. edulis			C. oleifera		S. sebiferum			M. pauhoi			
	[N]	N form	$[N] \times \text{form}$	[N]	N form	$[N] \times \text{form}$	[N]	N form	$[N] \times \text{form}$	[N]	N form	$[N] \times \text{form}$
Biomass	***	***	***	***	0.299	0.299	***	**	**	***	0.02	0.125
Root/Stem ratio	**	0.164	0.227	**	0.479	0.294	**	0.93	0.994	**	0.84	0.066
Seedling height	**	0.431	0.091	***	0.317	0.387	***	***	0.661	***	0.105	0.65
Leaf number	0.779	*	0.36	*	0.71	0.127	***	0.056	0.132	***	0.213	0.115
Leaf area	***	**	**	***	0.925	0.833	***	*	**	***	0.874	0.943
SPAD value	***	***	***	***	**	**	***	***	***	***	0.169	0.175
Root length (cm)	***	***	***	**	0.962	0.49	***	**	0.771	***	0.458	0.753
Surface area (cm <sup>2</sup> )	***	***	***	0.801	0.57	0.227	***	0.065	0.555	***	0.057	0.613
Avg diam (mm)	0.444	***	0.398	***	0.858	0.107	***	*	**	0.797	***	**
Root volume (cm <sup>3</sup> )	*	0.121	0.073	***	0.278	0.064	***	0.409	0.089	***	**	0.165
Root tips	***	***	***	**	0.793	0.088	***	0.38	0.12	***	0.926	0.637

\*\*\* indicates p < 0.001, \*\* indicates p < 0.01 and \* indicates p < 0.05.

different  $NH_4^+/NO_3^-$  ratios at either N concentration (Fig. 1 A, B). With elevated  $NH_4^+/NO_3^-$  ratios, root to shoot mass ratio significantly increased only in *P. edulis* at 8 mM N.

Similar to total biomass, the root biomass of *P. edulis* was lower than that of *C. oleifera* and *S. sebiferum* (Fig. 2A and B). However, comprised mainly by fibrous root, the *P. edulis* root system had much greater root surface area and root volume and more root tips than the taproot system of the tree species. The plasticity of the response in root morphology was much greater in *P. edulis*, with root total length, root surface area, root volume, and root tips all significantly increased at elevated N (NH<sub>4</sub><sup>+</sup>) concentrations compared to the native tree species (Fig. 2 C-D, Fig. S2).

### 3.2. Interspecific competitiveness between moso bamboo and native tree species

Under long-term  $NH_4^+$ -supply conditions, all species performed well (Fig. 3), and growth and biomass of *P. edulis* were significantly higher or

tended to be higher in mixed culture compared to monoculture conditions (Fig. 3 B, C, E, H; Fig. 4 A, B). However, the growth of all tree species was significantly inhibited, and biomass was significantly lower, in the bamboo-tree mixed culture compared to monoculture (Fig. 3 D-I; Fig. 4 A, B). The root-to-shoot mass ratio tended to decrease in S. sebiferum, while there was no significant difference in P. edulis and *C. oleifera* when interspecific competition under  $NH_4^+$ -rich conditions was imposed (Fig. 4 C, D). In terms of the annual growth dynamic, P. edulis displayed an earlier growth response, and plant height quickly accelerated from February to March, both in mono- and mixed-culture (Fig. 5). By contrast, the initiation of significant size increases occurred much later for the tree species, from April and May for S. sebiferum, and C. oleifera, respectively, in monoculture, and plant height was significantly suppressed in mixed culture (Fig. 3 F, I; Fig. 5). The results overall show P. edulis to be a superior competitor compared to the tree species under NH<sub>4</sub><sup>+</sup>-rich conditions.

When  $NO_3^-$  was supplied as the sole N source, P. edulis was



**Fig. 3.** Growth of moso bamboo and broadleaved trees in monoculture and mixed culture supplied with 8 mM NH<sub>4</sub><sup>+</sup>. Schematic diagram shows the cultivation systems for moso bamboo and broadleaved trees (A); Monoculture of *P. edulis* or mixed culture with broadleaved trees (B, C, E, H); Monoculture of *C. oleifera* or mixed culture with *P. edulis* (D, E, F); Monoculture of *S. sebiferum* or mixed culture with *P. edulis* (G, H, I).

significantly inhibited, and all bamboo seedlings eventually died both in the mono- and mixed cultures, with prolonged treatment; by contrast, the growth of *C. oleifera* was less affected, although growth in *C. fargesii*, known for its strong  $\rm NH_4^+$  preference, was also inhibited to some extent (Fig. 6).

# 3.3. $^{15}\text{NH}_4{}^+$ and $^{15}\text{NO}_3{}^-$ uptake kinetics of moso bamboo and native tree species

<sup>15</sup>N accumulation was significantly higher in all species when seedlings were treated with (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> rather than Na<sup>15</sup>NO<sub>3</sub> (Fig. 7, Fig. 3S; Table 3). The kinetic parameters of Vmax and Km for this influx system were determined by a non-linear curve-fitting model of Michaelis-Menten kinetics and several data-transformation methods, although the data seemed to be better fitted to the Hanes-Woolf equation as estimated by R<sup>2</sup> (Table 3). According to the Hanes-Woolf method, V<sub>max</sub> for NH<sub>4</sub><sup>+</sup> uptake was highest in *P. edulis*, which was approximately 1.55-, 5.16-, and 13.32-fold that in *S. sebiferum*, *C. oleifera* and *C. fargesii*, respectively (Table 3). In addition, *P. edulis* had a lower K<sub>m</sub> for the NH<sub>4</sub><sup>+</sup> substrate, about 22.34%, 29.45%, and 86.09% of that in *C. fargesii*, *C. oleifera*, and *S. sebiferum* respectively (Table 3). For NO<sub>3</sub><sup>-</sup>, *S. sebiferum* had the highest V<sub>max</sub>, followed by *P. edulis*, while *C. oleifera* and *C. fargesii* was the lowest (Table 3); in contrast, the K<sub>m</sub> was lowest in *S. sebiferum*, and followed by *C. fargesii*, *P. edulis* and *C. oleifera*. 3.4. Enzymes of moso bamboo and native tree species involved in N assimilation

NR and NiR activities varied in response to  $NO_3^-$  and  $NH_4^+$  in different organ tissues among species (Fig. 8). Two-way ANOVA indicated that N form and the interaction of N form and plant organs had no significant induction effects on NR and NiR activities in *P. edulis*, compared to the inducible response seen in native tree species when seedlings were fed with different N forms. NR activities were significantly induced by  $NO_3^-$  in stem tissue of *C. fargesii* and in root tissue of *C. oleifera*, increased by 1422.48% and 175.78%, respectively, compared to those under  $NH_4^+$ . NiR activities were significantly induced by  $NO_3^-$  in stem tissue of *P. edulis*, stem tissue of *C. oleifera*, and leaf tissue of *S. sebiferum*, increasing by 7.18%, 75.78%, and 13.41%, respectively, compared to those under  $NH_4^+$ .

Two-way ANOVA results showed that N form, plant organs, and the interaction of N forms and plant organs had highly significant impacts on GOGAT, GS, and GDH activities in *P. edulis*, GS and GDH activities in *C. fargesii*, GOGAT activities in *C. oleifera*, and GDH activities in *S. sebiferum* (Fig. 9). GOGAT activities were significantly induced by NH<sub>4</sub><sup>+</sup> in root tissue of *P. edulis*, root tissue of *S. sebiferum*, and leaf tissue of *C. oleifera*, increasing by 112.04%, 51.67%, and 68.09%, respectively, compared to those under NO<sub>3</sub><sup>-</sup>. GS activities were significantly induced by NH<sub>4</sub><sup>+</sup> in root and stem tissue of *P. edulis* and in stem tissue of



Fig. 4. Biomass accumulation and allocation in moso bamboo and native tree species grown in monoculture and mixed culture supplied with 8 mM  $NH_4^+$ . Data are the means from three replicates of 12–

36 seedlings measured at the end of the experiment, and error bars represent SE. Different letters indicate statistical differences among treatments and species (Fisher's LSD, P < 0.05).



**Fig. 5.** Height increment for seedlings of bamboo and native tree species grown in monoculture and mixed culture supplied with 8 mM  $NH_4^+$ . Data are the means from three replicates of 12–36 seedlings, and error bars represent SE. Different letters indicate statistical differences among treatments and species at the same time (Fisher's LSD, P < 0.05).

*C. fargesii*, increasing by 113.30%, 26.33%, and 299.20%, respectively. GDH activities were significantly induced by  $NH_4^+$  in root tissue of *P. edulis*, and in stem and leaf tissue of *C. fargesii*, increasing by 60.74%, 55.55%, and 167.86%, respectively.

### 4. Discussion

## 4.1. Phenotypic plasticity and interspecific competition between moso bamboo and native tree species under varied soil N chemistry

In our present study, native tree species exhibited markedly lower phenotypic plasticity, as seen in biomass accumulation and allocation and in root morphology, when compared to the more pronounced phenotypic plasticity of *P. edulis*, in response to increased soil N concentration and elevated  $NH_4^+/NO_3$  ratio (Figs. 1 and 2; Fig.S1-S2; Table 2). Growth and biomass accumulation of *C. fargesii* showed some  $NH_4^+$  preference (Fig. 6), which was consistent with our previous results (Zou et al., 2020 a), while *S. sebiferum* displayed slightly better growth on  $NO_3^-$ , and *C. oleifera* and *M. pauhoi* had no evident response to N-form variation (Figs. 1 and 2; Fig. 6; Table 2). Consistent and together with bamboo's previously documented strong  $NH_4^+$  preference (Zou et al., 2020 a,b), the results support our original hypothesis that there is a divergence in growth and phenotypic plasticity among tree species and moso bamboo in response to altered soil N chemistry. Although the biomass of *P. edulis* seedlings was lower than that of *C. oleifera* and *S. sebiferum* at the starting point, bamboo ultimately displayed higher

Moso bamboo



Native trees & Moso bamboo

Fig. 6. Growth of moso bamboo and broadleaved trees in monoculture and mixed culture supplied with different N forms. *C. oleifera* and *C. fargesii* seedlings were mono-cultured or mixed-cultured with moso bamboo and supplied with 8 mM  $NH_4^+$  or  $NO_3^-$ . Bar = 30 cm.



**Fig. 7.**  $^{15}$ NH<sub>4</sub><sup>+</sup> and  $^{15}$ NO<sub>3</sub><sup>-</sup> uptake in roots of bamboo and native tree species. *P. edulis (A), C. fargesii* (B), *C. oleifera* (C), and *S. sebiferum* (D) seedlings. The data shown are the means  $\pm$  SE of three replicates, and are fitted to the Michaelis-Menten equation.

### Table 3

|--|

N form		$\mathrm{NH_4}^+$			$NO_3^-$		
Calculation Method	Species	R <sup>2</sup>	$K_{\rm m}$ (µmol L <sup>-1</sup> )	$V_{\rm max}$ (µmol g <sup>-1</sup> DW h <sup>-1</sup> )	R <sup>2</sup>	$K_{\rm m}$ (µmol L <sup>-1</sup> )	$V_{\rm max}$ (µmol g <sup>-1</sup> DW h <sup>-1</sup> )
Michaelis-Menten	P. edulis	0.63	46.8	3.95	0.43	39.7	2.57
	C. fargesii	0.68	91.7	1.34	0.00	24.5	0.60
	C. oleifera	0.50	105.1	4.16	0.00	20.9	1.90
	S.sebiferum	0.71	81	11.70	0.85	59.5	6.92
Lineweaver-Burk	P. edulis	0.41	66.35	2.49	0.44	170.67	1.44
	C. fargesii	0.47	1724.97	4.09	0.26	42.15	0.26
	C. oleifera	0.76	381.51	2.78	0.33	136.42	0.39
	S.sebiferum	0.44	54.29	8.76	0.69	144.19	6.68
Eadie-Hofstee	P. edulis	0.24	42.40	2.521	0.05	26.30	1.16
	C. fargesii	0.08	47.90	0.80	0.08	25.80	0.28
	C. oleifera	0.25	197.50	2.38	0.06	58.50	0.42
	S.sebiferum	0.25	49.70	9.42	0.35	55.20	5.37
Hanes-Woolf	P. edulis	0.96	175.67	18.52	0.82	390.61	2.01
	C. fargesii	0.46	786.50	1.39	0.80	339.70	0.45
	C. oleifera	0.68	596.41	3.59	0.48	596.42	0.72
	S.sebiferum	0.87	204.06	11.93	0.95	140.51	6.33

The data shown are the means  $\pm$  SE of three replicates. DW: dry weight.

biomass under long-term  $NH_4^+$ -enriched conditions (Fig. 4 A, B), suggesting a more efficient metabolic conversion of available N ( $NH_4^+$ ) to organic N and higher biomass accumulation in *P. edulis* compared to the native species (Bobbink et al., 1998). In mixed culture, *P. edulis* tended to respond to competition with increasing biomass accumulation. In sharp contrast, biomass of the native trees was significantly reduced when interspecific competition with moso bamboo was introduced under  $NH_4^+$ -rich conditions (Fig. 4 A, B). Therefore, the results suggest a superior adaptation and competitive ability in moso bamboo to increased soil  $NH_4^+$  presence than in native tree species.

In our study, both moso bamboo and the native trees tended to increase root:shoot ratio in response to increased  $NH_4^+$  (Fig. 1C and D). P. edulis significantly increased biomass accumulation and allocation to roots, developed more extensive root system architectures, and root morphology displayed higher plasticity in response to increased ratios of  $NH_4^+/NO_3^-$  than what was observed in native trees at 8 mM N (Figs. 1 and 2; Fig. S2). In aboveground parts, leaf area, and the SPAD value were significantly increased in all species examined, although to a much greater degree in moso bamboo than in the native trees, with increasing  $NH_4^+$  (Fig. S1), which is expected to benefit the photosynthesis of the aboveground organs and favour biomass accumulation. Tillers were significantly increased under NH4+-rich conditions with extended treatment time in moso bamboo (Fig. 3), reminiscent of the rapid vegetative growth response of young bamboo culms from underground rhizomes observed under natural conditions (Kleinhenz and Midmore, 2001). With this capacity for rapid aboveground proliferation and producing increased plant density, P. edulis therefore also has competitive superiority in commanding growth space for light interception when co-existing with native tree species, in addition to the advantages in root-mediated underground resource competition.

Invasive plants are typically not able to exploit all habitats, and not all habitats are equally susceptible to invasion (Sher and Laura, 1999). When NO<sub>3</sub><sup>-</sup> was supplied as the sole N source, the growth of *P. edulis* was significantly inhibited compared to plants growing on NH<sub>4</sub><sup>+</sup>, and all seedlings eventually perished with increasing treatment time. By contrast, the broad-leaved tree species examined grew well under different N forms (Figs. 1–2, Fig. 6). Thus, the competitive advantage of moso bamboo over native tree species was only obtained under NH<sub>4</sub><sup>+</sup>- rather than NO<sub>3</sub><sup>-</sup>-enriched conditions (Fig. 3; Fig. 6). Therefore, our results indicate the central importance of soil N chemical composition in regulating the growth and competitive interaction of bamboo and native tree species. It can be inferred that management strategies aimed at affecting the conversion of soil N toward NO<sub>3</sub><sup>-</sup> domination might reduce the growth and competitiveness of invasive bamboo, thereby controlling bamboo invasions.

### 4.2. $NH_4^+$ and $NO_3^-$ uptake of moso bamboo and native tree species

Our <sup>15</sup>N-labelling analyses, conducted at the intact root level, showed significantly higher rates of <sup>15</sup>NH<sub>4</sub><sup>+</sup> than <sup>15</sup>NO<sub>3</sub><sup>-</sup> uptake in all species examined when the two N forms were supplied alone, although there were species-specific differential capacities: the highest average  $NH_4^+$  uptake occurred in S. sebiferum, followed by C. oleifera and P. edulis, and the lowest was observed in C. fargesii (Fig. 7; Table 3). A preference for NH<sub>4</sub><sup>+</sup> over NO<sub>3</sub><sup>-</sup> was seen in terms of biomass accumulation in P. edulis, but no consistent preferences were observed in the native tree species (Fig. 1). Uptake rates alone do not reveal N-source preferences (Britto and Kronzucker, 2013). NH<sub>4</sub><sup>+</sup>, in particular at lower levels of supply, is often the "preferred" N source, as the energy cost of uptake and assimilation is less than that for  $NO_3^-$  (Boudsocq et al., 2012), although this relationship shifts due to  $NH_4^+$  toxicity at higher levels of substrate supply (Britto et al., 2001; Britto and Kronzucker, 2002; Di et al., 2021). In our study, in S. sebiferum and C. oleifera, similar to what was observed in early-successional tree species examined elsewhere, net acquisition of  $NH_4^+$  compared to that of  $NO_3^-$  did not translate into a growth advantage (Min et al., 2000; Kronzucker et al., 2003). The present results may imply a breakdown in regulation at the higher levels of N provision where elevated NH<sub>4</sub><sup>+</sup> uptake rates in native tree species are not coordinated with elevated levels of N assimilation, and a futile N-uptake cycle may instead prevail (Britto et al., 2001; Kronzucker et al., 2003), although obvious toxicity responses were not observed, either in short- or longer-term  $NH_4^+$  experiments (Figs. 1–6).

The kinetic uptake parameters  $V_{\text{max}}$  and  $K_{\text{m}}$  have been shown to reflect evolutionary 'strategies', and are widely used to reveal differential capacities of species in uptake and affinity to specific ions (Kronzucker et al., 1995a,b, d; 1996, 1997, 2000; Zhang et al., 2019). Generally, in high-nutrient environments, K<sub>m</sub> can become large; in low-nutrient environments, Km can become small, and such lower values do appear to reveal an adaptation to nutrient-poor environments (Cacco et al., 1980). In our study, the  $V_{max}$  was much higher but the  $K_m$  was lower in P. edulis compared to the native tree species by using a Hanes-Woolf analysis, as shown in Table 3. These results suggest the adaptation of bamboo and the native species to relatively nutrient-poor and nutrient-rich soil NH4<sup>+</sup>-N habitats. Therefore, a reduced total soil-N mineralization rate and slowed N cycling that coincide with bamboo invasion appear to present another strategy of P. edulis to increase its relative competitive advantage against native tree species, reflected in lower K<sub>m</sub> for the NH<sub>4</sub><sup>+</sup> substrate and better physiological integration compared to the native tree species, besides the stronger NH<sub>4</sub><sup>+</sup> preference, higher NH4<sup>+</sup> tolerance, larger V<sub>max</sub> of *P. edulis*, which would tend to facilitate an increase in soil N availability and thereby gain an



**Fig. 8.** Activities of nitrate reductase in moso bamboo and native tree species in response to different N forms.

Nitrate reductase (NR) and nitrite reductase (NiR) in root, stem, and leaf tissue of *P. edulis* (A, B), *C. fargesii* (C, D), *C. oleifera* (E, F), and *S. sebiferum* (G, H) seedlings. Seedlings were treated with 8 mM  $\rm NH_4^+$  or  $\rm NO_3^-$  for 15 days, and then enzyme activities were measured. The data shown are the means  $\pm$  SE of three replicates. Different letters on the bars indicate significant difference. *P*-values of the ANOVAs of N form, parts, and their interaction are indicated. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.

a a

Leaf

ab

aٍb

ab

Leaf

Leaf

Leaf

ab

advantage over native species like many invasive plants (Zhang et al., 2019).

In addition to inorganic N in the forms of  $NH_4^+$  and  $NO_3^-$ , many plant species have also been reported to take up dissolved organic N (i.e. amino acids) directly from soils (Näsholm et al., 2009; Bueno et al., 2019). As well, N-form uptake preference can also change with growth stage, and plants sometimes show a stronger preference for  $NH_4^+$  in the early stages of growth, while  $NO_3^-$  is preferred by more mature plants (Abbès et al., 1995; Song et al., 2013; Min et al., 2021b). Furthermore, the uptake of different chemical forms of N by both native and invasive plants may also shift to effectively avoid competition by niche differentiation, including N acquisition at different times during the growing season (Simon et al., 2017). Thus, more work is clearly needed to focus on the N acquisition strategies of *P. edulis* and native tree species under natural conditions, especially those where native species coexist and compete with bamboo during invasion.

4.3. N metabolism in moso bamboo and native tree species in response to different N forms

NR is a substrate-inducible enzyme (Kronzucker et al., 1995a,c). The presence of NR and its response to  $NO_3^-$  availability serve as important indicators of plasticity with respect to the  $NO_3^-$  source of N (Kronzucker et al., 1995c, 2000; Min et al., 1998). In the present study, although root, stem, and leaf tissue of *P. edulis* all showed NR and NiR activities, there were no evident inducible effects on NR and NiR activities with  $NO_3^-$  provision. In contrast, NR and NiR activities in the native tree species showed a stronger response to  $NO_3^-$  treatment (Fig. 8). The lower response in NR and NiR activities in *P. edulis* compared with the native tree species suggests a limited capacity in  $NO_3^-$  assimilation. However,  $NO_3^-$  can clearly be taken up, and the  $V_{max}$  for  $NO_3^-$  uptake in *P. edulis* was larger than in *C. fargesii* and *C. oleifera* (Fig. 7; Table 3). Presumably, the growth inhibition and eventual death of *P. edulis* under prolonged



**Fig. 9.** Activities of enzymes of  $NH_4^+$  metabolism in moso bamboo and native tree species in response to different N forms. Glutamine synthetase (GS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH), in root, stem, and leaf tissue of moso bamboo (A–C), *C. fargesii* (D–F), *C. oleifera* (G–I), and *S. sebiferum* (J–L) seedlings. Seedlings were treated with 8 mM  $NH_4^+$  or  $NO_3^-$  for 15 days, and then enzyme activities were measured. The data shown are the means ± SE of three replicates. Different letters on the bars indicate significant difference. *P*-values of the ANOVAs of N form, parts, and their interaction are indicated. \**P* < 0.05; \*\**P* < 0.01; ns, not significant.

 $NO_3^-$  treatment may be related to the high internal build-up of unmetabolised  $NO_3^-$ , arising from insufficient regulation of  $NO_3^-$  uptake and limited assimilation through NR and NiR (Reddy and Menary, 1990).

Although NH<sub>4</sub><sup>+</sup> is the preferred N source for some species and is a natural intermediate in N metabolism, it is often harmful to plants when provided at high concentrations, and excessive NH4<sup>+</sup> accumulation in plant tissues frequently ensues, as NH4<sup>+</sup> absorbed by roots far exceeds the amount of assimilation (Britto et al., 2001; Britto and Kronzucker, 2002; Li et al., 2010, 2011, 2013, 2014, 2019; Sun et al., 2020). The capacity of NH4+-assimilatory enzymes in this metabolic detoxification is critical (Givan, 1979). In the present study, the activities of GS, GOGAT, and GDH were at much higher levels and significantly increased with NH4<sup>+</sup> provision in the roots of *P. edulis* (Fig. 9A–C). The results imply strong metabolic capacity for NH<sub>4</sub><sup>+</sup> in *P. edulis*, permitting the avoidance of NH<sub>4</sub><sup>+</sup> accumulation in tissues. By contrast, only the activities of GS and GDH in C. fargesii, and of GOGAT in C. oleifera and S. sebiferum, were responsive to  $NH_4^+$  treatment (Fig. 9D–L). The lower activities and responsiveness of NH4<sup>+</sup>-assimilation enzymes in native tree species may be one of the reasons why absorbed NH<sub>4</sub><sup>+</sup> cannot be efficiently converted into a growth advantage (Figs. 1 and 2; Fig.7). However, the <sup>15</sup>NO<sub>3</sub><sup>-</sup> and <sup>15</sup>NH<sub>4</sub><sup>+</sup> uptake rates and N-assimilation enzyme activities were determined in independent tests and involving different N concentrations, so no direct correlations between <sup>15</sup>N uptake and assimilation were established. Future studies will have to be designed to define the dynamic relationship between <sup>15</sup>NO<sub>3</sub><sup>-</sup> and <sup>15</sup>NH<sub>4</sub><sup>+</sup> uptake and N-assimilation enzyme activities in detail. The higher activities for enzymes of NH<sub>4</sub><sup>+</sup> assimilation in bamboo may be driven by the large quantity of N metabolites required for accelerated growth and biomass accumulation in response to NH<sub>4</sub><sup>+</sup> compared to the native trees (Figs. 1–6; Glass et al., 2001). Therefore, it can be speculated that the growth and expansion of P. *edulis* could be curtailed to some extent by inhibitors that block the incorporation of NH<sub>4</sub><sup>+</sup> into glutamine and the synthesis of glutamate, such as methionine sulfoximine, an inhibitor of GS, and azaserine, an inhibitor of GOGAT (Berger and Fock, 1983; Kusnan et al., 1987).

### 5. Conclusions

In conclusion, our study reveals the associations of species-specific morphologic and physiologic plasticity and interspecific competition between native tree species and exotic bamboo seedlings to changed soil N availability and provides important insight into soil N chemistry as a driving force in the success of bamboo invasion. Growth of native tree species displayed much lower phenotypic plasticity to altered soil N chemistry compared to the strong adaptive plasticity of P. edulis. Growth and interspecific competitive advantage of moso bamboo were significantly promoted under NH<sub>4</sub><sup>+</sup>-rich conditions but inhibited in an NO<sub>3</sub><sup>-</sup>dominated environment. All tested species showed significantly higher rates of <sup>15</sup>NH<sub>4</sub><sup>+</sup> than <sup>15</sup>NO<sub>3</sub><sup>-</sup> uptake, but the uptake preference for NH<sub>4</sub><sup>+</sup> over NO<sub>3</sub><sup>-</sup> only translated into biomass advantages in *P. edulis*. Both the  $V_{max}$  and the substrate affinity for  $NH_4^+$  was higher in *P. edulis* than in the native tree species. Furthermore, the enzyme activities of GS, GOGAT, and GDH in roots were significantly increased with NH4<sup>+</sup> provision in moso bamboo compared to a much more subdued, less plastic response in the native tree species. The considerable morphological and physiological plasticity of bamboo in response to NH4<sup>+</sup> may confer a significant growth and competitive advantage over native tree species in NH<sub>4</sub><sup>+</sup>-rich environments. The increased ratios of soil NH<sub>4</sub><sup>+</sup>/ NO<sub>3</sub><sup>-</sup> frequently seen in subtropical areas of China in the course of bamboo invasion, coupled to anthropogenic NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> deposition, may result in soil-nutritional habitats increasingly hospitable to bamboo expansion.

### CRediT authorship contribution statement

Huijing Chen: wrote the manuscript, All authors read and approved the final manuscript, carried out the experiments and performed the analyses. Xiaofeng Huang: wrote the manuscript, All authors read and approved the final manuscript, carried out the experiments and performed the analyses. Weiming Shi: wrote the manuscript, All authors read and approved the final manuscript, conceived and designed the research. Herbert J. Kronzucker: wrote the manuscript, All authors read and approved the final manuscript. Lihan Hou: carried out the experiments and performed the analyses. Haiyan Yang: carried out the experiments and performed the analyses. Qingni Song: carried out the experiments and performed the analyses. Jun Liu: carried out the experiments and performed the analyses. Jianmin Shi: carried out the experiments and performed the analyses. Qingpei Yang: carried out the experiments and performed the analyses. Na Zou: wrote the manuscript, All authors read and approved the final manuscript, conceived and designed the research.

### Declaration of competing interest

The authors declare they have no competing interests, and the manuscript has been approved by all authors.

### Acknowledgments

We thank Xianquan Weng (Xiayang State Forest Farm of Nanping of Fujian Province) for the kind provision of C. *fargesii* seeds, Ling Huang, Guochao Liu, Xiongde Tu and Tao Wen (College of Landscape and Art, Jiangxi Agricultural University), for their assistance in N metabolism enzyme activities and <sup>15</sup>N uptake testing, Tianchi Wang (New Zealand Institute for Plant & Food Research) for critical reading of the manuscript.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2021.153508.

#### Funding

This work was supported by the National Natural Science Foundation of China (32060254,31300521,41807028), the Natural Science Foundation of Jiangxi Province, China (20171ACB21027, 20203BBGL73227) and the Enterprise Academician Workstation Foundation

#### (BC20170014Z).

### References

- Abbès, C., Parent, L.E., Karam, A., Isfan, D., 1995. Effect of NH4<sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratios on growth and nitrogen uptake by onions. Plant and Soil 171, 289–296.
- Berger, M.G., Fock, H.P., 1983. <sup>15</sup>N and inhibitor studies on the photorespiratory nitrogen cycle in maize leaves. Photosynth. Res. 4 (1), 3–7.
- Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. J. Ecol. 86, 717–738.
- Boudsocq, S., Niboyet, A., Lata, J.C., Raynaud, X., Loeuille, N., Mathieu, J., Blouin, M., Abbadie, L., Barot, A.S., 2012. Plant preference for ammonium versus nitrate: a neglected determinant of ecosystem functioning? Am. Nat. 180, 60–69.
- Britto, D.T., Kronzucker, H.J., 2002. NH4+ toxicity in higher plants: a critical review. J. Plant Physiol. 159 (6), 567–584.
- Britto, D.T., Kronzucker, H.J., 2013. Ecological significance and complexity of N-source preference in plants. Ann. Bot. 112, 957–963.
- Britto, D.T., Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D., 2001. Futile transmembrane NH4(<sup>+</sup>) cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proc. Natl. Acad. Sci. U.S.A. 98 (7), 4255–4258.
- Bueno, A., Pritsch, K., Simon, J., 2019. Species-specific outcome in the competition for nitrogen between invasive and native tree seedlings. Frontiers in plant science 10, 337.
- Cacco, G., Ferrari, G., Saccomani, M., 1980. Pattern of sulfate uptake during root elongation in maize: its correlation with productivity. Physiol. Plantarum 48, 375–378.
- Callaway, R.M., Pennings, S.C., Richards, C.L., 2003. Phenotypic plasticity and interactions among plants. Ecology 84, 1115–1128.
- Di, D., Sun, L., Wang, M., Wu, J., Kronzucker, H., Fang, S., Chu, J., Shi, W.M., Li, G.J., 2021. WRKY46 promotes ammonium tolerance in Arabidopsis by repressing NUDX9 and IAA-conjugating genes and by inhibiting NH<sub>4</sub><sup>+</sup> efflux in the root elongation zone. New Phytol. https://doi.org/10.1111/nph.17554.
- Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6, 503–523.
- Fukushima, K., Usui, N., Ogawa, R., Tokuchi, N., 2015. Impacts of moso bamboo (*Phyllostachys pubescens*) invasion on dry matter and carbon and nitrogen stocks in a broad-leaved secondary forest located in Kyoto, western Japan. Plant Species Biol. 30, 81–95.
- Funk, J.L., Vitousek, P.M., 2007. Resource-use efficiency and plant invasion in lowresource systems. Nature 446, 1079.
- Givan, C.V., 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18, 375–382.
- Glass, A.D.M., Brito, D.T., Kaiser, B.N., Kronzucker, H.J., Kumar, A., Okamoto, M., Rawat, S.R., Siddiqi, M.Y., Silim, S.M., Vidmar, J.J., 2001. Nitrogen transport in plants, with an emphasis on the regulation of fluxes to match plant demand. J. Plant Nutr. Soil Sci. 164, 199–207.
- Glass, A.D.M., Erner, Y., Kronzucker, H.J., Schjoerring, J.K., Siddiqi, M.Y., Wang, M., 1997. Ammonium fluxes into plant roots: energetics, kinetics and regulation. Z. für Pflanzenernährung Bodenkunde 160 (3), 261–268.
- Huangfu, C., Li, H., Chen, X., Liu, H., Wang, H., Yang, D., 2016. Response of an invasive plant, *Flaveria bidentis*, to nitrogen addition: a test of form-preference uptake. Biol. Invasions 18, 1–16.
- Kleinhenz, V., Midmore, D.J., 2001. Aspects of bamboo agronomy. Adv. Agron. 74, 99–153.
- Knoepp, J.D., Turner, D.P., Tingey, D.T., 1993. Effects of ammonium and nitrate on nutrient uptake and activity of nitrogen assimilating enzymes in western hemlock. For. Ecol. Manag. 59, 179–191.
- Kronzucker, H.J., Glass, A.D.M., Siddiqi, M.Y., 1995. Nitrate induction in spruce: an approach using compartmental analysis. Planta 196, 683–690.
- Kronzucker, H.J., Glass, A.D.M., Siddiqi, M.Y., Kirk, G.J.D., 2000. Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: implications for rice cultivation and yield potential. New Phytol. 145 (3), 471–476.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1995a. Compartmentation and flux characteristics of nitrate in spruce. Planta 196 (4), 674–682.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1995b. Compartmentation and flux characteristics of ammonium in spruce. Planta 196 (4), 691–698.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1995d. Kinetics of NO3- influx in spruce. Plant Physiol. 109, 319–326.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1996. Kinetics of NH4+ influx in spruce. Plant Physiol. 110, 773–779.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59.
- Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., Britto, D.T., 2003. Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis. Physiol. Plantarum 117, 164–170.
- Kusnan, M.B., Berger, M.G., Fock, H.P., 1987. The involvement of glutamine synthetase/ glutamate synthase in ammonia assimilation by *Aspergillus nidulans*. J. Gen. Microbiol. 133, 1235–1242.
- Lavorel, S., Garnier, É., 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct. Ecol. 16, 545–556.
- LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.

#### H. Chen et al.

- Li, B., Li, G., Kronzucker, H.J., Baluška, F., Shi, W., 2014. Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci. 19, 107–114.
- Li, B., Li, Q., Su, Y., Chen, H., Xiong, L., Mi, G., Kronzucker, H.J., Shi, W., 2011. Shootsupplied ammonium targets the root auxin influx carrier AUX1 and inhibits lateral root emergence in *Arabidopsis*. Plant Cell Environ. 34, 933–946.
- Li, G., Li, B., Dong, G., Feng, X., Kronzucker, H.J., Shi, W., 2013. Ammonium induced shoot ethylene production is associated with the inhibition of lateral root formation in *Arabidopsis*. J. Exp. Bot. 64, 1413–1425.
- Li, G., Zhang, L., Wang, M., Di, D., Shi, W., 2019. The Arabidopsis AMOT1/EIN3 gene plays an important role in the amelioration of ammonium toxicity. J. Exp. Bot. 70, 1375–1388.
- Li, Q., Li, B., Kronzucker, H.J., Shi, W., 2010. Root growth inhibition by NH4+ in *Arabidopsis* is mediated by the root tip and is linked to NH4+ efflux and GMPase activity. Plant Cell Environ. 33, 933–946.
- Li, W., Sheng, H., Liu, Y., Chen, W., 2019. Responses of soil bacterial compositions to concentrations of nitrogen forms in the process of moso bamboo invasion. Ecol. Res. 34, 743–752.
- Li, Y., Li, Y., Chang, S.X., Xu, Q., Guo, Z., Gao, Q., Qin, Z., Yang, Y., Chen, J., Liang, X., 2017. Bamboo invasion of broadleaf forests altered soil fungal community closely linked to changes in soil organic C chemical composition and mineral N production. Plant Soil 418, 507–521.
- Li, Z., Zhang, L., Deng, B., Liu, Y., Kong, F., Huang, G., Zou, Q., Liu, Q., Guo, X., Fu, Y., Niu, D., Siemann, E., 2017. Effects of moso bamboo (*Phyllostachys edulis*) invasions on soil nitrogen cycles depend on invasion stage and warming. Environ. Sci. Pollut. Control Ser. 24, 1–11.
- Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., Li, B., 2008. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. New Phytol. 177, 706–714.
- Lima, R.A.F., Rother, D.C., Muler, A.E., Lepsch, I.F., Rodrigues, R.R., 2012. Bamboo overabundance alters forest structure and dynamics in the Atlantic Forest hotspot. Biol. Conserv. 147, 0-39.
- Liu, G., Yang, Y.B., Zhu, Z.H., 2018. Elevated nitrogen allows the weak invasive plant *Galinsoga quadriradiata* to become more vigorous with respect to inter-specific competition. Sci. Rep. 8, 3136.
- Liu, X., Siemann, E., Cui, C., Liu, Y., Guo, X., Zhang, L., 2019. Moso bamboo (*phyllostachys edulis*) invasion effects on litter, soil and microbial plfa characteristics depend on sites and invaded forests. Plant Soil 438, 85–99.
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105, 1141–1157.
- Matesanz, S., Horgan-Kobelski, T., Sultan, S.E., 2012. Phenotypic plasticity and population differentiation in an ongoing species invasion. PloS One 7 (9), e44955.
- Min, J., Sun, H.J., Kronzucker, H.J., Wang, Y., Shi, W.M., 2021a. Comprehensive assessment of the effects of nitrification inhibitor application on reactive nitrogen loss in intensive vegetable production systems. Agric. Ecosyst. Environ. 307, 107227. https://doi.org/10.1016/j.agee.2020.107227.
- Min, J., Sun, H.J., Wang, Y., Pan, Y.F., Kronzucker, H.J., Zhao, D.Q., Shi, W.M., 2021b. Mechanical side-deep fertilization mitigates ammonia volatilization and nitrogen runoff and increases profitability in rice production independent of fertilizer type and split ratio. J. Clean. Prod. 316, 128370. https://doi.org/10.1016/j. jclepro.2021.128370.
- Min, X., Siddiqi, M.Y., Guy, R.D., Glass, A.D.M., Kronzucker, H.J., 1998. Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine. Plant Cell Environ. 21, 1039–1046.
- Min, X., Siddiqi, M.Y., Guy, R.D., Glass, A.D.M., Kronzucker, H.J., 2000. A comparative kinetic analysis of nitrate and ammonium influx in two early-successional tree species of temperate and boreal forest ecosystems. Plant Cell Environ. 23 (3), 321–328.
- Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. New Phytol. 182, 31–48.

- Okutomi, K., Shinoda, S., Fukuda, H., 1996. Causal analysis of the invasion of broadleaved forest by bamboo in Japan. J. Veg. Sci. 7, 723–728.
- Parepa, M., Fischer, M., Bossdorf, O., 2013. Environmental variability promotes plant invasion. Nat. Commun. 4, 1604.
- Plett, D., Ranathunge, K., Melino, V.J., Kuya, N., Uga, Y., Kronzucker, H.J., 2020. The intersection of nitrogen nutrition and water use in plants: new paths toward improved crop productivity. J. Exp. Bot. 71, 4452–4468.
- Reddy, K.S., Menary, R.C., 1990. Nitrate reductase and nitrate accumulation in relation to nitrate toxicity in *Boronia megastigma*. Physiol. Plantarum 78, 430–434.
- Rennenberg, H., Dannenmann, M., Gessler, A., Kreuzwieser, J., Simon, J., Papen, H., 2009. Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biol. 11, 4–23.
- Ryser, P., Eek, L., 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. Am. J. Bot. 87, 402–411.
- Sher, A.A., Hyatt, L.A., 1999. The disturbed resource-flux invasion matrix: a new framework for patterns of plant invasion. Biol. Invasions 1, 107–114.
- Silverstein, T.P., 2019. When both Km and Vmax are altered, Is the enzyme inhibited or activated? Biochem. Mol. Biol. Educ. 47, 446–449.
- Simon, J., Dannenmann, M., Pena, R., Gessler, A., Rennenberg, H., 2017. Nitrogen nutrition of beech forests in a changing climate: importance of plant-soil-microbe water, carbon, and nitrogen interactions. Plant Soil 418, 89–114.
- Song, Q., Ouyang, M., Yang, Q., Lu, H., Yang, G., Chen, F., Shi, J., 2016. Degradation of litter quality and decline of soil nitrogen mineralization after moso bamboo (*Phyllostachys pubscens*) expansion to neighboring broadleaved forest in subtropical China. Plant Soil 404, 113–124.
- Song, Q., Yang, Q., Liu, J., Yu, D., Fang, K., Xu, P., He, Y., Ni, S., Pei, Y., Jun, L., 2013. Effects of *Phyllostachys edulis* expansion on soil nitrogen mineralization and its availability in evergreen broadleaf forest. Chin. J. Appl. Ecol. 24, 338–344 (in Chinese).
- Sun, L., Lu, Y.F., Yu, F.W., Kronzucker, H.J., Shi, W.M., 2016. Biological nitrification inhibition by rice root exudates and itsrelationship with nitrogen-use efficiency. New Phytol. 212, 646–656.
- Sun, L., Di, D.W., Li, G., Kronzucker, H.J., Wu, X., Shi, W., 2020. Endogenous ABA alleviates rice ammonium toxicity by reducing ROS and free ammonium via regulation of the SAPK9-bZIP20 pathway. J. Exp. Bot. 71, 4562–4577.
- Trinder, C.J., Brooker, R.W., Robinson, D., 2013. Plant ecology's guilty little secret: understanding the dynamics of plant competition. Funct. Ecol. 27, 918–929.
- Valliere, J.M., Allen, E.B., 2016. Nitrogen enrichment contributes to positive responses to soil microbial communities in three invasive plant species. Biol. Invasions 18, 1–16. Yu, P., White, P.J., Hochholdinger, F., Li, C., 2014. Phenotypic plasticity of the maize
- root system in response to heterogeneous nitrogen availability. Planta 240, 667–678. Zhang, L., Li, G., Dong, G., Wang, M., Di, D., Kronzucker, H.J., Shi, W., 2019.
- Characterization and comparison of nitrate fluxes in *Tamarix ramosissima* and cotton roots under simulated drought conditions. Tree Physiol. 39, 628–640.
- Zhang, L., Song, H., Li, B., Wang, M., Di, D.W., Lin, X.Y., Kronzcker, H.J., Shi, W.M., Li, G.J., 2021. Induction of *S-nirosoglutathione* reductase protects root growth from ammonium toxicity by regulating potassium homeostasis in Arabidopsis and rice. J. Exp. Bot. 72, 4548–4564.
- Zhang, P., Li, B., Wu, J., Hu, S., 2019. Invasive plants differentially affect soil biota through litter and rhizosphere pathways: a meta-analysis. Ecol. Lett. 22, 200–210.
- Zhao, Y., Qing, H., Zhao, C., Zhou, C., Zhang, W., Xiao, Y., An, S., 2010. Phenotypic plasticity of *Spartina alterniflora* and *Phragmites australis* in response to nitrogen addition and intraspecific competition. Hydrobiologia 637, 143–155.
- Zou, N., Shi, W., Hou, L., Kronzucker, H.J., Huang, L., Gu, H., Yang, Q., Deng, G., Yang, G., 2020 a. Superior growth, N uptake, and NH4+ tolerance in the giant bamboo *Phyllostachys edulis* over the broad-leaved tree *Castanopsis fargesii* at elevated NH4+ may underlie community succession and favor the expansion of bamboo. Tree Physiol. 44, 1606–1622.
- Zou, N., Huang, L., Chen, H., Huang, X., Song, Q., Yang, Q., Wang, T., 2020 b. Nitrogen form plays an important role in the growth of moso bamboo (*Phyllostachys edulis*) seedlings. PeerJ 8, e9938.