Superior growth, N uptake and NH$_4^+$ tolerance in the giant bamboo *Phyllostachys edulis* over the broad-leaved tree *Castanopsis fargesii* at elevated NH$_4^+$ may underlie community succession and favor the expansion of bamboo

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The unbridled expansion of bamboo has imposed serious threats on ecosystem processes and functions. Considerable evidence indicates that bamboo invasions can alter plant-available soil nitrogen (N) pools and rates of N cycling, but the consequences of altered N availability for plant growth and community structure have thus far been poorly characterized. The primary soil-accessible N forms for most plants are ammonium (NH$_4^+$) and nitrate (NO$_3^-$), but plants differ in their ability to use the different N forms, and these differences can be related to their ecological characteristics and drive community structure. In this context, we evaluated the growth response, N uptake and interspecific competition in two subtropical species, *Phyllostachys edulis* (Carrière) J. Houzeau (Synonym *Phyllostachys heterocycla* Carrière) and *Castanopsis fargesii* Franch., dominant species of bamboo and secondary evergreen broad-leaved forests, respectively, under changing N availability in seedlings supplied with different N concentrations and NH$_4^+$/NO$_3^-$ proportions, in vermiculite culture, in a controlled environment. The results show that (i) both species display an NH$_4^+$ preference at elevated N concentrations. The growth of *P. edulis* seedlings was strongly enhanced at increased ratios of NH$_4^+$ to NO$_3^-$ especially at higher N concentrations, but to a much lesser extent in *C. fargesii*. (ii) NH$_4^+$ preference at the level of N uptake in both species was confirmed by the Non-invasive Micro-test Technology and by examining $^{15}$N signatures. *Phyllostachys edulis* had higher NH$_4^+$ fluxes and N concentration under NH$_4^+$ treatments than *C. fargesii*. (iii) NH$_4^+$ at higher concentrations caused toxicity in both species as it inhibited root growth and even caused seedling death, but *P. edulis* had a higher NH$_4^+$-tolerance threshold (24 mM) than *C. fargesii* (16 mM). (iv) When mixed-species cultures were examined in an NH$_4^+$-rich medium, the growth of *C. fargesii*, but not *P. edulis*, was significantly inhibited compared with growth in monoculture. Therefore, *P. edulis* exhibited stronger plasticity and adaptation to changing N availability, whereas *C. fargesii* had low responsiveness and capacity to acclimate to soil N changes. *Phyllostachys edulis* displayed a significant competitive growth advantage compared with *C. fargesii* on NH$_4^+$-dominated substrates.

**Keywords:** bamboo expansion, *Castanopsis fargesii*, interspecific competition, NH$_4^+$ tolerance, nitrogen availability, nitrogen form, *Phyllostachys edulis*. 
Introduction

Moso bamboo (Phyllostachys edulis), Phyllostachys edulis (Carrière) J. Houzeau (Synonym Phyllostachys heterocycla Carrière) a member of the Poaceae and tree-like woody bamboo, widely predominates in subtropical China, possessing ecological, economic and cultural importance (Song et al. 2016). However, P. edulis is also notorious for its invasive character in Asian forests (Okutomi et al. 1996, Kleinhenz and Midmore 2001, Lima et al. 2012, Ying et al. 2016). Secondary evergreen broad-leaved forest, the typical vegetation type in subtropical areas, is the most vulnerable to expansion and can be readily displaced by bamboo (Okutomi et al. 1996, Song et al. 2016, Ying et al. 2016). The unbridled expansion of bamboo has caused substantial impacts on colonized forests, such as altered community structure and species composition (Okutomi et al. 1996, Lima et al. 2012), reduced biodiversity (Larp kern et al. 2011), altered soil processes and microbial composition (Song et al. 2013, 2016, 2017), and broken-up and degraded forest landscapes (Okutomi et al. 1996), and these, together, have been considered among the greatest contemporary threats to woody forests in China. According to previous studies, bamboo can become a dominant species in many forest communities by virtue of shading (Suzaki and Nakatsubo 2001, Lima et al. 2012), mechanical damage (Okutomi et al. 1996), litter suppression (Larp kern et al. 2011) and allelopathy (Chou and Yang 1982). Recently, many studies have reported the influence of P. edulis expansion on soil nutrients, such as soil organic matter, available soil nitrogen (N) pools and rates of N cycling, phosphorus status and dynamics, and soil potassium (K) (Song et al. 2013, 2016, 2017, Fukushima et al. 2015, Y. Li et al. 2017, Z. Li et al. 2017, Wu et al. 2018). However, little information has been gathered on the effects of these ecological processes to community structure, and the role of N has remained surprisingly understudied.

It has been proposed that soil N dynamics can be a driving force in forest succession (Kronzucker et al. 1997, 2003). Fukushima et al. (2015) assumed that P. edulis expansion into broadleaved forests changed the distribution pattern of carbon and N stored in plants and soil. It increased plant N accumulation but reduced soil N available pool, slowed the N cycling and reduced soil N mineralization rate (Song et al. 2016, 2017). Specifically, soil N mineralization changes in terms of both ammonification and nitrification occurred at similar rates, i.e., 3.82 and 3.31 mg kg⁻¹ (30 days)⁻¹ in secondary evergreen broad-leaved forest soil dominated by ammonification, that is 5.66 and 0.18 mg kg⁻¹ (30 days)⁻¹, respectively, in bamboo-dominated mixed forest after the expansion of bamboo (Song et al. 2013). Enhanced ammonification rates (soil NH₄⁺-N) but reduced nitrification rates (soil NO₃⁻-N) following bamboo invasion of broad-leaved forests have also been reported by other different laboratories in different sites (Y. Li et al. 2017, Z. Li et al. 2017). Changes in soil N chemistry are likely to be significant determinants of synecological changes in such forests, as they are in temperate Northern forests (Kronzucker et al. 2003). Although a considerable number of reports indicate that bamboo invasions can alter N content and the chemical speciation of inorganic N in forest soils, the consequences of altered soil N availability for forest plants are poorly understood in the bamboo and tree species coexisting in the affected areas.

Next to usually smaller quantities of organic N, the primary accessible forms of N for plant roots are ammonium (NH₄⁺) and nitrate (NO₃⁻) in most natural ecosystems (Britto and Kronzucker 2013), and species vary greatly in their ability to absorb different inorganic N forms (Britto and Kronzucker 2006, 2013). Over the course of their evolution and community construction, plants may have become adapted to the nitrogenous characteristics of their native forest soils (Kronzucker et al. 1997, 2003), that is, in environments dominated by NH₄⁺ or NO₃⁻ in soils, many plant species develop greater biomass or accumulate greater N quantities on one form of N versus the other, showing obvious, and at times stark, NH₄⁺ or NO₃⁻ preferences. For instance, trees occupying specific habitats of late-successional forests (mature forests) dominated by NH₄⁺ demonstrate more efficient N capture with NH₄⁺ relative to NO₃⁻, in both field and laboratory studies (Kronzucker et al. 1997, Li et al. 2015). Species-specific strategies and the differential capacity of plants to respond to, take up and metabolically process different sources of N is very important for species establishment on successional different sites (Kronzucker et al. 1997, Min et al. 1999, 2000, Kronzucker et al. 2003). For instance, in late-successional forests in the Northern temperate zone, disturbances, such as clear-cut harvesting, tend to convert soil N from NH₄⁺ to mostly NO₃⁻ and late-successional trees, often characterized by low NO₃⁻ uptake and utilization capacities (Kronzucker et al. 1995a, 1995b), as a consequence, become poor competitors for inorganic N on such soils, whereas the sites become dominated by nitrophiles (Kronzucker et al. 1997). On the other hand, in later successional stages, differential toxicity thresholds for NH₄⁺ can play a significant role in driving species composition in the opposite direction, away from domination by nitrophiles, as the latter succumb to NH₄⁺ toxicity (Kronzucker et al. 2003, Britto and Kronzucker 2013, and references therein). In many of the forest soils in Asia in which bamboo incursions have been recorded, soil NH₄⁺ tends to increase relative to NO₃⁻ during P. edulis expansion, but species-specific strategies in terms of uptake preference and growth response to different inorganic N forms have remained uncharacterized in bamboo and the evergreen broad-leaved forest species with which they compete.

The objectives of this study were to investigate species-specific N-use strategies of bamboo and tree species, and to explore the influence of altered N availability on plant growth. We addressed this question by using seedlings of the P. edulis, a dominant species of bamboo-dominant forests, and...
Castanopsis fargesii Franch. a dominant tree species of secondary evergreen broad-leaved forests in subtropical China (Cornelissen 1993, Song et al. 2016), which has been threatened in many parts of subtropical China by the expansion of moso bamboo (Okutomi et al. 1996, Song et al. 2016, Ying et al. 2016). Phyllostachys edulis typically grows on soils where N mineralization is dominated by ammonification, and, thus, NH₄⁺ is the even more dominant N form (Song et al. 2013, 2016, 2017, Z. Li et al. 2017). Soil ammonification and nitrification occur at similar rates in such evergreen broad-leaved forests, although soil nitrification is more pronounced during the principal growing season (from April to October), with soil NH₄⁺ exceeding NO₃⁻ (Song et al. 2013, 2016, 2017; Z. Li et al. 2017) or soil NO₃⁻ higher than NH₄⁺ (Y. Li et al. 2017) according to sites. Growth response and N uptake of the two species were studied by supplying seedlings with different N concentrations and NH₄⁺/NO₃⁻ proportions in vermiculite culture, and in controlled environments. The Non-invasive Micro-test Technology (NMT) and ¹⁵N isotope tracing were employed to assess acquisition of NH₄⁺ and NO₃⁻ in the test species. In view of soil N mineralization and the predominant N form in bamboo and evergreen broad-leaved forest soils, we hypothesized that the two species would display visible species-specific responses and competitiveness to altered N availabilities. Thus, we predicted that: (i) P. edulis will show NH₄⁺ preference, whereas C. fargesii will have improved performance and N uptake with NO₃⁻ or mixed N diets based on co-provision of NH₄⁺ and NO₃⁻; (ii) in NH₄⁺-rich conditions, P. edulis will demonstrate competitive advantages compared with C. fargesii; and (iii) at increased levels of NH₄⁺ supply, C. fargesii will succumb to NH₄⁺ toxicity more readily than P. edulis.

Materials and methods

Plants and treatments

Seeds of P. edulis were collected from Guanyang, Guangxi Province (25°14’N, 110°51’E, 354 m altitude) and were stored at 4 °C. The moso bamboo seeds were soaked overnight and sterilized using 20% NaClO (V/V) for 20 min, rinsed five times in sterile water and then germinated in plastic pots filled with vermiculite and 1 month afterwards three-foliate seedlings were selected for varying N treatments. Castanopsis fargesii seeds were collected from Xiayang State Forest Farm of Nanping, Fujian Province (26°45’N, 118°02’E) and were stratified with sand in a 3:1 (V/V) in greenhouse for 3 months after collection and before sowing in sand. Seedlings were germinated for ~3 months before transferring the three-foliate seedlings to different N treatments. Seedlings of both species were transplanted into plastic pots (diameter 150 mm, height 130 mm) filled with vermiculite and watered three times per week with deionized water until new roots were produced.

Nitrogen form preference experiment

Two weeks afterwards, uniform seedlings were selected for N treatments. Fifteen N treatments consisted of three N concentrations (0.1 mM, 2 mM, 8 mM) and five ratios (NH₄⁺/NO₃⁻ = 0/4, 1/3, 2/2, 3/1, 4/0). The nutrient solution, modified from Norisada and Kojima (2005), contained: 2.5 mM Ca²⁺, 3 mM K⁺, 0.25 mM MgSO₄·7H₂O, 0.6 mM Na₂HPO₄·10H₂O, 0.01 mM Fe-EDTA, 0.02 mM H₃BO₃, 2 μM MnCl₂·4H₂O, 2 μΜ ZnSO₄·7H₂O, 2 μΜ CuSO₄·5H₂O, 0.5 μΜ Mn₂MoO₄·2H₂O, 0.5 μΜ CoCl₂·6H₂O (Table 1). Different N forms and concentrations were supplied by KNO₃, Ca(NO₃)₂·4H₂O, (NH₄)₂SO₄ and K₂SO₄. The pH of each treatment nutrient solution was adjusted to pH 4.0 with 0.1 M NaOH or 0.1 M HCl. In total, 1 ml of 7 mM C₂H₄N₄ was dissolved in 500 ml deionized water added to 1000 ml of N-treatment nutrient solutions to prevent nitrification. Two seedlings per pot, and four pots placed inside another bigger pot as saucer, formed one replicate, and there were three replicates of 24 seedlings for each treatment. Seedlings were watered with nutrient solution twice a week from August to October in 2014. Plants were grown with 25/15
℃ ± 3 °C day/night temperature, 65–70% relative humidity and 14/10 h day/night photoperiod in a greenhouse at Jiangxi Agricultural University (28°41′N, 115°52′E). Pots were rotated every week to eliminate location effects. After 2 months, growth characteristics and root morphology were analyzed.

**Growth analysis and root morphology**

Height was measured with a ruler. Leaf number was determined at the end of the treatments. The chlorophyll content of the leaves was determined with a chlorophyll meter (SPAD-502, Minolta, Osaka, Japan). Root morphology characteristic indices, including total root length (RL), average diameter (AD), root surface (RS) area, root volume (RV) and leaf surface were determined by a root automatic scanning apparatus (EPSON color image scanner LA1600+, Toronto, Canada), equipped with WinRHIZO 2012 software (Regent Instruments, Quebec, Canada) after 2 months of treatments. When treatments were completed, plants were harvested and separated into foliage, stems and roots, dried at 105 °C for 30 min, and then to a constant weight at 60 °C for biomass determination.

**Measurement of net NH₄⁺ and NO₃⁻ fluxes with the NMT system**

Net NH₄⁺ and NO₃⁻ fluxes were measured using the NMT (NMT 100 Series, Younger USA LLC, Amherst, MA, Xuyue (Beijing) Science and Technology Co., Ltd, Beijing, China) and imFluxes V2.0 (Younger USA, LLC, Amherst, MA, USA) software. The NMT system and its application in ion flux detection are described in detail elsewhere (Li et al. 2010, Luo et al. 2013).

Uniform three-foliate seedlings of *P. edulis* and *C. fargesii* were selected and transplanted to plastic pots (diameter 150 mm, height 130 mm) with vermiculite. Two weeks after, seedlings were supplied with one of six ¹⁵N isotope-labeled solutions two times per week from September to November, in 2016. The six N solutions all contained 8 mM of N, with the three N sources provided in equimolar amounts (8 mM NaNO₃, 4 mM (NH₄)₂SO₄ or 4 mM NH₄NO₃), but differing in terms of which N source was labeled: (¹⁵NH₄)₂SO₄, Na¹⁵NO₃, NH₄¹⁵NO₃, NH₄¹⁵NO₃ or unlabeled N, provided as (NH₄)₂SO₄ and NaNO₃. There were three replicates of 36 seedlings per treatment.

After 2 months of N treatment, seedlings were harvested. The roots were washed to eliminate vermiculite and traces of ¹⁵N, twice with tap water and once with distilled water. Then, all fractions including the foliage, stems and roots were oven-dried at 60 °C for 48 h, weighed and finely ground with a mortar and pestle to pass through 0.5 mm sieve before measurement. The concentration of N and ¹⁵N abundance was measured using a Flash 2000 HT elemental analyzer interfaced to an Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Waltham, MA, USA), as described by Piao et al. (2017).

**NH₄⁺-tolerance experiments**

Uniform three-foliate seedlings of *P. edulis* and *C. fargesii* were selected and transplanted to plastic pots (diameter 150 mm, height 130 mm) containing vermiculite. After 15 days of growth, seedlings were treated with normal nutrient solution supplemented with varying concentrations of NH₄⁺ (2, 8, 16, 24, 32, 40 mM) supplied as (NH₄)₂SO₄ two times per week from July to September, in 2015. There were three replicates of 24 seedlings per treatment. The height of seedlings was monitored every 2 weeks. After 2 months, growth and root morphology were analyzed.

**Monoculture and mixed culture experiments**

Uniform three-foliate seedlings of *P. edulis* and *C. fargesii* were selected and transplanted to plastic pots (length 435 mm, width 200 mm, height 140 mm) with vermiculite. There were eight...
seedlings per pot to form the following three planting systems: _P. edulis_ monoculture (seedlings of the same species grown separately in containers), _C. fargesii_ monoculture, and _P. edulis_ and _C. fargesii_ mixed culture (seedlings of each species mixed in containers) to simulate the growth of two species in natural conditions and evaluate interspecific competitive responses in an NH₄⁺-rich environment. Two weeks after production of new roots, seedlings were treated with normal nutrient solution supplemented 4 mM (NH₄)₂SO₄ once a week from September 2017 to August 2018. The growth of seedlings was monitored by recording height monthly.

**Data analysis**

Data were statistically analyzed with SPSS version 13.0 (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) along with a Fisher’s least significance (LSD) test was applied to identify differences. Significant differences (P < 5%) between treatments are indicated by different letters. Sigma Plot 13.0 was used for the generation of graphs and Photoshop for photocomposition.

**Results**

**Growth analysis**

For seedlings of both species, negligible growth differences were found between N forms (ratios of NH₄⁺ to NO₃⁻) at 0.1 mM N, whereas, at greater N concentrations, seedlings displayed superior growth with NH₄⁺ or mixed N compared with that of NO₃⁻. For _P. edulis_, an increment in NH₄⁺ levels and the proportion of NH₄⁺ to NO₃⁻ at 8 mM N in the treatments significantly increased growth, root morphology and chlorophyll content (SPAD value) (Figures 1–3). The biomass of _P. edulis_ seedlings treated with 8 mM NH₄⁺ was significantly larger (3.03- and 8.80-fold) than in seedlings grown with the same N form at 2 and 0.1 mM, respectively, as opposed to _P. edulis_ seedlings grown with the same N form at 2 and 0.1 mM, respectively (Figure 1G). The growth of _C. fargesii_ seedlings was significantly less affected by N form at identical N concentrations in terms of most morphological parameters, except for biomass and chlorophyll content, which were higher with 8 mM NH₄⁺ (Figures 1H and 3B). The biomass of _C. fargesii_ seedlings treated with 8 mM NH₄⁺ were 1.78- and 1.66-fold, respectively, of seedlings grown on the same N form of 2 and 0.1 mM, as compared with the biomass of seedlings treated with 8 mM NO₃⁻, which was 1.29- and 1.24-fold, respectively, of that of seedlings treated with 2 mM and 0.1 mM NO₃⁻ (Figure 1H). Two-way ANOVA indicated that the effects of N concentration, N form and their interaction were significant for all morphological parameters of _P. edulis_ (Table 2). By contrast, the effect of N concentration was significant for most morphological parameters of _C. fargesii_ except for total RL, whereas the effects of N form and the interaction were significant only for biomass, RV and chlorophyll content (Table 2).

**NH₄⁺ and NO₃⁻ net fluxes**

The results show different net fluxes for NH₄⁺ and NO₃⁻ at the root tip in both species when exposed to different N forms (Figure 4). When treated with 8 mM NH₄⁺, net NH₄⁺ flux varied from −2839.11 (influx) to 992.22 (efflux) pmol cm⁻² s⁻¹ for _P. edulis_ and −609.20 to 601.46 pmol cm⁻² s⁻¹ for _C. fargesii_, and the maximal net NH₄⁺ uptake was detected at 0.3 mm from the root tip for both species (Figure 4A and B). When treated with 8 mM NO₃⁻, net NO₃⁻ flux ranged from −153.81 to 697.78 pmol cm⁻² s⁻¹ for _P. edulis_, −72.23 to 119.32 pmol cm⁻² s⁻¹ for _C. fargesii_ and, at 1 mm from the root tip, net NO₃⁻ displayed maximum influx in both species (Figure 4C and D). The maximal net fluxes of NH₄⁺ were 18.46- and 8.43-fold of those of NO₃⁻, respectively, in _P. edulis_ and _C. fargesii_. The maximal net NH₄⁺ and NO₃⁻ fluxes of _P. edulis_ were 4.66- and 2.13-fold, respectively, of those _C. fargesii_.

**Nitrogen concentration and δ¹⁵N in seedlings**

The N concentration of plant fractions and total seedlings varied with species and the ratio of NH₄⁺ to NO₃⁻ but did not differ between treatments with ¹⁵N-labeled solutions and unlabeled controls with the same N form for both species (Figure 5A and B). In _P. edulis_ seedlings, N concentration of plant fractions and total seedlings markedly increased with an increased proportion of NH₄⁺ to NO₃⁻, and N concentration in leaves was higher than in stem and root under the same treatment especially with NH₄⁺ or mixed N (NH₄NO₃) (Figure 5A). Moreover, variation in whole-plant N concentration was attributed to changes in N concentration in aboveground (stem and foliage), which was different among N forms, rather than root N concentration. For _C. fargesii_, seedlings grown with mixed N or NH₄⁺ showed higher N concentration than those NO₃⁻-treated seedlings (Figure 5B). In addition, N concentration in leaves was similar to that of root but larger than stem under the same treatment. Moreover, variation in plant fractions N concentration followed the same trend as that described at the plant level.

For both species, δ¹⁵N was higher in ¹⁴NH₄⁺-treated seedlings than in ¹⁵NO₃⁻-treated seedlings when supplied as either sole N-sources or in co-provision with ¹⁵NH₄NO₃ and NH₄¹⁵NO₃ (Figure 5C and D). δ¹⁵N of ¹⁴NH₄⁺-treated _P. edulis_ seedlings was 1.31-, 1.37- and 1.91-fold of ¹⁵NO₃⁻-treated seedlings, respectively, at 1 mm from the root tip, net NO₃⁻ displayed maximum influx in both species (Figure 4C and D). The maximal net fluxes of NH₄⁺ were 18.46- and 8.43-fold of those of NO₃⁻, respectively, in _P. edulis_ and _C. fargesii_. The maximal net NH₄⁺ and NO₃⁻ fluxes of _P. edulis_ were 4.66- and 2.13-fold, respectively, of those _C. fargesii_.

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**Table 2.** Comparison of biomass, root morphology and chlorophyll content (SPAD value) among seedlings grown in different N forms. The values followed by different letters (a, b, c) within the same treatment were significantly different (P < 0.05) with a Fisher’s least significance (LSD) test. Significant differences (P < 5%) between treatments are indicated by different letters. RV and chlorophyll content were different among N forms, whereas the effects of N form and the interaction were significant only for biomass, RV and chlorophyll content (Table 2).
Figure 1. Nitrogen concentrations and NH$_4^+$/NO$_3^-$ ratios on the growth of *P. edulis* and *C. fargesii*. Height (A and B), leaf number (C and D), leaf surface area (E and F) and biomass (G and H) of *P. edulis* (left) and *C. fargesii* (right) as affected by different N treatments. Data are means from three replicates of 24 seedlings, and bars represent the standard error (SE). Different letters indicate statistical differences among treatments for each species (Fisher's LSD, *P* < 0.05). To facilitate treatment comparison, mass scale is different for each species. This applies to following figures.
Figure 2. Nitrogen concentrations and NH$_4^+$/NO$_3^-$ ratios on root morphology of *P. edulis* and *C. fargesii*. Total RL (A and B), AD (C and D), RS area (E and F) and RV (G and H) of *P. edulis* (left) and *C. fargesii* (right) as affected by different N treatments. Data are means from three replicates of 24 seedlings, and bars represent the SE. Different letters indicate statistical differences among treatments for each species (Fisher’s LSD, *P* < 0.05).
of 15NH4+ treated seedlings was 1.19-, 1.50- and 1.58-fold in roots, stem and leaf tissue of that of 15NO3− treated seedlings with singular N sources, whereas δ15N in roots, stem, and leaf tissue was 5.22-, 3.45- and 5.35-fold of that with 15NO3− when NH4+ and NO3− were provided together (Figure 5D). By analyzing δ15N between (15NH4)2SO4 and 15NH4NO3, NH415N and Na15NO3, it emerged that the increased differences in δ15N were brought about not only by increased 15NH4+ uptake in the presence of NO3− but also by decreased uptake of 15NO3−, and 15NO3− uptake in the presence of NH4+ was reduced when compared with the single-N nutrient solutions in both species (Figure 5C and D).

**NH4+ tolerance**

Seedling growth differences began being evident after the 28th day of treatment (Figure 6). For *P. edulis*, differences in height growth among NH4+ treatments were visible from the 42nd day and accentuated with time, and, on the 56th day, the seedlings treated with 16–40 mM NH4+ were significantly larger than the seedlings treated with 2 mM NH4+ (Figure 6A). By contrast, differences in height growth of *C. fargesii* seedlings among NH4+ treatments were also accentuated with time, but there were no significant differences during the whole experiments (Figure 6B). For both species, the growth of aboveground parts such as leaf number, biomass, and SPAD value were increased with rising NH4+ concentrations (Figure 7).

Although aboveground growth in both species proceeded well at soil NH4+ concentrations from 2 to 40 mM, root growth suffered NH4+ toxicity symptoms in both *P. edulis* and *C. fargesii* with increasing NH4+ levels. Total RL, RS area and RV were significantly inhibited in *P. edulis* at NH4+ concentrations >24 mM compared with seedlings treated with 2–16 mM NH4+. By contrast, in *C. fargesii*, these root morphology indices were significantly inhibited at NH4+ concentrations >16 mM compared with seedlings treated at 2 mM NH4+ (Figure 8).

The results show different survival rates in the two species at increased NH4+ concentrations (Figure 9). In *P. edulis*, the survival rate of all NH4+ -treated seedlings was 100%, whereas, in *C. fargesii*, the survival rate sharply decreased with increasing NH4+, and the survival rate of 16–40 mM NH4+...
Figure 4. Effects of 8 mM different N treatment on NH4+ and NO3− flux along the root tip of *P. edulis* and *C. fargesii*. Three-foliate seedlings of *P. edulis* and *C. fargesii* were treated with 8 mM NH4+ (A and B) or NO3− (C and D) for 2 weeks and then subjected to NH4+ or NO3− flux measurements at 0–40 mm from the root cap junction. Data are means from two replicates of eight seedlings, and bars represent the SE. Different letters on the error bars in each panel indicate significant difference among the measured positions for each species. The measuring solution contained 2.5 mM CaCl2·2H2O, 1.5 mM K2SO4, 0.6 mM Na2HPO4·10H2O, 0.25 mM MgSO4·7H2O, pH 4.0, to which either 4 mM (NH4)2SO4 for NH4+ or 8 mM NaN3 for NO3− flux measurements were added.

Interspecific competition

As both species demonstrated a similar preference for NH4+, it was interesting to examine the niche complementarity of these species. Interspecific competition between *P. edulis* and *C. fargesii* under NH4+-rich conditions was detected by comparing results from monoculture and mixed culture experiments, as shown in Figure 10A and B. In *P. edulis*, the seedlings showed very similar height growth between the monoculture and mixed culture experiments (Figure 10C): during September 2017–February 2018, the growth rate was relatively flat, then growth suddenly accelerated, in March 2018; after that, the growth rate decreased, a suppression that lasted for the rest of the experiment. In *C. fargesii*, height growth was recorded from April 2018, accelerated, and lasted from May 2018 to August 2018 in the monoculture experiment, but growth was clearly inhibited in the mixed culture for the whole experiment (Figure 10C). Moreover, *P. edulis* displayed similar tillers, biomass and ratio of root to shoot between the monoculture and mixed culture experiments; however, biomass and branches of *C. fargesii* were significantly inhibited, whereas the ratio of root/shoot was higher in the mixed culture trial compared with monoculture (Figure 10D–F).

Discussion

**Both *P. edulis* and *C. fargesii* display NH4+ preference**

Our data support the view that substrate N concentration affects plant preference for N form (Britto and Kronzucker 2006), whereas the effects of N form were negligible at low concentrations for both species (Figures 1–3). Similar results were reported by Uscola et al. (2014) in two ecologically distinct Mediterranean forest trees that were fertilized with 1 mM N, and...
Figure 5. Nitrogen content and δ¹⁵N in root, stem, and leaf of *P. edulis* and *C. fargesii* under different NH₄⁺/NO₃⁻ ratios of 8 mM N. (A and B) Nitrogen content in whole plant and root, stem and leaf of *P. edulis* and *C. fargesii*, whole plant N concentration was calculated as the weighted average of N concentration in leaves, stems and roots taking into account the mass of the three compartments. (C and D) The δ¹⁵N signatures in plant roots, stem and leaves for species from *P. edulis* and *C. fargesii*. Data are means from three replicates of 36 seedlings, and bars represent SE. The different lowercase letters indicate significant differences in the means among treatments for either root, stem or foliar samples for each species using one-way ANOVA and Fisher’s LSD (*P* < 0.05).

Nitrogen acquisition strategy of invasive bamboo and native tree

by Warren and Adams (2002), who found negligible growth differences between N forms in seedlings of *Pinus pinaster*, fertilized with 0.125 and 0.5 mM N. The insignificant effects of N form at low concentrations is probably the consequence of plant depletion of supplied N (Uscola et al. 2014) and the fact that low N availability produces similar deficiency in seedlings at 0.1 mM regardless of N form, limiting growth in a similar fashion (Nacry et al. 2013). This is supported at least in part by the observation that increasing N concentrations in the nutrient solutions, especially in the case of NH₄⁺, significantly accelerated growth in both species (Figures 1–3, Table 2).

At increased N concentrations such as 8 mM, the growth of both species displayed an NH₄⁺ preference, but species had distinct performance to altered N availability: *P. edulis* responded very strongly to different ratios of NH₄⁺/NO₃⁻, and an increase in the proportion of NH₄⁺, even when NH₄⁺ was the only N source, significantly promoted growth, biomass, leaf chlorophyll and N concentration compared with seedlings on NO₃⁻ (Figures 1–3 and 5). For example, seedlings treated with NH₄⁺ displayed an increase of 61.47 and 449%, respectively, in biomass compared with seedlings with NH₄⁺/NO₃⁻ (2/2) and NO₃⁻ at 8 mM N (Figure 1G), similar to the species classified as 'ammonium specialists', such as late-successional Northern conifers (Kronzucker et al. 1997, Britto and Kronzucker 2006). By contrast, different ratios of NH₄⁺/NO₃⁻ had minor effects in *C. fargesii*, although biomass production (Figure 1H), leaf chlorophyll content (Figure 3B) and N concentration (Figure 5B) were higher with NH₄⁺ when supplied at 8 mM. For example, the biomass of 8 mM NH₄⁺ treated seedlings was increased only 14.73 and 23.33%, respectively, compared with NH₄⁺/NO₃⁻ (2/2) and NO₃⁻ treated seedlings at the same N concentration (Figure 1H). Although it was significantly larger than at 2 mM N (0/4, NO₃⁻ alone), the growth of *P. edulis* at 2 mM N (4/0, NH₄⁺ alone) was inhibited compared with
Figure 6. Effects of NH$_4^+$ concentrations on height increment of *P. edulis* and *C. fargesii* along with time course. Seedlings were grown with supply of different concentrations of NH$_4^+$. Heights are expressed relative to initial size. Data are means from three replicates of 24 seedlings, and bars represent the SE. Asterisk indicates statistical differences compared with the 0 day, different letters indicate statistical differences among treatments at the same time, and NS indicates no significant differences (Fisher’s LSD, *P* < 0.05).

Figure 7. Growth, biomass and chlorophyll content of aboveground parts in *P. edulis* and *C. fargesii* seedlings supplied with different concentrations of NH$_4^+$ (mM). Data are means from three replicates of 24 seedlings at the end of experiment, and bars represent SE. Different letters indicate statistical differences among treatments for each species (Fisher’s LSD, *P* < 0.05).
growth at 2 mM N (3/1, NH$_4^+$/NO$_3^-$) (Figures 1 and 2). We attributed the enhanced response to mixed N at 1.5 mM NH$_4^+$ + 0.5 mM NO$_3^-$ to a stimulatory effect of NO$_3^-$, for which there is much literature precedent (Kronzucker et al. 1999b, and references therein), whereas, at 2 mM of sole NH$_4^+$ supply, growth appears to still be suboptimal in _P. edulis_ (Figures 1–3 and 6–8). Although there appear to not be any stimulatory effects by NO$_3^-$ at concentrations of NO$_3^-$ <0.4 mM (Gu et al. 2016; Figures 1–3), or >2 mM, as seen when analyzing the treatments ranging from 2 mM N (0/4) and 8 mM N (0/4), growth was promoted with increased NH$_4^+$ concentrations. It is not unusual for NH$_4^+$/NO$_3^-$ ratios to have optimal response
points that differ with overall N concentration (Kronzucker et al. 1999b, Zhang et al. 2019). Ueda (1960) has reported that the application of ammonium sulfate strongly increased the production of new culms in the first year after fertilization in the groves of *P. edulis* and *P. reticulata*. Therefore, the data support a more generic NH$_4^+$ preference in bamboo.

A higher growth response to NH$_4^+$ than NO$_3^-$ both in *P. edulis* and *C. fargesii* might be associated with higher nutrient activation and increased absorption associated with NH$_4^+$ uptake than NO$_3^-$ because of increased H$^+$ extrusion and altered ion balance (van Beusichem et al. 1988, Britto and Kronzucker 2002); this could include phosphate, which is limiting for the growth of forest plants and net primary productivity both in bamboo and broad-leaved forest ecosystems (Yan et al. 2008, Du et al. 2016). In addition, similar to the report by Norisada and Kojima (2005), superior performance with NH$_4^+$ treatment in both species might also be associated with the higher photosynthetic capacity of a larger overall photosynthetic apparatus (leaf number, leaf area), higher leaf chlorophyll and higher leaf N concentration of NH$_4^+$-treated seedlings (Figures 1, 3 and 5). Furthermore, the size and architecture of the root system are significant factors in determining the ability of a plant to interception and acquisition of N (Nacry et al. 2013). In our experiments, increased NH$_4^+$/NO$_3^-$ at higher N concentrations significantly promoted root growth in *P. edulis* seedlings as seen in root biomass and root system architecture (Figures 1G and 2A, C, E and G, Table 2), and seedlings achieved higher aboveground growth and biomass accumulation under such treatments (Figure 1A, C, E and G). However, the architecture of the straight root of *C. fargesii* was less affected by N forms (Figure 2B, D, F and H, Table 2), compared with the fibrous adventitious root of *P. edulis*.

Nitrogen deposition in China has been dramatically enhanced by anthropogenic emissions. In Jiangxi red soil forestland, one typical site of moso bamboo and broad-leaved forest, the total inorganic N deposition was 83.7 kg ha$^{-1}$ year$^{-1}$ in 2004 and 81.3 kg ha$^{-1}$ year$^{-1}$ in 2005, with reduced N compounds accounting for 78.3% of total N deposition (Fan et al. 2009). The increased deposition of atmospheric NH$_3$/NH$_4^+$ would lead to the accumulation of excess NH$_4^+$ in forest soil. Nitrogen concentrations of NH$_4^+$ and NO$_3^-$ in soil solutions >2.0 mM have been reported, [NH$_4^+$] in forest-floor soil solutions can range from 0.4 to 4 mM (Britto and Kronzucker 2006), and, in some soils, NH$_4^+$ concentrations of ~40 mM are achieved (Britto and Kronzucker 2002), whereas [NO$_3^-$] may range 1000-fold over a distance of centimeters and over the course of hours (Bloom 2015). Furthermore, N availability is highly heterogeneous both spatially and temporally, and varies greatly among sites and with the seasons (Britto and Kronzucker 2006, Wang et al. 2007, Song et al. 2016, Y. Li et al. 2017, Z. Li et al. 2017). Therefore, such N concentrations are likely on some soils where *P. edulis* and *C. fargesii* are found, in subtropic forest ecosystems. Increased NH$_4^+$ would benefit both *C. fargesii* and *P. edulis* given their N-source preference. Due to the advantages of *P. edulis* in interspecific competition and the higher NH$_4^+$-tolerance threshold compared with *C. fargesii*, increased soil ammonification accompanying bamboo invasion coupled with NH$_4^+$-dominated N deposition can be inferred as an important driving force for the bamboo expansion success in subtropical China. Due to NH$_4^+$ preference, NH$_4^+$-N should also be the main form of fertilizer in the seedling cultivation of the two species.

Both species display NH$_4^+$ preference but *P. edulis* has higher NH$_4^+$ net fluxes and N concentration than *C. fargesii*

By using NMT, we observed that the spatial variability and net influxes of NH$_4^+$ and NO$_3^-$ were largest at 0.3 and 1 mm, respectively, from the root tips of *P. edulis* and *C. fargesii* (Figure 4), and distinct net fluxes of NH$_4^+$ and NO$_3^-$ in different zones have also been seen in other woody plants (Luo et al. 2013). The apical region of the root is characterized by the root cap, the meristematic region, the cell elongation zone and the maturation zone (Li et al. 2010), and the net fluxes of NH$_4^+$ and NO$_3^-$ can reflect results of N assimilation and uptake kinetics of these ions into root cells (Britto and Kronzucker 2006, Luo et al. 2013). So, our results suggest that the root tip and the meristematic and cell elongation zones (0.3 ~ 1 mm) are more important than the maturation zone for uptake of NH$_4^+$ and NO$_3^-$, possibly because of cytosolic concentrations of NH$_4^+$ and NO$_3^-$ in those zones falling below the thresholds needed for N assimilation to support the fast-growing in the species. Although effects by root-cutting on
ions fluxes cannot be discounted, the more efficient N capture with \( \text{NH}_4^+ \) relative to \( \text{NO}_3^- \) in both species and higher maximal net \( \text{NH}_4^+ \) fluxes in *P. edulis* than *C. fargesii* displayed by NMT were also consistent with and supported by \( \delta^{15}\text{N} \) experiments and N concentration in both plants (Figure 4). Both bamboo-dominated forests and *C. fargesii*-dominated secondary evergreen broad-leaved forests contained more \( \text{NH}_4^+ \) than \( \text{NO}_3^- \) (Song et al. 2016); therefore, \( \text{NH}_4^+ \)-uptake preference appears to reflect adaptation to the most abundant N form in the soil habitat.

The present study shows an inhibition of \( \text{NO}_3^- \) uptake by \( \text{NH}_4^+ \), as is classically observed (Kronzucker et al. 1999a,
NH$_4^+$ toxicity differs between the species, and _P. edulis_ has superior NH$_4^+$ tolerance than _C. fargesii_ at elevated soil NH$_4^+$

Despite the fact that NH$_4^+$ is the preferred N source for many plants, toxicity symptoms, such as a stunted root system and leaf chlorosis, emerge readily in most species, even those frequently labeled as ‘NH$_4^+$ specialists’ at increased levels of NH$_4^+$ supply (Britto and Kronzucker 2006, Li et al. 2014). In our study, NH$_4^+$ at higher concentrations also caused toxicity in both species, but _P. edulis_ was distinctly more NH$_4^+$-tolerant than _C. fargesii_: NH$_4^+$ toxicity thresholds on belowground were higher in _P. edulis_ (≥24 mM) than in _C. fargesii_ (≥16 mM) (Figure 8); in addition, _C. fargesii_ was more susceptible, and survival rate significantly decreased with increased NH$_4^+$ concentrations compared with that of _P. edulis_ (Figure 9). Therefore, results support our second hypothesis that _P. edulis_ had stronger NH$_4^+$ tolerance than _C. fargesii_.

Recent studies have demonstrated that shoot-supplied NH$_4^+$ and root-supplied NH$_4^+$ sources suppress plant growth differentially by targeting different tissues (Li et al. 2014). Specifically, root-supplied NH$_4^+$ principally targets root system development, and such responses seemed to be controlled by independent genes and pathways that are enriched in root tips (Li et al. 2010, 2011, 2013). In agreement with this view, in our root-supplied NH$_4^+$ experimental system, although the root system was suppressed under increased NH$_4^+$ concentrations (Figure 8), aboveground variables such as leaf number and leaf chlorophyll content were not inhibited but even increased, in both species (Figure 7).

*Phyllostachys edulis* has a significant competitive growth advantage compared with _C. fargesii_ under co-existence conditions in an NH$_4^+$-rich environment

The similarity in NH$_4^+$ preference implies the absence of fundamental niche complementarity for N uptake, whereas a consistency in the growth of _P. edulis_ between monoculture and mixed culture systems suggests negligible effects of interspecific competition on _P. edulis_’ overall performance (Figure 10B–F). On the other hand, _C. fargesii_ exhibited inhibited height growth, branching number and biomass in mixed culture systems compared with monoculture (Figure 10B–F). Therefore, _P. edulis_ had higher competitiveness compared with _C. fargesii_ in the NH$_4^+$-rich environment, which supported our third hypothesis.

Fast-growth properties of _P. edulis_ compared with _C. fargesii_ are a general characteristic of the species under suitable growth conditions. In _P. edulis_, strong height growth occurred in February and March (Figure 10C), similar to the observed annual growth dynamic of aboveground culms (Kleinhenz and Midmore 2001). However, in _C. fargesii_, maximum growth occurred from May to July (Figure 10C), largely consistent with the reports of Hou (2015), who showed that _C. fargesii_ seedlings grew rapidly from April to September during the growth season (Hou 2015, Song et al. 2016). It has been reported that changes in plant height have important consequences for capturing light resources on undergrowth plants (Stevens and Carson 1999). In addition, when light intensity changes, soil humidity also changes. Therefore, in addition to soil nutrient competition, accompanying environmental factors, such as low light intensity and high soil moisture, in the presence of competing and shading plant canopies may also contribute to the low competitiveness of _C. fargesii_ in mixed culture with _P. edulis_. Particularly, we have to mention that the seedlings we used to test the influences of N availability on plant growth are not entirely representative of that from adult bamboo and trees in natural conditions. In natural settings, _P. edulis_’ active rhizomes, and their vigorous lateral expansion, further contribute to rapid invasion into neighboring forests. In addition, young shoots developed from buds on rhizomes quickly reach full heights of 10–20 m, matching those of well-developed canopy trees, are connected with mother plant (Fukushima et al. 2015), competing with trees both aboveground and belowground. Furthermore, N, phosphorus and carbohydrates can readily translocate within clonal fragments inform mother bamboo (Li et al. 2000, Saitoh et al. 2006, Wang et al. 2016, Sun et al. 2019), not only improving the adaptability of bamboo to adverse environments, but also facilitating group competitiveness compared with trees.

In conclusion, this study demonstrates species-specific strategies and the differential capacity of _P. edulis_ and _C. fargesii_ to altered soil N availabilities. The growth of both species shows a clear NH$_4^+$ preference at higher N concentrations, as verified both by the NMT approach and by examining $^{15}$N signatures. However, the responses of growth and root system architecture were much stronger in _P. edulis_ than those of _C. fargesii_ when confronted with increased proportions of NH$_4^+$/NO$_3^-$ and with increased NH$_4^+$ levels. An increase in the ratio of NH$_4^+$/NO$_3^-$ significantly increased N concentration in _P. edulis_ but not in _C. fargesii_, as compared with the NH$_4$NO$_3$ treated seedlings, at 8 mM N. *Phyllostachys edulis* demonstrated a significant
competitive growth advantage compared with *C. fargesii* on NH₄⁺-rich substrates. In addition, *Phyllostachys edulis* was more NH₄⁺-tolerant than *C. fargesii*. Therefore, it can be inferred that, in broad-leaved forests, soil N is converted from a mixture of NH₄⁺ and NO₃⁻ to mostly NH₄⁺ in the process of bamboo invasion, and the previously dominant tree species are unable to compete with the NH₄⁺ specialist bamboo, which, intern becomes dominant. The differential toxicity thresholds for NH₄⁺ in *Phyllostachys edulis* (24 mM) and *C. fargesii* (16 mM) may play a role in community structure succession, as the latter species is more susceptible to NH₄⁺ toxicity and its survival rate sharply decreases with increasing NH₄⁺. Our experiment also provides important insights for the nursery cultivation of these species.

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