Tree Physiology 44, 1606–1622
doi:10.1093/treephys/tpaa086

Research paper

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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Received December 2, 2019; accepted June 19, 2020 ; handling Editor David Whitehead

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^+ net 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 (Larpkern 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 (Larpkern 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 NH_4^+-N) but reduced nitrification rates (soil NO_3^--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 nnumber 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 (NH_4^+) and nitrate (NO_3^-) 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 NH_4^+ or NO_3^- 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, NH_4^+ or NO_3^- preferences. For instance, trees occupying specific habitats of late-successional forests (mature forests) dominated by NH_4^+ demonstrate more efficient N capture with NH_4^+ relative to NO_3^-, 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 successionally 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 NH_4^+ to mostly NO_3^- and late-successional trees, often characterized by low NO_3^- uptake and utilization capacities (Kronzucker et al. 1995*a*, 1995*b*), 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 NH_4^+ can play a significant role in driving species composition in the opposite direction, away from domination by nitrophiles, as the latter succumb to NH_4^+ 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 NH_4^+ tends to increase relative to NO_3^- 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

Table 1. Nutrient solution composition of different N concentrations [N] and NH_4^+/NO_3^- ratios.

[N]	0.1 mM					2 mM					8 mM				
NH_4^+/NO_3^-															
	0/4	1/3	2/2	3/1	4/0	0/4	1/3	2/2	3/1	4/0	0/4	1/3	2/2	3/1	4/0
KNO_3	0.1	0.075	0.05	0.025	0	2	1.5	1	0.5	0	3	1	0	0	0
$Ca(NO_3)_2.4H_2O$	0	0	0	0	0	0	0	0	0	0	2.5	2.5	2	1	0
$(NH_4)_2SO_4$	0	0.0125	0.025	0.0375	0.05	0	0.25	0.5	0.75	1	0	1	2	3	4
K_2SO_4	1.45	1.4625	1.475	1.4875	1.5	0.5	0.75	1	1.25	1.5	0	1	1.5	1.5	1.5
$CaCl_2.2H_2O$	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0	0	0.5	1.5	2.5
$MgSO_4.7H_2O$	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
$Na_2HPO_4.10H_2O$	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	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	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
H_3BO_3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
$MnCl_2.4H_2O$	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
$ZnSO_4.7H_2O$	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
$CuSO_4.5H_2O$	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
$Na_2MoO_4.2H_2O$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
$CoCl_2.6H_2O$	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005

Concentrations are in mM.

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, NH_4^+ 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 NH_4^+ exceeding NO_3^- (Song et al. 2013, 2016, 2017; Z. Li et al. 2017) or soil NO_3^- higher than NH_4^+ (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 NH_4^+/NO_3^- proportions in vermiculite culture, and in controlled environments. The Non-invasive Micro-test Technology (NMT) and ^{15}N isotope tracing were employed to assess acquisition of NH_4^+ and NO_3^- 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 NH_4^+ preference, whereas *C. fargesii* will have improved performance and N uptake with NO_3^- or mixed N diets based on co-provision of NH_4^+ and NO_3^-; (ii) in NH_4^+-rich conditions, *P. edulis* will demonstrate competitive advantages compared with *C. fargesii*; and (iii) at increased levels of NH_4^+ supply, *C. fargesii* will succumb to NH_4^+ 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 ($NH_4^+/NO_3^- = 0/4, 1/3, 2/2, 3/1, 4/0$). The nutrient solution, modified from Norisada and Kojima (2005), contained: 2.5 mM Ca^{2+}, 3 mM K^+, 0.25 mM $MgSO_4 \cdot 7H_2O$, 0.6 mM $Na_2HPO_4 \cdot 10H_2O$, 0.01 mM Fe-EDTA, 0.02 mM H_3BO_3, 2 μM $MnCl_2 \cdot 4H_2O$, 2 μM $ZnSO_4 \cdot 7H_2O$, 2 μM $CuSO_4 \cdot 5H_2O$, 0.5 μM $Na_2MoO_4 \cdot 2H_2O$, 0.5 μM $CoCl_2 \cdot 6H_2O$ (Table 1). Different N forms and concentrations were supplied by KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2SO_4$ and K_2SO_4. 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 $C_2H_4N_4$ (294 mg $C_2H_4N_4$ was dissolved in 500 ml deionized water) was 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

°C ± 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 NH_4^+ and NO_3^- fluxes with the NMT system

Net NH_4^+ and NO_3^- fluxes were measured using the NMT (NMT100 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 8 mM of NH_4^+ or NO_3^-. After treatment for 2 weeks from June to July in 2016, the white fine roots were selected and cut from the root system of *P. edulis* and *C. fargesii*. The excised roots were immediately equilibrated in measuring solution for 20–30 min. Eight plants for NH_4^+ and another eight plants for NO_3^- were used for ion flux analyses. Each plant was measured once. The equilibrated roots were then transferred to the measuring chamber containing 2–3 ml of fresh measuring solution. The measuring solution was composed of 2.5 mM $CaCl_2{\cdot}2H_2O$, 1.5 mM K_2SO_4, 0.6 mM $Na_2HPO_4{\cdot}10H_2O$, 0.25 mM $MgSO_4{\cdot}7H_2O$, 4 mM $(NH_4)_2SO_4$ for NH_4^+ and 2.5 mM $CaCl_2{\cdot}2H_2O$, 1.5 mM K_2SO_4, 0.6 mM $Na_2HPO_4{\cdot}10H_2O$, 0.25 mM $MgSO_4{\cdot}7H_2O$, 8 mM $NaNO_3$ for NO_3^- and pH 4.0 was adjusted by HCl-NaOH. The final flux values at each point were the means of eight individual plants from a given treatment.

Pre-pulled and silanized microsensor (Φ9 ± 1 μm, XY-CGQ-01, Younger USA) were first filled with a backfilling solution (NH_4^+,100 mM NH_4Cl, NO_3^-, 10 mM KNO_3) to a length of ~1.0 cm from the tip. Then, the micropipettes were front-filled with 40–50 μm or 80 μm columns of selective liquid ion-exchange cocktails (NH_4^+ LIX, XY-SJ-NH4, NO_3^- LIX, XY-SJ-NO3, Younger USA) for NH_4^+ and NO_3^-, respectively. YG003-Y11 (Younger USA) was used as the reference microsensor. Before each flux measurement, the microelectrodes were calibrated using NH_4^+ (NO_3^-) at concentrations of 0.5, 1.0, 5.0 and 10 mM. Only microsensors with a Nernstian slope >50 mV/decade for NH_4^+ and <−50 mV/decade for NO_3^- were used in the study.

^{15}N-tracer experiments and determination of total N and $\delta^{15}N$

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 ^{15}N 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 $NaNO_3$, 4 mM $(NH_4)_2SO_4$ or 4 mM NH_4NO_3), but differing in terms of which N source was labeled: $(^{15}NH_4)_2SO_4$, $Na^{15}NO_3$, $^{15}NH_4\ NO_3$, $NH_4{}^{15}NO_3$, or unlabeled N, provided as $(NH_4)_2SO_4$ and $NaNO_3$. 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 ^{15}N, 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 ^{15}N 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).

NH_4^+-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 NH_4^+ (2, 8, 16, 24, 32, 40 mM) supplied as $(NH_4)_2SO_4$ 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 NH_4^+-rich environment. Two weeks after production of new roots, seedlings were treated with normal nutrient solution supplemented 4 mM $(NH_4)_2SO_4$ 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 NH_4^+ to NO_3^-) at 0.1 mM N, whereas, at greater N concentrations, seedlings displayed superior growth with NH_4^+ or mixed N compared with that of NO_3^-. For *P. edulis*, an increment in NH_4^+ levels and the proportion of NH_4^+ to NO_3^- 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 NH_4^+ 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 seedlings treated with 8 mM NO_3^-, which were similar (1.06- and 1.61-fold) to the seedlings with NO_3^- 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 NH_4^+ (Figures 1H and 3B). The biomass of *C. fargesii* seedlings treated with 8 mM NH_4^+ 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 NO_3^-, which was 1.29- and 1.24-fold, respectively, of that of seedlings treated with 2 mM and 0.1 mM NO_3^- (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).

NH_4^+ and NO_3^- net fluxes

The results show different net fluxes for NH_4^+ and NO_3^- at the RS along the root tip in both species when exposed to different N forms (Figure 4). When treated with 8 mM NH_4^+, net NH_4^+ 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 NH_4^+ uptake was detected at 0.3 mm from the root tip for both species (Figure 4A and B). When treated with 8 mM NO_3^-, net NO_3^- 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 NO_3^- displayed maximum influx in both species (Figure 4C and D). The maximal net fluxes of NH_4^+ were 18.46- and 8.43-fold of those of NO_3^-, respectively, in *P. edulis* and *C. fargesii*. The maximal net NH_4^+ and NO_3^- fluxes of *P. edulis* were 4.66- and 2.13-fold, respectively, of those *C. fargesii*.

Nitrogen concentration and $\delta^{15}N$ in seedlings

The N concentration of plant fractions and total seedlings varied with species and the ratio of NH_4^+ to NO_3^- but did not differ between treatments with ^{15}N-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 NH_4^+ to NO_3^-, and N concentration in leaves was higher than in stem and root under the same treatment especially with NH_4^+ or mixed N (NH_4NO_3) (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 NH_4^+ showed higher N concentration than those NO_3^- 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, $\delta^{15}N$ was higher in $^{15}NH_4^+$-treated seedlings than in $^{15}NO_3^-$-treated seedlings when supplied as either sole N-sources or in co-provision with $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ (Figure 5C and D). $\delta^{15}N$ of $^{15}NH_4^+$-treated *P. edulis* seedlings was 1.31-, 1.37- and 1.91-fold of $^{15}NO_3^-$-treated seedlings in, respectively, root, stem and leaf tissue when different N-sources were provided with $(^{15}NH_4)_2SO_4$ and $Na^{15}NO_3$, compared with a 2.18-, 1.65- and 2.22-fold in root, stem and leaf tissue when N co-provision as $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ was imposed (Figure 5C). For *C. fargesii*, $\delta^{15}N$

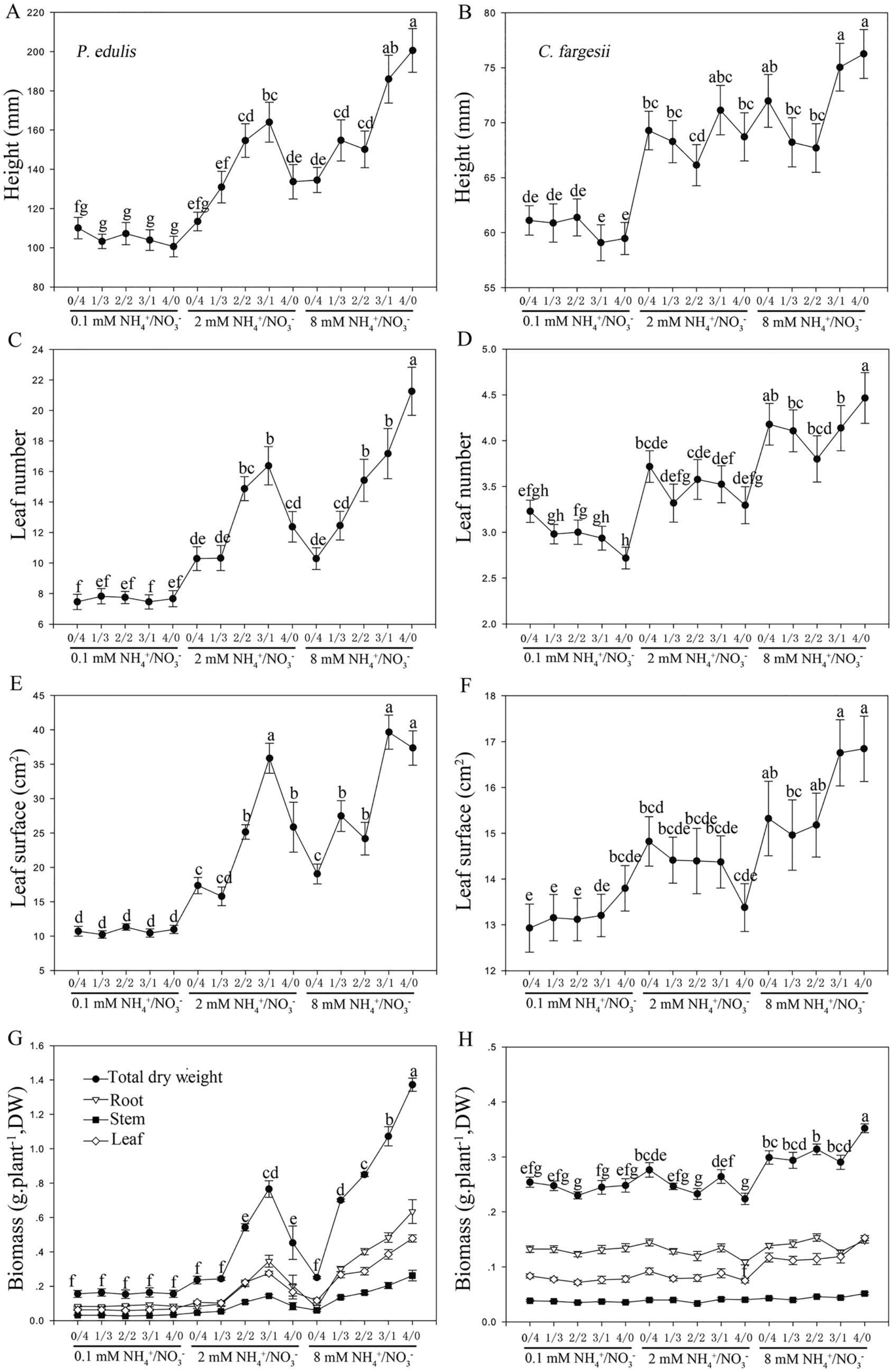

Figure 1. Nitrogen concentrations and NH_4^+/NO_3^- ratios on the growth of *P. edulis* and *C. fargesii*. Height (A and B), leaf number (C and D), leaf surface area (E and F) and biomass (G and H) of *P. edulis* (left) and *C. fargesii* (right) as affected by different N treatments. Data are means from 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.

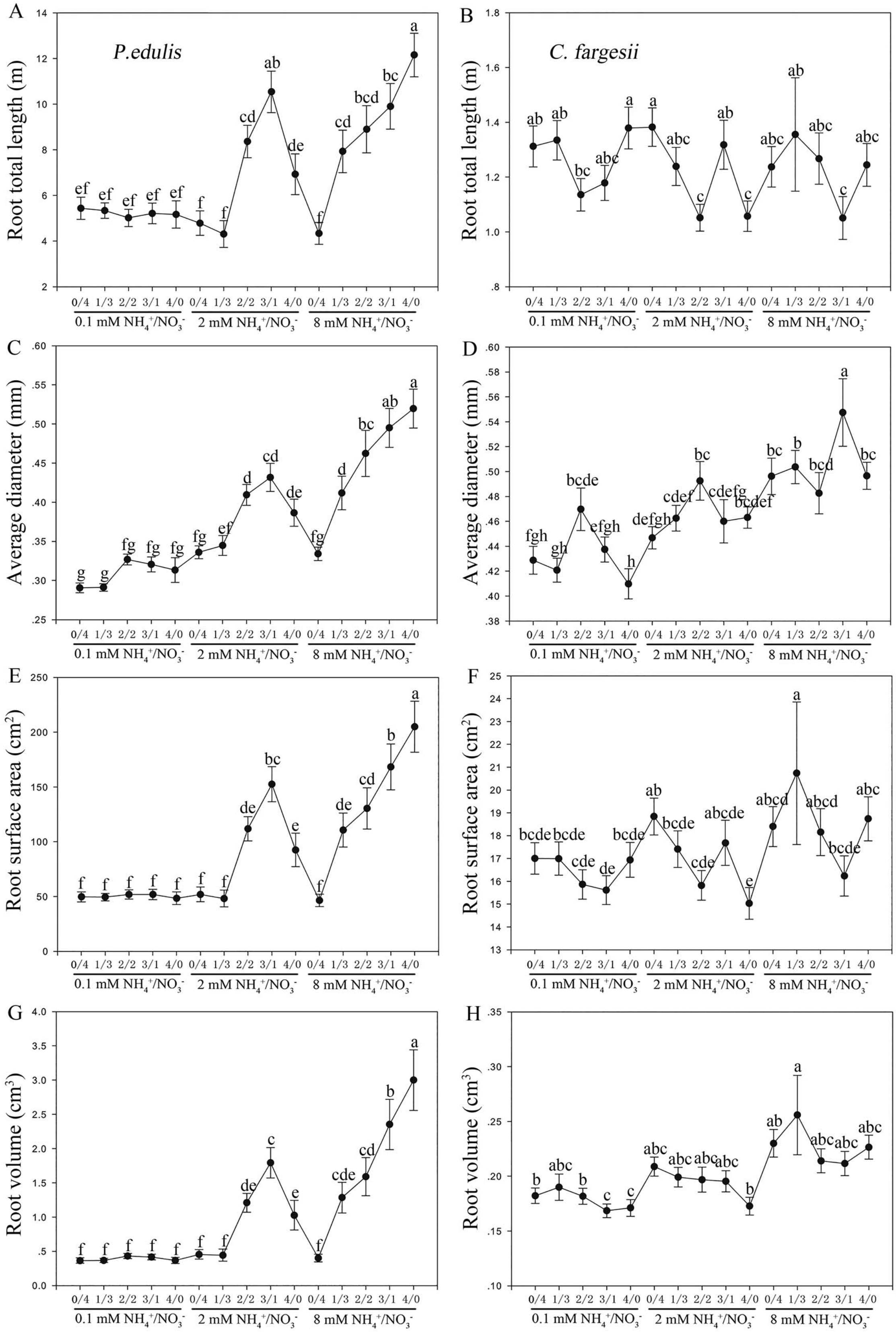

Figure 2. Nitrogen concentrations and NH_4^+/NO_3^- ratios on root morphology of *P. edulis* and *C. fargesii*. Total RL (A and B), AD (C and D), RS area (E and F) and RV (G and H) of *P. edulis* (left) and *C. fargesii* (right) as affected by different N treatments. Data are means from three replicates of 24 seedlings, and bars represent the SE. Different letters indicate statistical differences among treatments for each species (Fisher's LSD, $P < 0.05$).

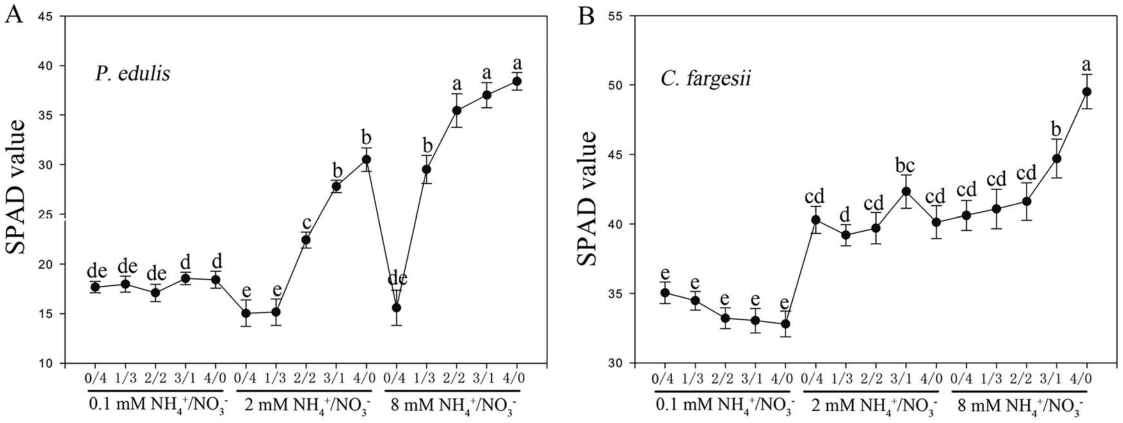

Figure 3. Nitrogen concentrations and NH_4^+/NO_3^- ratios on chlorophyll content (SPAD value) of fully developed leaves in *P. edulis* and *C. fargesii*. Chlorophyll content is shown as value from SPAD meter. Data are means from three replicates of 24 seedlings, and bars represent the SE. Different letters indicate statistical differences among treatments for each species (Fisher's LSD, $P < 0.05$).

Table 2. Summary of two-way ANOVA (*P*-value) for N concentrations and NH_4^+/NO_3^- ratios on growth indices of *P. edulis* and *C. fargesii* seedlings.

Growth index	*P*-value (*P. edulis*)			*P*-value (*C. fargesii*)		
	[N]	NH_4^+/NO_3^-	[N] × NH_4^+/NO_3^-	[N]	NH_4^+/NO_3^-	[N] × NH_4^+/NO_3^-
Seedling height	<0.001	<0.001	<0.001	<0.001	0.148	0.101
Leaf number	<0.001	<0.001	<0.001	<0.001	0.619	0.285
Leaf surface area	<0.001	<0.001	<0.001	<0.001	0.671	0.21
Biomass	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total RL	<0.001	<0.001	<0.001	0.524	0.077	0.034
RS area	<0.001	<0.001	<0.001	0.011	0.095	0.176
AD	<0.001	<0.001	<0.001	<0.001	0.052	0.026
RV	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SPAD value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Nitrogen concentrations (0.1, 2 and 8 mM) and ratios of NH_4^+/NO_3^- (0/4, 1/3, 2/2, 3/1, 4/0) are the same as shown in Table 1. [N] × NH_4^+/NO_3^- indicates the interaction effect of N concentrations and NH_4^+/NO_3^- ratios.

of $^{15}NH_4^+$-treated seedlings was 1.19-, 1.50- and 1.58-fold in roots, stem and leaf tissue of that of $^{15}NO_3^-$-treated seedlings with singular N sources, whereas $\delta^{15}N$ in roots, stem, and leaf tissue was 5.22-, 3.45- and 5.35-fold of that with $^{15}NO_3^-$ when NH_4^+ and NO_3^- were provided together (Figure 5D). By analyzing $\delta^{15}N$ between $(^{15}NH_4)_2SO_4$ and $^{15}NH_4NO_3$, $NH_4{}^{15}NO_3$ and $Na^{15}NO_3$, it emerged that the increased differences in $\delta^{15}N$ were brought about not only by increased $^{15}NH_4^+$ uptake in the presence of NO_3^- but also by decreased uptake of $^{15}NO_3^-$, and $^{15}NO_3^-$ uptake in the presence of NH_4^+ was reduced when compared with the single-N nutrient solutions in both species (Figure 5C and D).

NH_4^+ *tolerance*

Seedling growth differences began being evident after the 28th day of treatment (Figure 6). For *P. edulis*, differences in height growth among NH_4^+ treatments were visible from the 42nd day and accentuated with time, and, on the 56th day, the seedlings treated with 16–40 mM NH_4^+ were significantly larger than the seedlings treated with 2 mM NH_4^+ (Figure 6A). By contrast, differences in height growth of *C. fargesii* seedlings among NH_4^+ 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 NH_4^+ concentrations (Figure 7).

Although aboveground growth in both species proceeded well at soil NH_4^+ concentrations from 2 to 40 mM, root growth suffered NH_4^+ toxicity symptoms in both *P. edulis* and *C. fargesii* with increasing NH_4^+ levels. Total RL, RS area and RV were significantly inhibited in *P. edulis* at NH_4^+ concentrations >24 mM compared with seedlings treated with 2–16 mM NH_4^+. By contrast, in *C. fargesii*, these root morphology indices were significantly inhibited at NH_4^+ concentrations >16 mM compared with seedlings treated at 2 mM NH_4^+ (Figure 8).

The results show different survival rates in the two species at increased NH_4^+ concentrations (Figure 9). In *P. edulis*, the survival rate of all NH_4^+-treated seedlings was 100%, whereas, in *C. fargesii*, the survival rate sharply decreased with increasing NH_4^+, and the survival rate of 16–40 mM NH_4^+

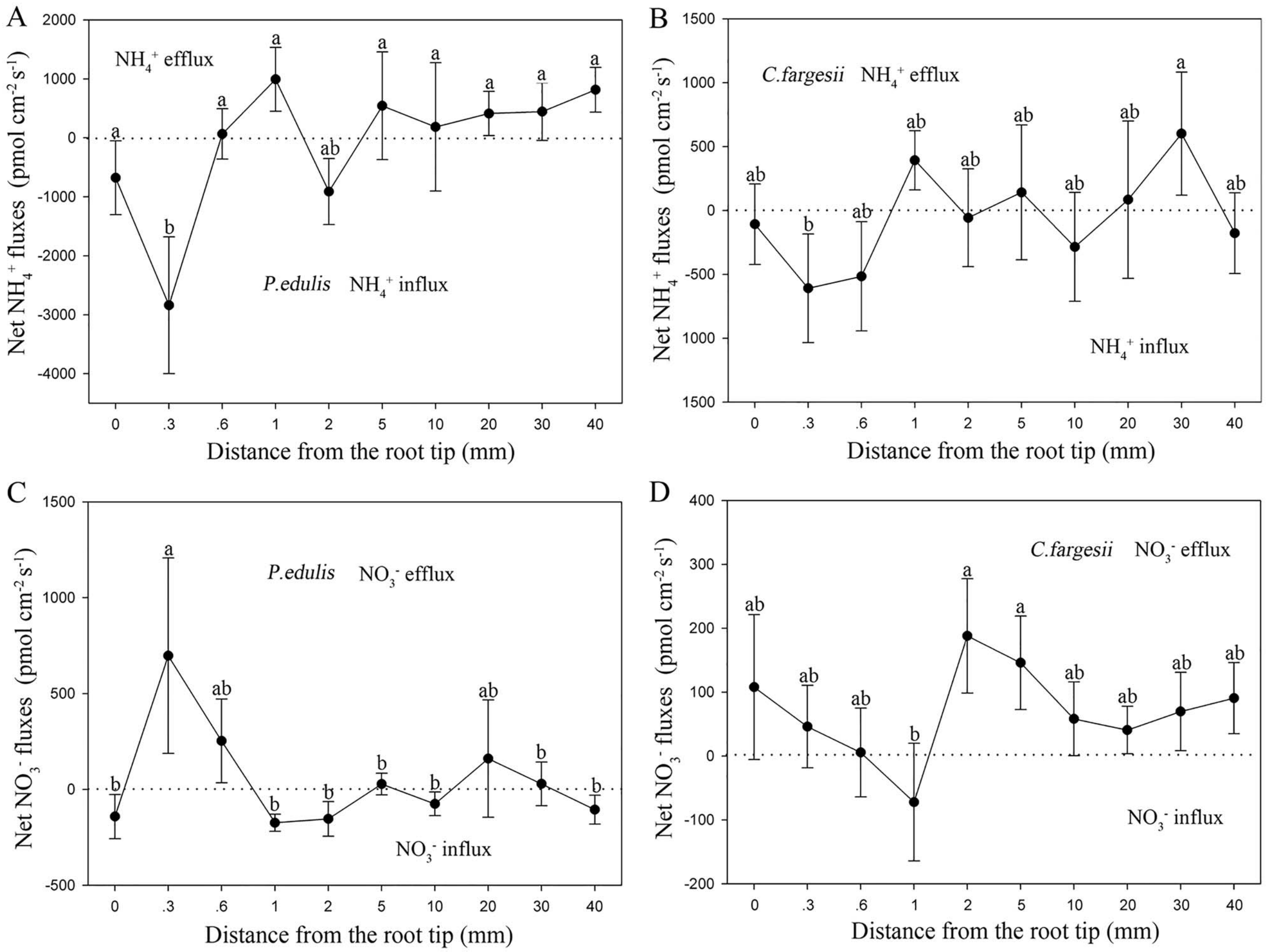

Figure 4. Effects of 8 mM different N treatment on NH_4^+ and NO_3^- flux along the root tip of *P. edulis* and *C. fargesii*. Three-foliate seedlings of *P. edulis* and *C. fargesii* were treated with 8 mM NH_4^+ (A and B) or NO_3^- (C and D) for 2 weeks and then subjected to NH_4^+ or NO_3^- flux measurements 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 $CaCl_2{\cdot}2H_2O$, 1.5 mM K_2SO_4, 0.6 mM $Na_2HPO_4{\cdot}10H_2O$, 0.25 mM $MgSO_4{\cdot}7H_2O$, pH 4.0, to which either 4 mM $(NH_4)_2SO_4$ for NH_4^+ or 8 mM $NaNO_3$ for NO_3^- flux measurements were added.

treated seedlings was significantly lower than that treated with 2 mM NH_4^+.

Interspecific competition

As both species demonstrated a similar preference for NH_4^+, it was interesting to examine the niche complementarity of these species. Interspecific competition between *P. edulis* and *C. fargesii* under NH_4^+-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 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 NH_4^+ 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

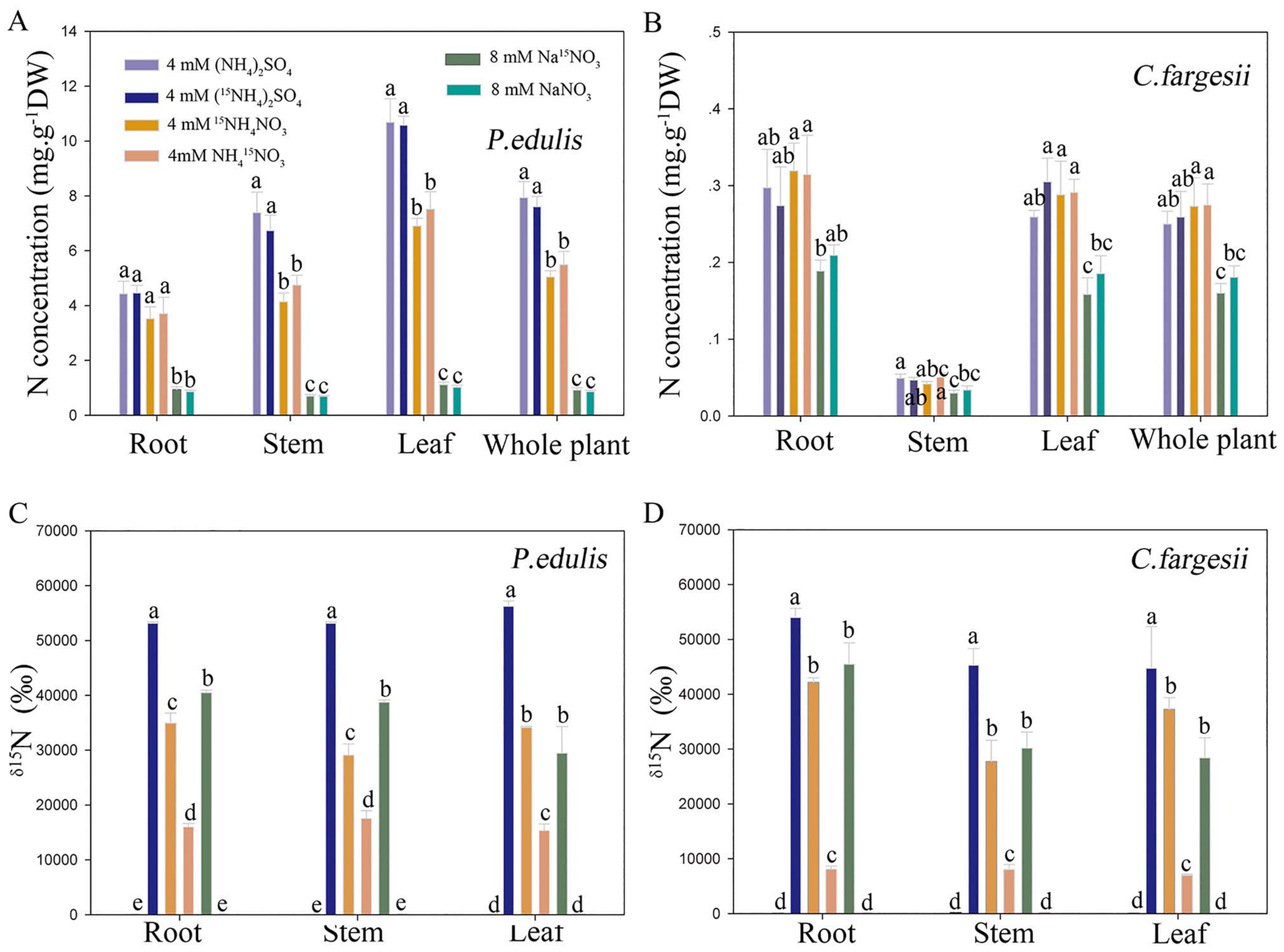

Figure 5. Nitrogen content and δ^{15}N in root, stem, and leaf of *P. edulis* and *C. fargesii* under different NH$_4^+$/NO$_3^-$ ratios of 8 mM N. (A and B) Nitrogen content in whole plant and root, stem and leaf of *P. edulis* and *C. fargesii*, whole plant N concentration was calculated as the weighted average of N concentration in leaves, stems and roots taking into account the mass of the three compartments. (C and D) The δ^{15}N 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 ($P < 0.05$).

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 NH$_4^+$, 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 NH$_4^+$ preference, but species had distinct performance to altered N availability: *P. edulis* responded very strongly to different ratios of NH$_4^+$/NO$_3^-$, and an increase in the proportion of NH$_4^+$, even when NH$_4^+$ was the only N source, significantly promoted growth, biomass, leaf chlorophyll and N concentration compared with seedlings on NO$_3^-$ (Figures 1–3 and 5). For example, seedlings treated with NH$_4^+$ displayed an increase of 61.47 and 449%, respectively, in biomass compared with seedlings with NH$_4^+$/NO$_3^{-(2/2)}$ and NO$_3^-$ 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 NH$_4^+$/NO$_3^-$ had minor effects in *C. fargesii*, although biomass production (Figure 1H), leaf chlorophyll content (Figure 3B) and N concentration (Figure 5B) were higher with NH$_4^+$ when supplied at 8 mM. For example, the biomass of 8 mM NH$_4^+$ treated seedlings was increased only 14.73 and 23.33%, respectively, compared with NH$_4^+$/NO$_3^{-(2/2)}$ and NO$_3^-$ treated seedlings at the same N concentration (Figure 1H). Although it was significantly larger than at 2 mM N (0/4, NO$_3^-$ alone), the growth of *P. edulis* at 2 mM N (4/0, NH$_4^+$ alone) was inhibited compared with

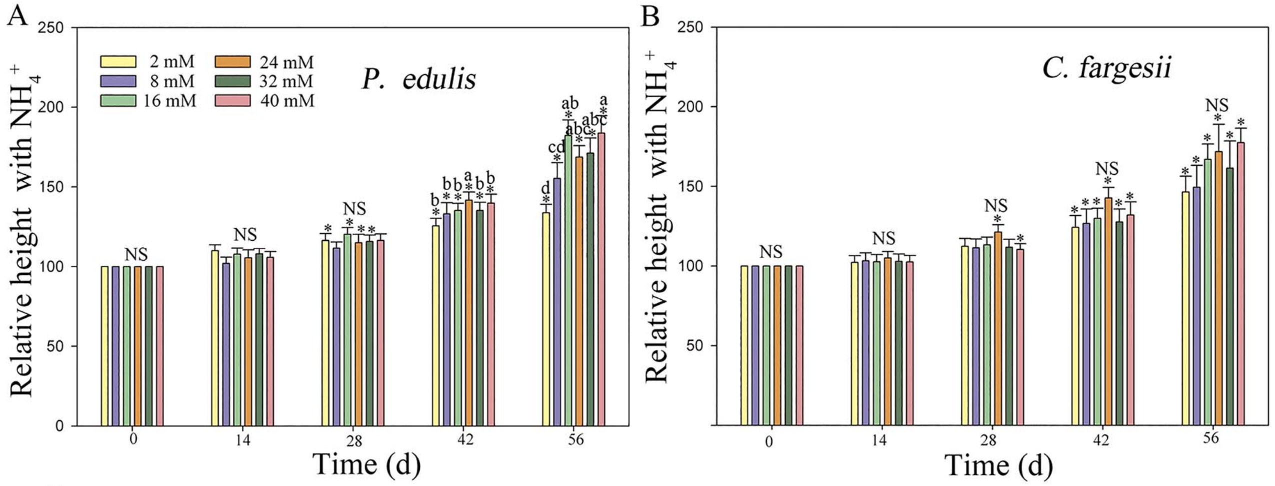

Figure 6. Effects of NH_4^+ concentrations on height increment of *P. edulis* and *C. fargesii* along with time course. Seedlings were grown with supply of different concentrations of NH_4^+. Heights are expressed relative to initial size. Data are means from three replicates of 24 seedlings, and bars 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, $P < 0.05$).

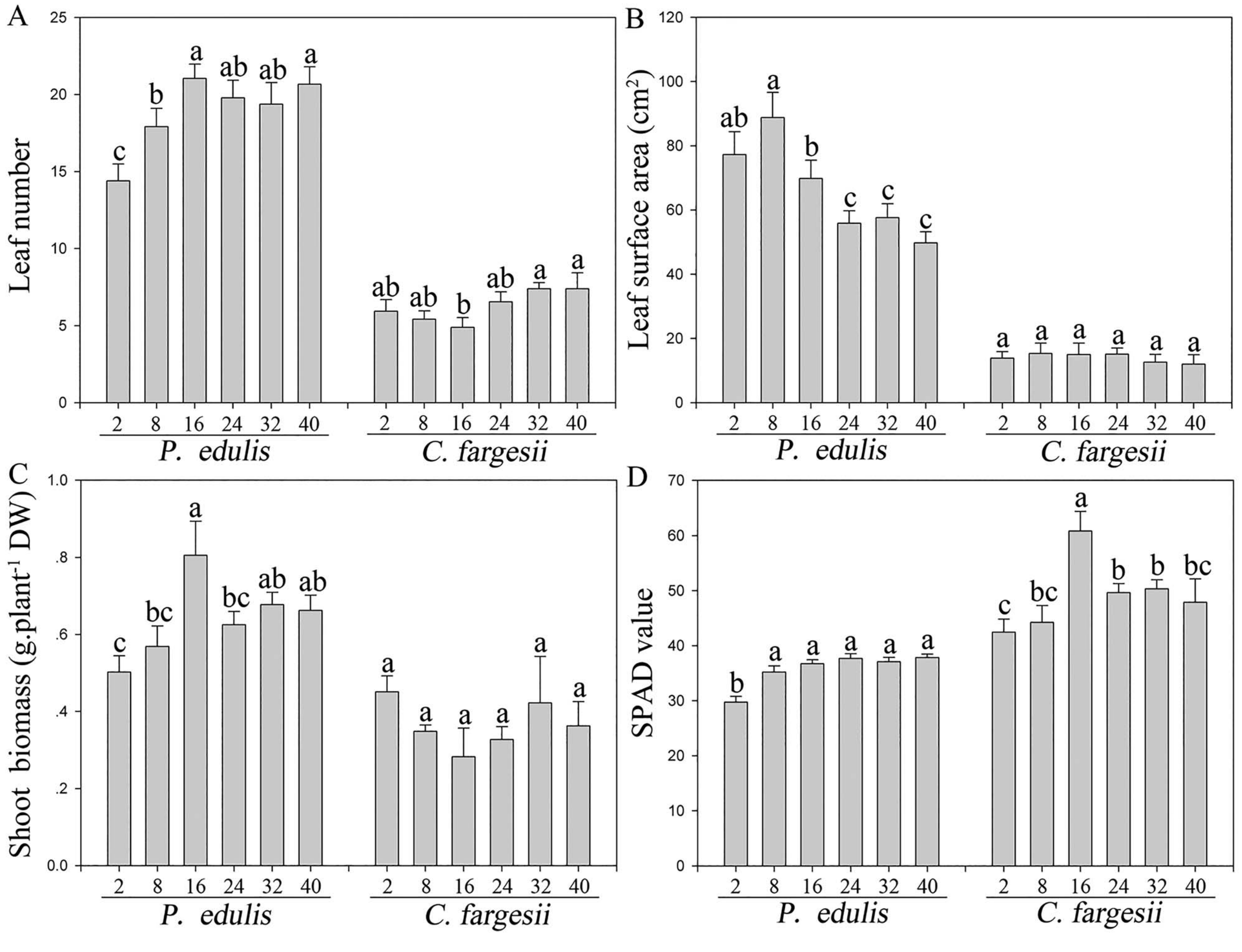

Figure 7. Growth, biomass and chlorophyll content of aboveground parts in *P. edulis* and *C. fargesii* seedlings supplied with different concentrations of NH_4^+ (mM). Data are means from three replicates of 24 seedlings at the end of experiment, and bars represent SE. Different letters indicate statistical differences among treatments for each species (Fisher's LSD, $P < 0.05$).

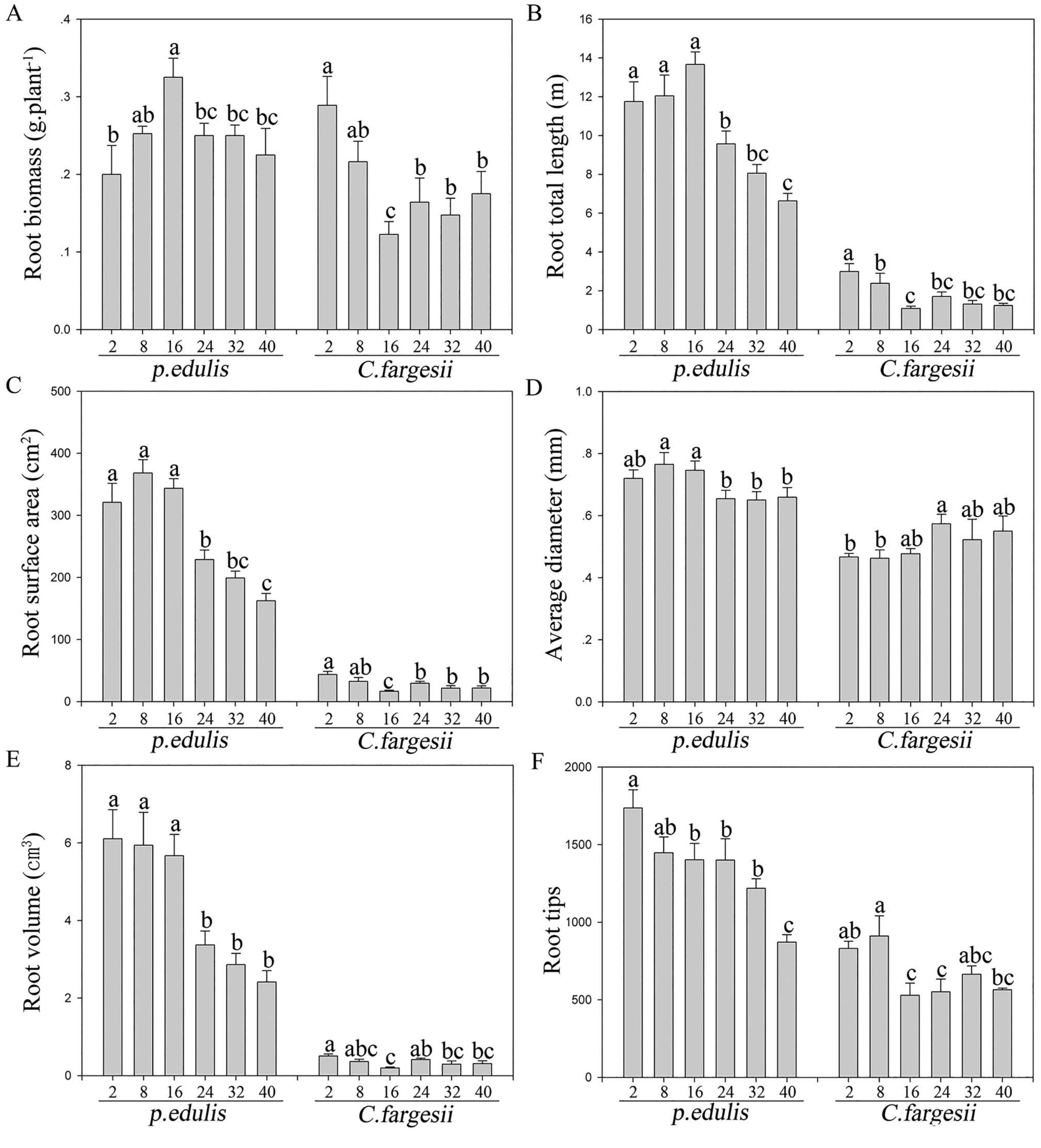

Figure 8. Root biomass and morphology of *P. edulis* and *C. fargesii* seedlings supplied with different concentrations of NH_4^+ (mM). Data are means from three replicates of 24 seedlings at the end of the experiment, and bars represent the SE. Different letters indicate statistical differences among 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. 1999*b*, 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

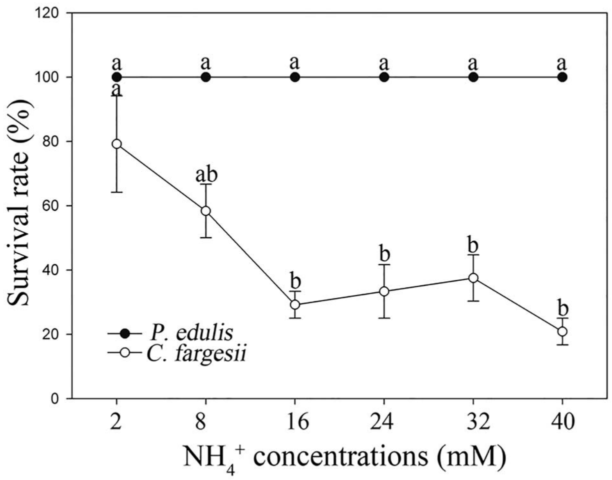

Figure 9. Effects of NH_4^+ concentrations on survival rate of *P. edulis* and *C. fargesii*. Seedlings were grown with different concentrations of NH_4^+, survival rates were surveyed at the end of experiments. Data are means from three replicates of 24 seedlings, and bars represent the SE. Different letters indicate statistical differences among treatments (Fisher's LSD, $P < 0.05$).

points that differ with overall N concentration (Kronzucker et al. 1999*b*, 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

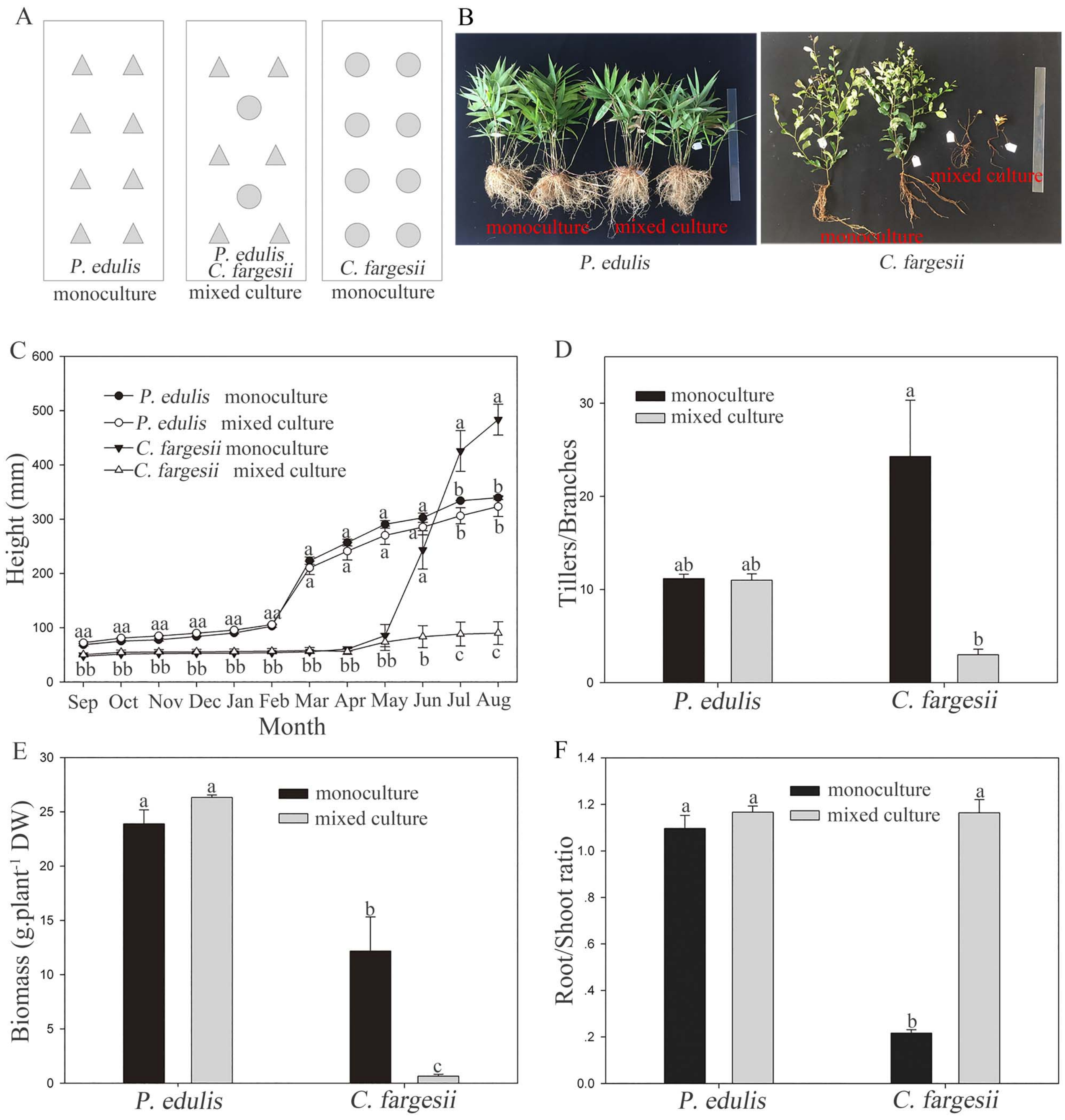

Figure 10. Growth and response variables for seedlings of *P. edulis* and *C. fargesii* grown in monoculture and mixed culture experiments supplied with 8 mM NH_4^+. (A) Layout of the monoculture and mixed culture experiment with spatial distribution of seedlings, (B) seedlings of *C. fargesii* and *P. edulis* in monoculture and mixed culture, (C) height increment of *P. edulis* and *C. fargesii* seedlings in response to different plantation systems, (D) tiller and branches, (E) biomass and (F) ratios of root/shoot in *P. edulis* and *C. fargesii* seedlings at the end of the experiment. Data are means from three replicates of 12 to 36 seedlings, and bars represent the SE. Different letters indicate statistical differences among treatments and species (Fisher's LSD, $P < 0.05$).

ions fluxes cannot be discounted, the more efficient N capture with NH_4^+ relative to NO_3^- in both species and higher maximal net NH_4^+ fluxes in *P. edulis* than *C. fargesii* displayed by NMT were also consistent with and supported by $\delta^{15}N$ experiments and N concentration in both plants (Figure 4). Both bamboo-dominated forests and *C. fargesi*-dominated secondary evergreen broad-leaved forests contained more NH_4^+ than NO_3^- (Song et al. 2016); therefore, 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 NO_3^- uptake by NH_4^+, as is classically observed (Kronzucker et al. 1999*a*, Luo et al. 2013), and a promotion of NH_4^+ uptake by NO_3^- (Figure 5C and D), similar to what has been reported for the NH_4^+ specialist rice (Kronzucker et al. 1999*b*) and poplar trees (Luo et al. 2013). Possibly, the enhancement of net NH_4^+ uptake by the co-presence of NO_3^- overcomes the suppressive effects of NH_4^+ on NO_3^- uptake in *C. fargesii* but not in *P. edulis*, resulting in improved N acquisition and accumulation in *C. fargesii* under mixed N, as compared with provision with NO_3^- or NH_4^+ alone (Figure 5A and B). Therefore, the balance in soil N mineralization between ammonification and nitrification is shifted toward ammonification in bamboo-dominated forests after the expansion of bamboo (Song et al. 2013, 2016), which would be a disadvantage for N capture by *C. fargesii* rather than *P. edulis*, taking into consideration the interactions between the inorganic N ions.

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* ($\geqq$24 mM) than in *C. fargesii* ($\geqq$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_4NO_3 treated seedlings, at 8 mM N. *Phyllostachys edulis* demonstrated a significant competitive growth advantage compared with *C. fargesii* on NH_4^+-rich substrates. In addition, *P. edulis* was more NH_4^+-tolerant than *C. fargesii*. Therefore, it can be inferred that, in broad-leaved forests, soil N is converted from a mixture of NH_4^+ and NO_3^- to mostly NH_4^+ in the process of bamboo invasion, and the previously dominant tree species are unable to compete with the 'NH_4^+ specialist' bamboo, which, intern becomes dominant. The differential toxicity thresholds for NH_4^+ in *P. edulis* (24 mM) and *C. fargesii* (16 mM) may play a role in community structure succession, as the latter species is more susceptible to NH_4^+ toxicity and its survival rate sharply decreases with increasing NH_4^+. Our experiment also provides important insights for the nursery cultivation of these species.

Acknowledgments

We are grateful to Professor Xianquan Weng (Xiayang State Forest Farm of Nanping of Fujian Province) for the kind provision of *C. fargesii* seeds. We thank Professors Fusheng Chen, Jianmin Shi from Jiangxi Provincial Key Laboratory for Bamboo Germplasm Resources and Utilization, China and Tianchi Wang from the New Zealand Institute for Plant and Food Research for their critical reading of the manuscript. We also thank other members of our team and members in Jiangxi Provincial Key Laboratory for Bamboo Germplasm Resources and Utilization for their assistance. This work was supported by the National Natural Science Foundation of China (31300521), the Natural Science Foundation of Jiangxi Province, China (20171ACB21027), 'first-class discipline' construction fund of forestry (9010020-09003) and the Enterprise Academician Workstation Foundation (BC20170014Z).

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