

Effects of simulated nitrogen deposition on the ecophysiological responses of *Populus beijingensis* and *P. cathayana* under intra- and interspecific competition

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Abstract

Aims N deposition has become a major driver of a decline in species richness and biodiversity loss. We aim to discover whether N deposition could affect competitive relationships between poplars to predict threats posed by the introduction of exotic hybrids to native relative species.

Methods Intra- and interspecific competition was investigated for an introduced hybrid poplar (*Populus beijingensis*) and the native paternal species *P. cathayana* under two N deposition regimes (with and without N deposition).

Results Under control conditions, *P. cathayana* grown under either of the competition modes showed consistently greater above-ground biomass, root-to-shoot ratio (R/S), photosynthetic capacity, higher activities of N-assimilation enzymes in leaves, and preference for N-NO_3^- than corresponding *P. beijingensis*. Interspecific competition increased the leaf area (LA), specific leaf area (SLA), R/S, and photosynthetic nitrogen use efficiency (P_{NUE}) in *P. cathayana*, but decreased P_{NUE} in *P. beijingensis* compared to the values under monoculture. Thus, *P. cathayana* can gain a competitive advantage over *P. beijingensis* at low N availability. In contrast, under N deposition, *P. beijingensis* grown in a mixture showed more positive responses in growth, higher plasticity in biomass allocation, more flexible fine root traits, and the shift for N-NO_3^- preference when compared with *P. cathayana*, which resulted in relatively higher values in LA, SLA, and biomass accumulation in *P. beijingensis*.

Conclusions Our results suggest that N deposition could reverse competitive relationships between the poplars, and exotic *P. beijingensis* has the potential to outcompete and displace native *P. cathayana* under growing N deposition.

1. Introduction

Competitive interactions are recognized as an important determinant for the performance of individuals, structures, compositions, and dynamics of plant communities, because plant–plant competition has great potential for shaping distribution patterns, plant species abundance, and functional trait evolution (Eckstein et al., 2010; Goldberg and Barton, 1991; Kraft et al., 2015). Functional traits' differentiation between competitors not only drives stabilizing niche differences and competitive coexistence and maintains species diversity (Kraft et al., 2015), but also underlies average fitness differences, promoting competitive exclusion (Godoy et al., 2014). It is commonly assumed that competition intensity can vary over particular types of environmental gradients (Casper et al., 1997), and competition effects are highly context dependent (Cahill and McNickle, 2011). However, belowground competitive ability is highly correlated with functional traits, including fine root density, surface area, and architectural plasticity involved in nutrient uptake (Aschehoug and Callaway, 2014; Kembel and Cahill, 2005), whereas aboveground competitive ability depends on leaf growth rate and ability to occupy aerial volume (Eckstein, 2010). For example, for slow-growth plants in nutrient-poor habitats, competitive superiority can be achieved by allocating more biomass to root systems and adjusting root morphology, such as increasing root length per unit root mass, while in microsites with high nutrient availability, the roots of fast-growing species react rapidly by increasing nutrient uptake kinetics, conferring a competitive advantage (Aerts, 1999). However, plants that allocate more photosynthate to root production enable them to capture soil nutrients more efficiently at the expense of aboveground parts' growth, resulting in trade-offs in competitive ability for other resources, which affects competition outcomes under different conditions (Aschehoug et al., 2016; Tilman, 1987).

There is much discussion about differences in competition intensity and competition relationships (positive, negative, or no effect) among conspecifics versus heterospecifics. Theoretically, conspecifics compete more strongly than heterospecifics in view of the same requirements for and ability to access resources, although no consistent pattern was found (Goldberg and Barton, 1991). In fact, intra- and interspecific competition outcomes not only depend on resource availability but can also be readily influenced by abiotic and biotic factors. For instance, Guo et al. (2016) observed that *Larix kaempferi* performed better under interspecific competition with *L. olgensis* compared with monocultures under elevated nitrogen (N) fertilization, which was not discovered under treatments without N addition. Yu et al. (2019) found that temperature elevation induced an asymmetric competition pattern in which *Picea purpurea* benefited from the presence of *Abies faxoniana* under interspecific competition but not vice versa. In contrast, the species-specific detection and avoidance system in the roots of *Ambrosia dumos*, not in those of *Larrea tridentat*, may allow *A. dumos* to grow in clumped distribution with little intraspecific competition for water (Mahall and Callaway, 1992). The net effect of the interspecific interaction between *Elymus elymoides* and *Taeniatherum caputmedusae* was negative for both species, and both species displayed intraspecific facilitation (Sheley and James, 2014). Under shifting environmental conditions, competitive effects or the responses of target plants in the face of distinct neighbors can be reflected by their plasticity in biomass and physiology. Moreover, we should place more emphasis on the role of belowground competition and the interaction between roots and shoots to understand the overall effects of competition and competition outcomes (Casper et al., 1997; Foxx and Fort, 2019).

In recent decades, due to intensive agriculture and fossil fuel combustion, atmospheric N deposition has increased continuously on a global scale, and this trend is predicted to continue in the coming decades. It is generally believed that excessive N deposition may exert profound effects on terrestrial ecosystem processes. Long-term and excessive N deposition has become a major driver of a decline in species richness and biodiversity loss (Stevens et al., 2019). A large body of evidence has shown that increasing N deposition can alter resource availability and induce asymmetric competition between species, which benefits nitrophilous species but threatens species adapted to infertile conditions (Elias and Agrawal, 2021), although a few studies found that N addition alleviated competitive effects (Luo et al., 2014) or that elevated N availability may not reinforce competitive advantages (Bradford et al., 2007). In general, for N-deficient ecosystems, N deposition often causes plants to invest more in shoots than in roots when other nutrients and water are not limited (Leith et al., 1999), thus increasing competition for light and living space (Hautier et al., 2009). However, N deposition accelerates the N cycle, increasing N availability, while other essential nutrients (such as P, K, Ca, and Mg) may become limiting factors (Sardans et al., 2016). At the individual species' level, N deposition is expected to have a negative impact on the balance of mineral nutrients (such as higher foliar N but lower Ca and Mg, or skewed N:P) and susceptibility to stress conditions (Friedrich et al., 2012; Mao et al., 2018), therefore altering plant performance, survival, and fitness (Wedlich et al., 2016). Nonetheless, most of these studies mainly focused on biomass accumulation, growth plasticity, and the morphological reaction of plants to reflect the shift of competition

patterns in response to N deposition. Therefore, more knowledge about N allocation and N assimilation physiology is necessary to reveal N use strategies adopted by competitive counterparts under N deposition.

Populus species, a fast-growing and widely distributed tree species in the northern hemisphere, have become one of the most intensive plantation species in China. Under natural conditions, several species of the genus *Populus* are broadly sympatric and are known to hybridize extensively (Hamzeh et al., 2004). Natural hybridization among members of poplars has long been recognized as a crucial driving force for genetic exchange, genetic variation and evolution, the origin of new species, and population genetic structure (Abbott et al., 2016). However, some excellent hybrid cultivars of poplars originating from anthropogenic hybridization between exotic and native poplar species have been subjected to rigorous breeding processes and have been demonstrated to have over-parent heterosis. It has been realized that intensive application of these poplar cultivars may pose a threat to native poplar species not only by occupying their habitats but also by influencing the genetic integrity of native poplar species via introgressive hybridization and gene flow (Broeck et al., 2005; Meirmans et al., 2010; Paffetti et al., 2018; Talbot et al., 2012). More importantly, along with the growing habitat fragmentation of the native poplar species due to agriculture development and urbanization, the opportunities for contact between introduced poplar hybrids and the native species greatly increase. This increasing contact may build direct competitive relationships between them, alter the niche of native poplar species, and endanger the growth, survival, and reproduction of native poplars. However, little information is available about the competitive interactions between them, especially in the context of growing N deposition.

Populus beijingensis (*P. nigra* var. *italica* × *P. cathayana*) is an excellent hybrid and is commonly used as a major tree species for shelterbelt afforestation and road greening in central, northwest, and parts of northeast China. With higher requirements for nutrients and resources, this species grows faster than native poplar species in sites with sufficient nutrients and water. *Populus beijingensis* has a similar ecological niche to the native paternal species *P. cathayana*, which results in competition between the poplars for living space and nutrients. Therefore, in the present study, we evaluated the competitive relationships between sympatric species and examined how N deposition affects intra- and interspecific competition by detecting growth characteristics, morphological traits, photosynthetic rates, N metabolism physiology, N preference, and long-term water use efficiency. We tested the following hypotheses: (i) There are species-specific differences in adaptive strategies reflected by morphological and physiological adjustments under intra- and interspecific competition. (ii) Under N deposition, *P. beijingensis* exhibits competitive advantages over *P. cathayana* due to its higher resource requirements.

2. Materials And Methods

2.1 Study site and plant material

This study was conducted in a naturally lit greenhouse providing only shelter from rainfall at the Chengdu campus of Sichuan Agricultural University, China (30°42' N, 103°51' E). The mean annual rainfall, temperature, transpiration, and duration of the frost-free period in the region are 966.1 mm, 16°C, 838 mm, and 294 days, respectively.

Healthy 1-year-old cuttings of *P. cathayana* and *P. beijingensis* were selected from a state forest farm under normal conditions in Qinghai Province (Datong, 101°35'E, 35°56'N), P.R., China. After rinsing in a sterilizing agent (1.25% carbendazol, w/w) for a half hour, the cuttings were planted in a seedbed. For each species, 72 healthy cuttings approximately 15 cm in height and 10 cm in root length were chosen for use in the below competition experiment.

2.2 Experimental design

Two cuttings were transplanted to each cylindrical plastic pot, with a diameter of 40 cm and height of 35 cm, and filled with 45 kg homogenized soil. The cuttings in each pot were arranged along a diametrical line, and the distance between each cutting and the center of the pot was 10 cm. All cuttings were planted at a similar soil depth (10–15 cm). The physical and chemical soil properties were as follows (kg⁻¹ dry soil): pH 7.86, organic matter 16.12 g, available potassium 21.62 mg, available phosphate 13.78 mg, ammonium nitrogen 2.31 mg, and nitrate nitrogen 0.35 mg. We designed three types of competition patterns: *P. cathayana* + *P. cathayana* (CC, intraspecific competition between *P. cathayana* cuttings); *P. beijingensis* + *P. beijingensis* (BB, intraspecific competition between *P. beijingensis* cuttings); and *P. cathayana* + *P. beijingensis* (CB, interspecific competition between *P. cathayana* cuttings and *P. beijingensis* cuttings). Then, two regimes of N deposition (i.e., N⁻ and N⁺) were arranged in each competition pattern. N⁻ and N⁺ represent the control conditions and simulated N deposition, respectively.

The experimental layout was a completely randomized design with three factors (species, competition pattern, and N deposition). For each species, there were four treatments according to the combination of competition pattern and N deposition. Twelve pots per treatment (three replicates with four pots per replicate) were included in the experiment. One month after being transplanted, the plants were subjected to different N treatments. For the N⁺ treatment, N amount was determined based on the area of the soil surface and N deposition level (about 15 g N m⁻² a⁻¹) in local habitats according to the local environmental monitoring bureau. All N was divided 17 times to add to the surface of the soil. Each time, 235 ml of 3.75 mM NH₄NO₃ solution was evenly sprayed onto the potted soil surface each week during the treatment period. The treatment without an additional N supply was watered with 235 ml of deionized water at the same interval. The treatments were conducted under climate conditions with a daytime temperature of 14–22°C, a night-time temperature of 11–18°C, and an average relative humidity of 75% for 17 weeks at Sichuan Agriculture University.

2.3 Growth measurement

At the end of the experiment, five cuttings from each treatment were selected randomly to measure plant height (PH) and basal diameter (BD). Then, all plants were harvested and divided into leaves, stems, and roots. Furthermore, roots were isolated gently and strictly by branch order, following the methods of Pregitzer et al. (2002), i.e., the distal roots were designated as the first order (R1), roots from which two first-order roots branched were categorized as the second order (R2), and so on. A total of five orders (i.e., R1, R2, R3, R4, and R5) were identified in our study according to a commonly used method for the root

rank division of woody plants. Root samples of different orders, as well as stems and leaves, were dried at 75°C to a constant weight, and root mass (RS), leaf mass (LS), and stem mass (SM) were determined separately. The root/shoot ratio (R/S) was calculated as $RS / (LS + SM)$. The total leaf area (LA) was determined using a portable laser leaf area meter (CI-203, CID Inc., Camas, WA). The specific leaf area (SLA, leaf area per unit dry mass) was calculated as the ratio of the leaf area to its dry mass.

2.4 Fine root morphology determination

Before the root biomass assessment, different root orders were positioned to minimize overlap and placed on a flatbed scanner. Root morphological parameters, including total root length (TRL), total root surface area (TRSA), average length of individual root (ALIR), and average diameter of individual root (ADIR) for different orders, were measured using WinRHIZO image analysis software (Regent Instruments, Quebec, QC, Canada). Then, the specific root area (SRA) was calculated by dividing the root surface area by the dry weight of the roots used for scanning.

2.5 Determination of net photosynthetic rate and photosynthetic N-use efficiency

At the end of the experiment, for each treatment, the fourth fully expanded and intact leaf from five randomly chosen individuals was used to measure the net photosynthetic rate using a portable photosynthesis system (LI-6400; LI-COR Inc. Lincoln, NE, USA). The net photosynthetic rate (P_n) and stomatal conductance (G_s) was measured under the following conditions: ambient CO₂ concentration, 400 $\mu\text{mol mol}^{-1}$; leaf temperature, 25°C; photosynthetic photon flux density (PPFD), 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$; and relative air humidity, 70%. The photosynthetic N use efficiency (P_{NUE}) was determined as the ratio of the P_n to the leaf N content per area.

2.6 Analysis of N assimilation enzymes and N status in leaves and absorptive roots

At the end of the experiment, activity assays for N assimilation-related enzymes were conducted for the third fully expanded and intact leaf from five randomly chosen cuttings from each treatment. Fresh leaves (0.3 g) were ground to a fine powder in liquid nitrogen. The powder was extracted in a 2 mL ice-cold extraction buffer consisting of 50 mM Tris-HCl buffer (pH 7.5), 0.5 mM EDTA, 1 mM MgCl₂, 10 mM β -mercaptoethanol, and 0.5% PVPP (Polyvinylpyrrolidone). The extract was centrifuged at 12,000 g for 10 min at 4°C as the crude enzyme extract.

Nitrate reductase (NR, EC 1.6.6.1) activity was measured according to the method described by Datta and Sharma (1999). NR activity was determined by monitoring the absorbance at 540 nm using a spectrophotometer (Unicam UV-330, Unicam, Cambridge, UK). The consumed NO₂⁻ was expressed as nmol per minute per g of fresh material.

For glutamine synthetase (GS, EC 6.3.1.2) activity measurement, 0.1 mL of the crude enzyme extract was added to a 1.9 mL assay mixture, including 50 mM Tris-HCl buffer (pH 7.5), 13 mM hydroxylamine, 50 mM glutamic acid-Na, 1 mM ATP, 20 mM AsNa₃O₄, and 20 mM MgCl₂. The mixture was incubated for 30 min at 37°C, and the reaction was stopped by adding 1 mL acidic FeCl₃. The mixture was centrifuged at 5000 g for 15 min, and the absorbance of the supernatant was assayed at 540 nm. GS activity was expressed as nmol γ -glutamyl-hydroxamate (γ -GHM) produced per minute per g of fresh tissue.

Glutamate synthetase (GOGAT, EC 1.4.7.1) activity was assayed according to Chiu and Shargool (1979). The activity was expressed as nmol NADH consumed per minute per g of fresh tissue based on the absorbance at 340 nm.

For the measurement of aspartate aminotransferase (AspAT) activity, 100 μL of crude enzyme extract was added to 1.4 mL of an assay mixture consisting of 50 mM Tris-HCl buffer (pH 7.5), 5 mM EDTA, 0.2 mL of aspartic acid, 12 mM of α -ketoglutaric acid, 0.15 mM NADH, 0.5 M l-alanine, and 5 units of malic dehydrogenase. After the reaction started, the extinction value was detected in a 30 s interval at 340 nm using a spectrophotometer and was recorded continuously for 450 s. The slope of the scanning curve was used to calculate AspAT activity. AspAT activities were expressed as mmol NADH per minute per g of fresh tissue.

Deaminating glutamate dehydrogenase (NAD-GDH) (EC 1.4.1.2) activity was measured according to Gao et al. (2013). The crude enzyme extract (0.1 mL) was added to a 1.9 mL assay mixture containing 50 mM Tris-HCl buffer (pH 8.8), 80 mM L-glutamic acid, and 0.2 mM NAD⁺. The absorbance of the mixture was monitored at 340 nm for 450 s. The NAD-GDH activity was expressed as nmol NADH produced per minute per g of fresh leaves.

For NO₃⁻-N determination, frozen dried leaf samples (0.2 g) were ground to a powder and extracted in 50 mL of 2 mol·L⁻¹ KCl for 30 min. After filtering, 2 mL of the solution was mixed with 1 mL of 1 mol·L⁻¹ HCl. The NO₃⁻-N content was determined when monitored at 220 nm using a spectrophotometer. The values were quantified according to a standard curve. Dried samples (0.1 g) of leaves and absorptive roots (R1 and R2) were used for the N concentration measurements. The N concentration was determined using the Kjeldahl method.

2.7 ¹³C and ¹⁵N analysis

At the end of the experiment, the carbon isotope composition (¹³C/¹²C), as expressed by $\delta^{13}\text{C}$, was determined for the dried leaves. The dried leaves were ground into fine powder in a ball mill and passed through a 100-mesh sieve. Stable carbon isotope abundance in the combusted samples was determined with a mass spectrometer (Thermo Fisher Scientific, Inc., USA). The overall precision of the δ -values was better than 0.1‰, as determined from repetitive samples. The entire analysis was performed in the Stable Isotope Laboratory for Ecological and Environmental Research (SILEER), CAS.

At the end of the experiment, five randomly chosen cuttings per treatment were supplied with either labeled ¹⁵NO₃⁻-N as NH₄¹⁵NO₃ or labeled ¹⁵NH₄⁺-N as ¹⁵NH₄NO₃ (30 mg per plant). Then, 72 h later, the third fully expanded and intact leaf from the upper position of the cuttings was harvested, dried in an oven at 75°C for 48 h, and ground into fine powder for the ¹⁵N isotope composition analysis. The ¹⁵N/¹⁴N ratios, as expressed by $\delta^{15}\text{N}$, were determined by an Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Inc., USA). The overall precision of $\delta^{15}\text{N}$ was better than 0.1‰, as determined by repetitive samples.

2.8 Relative competitive intensity

The relative competitive index (RCI) of *P. cathayana* and *P. beijingensis* under control and N deposition conditions was calculated according to the methods of Chen et al. (2017): $RCI = (B_{inter} - B_{intra}) / B_{intra}$, where B_{inter} stands for the average dry matter accumulation of a kind of organ or total biomass of cuttings from interspecific competition, and B_{intra} stands for the average dry matter accumulation of the corresponding organ or total biomass of cuttings from intraspecific competition.

2.9 Statistical analyses

All data were analyzed with the Statistical Package for the Social Sciences software (SPSS, Chicago, IL, USA) version 20.0. One-way analysis of variance (anova) was performed for all parameters, and post hoc comparisons were applied using Tukey's test at a significance level of $\alpha = 0.05$. All data were tested for normality and homogeneity of variances and log-transformed to correct deviations from these assumptions when needed. A paired-sample *t*-test was used to evaluate the significance of differences between the N treatments ($P < 0.05$). Three-way ANOVA was employed to test the interactive effects of species, N deposition, and competition mode on morphological, physiological, and biochemical parameters. The effects were considered to be significant if $P < 0.05$.

3. Results

3.1 Growth characteristics

The PH and BD of both species were significantly increased after N addition, regardless of any competition pattern, except that N application did not induce a significant change in the BD of *P. cathayana* under monoculture mode (Table 1). Under either N⁻ or N⁺ conditions, there were no significant differences between the species in PH and BD when they grew in either competition mode. However, N addition significantly increased the LA of *P. beijingensis* in both competition modes, which did not occur in *P. cathayana*. Under N⁻ conditions, there was no difference in both LA and SLA between the species from the interspecific competition mode, whereas *P. beijingensis* had significantly higher LA and SLA than *P. cathayana* in the intraspecific competition mode. In contrast, under N⁺ conditions, there was a significantly higher LA and SLA in *P. beijingensis* than in *P. cathayana* in both competition modes.

Table 1

The effects of N addition on growth, biomass accumulation and allocation of *P. cathayana* and *P. beijingensis* under intra- and interspecific competition

Treatments	PH (cm)	BD (mm)	LA (dm ²)	SLA (cm ² /g)	LM (g plant ⁻¹)	SM(g plant ⁻¹)	RM(g plant ⁻¹)	TB (g plant ⁻¹)	R/S
C/CC	160.80 ± 2.37 ^a	13.22 ± 0.33 ^a	21.65 ± 1.04 ^b	69.66 ± 7.53 ^b	31.52 ± 2.36 ^a	36.54 ± 2.71 ^a	8.24 ± 0.39 ^a	76.31 ± 5.38 ^a	0.13 ± 0.01 ^c
B/BB	165.20 ± 3.51 ^a	13.31 ± 0.31 ^a	30.65 ± 0.85 ^a	122.20 ± 2.45 ^a	25.13 ± 1.13 ^{ab}	20.61 ± 1.41 ^b	8.90 ± 0.62 ^a	54.64 ± 3.00 ^{bc}	0.19 ± 0.01 ^{ab}
C/CB	161.20 ± 1.71 ^a	12.69 ± 0.60 ^a	25.21 ± 2.18 ^{ab}	92.96 ± 16.70 ^{ab}	28.00 ± 2.65 ^{ab}	32.95 ± 2.49 ^a	9.76 ± 0.27 ^a	70.71 ± 5.35 ^{ab}	0.16 ± 0.01 ^b
B/CB	163.00 ± 5.03 ^a	11.60 ± 0.45 ^a	25.99 ± 2.81 ^{ab}	124.30 ± 9.46 ^a	20.87 ± 1.17 ^b	16.95 ± 1.30 ^b	8.70 ± 0.48 ^a	46.51 ± 2.85 ^c	0.23 ± 0.01 ^a
C/CC-N	176.20 ± 1.93 ^{A**}	13.48 ± 0.35 ^A	25.18 ± 1.09 ^B	60.42 ± 3.60 ^B	41.76 ± 0.74 ^{AB}	47.37 ± 0.97 ^A	10.55 ± 0.64 ^A	99.68 ± 3.71 ^{AB}	0.12 ± 0.01 ^B
B/BB-N	181.00 ± 1.79 ^{A**}	14.08 ± 0.11 ^{A*}	49.98 ± 2.10 ^{A**}	106.82 ± 4.42 ^A	46.87 ± 1.82 ^{A*}	47.31 ± 1.89 ^{A**}	11.09 ± 0.21 ^A	105.27 ± 3.69 ^{A*}	0.12 ± 0.01 ^{B*}
C/CB-N	175.60 ± 2.73 ^{A*}	13.64 ± 0.43 ^{A*}	25.88 ± 0.45 ^B	69.25 ± 3.15 ^B	37.58 ± 2.39 ^{B**}	37.02 ± 2.21 ^B	11.81 ± 1.04 ^A	86.42 ± 5.07 ^B	0.16 ± 0.01 ^A
B/CB-N	183.60 ± 1.78 ^{A*}	14.40 ± 0.30 ^{A**}	53.99 ± 1.17 ^{A*}	117.63 ± 6.61 ^A	46.06 ± 1.54 ^{A**}	45.61 ± 1.29 ^{A**}	12.17 ± 0.80 ^{A*}	103.85 ± 3.38 ^{A**}	0.13 ± 0.01 ^{AB**}
<i>P.</i> F _S	*	ns	**	**	ns	**	ns	ns	**
<i>P.</i> F _N	**	**	**	**	**	**	**	**	**
<i>P.</i> F _C	ns	ns	ns	*	*	**	ns	*	**
<i>P.</i> F _{S×N}	ns	*	**	ns	**	**	ns	**	**
<i>P.</i> F _{S×C}	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>P.</i> F _{N×C}	ns	*	*	ns	ns	ns	ns	ns	ns
<i>P.</i> F _{S×N×C}	ns	ns	ns	ns	ns	ns	ns	ns	ns

Each value is the mean ± SE (n = 5). Values followed by the different lowercase letters in the same column represent for significant differences between the treatments under N⁻ conditions, while values followed by the different uppercase letters in the same column represent for significant differences between the treatments under N⁺ conditions according to Tukey's test (*P* < 0.05). The asterisks designate significant differences according to the paired-samples *t*-test between the N treatments (*, 0.01 < *P* < 0.05; **, *P* ≤ 0.01). PH, plant height; BD, basal diameter; LA, leaf area; SLA, specific leaf area; LM, leaf mass; SM, stem mass; RM, root mass; TB, total biomass; R/S, root/shoot ratio. F_S, species effect; F_N, N effect; F_C, competition mode effect; F_{S×N}, the interactive effect of species and N; F_{S×C}, the interactive effect of species and competition mode; F_{N×C}, the interactive effect of N and competition mode; F_{S×N×C}, the interactive effect of species, N and competition mode. ns, not significant; *, 0.01 < *P* < 0.05; **, *P* ≤ 0.01. C/CC, *P. cathayana* cuttings from intraspecific competition; B/BB, *P. beijingensis* cuttings from intraspecific competition; C/CB, *P. cathayana* cuttings from interspecific competition; B/CB, *P. beijingensis* cuttings from interspecific competition; C/CC-N, *P. cathayana* cuttings from intraspecific competition under N deposition; B/BB-N, *P. beijingensis* cuttings from intraspecific competition under N deposition; C/CB-N, *P. cathayana* cuttings from interspecific competition under N deposition; B/CB-N, *P. beijingensis* cuttings from interspecific competition under N deposition. The same as below.

N addition significantly increased LM, SM, and TB of *P. beijingensis* but decreased R/S of *P. beijingensis* in both competition modes, whereas N addition did not change such parameters of *P. cathayana*, except that an increase in LM occurred in interspecific competition mode when exposed to N addition. Under N⁻ conditions, *P. cathayana* had significantly higher SM and TB but lower R/S than *P. beijingensis* in both competition modes. However, under N⁺ conditions, *P. beijingensis* showed a significantly higher LM, SM, and TB than *P. cathayana* in the interspecific competition pattern, while there was no significant difference in such indexes between the species in the intraspecific competition mode.

Based on ANOVA, PH, LA, SLA, SM, and R/S were significantly affected by species as independent factors. All growth and morphological parameters were significantly affected by N application. Competition patterns significantly affected SLA, LM, SM, TB, and R/S. In addition, both BD and LA were significantly affected by the interactive effect of species × N and N × competition mode.

3.2 Morphology and biomass of different root orders

Under all treatments, TRL, TRSA, and SRA showed a decreased trend along with an increase in root order for both species. In contrast, an opposite trend for root biomass, ALIR, and ADIR was observed with increasing root order. Regardless of the competition mode, a significant increase in TRL, TRSA, and root biomass of R1 and R2 was observed in *P. beijingensis* but not in *P. cathayana* when exposed to N addition. Additionally, N addition caused an increase in TRL, TRSA, and root biomass of both R3 and R4 for *P. beijingensis* under mixed culture mode but caused a decrease in ALIR of R1 for *P. beijingensis* under pure culture mode.

Under N⁻ conditions, there was no significant difference in root morphological indexes or root biomass between *P. cathayana* and *P. beijingensis* under either pure cultivation or mixed culture, except that *P. cathayana* had a greater TRSA and biomass in R3 but lower ADIR in R2 than in *P. beijingensis* under the interspecific competition mode. However, under N⁺ conditions, *P. cathayana* in mixed culture mode had greater TRL and root biomass of R1 but lower ADIR of R2 than those in pure culture mode, whereas *P. beijingensis* in mixed culture mode showed greater TRL, TRSA, and biomass of R2 than those under pure culture conditions. Regardless of any competition mode, *P. beijingensis* exhibited larger TRL, TRSA, and root biomass of both R1 and R2 than *P. cathayana*. In addition, in the mixed culture mode, *P. beijingensis* had a greater TRSA of R3 and R4 and a bigger ADIR and SRA of both R1 and R2 than *P. cathayana*, whereas there were no significant differences in such parameters between the species under the pure culture mode.

Furthermore, based on ANOVA, species, N, and competition mode as independent factors all significantly affected the TRL, TRSA, and root biomass of both R1 and R2, as well as the SRA of R1 and R4. Both species and competition mode significantly affected the ADIR of R1 and R2, and only N as an individual factor significantly affected the ALIR of R1, R2, and R3. Competition mode as an independent factor exerted a significant effect on the TRL, ADIR, and biomass of R3. Both species and N significantly affected TRL and TRSA of R4 and SRA of R2 and R5, while ADIR and biomass of R4 were significantly affected by species and competition mode and by N deposition, respectively. N deposition as an individual factor significantly affected the TRL, TRSA, and root biomass of R5, and competition mode significantly affected both the TRL and ADIR of R5. N, as an independent factor, did not significantly affect the ADIR of all roots, and the ALIR of all roots was not significantly affected by species and competition mode as independent factors.

3.3 Gas exchange rate and N and water use efficiency

N supply induced a significant increase in P_n and G_s in both species under both competition patterns, except for P_n of *P. cathayana* in the interspecific competition mode, while N application did not significantly influence P_{NUE} of either species in both competition patterns. Under N⁻ conditions, compared with *P. beijingensis*, *P. cathayana* showed a significantly higher P_n , and in both competition modes, while under N⁺ conditions, P_n of *P. cathayana* was greater than that of *P. beijingensis* in intraspecific competition mode but not in interspecific competition mode. Compared to P_n , N deposition induced similar alterations in G_s and differences in G_s between the species, except that G_s of *P. cathayana* was lower than that of *P. beijingensis* under interspecific competition. Under N⁺ conditions, *P. beijingensis* in the mixed culture mode exhibited a higher P_n than those in the pure cultivation mode, whereas the opposite results were found under the N⁻ condition. Under N⁻ conditions, *P. cathayana* showed a significantly lower P_{NUE} than *P. beijingensis* in the intraspecific competition mode, while there was no significant difference between the species under interspecific competition due to the decrease in P_{NUE} in *P. beijingensis* when compared to the values under monoculture. Under N⁺ conditions, *P. beijingensis* showed a slightly higher P_{NUE} than *P. cathayana* under both competition modes. N deposition induced an increased $\delta^{13}C$ in leaves of *P. beijingensis* under monoculture and in the leaves of *P. cathayana* under mixture. However, there was no significant difference in this parameter between the species under all treatments.

Additionally, both P_n and G_s was significantly affected by species, N, competition mode, and the interaction of species \times N and species \times competition mode. P_{NUE} was significantly affected by species, the interactive effects of species \times competition mode and species \times N \times competition mode. $\delta^{13}C$ was significantly affected by N and the interactive effects of species \times N \times competition mode.

3.4 N assimilation and N status

The application of N induced increases in the activities of N assimilation enzymes in the leaves of both species to varying degrees, except for GS activities. For example, there was a significant increase in GOGAT and Aspat activities in both species when N was supplied, regardless of the competition mode. In addition, when exposed to N addition, NR activities in the leaves of *P. beijingensis* increased significantly in the pure cultivation mode. N application also increased NO₃⁻ concentration in the leaves of *P. beijingensis* under both competition modes but not in those of *P. cathayana*. N deposition induced a significant increase in N content in leaves, R1 and R2 of *P. beijingensis* under either of the competition patterns, while N deposition only increased the N concentration of leaves and R1 in *P. cathayana* under monoculture. With or without N supply, there were consistently higher enzyme activities of NR, GOGAT, AspAT, and NAD-GDH in the leaves of *P. cathayana* than those of *P. beijingensis* in both competition modes. Moreover, under N addition, NO₃⁻ concentration in the leaves of *P. beijingensis* was greater than those in the leaves of *P. cathayana* in pure culture mode, whereas there was no significant difference in this parameter between the species in mixed culture mode. In interspecific competition mode, *P. cathayana* had a significantly higher N content in R2 than in *P. beijingensis* under N⁻ conditions, whereas the opposite was true under N⁺ conditions.

Additionally, based on ANOVA, these parameters were significantly affected by species, N, and competition pattern, except for NAD-GDH activity, N content in leaves, and N in R1, which was not affected significantly by N, competition mode, and species as independent factors, respectively. GS activity was significantly affected by the interactive effects of species \times competition mode and N \times competition mode. AspAT activity was significantly affected by the interactive effects of species \times N, species \times competition mode, N \times competition mode, and species \times N \times competition mode. Both NO₃⁻ concentration in leaves and N content in R1 were significantly affected by the interactive effects of species \times N \times competition mode. N content in R2 was affected significantly by the interactive effects of species \times N, species \times competition mode and N \times competition mode.

3.5 N isotope concentrations in leaves

For both species, N addition significantly increased both $\delta^{15}NO_3^-$ -N and $\delta^{15}NH_4^+$ -N under either of the competition modes, except for $\delta^{15}NH_4^+$ -N in leaves of *P. beijingensis* from the mixture. Under N⁻ conditions, when compared to *P. cathayana*, *P. beijingensis* had significantly higher $\delta^{15}NO_3^-$ -N in monoculture conditions, and higher $\delta^{15}NH_4^+$ -N in mixed culture conditions. In contrast, under N addition, *P. beijingensis* had significant higher $\delta^{15}NH_4^+$ -N in monoculture conditions and higher $\delta^{15}NO_3^-$ -N than *P. cathayana* in mixture conditions. In addition, both $\delta^{15}NO_3^-$ -N and $\delta^{15}NH_4^+$ -N were significantly affected by species, N, and competition mode as independent factors and the interactive effects of N \times competition mode and species \times N \times competition mode.

3.6 Relative competition intensity

As shown in Fig. 4, under N⁻ conditions, the RCI of all organs in both species was negative, except for a positive value detected in the roots of *P. cathayana*, and such negative effects exerted on *P. beijingensis* were more obvious compared to *P. cathayana*. However, under N deposition conditions, for both species, the RCIs of aboveground organs (RCI-leaf and RCI-stem) and total biomass (RCI-total) were still negative, but the RCI roots of both species were positive. In contrast, the negative effects exerted on *P. cathayana* were more apparent when compared to *P. beijingensis*.

Table 2 The effects of N addition on activities of N assimilation-related enzymes and N status in *P. cathayana* and *P. beijingensis*

under intra- and interspecific competition

Treatments	NR (nmol NO ₂ ⁻ min ⁻¹ g ⁻¹ FW)	GS (nmol γ-glutamylhydroxamate min ⁻¹ g ⁻¹ FW)	GOGAT (nmol NADH min ⁻¹ g ⁻¹ FW)	AspAT (mmol NADH min ⁻¹ g ⁻¹ FW)	NAD-GDH (nmol NADH min ⁻¹ g ⁻¹ FW)	NO ₃ ⁻ -N (μmol g ⁻¹ FW)	N content in leaves (mg g ⁻¹ DW)	N content in the first root order (mg g ⁻¹ DW)
C/CC	9.61±0.48 ^a	2.09±0.03 ^{bc}	10.97±0.94 ^a	0.20±0.002 ^a	101.89±14.77 ^a	8.28±0.32 ^a	4.33±0.04 ^a	4.63±0.18 ^b
B/BB	4.42±0.23 ^b	2.04±0.01 ^c	6.72±0.84 ^b	0.01±0.001 ^c	17.71±1.46 ^b	8.80±0.34 ^a	3.60±0.14 ^a	4.71±0.11 ^{ab}
C/CB	10.73±0.23 ^a	2.24±0.02 ^a	17.79±1.52 ^a	0.19±0.005 ^a	126.53±14.24 ^a	8.95±0.46 ^a	4.57±0.57 ^a	5.27±0.12 ^a
B/CB	5.39±0.39 ^b	2.21±0.04 ^{ab}	7.62±1.46 ^b	0.07±0.003 ^b	76.11±14.59 ^a	7.67±0.40 ^a	4.02±0.30 ^a	4.83±0.10 ^{ab}
C/CC-N	10.62±0.30 ^A	2.22±0.02 ^B	22.00±1.83 ^{A**}	0.24±0.008 ^{A*}	135.47±7.90 ^A	9.28±0.39 ^B	5.92±0.09 ^{A**}	5.91±0.19 ^{AB}
B/BB-N	5.45±0.32 ^{B*}	2.02±0.03 ^C	13.69±0.86 ^{B*}	0.02±0.001 ^{C*}	33.41±8.22 ^B	11.67±0.41 ^{A**}	4.92±0.43 ^{A*}	5.44±0.21 ^{B*}
C/CB-N	12.19±0.44 ^A	2.41±0.05 ^A	21.87±0.97 ^{A*}	0.26±0.004 ^{A**}	151.53±14.51 ^A	9.62±0.18 ^B	5.32±0.49 ^A	5.53±0.21 ^{AB}
B/CB-N	6.43±0.41 ^B	2.37±0.05 ^{AB}	15.29±1.50 ^{B*}	0.15±0.006 ^{B**}	67.97±8.58 ^B	9.09±0.52 ^{B*}	5.64±0.01 ^{A*}	6.41±0.21 ^{A*}
<i>P</i> : F _S	**	**	**	**	**	**	*	ns
<i>P</i> : F _N	**	**	**	**	ns	**	**	**
<i>P</i> : F _C	**	**	*	**	**	*	ns	*
<i>P</i> : F _{S×N}	ns	ns	ns	*	ns	ns	ns	ns
<i>P</i> : F _{S×C}	ns	*	ns	**	ns	ns	ns	ns
<i>P</i> : F _{N×C}	ns	*	ns	**	ns	ns	ns	ns
<i>P</i> : F _{S×N×C}	ns	ns	ns	**	ns	**	ns	**

Each value is the mean ± SE (n=5). NR, nitrate reductase; GS, glutamine synthetase; GOGAT, glutamate synthetase; AspAT, aspartate aminotransferase; NAD-GDH, deaminating glutamate dehydrogenase.

4. Discussion

In our study, we found that the effects of competitive interaction on the species-specific growth of poplars depend on N regimes. Under N⁻ conditions, *P. cathayana* grown under either of the competition modes always showed a better performance in growth than its counterpart, as seen in the consistently higher LM, SM, TB, and P_n, suggesting that *P. cathayana* has a relatively lower N demand. Previous studies have found that the bulk of neighbor effects were mainly belowground at low N availability (Wilson and Tilman, 1991). In our study, investing more in belowground growth, as shown by the significantly increased R/S, can be considered a strategy adopted by *P. cathayana* under heterospecific competition, which enables *P. cathayana* to capture more nutrients from the soil. The positive RCI roots also suggested that the growth of roots in *P. cathayana* benefited from the presence of *P. beijingensis*, which may help *P. cathayana* to be a stronger competitor in belowground competition than *P. beijingensis* at low N availability (Kołodziejek, 2019). However, *P. beijingensis* under the mixture had greater growth responses to N deposition, with a significant increase in PH, BD, LA, LM, SM, RM, TB, and R/S, whereas the corresponding *P. cathayana* was comparatively insensitive to N deposition, especially dry mass accumulation. Therefore, *P. beijingensis* showed competitive superiority over *P. cathayana* under N deposition, as seen in the higher values in LA, SLA, LM, SM, and TB. The relatively greater size of *P. beijingensis* could confer stronger competitiveness by shading neighbors (Valladares et al., 2016), and the higher LA and SLA of *P. beijingensis* may compensate for inferiority in carbon assimilation in the unit leaf area. Furthermore, under N deposition, *P. beijingensis* competed for soil nutrients by increasing the absorbing power of the roots through architectural adjustment, including more production of absorptive roots (R1 and R2), rather than through investing more into roots, as shown by the

decrease of R/S. Considering the optimal foraging theory (Gedroc et al., 1996), such decreased R/S meant that more carbon and nutrients can be allocated for aboveground growth to enhance plant competitive capacity. Our results are also in line with the findings that plants with higher plasticity in biomass allocation upon N fertilisation have a competitive advantage (Guo et al., 2016; Song et al., 2017). Taken together, our results suggest that species-specific differences in growth are not only regulated by N availability but also dependent on competitive relationships.

Nutrient availability has been found to affect the competitive ability of plants and consequent species composition in communities because of interspecific differences in N uptake and use efficiency (Song et al., 2017). In our study, P_{NUE} was mainly determined by species and indirectly affected by N availability and competition mode. Compared to the values under intraspecific competition, interspecific competition increased P_{NUE} of *P. cathayana* but decreased P_{NUE} of *P. beijingensis*, which could benefit the competitive performance of *P. cathayana*. However, we detected species-specific physiological reactions in N assimilation in response to N deposition and competition mode, which may partly contribute to the competitive ability of poplar. In accordance with responses in the activities of N assimilation-related enzymes to N addition (Li et al., 2015; Luo et al., 2013), we found that N deposition increased the activities of all N-related enzymes in both *P. beijingensis* and *P. cathayana* to varying degrees. Interestingly, *P. cathayana* exhibited consistently higher activity of these enzymes than *P. beijingensis* in both competition modes, regardless of N availability. It is reasonable to suppose that *P. cathayana* has a higher constitutive expression of specific genes coding for the N assimilating pathway (Tischner, 2000), conferring an inherently greater capacity for N assimilation under low N availability. Thus, under N^- conditions, the higher ability in N assimilation can facilitate *P. cathayana* to acquire more inorganic N under interspecific competition, which can be reinforced by the fact that the N content in R1 and R2 increased in *P. cathayana*, not in *P. beijingensis*, under mixture when compared to the values under monoculture. Nevertheless, under N deposition, mixture cultivation induced greater increases in both GS and AspAT activities in *P. beijingensis* than in *P. cathayana* when compared to monoculture, which may be responsible for the decrease in NO_3^- -N but a slight increase in N content in the leaves of *P. beijingensis*. In view of greater LA and LM, these results indicate that *P. beijingensis* from the mixture assimilates more inorganic N in leaves than *P. cathayana* under N deposition. In addition, we found that interspecific competition increased the N content of both R1 and R2 in *P. beijingensis*, but not in those of *P. cathayana* under N deposition, when compared to individuals subjected to intraspecific competition. Therefore, these results collectively suggest that *P. beijingensis* grown in mixture conditions is more sensitive to N deposition and exhibits a higher capability to utilize the increasing N in soils than its counterpart.

Many studies emphasize the ecological significance of belowground competition (Casper and Jackson, 1997; Fajardo and Piper, 2019), which drives the trade-off in biomass allocation and affects competitive outcomes. Belowground competition intensity between individual plants usually decreases as nutrient availability increases, but the importance of belowground competition that structures communities may increase with resource availability (Schenk, 2006). A growing number of studies suggest that morphological and architectural traits of roots, which are shaped by myriad environmental signals (Morris et al., 2017), play a major role in mediating plant–plant interactions and resource capture from soils (Colom and Baucom, 2020), and can be used to predict the ability of plants to tolerate their competitors (Semchenko et al., 2018). In our study, when exposed to N^- conditions, there are rarely species-specific divergences in biomass and morphology at the root rank scale, especially for absorptive roots (i.e., R1 and R2), under both competition modes. Such results confirm that species-specific differences in N assimilation rate and N use efficiency, rather than N uptake capacity, may contribute to *P. cathayana* outcompeting *P. beijingensis* under N^- conditions. However, under either of the competition modes, *P. beijingensis* exhibited higher plasticity in biomass allocation than *P. cathayana*, as shown by the decrease in R/S and increases in RB, TL, and TRSA of absorptive fine roots (i.e., R1 and R2) in response to N deposition. Combined with the higher ADIR and SRA in both R1 and R2, it can be inferred that *P. beijingensis* from the mixture can take up more space and explore more nutrients by altering root architecture, and thereafter increase its belowground competitive ability (Lei et al., 2012). Lower ranks of roots, especially R1 and R2, were more sensitive to N deposition and the competition mode than higher ranks of roots, which agrees with the findings of Salahuddin et al. (2018). Taken together, we presumed that *P. beijingensis* is more sensitive to N deposition and readily gains a competitive advantage over *P. cathayana* by regulating root biomass allocation and architecture.

Our results revealed that N deposition affects carbon gain and long-term water use efficiency by regulating stomatal behavior. Under N^- conditions, *P. beijingensis* from the mixture showed a lower P_n but a higher G_s than those from monoculture, suggesting that suppression in photosynthetic capacity in *P. beijingensis* may result from a decline in carboxylation capacity rather than stomatal limitation. Previous studies on the effects of N deposition on water use efficiency have revealed variable results; for example, N deposition can lead to increased (Lu et al., 2019), decreased (Elhani et al., 2005; Huang et al., 2016), or invariant foliar iWUE (Diao et al., 2021). Such variable results can be explained by the fact that the effects of N on water use efficiency may depend on the extent and duration of N addition, as well as on the sensitivity of plants to N and the background N availability in soil. In our study, N deposition induced an increased $\delta^{13}C$ in leaves of both species, regardless of competition mode, accompanied by increases in P_n and G_s , which agrees with the findings of Guo et al. (2016) and Song (2017). These results indicate that the increased carboxylation rate, rather than stomatal limitation, contributed to the increased long-term water use efficiency under N deposition, as shown by the increased $\delta^{13}C$. In addition, we noted that N deposition resulted in an increased $\delta^{13}C$ value for the conspecific *P. beijingensis* and for the heterospecific *P. cathayana* when compared with individuals under N^- conditions, possibly because *P. beijingensis*, with a relatively larger transpiring area, depleted water availability in the soil and forced their neighbors to promote water use efficiency. Consistently, we observed that competition with *P. beijingensis* induced stomatal closure of *P. cathayana*, which may be responsible for the increased water use efficiency of *P. cathayana*.

Previous studies have demonstrated that the preference for N resources in plants depends on species, plant sex, and soil nature, such as pH (Li et al., 2015). Some poplars have been proven to prefer $N-NO_3^-$ to $N-NH_4^+$ because $N-NO_3^-$ can be highly efficient for uptake and assimilation (Min et al., 1998). In our study, we observed that both poplars prefer $N-NO_3^-$ over $N-NH_4^+$ because both species showed higher content of $N-NO_3^-$, which is in line with the findings of Chen (2014). More interestingly, we found that competition mode exerted an impact on poplars' preference for N forms, as reflected by the changing $\delta^{15}N-NO_3^-$ and $\delta^{15}N-NH_4^+$ in poplar leaves. Overall, under N^- conditions, when compared to monoculture, interspecific competition increased $\delta^{15}N-NO_3^-$ in *P. cathayana* leaves but decreased the $\delta^{15}N-NO_3^-$ content in *P. beijingensis* leaves. The concomitant increase in $\delta^{15}N-NH_4^+$ indicates that *P. beijingensis* may absorb more

N-NH₄⁺, whereas *P. cathayana* showed a preference for N-NO₃⁻. However, under N⁺ conditions, the opposite pattern of N preference between the poplars was detected. In view of the findings that poplars with better adaptation to N-NO₃⁻ are prone to achieve a competitive advantage (Chen et al., 2014), our results suggest that selectively absorbing more N-NO₃⁻ is a possible cause of the competitive advantage of *P. cathayana* under N⁻ conditions and *P. beijingensis* under N deposition.

High niche overlap could lead to fierce competition between vigorously introduced hybrids and closely related native species that occupy the sympatric range (Eckhart et al., 2017), thereby exerting adverse effects on species coexistence. Novel recombinant genotypes with wide niche breadth have shown the potential to outcompete and displace the parent species in the introduced habitats (McCartney et al., 2019), and ultimately decrease biodiversity (Thum and Lennon, 2010). In our study, under both N conditions, we detected an adverse competition relationship between the species, as shown by the negative RCI values, suggesting that there is high niche overlap between the poplars, and interspecific competition may ultimately decrease the fitness of both species. Under N⁻ conditions, growing with *P. cathayana* exerted more negative effects on *P. beijingensis*, as indicated by the more negative values in the RCI of all organs, which could place *P. beijingensis* at a disadvantage. *Populus cathayana* may benefit from the presence of *P. beijingensis* with respect to the exploration of soil resources, as shown by the positive root RCI in *P. cathayana*. Nevertheless, under N deposition, *P. cathayana* suffered more negative effects from the distinct neighbor, and aboveground growth was obviously inhibited, as reflected by the negative leaf and stem RCI values, while *P. beijingensis* was slightly affected in the presence of *P. cathayana*. Such results also indicate that *P. beijingensis* may gain a competitive advantage over *P. cathayana* under N deposition.

In conclusion, our results provide insight into the early competitive performance of two widely planted poplar species in two N regimes, which is helpful for predicting the effects of the introduced exotic hybrid poplar on native poplar species under N deposition. Our study indicates that the competition mode could affect species-specific differences between *P. cathayana* and *P. beijingensis* in growth traits, biomass allocation, root architecture, photosynthetic rate, N-related metabolism, long-term water use efficiency, and preference for N forms in response to two N regimes. Under N⁻ conditions, native *P. cathayana* is a competitively superior species, illustrated by its greater biomass, photosynthetic capacity, P_{NUE} , and higher N-assimilation capability. In contrast, simulated N deposition induced an opposite competition pattern between the poplars. Under N⁺ treatment, the exotic *P. beijingensis* showed more positive responses in growth, better light capture ability, and higher flexibility in fine root architecture, as well as a shift for N-NO₃⁻ preference compared with *P. cathayana*, suggesting that *P. beijingensis* has the potential to outcompete *P. cathayana* when exposed to N deposition. Therefore, it is expected that native species may be replaced at an accelerated rate by species with a suite of functional traits beneficial for using excessive N in soils, thus showing a more sensitive reaction to growth under the background of growing N deposition. Long-term observation of this interactive relationship should be performed to estimate the competitive outcomes, and the ecological consequences of introduced tree species thriving in afforestation on the native species.

Declarations

Author contribution statement

Lianghua Chen: Conceptualization, Methodology, Investigation, Writing – original draft. **Jiaxuan Mi:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Linting Hao:** Methodology, Formal analysis, Writing – review & editing. **Fang He:** Formal analysis, Writing – review & editing. **Hanbo Yang:** Resources, Methodology, Writing – review & editing. **Xueqin Wan:** Resources, Writing – review & editing. **Fan Zhang:** Resources, Writing – review & editing. **Yang Liu:** Resources, Methodology, Writing – review & editing. **TianTian Lin:** Supervision, Conceptualization, Writing – original draft, Writing – review & editing.

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Figures

Figure 1

The effects of N addition on biomass and morphological traits of different root orders in *P. cathayana* and *P. beijingensis* under intra- (monoculture) and interspecific competition (mixture).

(a) root biomass; (b) TRL, total root length; (c) TRSA, total root surface area; (d) ALIR, average length of individual root; (e)ADIR, average diameter of individual root; and (f) SRA, specific root surface area. Different lowercase letters above the bars represent for significant differences between the treatments under N⁻ conditions, while different uppercase letters above the bars represent for significant differences between the treatments under N⁺ conditions according to Tukey's test ($P < 0.05$). The asterisks designate significant differences according to the paired-samples *t*-test between the N treatments (*, $0.01 < P < 0.05$; **, $P \leq 0.01$). S, species effect; N, N effect; C, competition mode effect; S×N, the interactive effect of species and N; S×C, the interactive effect of species and competition mode; N×C, the interactive effect of N and competition mode; S×N×C, the interactive effect of species, N and competition mode. ns, not significant, *, $0.01 < P < 0.05$; **, $P \leq 0.01$. Values are given as mean ± SE (n=5).

Figure 2

The effects of N addition on net photosynthetic rate (P_n) (a), stomatal conductance (G_s) (b), photosynthetic nitrogen use efficiency (P_{NUE}) (c), foliar carbon isotope composition ($\delta^{13}C$) (d) of *P. cathayana* and *P. beijingensis* under intra- (monoculture) and interspecific competition (mixture).

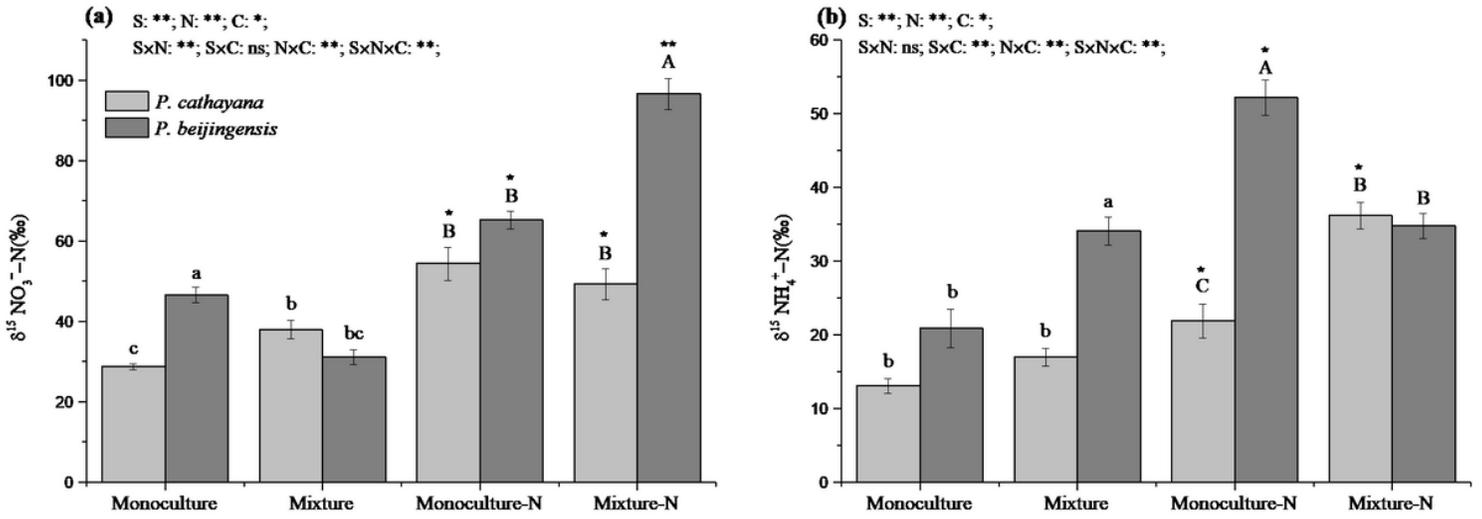


Figure 3

The effects of N addition on $\delta^{15}\text{NO}_3^- \text{-N}$ concentration (a) and $\delta^{15}\text{NH}_4^+ \text{-N}$ concentration (b) in leaves of *P. cathayana* and *P. beijingensis* under intra- (monoculture) and interspecific competition (mixture).

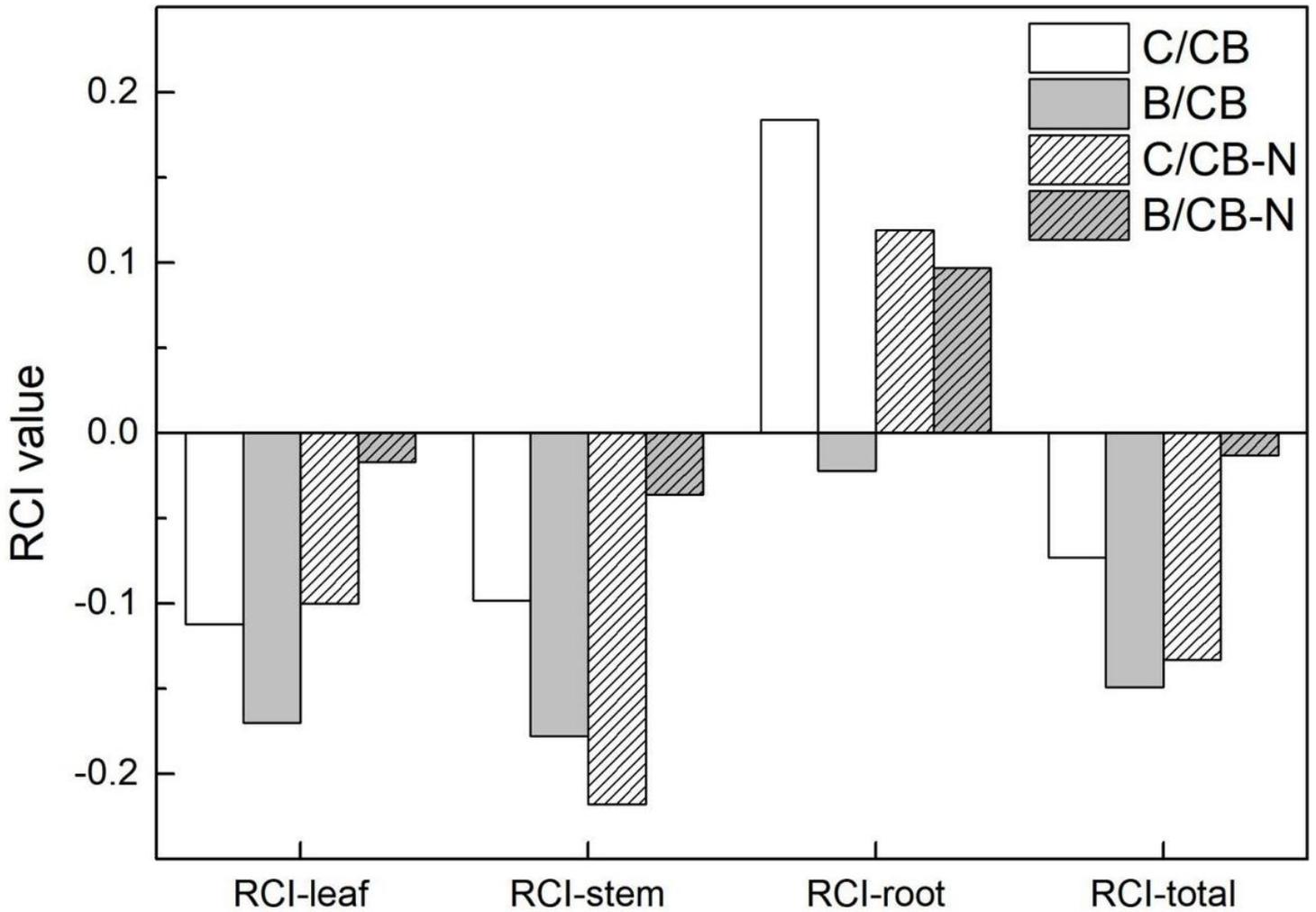


Figure 4

Relative competitive intensity of *P. cathayana* and *P. beijingensis* exposed to interspecific competition and N deposition.

RCI-leaf, relative competitive intensity calculated from responses in leaf mass. RCI-stem, relative competitive intensity calculated from responses in stem mass. RCI-root, relative competitive intensity calculated from responses in root mass. RCI-total, relative competitive intensity calculated from responses in total biomass.