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# Ecophysiological responses of two poplar species to intraspecific and interspecific competition under different nitrogen levels

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## Abstract

***Aims*** *Populus deltoides* and *P. euramericana* are widely used in China as major forestry species. At present, little is known about the responses of these two species to nitrogen deficiency when grown in monocultures or mixed plantations. The aim of this investigation was to analyze the growth, and morphological and physiological responses of *P. deltoides* and *P. euramericana* to different nitrogen levels under competition conditions.

***Methods*** We employed two *Populus* species (*P. deltoides* and *P. euramericana*) as research materials to discover how N deficiency affects plant traits under different competition types (*P. deltoides* × *P. deltoides*, intraspecific competition; *P. euramericana* × *P. euramericana*, intraspecific competition; *P. deltoides* × *P. euramericana*, interspecific competition). Potted seedlings were exposed to two nitrogen (N) levels (normal N, N deficiency), and nitrogen- and competition-driven differences in growth, morphology and physiology were examined.

***Important Findings*** Under normal N conditions, interspecific competition significantly decreased the total root weight, root mass fraction (RMF), root-shoot ratio (R/S) and carbon/nitrogen ratio (C/N), and increased the leaf dry weight, leaf mass fraction (LMF) and total leaf area (TLA) of *P. euramericana* compared to intraspecific competition. The same conditions significantly affected most growth and morphological variables of *P. deltoides,* except for the dry weight of fine roots (FR), R/S, specific leaf area (SLA), RMF, total nitrogen content and C/N compared to intraspecific competition. In addition, chlorophyll a (Chla), total chlorophyll (Tchl), carotenoid contents (Caro) and the carbon isotope composition ($\delta^{13}C$) of *P. deltoides* were significantly lower in interspecific competition than in intraspecific competition, but no difference were detected in *P. euramericana*. The effects of N deficiency on *P. deltoides* under intraspecific competition were stronger than under interspecific competition. In contrast, the effects of N deficiency on *P. euramericana* between intraspecific and interspecific competition were not significantly different. In addition, N deficiency significantly increased the relative competitive intensity (RCI) of *P. deltoides*. Overall, these data demonstrated that under normal N conditions, compared with intraspecific competition, interspecific competition will affect the performances of both species, the effects being stronger in *P. deltoides* than in *P. euramericana*. On the other hand, N deficiency affected negatively the performance of both species, but the differences between intraspecific and interspecific competition were smaller than under normal N conditions.

***Keywords***: competition; nutrient resorption efficiency; photosynthesis capacity; competition intensity index; N deficiency

## Introduction

In forest environments, plants will face the pressure caused by their neighbors, such as interspecific and intraspecific competition (Michelle and Janos 2004; Oksanen et al. 2006; Yamawo 2015). Adler et al. (2013) have proposed that species with different morphological and physiological traits will compete less intensely than species with similar traits, because they have different resource requirements. It has been shown that mixed-species plantations have a higher productivity than monocultures (Lovelock and Ewel 2005; Richards et al. 2010; Guo et al. 2019). One important reason is that species mixtures contain multiple functional groups (Reich et al. 2004; Mouillot et al. 2011) that possess different traits and may use resources in a complementary way; e.g., phenological differences lead to temporal light partitioning (Sapijanskas et al. 2014; Forrester and Pretzsch 2015; Forrester and Bauhus 2016).

Plants can adjust their morphological and physiological traits proactively to facilitate the optimization of their performance in various environments (Callaway et al. 2003; Anten et al. 2005; Guo et al. 2017; Yu et al. 2019a). Song et al. (2017) have investigated chemical and physiological determinants, and they have demonstrated that *Salix rehderiana* individuals subjected to interspecific competition benefited from the presence of *Populus purdomii* plants. When studying coniferous trees, Yu et al. (2017) observed that *Abies fabri* individuals exposed to interspecific competition showed a stronger competitive ability when compared to *Picea brachytyla* individuals. Furthermore, Guo et al. (2016) have showed that competition promoted N and carbohydrate storage capacity in two coniferous tree species and the differences between species in carbohydrate metabolism may contribute to their coexistence.

Nitrogen (N) is an essential mineral element that plants need in great amounts and itis a limiting factor for growth and development (Frink et al. 1999). With increasing anthropogenic activities and soil erosion, nitrogen deficiency has become more common, especially in alpine forests (Korner 1999). Nitrogen deficiency will result in a series of harmful physiological and chemical responses in plants (Zhang et al. 2014). First, it negatively affects leaf development, photosynthesis and metabolic processes, and plant growth (Boyce et al. 2006; Kant et al. 2011). Furthermore, these changes affect the nutrition investment of organs for capturing resources and the competitive ability of plants (Venterink and Gusewell 2010; Xia et al. 2020). There are many competition-related studies on the productivity of mixed-species plantations and monocultures, potential underlying mechanisms and long-term consequences of neighbor effects for competition. However, studies on how N deficiency affects the performance of deciduous broadleaf plants exposed to different interactions with their neighbors are still limited.

Poplars are commonly used as models in studies on the physiology of woody plants. *Populus deltoides* (D) and *P. euramericana* (E) arewidely used in China as major forestry species because of their fast growth, disease resistance and environmental adaptability. However, land degradation and lack of nitrogen in poplar plantation soil is an important problem for the sustainable development of poplar plantations. In this study, we investigated the performance of two deciduous broadleaf species, *P. deltoides* and *P. euramericana* to examine the effect of N deficiency on plant traits under intraspecific and interspecific competition environments in order to explore the responses of these two species to low N in pure forest and mixed forest conditions.

These two plants have closely similar life histories and morphological traits. However, species-specific response strategies related to competitive ability under N deficiency environments are poorly known. In this study, our aim is to gain an insight into growth, and morphological and physiological traits that affect the competitive capacity of *P. deltoides* and *P. euramericana* under N deficiency. The following hypotheses were tested: (i) The performances of the two species under two competition types are different. (ii) The species which copes better under N limitation is more competitive in N deficiency conditions.

## Materials and methods

### *Plant material and experimental design*

Healthy annual shoots of *P. deltoides* and *P. euramericana* trees were collected from the germplasm nursery at the Communist Youth League farm located in the Jingkou District of Zhenjiang in the Jiangsu Province, China (32°20′ N, 119°47′ E). The experiment was performed at the Mianyang Normal University in the Sichuan province (33°03′ N, 105°43′ E). Cuttings were planted separately in a greenhouse in March 2016. After sprouting and growing for 6 weeks, healthy seedlings of an approximately identical crown size and equal height (~20 cm) were selected for the experiment. The seedlings were grown in a greenhouse under ambient conditions. The day-time temperature was 19-28 °C, the nighttime temperature was 12-18 °C, the relative air humidity was 50-75%, and the light conditions were natural.

The experiment was completely randomized and included three factors (species, nitrogen treatment and competition) as follows: two species (*P. deltoides* (D) and *P. euramericana* (E)), two nitrogen treatments (normal N and N-deficiency) and two competition types (*P. deltoides* × *P. deltoides*, DD, intraspecific competition; *P. euramericana* × *P. euramericana*, EE, intraspecific competition; *P. deltoides* × *P. euramericana*, DE, interspecific competition). Sixteen replicates per treatment were included in the experiment. On 5 May 2016, healthy seedlings of *P. deltoides* and *P. euramericana* were chosen and transplanted into 30-L plastic pots filled with a vermiculite and perlite mixture (1:1 v/v). The mixture contained no added nutrients. Two seedlings were planted per pot (two *P. deltoides*, two *P. euramericana*, or one *P. euramericana* and one *P. euramericana*) and, thereafter, watered with a nutrient solution. The pots with a normal nitrogen treatment were watered with the modified Hoagland solution (normal N), which allows normal growth, containing 1.25 mM $KNO_3$, 1.25 mM $Ca(NO_3)2\cdot 4H_2O$, 0.5 mM $MgSO_4\cdot 7H_2O$, 0.25 mM $KH_2(PO_4)$, 11.6 μM $H_3BO_3$, 4.6 μM $MnCl_2\cdot 4H_2O$, 0.19 μM $ZnSO_4\cdot 7H_2O$, 0.12 μM $Na_2MoO_4\cdot 2H_2O$, 0.08 $CuSO_4\cdot 5H_2O$ and 10 μM Fe supplied as Fe(III)-EDTA (Fodor et al., 2005; Zhang et al., 2014; Li et al., 2015). In the nitrogen deficiency treatment (N deficiency), $NO_3^-$ was replaced by $Cl^-$ (without N) (Zhang et al., 2014). The plants were harvested on 20 August 2016. During the experiment, each pot was watered with 1000 ml of water combined with the corresponding nutrient solution every day.

### *Growth measurements and analysis of morphological characteristics*

On 20 August 2016, all seedlings were used to measure the plant height and stem width (SW). Height growth measurements were based on the length of the stem from the collar to the apex. Stem base width measurements were based on the stem diameter. The total leaf area was determined by a Portable Laser Area Meter (CI-203, CID Inc., Camas, WA, USA). Five randomly selected seedlings from each treatment were used for the green leaf and senesced leaf collection. Four leaves from the top of each seedling were sampled as the green leaf group on 20 August 2016 and four leaves from the bottom of each seedling were sampled as the group of senesced leaves 20 days later. Other seedlings were harvested on 20 August 2016 and then divided into leaves (green leaves), stems, fine roots (<2 mm) and coarse roots (>2 mm). All plant parts were dried to a constant mass at 75 °C, and the dry weight of leaves, stems, fine roots (FR) and coarse roots (CR), as well as the total root weight (TR) were measured. The root-shoot ratio (R/S) was calculated as belowground dry matter weight / aboveground dry matter weight. The fine root/total root (FR/TR) was calculated as FR / (FR+ CR). Specific stem length (SSL) was calculated as the ratio of plant height to stem mass. Specific leaf area (SLA) was calculated as the ratio of leaf area to dry matter. The biomass partitioning of leaves, stems and roots equals to the ratio of leaf dry matter weight, stem dry matter weight and total root dry matter weight to total dry matter weight, as expressed, respectively, as leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF).

### *Gas exchange and pigment content measurements*

On 18 and 19 August 2016, the photosynthetic characteristics of the third fully expanded and intact leaves from five randomly selected seedlings in each treatment were measured using a portable LI-6400 photosynthesis system with the standard leaf chamber (2×3 $cm^2$ window area; Li-Cor Inc., Lincoln, NE, USA). The measurements were conducted between 08:00 and 11:30 h. The photosynthetically active photon flux density was provided by the LI-6400 Light Emitting Diode light source and kept at 1400 $\mu mol\ m^{-2}s^{-1}$. The leaf temperature was set at 25 °C, air flow rate through the sample chamber was adjusted to 500 $\mu mol\ s^{-1}$, and the $CO_2$ concentration of the chamber was adjusted to $400 \pm 5\ \mu mol\ mol^{-1}$.

The leaves used for photosynthesis measurements were sampled for the determination of leaf pigment contents. Ten pieces of leaf disks of 0.8 cm diameter were cut and immersed into 10 ml 80% chilled acetone (v/v). After 6 days of extraction in dark conditions, the extract was used for the measurements. The absorbance of extracts at 663, 646 and 470 nm was measured using a spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan). Chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoid contents (Caro) were calculated using the equations of Porra et al. (1989). The total chlorophyll content (Tchl) was the sum of Chla and Chlb.

### *Determination of carbon isotope composition*

The carbon isotope composition ($\delta^{13}C$) was determined for leaves with the same position as in leaves used for the photosynthesis measurements. Leaf samples were oven-dried at 75 °C for 72 h and ground to a fine powder. The $^{13}C/^{12}C$ ratios were determined using an Isotope Ratio Mass Spectrometer (DELTA V Advantage; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) according to the method of Li et al. (2004) at the Stable Isotope Laboratory for Ecological and Environmental Research (SILEER) of CAS. The carbon isotope composition is expressed as a $\delta^{13}C$ value, relative to the standard Pee Dee Belemnnite and determined as follows: $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$, where $R_{sample}$ is the $^{13}C/^{12}C$ ratio of the leaf sample and $R_{standard}$ is that of the standard. The overall precision of the δ values was better than 0.1‰, as determined from repeated samples.

### *Leaf element contents and N and P resorption efficiency*

Leaf samples from all harvested seedlings were used for the analyses of total carbon (C), total N and total phosphorus (P) concentrations. The samples were oven-dried at 75 °C, ground to a fine powder and passed through a 100-mesh screen. Then, 0.5 g plant samples were acidified with 8 ml ultrapure concentrated mixture of 2.5 ml ($HNO_3$) + 4 ML (HF) + 1.5 ml ($HClO_4$) (He et al. 2016). All samples were solubilized in 50 ml teflon centrifuge tubes and digested in a microwave digestion system prior to elemental analysis. The concentrations of N and C were determined by the Vario MAX CN element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and the P concentration was analyzed by induced plasma emission spectroscopy (Hotscher and Hay 1997).

Nutrient resorption efficiency (NuRE) was defined as the proportion of the nutrient pool in mature leaves as follows (Lu et al. 2013):

$NuRE = (1\text{-}Nutrient_{senesced}/Nutrient_{green}) \times 100\%$, where $Nutrient_{senesced}$ and $Nutrient_{green}$ are N or P concentrations of the senesced and green leaves, respectively. N and P concentrations were expressed on a dry mass basis. Nitrogen (NRE) or phosphorus resorption efficiency (PRE) was expressed as N or P concentrations in senesced leaves (Killingbeck 1996).

### Analysis of relative competitive intensity

The relative competitive intensity (RCI) of *P. deltoides* and *P. euramericana* when exposed to different competition and nitrogen levels was determined according to the methods of Grace (1995) and Chen et al. (2017): $RCI = (B_{inter} - B_{intra}) / B_{intra}$, where $B_{inter}$ represents the total dry matter weight of a seedling from interspecific competition, and $B_{intra}$ represents the total dry matter weight of a seedling from intraspecific competition.

### Statistical analyses

Statistical analyses were conducted with the IBM SPSS 19.0 statistical software package (SPSS, Inc., Chicago, IL, USA). Before ANOVAs, the data were checked for the normality and homogeneity of variances and transformed to correct deviations from these assumptions when needed. For each species, we performed two-way ANOVAs for the effects of competition, nitrogen and their interactions on each variables. The significances of differences between the treatments for each species were tested using Duncan's test. All statistical effects were considered significant at a level of $P < 0.05$.

## Results

*Growth and morphological characteristics*

In *P. deltoides*, compared with intraspecific competition, interspecific competition significantly decreased the plant height, SW and biomass accumulation, except for FR, TLA and SMF, while significant increases were detected in FR/TR, SSL and LMF under a normal N condition. N deficiency significantly inhibited the plant height, SW, CR, dry weight of leaves and stem, TR, total dry matter weight, TLA and SMF, and increased SSL of D/DD, the changes equaling 54.9%, 19.1%, 78.0%, 67.3%, 78.0%, 66.7%, 71.9%, 63.3%, 22.7% and 105.0%, respectively. On the other hand, N deficiency significantly decreased the plant height, dry weight of leaves and stem, total dry matter weight, TLA and LMF, and increased R/S and RMF of D/DE; the changes equaling 34.7%, 60.8%, 40.7%, 41.7%, 62.3%, 32.1%, 216.7% and 145.5%, respectively. The differences between D/DD and D/DE under N deficiency were significant only for TLA, R/S, LMF and RMF. In *P. euramericana*, compared with intraspecific competition, interspecific competition significantly increased the dry weight of leaves, TLA and LMF, but decreased TR, R/S and RMF under a normal N condition. N deficiency significantly decreased the plant height, dry weight of leaves and stem, and total dry matter weight in both competition types. However, a significant difference between intraspecific and interspecific competitions was found only for SLA under N deficiency (Table 1, Fig. 1, Fig. 2, Fig. 3).

The statistical analysis showed that both competition and N deficiency significantly affected the dry weight of leaves, stems and roots, total dry matter weight and TLA of *P. deltoides*, and plant height, SMF, RMF, TLA, SLA and FR/TR were affected by N deficiency alone. In *P. euramericana*, both competition and N deficiency significantly affected the plant height, dry weight of leaves, TLA, SSL, CR and R/S. SW and LMF were affected by competition, and the dry weight of stems, total dry matter weight and RMF were affected by N deficiency. The C×N interaction significantly affected the dry weight of leaves, stems and roots, total dry matter weight, SLA, LMF, SMF and RMF of *P. deltoides,* and the plant height, dry weight of leaves, CR, FR/TR, TLA and SSL of *P. euramericana*.

### *Gas exchange and pigment contents*

Interspecific competition significantly reduced Chla, Tchl and Caro in *P. deltoides*, but no significant effects were found in *P. euramericana* under normal N conditions (Table 2). N deficiency significantly decreased net photosynthetic rate ($P_{\mathrm{n}}$), Chlb and Caro in D/DD and D/DE, Chla and Tchl in D/DD, and stomatal conductance ($g_s$) in D/DE compared with a normal N condition. Under N deficiency, a significant difference between intraspecific and interspecific competition was found only in $g_s$. In *P. euramericana*, N deficiency significantly decreased Chla, Chlb, Caro and Tchl of both E/EE and E/DE, while it increased the intercellular $CO_2$ concentration ($C_i$) and transpiration rate ($E$) of E/EE compared with a normal N condition, but no significant differences between E/EE and E/DE were found in these indexes under N deficiency (Table 2).

Both competition and N deficiency significantly affected Chla, Tchl, Caro and $P_{\mathrm{n}}$ of *P. deltoides*, while competition affected $g_s$ and N deficiency affected Chlb. $P_{\mathrm{n}}$ was significantly affected by competition, while $g_s$, $C_i$, $E$, Chla, Chlb, Tchl and Caro were affected by N deficiency in *P. euramericana*. The C×N interaction significantly affected $g_s$, Chla, Tchl, Caro of *P. deltoides* and Chlb of *P. euramericana* (Table 2).

### *Carbon isotope composition*

As shown in Table 3, in a normal N condition, $\delta^{13}C$ of D/DE was significantly lower than that of D/DD. N deficiency significantly decreased $\delta^{13}C$ of D/DD, but no significant change was found in D/DE. Under N deficiency, the difference between intraspecific and interspecific competition on $\delta^{13}C$ was not significant. In *P. euramericana*, $\delta^{13}C$ of D/DE was significantly lower than that of D/DD in both N conditions. Competition, N deficiency and the C×N interactionsignificantly affected $\delta^{13}C$ in both *P. deltoides* and *P. euramericana* (Table 3).

### *Nutrition utilization*

Under normal N conditions, NRE and NRE/PRE of D/DE were significantly lower but PRE and N/P higher than those of D/DD. N deficiency significantly inhibited all these indexes in both competition conditions, except for NRE in D/DE. In addition, significant differences between D/DD and D/DE were found in PRE and NRE/PRE under N deficiency. In *P. euramericana*, interspecific competition significantly decreased C/N compared with intraspecific competition. N deficiency significantly inhibited the total N content, C/N, N/P and NRE, but increased C/N in both competition conditions compared with a normal N condition, but the differences between E/EE and E/DE were not significant (Table 3, Figure 4).

Competition significantly affected NRE, NRE/PRE and N/P of *P. deltoides,* and C/N of *P. euramericana*. N competiton signficantly affected all indexes in *P. deltoides,* and NRE, PRE, NRE/PRE, C/N and N/P in *P. euramericana*. The C×N interactionsignificantly affected NRE, PRE, NRE/PRE and N/P in *P. deltoides*, and C/N in *P. euramericana*.

### *Relative competition intensity*

As shown in Figure 5, RCI of *P. deltoides* under normal N showed a negative value, while it showed a positive value under N deficiency. However, the difference between normal N and N deficiency in RCI of *P. deltoides* was significant. RCI of *P. euramericana* showed a positive value under a normal N condition and a negative value under N deficiency, but the difference between them was not significant.

## Discussion

### *The effects of N deficiency on growth characteristics are stronger in intraspecific competition than in interspecific competition.*

Previous studies have showed that mixed-species plantations have higher rates of above-ground biomass production and carbon sequestration than monocultures (Erskine et al. 2006; Piotto 2008; Pretzsch and Schütze 2009), because of the complementary use of resources and facilitation (Richards et al. 2010). In our investigation, under a normal N condition, *P. deltoides* grew better in intraspecific competition than in interspecific competition, visible as a significantly higher plant height, SW, CR, TR, dry weight of leaves and stems, and total dry matter weight. The two studied *Populus* species have closely similar life histories, phenology and morphological traits, and the complementary effects are not obvious. However, there are species-specific responses to competition (Guo et al. 2016, 2017; Yu et al. 2017). In *P. euramericana*, TR was significantly lower, and leaf dry weight significantly higher in interspecific competition than in intraspecific competition. These results were consistent with previous results indicating that interspecific competition decreases the total biomass of one species but shows no negative effects on another species (Yu et al. 2017). Previous studies have suggested that species can identify conspecific or heterospecific individuals through a complex neighbor detection mechanism, such as root growth of one species may inhibit the root growth of another species, reflecting classical competition exclusion (Bais et al. 2006; Kegge and Pierick 2010; Gagliano et al. 2012; Mommer et al. 2012; Guo et al. 2016). However, a stimulation effect and no effect of neighborhood on growth have also been reported (Li et al. 2006; Cahill et al.

2010; Mahall and Callaway 1991; Dudley and File 2007; Semchenko and Hutchings 2007). In our study, the growth responses of *P. deltoides* and *P. euramericana* to heterospecific neighbors were different under a normal N condition.

N is a mineral element that plants require in great amounts and it is often the growth limiting nutrient (Antal et al. 2010). In our study, N deficiency significantly decreased the plant height, SW and biomass accumulation of *P. deltoides*, more strongly in intraspecific competition than in interspecific competition, especially in the case of root biomass accumulation. It is evident that when the environment changes, the competitiveness of plants will change, as reported in previous studies as well (Dormann et al. 2004; Fotelli et al. 2005; van der Waal et al. 2009). Our results clearly suggested that N limitation stimulates intraspecific competition in *P. deltoides* compared with a normal N condition. Similarly, Song et al. (2017) have reported that the competitive ability of *S. rehderiana* changed under N-poor conditions. In our study, *P. euramericana* showed root growth advantage in intraspecific competition but leaf growth advantage in interspecific competition. Compared with a normal N condition, N deficiency significantly reduced the leaf area and increased R/S and RMF in interspecific competition, but no changes were found in intraspecific competition. Previously, N limitation has been found to reduce the leaf area and increase biomass allocation to roots (Ingestad and Agren 1991). Overall, no significant differences between intraspecific and interspecific competition were found under a N limitation condition. It means that *P. euramericana* showed a different growth performance in intraspecific and interspecific competition under a normal N condition, but the difference disappeared under N deficiency.

### *N deficiency affects morphological traits in both competition types*

Previous studies have indicated that N limitation generally reduces the leaf area and increases biomass allocation to roots (Venterink and Güsewell 2010). Under a normal N condition, interspecific competition significantly decreased TLA of *P. deltoides*. N deficiency inhibited TLA but increased allocation to roots in interspecific competition compared with a normal N condition. This result was consistent with previous research (Güsewell 2005a, b). However, *P. euramericana* had different strategies in responses to competition and N deficiency. Under a normal N condition, TLA and LMF were significantly higher but R/S and RMF significantly lower in interspecific competition than in intraspecific competition. This was consistent with the observed growth performance of roots and leaves. N deficiency decreased TLA but increased R/S and RMF in interspecific competition compared with a normal N condition. As detected in previous studies, plants will decrease the belowground/aboveground ratio and allocate more carbon and nutrients to root growth to enhance nutrient acquisition and competitive capacity (Enquist 2003; Kleczewski et al. 2010; Bennett et al. 2012). Overall, our study revealed a different growth performance in *P. deltoides* and *P. euramericana* under competition and different N levels.

### *Differences between intraspecific and interspecific competition in physiological traits are affected by N deficiency*

Nitrogen and phosphorus are important nutrients and they control many biogeochemical processes (Li et al. 2016). Nutrient resorption efficiency can be used to quantify nutrient resorption from senescing plant tissues, which is one of the most important mechanisms of nutrient conservation (Killingbeck 1996; Lu et al. 2012). In line with our observations, NRE has been found to be positively correlated with plant growth and biomass accumulation. In our study, the conspecific neighbor had a positive effect on NRE in *P. deltoides*, while a different neighboring species had a negative effect. This phenomenon was similar to a previous discovery showing that the N acquisition capacity of beech can be impaired by the effect of blackberry (Fotelli et al. 2005). In addition, $\delta^{13}C$ of plant organic tissues can be a useful indicator of water use efficiency (WUE) (Farquhar et al. 1989), which showed similar changes as NRE of *P. deltoides* under different competition types. According to previous reports, an explanation for this phenomenon may be that water availability can affect the nutrient absorption efficiency of *P. deltoides* (Van Heerwaarden et al., 2003; Lü and Han, 2010). In *P. euramericana*, interspecific competition significantly decreased $\delta^{13}C$ and C/N, but no significant change was found in nutrition absorption efficiency. Species-specific responses of nutrient use efficiency to different competition types may be affected by other factors as well, such as plant height or the relative growth rate (Poorter et al. 1990; Garnier et al. 1995).

Chlorophyll concentrations and photosynthesis rates have been observed to decrease with N deficiency (Evans and Terashina 1987; Marschner 1995). In our study, N deficiency significantly suppressed the content of photosynthetic pigments in both species and $P_n$ in *P. deltoides* under both competition types, but no differences in photosynthetic rates were detected between intraspecific and interspecific competition. Aerts (1996) has reported that nutrient resorption efficiency does not respond to an increased nutrient supply, while other studies have indicated that N and P resorption efficiencies can be altered by nutrient supplies (Van Heerwaarden et al. 2003; Lu and

Han 2010; Wang et al. 2014). Our results on both *Populus* species were consistent with previous results indicating that N deficiency results in N limitation (N/P< 14) and significantly decreases the total N content of leaves, and N and P resorption efficiencies in both species (Zechmeister-Boltenstern et al. 2015). Therefore, nutrient resorption can be sensitive to the soil nitrogen content and competition, and possibly provides an important strategy for nutrient conservation, and affects growth and competition (Li et al. 2016).

RCI is one of the indicators used to measure the intensity of competition, and it can reflect the competitive effect of neighboring trees (Grace 1995). Chen et al. (2017) calculated RCI to analyze sex-specific competitiveness. Their results indicated that the sensitivity to neighboring plants is an important factor driving sex-specific growth patterns. Based on our results, N deficiency affected the competitiveness of *P. euramericana* in relation to *P. deltoides*, but no significant effects were visible on the competitiveness of *P. deltoides* in relation to *P. euramericana*. Thus, the difference between *P. deltoides* and *P. euramericana* in the sensitivity to heterospecific competitors under different N levels can explain the growth performance of these two species.

## Conclusions

In conclusion, this study revealed the effects of intraspecific and interspecific competition and N deficiency on the growth, morphological traits and physiological traits of *Populus deltoides* and *P. euramericana*. Under a normal N condition, the differences between intraspecific and interspecific conditions were stronger in *P. deltoides* than in *P. euramericana*. N deficiency affected these characteristics in *P. deltoides*, the effects resulting from N deficiency being stronger under intraspecific competition than under interspecific competition. In addition, the effects of heterospecific neighbors were more negative on *P. deltoides* than on *P. euramericana*. These findings indicate that *P. deltoides* is expected to gain an advantage in monocultures rather than in mixtures with *P. euramericana* under a normal N condition. Under N deficiency, the growth performance of *P. euramericana* was more stable than that of *P. deltoides* under both cultivation modes.

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**Author contributions** Yan Li had the main responsibility for data collection, analysis and writing, Jieyu Kang and Zhijun Li contributed to data analysis, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

**Conflict of interest** The authors declare that they have no conflict of interest.

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**Table 1.** Growth and morphological traits of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency.

| Nitrogen treatment | Competition pattern | Plant height (cm) | SW (mm) | CR (g) | FR (g) | FR/TR | R/S | TLA ($cm^2$) | SLA ($cm^{-2}$ $g^{-1}$) | SSL($cm$ $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *P. deltoides* | | | | | | | | | | |
| Normal N | D/DD | 109.03±4.05a | 8.24±0.48a | 1.82±0.21a | 1.81±0.74a | 0.46±0.11b | 0.17±0.01b | 1963.23±170.68a | 189.11±0.73a | 10.27±0.97b |
| | D/DE | 79.67±3.06b | 6.21±0.26b | 0.21±0.11b | 1.09±0.06a | 0.85±0.06a | 0.12±0.02b | 1298.84±19.98b | 204.38±27.69a | 17.89±1.02a |
| N deficiency | D/DD | 49.17±0.44c | 6.67±0.61b | 0.40±0.18b | 0.81±0.02a | 0.70±0.09ab | 0.21±0.03b | 721.47±7.97c | 213.17±5.57a | 21.05±1.55a |
| | D/DE | 52.00±1.53c | 6.08±0.15b | 0.69±0.06b | 1.28±0.20a | 0.65±0.02ab | 0.38±0.06a | 489.39±23.05d | 189.99±12.68a | 19.51±0.83a |
| | $P > F_C$ | ns | ns | ns | ns | ns | ns | *** | ns | ns |
| | $P > F_N$ | *** | ns | ns | ns | * | ns | *** | ns | ns |
| | $P > F_{C \times N}$ | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| *P. euramericana* | | | | | | | | | | |
| Normal N | E/EE | 84.83±0.88a | 6.84±0.19a | 0.38±0.16a | 1.72±0.24a | 0.83±0.03a | 0.20±0.05a | 896.49±38.76b | 184.56±6.17b | 14.88±0.74a |
| | E/DE | 91.33±4.33a | 7.01±0.29a | 0.12±0.04a | 0.99±0.09a | 0.89±0.04a | 0.09±0.00b | 1289.37±50.77a | 185.59±2.34b | 17.16±0.77a |
| N deficiency | E/EE | 66.33±1.36b | 7.04±0.40a | 0.59±0.15a | 1.13±0.29a | 0.64±0.12a | 0.22±0.03a | 834.86±43.31b | 212.17±5.27a | 17.38±2.07a |
| | E/DE | 62.33±1.33b | 6.69±0.47a | 0.58±0.22a | 0.95±0.26a | 0.62±0.12a | 0.20±0.03a | 803.41±43.05b | 191.71±1.93b | 18.10±0.67a |
| | $P > F_C$ | *** | * | ** | ns | ns | * | *** | ns | * |
| | $P > F_N$ | *** | ns | * | ns | ns | * | *** | ** | *** |
| | $P > F_{C \times N}$ | *** | ns | *** | ns | * | ns | * | * | ** |

$F_C$, competition effect; $F_N$, nitrogen effect; $F_{C \times N}$, the interaction of competition and nitrogen effect; D/DD, *P. deltoides* from intraspecific competition; E/EE, *P. euramericana* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/DE, *P. euramericana* from interspecific competition. Different letters above the bars indicate a significant difference among treatments within species. Values followed by the same letters in the same column are not significantly different at the $P < 0.05$ level according to Duncan's test. Each value represents the mean of five replicates ± standard error (the mean ± SE). The significance values of the factorial analysis (ANOVA) are listed. SW, stem base width; CR, weight of coarse roots; FR, weight of fine roots; FR/TR, the ratio of fine root weight to total root weight; R/S, root-shoot ratio; TLA, total leaf area; SLA, specific leaf area; SSL, specific stem length; . ns, non-significant difference; *$0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$.

**Table 2.** Photosynthetic characteristics and pigment contents of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency.

| Nitrogen treatment | Competition pattern | *P*n ($\mu$mol m$^{-2}$ s$^{-1}$) | $g_s$ (mol m$^{-2}$ s$^{-1}$) | *C*i ($\mu$mol mol$^{-1}$) | *E* (mmol mol$^{-1}$) | Chl a (mg dm$^{-2}$) | Chl b (mg dm$^{-2}$) | Tchl (mg dm$^{-2}$) | Caro (mg dm$^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|
| *P. deltoides* | | | | | | | | | |
| Normal N | D/DD | 20.15±0.65a | 1.27±0.22ab | 407.11±3.31a | 5.66±0.31a | 4.99±0.62a | 7.62±0.12a | 12.61±0.75a | 2.82±0.20a |
| | D/DE | 17.26±1.41a | 1.14±0.38ab | 401.67±15.00a | 5.75±0.45a | 1.48±0.10b | 7.38±0.27a | 8.85±0.36b | 1.61±0.02b |
| N deficiency | D/DD | 11.40±0.55b | 2.14±0.24a | 431.41±1.79a | 6.67±0.14a | 2.28±0.31b | 4.94±0.73b | 7.22±0.85b | 1.19±0.07c |
| | D/DE | 8.77±0.94b | 0.46±0.10c | 403.48±4.59a | 5.21±0.84a | 1.59±0.23b | 5.31±0.24b | 6.90±0.35b | 1.13±0.08c |
| | $P > F_C$ | * | * | ns | ns | *** | ns | * | ** |
| | $P > F_N$ | *** | ns | ns | ns | * | *** | *** | *** |
| | $P > F_{C\times N}$ | ns | * | ns | ns | * | ns | * | ** |
| *P. euramericana* | | | | | | | | | |
| Normal N | E/EE | 12.93±0.41b | 0.55±0.26a | 383.17±17.79b | 3.47±0.83b | 6.60±0.24a | 8.19±0.22a | 14.79±0.47a | 2.75±0.12a |
| | E/DE | 16.58±1.18a | 1.15±0.50a | 403.47±11.67ab | 5.01±1.03ab | 6.30±0.58a | 8.82±0.33a | 15.11±0.24a | 2.79±0.38a |
| N deficiency | E/EE | 11.90±1.00b | 1.88±0.28a | 429.79±1.26a | 6.74±0.38a | 1.25±0.17b | 5.93±0.28b | 7.18±0.25b | 1.34±0.09b |
| | E/DE | 13.13±1.22b | 2.95±1.73a | 425.72±5.60a | 7.25±0.45a | 2.58±0.53b | 5.25±0.11b | 7.84±0.62b | 1.42±0.12b |
| | $P > F_C$ | * | ns | ns | ns | ns | ns | ns | ns |
| | $P > F_N$ | ns | * | * | ** | *** | *** | *** | *** |
| | $P > F_{C\times N}$ | ns | ns | ns | ns | ns | * | ns | ns |

$F_C$, competition effect; $F_N$, nitrogen effect; $F_{C \times N}$, the interaction of competition and nitrogen effect; D/DD, *P. deltoides* from intraspecific competition; E/EE, *P. euramericana* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/DE, *P. euramericana* from interspecific competition. Different letters above the bars indicate a significant difference among treatments within species. Values followed by the same letters in the same column are not significantly different at the $P < 0.05$ level according to Duncan's test. Each value represents the mean of five replicates ± standard error (the mean ± SE). The significance values of the factorial analysis (ANOVA) are listed. $P_n$, net photosynthetic rate; $g_s$, stomatal conductance; $C_i$, intercellular $CO_2$ concentration; $E$, transpiration rate; Chla, chlorophyll a; Chlb, chlorophyll b; Tchl, total chlorophyll content; Caro, carotenoid contents; *$0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$.

**Table 3.** The nutrient element contents and carbon isotope composition of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency.

| Nitrogen treatment | Competition pattern | $\delta^{13}C$ (‰) | Total N (mg $g^{-1}$) | N/P | C/N |
|---|---|---|---|---|---|
| *P. deltoides* | | | | | |
| Normal N | D/DD | -29.41±0.04a | 23.45±0.35b | 12.84±0.25b | 17.49±0.32b |
| | D/DE | -31.33±0.02b | 24.52±0.09b | 15.41±0.45a | 15.96±0.07b |
| N deficiency | D/DD | -31.32±0.02b | 14.54±0.48a | 9.77±0.08c | 26.18±1.21a |
| | D/DE | -31.25±0.10b | 15.00±0.08a | 10.16±0.10c | 25.24±0.09a |
| | $P > F_C$ | *** | ns | *** | ns |
| | $P > F_N$ | *** | *** | *** | *** |
| | $P > F_{C\times N}$ | *** | * | ** | ns |
| *P. euramericana* | | | | | |
| Normal N | E/EE | -30.10±0.03a | 23.41±0.34 | 13.57±0.70a | 17.37±0.25b |
| | E/DE | -31.07±0.02b | 24.32±0.10 | 13.90±0.11a | 15.96±0.06c |
| N deficiency | E/EE | -31.14±0.01b | 14.43±0.13 | 9.73±0.63b | 26.79±0.15a |
| | E/DE | -31.46±0.02c | 14.33±0.12 | 9.50±0.05b | 26.64±0.20a |
| | $P > F_C$ | *** | ns | ns | ** |
| | $P > F_N$ | *** | *** | *** | *** |
| | $P > F_{C\times N}$ | *** | * | ns | ** |

$F_C$, competition effect; $F_N$, nitrogen effect; $F_{C\times N}$, the interaction of competition and nitrogen effect; D/DD, *P. deltoides* from intraspecific competition; E/EE, *P. euramericana* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/DE, *P. euramericana* from interspecific competition. Different letters above the bars indicate a significant difference among treatments within species. Values followed by the same letters in the same column are not significantly different at the $P < 0.05$ level according to Duncan's test. Each value represents the mean of five replicates ± standard error (the mean ± SE). The significance values of the factorial analysis (ANOVA) are listed. $\delta^{13}C$, carbon isotope composition; Total N, the total nitrogen content in the leaves; N/P, the ratio of nitrogen and phosphorus contents in the leaves; C/N, the ratio of carbon and nitrogen contents in leaves; ns, non-significant difference; $*0.01<P<0.05$; $** 0.001<P<0.01$; $*** P<0.001$.

## Figure legends

**Figure 1.** Biomass accumulation of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency. Values are given as mean ± SE (n=5). Different letters above the bars indicate a significant difference among treatments within species. D/DD, *P. deltoides* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/EE, *P. euramericana* from intraspecific competition; E/DE, *P. euramericana* from interspecific competition. C, competition effect; N, nitrogen effect; C×N, the interaction of competition and nitrogen effect; ND, N deficiency. ns, non-significant difference; *0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001.

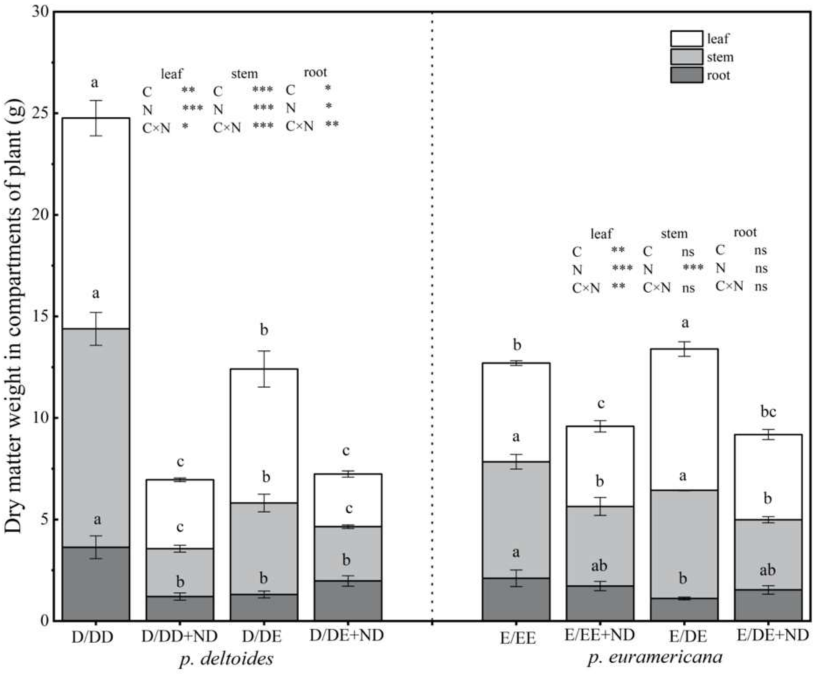
**Figure 2.** The total dry matter weight of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency. Values are given as mean ± SE (n=5). Different letters above the bars indicate a significant difference among treatments within species. D/DD, *P. deltoides* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/EE, *P. euramericana* from intraspecific competition; E/DE, *P. euramericana* from interspecific competition. C, competition effect; N, nitrogen effect; C×N, the interaction of competition and nitrogen effect; ND, N deficiency. ns, non-significant difference; *0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001.

**Figure 3.** Biomass partitioning of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency. Values are given as mean ± SE (n=5). Different letters above the bars indicate a significant difference among treatments within species. D/DD, *P. deltoides* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/EE, *P. euramericana* from intraspecific competition; E/DE,

*P. euramericana* from interspecific competition. ND, N deficiency; C, competition effect; N, nitrogen effect; C×N, the interaction of competition and nitrogen effect; ns, non-significant difference; *0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001.

**Figure 4.** Nutrient resorption efficiency of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency. Values are given as mean ± SE (n=5). Different letters above the bars indicate a significant difference among treatments within species. NRE, nitrogen resorption efficiency; PRE, phosphorus resorption efficiency; NRE/PRE, the ratio of NRE to PRE; D/DD, *P. deltoides* from intraspecific competition; D/DE, *P. deltoides* from interspecific competition; E/EE, *P. euramericana* from intraspecific competition; E/DE, *P. euramericana* from interspecific competition. ND, N deficiency. C, competition effect; N, nitrogen effect; C×N, the interaction of competition and nitrogen effect; ns, non-significant difference; *0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001.

**Figure 5.** Relative competitive intensity analysis of *P. deltoides* and *P. euramericana* exposed to competition and nitrogen deficiency. Values are given as mean ± SE (n=5). Different letters above the bars indicate a significant difference. RCI, relative competitive intensity. ND, nitrogen deficiency. ND, N deficiency.


Dry matter weight in compartments of plant (g)
30
25
20
15
10
5
0
leaf
stem
root
leaf
stem
root
C ** C *** C *
N *** N *** N *
C×N * C×N *** C×N **
leaf
stem
root
C ** C ns C ns
N *** N *** N ns
C×N ** C×N ns C×N ns
a
a
a
c
c
b
b
b
b
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b
ab
D/DD
D/DD+ND
D/DE
D/DE+ND
p. deltoides
E/EE
E/EE+ND
E/DE
E/DE+ND
p. euramericana

**Figure 1**

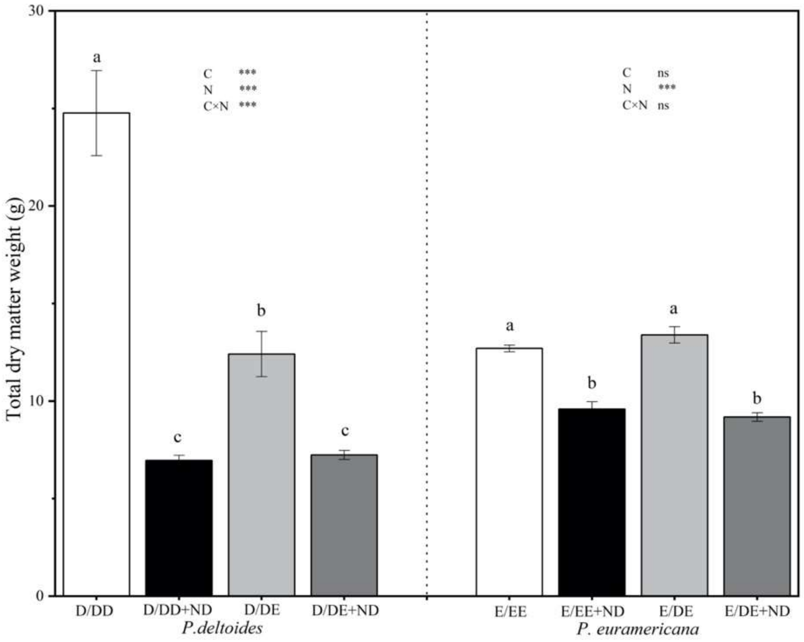
Total dry matter weight (g)
0
10
20
30
a
b
c
c
C ***
N ***
C×N ***
C ns
N ***
C×N ns
a
b
a
b
D/DD
D/DD+ND
D/DE
D/DE+ND
P.deltoides
E/EE
E/EE+ND
E/DE
E/DE+ND
P. euramericana

**Figure 2**

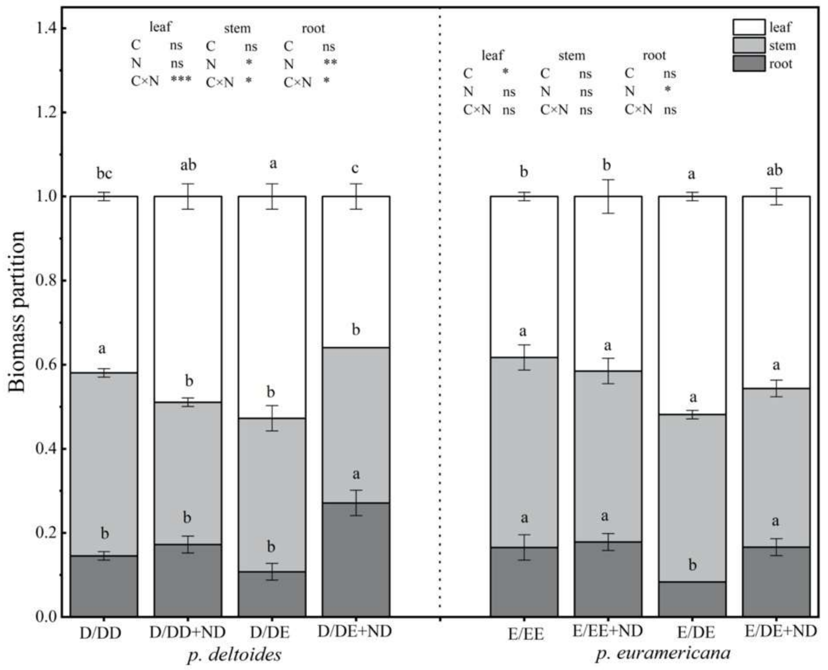
leaf
C ns
N ns
C×N ***
stem
C ns
N *
C×N *
root
C ns
N **
C×N *
leaf
C *
N ns
C×N ns
stem
C ns
N ns
C×N ns
root
C ns
N *
C×N ns
leaf
stem
root
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Biomass partition
bc
ab
a
c
b
b
a
ab
a
b
b
b
a
a
a
a
b
b
b
a
a
a
b
a
D/DD
D/DD+ND
D/DE
D/DE+ND
p. deltoides
E/EE
E/EE+ND
E/DE
E/DE+ND
p. euramericana

**Figure 3**

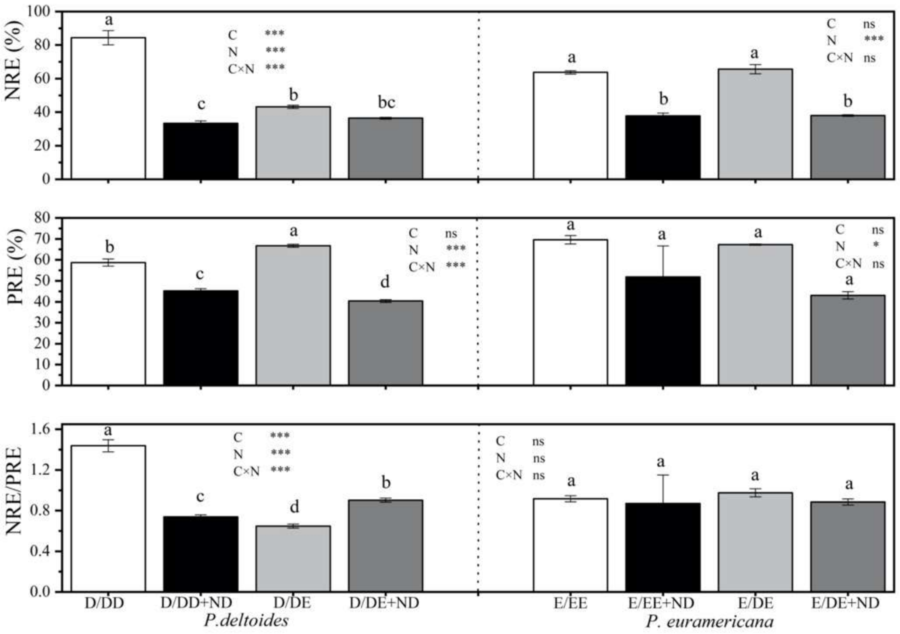
NRE (%)
0
20
40
60
80
100
a
c
b
bc
C ***
N ***
C×N ***
a
b
a
b
C ns
N ***
C×N ns
PRE (%)
0
10
20
30
40
50
60
70
80
b
c
a
d
C ns
N ***
C×N ***
a
a
a
C ns
N *
C×N ns
a
NRE/PRE
0.0
0.4
0.8
1.2
1.6
a
c
d
b
C ***
N ***
C×N ***
C ns
N ns
C×N ns
a
a
a
a
D/DD
D/DD+ND
D/DE
D/DE+ND
P.deltoides
E/EE
E/EE+ND
E/DE
E/DE+ND
P. euramericana

**Figure 4**

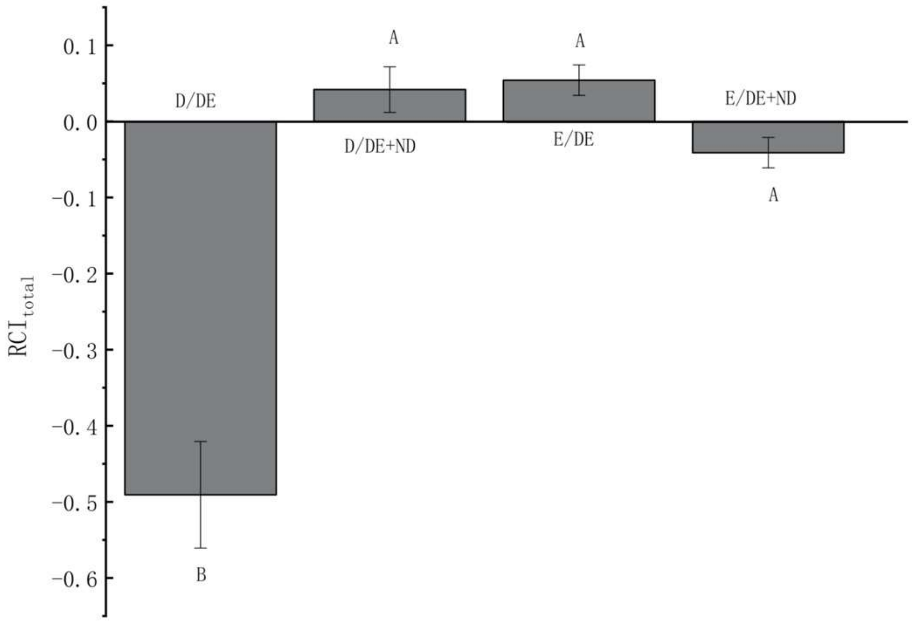

**Figure 5**