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Elevated CO_2 causes different growth stimulation, water and nitrogen use efficiencies and leaf ultrastructure responses in two conifer species under intra- and interspecific competition

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Head title: Elevated CO_2 affects plant-plant competition

Abstract The continuously increasing atmospheric carbon dioxide concentration ([CO_2]) has substantial effects on plant growth, and on the composition and structure of forests. However, how plants respond to elevated [CO_2] (e[CO_2]) under intra- and interspecific competition has been largely overlooked. In this study, we employed *Abies faxoniana* and *Picea purpurea* seedlings to explore the effects of e[CO_2] (700 ppm) and plant-plant competition on plant growth, physiological and morphological traits, and leaf ultrastructure. We found that e[CO_2] stimulated plant growth, photosynthesis and nonstructural carbohydrates (NSC), affected morphological traits and leaf ultrastructure, and enhanced water and nitrogen use efficiencies in *A. faxoniana* and *P. purpurea*. Under interspecific competition and e[CO_2], *P. purpurea* showed a higher biomass accumulation, photosynthetic capacity and rate of ectomycorrhizal infection, and higher water and nitrogen use efficiencies compared with *A. faxoniana*. However, under intraspecific competition and e[CO_2], the two conifers showed no differences in biomass accumulation, photosynthetic capacity, and water and nitrogen use efficiencies. In addition, under interspecific competition and e[CO_2], *A. faxoniana* exhibited higher NSC levels in leaves as well as more frequent and greater starch granules, which may indicate carbohydrate limitation. Consequently, we concluded that under interspecific competition, *P. purpurea* possesses a positive growth and adjustment strategy (e.g. a higher photosynthetic capacity and rate of ectomycorrhizal infection, and higher water and nitrogen use efficiencies), while *A. faxoniana* likely suffers from carbohydrate limitation to cope with rising [CO_2]. Our study highlights that plant-plant competition should be taken into consideration when assessing the impact of rising [CO_2] on the plant growth and physiological performance.

Keywords: elevated CO_2, plant-plant competition, water use efficiency, nitrogen use efficiency, conifer species

Introduction

The atmospheric CO_2 concentration ($[CO_2]$) has increased from ~280 ppm during the 19th century industrial revolution to current ~400 ppm (IPCC 2013), and it is predicted to exceed 1,000 ppm by the year 2100, if the current CO_2 emission trend continues (IPCC 2014). Due to the importance of CO_2 to plant photosynthesis, elevated $[CO_2]$ (e$[CO_2]$) would have profound effects on the physiology and growth of individual plants, and on the structure and function of terrestrial ecosystems. Most earlier studies have demonstrated that e$[CO_2]$ stimulates plants' photosynthesis, growth and biomass accumulation, e.g., investigations on *Telopea speciosissima* seedlings (Huang et al. 2015) and *Eucalyptus camaldulensis* cuttings (Blackman et al. 2016, Aspinwall et al. 2018) under controlled glasshouse conditions, and on mature eucalypt trees in the EucFACE experiment (Gimeno et al. 2016). On the other hand, Inauen et al. (2012) conducted free air CO_2 enrichment (FACE) for three seasons in the Swiss Alps and found that e$[CO_2]$ caused no growth stimulation and did not enhance the total biomass in high-elevation pioneer species, but decreased the aboveground biomass, which was associated with a higher root biomass allocation. In addition, van der Sleen et al. (2015) analyzed the stable carbon isotope composition and growth rings of 1,100 trees in tropical forests and found that no growth stimulation but increasing water use efficiencies have occurred in tropical trees during the past 150 years of rising $[CO_2]$.

Typically, e$[CO_2]$ changes biomass allocation and leads to a greater allocation to roots, which helps plants to cope with different environments (Inauen et al. 2012, Way et al. 2015, Apgaua et al. 2019). Concerning leaf gas exchange, e$[CO_2]$ generally increases photosynthesis, while it decreases stomatal conductance (g_s) and transpiration, as observed in *Eucalyptus* species (Gimeno et al. 2016, Aspinwall et al. 2018), *Populus cathayana* (Chen et al. 2021), and *Pinus halepensis* (Gattmann et al. 2021). In terms of leaf morphology, the enhanced leaf thickness and leaf mass per area (decreased specific leaf area, SLA) have been observed under e[CO_2] (Leakey et al. 2009, Duan et al. 2019, Wang et al. 2020). Furthermore, previous studies have demonstrated that e[CO_2] also affects plant root traits (Pritchard et al. 2008, Mueller et al. 2018, Wang et al. 2020). For instance, a meta-analysis of 110 case studies found that e[CO_2] increases both fine and coarse root biomass as well as root length and diameter (Nie et al. 2013).

The storage and remobilization of nonstructural carbohydrates (NSC) play key roles in plant survival and growth, and NSC buffer stress and disturbance in resource supplies, and act as indicators of the balance between source (photosynthesis) and sink (growth, respiration, defense etc.) (Niinemets 2010, Sala et al. 2012, Palacio et al. 2014, Wiley et al. 2017, 2019). Typically, e[CO_2] increases the accumulation of NSC, which may result in unbalanced C sinks and sources, and in the downregulation of photosynthesis (Ainsworth and Rogers 2007). For example, Zhao et al. (2012) found that *Populus cathayana* females show carbohydrate limitation in mature leaves under e[CO_2]. This may be explained by the declined activity of the primary carboxylating enzyme Rubisco, associated with the decreased leaf N concentration (Rogers and Ainsworth 2006). In addition, the accumulation of starch grains in leaf tissues can result in deformed chloroplasts (Nakano et al. 2000) and altered leaf ultrastucture (Zhao et al. 2012), which decrease the conductance of CO_2 diffusion from the intercellular space to Rubisco and induce the suppression of photosynthesis.

Competition is a biotic factor that may induce changes in the photosynthetic capacity, nonstructural carbohydrates (Chen et al. 2015, Li et al. 2016, Song et al. 2017), water use efficiency (Chen et al. 2014, Yu et al. 2017) and nitrogen absorption (Miller et al. 2007, Duan et al. 2014), which, in turn, optimize plants' performance to cope with varied environments. In natural ecosystems, plant-plant competition usually interacts with abiotic factors (e.g. resource availability and temperature), which can induce unique responses (Niinemets 2010, Guo et al. 2016, Yu et al. 2019). For example, e[CO_2] enhances carbohydrate contents of *Fagus sylvatica* under intraspecific competition, but the stimulation is suppressed when *F. sylvatica* saplings grow mixed with *Picea abies*. On the other hand, the stimulation of carbohydrates by e[CO_2] in *P. abies* saplings is similar under intra- and interspecific competition (Liu et al. 2004). These data revealed that different species have different responses to e[CO_2] (Norby et al. 1999), which may result in changes in plants' competitive relations, thus influencing the species composition and structure, and ecosystem productivity (Anderson-Teixeira et al. 2013).

Abies faxoniana and *Picea purpurea* are evergreen coniferous species naturally distributed in the northern part of the Sichuan province, China (Wang 2004, Taylor et al. 2006). These two conifers are dominant tree species of subalpine coniferous forests, and possess different life history traits. *P. purpurea* is characterized as having high longevity (> 500 years) and survivorship, while *A. faxoniana* has shorter longevity (usually less than 350 years) and higher shade tolerance (Wang 2004, Taylor et al. 2006). In several studies, they have been employed to investigate the effects of climate change, primarily rising [CO_2] and temperature (Ran et al. 2013, Li et al. 2015, Yu et al. 2019). An earlier study by Li et al. (2015) on *A. faxoniana* seedlings grown under e[CO_2] (700 μmol mol^{-1}) found that e[CO_2] increases the net photosynthetic rate, nonstructural carbohydrate content and photosynthetic N use efficiency. However, the present knowledge of the responses of *A. faxoniana* and *P. purpurea* to e[CO_2], and of the interactions of plant-plant competition and e[CO_2] are still limited. In addition, previous studies have observed that plants from high elevation sites show a greater sensitivity to rising [CO_2] (Körner and Diemer 1987, Körner 2003, Inauen et al. 2012).

In this study, we explored how e[CO_2] influences the physiological and functional characteristics of *A. faxoniana* and *P. purpurea* exposed to intra- and interspecific competition. The biomass accumulation and allocation, root morphological traits, photosynthetic capacity, leaf ultrastructure, water and nitrogen use efficiencies, and non-structural carbohydrate contents of these two conifers were determined from e[CO_2] and intra- and interspecific competition experiments. We hypothesized that (1) e[CO_2] causes changes in growth, water and nitrogen use efficiencies, and morphological and leaf ultrastructure traits, and those are affected by plant-plant competition; (2) e[CO_2] affects the competition relationship and life history strategies of *A. faxoniana* and *P. purpurea*.

Materials and methods

Plant material and experimental design

The study was conducted in an experimental area (2600 m above sea level) of the

Wanglang National Nature Reserve, Pingwu County, western Sichuan Province, Southwest China (32°49'-33°02'N, 103°55'-104°10'E, altitude range 2,300-4,980 m). The long-term climatic conditions and plant composition in the Reserve are described in our previous study (Yu et al. 2019). *A. faxoniana* and *P. purpurea* seedlings with a uniform size and height (~ 30 cm) were used for the study. At the end of September 2014, two conifer seedlings (10 cm apart from each other) were planted in each plastic pot (diameter 36 cm, height 40 cm) with homogenized soil (pH 6.03, C 58.19 g kg^{-1}, N 1.38 g kg^{-1}, P 0.60 g kg^{-1}) from their natural habitats. The study included fifteen replicates per treatment. The experimental design was completely randomized with three factors (species, competition and [CO_2]). There were two competition patterns, i.e. pure plantations (P) of *A. faxoniana* + *A. faxoniana* and *P. purpurea* + *P. purpurea* (intraspecific competition), and mixed plantations (M) of *A. faxoniana* + *P. purpurea* (interspecific competition). PC and MC were pure plantation and mixed plantation under e[CO_2]. CO_2 concentrations were elevated to 700 ppm (e[CO_2]) in growth chambers, as described in our previous study (Yu et al. 2019). Experimental treatments lasted from early May 2015 until the end of August 2016. All seedlings were hand-watered daily or every other day to ensure no limiting soil moisture.

Growth characteristic measurements

Five pots of each treatment were randomly chosen and harvested on 30 August 2016. All harvested seedlings were divided into leaves, stems and roots, then dried at 70 °C for 72 h to measure their biomass. Leaf mass fraction (LMF; g g^{-1}), stem mass fraction (SMF; g g^{-1}) and root mass fraction (RMF; g g^{-1}) were analyzed as the organ biomass/total plant biomass. The root/shoot ratio (R/S ratio) was analyzed as root biomass/the sum of leaf and stem biomass. The specific leaf area (SLA, cm^2 g^{-1}) was calculated as leaf area/leaf biomass. The WinRHIZO root-scanning software (Regent Instruments Inc., Ottawa, ON Canada) was used for root trait measurements. Specific root length (SRL) was analyzed as root length/root mass and specific root tip density (SRD) was analyzed as root tip/root length (Dong et al. 2016, Guo et al. 2016). In addition, the rate of ectomycorrhizal infection (ECM) was indicated as the total number of mycorrhizal tips per seedling (Yin et al. 2013).

Leaf gas exchange measurements and transmission electron microscopy

Healthy current-year leaves from five randomly chosen individuals in each treatment were selected for light-saturated net photosynthetic rate (P_n) and stomatal conductance (g_s) measurements by the LI-COR 6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) with a conifer type chamber (PLC-conifer, PP Systems), conducted between 08:00 a.m. and 11:30 a.m. in the middle of August 2016. The measurements were conducted in following conditions: leaf temperature, 25 °C; air vapor pressure deficit, 1.5 ± 0.5 kPa; relative humidity, 50%; light intensity (PPFD), 1500 μmol m^{-2} s^{-1}; and CO_2 concentration, 400 ± 5 μmol mol^{-1}. Intrinsic water use efficiency (iWUE) was calculated as the ratio of leaf photosynthesis to stomatal conductance. In addition, healthy and intact leaves were sampled for transmission electron microscope analyses by H-600IV TEM (Hitachi, Tokyo, Japan), conducted according to the methodology of Zhao et al. (2012). Briefly, small leaf sections (1-2 mm in length) were fixed with 3% glutaraldehyde (v/v) in 0.1 M phosphate buffer (pH 7.2) for 6-8 h under 4 °C, post-fixed in 1% osmium tetroxide for 1 h and immersed in 0.1 M phosphate buffer (pH 7.2) for 1-2 h. Then, the leaflets were dehydrated in ethanol and embedded in epon-araldite.

Determination of leaf N concentration and carbon isotopic composition

Fine power of leaf samples was used for leaf N measurements by the semi-micro Kjeldahl method (Mitchell 1998). In addition, the mass-based photosynthetic N use efficiency (PNUE) was calculated as the mass-based photosynthetic rate (P_n)/leaf N concentration. Leaf carbon isotopic composition was expressed as $\delta^{13}C$ values (relative to Pee Dee Belemnite), measured by a DELTA V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and given as: $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$, where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ that of the standard.

Non-structural carbohydrate measurements

Five randomly chosen plant samples (leaf, stem and root) from each treatment were selected and grounded into fine powder for soluble sugar and starch determination according to the anthrone-sulfuric acid method (Yemm and Willis 1954). About 50 mg fine powder was put into 10-ml centrifuge tubes, extracted in 4 ml 80% (v/v) ethanol at 80 °C for 30 min, and then centrifuged at 5000 g for 10 min, after which the supernatant was transferred to a 10-ml centrifuge tube. Then, 2 ml 80% (v/v) ethanol was added to the sediment, and centrifuged at 5000 g for 10 min. This procedure was repeated again, the supernatants were combined, and 80% (v/v) ethanol was added to bring the final volume to 10 ml. Soluble sugar and starch were detected colorimetrically at 625 nm and 620 nm, respectively.

Statistical analyses

Before analyses, data were checked for normality and homogeneity of variances and log-transformed to correct for deviations from these assumptions when needed. Tukey's tests of one-way ANOVAs were used to compare individual traits among treatments at a significance level of $P < 0.05$. Three-way ANOVAs were used to test the effects of species, competition, [CO_2] and their interactions. All analyses were carried out with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0. A principal component analysis (PCA) was used to test coordination among studied traits, using the R software and RStudio (R version 4.0.3 for Windows, packages readxl, ggplot2, ggpubr and vegan were used).

Results

Growth characteristics

Compared with control treatments, leaf, stem, root and total biomass of both conifers significantly increased by e[CO_2] under both competition patterns (Figure 1 a-d). Under e[CO_2] and intraspecific competition, the two conifers showed no differences in organ (leaf, stem and root) and total biomass (Figure 1 a-d). However, *P. purpurea* showed higher organ and total biomass compared to *A. faxoniana* under e[CO_2] and interspecific competition, suggesting a significant species × competition × [CO_2] interaction (Table 1). [CO_2] significantly affected leaf, stem and root biomass, and the R/S ratio (Table 1). The stem mass fraction (SMF) showed a decreasing tendency, whereas the root mass fraction (RMF) and R/S ratio significantly increased in both conifers under e[CO_2] (Figure 2). The e[CO_2] condition decreased SRL but increased AD in both conifers under both competition patterns (Figure 3a,b), showing a significant [CO_2] effect on these two parameters (Table 1). SRD showed no differences among different treatments (Figure 3c), but species, competition and [CO_2] had significant effects on ECM (Table 1). The e[CO_2] condition increased ECM in both conifers, and *P. purpurea* had higher ECM than *A. faxoniana* under interspecific competition and e[CO_2] (Figure 3d).

Gas exchange and leaf ultrastructure

The e[CO_2] condition significantly decreased g_s, the leaf N content and SLA but increased P_n in both conifers under both competition patterns (Figure 4a-d). Under intraspecific competition and e[CO_2], the two conifers showed no differences in P_n. However, when exposed to interspecific competition and e[CO_2], P_n of *P. purpurea* was significantly higher than that of *A. faxoniana*. In addition, species, competition and

[CO_2] had significant effects on these four parameters, and species × [CO_2] interaction significantly affected P_n and the leaf N concentration (Table 1). In addition, ultrastructural alterations were detected in leaves of both conifers. Under control treatments, the mesophyll cells of both conifers had smooth and continuous cell membranes and cell walls, and less starch accumulation and fewer granules (Figure 5a-d). However, under e[CO_2], both conifers exhibited more frequent and greater starch granules in chloroplasts (Figure 5e-h). Furthermore, under interspecific competition and e[CO_2], *A. faxoniana* showed profound modifications with greater starch granules and swollen chloroplasts, which implied serious damage on cellular ultrastructure (Figure 5g).

Resource use efficiency

The e[CO_2] condition significantly increased PNUE, iWUE and $\delta^{13}C$ in both conifers under both competition patterns (Figure 6a-c), suggesting that e[CO_2] increases water and nitrogen use efficiencies (Table 1). Under intraspecific competition and e[CO_2], the two conifers showed no differences in PNUE, iWUE and $\delta^{13}C$. However, *P. purpurea* showed significantly higher PNUE and iWUE than *A. faxoniana* under interspecific competition and e[CO_2]. Furthermore, species × [CO_2] and competition × [CO_2] interactions were significant for PNUE and iWUE. Overall, under e[CO_2], these two parameters increased more in *P. purpurea* exposed to interspecific competition (Table 1).

Nonstructural carbohydrates

In general, e[CO_2] increased soluble sugar, starch and NSC concentrations in both conifers under both competition patterns (Figure 7). Under interspecific competition and e[CO_2], *A. faxoniana* exhibited significantly higher soluble sugar, starch and NSC concentrations in leaves, while *P. purpurea* showed significantly higher soluble sugar, starch and NSC contents in stems and roots. In addition, species × competition × [CO_2] interactions were significant for soluble sugar, starch and NSC concentrations in all organs, except for leaf soluble sugar and stem starch concentrations (Table 2).

PCA

The PCA model of two components explained 69.38% of the total variance of studied traits in both conifers in response to plant-plant competition and e[CO_2] (Figure 8). Control and e[CO_2] treatments were separated along the first PCA axis. Under e[CO_2], *A. faxoniana* and *P. purpurea* exposed to interspecific competition were well separated from each other (Figure 8). PC1 was strongly influenced by leaf, stem, root and total biomass, stem soluble sugar, ECM, P_n, PNUE, iWUE, RMF and R/S ratio, which were negatively correlated with SMF, g_s and SRL. PC2 was strongly influenced by leaf soluble sugar, LMF, leaf, stem and root starch, SRD, and leaf and root NSC. Furthermore, PNUE, iWUE and ECM showed positive correlations with leaf, stem, root and total biomass, and P_n.

Discussion

e[CO_2] improved growth and performance but plant-plant competition effects were found

Our data revealed that competition and e[CO_2] significantly affect biomass accumulation and allocation (e.g. leaf, stem, root and total biomass, SMF, RMF and R/S ratio; Figures 1,2), as well as morphological characteristics (SRL, AD and SLA; Figures 3,4) of *A. faxoniana* and *P. purpurea*. These results were in line with previous studies (Huang et al. 2015, Duan et al. 2019, Dusenge et al. 2019, Wang et al. 2020). In addition, under a[CO_2] conditions (control treatments), the total biomass of either conifer showed no differences between pure and mixed plantations (P versus M) (Figure 1d). Under e[CO_2], *P. purpurea* showed higher organ (leaf, stem, root) and total biomass in a mixed plantation than in a pure plantation, but *A. faxoniana* showed no differences between the two plantation types (PC versus MC). These results implied that the growth of *P. purpurea* had an advantage compared with *A. faxoniana* under e[CO_2], which was further supported by the significant interactive effects of species × competition ×[CO_2] on organ and total biomass (Figure 1). Thus, under interspecific competition, the organ and total biomass of *P. purpurea* increased more under e[CO_2]. Furthermore, e[CO_2] significantly increased RMF and R/S ratio of both conifers, thus indicating that they show plasticity in their biomass allocation to cope with changing environments, which confirms previous studies (Wang et al. 2012, Apgaua et al. 2019, Fan et al. 2020). Plant roots are the most important organ for water and nutrient uptake, and plasticity allows plants to invest relatively more into roots to improve the capture of soil nutrients, and the transportation of water and carbohydrates (Portsmuth and Niinemets 2007).

Earlier studies have reported that the root diameter increases with rising [CO_2] (Nie et al. 2013, Wang et al. 2020), and our data confirmed these results, as e[CO_2] significantly increased AD of both conifers under both competition patterns (Figure 3b). In contrast to AD, e[CO_2] significantly decreased SRL of both conifers (Figure 3a), which implied a lower cost-benefit ratio as well as a superior carbon utilization strategy (Anderson et al. 2010, Pokorný et al. 2013). Furthermore, the increased root diameter under e[CO_2] is generally associated with an enhanced absorption surface area (Eissenstat 1992). Such increased absorption capacity could offset the negative effect resulting from decreased SRL under e[CO_2] to some extent. In addition to root morphological traits, rising [CO_2] usually enhances the level of mycorrhizal infection (Norby et al. 1987, Treseder 2004, Pandey et al. 2015). For instance, a free air CO_2 enrichment (FACE) conducted in a temperate forest revealed that e[CO_2] enhanced root colonization by 14% in ECM (Garcia et al. 2008). In accordance with earlier studies, our results showed that e[CO_2] increased the level of ECM in both conifers, and *P. purpurea* had higher ECM than *A. faxoniana* under interspecific competition and e[CO_2] (Figure 3d). It is well known that mycorrhiza can promote nutrient absorption and plant growth, and they play important roles in helping plants to cope with stressful environments (van der Heijden et al. 1998, Nadeem et al. 2014, Fernandez et al. 2017). Our results indicated that *P. purpurea* with higher ECM may have a greater capacity of nutrient absorption compared with *A. faxoniana* under e[CO_2].

In this study, e[CO_2] significantly increased P_n but decreased g_s in both conifers under both competition patterns (Figure 4a,b), which is in good agreement with earlier studies

(Franks et al. 2013, Duan et al. 2019, Dusenge et al. 2019). Rising [CO_2] generally results in enhanced carboxylation, and suppression of oxygenase reactions and photorespiration, thus increasing plants' photosynthesis and growth, particularly in C3 plants (Jordan and Ogren 1981, Feng et al. 2014, Pandey et al. 2015). In addition, the significant interaction of species × [CO_2] in P_n indicated that P_n of *P. purpurea* increased more compared with *A. faxoniana* under e[CO_2] (Table 1). In terms of leaf morphology, SLA as an important trait of plants' carbon economy and construction consumption, generally decreased under e[CO_2] (Figure 4d), which was in line with earlier studies (Li et al. 2013, Wang et al. 2020). Furthermore, Wright et al. (2004) have reported that plants with smaller SLA may produce leaves with higher construction costs under e[CO_2], which may imply longer leaf lifespan. In addition, according to the PCA analysis, there were positive associations among organ and total biomass, P_n, PNUE, IWUE and ECM (Figure 8). Moreover, under e[CO_2], *A. faxoniana* and *P. purpurea* exposed to interspecific competition were well separated from each other. These results collectively demonstrated that there were species-specific responses in growth and performance to e[CO_2], and those were affected by plant-plant competition.

e[CO_2] increased resource use efficiency but interspecific variation was detected

Photosynthetic nitrogen use efficiency (PNUE), defined as the ratio of net photosynthesis per unit nitrogen in a leaf, is closely related to the ecological and economic characteristics of leaves (Niinemets 1999, Takashima et al. 2004, Hidaka and

Kitayama 2009). It has been suggested that e[CO_2] increases PNUE in tree species (Leakey et al. 2009, Zhao et al. 2012, Xu et al. 2013). Our results were consistent with previous studies showing that e[CO_2] increases P_n and decreases leaf N concentrations, thus resulting in enhanced PUNE in both conifers (Figures 4a,b; 6a). The decreased leaf N concentration under e[CO_2] may be due to the "dilution" effect mediated by accumulated carbohydrates (Tissue et al. 1999, Tausz-Posch et al. 2014). Under interspecific competition and e[CO_2], *P. purpurea* had significantly higher PNUE than *A. faxoniana* (Figure 6a). This was further supported by the significant interactive effects of species × competition, species × [CO_2] and competition × [CO_2] on PNUE, which collectively indicated that PNUE of *P. purpurea* increased more compared with *A. faxoniana* under e[CO_2] and interspecific competition (Table 1).

Plants' water use strategies play key roles in survival, growth, reproduction and successful establishment, and climate change (e.g. rising [CO_2]) strongly affects plants' water use traits (Sandquist et al. 2003, Soh et al. 2019, Driscoll et al. 2020). For example, previous studies have reported that e[CO_2] results in the closure of stomata (decreased g_s) and increased water use efficiency (WUE) (Zhao et al. 2012, Silva et al. 2013, Van der Sleen et al. 2015, Gimeno et al. 2016, Soh et al. 2019). Our results were consistent with above statements that e[CO_2] increases P_n, along with the closure of stomata (decreased g_s), thus increasing the intrinsic water use efficiency (iWUE) (Figures 4a,b; 6b). Furthermore, the long-term water use efficiency, as revealed by the stable carbon isotope composition ($\delta^{13}C$, discussed by Farquhar et al. 1989 and Li 1999), also increased under e[CO_2] (Figure 6c). The reason may be that rising [CO_2] induced the closure of stomata (decreased g_s) and enhanced the fixation of ^{13}C, consequently resulting in increased $\delta^{13}C$. In addition, the significant interactive effects of species × [CO_2] and competition × [CO_2] on iWUE collectively indicated that iWUE of *P. purpurea* increased more compared with *A. faxoniana* under e[CO_2] and interspecific competition (Table 1). Driscoll et al. (2020) have demonstrated that enhanced iWUE in response to e[CO_2] potentially alleviates drought stress, as plants could maintain similar carbon assimilation rates with a decreased water demand. In the present study, under e[CO_2], increased P_n and decreased g_s as well as the decreased transpiration rate (data not shown) resulted in an enhanced water use efficiency, implying that rising [CO_2] may improve both conifers' tolerance under increased aridity (review by Sekhar et al. 2020).

Carbon source-sink relationships altered by e[CO_2] and plant-plant competition

A review written by Ainsworth and Rogers (2007) has demonstrated that different plants have different capacities to deal with excess carbohydrates. For example, poplar has a large sink capacity when grown under e[CO_2], and it could transport most photosynthates (>90%) during the day (Stitt and Quick 1989, Davey et al. 2006). On the other hand, e[CO_2] increases the accumulations of NSC, resulting in carbohydrate limitation associated with declined photosynthesis, which may be induced by the decreased activity of the primary carboxylating enzyme Rubisco (Ainsworth and

Rogers 2007). In addition, the N concentration of leaves often decreases under carbohydrate limitation, because N is closely related to Rubisco and transferred to other parts of the plant (Herms and Mattson 1992). Under interspecific competition and e[CO_2], *A. faxoniana* exhibited significantly higher soluble sugar, starch and NSC concentrations in leaves associated with lower photosynthesis compared with *P. purpurea*, which implied that *A. faxoniana* may suffer from carbohydrate limitation that leads to decreased potential for C acquisition. In addition, this result was supported by the more frequent and greater starch granules of *A. faxoniana* observed under interspecific competition and e[CO_2] (Figure 5g). Furthermore, under interspecific competition and e[CO_2], the greater starch granules and swollen chloroplasts indicated that leaf ultrastructure was seriously damaged in *A. faxoniana*, which may cause inhibited photosynthesis (Zhao et al. 2012). On the other hand, *P. purpurea* accumulated less carbohydrates in leaves but more in stems and roots under interspecific competition and e[CO_2]. Thus, plant-plant competition potentially altered carbon source-sink relationships in *A. faxoniana* and *P. purpurea* under rising [CO_2] connected with climate change.

Conclusions

Our study showed that e[CO_2] stimulates plant growth and performance, affects morphological traits and leaf ultrastructure, and increases water and nitrogen use efficiencies in *A. faxoniana* and *P. purpurea*. However, we also found interspecific variation in both conifers' responses to plant-plant competition and e[CO_2]. *P. purpurea,* in general, possesses a positive growth and adjustment strategy (e.g. a higher photosynthetic capacity and rate of ectomycorrhizal infection, higher water and nitrogen use efficiencies), while *A. faxoniana* likely suffers carbohydrate limitation under interspecific competition and e[CO_2]. Our results demonstrated that different species possess different growth and adjustment strategies to cope with rising [CO_2], which potentially changes competitive relations, thus influencing species composition and ecosystem productivity in subalpine coniferous forests.

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Author contributions Lei Yu had the main responsibility for data collection, analysis and writing, Haojie Dong and Zongdi Huang had a significant contribution to data analysis, Helena Korpelainen had a significant contribution 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.

References

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258-270.

Anderson LJ, Derner JD, Polley HW, Gordon WS, Eissenstat DM, Jackson RB (2010) Root responses along a subambient to elevated CO_2 gradient in a C_3-C_4 grassland. Global Change Biol 16:454-468.

Anderson-Teixeira KJ, Miller AD, Mohan JE, Hudiburg TW, Duval BD, DeLucia EH (2013) Altered dynamics of forest recovery under a changing climate. Global Change Biol 19:2001-2021.

Apgaua Deborah MG, Tng DYP, Forbes SJ, Ishida YF, Vogado NO, Cernusak LA, Laurance SGW (2019) Elevated temperature and CO_2 cause differential growth stimulation and drought survival responses in eucalypt species from contrasting habitats. Tree Physiol 39:1806-1820.

Aspinwall MJ, Blackman CJ, de Dios VR, Busch FA, Rymer PD, Loik ME et al. (2018) Photosynthesis and carbon allocation are both important predictors of genotype productivity responses to elevated CO_2 in *Eucalyptus camaldulensis*. Tree Physiol 38:1286-1301.

Blackman CJ, Aspinwall MJ, Resco de Dios V, Smith RA, Tissue DT (2016) Leaf photosynthetic, economics and hydraulic traits are decoupled among genotypes of a widespread species of eucalypt grown under ambient and elevated CO_2. Funct Ecol 30:1491-1500.

Chen J, Dong TF, Duan BL, Korpelainen H, Niinemets Ü, Li CY (2015) Sexual competition and N supply interactively affect the dimorphism and competiveness of opposite sexes in *Populus cathayana*. Plant Cell Environ 38:1285-1298.

Chen J, Duan BL, Wang ML, Korpelainen H, Li CY (2014) Intra- and inter-sexual competition of *Populus cathayana* under different watering regimes. Funct Ecol 28:124-136.

Chen J, Liu Q, Yu L, Korpelainen H, Niinemets Ü, Li CY (2021) Elevated temperature and CO_2 interactively modulate sexual competition and ecophysiological responses of dioecious *Populus cathayana*. For Ecol Manage 481:118747.

Davey PA, Olcer H, Zakhleniuk O, Bernacchi CJ, Calfapietra C, Long SP, Raines CA (2006) Can fast-growing plantation trees escape biochemical down-regulation of photosynthesis when grown throughout their complete production cycle in the open air under elevated carbon dioxide? Plant Cell Environ 29:1235-1244.

Dong TF, Duan BL, Zhang S, Korpelainen H, Niinemets Ü, Li CY (2016) Growth, biomass allocation and photosynthetic responses are related to intensity of root severance and soil moisture conditions in the plantation tree *Cunninghamia lanceolata*. Tree Physiol 36:807-817.

Driscoll AW, Bitter NQ, Sandquist DR, Ehleringer JR (2020) Multidecadal records of intrinsic water-use efficiency in the desert shrub *Encelia farinosa* reveal strong responses to climate change. Proc Natl Acad Sci USA 117:18161-18168.

Duan BL, Dong TF, Zhang XL, Zhang YB, Chen J (2014) Ecophysiological responses of two dominant subalpine tree species *Betula albo-sinensis* and *Abies faxoniana* to intra- and interspecific competition under elevated temperature. For Ecol Manage 323:20-27.

Duan HL, Ontedhu J, Milham P, Lewis JD, Tissue DT (2019) Effects of elevated carbon dioxide and elevated temperature on morphological, physiological and anatomical responses of *Eucalyptus tereticornis* along a soil phosphorus gradient. Tree Physiol 39:1821-1837.

Dusenge ME, Duarte AG, Way DA (2019) Plant carbon metabolism and climate change:

elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. New Phytol 221:32-49.

Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. J Plant Nutr 15:763-782.

Fan XD, Cao X, Zhou HR, Hao LH, Dong W, He CL, Xu M, Wu HX, Wang LS, Chang ZJ, Zheng YP (2020) Carbon dioxide fertilization effect on plant growth under soil water stress associates with changes in stomatal traits, leaf photosynthesis, and foliar nitrogen of bell pepper (*Capsicum annuum* L.). Environ Exp Bot 179:104203.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Biol 40:503-537.

Feng GQ, Li Y, Cheng ZM (2014) Plant molecular and genomic responses to stresses in projected future CO_2 environment. Crit Rev Plant Sci 33:238-249.

Fernandez CW, Nguyen NH, Stefanski A et al. (2017) Ectomycorrhizal fungal response to warming is linked to poor host performance at the boreal-temperate ecotone. Global Change Biol 23:1598-1609.

Franks PJ, Adams MA, Amthor JS et al. (2013) Sensitivity of plants to changing atmospheric CO_2 concentration: from the geological past to the next century. New Phytol 197:1077-1094.

Garcia MLG, Di Massimo G, Manjon JL, Garcia-Canete J (2008) Effect of *Sphaerosporella brunnea* mycorrhizas on mycorrhization of *Quercus ilex* × *Tuber melanosporum*. N Z J Crop Hortic Sci 36:153-158.

Gattmann M, Birami B, Nadal Sala D, Ruehr NK (2021) Dying by drying: Timing of physiological stress thresholds related to tree death is not significantly altered by highly elevated CO_2. Plant Cell Environ 44:356-370.

Gimeno TE, Crous KY, Cooke J et al. (2016) Conserved stomatal behaviour under elevated CO_2 and varying water availability in a mature woodland. Funct Ecol 30:700-709.

Guo QX, Li JY, Zhang YX, Zhang JX, Lu DL, Korpelainen H, Li CY (2016) Species-specific competition and N fertilization regulate non-structural carbohydrate contents in two *Larix* species. For Ecol Manage 364:60-69.

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283-335.

Hidaka A, Kitayama K (2009) Divergent patterns of photosynthetic phosphorus-use efficiency versus nitrogen-use efficiency of tree leaves along nutrient-availability gradients. J Ecol 97:984-991.

Huang GM, Rymer PD, Duan HL, Smith RA, Tissue DT (2015) Elevated temperature is more effective than elevated [CO_2] in exposing genotypic variation in *Telopea speciosissima* growth plasticity: implications for woody plant populations under climate change. Global Change Biol 21:3800-3813.

Inauen N, Körner C, Hiltbrunner E (2012) No growth stimulation by CO_2 enrichment in alpine glacier forefield plants. Global Change Biol 18:985-999.

Jordan DB, Ogren WL (1981) Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature 291:513-515.

Körner C (2003) Carbon limitation in trees. J Ecol 91:4-17.

Körner C, Diemer M (1987) In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. Funct Ecol 1:179-194.

Leakey AD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot 60:2859-2876.

Li C (1999) Carbon isotope composition, water-use efficiency and biomass productivity of *Eucalyptus microtheca* populations under different water supplies. Plant Soil 214:165-171.

Li Y, Duan BL, Chen J, Korpelainen H, Niinemets Ü, Li CY (2016) Males exhibit competitive advantages over females of *Populus deltoides* under salinity stress. Tree Physiol 36:1573-1584.

Li YP, Dong TF, Duan BL, Zhang YB (2015) Physiological responses of *Abies faxoniana* populations from different elevations to increased CO_2 and N application. Acta Physiol Plant 37:1724.

Li YP, Zhang YB, Zhang XL, Korpelainen H, Berninger F, Li CY (2013) Effects of elevated CO_2 and temperature on photosynthesis and leaf traits of an understory dwarf bamboo in subalpine forest zone, China. Physiol Plant 148:261-272.

Liu XP, Kozovits AR, Grams TEE, Blaschke H, Rennenberg H, Matyssek R (2004) Competition modifies effects of enhanced ozone/carbon dioxide concentrations on carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. Tree Physiol 24:1045-1055.

Miller AE, Bowman WD, Suding KN (2007) Plant uptake of inorganic and organic nitrogen: Neighbor identity matters. Ecology 88:1832-1840.

Mitchell AK (1998) Acclimation of Pacific yew (*Taxus brevifolia*) foliage to sun and shade. Tree Physiol 18:749-757.

Mueller KE, LeCain DR, McCormack ML, Pendall E, Carlson M, Blumenthal DM (2018) Root responses to elevated CO_2, warming and irrigation in a semi-arid grassland: integrating biomass, length and life span in a 5-year field experiment. J Ecol 106:2176-2189.

Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stress-full environments. Biotechnol Adv 32:429-448.

Nakano H, Muramatsu S, Makino A, Mae T (2000) Relationship between the suppression of photosynthesis and starch accumulation in the pod-removed bean. Aust J Plant Physiol 27:167-173.

Nie M, Lu M, Bell J, Raut S, Pendall E (2013) Altered root traits due to elevated CO_2: a meta-analysis. Global Ecol Biogeogr 22:1095-1105.

Niinemets Ü (1999) Components of leaf dry mass per area thickness and density alter leaf photosynthetic capacity in reverse directions in woody plants. New Phytol 144:35-47.

Niinemets Ü (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. For Ecol Manage 260:1623-1639.

Norby RJ, O'Neill EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiol 3:203-210.

Norby RJ, Wullschleger SD, Gundeson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683-714.

Palacio S, Hoch G, Sala A, Körner C, Millard P (2014) Does carbon storage limit tree growth? New Phytol 201:1096-1100.

Pandey R, Zinta G, AbdElgawad H, Ahmad A, Jain V, Janssens IA (2015) Physiological and molecular alterations in plants exposed to high [CO_2] under phosphorus stress. Biotechnol Adv 33:303-316.

Pokorný R, Tomášková I, Marek MV (2013) Response of Norway spruce root system to elevated atmospheric CO_2 concentration. Acta Physiol Plant 35:1807-1816.

Portsmuth A, Niinemets Ü (2007) Structural and physiological plasticity to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. Funct Ecol 21:61-77.

Pritchard SG, Strand AE, McCormack ML, Davis MA, Finzi AC, Jackson RB et al. (2008) Fine root dynamics in a loblolly pine forest are influenced by free-air-CO_2-enrichment: a six-year minirhizotron study. Global Change Biol 14:588-602.

Ran F, Zhang XL, Zhang YB, Korpelainen H, Li CY (2013) Altitudinal variation in growth, photosynthetic capacity and water use efficiency of *Abies faxoniana* Rehd. et Wils. seedlings as revealed by reciprocal transplantations. Trees 27:1405-1416.

Rogers A, Ainsworth EA (2006) The response of foliar carbohydrates to elevated [CO_2]. In: Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (eds) Managed ecosystems and CO_2: case studies, processes and perspectives. Springer, Berlin, pp 293-330.

Sala A, Woodruff DR, Meinzer FC (2012) Carbon dynamics in trees: feast or famine? Tree Physiol 32:764-775.

Sandquist DR, Ehleringer JR (2003) Carbon isotope discrimination differences within and between contrasting populations of *Encelia farinosa* raised under common environment conditions. Oecologia 134:463-470.

Sekhar KM, Kota VR, Reddy TP, Rao KV, Reddy AR (2020) Amelioration of plant responses to drought under elevated CO_2 by rejuvenating photosynthesis and nitrogen use efficiency: implications for future climate-resilient crops. Photosynth Res. https://doi.org/10.1007/s11120-020-00772-5.

Silva LCR, Anand M (2013) Probing for the influence of atmospheric CO_2 and climate change on forest ecosystems across biomes. Global Ecol Biogeogr 22:83-92.

Soh WK, Yiotis C, Murray M, Parnell A, Wright IJ, Spicer RA, Lawson T, Caballero R, McElwain JC (2019) Rising CO_2 drives divergence in water use efficiency of evergreen and deciduous plants. Sci Adv 5:eaax7906.

Song MY, Yu L, Jiang YL, Lei YB, Korpelainen H, Niinemets Ü, Li CY (2017) Nitrogen-controlled intra- and interspecific competition between *Populus purdomii* and *Salix rehderiana* drive primary succession in the Gongga Mountain glacier retreat area. Tree Physiol 37:799-814.

Stitt M, Quick P (1989) Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. Physiol Plant 77:663-641.

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant Cell Environ 27:1047-1054.

Tausz-Posch S, Armstrong R, Tausz M (2014) Nutrient use and nutrient use efficiency of crops in a high CO_2 atmosphere. In: Hawkesford MJ, De Kok LJ, Kopriva S (Eds.), Nutrient Use Efficiency in Plants. Series; Plant Ecophysiology. Springer International Publishing, Basel, Switzerland, pp 229-252.

Taylor AH, Jang SW, Zhao LJ, Liang CP, Miao CJ, Huang J (2006) Regeneration patterns and tree species coexistence in old-growth *Abies-Picea* forests in southwestern China. For Ecol Manage 223:303-317.

Tissue DT, Griffin KL, Ball JT (1999) Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO_2. Tree Physiol 19:221-228.

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347-355.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T et al. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

van der Sleen P, Groenendijk P, Vlam M, Anten NP, Boom A, Bongers F, Pons TL, Terburg G, Zuidema PA (2015) No growth stimulation of tropical trees by 150 years of CO_2 fertilization but water-use efficiency increased. Nat Geosci 8:24-28.

Wang D, Heckathorn SA, Wang XZ, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia 169:1-13.

Wang KY (2004) Processes of Subalpine Forest Ecosystem in the West of Sichuan. Sichuan University Press, Chengdu, China.

Wang N, Gao GQ, Wang Y, Wang DN, Wang ZQ, Gu JC (2020) Coordinated responses of leaf and absorptive root traits under elevated CO_2 concentration in temperate woody and herbaceous species. Environ Exp Bot 179:104199.

Way DA, Oren R, Kroner Y (2015) The space-time continuum: the effects of elevated CO_2 and temperature on trees and the importance of scaling. Plant Cell Environ 38:991-1007.

Wiley E, Hoch G, Landhäusser SM (2017) Dying piece by piece: carbohydrate dynamics in aspen (*Populus tremuloides*) seedlings under severe carbon stress. J Exp Bot 68:5221-5232.

Wiley E, King CM, Landhäusser SM (2019) Identifying the relevant carbohydrate storage pools available for remobilization in aspen roots. Tree Physiol 39:1109-1120.

Wright IJ, Reich PB, Westoby M et al. (2004) The worldwide leaf economics spectrum. Nature 428:821-827.

Xu ZZ, Shimizu H, Yagasaki Y, Ito S, Zheng YR, Zhou GS (2013) Interactive effects of elevated CO_2, drought, and warming on plants. J Plant Growth Regul 32:692-707.

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508-514.

Yin HJ, Xiao J, Li YF, Chen Z, Cheng XY, Zhao CZ, Liu Q (2013) Warming effects on root morphological and physiological traits: The potential consequences on soil C dynamics as altered root exudation. Agri Forest Meteorol 180:287-296.

Yu L, Song MY, Lei YB, Duan BL, Berninger F, Korpelainen H, Niinemets Ü, Li CY (2017) Effects of phosphorus availability on later stages of primary succession in Gongga Mountain glacier retreat area. Environ Exp Bot 141:103-112.

Yu L, Song MY, Xia ZC, Korpelainen H, Niinemets Ü, Li CY (2019) Elevated temperature differently affects growth, photosynthetic capacity, nutrient absorption and leaf ultrastructure of *Abies faxoniana* and *Picea purpurea* under intra- and interspecific competition. Tree Physiol 39:1342-1357.

Zhao HX, Li YP, Zhang XL, Korpelainen H, Li CY (2012) Sex-related and stage-dependent source-to-sink transition in *Populus cathayana* grown at elevated CO_2 and elevated temperature. Tree Physiol 32:1325-1338.

Table 1. Main and interactive effects of species, competition and [CO$_2$] on growth and photosynthetic parameters of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO$_2$] levels.

	Main effects			Interactions			
Parameters	*Species(S)*	*Competition(C)*	*[CO$_2$]*	*S×C*	*S×[CO$_2$]*	*C×[CO$_2$]*	*S×C×[CO$_2$]*
Leaf biomass	**0.000**	**0.000**	**0.000**	**0.003**	0.504	0.102	**0.004**
Stem biomass	**0.000**	**0.000**	**0.000**	**0.000**	0.301	**0.000**	**0.000**
Root biomass	**0.000**	**0.000**	**0.000**	**0.000**	0.739	**0.000**	**0.000**
Total biomass	**0.000**	**0.000**	**0.000**	**0.000**	0.915	**0.000**	**0.000**
LMF	0.218	0.302	**0.009**	0.905	0.285	0.068	0.715
SMF	0.045	0.957	**0.000**	0.984	**0.036**	**0.049**	0.171
RMF	0.353	0.071	**0.000**	0.873	0.182	0.848	0.165
R/S ratio	0.382	0.072	**0.000**	0.926	0.189	0.921	0.162
SRL	0.887	0.410	**0.000**	0.659	0.958	0.821	0.135
AD	0.868	**0.010**	**0.000**	0.788	0.863	**0.015**	0.485
SRD	0.736	0.381	0.780	0.591	0.990	0.395	0.701
ECM	**0.000**	**0.039**	**0.000**	0.846	0.560	0.179	0.087
P_n	**0.000**	0.299	**0.000**	0.068	**0.027**	**0.001**	0.314
g_s	0.290	0.554	**0.000**	0.409	0.873	0.873	0.478
Leaf N content	**0.000**	**0.006**	**0.000**	0.520	**0.028**	0.460	0.968
SLA	0.103	0.090	**0.007**	0.751	0.963	0.729	**0.039**
PNUE	0.812	0.058	**0.000**	**0.003**	**0.000**	**0.000**	0.374
iWUE	**0.001**	**0.029**	**0.000**	0.260	**0.034**	**0.009**	0.141
$\delta^{13}C$	**0.003**	0.190	**0.000**	0.104	0.987	**0.000**	0.560

LMF, leaf mass fraction; SMF, stem mass fraction; RMF, root mass fraction; R/S ratio, root/shoot ratio; SRL, specific root length; AD, average root diameter; SRD, specific root tip density; ECM, the rate of ectomycorrhizal infection; P_n, net photosynthetic rate; g_s, stomatal conductance; SLA, specific leaf area; PNUE, photosynthetic N use efficiency. Significant values ($P < 0.05$) are shown in bold.

Table 2. Main and interactive effects of species, competition and [CO_2] on carbohydrate parameters of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels.

	Main effects			Interactions			
Parameters	*Species(S)*	*Competition(C)*	*[CO₂]*	*S×C*	*S×[CO₂]*	*C×[CO₂]*	*S×C×[CO₂]*
Leaf soluble sugar	**0.000**	0.378	**0.000**	**0.000**	**0.000**	**0.000**	0.503
Stem soluble sugar	**0.000**	**0.000**	**0.000**	0.058	**0.000**	**0.000**	**0.002**
Root soluble sugar	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.034**	**0.000**
Leaf starch	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**
Stem starch	**0.000**	**0.000**	**0.000**	0.750	**0.014**	0.068	0.134
Root starch	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.301	**0.000**
Leaf NSC	0.121	**0.000**	**0.000**	**0.000**	**0.000**	**0.019**	**0.000**
Stem NSC	**0.000**	**0.000**	**0.000**	0.522	**0.000**	0.660	**0.002**
Root NSC	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.333	**0.000**

Significant values ($P < 0.05$) are shown in bold.

Figure legends

Figure 1. (a) Leaf biomass, (b) stem biomass, (c) root biomass and (d) total biomass of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. Each value is the mean ± SE (*n* = 5). Different letters indicate significant differences among treatments according to Tukey's tests (*P* < 0.05). P, intraspecific competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 2. Biomass allocation of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. (a) Leaf biomass fraction (LMF), (b) stem biomass fraction (SMF), (c) root biomass fraction (RMF) and (d) root to shoot ratio (R/S ratio). Each value is the mean ± SE (*n* = 5). Different letters indicate significant differences among treatments according to Tukey's tests (*P* < 0.05). P, intraspecific competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 3. Root traits of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. (a) Specific root length (SRL), (b) average root diameter (AD), (c) specific root tip density (SRD) and (d) the rate of ectomycorrhizal infection (ECM). Each value is the mean ± SE (*n* = 5). Different letters indicate significant differences among treatments according to Tukey's tests (*P* < 0.05). P, intraspecific

competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 4. (a) Net photosynthetic rate (P_n), (b) stomatal conductance (g_s), (c) leaf N concentration and (d) specific leaf area (SLA) of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. Each value is the mean ± SE ($n = 5$). Different letters indicate significant differences among treatments according to Tukey's tests ($P < 0.05$). P, intraspecific competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 5. Mesophyll cell observations of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels by transmission electron microscopy (TEM). (a-d) control treatments: (a) A/AA, (b) P/PP, (c) A/AP, (d) P/AP. (e-h) e[CO_2] treatments: (e) A/AA, (f) P/PP, (g) A/AP, (h) P/AP. The scale bar indicates 2 μm (a). C, chloroplast; CW, cell wall; S, starch granule. A/AA, *A. faxoniana* individuals from intraspecific competition; A/AP, *A. faxoniana* individuals from interspecific competition; P/AP, *P. purpurea* individuals from interspecific competition; P/PP, *P. purpurea* individuals from intraspecific competition.

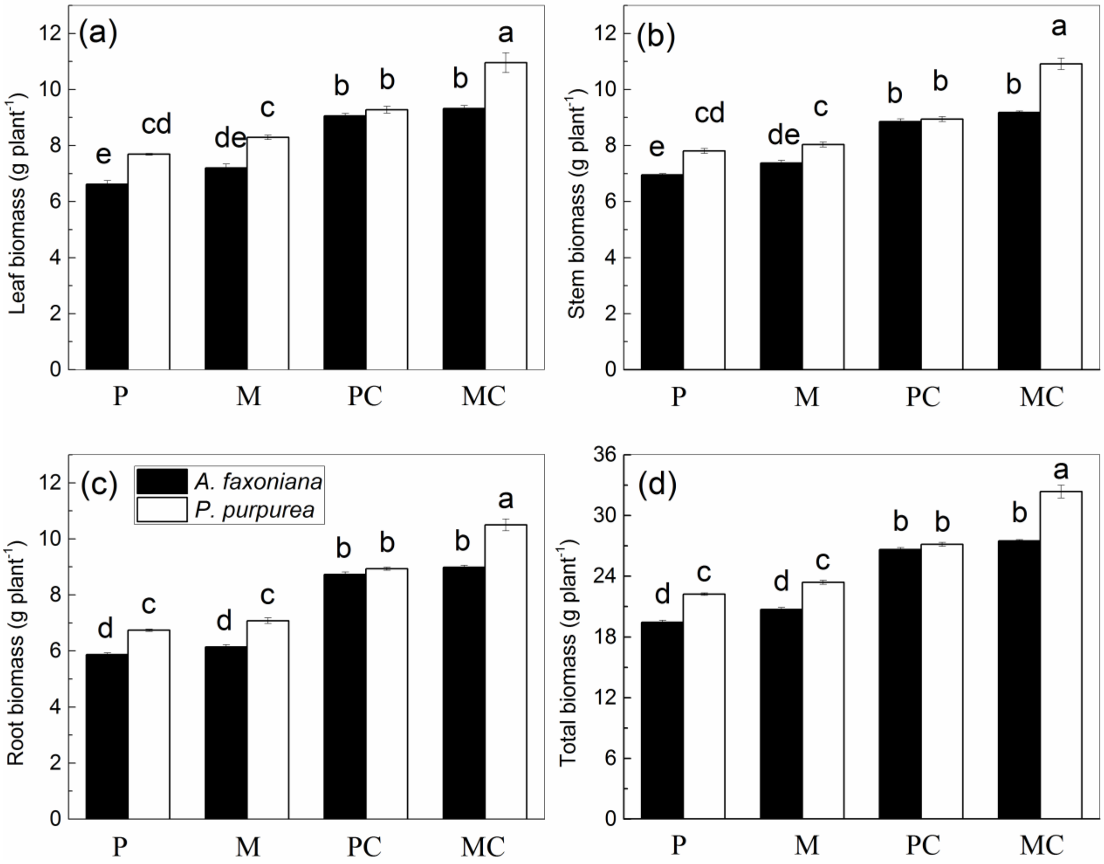
Figure 6. (a) Photosynthetic nitrogen use efficiency (PNUE), (b) Intrinsic water use efficiency (iWUE) and (c) carbon isotope composition ($\delta^{13}C$) of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. Each value is the mean

± SE (n = 5). Different letters indicate significant differences among treatments according to Tukey's tests ($P < 0.05$). P, intraspecific competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 7. Non-structural carbohydrate concentrations in different organs of *A. faxoniana* and *P. purpurea* under different competition patterns and [CO_2] levels. (a) Soluble sugar concentration, (b) starch concentration and (c) NSC concentration. Each value is the mean ± SE (n = 5). Different letters indicate significant differences among treatments according to Tukey's tests ($P < 0.05$). The bars with and without oblique lines denote *P. purpurea* and *A. faxoniana*, respectively. The white, grey and dark grey portions denote leaf, stem and root, respectively. P, intraspecific competition; M, interspecific competition; PC, intraspecific competition under e[CO_2]; MC, interspecific competition under e[CO_2].

Figure 8. The PCA based on ecophysiological characteristics of *Abies faxoniana* and *Picea purpurea* among different competition patterns and [CO_2] levels. The circle, triangle, square and cross indicate A/AA, A/AP, P/AP and P/PP, respectively. The grey and black symbols indicate control and e[CO_2] treatments, respectively. P_n, net photosynthetic rate; g_s, stomatal conductance; Leaf N, leaf N concentration; SLA, specific leaf area; PNUE, photosynthetic nitrogen use efficiency; iWUE, intrinsic water use efficiency; LMF, leaf biomass fraction; SMF, stem biomass fraction; RMF, root

biomass fraction; RS, R/S ratio; SRL, specific root length; AD, average root diameter; SRD, specific root tip density; ECM, the rate of ectomycorrhizal infection; SS, soluble sugar; ST, starch. A/AA, *A. faxoniana* individuals from intraspecific competition; A/AP, *A. faxoniana* individuals from interspecific competition; P/AP, *P. purpurea* individuals from interspecific competition; P/PP, *P. purpurea* individuals from intraspecific competition.


(a)
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Stem biomass (g plant-1)
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b
b
a
(c)
A. faxoniana
P. purpurea
Root biomass (g plant-1)
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Total biomass (g plant-1)
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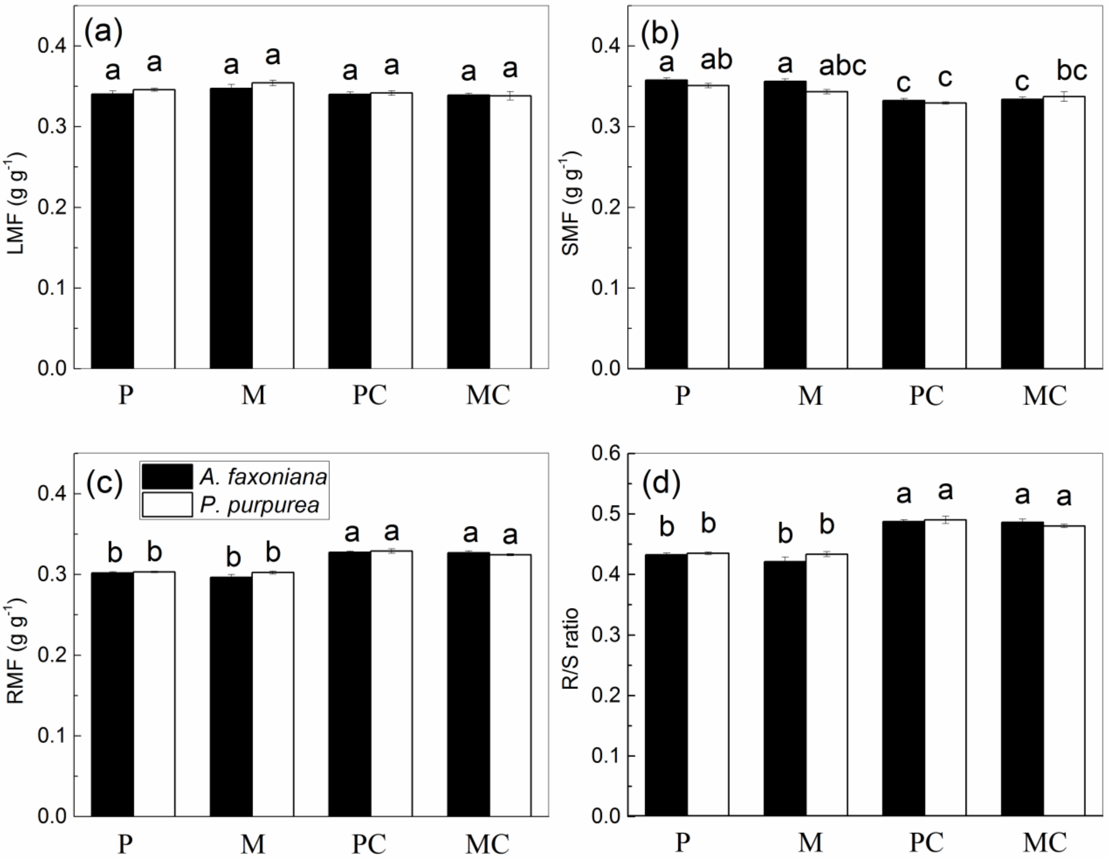
Figure 1


(a)
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MC
(b)
SMF (g g^{-1})
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0.1
0.2
0.3
0.4
a ab a abc c c c bc
P
M
PC
MC
(c)
RMF (g g^{-1})
0.0
0.1
0.2
0.3
0.4
A. faxoniana
P. purpurea
b b b b a a a a
P
M
PC
MC
(d)
R/S ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
b b b b a a a a
P
M
PC
MC

Figure 2

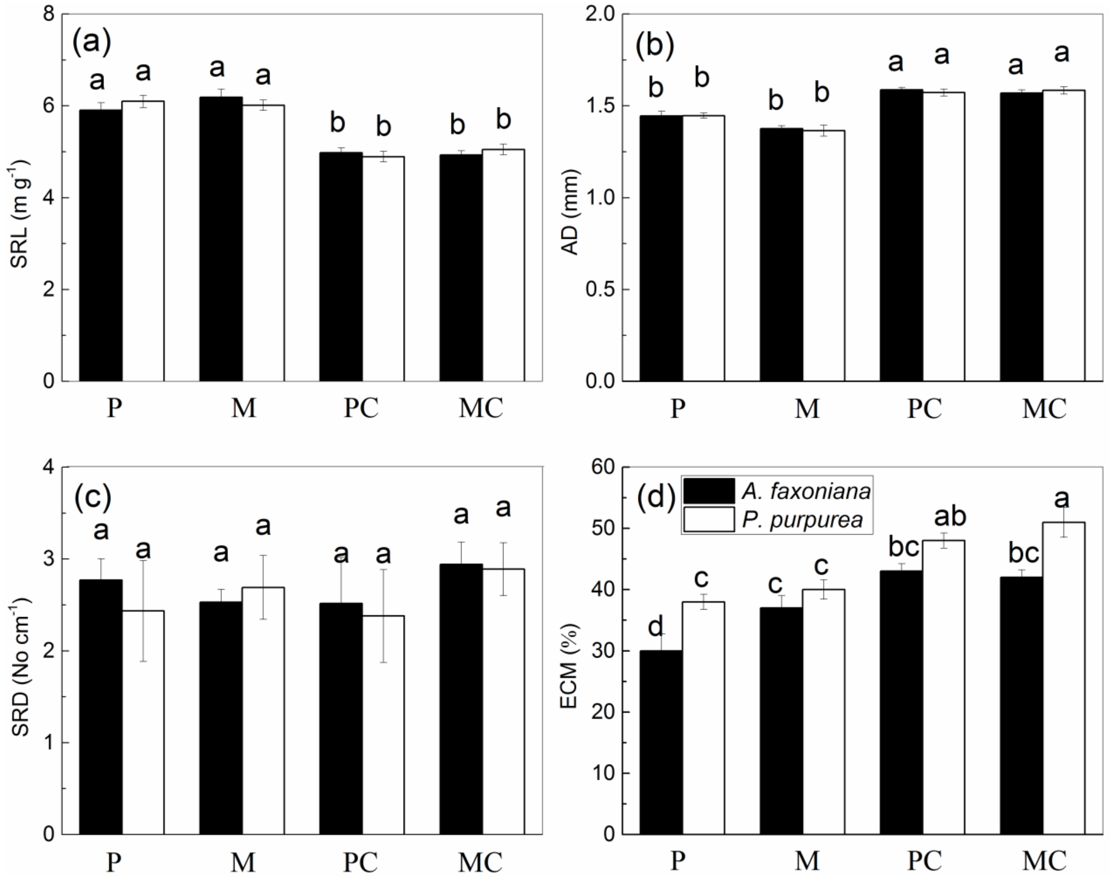

(a)
SRL (m g-1)
8
6
4
2
0
a a a a b b b b
P M PC MC
(b)
AD (mm)
2.0
1.5
1.0
0.5
0.0
b b b b a a a a
P M PC MC
(c)
SRD (No cm-1)
4
3
2
1
0
a a a a a a a a
P M PC MC
(d)
ECM (%)
60
50
40
30
20
10
0
A. faxoniana
P. purpurea
d c c c bc ab bc a
P M PC MC

Figure 3

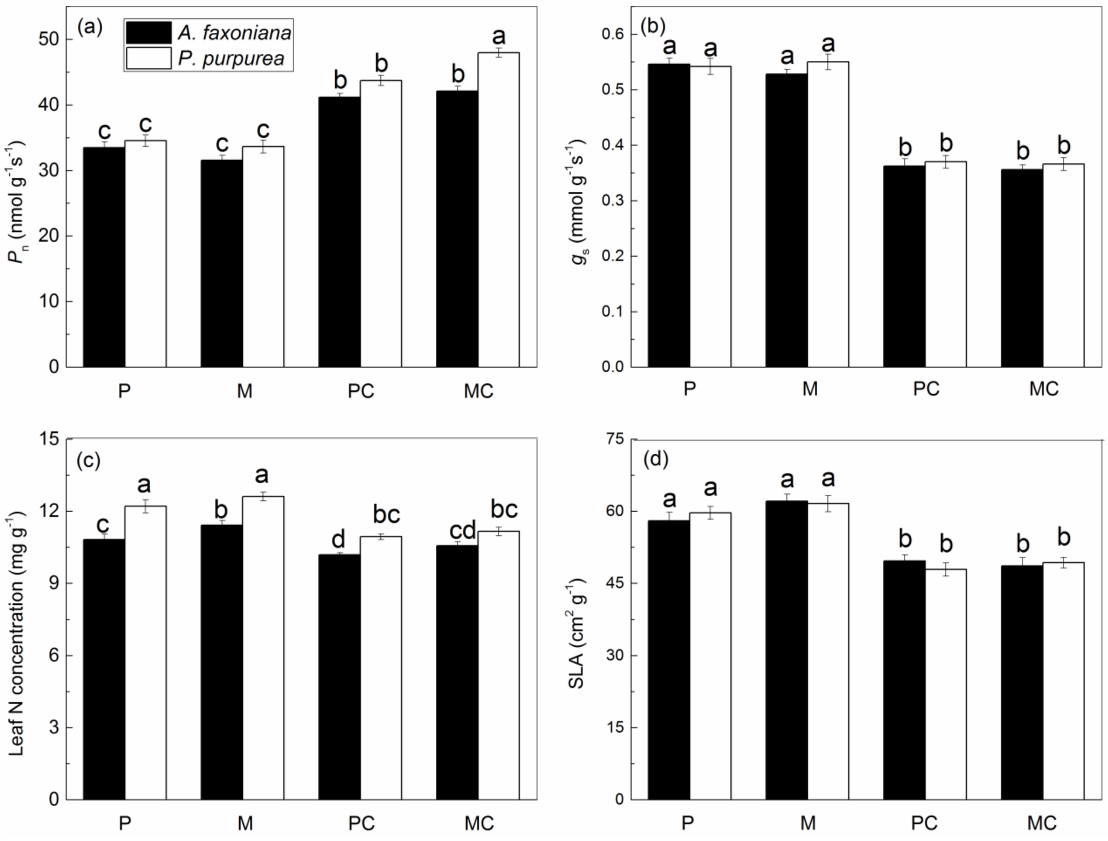

(a)
A. faxoniana
P. purpurea
P_n (nmol g^{-1} s^{-1})
0
10
20
30
40
50
c
c
c
c
b
b
b
a
P
M
PC
MC
(b)
g_s (mmol g^{-1} s^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
0.6
a
a
a
a
b
b
b
b
P
M
PC
MC
(c)
Leaf N concentration (mg g^{-1})
0
3
6
9
12
15
c
a
b
a
d
bc
cd
bc
P
M
PC
MC
(d)
SLA (cm^2 g^{-1})
0
15
30
45
60
75
a
a
a
a
b
b
b
b
P
M
PC
MC

Figure 4

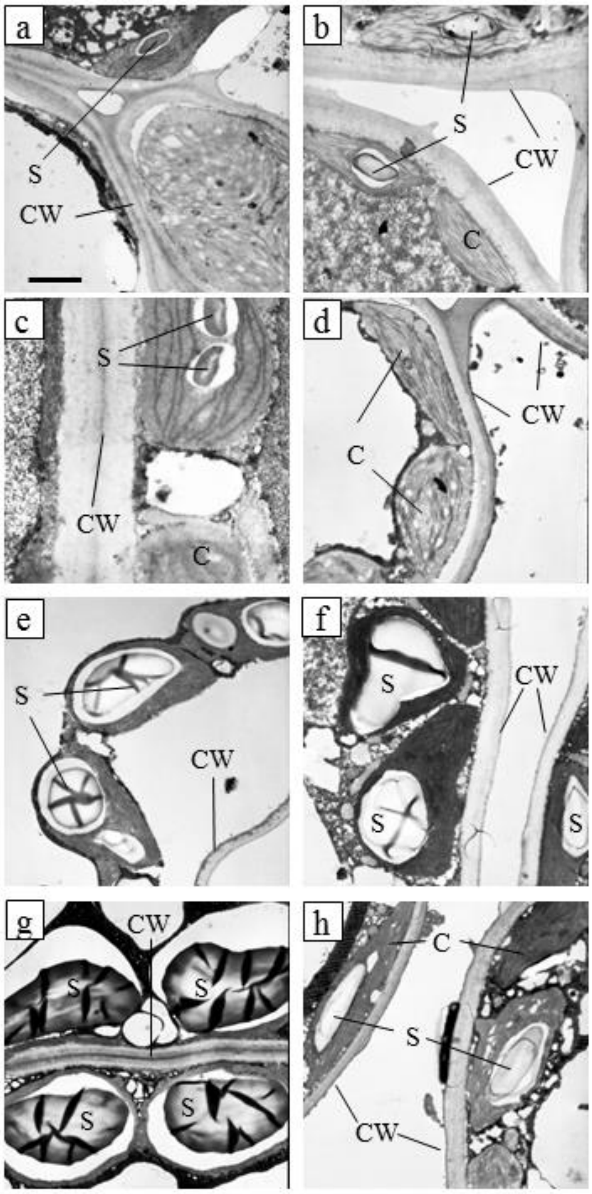
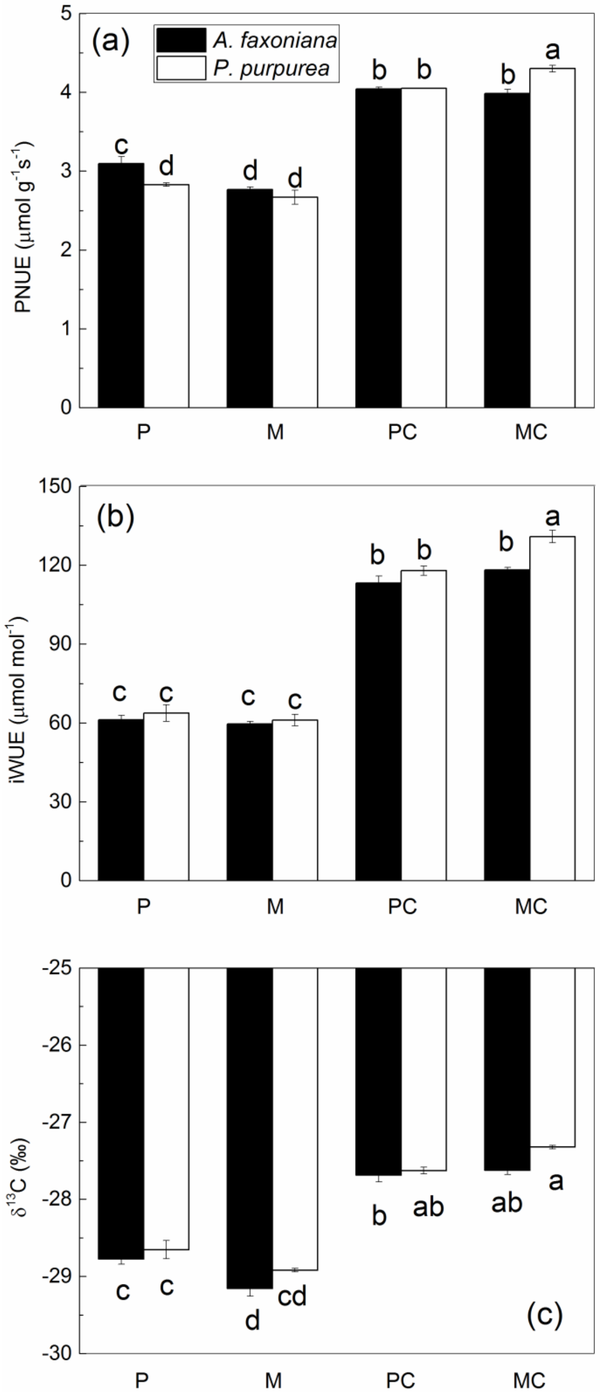


Figure 5



(a)
A. faxoniana
P. purpurea
PNUE (μmol g⁻¹s⁻¹)
0
1
2
3
4
5
c
d
d
d
b
b
b
a
P
M
PC
MC
(b)
iWUE (μmol mol⁻¹)
0
30
60
90
120
150
c
c
c
c
b
b
b
a
P
M
PC
MC
(c)
δ13C (‰)
-25
-26
-27
-28
-29
-30
c
c
d
cd
b
ab
ab
a
P
M
PC
MC

Figure 6

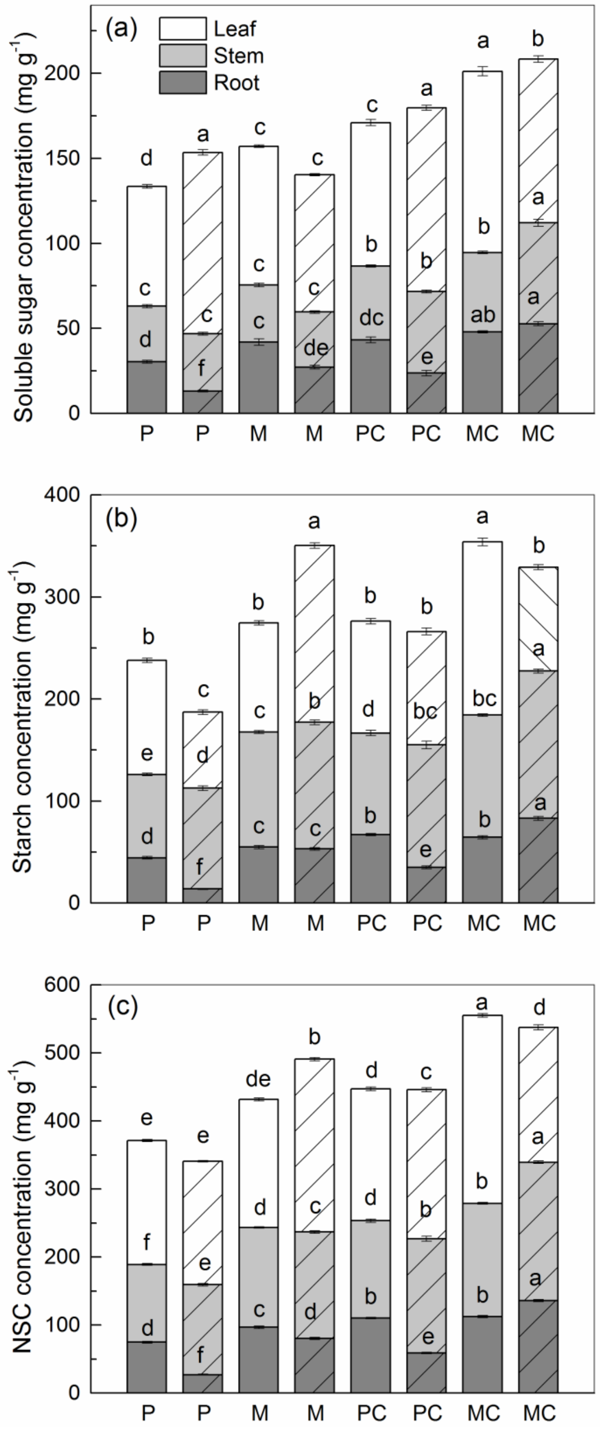

(a)
Leaf
Stem
Root
Soluble sugar concentration (mg g^{-1})
0
50
100
150
200
d c d
a c f
c c c
c c de
c b dc
a b e
a b ab
b a a
(b)
Starch concentration (mg g^{-1})
0
100
200
300
400
b e d
c d f
b c c
a b c
b d b
b bc e
a bc b
b a a
(c)
NSC concentration (mg g^{-1})
0
100
200
300
400
500
600
e f d
e e f
de d c
b c d
d d b
c b e
a b b
d a a
P
P
M
M
PC
PC
MC
MC

Figure 7

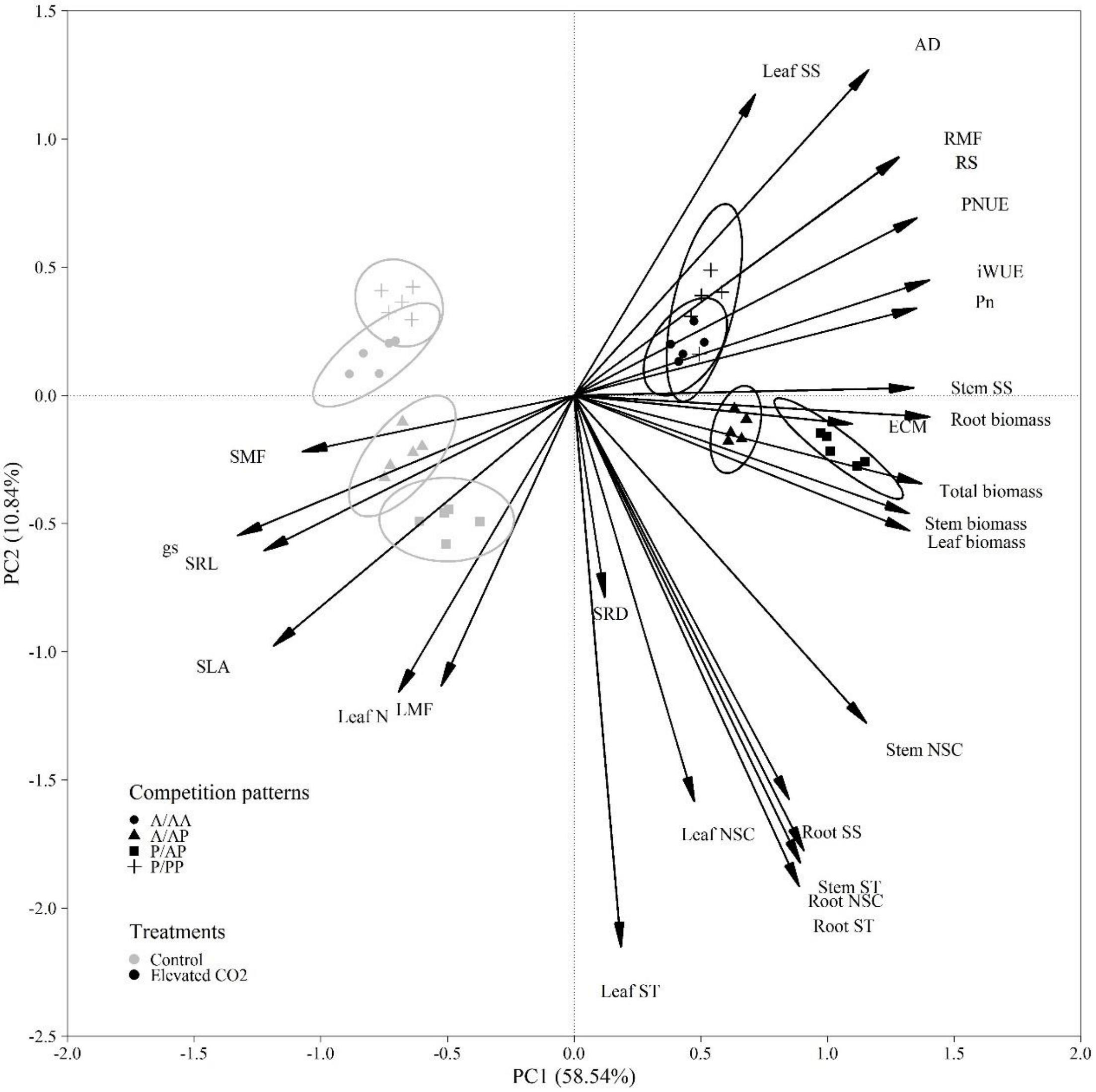

AD
Leaf SS
RMF
RS
PNUE
iWUE
Pn
Stem SS
Root biomass
ECM
Total biomass
Stem biomass
Leaf biomass
SMF
gs
SRL
SLA
Leaf N
LMF
SRD
Stem NSC
Leaf NSC
Root SS
Stem ST
Root NSC
Root ST
Leaf ST
Competition patterns
● A/AA
▲ A/AP
■ P/AP
+ P/PP
Treatments
● Control
● Elevated CO2
PC2 (10.84%)
PC1 (58.54%)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0

Figure 8