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Elevated temperature and CO_2 interactively modulate sexual competition and ecophysiological responses of dioecious *Populus cathayana*

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Running title: Climate change modulates sexual competition

Abstract It remains unclear how global climate change affects dioecious plants that may be especially vulnerable to climate drivers, because they often exhibit skewed sex ratios and eco-physiological specialization in certain microhabitats. In this study, female and male saplings of *Populus cathayana* were employed to explore sex-specific responses and the effects of sexual competition under elevated temperature (ET), elevated CO_2 (EC) and combination of elevated temperature and CO_2 (ETC). The results demonstrated that elevated temperature and CO_2 interactively modulated sexual competition and responses of *P. cathayana*. Moreover, competition patterns affected the eco-physiological responses of *P. cathayana* to climate change treatments. Under both intra- and inter-sexual competition, biomass components, photosynthetic parameters and carbon-related metabolites of females were most strongly affected by ET, while males exhibited a higher photosynthesis and resource use efficiency, and a better biomass accumulation and carbon balance mechanism when compared to females when experiencing intra-sexual competition under EC. The competitive pressure of females on males in inter-sexual competition was intensified by ET, while it was alleviated by ETC. We conclude that climate change drivers and competition patterns differently regulate the sex-specific responses and competitive intensity of males and females, which may have a crucial effect on sex ratios, spatial sexual segregation, biomass production and carbon sequestration in dioecious species in the future.

Keywords: nitrogen use efficiency; sexual competition; climate change; non-structural carbohydrates; structural carbohydrates; photosynthetic capacity

1. Introduction

Dioecious species, distributed among 959 plant genera, represent 6% of angiosperm plants (Renner and Ricklefs, 1995) and play an important role in terrestrial ecosystems. Many of them express sexual dimorphism that involves sex-related phenotypic differences, including differences in morphology, physiological processes, life-history (Dawson and Ehleringer, 1993; Geber *et al*., 1999; Correia and Barradas, 2000; Retuerto *et al*., 2000), and responses to environmental stresses (Chen *et al*., 2010; Zhang *et al*., 2012; Juvany and Munné-Bosch, 2015). However, there is still limited information on how opposite sexes of dioecious plants respond to global climate drivers, such as elevated temperature and CO_2 concentration. The average surface temperature of our planet will continue to increase by 1.4-4.8 ºC and the CO_2 concentration will increase to 670-936 ppm by the end of the current century (IPCC, 2013), which will have major effects on ecosystems.

Some dioecious trees express sexual differences in photosynthesis, xylem anatomy and tree ring growth under climate change (Wang and Curtis, 2001; Rozas *et al*., 2009; Hultine *et al*., 2016; Olano *et al*., 2017). The flower buds of *Populus tomentosa* females show better adaptation to high temperature compared to males (Song *et al*., 2014), while *P. trichocarpa* shows no significant sexual difference under elevated temperature (McKown et al., 2017). According to the principle of optimal carbon balance and resource allocation, plants would regulate their investment in reproduction, growth and defense in a changing environment (Fernandez *et al*., 2016; Guo *et al*., 2018). Knowledge of the dynamics of primary carbohydrates and secondary metabolic substances, such as tannins and phenolics etc., are important when explaining plant adaptation and carbon cycling under climate change (Blaschke *et al*., 2002; Randriamanana *et al*., 2015; Cha *et al*., 2017; Ghimire *et al*., 2018). This may also provide a potential mechanistic explanation for differences in climate change effects on dioecious plants. It has been reported that the proportion of males in *Valeriana edulis* has increased by warming climate (Petry *et al*., 2016). The effects of climate change on dioecious populations may counteract the Fisherian sex-ratio balance effect (Loarie, 2011). Sex-specific responses to climate change can alter sex ratios and eventually lead to a population decline due to decreased reproductive success and fitness (Hultine *et al*., 2016; Petry *et al*., 2016). Therefore, sexual dimorphism in responses of some dominant dioecious woody species may be of particular importance for the productivity and carbon sequestration in terrestrial ecosystems under global climate change (Norby *et al*., 2005; Zhao *et al*., 2012; Thom *et al*., 2017).

Climate change can affect the community composition and structure, and species diversity by modifying the direction and intensity of species interactions, including competition and facilitation (Michalet, 2006; Maestre *et al*., 2009; Dijkstra *et al*., 2010; Levine *et al*., 2010; Kleynhans *et al*., 2016; Olsen *et al*., 2016). Some studies have demonstrated asymmetric competition and plasticity in reproductive or resource allocation between females and males, while the effect of sexual competition on the eco-physiological responses has been largely neglected (Hesse and Pannell, 2011; Tonnabel *et al*., 2017). Although biased sex ratios and spatial segregation of the sexes (SSS) associated with microhabitat differences in dioecious species have been observed in the nature (Eppley, 2006; Garbarino *et al*., 2015; Petry *et al*., 2016), it remains unclear whether sexual competition at the seedling or adult stage is the primary reason for sex ratio biases and SSS in natural populations. Climate change may result in an imbalance of reproduction frequency and influence population structure and distribution in dioecious plants (Miller *et al*., 2011; Miller and Inouye, 2013). A key question is how global change drivers alter intra- and intersexual competition patterns in dioecious species. Plant interactions may lag or supersede the effects of climate change (Stocker *et al*., 1999; Bloor *et al*., 2008; Alexander *et al*., 2015; Arndal *et al*., 2018). Therefore, sex-specific responses and competitive interactions of females and males in dioecious species might be critical determinants when adapting to climate change.

Populus, a dioecious tree genus belonging to Salicaceae, is distributed widely in boreal and temperate ecosystems and used as an important forest plantation species (Zhang *et al*., 2012). *Populus* forests form an important part of carbon budget in terrestrial ecosystems, and poplar is regarded as a suitable model tree to study the responses of dioecious species to climate change. Previous studies have indicated that *P. cathayana* females and males show significant differences in their responses to stressful environments (Zhao *et al*., 2009; Zhang *et al*., 2011, 2014, 2019; Xia *et al*., 2020). Moreover, females appear more sensitive to elevated temperature and drought (Xu *et al*., 2008), while males are positively affected by CO_2 enrichment (Zhao *et al*., 2012). However, these studies have been mostly conducted in non-competitive conditions without plant interactions. It is currently unclear, whether females are more sensitive to competition or whether they are better competitors than males under climate change conditions. It may be predicted that female sensitivity could be reinforced by sexual competition over resources under a given stress environment (Chen *et al*., 2014, 2015). However, females with greater carbon requirements for reproduction could develop enhanced carbon acquisition and a stronger allocation to aboveground biomass under elevated temperature and CO_2, which may improve females' competitive abilities. Therefore, by elucidating the effects between sexual competition and climate drivers, the afforestation and management of dioecious species will be promoted in the future.

The present study was designed to explore intra- and intersexual competition and sex-specific responses in *P. cathayana* under different climate change scenarios, including elevated temperature and CO_2 concentration alone or their combination. We hypothesized that climate drivers and competition patterns would modulate the adaptability of the two sexes by changes in resource acquisition and carbon-related metabolism processes. Moreover, there might be interactive effects caused by elevated temperature and CO_2 concentration. The promoting effect of CO_2 on females or males may be regulated by elevated temperature. In the present study, the biomass accumulation and partitioning, photosynthetic characteristics, water and nitrogen use efficiencies, structural and non-structural carbohydrates and secondary metabolites of *P. cathayana* females and males were determined from different competition experiments (female × female, FF; female × male, FM, male × male, MM). The aims of the study were as follows: (1) to identify the competition relationship of *P. cathayana* females and males under different climate change scenarios, and to reveal, whether climatic factors affect the competition intensity and competiveness of the sexes; (2) to identify the effects of sexual competition, elevated CO_2 and temperature alone and their combination on the eco-physiological responses of *P. cathayana* females and males, and to reveal, whether competition patterns regulate the effect of climate change on dioecious plants.

2. Materials and Methods

2.1. Plant material and experimental design

Male and female cuttings of *P. cathayana* were collected from 60 different trees sampled from 15 populations (four adult trees per population), including 30 males and 30 females, in riparian and valley flat habitats (Datong, 35°56′N, 101°35′E, altitude of 2450 m, annual temperature of 3.7 ºC, annual precipitation of 350 mm) in the Qinghai Province, China. In April 2015, the *P. cathayana* cuttings were planted in plastic pots. After growing for about one month, 288 healthy cuttings (144 males and 144 females) with approximately the same crown size of 15 cm and height of 25 cm were chosen and replanted (two cuttings in each pot according to three different competition patterns: female × female, female × male, male × male) in 30-L plastic pots. The pots were filled with 25 kg homogenized surface sandy soil (0-30 cm) taken from the experimental site. The saplings were moved to 12 growth chambers with an automatic control of air temperature and CO_2 concentration allowing a simulation of climate change conditions. According to the IPCC report (IPCC, 2013), the climate change treatments were selected as follows: temperature elevated by 2 ºC (ET)， CO_2 concentration elevated to 700 ppm (EC), and the combination of elevated temperature and CO_2 concentration (ETC). The control treatment had ambient temperature and CO_2 concentration

(CK). The experiment followed a fully randomized design. The experimental design is shown in Figure 1.

The climate-controlled growth chambers were located at the ecological station of the Wanglang Nature Reserve, Sichuan province of China (104°9′E, 32°54′N, altitude of 2461 m). The site has a temperate monsoon climate with distinct dry and wet seasons. The annual accumulated temperature is about 1056 ºC, the average precipitation varies from 801 to 825 mm, and the CO_2 concentration is about 348.2 ppm. The plant growth chambers are nearly cylindrical structures with 11 walls. The top of each growth chamber has a hollow sunlight plate, and the sides are closed by hollow tempered glass with light transmittance greater than 85%. The temperature was regulated by computer-controlled heating and cooling units, and the CO_2 concentration was monitored and controlled by CO_2 sensors and injectors in the growth chambers (Figure 1). The experiment lasted from early May 2015 to 28 September 2015. The ambient day temperature varied between 14-28 °C and the night temperature between 9-15 °C during the experiment. The elevated temperature treatments had temperatures increased by 2 °C compared to the ambient temperature. The growth chambers had a relative air humidity range of 50-70% during the experiment. Into each pot, 12 g slow release fertilizer (13% N, 10% P and 14% K) was added, and the pots were watered every two days during the experiment. The position of saplings in the growth chambers was frequently changed to decrease the position effect on plant growth. During the experiment, a total of six saplings were randomly sampled from each treatment (2 replicates of each competition pair from 3 replicates of each climate change treatment) and the photosynthetic characteristics were measured. These same six cuttings were sampled to determine plant dry mass and metabolite contents at the end of the experiment.

2.2. Determination of biomass accumulation

At the end of the experiment, the same six saplings used for the measurement of photosynthetic characteristics were harvested (excluding cuttings that were used at the beginning of the experiment). The sampled plants were divided into leaves, stems and roots. Biomass samples were dried at 70 °C for 48 h and their dry mass was determined. The root/shoot ratio (RS ratio) was calculated as root biomass/(leaf biomass + stem biomass), and the root/leaf ratio (RL ratio) as root biomass/leaf biomass. The relative growth rate (RGR) of aboveground and belowground biomass was calculated as follows:

$$RGR_a=(\ln DWa_{t+1}-\ln DWa_t)/\Delta t \qquad \text{(Eq. 1)}$$

$$RGR_b=(\ln DWb_{t+1}-\ln DWb_t)/\Delta t \qquad \text{(Eq. 2)}$$

DWa_t and DWa_{t+1} are the aboveground biomasses of the two sexes, while DWb_t and DWb_{t+1} are the belowground biomasses of the two sexes from initial to final t+1 time under each treatment, Δt was the time interval of initial and ending time in the experiment.

2.3. Determination of gas exchange characteristics

Leaf photosynthetic characteristics were estimated for the fourth fully expanded intact leaf from six randomly chosen individuals from each treatment using a LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA) between 08:00 and 11:30 h on 15 and 17 August 2015. Net photosynthetic rate (P_n, μmol m^{-2} s^{-1}) and transpiration rate (E, mmol m^{-2} s^{-1}) were measured after leaf gas exchange rates had stabilized under the following conditions: leaf temperature 25 °C; leaf-to-air vapor pressure deficit 1.5 ± 0.5 kPa; light intensity 1500 μmol m^{-2} s^{-1}; relative air humidity 50%; and ambient CO_2 concentration 400 ± 5 μmol mol^{-1}. From these measurements, instantaneous photosynthetic water-use efficiency (WUE_i, μmol mol^{-1}) was calculated as the ratio of P_n to E. The same leaves used for gas exchange measurements were sampled. Their area was estimated by a scanner, then the leaves were oven dried at 70 °C and ground for the N analysis. The N content (%N g^{-1} dry weight) was estimated by a semi-micro Kjeldahl method (Mitchell, 1998). About 25 mg of dry leaf tissue were weighed and transferred to a microdigestion tube, then digested with 5 ml of H_2SO_4 and a few mg of 3:1 $CuSO_4$-K_2SO_4 mixture. The acid digest was diluted with 1 ml ammonia-free water and washed into the distillation apparatus. The ammonia was collected in 10 ml of 0.01 M H_2SO_4. Distillates were boiled and titrated with 0.01 M NaOH to a methyl red endpoint, and then the N content was calculated. Photosynthetic nitrogen-use efficiency (PNUE, μmol $g^{-1}Ns^{-1}$) was calculated as the ratio of P_n to leaf N content.

2.4. Determination of non-structural and structural carbohydrate contents

The leaf and root samples for starch and sucrose analyses were sampled from the same six individuals harvested to estimate biomass accumulation at the end of the experiment.

Approximately 0.2 g of dried leaf and root tissue was incubated in 80% (v/v) ethanol at 80 °C for 30 min, and centrifuged at 5000 g for 10 min. The ethanol extract was used for the determination of the sucrose according to the method of Green, Mitchell and Gjerstad (1994). The residue was further hydrolyzed with 9.2 mol l^{-1} $HClO_4$ for 30 min, and centrifuged at 5000 g for 15 min. The extract solution was used for the analysis, and the starch content in glucose equivalents was determined according to the anthrone reagent method of Yemm and Willis (1954). For the determination of cellulose and lignin contents, 0.5 g of dried leaf powder was extracted with 1 M cetyltrimethylammonium bromide (CTAB)-H_2SO_4 solution. The cellulose and lignin contents were determined by the Fibertec™ M61020/1021 fiber analyzer (Foss, Hillerød, Denmark), according to the manufacturer's standard analytical procedures for acid detergent fiber and acid detergent lignin.

2.5. Determination of total polyphenol and anthocyanidin contents

Leaf samples of the same six individuals of each treatment harvested at the end of the experiment, were analyzed for total polyphenol and anthocyanidin contents. For that, 0.25 g of dried leaf powder was extracted with 80% (v/v) aqueous methanol. The polyphenol content was determined by the Folin-Ciocalteu method using gallic acid as a standard (Astill *et al.*, 2001). For analyzing the anthocyanidin content, 0.2 g leaf powder was placed into a 50-ml beaker. After adding 10 ml of 2% hydrochloric acid methanol solution, the beaker was air-tightly closed with a sealing membrane to prevent volatilization. After extracting for 2 h at room temperature in the dark, the solution was filtered to a 50-ml volumetric flask and constant volume with 2% hydrochloric acid methanol solution. The absorbance of the solution was analyzed by a UV-330 spectrophotometer (Unicam, Cambridge, UK) at 530 nm.

2.6. Determination of relative competition index (RCI)

The influence of different neighbors on the responses of the two sexes to climate change was assessed by the relative competition index (RCI) estimated for different types of competition ($RCI_{f/fm}$, competition effect of males on females in inter-sexual competition; $RCI_{m/fm}$, competition effect of females on males in inter-sexual competition) according to the following formulas (Weigelt and Jolliffe, 2003; Manea and Leishman, 2011).

$$RCI_{f/fm} = (Y_{f/fm} - Y_{f/ff}) / Y_{f/ff} \qquad \text{(Eq. 3)}$$

$$RCI_{m/fm} = (Y_{m/fm} - Y_{m/mm}) / Y_{m/mm} \qquad \text{(Eq. 4)}$$

$Y_{f/fm}$ and $Y_{m/fm}$ are the biomasses of the two sexes under inter-sexual competition, and $Y_{f/ff}$ and $Y_{m/mm}$ are the biomasses of the two sexes under intra-sexual competition.

2.7. Statistical analysis

One-way and three-way analyses of variance (ANOVAs) were performed using the SPSS 19.0 for windows statistical software package (SPSS, Chicago, IL, USA). Before conducting ANOVAs, data were checked for normality and homogeneity of variances, and log-transformed to correct for deviations from these assumptions when needed. The differences among means within treatments were separated by Tukey's tests following one-way ANOVAs. Independent-sample *t*-tests were employed to determine the significance of differences between inter- and inter-sexual competition under different climate change treatments. Three-way ANOVAs were used to evaluate the effects of sex competition, elevated temperature and CO_2 and their interactions. All statistical tests were considered significant at $P < 0.05$. A principal component analysis (PCA) was further undertaken using SPSS 19.0 to gain insight into the trait loadings as dependent on the interactive effect of sexual competition and climate change, and to distinguish the differences in competitiveness and adaptation abilities among the sexes under different climate change scenarios.

3. Results

3.1. Effects of simulated climate change treatments and competition on biomass partitioning

Compared with ambient environment, ET treatments increased the total biomass of F/FM and F/FF, EC treatments increased the biomass of M/FM and M/MM (Figure 2a, b, c, d), and ETC treatment increased the total biomass of F/FM, F/FF, M/FM and M/MM. However, no significant difference was observed between ambient environment and ET treatment in M/MM and M/FM. In the inter-sexual competition treatment, compared with males, females showed a higher biomass, RGR_a and RGR_b under ET and ETC and higher RS under EC, while lower RL and RS under ET (Fig. 2a-f). In intra-sexual competition, compared with males, females (F/FF) had a lower leaf, root and total biomass, RGR_a and RGR_b under EC and ETC treatments, and a lower RS ratio under ETC (Fig. 2a-f). In females, F/FM showed a higher leaf and total biomass, and RGR_a, and lower RL than F/FF under ET, while a higher biomass and RGR_a under ETC, and a higher biomass, RL and RS under EC. In males, compared to M/FM, M/MM showed a higher biomass and RGR_a under all climate change treatments. Three-way ANOVAs indicated that CO_2, temperature and competition alone affected leaf, stem, root and total biomass, RGR_a and RGR_b. The interaction between CO_2 and temperature affected growth traits, except for stem biomass, RL and RS ratio. The interaction between competition and CO_2 or temperature affected all growth traits, while the interaction of CO_2, temperature and competition affected root and total biomass, RGR_b, RS and RL ratio.

3.2. Effects of climate change treatments and competition on gas exchange characteristics

Compared with ambient environment, ET and ETC treatments increased P_n, WUE_i and PNUE of females. EC and ETC increased P_n, WUE_i and PNUE, while they decreased E of males under intra- and inter-sexual competition. In intersexual competition, females showed higher P_n, WUE_i and PNUE, and lower E under ET, higher P_n under ETC, while lower P_n, WUE_i and PNUE, and a higher E under EC than males (Table 1). In inter-sexual competition, M/MM had higher P_n, WUE_i and PNUE under EC and ETC treatments, and a lower E value under EC than F/FF (Table 1). Moreover, F/FM showed higher P_n, WUE_i and PNUE than F/FF under ET and ETC treatments. M/MM showed lower E and higher WUE_i under EC, and higher P_n and WUE_i than M/FM under ETC. According to the statistical analysis, CO_2, temperature and competition alone, the interaction between competition and CO_2 or temperature, and the interaction of CO_2, temperature and competition affected all gas exchange characteristics. The interaction between CO_2 and temperature affected PNUE and *E*.

3.3. Effects of climate change treatments and competition on the content of carbohydrates

Compared with controls, EC, ET and ETC treatments increased leaf starch and sucrose contents of females from intra- and inter-sexual competition. However, EC, ET and ETC treatments had no significant effect on the root starch of females, and only ETC significantly increased the root sucrose of females from intra- and inter-sexual competition (Figure 3a-d). On the other hand, ET significantly increased the leaf starch content, while EC, ET and ETC treatments significantly increased leaf sucrose contents of males from inter-sexual competition. Moreover, EC and ETC treatments significantly increased root starch and sucrose contents of males from intra- and inter-sexual competition. In inter-sexual competition, females had lower leaf starch, and higher leaf and root sucrose and root starch contents under ambient environment and ET, but a lower root sucrose content under ETC, while a higher leaf starch content under EC and ETC when compared to males. In intra-sexual competition treatments, M/MM showed higher leaf and root sucrose levels under EC and ETC treatments, and higher root starch contents under ETC than F/FF, while F/FF had a higher leaf sucrose content under ET, and a higher leaf starch content under EC and ETC treatments. In addition, F/FM showed a lower leaf starch content than F/FF under EC and ETC, and a higher leaf sucrose content under ETC than F/FF, while M/MM showed a higher leaf sucrose level under EC and ETC treatments, a higher root starch content under ETC and a higher root sucrose content under EC than M/FM (Figure 3a-d). Compared with ambient environment, ET, EC and ETC treatments increased leaf cellulose and lignin contents of females and males from inter-sexual competition, while EC and ETC increased leaf cellulose of F/FF and leaf lignin of M/MM, while only ETC increased leaf lignin of F/FF. In the inter-sexual competition, females had more cellulose than males under all climate change treatments, except for ETC, a higher leaf lignin content under ET and a lower leaf lignin content under EC and ETC than males (Figure 4a, b). In the intra-sexual competition, F/FF had more cellulose under all climate change treatments, while less leaf lignin than M/MM under EC and ETC. Moreover, F/FM showed a lower cellulose content under ETC and higher lignin contents under ET and ETC when compared to F/FF, while M/MM showed higher lignin contents under EC and ETC than M/FM. CO_2, temperature and competition alone, and the interaction between competition and CO_2 or temperature affected all observed carbohydrate variables. The interaction between CO_2 and temperature affected carbohydrates, except for root starch. The interaction of CO_2, temperature and competition affected carbohydrates, except for the leaf lignin content.

3.4. Effects of climate change treatments and competition on leaf anthocyanidin and total polyphenol contents

Compared to ambient environment, EC and ETC treatments increased the leaf polyphenol content of both females and males, while ET increased the leaf polyphenol content of males in both intra- and intersexual competition. ET and ETC treatments increased the leaf anthocyanidin content of F/FM, and EC and ETC treatments increased the leaf anthocyanidin content of M/FM, while ET, EC and ETC treatments increased leaf anthocyanidin contents of F/FF and M/MM. In inter-sexual competition, females had more leaf anthocyanidin under ambient environment and ET, but less under EC and ETC, and less polyphenols under ET and EC treatments than males (Figure 4c, d). In intra-sexual competition, F/FF had more leaf anthocyanidin under ET, and less anthocyanidin in EC and ETC treatments compared with M/MM. Among males, M/MM showed higher leaf anthocyanidin amounts than M/FM under ET, EC and ETC. According to three-way ANOVAs, CO_2, temperature and competition alone, the interaction between competition and CO_2 or temperature as well as the interaction of CO_2, temperature and competition affected leaf anthocyanidin and total polyphenol, while the interaction between CO_2 and temperature affected leaf total polyphenol.

3.5. Relative competition index (RCI) and principal component analysis (PCA)

F/FM had positive RCI values, while M/FM had negative RCI_{leaf}, RCI_{stem}, RCI_{root} and RCI_{total} values under ET, EC and ETC treatments (Figure 5a-d), indicating that females benefitted from the presence of males (compared to the presence of females), while males were suppressed by females. Moreover, RCI_{root} and RCI_{total} of F/FM were higher under EC and ETC than under ET and ambient conditions, which indicated that ETC and EC treatments promoted the root and total biomass of females in inter-sexual competition more than ET. On the other hand, RCI_{stem} of M/FM was higher in EC and ETC treatments than under ET, and

RCI_{root} of M/FM was higher in EC and ETC treatments than in ambient conditions, indicating that EC and ETC treatments had a more positive effect on M/FM.

The PCA analysis highlighted the presence of a considerable distance between F/FM and M/FM under the ambient environment, and ET and EC treatments. Moreover, ET further separated F/FM and M/FM (Figure 6a), underscoring the major difference in how inter-sexual competition affected males and females. However, ETC reduced the distance between inter-sexual competition treatments, indicating that ETC benefitted males relatively more, resulting in a reduction in the competitive pressure of females on males. In addition, in intra-sexual competition under ambient environment, females and males were positioned close to each other in PCA (Figure 6b), indicating similar competitive abilities in both sexes. However, ET, EC and ETC treatments expanded the distance between them, indicating the importance of the global climate change on competitive abilities. M/MM had higher PC1 factor scores than females under EC and ETC.

In inter-sexual competition, PC1 and PC2 explained 74.8% of the total variation. PC1 explained 60.3.0% of the variation，being affected by P_n, E, WUE_i, PNUE, root starch and sucrose, leaf sucrose, leaf anthocyanidin, leaf lignin, RGR_a, RGR_b, and leaf, stem, root and total biomass, while PC2 explained 14.5%，being affected by E, RL and RS (Figure 6c). In intra-sexual competition, PC1 and PC2 explained 71.6% of the total variation. PC1 explained 54.0% of the variation，being affected by P_n, E, WUE_i, PNUE, root starch and sucrose, leaf sucrose, leaf anthocyanidin, lignin, RGR_a, RGR_b, and leaf, stem, root and total biomass, while PC2 explained 17.6% of variation, being affected by leaf starch, leaf polyphenol contents, RL and RS ratio (Figure 6d).

4. Discussion

4.1. Interactive effects of climate change and sexual competition on P. cathayana

Only few studies have reported sexual dimorphism and sex ratio biases in responses to climate change in dioecious plants (Tognetti, 2012; Álvarez-Cansino *et al*., 2013; Hultine *et al*., 2016). Our study further indicated that the key climate drivers and sexual competition patterns interactively affect growth, photosynthetic capacity, and the dynamics of non-structural and structural carbohydrates and secondary metabolites in the two sexes, which may alter biomass productivity and carbon sequestration of *P. cathayana* forests in climate change conditions. In intra- and inter-sexual competition, elevated temperature (ET) promoted female performance more, as shown as significant increases in biomass accumulation, P_n, WUE_i and PNUE in females. Moreover, under the combined treatment of ET and inter-sexual competition, females showed a higher total biomass and photosynthetic capacity, and greater contents of leaf sucrose, root starch and sucrose, leaf cellulose, lignin and anthocyanidin, indicating superior carbon assimilation and adaptability when compared to males. However, in several single-plant experiments, opposite responses have been found, in particular that females suffer greater negative effects from elevated temperature (Xu *et al*., 2008; Zhao *et al*., 2012). The differences in results imply that sexual competition patterns change the responses of the two sexes to climate change. The identity of neighbors significantly affects the ability of roots to capture resources (Rogers and Eppley, 2012), and sexual differences in sensitivity to adjacent plants may be an important factor regulating the growth patterns of dioecious plants under a given habitat (Zhang *et al*., 2009).

Our study showed that elevated CO_2 (EC) significantly increased biomass accumulation, photosynthetic capacity, and water and N use efficiency, with stronger positive effects on males than on females under intra-sexual competition. It has been reported that *Salix arctica* and *P. tremuloides* males show higher photosynthetic rates than females under elevated CO_2 concentration (Jones et al., 1999; Wang and Curtis, 2001). Males have lower resource demands and higher resource utilization efficiencies (Li *et al*., 2007; Zhao *et al*., 2011). Moreover, the gas exchange, N content per leaf area and soluble protein content of mature leaves of *P. cathayana* males increase with elevated CO_2 (Zhao et al., 2012), which may enhance competitiveness and fitness of males under elevated CO_2. The combination of elevated CO_2 and temperature has been shown to promote photosynthesis and carbon transformation from source to sink (Luo and Polle, 2009). Our results indicated that the combined elevated temperature and CO_2 treatment significantly increased the leaf and total biomass, P_n, WUE_i, PNUE, and the contents of carbohydrates and secondary metabolism of both sexes in all competition patterns. Thus, the data collectively indicated that climate drivers alone and in combination show different effects on the two sexes, which may potentially affect the distribution and population dynamics of dioecious plants in the future.

There is a trade-off among reproduction, growth and defense functions in plants according to the optimum resource allocation principles and carbon balance hypothesis (Fernandez *et al*., 2016; Guo *et al*., 2018). It has been previously reported that elevated temperature affects the secondary metabolism of *P. davidiana* and that *Salix myrsinifolia* females increase leaf flavonoid and tannin contents more than males under increased temperature (Randriamanana *et al*., 2015). Our results also showed that elevated temperature, elevated CO_2 and their combination significantly affected carbon metabolism and balance. Higher leaf cellulose, lignin and anthocyanidin contents of both sexes were observed under ETC, which may alleviate the suppressing effect on photosynthesis caused by excess carbohydrates in chloroplasts (Zhao *et al*., 2012).

Males can sustain a higher rate of carbon assimilation with greater sink activities (Wang and Curtis, 2001; Zhao *et al*., 2012), and they have greater adaptability and greater carbohydrate use efficiency than females under CO_2 enrichment (Zhao *et al*., 2011). In the present study, males possessed lower leaf starch, and higher root starch and leaf polyphenol contents when compared to females under intra-sexual competition, thus indicating a more efficient C allocation under elevated CO_2 (Zhao *et al*., 2012). Our results showed that the greatest increase in polyphenols, anthocuanidins and lignins of both sexes occurs under EC and ETC, while males from inter-sexual competition accumulate more leaf starch and less cellulose than females under ET, indicating switching from growth to non-structural C storage that in consistent with low photosynthesis and biomass production. It has been reported that climate drivers affect tree lignification (Blaschke *et al*., 2002), the content of tannins and phenolics

(Ghimire *et al*., 2018), and litter decomposition (Cha *et al*., 2017), implying an indirect effect on soil and plant-herbivore interactions (Cornelissen and Stilling, 2005; Kelley *et al*., 2011), which can further affect plant adaptation and competitiveness under future climate change. In all, climate drivers and sexual competition of dioecious species may interactively affect the C-related nutrient balance between source and sink, and carbon sequestration in terrestrial ecosystems.

4.2. Competition relationships of P. cathayana under different climate change scenarios

Our study demonstrated that the two key climate change drivers, elevated temperature and CO_2 alone and in combination affected the intensity of sexual competition in *P. cathayana,* which means that sex ratios and population structures might be altered in the future. Previous studies have found that the growth of plant species is modulated by elevated CO_2 depending on inter- and intra-specific relationships and interactions with abiotic factors (Ward *et al*., 1999; Ainsworth, 2016; Flexas *et al*., 2016; Sánchez-Gómez *et al*., 2017), or on competitors that affect available soil resources by responding to chemical root signals and mechanical touching from neighboring plants (Poorter and Navas, 2003; Gruntman and Novoplansky, 2004; Messier *et al*., 2009; Alexander *et al*., 2015; Arndal *et al*., 2018). Some studies on dioecious grasses have shown that there are sex ratio biases and sexual space segregation under environmental gradients and that females have a greater competitive ability than males (Eppley, 2006; Mercer and Eppley, 2010). Our results indicated that the sexual identity of the neighbor significantly affects competitive abilities under all tested climate conditions.

The PCA and competition indexes showed that males had a greater competitive capacity under intra-sexual competition under EC and ETC conditions. Elevated temperature intensified the competitive pressure of females on males in inter-sexual competition. However, the combined elevated temperature and CO_2 treatment alleviated the competition pressure. In addition, there were more positive effects on males than females in intra-sexual competition under EC and ETC. Such interactions implied that the two sexes have different eco-physiological response plasticities and trade-offs among climate drivers and niche differentiation under climate change conditions, as reported in previous studies as well (Epply 2006; Niinemets and Valladares, 2006; Nicotra *et al*., 2010; Laanisto and Niinemets, 2015). The alleviated competitive intensity under ETC may be related to niche differentiation. Sex-specific resource allocation and utilization patterns may cause spatial segregation and alleviate sexual competition (Sάnchez-Vilas *et al*., 2011; Xia *et al*., 2020). The niche differentiation hypothesis proposes that females and males have different niches due to inter-sexual competition intensity being greater than intra-sexual competition, while the sex-specific cost of reproduction hypothesis suggests that differences in costs of reproduction may cause adaptation to different habitats (Bierzychudek and Eckhart, 1988; Eppley, 2006; Stehlik *et al*., 2008).

Sex ratio biases have been reported in many dioecious species, often towards males, especially in less optimal habitats (Stehlik *et al*., 2008; Hesse and Pannell, 2011). Climate change may contribute to skewed sex ratios due to differential tolerance between sexes to climatic factors (Tognetti, 2012; Hultine *et al.*, 2016). Elevated temperature intensified the competitive advantage of *P. cathayana* females, which would eventually lead to a negative effect on the reproductive success at the population scale (Hultine *et al.*, 2016). A female-biased sex ratio triggered by an increased competitive success of females may contradict previous thinking of the effect of climatic change drivers on dioecious species, implying that competitive interactions between sexes may counteract factors that generate male-biased sex ratios. Also, male-biased or female-biased sex ratios may further impact the intensity of sexual selection and mate success.

The balance of facilitation and competition appears to vary with the life stages of interacting species and with environmental gradients (Callaway and Walker, 1997; Michalet, 2006; Brooker *et al.*, 2008; Olsen *et al.*, 2016). It is predicted that competition and facilitation between males and females in dioecious plants may vary with the stage of climate change. In our study, there was an increasing competitive asymmetry between males and females when exposed to inter-sexual competition under elevated temperature. Compared with intra-sexual competition, females showed more facilitation and less competitive pressure in inter-sexual competition under elevated temperature and CO_2 and their combination. The outcome of competitive interactions and the change from competition to facilitation depend on resource levels under a given environment (Tilman, 1982, 1988; Grime, 2006). In particular, elevated CO_2 and temperature may result in synergistic and antagonistic responses when interacting with each other or with other biotic and abiotic factors (Alexander *et al.*, 2015; Sánchez-Gómez *et al.*, 2017; Arndal *et al.*, 2018). If competitive interactions are neglected, the effect of climate change on the productivity of ecosystems will be over-estimated.

5. Conclusions

The current study demonstrates that the two key climate change drivers, elevated temperature and CO_2, affect the eco-physiological responses of males and females and the intensity of sexual competition in *P. cathayana*. Females exposed to inter- and intra-sexual competition showed greater responses to elevated temperature, while males from intra-sexual competition were better adapted to elevated CO_2. The competitive pressure of females on males in inter-sexual competition was intensified by elevated temperature, while it was alleviated by the combination of elevated temperature and CO_2. Females suffered more from intra-sexual competition pressure under elevated CO_2. The main implications for afforestation and forest management are that the effects of climate drivers and sexual competition should be considered in afforestation and management of dioecious tree species to enhance productivity and carbon sequestration under future climate change. The two sexes should be grown in proper combinations in order to decrease the inter- and intra-sexual competition pressure that is regulated by elevated temperature and CO_2. Furthermore, effects of climate change drivers on competition may further modulate sex ratios and spatial segregation of sexes in dioecious plants in the future. We present that our results contribute to competition models that are central for estimating the impact of climate change on dioecious species and consequent effects on terrestrial ecosystems.

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Author contributions Juan Chen had the main responsibility for data collection, analysis and writing, Quan Liu and Lei Yu contributed to data analysis, Helena Korpelainen and Ülo Niinemets 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 Photosynthetic characteristics of *P. cathayana* females and males under different competition patterns and climate change scenarios.

Competition	Climate change treatment	P_n (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	WUE_i (μmol mol^{-1})	PNUE (μmol g^{-1}Ns^{-1})
Inter-sexual					
F/FM	CK	14.75±0.41c**	5.59±0.23bc	2.66±0.14c*	28.09±0.97bc**
	ET	21.79±0.43a***	5.49±0.22bc	4.00±0.19ab***	41.25±1.32a***
	EC	13.85±0.19c*	6.72±0.15a*	2.06±0.04d	25.08±0.86bc*
	ETC	22.63±0.39a***	5.42±0.19bc	4.20±0.14a***	47.05±1.82a***
M/FM	CK	11.21±0.38d**	5.78±0.15b	1.94±0.04d**	21.99±1.13c**
	ET	15.27±0.32c	6.87±0.15a	2.23±0.08cd	31.08±1.26b
	EC	17.95±0..49b	4.98±0.12bc**	3.62±0.15b**	41.4±1.52a*
	ETC	18.61±0.31b***	4.94±0.19c	3.79±0.15ab***	45.31±2.16a
Intra-sexual					
F/FF	CK	11.83±0.41D	5.89±0.19AB	2.02±0.12C	21.82±0.97C
	ET	17.18±0.36B	5.96±0.25AB	2.90±0.10B	30.08±1.08BC
	EC	12.37±0.34D	5.78±0.27AB	2.15±0.06C	21.05±1.54C
	ETC	14.63±0.35C	5.55±0.19B	2.66±0.14BC	27.97±1.23BC
M/MM	CK	14.57±0.58C	5.71±0.19B	2.57±0.13BC	27.61±0.79C
	ET	15.99±0.41BC	6.67±0.14A	2.41±0.09BC	25.07±4.47C
	EC	17.61±0.24B	3.87±0.20C	4.61±0.23A	36.83±0.72B
	ETC	25.52±0.50A	5.07±0.12B	5.05±0.18A	50.09±2.27A

Each value is the mean ± SE ($n = 6$). Different lowercase letters within the column indicate significant differences between the sexes from inter-sexual competition, while different uppercase letters within the column indicate significant differences between the sexes from intra-sexual competition under different climate change scenarios according to Tukey's test ($P<0.05$). Asterisks indicate significant differences according to independent-samples *t*-tests between inter- and intra-sexual competition within each climate change treatment (*$0.01 < P < 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$). CK: ambient temperature and CO_2 concentration, ET: elevated temperature, EC: elevated CO_2 concentration, ETC: combined elevated temperature and CO_2 concentration treatment. F/FM, female individual from inter-sexual competition; M/FM, male individual from inter-sexual competition; F/FF, female individual from intra-sexual competition; M/MM, male individual from intra-sexual competition.

Table 2 Statistical effects of elevated CO_2, elevated temperature and sexual competition treatments and their interactions on the ecophysiological characteristics of *P. cathayana* females and males.

Characteristic	$P_{CO2.}$	P_{T}	$P_{comp.}$	$P_{CO2.\times T}$	$P_{CO2.\times comp.}$	$P_{T\times comp.}$	$P_{CO2.\times T\times comp.}$
P_n	0.00^{***}	0.00^{***}	0.00^{***}	0.270^{ns}	0.00^{***}	0.00^{***}	0.00^{***}
E	0.00^{***}	0.031^{*}	0.002^{**}	0.002^{**}	0.00^{***}	0.00^{***}	0.022^{*}
WUE_i	0.00^{***}	0.00^{***}	0.00^{***}	0.091^{ns}	0.00^{***}	0.00^{***}	0.007^{**}
PNUE	0.00^{***}	0.00^{***}	0.00^{***}	0.012^{*}	0.00^{***}	0.00^{***}	0.00^{***}
Leaf starch	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}
Leaf sucrose	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}
Root starch	0.00^{***}	0.00^{***}	0.00^{***}	0.068^{ns}	0.00^{***}	0.017^{*}	0.001^{**}
Root sucrose	0.00^{***}	0.00^{***}	0.00^{***}	0.015^{*}	0.00^{***}	0.00^{***}	0.00^{***}
Leaf cellulose	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}
Leaf lignin	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.666^{ns}
Anthocyanidin	0.00^{***}	0.00^{***}	0.00^{***}	0.062^{ns}	0.00^{***}	0.00^{***}	0.00^{***}
Total polyphenol	0.00^{***}	0.00^{**}	0.004^{**}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}
Leaf biomass	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.190^{ns}
Stem biomass	0.00^{***}	0.00^{***}	0.00^{***}	0.369^{ns}	0.00^{***}	0.00^{***}	0.084^{ns}
Root biomass	0.00^{***}	0.00^{***}	0.00^{***}	0.004^{**}	0.00^{***}	0.00^{***}	0.00^{***}
Total biomass	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}
RL	0.147^{ns}	0.721^{ns}	0.624^{ns}	0.952^{ns}	0.00^{***}	0.001^{**}	0.016^{*}
RS	0.990^{ns}	0.042^{*}	0.00^{***}	0.548^{ns}	0.00^{***}	0.010^{**}	0.00^{***}
RGR_a	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.003^{**}	0.712^{ns}
RGR_b	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}	0.00^{***}

Note: P_{CO2}, elevated CO_2 effect; P_T, elevated temperature effect; P_{comp}, sexual competition effect; $P_{CO2.\times T}$, interactive effect of elevated CO_2 and temperature; $P_{CO2.\times comp}$, interactive effect of elevated CO_2 and sexual competition; $P_{T\times comp.}$, interactive effect of elevated temperature and sexual competition; $P_{CO2.\times T\times comp.,}$ tinteractive effect of elevated CO_2, elevated temperature and sexual competition. *$0.01 < P < 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$, ns, non-significant.

Figure legends

Figure 1 Schematic diagram of the experimental design. F, *P. cathayana* females; M, *P. cathayana* males. CK: control treatment - ambient temperature and CO_2 concentration, ET: elevated temperature treatment; EC: elevated CO_2 concentration treatment; ETC: combined elevated temperature and CO_2 treatment. Competition patterns, female × female, FF; female × male, FM; male × male, MM. Each competition pair (FF, MM and FM) and climate change treatment (CK, ET, EC and ETC) included 12 replicates. A total of 288 saplings (144 males and 144 females) were kept in 12 growth chambers with an automatic control of air temperature and CO_2 concentration allowing a simulation of climate change conditions.

Figure 2 Biomass accumulation and partitioning and relative growth rates in *P. cathayana* females and males under different competition patterns and climate change scenarios. (a) Leaf biomass, (b) stem biomass, (c) root biomass, (d) total biomass, (e) root/shoot (RS) ratio, (f) root/leaf (RL) ratio, (g) relative growth rate of aboveground biomass (RGR_a) and (h) relative growth rate of belowground biomass (RGR_b). Each value is the mean ± SE ($n = 6$). Different lowercase letters within the bars indicate significant differences between sexes from inter-sexual competition, while different uppercase letters within the bars indicate significant differences between sexes from intra-sexual competition under different climate change scenarios according to Tukey's test ($P<0.05$). Significant differences according to independent-samples *t*-test between inter- and intra-sexual competition pattern within each climate change scenarios are shown as follows: * $0.01 < P < 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$. CK: ambient temperature and CO_2 concentration, ET: elevated temperature, EC: elevated CO_2 concentration, ETC, combined elevated temperature and CO_2 concentration treatment. F/FM, female individual from inter-sexual competition; M/FM, male individual from inter-sexual competition; F/FF, female individual from intra-sexual competition; M/MM, male individual from intra-sexual competition.

Figure 3 Non-structural carbohydrate contents of *P. cathayana* females and males under different competition patterns and climate change scenarios. (a) Leaf starch content, (b) leaf sucrose content, (c) root starch content and (d) root sucrose content. Each value is the mean ± SE ($n = 6$). Competition treatment codes, climate change codes and statistical significance codes are as in Figure 2.

Figure 4 Leaf structural carbohydrate, anthocyanidin and polyphenol contents in *P. cathayana* males and females under different competition patterns and climate change scenarios. (a) Leaf cellulose content, (b) leaf lignin content, (c) leaf anthocyanidin content and (d) leaf polyphenol content. Each value is the mean ± SE ($n = 6$). Competition treatment codes, climate change codes and statistical significance as in Figure 2.

Figure 5 Relative competition index (RCI) in *P. cathayana* males and females under different climate change and competition treatments. (a) RCI_{leaf}, (b) RCI_{stem}, (c) RCI_{root} and (d) RCI_{total}.

Each value is the mean ± SE (*n* = 6). Different letters in the same column indicate significant differences among treatments according to Tukey's test (P<0.05). Competition treatment codes and climate change codes as in Figure 2.

Figure 6 Results of principal component analysis of *P. cathayana* males and females from different competition and climate change treatments. (a) Factor score plot of *P. cathayana* from inter-sexual competition under different climate change treatments, (b) factor score plot of *P. cathayana* from intra-sexual competition under different climate change treatments, (c) factor loading plots of all ecophysiological characteristics of *P. cathayana* in inter-sexual competition and climate change treatments, (d) factor loading plots of all ecophysiological characteristics of *P. cathayana* in intra-sexual competition and climate change treatments. The acronyms are as follows: P_n, light-saturated net photosynthetic rate; PNUE, photosynthetic N-use efficiency; E_{value}, transpiration rate; WUE_i, instantaneous water use efficiency; L_{sta}, leaf starch content; L_{suc}, leaf sucrose content; R_{sta}, root starch content; R_{suc}, root sucrose content; Poly, leaf total polyphenol content; Cel, leaf cellulose content; Lig, leaf lignin content; Ant, leaf anthocyanidin content; LB, leaf biomass; SB, stem biomass; RB, root biomass; TB, total biomass; RS, root to shoot ratio; RL, root to leaf ratio; RGR_a, relative growth rate of aboveground biomass; RGR_b, relative growth rate of belowground biomass. F/FM and M/FM, respectively, indicate females and males from inter-sexual competition pattern under ambient temperature and CO_2 concentration; F/FF and M/MM, respectively, indicate females and males from intra-sexual competition pattern under ambient temperature and CO_2 concentration; TF/FM and TM/FM, respectively, indicate females and males from inter-sexual competition pattern under elevated temperature; TF/FF and TM/MM, respectively, indicate females and males from intra- sexual competition pattern under elevated temperature; CF/FM and CM/FM, respectively, indicate females and males from inter-sexual competition pattern under elevated CO_2 concentration; CF/FF and CM/MM, respectively, indicate females and males from intra-sexual competition pattern under elevated CO_2 concentration; TCF/FM and TCM/FM, respectively, indicate females and males from inter-sexual competition pattern under combined elevated temperature and CO_2 concentration. TCF/FF and TCM/MM, respectively, indicate females and males from intra-sexual competition pattern under combined elevated temperature and CO_2 concentration.

Figure 1

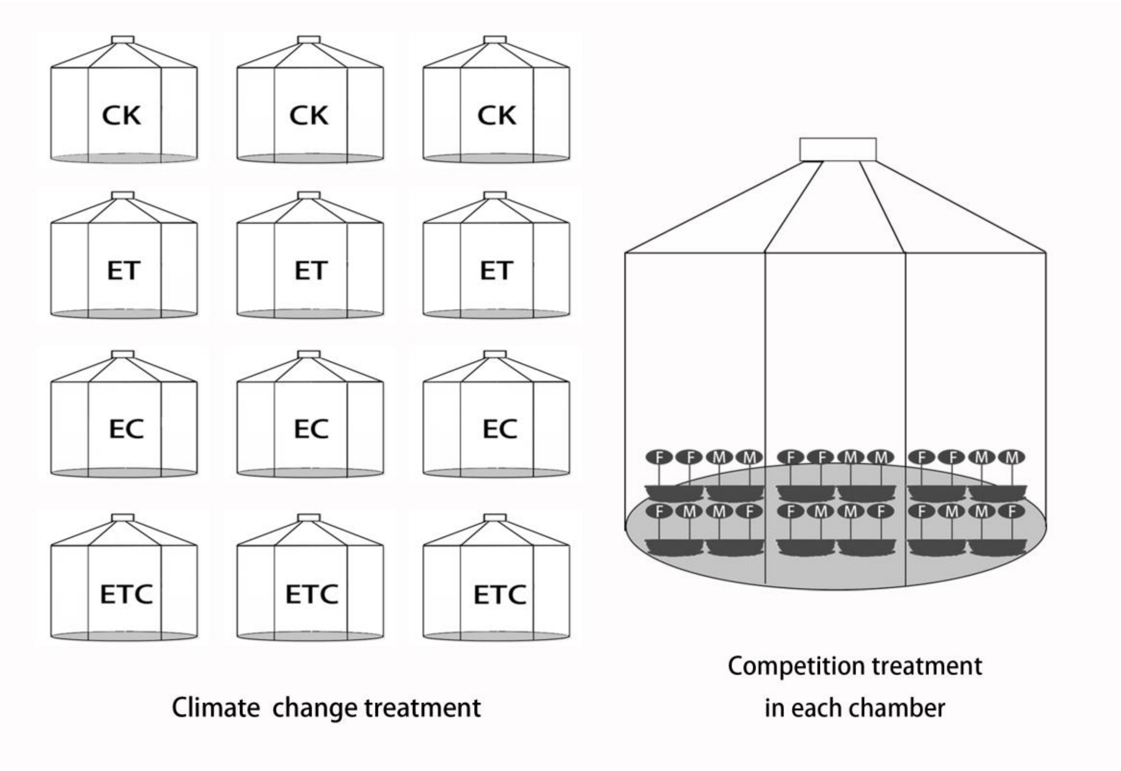

CK
CK
CK
ET
ET
ET
EC
EC
EC
ETC
ETC
ETC
Climate change treatment
F F M M F F M M F F M M
F M M F F M M F F M M F
Competition treatment
in each chamber

Figure 2

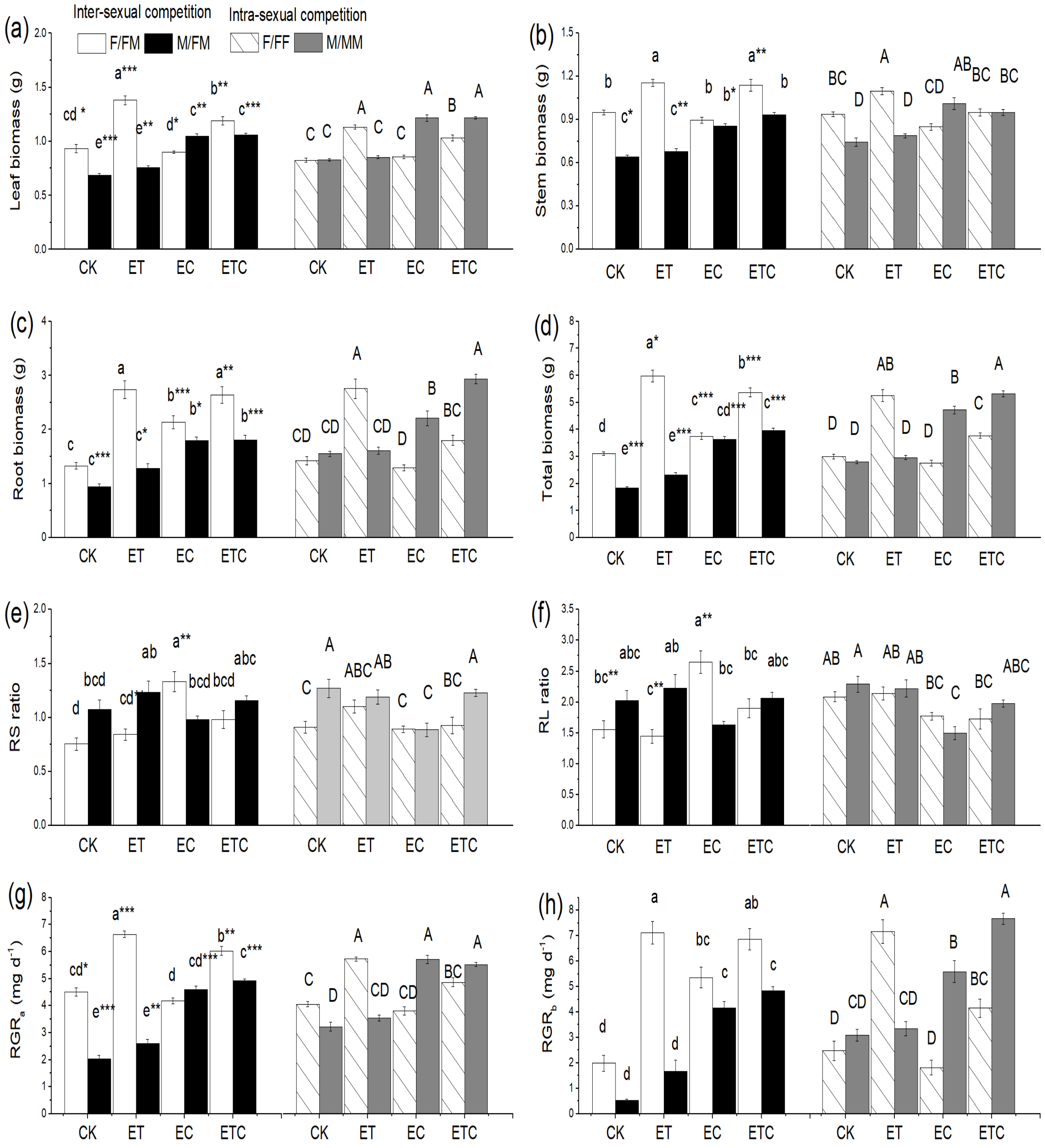
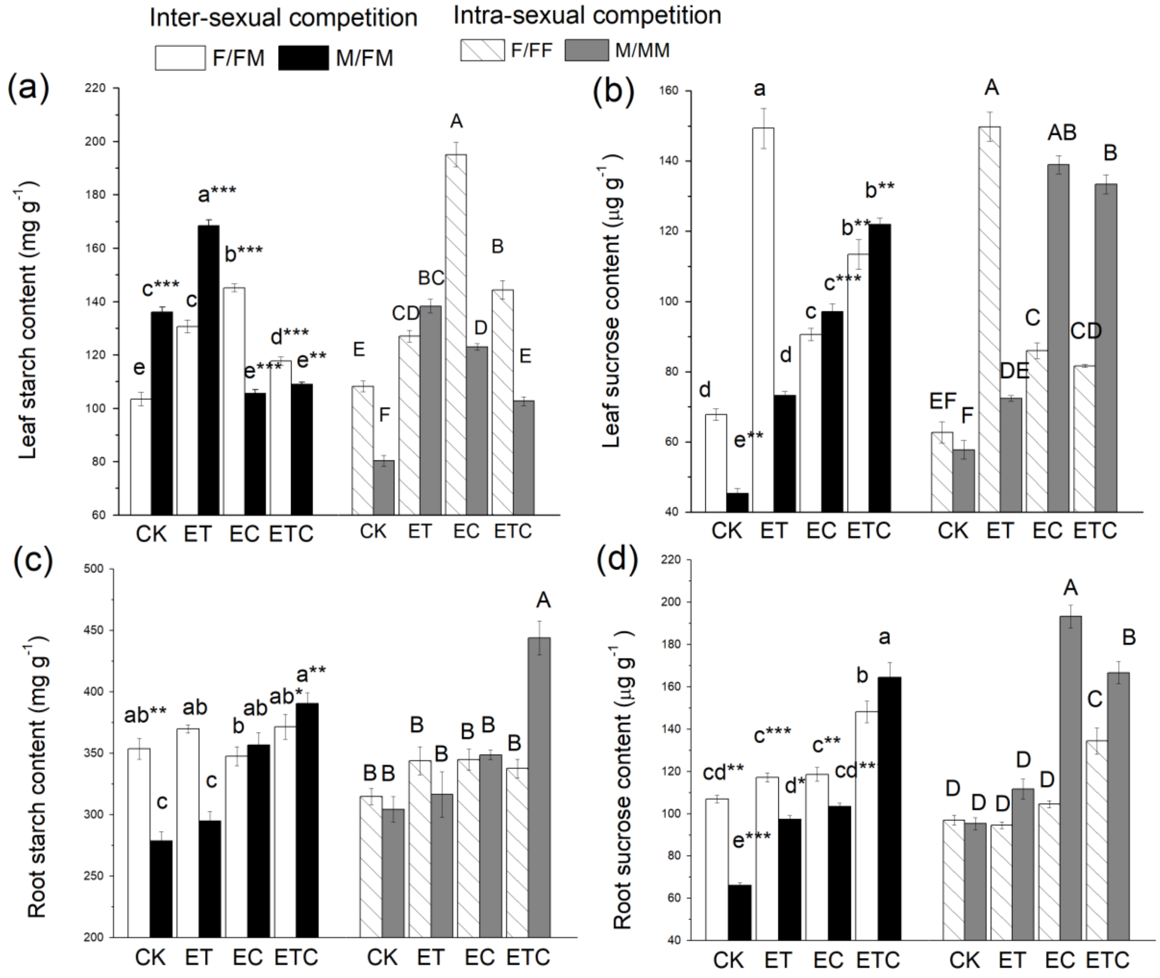
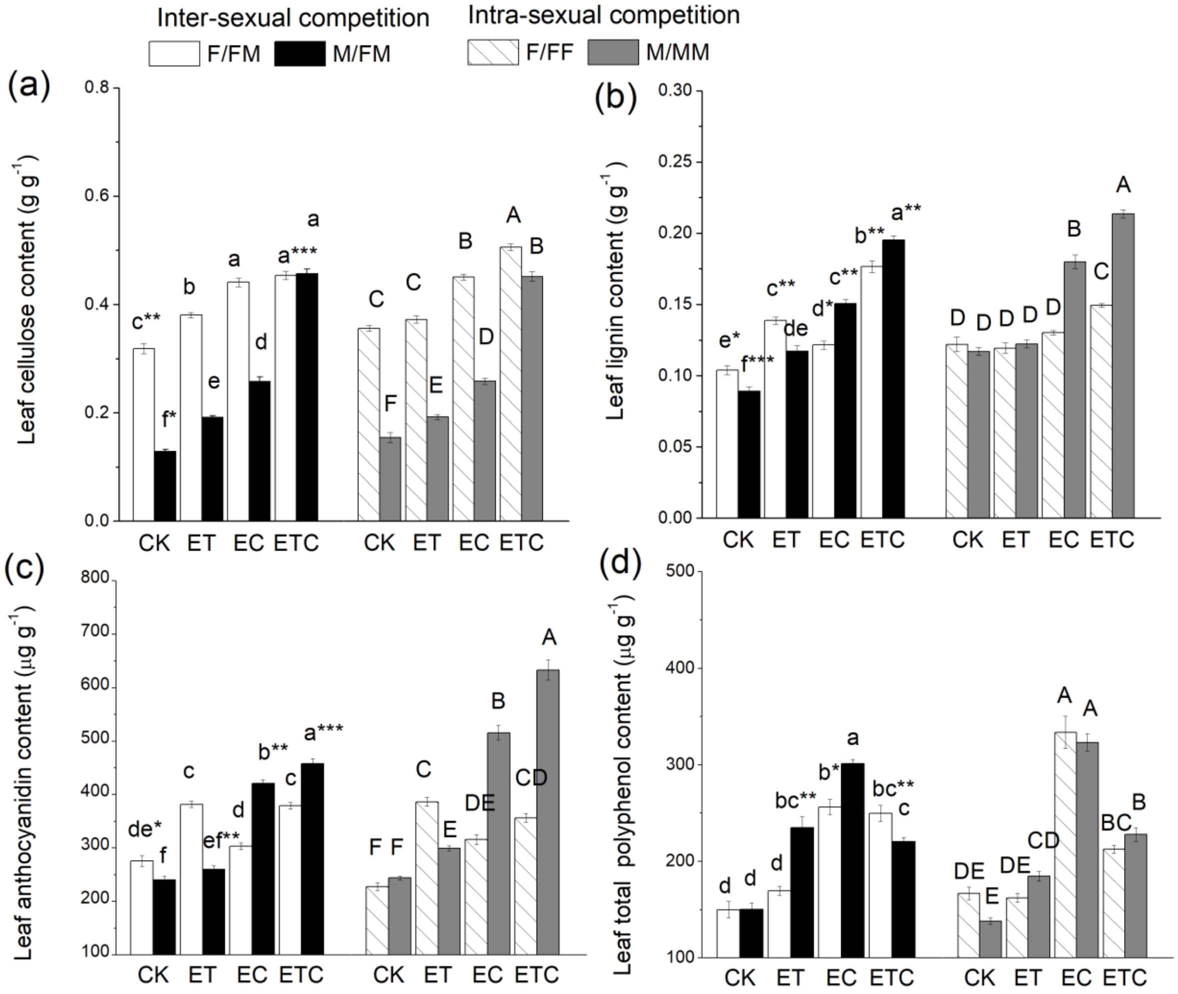

Inter-sexual competition
F/FM
M/FM
Intra-sexual competition
F/FF
M/MM
(a)
Leaf biomass (g)
0.0
0.5
1.0
1.5
2.0
cd *
e***
a***
e**
d*
c**
b**
c***
C
C
A
C
C
A
B
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(b)
Stem biomass (g)
0.0
0.3
0.6
0.9
1.2
1.5
b
c*
a
c**
b
b*
a**
b
BC
D
A
D
CD
AB
BC
BC
CK
ET
EC
ETC
CK
ET
EC
ETC
(c)
Root biomass (g)
0
1
2
3
4
c
c***
a
c*
b***
b*
a**
b***
CD
CD
A
CD
D
B
BC
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(d)
Total biomass (g)
0
1
2
3
4
5
6
7
8
d
e***
a*
e***
c***
cd***
b***
c***
D
D
AB
D
D
B
C
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(e)
RS ratio
0.0
0.5
1.0
1.5
2.0
d
bcd
cd**
ab
a**
bcd
bcd
abc
C
A
ABC
AB
C
C
BC
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(f)
RL ratio
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
bc**
abc
c**
ab
a**
bc
bc
abc
AB
A
AB
AB
BC
C
BC
ABC
CK
ET
EC
ETC
CK
ET
EC
ETC
(g)
RGR_a (mg d^{-1})
0
1
2
3
4
5
6
7
8
cd*
e***
a***
e**
d
cd***
b**
c***
C
D
A
CD
CD
A
BC
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(h)
RGR_b (mg d^{-1})
0
1
2
3
4
5
6
7
8
d
d
a
d
bc
c
ab
c
D
CD
A
CD
D
B
BC
A
CK
ET
EC
ETC
CK
ET
EC
ETC

Figure 3



Inter-sexual competition
F/FM
M/FM
Intra-sexual competition
F/FF
M/MM
(a)
Leaf starch content (mg g^{-1})
60
80
100
120
140
160
180
200
220
e
c***
c
a***
b***
e***
d***
e**
E
F
CD
BC
A
D
B
E
CK
ET
EC
ETC
CK
ET
EC
ETC
(b)
Leaf sucrose content (μg g^{-1})
40
60
80
100
120
140
160
d
e**
a
d
c
c***
b**
b**
EF
F
A
DE
C
AB
CD
B
CK
ET
EC
ETC
CK
ET
EC
ETC
(c)
Root starch content (mg g^{-1})
200
250
300
350
400
450
500
ab**
c
ab
c
b
ab
ab*
a**
B
B
B
B
B
B
B
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(d)
Root sucrose content (μg g^{-1})
40
60
80
100
120
140
160
180
200
220
cd**
e***
c***
d*
c**
cd**
b
a
D
D
D
D
D
A
C
B
CK
ET
EC
ETC
CK
ET
EC
ETC

Figure 4



Inter-sexual competition
F/FM
M/FM
Intra-sexual competition
F/FF
M/MM
(a)
Leaf cellulose content (g g^{-1})
0.0
0.2
0.4
0.6
0.8
c**
f*
b
e
a
d
a***
a
C
F
C
E
B
D
A
B
CK
ET
EC
ETC
CK
ET
EC
ETC
(b)
Leaf lignin content (g g^{-1})
0.00
0.05
0.10
0.15
0.20
0.25
0.30
e*
f***
c**
de
d*
c**
b**
a**
D
D
D
D
D
B
C
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(c)
Leaf anthocyanidin content (μg g^{-1})
100
200
300
400
500
600
700
800
de*
f
c
ef**
d
b**
c
a***
F
F
C
E
DE
B
CD
A
CK
ET
EC
ETC
CK
ET
EC
ETC
(d)
Leaf total polyphenol content (μg g^{-1})
100
200
300
400
500
d
d
d
bc**
b*
a
bc**
c
DE
E
DE
CD
A
A
BC
B
CK
ET
EC
ETC
CK
ET
EC
ETC

Figure 5

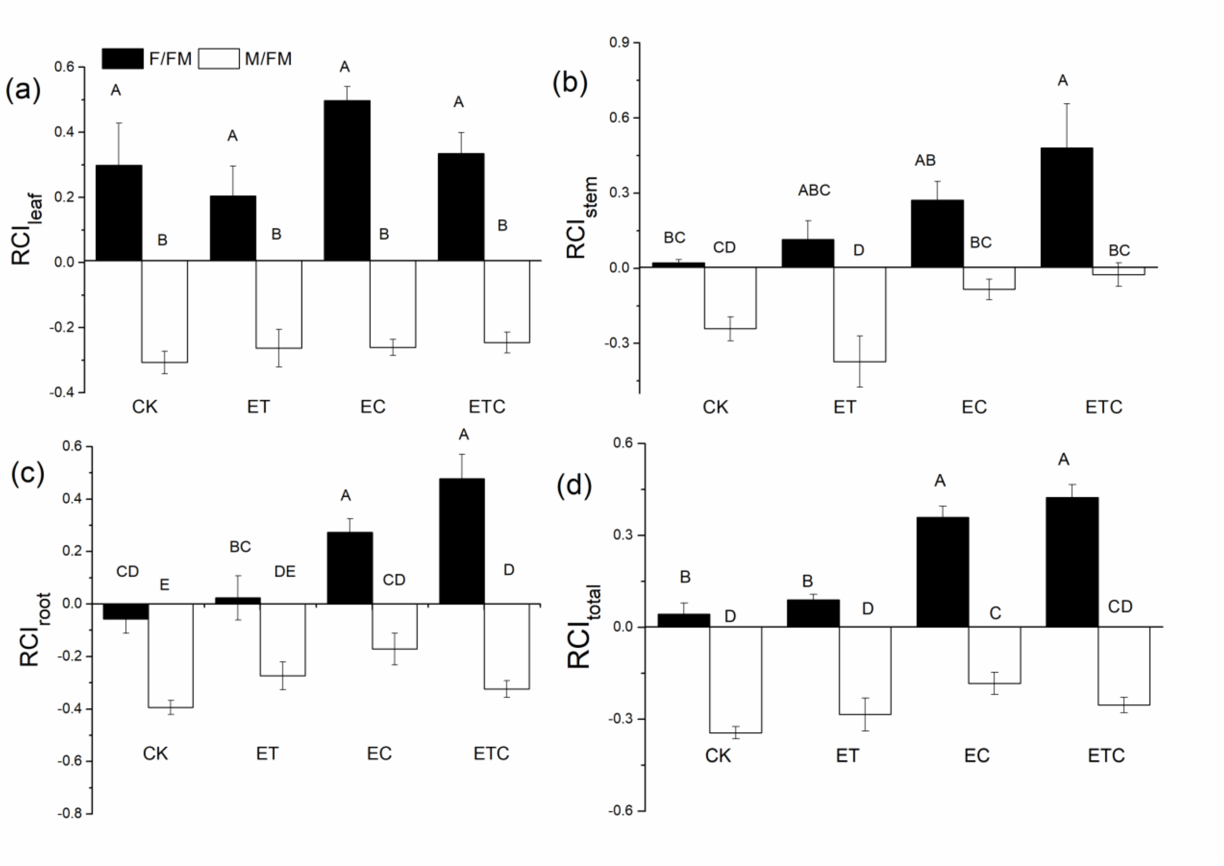
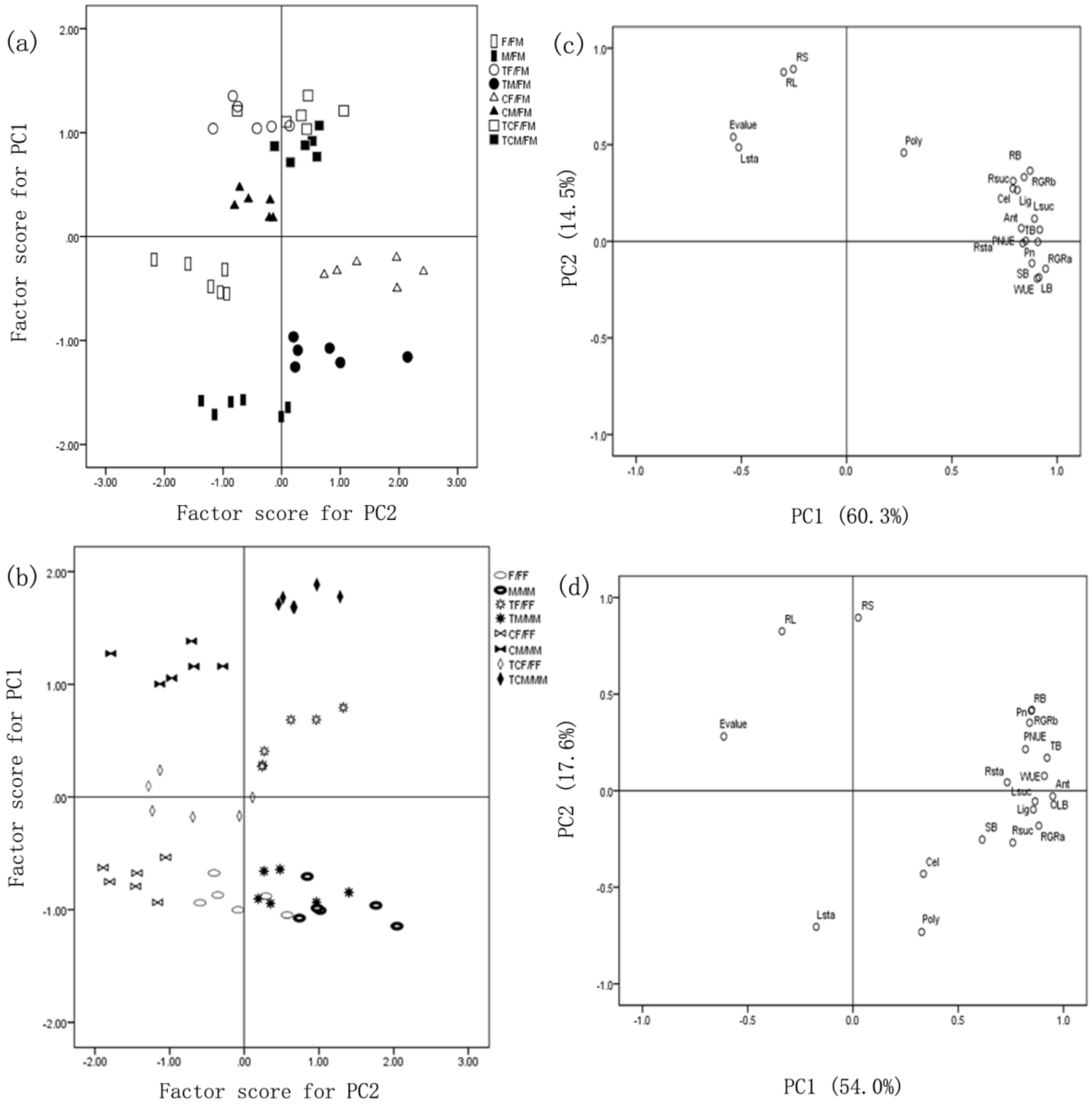

F/FM
M/FM
(a)
RCI$_{leaf}$
0.6
0.4
0.2
0.0
-0.2
-0.4
A
B
A
B
A
B
A
B
CK
ET
EC
ETC
(b)
RCI$_{stem}$
0.9
0.6
0.3
0.0
-0.3
BC
CD
ABC
D
AB
BC
A
BC
CK
ET
EC
ETC
(c)
RCI$_{root}$
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
CD
E
BC
DE
A
CD
A
D
CK
ET
EC
ETC
(d)
RCI$_{total}$
0.6
0.3
0.0
-0.3
-0.6
B
D
B
D
A
C
A
CD
CK
ET
EC
ETC

Figure 6


(a)
Factor score for PC1
Factor score for PC2
2.00
1.00
.00
-1.00
-2.00
-3.00
-2.00
-1.00
.00
1.00
2.00
3.00
F/FM
M/FM
TF/FM
TM/FM
CF/FM
CM/FM
TCF/FM
TCM/FM
(b)
Factor score for PC1
Factor score for PC2
F/FF
M/MM
TF/FF
TM/MM
CF/FF
CM/MM
TCF/FF
TCM/MM
(c)
PC2 (14.5%)
PC1 (60.3%)
1.0
0.5
0.0
-0.5
-1.0
RS
RL
Evalue
Lsta
Poly
RB
Rsuc
RGRb
Cel
Lig
Lsuc
Ant
TB
PNUE
Rsta
Pn
RGRa
SB
WUE
LB
(d)
PC2 (17.6%)
PC1 (54.0%)
RS
RL
Evalue
RB
Pn
RGRb
PNUE
TB
Rsta
WUE
Ant
Lsuc
Lig
LB
SB
Rsuc
RGRa
Cel
Lsta
Poly