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Sex-specific competition differently regulates ecophysiological responses and phytoremediation of *Populus cathayana* under Pb stress

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Abstract
Background and aims Lead (Pb) contamination seriously threatens agroforestry production and safety. We aim to determine the interactive influence of Pb and sexual competition on the growth performance, photosynthetic and biochemical traits, ultrastructure and phytoremediation-related parameters of males and females.
Methods In the present study, eco-physiological responses and phytoremediation traits of *Populus cathayana* females and males were evaluated under interactive treatments of Pb and competition.
Results There were significant sex-specific competition effects on biomass partition, photosynthetic activities, carbohydrate contents, nitrogen and phosphorus use efficiencies, ultrastructure and phytoremediation under Pb stress. When competition within the same sex was compared, females were more sensitive to Pb stress, while males possessed greater Pb contents, and a higher bioconcentration factor and tolerance index. Under inter-sexual competition, males alleviated competition effects through greater Pb absorption, and lower photosynthetic rates, nutrient use efficiencies and biomass accumulation. Moreover, Pb stress altered competition intensities of both sexes.
Conclusions Sex-specific competition and neighbor effects may regulate responses and phytoremediation under heavy metal stress in dioecious plants. In the future, more attention should be paid on the effects of inter- and intra-sexual competition on dioecious species in the process of forestation and restoration of contaminated soil.

Keywords Dioecy · Competition · Lead · Photosynthetic capacity · Phosphorus and nitrogen use efficiency · Carbohydrates

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Introduction

Dioecy exists in 157 flowering plant families and about 6% of the 240,000 angiosperm species are dioecious (Renner and Ricklefs 1995). It is well known that spatial segregation of the sexes (SSS) is associated with micro-habitat differences in dioecious species (Onyekwelu and Harper 1979; Korpelainen 1991; Dudley 2006; Li et al. 2007). Females are more common in favorable habitats, while males are more common in habitats with limited

resources (Dawson and Ehleringer 1993; Stehlik et al. 2008). Many morphological, physiological and ecological differences between sexes have been observed in dioecious plants under environmental stresses, including, e.g., drought, salinity, nutrient deficiency and heavy metal stress (Xu et al. 2008; Chen et al. 2010; Chen et al. 2011; Zhang et al. 2014; Juvany and Munné-Boch 2015). However, these sex-related studies have rarely considered biotic factors, such as competition. Competition causes significant effects on plant growth, species diversity, community structures and nutrient cycles in local ecosystems. The type of plant–plant competition (positive, negative or neutral) frequently shifts along with different environmental factors (Fargione et al. 2003; Liancourt et al. 2005; Maestre et al. 2009). Tilman's competition theory (1982) suggests that competition is stronger at low nutrient levels rather than at high nutrient levels, while the stress gradient hypothesis (SGH) suggests that plants may negatively compete for resources in a productive environment but may positively compete with neighbors in a severe environment (Bertness and Callaway 1994).

The classic ecological niche theory predicts that the real niche is always smaller than the fundamental niche due to competitive effects (Dybzinski and Tilman 2007; Bloor et al. 2008). Herrera (1988) observed that sex-specific competition of *Osyris quadripartite* is asymmetrical, and males are inhibited more when competing with neighboring females. Nanami et al. (2005) showed that both intra- and inter-sexual competition effects on growth rates are significant in female *Podocarpus nagi* trees, while Sánchez-Vilas et al. (2011) found that both intra- and inter-sexual competition affect the aboveground growth of males but not the growth of females in *Mercurialis annua*. Therefore, sex-related responses to competition are species-specific, possibly related to the presence of neighboring competitors (Nanami et al. 2005; Zhang et al. 2009) and to given environmental conditions (Eppley 2006). However, sex-related competitive interactions and neighbor effects under abiotic stresses have been largely neglected in studies on dioecious plants (Herrera 1988; Eppley 2006; Rogers and Eppley 2012). Moreover, previous studies have mostly focused on changes in growth and morphology instead of exploring intrinsic physiological processes under different competition patterns (Nicotra 1999; Eppley 2006).

Heavy metals seriously threaten the growth and survival of plants and there may be special consequences on dioecious species with sex-specific responses (Chen et al. 2011; Han et al. 2013; Jiang et al. 2013). Lead (Pb) contamination has become a serious problem in agroforestry production and safety in China due to high toxicity and long-lasting persistence in soil (Shahid et al. 2011). Recent studies have indicated that some dioecious trees have potential for phytoremediation, i.e. removal of soil contaminants (Han et al. 2013; He et al. 2013; Ermakov et al. 2015; Qasim et al. 2016). However, it remains unclear, whether there are interactive effects between heavy metal stress and sexual competition on ecophysiological responses and phytoremediation traits of dioecious species. It is well known that intrinsic physiological processes as well as extrinsic growth traits can reflect the response mechanism of plants to heavy metal stress. Photosynthesis inhibition and damage to leaf ultrastructure are common symptoms of Pb toxicity (Kalaji and Loboda 2007; Islam et al. 2008; Han et al. 2013). Proper N application may alleviate toxic effect by increasing stromal proteins and photosynthetic capacity; moreover, non-structural carbohydrate storage can enhance plants' ability to resist stress conditions (Myers and Kitajima 2007; O'Brien et al. 2014). It is recognized that the bioconcentration factor (BCF), translocation factor (TF) and tolerance index (TI) can describe the extent of the phytoremediation ability (Baker et al. 1994; Zayed et al. 1998). Therefore, these ecophysiological parameters can be used as indexes to evaluate plant adaptation and phytoremediation under Pb stress.

Populus cathayana Rehd, a dioecious species widely distributed in China, has a high economical value and an important ecological role in local ecosystems. In the present study, we hypothesize that the responses of *P. cathyana* males and females under Pb stress might be affected by sexual competition, which may explain spatial segregation of sexes in heavy metal contaminated regions. To test these hypotheses, our study aims (i) to determine the interactive influence of Pb and sexual competition on the growth performance, photosynthetic and biochemical traits, ultrastructure and phytoremediation-related parameters of males and females, (ii) to evaluate, whether a specific sex of neighbors changes the responses and phytoremediation traits of *P. cathayana* under Pb stress, and (iii) to investigate, whether Pb stress changes competition intensities and interactions between the two sexes.

Materials and methods

Experimental design

The experiment followed a completely randomized design with three factorial combinations, including sex, competition and Pb stress. Two Pb treatments (0, 500 μM), two sexes (females and males) and three competition patterns (female × female, FF; female × male, FM; male × male, MM) were employed. Twenty replicates per treatment were included in the experiment. On 19 March 2015, 240 healthy cuttings (120 females and 120 males) of *P. cathayana* were chosen and transplanted into 30-L plastic pots filled with 25 Kg homogenized brown soil (two cuttings per pot; two females, two males, or a female and a male). After 8 weeks of growth, the plants were subjected to Pb stress for 12 weeks. In the treatment, Pb was applied into soil by evenly adding 5.33 mmol L^{-1} $Pb(NO_3)_2$ solution to the pots every day during the first 15 days of the treatment. The final Pb level was 500 μM Pb kg^{-1} dry soil. The Pb concentrations used in this study were chosen according to the Pb content of soil around the neighbouring lead mines. 12 g of slow-release fertilizer (13% N, 10% P and 14% K) was added to each pot during the experiment. The treatments started on 15 May 2015, and the plants were harvested on 15 August 2015.

Growth measurements

At the end of the experiment, five cuttings from each sex and treatment were used for the biomass measurements. The cuttings were harvested and separated into leaves, stems and roots, and separately oven-dried at 70 °C for 48 h to constant weight and weighed. Total dry matter weight (TDW) equals the sum of leaf, stem and root dry matter weight. The biomass partition of leaves, stems and roots equals the ratio of dry matter weight of leaves, stems and roots to total dry matter weight, as expressed respectively as LDW/TDW, SDW/TDW and RDW/TDW.

Gas exchange measurements

The photosynthetic gas exchange measurements were conducted for the fourth fully expanded and intact leaf from five randomly chosen individuals from each treatment between 08:00 and 11:30 a.m. in July 2015 using a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA). The saturated photosynthetic photon flux density (PPFD) was determined by preliminary experiments. Prior to measurements, the samples were illuminated with saturated PPFD provided by the LED light source for 10–30 min to achieve a full photosynthetic induction. The net photosynthetic rate (P_n) and internal CO_2 concentration of leaves (C_i) were measured under following conditions: leaf temperature, 25 °C; leaf-to-air vapour pressure deficit, 1.5 ± 0.5 kPa; PPFD, 1500 μmol m^{-2} s^{-1}; relative air humidity, 50%; and ambient CO_2 concentration, 400 ± 5 μmol mol^{-1}. Once the apparent steady-state gas exchange was achieved, the steady-state data were recorded. The net photosynthetic responses to PPFD (P_n -PPFD curve) were measured at 0, 50, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, 1500, 1800 μmol m^{-2} s^{-1}. Response curves were modeled by a non-rectangular hyperbola, as described by Prioul and Chartier (1977). The light-saturated photosynthetic rate (P_{max}) and dark respiration rate (R_D) were determined by fitting data to the model function, and the light compensation point (LCP) was derived by the linear regression of P_n against PPFD. For the response curves of P_n to C_i (P_n- C_i curve), leaves were first exposed to their ambient CO_2 concentration until steady-state gas exchange was observed. CO_2 concentration was then reduced stepwise to 0 μmol mol^{-1} (400, 300, 200, 150, 100, 50, 0) and increased stepwise to 1500 μmol mol^{-1} (400, 600, 800, 1000, 1200 and 1500 μmol mol^{-1}). The net photosynthetic rate versus C_i curves over the range of 0–200 μmol $mol^{-1}$was determined, and the regression of CO_2 and P_n was used to determine the CO_2 compensation point (CCP) and carboxylation efficiency (CE) (Olsson and Leverenz 1994). The maximum rate of electron transport driving regeneration of RuBP (J_{max}) and maximum rate of RuBP carboxylation (V_{cmax}) were determined according to Long and Bernacchi (2003).

Determination of pigment and total polyphenol contents of leaves

Pigments were determined for the same leaves, which were used for gas exchange measurements, employing the methods described by Lichtenthaler (1987). The leaf

samples were extracted in 80% chilled acetone (*v*/v). Absorbance of extracts was measured using spectrophotometry (Unicam UV-330; Unicam, Cambridge, UK) at 470, 646 and 663 nm. The total chlorophyll content was expressed as mg g^{-1} on the basis of fresh weight (FW). The total chlorophyll content ($chlorophyll_{ab}$) was the sum of chlorophyll a and chlorophyll b. The total polyphenol content was determined by the Folin-Ciocalteu method using gallic acid as a standard (Julkunen-Tiitto 1985).

Determination of starch and soluble sugars in leaves and roots

About 50 mg of dried fine powder from leaf and root samples in 4 ml 80% (v/v) ethanol was placed in a water bath at 80 °C for 30 min, followed by centrifugation at 7000 g for 5 min, after which the supernatant was transferred to a 10-ml centrifuge tube. Then, another 2 ml 80% (v/v) ethanol was added to the sample, followed by centrifugation at 7000 g for 5 min. This procedure was repeated again and the supernatants were pooled into the first 10-ml centrifuge tube. The content of total soluble sugars was detected colorimetrically at 625 nm following the anthrone–sulfuric acid method. Residues left in the centrifuge tubes after sugar extraction were used to determine starch in glucose equivalents by using the anthrone reagent (Yemm and Willis 1954).

Determination of N and P contents in leaves and roots

Dried leaf and root samples (0.2 g) were used for N and P content measurements. N was determined by the semimicro Kjeldahl method (Mitchell 1998) and P was determined by induced plasma emission spectroscopy (Hötscher and Hay 1997). Photosynthetic N-use efficiency (PNUE) is expressed as P_{max} divided by the foliar N content per area. Photosynthetic P-use efficiency (PPUE) is expressed as P_{max} divided by the foliar P content per area.

Determination of Pb content and phytoremediation-related index

Dried leaf, stem and root samples were ground to fine powder and passed through a 100-mesh screen. The determination of the Pb content was made by graphite furnace atomic absorption spectrometry (Analyst 300; Perkin Elmer, Uberlingen, Germany). The bioconcentration factor (BCF) is defined as the ratio of the metal concentration in the plant to the metal concentration in the soil (Zayed et al. 1998). The translocation factor (TF) is used to express the ability of plants to translate heavy metals from roots to harvestable aerial parts, and it is the ratio of the metal concentration in aboveground parts to the metal concentration in roots. The tolerance index (TI) is calculated as the mean dry matter weight of a plant grown under metal stress conditions divided by the mean weight of a control plant (Baker et al. 1994).

Transmission electron microscopy observations

Transmission electron microscopy (TEM) was performed on small sections (1–2 mm in length) of the fifth fully expanded leaves. Leaflets 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 a graded ethanol series (50, 60, 70, 80, 90, 95 and 100%) and embedded in epon-araldite. Ultrathin sections (80 nm) were sliced, stained with uranyl acetate and lead citrate, and mounted on copper grids for viewing in H-600IV TEM (Hitachi, Tokyo, Japan).

Analysis of relative competitive intensity

The relative competitive intensity (RCI) of males and females when exposed to different competition and Pb treatments was determined, according to the following formula described by Grace (1995): $RCI = (B_{inter} - B_{intra})/B_{intra}$.

where B_{inter} represents the leaf, stem, root and total dry matter weight of an individual from inter-sexual competition, and B_{intra} represents the leaf, stem, root and total dry matter weight of corresponding seedlings from intra-sexual competition.

Statistical analysis

All measurements were tested by a three-way ANOVA for the effects of sex, Pb and competition by using the SPSS 16.0 for windows statistical software package (SPSS, Inc., Chicago, IL, USA). Before ANOVAs, the

data were checked for normality and the homogeneity of variances and log-transformed to correct deviations from these assumptions when needed. Differences between treatments were tested using Tukey's test at a significance level of $P < 0.05$.

Results

Sex-specific competition induced responses to Pb in biomass accumulation and partition

Competition differently affected the biomass accumulation and partition of females and males exposed to Pb stress. The Pb treatment significantly decreased biomass accumulation in both sexes (Fig. 1a, c): in F/FM, F/FF, M/FM and M/MM, the total biomass decreased by 26.98%, 21.09%, 18.45% and 16.94%, respectively, and the root biomass decreased by 35.75%, 30.88%, 4.68% and 14.82%, respectively, which indicates a greater Pb-induced inhibition effect on females than males. Moreover, in the Pb treatment, females showed a lower root biomass and R/S ratio than did males from intra- and inter-sexual competition. Concerning biomass partition, the LDW/TDW and SDW/TDW ratios of both sexes from different competition patterns showed no significant changes under a Pb treatment compared with controls. However, under Pb stress, the RDW/TDW and R/S ratios of F/FM and F/FF significantly decreased (Fig. 1b, d), while they increased in M/FM. The statistical analysis showed that the interaction of sex × Pb significantly

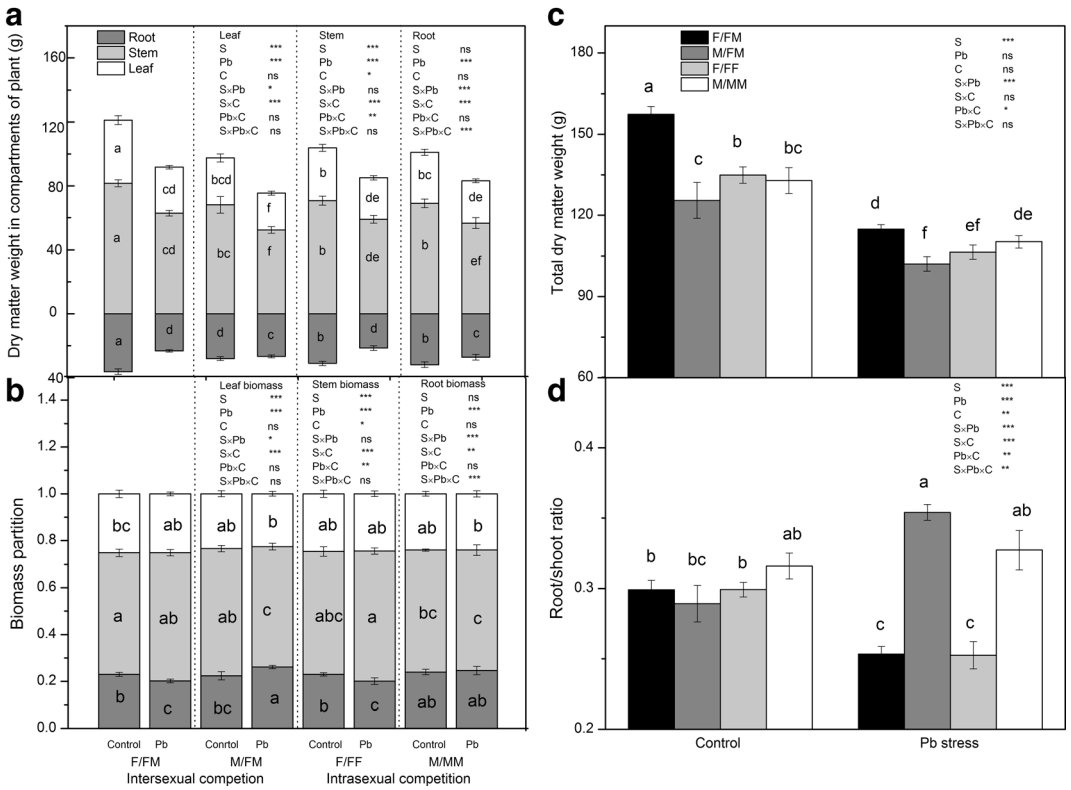


Fig. 1 Biomass accumulation and allocation traits of *P. cathayana* males and females exposed to Pb and sexual competition. (**a**) Dry matter weight in different parts of plants, (**b**) biomass partition, (**c**) total dry matter weight, (**d**) root/shoot ratio. Different letters above bars denote statistically significant differences between treatments at P < 0.05 level according to Tukey's test. Values are expressed as means ± SE, $n = 5$. *0.01 ≤ P < 0.05; **0.001 ≤ P < 0.01; ***P < 0.001; ns, nonsignificant. Sex, sex effect; Pb, Pb effect; C, competition effect; sex × Pb, and sex × C the interaction effect of sex and Pb, and sex and competition, respectively. Pb × C, the interaction effect of Pb and competition; sex × Pb × C, the interaction effect of sex, Pb and competition

affected leaf, root and total biomass, LDW/TDW, RDW/TDW and R/S ratios, and the sex × competition interaction significantly affected all growth parameters, except for the total biomass. The interaction of Pb × competition significantly affected the stem and total biomass, SDW/TDW and R/S ratio. The interaction of sex × Pb × competition significantly affected the root biomass, RDW/TDW and R/S ratio (Fig. 1).

Sex-specific competition induced responses to Pb in gas exchange

The sex-specific competition patterns induced different photosynthetic responses to Pb stress in the two sexes. As shown in Table 1, significant decreases in P_{max} (range 36.7–57.4%) were detected in both sexes under Pb stress, especially in females from intra-sexual competition (57.4%) compared with the controls. Under inter-sexual competition, males showed lower P_{max} than females under both control and Pb treatments. Among all competition patterns, F/FF showed the highest LCP and R_D values, while M/MM had the highest P_{max} and lowest LCP, CCP and R_D in control conditions. The Pb treatment significantly inhibited CE and increased CCP of both sexes, except for F/FM. The Pb treatment significantly inhibited LCP, V_{cmax} and J_{max} of both sexes. Among all competition patterns, females from intra-sexual competition showed the highest R_D and lowest CE values under Pb conditions. The statistical analysis showed that the interaction of sex × Pb significantly affected CE, LCP, R_D, V_{cmax} and J_{max}, and sex × competition significantly affected P_{max}, CE, CCP, LCP and R_D. The interaction of Pb × competition significantly affected P_{max}, CCP, V_{cmax} and J_{max}, and the interaction of sex × Pb × competition significantly affected P_{max}, CE, CCP, LCP, R_D, V_{cmax} and J_{max}.

In control conditions and under inter-sexual competition, females showed higher PNUE and PPUE than did males. No significant sex-related differences were observed in PNUE and PPUE under intra-sexual competition. The Pb treatment significantly decreased PNUE and PPUE in both sexes, especially in M/FM and F/FF (Fig. 2a, b). The interaction of sex × competition significantly affected PNUE and PPUE, while the interaction of sex × Pb × competition significantly affected PNUE.

Sex-specific competition induced responses to Pb in pigment and total polyphenol contents

In control conditions and under inter-sexual competition, females possessed a higher total $chlorophyll_{ab}$ content than did males. No significant difference in total $chlorophyll_{ab}$ contents was observed in males and females under intra-sexual competition. The Pb treatment significantly decreased the total $chlorophyll_{ab}$ content, especially in F/FF (Fig. 2c). The Pb stress significantly increased the total polyphenol content of both sexes, but males showed greater increases in all competition treatments (Fig. 2d). The interaction of sex × competition and sex × Pb significantly affected total $chlorophyll_{ab}$ and polyphenol contents.

Sex-specific competition induced responses to Pb in nutrient elements and carbohydrates

In control conditions and under inter-sexual competition, females showed higher P and N contents in leaves and roots than did males. The Pb treatment had no significant effect on the root and leaf P content of either sex under intra-sexual competition when compared with the controls. The combined treatment of inter-sexual competition and Pb stress caused no significant effect on the root P content, while it changed the leaf P content of both sexes, more in males than females. Pb stress increased the leaf N contents of F/FF and M/FM and decreased the root N content of females, while it had no significant effect on males under intra- and inter-sexual competition (Fig. 3a, b). The interaction of sex × Pb significantly affected leaf P and root P contents and the root N content. The interaction of sex × competition significantly affected the root N content, and the interaction of sex × Pb × competition significantly affected the root P and leaf N content.

In control conditions, F/FM showed the lowest leaf starch content among all competition treatments. The Pb stress increased leaf starch contents of both sexes, except for M/MM, and increased also root starch contents of both sexes, except for F/FF. Moreover, F/FF had the highest leaf starch content, while M/MM had the highest root starch content among all competition patterns. The Pb treatment significantly increased the soluble sugar contents of leaves and roots of both sexes, except for F/FF and M/MM, respectively. In addition, M/FM possessed higher levels of leaf and root soluble sugars than

Table 1 Photosynthetic parameters of *P. cathayana* males and females exposed to Pb and competition

Treatment	Competition	P_{max} (μmol m^{-2} s^{-1})	CE (mol m^{-2} s^{-1})	CCP (μmol mol^{-1})	LCP (μmol m^{-2} s^{-1})	R_D (μmol m^{-2} s^{-1})	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})
Control	F/FM	29.55 ± 0.72b	0.033 ± 0.003cde	174.10 ± 2.18b	34.67 ± 1.11de	2.28 ± 0.20c	52.62 ± 0.36ab	55.56 ± 0.38ab
	M/FM	21.29 ± 0.88d	0.067 ± 0.003a	82.41 ± 1.21f	52.99 ± 0.77b	2.45 ± 0.22c	46.17 ± 0.53c	48.67 ± 0.57c
	F/FF	25.51 ± 0.71c	0.038 ± 0.003bc	144.02 ± 1.08d	72.92 ± 0.72a	3.74 ± 0.07a	50.63 ± 0.86b	53.24 ± 0.90b
	M/MM	34.27 ± 0.67a	0.048 ± 0.003b	69.91 ± 1.10g	22.12 ± 0.56g	1.46 ± 0.05d	54.69 ± 0.46a	57.46 ± 0.50a
Pb stress	F/FM	18.69 ± 0.44d	0.024 ± 0.002de	165.66 ± 3.16c	27,53 ± 0.37 fg	2.29 ± 0.19c	38.92 ± 1.22d	40.86 ± 1.29d
	M/FM	11.83 ± 1.02e	0.034 ± 0.003cd	96.79 ± 0.94e	39.19 ± 1.45cd	2.61 ± 0.11bc	27.62 ± 0.47e	28.87 ± 0.53e
	F/FF	10.88 ± 0.78f	0.022 ± 0.002e	195.67 ± 1.54a	45.42 ± 3.44c	3.09 ± 0.05b	26.04 ± 0.49e	27.32 ± 0.54e
	M/MM	17.31 ± 0.72d	0.032 ± 0.003cd	90.30 ± 0.50e	32.34 ± 0.45ef	2.07 ± 0.08c	39.13 ± 1.13d	41.09 ± 1.19d
P	Sex	ns	***	***	***	***	ns	ns
	Pb	***	***	***	***	ns	***	***
	C	***	*	***	***	*	*	*
	Sex × Pb	ns	**	ns	***	***	***	***
	Sex × C	***	**	***	***	***	ns	ns
	Pb × C	***	ns	***	ns	ns	**	**
	Sex ×Pb × C	***	**	***	***	**	***	***

Each value is the mean ± SE (n = 5). Values followed by a different letter in the same column denote statistically significant differences between treatments at $P < 0.05$ level according to Tukey's test. Sex, sex effect; Pb, Pb effect; C, competition effect; sex × Pb, interaction effect of sex and Pb; sex × C, interaction effect of sex and competition; Pb × C, interaction effect of Pb and competition; sex ×Pb × C, interaction effect of sex, Pb and competition

ns, non-significant; *F/FM and M/FM*, females and males from inter-sexual competition; *F/FF and M/MM*, females and males from intra-sexual competition

*$0.01 \le P < 0.05$, **$0.001 \le P < 0.01$, ***$P < 0.001$

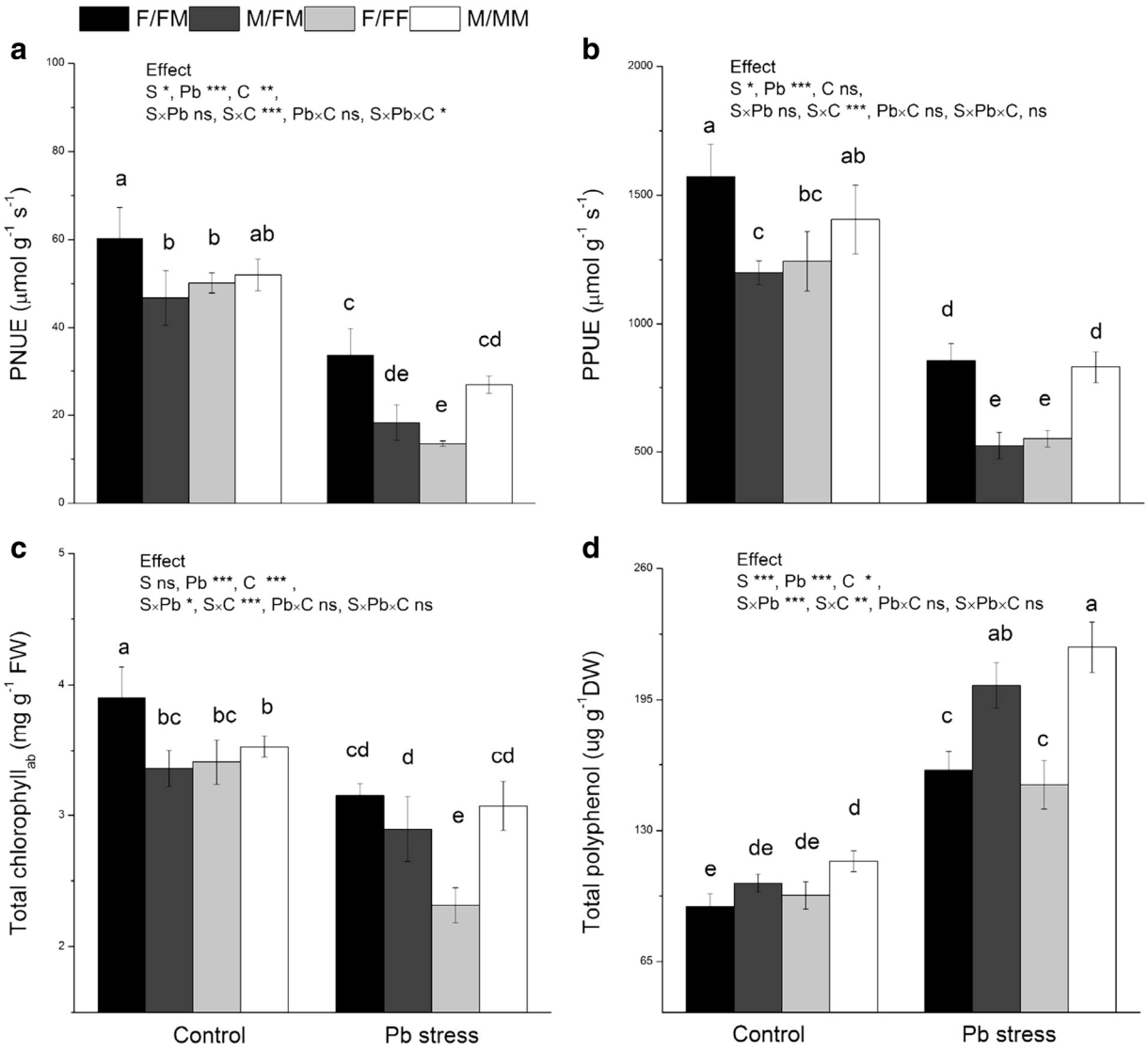


Fig. 2 Leaf-related traits of *P. cathayana* males and females exposed to Pb and sexual competition. (**a**) Photosynthetic nitrogen use efficiency (PNUE), (**b**) photosynthetic phosphorus use efficiency (PPUE), (**c**) total chlorophyll$_{ab}$ content, (**d**) total polyphenol content. Data presentation and statistical significances as in Fig. 1

F/FM. The interaction of sex × Pb, sex × competition and sex × Pb × competition significantly affected leaf starch and root soluble sugar contents. Additionally, the interaction of sex × Pb, sex × competition and Pb × competition significantly affected root starch and leaf soluble sugar contents.

Sex-specific competition induced responses to Pb uptake and phytoremediation parameters

Competition patterns significantly affected the Pb uptake and phytoremediation parameters (Table 2). Under Pb treatment and inter-sexual competition, females showed higher levels of leaf and stem Pb and TF but lower root and total Pb contents when compared to males. No significant differences were observed between the sexes in root and total Pb contents under intra-sexual competition. In addition, females from intra- and inter-sexual competition showed higher leaf and stem Pb contents, BCF_{leaf} and BCF_{stem} when compared to males, while M/FM showed the highest root Pb content, BCF_{root}, BCF_{total} and TI_{root} among Pb treatments. However, males and females had no significant differences in TI_{leaf} and TI_{stem}.

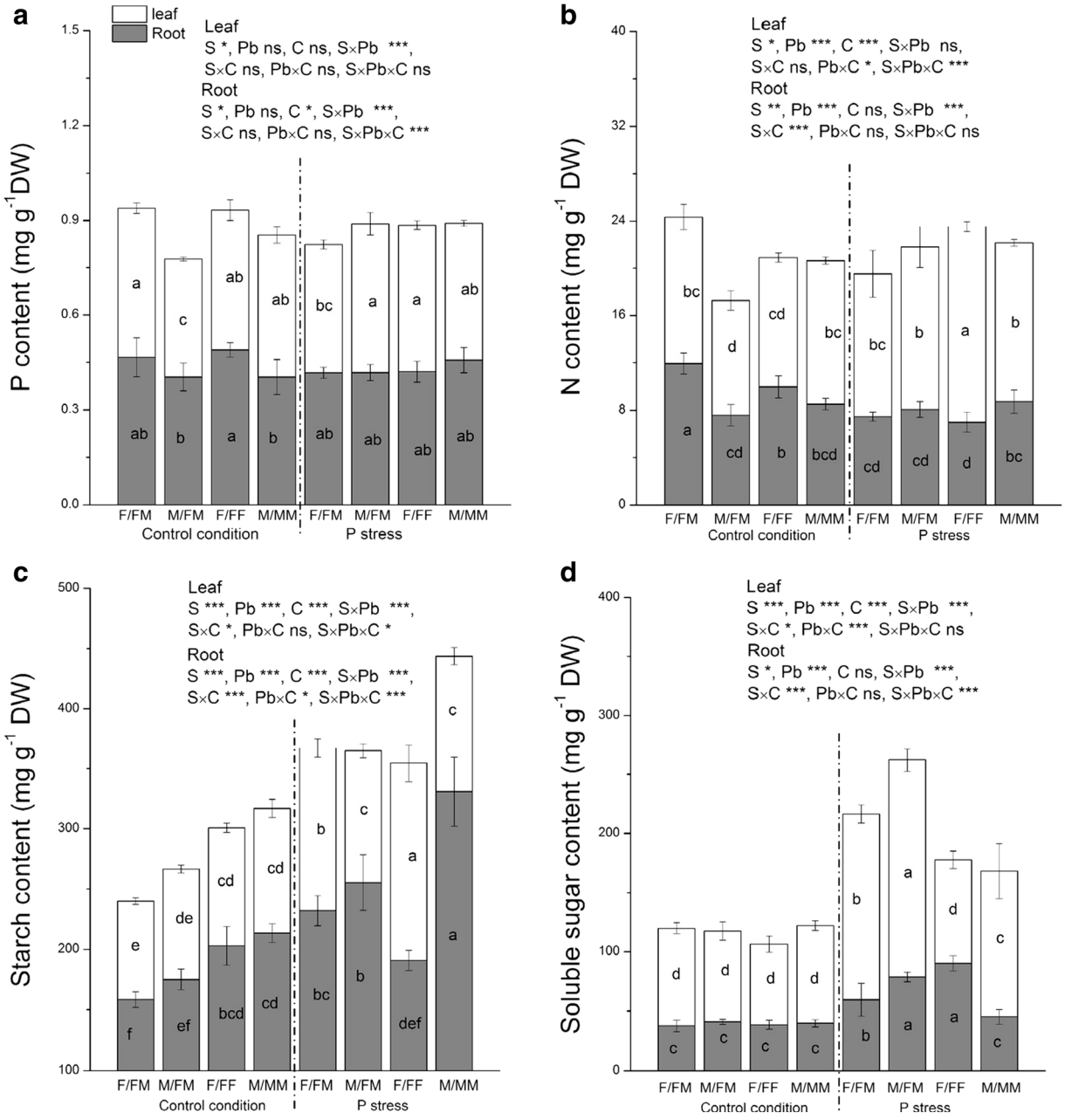


Fig. 3 Nutrient elements and carbohydrates in leaves and roots of *P. cathayana* males and females exposed to Pb and sexual competition. (**a**) P content, (**b**) N content, (**c**) starch content and (**d**) soluble sugar content. Data presentation and statistical significances as in Fig. 1

Sex-specific competition induced responses to Pb in cellular ultrastructure

Sex-related differences in cellular ultrastructure are presented in Fig. 4. In control conditions, both females and males had smooth, clean and continuous cell membranes, and a granular cytoplasm densely filled with numerous organelles. The chloroplasts exhibited a lenticular shape, and mitochondria had a typical structure and clear cristae. The combination of Pb and competition had severe effects on the ultrastructure of leaf cells in both sexes, such as abnormal cell walls, and seriously distorted chloroplasts with large starch granules and disintegrated

Table 2 Pb content, bioconcentration factor (BCF), tolerance index (TI) and translation factor (TF) of *P. cathayana* females and males exposed to Pb and competition

	Pb treatment			
	Inter-sexual competition		Intra-sexual competition	
Parameter	F/FM	M/FM	F/FF	M/MM
Leaf Pb (mg g^{-1} DW)	16.73 ± 1.20a	12.31 ± 1.57bc	14.54 ± 1.35ab	11.56 ± 0.69c
Stem Pb (mg g^{-1} DW)	12.28 ± 0.89a	9.58 ± 1.26b	13.71 ± 0.79a	10.51 ± 0.62b
Root Pb (mg g^{-1} DW)	24.47 ± 4.01c	76.92 ± 7.79a	53.53 ± 3.59b	55.75 ± 10.13b
Total Pb (mg g^{-1} DW)	15.86 ± 0.32c	27.76 ± 1.05a	21.95 ± 0.48b	21.94 ± 1.31b
BCF_{leaf}	0.16 ± 0.01a	0.12 ± 0.02bc	0.14 ± 0.01a	0.11 ± 0.01c
BCF_{stem}	0.12 ± 0.01a	0.09 ± 0.01b	0.13 ± 0.01a	0.10 ± 0.01b
BCF_{root}	0.24 ± 0.04c	0.74 ± 0.08a	0.52 ± 0.03b	0.54 ± 0.10b
BCF_{total}	0.15 ± 0.000c	0.27 ± 0.02a	0.21 ± 0.01b	0.21 ± 0.03b
TI_{leaf}	0.16 ± 0.01a	0.12 ± 0.02a	0.14 ± 0.01a	0.11 ± 0.01a
TI_{stem}	0.12 ± 0.01a	0.09 ± 0.01a	0.13 ± 0.01a	0.10 ± 0.01a
TI_{root}	0.64 ± 0.05c	0.95 ± 0.05a	0.69 ± 0.06c	0.85 ± 0.05b
TI_{total}	0.15 ± 0.01b	0.27 ± 0.02a	0.21 ± 0.01ab	0.21 ± 0.03a
TF	1.22 ± 0.25a	0.29 ± 0.02c	0.53 ± 0.05b	0.41 ± 0.08bc

Each value is the mean ± SE (n = 5). Values followed by a different letter in the same row denote statistically significant differences between treatments at $P < 0.05$ level according to Tukey's test

mitochondrial cristae (Fig. 4c-f). Females from intra-sexual competition and males from inter-sexual competition possessed fewer normal and more deformed chloroplasts, incorrectly arranged inner membranes, vacuolization and decreased organellar density in the cytoplasm compared with other competition patterns when exposed to Pb stress (Fig. 4d, e).

Analysis of competition index

As shown in Fig. 5, females from inter-sexual competition showed positive values, while neighboring males had negative values of RCI_{leaf}, RCI_{stem}, RCI_{root} and RCI_{total} under control and Pb treatments. This means that females grow better than do males under inter-sexual competition. Pb stress did not change the competitive trend, but it significantly altered the degree of leaf, root and total competitive pressure and intensity.

Discussion

Competition differently affects eco-physiological parameters and phytoremediation in *P. cathayana* males and females

Previous studies have reported that there are sex-related differences in *P. cathayana* in responses to heavy metal treatments, and males possess better tolerance when compared to females (Chen et al. 2011; Han et al. 2013). Our results indicated that sexual competition significantly affects biomass accumulation and partition, photosynthetic capacity, carbohydrates, N and P contents, and ultrastructure in *P. cathayana* males and females under Pb stress. Males were better adapted, visible as higher root biomass, RDW/TDW, R/S ratio, P_{max}, CE, V_{cmax}, J_{max}, PNUE, PPUE, and leaf total $chlorophyll_{ab}$ and polyphenol contents, and as lower CCP, LCP and leaf starch content, and less damaged ultrastructure when compared to females under intra-

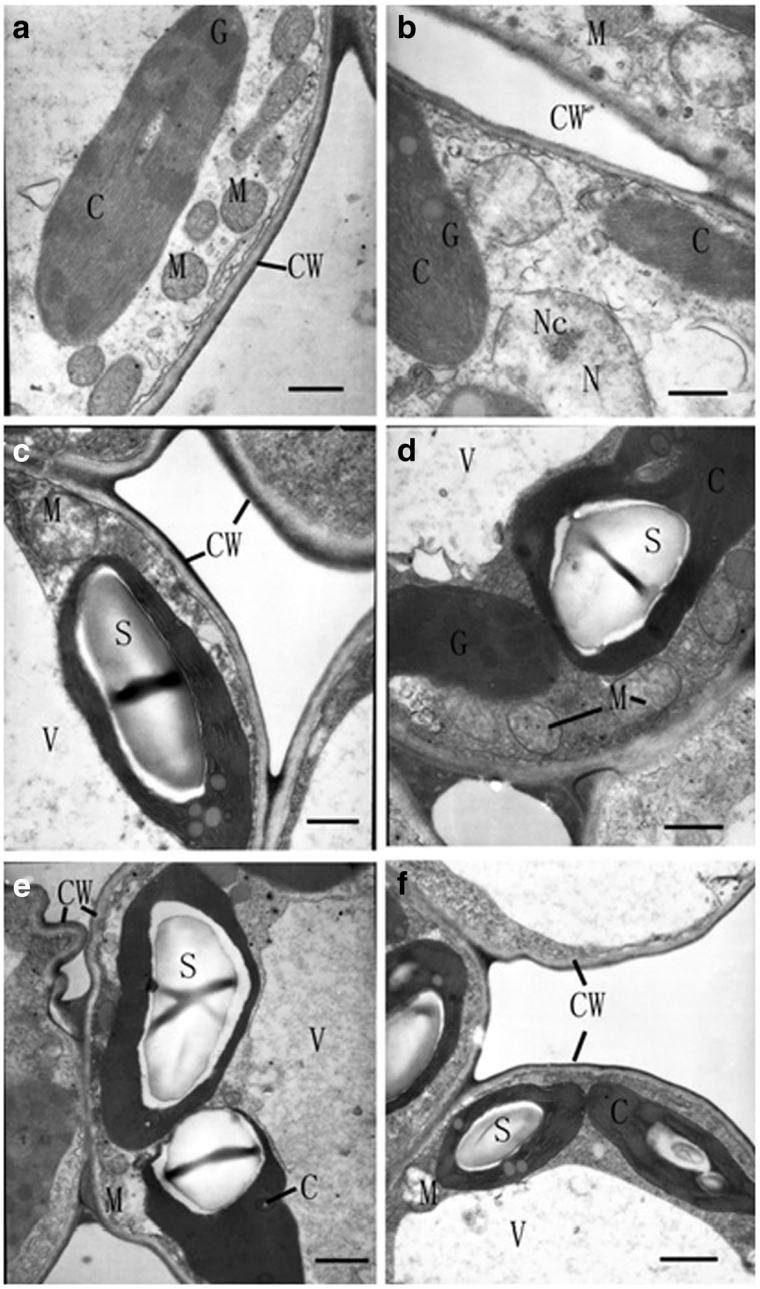


Fig. 4 Transmission electron microscopy observations of mesophyll cells in *P. cathayana* males and females exposed to Pb and sexual competition. (**a**) Mesophyll cells of control females; (**b**) mesophyll cells of control males; (**c**) mesophyll cells of females exposed to Pb and inter-sexual competition; (**d**) mesophyll cells of males exposed to Pb and inter-sexual competition; (**e**) mesophyll cells of females exposed to Pb and intra-sexual competition; (**f**) mesophyll cells of males exposed to Pb and intra-sexual competition. The bars shown are 1 μm. C, chloroplast; CW, cell wall; S, starch granule; G, granum; N, nucleus; M, mitochondrion; N_C, nucleolus; V, vacuole

sexual competition and Pb stress. In control conditions, females from inter-sexual competition grew better than males, while the Pb treatment significantly decreased biomass accumulation, more in females than in males. Moreover, males caused a positive effect on females under inter-sexual competition by accumulating more Pb in roots. Males also had higher BCF and a lower competitive ability, as shown as lower biomass accumulation, PPNE, PNUE, P_{max}, V_{cmax} and J_{max}, all indicating lower resource capture for light and nutrients.

Plant growth is linked closely with intrinsic physiological processes, including photosynthesis, nutrient uptake and metabolism. In our study, the inhibited V_{cmax} and J_{max} values under Pb stress supported the view that the limitation of Rubisco carboxylation and RuBP regeneration caused the decreases in the photosynthetic rate and carbon

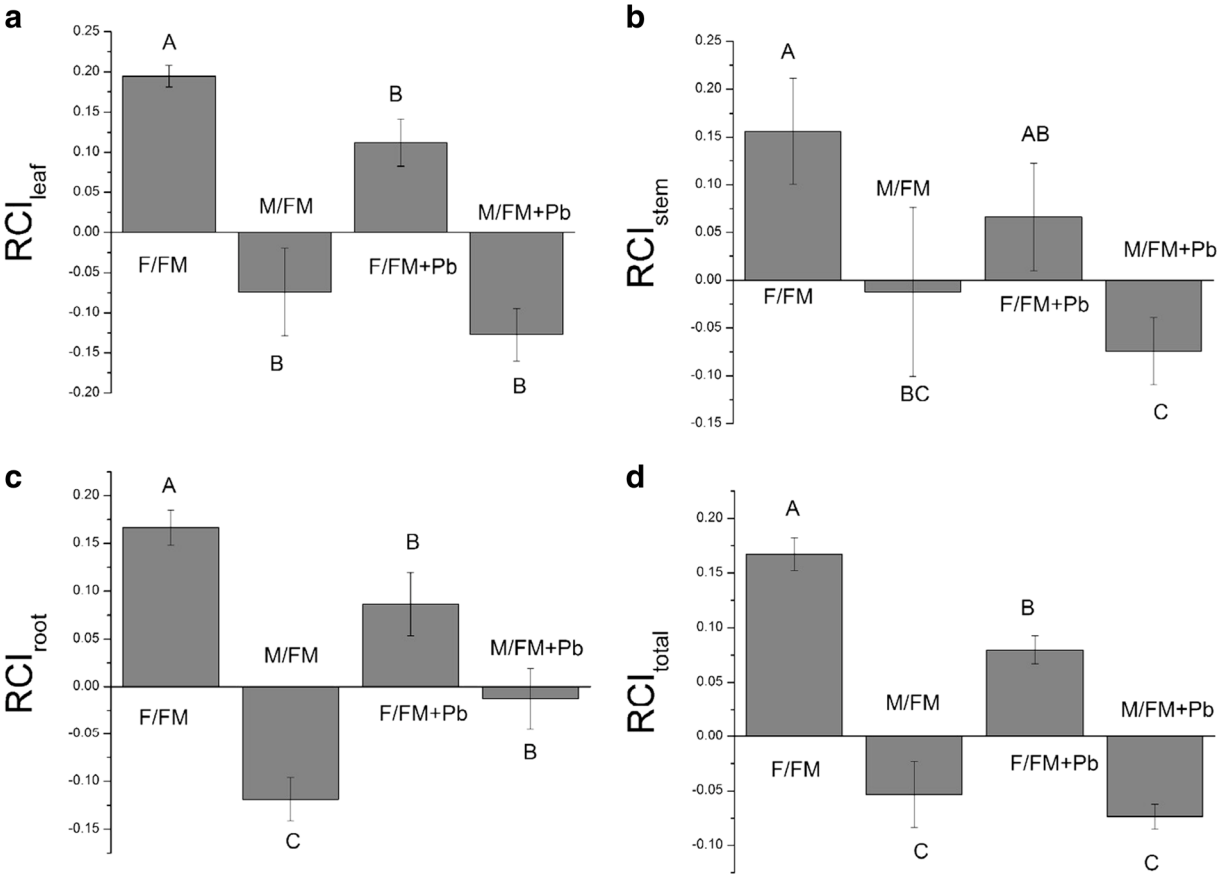


Fig. 5 RCI analysis of *P. cathayana* males and females exposed to Pb and sexual competition. Different letters above bars denote statistically significant differences between treatments at $P < 0.05$ level according to Tukey's test. Values are expressed as means ± SE, n = 5

assimilation (Long and Bernacchi 2003). Males exhibited a greater plasticity in the photosynthetic capacity compared with females under Pb stress combined with intra-sexual competition, which may induce more starch and biomass accumulation in male roots. The high P_{max} and lower R_D values detected in males indicate a greater photosynthetic return for carbon used for dark respiration, while higher CE, V_{cmax} and J_{max} in males indicate less reduced rubisco activity and high carbon-use efficiency compared with females under Pb stress and intra-sexual competition. These results are similar to the responses previously discovered in single-plant experiments (Han et al. 2013). However, under inter-sexual competition, males showed lower P_{max}, V_{cmax}, J_{max}, CCP, less leaf, stem and total biomass than did females under control and Pb stress conditions, which indicates that competition patterns significantly affect the photosynthetic function and growth traits in both sexes. Pb had severe effects on the ultrastructure of leaf cells in both sexes, as shown as a serious distortion of chloroplasts with larger starch granules and disintegrated mitochondrial cristae, which supports the observation reported in previous studies on poplars (Chen et al. 2011; Han et al. 2013). Compared with other competition patterns combined with Pb stress, females from intra-sexual competition and males from inter-sexual competition had more deformed chloroplasts. Simultaneously, females from intra-sexual competition showed the lowest $chlorophyll_{ab}$ content among all competition patterns under Pb treatment. These different responses in photosynthetic-related traits further explained the divergence in biomass accumulation in females and males under Pb stress combined with competition.

Plants' competitive abilities and interactions vary along with the availability of N or P (Trinder et al. 2012; Ahmad-Ramli et al. 2013; Mayor et al. 2014). The growth-rate hypothesis (GRH) states that organisms require relatively more investment in phosphorus-rich ribosomes and rRNA to support the rapid protein

synthesis and thus exhibit lower tissue N: P and C: P ratios (Sterner and Elser 2002). In our study, males and females showed significant differences in N and P concentrations and allocation to roots and leaves, while no significant changes in N: P ratios were observed, which did not totally support GSH. Competition changes the availability and transformation of essential nutrients that may affect plants' responses to abiotic stresses and also the nutrient cycle in the local ecosystem. Our results indicated that females have a greater leaf P content and higher PNUE and PPUE than do males in control conditions and under inter-sexual competition, which supports greater biomass accumulation in females. The Pb treatment significantly decreased the root N content of females but not that of males under intra-and inter-sexual competition. In addition, Pb stress significantly decreased PNUE and PPUE of both sexes. Moreover, M/FM and F/FF had lower PPNU under Pb stress, which may cause their lower biomass accumulation among competition patterns. In all, these results indicated that the type of neighbors affects the nutrient absorption and use efficiency, but males and females display different responses to Pb stress.

In addition, non-structural carbohydrates (NSC) play a key role in serving as signaling molecules, providing basic C energy to growth and respiration, and maintaining osmoregulation (Secchi and Zwieniecki 2011). It has been found that negative and positive competition patterns have different impacts on species' NSC and modulate the accumulation of starch and soluble sugars (Liu et al. 2004; Guo et al. 2016). In our study, Pb stress was found to significantly alter starch and soluble sugar contents of leaves and roots. The highest starch contents of leaves and roots among all competition patterns were observed in females and males under intra-sexual competition. This may result in changes in the efficiency of photosynthesis in leaves, and in the regulation of carbohydrate storage and nutrient uptake in roots.

According to the carbon–nutrient balance hypothesis, the amounts of nonstructural carbohydrates and C-based secondary metabolites, such as polyphenols, are determined by environmental conditions and plant growth strategy; stressful conditions are expected to enhance C investments in storage and defense (Paul-Victor et al. 2010; Millard and Way 2011). Heavy metal toxicity is well known to be related to the polyphenols in plants (Llugany et al. 2013). Polyphenols, such as tannins and phenolics, act as organic ligands for metal chelation to decrease the toxicity of heavy metals (He et al. 2013). A previous study has indicated that competition influences the content of phenolic glycosides, consequently possibly affecting plant tolerance to heavy metal stress (Donaldson et al. 2006). As shown in Fig 2d, males possessed a higher leaf polyphenol content when compared to females under Pb stress, which may help males to alleviate the suppression of photosynthesis caused by Pb stress. Our results showed that sex-specific competition has a significant effect on the phytoremediation traits. Under Pb stress, females from intra- and inter-sexual competition showed higher leaf and stem Pb contents, and BCF_{leaf} and $BCF_{stem}$when compared to males, while M/FM had the highest root Pb content, BCF_{root}, BCF_{total} and TI_{root}, which, thus, indicates different Pb allocation and tolerance strategies in the aboveground and belowground parts of *P. cathayana*.

Sex-specific competition intensities altered by Pb stress

Positive and negative interactions among plant species vary along environmental factors and gradients (Maestre et al. 2009). In addition, plants' responses may depend on the interacting neighbors. In the present study, females from inter-sexual competition showed intensive competition pressure on males in control conditions, while Pb stress changed the intensity and pattern of inter-sexual competition (Fig. 5). The Pb stress and intra-sexual competition showed greater negative effects on the growth and physiological parameters of females than on those of males. Individuals of the same sex usually have similar demands for specific environmental resources, and this will induce a greater competition pressure under an environmental stress. It has been reported that species can distinguish con- and hetero-specific individuals (Kegge and Pierik 2010), and this may affect plant interactions (Miller et al. 2007; Duan et al. 2014). Therefore, we assumed that the two sexes of dioecious species may distinguish the same or opposite sex in the neighborhood, consequently altering their root distribution and investment for resource capture, possibly leading to changes in the intensity and pattern of competition.

Neighboring trees compete for available light aboveground and for water and nutrients belowground, which may influence the intensity of nutrient cycling rates and ecosystem functioning (Deyn and van der Putten 2005; Santiago et al. 2012). In our study, Pb stress and the presence of neighbors changed aboveground and belowground competitive interactions of the two sexes. A positive RCI value in females under control and Pb

stress conditions stands for a better growth with a neighbor of the opposite sex than with a neighbor of the same sex. Males showed negative CRI in the leaf, stem, root and total biomass under inter-sexual competition and control conditions. However, Pb stress significantly decreased competitive intensities of both sexes (Fig. 5).

The stress gradient hypothesis indicates that facilitation is most common in severe environments (Callaway and Walker 1997). These amelioration or deterioration effects of interacting plants in local microhabitats vary along with the abiotic stress. In our study, males and females showed different responses when competing with individuals of the same or opposite sex. Females exerted a negative effect on the growth performance of male seedlings under control conditions, mainly due to the high growth rate of females. Under the Pb treatment, females benefited from the amelioration effect by males in inter-sexual competition, because neighboring males absorbed more Pb and possessed a lower resource use and biomass accumulation. The intra-sexual competition had a greater negative influence on females than on males under Pb stress. Males and females adjusted the soil resource levels and the outcome of competitive interactions, which might be relative to niche modifications (McKane et al. 2002). It has been found that sexual competition patterns affect the root: shoot ratio and function for capturing resources (Rogers and Eppley 2012). Therefore, differences between males and females in the sensitivity to neighboring plants are considered to be an important factor driving sex-specific growth patterns, physiological processes and the intensity of competition. Our results suggest that sexual competition should be considered when developing strategies for forestation and restoration of contaminated land. In Pb contaminated soils, a mixture of *P. cathyana* males and females should be in proper ratios and space to decrease the inter-sexual competitive pressure on males, and more males could be utilized for phytoremediation due to their better tolerance and lower intra-sexual competition pressure.

Conclusions

Our results indicated that sexual competition patterns alter the responses and phytoremediation of males and females under Pb stress. The sensitivity of males and females to Pb stress depends on the sex of their competitor. Compared with the control conditions, Pb stress changed the intensity of competition in males and females. The dynamics of the competitive index, photosynthetic parameters, PNUE, PPUE, the contents of carbohydrates, N, P, chlorophyll$_{ab}$ and polyphenols, and alterations in ultrastructure and phytoremediation-related parameters, including BCF, TI and TF, indicated that sexual competition patterns significantly affect the responses and adaptation of *P. cathayana* seedlings to Pb stress. These different responses of seedlings would have long-term effects on the growth, reproduction investment and competitiveness of adult female and male plants. We suggest that sexual competition may partially explain the growth differences and spatial segregation of sexes in heavy metal contaminated regions. In the process of forestation and restoration of contaminated soil, more attention should be paid in the future on the effects of inter- and intra-sexual competition on dioecious species.

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Author's contribution Juan Chen had the main responsibility for data collection, analysis and writing, Qingquan Han and Baoli Duan had a significant contribution to data collection and 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.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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