

# Sex-specific strategies of phosphorus (P) acquisition in *Populus cathayana* as affected by soil P availability and distribution

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## Summary

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- Soil phosphorus (P) availability and its distribution influence plant growth and productivity, but how they affect the growth dynamics and sex-specific P acquisition strategies of dioecious plant species is poorly understood.
- In this study, the impact of soil P availability and its distribution on dioecious *Populus cathayana* was characterized. *P. cathayana* males and females were grown under three levels of P supply, and with homogeneous or heterogeneous P distribution.
- Females had a greater total root length, specific root length (SRL), biomass and foliar P concentration under high P supply. Under P deficiency, males had a smaller root system than females but a greater exudation of soil acid phosphatase, and a higher colonization rate and arbuscular mycorrhizal hyphal biomass, suggesting a better capacity to mine P and a stronger association with arbuscular mycorrhizal fungi to forage P. Heterogeneous P distribution enhanced growth and root length density (RLD) in females. Female root proliferation in P-rich patches was related to increased foliar P assimilation. Localized P application for increasing P availability did not enhance the biomass accumulation and the morphological plasticity of roots in males, but it raised hyphal biomass.
- The findings herein indicate that sex-specific strategies in P acquisition relate to root morphology, root exudation and mycorrhizal symbioses, and they may contribute to sex-specific resource utilization patterns and niche segregation.

## Introduction

Males and females of dioecious trees meet different reproductive demands and selective pressures. Different resource-related trade-offs between female and male trees can be attributed to the difference in the allocation of more resources for reproduction by females than by males (Obeso, 2002; Tognetti, 2012; Hultine *et al.*, 2016). For instance, *Populus purdomii* females show a higher reproductive investment, construction cost and payback time (the period that a leaf must photosynthesize to pay off the cost of reproductive organs) when compared to males (Lei *et al.*, 2017). Nevertheless, life history traits with physiological differences related to sexes could be observed before reproductive maturity, indicating that many sex-specific differences may be inherently present and not only a consequence of unequal reproductive costs between genders (Montesinos *et al.*, 2012; Randriamanana *et al.*, 2014).

Functional sex-related trait differences of dioecious plant species become greater in responses to stressful environments (Xu *et al.*, 2008; Graff *et al.*, 2013; Li *et al.*, 2016), as males are often more tolerant to both abiotic and biotic stresses (Hultine *et al.*, 2016; Melnikova *et al.*, 2017; Retuerto *et al.*, 2018). Most previous studies have focused on gender responses to nutrient

availability (Robakowski *et al.*, 2018; Song *et al.*, 2018). For example, Zhang *et al.* (2014) found that the photosynthetic rate of males was higher than that of females, when *Populus* was grown under nutrient-deficient conditions. Males showed a smaller accumulation of starch grains in the chloroplast, illustrating that males could better adjust the physiological leaf-level processes to reduce PSII damage. However, the responses of different genders to nutrient stress at the root level and related adaptation mechanisms are largely unknown.

Plants have evolved various key strategies to acquire resources in barren soils (Shen *et al.*, 2011; Zemunik *et al.*, 2015). For instance, plants can develop highly specialized morphological root traits to enhance soil exploration efficiency for phosphorus (P) assimilation, such as increasing the specific root length (SRL), and density and length of lateral roots (Zhu & Lynch, 2004). Also, plants can enhance P availability by mining P in the rhizosphere via the secretion of inorganic phosphate (Pi)-solubilizing root exudates, such as organic acids, enzymes and hydrogen protons, which can mobilize Pi and improve P-acquisition efficiency (Lambers *et al.*, 2009; Richardson *et al.*, 2011; Wang & Lambers, 2019). Moreover, a symbiotic relationship between roots and mycorrhizal fungi is the most common strategy (Smith & Read, 2008), which

increases a plant's P uptake capacity through the increased soil volume exploration via a hyphal network.

As a result of spatiotemporal fluctuations in the decomposition of organic matter, the natural distribution of soil P seems to be patchy or heterogeneous (Rodríguez *et al.* 2009). Plants should exploit and utilize localized soil nutrient patches through root proliferation or mycorrhizal hyphal foraging (Hodge, 2004; Wang *et al.*, 2006). The modular structure of the root system facilitates this exploitation by providing great structural flexibility and allowing roots to proliferate in nutrient-rich areas. In addition, physiological responses of roots can be observed in response to locally enriched nutrients in certain plant species (Jackson *et al.*, 1990; Eissenstat *et al.*, 2015; Kudoyarova *et al.*, 2015; Liu *et al.*, 2015). This can be a compensatory response to adapt to spatiotemporal variability in P availability (Richardson *et al.*, 2009; Peret *et al.*, 2011). Thus, foraging for nutrients appears determined by plasticity in root traits and allocation to roots and their mycorrhizal partners. In fact, belowground success of plants is ultimately determined by these mechanisms. It has been shown that responses and adaptation strategies to the changing availability and distribution of soil P can vary widely across plant species (Zhang *et al.*, 2012; Adams *et al.*, 2013; Hou *et al.*, 2016; McNickle *et al.*, 2016).

Different habitats may have divergent P-capture strategies (Barot *et al.*, 2016; Li *et al.*, 2019); species from nutrient-rich habitats, particularly in P-rich zones, have a strong root proliferation capacity (Grime, 1994), whereas species from habitats that are nutrient-poor may better adapt to low P availability. As carbon is needed for all strategies, plants are likely to enhance the dependence on a particular mechanism at the cost of another one (Barber, 1995). There is a balance or complementarity in P acquisition strategies across plant species (Fort *et al.*, 2015). For instance, Lyu *et al.* (2016) found that *Zea mays* and *Triticum aestivum* have higher root:shoot biomass ratios than legumes, whereas legumes have a higher degree of carboxylate exudation than *Z. mays* and *T. aestivum* under a decreasing soil P supply. Wen *et al.* (2019) found in 16 studied plant species that a range of trade-offs exist in root morphology, metabolic regulation and mycorrhizal associations. Also, root diameter is an important trait to predict acquisition strategies: species with thinner roots exhibit a stronger response as SRL, whereas species with thicker roots show stronger associations with arbuscular mycorrhizal fungi (AMF) and more P-mobilizing exudates in the rhizosphere. Likewise, plant species with a higher root foraging sensitivity or precision react highly positively to P heterogeneity and obtain large growth benefits through morphological plasticity; these species usually have thin roots (Mommer *et al.*, 2011). By contrast, species with more coarse roots rely mainly on the foraging nutrients of mycorrhizal fungi and they are accompanied by a higher mycorrhizal colonization (Chen *et al.*, 2016), but their benefits are often limited (Fitter, 1994).

Dioecious species exhibit a strong spatial habitat segregation of sexes under natural conditions: males with high resource-use efficiency usually being prevalent in nutrient-poor environments, whereas females may be adapted to nutrient-rich conditions (Hultine *et al.*, 2016). In high-resource habitats, females often

show a stronger photosynthesis ability and develop a higher specific leaf area (Hultine *et al.*, 2008). Based on assumed trait coordination between leaves and roots, it has been hypothesized that dioecious species have sex-specific strategies that involve distinct root traits, which results in different female and male performance under variable soil P environments. *Populus cathayana* is an endemic and dioecious tree species, which is very important in the forestation and maintenance of ecological stability in high altitude regions of China. Its sex ratio varies along nutrient gradients (Meng *et al.*, 2018).

In order to investigate sex-specific adaptive strategies, experiments were conducted with varying P supply levels and with both homogeneous or heterogeneous P application to test three hypotheses: (1) females respond more positively to a rich availability and heterogeneous distribution of P than males, but males are less impacted by limited P; (2) a trade-off is found among root morphology, metabolic regulation and mycorrhizal associations for P acquisition between males and females: strong root exudation and associations with mycorrhizal fungi make males more resistant to low P when compared to females, whereas females with higher SRL mainly depend on their root morphology to enhance P acquisition; and (3) the P acquisition strategies of two sexes in varying soil P availability and distribution respond by showing distinct functional traits and varied plasticity of roots.

## Materials and Methods

### Plant materials and soils

*Populus cathayana* Rehd. cuttings were collected from 20 different trees of each sex, sampled from five populations (four adult trees per population of each sex) in riparian and valley flat habitats (Datong, 35°56'N, 101°35'E) located in Qinghai, China. To ensure the randomness and independence of experimental treatments, each cutting was obtained from the different individual parent tree. The cuttings were then grown in a glasshouse. After sprouting and growth for 4 wk, 40 healthy seedlings (20 females, 20 males) of approximately identical crown sizes and equal heights (~20 cm) were selected for the experiments. The sandy soil used was collected from the experimental site at the Hangzhou Normal University, Zhejiang Province, China, air-dried and sieved (2 mm). The soil had 2.82 g kg<sup>-1</sup> organic matter content, 0.28 g kg<sup>-1</sup> total nitrogen (N), 2.62 mg kg<sup>-1</sup> available P and 90.65 mg kg<sup>-1</sup> available K, and pH 8.64. The experiments were conducted in a glasshouse with the temperature at 21–25°C during the day and 15–18°C at night, with 12–14 h photoperiod throughout the growth period at the Hangzhou Normal University in Zhejiang.

### Experimental set-up

The first experiment was to investigate how *P. cathayana* males and females respond to changing P availability. A pot experiment was established in a glasshouse with two genders and three soil P supply level concentrations: 0, 50 and 200 mg kg<sup>-1</sup> P, added as

$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ . There were six treatment combinations, with four replicates per treatment. The pots were filled with 10 kg air-dried sandy soil. To ensure that the nutrient supply was adequate for plant growth, soil was fertilized as follows ( $\text{mg kg}^{-1}$ ):  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  804;  $\text{K}_2\text{SO}_4$  10;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  13;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  5;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  7.5; and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  1.5. The arrangement of pots was done in a completely randomized design which additionally was randomized weekly. The plants were watered daily to maintain field capacity. The plants were harvested 6 months after transplanting (from March to August 2018) and separated into leaves, stems and roots. After the excavation of the roots, rhizosphere soil was identified as the soil adhering to the roots and measurements of the phospholipid fatty acid (PLFA), pH and soil acid phosphatase were recorded. The extramatrical arbuscular mycorrhizal (AM) hyphal biomass was estimated using the PLFA biomarker 16:1 $\omega$ 5c.

The second experiment was to assess sex-specific variation between *P. cathayana* males and females in response to the spatial distribution of P. A factorial experiment was set up comprising two genders and two P supply patterns, with four replications for each treatment. Again P was supplied as  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  in all treatments with the total amount of P being equal. The experiment was conducted using a specific root segregation device made of PVC material (length 20 cm  $\times$  width 20 cm  $\times$  height 30 cm), divided into two equal compartments by rigid plastic (Supporting Information Fig. S1). The partition in the middle prevented the movement of nutrients between the two compartments. Each root device was filled with 10 kg soil. To ensure natural growth of roots in the compartments, a buffer zone was created by placing a 3-cm-deep clean sand layer on the soil surface. For the heterogeneous P treatment, 2 g P was manually mixed into one side of the root box as a P-rich patch, and the other side was left without added P. For the homogeneous P treatment, 2 g P was spread evenly throughout the soil of both compartments. To ensure nutrient supply for plant growth, the same amounts of other nutrients as in Experiment 1 were supplemented in the soil and mixed thoroughly. One cutting of either sex was planted at the centre of each root segregation device. At the end of the experiment, the seedlings were carefully harvested and segregated into roots, stems and leaves (from March to August 2018). In the heterogeneous P treatments, roots grown inside vs outside the P-rich patch were sampled, and corresponding roots at the same location were sampled in the homogeneous treatments. Following root excavation, the soil was subsampled for an extramatrical AM hyphal biomass analysis.

### Root trait measurements

After careful washing, the root system of each plant, a part of the roots (15–30 pieces of 15-mm-long fine roots segments per plant) from each plant was excised from the root system, then analyzed for the arbuscular mycorrhizal fungi (AMF) colonization rate, as described by Vierheilig *et al.* (1998). Root segments (15 mm long) were immersed in a fixative FAA for 4 h after being sampled randomly and washed. Root segments were bleached in 10% KOH for 1 h and stained in ink and vinegar (95% vinegar

and 5% ink) for 3 min at 90°C. All stained root segments were randomly selected for microscopic observations to calculate the colonization rate (Col). Subsequently, other samples were scanned and analyzed for morphological root parameters. Fine roots (< 2 mm) were separated from coarse roots and scanned using a 400 dpi Epson root scanner (Expression1600 pro, Model EU-35; Epson, Tokyo, Japan). The Win-RHIZO system (WINRHIZO PRO2004B software, v.5.0, Regent Instruments Inc., Quebec, QC, Canada) was used in analyzing the total length of roots (both coarse and fine roots). Subsequently, collection of all roots was carried out. All roots were dried for 72 h at 75°C and weighed for determining the specific root length.

### Determination of P concentration in leaves

The P concentration in leaves was determined after digestion with a mixture of 5 ml concentrated sulphuric acid and 8 ml 30% v/v  $\text{H}_2\text{O}_2$ . P was analyzed by the molybdovanadophosphate method with spectrophotometry at 440 nm (Varian Vista-Pro CCD; Johnson & Ulrich, 1959).

### Soil pH, acid phosphatase and extramatrical AM hyphal biomass measurements

The pH of the rhizosphere solutions was measured using a pH meter. Soil acid phosphatase activity in the rhizosphere soil was analyzed according to Neumann (2006). A soil suspension of 0.5 ml (2 ml deionized water mixed with 0.5 g rhizosphere soil) was placed into the 2-ml Eppendorf vials. Then, 0.1 ml substrate (pNPP (p-nitrophenylphosphate); Sigma-Aldrich) and 0.4 ml acetate buffer (pH 5.2) were added. Vials were incubated after being shaken gently at 30°C for 30 min. The reaction was ended with 0.5 ml 0.5 M NaOH, and the centrifuging of the mixture at 12 000 g for 10 min. In the case of controls, NaOH was added before incubation. A spectrophotometer at 405 nm was used to measure the absorbance of this solution. For the PLFA analysis, c. 5 g of freeze-dried soil was used for fatty acid extraction, and PLFAs of different biomarkers were quantified by gas chromatography (Frostegård & Bååth, 1996). For measuring the AM hyphal biomass, the Fatty acid 16:1 $\omega$ 5c, an AM fungal biomarker identified from a previous study, was used (Chen *et al.*, 2016).

### Data analysis

Differences in the index of P stress intensity between two sexes were identified by independent-samples *t*-test. Tukey's Honestly Significant Difference (HSD) tests after one-way ANOVAs were used to compare individual differences among means at the significance level of  $P < 0.05$ . Plant growth (biomass and foliar P concentration), root traits (total root length, specific root length (SRL) and root length density (RLD)), AMF associations (AMF colonization rate, extramatrical AM hyphal biomass) and other parameters (soil pH and acid phosphatase) were subjected to two-way ANOVAs to evaluate the impact of gender, soil P supply and their

interaction in Experiment 1, as well as the effects of gender and heterogeneous/homogeneous P supply in Experiment 2.

## Results

### Soil properties, average diameter at breast height (DBH) and sex ratio of *P. cathayana* populations

Soil properties were similar in the five *P. cathayana* populations, and the coefficients of variation of all parameters were small (Table S1). Furthermore, the content of soil nutrients was low, especially that of available P. In addition, DBH values of all populations were similar, but the coefficient of variation was slightly higher in females than in males (Fig. S2a). The sex ratios were male-biased in the five *P. cathayana* populations (Fig. S2b).

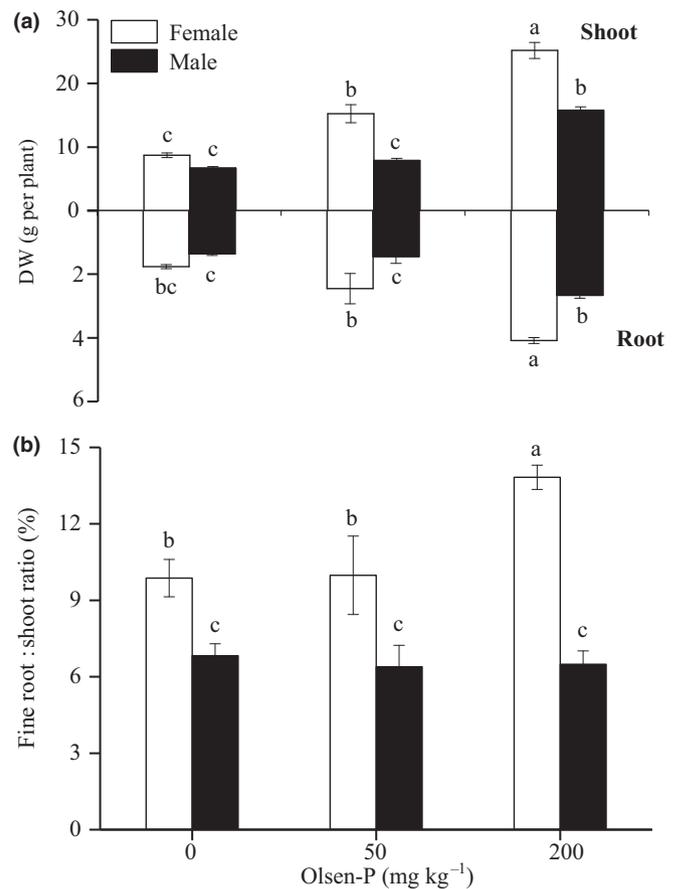
### Sexual differences in *P. cathayana* under variable soil P supply (Experiment 1)

The shoot and root biomass of *P. cathayana* increased with soil P supply, except for males at 0–50 mg kg<sup>-1</sup> P supply (Fig. 1). The biomass of *P. cathayana* females increased more and was greater than that of males, the largest growth difference being found under 200 mg kg<sup>-1</sup> P supply. However, the growth responses of females were more strongly suppressed by P shortage than those of males (Fig. S3). High P application increased fine root allocation of females, but not that of males (Fig. 1). Likewise, P application significantly increased the leaf P concentration of females (Fig. 2), whereas the leaf P concentration of males was unaffected by the P supply level.

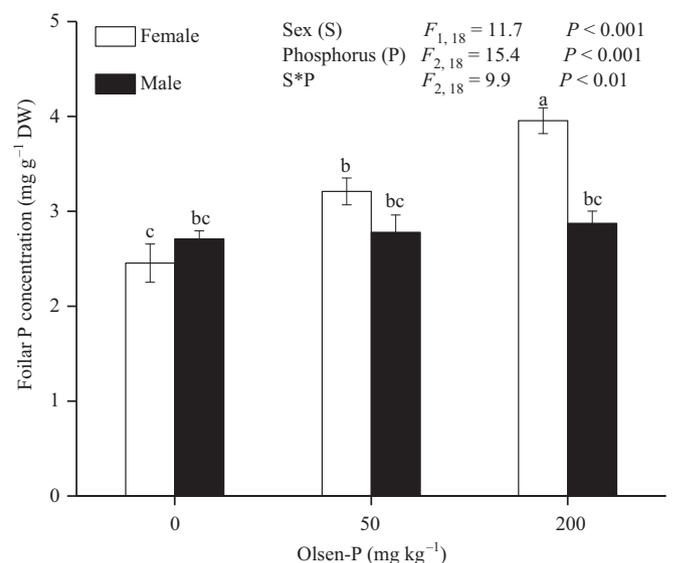
Soil P level significantly enhanced root growth in both sexes only at 200 mg kg<sup>-1</sup> (Fig. 3a). Females displayed greater increase in both total root length and fine root length compared with males; total root length and fine root length both were maximized at the 200 mg kg<sup>-1</sup> P supply level (Figs 3a, S4a). By contrast, males exhibited less definite P application effects on total root length or fine root length. SRL of total roots showed significant differences between the sexes, but not among P supply levels (Fig. 3b). Females possessed higher SRL of total roots than did males at all P supply levels, the value decreasing with an increasing soil P supply. By contrast, SRL of total roots in males remained unchanged. In addition, SRL of fine root of females decreased with P supply levels (Fig. S4b), but no significant effect was detected in males. Furthermore, a gender difference in SRL of fine roots was only found under no P addition.

Rhizosphere soil pH was significantly lower under P deficiency (Fig. 4a). However, no significant differences were observed between the sexes at any P supply level. By contrast, the acid phosphatase activity in the rhizosphere decreased with an increasing P supply (Fig. 4b). Males exhibited significantly higher acid phosphatase activities under P deficiency compared to females.

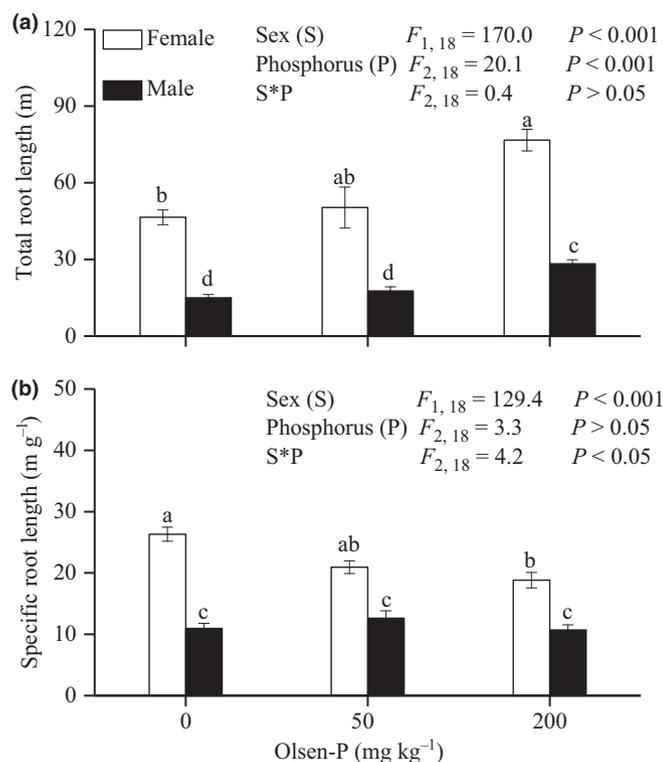
Phosphorus application significantly reduced the colonization rate and AM hyphal biomass of *P. cathayana* (Fig. 5a,b). Under P shortage, AM hyphal biomasses and colonization rates were found to be higher in males than in females, whereas no



**Fig. 1** Effects of phosphorus (P) application on biomass accumulation (a) and allocation (b) of *Populus cathayana* females and males. Error bars, ±SE. Bars with the same letter are not significantly different at  $P < 0.05$ , according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.



**Fig. 2** Effects of phosphorus (P) application on the foliar P concentration of *Populus cathayana* females and males. Error bars, ±SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.



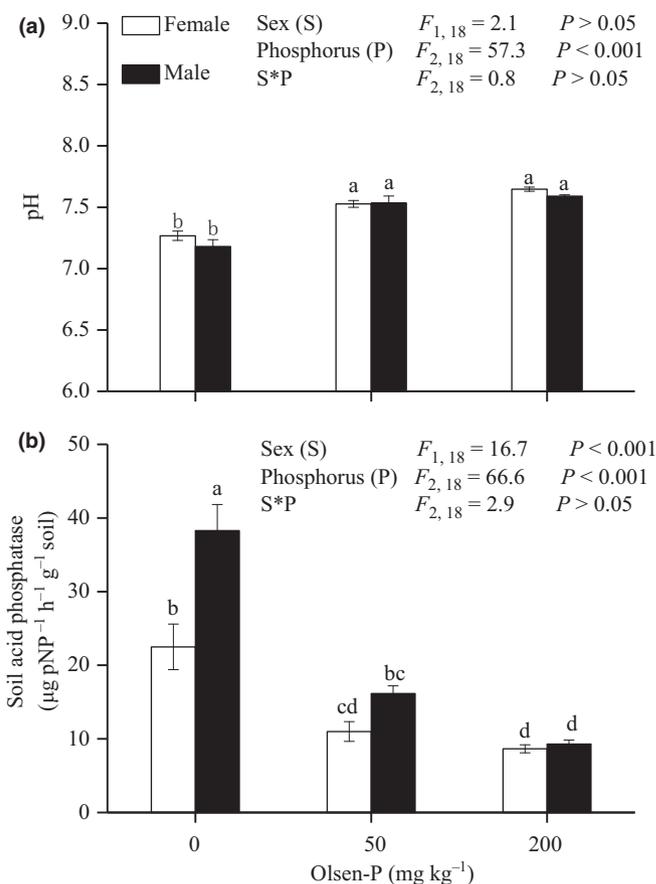
**Fig. 3** The total root length (a) and specific root length (b) of *Populus cathayana* females and males supplied with three concentration levels of phosphorus (P). Error bars,  $\pm$  SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.

significant variations were observed in these parameters under higher supply of P (200 mg kg<sup>-1</sup> P) between the sexes.

### The impact of spatial variability in P supply on dioecious *P. cathayana* (Experiment 2)

The shoot biomass of *P. cathayana* was significantly affected by the spatial distribution of P (Fig. 6a). Also sex-related differences in biomass were found. On the one hand, when soil P was supplied heterogeneously, there was a 37% higher of female biomass compared with the homogeneous P application, whereas males showed a slightly lower biomass in the heterogeneous treatment than in the homogeneous treatment. On the other, the only statistically significant difference in the foliar P concentration between the sexes appeared under a heterogeneous P supply (Fig. 6b).

Root length density in the P-rich soil of *P. cathayana* females was larger in the heterogeneous treatment compared with the homogeneous treatment (Fig. 7a). However, RLD of males did not differ between the heterogeneous and homogeneous environments. The PLFA biomarker 16:1 $\omega$ 5c was used to represent the external fungal proliferation of AMF. The external biomass of fungi in the two sexes showed no significant differences between the homogeneous and the heterogeneous environment in the P-rich patch (Fig. 7b). Overall, the RLD of *P. cathayana* in the P-rich patch was significantly influenced by sex and spatial



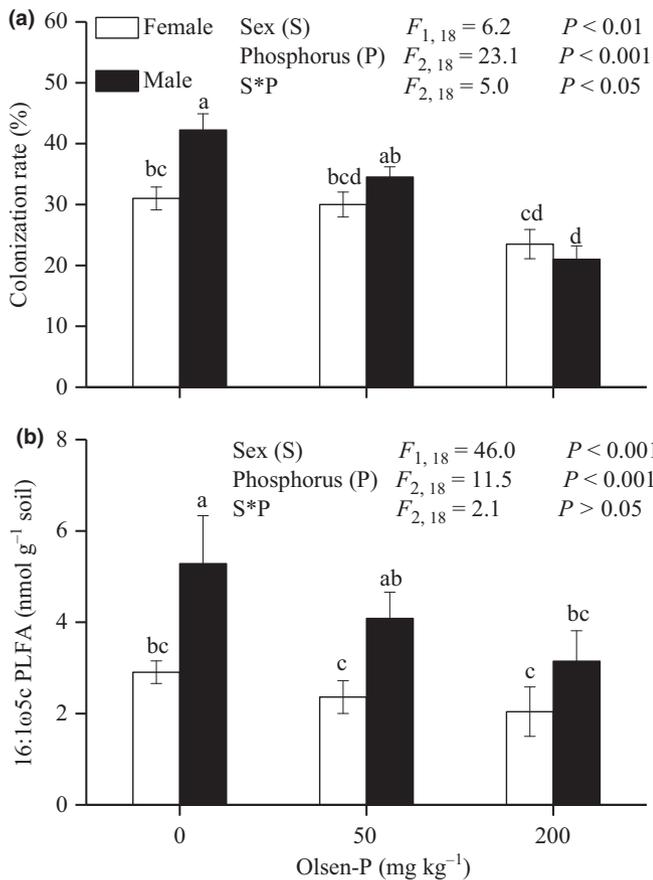
**Fig. 4** Soil pH (a) and acid phosphatase activity (b) in the rhizosphere of *Populus cathayana* females and males supplied with three concentration levels of phosphorus (P). Error bars,  $\pm$  SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.

distribution of P, but the external fungal biomass was unaffected (Table 1).

The spatial distribution of P did not have any significant impact on the RLD of *P. cathayana* outside the P-rich soil. However, the impact on the external fungal biomass was significant (Fig. 7c,d; Table 1). Unlike females, males showed a higher mycorrhizal hyphal proliferation in the heterogeneous environment outside the P-rich patch when compared to the same position in the homogeneous environment (Fig. 7d).

## Discussion

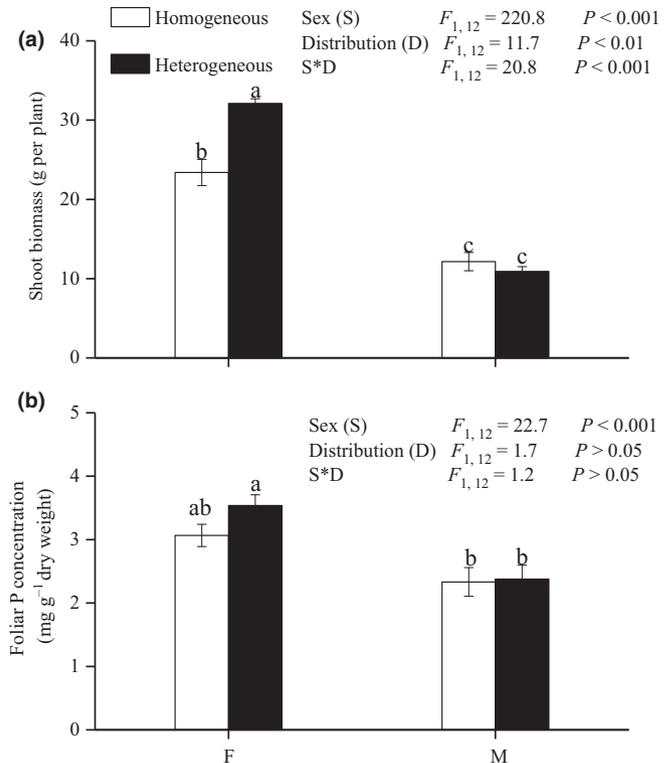
To our knowledge, the present study is the first to provide evidence for sex-specific strategies in phosphorus (P) acquisition of *Populus cathayana* under a variable soil P concentration and distribution. It showed that a strong trade-off exists between the fast biomass accumulation under high P and ability to tolerate low P. In particular, there appears to be distinct pathways: males can enhance root exudation and depend on stronger mycorrhizal associations to better tolerate P deficiency, whereas females show more morphological adjustment for enhanced P acquisition to ensure fast growth under a high and heterogeneous P supply.



**Fig. 5** Colonization rates (a) and phospholipid fatty acid (PLFA) biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass (b) of *Populus cathayana* females and males supplied with three concentration levels of phosphorus (P). Error bars, ± SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.

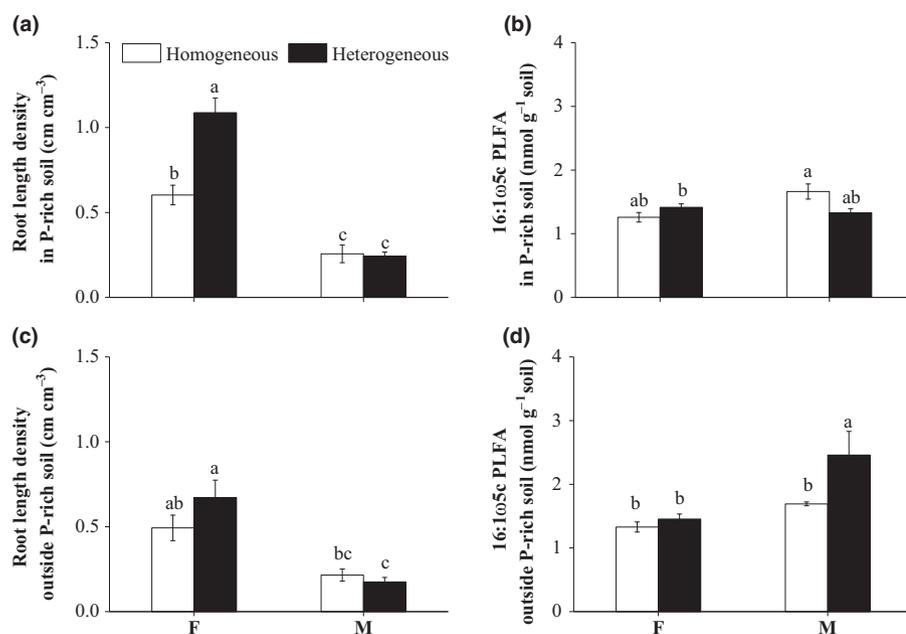
*Populus* trees are fast-growing dioecious species, although males and females display different growth rates under different environmental context (Randriamanana *et al.*, 2014; Zhang *et al.*, 2014; Chen *et al.*, 2015). Herein, it was found that there is sex-specific variation in the growth rates of *P. cathayana* depending on the availability and distribution of soil P. Furthermore, under added P, the biomass accumulation of females was greater than that of males. The rate of plant growth usually depends on the availability of a sufficient supply of nutrients in soil (Chen *et al.*, 2018). Therefore, females are more sensitive to P application, whereas relatively slowly growing males are more tolerant to P deficiency. Considering the distribution of P in soil, no significant difference in male biomass was observed between homogeneous and heterogeneous P supply patterns. However, the biomass accumulation of females was much higher under heterogeneous P supply than that in an homogeneous P condition.

Plants can express morphological adjustment in their root systems to adapt to variable soil P environments. In response to P shortage, plants can inhibit the growth of their primary roots and promote the development of lateral roots (López-Bucio *et al.*, 2002; Niu *et al.*, 2012). It was discovered herein that



**Fig. 6** Effects of homogeneous vs heterogeneous phosphorus (P) supply on the shoot biomass (a) and foliar P concentration (b) of *Populus cathayana* females and males. Error bars, ± SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.

*P. cathayana* females increase specific root length (SRL) more under P shortage conditions. Having a large contact area between roots and soil is expected to enhance P acquisition, whereas added P allows females to increase the total root length and reduce SRL of total roots. It also is worth noting that high P application significantly reduced female SRL of fine roots, thus suggesting that the change in the construction cost of the whole root system was not only caused by the promotion of coarse roots. Previous studies have found that plants with a greater root length may possess more absorptive roots, which can increase nutritional capture under high nutrient supply conditions (Hodge, 2004; Kavka & Polle, 2016; He *et al.*, 2017; Chen *et al.*, 2018). Hence, the greater root length and root surface area of females in comparison with males suggest that females probably have more absorptive roots, which may facilitate P acquisition under high P supply. However, morphological plasticity in biomass allocation is considered to be an important feature for adapting to soil nutrient availability (Freschet *et al.*, 2018). It was observed herein that the proportion of fine root biomass abruptly increased in fast-growing females from medium to high P supply, which indicated that females have greater plasticity in root morphology under high P supply, and the increased fine root allocation would be helpful for promoting P absorption and the maintenance of rapid growth. By contrast, males showed no changes at any P supply level, which indicated that they have lower morphological plasticity in response to P availability,



**Fig. 7** Effects of homogeneous/heterogeneous phosphorus (P) supply on the root length density and phospholipid fatty acid (PLFA) biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass of *Populus cathayana* females and males in (a, b) and outside P enriched soil (c, d). Error bars, ± SE. Columns with the same letter are not significantly different at  $P < 0.05$  according to ANOVA, followed by Tukey's honestly significant difference (HSD) tests.

**Table 1** Effects of soil phosphorus (P) distribution and sex on root length density and phospholipid fatty acid (PLFA) biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass of *Populus cathayana* in and outside a P-rich patch.

Factors	Root length density in soil (cm cm <sup>-3</sup> )				16:1ω5c PLFA in soil (nmol g <sup>-1</sup> )			
	In P-rich soil		Outside P-rich soil		In P-rich soil		Outside P-rich soil	
	F	P	F	P	F	P	F	P
Sex	99.5	<b>&lt;0.001</b>	33.1	<b>&lt;0.001</b>	3.8	>0.05	22.2	<b>&lt;0.01</b>
Distribution	15.6	<b>&lt;0.01</b>	1.1	>0.05	1.2	>0.05	7.6	<b>&lt;0.05</b>
Sex × Distribution	17.4	<b>&lt;0.01</b>	2.7	>0.05	8.8	<b>&lt;0.05</b>	2.5	>0.05

F-values from ANOVA are given with their significance levels. Statistically significant P-values are shown in bold.

whereas their increased biomass was partly driven by a higher photosynthetic capacity under a high P supply.

An increased SRL is not a universal response to a low P supply (Pang *et al.*, 2010; Lyu *et al.*, 2016). Indeed, there was no effect of P deficiency on SRL of males, but males were less impacted by P shortage compared to females. Strengthening root exudation is an important mechanism by which males adapt to soil P deficiency. It has been reported previously that the release of acid phosphatase increases in woody Chinese fir under a resource-use environment (Chen *et al.*, 2018). Additionally, plants can release protons to induce root acidification, which would enhance the hydrolyzation of organic P by increasing phosphatase activity (Yan *et al.*, 2002; Valentinuzzi *et al.*, 2015). The present study found that protons and soil acid phosphatase concentrations increased in the low-P environment, but also that significant sex-specific differences were found. Males have stronger rhizospheric soil acid phosphatase activities compared to females, thereby having a better capacity to increase the release of soil acid phosphatase to mine organic P in order to compensate the negative impact of P limitation.

Moreover, it was found herein that under a low-P conditions, the AM hyphal biomass of males is higher, which potentially indicates that males also may acquire more P through mycorrhizal hyphae proliferation in a complementary fashion. A recent study by Wu *et al.* (2018) found sex-specific responses to mycorrhiza in dioecious *P. cathayana*. The results also indicated that males have a higher colonization level compared to females. Although studies on several species have found that females show a higher dependence on mycorrhizal fungi in high-resource habitats (Verdú & García-Fayos, 2003; Varga & Kytöviita, 2008; Varga *et al.*, 2017), *P. cathayana* males perform better than females in response to stress and arbuscular mycorrhizal fungi (AMF) inoculation (Li *et al.*, 2015; Wu *et al.*, 2015). Thus, it is concluded that sex-specific associations between roots and mycorrhizal fungi depend on plant identity and AM fungal species, as well as on environmental context. Taken together, males can adapt to P-impo- verished soils through a possible compensatory strategy by changing physiological root features and the function of asso- ciated mycorrhizal fungi.

The growth responses of plants to P distribution often show species-specific effects (Hodge, 2004, 2006). However, the present study is the first one to report in a dioecious plant that there are gender-related differences in responses to P distribution within the same population. As illustrated earlier, the benefits from heterogeneous nutrient distribution are much higher in plants with high rate of root growth and comparatively thinner roots (Tamme *et al.*, 2016). On the one hand, it was found that at all P supply levels, females have greater root biomass than males and they can acquire more P in nutrient-rich patches, largely depending on their foraging sensitivity or precision. On the other, a higher biomass of AM hyphae was found outside P-rich patches in males, which indicates that AM hyphal proliferation compensates for a lower ability to proliferate roots.

Dioecy effectively increases opportunities of survival and growth in a population (Charlesworth, 2002; Barrett & Hough, 2012). Females have higher reproductive costs than males and need more P to support them (Zhang *et al.*, 2014; Hultine *et al.*, 2016; Lei *et al.*, 2017). Then, females produce thin roots (high SRL) with relatively low carbon costs. Consequently, the soil around their roots may not be intensively explored, but they build longer roots with a similar C budget compared to males. Plants with high SRL can absorb more P and are better able to adapt to high or heterogeneous P environments, resulting in greater growth benefits (Hodge, 2004, 2006). However, this acquisitive strategy may incur a long-term cost, because roots with such features tend to have short lifespans, which is not a good adaptation strategy in infertile soils in a long term (Freschet *et al.*, 2018). By contrast, males with lower SRL but a high root tissue density possess high root construction costs, especially under resource-use conditions. In the present study, males showed stronger physiological or mycorrhizal symbiosis but weaker morphological responses of roots compared to females when adapting to low-P environments. This represents an economic strategy, because it is generally believed that the cost of spending C on higher metabolic rates is less than the construction of longer and thicker roots (Le Roux *et al.*, 2009; Funayama-Noguchi *et al.*, 2015). However, species with low SRL but a high degree of root colonization by mycorrhizal fungi can better resist herbivore and pathogen attacks, which may be beneficial in unfertile soils (Laliberte *et al.*, 2015). Actually, this may be another reason for males being less impacted by P limitation.

Sexual dimorphism may result in niche segregation, bias in sex ratios, and spatial segregation of the sexes (SSS) across environmental gradients (Eppley, 2006; Li *et al.*, 2007). Earlier evidence had illustrated that there was less stress in majority of the habitats with female-biased sex ratios, whereas males tend to be more abundant than females under adverse environmental conditions (Juvany & Munne-Bosch, 2015; Hultine *et al.*, 2016; Melnikova *et al.*, 2017). Sex ratio biases may reflect trade-offs between strategies of resource exploration across resource gradients (Lei *et al.*, 2017), whereby females are selected for a higher resource uptake to satisfy the higher cost of reproduction through root morphology (Eppley *et al.*, 2009), and males are selected for an effective storage of nutrients to maximize stress tolerance and reproducing

opportunities in low-resource habitats through root physiology to enhance the acquisition of soil organic P or AM hyphal proliferation (Hultine *et al.*, 2016; Graff *et al.*, 2018). In general, dioecious plants are more vulnerable to rapid climate change because of SSS across resource gradients (Hultine *et al.*, 2016). Secondary trait specialization in morphology and physiology vary between the sexes in different habitats. Differences in resource uptake, allocation and utilization strategies between males and females may lead to a situation that one sex is more prone to future changes than the other one. Thus, sex-specific strategies in nutrient foraging would be helpful for adjusting sex ratios, thereby maintaining the ecosystem community structure and stability.

The studies herein were conducted in glasshouse conditions with inevitable limitations. Some issues still need to be addressed in the future: (1) despite the low P content in five *P. cathayana* populations, which indicates male-biased sex ratios, the effect of P on SSS remains to be verified across a series of P gradients; (2) natural, diverse P acquisition strategies in root morphology, root exudation and associations with mycorrhiza fungi in *P. cathayana* males and females are converged or diverged under different soil P concentrations and distribution. These questions will encourage us to conduct further field experiments to explore sex-specific strategies in P acquisition.

## Conclusions

This study sheds light on the growth consequences of sexual dimorphism in dioecious *P. cathayana* to cope with a variable soil P concentration and distribution. On the one hand, males are less sensitive to the P availability and distribution, and they have a good ability to regulate physiological and mycorrhizal processes of roots, thereby increasing the acquisition of P in resource-use conditions. On the other, females have larger root systems, which can increase the foraging region of roots and P acquisition. Therefore, when the amount of soil P is high and heterogeneously distributed, females grow better when compared to males. The results of this study would improve the predictions for potential climate change impacts on dioecious *P. cathayana* populations and allow effective action to be taken for restoration programmes that underline prospective migration to maintain the dioecious population structure of great values.

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## Author contributions

ZX had the main responsibility for data collection, analysis and writing; YH, LY and RL contributed to data collection and analysis; HK contributed to the interpretation of data and manuscript preparation; and CL (the corresponding author) had overall responsibility for experimental design and project management.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Schematic diagram of a root segregation device.

**Fig. S2** The average DBH and sex ratio of *P. cathayana* populations.

**Fig. S3** Effects of P shortage on total biomass production in *P. cathayana* females and males.

**Fig. S4** The fine root length and SRL of fine roots in *P. cathayana* females and males supplied with P.

**Table S1** Soil properties and their coefficients of variation in the locations of *P. cathayana* populations.

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