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

Zhichao Xia [1], Yue He [1], Lei Yu [1], Rubing Lv [1],
Helena Korpelainen [2] and Chunyang Li [1, *]

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, Zhejiang, China

[2] Department of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, P.O. Box 27, FI-00014, Finland

* Corresponding author: Chunyang Li, E-mail address: licy@hznu.edu.cn

Highlights

- Sexual differences in P acquisition are affected by soil P level and distribution.
- *P. cathayana* females benefit more from high P level and heterogeneous distribution.

strategies.

Abstract 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 effects of soil P availability and its distribution on dioecious *Populus cathayana* Rehd. were characterized. *P. cathayana* males and females were grown under three levels of phosphorus (P) supply, and with homogeneous or heterogeneous P distribution. Females had a greater total root length, specific root length (SPL), biomass and foliar P concentration. 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 availability enhanced growth and root length density in females. Female root proliferation in P-rich patches was associated with increased foliar P assimilation. Increased P availability by localized P application did not enhance the biomass accumulation and the morphological plasticity of roots in males, but increased hyphal biomass. Our results suggest that *P. cathayana* displays sexually different P acquisition strategies, which explain the performance of females and males under variable soil P environments.

Keywords: dioecy; P deficiency; heterogeneous phosphorus supply; rhizosphere processes; root morphological plasticity; mycorrhizal associations.

Introduction

Males and females of dioecious trees meet different reproductive demands and selective pressures. Female plants often allocate more resources to reproductive effort than males, which results in different resource-related trade-offs between males and females (Tognetti 2012; Juvany and Munne-Bosch 2015; Hultine et al. 2016). For instance, *Populus purdomii* females have showed higher reproductive investment, construction cost and payback time when compared to males (Lei et al. 2017). However, sex related differences in physiological and life-history traits can be observed already 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 trait differences of dioecious plant species become greater in responses to stressful conditions (Xu et al. 2008; Graff et al. 2013; Li et al. 2016), when males tend to be 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 when *Populus* confronted nutrient shortage, the photosynthetic rate of males was higher than that of females. Males showed a smaller accumulation of starch grains in the chloroplast, indicating that males could better adjust their physiological leaf-level processes to reduce growth damage. However, the responses of different genders to nutrients stress on the root level and related adaptation mechanisms are largely unknown.

Plants have evolved a suite of adaptive strategies to acquire resources in severely impoverished soils (Lambers et al. 2008; 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 and Lynch 2004). Also, plants can enhance P availability by mining P in the rhizosphere via the release of Pi-solubilizing root exudates, such as organic acids, enzymes and hydrogen protons, which can liberate Pi from inaccessible soil complexes and phosphorylated compounds (Lambers et al. 2009; Neumann and Martinoia 2002; Richardson et al. 2011). Moreover, a symbiotic relationship between roots and mycorrhizal fungi is the most common strategy (Smith and Read 2008), which increases a plant’s acquisition of soil P through the increased soil volume the plant can explore via a hyphal network. Because all strategies require significant amounts of carbon, plants may increase the expression of one mechanism at the expense of the other one (Barber 1995).

There is a balance or complementarity between the morphological and physiological responses of roots to variable P-limited environments across plant species (Fort et al. 2015). For instance, Lyu et al. (2016) found that species with fibrous roots have higher root/shoot biomass ratios than legumes, whereas legumes have a higher degree of carboxylate exudation than species with fibrous roots under deacreasing soil P supply.

Soil nutrient heterogeneity can greatly affect root plasticity. Plants should exploit and utilize localized soil nutrient patches through root proliferation or mycorrhizal hyphal foraging (Hodge 2004; Wang et al. 2006). In addition, some plant species show physiological responses of roots to localized nutrient enrichment (e.g. an increase in the nutrient uptake capacity per root length unit; Jackson et al., 1990). This is an effective strategy to adapt spatiotemporal variability in P availability, often considered to be a compensatory response (Richardson et al. 2009; Peret et al. 2011). Thus, foraging for nutrients appears determined by root plasticity dynamics in response to soil P variability. Plants' 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). However, it is unclear, how dioecious plants respond to a P deficiency and varying local distribution of soil P.

*Populus cathayan*a is a typically rapidly growing dioecious species, which plays a very important role in the forestation and maintenance of ecological stability in high altitude regions of China. It also exhibits a strong spatial habitat segregation of the sexes under natural conditions, with males usually being prevalent in nutrient-poor environments while females favor nutrient-rich conditions (Meng et al. 2019). Some studies have indicated that contrasting genotypes dominating in different habitats may have divergent P-capture strategies (Barot et al. 2016): species from nutrient-rich habitats have a strong root proliferation capacity, especially in P-rich zones (Grime 1994), whereas species from nutrient-poor habitats may have the capacity to adapt to P shortage conditions. In the present study, in order to investigate sex-specific adaptive strategies, we conducted experiments with varying P supply levels and with both homogeneous or heterogeneous P distribution to test the hypothesis that females show a more positive response to rich and heterogeneous P environments compared to males, because females have a high capacity for the morphological proliferation of roots, resulting in improved biomass growth and nutrient uptake. In contrast, males would be favored by a low P availability due to their compensatory strategies associated with significant physiological and mycorrhizal plasticity of roots.

Methods and materials

Plant materials and soils

Populus cathayana cuttings were collected from 20 different trees, including 10 females and 10 males, sampled from 5 populations (four adult trees per population) in riparian and valley flat habitats (Datong, 35°56′N, 101°35′E) located in the Qinghai Province, China. The cuttings were then grown in a naturally lit greenhouse. After sprouting and growing for 4 weeks, 60 healthy seedlings (30 females and 30 males) of approximately identical crown sizes and equal heights (~20 cm) were selected for the experiments. The used sandy soil was collected from the experimental site at the Hangzhou Normal University, Zhejiang Province, China, air-dried and passed through a 2-mm sieve. Soil properties were as follows: soil organic matter content of 2.82 g kg^{-1}, total N of 0.28 g kg^{-1}, available P of 2.62 mg kg^{-1} and available K of 90.65 mg kg^{-1}, pH 8.64 (the ratio of soil to $CaCl_2$ solution was 1:2.5).

Experimental set-up

Experiment 1 In order to investigate how *P. cathayana* males and females respond to changing P availability, a pot experiment was conducted in a glasshouse with two genders and three soil P supply levels: 0 mg kg^{-1}, 50 mg kg^{-1}and 200 mg kg^{-1}. P was applied as $Ca\ (H_2PO_4)_2.\ H_2O$. There were 6 treatment combinations, with four replicates per treatment. The pots were filled with 10 kg of air-dried sandy soil. To ensure that the nutrient supply was adequate for plant growth, soil was also fertilized with nutrients as follows (mg per pot): $Ca(NO_3)_2 . 4H_2O$ 8040; K_2SO_4 100; $MgSO_4.7H_2O$ 130; $MnSO_4.H_2O$ 50; $ZnSO_4.7H_2O$ 75; $CuSO_4.5H_2O$ 15. All pots were arranged in a completely randomized design and additionally randomized weekly. The plants were watered every day to maintain field capacity (18%, w/w). The plants were harvested 6 months after transplanting (from March to August 2018) and separated into leaves, stems and roots. Following root excavation, the soil adhering to roots was defined as rhizosphere soil and sub-sampled for pH, soil acid phosphatase and phospholipid fatty acid (PLFA) measurements. Extramatrical arbuscular mycorrhizal (AM) hyphal biomass was estimated using the PLFA biomarker 16:1ω5c

Experiment 2 In order to assess sex-specific variation between *P. cathayana* males and females in response to the spatial distribution of P, we set up a factorial experiment comprising two genders and two P supply patterns, with four replications for each treatment. P was supplied as $Ca (H_2PO_4)_2 . H_2O$ 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 × width 20 cm × height 30 cm), divided into two equal compartments by rigid plastic. The partition in the middle prevented the movement of nutrients between the two compartments. Each root device was filled with 10 kg soil. A clean 3-cm deep sand layer was placed on the top of the soil as a buffer zone to enable natural root growth in the compartments. For the heterogeneous P treatment, 2 g of 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 of 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 added to the soil, followed by thorough mixing. One cutting of each sex was planted at the center of each root segregation device. All seedlings were harvested at the end of the experiment and divided into leaves, stems and roots (from March to August 2018). In the heterogeneous P treatments, roots grown inside vs outside the P-rich patch were sampled, and the corresponding roots at the same location were sampled in the homogeneous treatments. Following root excavation, the soil was sub-sampled for an extramatrical AM hyphal biomass analysis.

Root trait measurements

The root system of each plant was first washed carefully. 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 AMF colonization rate, as described by Vierheilig et al. (1998). Roots segments (15 mm long) were randomly sampled from each treatment, washed in distilled water, and then immersed in a FAA fixative for 4 h. 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. Roots were scanned with an EPSON root scanner at 400 dots per inch (Epson Expression1600 pro, Model EU-35, Tokyo, Japan). The total root length was analyzed using software Win-RHIZO (WinRhizo Pro2004b, version 5.0, Regent Instruments Inc., Quebec, QC, Canada). Afterwards, all roots were collected and dried at 75 °C for 72 h and weighed to calculate the specific root length.

Determination of leaf and root P contents

The phosphorus concentration of shoots and roots was determined after digestion with a mixture of 5 ml of concentrated sulphuric acid and 8 ml of 30% v/v H_2O_2. P was analysed by the molybdovanadophosphate method by spectrophotometry at 440 nm (Varian Vista–Pro CCD; Johnson & Ulrich, 1959).

Determination of soil pH, acid phosphatase, and extramatrical AM hyphal biomass

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). Briefly, 0.5 mL soil suspension (0.5 g rhizosphere soil mixed with 2 mL deionized water) was placed into 2-mL Eppendorf vials, followed by the addition of 0.4 mL acetate buffer (pH 5.2) and 0.1 mL substrate [pNPP (pnitrophenylphosphate); Sigma St. Louis, MO, USA]. Vials were gently shaken and incubated for 30 min at 30 °C. The reaction was terminated with 0.5 mL 0.5 M NaOH, and the mixture was centrifuged for 10 min at 12,000×g. In the case of controls, NaOH was added before incubation. The absorbance of this solution was measured with a spectrophotometer at 405 nm. For the PLFA analysis, about 5 g of freeze-dried soil was used for fatty acid extraction, and PLFAs of different biomarkers were quantified by gas chromatography (Frostegård and Bååth 1996). Fatty acid 16:1ω5c, identified as an AM fungal biomarker in a previous study, was used to measure AM hyphal biomass (Chen et al. 2016).

Data analysis

Biomass, total root length, SPL, root length density (RLD), foliar P concentration, AMF colonization rate, extramatrical AM hyphal biomass, soil pH and acid phosphatase were analyzed with one- or two-way analysis of variance (ANOVA) according to each experiment. All treatments were regarded as fixed factors (i.e. gender, P level, P distribution, as well as the interactions between gender and P level or distribution). Tukey’s honestly significant difference test was used for multiple comparisons when ANOVA terms were significant using SPSS 16.0 for Windows (SPSS Inc. Chicago, Illinois, USA).

Results

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 (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^{-1} P supply conditions. Similarly, P application significantly increased the leaf P concentration of females (Fig. 2). However, the leaf P concentration of males was unaffected by the P supply level.

P application significantly enhanced root growth in both sexes (Fig 3a). The females exhibited greater responses in total root length compared with males; the total root length was maximized at 200 mg kg^{-1} P supply level. In contrast, males exhibited less definite P application effects on the total root length. SPL showed significant differences between the sexes, but not among P supply levels (Fig 3b). Moreover, females possessed higher SRL than did males at all P application levels, the value decreasing with an increasing soil P supply. In contrast, SRL of males remained unchanged.

Rhizosphere soil pH was significantly lower under P deficiency (Fig. 4a). However, there were no significant differences between the sexes at any P 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.

P application significantly reduced the colonization rate and AM hyphal biomass of *P. cathayana* (Fig. 5a, b). Males had higher colonization rates and AM hyphal biomasses than did females under P shortage, while there were no significant differences in these parameters between the sexes under high P supply (200 mg kg^{-1} P).

The effect 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. When soil P was supplied heterogeneously, the female biomass was 37% higher than under homogeneous application, whereas males showed a slightly lower biomass in the heterogeneous treatment than in the homogeneous treatment. On the other hand, sex influenced the foliar P concentration of *P. cathayana*, but the spatial distribution of P did not (Fig. 6b).

RLD of *P. cathayana* females was greater in the P-rich soil in the heterogeneous treatment compared with the homogeneous treatment (Fig.7a). However, RLD of males did not differ between the heterogeneous and homogeneous environment. We used the PLFA biomarker 16:1ω5c 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, RLD of *P. cathayana* in the P-rich patch was significantly influenced by sex and spatial distribution of P, but the external fungal biomass was not affected (Table 1).

Outside the P-rich soil, RLD of *P. cathayana* was not significantly affected by the spatial distribution of P, but the effect was significant on the external fungal biomass (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

Populus trees are fast-growing dioecious species, although males and females display different growth rates under different environmental conditions (Randriamanana et al. 2014; Zhang et al. 2014; Chen et al. 2015). In this study, we found that there is sex-specific variation in the growth rates of *P. cathayana* under a different availability and distribution of soil P. Furthermore, under added P conditions, the biomass accumulation of females is greater than that of males, which indicates that females grow more rapidly than males. The growth speed of plants 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, while relatively slowly growing males are more tolerant to low P conditions. Considering the distribution of P in soil, there was no significant difference in male biomass between homogenous and heterogeneous P supply patterns. However, the biomass accumulation of females was much higher under heterogeneous P supply than that in a homogenous P condition, which showed that the heterogeneous P distribution was more conducive to the growth of females.

Plants can adjust the morphological plasticity of 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 (Williamson et al. 2001; López-Bucio et al. 2002). We discovered that *P. cathayana* females increase SPL 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 SRL. A previous study found that plants with a greater root length may possess more lateral roots, which can facilitate nutritional foraging under high nutrient supply conditions (Kavka and Polle 2016; He et al. 2017; Chen et al. 2018). Thus, 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.

An increase in 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 still showed a stronger ability to tolerate P shortage. Strengthening the rhizosphere processes is an important mechanism through 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 low P environment (Chen et al. 2018). Additionally, plants can burst out 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). In this study, protons and soil acid phosphatase levels increased in the low P environment, but also significant sex-specific differences were found. Males have stronger rhizosphere processes 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, we found that under a low P condition, the AM hyphal biomass of males is higher, which potentially indicates that males 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 showed 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 and García-

Fayos 2003; Varga and KytovIita 2008; Varga et al. 2017), *P. cathayana* males perform better than females when subjected to stress and AMF (Li et al. 2015; Wu et al. 2015). Thus, we conclude that sex-specific associations between roots and mycorrhizal fungi depend on the identity of the plant and AM fungal species, as well as on environmental conditions. Taken together, males can adapt to P-impoverished soils through a possible compensatory strategy by changing physiological root features and the function of associated mycorrhizal fungi.

The growth responses of plants to P distribution often show species-specific effects (Hodge 2004, 2006). However, our 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. Previously, it has been shown that plants with a larger root biomass often benefit more from a heterogeneous nutrient distribution (Tamme et al. 2016). We discovered that at all P supply levels, females have more root biomass than males and they can acquire more P in nutrient-rich patches, yet depending on their foraging sensitivity or precision. On the other hand, we found a higher biomass of AM hyphae outside P-rich patches in males, which indicates that AM hyphal proliferation compensates for the relative inefficiency to proliferate roots.

Dioecy effectively increases opportunities of survival and growth in a population. 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). Therefore, females produce thin roots (high SRL) with relatively low carbon costs. Consequently, the soil around their roots is not 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 result in a long-term cost, since roots with such characteristics have short lifespans, which is not a good adaptation strategy in infertile soils in a long term (Freschet et al. 2018). In contrast, males with low SPL but a high root tissue density possess high root construction costs, especially under low P conditions.

In the present study, males showed stronger physiological or mycorrhizal 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 roots (Le Roux et al. 2009; Funayama-Noguchi et al. 2015). On the other hand, species with low SPL but a high degree of root colonisation 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 the relatively good male performance.

Sexual dimorphism results in niche divergence, variation in sex ratios, and spatial segregation of the sexes (SSS) across environmental gradients (Eppley 2006; Li et al.

2007). Previously, it has been showed that habitats with female-biased sex ratios are less stressful than those with male-biased sex ratios, while males are more abundant than females under adverse environmental conditions (Juvany and Munne-Bosch 2015; Hultine et al. 2016; Melnikova et al. 2017). Sex ratio biases across resource gradients may reflect trade-offs between resource exploration strategies (Lei et al. 2017), whereby females are selected for a higher resource uptake to meet 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 mating 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). However, our studies were conducted in greenhouse conditions with inevitable limitations, and field work is being carried out to further verify our conclusions.

Conclusions

This study provided new insights into the growth consequences of sexual dimorphism in dioecious *P. cathayana* to cope with a variable soil P level and distribution. Males are not 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 low P conditions. On the other hand, 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.

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Figure legends

Figure 1 Effects of P application on the performance of *P. cathayana* females and males. Bars with the same letter are not significantly different at $P < 0.05$, according to

ANOVA, followed by Tukey HSD tests.

Figure 2 Effects of P application on the foliar P concentration of *P. cathayana* females and male. Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 3 The total root length (a) and specific root length (b) of *P. cathayana* females and males supplied with three levels of P. Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 4 Soil pH (a) and acid phosphatase activity (b) in the rhizosphere of *P. cathayana* females and males supplied with three levels of P. Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 5 Colonization rates (a) and PLFA biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass (b) of *P. cathayana* females and males supplied with three levels of P. Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 6 Effects of homogeneous vs heterogeneous phosphorus (P) supply on the shoot biomass (a) and foliar P concentration (b) of *P. cathayana* females and males. Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 7 Effects of homogeneous/heterogeneous phosphorus (P) supply on the root length density and PLFA biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass of *P. cathayana* females and males within (a-b) and outside P enriched soil (c-d). Columns with the same letter are not significantly different at $P < 0.05$ according to ANOVA, followed by Tukey HSD tests.

Figure 1

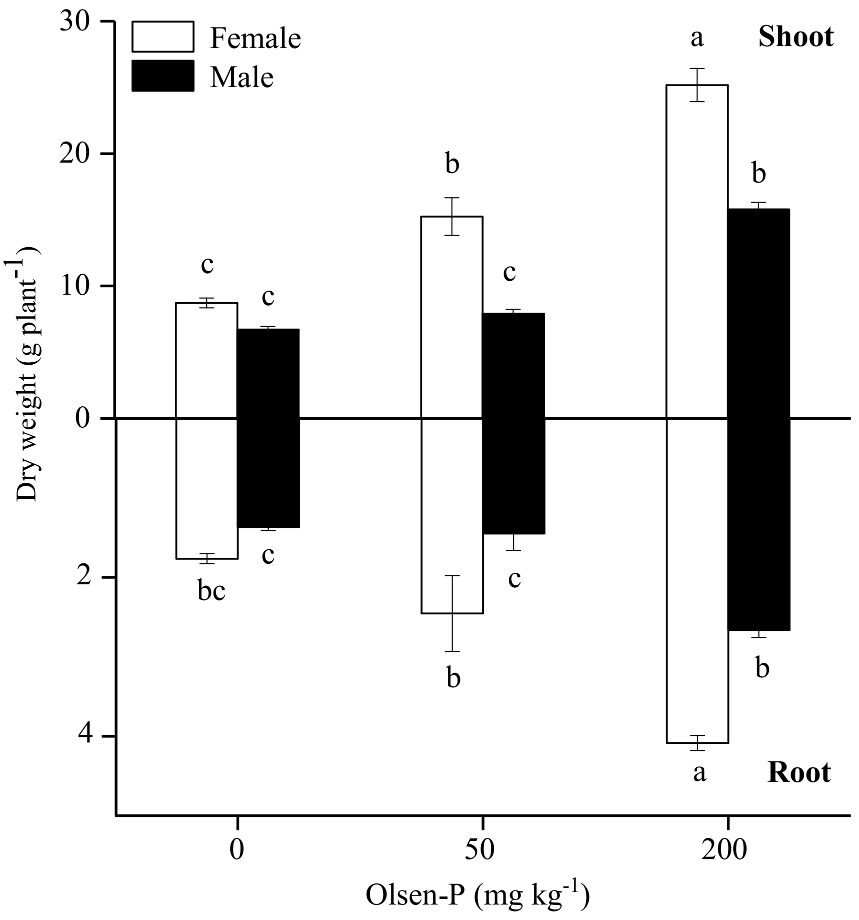
Female
Male
Shoot
Root
Dry weight (g plant-1)
Olsen-P (mg kg-1)
30
20
10
0
2
4
0
50
200
c
c
b
c
a
b
bc
c
b
c
a
b

Figure 2

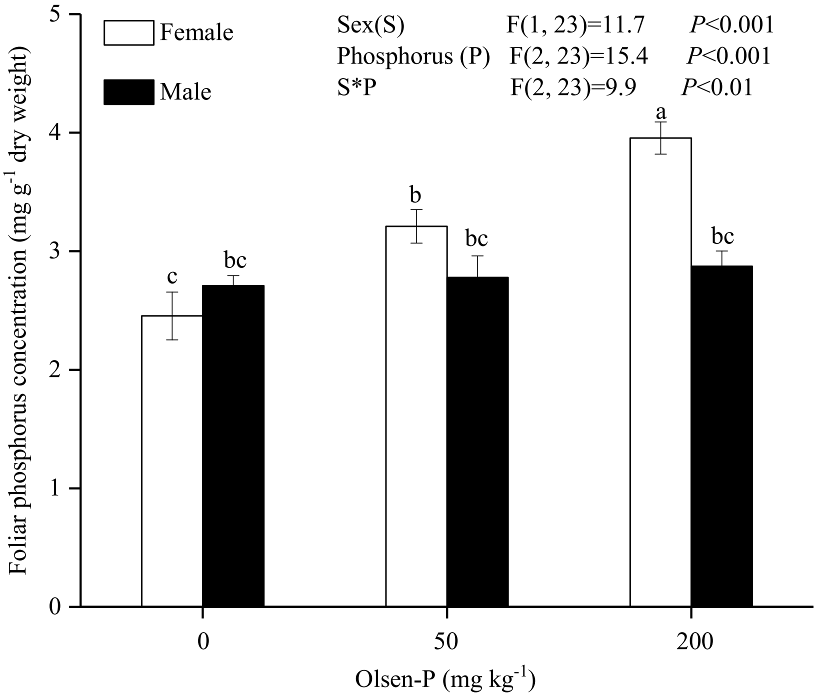
Female
Male
Sex(S) F(1, 23)=11.7 P<0.001
Phosphorus (P) F(2, 23)=15.4 P<0.001
S*P F(2, 23)=9.9 P<0.01
a
b
bc
c
bc
bc
Foliar phosphorus concentration (mg g-1 dry weight)
0
1
2
3
4
5
0
50
200
Olsen-P (mg kg-1)

Figure 3

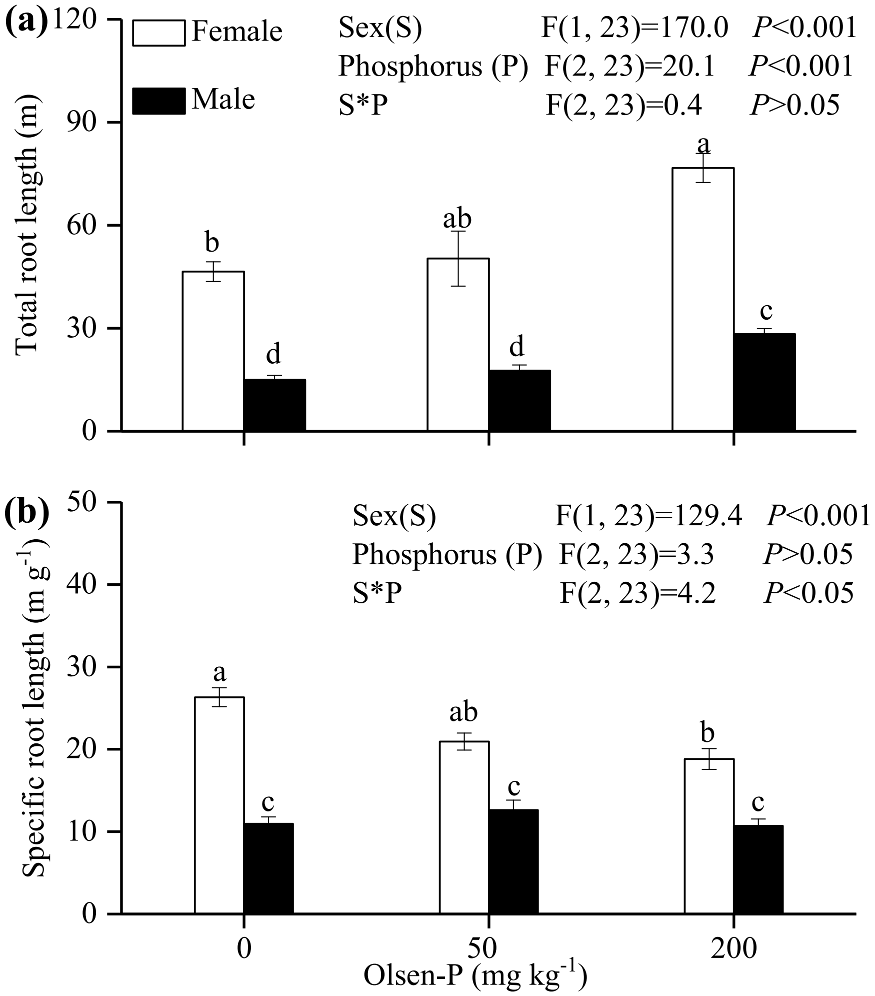
(a)
Total root length (m)
120
90
60
30
0
Female
Male
Sex(S) F(1, 23)=170.0 P<0.001
Phosphorus (P) F(2, 23)=20.1 P<0.001
S*P F(2, 23)=0.4 P>0.05
b
d
ab
d
a
c
(b)
Specific root length (m g-1)
50
40
30
20
10
0
Sex(S) F(1, 23)=129.4 P<0.001
Phosphorus (P) F(2, 23)=3.3 P>0.05
S*P F(2, 23)=4.2 P<0.05
a
c
ab
c
b
c
0
50
200
Olsen-P (mg kg-1)

Figure 4

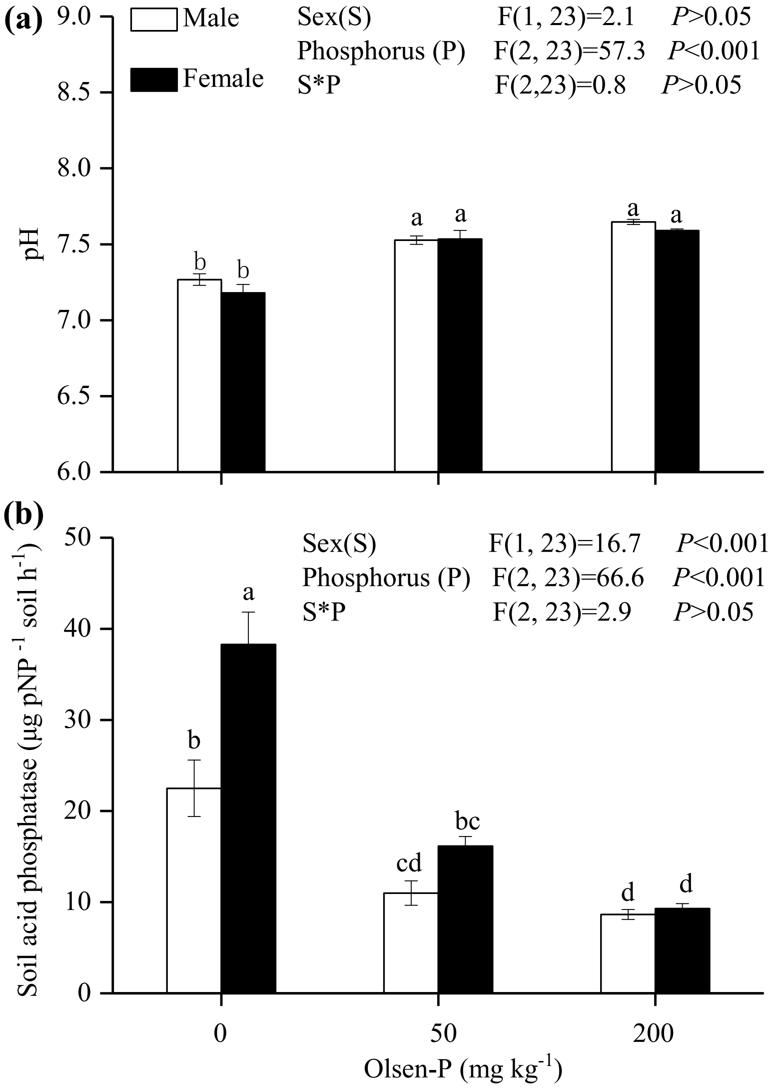
(a)
Male
Female
Sex(S) F(1, 23)=2.1 P>0.05
Phosphorus (P) F(2, 23)=57.3 P<0.001
S*P F(2,23)=0.8 P>0.05
pH
9.0
8.5
8.0
7.5
7.0
6.5
6.0
b
b
a
a
a
a
(b)
Sex(S) F(1, 23)=16.7 P<0.001
Phosphorus (P) F(2, 23)=66.6 P<0.001
S*P F(2, 23)=2.9 P>0.05
Soil acid phosphatase (µg pNP -1 soil h-1)
50
40
30
20
10
0
a
b
bc
cd
d
d
0
50
200
Olsen-P (mg kg-1)

Figure 5

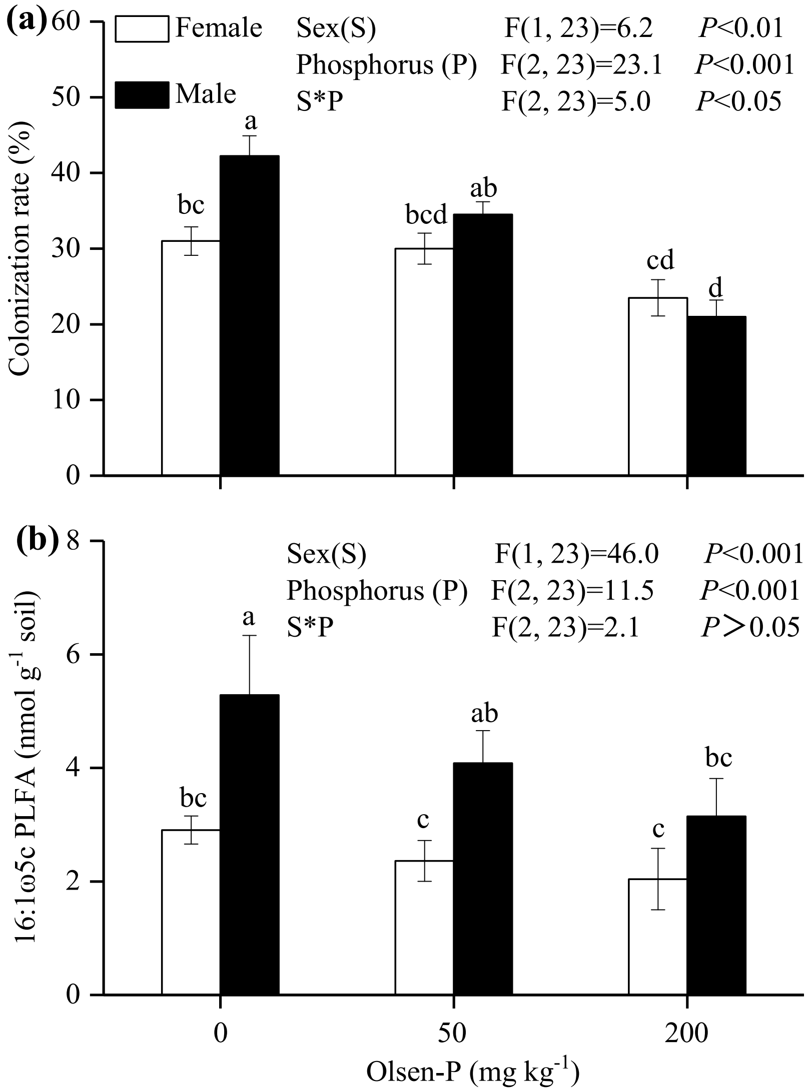
(a)
Female
Male
Sex(S) F(1, 23)=6.2 P<0.01
Phosphorus (P) F(2, 23)=23.1 P<0.001
S*P F(2, 23)=5.0 P<0.05
Colonization rate (%)
0
10
20
30
40
50
60
bc
a
bcd
ab
cd
d
(b)
Sex(S) F(1, 23)=46.0 P<0.001
Phosphorus (P) F(2, 23)=11.5 P<0.001
S*P F(2, 23)=2.1 P>0.05
16:1ω5c PLFA (nmol g-1 soil)
0
2
4
6
8
a
bc
ab
c
bc
c
0
50
200
Olsen-P (mg kg-1)

Figure 6

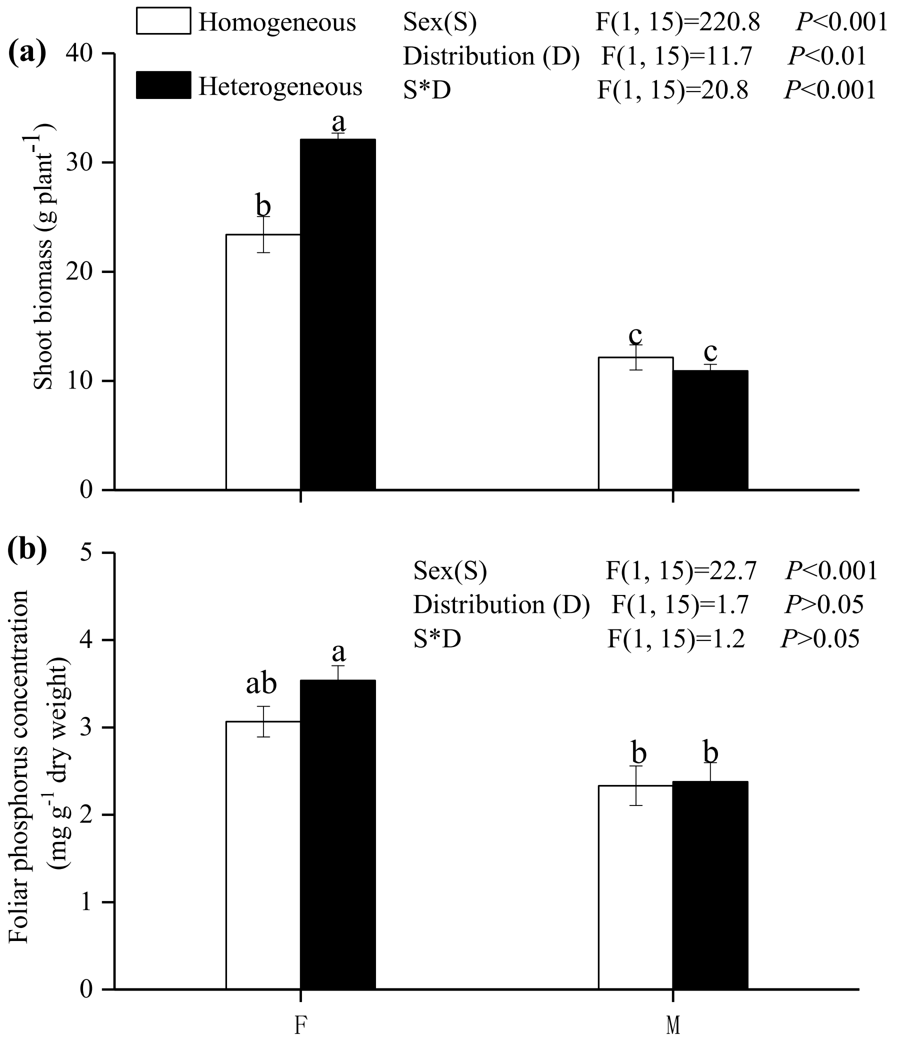
(a)
Homogeneous
Heterogeneous
Sex(S) F(1, 15)=220.8 P<0.001
Distribution (D) F(1, 15)=11.7 P<0.01
S*D F(1, 15)=20.8 P<0.001
Shoot biomass (g plant-1)
0
10
20
30
40
a
b
c
c
(b)
Sex(S) F(1, 15)=22.7 P<0.001
Distribution (D) F(1, 15)=1.7 P>0.05
S*D F(1, 15)=1.2 P>0.05
Foliar phosphorus concentration (mg g-1 dry weight)
0
1
2
3
4
5
ab
a
b
b
F
M

Figure 7

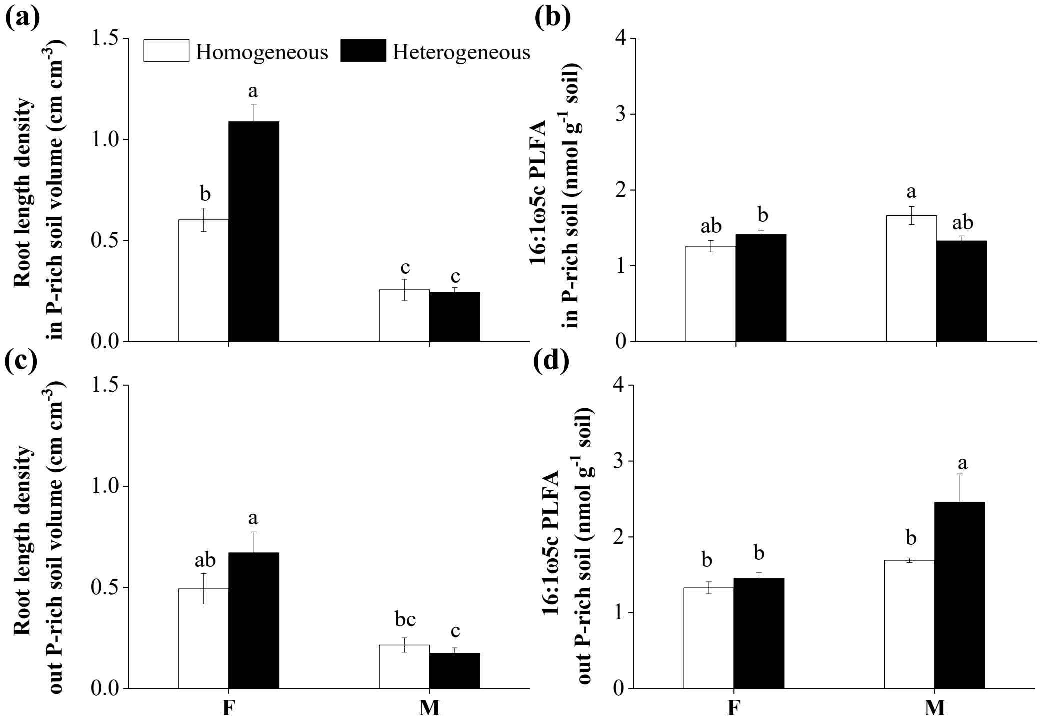

Table 1 Effects of soil P distribution and sex on root length density and PLFA

biomarkers 16:1ω5c representing extramatrical mycorrhizal hyphal biomass of *P. cathayana* inside and outside P-rich soil.

Factors	Root length density in soil (cm cm^{-3})				16:1ω5c PLFA in soil (nmol g^{-1})			
	Inside P-rich patch		Outside P-rich patch		Inside P-rich patch		Outside P-rich patch	
	F	*P*	F	*P*	F	*P*	F	*P*
Sex	99.5	**<0.001**	33.1	**<0.001**	3.8	>0.05	22.2	**<0.01**
Distribution	15.6	**<0.01**	1.1	>0.05	1.2	>0.05	7.6	**<0.05**
Sex ×Distribution	17.4	**<0.01**	2.7	>0.05	8.8	**<0.05**	2.5	>0.05

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