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# Sex-specific phosphorus acquisition strategies and cycling in dioecious *Populus euphratica* forests along a natural water availability gradient

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## Abstract

Soil phosphorus (P) availability affects plant growth and distribution. However, it is still unknown how sex-specific variation in functional traits affects plants' P acquisition and soil P transformation. On wet sites, female poplars had a greater specific root length (SRL), and a higher diversity of arbuscular mycorrhizal fungi (AMF) and phosphate-solubilizing bacteria (PSB). Male poplars living on wet sites increased the abundance of AMF and PSB communities and enhanced moderately labile and highly resistant organic P mineralisation via increased phosphatase activity. In contrast, on the dry site, the abundance and diversity of AMF and PSB communities increased in females, enhancing moderately labile and highly resistant organic P mineralisation via elevating phosphatase activities. Males maintained greater SRL and promoted Ca-P mobilisation via the release of root carboxylic acids and rhizosphere acidification on the dry site. The AMF community diversity followed a similar pattern as that of the PSB community when altering the P availability of different-sex plants. Our results indicated that organic P and Ca-P are the major sources of plant-available P in natural *P. euphratica* forests. Seasonal shifts and geographic locations affected the share of organic and inorganic P pools, and AMF and PSB diversities, ultimately altering sex-specific P acquisition strategies of plants.

**KEYWORDS**
dioecy, drought, phosphate-solubilizing bacteria, phosphorus mobilisation

## 1 | INTRODUCTION

The evolutionary origin of dioecious plants is commonly considered to be related to sexual dimorphism in their morphological and physiological characteristics, reflecting sex-specific adaptations to different resource demands (Dawson & Geber, 1999; Hultine et al., 2016; Obeso, 2002). Among dioecious plants, females usually exhibit greater nutrient acquisition in favourable environments but lower tolerance to stressors, while males maximise nutrient utilisation and survival in poor environments (Hultine et al., 2016; Liu et al., 2021, 2022a, 2022b). Some dioecious plant species may be

vulnerable to climate change if climatic modifications affect differently the performance of the opposite sexes and/or lead to the spatial segregation of sexes (Hultine et al., 2016). Therefore, examining sex-specific strategies for nutrient utilisation to predict the effects of climate change on plant growth and community stability is crucial.

Soil P is commonly a major limiting factor for plant growth, primarily because of the slow diffusion and occlusion of soil minerals, soil organic matter and fixation in the soil microbiome (Amadou et al., 2021; Harrison, 1987; Shen et al., 2011). Most soil P is fixed in inorganic and organic P complexes through complex biochemical and

geochemical processes, and it is not immediately available to plants (Huang et al., 2017; Malik et al., 2012). Most inorganic P is locked in moderately occluded minerals and recalcitrant P minerals with calcium, iron, and aluminum as primary and secondary P minerals (Harrison, 1987; Šantrůčková et al., 2004). Soil organic P is usually present as labile orthophosphate monoesters and organic polyphosphates (Šantrůčková et al., 2004; Tiecher et al., 2012). Roots undergo multiple morphological and physiological changes to enhance P uptake and bioavailability (Aslam et al., 2022; Gahoonia & Nielsen, 2003). Such changes include alterations in the root architecture and in the symbiosis of roots with arbuscular mycorrhizal fungi (AMF) to acquire P, rhizosphere acidification, carboxylate exudation, and the release of phosphatases to mobilise P (Lambers, 2022; Zhao et al., 2021; Richardson et al., 2000; Zhang et al., 2010). Recent evidence suggests that AMF play a major role in enhancing organic P mineralisation and capture (Jiang et al., 2021). In particular, AMF can harbour phosphate-solubilizing bacteria (PSB) on the hyphal surface, enhancing the efficacy of AMF to capture soil P (Jiang et al., 2021). However, less attention has been paid to the relative contributions of organic and inorganic P mobilisation, or to the interaction of AMF and PSB in controlling the transformation of soil P to provide available P pools. Furthermore, how AMF with PSB can alter P availability of female and male plants is not known. However, there is evidence that females and males of dioecious plants, such as poplars, support different soil microbial communities and have different root exudation rates (Xia et al., 2022). Thus, it is plausible that there are important sexual dimorphisms in P nutrition traits.

Soil inorganic and organic P transformation and P availability to plants are strongly correlated with soil water availability (Austin & Vitousek, 1998; Chen et al., 2021; Wood et al., 2016). Drought directly limits phosphatase activity via substrate diffusion and affinity and indirectly affects microbial activity and organic acid-mediated P solubility in the rhizosphere (Allison et al., 2011; Schimel, 2018). For example, drought decreases the concentration of soil inorganic P but increases the content of soil organic P (O'Connell et al., 2018). Another study argues that soil aridity is positively correlated with soil inorganic P concentrations in global drylands (Delgado-Baquerizo et al., 2018). Overall, these studies suggest that drought has complex effects on soil P cycling. Furthermore, interactions between bacteria and fungi, and their seasonal timing promote organic P mineralisation and capture of available P, thereby promoting plant survival and growth under water deficiency conditions (Chen et al., 2020; Zhang et al., 2023; Zhou et al., 2022). However, sex-specific responses of soil P cycling and acquisition strategies to natural soil-water gradients are largely unclear in dioecious plants.

Dioecious *Populus euphratica* Oliv. is an important component of the desert ecosystems in Xinjiang, China with vast arid terrain. The area is characterised by strong dryness and winds. The survival and productivity of *P. euphratica* communities become challenging during frequent and extensive droughts (Hultine et al., 2016). The major environmental variables affecting the groundwater level on the northwestern sites with an extremely dry desert climate cause a heterogeneous distribution of soil moisture (Yuanming et al., 2003). However, whether and how sex-specific nutrient acquisition strategies and soil P cycling are affected by the extensive variability of soil moisture in natural *P. euphratica* forests remain unclear. In this study, we assessed the relative contributions of insoluble inorganic and organic P in supplying bioavailable P and sex-specific P mobilisation using a natural soil water gradient in 30-year-old natural *P. euphratica* forests, and followed the process over an entire growing season. Our study aimed to answer the following questions: (1) How do soil water conditions affect the relative contributions of immobile inorganic P and organic P to the availability of P for plants during the growing season? (2) What is the sex-specific mechanism underlying soil P mobilisation and how it is affected by soil water availability during the entire growing season? (3) How do sex-specific functional traits affect sex-specific responses to water limitation and P acquisition strategies in dioecious plant species? Our results contribute to the understanding of how climate change-driven decreases in soil water availability alter biogeochemical processes of soil P in natural *P. euphratica* forests.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and sample collecting

The study site was located in Xinjiang, China. The site has a temperate continental arid climate with a mean annual precipitation of 300–60 mm, annual evaporation capacity of over 2800 mm, and mean temperature of 4–20°C. Three undisturbed *P. euphratica* forest communities were selected based on the soil water gradient at 1 m soil depth: Shigongli (SGL; soil water content 32%, 40°76′ N, 82°05′ E; very wet site), Sanhe (SH; soil water content 18%, 40°42′ N, 80°91′ E; moderately wet site), and Shaya (SY; soil water content 8%, 40°56′ N, 83°04′ E; dry site). Considering the low precipitation in Xinjiang, the natural soil water gradient primarily results from natural groundwater gradients (Hao et al., 2009). *Tamarix sp.* is the dominant shrub, sparsely distributed on these sites. *P. euphratica* females and males were identified based on flower traits during the spring. The soil traits on the three sites are listed in Table S1.

The study was conducted in mid-July and mid-October 2021. Eight 20 × 20 m plots (two plant sexes × four replicates) were randomly established on each of the three sites, a total of 24 plots. Soil samples were collected from a depth of 90–130 cm at the four cardinal points from a selected trunk (as the root development of *P. euphratica* always occurs below 90 cm). Four female and male *P. euphratica* samples were selected from each plot on each date, resulting in 48 samples (two sampling dates × two plant sexes × three sites × four replicates). Roots with a diameter less than 2 mm were separated from the soil, and the rhizosphere soil was detached from the fine roots. The roots were then gently washed with deionized water and scanned using a Win-RHIZO scanner to measure the total root length. All root samples were dried at 70°C for 72 h to calculate

the specific root length (the ratio of root length to root dry mass; SRL). The rhizosphere soil samples were pooled for microbial community analyses. The remaining soil samples were air-dried and sieved (2 mm) before the physicochemical analysis.

## 2.2 | Plant P content measurements

Root and leaf samples were dried at 75°C, and about 0.2 g of the dry root and leaf samples were digested with $H_2SO_4$-$H_2O_2$. The P concentration was measured with an inductively coupled plasma-optical emission spectrometer (Model 5300DV, Perkin-Elmer).

## 2.3 | Soil P fractions

Soil P fractions were sequentially extracted from soil samples (0.5 g) and measured according to a modified Hedley method (Hedley et al., 1982). (1) For resin-P (water-soluble P), soil samples were extracted with 1 g of resin (717 anion exchange resin). (2) $NaHCO_3$-P ($Ca_2$-P) was extracted with 30 mL of 0.5 mol $L^{-1}$ $NaHCO_3$ (pH 8.5). One part of the supernatant was added to 6 mL of 0.9 mol $L^{-1}$ $H_2SO_4$, and the concentration of inorganic $NaHCO_3$-$_{Pi}$ was measured. The other part of the supernatant was added to 0.5 g of $(NH_4)_2S_2O_8$ and 10 mL of 0.9 mol $L^{-1}$ $H_2SO_4$, and the concentration of total $NaHCO_3$-$P_t$ was measured. (3) NaOH-P (Al, Fe-binding P) was extracted with 30 mL of 0.1 mol $L^{-1}$ NaOH. One part of the supernatant was added to 1.6 mL of 0.9 mol $L^{-1}$ $H_2SO_4$, and the concentration of inorganic $NaHCO_3$-$_{Pi}$ was measured. The other part of the supernatant was added to 0.5 g of $(NH_4)_2S_2O_8$ and 10 mL of 0.9 mol $L^{-1}$ $H_2SO_4$ and autoclaved for 90 min to measure the total NaOH-$P_t$. (4) HCl-P ($Ca_8$-P and $Ca_{10}$-P) was extracted with 10 mL of HCl. (5) Residual P (refractory minerals and organic matter-binding P) was calculated as the difference between the total soil P and the first four P fractions. Moderately occluded P was calculated as the sum of the $NaHCO_3$-P and NaOH-P fractions, and recalcitrant P was the sum of the HCl-P and residual P (Wang et al., 2022).

## 2.4 | Soil physiological traits and measurement of phosphatase activity

Soil pH was measured from a soil and water mixture (1:2.5) using a pH metre (150 PHBJ-260). Total soil nitrogen (TN) and carbon (TC) contents were measured with a C and N analyser (Multi C/N 152 3100; Jena Analytics). Soil ammonium ($NH_4^+$-N) and nitrate ($NO_3^-$) nitrogen concentrations were determined according to the KCl extraction-colorimetric method (Doane & Horwáth, 2003). Soil cation exchange capacity (CEC) was determined according to the ammonium acetate method (Kitsopoulos, 1999). Soil-available potassium (AK) was determined with flame spectrometry (PFP7 flame photometer) after extraction with $HNO_3$ (Zhang & Gong, 2012). Soil extracellular acid phosphatase, neutral phosphatase, and alkaline

phosphatase activities were determined using p-nitrophenyl phosphate as a substrate at pH 5, 7, and 9.4, respectively, at 37°C (Tabatabai, 1994).

## 2.5 | Root exudate collecting and exudate chemical analyses

Root exudates were extracted as described by Michalet et al. (2013). Briefly, the roots were carefully excavated and rinsed with deionized water. Thereafter, the roots were placed on a wet geotextile tissue for 72 h. Subsequently, the roots were placed in 50-mL syringes filled with an osmotic solution (3 mM $CaCl_2$) and glass beads. After 72 h, the root exudates in the syringes were collected and filtered with a 0.2-μm membrane. The collected root exudates were frozen in liquid nitrogen and freeze-dried in a vacuum freeze-dryer. The resulting pellets were resuspended in 200 μL methanol and centrifuged at 12 000 rpm for 10 min at 4°C. Thereafter, the mixtures were transferred to sample bottles and measured with ultra-performance lipid chromatography (UPLC, SHIMADZU Extra X2, Shimadzu) and tandem mass spectrometry (Applied Biosystems 4500 QTRAP) under the following conditions: 4 μL injection volume, 0.35 mL $min^{-1}$ flow rate, 40°C column temperature. The mobile phase consisted of 0.1% formic acid in both pure water (solvent A) and acetonitrile (solvent B). Linear ion trap and triple quadrupole scans were performed with a triple quadrupole-linear ion-trap mass spectrometer.

## 2.6 | Sequencing and bioinformatics

DNA was extracted from fresh soil samples (50 mg) using a DNA extraction kit (MO BIO Laboratories Inc.) according to the manufacturer's instructions. The ITS2 site (fITS7/ITS4) of the PSB community harbouring phoD gene, was amplified using the primers F733 (5'-TGGGAYGA TCAYGARGT-3') and R1083 (5'-CTGSGCSAKSACRTTCCA-3' (Ragot et al., 2015). The PCR reaction in a volume of 50 μL comprised 25 μL 2× Premix Taq, 1 μL of each primer (10 μM), 3 μL DNA template, and 21 μL nuclease-free water. The PCR was performed with a Bio-Rad S1000 (Bio-Rad Laboratory). PCR products were detected using 1% agarose gel electrophoresis and purified using an EZNA Gel Extraction Kit (Axygen Biosciences). Sequencing libraries were constructed using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (New England Biolabs) according to the manufacturer's instructions, following the addition of index codes. After an assessment with a Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific), the library was sequenced with an Illumina Nova6000 platform (Illumina), which generated 250 bp paired-end reads. Clean paired-end reads were obtained by removing the primers located at each end of the sequence. Subsequently, the clean paired-end reads were assembled according to the fastq-merged pair method (version 10, http://www.drive5.com/usearch/). Raw tag quality control was performed with FASTQ (version 0.14.1; https://github.com/OpenGene/fastp). Amplicon sequence variants (ASVs) were assigned from the sequencing data at 97% nucleotide identity with UPARSE

(Edgar, 2013). We produced an average of 93 291 and 124 607 PSB and AMF community ASVs, respectively, for subsequent analyses. Fungal sequences were analysed with UNITE (V.8.0) as the reference database (Callahan et al., 2016; Gweon et al., 2015; Nilsson et al., 2019). Raw DNA sequence files and associated metadata were deposited in the NCBI with the accession number PRJNA1015180.

## 2.7 | Statistical analysis

A three-way analysis of variance was performed to examine the effects of site, season, and plant sex using SPSS (version 22.0). Differences between mean values were analysed with the Duncan test after one-way analysis of variance. The α-diversity indices of the PSB community were analysed with R ver. 0.4.0 and visualised with the "ggplot2" package. The β-diversity indices of PSB communities were calculated by nonmetric multidimensional scaling (NMDS) using the Bray–Curtis dissimilarity with the "ordinate" function of the Phyloseq package (McMurdie & Holmes, 2013). Structural equation modelling (SEM) was used to analyse the correlation network between plant biomass P, soil organic P, PSB community (community diversity and compositional indices), soil factors (including TN, TC, AK, CEC, pH, $NO_3^-$, and $NH_4^+$), and soil acidic compounds (organic acids and phenolics in the rhizosphere). SEM was performed with AMOS 21.0 (Amos Development Corporation). The principal component analysis (PCA) was performed to develop the multivariate functional indices using R. The first component was used as the new variable for subsequent analyses (Chen et al., 2019). The partial Mantel test was used to determine the correlation between environmental distances and similarities in the rhizosphere microbial communities. The best-fit model was evaluated using the $\chi^2$ test and root mean square error of approximation (RMSEA) (Grace et al., 2016). In the Chi-square test, $p > 0.5$ and the RMSEA ≤0.05 indicate a good fit (Schermelleh-Engel et al., 2003). The Mantel test and $\chi^2$ test were carried out with Mantel 3.0. All statistical tests were considered significant at $p < 0.05$.

## 3 | RESULTS

### 3.1 | Leaf and root P contents

The leaf P content of *P. euphratica* trees decreased from summer to autumn in both male and female plants (Figure 1a). There were significant season × site, season × sex and season × site × sex interactions, reflecting a greater overall seasonal reduction in leaf P during the season in SH and SY, and a lower reduction in P in females than in males in SY (Figure 1a). In the case of roots, the seasonal reduction in the P content was observed only in males in SH, and in females in SY, whereas the male roots in SY had a greater P content in autumn than in summer (Figure 1b).

Sex-specific differences in the leaf and root P contents were detected only on the dry site (SY, Figure 1a,b). On this site, males exhibited a higher leaf P content in summer but a lower P content in autumn compared to females (Figure 1a). The root P content was higher in females than in males in summer, but this difference disappeared in autumn (Figure 1b). The SRL of both sexes increased on the dry site (SY) relative to the other two sites. On this site, males had a greater SRL than females in summer, while the opposite was observed in autumn (Figure 1c). On the moderately wet site (SH), the SRL of females was higher than that of males in summer, whereas no sexual difference was found in autumn (Figure 1d). In summer, the total AMF abundance was greater on the dry site (SY) in both sexes compared to the other two sites, and females had a greater AMF abundance than males. In contrast, in autumn, the AMF abundance was higher in SH than in SY, and the AMF abundance was greater in males than in females on these two sites (Figure S1).

### 3.2 | Soil P fractions

There were no significant sex-related differences in the rhizosphere resin-P and $NaHCO_3$-P contents in summer, regardless of soil water conditions (except for resin-P in SGL; Table 1). In autumn, the rhizosphere of female poplars showed higher resin-P and $NaHCO_3$-P contents than that of male poplars in SY, whereas no significant sex-related differences in these traits were observed between SGL and SH. Sex-specific differences in rhizosphere NaOH-P and HCl-P contents were detected only in SY. The rhizosphere of male poplars showed a greater increase from summer to autumn in these soil P fractions compared to females (Table 1). The rhizosphere residual P content decreased during the season, and this decrease was greater in male plants in SH, while the sex differences were the opposite on the dry (SY) site during the entire growing season. The sex-specific differences in organic P contents were independent of the soil water status, and the rhizospheres of female poplars had greater organic P contents than those of males during the entire growing season (Table 1).

### 3.3 | Soil PSB and AMF communities and the dominant phyla

The rhizosphere of female poplars showed a higher Chao1 index of the PSB community compared to males on moderately wet (SH) and dry (SY) sites, regardless of the growth season (Figure 2a). The sex-specific difference in the Chao1 index of the PSB community was greater in autumn on the moderately wet site (SH), but it was greater in summer on the dry site (SY) (Figure 2c). The Chao1 index of the AMF community was higher in the rhizosphere of females than in the rhizosphere of males on the dry (SY) site regardless of the growth season (Figure 2c). On the moderately wet (SH) site, a sexual difference in the Chao1
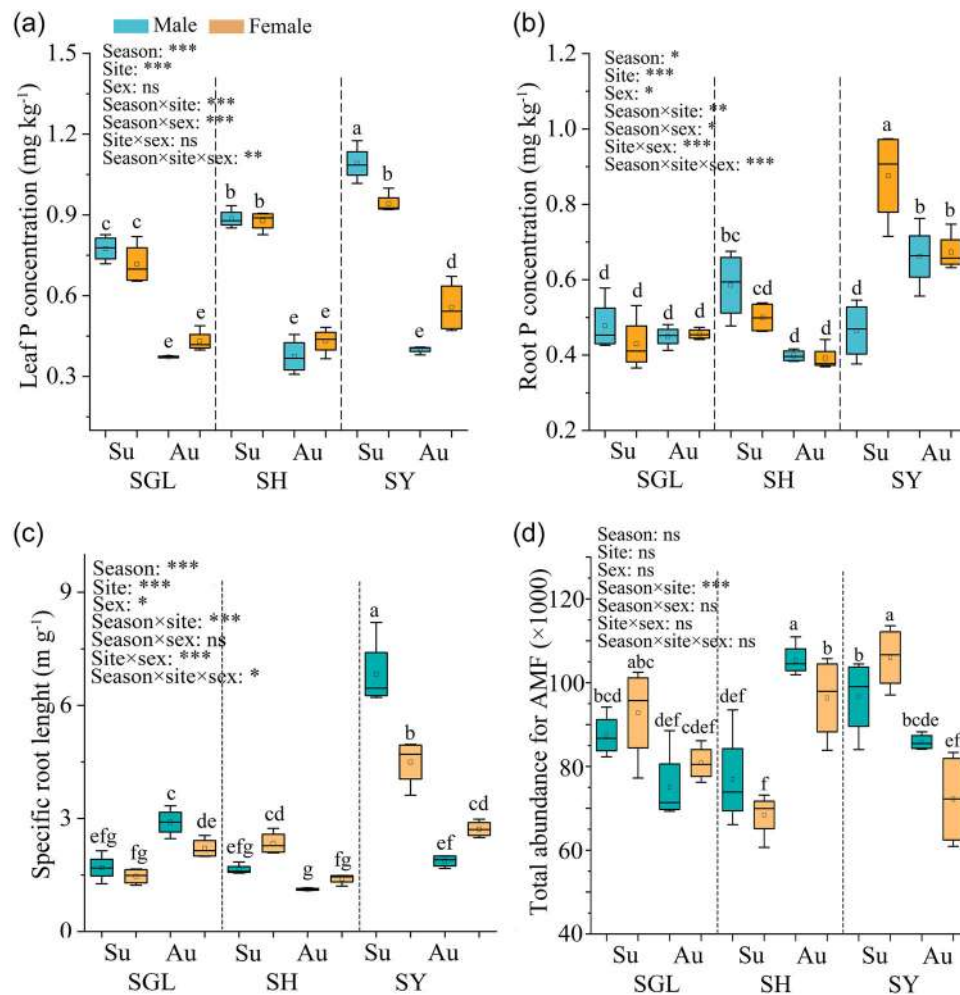
**FIGURE 1** Phosphorus (P) contents in leaves (a) and roots (b), specific root length (SRL) (c) and the relative abundance of arbuscular mycorrhizal fungi (AMF) (d) of *P. euphratica* females and males growing under three natural soil water conditions (measured at 1 m soil layer depth): Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%) and Shaya (SY, soil water content 8%). Differences between treatments were analysed with Duncan's tests after one-way ANOVA. Values are showed as means ± SE (n = 4). Lowercase letters indicate significant differences between treatments (p < 0.05). Blue columns represent males and yellow ones represent females. The horizontal line in the boxes represents median. Su, summer; Au, autumn. Season, season main effect; site, site main effect; sex, sex main effect; season × site, the interaction of season and site; season × sex, the interaction of season and sex; sex × site, the interaction of sex and site; season × sex × site, the interaction of season, sex and site. ns, not significant; *0.01 < $p \leq 0.05$; **0.001 < $p \leq 0.01$; and ***$p \leq 0.001$. ANOVA, analysis of variance; Au, autumn; Su, summer. [Color figure can be viewed at wileyonlinelibrary.com]

index was detected only in autumn, and the rhizosphere of females had a higher Chao1 index than the rhizosphere of males. The NMDS analysis showed that the PSB community showed a sex-related difference in its structure in the poplar rhizosphere on the dry (SY) site in summer (Figure 2d). Large sex-dependent differences in community structure of the PSB community were found on the moderately wet (SH) site in autumn (Figure 2d). Concerning the dominant phyla of the AMF community on the moderately wet (SH) site, the rhizosphere of females had higher abundances of Tubilinea in summer and Mucoromycota in autumn (Figure 2e). In contrast, on the dry (SY) site, the rhizosphere of females had a greater abundance of Mucoromycota, whereas Basidiomycota and Ascomycota were most abundant in the rhizosphere of males regardless of the growth season (Figure 2f).

## 3.4 | Phosphatase activity and its correlation with organic and residual P in the rhizosphere

Differences in sex-specific alkaline, neutral, and acid phosphatase activities were more significant in SY, irrespective of the growing season, the rhizosphere of females having a greater phosphatase activity than that of males (Figure 3a–d). However, on the well-watered sites (SH and SGL), all types of phosphatase activities were greater in the rhizosphere of male poplars (Figure 3a–c). Phosphatase activities generally decreased in autumn, except for SY in the rhizosphere of females (Figure 3a). The rhizosphere ratio of moderately labile to recalcitrant P was higher in poplar females and males on the dry site than on the wet sites (Figure 3d). Season and plant sex did not affect the ratio of moderately labile to recalcitrant P

**TABLE 1** Soil phosphorus (P) fractions (mg/kg): Resin-P, $NaHCO_3$-$P_t$, $NaHCO_3$-$_{Pi}$, NaOH-$P_t$, HCl-P, Residual P and organic P in the rhizosphere of *P. euphratica* females (F) and males (M) in summer (Su) and autumn (Au) growing in three natural soil water conditions: Shigongli (SGL, volumetric soil water content of 32% at 1 m depth), Sanhe (SH, soil water content of 18% at 1 m depth) and Shaya (SY, soil water content of 8% at 1 m depth).

| Treatment | Resin-P (mg kg$^{-1}$) | $NaHCO_3$-$P_t$ (mg kg$^{-1}$) | NaOH-$P_t$ (mg kg$^{-1}$) | HCl-P (mg kg$^{-1}$) | Residual P (mg kg$^{-1}$) | Organic P (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| SuSGLM | 2.48 ± 0.60ab | 1.24 ± 0.40bc | 4.69 ± 0.18def | 485.3 ± 23.3a | 120.6 ± 4.0ab | 45.5 ± 5.2cd |
| SuSGLF | 1.88 ± 0.28cd | 0.59 ± 0.01c | 3.63 ± 0.28efg | 475.6 ± 17.6a | 113.9 ± 10.4abc | 49.9 ± 2.3bc |
| SuSHM | 1.23 ± 0.13e | 1.02 ± 0.40bc | 2.18 ± 0.54g | 492.3 ± 12.2a | 112.3 ± 5.9bc | 38.0 ± 5.7e |
| SuSHF | 1.30 ± 0.17e | 0.65 ± 0.31c | 2.76 ± 0.85fg | 479.9 ± 23.3a | 102.3 ± 10.2de | 53.4 ± 6.8b |
| SuSYM | 2.29 ± 0.31bc | 2.52 ± 0.90a | 6.57 ± 1.32cd | 464.7 ± 12.6a | 95.1 ± 2.8efg | 40.8 ± 3.8de |
| SuSYF | 2.20 ± 0.35bc | 2.86 ± 0.70a | 8.81 ± 2.47ab | 432.3 ± 15.9b | 122.8 ± 9.8a | 59.2 ± 6.4a |
| AuSGLM | 0.98 ± 0.11e | 1.09 ± 0.25bc | 5.06 ± 1.24de | 485.3 ± 23.9a | 101.3 ± 6.1def | 20.8 ± 2.3f |
| AuSGLF | 1.15 ± 0.12e | 1.01 ± 0.17bc | 5.31 ± 0.30de | 477.3 ± 18.3a | 108.0 ± 6.2cd | 18.7 ± 2.2fg |
| AuSHM | 1.11 ± 0.89e | 1.09 ± 0.80bc | 2.78 ± 0.90fg | 482.3 ± 18.0a | 88.2 ± 91.5gh | 13.5 ± 1.1gh |
| AuSHF | 1.15 ± 0.12e | 1.12 ± 0.22bc | 4.06 ± 0.67efg | 484.9 ± 14.3a | 91.5 ± 3.6fgh | 12.8 ± 1.6gh |
| AuSYM | 1.45 ± 0.38de | 1.57 ± 0.68b | 10.10 ± 1.28a | 431.2 ± 11.2b | 85.5 ± 4.2h | 12.0 ± 0.6h |
| AuSYF | 3.00 ± 0.89a | 2.5 ± 0.76a | 7.95 ± 2.83bc | 426.9 ± 5.8b | 98.1 ± 3.3defg | 16.8 ± 1.0fgh |
| $p_{Season}$ | *** | ns | ** | ns | *** | *** |
| $p_{site}$ | *** | *** | *** | *** | *** | * |
| $p_{sex}$ | Ns | ns | ns | * | ** | *** |
| $p_{season × site}$ | *** | ns | ns | ns | ns | * |
| $p_{season × sex}$ | ** | * | ns | ns | *** | *** |
| $p_{site × sex}$ | ** | ** | ns | ns | ns | ** |
| $p_{season × site × sex}$ | * | ns | ** | ns | ** | ns |

*Note*: Treatment codes combine season, site and sex. For example, SuSGLM stands for summer, SGL site and males. Differences between treatments were revealed by Duncan tests after one way-ANOVA. Values are showed as means ± *SD* (*n* = 4). Different lowercase letters indicate significant differences between treatments (*p* < 0.05). $p_{Season}$, season main effect; $p_{site}$, site main effect; $p_{sex}$, plant sex main effect; $p_{season × sex}$, the interaction of season and plant sex; $p_{season × site}$, the interaction of season and site; $p_{site × sex}$, the interaction of site and plant sex; $p_{season × site × sex}$, the interaction of season, site and plant sex. Pi, inorganic P, $P_o$, organic P, Pt, total P; Resin-extracted total P, resin-$P_t$; $NaHCO_3$-extracted total P, $NaHCO_3$-$P_t$; NaOH-extracted total P, NaOH-$P_t$; HCl-extracted total P, HCl-$P_t$. ns, not significant;

*0.01 < *p* ≤ 0.05;

**0.001 < *p* ≤ 0.01; and

***$p$ ≤ 0.001.

on the wet sites (SGL and SH, except for SH in summer). On the dry site (SY), the rhizosphere ratio of moderate-labile to recalcitrant P was greater in females than in males in summer, whereas there was no sexual difference in this ratio in autumn (Figure 3d).

### 3.5 | Correlations among different phosphate activities, and phosphatase activity with moderately labile organic P and highly resistant residual P

Positive correlations among different phosphate activities were significant in the rhizosphere of both poplar sexes (Figure 4a–c). Correlations were greatest between acid and neutral phosphatase activities, and smallest between alkaline and neutral phosphatase

activities (Figure 4a–c). In addition, phosphatase activities showed a significant positive correlation with the organic P and residual P contents in the rhizosphere of both poplar sexes (Figure 4d).

### 3.6 | Relationships between the PSB community dissimilarities, and soil exudates and P fractions

Across seasons, the correlations of rhizosphere AMF and PSB community compositions with soil traits were stronger in the rhizospheres of male poplars than in those of females (Figure 5a,b). Soil water and $NO_3^-$ contents were responsible for PSB and AMF community compositions in the rhizospheres of both sexes (Figure 5a,b). The AMF community diversity was more strongly
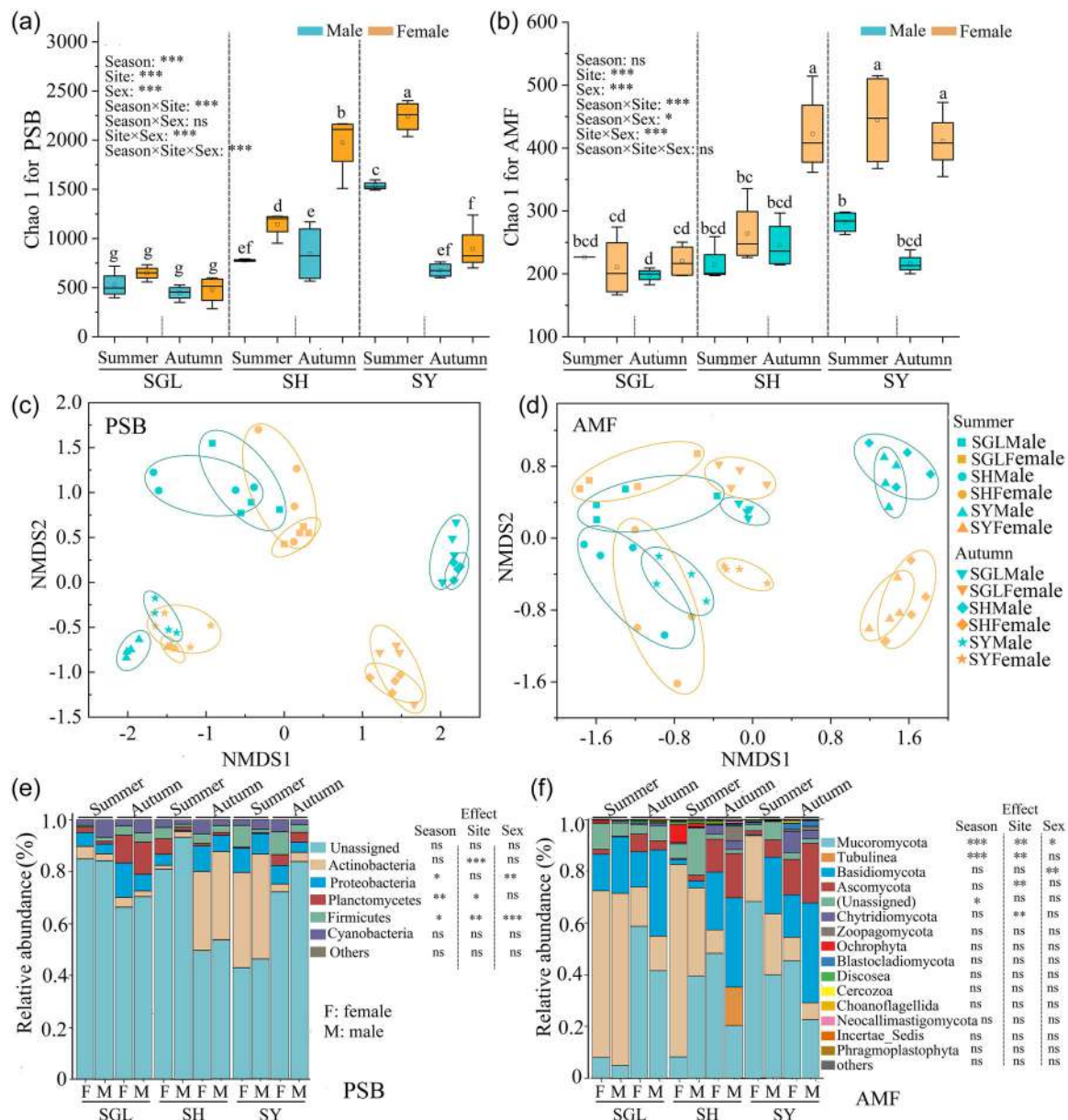
**FIGURE 2** The alpha diversity indicated by the Chao index (a, b), the beta diversity indices (c, d) and the dominant phyla (e, f) of phosphate solubilizing bacterial (PSB) communities (harbouring *phoD* genes) (a, c, e) and arbuscular mycorrhizal fungi (AMF) (b, d, f) in *P. euphratica* females and males growing under three natural soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%) and Shaya (SY, soil water content 8%). For (a) and (b), blue columns represent males and yellow ones represent females. The beta diversity indices were constructed by the nonmetric multidimensional scaling method. The dominant phyla were defined as those with a relative abundance of >1%, and phyla with a relative abundance of <1% were denoted as "others". Season, season main effect; sex, sex main effect; site, site main effect; ns, not significant; *$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; and ***$p \leq 0.001$. Au, autumn; F, females; M, males; Su, summer. [Color figure can be viewed at wileyonlinelibrary.com]

affected by root exudates and phosphatase activities in the rhizosphere of male poplars (Figure 5b). The rhizosphere AMF and PSB community diversities positively influenced each other in both sexes. The PSB community diversity was strongly correlated with residual P and neutral phosphatase activities in the rhizosphere of male poplars and with lipids and neutral phosphatase activities in the rhizosphere of females (Figure 5a). However, during the entire growth season, the rhizospheres of female poplars showed stronger correlations among soil physicochemical factors, soil P fraction, root exudates, SRL, and phosphatase activities than the rhizosphere of males (Figure S2).

The PSB community diversity in the rhizosphere was strongly affected by the soil physicochemical factors, SRL, and phosphatase activity, regardless of the growth season. The root exudates and P fractions were responsible for the PSB community composition in the rhizosphere of female poplars mainly in autumn (Figure S2).
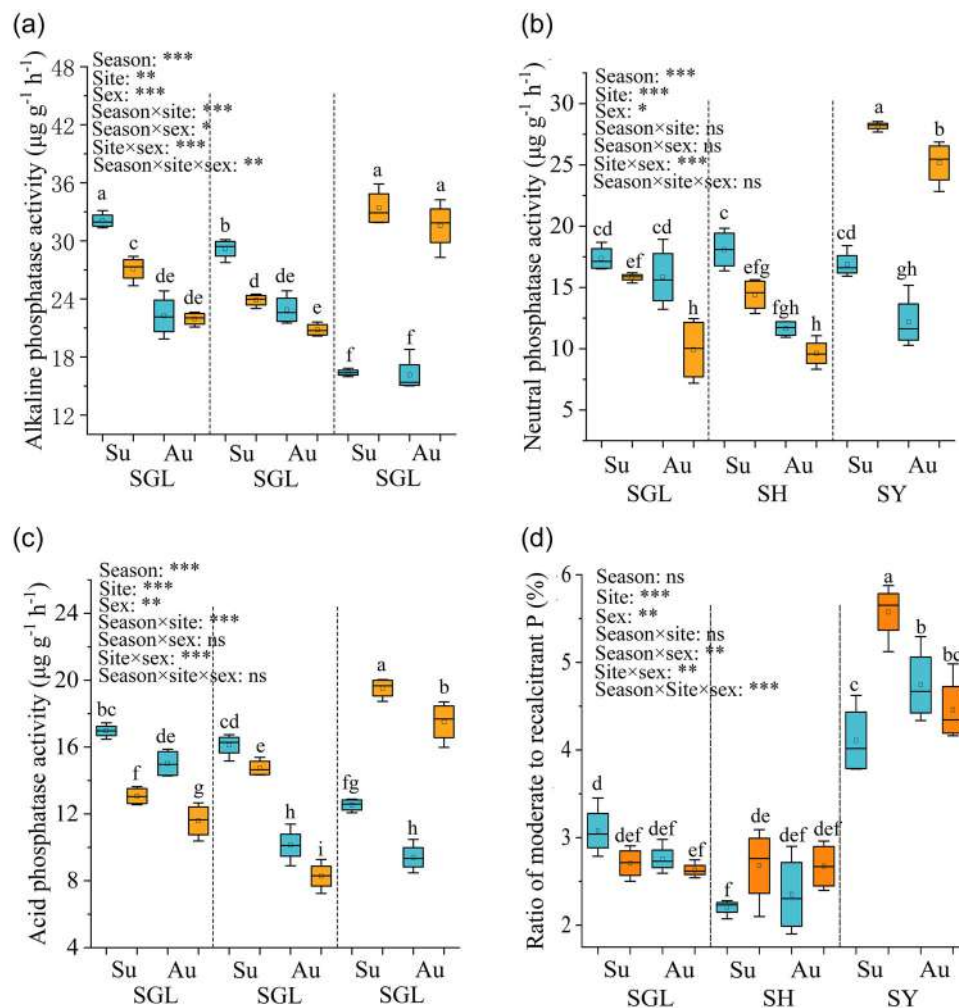
**FIGURE 3** The alkaline (a), neutral (b), and acid phosphatase activities (c) and the ratio of moderately occluded phosphorus (P) to recalcitrant P (d) in the rhizospheres of *P. euphratica* females and males on three sites with natural differences in soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%), and Shaya (SY, soil water content 8%). Differences between treatments were analysed using Duncan's tests after one-way ANOVA. Values are presented as the mean ± *SE* (*n* = 4). Blue columns represent males and yellow ones represent females. Lowercase letters indicate significant differences between treatments (*p* < 0.05). Su, summer; Au, autumn. Season, season main effect; Site, site main effect; Sex, plant sex main effect; Season × sex, interaction between season and plant sex; Season × site, interaction between season and site; Site × sex, interaction between site and plant sex; Season × site × sex, interaction between season, site, and plant sex; ns, not significant; *0.01 < *p* ≤ 0.05; **0.001 < *p* ≤ 0.01; and ***p* ≤ 0.001. ANOVA, analysis of variance. [Color figure can be viewed at wileyonlinelibrary.com]

The AMF community composition in female poplars was strongly affected by the root exudates during autumn. In the male poplar rhizosphere, the PSB community was strongly correlated with root exudates and soil physicochemical factors, regardless of the growth season (Figure S2). However, most soil P fractions in male poplars were responsible for the PSB community composition in autumn. The AMF community composition was correlated with SRL and phosphatase activities during the entire growth season in the rhizosphere of male poplars. In addition, the rhizosphere pH in females positively affected HCl-extracted P, AK and $NO_3^-$. Rhizosphere $NaCO_3$-P and NaOH-P also showed positive associations with soil phosphatases in females. In contrast, organic P and residual P interacted positively with phosphatase activities in the rhizosphere of males.

## 3.7 | Relationships of soil characteristics with AMF and PSB community diversity

The SEM analysis revealed that root exudates indirectly affected PSB-regulated soil P cycling by altering the rhizosphere nutrient availability in summer, whereas root exudates directly controlled the diversity of the rhizosphere PSB bacterial community to regulate soil P cycling in autumn (Figure 6). Additionally, the SEM model revealed that plant-available P was dominantly and directly controlled by moderately occluded P, soil factors, and the abundance of soil organic acid, and indirectly controlled by soil organic P and soil factors (Figure 6). Moderately occluded P was positively affected by the abundance of soil organic acid and organic P concentration, but negatively affected by soil factors and soil recalcitrant P. Among
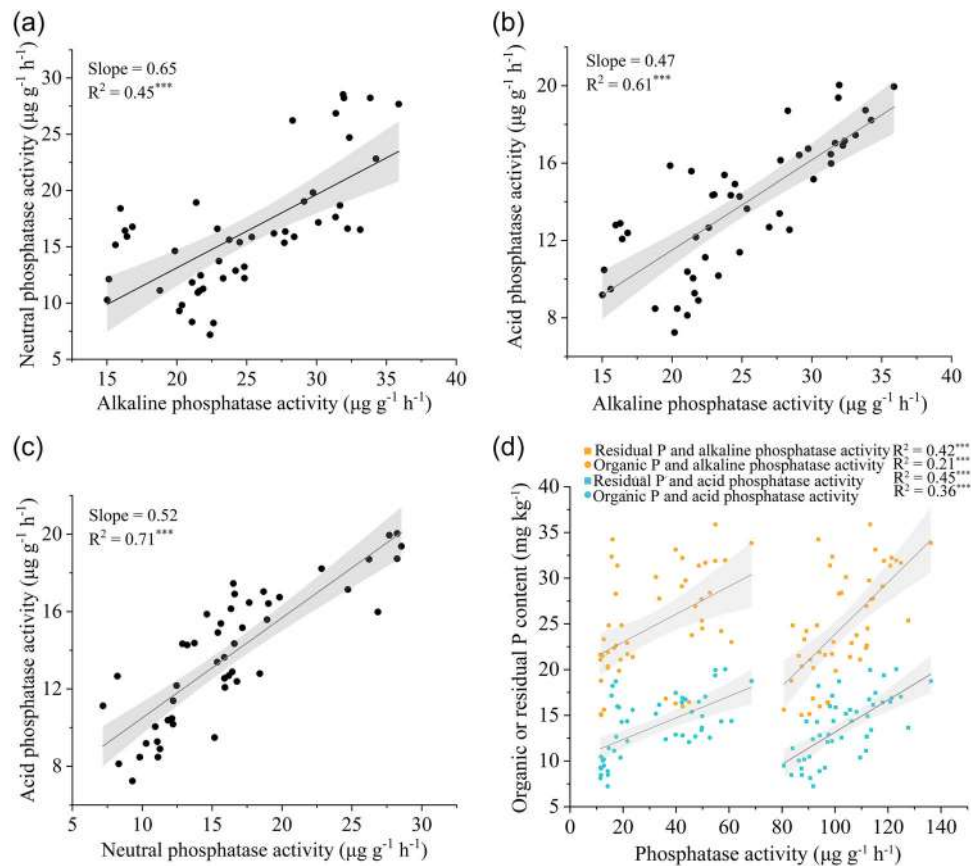
**FIGURE 4** The correlation of alkaline phosphatase with neutral phosphatase activity (a), alkaline phosphatase with acid phosphatase activity (b), neutral phosphatase with acid phosphatase activity (c), and phosphatase activity with soil inorganic phosphorus (P) content (d) in the rhizosphere of *P. euphratica* females and males on three sites with natural differences in soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%), and Shaya (SY, soil water content 8%). Differences between treatments were analysed using Duncan's tests after one-way ANOVA. Values are presented as the mean ± *SE* (*n* = 4). Lowercase letters indicate significant differences between treatments (*p* < 0.05). Su, summer; Au, autumn. Season, season main effect; Site, site main effect; Sex, plant sex main effect; Season × sex, interaction between season and plant sex; Season × site, interaction between season and site; Site × sex, interaction between site and plant sex; Season × site × sex, interaction between season, site, and plant sex; ns, not significant; *0.01 < *p* ≤ 0.05; **0.001 < *p* ≤ 0.01; and ***$p$ ≤ 0.001. [Color figure can be viewed at wileyonlinelibrary.com]

these factors, the soil organic P had the greatest direct effect on moderately occluded P. In addition, recalcitrant P was positively affected by soil factors (0.42) and negatively affected by the abundance of soil organic acids (−0.47; Figure 6).

# 4 | DISCUSSION

## 4.1 | Sex-specific P uptake strategies along a soil water gradient

Root morphogenesis is steered by soil water heterogeneity, and increased root growth facilitates water and nutrient acquisition (von Wangenheim et al., 2020). *P. euphratica* has deep vertical root systems, and lower groundwater levels create heterogeneous soil moisture environments around the roots (Hao et al., 2013; Yu et al., 2013). On the dry site, SRL increased in both female and male poplars, and the increase was greater in males than in females

(Figure 1c). An increased absorption surface area between the soil and roots may enhance P and water uptake (Hodge, 2004). Larger SRL in plants enhances their nutritional exploration in heterogeneous environments (Mommer et al., 2011). Hence, the larger SRL of males compared with females means that males have more absorptive roots, which may promote P acquisition on the dry site (Figures 1c and 7).

However, female poplars had higher P levels than males, implying that P acquisition pathways other than roots were involved in P acquisition. In addition to modifications in the root architecture, colonisation by AMF and their interaction with PSB taxa potentially promote P acquisition (Neumann & Röm-held, 2002; Zhang et al., 2010). Plants can recruit different rhizosphere microbiomes, which facilitate plant adaptation to their environment (Hacquard, 2016; Thiergart et al., 2020). The SRL of female poplars was smaller than that of males, which enhanced AMF symbiosis and aided exploiting larger soil volumes beyond the root P-depletion zone (Laliberté, 2017; Ma
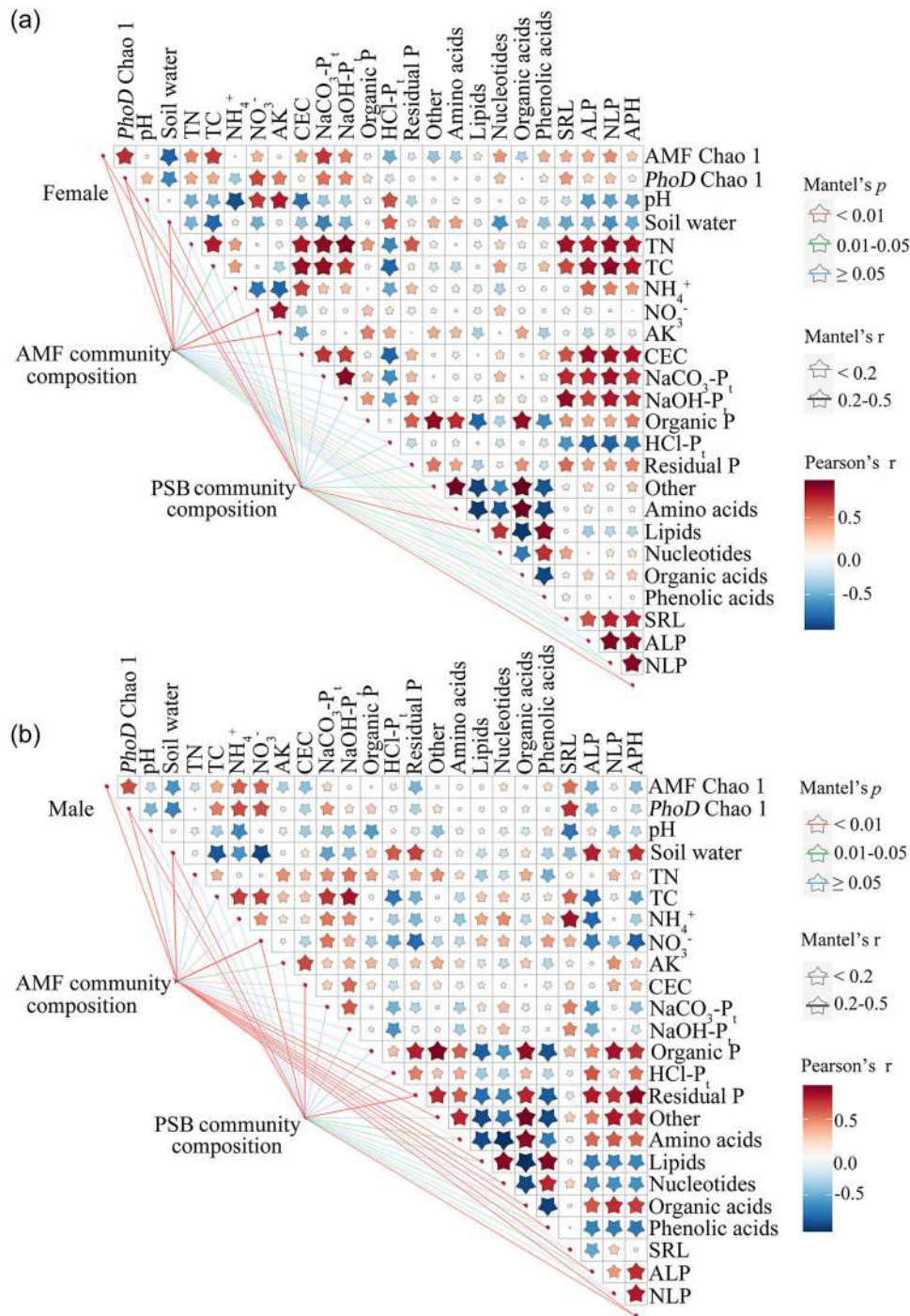
**FIGURE 5** Correlations of soil physiochemical traits, compositions of arbuscular mycorrhizal fungal (AMF) and PSB (harbouring *phoD* genes) communities in the rhizosphere of *P. euphratica* females (a) and males (b) on three sites with natural differences in soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%), and Shaya (SY, soil water content 8%). The microbial community composition matrix was calculated based on the Bray-Curtis distance with a partial Mantel test. The line colour represents statistical significance with 999 permutations, and the line width scale represents the size of partial Mantel r value. The colour bar provides the scale for the Pearson correlation coefficient. Resin-P$_t$, resin-extracted total P; NaHCO$_3$-P$_t$, NaHCO$_3$-extracted total P; NaOH-P$_t$, NaOH-extracted total P; HCl-P$_t$, HCl-extracted total P. AK, available potassium content; ALP, alkaline phosphatase activity; AP, available P content; APH, acidic phosphatase activity; CEC, cation exchange capacity; NH$_4^+$, ammonium N content; NO$_3^-$, nitrate N content; OP, organic phosphorus content; Other, other root exudates; NLP, neutral phosphatase activity; Other, other root exudates; SRL, specific root length; SW, soil volumetric water content; TC, total soil carbon content; TN, total soil nitrogen content; amino acids, lipids, nucleotides, organic acids and phenolic acids represent their contents. [Color figure can be viewed at wileyonlinelibrary.com]
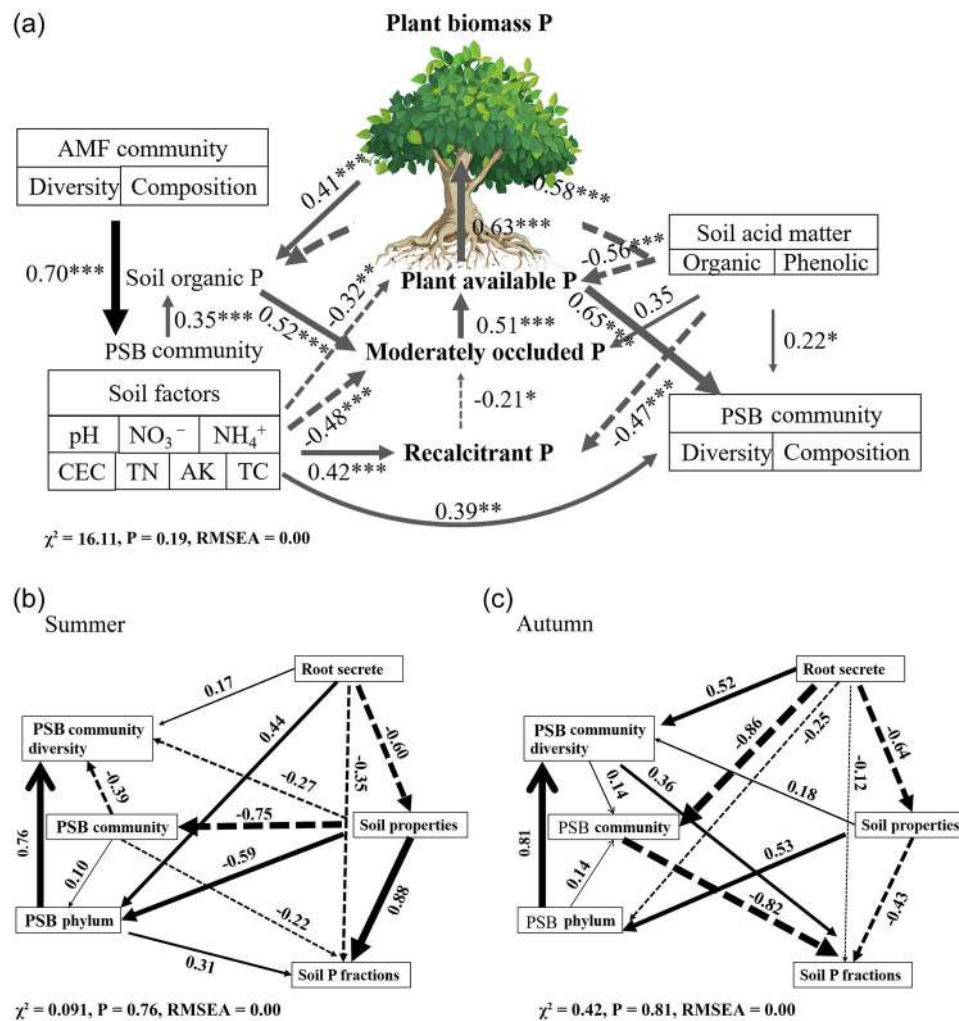
**FIGURE 6** Structural equation model of correlations between root exudates, PSB (harbouring *phoD* genes) composition and diversity, main PSB phylum, soil phosphorus (P) fractions, and rhizosphere soil properties in the rhizospheres of *P. euphratica* females and males during the entire growth season (a), in summer (b) and autumn (c) in three natural soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%), and Shaya (SY, soil water content 8%). The standardised coefficients are presented alongside the arrow. The line width is proportional to the standardised coefficients. The dashed and dotted lines represent positive and negative correlations, respectively. Multiple-layer rectangles represent the first main principal component of root exudate profiles, soil properties (soil water content, TC, pH, TN, AK, $NO_3^-$, $NH_4^+$, and CEC), and P fractions (P-resin, $P_t$-NaHCO$_3$, $P_i$-NaHCO$_3$, $P_t$-NaOH, $P_i$-NaOH, P-HCl, and residual P). AK, available potassium content; CEC, cation exchange capacity; $NH_4^+$, ammonium N content; $NO_3^-$, nitrate N content; Plant P, total P contents (in leaves and roots); TC, total soil carbon content; TN, total soil nitrogen content. [Color figure can be viewed at wileyonlinelibrary.com]

et al., 2018). The greater AMF diversity and abundance of female poplars in summer compared to males on the dry site suggests that females have more symbiotic AMF, which may facilitate P foraging from soils and increase plant P levels. Plant roots have a spatial disadvantage when competing with soil microbes for organic P. Therefore, the AMF pathway is expected to be dominant in foraging for organic P (Zhang et al., 2010). Notably, the AMF acquisition of P from organic P patches depends on the ability of PSB to secrete phosphatase enzymes (Zhang et al., 2020). On the dry site, female poplars had a greater capacity to mineralise organic and residual P via enhanced phosphatase activity (including alkaline, neutral and acid phosphatases) (Figures 2 and 7; Table 1). The community diversity and

abundance of AMF followed patterns highly similar to those of the PSB community when being affected by plant sex and soil moisture. The hyphae of AMF act as a "highway" for PSB to reach organic P patches and enhance organic P utilisation (Zhang et al., 2020), which may facilitate P acquisition by female poplars on a dry site.

## 4.2 | Sex-specific P mobilisation and availability from different P pools

Sex-specific P mobilisation from inorganic and organic P pools largely depends on the soil water status on the three sites of the
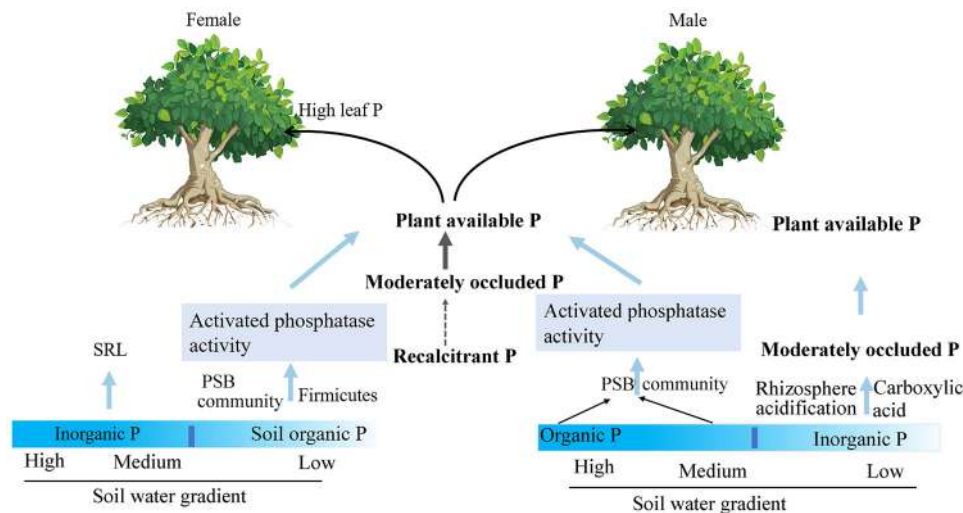
**FIGURE 7** Conceptual frameworks for soil phosphorus (P) cycling in *P. euphratica* females and males in summer and autumn on three sites with natural differences in soil water conditions: Shigongli (SGL, soil water content 32%), Sanhe (SH, soil water content 18%), and Shaya (SY, soil water content 8%). The red arrows represent an increase and blue arrows a decrease in key traits in the rhizosphere of females compared to males. The brown arrows represent an increase and green arrows a decrease in key traits in autumn compared to summer. AMF, arbuscular mycorrhizal fungi; ns, no significant difference; PSB community, the alkaline phosphomonoesterase (alkaline PAse)-encoding gene *phoD*-harbouring bacterial community; SRL, specific root length. [Color figure can be viewed at wileyonlinelibrary.com]

natural dioecious *P. euphratica* forests. Soil organic P is an important P source for plants and its transformation has a great influence on overall soil P bioavailability (Bünemann, 2015; Turner et al., 2007) via the secretion of phosphatase enzymes and associations with fungi (Alvarez et al., 2012; Jiang et al., 2021). Consistently, organic P was the major source for supplying plant-available P in female and male poplars among the three sites. This was supported by the depletion of rhizosphere organic P (Table 1). As a proxy for organic P mineralisation, higher phosphatase activities in the rhizosphere of both poplar sexes in summer than in autumn showed the presence of an accelerated microbial mineralisation of organic P (Figure 2). The increase in the leaf P content and its positive correlation with PSB community dissimilarities are also consistent with the greater organic P mineralisation during the growing season (Figures 1 and 6). Soil organic P in forest soils represents a dynamic P pool that is potentially available to plants through a variety of mechanisms, including the synthesis of phosphatase enzymes, secretion of organic acids, and association with mycorrhizal fungi (Attiwill & Adams, 1993). Secondly, soil organic P occurs in a broad spectrum of compounds that differ markedly in their behaviour in soil and, therefore, in the degree to which they are available to organisms. The P content in two poplar sexes was significantly higher on the dry site than on the moderately wet site, whereas the organic P mineralisation did not increase with the decreasing soil water on the three sites (Table 1). The results suggest that plant growth might be more strongly limited by water availability than P uptake. In addition, increases in other P sources as the result of altered P transformations could

contribute to securing the plant P supply. Except for organic P, recalcitrant P (including Ca-P and residual P) is also an important P source for the plant-available P pools on the dry site, as evidenced by the increased ratio of moderately occluded P to recalcitrant P (Table 1; Figure 3d). This is consistent with previous studies showing that environmental disturbances intensify the mobilisation of recalcitrant P fractions into more labile P fractions (Zhou et al., 2021).

Ca-P is the largest P reservoir in northern alkaline soils (Lindsay et al., 1989). Consistently, Ca-P accounted for the majority of total P (decreasing significantly with limited soil water) and for the higher plant P content in *P. euphratica* forest soils (Table 1; Figure 1). The increased Ca-P mobilisation is likely associated with the drought-induced mineral-bound P mobilisation (Haynes & Swift, 1989; McLaughlin et al., 1981), and increased microbial activity and metabolism secretion (Schimel, 2018). Drought conditions on the dry site increased the PSB community diversity in the rhizosphere of male poplars (Figure 2). The positive correlation of the PSB community composition with drought-induced root exudates also reflected the utilisation of Ca-P to some extent (Figure 5). Furthermore, the sex-specific intensified transformation of residual P (recalcitrant P) relied on the soil water status in the three *P. euphratica* forests. On the moderately wet (SH) site, the transformation of rhizosphere residual P was enhanced in male poplars more than in females (Table 1). Therefore, female poplars are more dependent on adequate soil water for residual P translocation compared to males, which is consistent with their higher phosphatase activity compared to males under limited soil water conditions.

## 4.3 | Sex-specific P mobilisation and acquisition mechanism in the rhizosphere of female and male poplars along a soil water gradient

Modified root architecture, symbiosis with AMF and their interaction with PSB, and the release of P-mobilising exudates are effective strategies for enhancing P acquisition (Lambers, 2022; Neumann & Römheld, 2002). However, sex-specific P acquisition depends on the soil water availability and growth season, as sex-related functional traits become more visible with an increasing soil drought (Chen et al., 2014; Zhao et al., 2023). On the wet SGL and SH sites, female and male poplars enhanced the mobilisation of moderately occluded P and the acquisition of available P, and no sex-related differences were detected in SRL (Table 1; Figures 1 and 7). However, sex-specific variation in the mineralisation of organic P and root symbiosis with AMF was greater on the very wet and on the dry site than on the moderately wet site (Table 1; Figure 1). These results are consistent with previous studies showing that sex-specific differences in functional traits are enhanced in response to stressful environments, but the trait differences disappear in humid (favourable) conditions (Chen et al., 2014). Female poplars showed increased AMF and PSB community diversities, whereas males showed elevated AMF and PSB abundances in autumn (Figures 1 and 2). The elevated microbial diversity largely determines the stability of community structures and activities (Tardy et al., 2014). The greater diversity of AMF and PSB in the rhizosphere of females compared to males may facilitate the mobilisation of organic P (Figure 2; Table 1).

Root-induced chemical and biological changes, including carboxylate exudation and rhizosphere acidification, are potentially an efficient strategy to mobilise recalcitrant Ca-P (Del-Saz et al., 2018; Zhou et al., 2021). An increased release of small-molecule carboxylic acids, such as citrate, is a critical mechanism of inorganic P mobilisation, which enhances soil P availability via ligand exchange and cation chelation (Ding et al., 2021; Mitra et al., 2022). In the present study, drought stimulated the release of citrate and glutaric acid by the roots of both poplar sexes on the dry site (Table S2). The rhizosphere carboxylic acid release-induced acidification decreases the pH relative to that of the bulk soil and causes the dissolution of sparingly available P (Shen et al., 2011). A decreased soil pH was observed in the rhizospheres of both poplar sexes on the dry site (Table S1). The root exudate release and rhizosphere pH also vary depending on plant sex and the rhizosphere environment (Liu et al., 2023). The rhizosphere organic acid release was greater in male poplars than in females, as evidenced by the higher relative abundance and lower rhizosphere pH in males than in females on the dry site (Tables S1, S2). Interestingly, citrate released by the roots was detected in the rhizosphere of both sexes on all three sites only in the summer, suggesting that citrate release is dependent on the season. Seasonal changes in the release of root exudates, including organic acids, have been observed in many other plant species (Edwards et al., 2018). In autumn, malonic acid release dominated rhizosphere acidification and P mobilisation. The sex-specific abundance of most organic acids was reduced, and female poplars had greater values than males (Figure 6; Table S2), thus underlining the greater potential for soil P dissolution via organic acids in the rhizospheres of both poplar sexes on the dry site in summer. However, the roles of root exudate species in soil Ca-P mobilisation must be further identified and explored.

In conclusion, female poplars had a greater ability to mineralise organic P in the rhizosphere than males, regardless of soil water availability in natural *P. euphratica* forests. The sex-specific P acquisition strategy relies on the temporo-spatial dynamics of soil P pools. Morphological and physiological adjustments by poplars showed distinct sex-related and temporal patterns on the dry site during the entire growing stage, and female poplars increased the symbiosis of roots along with the AMF and PSB abundance and diversity. Moreover, female poplars showed an enhanced mineralisation of labile and highly resistant organic P via an elevated phosphatase activity on the dry site. In contrast, SRL was greater in male poplars than in females, and the transformation of Ca-P to more available forms of P occurred via an organic acid release and decreased soil pH in the rhizosphere. Such sex-specific P mobilisation and acquisition were greater during the earlier growth season. During the later growth season, female poplars still had higher phosphatase activities, AMF diversity, and stimulated carboxylic acid release. Therefore, the P concentration in female poplars was significantly higher than that in males on the dry site, regardless of the growth season. The results imply that the temporal and site-specific dynamics of soil P affects sex-specific P acquisition strategies and modify soil P cycling. Our results provide a scientific basis for predicting the impacts of a climate change-dependent reduction in the water availability on the soil P biogeochemical processes and plant sex ratio segregation in natural *P. euphratica* forests.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [repository name] at [DOI], reference number [reference number]. The data that support the findings of this study are available from the corresponding author upon reasonable request. Raw DNA sequence files and associated metadata were deposited in the NCBI with the accession number PRJNA1015180.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.