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ORIGINAL PAPER

Phosphorus (P) mobilisation from inorganic and organic P sources depends on P-acquisition strategies in dioecious *Populus euphratica*

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Abstract

Dioecious species have secondary trait dimorphism in resource acquisition, allocation, and a skewed sex ratio. Yet, it is unclear how their sex-specific nutrient acquisition strategy affects the contributions of inorganic and organic phosphorus (P) soil pools to plant-available P. Here, the contribution of inorganic and organic P sources to available P in soil and sex-specific P acquisition during the whole growing season (from June to October) was assessed in a 20-year-old *Populus euphratica* plantation via analysing the transformation of soil P pools. Poplar females obtain available inorganic P by increasing specific root length (by 71% compared with males) and releasing organic acids to mobilise P from precipitated P (HCl-P), thus obtaining higher P than males during the mid-growing season (June). The increased mobilisation of moderately precipitated P in the rhizosphere was more significant in females during the whole growing season. During the late-growing season, males showed increased alkaline phosphatase activities (by 25% compared with females) and maintained a higher abundance of arbuscular mycorrhiza fungi to obtain P via higher consumption of organic and residual P (decreased by 68% and 24% from June to October). These changes in P acquisition strategies reflect the temporal niche differentiation: females acquire inorganic P mainly during the beginning and middle of the season, whereas males take up organic P and HCl-P, preferably in the second half of the season. The strategic adjustment of sex-specific P acquisition modulated the transformation of organic and inorganic P sources in soil towards plant-available P, increasing resource niche partitioning between two poplar sexes to maintain P supply.

Keywords Plant dioecy · Phosphorus mobilisation and acquisition · Root exudates · Rhizosphere processes · Plant-mycorrhizal interactions · Soil phosphorus forms

Introduction

Dioecious plants are those where male and female sexual organs are possessed by different individuals of the same species. Female plants tend to have greater reproductive costs than their male counterpart due to the demands for producing seeds and fruits. Such sex-specific resource and energy costs for reproduction may cause secondary trait dimorphism in resource acquisition and allocation, which may cause sex ratio biases in response to environmental pressures (Hultine et al. 2016).

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Phosphorus (P) is a common plant growth-limiting nutrient present in most terrestrial ecosystems, primarily due to its strong fixation in soils and very slow diffusion. Plants have evolved multiple functional traits to acquire available P from soils, including the increased specific root length and length and density of lateral roots and root hairs (Niu et al. 2013; Bindraban and Dimkpa 2020). The symbioses of roots with arbuscular mycorrhizal fungi (AMF) are the most common strategy to improve P nutrition, which can increase the root absorbing surface area and exploit larger soil volume via the formation of the hyphal network (Bolan 1991; Andrino et al. 2021). Poplar females and males exhibit sex-specific P acquisition strategies: females rely on the root system to obtain P, while males have a better capacity to form symbiotic relationships with AMF to forage P (Xia et al. 2022). There may exist a trade-off of resource exploration strategies between sexes in dioecious plant species; that is, females select higher resource uptake to satisfy their higher reproductive cost via the root morphology (Eppley et al. 2009), while males select more effective nutrient availability strategy via the symbiosis with AMF (Hultine et al. 2016; Graff et al. 2018). Due to the timing of energy investment in reproductive organs, the exploration strategies and the dynamics over the vegetation period may differ between females and males. It remains unclear how the selection of root and AMF by females and males changes with the season in mature *P. euphratica* plantations.

Maintaining a proper P-supplying level in the rhizosphere can maximise the efficiency of plant roots (Shen et al. 2011; De-Bashan et al. 2022). However, most soil P is locked in moderately and recalcitrant occluded P, with insoluble P in aluminium (Al), iron (Fe), or calcium (Ca) compounds (Holford 1997; Oelkers and Valsami-Jones 2008; Zhai et al. 2015), and organic P with labile orthophosphate monoesters, labile orthophosphate monoesters, and organic polyphosphates (Harrison 1987; Tiecher et al. 2012; Nannipieri et al. 2011, 2020). Plant P acquisition largely depends on the P released from inorganic and organic reservoirs (Peng et al. 2021; Ibrahim et al. 2022). Rhizosphere processes, such as the release of protons and phosphatases or phytases from roots, as well as organic anion exudation of anions of carboxylic acids, are the most common inorganic or organic P mobilisation mechanisms (Tian et al. 2016; Fan et al. 2018). Rhizosphere microorganisms directly drive P mobilisation and recycling from various pools via acidification, carboxylate production (Chen et al. 2021; Koester et al. 2021; De-Bashan et al. 2022), and the release of enzymes (Sun et al. 2018; Wei et al. 2019; Peng et al. 2022) at the soil profile and ecosystem levels (Wu et al. 2020; Lang et al. 2021). Plant P-acquisition strategies largely affect the contribution of inorganic and organic P in supplying plant-available P. For example, Zhou et al. (2021) proposed that plant Ca-P was the primary source of plant-available P for sedges and forbs related to carboxylate-releasing P-acquisition plant strategies. Organic P mineralisation regulated by alkaline phosphatase plays a critical role in seasonal P dynamics and influences the availability in soils with slightly alkaline pH (Li et al. 2021). Given the differential temporal dynamics of the P pool, the dominance of specific P pools in soils governs P availability and acquisition across seasons. However, the sex-specific sources for available P and the relative contributions of roots and microbiome to P mineralisation and acquisition have yet to be elucidated.

Populus euphratica trees dominate the extremely arid ecosystems in northwest China. Poplar trees have sexual dimorphism in nutrient uptake, allocation, and utilisation (Liu et al. 2020, 2021, 2022; Xia et al. 2022; Zhao et al. 2023). Here, we conducted a long-term field experiment to study sex-specific P mineralisation and acquisition in 20-year-old female and male *P. euphratica* during the whole growing season (from June to October). In this study, we address the following questions: (1) What are the contributions of inorganic and organic P pools to plant-available P in soil? (2) How do sex-specific P acquisition strategies change during vegetation season? (3) What are the mechanisms underlying soil P mobilisation with the changed root acquisition strategies with seasonal shifts? This study provides new insights into the spatial P mobilisation and adaptative regulation to P-acquisition strategies in dioecious *P. euphratica* plantations.

Materials and methods

Field experiment design and sampling

The experiment was conducted in the north-western reaches of the Tarim River in the southern Xinjiang Autonomous Region, China. The mean annual temperature is 11 °C, with approximately 42 mm of annual precipitation and an annual evaporation capacity of over 2800 mm (Guo et al. 2021). The soil properties are listed in Table S1. We performed a field experiment to examine the soil P dynamics and plant P acquisition in a 20-year-old *Populus euphratica* plantation. In 2001, *P. euphratica* seedlings were cultivated for vegetation restoration, with nine 15 × 10-m plots established at the upper reaches of the Tarim River. The trees were spaced at 1.5 × 1.5 m. The plots were separated by at least 10 rows of buffer trees. The sex of each tree was determined according to Xia et al. (2022), and the sex ratio (females to males) was 0.86.

A sampling of rhizosphere soils and plants was performed for both sexes in each plot during July (the earlier growing season) and October (the later growing season) of 2021. Six *P. euphratica* females or males were selected as one replicate within a plot. For each tree, four leaves from the top whorls

of the trees in the four cardinal directions were harvested as one sample, while four samples from the surrounding of the trunk in the four cardinal points were collected for each tree. The four soil samples collected per tree were pooled into a single composite sample. The roots and rhizosphere soil of chosen trees were identified by tracing the connections of secondary roots to the main roots. Roots were separated from the rhizosphere soils, and rhizosphere soil samples were collected from the detached roots and stored at 4 °C within 2 weeks. One of the rhizosphere aliquots was stored at − 80 °C until analysis. The remaining soil samples were air-dried and sieved to 2 mm for physical and chemical analyses. Root and leaf samples were dried at 75 °C to a constant weight.

Soil physicochemical traits

Soil pH was measured using a glass electrode with a 150 PHBJ-260 pH meter at a ratio of 1:2.5 soil to H_2O. The soil total C (TC) and N (TN) were determined using a chemiluminescence detector (CLD-TOC) coupled to a C and N analyser (multi C/N 152 3100; Jena Analytics, Jena, Germany). Air-dried soil was digested with 2 mol l^{-1} HNO_3 (20:1) and analysed by plasma-atomic emission spectrometry (ICPS-7500, Shimadzu, Japan). The air-dried soil samples were extracted with 1 mol l^{-1} KCl, and the ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^-) contents were determined using a continuous flow analytical system (SEAL Analytical Auto Analyzer 3; Sahrawat and Prasad 1975; Sims and Jackson 1971). The soil cation exchange capacity (CEC) was determined following the ammonium acetate method (Kitsopoulos 1999). The soil-available potassium (AK) was measured using flame spectrometry (PFP7 flame photometer) after extraction with 2 mol l^{-1} HNO_3 (20:1; Zhang and Gong 2012).

Soil P forms were extracted as previously described (Hedley et al. 1982). Sieved soil samples were extracted as follows: (1) Resin-P with soil sample was extracted with 1 g of resin to obtain water-soluble and plant-available P; (2) $NaHCO_3$-P (plant-available P) with the other soil samples were extracted with 30 ml of 0.5 mol l^{-1} $NaHCO_3$ (pH 8.5) and centrifuged at 4500 × *g* for 10 min at 4 °C, 10 ml of clear supernatant was added to 6 ml of 0.9 mol l^{-1} H_2SO_4, and $NaHCO_3$-P_i (inorganic P of $NaHCO_3$) was measured using molybdenum antimony colourimetry. A total of 5 ml of clear 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. The mixed solution was autoclaved for sterilisation for 60 min, and the $NaHCO_3$-P_t (total P of $NaHCO_3$) was measured using molybdenum antimony colourimetry. The organic P of $NaHCO_3$ (presumed $NaHCO_3$-P_o) was determined as the difference between the total P in the form of $NaHCO_3$ and the inorganic P in the form of $NaHCO_3$. (3) NaOH-P (presumed Al, Fe-binding P, moderately occluded P21) with the other soil samples were extracted with 30 ml of 0.1 mol l^{-1} NaOH and centrifuged at 25,000 × *g* for 10 min at 4 °C. A 10 ml clear supernatant was added to 1.6 ml of 0.9 mol l^{-1} H_2SO_4, and NaOH-P_i (presumed inorganic P of NaOH) was measured using molybdenum antimony colorimetry; 5 ml of clear 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. The mixed solution was autoclaved for sterilisation for 90 min, and the NaOH-P_t (total P of NaOH) was measured using molybdenum antimony colourimetry. The organic P of $NaHCO_3$ (NaOH-P_o) was determined as the difference between NaOH-P_t and NaOH-P_i. (4) HCl-P (recalcitrant P) with the other soil samples were extracted with 10 ml of concentrated HCl and centrifuged at 25,000 × *g* for 10 min at 4 °C. A 10 ml clear supernatant was measured using molybdenum antimony colourimetry. (5) Residual P (refractory minerals and organic matter-binding P) with the difference between the total soil P and the first four forms of P. The moderately occluded P was defined as the sum of presumed Al, Fe–P, and Ca_2-P, while the recalcitrant P was considered the sum of HCl-P and the residual P (Wang et al. 2022). The moderately occluded P was calculated as the sum of $NaHCO_3$-P and NaOH-P fractions, while the recalcitrant P was the sum of HCl-P and residual P (Wang et al. 2022).

Soil microbial C, N, and P measurements

The soil microbial biomass C (MBC), N (MBN), and P (MBP) were determined by the chloroform fumigation extraction method (Brookes et al. 1985; Joergensen et al. 1995; Jenkinson et al. 2003). Five grams of fresh soil samples (sieved < 2 mm) were fumigated with $CHCl_3$ for 24 h. The difference in the organic C, total N, and total P between the fumigated and unfumigated soil samples was calculated for the microbial biomass. The fumigated and unfumigated soil samples were extracted with 20 mL of 0.5 mol l^{-1} K_2SO_4 (soil:water, 1:4) for 30 min. The solutions were centrifugated at 5000 g for 10 min. The clear supernatant was used to measure the content of organic C with a TOC analyser (Shimadzu Model TOC-500, Japan). The fumigated and unfumigated soil samples were used to measure the total N content by the ninhydrin colorimetric method. The microbial P was determined by molybdenum antimony colorimetry. The conversion factors of MBC, MBN, and MBP were 2.22, 2.22, and 2.5, respectively (Jenkinson et al. 2003). For MBP measurement, we have a control (unfumigated soil sample) when we added inorganic P to soil for determining the recovery of extracted P.

Soil phosphatase activity measurement

Soil acid phosphomonoesterase and alkaline phosphomonoesterase activities were determined using p-nitrophenyl

phosphate as a substrate at pH 6.5 and 11, respectively, at 37 °C (Tabatabai 1994). The produced PNP was colorimetrically measured at 405 nm.

Plant P content

The root and leaf samples were dried to a constant weight at 75 °C for 48 h. The dried samples were digested with H_2SO_4-H_2O_2. The clear supernatant was analysed using an inductively coupled plasma–optical emission spectrometer (Model 5300DV).

Rhizosphere soil exudate profile collection and analysis

Soil exudates were collected following the method reported by Michalet et al. (2013). Briefly, the roots were excavated and rinsed with deionised water and then placed in geotextile tissue surrounded by soil. After 72 h, the roots were placed in 50-mL syringes filled with osmotic solution (3 mM $CaCl_2$) and glass beads. The root exudates were collected and immediately filtered at 0.2 µm after another 72 h, following freezing in liquid nitrogen. About 100 g of fresh soil sample was added to 500 mL of ultrapure water. The soil solutions were then centrifuged at 7000 × *g* for 10 min at 4 °C. The supernatant was filtered through 0.22-µm syringes to measure the auxin in the rhizosphere soil solution. The supernatants from the root exudate and rhizosphere soil solution were freeze-dried with a vacuum freeze-drier. The dried pellets were resuspended in 200 µl of internal standard methanol extract and centrifuged at 12,000 r min^{-1} for 10 min at 4 °C.

The mixed solution was stored in sample bottles and analysed by ultra-performance lipid chromatography (UPLC, SHIMADZU Extra X2) and an Applied Biosystems 4500 QTAP. The flow rate was set to 0.35 mL min^{-1}, and the injection volume was 4 µl. The mobile phase was composed of solvent A (0.1% pure water) and solvent B (acetonitrile with 0.1% formic acid). The gradient of solvent B was set as 5% at 0 min to 95% within 9 min, 5% within 1 min, and kept at 5% for 5 min. The flow rate was set to 0.35 ml s^{-1}. The temperature of the column was set to 40 °C, and the injection volume was 4 µl. The triple quadrupole scans and LIT were obtained using a triple quadrupole–linear ion-trap mass spectrometer and an AB4500Q TRAP UPLC/MC/MS system equipped with an ESI Turbo Ion-Spray interface.

Root trait measurement

Fine root samples were selected to measure root parameters. The root samples were washed carefully with deionised water and scanned with a root scanner (Epson V700) linked to a Win-RHIZO system (Regent Instrument Inc.). Root images were analysed using the Win-RHIZO system. The root samples were then dried at 75 °C for 48 h. The specific root length were calculated as the ratio of the total root length to root dry weight and the ratio of the dry mass to the root volume, respectively (Xia et al. 2022).

Sequencing data and bioinformatics

We extracted DNA from 50 mg of fresh rhizosphere soil samples using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc. Carlsbad, CA, USA) following the manufacturer's instructions. The V3-V4 hypervariable regions of the 16S rRNA gene were used to target bacteria using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The ITS2 region (fITS7/ITS4) was amplified in fungi using the primers ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). The polymerase chain reaction (PCR) was performed using a 25 µl reactive system containing 10 ng template DNA, 0.25 µl of each primer (10 µmol l^{-1}), 0.5 µl of 10 ng template DNA, 3.8 µl of nuclease-free water, and 5.2 µl of SYBR Green and ROX mixture (2×, Takara Bio Inc., Shiga, Japan). The conditions were as follows: 95 °C for 2 min; 40 cycles of 95 °C for 10 s and 56 °C for 30 s; an extension phase of 72 °C for 20 s; and 55 °C to 95 °C for melting curve analyses. The PCR products were purified using an NA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's instructions. Subsequently, the PCR products were quantified using a NanoDrop device (NanoDrop 2000C, Thermo Scientific). Amplicon libraries were distributed on picotiter plates, and paired-end sequencing was performed using an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) with a read length of 300 bp. The sequencing ASVs were assigned using the primer-specific classifiers (Silva132 at 99% similarity and UNITE v8 dynamic) with the QIIME2 plugin "classify-sklearn" (Jin et al. 2022). The sequencing reads were paired and filtered with less than 200 bp. The amplicon sequence variants (ASVs) were assigned from the sequencing data at 97% nucleotide identity with UPARSE (Edgar 2013). We produced an average of 107,947 bacterial and 93,291 fungal ASVs for subsequent analysis. Fungal and bacterial sequences were analysed with UNITE (V.8.0) and SILVA (v.138) as reference databases, respectively (Gweon et al. 2015; Callahan et al. 2016; Nilsson et al. 2019). Raw DNA sequence files and associated metadata were deposited in the NCBI with the accession number SUB13799965.

Statistical analysis

Mixed linear models were used to analyse the main effects of sex and season, and their interactive effects on the soil

bacterial and fungal community and root exudate profiles. After checking for variance, statistical analysis was performed in SPSS software, followed by Duncan's test (version 22.0) ($p < 0.05$). The α-diversity indices were calculated in R and visualised using the "*ggplot2*" package33. The β-diversity indices were calculated by nonmetric multidimensional scaling (NMDS) based on the distance matrix of Bray–Curtis dissimilarities based on the "ordinate" function of the phyloseq package (McMurdie and Holmes 2013).

Permutational multivariate analysis of variance (PERMANOVA) was performed to explain the variation attributed to season, sex, and their interactions based on the Bray–Curtis distance matrix with the "adonis" package in R according to the 999 permutations (Oksanen et al. 2007). The dominant bacterial and fungal phyla were defined with a relative abundance of more than 10%, and phyla with less than 1% relative abundance were regarded as "others".

Results

Plant P contents

Growth season affected leaf and root P contents of *P. euphratica* females and males. At the later growing season, the leaf P content decreased by 42% in females, while significant differences were observed in males between the two growing seasons (*i.e.* earlier and later) (Fig. 1). The root P content increased by 62% in males, but no difference was detected in roots of females between two seasons (Fig. 1). At the earlier growing season, females had higher P contents of leaves and roots than males, whereas during the later growing season, the leaf P content was higher in males, but there was no sexual difference in root P (Fig. 1).

Soil P fractions

Growth season affected soil P specification, except for HCl-extracted Ca-P. The resin-P was increased by 36% in the rhizosphere of females and decreased by 28% in males at the earlier rather than later growing season (Fig. 2). Females had a higher content of resin-P in the rhizosphere than males at the later stage of growing season, but no sexual difference was detected at the earlier growing season (Fig. 2). Compared with the later stage of growing season, the rhizosphere $NaHCO_3$-P and NaOH-P in females and males at the earlier growing season were decreased by 40% and 35% and by 26% and 27%, respectively. Similarly, the refractory P and organic P in females and males decreased by 14% and 56% and by 24% and 68%, respectively (Fig. 2). There was no sexual difference in $NaHCO_3$-P, NaOH-P, refractory P, and organic P in the rhizosphere at the earlier stage, whereas females had higher contents of $NaHCO_3$-P, refractory P, and organic P than males at the later growing season (Fig. 2). The rhizosphere HCl-P was increased in females but decreased in males at the earlier than the later growing season stage. Sex-specific differences in HCl-P were influenced by plant growing season: females had a higher value than males at the earlier stage, with no sexual difference at the later stage (Fig. 2).

Root traits and AMF community composition

Growing season and sex affected the specific root length of *P. euphratica* females and males; indeed, they were lower at the later than the earlier growing season. Females had greater specific root length than males at the earlier growing season, with no difference in specific root length between sexes at the later stage (Fig. 3a). The total relative abundance of AMF

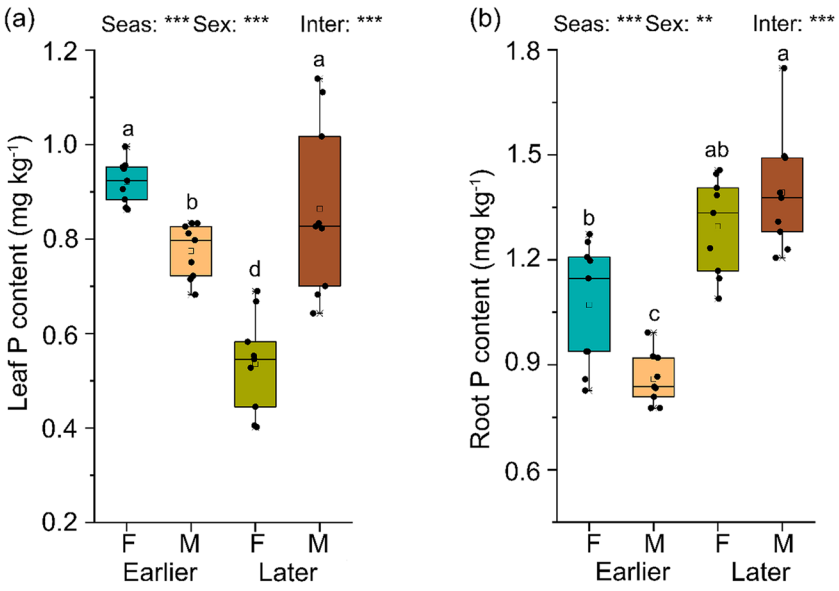

Fig. 1 Phosphorus (P) concentration in leaves (**a**) and roots (**b**) of *Populus euphratica* females and males during the earlier (summer) and later (autumn) growing seasons. Differences among treatments were analysed using Duncan's tests. Data are means ± SD ($n = 9$). Lowercase letters indicate significant differences among treatments ($p < 0.05$). F, females; M, males

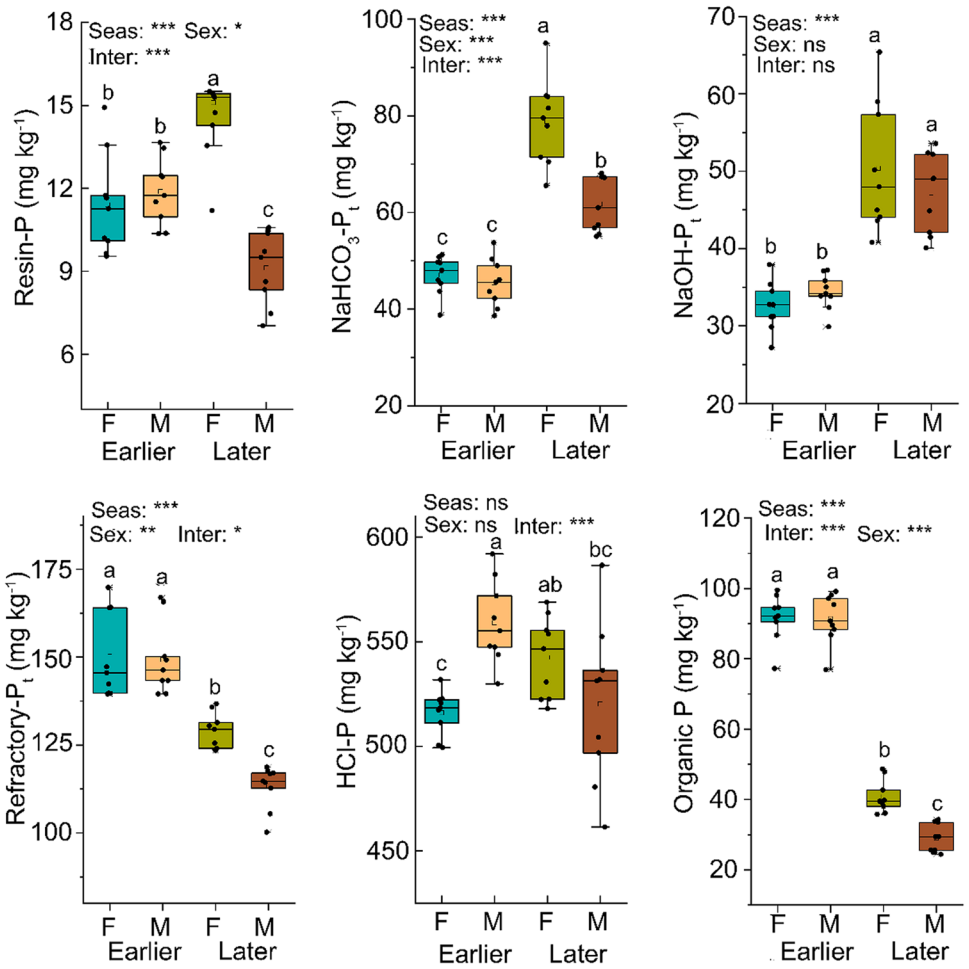

Fig. 2 Soil phosphorus (P) fractions in the rhizosphere of *Populus euphratica* females and males during the earlier (summer) and later (autumn) growing seasons. Differences between treatments were analysed using Duncan's tests. Data are presented as means ± SD ($n = 9$). Lowercase letters indicate differences between groups ($p < 0.05$). Season, season main effect; sex, sex main effect; Inter, interactive effect of season and sex; ns, not significance; P_i, inorganic P; P_o, organic P; P_t, total P; *$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; and ***$p \leq 0.001$. 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. F, females; M, males

phyla was higher at the later than the earlier growing season, while AMF abundance for male plants was greater at the later growing season (Fig. 3b). The NMDS results indicated that the AMF community composition in the rhizosphere of both sexes showed great seasonal variation, but no sex-based variation (Fig. 3d). The dominant AMF phyla in the rhizosphere of both sexes showed a greater seasonal variation than sex-based variation. Both sexes had a higher relative abundance of AMF at the earlier growing season, including Discosea, whereas the Rotifera, Mucoromycota, Basidiomycota, and Arthropoda phyla had greater relative abundances in the rhizospheres of both sexes at the later growing season (Fig. 3d; Table S3). Plant sex had a greater effect on the relative abundance of AMF phyla at the later growing season and was typically higher in poplar females than males, including the Preaxostyla, Choanoflagellida, and Cryptomycota phyla.

Soil microbiome related to soil phosphatase activities

The dominant bacterial phyla associated with P cycling were more affected by the season than plant sex and their interaction (Fig. 4a). The dominant bacterial phyla, including Planctomycetes, Proctomycetes, Gemmatimonadetes, Bacteroidetes, and Verrucomicrobia, had a higher relative abundance at the later growing season, whereas the relative abundances of Actinobacteria, Firmicutes, and Chloroflexi phyla were higher at the earlier growing season (Fig. 4a, b; Table S2). Poplar females had higher relative abundances of some dominant bacterial phyla in their rhizospheres than males, including Planctomycetes, Gemmatimonadetes, and Verrucomicrobia at the earlier stage of the growing season, and Firmicutes and Chloroflexi at the later stage. The relative abundances of the remaining dominant bacterial phyla in the rhizosphere did not differ between females and males.

The relative abundances of saprotrophic–symbiotrophic fungi were greater at the later than earlier growing season, regardless of plant sex, while there was no difference in either the relative abundance of saprotrophic fungi between seasons or sexes (Fig. 4c). The abundance of symbiotrophic fungi was higher in the rhizosphere of males than females. The alkaline phosphatase activities were the highest than the acid phosphatase activities in the rhizosphere of both sexes,

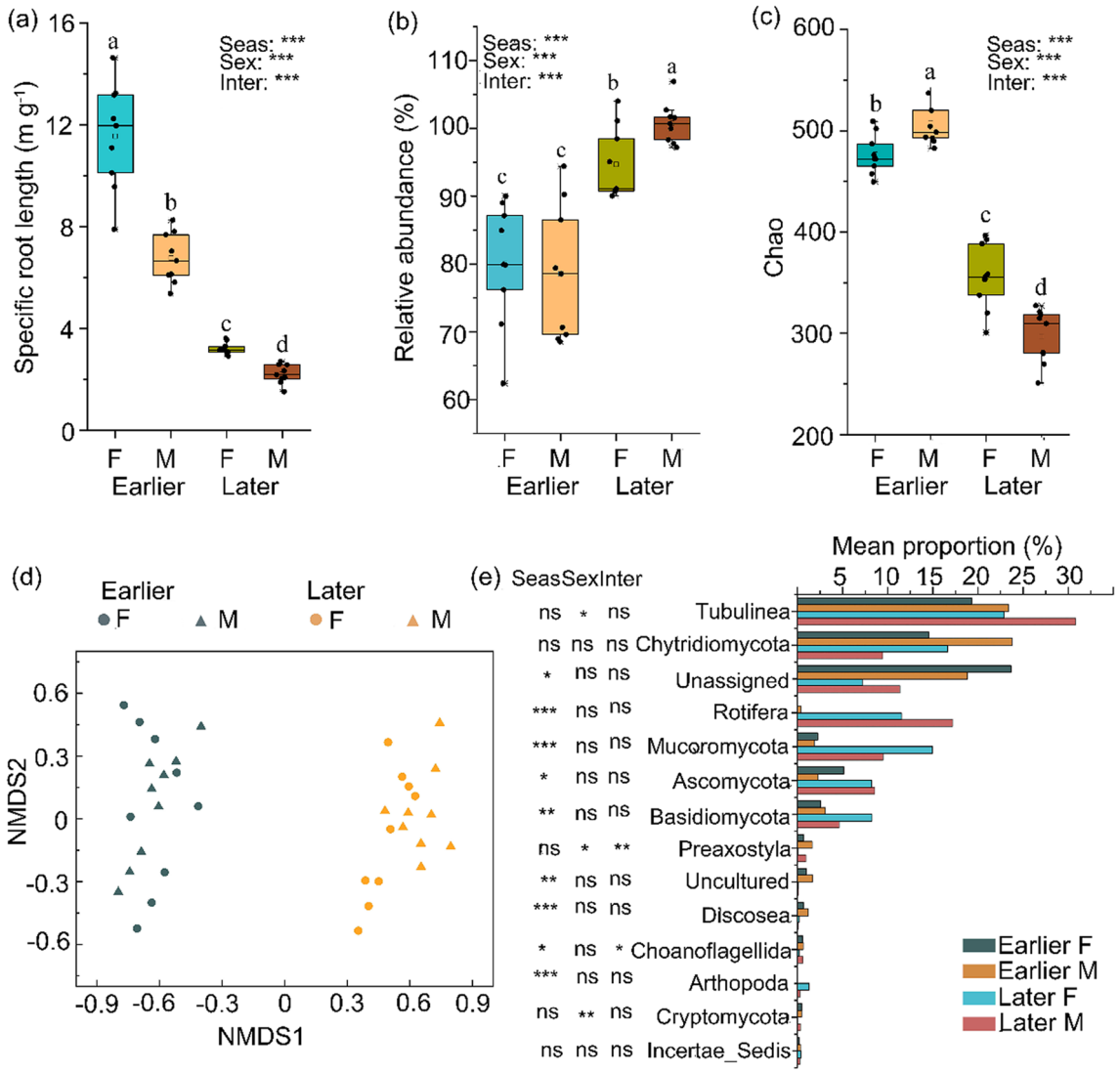

Fig. 3 Specific root length (**a**), total relative abundance (**b**), and α-diversity indices (**c**, Chao indices) of the AMF community, (**d**) β-diversity indices of the AMF community, and (**e**) species difference analysis with Kruskal–Wallis of variance by ranks for the dominant phyla of the AMF community in the rhizosphere of *Populus euphratica* females and males during the earlier (summer) and later (autumn) growing seasons. The β-diversity indices were calculated using the Bray–Curtis distance matrix. 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". Data are presented as the means ± SD ($n = 9$). Lowercase letters indicate differences among treatments ($p < 0.05$). Season, season main effect; sex, sex main effect; Inter, the interactive effect of season and sex; ns, not; *$0.01 < p \le 0.05$; **$0.001 < p \le 0.01$; ***$p \le 0.001$. F, females; M, males

regardless of the season (Fig. 4d). Further, males had higher phosphatase activities than females in the rhizosphere.

Organic acids released by roots and correlation of root exudate with microbial community composition and soil nutrients

Most root organic acids were higher in females at the earlier growing season and in males at the later stage of the growing season relative to the opposite sex (Fig. 5a, b). The bacterial and fungal community composition in the rhizosphere soil of both sexes showed great variability across seasons (Fig. 5). RDA analysis showed that the bacterial and fungal community compositions have a greater correlation with soil P fractions (RDA1 90.1% for bacteria and 77.2% for fungi) (Fig. 4a). The bacterial community dissimilarity was positively associated with the plant availabilities of P ($NaHCO_3$-P) and NaOH-P at the earlier growing season, and the residual P and HCl-P fractions at the later stage; the contrary was true for the correlation between the fungal community and soil P fractions in the two seasons.

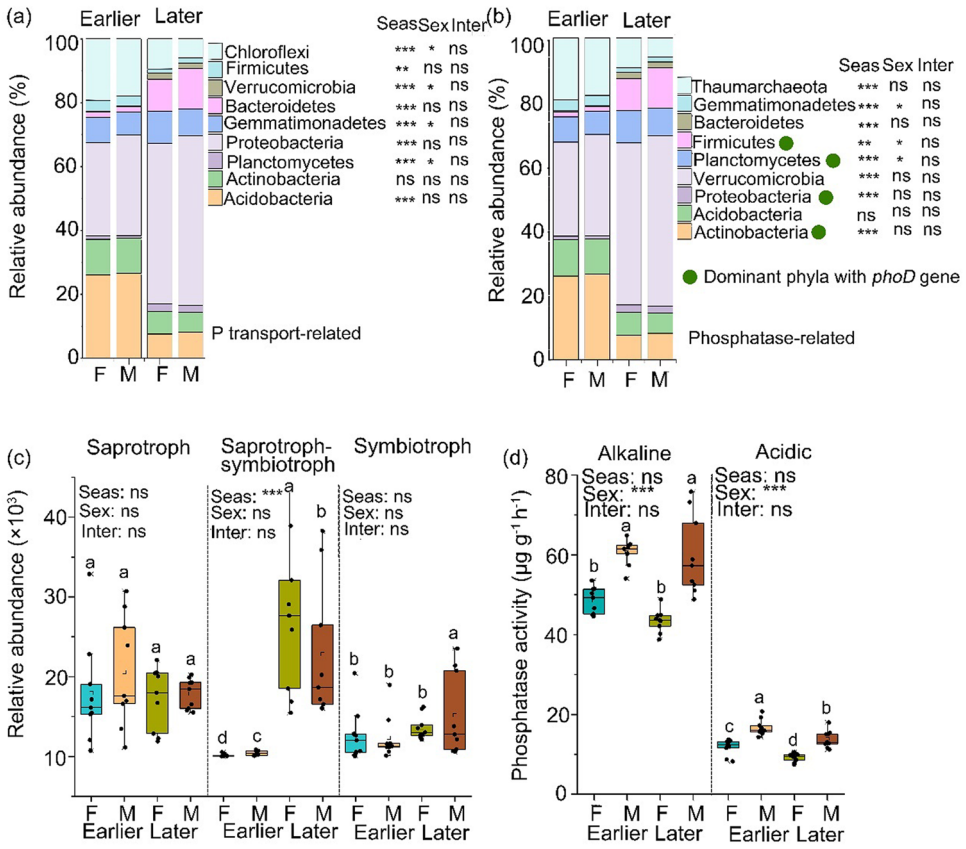

Fig. 4 Dominant phyla of bacterial communities related to P transport (**a**) and phosphatase activities (**b**), the predicted relative abundance of saprotroph, saprotroph-symbiotroph, and symbiotroph from the fungal communities (**c**), and the alkaline and acid phosphatase activities (**d**) in the rhizosphere of *Populus euphratica* females and males during the earlier (summer) and later (autumn) growing seasons. Green cycles mean the dominant bacterial phyla with *phoD* alkaline phosphatase genes identified by *phoD* gene amplification in this study. Differences between groups were analysed using Duncan's tests. 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". Data are presented as means ± SD ($n = 9$). Lowercase letters indicate differences between groups ($p < 0.05$). Season, season main effect; sex, sex main effect; Inter, interactive effect of season and sex. *$0.01 < p \le 0.05$; **$0.001 < p \le 0.01$; and ***$p \le 0.001$. F, females; M, males

Linking root exudate, microbial community composition, and soil nutrients

Our structural equation modelling analysis fit the significance criteria ($\chi^2 = 8.17$, $p = 0.23$, and RMSEA = 0.10) (Fig. 6). The plant-available P was dominantly and directly controlled by the moderately occluded soil P (0.45) and indirectly by the soil traits (including pH, –0.42) and soil acids (organic and phenolic acids, 0.51). Soil acids also directly affected the plant-available P. Plant P acquisition in both sexes was positively affected by the soil-available P (0.58 with a direct effect) and moderately by occluded soil P (0.45 with an indirect effect) but negatively correlated with soil recalcitrant P (–0.23, including insoluble HCl-P and residual P). The soil recalcitrant P was directly positively affected by soil traits (0.33).

Discussion

The dominance of organic and inorganic P sources for available P and plant P acquisition strategy

Poplar females and males rely on rhizosphere inorganic P mobilisation to supply available P at the earlier growing season and on the organic and refractory P during the later stages of the growing season (Fig. 7). This is supported by the decreased rhizosphere moderately occluded P pools ($NaHCO_3$-P and NaOH-P) in females and males (Fig. 2). Poplars have a great demand for N and P during the vigorous growing season (Yu et al. 2022). Similarly, poplar females and males had a higher P level at the vigour growth season than non-growing season (Fig. 1). The increased nutrient demand and acquisition in both poplar sexes would cause

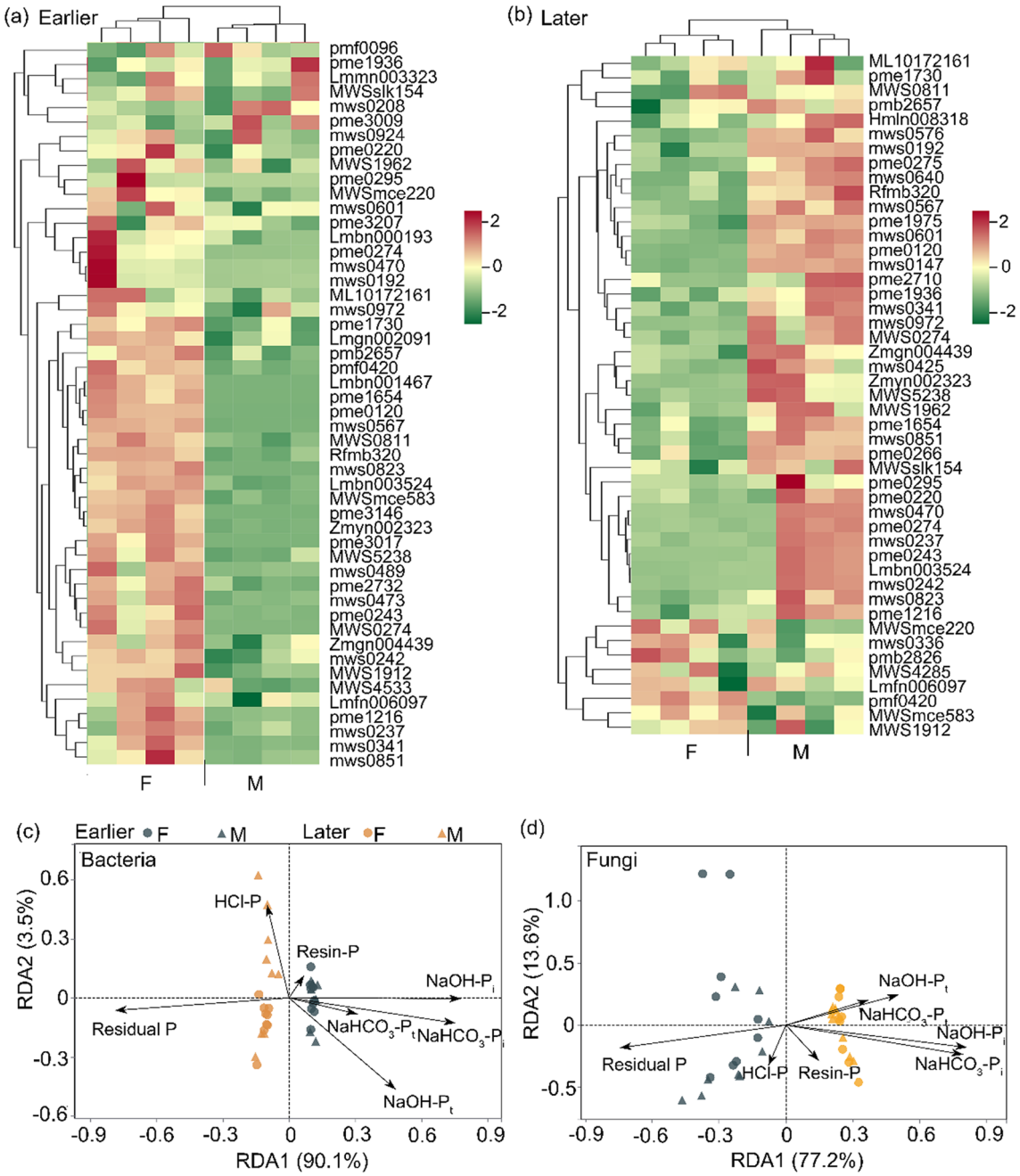

Fig. 5 Heatmaps of organic acids by roots (**a**, **b**) and correlations between the bacterial (**c**) and fungal (**d**) community compositions with soil P fractions in *Populus euphratica* females and males during the earlier (summer) (**b**) and later (autumn) (**b**) growing seasons. Resin-extracted 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. F, females; M, males

rapid depletion of rhizosphere available P, which required other P source transformations to secure available P supply (Shen et al. 2011). Therefore, the increased translocation of moderately occluded P to available P could maintain great demand for nutrients in females and males at the vigour growth season. In dioecious plants, females usually rely more on P to produce fruits and seeds (Bürli et al. 2022). Similarly, poplar females had a higher P content and strengthened the translocation of occluded P into moderately occluded P than males at the early growing season (Fig. 1). Mobilisation of HCl-P is a major source of available P in alkaline soils (Zhou et al. 2021). The higher transformation of HCl-P was expected to supply more available P for females at the early growth season and for males at the later

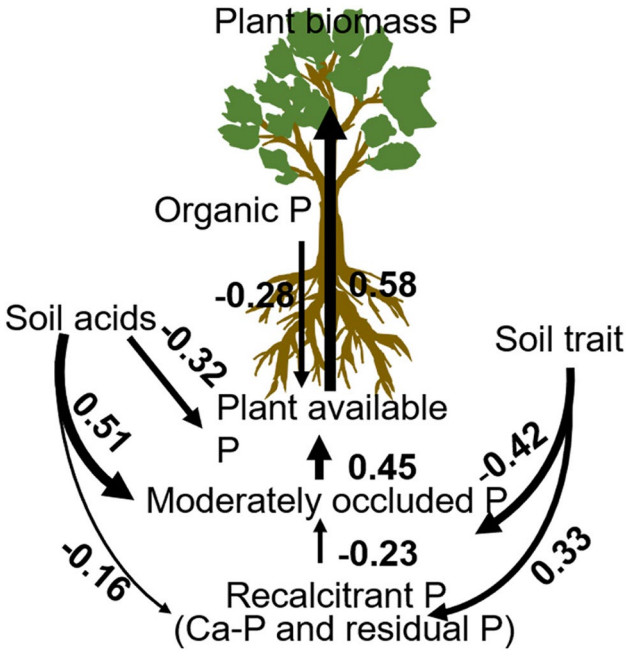

Fig. 6 Structural equation model of the acceleration of soil phosphorus (P) cycling in the rhizosphere of *euphratica* females and males during the earlier (summer) and later (autumn) growing seasons. Arrows represent pathway coefficients. Standardised coefficients are presented alongside the arrow. Line width is proportional to the standardised coefficients. Multiple-layer rectangles represent the first main component of the principal component analysis conducted for root organic acid profiles and soil traits (TC, pH, TN, AP, AK, NO_3^-, NH_4^+, and CEC). Upward and downward arrows represent increase and decrease, respectively, in autumn than in summer. TC, soil total carbon; TN, soil total nitrogen; AK, available potassium; AP, available P; CEC, cation exchange capacity; NH_4^+, ammonium N; NO_3^-, nitrate N

growth season, as shown by the rhizosphere depletion of HCl-P.

As inorganic P depletion, accumulated organic P could be a supplementary P source during the non-growing season (Magid and Nielsen 1992). Indeed, both female and male plants increased the depletion of organic and residual P fraction and the enhanced translocation ratio of moderately occluded P into moderately occluded and available P (Fig. 2). However, there were sex-specific variations in plant P content and P solubility depending on the growing season. Unlike female plants, males increased the consumption of rhizosphere organic P and residual P and elevated P content at the later growing season. Male plants had a lower leaf P resorption efficiency than females in *P. euphratica* (Yu et al. 2022). In addition, our study observed a longer growth period for poplar males than females. Therefore, male plants tended heavily to soil nutrient supplies than females at the later of the growing season. These results suggested that soil P cycling was mainly driven by sex-specific P demand in the *P. euphratica* plantations.

Mechanism underlying inorganic and organic P mobilisation and sex-specific P acquisition

Our study suggested that poplar relied on sex-specific carboxylic acid exudation to mobilise the sparsely available soil P and microbial-regulated organic P mineralisation with the shift from the earlier to later growing season (Fig. 7). The increased release of carboxylic acids by roots is a key mechanism underlying HCl-P mobilisation through chelating cations or ligand exchange (Gerke 1994; Zhou et al. 2021). Among carboxylic acids, oxalic acid was detected in root exudates of females and males at the earlier rather than

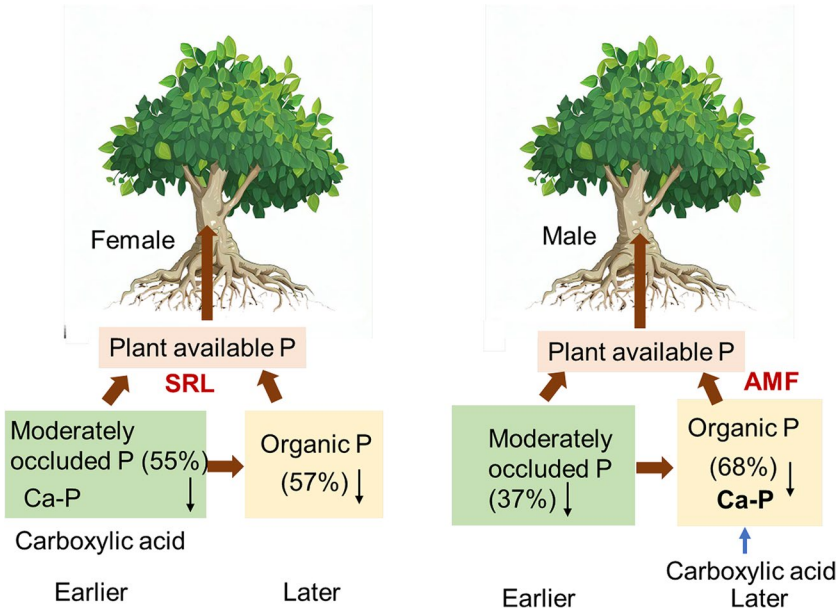

Fig. 7 Conceptual framework for phosphorus (P) mobilisation and P acquisition strategies of *Populus euphratica* females and males during the earlier (summer) and later (autumn) growing season. SRL, specific root length; AMF, Arbuscular mycorrhizal fungi. Moderately occluded P was composed of $NaCO_3$-extracted P and NaOH-extracted P

later growing season. Oxalic acid efficiently promotes the mobilisation of moderately occluded P (Fox and Comerford 1990; Gerke 1994; Koester et al. 2021). Thus, the oxalic acid released by roots of both poplar sexes may mobilise moderately precipitated P and HCl-P at the earlier growing season (Fig. 2). However, sex-specific HCl-P mobilisation via the carboxylate-releasing P-acquisition strategies varied with the growth period. Poplar females released more diverse and greater amounts of organic acids than males at the early growth season (Fig. 5; Table S4). In contrast, the release of organic acids and HCl-P mobilisation was more intense in males than females at the later growing season (Fig. 2), meaning the carboxylic acid-induced P solubility of HCl-P mainly can supple more available P at the later growing season. These results reflect the temporal HCl-P solubility induced by carboxylate-releasing P-acquisition strategies in dioecious females and males. Notably, for root exudate collection, root systems are submerged in an osmotic solution to maintain the diffusion gradient between the trap solution and root cells, which better reflect the rhizosphere microenvironment (Phillips et al. 2008). However, the osmotic solution has a limitation that does not uniformly collect root exudates following the preferential flow paths (Phillips et al. 2008). In addition, the greater requirement for volume of solution has a disadvantage in post-collection steps (Phillips et al. 2008). Thus, the new approach was urgent to estimate the roles of root-secreted carboxylates in mobilising soil HCl-P in poplar females and males.

Beside the direct mobilisation of inorganic P, root exudates affected the selection of hosts for rhizosphere microbiome which in turn may drive nutrient cycling process (Nannipieri et al. 2023). Plants or microbiomes secreted phosphatase enzymes to promote organic P mineralisation (Quiquampoix and Mousain 2005; Nannipieri et al. 2011; Ma et al. 2021). The alkaline and acidic phosphatase activities were higher in the rhizosphere of poplar males than females, regardless of growth season, consistent with previous studies (Liu et al. 2023; Xia et al. 2022; Zhao et al. 2023). Thus, male plants would enhance the mineralisation of organic P by secreting phosphatase, which may be the main source of available P throughout the growth cycle. Interestingly, the alkaline, rather than acid phosphatase activities, played critical roles in organic P mineralisation (Fig. 2). This was supported by Li et al. (2021), in which alkaline phosphatase activity accounted for the seasonal dynamics of available P from organic P pools in the soil of a subalpine forest ecosystem. In contrast, for females, the rhizosphere bacterial community composition showed a greater positive correlation with recalcitrant P, especially in the rhizosphere of females at the early stage of the growing season, implying the possibility of soluble P into insoluble status. It was also reported that soluble P is immobilised by microorganisms and subsequently transformed into organic and/or recalcitrant P (Oberson and Joner 2005; Liebisch et al. 2014). Therefore, the rhizosphere microbiome in the rhizosphere of females probably increased the recalcitrant P formation except for mineralizing organic P.

Plant soil P cycling and relative dominance of organic and inorganic P pools may impact the plant P acquisition strategies. There were trade-offs and covariations among P acquisition in dioecious plants (Xia et al. 2022). Plants with a higher specific root length have more fine roots and a greater ability to explore soil volume for nutrients (Weemstra et al. 2020; Xia et al. 2022). Females have a greater specific root length than males, especially at the vigorous season (Fig. 3a). Larger specific root length in female plants allows them to explore soil regions widely and efficiently increase nutrient absorption (Xia et al. 2022). In contrast, smaller specific root length means a slightly larger root diameter, which easily forms a symbiosis with AM fungi and increases root absorbing surface area (Ma et al. 2018). Plants rely heavily on the assistance of AMF to acquire organic nutrient rather than their root system per se with the shift from the earlier growing season to the later stage (Craine et al. 2009; Zhang et al. 2019). The smaller specific root length and higher symbiosis with AMF, including Preaxostyla, Choanoflagellida, and Cryptomycota phyla, in males probably explained the higher P levels than females at the later growing season (Figs. 1 and 2).

Additionally, we examined the P cycling with the different P fractions according to Hedley et al. (1982). Our results would reflect some main information about soil P dynamic, although some concerns have been raised for many years about the results of fractionations concerning P cycling and soil P chemistry with the Hedley fractionation (Condron and Newman 2011; Barrow et al. 2021). The chemical precision with traditional P fractionation was proven with some advanced techniques, such as P k-edge XANES (x-ray adsorption near edge structure) spectroscopy (Kar et al. 2011; Klotzbücher et al. 2019). However, the labile P pools of modified Hedley fractionations are thought to mimic natural P acquisition and can be better quantified than P XANES spectroscopy (Klotzbücher et al. 2019; Gu and Margenot 2021). Moreover, organic P measure with Hedley fractionations is regarded as a complement of XANES spectroscopy, since some XANES spectroscopy did not differentiate inorganic and organic P (Prietzel et al. 2016; Gu and Margenot 2021). Large concerns mainly focused on acid-extractable fractions as Ca-P for soil P cycling and discriminated and the existence of Fe, Al, and Ca-P (Gu et al. 2020; Barrow et al. 2021). In this study, we defined moderately occluded P as the sum of Al, Fe–P, and Ca_2-P, and the recalcitrant P was defined as the sum of HCl-P and the residual P (Wang et al. 2022). We measured the content differences of specific P fractions between the early and later growth seasons. Our results focused more on the changes in

the contents of different P fractions instead of the species of each P fractions. Moreover, some individual fractions were complexes and appeared to depend on fertiliser type and soil parameters, meaning that it is difficult to discriminate the specific chemical forms (Wang et al. 2019; Gu et al. 2020). Certainly, more accurate methods for P fraction also need to be modified to mitigate artifacts in future study.

Conclusions

This study demonstrated that the contribution shifted from inorganic P sources to organic and recalcitrant P pools with the shift from the earlier to the later growing season. Females had greater specific root length with more acquisition root (71%), thus increased P uptake and accumulation (20%) than males during the early growing season mainly from the mobilisation of moderately occluded P (decreased by 40% and 35, respectively, for $NaHCO_3$-P and NaOH-P, respectively, than a later growing season). In contrast, males established a symbiotic relationship with AMF to acquire P from organic P (decreased by 68% than the earlier growing season) and recalcitrant Ca-P (decreased by 11% than the earlier growing season), mainly via the maintenance of higher alkaline phosphatase activity and secretion of organic acids by roots with the shift from middle to late growing season. During the late growing season, fungal pathways contributed more to P acquisition mainly from degradation-resistant organic matter and recalcitrant P by males than females. These results suggest that different plant sexes adjust their P mobilisation and acquisition strategies with temporal niche differentiation. Taken together, our findings provide a scientific basis for predicting the impact of seasonal variations on plant nutrient acquisition and soil biogeochemical processes in *P. euphratica* and plantation management by considering plant sex differences.

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Author contribution Kaimin Lan had the main responsibility for data collection, analysis, and writing, and Yijin Li and Yiwei Shuai contributed to data collection; Juntuan Zhai contributed to the interpretation of data; Qingxu Ma and Yakov Kuzyakov contributed to manuscript revision, and Miao Liu (the corresponding author) had the overall responsibility for experimental design and project management.

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Data Availability Data are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare no competing interests.

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