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RESEARCH ARTICLE

Rhizosphere microbe populations but not root traits induced by drought in *Populus euphratica* males

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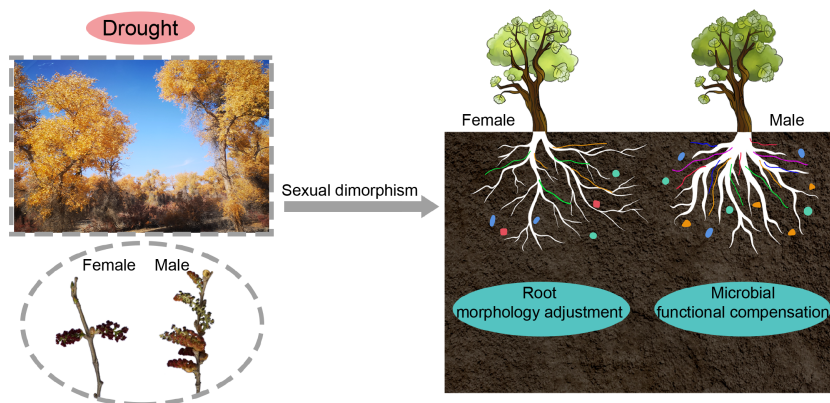
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HIGHLIGHTS

- Sexually dimorphic belowground responses to cope with drought.
- Females show more morphological plasticity in response to water deficiency.
- Males influence rhizosphere microorganisms to compensate for resource acquisition.
- Microbial responses are associated with root trait adjustments to drought.

GRAPHICAL ABSTRACT



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ABSTRACT

How sex-related root traits and soil microbes and their interactions respond to drought remains unclear. Here, we investigated how fine root traits and the composition of rhizosphere microbial communities in *Populus euphratica* females and males respond to drought in concert in 17-year-old plantations. Females increased specific root length (SRL) in response to drought. However, males showed no changes in their roots but significant increases in arbuscular mycorrhizal hyphal biomass and population of Gram-negative bacteria in the rhizosphere. Also, fungal symbiotroph communities associated with root systems in males differed from those in females under drought. We further demonstrated that the Gram-positive to Gram-negative bacteria ratios positively correlated with the SRL, while fungi to bacteria ratios were negatively correlated. Meanwhile, the relative abundance of symbiotrophs was negatively correlated with the SRL, while saprotroph abundance was positively correlated. Nevertheless, the relative abundance of symbiotrophs was positively correlated with the RCC. These findings indicate that microbial responses to drought depend highly upon the sex of the plant and microbial group and are related to root trait adjustments to drought. This discovery also highlights the role of plant-microbial interactions in the ecosystems of *P. euphratica* forest plantations.

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1 Introduction

About 6% of angiosperms are dioecious, with females and

males facing distinct resource demands and reproductive pressures (Renner, 2014). Differences in resource acquisition and utilization trade-offs between genders can be attributed to females' greater investment in reproduction (Randriamanana et al., 2014; Lei et al., 2017; Liu et al., 2021a). Dioecious plants typically have sex-specific

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1 resource acquisition strategies, resulting in a markedly
 different performance in females and males even in the
 same environment (Juvany and Munne-Bosch, 2015; Xia
 et al., 2020a; Nowak et al., 2021). Males are generally more
 5 tolerant than females under abiotic stresses (Hultine et al.,
 2016; Melnikova et al., 2017), and differences in functional
 traits between males and females become larger in adverse
 environments (Hultine et al., 2016; Liu et al., 2021a; Xia
 et al., 2020a; Wu et al., 2021). Numerous studies have
 10 investigated how the foliar level of male and female plants
 responded to drought (Xu et al., 2008a; Liu et al., 2020; Xia
 et al., 2020b; Zhang et al., 2021). For example, Liu et al.
 (2020) discovered that when poplars were grown in
 drylands, male plants could better regulate the physiologic
 15 processes of leaves and mitigate drought damage.
 However, the root-level responses of dioecious plants to
 drought in nature are largely unknown.

Tree roots can avoid and tolerate drought via various
 strategies (Olmo et al., 2014; Nikolova et al., 2020). These
 20 adaptive advantages include root biomass modifications,
 anatomical adjustments and physiologic acclimations
 (Bhusal et al., 2021). Root traits respond to drought in a
 species-specific way related to resource allocation patterns
 (Balachowski and Volaire, 2018; Lozano et al., 2021). Certain
 25 species have smaller mean root diameters and longer
 specific root lengths (SRL), which allow for a greater water
 uptake with a lower carbon investment under water
 deficiency (Debinski et al., 2010). In comparison, plants with
 thicker roots reduce the risk of hydraulic fracturing and form
 30 stronger associations with symbiotic arbuscular mycorrhizal
 fungi (AMF), thus increasing water capture efficiency
 (Zufferey et al., 2011). Moreover, the soil microflora is
 affected by species-specific root traits associated with
 different nutrient content or root exudation (Xia et al., 2019;
 35 Sweeney et al., 2021). In addition, during drought, beneficial
 microorganisms can significantly help roots in absorbing
 water and nutrients, thereby promoting tree health and
 disease control (Schimel, 2018; de Vries et al., 2020).

Dioecious plants exhibit skewed sex ratios along resource
 40 gradients (Hultine et al., 2016). Males' low resource
 demands are accompanied by relatively conserved traits
 (lower SRL) but stronger associations with AMF, which
 frequently predominate in resource-limited environments
 (Xia et al., 2020a; Liu et al., 2021a). In high-resource
 45 environments, females tend to show stronger photosynthetic
 capacities and develop higher SRL, but they are less
 correlated with AMF (Xia et al., 2020a).

Inherent sex genes may drive the assembly of gender-
 specific rhizosphere microbiomes by sexual rhizodeposition
 and root exudation patterns (Doniger et al., 2020; Xia et al.,
 50 2021b). Guo et al. (2021) discovered that the distinct
 microbial communities of the rhizosphere in female and
 male poplars resulted in sex-specific nutrient biogeochem-
 ical cycles. However, the combined effects of intrinsic sex
 55 and external drought, and their relative contributions to the
 rhizosphere microbial communities of male and female

plants are still unclear. It is also unknown how dioecious
 plants' root functional traits are associated with rhizosphere
 microorganisms within the same population.

Populus euphratica is a typical dioecious plant and is the
 only tree species living in certain deserts. The distribution
 5 region of *P. euphratica* in the Tarim Basin exceeds 650 000 ha,
 covering more than 90% of the total area of *P. euphratica*
 in China. Water scarcity resulted in a 75% decrease in the
 growth area of the *P. euphratica* forest over the 40-year
 period 1950–1990. Therefore, to deal with these changes
 10 and ensure effective increases in primary production in
 managed ecosystems, a deeper understanding of *P.*
euphratica's drought-stressed growth strategies is required.

To investigate sex-specific adaptive strategies of *P.*
euphratica to drought events, experiments were conducted
 15 with two water management conditions in *P. euphratica*
 plantations that resulted in different soil humidity levels. We
 collected data sets, including root functional traits,
 phospholipid fatty acid (PLFA) contents of microbial groups,
 and functional groups of fungi in the rhizosphere of *P.*
 20 *euphratica* males and females to test three hypotheses: (1) as
 rhizosphere microorganisms can be affected by inherent
 genes and external environment, we hypothesize that the
 rhizosphere microbial communities of *P. euphratica* females
 and males respond differently to drought due to sex-related
 25 differences; (2) drought affects root traits of *P. euphratica*
 in a sex-specific way. As acquisitive species are considered
 more responsive to environmental stresses, we assume that
 the root morphology of females responds more positively to
 drought than that of males; (3) the adjustment of functional
 30 traits of roots affects the relationships between plants and
 microbial taxa, because roots and soil microbes are
 intrinsically linked in time and space within the rhizosphere.

2 Materials and methods

2.1 Field sites

The study area was at the north-western border of the Tarim
 Basin in Xinjiang Uygur Autonomous Region, China. The
 40 annual mean air temperature is 10.8°C, with 50 mm
 precipitation. The soil is classified as calcic xerosol. The
 plantations were situated in the upper reaches of the Tarim
 River (81°17' E, 40°32' N–81° 7' E, 40°56' N). *P. euphratica*
 45 individuals were planted in 2003. Two water management
 conditions were selected with two genders each to produce
 four treatment combinations: Female, well-watered and
 drought; Male: well-watered and drought, each with six
 replicates (Xia et al., 2021b). During the first five years, all
 50 plots were irrigated. Half of the plots were randomly
 assigned to drought and depended on natural rainfall, while
 the other half were later irrigated from 2009 to 2019 in
 March and April for half of a month. This irrigation was set at
 8 h per day at about 50 m³ h⁻¹ and the soil relative water
 55 content, throughout the top 60 cm m 60 cm depth, was

maintained at 90%. Each replicated plot was 100 m², with distance of 10–15 m apart from replicate plots. We identified the sex of each tree throughout the blooming season based on the flower morphology (Fig. S1).

2.2 Sampling and storage

We sampled fine roots from well-watered and drought plots, respectively, in August 2019. Three *P. euphratica* females or males were selected from each well-watered and each drought plot. Fine root (average diameter < 2 mm) samples were taken from the rhizosphere of each tree. Soils from the rhizosphere were carefully separated from fine roots. Within each plot, three soil samples (root or rhizosphere soil) from each sex formed a mixed sample. Soil samples were stored at -80°C until the sequencing of soil microbes were performed.

2.3 Root trait measurements

After harvest, the root samples containing more than three root orders were selected (Goebel et al., 2011). They were washed carefully using deionized water, and the fresh weight of root segments was measured. Then the root samples were scanned by the scanner (Epson V700), and the Win-RHIZO system (Régent instrument Inc., Québec, Canada) was used to analyze the total root length, root volume, root tips and forks (Liu et al., 2015). All samples were dried at 70°C for 48 h to determine the specific root length (SRL). The root tissue density (RTD) was the ratio of fresh weight of root segments to root volume, the root branching intensity (RBI) refers to the number of first-order roots per unit length of second-order root, and the root branching ratio (RBT) was the number of first-order roots divided by the number of second-order roots within the selected root segments. The root nitrogen content (RNC) was determined by the Kjeldahl method (Liu et al., 2018), the root carbon content (RCC) was determined by an element analyzer (FlashSmart; Thermo Scientific, Milan, Italy), and the root carbon to nitrogen ratio (CNRatio) was the ratio of RCC to RNC.

2.4 Phospholipid fatty acid analysis

About 10 g of fresh rhizosphere soil was collected for freeze-drying, and soil PLFA was determined by the method mentioned in Xia et al. (2019). Briefly, main soil microbial groups including Gram-positive bacteria, Gram-negative bacteria, bacteria, fungi, AMF, actinomycetes, were used. For detailed information of the biomarkers see Supplementary S1.

2.5 Fungal communities

The fungal community member identities were determined

by high-throughput sequencing. The procedures of DNA sequencing see Supplementary S2. The original data obtained by sequencing were spliced and filtered to acquire effective data. Then, the data were clustered by Uparse software (Uparse v7.0.1001). The sequences were automatically grouped into operational taxonomic units (OTUs) with 97% consistency. The BLAST method in the Qiime software (version 1.9.1) and Unite database were used for species annotations. The functional annotation of fungal communities among samples was carried out using functional guild data with a specific taxonomic level from the database FUNGuild (Nguyen et al., 2016).

2.6 Statistical analyses

Differences in seven root traits among the four groups were determined by One-way ANOVAs. Following One-way ANOVAs, Tukey's Honestly Significant Difference (HSD) tests were employed for multiple comparisons. The effects of water, sex and their interactions were determined by two-way ANOVAs. The principal component analysis (PCA) for seven root traits was conducted by psych, ggplot2 and ggbiplot R packages (Vu, 2016; Wickham, 2016; Revelle, 2017). In addition, vegan package was used to perform PerMANOVA to test root traits among the four groups (Oksanen et al., 2015). For the data set of PLFA, individual *t*-tests were used to analyze the water and sex effects.

Fungal functional groups were categorized into three types: symbiotroph, pathotroph and saprotroph based on the FunGuild database (Nguyen et al., 2016). Then, these classification results were used to calculate the relative abundances of the three functional group types in all samples and analyzed the difference between water and sex effects by individual *t*-tests. Principal-coordinate analyses (PCoAs) utilizing the Bray-Curtis dissimilarity index were used to visualize beta-diversities among fungal communities related to drought and sex impacts. The PCoAs were performed, including all OTUs (total fungi), and the OTUs assigned to a specific functional group type. In addition, PerMANOVA tests were used for total fungal communities and three fungal functional group types. Then, the values of the axis 1 and axis 2 of PCoAs under drought and sex effects were tested by two-way ANOVAs. Finally, Pearson correlation analyses were performed to show relationships between fine root traits, PLFA biomarkers and fungal guilds.

Before performing the canonical correspondence analysis (CCA), we used the ggvegan package to conduct a covariance analysis for seven root traits and for fungal communities at the order level (Simpson, 2015). Subsequently, six root traits were used in CCA, the CN ratio being omitted. The vegan and ggplot2 packages were used for CCA results and to examine the correlation between root traits and fungal community changes. To determine whether water management conditions or sexes influence the composition of fungal communities, we utilized the Scheirer-

Ray-Hare test using rcompanion package (Mangiafico and Mangiafico, 2017) and visualized them by the ggplot2 package.

3 Results

3.1 Sexual differences in fine root traits at two different soil water levels

The soil water content of well-watered treatments was always higher than that of drought treatments at any depth (Fig. S2). Drought significantly affected most morphological and physiologic traits of roots (Table 1). In contrast, sex and its interaction with water management only affected the RTD and SRL ($p < 0.05$; Table 1). In comparison to males, females significantly increased the SRL under drought conditions ($p < 0.05$; Table 1). PCA revealed variation in the root traits of *P. euphratica* females and males in response to drought; the first and second PCA axes explained 43.1% and 20.5% of the total variation, respectively (Fig. 1). The SRL was negatively correlated with the RTD, RCC, RBI, and CNRatio (Fig. 1). On the contrary, the root traits of *P. euphratica* females and males showed no significant differences under the well-watered condition. In contrast, under drought, females and males were positioned along the first and second PC axis, respectively (Fig. 1).

3.2 PLFA biomarkers and fungal functional guilds of dioecious *P. euphratica* under drought

Drought significantly affected most PLFA groups, including bacteria, fungi, AMF, and Gram-positive bacteria and Gram-negative bacteria ($p < 0.05$; Fig. 2). However, the difference in PLFA groups between sexes was observed only under drought conditions ($p < 0.05$; Fig. 2). Furthermore, males had more AMF and Gram-negative bacteria than females. In

contrast, the ratio of Gram-positive bacteria to Gram-negative bacteria was significantly lower in males than in females ($p < 0.05$; Fig. 2).

The taxonomic composition of fungal communities at the phylum and genus level (relative abundance $> 0.01\%$) is shown in Fig. 3. The abundances of the 9 phyla were in the following order: *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Chytridiomycota*, *Kickxellomycota*, *Zoopagomycota*, *Aphelidiomycota* and *Monoblepharomycota*. Only the relative abundance of *Mucoromycota* was significantly reduced by drought, while sex did not have a significant effect on the relative abundance of the 9 phyla. The abundances of the 13 genera were in the following order: *Geopora*, *Helvella*, *Verticillium*, *Mortierella*, *Lecanicillium*, *Penicillium*, *Metarhizium*, *Trematosphaeria*, *Fusarium*, *Preussia*, *Acrostalagmus*, *Acremonium* and *Aspergillus*. Drought significantly reduced the relative abundance of *Helvella* and *Metarhizium*, while it increased the relative abundance of *Acrostalagmus* and *Aspergillus* ($p < 0.05$; Fig. 3). However, in males the relative abundance of *Helvella* ($p < 0.05$; Fig. 3) significantly increased, which is a type of ectomycorrhizal fungi. The relative abundance of symbiotrophs in *P. euphratica* was significantly reduced by drought, but not that of pathotrophs and saprotrophs ($p < 0.05$; Fig. 4). Likewise, the relative abundance of symbiotrophs in males was significantly higher than that in females regardless of drought ($p < 0.05$; Fig. 4). However, there was no difference in the relative abundance of pathotrophs and saprotrophs between females and males (Fig. 4).

The response of fungal communities to water management and sex depended on the functional guilds (Table 2; Fig. S3). The composition of the total fungal community and the symbiotroph community were significantly different among the four treatments ($p < 0.05$; Table 2; Fig. S3). Furthermore, the structure and composition of the symbiotroph community was more

Table 1 Mean fine root trait values and standard errors ($n = 6$) of *P. euphratica* females and males under well-watered and drought conditions.

Variation		SRL (m g ⁻¹)	RTD (g cm ⁻³)	RBI (cm ⁻¹)	RBT	RNC (mg g ⁻¹)	RCC (mg g ⁻¹)	CNRatio
Site	Sex							
Well-watered	Females	10.49 ± 1.66 b	0.55 ± 0.10 ab	3.03 ± 0.19 a	1.35 ± 0.22 a	8.70 ± 0.28 a	426.92 ± 8.09 a	49.42 ± 2.23 a
	Males	8.52 ± 0.85 b	0.62 ± 0.07 a	2.60 ± 0.29 ab	1.02 ± 0.17 ab	8.85 ± 0.70 a	427.57 ± 12.39 a	49.82 ± 4.04 a
Drought	Females	19.48 ± 0.86 a	0.28 ± 0.06 b	2.10 ± 0.22 b	0.78 ± 0.05 ab	9.26 ± 0.62 a	381.25 ± 15.39 a	41.58 ± 1.36 a
	Males	12.29 ± 0.77 b	0.53 ± 0.06 ab	2.39 ± 0.23 ab	0.62 ± 0.08 b	7.61 ± 0.36 a	387.79 ± 18.68 a	51.99 ± 4.89 a
Two-way ANOVA test								
Water		***	*	*	*	ns	**	ns
Sex		***	*	ns	ns	ns	ns	ns
Water × Sex		*	ns	ns	ns	ns	ns	ns

The seven root traits include specific root length (SRL), root tissue density (RTD), root branching intensity (RBI), root branching ratio (RBT), root nitrogen content (RNC), root carbon content (RCC) and root carbon to nitrogen ratio (CNRatio). The significance levels are marked with * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $p \leq 0.001$; ns, not significant. Different letters indicate significant differences among treatments at $p < 0.05$, ANOVA with Tukey's honestly significant difference test.

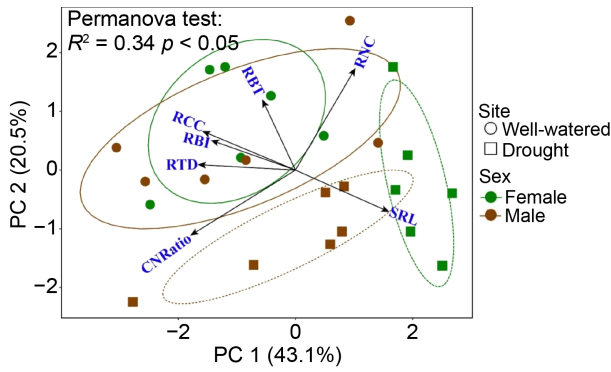


Fig. 1 Principal component analysis of fine root traits of *P. euphratica* females and males under well-watered and drought conditions. Circles represent root traits under well-watered conditions and boxes represent root traits under drought conditions. Green colors represent root traits of *P. euphratica* females and red colors represent root traits of *P. euphratica* males. Fine root traits corresponding to the arrows are shown in blue font: specific root length (SRL), root tissue density (RTD), root branching intensity (RBI), root branching ratio (RBT), root nitrogen content (RNC), root carbon content (RCC) and root carbon to nitrogen ratio (CNRatio).

sensitive to water and sex effects ($p < 0.05$; Table 2). In addition, the community composition and structure of total fungi were significantly affected by water management but not by sex ($p < 0.05$; Table 2). However, the community

composition and structure of pathotrophs and saprotrophs may not be sensitive to drought and sex because PCoA 2 values were only slightly affected by drought (Table 2).

3.3 Associations between fine root traits and rhizosphere microbes

The SRL was correlated with many PLFAs groups and functional fungal guilds, among which the concentration of AMF, the ratio of fungi and bacteria, and the relative abundance of symbiotrophs were negatively correlated, while the ratio of Gram-positive bacteria and Gram-negative bacteria, and the relative abundance of saprotrophs were positively correlated ($p < 0.05$; Fig. 5). The RTD and RCC were both significantly positively correlated with the concentration of AMF and the relative abundance of symbiotrophs, and the RCC was slightly positively correlated with the ratio of fungi and bacteria ($p < 0.05$; Fig. 5). However, the RBI was slightly negatively correlated with saprotrophs, while the RBT was slightly negatively correlated with pathotrophs ($p < 0.05$; Fig. 5). In addition, the root nitrogen content was slightly negatively correlated with the ratio of fungi and bacteria ($p < 0.05$; Fig. 5).

Collectively, the fine root traits in CCA explained almost 80% of the variation in the relative abundances of fungal

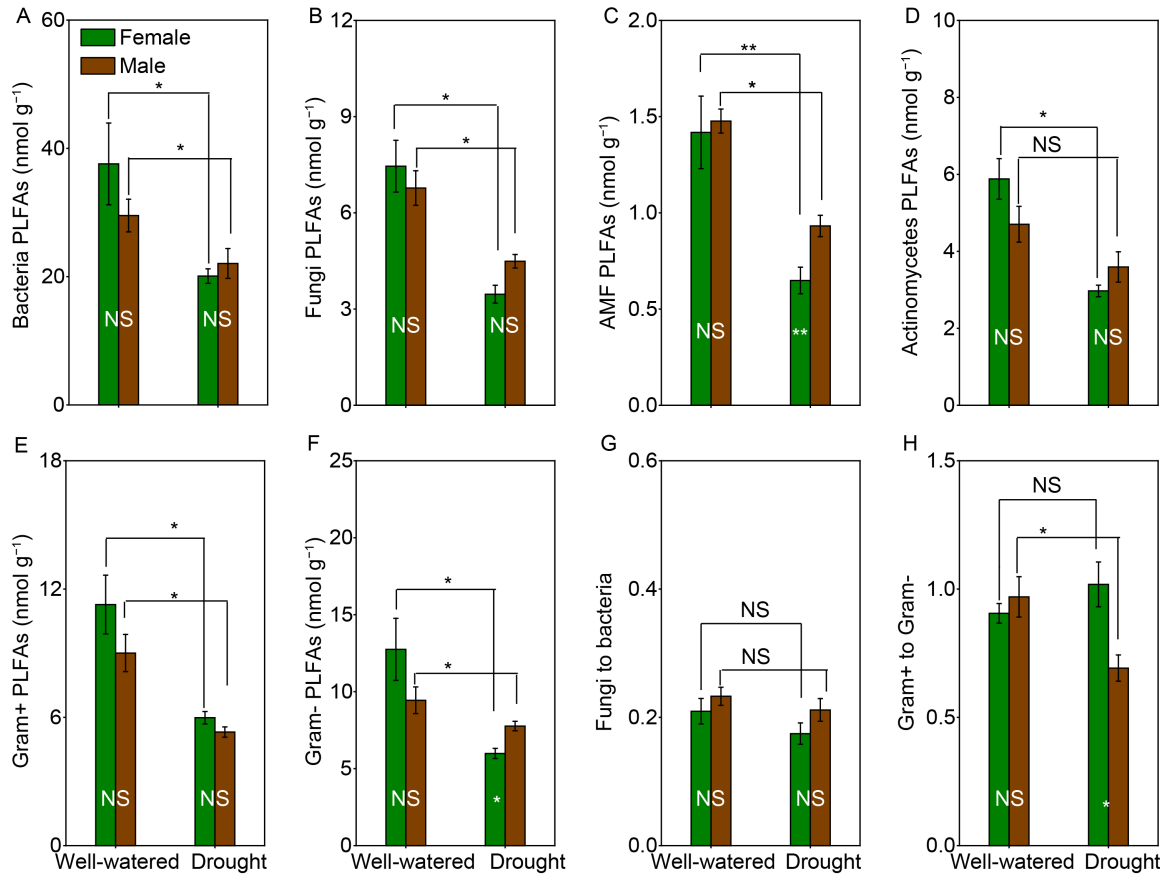


Fig. 2 Concentrations of PLFA biomarkers in the rhizosphere of *P. euphratica* females and males under well-watered and drought conditions. Values are means \pm standard errors. Significances are marked as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

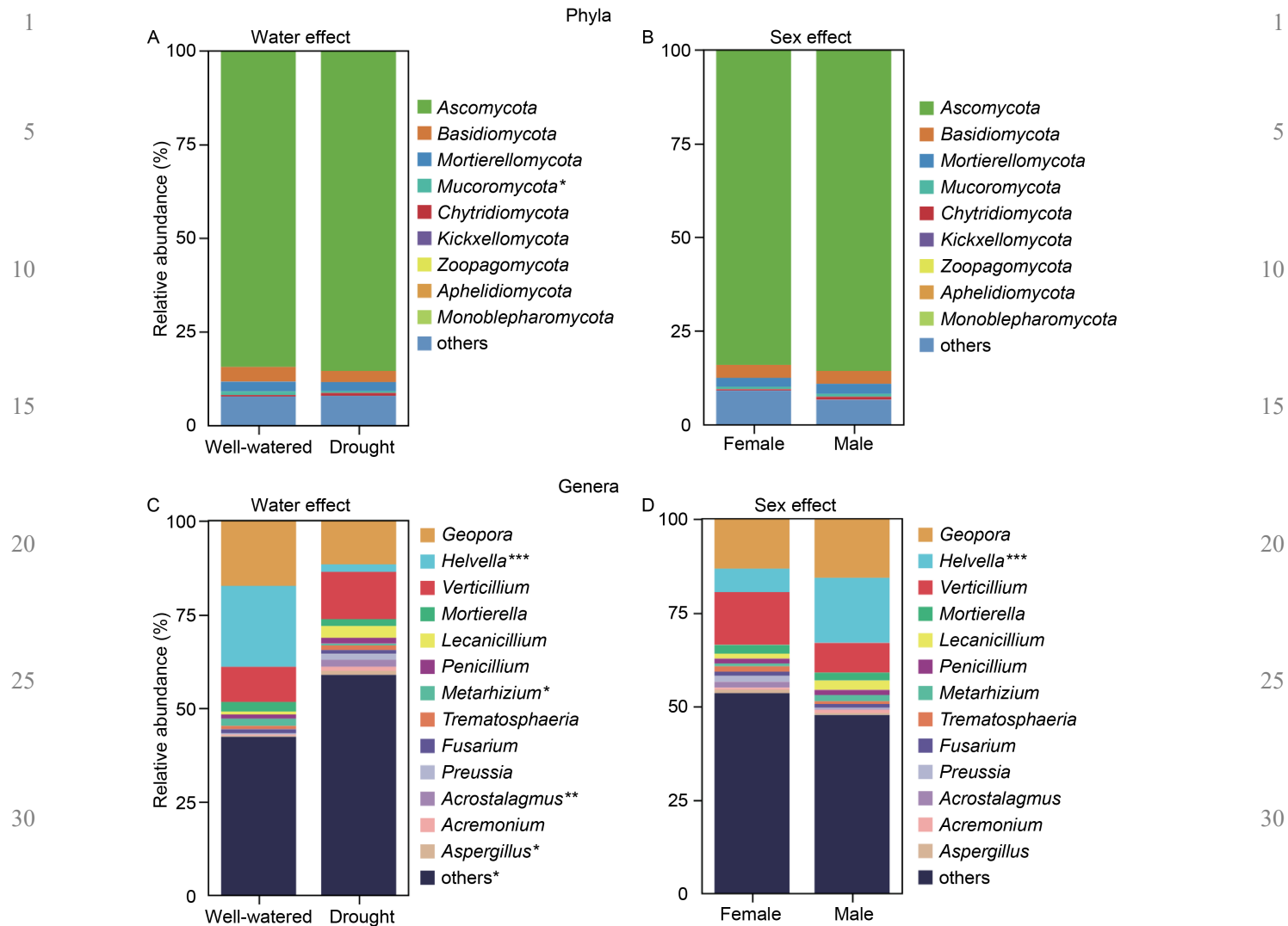


Fig. 3 Comparisons of relative abundances of dominant fungal phyla and genera between water management conditions (a, b) and between sexes (c, d). Asterisks denote significant differences in relative abundances between water management conditions and sexes, as generated by two-way ANOVA models. Relative abundances are represented by means and standard errors. Significances are marked as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

orders (Fig. 6A). Two root traits, i.e., the SRL and RCC, significantly explained the variation in the relative abundance of fungal orders ($p < 0.05$ and $p < 0.01$, respectively; Fig. 6B). However, the relative abundances of *Glomerellales*, *Sordariales* and *Pleosporales* orders were associated with higher values of SRL and lower values of RBI (Fig. 6A). The relative abundance of *Agaricales* was associated with a higher RCC, while the relative abundances of *Hypocreales* and *Helotiales* were associated with a lower RCC (Fig. 6A).

4 Discussion

Our study showed that sex-specific strategies in response and adaptation to drought of *P. euphratica* depend on different microorganisms that relate to root trait adjustments

to drought. In particular, (1) drought influenced the attributes and syndromes of root functional traits of *P. euphratica*, but the magnitude and orientation of these changes varied depending on sex; (2) the relative abundance and community composition of rhizosphere microbes in *P. euphratica* were affected by drought, but the magnitude of these effects were related to the broad microbial groups, fungal functional guilds and sexual attributes; (3) microbial responses were associated with root trait adjustments to drought, particularly with the SRL and RCC.

Although *P. euphratica* is a drought-tolerant desert tree, we observed distinct sex-related adaptations of roots to drought. In response to soil water deficiency, roots often lower the root branching intensity and ratio while increasing taproot depth and SRL (Brunner et al., 2015). Females have a larger SRL with a reduced root branching intensity and ratio under drought compared with males. Likewise, PCA revealed that the functional characteristics of female and

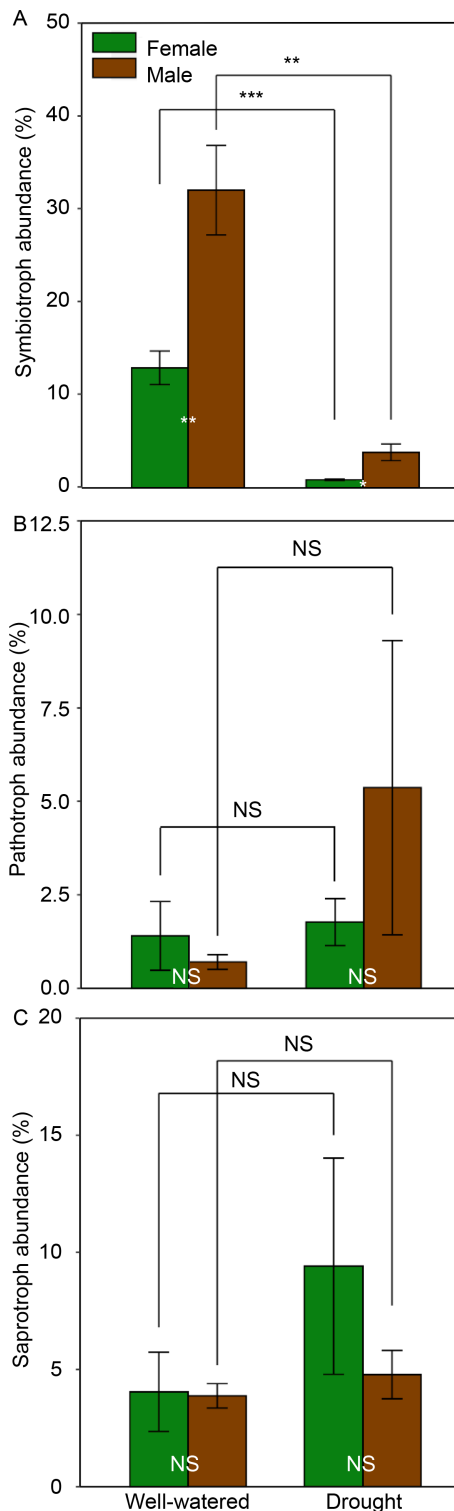


Fig. 4 Relative abundances (percentages calculated based on the total number of sequences) of (a) symbiotrophs, (b) pathotrophs and (c) saprotrophs associated with *P. euphratica* females and males growing under well-watered and drought conditions. Relative abundances are represented by means and standard errors. Significances are marked as ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

male roots responded differently to drought. Males, with a lower SRL, reduced the risk of hydraulic fracturing,

suggesting a weaker morphological plasticity of roots in response to drought. However, the abundance of AMF and Gram-negative bacteria in the rhizosphere of males was higher than in that of females. Drought-induced changes in soil microorganisms may affect plants' resource acquisition and growth (Kannenberg and Phillips, 2017; Schimel, 2018; de Vries et al., 2020). A higher mycelium biomass can provide males with improved water capture efficiency during drought. Similarly, Gram-negative bacteria can tolerate various abiotic stresses (Naylor and Coleman-Derr, 2018; Spitzer et al., 2021), alleviating, in males, to some extent the resource shortage caused by water stress. Taken collectively, males can adapt to drought through favorable microbial processes due to the activities of associated AMF and functional bacteria.

Diocycy increases a population's opportunities for survival and expansion. The trade-off difference between the two sexes is the theoretical underpinning for establishing a framework between functional traits and acquisition strategies of roots. Females require more resources, since their reproductive costs are higher than those of males, especially under limited resources (Juvany and Munne-Bosch, 2015; Lei et al., 2017; Retuerto et al., 2018). Females produce thin roots (high SRL) that reduce a plant's reliance on AMF to absorb water (Lin et al., 2015). They can increase water absorption efficiency at a lower C cost by exploring a more extensive territory. However, this aggressive acquisition may incur a long-term and expensive cost. Conversely, males (lower SRL but a high RTD) typically rely on AMF to explore and acquire nutrients in soils (Brundrett, 2002; Lin et al., 2015), and they are more resistant to herbivore and disease attacks in infertile soils (Laliberté, 2015).

Microbial richness, and community composition and structure in the plant rhizosphere are affected by genes and environment (Guo et al., 2021; Liu et al., 2021b; Xia et al., 2022). In our study, we found that the composition and structure of total fungal communities and fungal symbiotrophs were affected by drought. In addition, fungal communities associated with females showed considerable differences between well-watered and drought sites. In contrast, fungal communities associated with males with better resistance to drought were similarly independent of water management. The morphological root trait modifications to drought events is sex-specific and that may reflect in the metabolic file of root exudates and in the variation of fungal communities (Broeckling et al., 2008; Huang et al., 2020). In addition, water shortage could affect C assimilation and storage differently in females and males, as females have a greater water uptake than males (Xu et al., 2008b).

Sex affected the rhizosphere symbiotic community of *P. euphratica*, which may be related to the differences in the accumulation of defensive compounds. The content and richness of phenolic compounds in female poplar roots are significantly higher than those in male roots (Xia et al.,

Table 2 Sex and drought effects on fungal attributes (structure and composition) of the total fungal community, symbiotroph, pathotroph and saprotrophs. Sex, water management and their interactions were considered as fixed factors. The composition was analyzed through permutational multivariate ANOVA (PERMANOVA) and by linear models using the first two principal coordinate axes (PCoAs) as representative of the fungal composition. F and *p* values are shown in parentheses. *p* < 0.05 in bold; *p* < 0.1 in bold italic.

Fungal group	Attribute	Water	Sex	Water × Sex
Total community	Structure (PERMANOVA)	3.27 (<0.01)	1.21 (0.25)	1.39 (0.17)
	Composition (PCoA1)	5.48 (<0.05)	2.72 (0.12)	0.85 (0.37)
	Composition (PCoA2)	19.27 (<0.001)	2.54 (0.13)	1.35 (0.26)
Symbiotroph	Structure (PERMANOVA)	35.83 (<0.001)	5.93 (<0.01)	12.63 (<0.001)
	Composition (PCoA1)	9.68 (<0.01)	3.57 (0.07)	3.23 (0.09)
	Composition (PCoA2)	0.43 (0.52)	0.79 (0.38)	0.29 (0.60)
Pathotroph	Structure (PERMANOVA)	1.17 (0.28)	0.80 (0.58)	0.98 (0.42)
	Composition (PCoA1)	0.01 (0.94)	1.99 (0.17)	0.55 (0.47)
	Composition (PCoA2)	4.48 (<0.05)	0.09 (0.77)	0.88 (0.36)
Saprotroph	Structure (PERMANOVA)	1.22 (0.24)	0.64 (0.88)	0.68 (0.79)
	Composition (PCoA1)	0.24 (0.63)	0.13 (0.73)	0.11 (0.74)
	Composition (PCoA2)	7.32 (<0.05)	0.01 (0.94)	0.45 (0.51)

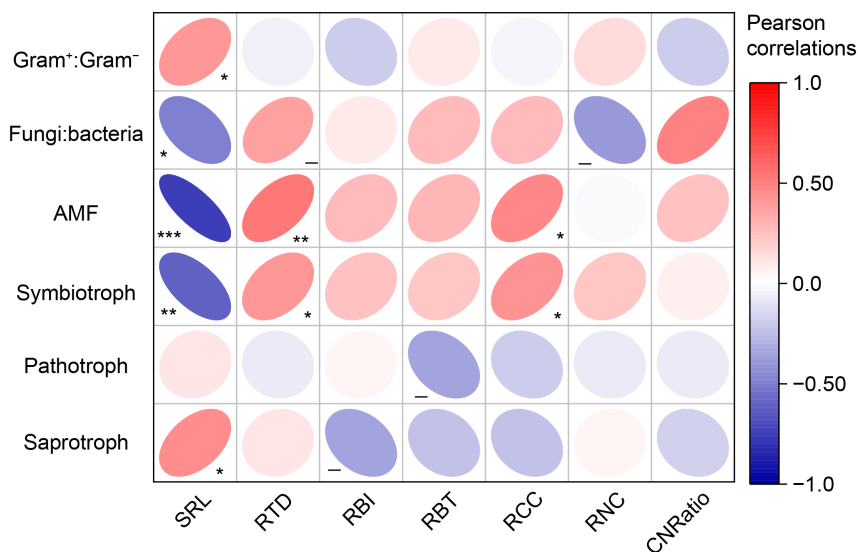


Fig. 5 Pearson correlations between fine root traits and absolute abundances of PLFA biomarkers and fungal functional guilds. Abbreviations of fine root traits can refer to Figure 1. Gram+: Gram- means the ratio of Gram-positive to Gram-negative bacteria. AMF represents arbuscular mycorrhizal hyphal biomass of PLFA biomarkers 16:1 ω5c. Significances are marked as ***, *p* ≤ 0.001; **, *p* ≤ 0.01; *, *p* ≤ 0.05; '-' indicates 0.05 < *p* ≤ 0.1.

2021b). These defensive chemical compounds could weaken the association between symbiotic fungi and roots (Xia et al., 2021a), thereby potentially affecting the richness, community composition and structure of symbiotic fungi. On the other hand, drought and sex had no effect on the richness, composition and structure of the rhizosphere pathotrophs and saprotrophs in *P. euphratica*, indicating that the two functional guilds are conserved in desert areas. *P. euphratica* has a long lifespan and can survive for thousands of years in a desert. Possibly, the root system of *P. euphratica* could control the richness, community composition and structure of soil pathogenic fungi and saprophytic fungi through physical, physiologic, and

biochemical ways to maintain homeostasis (Tang et al., 2013; Yu et al., 2020).

Although the correlation between root traits and microorganisms has been revealed in trees and herbs in various ecosystems (Lozano et al., 2021; Sweeney et al., 2021; Spitzer et al., 2021; Wan et al., 2021), to our knowledge our study is the first one to find that variation in functional traits of fine roots in *P. euphratica* populations is associated with microbial groups. The positive correlations between Gram-positive bacteria to Gram-negative bacteria ratios and SRL may be due to Gram-positive bacteria increasing nutrients by enabling the decomposition of more complex carbon forms (Orwin et al., 2018; Fanin et al.,

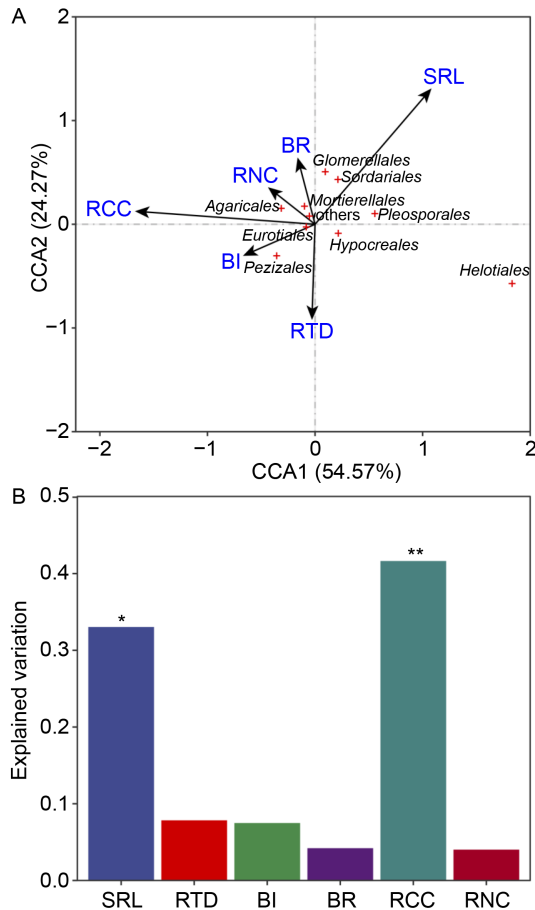


Fig. 6 Canonical correspondence analysis of fungal orders constrained by fine root traits across all experimental units (a) and variation in fine root traits explaining fungal orders (b). The black arrows are fine root traits. Red crosses represent fungal orders. Significances are marked as **, $p \leq 0.01$; *, $p \leq 0.05$. Abbreviations of fine root traits can refer to Figure 1.

2019). In addition, longer SRL may provide more N to Gram-positive (Spitzer et al., 2021). In comparison to conservative root traits (larger root average diameter and RTD), acquisitive root traits often involve a higher SRL (smaller root diameter) and a rhizosphere with a lower fungi and bacteria ratio. Additionally, thinner roots promote saprotrophic activity in the rhizosphere through a release of easily degradable carbohydrates (Semchenko et al., 2018). Similarly, relationships were found between fungal functional groups and root traits. The variation in root functional traits is primarily explained by root-mycorrhizal connections, emphasizing the trade-off between “do it yourself” and “outsourcing” resource acquisition (Freschet et al., 2021a; Freschet et al., 2021b). The relative abundance of fungal symbiotroph was positively associated with RCC. This could be because of conservative root traits, which rely more on AMF for nutrient exploration and acquirement under resource-limited conditions.

Since 1990, the Chinese government has effectively increased the growth area of *P. euphratica* by establishing forest plantations and protecting natural forests. However,

frequent extreme events still threaten the survival of *P. euphratica*. Our study found how the roots of dioecious *P. euphratica* and coexisting microorganisms respond to and tolerate drought stress and how their synergy functions. Although sexual dimorphism effectively increases a population's chances of survival in the long-term, sex-specific differences in resource allocation and utilization strategies may lead to one sex being more vulnerable to extreme climate than the other (Hultine et al., 2016). Therefore, the knowledge of sexual dimorphism under extreme conditions in acquiring resources would help adjust sex ratios to maintain the community structure and stability of desert ecosystems.

5 Conclusions

This study sheds light on the sexually dimorphic below-ground responses of dioecious *P. euphratica* to cope with drought. On the one hand, females show morphological plasticity, as their SRL increases in response to water deficiency, thereby increasing water acquisition in resource-use conditions. The morphological plasticity of males is not as responsive as that of females. However, males significantly increase the content of Gram-negative bacteria and AMF in the rhizosphere to resist drought. The symbiotic fungal communities associated with males differ from those associated with females during drought. Above all, males have a good ability to influence rhizosphere microorganisms to compensate for their resource acquisition under environmental stress. We found that root trait adjustments under drought, such as SRL and RCC, may affect rhizosphere microbes in *P. euphratica* populations. Our results also show the presence of sex-specific root-microbe interactions under drought. The results will be helpful for predicting the consequences of global change on dioecious *P. euphratica*, and they allow effective actions for restoration programs to maintain *P. euphratica* populations in ecologically fragile regions.

Conflict of interest

The authors declare that they have no conflict of interest.

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Electronic supplementary material

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