Revealing interactions between root phenolic metabolomes and rhizosphere bacterial communities in Populus euphratica plantations

Xia, Zhichao

2021-04

Xia , Z , He , Y , Yu , L , Li , Z , Korpelainen , H & Li , C 2021 , ' Revealing interactions between root phenolic metabolomes and rhizosphere bacterial communities in Populus euphratica plantations ' , Biology and Fertility of Soils , vol. 57 , no. 3 , pp. 421-434 . https://doi.org/10.1007/s00374-0

http://hdl.handle.net/10138/342258
https://doi.org/10.1007/s00374-020-01527-z

acceptedVersion

The revision of ***BFSO-D-20-00561***

Revealing interactions between root phenolic metabolomes and rhizosphere bacterial communities in *Populus euphratica* plantations

Zhichao Xia [1], Yue He [1], Lei Yu [1], Zhijun Li [2],

Helena Korpelainen [3] and Chunyang Li [1,*]

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

[2] College of Life Sciences, Tarim University, Alar 843300, China

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

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

Abstract How the root metabolic profiles and rhizosphere bacterial communities of dioecious plants respond to soil properties and sex identity is largely unknown. In this study, we analyzed root phenolic metabolomes and rhizosphere bacterial microbiomes of *Populus euphratic* females and males in two *P. euphratica* plantations with different soil properties to reveal the relative importance of soil and sex effects, and to decipher associations of certain phenolic compounds with specific bacterial taxa. We found that the relative abundances of bacterial OTUs and phenolic metabolites were closely linked to soil properties and sex identity. Soil is the main filter influencing the root phenolic metabolic profiles and rhizosphere bacterial communities of *P. euphratica*, while sexes and their interactions with soil properties are secondary factors. Differences in the diversity and evenness of phenolic metabolites were affected by plant sex, but not by soil properties. Conversely, the diversity and evenness of bacterial communities were affected by soil properties independent of plant sex. A multiple regression model indicated the presence of associations between root phenolic metabolites and specific soil bacteria taxa. Furthermore, all bacterial phyla and families correlated with at least one phenolic metabolite. Especially, both *Nitrosomonadaceae* and *Cytophagaceae* positively correlated with salicylic acid. Thus, our study provides new insights into the ecological mechanism that maintains rhizosphere bacterial communities in *P. euphrasica* plantations in the desert area.

Keywords: Dioecious plants; Bacterial community assembly; Microbial diversity; Soil properties; Phenolic metabolome; *Populus euphratica* plantation

Introduction

During the process of co-evolution, microbiomes and their hosts form dynamically balanced ecosystems. Plants provide suitable habitats and nutrients for microbes. Reciprocally, microbes can significantly improve plant growth, nutrient absorption, photosynthesis, and stress and disease resistance (Compant et al. 2019; Yu et al. 2019; de la Fuente et al. 2020). Studies on rhizosphere microbiomes have focused on the effects of plant gene expression on the microbes present in the soil matrix in different environments (Lareen et al. 2016; Veach et al. 2019), but the relative contributions of the environment and plant genes need to be further explored. An accurate assessment of the relative role of these factors within the framework of root-soil-microbe interactions would be critical when predicting the microbiome regulation of plants' nutrition and health.

Changes in physicochemical properties of soil, such as pH, nutrients and moisture, can affect not only the colonization of microbes but also the overall community composition of microbes and even the preferences of specific functional microbial groups in the rhizosphere (Bais et al. 2006; Hinsinger et al. 2009; Guo et al. 2019). On the other hand, plants can shape the surrounding microbial community through root exudation or rhizodeposition, which can enrich specific microbial populations and promote their activity (Paterson et al. 2007; de Graaff et al. 2010; Zhalnina et al. 2018). Over time, this process can lead to plants selecting specific beneficial rhizosphere

67 microbes (Yu et al. 2019). Root phenolic compounds, as secondary metabolites in roots,
68 are often regarded as an important chemical defense system for plants to prevent
69 pathogen infections and soil fauna from feeding on them (Barbehenn and Constabel
70 2011; Boeckler et al. 2011). In addition, phenolic compounds can increase the relative
71 abundance of certain microbes, thus providing growth benefits and immune resistance
72 for plants (Shi et al. 2011; Voges et al. 2019).
73
74 Generally, phenolic metabolites affect bacteria more than fungi (Gu et al. 2020).
75 *Arabidopsis thaliana* genotypes were found to increase the density of *Pseudomonas*
76 spp. by manipulating the expression of salicylic acid signals and they may regulate the
77 colonization of specific bacterial taxa, such as *Streptomytidae* (Lebeis et al. 2015).
78 Badri et al. (2013) discovered that phenolic compounds in root exudates were
79 significantly positively correlated with the number of bacterial OTUs. The phenolic
80 profiles vary greatly among different plants. For instance, Veach et al. (2019) suggested
81 that the phenolic profile in *Populus* roots was diverse and abundant, and the production
82 of salicylic acid derivatives varied among *Populus* clones under specific soil conditions,
83 leading to differences in the composition of bacterial communities. Consequently,
84 variation in plants' phenolic profiles may potentially induce changes in bacterial
85 microbiomes, thus affecting plant-soil interactions.
86
87 Natural selection may drive sexual differentiation among dioecious plants, leading to
88 sex-specific growth strategies (Obeso 2002). Female and male plants exhibit sexual

dimorphism not only in reproductive organs but also in vegetative traits, such as leaf morphology, secondary chemistry and phenology (Randriamanana et al. 2014; Nissinen et al. 2018; Rozas et al. 2019). Due to different reproductive costs, sexual differences in chemical defenses often occur in dioecious plants (Cornelissen and Stiling 2005; Avila-Sakar et al. 2012). Females typically invest more in chemical defense than males, because they produce fruit and seeds. Total phenolic content in female leaves is higher than that in male leaves, and the leaf phenolic profile of females is different from that of males, which results in stronger herbivore and disease resistance in females (ZC Xia, unpublished data).

To our best knowledge, no previous studies have explored sexual differences in root phenolic profiles and their associations with root-related microbes in nature. Generally, males and females show plasticity in the secondary metabolism and root microbiome, which could be adjusted according to external environmental variation (Wei and Ashman 2018; Unc et al. 2019; Zhang et al. 2019). For instance, additions of different ratios of nitrogen (N) and phosphorus (P) were found to lead to sex-specific differences in the secondary metabolism of *P. tremula* leaves (Randriamanana et al. 2014), and drought and P deficiency contributed to the differentiation of microbial communities in *P. cathayana* females and males, thus forming sex-specific resistance in microbial communities (Xia et al. 2020b). Yet, it is poorly known, whether and how the root metabolome modulates the rhizosphere microbiome in a sex-specific way and, consequently, how dioecious plants fine-tune these complex belowground interactions.

Populus euphratica is a unique and precious forest resource in the desert area. Most studies on it have focused on the physiological characteristics and molecular mechanisms of drought and salt resistance (Li et al. 2011; Zhao et al. 2016; Yu et al. 2020), while sex specificity of its traits is largely unknown. Overall, sex specificity has been found in morphology, physiology, phenology, resistance to environmental stresses and even in the rhizosphere microbiome in many *Populus* species (Chen et al. 2016; Sobuj et al. 2018; Wu et al. 2019; Xia et al. 2020a, b). Our present paper reports here the first study on sexual dimorphism in *P. euphratica*. Two *P. euphratica* plantations with different soil properties were selected to investigate the root phenolic metabolomes and rhizosphere bacterial communities of female and male plants. We aimed to test the following three hypotheses: (1) differences in soil properties can affect phenolic metabolic profiles and corresponding rhizosphere bacterial communities; (2) there are sexual differences in phenolic metabolomes and rhizosphere bacterial communities; (3) certain phenolic compounds may drive changes in specific bacteria taxa.

Materials and Methods

Field experiment

The experiment was conducted at the northwestern margin of the Tarim Basin in the Xinjiang province, China. The experimental sites have calcic xerosol soil. The climate is a typical temperate desert climate with a mean annual air temperature of 10.8 °C and precipitation of 50 mm.

The experiment was established on two *P. euphratica* plantation sites with two genders. There were four treatment combinations, with four replicates per treatment. The plantations are located in “Dongmen (DM)” (81°17'E, 40°32'N) and “Jiutuan (JT)” (81°17'E, 40°56'N). The selected field sites differ in soil properties. *P. euphratica* individuals were planted as vegetation restoration trees in 2003. Each replicated plot had an area of 100 m^2 and row spacing of 1.2 m × 4.2 m. During the flowering period, we marked the sex of each tree based on the morphology of flowers. Five *P. euphratica* females or males were selected within each plot in DM and JT (Fig. S1). We sampled fine roots (average diameter < 2 mm) from each site during July 2019. After root excavation, we separated rhizosphere soil (soil adhering to fine roots) from bulk soil (non-adhering soil). The five samples (root or rhizosphere soil) of each sex from each plot resulted in a mixed sample that was considered as a replicate. Bulk soil samples were sieved to pass a 2 mm mesh for measuring the physiochemical properties of soil.

155 The physiochemical analyses were conducted as described in Appendix.

156

157 Root samples for the phenolic metabolic analysis were immediately frozen on dry ice,
158 after which they were transferred to a -80 °C freezer. Each sample was ground to a fine
159 powder in liquid nitrogen and freeze-dried. Rhizosphere soil samples were frozen at -
160 80 °C until processed for DNA extractions.

161

162 *Phenolic metabolome analysis of roots*

163

164 In brief, the metabolome of sixteen samples (four biological replicates per treatment)
165 were analyzed by LC-MS (Ultimate 3000LC, Q Exactive, Thermo) with Hyper gold
166 C18 column (2.1 × 100 mm × 1.9 μm) kept at 40 °C. For the metabolomics analysis, 50
167 mg of freeze-dried sample was extracted with 800 μl of 80% methanol and 5 μl of
168 internal standard (2.8 mg ml^{-1}, DL-*o*-chlorophenylalanine), and then vortexed for 30 s.
169 Each sample was ground to fine powder using a grinding mill (ShangHai, JingXin)
170 operated at 30 Hz for 120 s. Then, the samples were ultrasonicated for 15 min with 40
171 KHz with ultrasonic cleaner and kept for 1 hour at -20 °C. The samples were centrifuged
172 at 12000 ×g and 4 °C for 10 min. Supernatants were transferred to 1.5 ml Eppendorf
173 tubes, and kept for 1 hour at -20 °C, and transferred to a vial for LC-MS analysis. The
174 elution was carried out with a binary solvent system consisting of 5% acetonitrile and
175 0.1% formic acid in H_2O (solvent A), and 0.1% formic acid in acetonitrile (solvent B)
176 at a constant flow rate of 0.3 ml min^{-1}. Simultaneous separations were completed using

177 a gradient elution of 0.0 min/100% A, 1.5 min/80% A, 9.5 min/100% B, 14.5 min/100%
178 B, 14.6 min/100% A, and 18 min/100% A. The typical conditions (ESI+ and ESI-) were
179 as follows: heater temperature of 300 °C; sheath gas flow rate of 45 arbitrary units; aux
180 gas flow rate of 15 arbitrary units; sweep gas flow rate of 1 arbitrary unit; spray voltage
181 of 3.0 KV; capillary temperature of 350 °C; S-Lens RF level of 30%. The Compound
182 Discoverer software version 3.0. (Thermo) was used to extract and process the LC/MS
183 detection data and organize them into a two-dimensional data matrix. According to the
184 mass value obtained from the test results, the phenolic compounds were identified by
185 full spectrum identification based on the network database Metlin
186 (https://metlin.scripps.edu).
187
188 *Bacterial microbiome analysis*
189
190 Soil DNA extraction was performed from 0.5 g frozen soil samples with GenElute™
191 Soil DNA Isolation Kit (Sigma-Aldrich), as described by Schöler et al. (2017). DNA
192 integrity and purity were detected by 1% agarose-gel electrophoresis, and DNA
193 concentration and purity were detected by NanoDropOne (Thermo Fisher, US).
194 Negative controls were carried out to confirm the absence of contamination in the used
195 kits and solutions (Vestergaard et al. 2017). For PCR amplification and product
196 electrophoresis detection, genomic DNA was used as a template. Based on the selection
197 of the sequencing region, primers with a barcode and PremixTaq (TaKaRa) were used
198 in PCR amplifications. In detail, the V4-V5 region of the bacterial 16s rDNA was

amplified by PCR with primers 515F (5'- GTGCCAGCMGCCGCGGTAA-3') and 907R (5'- CCGTCAATTCMTTTRAGTTT-3') (Caporaso et al. 2012; Li et al. 2014). After comparing the concentrations of PCR products with Genetools Analysis Software (version 4.03.05.0, Syngene), the required volume of each sample was calculated according to the principle of equal mass, and the PCR products were mixed. The PCR mixture was recovered using the E.Z.N.A.®Gel Extraction Kit (Omega), and the target DNA fragments were eluted with TE buffer. The subsequent database construction was carried out according to the standard process of NEBNext®Ultra TMDNA Library Prep Kit for Illumina®, and sequencing was conducted on the high-throughput sequencing platform Hiseq. The original image data file obtained by sequencing was transformed into raw sequences (Raw Reads) by base calling analysis. The results were stored in the fastq file format, which contained the sequences (reads) and corresponding sequencing quality information.

Before further analyses, the original PE reads obtained by sequencing were cut and filtered to remove the low-quality reads to obtain the clean reads. Using the UPARSE software (version 7.1, http://drive5.com/uparse/), OTU clustering was carried out on the sequences according to 97% similarity, and single sequences and chimeras were removed in the clustering process. The number of reads per sample was rarefied to 30000. RDP classifier (http://rdp.cme.msu.edu/) was employed to annotate the species classification of each sequence, and representative sequences were assigned by comparing to Silva database (ssu123), with the comparison threshold being 70%, in

order to obtain the classification information of bacterial communities. Bacteria were classified by phylum and family.

The analysis of the composition of bacterial communities was performed to calculate the relative abundance of each taxon by counting the sequence information at the phylum and family level. At the same time, the taxa with relative abundances more than 0.01% were selected and the top 15 categories were classified to draw the relative abundance distribution map.

Data analysis

Differences in the physiochemical properties of soil between two *P. euphratica* plantations were identified by independent samples t-test. A permutational multivariate analysis of variance (PERMANOVA) was applied to the physiochemical properties of soil to show differences among soil samples. To visualize the separation between root phenolic metabolomes in different treatments, an ordination using non-metric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix was used. Then, we ran a two-way PERMANOVA with ‘sex’ and ‘soil properties’ as fixed factors. For this model, we tested variation in root phenolic metabolomes as explained by sex, soil properties and interactions. The R language Pheatmap package was used to cluster samples (the ratio of the phenolic concentration of each group was clustered) using Euclidean Distances. A two-way ANOVA model performed with soil properties

and sex were used for the analysis of root phenolic alpha-diversity and evenness (Huberty et al. 2020).

Bray-Curtis dissimilarities were calculated and implemented in NMDS to visualize bacterial community differences between sexes and soil properties. A PERMANOVA model was also implemented to discern the amount of variation attributed to soil properties, sex and interactions. For microbial taxon abundances, raw counts of bacterial phyla and families were normalized. Two-way ANOVAs were used to analyze how soil properties, sex and their interactions influence taxon abundances. The R language Pheatmap package was used to cluster samples (the ratio of bacterial family of each group was clustered) using Euclidean Distances. A two-way ANOVA was performed to reveal microbial alpha-diversity and evenness by using soil properties and sex as explanatory variables.

A multiple regression model with a stepwise selection and Akaike's Information Criterion (AIC) minimization approach was performed to determine, whether the phenolic metabolome profiles of roots correlate with soil bacterial OTUs. We screened crucial root phenolic variables to improve the prediction of specific bacterial taxa.

Results

Physicochemical properties of soil in situ

There were significant differences in total phenolic and nutrient contents, except for soil pH, K and available P, between DM and JT (Table 1). The DM soil showed a relatively high fertility, while JT was extremely low in soil fertility. The results of PERMANOVA clearly distinguished the soil properties of *P. euphratica* plantations DM and JT (F=485, p=0.03).

Phenolic metabolome of roots

We conducted a non-metric multidimensional scaling (NMDS) for DFR, DMR, JFR and JMR (Fig. 1a). In this analysis, two axes were obtained separately with a stress value 0.014. The separation of the metabolic profiles between DFR and DMR, JFR and JMR, DFR and JFR, and DMR and JMR was clear, indicating significant differences in phenolic metabolic profiles (Fig. 1a**).** Two-way PERMANOVA revealed that the soil effect explained 69.1% of the variation in the root metabolome (F = 50.78, P= 0.001), and sex and its overlap with soil also had significant effects (Fig. 1b).

A total of 68 phenolic metabolites were detected. Then, four sets of comparisons were made to distinguish between soil effects (DFR/JFR and DMR/JMR) and sex effects (DFR/DMR and JFR/JMR), as marked in red or blue color corresponding to up-

regulation or down-regulation, respectively, in Fig. 2a. Four phenolic metabolites were down-regulated by “soil effect” but up-regulated by “sex effect”, the latter showing a positive effect on soil-inhibited metabolites. Conversely, 24 phenolic metabolites were up-regulated by “soil effect” but down-regulated by “sex effect”, the latter showing a negative effect on soil-induced metabolites (Fig. 2b).

Composition of bacterial communities

Bacterial β-diversity patterns were visualized with NMDS plots with the stress value 0.02 (Fig. 3a). The horizontal axis clearly distinguished the bacterial community patterns of DM and JT. In addition, the vertical axis differentiated the bacterial community patterns of females and males in JT. Although the difference between the bacterial community composition of females and males in DM was not evident, it could still be seen that sex ($F=3.63$, $p=0.01$) and sex × soil interactions ($F=4.27$, $p=0.02$) play a moderate, yet significant, role in explaining the bacterial β-diversity. The soil effect explained the bacterial community composition to a good strength as well.

The sequences were analyzed by the RDP Classifier algorithm against the SILVA 16S rRNA database using a confidence threshold 80%. The taxonomic composition of the bacterial communities at the phylum and family level (relative abundance > 0.01%) are shown in Fig 4a-d. Based on the classifiable sequences, the bacterial reads were mostly assigned to 14 phyla and 13 families. The abundance of the 14 phyla were in the

following order: *Proteobacteria*, *Bacteroidetes*, *Euryarchaeota*, *Actinobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Thaumarchaeota*, *Parcubacteria*, *Verrucomicrobia*, *Nanohaloarchaeota*, *Firmicutes* and *JL-ETNP-Z39*. Almost all bacterial phyla differed between soil types, except *Actinobacteria*, *Parcubacteria* and *Firmicutes*, while only *Proteobacteria* and *Firmicutes* differed between sexes (Fig. 4a, c). The DM soil had significantly lower abundances of *Bacteroidetes* and *Euryarchaeota*, but higher abundances of *Planctomycetes*, *Gemmatimonadetes*, *Chloroflexi* and *Acidobacteria* (Fig. 4a). The 13 families were in the following order: *Halobacteriaceae*, *Halomonadaceae*, *Planctomycetaceae*, *OM1_clade*, *Nitrosomonadaceae*, *Rhodospirillaceae*, *Alteromonadaceae*, *Anaerolineaceae*, *Cytophagaceae*, *Xanthomonadaceae*, *Cellvibrionaceae*, *Rhodobacteraceae* and *Gemmatimonadaceae*. All 13 dominant bacterial families differed in abundance between soil types, while *Halomonadaceae*, *OM1_clade*, *Cellvibrionaceae* and *Rhodobacteraceae* differed between sexes (Fig. 4b, d). The JT soil had significantly greater abundances of *Halobacteriaceae* and *Halomonadaceae*, but lower abundances of *Planctomycetaceae* and *Nitrosomonadaceae* (Fig. 4b). On the other hand, the female rhizosphere had a lower abundance of *Rhodobacteraceae* *Halomonadaceae*, *Cellvibrionaceae* and *OM1_clade* than the male rhizosphere (Fig. 4d). A total of 7 known bacterial families were up-regulated by “soil effect” but down-regulated by “sex effect”. Only one family, *Halobacteriaceae,* was down-regulated by “soil effect” but up-regulated by “sex effect” (Fig. S2).

Phenolic alpha diversity of roots and microbial alpha diversity

Phenolic Shannon diversity and evenness were significantly influenced by sex, but not by soil or soil × sex interactions (Table 2). Phenolic diversity and evenness were greater in females than in males (Fig. 5a-b). Bacterial Shannon diversity and evenness differed between DM and JT, but were not affected by sex or soil × sex interactions. Bacterial diversity and evenness were greater in DM than in JT.

Relations between root phenolic metabolites and specific bacterial taxa

Abundances of all bacterial phyla correlated with at least one phenolic metabolite (Table 3). Abundances of *Actinobacteria, Acidobacteria, Parcubacteria* and *Firmicutes* correlated with homodihydrocapsaicin-I (Table 3). Abundances of *Bacteroidetes, Planctomycetes, Chloroflexi* and *JL-ETNP-Z39* correlated with (-)-fustin. These phyla increased in abundance with increasing (-)-fustin concentrations, except abundances of *Bacteroidetes* that exhibited a negative correlation with (-)-fustin (Table 3). In addition, abundances of *Actinobacteria, Planctomycetes* and *Acidobacteria* correlated positively with penbutolol, abundances of *Euryarchaeota* and *Nanohaloarchaeota* with 4-coumaric acid, and abundances of *Acidobacteria* and *Verrucomicrobia* with catechin, while abundances of *Planctomycetes* and *Parcubacteria* correlated negatively with caffeic acid (Table 3). In addition, abundances of all bacterial families correlated with at least one phenolic metabolite (Table S1). Abundances of several bacterial families

correlated positively with abyssinone I (*Anaerolineaceae, Xanthomonadaceae* and *Gemmatimonadaceae*; Table S1). In addition, abundances of these families as well as *Planctomycetaceae* correlated positively with homodihydrocapsaicin-I (Table S1). Abundances of both *Nitrosomonadaceae* and *Cytophagaceae* correlated positively with salicylic acid (Table S1). Moreover, abundances of *Halobacteriaceae, OM1_clade* and *Anaerolineaceae* correlated with 4-coumaric acid (Table S1).

Discussion

In our study, we provide evidence that soil properties representing the soil nutrient status mainly affect root-associated bacterial microbiomes, followed by plant sex and, to some extent, the chemotype. There were sex-specific differences in the phenolic metabolomes of plant roots and bacterial communities, and we observed preferences for specific bacterial taxa along the phenolic metabolite gradients in the rhizosphere.

Effects of soil properties on root phenolic metabolic profiles and rhizosphere bacterial microbiomes

Compared with the sex effect, changes in soil properties visible in the soil nutrient status largely explained variation in root phenolic metabolism profiles and rhizosphere bacterial communities. The production and accumulation of phenolic compounds always shows plasticity under different environmental conditions (Cheynier et al. 2013; Sampaio et al. 2016). The phenolic profiles of six wild growing *Marrubium vulgare* L. (Lamiaceae) populations have demonstrated remarkably significant quantitative variation in responses to variation in soil properties (Boulila et al. 2015). In addition, eight *Salix* species/hybrids collected from two sites with different soil conditions showed differences in the leaf phenolic composition (Gąsecka et al. 2017). Differences in soil resources would result in unequal allocation between growth and reproduction, and production of defense compounds, thus regulating the carbon flux between primary and secondary metabolism to obtain better adaptation to environmental stress. We

discovered that the rhizosphere bacterial communities of *P. euphratica* were affected by soil properties. Liu et al. (2020) have found that soil properties have a greater effect on bacterial communities than plant species identity. Yet, our two study sites were ten kilometers apart, and had similar soil phylogenetic distances and planting history. There were minor differences in the microbe pools caused by different soil origins, but the difference was mostly due to the nutrient status. There is no doubt that the nutrient status of DM was better than that of JT. This may be mainly due to the artificial irrigation of DM during the early growing seasons, which left a longer lasting effect on the soil nutrient status. JT tended to have just a small amount of natural rainfall. Evidently, the difference in the soil nutrient status caused by soil water greatly influenced the bacterial communities of *P. euphratica*.

Sex-specific effects on root phenolic metabolomes and bacterial communities

Male and female plants show dimorphism after a period of sexual differentiation (Barrett and Hough 2013). Bioactivities of certain metabolites are involved in morphogenesis and sex determination (Di Carlo et al. 1999), and metabolites vary between the sexes (Cornelissen and Stiling 2005). Previous studies have shown that females have greater investment costs on chemical defense than males (Juvany and Munné-Bosch 2015; Hultine et al. 2016). However, our study found that chemical defense was not only reflected in quantity, but also in the higher diversity and evenness of chemical compounds in females. We propose that future studies are needed to

evaluate the chemical defense of males and females from a multi-dimensional perspective.

It is evident that sexual differentiation is also regulated by genes (Li et al. 2020), but studies on sex-specific differences in the microbiomes of dioecious plants have been mostly neglected. Yet, Wei et al. (2018) have detected strong sexual dimorphism in flower and leaf microbiomes in sexually polymorphic *Fragaria*. In addition, microbial communities in the rhizosphere of *P. cathayana* change along with variation in soil salinity, and potential differences in the microbial communities exist between males and females (Wu et al. 2019). However, sex has only a minor impact on the bacterial diversity of the dioecious desert plant *Acanthosicyos horridus* (Unc et al. 2019). Our results on the dioecious *P. euphratica* supported the view of sex-specific variation in bacterial microbiomes. Abundances of *Rhodobacteraceae*, *Halomonadaceae*, *Cellvibrionaceae* and *OM1_clade* were significantly higher in the male rhizosphere compared with the female rhizosphere. Members of *Rhodobacteraceae* and *Halomonadaceae* show a denitrification effect, which can enhance adaptation to extreme soil environments (Garcia et al. 2004; Wang et al. 2017).

Another interesting contrast was that sex-specific differences in bacterial taxa varied along with soil properties. Compared with JT, females enriched abundances of rhizosphere *OM1_clade* and *Nitrosomonadaceae* in DM, while males increased abundances of several other bacterial taxa, such as *Planctomycetaceae*,

Xanthomonadaceae, *Cytophagaceae* and *Gemmatimonadaceae*. Indeed, females are more likely to form a fertility island effect in areas with high nutrient status, because some long lived dioecious plants produce nectar drops from their cones to attract pollinating insects, which could affect the soil biota underneath (Hultine et al. 2016). On the other hand, a male-biased sex ratio is often observed in dioecious plant populations, which may be related to environmental stress, as more male than female plants occur on sites with poor fertility (Ortiz et al. 2002; Song et al. 2018). Xia et al. (2020b) have found that the rhizosphere microbiome of *P. cathayana* males is more plastic, which makes it easier to accumulate plant growth -promoting bacteria in adverse conditions. Plant growth is considered to be closely associated with coexisting microbes (Bulgarelli et al. 2015; Sarr et al. 2020). These results indicate that there are differences between sexes in plant protection strategies mediated by microbes. In future studies, we should pay more attention to the relations between the regulation of the rhizosphere microbiome and adaptive plasticity of male and female plants.

Associations between phenolic compounds and specific bacterial taxa

Although the soil effect is the main driver of the rhizosphere microbiome (Guo et al. 2019; Veach et al. 2019), the root phenolic metabolome regulates bacterial colonization and assembly to a certain level. Furthermore, we found that the majority of bacterial taxa correlated with at least one phenolic metabolite. Previously, Lebeis et al. (2015) have showed that *Arabidopsis thaliana* mutants lacking SA signals present significant

changes in the endophytic bacterial communities of roots, including significant decreases in abundances of *Firmicides* and *Proteobacteria*. In our study, we found that the content of homodihydrocapsaicin-I was positively correlated with the abundance of *Firmicides.* In the case of pathogen infections, plants can gain resistance to phytopathogens by regulating the SA signaling pathway (Loake and Grant 2007). Similarly to other studies, we found that abundances of specific *Nitrosomonadaceae* and *Cytophagaceae* respond positively to SA production.

Complex microbial communities in natural settings may respond differentially to diverse phenolic metabolites. Some phenolic compounds may act as metal chelators in the rhizosphere, affect microbial metabolism and influence relevant enzyme activities (Bais et al. 2006). Voges et al. (2019) have demonstrated that coumarin, as an iron-mobilizing compound, functions in the structuring of the rhizobiome and its release could inhibit the growth of *Pseudomonas*. We observed negative correlations between phenolic compounds and abundances of specific bacterial taxa (between caffeic acid and abundances of *Planctomycetes* or *Parcubacteria*; between (-)-fustin and abundances of *Bacteroidete*). Some other phenolic compounds can also be used as antimicrobial allelochemicals (Lanoue et al. 2010), thus directly suppressing microorganisms *via* antibiosis (Zwetsloot et al. 2020). Zhou et al. (2018) have found that p-coumaric acid inhibits the relative abundances of *Lysobacter*, *Haliangium* and *Gymnoascus* spp., which could have pathogen-antagonistic and/or plant-growth -promoting effects. In conclusion, further evidence is needed to verify the associations

between specific phenolic compounds and rhizosphere bacterial taxa through controlled experiments.

Conclusions

Plant-soil-microbe interactions and the role of phenolic production are largely unknown in relation to rhizosphere microbiomes. This study confirms the relative importance of the external environment and plant itself for the assembly of soil microbiomes and evaluates the contribution of sex and chemotype. Our results indicate that rhizosphere bacterial community composition is mainly determined by soil properties followed by the plant sex effect. Specifically, phenolic metabolic profiles and specific phenolic metabolites affect the assembly of bacterial communities and specific bacterial taxa. The use of *P. euphratica* as a soil restoration tree species in desert areas not only needs to consider the influence of soil properties on bacterial communities, but also needs to consider the sex identity of *P. euphratica*. Our results provide key information for future studies on plant metabolomes and soil microbiomes, and on biotic interactions.

Acknowledgements The authors thank Juntuan Zhai for assistance in the field work. We also thank Lin Zhang and Tianle Xu for assistance in data analysis. This work was supported by the Natural Science Foundation of China (U1803231) and the Talent Program of the Hangzhou Normal University (2016QDL020).

Author contributions Zhichao Xia had the main responsibility for data collection, analysis and writing, Yue He, Lei Yu and Zhijun Li performed the experiment, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

Conflict of interest The authors declare that they have no conflict of interest.

References

Avila-Sakar G, Romanow CA (2012) Divergence in defence against herbivores between males and females of dioecious plant species. Int J Biol Sc 2012:897157

Badri DV, Chaparro JM, Zhang R, Shen Q, Vivanco JM (2013) Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J Biol Chem 288:4502-4512

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233-266

Barbehenn RV, Peter Constabel C (2011) Tannins in plant-herbivore interactions. Phytochemistry 72:1551-1565

Barrett SC, Hough J (2013) Sexual dimorphism in flowering plants. J Exp Bot 64:67-82

Boeckler GA, Gershenzon J, Unsicker SB (2011) Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. Phytochemistry 72:1497-1509

Boulila A, Sanaa A, Salem IB, Rokbeni N, M'Rabet Y, Hosni K, Fernandez X (2015) Antioxidant properties and phenolic variation in wild populations of *Marrubium vulgare* L. (Lamiaceae). Ind Crop Prod 76:616-622

Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC, Schulze-Lefert P (2015) Structure and function of the bacterial root microbiota in wild and domesticated Barley. Cell Host Microbe 17:392-403

551 Caporaso J, Lauber C, Walters W. et al. (2012) Ultra-high-throughput microbial
552 community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621-
553 1624
554 Chen L, Zhang D, Yang W, Liu Y, Zhang L, Gao S (2016) Sex-specific responses of
555 *Populus deltoides* to *Glomus intraradices* colonization and Cd pollution.
556 Chemosphere 155:196-206
557 Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S (2013) Plant phenolics:
558 recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol
559 Biochem 72:1-20
560 Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome:
561 ecology, functions, and emerging trends in microbial application. J Adv Res 19:29-
562 37
563 Cornelissen T, Stiling P (2005) Sex-biased herbivory: a meta-analysis of the effects of
564 gender on plant-herbivore interactions. Oikos 111:488-500
565 de Graaff MA, Classen AT, Castro HF, Schadt CW (2010) Labile soil carbon inputs
566 mediate the soil microbial community composition and plant residue
567 decomposition rates. New Phytol 188:1055-1064
568 de la Fuente CC, Simonin M, King E, Moulin L, Bennett MJ, Castrillo G, Laplaze L
569 (2020) An extended root phenotype: the rhizosphere, its formation and impacts on
570 plant fitness. Plant J 103:951-964
571 Di Carlo G, Mascolo N, Izzo AA, Capasso F (1999) Flavonoids: old and new aspects
572 of a class of natural therapeutic drugs. Life Sci 65:337-353

García MT, Mellado E, Ostos JC, Ventosa A (2004) *Halomonas organivorans* sp. nov. a moderate halophile able to degrade aromatic compounds. Int J Syst Evol Micr 54:1723-1728

Gąsecka M, Mleczek M, Jutrzenka A, Goliński P, Stuper-Szablewska K (2017) Phenolic compounds in leaves of *Salix* species and hybrids growing under different soil conditions. Chem Ecol 33:196-212

Gu Y, Wang X, Yang T, Friman VP, Geisen S, Wei Z, Xu Y, Jousset A, Shen Q (2020) Chemical structure predicts the effect of plant-derived low molecular weight compounds on soil microbiome structure and pathogen suppression. Funct Ecol 34:2158-2169

Guo Q, Yan L, Korpelainen H, Niinemets Ü, Li C (2019) Plant-plant interactions and N fertilization shape soil bacterial and fungal communities. Soil Biol Biochem 128:127-138

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117-152

Huberty M, Choi YH, Heinen R, Bezemer TM (2020) Above-ground plant metabolomic responses to plant-soil feedbacks and herbivory. J Ecol 108:1703-1712

Hultine KR, Grady KC, Wood TE, Shuster SM, Stella JC, Whitham TG (2016) Climate change perils for dioecious plant species. Nat Plants 2:1-8

Juvany M, Munné-Bosch S (2015) Sex-related differences in stress tolerance in dioecious plants: a critical appraisal in a physiological context. J Exp Bot 66:6083-

595 6092
596 Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse US (2010) De novo
597 biosynthesis of defense root exudates in response to *Fusarium* attack in barley.
598 New Phytol 185:577-588
599 Lareen A, Burton F, Schafer P (2016) Plant root-microbe communication in shaping
600 root microbiomes. Plant Mol Biol 90:575-587
601 Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, ... Dangl
602 JL (2015) Salicylic acid modulates colonization of the root microbiome by specific
603 bacterial taxa. Science 349:860-864
604 Li B, Qin Y, Duan H, Yin W, Xia X (2011) Genome-wide characterization of new and
605 drought stress responsive microRNAs in *Populus euphratica*. J Exp Bot 62:3765-
606 3779
607 Li W, Wu H, Li X, Chen Y, Yin T (2020) Fine mapping of the sex locus in *Salix triandra*
608 confirms a consistent sex determination mechanism in genus *Salix*. Hortic Res
609 7:64
610 Li XZ, Rui JP, Mao YJ, Yannarell A, Mackie R (2014) Dynamics of the bacterial
611 community structure in the rhizosphere of a maize cultivar. Soil Biol Biochem
612 68:392-401
613 Liu L, Huang X, Zhang J, Cai Z, Jiang K, Chang Y (2020) Deciphering the relative
614 importance of soil and plant traits on the development of rhizosphere microbial
615 communities. Soil Biol Biochem 148:107909
616 Loake G, Grant M (2007) Salicylic acid in plant defence - the players and protagonists.

617 Curr Opin Plant Biol 10:466-72
618 Nissinen K, Virjamo V, Mehtatalo L, Lavola A, Valtonen A, Nybakken L, Julkunen-
619 Tiitto R (2018) A seven-year study of phenolic concentrations of the dioecious
620 *Salix myrsinifolia*. J Chem Ecol 44:416-430
621 Obeso JR (2002) The costs of reproduction in plants. New Phytol 155:321-348
622 Ortiz PL, Arista M, Talavera S (2002) Sex ratio and reproductive effort in the dioecious
623 *Juniperus communis* subsp. alpina (Suter) Čelak. (Cupressaceae) along an
624 altitudinal gradient. Ann Bot 89:205-211
625 Paterson E, Gebbing T, Abel C, Sim A, Telfer G (2007) Rhizodeposition shapes
626 rhizosphere microbial community structure in organic soil. New Phytol 173:600-
627 610
628 Randriamanana TR, Nybakken L, Lavola A, Aphalo PJ, Nissinen K, Julkunen-Tiitto R
629 (2014) Sex-related differences in growth and carbon allocation to defence in
630 *Populus tremula* as explained by current plant defence theories. Tree Physiol
631 34:471-487
632 Rozas V, Le Quesne C, Rojas-Badilla M, González-Reyes Á, Donoso S, Olano JM
633 (2019) Climatic cues for secondary growth and cone production are sex-dependent
634 in the long-lived dioecious conifer *Araucaria araucana*. Agric Forest Meteorol
635 274:132-143
636 Sampaio BL, Edrada-Ebel R, Da Costa FB (2016) Effect of the environment on the
637 secondary metabolic profile of *Tithonia diversifolia*: a model for environmental
638 metabolomics of plants. Sci Rep 6:29265

639 Sarr PS, Ando Y, Nakamura S, Deshpande S, Subbarao GV (2020) Sorgoleone release
640 from sorghum roots shapes the composition of nitrifying populations, total bacteria,
641 and archaea and determines the level of nitrification. Biol Fertil Soils 56:145-166
642 Shi S, Richardson AE, O'Callaghan M, DeAngelis KM, Jones EE, Stewart A, Firestone
643 MK, Condron LM (2011) Effects of selected root exudate components on soil
644 bacterial communities. FEMS Microbiol Ecol 77:600-610
645 Sobuj N, Virjamo V, Zhang Y, Nybakken L, Julkunen-Tiitto R (2018) Impacts of
646 elevated temperature and CO_2 concentration on growth and phenolics in the
647 sexually dimorphic *Populus tremula* (L.). Environ Exp Bot 146:34-44
648 Song H, Lei Y, Zhang S (2018) Differences in resistance to nitrogen and phosphorus
649 deficiencies explain male-biased populations of poplar in nutrient-deficient
650 habitats. J Proteomics 178:123-127
651 Unc A, Maggs-Kölling G, Marais E, Sherman C, Doniger T, Steinberger Y (2019) Soil
652 bacterial community associated with the dioecious *Acanthosicyos horridus* in the
653 Namib Desert. Biol Fert Soils 55:393-403
654 Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, Tschaplinski TJ,
655 Schadt CW (2019) Rhizosphere microbiomes diverge among *Populus trichocarpa*
656 plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome
657 7:76
658 Voges MJ, Bai Y, Schulze-Lefert P, Sattely ES (2019) Plant-derived coumarins shape
659 the composition of an *Arabidopsis* synthetic root microbiome. Proc Natl Acad Sci
660 USA 116:12558-12565

661 Wagner MR, Lundberg DS, Del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T (2016)
662 Host genotype and age shape the leaf and root microbiomes of a wild perennial
663 plant. Nat Commun 7:12151
664 Wang X, Zheng Q, Yuan Y, Hai R, Zou D (2017) Bacterial community and molecular
665 ecological network in response to Cr_2O_3 nanoparticles in activated sludge system.
666 Chemosphere 188:10-17
667 Wei N, Ashman TL (2018) The effects of host species and sexual dimorphism differ
668 among root, leaf and flower microbiomes of wild strawberries in situ. Sci Rep
669 8:5195
670 Wu N, Li Z, Wu F, Tang M (2019) Microenvironment and microbial community in the
671 rhizosphere of dioecious *Populus cathayana* at Chaka Salt Lake. J Soil Sediment
672 19:2740-2751
673 Vestergaard G, Schulz S, Schöler A, Schloter M (2017) Making big data smart-how to
674 use metagenomics to understand soil quality. Biol Fertil Soils 53:479-484
675 Xia Z, He Y, Yu L, Lv R, Korpelainen H, Li C (2020a) Sex-specific strategies of
676 phosphorus (P) acquisition in *Populus cathayana* as affected by soil P availability
677 and distribution. New Phytol 225:782-792
678 Xia Z, He Y, Zhou B, Korpelainen H, Li C (2020b) Sex-related responses in rhizosphere
679 processes of dioecious *Populus cathayana* exposed to drought and low phosphorus
680 stress. Environ Exp Bot 175:104049
681 Yu K, Pieterse CMJ, Bakker P, Berendsen RL (2019) Beneficial microbes going
682 underground of root immunity. Plant Cell Environ 42:2860-2870

683 Yu L, Dong H, Li Z, Han Z, Korpelainen H, Li C (2020) Species-specific responses to
684 drought, salinity and their interactions in *Populus euphratica* and *P. pruinosa*
685 seedlings. J Plant Ecol 13:563-573.
686 Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, Cho H, Karaoz U,
687 Loque D, Bowen BP, Firestone MK, Northen TR, Brodie EL (2018) Dynamic root
688 exudate chemistry and microbial substrate preferences drive patterns in
689 rhizosphere microbial community assembly. Nat Microbiol 3:470-480
690 Zhang S, Tang D, Korpelainen H, Li C (2019) Metabolic and physiological analyses
691 reveal that *Populus cathayana* males adopt an energy-saving strategy to cope with
692 phosphorus deficiency. Tree Physiol 39:1630-1645
693 Zhao L, Wang L, Cernusak LA, Liu X, Xiao H, Zhou M, Zhang S (2016) Significant
694 difference in hydrogen isotope composition between xylem and tissue water in
695 *Populus euphratica*. Plant Cell Environ 39:1848-1857
696 Zhou X, Zhang J, Pan D, Ge X, Jin X, Chen S, Wu F (2018) p-Coumaric can alter the
697 composition of cucumber rhizosphere microbial communities and induce negative
698 plant-microbial interactions. Biol Fertil Soils 54:363-372
699 Zwetsloot MJ, Ucros JM, Wickings K, Wilhelm RC, Sparks J, Buckley DH, Bauerle
700 TL (2020) Prevalent root-derived phenolics drive shifts in microbial community
701 composition and prime decomposition in forest soil. Soil Biol Biochem
702 145:107797

Figure legends

Figure 1 The ordination using non-metric multidimensional scaling (NMDS) of root phenolic metabolome in *P. euphratica* females or males grown in different types of soil according to a LC/MS analysis (a). Variation in root phenolic metabolome profiles explained by soil and sex, obtained as R^2 from PERMANOVA models (b). *, **, *** indicate significant effects in the PERMANOVA tests at $P< 0.05$, $P< 0.01$, $P< 0.001$ respectively. (a) DFR, female root in DM; DMR, male root in DM; JFR, female root in JT; JMR, male root in JT.

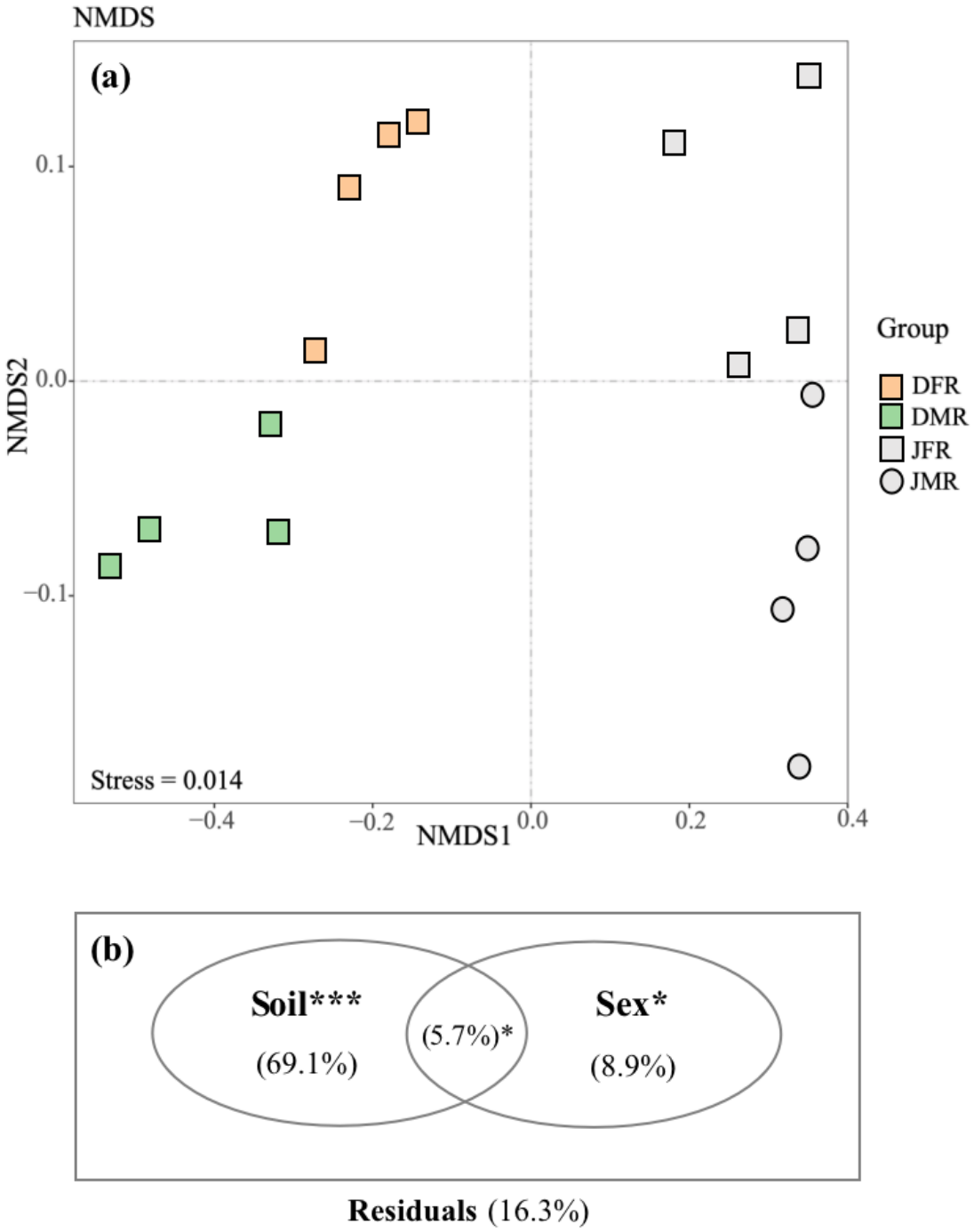
Figure 2 Hierarchical clustering of 68 root phenolic metabolites in the intersection of four comparative groups. The ratio of phenolic content of each group was clustered by Euclidean distance, and the color of each grid represents the relative abundance of phenolic substances on the corresponding scale.

Figure 3 Non-metric dimensional scaling ordination for bacteria in *P. euphratica* females or males grown in different types of soil (a). Variation in bacterial communities explained by soil and sex (b). Other treatment codes and statistical analyses as in Fig. 1.

Figure 4 The relative abundance of dominant bacterial phyla and families between soil types (a, b) and between sexes (c, d). Asterisks denote significant differences in abundances between soil types and sexes, as generated by two-way ANOVA models.

Figure 5 Phenolic diversity (Shannon's Diversity: 1-D) and Shannon's Evenness between sexes (a, b), and bacterial diversity (Shannon's Diversity: 1-D) and Shannon's Evenness between soil types (c, d).

728 **Figure 1**



729

730

731

732

733

734 **Figure 2**

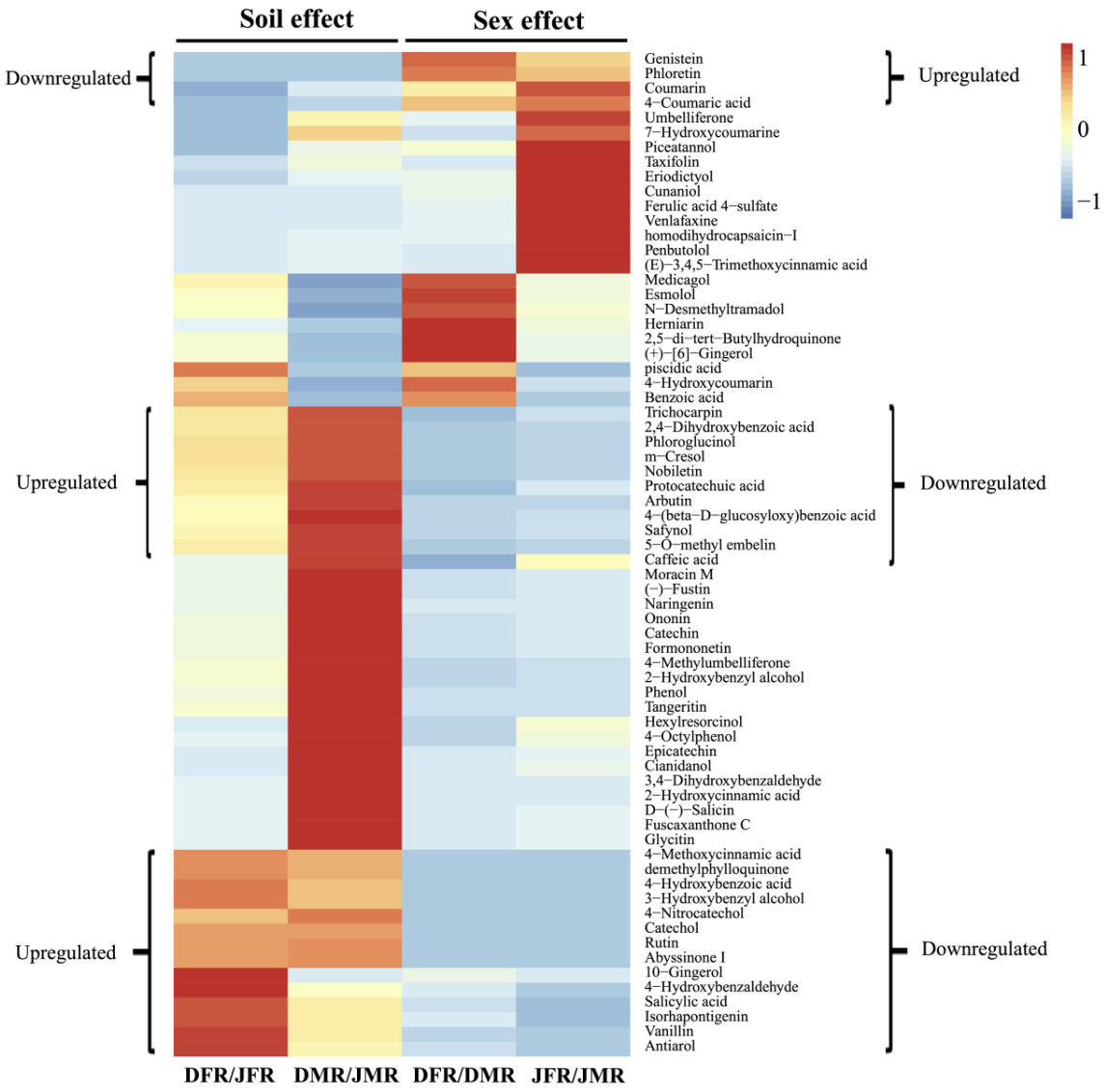
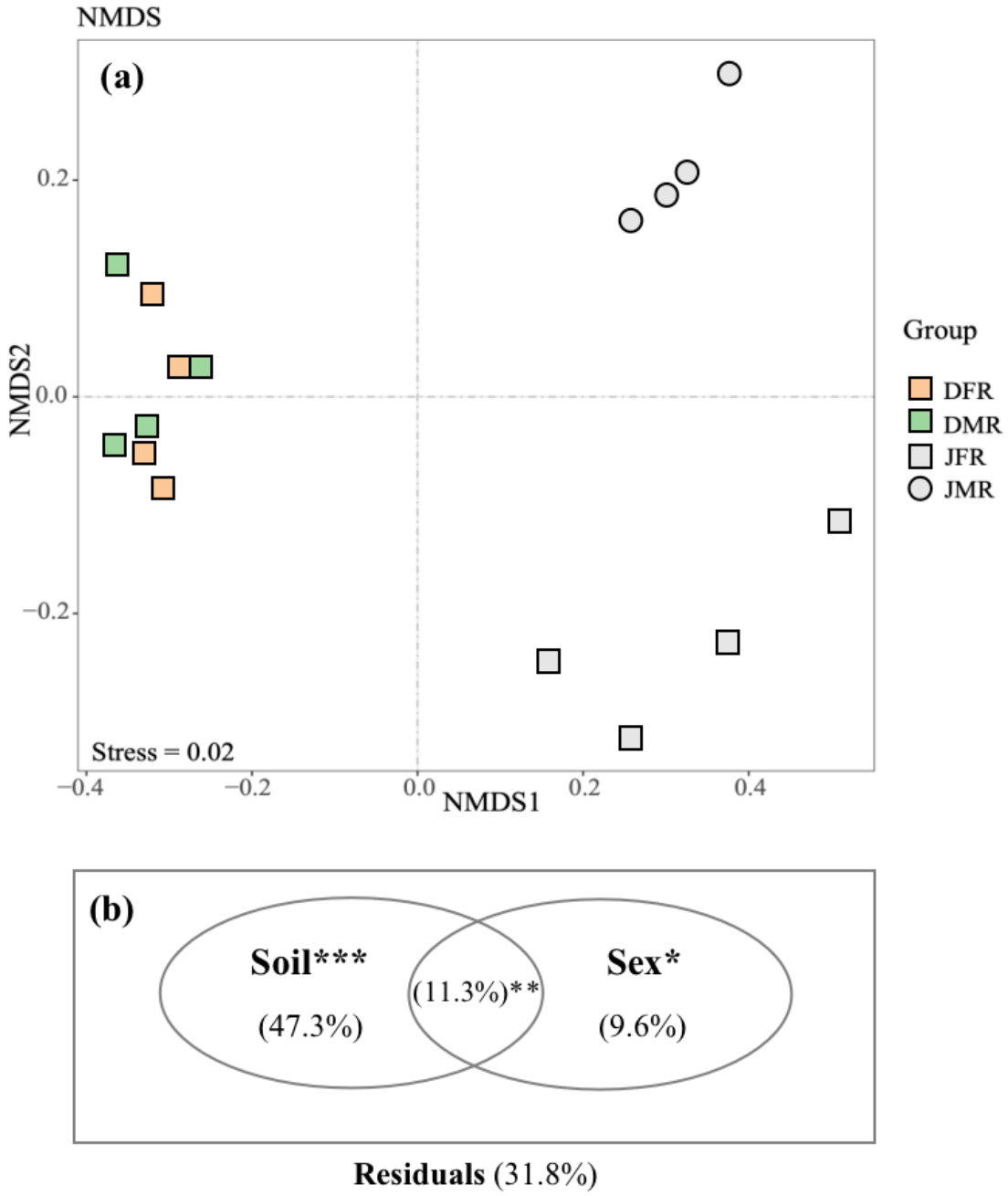

Soil effect
Sex effect
Downregulated
Upregulated
Upregulated
Downregulated
Upregulated
Downregulated
1
0
−1
Genistein
Phloretin
Coumarin
4−Coumaric acid
Umbelliferone
7−Hydroxycoumarine
Piceatannol
Taxifolin
Eriodictyol
Cunaniol
Ferulic acid 4−sulfate
Venlafaxine
homodihydrocapsaicin−I
Penbutolol
(E)−3,4,5−Trimethoxycinnamic acid
Medicagol
Esmolol
N−Desmethyltramadol
Herniarin
2,5−di−tert−Butylhydroquinone
(+)−[6]−Gingerol
piscidic acid
4−Hydroxycoumarin
Benzoic acid
Trichocarpin
2,4−Dihydroxybenzoic acid
Phloroglucinol
m−Cresol
Nobiletin
Protocatechuic acid
Arbutin
4−(beta−D−glucosyloxy)benzoic acid
Safynol
5−O−methyl embelin
Caffeic acid
Moracin M
(−)−Fustin
Naringenin
Ononin
Catechin
Formononetin
4−Methylumbelliferone
2−Hydroxybenzyl alcohol
Phenol
Tangeritin
Hexylresorcinol
4−Octylphenol
Epicatechin
Cianidanol
3,4−Dihydroxybenzaldehyde
2−Hydroxycinnamic acid
D−(−)−Salicin
Fuscaxanthone C
Glycitin
4−Methoxycinnamic acid
demethylphylloquinone
4−Hydroxybenzoic acid
3−Hydroxybenzyl alcohol
4−Nitrocatechol
Catechol
Rutin
Abyssinone I
10−Gingerol
4−Hydroxybenzaldehyde
Salicylic acid
Isorhapontigenin
Vanillin
Antiarol
DFR/JFR
DMR/JMR
DFR/DMR
JFR/JMR

Figure 3



744

745

746

747

748

749

750

751 **Figure 4**

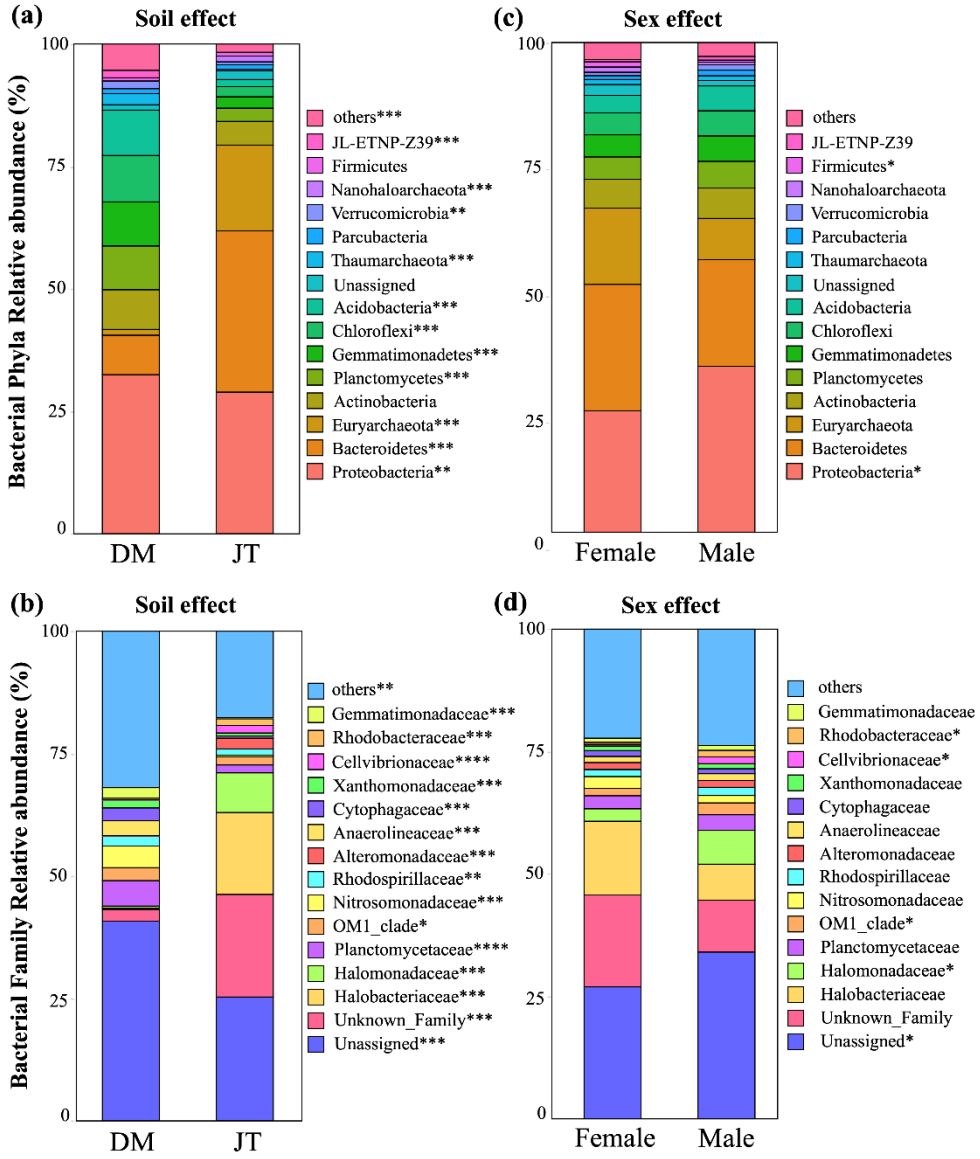
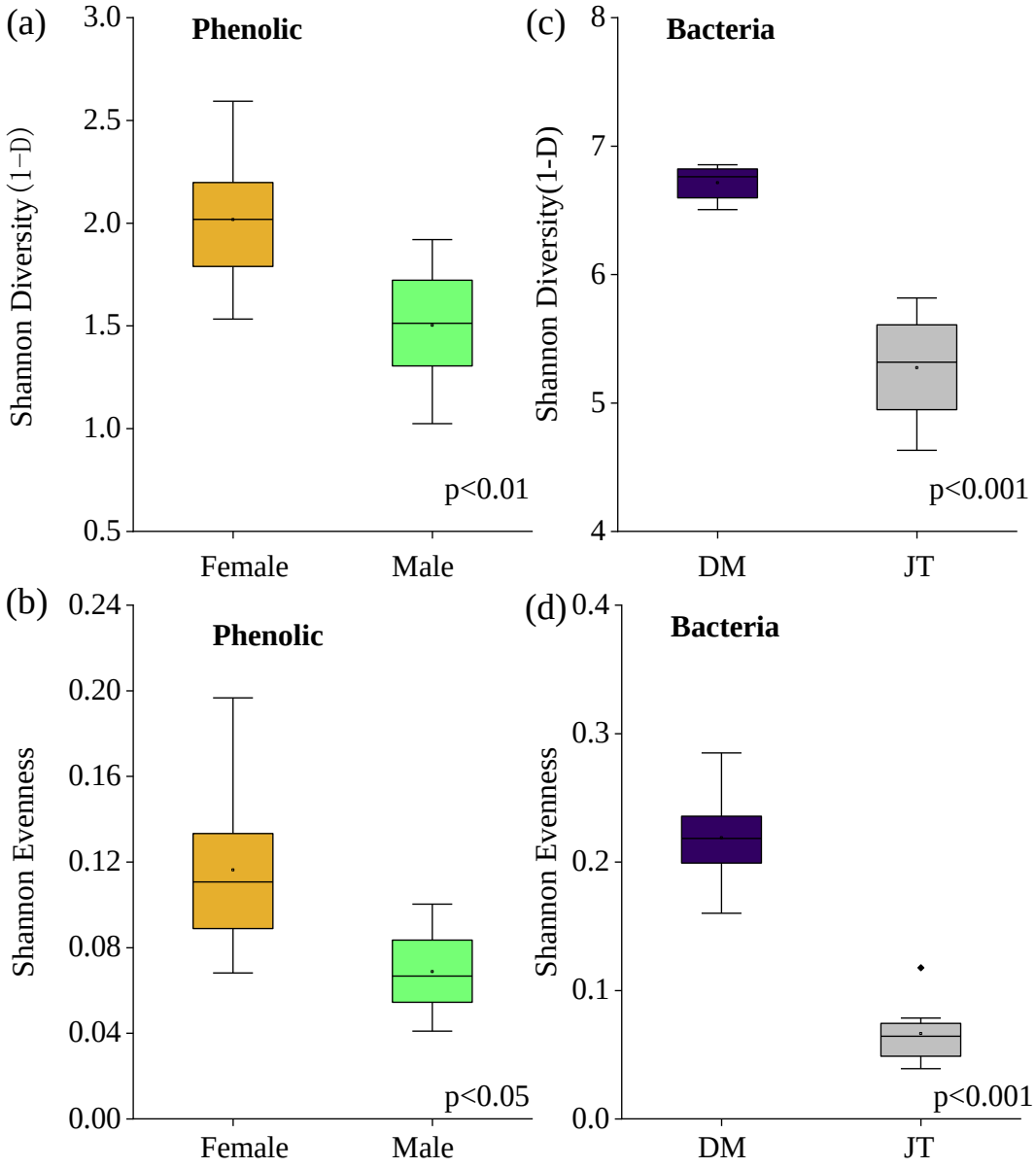

(a)
Soil effect
Bacterial Phyla Relative abundance (%)
100
75
50
25
0
DM
JT
others***
JL-ETNP-Z39***
Firmicutes
Nanohaloarchaeota***
Verrucomicrobia**
Parcubacteria
Thaumarchaeota***
Unassigned
Acidobacteria***
Chloroflexi***
Gemmatimonadetes***
Planctomycetes***
Actinobacteria
Euryarchaeota***
Bacteroidetes***
Proteobacteria**
(c)
Sex effect
Female
Male
others
JL-ETNP-Z39
Firmicutes*
Nanohaloarchaeota
Verrucomicrobia
Parcubacteria
Thaumarchaeota
Unassigned
Acidobacteria
Chloroflexi
Gemmatimonadetes
Planctomycetes
Actinobacteria
Euryarchaeota
Bacteroidetes
Proteobacteria*
(b)
Soil effect
Bacterial Family Relative abundance (%)
DM
JT
others**
Gemmatimonadaceae***
Rhodobacteraceae***
Cellvibrionaceae****
Xanthomonadaceae***
Cytophagaceae***
Anaerolineaceae***
Alteromonadaceae***
Rhodospirillaceae**
Nitrosomonadaceae***
OM1_clade*
Planctomycetaceae****
Halomonadaceae***
Halobacteriaceae***
Unknown_Family***
Unassigned***
(d)
Sex effect
Female
Male
others
Gemmatimonadaceae
Rhodobacteraceae*
Cellvibrionaceae*
Xanthomonadaceae
Cytophagaceae
Anaerolineaceae
Alteromonadaceae
Rhodospirillaceae
Nitrosomonadaceae
OM1_clade*
Planctomycetaceae
Halomonadaceae*
Halobacteriaceae
Unknown_Family
Unassigned*

Figure 5



761

762

763

764

765

766

767

768

769 **Table 1** Soil properties of the two *P. euphratica* plantations.

Soil properties	DM	JT
pH	8.44±0.01	8.68±0.09
Total N (g kg^{-1})	1.11± 0.04***	0.48 ± 0.02
Organic matter content (g kg^{-1})	120.28 ± 0.41***	72.00± 1.00
NO_3^-- N (mg kg^{-1})	3.67 ± 0.21***	2.07 ± 0.12
NH_4^-- N (mg kg^{-1})	2.16 ± 0.13***	0.38 ± 0.01
K (g kg^{-1})	0.41 ± 0.11	0.19 ± 0.03
Total P (g kg^{-1})	0.81± 0.02***	0.59 ± 0.01
Available P (mg kg^{-1})	1.24 ± 0.14	1.19 ± 0.07
Na^+ (g kg^{-1})	1.87 ± 0.05**	1.30 ± 0.10
Ca^{2+} (g kg^{-1})	15.81 ± 0.39**	12.52 ± 0.45
Mg^{2+} (g kg^{-1})	0.88 ± 0.04***	0.38 ± 0.03
Total phenolic (mg g^{-1})	14.89 ± 0.20*	12.82 ± 0.69

* Indicates significantly different values at $P < 0.05$ from t-test.

Table 2 A two-way ANOVA model summary for responses of Shannon-Wiener's Diversity and Evenness for the root phenolic composition and bacterial communities,

and explanatory variables of soil effect, sex effect and their interaction. Statistically significant explanatory variables are bolded.

Response variable	Explanatory variable	df	F-value	*p*-value
Phenolic diversity	Soil effect	1	1.25	>0.05
	Sex effect	**1**	**9.65**	**<0.01**
	Interactions	1	0.01	>0.05
Phenolic evenness	Soil effect	1	0.67	>0.05
	Sex effect	**1**	**7.74**	**<0.05**
	Interactions	1	0.02	>0.05
Bacteria diversity	**Soil effect**	**1**	**86.53**	**<0.001**
	Sex effect	1	0.32	>0.05
	Interactions	1	2.40	>0.05
Bacteria evenness	**Soil effect**	**1**	**105.70**	**<0.001**
	Sex effect	1	1.82	>0.05
	Interactions	1	1.93	>0.05

794 **Table 3** Multiple stepwise regression model results for dominant bacterial phyla that are significantly correlated with a phenolic metabolite.

Microbial phylum	Explanatory variable	T	*p*-value	Full model statistics		
				F-statistic	Adj.R^2	*p*-value
Proteobacteria	Intercept	20.78	<0.01	6.99	0.44	<0.01
	cunaniol	**-2.88**	**0.01**			
	(E)-3,4,5-trimethoxycinnamic acid	**-2.66**	**0.02**			
Bacteroidetes	Intercept	2.69	0.02	42.80	0.92	<0.01
	(-)-fustin	**-3.46**	**<0.01**			
	genistein	**6.04**	**<0.01**			
	umbelliferone	**3.60**	**<0.01**			
	piceatannol	**2.85**	**0.02**			
Euryarchaeota	Intercept	8.32	<0.01	335.32	0.99	<0.01
	4-coumaric acid	**26.82**	**<0.01**			
	ferulic acid 4-sulfate	**20.53**	**<0.01**			
	4-hydroxycoumarin	**-12.18**	**<0.01**			
	10-gingerol	**7.82**	**<0.01**			
	demethylphylloquinone	**-11.35**	**<0.01**			
	eriodictyol	**-9.98**	**<0.01**			
	tangeritin	**-8.27**	**<0.01**			
	cianidanol	**6.24**	**<0.01**			
	antiarol	**2.88**	**0.03**			
Actinobacteria	Intercept	5.80	<0.01	33.16	0.93	<0.01
	cianidanol	**3.04**	**0.01**			
	homodihydrocapsaicin-I	**6.92**	**<0.01**			
	penbutolol	**-5.79**	**<0.01**			
	abyssinone I	**5.50**	**<0.01**			

	m-cresol	**4.51**	**<0.01**			
	4-(beta-D-glucosyloxy)benzoic acid	**-2.42**	**0.04**			
Planctomycetes	Intercept	6.42	<0.01	43.44	0.92	<0.01
	m-cresol	**7.07**	**<0.01**			
	(-)-fustin	**5.02**	**<0.01**			
	penbutolol	**3.31**	**<0.01**			
	caffeic acid	**-2.24**	**0.04**			
Gemmatimonadetes	Intercept	-0.84	0.42	84.67	0.97	<0.01
	vanillin	**2.50**	**0.03**			
	4-methoxycinnamic acid	**8.08**	**<0.01**			
	5-O-methyl embelin	**-5.47**	**<0.01**			
	4-nitrocatechol	**3.47**	**<0.01**			
	benzoic acid	**3.45**	**<0.01**			
Chloroflexi	Intercept	4.08	<0.01	32.71	0.81	<0.01
	(-)-fustin	**3.75**	**<0.01**			
	2-hydroxybenzyl alcohol	**3.29**	**<0.01**			
Acidobacteria	Intercept	116.15	<0.01	1499491.97	1.00	<0.01
	antiarol	**332.13**	**<0.01**			
	catechin	**1096.77**	**<0.01**			
	coumarin	**-363.35**	**<0.01**			
	penbutolol	**196.79**	**<0.01**			
	3-hydroxybenzyl alcohol	**-140.84**	**<0.01**			
	ferulic acid 4-sulfate	**-19.09**	**<0.01**			
	homodihydrocapsaicin-I	**-163.07**	**<0.01**			
	vanillin	**114.43**	**<0.01**			
	isorhapontigenin	**-42.06**	**<0.01**			
	medicagol	**-18.31**	**<0.01**			
	4-hydroxycoumarin	**7.24**	**<0.01**			
	taxifolin	**-4.38**	**0.02**			
Thaumarchaeota	Intercept	2.74	0.03	3817.26	1.00	<0.01

	safynol	**49.33**	**<0.01**			
	catechol	**56.02**	**<0.01**			
	2-hydroxycinnamic acid	**-45.62**	**<0.01**			
	3,4-dihydroxybenzaldehyde	**20.01**	**<0.01**			
	10-gingerol	**17.50**	**<0.01**			
	phloroglucinol	**18.29**	**<0.01**			
	eriodictyol	**-7.76**	**<0.01**			
	4-hydroxybenzaldehyde	**-4.63**	**<0.01**			
Parcubacteria	Intercept	8.01	<0.01	175.98	0.99	<0.01
	benzoic acid	**22.46**	**<0.01**			
	homodihydrocapsaicin-I	**-17.92**	**<0.01**			
	caffeic acid	**-18.19**	**<0.01**			
	genistein	**-12.54**	**<0.01**			
	catechol	**4.13**	**<0.01**			
	herniarin	**9.83**	**<0.01**			
	abyssinone I	**-4.89**	**<0.01**			
	D-(-)-Salicin	**3.10**	**0.02**			
Verrucomicrobia	Intercept	3.52	<0.01	28.23	0.88	<0.01
	tangeritin	**3.77**	**<0.01**			
	herniarin	**4.74**	**<0.01**			
	catechol	**3.35**	**<0.01**			
	catechin	**2.69**	**0.02**			
Nanohaloarchaeota	Intercept	-2.54	0.03	74.43	0.91	<0.01
	4-coumaric acid	**5.44**	**<0.01**			
	cunaniol	**4.38**	**<0.01**			
Firmicutes	Intercept	6.80	<0.01	62.34	0.80	<0.01
	homodihydrocapsaicin-I	**7.90**	**<0.01**			
JL-ETNP-Z39	Intercept	-3.36	<0.01	437.57	0.99	<0.01
	4-nitrocatechol	**22.45**	**<0.01**			
	2,4-dihydroxybenzoic acid	**10.83**	**<0.01**			

	2-hydroxybenzyl alcohol	**-8.01**	**<0.01**			
	(-)-fustin	**5.50**	**<0.01**			
others	Intercept	4.87	<0.01	20.75	0.73	<0.01
	nobiletin	**3.06**	**<0.01**			
	4-coumaric acid	**-2.62**	**0.02**			
Unassigned	Intercept	10.22	<0.01	74.65	0.91	<0.01
	cunaniol	**11.97**	**<0.01**			
	homodihydrocapsaicin-I	**-4.01**	**<0.01**			

795