



Rhizosphere bacterial communities and soil nutrient conditions reveal sexual dimorphism of *Populus deltoides*

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Received: 18 November 2021 / Accepted: 31 March 2022 / Published online: 22 July 2022
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Abstract Sexual dimorphism of plants shapes the different morphology and physiology between males and females. However, it is still unclear whether it influences below-ground ecological processes. In this study, rhizosphere soil of male and female *Populus deltoides* and bulk soil were collected from an 18-year plantation (male and female trees mix-planted) and grouped into three soil compartments. Soil carbon (C), nitrogen (N) and phosphorus (P) levels were determined, and soil bacterial communities were analyzed by high-throughput sequencing. The results showed the less total carbon and total organic carbon, the more nutrients (available phosphorus, nitrate nitrogen and ammonium nitrogen) available in the rhizosphere soils of female poplars than

soils of males. However, α -diversity indices of the rhizosphere bacterial communities under male plants were significantly higher. Principal component analysis showed that the bacterial communities were significantly different between the male and female soil compartments. Further, the bacterial co-occurrence network in soil under male trees had more nodes and edges than under females. BugBase analysis showed the more functional bacteria taxa related to biofilm formation and antioxidation under males. The results indicate that soils under male poplars had more diverse and more complex co-occurrence networks of the rhizosphere bacterial community than soils under female trees, implying that male poplars might have better environmental adaptability. The study provides insight into the different soil-microbe interactions of dioecious plants. More details about the influencing mechanism of sexual dimorphism on rhizosphere soil bacterial communities need to be further studied.

Qiliang Zhu and Kun Yan contributed equally to this work.

Project funding: The work was supported by the National Natural Science Foundation of China (32071751), the National key research and development program (2021YFD220120102), the Natural Science Foundation of Shandong Province (ZR2018ZC08N3) and the funds of the Shandong Double Tops Program (Grant No. SYL2017XTTD03).

The online version is available at <http://www.springerlink.com>.

Corresponding editor: Tao Xu.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11676-022-01517-x>.

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Keywords Dioecious poplar · Rhizosphere bacterial community · High-throughput sequencing · Bacterial function prediction

Introduction

Dioecious plants with male and female flowers on different plants, are approximately 6% of terrestrial angiosperm species, and play an important role in biodiversity conservation and terrestrial ecosystem functions (Tognetti 2012; Song et al. 2019). Sexual dimorphism reveals the unique evolutionary history and adaptability of some plant species. Studies have shown that male and female plants differ significantly in morphology and physiology (Barrett and Hough 2012), and they have specific demands for resources (Forrest 2014). Specially, different adaptabilities have been shown by

many dioecious herbaceous and woody plants (Dudley and Galen 2007; Tonnabel et al. 2017), and most male plants demonstrate greater tolerance to stress than female plants (Juvany and Munné-Bosch 2015). However, their different adaptability mechanisms to the environment have not been fully clarified.

Rhizosphere microorganisms play key roles in plant growth and environmental adaption (Ren et al. 2020). Their communities are well-shaped by root-soil interactions (Wang et al. 2018), such as root secretion and nutrient availability (Delgado-Baquerizo et al. 2017). For example, root-secreted carbon serves as a substrate for microbial growth and reproduction (Nyawade et al. 2019). Soil nitrogen (N) and phosphorus (P) required for microbial metabolism and morphogenesis (e.g., ATP, DNA, RNA; Peñuelas and Sardans 2009; Lu et al. 2019) influence rhizosphere bacterial community assemblies (Sturner and Elser 2002; Nyawade et al. 2019). Different microorganisms are recruited to improve the mineralization of rhizosphere nutrients and to enhance plant growth (van der Voort et al. 2016; Bakker et al. 2018). Therefore, nutrients in the rhizosphere soil are closely related to the composition and structure of microbial communities (Williams and de Vries 2020).

However, interactions between plant roots and microorganisms are complex and elaborate (Lladó et al. 2017; Hartman and Tringe 2019). Root exudates from fine roots into the surrounding soil show host-specific effects (Matthews et al. 2019), which influence the recruitment and assembly of specific bacterial communities (Bakker et al. 2018; Hassan et al. 2019). In particular, rhizosphere microorganism communities are readily influenced by root-secreted metabolites (Berendsen et al. 2018; Liu et al. 2020) under environment stress (Wei et al. 2019). Therefore, large quantities of special functional microorganisms are recruited in the rhizosphere to improve plant tolerance to stress (van der Voort et al. 2016; Bakker et al. 2018). Often plants with superior environmental adaptability and nutrient utilization have common microbial community structures in the rhizosphere, suggesting that there is a close relationship between plant phenotype and rhizosphere microbial function (Liu et al. 2020). However, the differentiation of these microbial communities between male and female plants is largely obscure. The exploration of the rhizosphere bacterial communities of dioecious plants may reveal the different adaptabilities of male and female plants in specific environments.

Poplars (*Populus* spp.) are dioecious woody plants. Studies have examined sexual dimorphism under drought (30% of field capacity), saline-alkali levels (0.2% (w/w) of sodium chloride), heavy metals (5 mg kg⁻¹ of cadmium) and nutrient deficiencies (50 and 200 mg kg⁻¹ Calcium superphosphate (Ca(H₂PO₄)₂·H₂O)), including morphology, physiology, proteomes and gene expression (Xu et al. 2008; Yang et al. 2011; Hao et al. 2020; Hu et al. 2020; Xia et al. 2020a).

Similarly, much of the literature indicates that male poplars usually demonstrate more tolerance to stress than female ones (Melnikova et al. 2017), indicating that they have better adaptability to environment changes. However, these studies have focused on the aboveground parts of dioecious poplars, and belowground parts have rarely been considered (Wu et al. 2019; Chen et al. 2020). In particular, the differences in rhizosphere microbial communities and soil nutrients between male and female poplar are still obscure.

In this study, roots of male and female *Populus deltoides* in an 18-year-old plantation were collected and the rhizosphere bacterial community and soil nutrient levels determined. The objective was to: (1) clarify the carbon, nitrogen, and phosphorus levels of rhizosphere soils under male and female poplars; and (2) identify the differences of rhizosphere bacterial community structure under male and female poplars. It was hypothesized that sexual dimorphism would result in the differentiation of the root-associated bacterial communities.

Materials and methods

Study site, root, and soil sampling

The study site is on the Dashahe National Forest Farm (34°79' N, 116°08' E) of Shandong Province, China. The site has a warm temperate, continental monsoon climate with an average annual temperature of 13.9 °C. The average annual frost-free period is approximately 206 days, and the average annual precipitation is 737.1 mm. Soils have poor water retention capacity and low organic matter. The study plot was established in an 18-year-old *P. deltoides* plantation with an average diameter breast height (DBH) of 25.0 cm (male and female trees were mix-planted). The plantation density was 500 trees/ha, with a spacing of 4-m, and a 5-m row spacing (Fig. 1). No fertilization or irrigation had been undertaken since plantation establishment. The understory is approximately 60% cover and mainly consists of annual and perennial herbs, such as *Setaria viridis* Beauv., *Amaranthus tricolor* Linn., and *Chenopodium serotinum* Linn..

Six 30 m × 30 m quadrates (Q1–Q6) were established (Fig. 1a). In each quadrate, based on an afforestation map, male and female trees were identified and three of each with similar heights, DBH and crown size were selected for root sampling. The sample trees were remote from each other to avoid interference with root sampling (Fig. 1b). Considering the possible heterogeneity of the soils and distribution of roots, four soil blocks (50 cm × 50 cm and 20 cm deep) were selected around the trunk at 0.5-m (Zhu et al. 2018) for root sampling (Fig. 1c). The soil block included coarse roots to ensure that fine roots were specifically from the sample

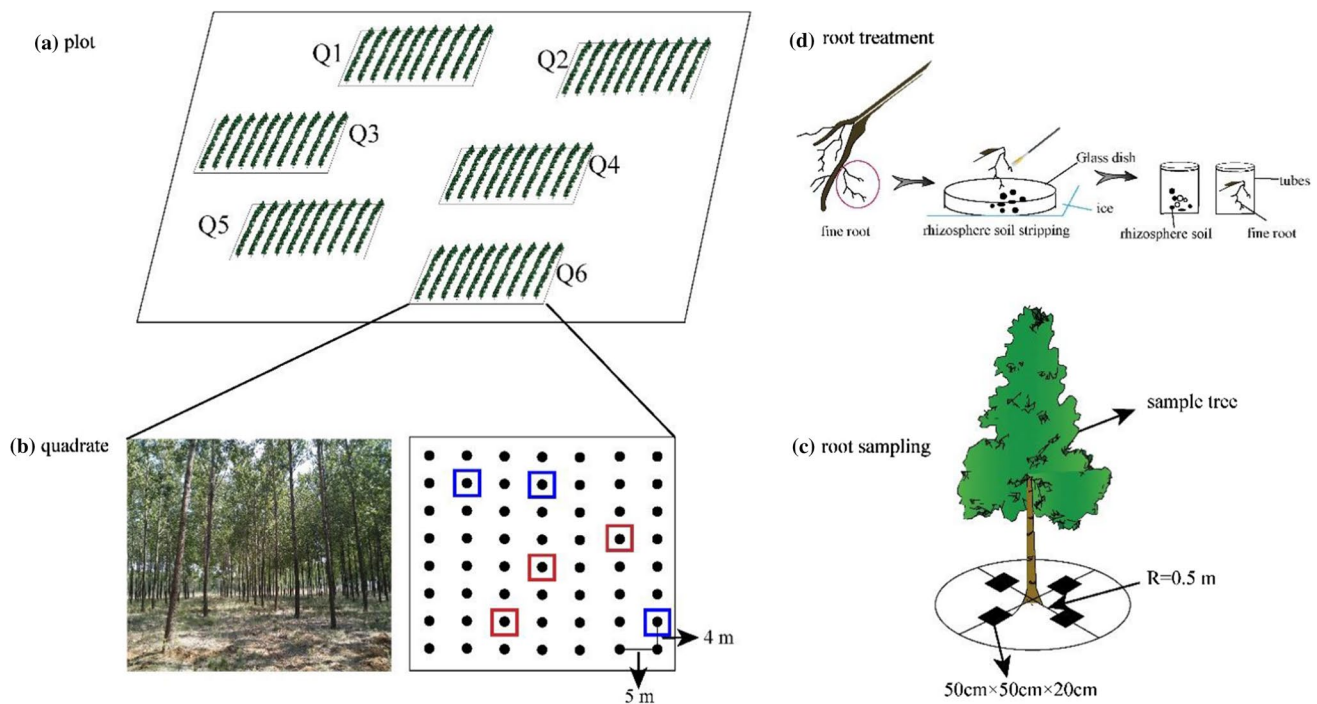


Fig. 1 Schematic diagram of plot selection and root sampling; **a** six quadrates were randomly selected, **b** red and blue squares represent the sample male and female poplars, respectively; **c** considering

uneven distribution of poplar roots, four soil blocks were excavated around each sample tree; **d** all live roots of poplar were collected to obtain the rhizosphere soils

tree. Before collecting the roots, surface vegetation was removed. Based on color and flexibility, live fine roots were easily distinguished and stored at approximately 4 °C. Bulk soils of each quadrat were also taken. All roots of three male and female trees from the same quadrat were grouped into one sample. In the laboratory, soils adhering to the roots were collected with a hairbrush as rhizosphere soil and stored in tubes (Fig. 1d). A total of 18 soil samples (six rhizosphere soils of the male poplar, six of the female poplar, and six bulk soils) were used for nutrient analysis and bacteria genomic DNA extraction. All samples were grouped into three compartments marked as male, female and BS.

Soil nutrients analysis

Total carbon (TC), total nitrogen (TN) and total organic carbon (TOC) were determined by an elemental analyzer (vario MACRO cube, Elementar, Germany). Total phosphorus (TP) and available phosphorus (AP) were measured by an automatic intelligent chemistry analyzer (Smartchem 200, AMS/Westco, Italy). Soil nitrate (NO_3^- -N) and ammonium (NH_4^+ -N) contents were determined with an automatic flow analyzer (SEAL AA3, Germany). Soil stoichiometric characteristics were calculated.

Soil bacterial community analysis

Extraction and amplification of soil bacterial DNA

Microbial DNA was extracted from 100 mg soil using Soil DNA Extraction Kits (Omega, USA), and the quality of DNA was checked using an RS232G UV spectrophotometer (Eppendorf Company, Hamburg, Germany). The bacterial V3–V4 region of 16S rDNA was amplified by specific primers. The primer sequences were 338F (5'-ACTCCTACG GGAGGCAGCA-3') and 806R (5'-GGACTACHVGGG TWTCTAAT-3'). A unique 7-mer barcode sequence was added between the sequencing adapter and the forward primer to differentiate between samples. The amplified products were sequenced with an Illumina platform (Illumina MiSeq Sequencer, Illumina Inc., San Diego, CA, USA) using paired-end 250 bp sequencing.

Sequence data filtration

The raw data were trimmed by the Trimmomatic method in paired-end mode (Bolger et al. 2014). The sliding window method screened the double-ended sequences of the FASTQ format one by one, with a window size of 10 bp and a step size of 1 bp. Using FLASH software (v1.2.7, <http://ccb.jhu.edu/software/FLASH/>) (Magoč and Salzberg 2011), the filtered reads were spliced to get the paired end sequence.

Finally, the valid sequence was identified according to the index information (i.e., the barcode sequence) corresponding to each sample, requiring the index sequence to match exactly. The chimera sequence was removed using QIIME software (Quantitative Insights into Microbial Ecology, v1.8.0, <http://qiime.org/>) (Caporaso et al. 2010) and USEARCH (v5.2.236, <http://www.drive5.com/usearch/>).

OTU clustering and annotation

Using UCLUST in QIIME clusters, high-quality sequences with 97% similarity (Edgar 2010) were identified and considered as operational taxonomic units (OTUs), and the most abundant sequence in each OTU was selected as the representative sequence. To ensure the accuracy of the results, OTUs with relative abundance < 0.001% were discarded (Bokulich et al. 2013). After filtration, 594,746 sequences were retained in the bacterial communities. BLAST QIIME compared the sequence databases to gain taxonomic information of each representative sequence. Greengenes database was employed to obtain the bacteria species information (Release 13.8, <http://greengenes.secongenome.com/>) (DeSantis et al. 2006). The sequence data were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number SRP313924.

Statistical analysis

Volcano plots, heatmap, bacteria cooccurrence networks, and PCA biplots were finished based on the relative abundance of OTUs by R version 3.6.1 (R Core Team 2020). In addition, four α -diversity indices (Ace, Chao1, Shannon and Simpson) were calculated for each soil compartment using MOTHUR ver. 1.8.0. Linear discriminant effect size (LEfSe) analysis was conducted to show the biomarkers at different taxonomic levels (<http://huttenhower.sph.harvard.edu/galaxy/>). The Psych R package (v.2.0.9) was used for correlation analysis (<https://CRAN.R-project.org/package=psych>), and the results were visualized with the corrplot R package (v.0.84) (<https://github.com/taiyun/corrplot>). One-way analysis of variation (ANOVA) and least significant difference (LSD) methods ($\alpha = 0.05$) assessed the significant differences of the relative abundance of soil bacteria at the genus level among the soil compartments. Co-occurrence network of bacteria in soil compartment was visualized and analyzed by via Gephi software (v.0.9.2) (Bastian et al. 2009). BugBase website (<http://bugbase.cs.umn.edu>) performed phenotypic classification such as Gram-positive, Gram-negative, and biofilm formation (Ward et al. 2017). PICRUST and FAPROTAX were used to predict the functional profiles of rhizosphere bacteria communities. PICRUST predicted bacterial function based on gene sequence according to the KEGG database (Langille et al. 2013), and

FAPROTAX performed prokaryotic function annotation based on the current literature of cultivable bacteria (Louca et al. 2016).

Results

C, N and P nutrients in soil compartments

The TC, TOC, TN, and TP in the soil rhizosphere of male poplar and bulk soil were more than those of soil rhizosphere of female poplar. In contrast, the contents of AP, NO_3^- -N and NH_4^+ -N in soils under male trees were less than under female trees (Table 1). The rhizosphere soil of female poplar showed significantly higher TC:TN ratios while lower TC:TP and TN:TP ratios than for soil under male poplars. Rhizosphere soils of male and female poplars had higher TC:TN:TP ratios than the bulk soil. All the results indicate that soil properties were different in soils between the male and female poplars, implying that sexual dimorphism might affect rhizosphere nutrients.

Differences of bacterial communities in soils under male and female trees, and bulk soil

Soil bacterial communities under male trees were more diverse than those under female trees and in bulk soil (Fig. 2). Principal component analysis (PCA) indicated that bacterial communities were significantly different among soils under the male and female trees and in the bulk soil (Fig. 3, $P = 0.001$). The operational taxonomic unit (OTU) composition of the bacterial community differed

Table 1 Soil C, N P nutrient conditions (mean $\pm$ SE)

Soil	Female	Male	BS
TC ($\text{mg}\cdot\text{g}^{-1}$)	11.8 ± 0.7^a	13.4 ± 0.7^b	13.5 ± 1.0^b
TOC ($\text{mg}\cdot\text{g}^{-1}$)	5.3 ± 0.7^a	5.5 ± 0.6^a	8.0 ± 1.3^b
TN ($\text{mg}\cdot\text{g}^{-1}$)	0.6 ± 0.1^a	0.7 ± 0.1^{ab}	0.8 ± 0.1^b
TP ($\text{mg}\cdot\text{g}^{-1}$)	0.5 ± 0.0^a	0.5 ± 0.0^a	0.5 ± 0.0^a
AP ($\text{ug}\cdot\text{g}^{-1}$)	1.9 ± 0.5^a	0.8 ± 0.4^b	1.9 ± 0.7^a
NO_3^- -N ($\text{ug}\cdot\text{g}^{-1}$)	31.7 ± 3.0^a	26.4 ± 2.2^{ab}	19.8 ± 1.5^b
NH_4^+ -N ($\text{ug}\cdot\text{g}^{-1}$)	15.5 ± 2.0^a	12.1 ± 1.6^b	17.6 ± 1.0^a
NH_4^+ -N: NO_3^- -N	0.5 ± 0.1^a	0.5 ± 0.1^a	0.9 ± 0.1^b
TC:TN	22.4 ± 1.3^a	18.8 ± 0.7^b	17.3 ± 1.1^b
TC:TP	25.7 ± 1.6^a	30.0 ± 0.9^b	29.0 ± 2.3^b
TN:TP	1.2 ± 0.2^a	1.6 ± 0.1^b	1.8 ± 0.3^b
TC:TN:TP	48.4 ± 2.7^a	42.5 ± 2.9^{ab}	36.9 ± 2.1^b

Letters in the same column mean significant differences among soil compartments at the 0.05 level. Abbreviations: total carbon (TC), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP) and ratio of NH_4^+ -N to NO_3^- -N (NH_4^+ -N: NO_3^- -N)

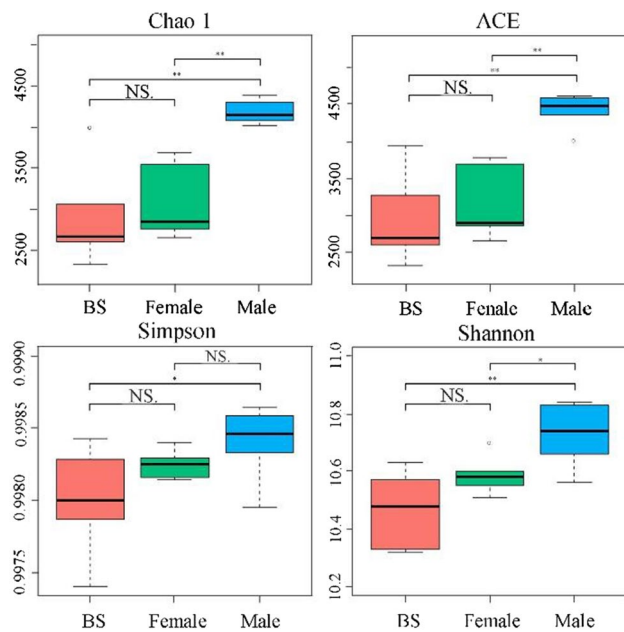


Fig. 2 Box plots for bacterial community diversity in three soil compartments; significant differences in soils under male and female trees, and in bulk soil (BS) are indicated with * at 0.05. The significant difference at 0.01 is marked with **

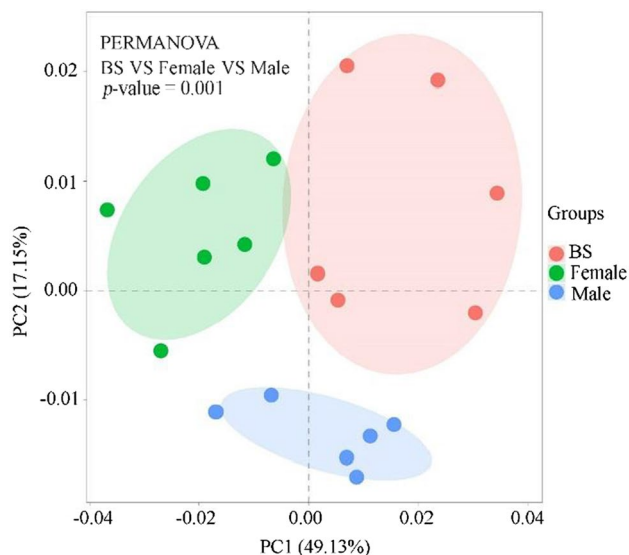


Fig. 3 Principal component analysis (PCA) on the bacterial communities of three soil compartments

significantly among the three soil compartments (Fig. S1). Heatmap analysis on the top 50 abundant bacteria genera also showed some differences among the soil compartments (Fig. S2), and some bacteria (i.e., *RB41*, *Sphingomonas*, *Cupriavidus*, *Haliangium*) exhibited significant differences in relative abundance (Table 2). LEfSe analysis indicated that four genera (*Bacillus*, *Ensifer*, *Pseudomonas*,

Table 2 Relative abundance of dominant bacteria genera in three soil compartments (mean $\pm$ SE)

Genus	Female (%)	Male (%)	BS (%)
RB41	6.6 $\pm$ 0.5 ^a	8.4 $\pm$ 0.5 ^b	10.1 $\pm$ 0.5 ^c
<i>Sphingomonas</i>	2.4 $\pm$ 0.2 ^a	4.1 $\pm$ 0.2 ^b	3.0 $\pm$ 0.3 ^a
<i>Iamia</i>	2.3 $\pm$ 0.4 ^a	1.7 $\pm$ 0.2 ^a	2.2 $\pm$ 0.1 ^a
<i>Streptomyces</i>	2.1 $\pm$ 0.3 ^a	1.4 $\pm$ 0.3 ^a	2.0 $\pm$ 0.4 ^a
<i>Cupriavidus</i>	1.3 $\pm$ 0.3 ^{ab}	0.3 $\pm$ 0.2 ^a	1.5 $\pm$ 0.5 ^b
11–24	0.8 $\pm$ 0.1 ^a	0.8 $\pm$ 0.1 ^a	1.1 $\pm$ 0.2 ^a
<i>Haliangium</i>	0.9 $\pm$ 0.1 ^a	1.1 $\pm$ 0.1 ^b	1.0 $\pm$ 0.1 ^{ab}
<i>Woodsholea</i>	1.1 $\pm$ 0.1 ^a	1.2 $\pm$ 0.1 ^a	0.8 $\pm$ 0.2 ^a
H16	1.0 $\pm$ 0.1 ^a	1.0 $\pm$ 0.1 ^a	0.8 $\pm$ 0.1 ^a

Based on the annotation of OTUs, only nine bacteria genera were more than 1% abundance. Different letters in same column mean significant differences among different soil compartments at the 0.05 level

Lysobacter) were significantly enriched in soils under female trees, two genera (*Terrimonas*, *Sphingomonas*) enriched in soils under male trees, and five genera (*Blastocatella*, *RB41*, *Kribbella*, *Solirubrobacter*, *Cupriavidus*) enriched in bulk soil (Fig. S3).

The cooccurrence network of bacteria demonstrated that soils under male trees had more nodes and edges than soils under female trees and in bulk soil (Fig. 4; Table S1). The correlation among the bacterial groups in soil under male trees was much closer than in soils under female trees (Fig. S4a). The degree of a node was the number of one node directly connected to other nodes. Similarly, the degree of network nodes and eigenvector centrality in soils under male trees were significantly higher (Fig. S4b–c). Therefore, the cooccurrence networks of soil bacteria in soils under male poplar appeared more complex than those under female poplar.

Correlation between bacterial community and soil nutrients

Among the top 50 abundant bacterial genera, fifteen in male soils and six in female soils were significantly correlated with soil nutrients (Fig. 5), implying that rhizosphere bacteria might play different roles in soil nutrient cycling of male and female poplars. Heatmap analysis showed that the genome function, relative to metabolism, exhibited significant differences among soils under male and female poplars and in bulk soil (Fig. S5a), indicating significantly different relationships between the soil bacterial communities and nutrients among the three soil compartments. For example, 20 and 12 metabolic pathways were correlated with NO_3^- -N and TC:TN in soils, respectively (Fig. S5b), which indicates that these might be important factors affecting the interaction of the rhizosphere bacterial community and soil

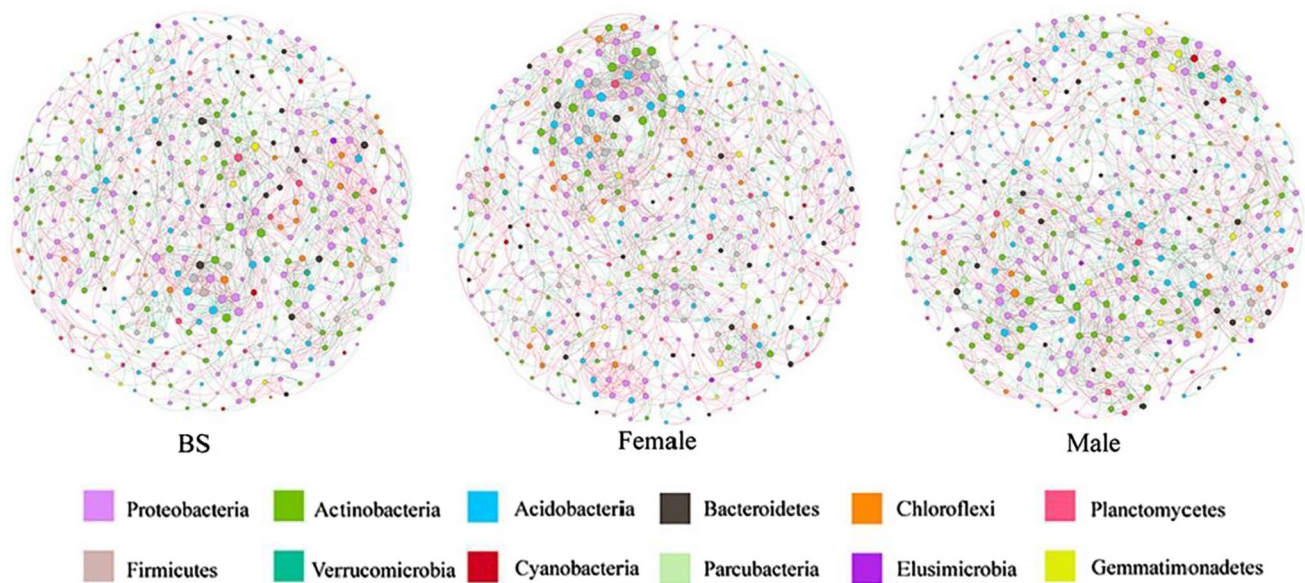


Fig. 4 The co-occurrence networks of bacteria in three soil compartments; dominant genera represented by different colors; red and green lines indicate positive and negative correlations ($P < 0.01$)

nutrients for male and female poplars. Further, BugBase analysis showed that there were significantly more bacterial taxa related to biofilm formation and antioxidation in soils under male trees than under females (Fig. 6), implying that the environmental adaptability of male poplars might be better than female poplars..

Discussion

Relationships between rhizosphere bacterial communities and nutrients

Soil microbes play important roles in depolymerization and mineralization of soil carbon, nitrogen, and phosphorus (Jacoby et al. 2017). The relationship between the soil bacterial community and soil nutrients is mutual and complex. This study showed that the rhizosphere soil of male poplars had more carbon (TC and TOC) but less available N and P (NO_3^- -N, NH_4^+ -N, AP) (Table 1), indicating that male poplars utilized available nutrients more than female trees. The preferential substrate utilization hypothesis (Dijkstra et al. 2013) indicates that soil microbes prefer to utilize easily decomposed root exudates. Consequently, both the decomposition of soil organic matter and the secretion of extracellular enzymes decreased in soils with higher available nutrients (Kuzakov and Cheng 2004). Moreover, higher nutrient availability would also reduce carbohydrate allocation to roots (Phillips et al. 2011) and decrease the richness of rhizosphere microorganisms. Thus, the more available nutrients inhibited the mineralization of soil organic matter,

resulting in negative rhizosphere priming effects (Dijkstra et al. 2013). In this study, the more diverse the rhizosphere bacterial community in soils under male poplars was closely related to greater soil C and less N and P nutrients (Figs. 2, 5). Additionally, sexual dimorphism in dioecious plants might be responsible for sex-specific resource requirements for reproduction (i.e., high carbon requirements for ovules and high nitrogen demands for pollen). Therefore, male and female trees may acquire and utilize nutrients differently with sex-specific morphologies (Tonnabel et al. 2019) and take different pathways to adjust their carbon resource allocation (Tonnabel et al. 2017). Female plants tend to produce sufficient carbon through photosynthesis for reproductive demand, while male plants allocate more carbon into the roots to forage more nitrogen. Therefore, the different C:N ratios in the rhizosphere impacted the rhizosphere bacterial community, and some studies have also proposed that soil stoichiometry drives the changes in microbial community structure (Delgado-Baquerizo et al. 2017).

In addition, phosphate-solubilizing bacteria play important roles in functional requirements of plants for phosphorus (Ren et al. 2020). Studies have shown that female plants of *Populus cathayana* Rehd. required more phosphorus (Xia et al. 2020a) while male plants demonstrated a greater ability to forage phosphorus nutrients (Xia et al. 2020b). In the present study, enriched *Bacillus* in the rhizosphere soils (Fig. S3) had the function of phosphate solubilization (Alori et al. 2017), which would improve the available phosphorus for poplar. Several other genera, such as *Nocardioides*, *Variibacter*, *Acidovorax*, and *Chthoniobacter*, also showed positive correlations with

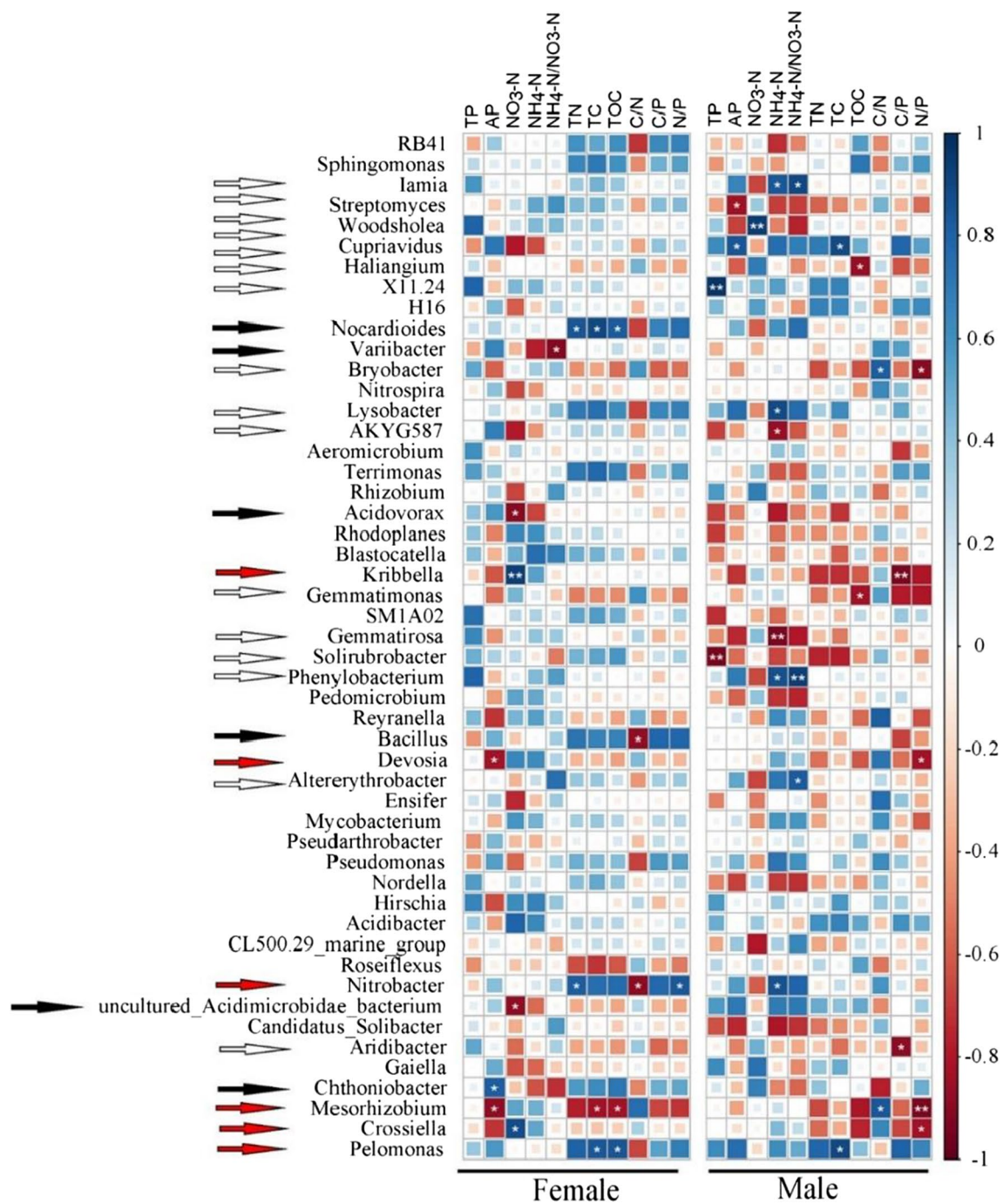


Fig. 5 Correlation between soil nutrients and bacteria genera with relative abundance over 0.1%; Blank and solid black arrows indicate genera significantly correlated with soil nutrients under male and

female trees, respectively; solid red arrows indicate genera significantly correlated with soil nutrients under both male and female trees; ** and * show significant correlations at the 0.01 and 0.05 levels

available phosphorous in female plants (Fig. 5). Therefore, the functional bacteria related to soil nutrients showed differences in the rhizosphere soils, which might influence the soil nutrients conditions of male and female poplars.

Different cooccurrence networks of rhizosphere bacterial communities of dioecious plants

Plants have developed considerable phenotypic plasticity with

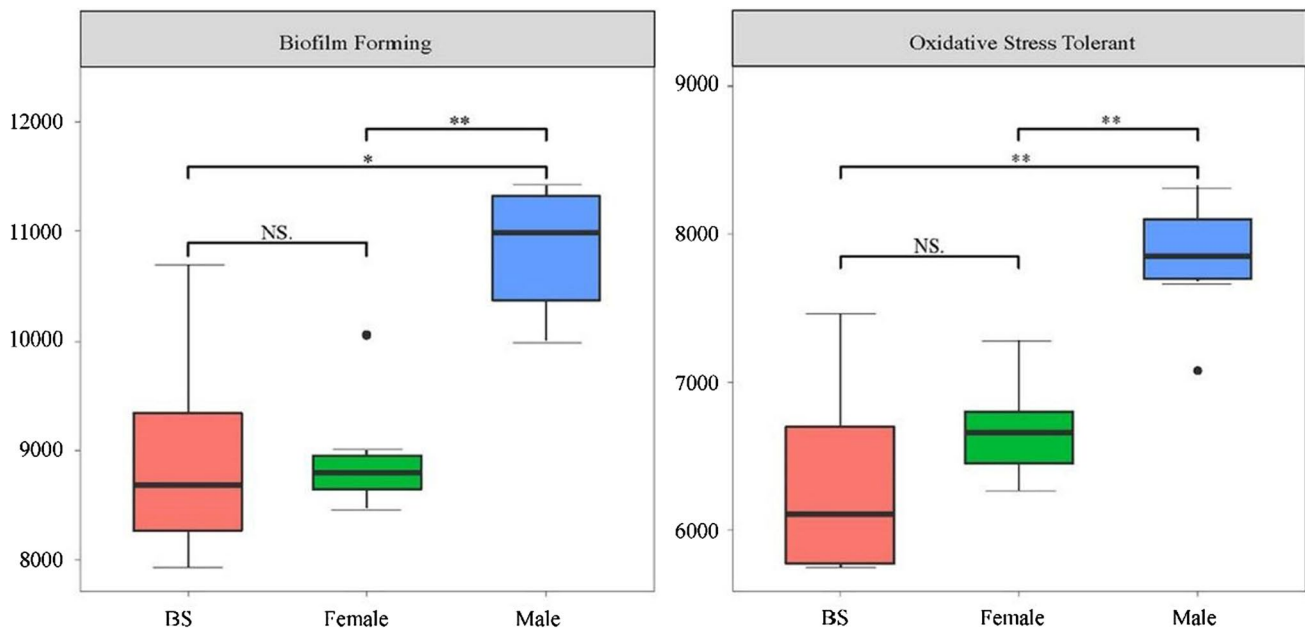


Fig. 6 Box plots for the prediction of bacteria communities' function in three soil compartments; ** and * show significant correlations at 0.01 and 0.05 levels; NS is non-significant difference ($P > 0.05$)

regards to plant-microbial interactions (Saleem et al. 2018). Activities of soil bacterial communities are closely related to plant growth (Wei et al. 2018), and their complexity contributes to the greater adaptability of plants to their environment. Healthy plants have more complex cooccurrence networks of bacterial communities in rhizosphere soils (Bonacich 2007; Wei et al. 2019). Xia et al. (2020b) reported that sexual dimorphism resulted in different morphological and physiological traits between male and female trees. This study further that sexual dimorphism also affects root-soil interactions, and male poplars developed more complex bacterial communities with larger total nodes, degree and eigenvector centrality than female poplars (Table S1, Fig. S4), indicating that male trees are more adaptable to environmental changes. In addition, under changing environments, the positive links of bacterial communities make synchronous changes and cause co-oscillation of the entire community, while negative links decrease co-oscillation and promote the community stability (Coyte et al. 2015; de Vries et al. 2018). Therefore, competitive interaction promotes soil nutrient utilization by rhizosphere bacteria and reduces the colonization of harmful pathogens (Li et al. 2019). In this study, the bacterial cooccurrence networks in male poplars had a greater proportion of negative interactions (Table S1), implying that male poplars have greater adaptability.

Functional differences of rhizosphere bacteria under dioecious plants

Male plants exhibit much better growth than females, especially under stress (Melnikova et al. 2017), which might be attributed to specific microbes. There are three general mechanisms to explain the positive correlations of rhizosphere bacteria with plants: (1) manipulating plant hormonal signaling (Verbon and Liberman 2016); (2) repelling pathogenic microbial strains (Mendes et al. 2013); and, (3) increasing the availability of soil nutrients (van der Heijden et al. 2008). This study showed that approximately 20 microbial metabolisms were significantly correlated with NO_3^- -N levels in the rhizosphere (Fig. S5), indicating that NO_3^- -N is an important factor affecting the function of rhizosphere bacteria in soils of male and female poplars. Specially, two dominant bacterial genera (*Sphingomonas* and *Haliangium*) associated with male trees were significantly more abundance than soils of female trees (Table 2). *Sphingomonas* produces indole-3-acetic acid (Shi et al. 2021) to mitigate heavy metal stress in plants (Wei et al. 2021) and degrades organic pollutants (Lu et al. 2021; Song et al. 2021), while *Haliangium* produces haliangicin that inhibits wide-spectrum fungi growth (Ling et al. 2014). Therefore, these two genera with higher abundance

in soils of male poplars play important roles in improving their growth.

In addition, biofilm formation is induced by chemical signals released from roots which protects plants from various soil-borne diseases (Chen et al. 2013) and promotes resistance to abiotic stresses like salinity (Pandit et al. 2020), drought (Kumar et al. 2016), and inorganic/organic pollutants (Zhou and Gao 2019). Based on BugBase phenotypic classification, this study found a stronger biofilm formation and antioxidation capacity of rhizosphere bacteria in soils under male trees than under females, and the gene function prediction showed that flavonoid biosynthesis in soils under male poplars was also greater (Fig. 6; Fig. S5). The results indicate that male poplars show better environmental adaptability than females.

Conclusions

The less C but more available N and P were examined in the rhizosphere soil of female poplar. The more diverse and complex cooccurrence networks of bacterial communities were found in the rhizosphere soils of male poplars. Sexual dimorphism influenced the utilization patterns of soil nutrients and shaped the bacteria community structure in the rhizosphere soils of male and female poplars. Male poplars were more adaptable to environmental changes than female poplars. The results provide more insight into the patterns of soil-microbial interactions of dioecious plants. However, more research on the recruitment and assembly of soil bacteria in rhizosphere soils under dioecious plants need to be carried out.

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