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Intra- and intersexual interactions shape microbial community dynamics in the
rhizosphere of *Populus cathayana* females and males exposed to excess Zn

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Abstract Although increasing attention has been paid to rhizospheric bacteria in plants in relation to the bioavailability and tolerance of heavy metals, interactive effects between sex and microbiological processes on phytoextraction have been overlooked, especially in dioecious plants. In this study, we intended to investigate the responses of rhizospheric bacterial communities of *Populus cathayana* Rehder to excess Zn under different planting patterns. The results suggested that intersexual and intrasexual interactions strongly affect plant growth and Zn extraction in both sexes, as well as rhizosphere-associated bacterial community structures. Females had a higher capacity of Zn accumulation and translocation than males under all planting patterns. Males had lower Zn accumulation and translocation under intersexual than under intrasexual interaction; the contrary was true for females. Females harbored abundant *Streptomyces* and *Nocardioides* in their rhizosphere, similarly to males under intersexual interaction, but differed from single-sex males under excess Zn. Conversely, intersexual interaction increased the abundance of key taxa Actinomycetales and Betaproteobacteria in both sexes exposed to excess Zn. Males improved the female rhizospheric microenvironment by increasing the abundance of some key tolerance taxa of Chloroflexi, Proteobacteria and Actinobacteria in both sexes under excess Zn in intersexual interaction. This was associated with metal activation and bioavailability. Females harbored abundant *Methanothermobacter*, while males had abundant *Mycobacterium* in their rhizosphere under intrasexual interaction. These results indicated that the sex of neighboring plants affected sexual differences in the choice of specific bacterial colonizations for phytoextraction and tolerance to Zn-contaminated

soils, which might regulate the spatial segregation and phytoremediation potential of *P. cathayana* females and males under heavy metal contaminated soils.

Key words: sexual interaction; excess Zn; bacterial abundance; bacterial community; rhizosphere.

Graphical abstract

1. Introduction

Males and females of dioecious plant species differ in their sexual and vegetative traits, including physiology, phenology, and allocation to reproduction and defense (Chen et al., 2014; Tonnabel et al., 2017; Xia et al., 2020). Sex-related differences in these traits are probably associated with differences in reproductive costs. Females allocate more resources to reproduction than males, since females produce not only flowers but also fruits and seeds (Juvany and Munné-Bosch, 2015). The maintenance of reproductive vigor usually limits vegetative growth and defense investment. Thus, when resources are limited, reproduction directly competes with the other two processes, leading to reduced stress tolerance (Mercer and Eppley, 2010; Tonnabel et al., 2017). It has been shown that females generally allocate more resources to reproduction and males often increase investment into defensive responses under natural conditions (Juvany and Munné-Bosch, 2015). Our previous studies have suggested that males usually exhibit a higher tolerance to stressful environments when compared with females (Zhao et al., 2012; Chen et al., 2014; Li et al., 2016; Zhang et al., 2019).

Zinc (Zn) is an essential micronutrient with a series of critical roles in living organisms; yet, Zn can be toxic at elevated concentrations (Broadley et al., 2007; Lu et al., 2013). It has been suggested that Zn toxicity symptoms usually appear when the leaf Zn content is $> 30 \text{ mg} \cdot \text{kg}^{-1}$ of leaf dry weight, although the toxicity threshold may be highly variable, even in the same species (Ayangbenro and Babalola, 2017). Excess Zn affects

77 plant growth and development, as well as threatens the health of animals due to
78 excessive amounts of Zn ending up into the food chain (Duruibe et al., 2007; Carruthers,
79 2016). The threat of excess Zn to the health of living organisms in the environment is
80 exacerbated by its persistent nature (Ayangbenro and Babalola, 2017). Therefore, it is
81 critical to remediate Zn-polluted soils. Several fast-growing trees, including poplars,
82 are regarded as promising candidates for phytoremediation of heavy metal-polluted
83 soils due to their high photoabsorption capacity and biomass, as well as reduced impact
84 on the food chain (Chen et al., 2011; He et al., 2015; Li et al., 2017).

85
86 The migration and transformation of heavy metals in rhizospheric soils largely depend
87 on the physical and chemical properties of soil (Rasmussen et al., 2000; Weng et al.,
88 2014), plant types (Hou et al., 2017), rhizospheric microorganisms (Ayangbenro and
89 Babalola, 2017) and exudates (Tao et al., 2020). Among these, rhizospheric microbial
90 interactions with heavy metals play a critical role in heavy metal uptake and tolerance
91 (Rajkumar et al., 2012). Microorganisms have developed variable mechanisms for
92 maintaining heavy metal homeostasis and resistance, including biomineralization,
93 bioaccumulation, biotransformation and biosorption (Navarro-Noya et al., 2010;
94 Mishra et al., 2017). Heavy metals have been shown to suppress microorganisms'
95 growth, and alter their cell morphology and biochemical characteristics, which reduces
96 microbial biomass and diversity (Ayangbenro and Babalola, 2017). Still,
97 microorganisms have evolved diverse defense mechanisms that help them cope with
98 the toxic effect of heavy metals. Burd et al. (2000) have suggested that a metal-resistant

bacterium *Kluyvera ascorbate* SUD 165 alleviates growth inhibition caused by a high level of nickel in *Brassica campestris*. Hou et al. (2017) have also reported that the rhizospheric bacterium *Streptomyces* probably promotes Cd accumulation in the hyperaccumulator plant *Sedum alfredii*. Therefore, the coexistence of plant and microbial systems not only facilitates survival in heavy-metal contaminated soils, but also the removal of heavy metal.

Interspecific and/or intraspecific neighbor interactions of plants play critical roles in determining the structure and function of biological communities (Chen et al., 2014). Intra- and interspecific competitive capacities have important implications for phytoremediation in metal-hyperaccumulating species (Arthur et al., 2005; Pilon-Smits and Freeman, 2006). Interactions have been shown to alter plants' responses to nickel and Zn (Koelbener et al., 2008). Zhao et al. (2017) have found that interspecific interactions can enhance antioxidant enzyme activities that increase survival and fitness in plants exposed to multiple metal stresses. Chen et al. (2016) have also shown that resource competition between consensual and heterosexual neighboring plants affect Cd allocation, biomass partitioning and carbon-nutrient balance in poplars exposed to Cd stress. Interactions not only alter plants' morphological and physiological responses, such as nutrient uptake, heavy metal availability and root exudate secretion, but also affect microbial communities in soil (Guo et al., 2019). So far, the effects of intersexual and intrasexual interactions on belowground microbial communities have been largely overlooked, especially under heavy metal stress.

Populus cathayana Rehder is widely distributed in China and is regarded as a major forestry species with a high commercial and ecological value. *P. cathayana* is a common dioecious plant that displays sexual dimorphism (Chen et al., 2014; Zhang et al., 2019; Xia et al., 2020). Recently, some studies have suggested that poplar species have different tolerance mechanisms and phytoremediation potential to heavy metals, mainly based on genetic differences (Chen et al., 2017; Bi et al., 2020; Liu et al., 2020b). In addition, *Populus* females and males display sexually different physiological responses to heavy metal stress, and males usually show a stronger tolerance when compared to females (Chen et al., 2016, 2017; Liu et al., 2020a, b). Sex-specific responses to abiotic factors are affected by inter- and/or intraspecific interactions (Chen et al., 2016, 2017). However, sex-specific responses to excess Zn stress and neighbor effects have been largely overlooked in *P. cathayana*, especially in relation to rhizospheric microbial community structures. In the present study, *P. cathayana* was used to examine sexual differences in competitive ability under excess Zn conditions. We aimed to address the following questions: (i) Does excess Zn affect intra- and intersexual interactions? (ii) How do intra- and intersexual interactions affect rhizospheric bacterial communities? (iii) Do interactive effects between excess Zn and sexual interactions affect plant growth and rhizospheric bacterial community composition and diversity?

2. Materials and methods

2.1. Plant materials and experimental design

Cutting of *P. cathayana* females and males were collected from 60 different trees sampled in 15 populations in the riparian and valley flat habitats of the Qinghai Province, China. Cuttings were rooted as described by Chen et al. (2016). The experimental design was completely randomized with three factors (sex, excess Zn and plantation type), i.e. two sexes (females, males), two Zn regimes (no-Zn, Zn) and five plantation types. After sprouting and growing for 4 weeks, uniform seedlings were planted into 60-L plastic pots filled with *c.* 40 kg of homogenized soil. The soil was collected from the poplar plantation at the Hangzhou Normal University, Zhejiang Province, China (30.03° N, 120.12° E). Soil samples were air-dried and sieved through 2 mm sieve. The properties were as follows: 1.77 g kg⁻¹ total N, 1.80 g kg⁻¹ total P, 7.91 g kg⁻¹ total K, and 0.1 g kg⁻¹ total Zn. For the excess Zn treatment, 1 L deionized water containing 100 µM ZnSO₄ was used to evenly irrigate the pots every two days until the final Zn level of 50 mg ZnSO₄ kg⁻¹ dry soil was reached, while the control plants were irrigated with equal quantities of deionized water (Chen et al., 2016).

The five plantation types were as follows: F, single-cultivated females; M, single-cultivated males; FF, female × female; MM, male × male; FM, female × male. Intrasexual neighboring plants were denoted as M/MM for males and F/FF for females. Intersexual neighboring plants were denoted as M/FM for males and F/FM for females. For single-plant cultivation, one cutting was planted per pot (a female or a male); for

interactions, two plants (two females, two males or a female and a male) were cultivated 10 cm apart from each other in a plastic pot (external diameter 52 cm and height 35 cm). All pots were arranged randomly and each treatment was replicated four times. The experiment was performed at the Hangzhou Normal University. The plants were cultured for 120 d in a semi-controlled greenhouse at the Hangzhou Normal University (China, 30.03° N, 120.12°E) with a relative humidity of 76%-81%, a daytime temperature of 21-25 °C, a night-time temperature of 15-18 °C and a photoperiod of 12-14 h throughout the growth period.

2.2. Gas exchange and fluorescence measurements

The fully developed young leaves from each plant were used to measure gas exchange and chlorophyll fluorescence. Net CO₂ assimilation rate (A) and stomatal conductance (g_s) were measured with a portable photosynthesis measuring system (LI-6400), as described previously by Chen et al. (2011). Chlorophyll fluorescence kinetics parameters (ETR , electron transport rate; quantum yield of photochemical energy conversion in $PS II$, $Y(II)$; quantum yield of regulated non-photochemical energy loss in $PS II$, $Y(NPQ)$; F_v/F_m , variable and maximum fluorescence) were measured with a PAM chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany). These parameters were calculated according to the method of Van Kooten and Snel (1990).

2.3. Soil sampling, plant harvesting and element measurements

187

188 After the measurement of photosynthesis, rhizosphere soil was collected by gently

189 shaking a plant root; the soil adhering to the root was collected as a sample of

190 rhizospheric soil. In total, 40 soil samples (4 replicates $\times$ 5 interaction patterns $\times$ 2

191 treatments) were immediately sieved (4 mm) in the laboratory. The rhizospheric soils

192 were divided into two subsamples. One subsample was oven dried at 75 °C and used

193 for analyzing soil properties, and another subsample was stored at -80 °C until DNA

194 extraction.

195

196 The plants were separated into leaves, stems and roots, and washed with deionized

197 water. Dried leaves and roots were finely ground, and about 0.4 g samples (< 1 mm)

198 were dissolved in 3:1 (v/v) of HNO₃ and HClO₄. The mixtures were carefully shaken

199 and predigested at room temperature for 30 min. The vessels used for digestion were

200 sealed and placed into a microwave digestion system. The microwave heating program

201 was performed as follows: (1) 10 min at 170 °C; (2) 10 min at 190 °C; (3) 10 min at

202 210 °C (Tokaloğlu et al., 2018). The vessels were cooled down to room temperature to

203 avoid splashing and foaming. Clear digested solutions were transferred to 25 ml

204 polyethylene tubes, which were filled to a volume of 25 ml with ultra-pure water for

205 further ICP-MS analysis. Total Zn and nutrient elements were measured with ICP-MS

206 (inductively coupled plasma mass spectrometer; Agilent 7500a; Agilent Technologies).

207 For quality assurance/quality control (QA/QC) purposes, a blank control and standard

208 reference material GBW10020 (GSB-11) of citrus leaves were used to validate

209 quantification according to Xie et al. (2020). Blank control and reference materials were

treated under the same conditions as the experimental samples. The recovery of the standard at a medium calibration level was checked with every 10 samples. The method detection limits (mg kg^{-1}) were 0.2 mg l^{-1} for Zn and the recovery rates were 80-90%. All data are presented as means $\pm$ the standard deviations, unless specified differently.

The translocation factor (T_f) was defined as the Zn concentration in a shoot divided by the Zn concentration in roots (He et al., 2013b). The bio-concentration factor BCF was calculated as the Zn concentration in roots or shoots divided by the Zn concentration in the soil (Shi et al., 2010; He et al., 2013b).

2.4. Statistical analysis

Statistical analyses were carried out using the SPSS software package (version 22.0). All data were checked for normality before analyses of variance (ANOVAs). Differences between means were analyzed by Duncan's tests following three-way ANOVAs, which were used to evaluate sexual interaction and excess Zn treatment effects.

2.5. DNA extraction, 16S amplification and MiSeq sequencing

Approximately 0.5 g of each soil sample was used to extract genomic DNA utilizing the PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc. Carlsbad, USA) following

the manufacturer's instructions. The 16S rRNA genes were amplified using the primer pair 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Approximately 10 ng of purified DNA was amplified with 25 µl of the PCR reaction system under the following conditions: at 95 °C 2 min for denaturation, followed by 40 cycles of 10 s at 95 °C for denaturation, 30 s at 56 °C for annealing, and 20 s at 72 °C for extension, with 55 to 95 °C for melting curve analyses. The PCR products were pooled and purified with a MicroElute Cycle-Pure Kit (Omega), and high-throughput sequencing was performed using the Hiseq 2500 PE250 platform with 2×250 bp according to the manufacturer's manual.

2.6. Processing of high-throughput sequencing data and analysis

Amplicon sequences were processed using the QIIME 2 version 2017.12 (<https://qiime2.org/>). All sequences of 16S rRNA raw data were demultiplexed, and quality control was carried out using DADA2 with the “consensus” method, to remove chimeric and low-quality sequences (Callahan et al., 2017; Yuan et al., 2018). When the paired-ends were joined, the unreliable and low-quality sequences were detected based on the low sequence quality of the 3'-ends of the reads (Merloti et al., 2019). After that, the Amplicon Sequence Variants (ASVs) were created using the Deblur tool. The resulting final ASV table contained only high-quality reads. The taxonomic identification of ASVs (with 99% of similarity) was conducted with the VSEARCH consensus taxonomy classifier implemented in Qiime2 and the SILVA 16S rRNA

database. The archaeal and chloroplast sequences were removed. Statistical analyses of the 16S rRNA microbiome sequencing data were performed using the generated taxonomic matrices. The sequencing data were submitted to NCBI (BioProject accession number: PRJNA644210).

2.7. Statistical analysis of sequencing data

The α -diversity of bacteria was characterized by the Simpson's diversity index and the effective number of species, and the data were analyzed with ANOVA using Duncan's test ($P < 0.05$). β -diversity was calculated based on weighted-UniFrac distance metrics. Principal coordinates analysis (PCoA) was used to separate the overall bacterial community structure based on the weighted-UniFrac distance with the "pcoa" function of the "ape" package in R (v3.2.2). Permutational multivariate analysis of variance (PERMANOVA) was performed to separate and evaluate the effects of sex, plantation mode and Zn treatment and their interactions on the rhizospheric bacterial communities using the "anosims" function in "vegan" package in R (v3.2.2). The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to analyze the relative bacterial abundance in different treatments, irrespective of sexual interactions (Class: Zn treatment; Subclass: interaction patterns), as well as in different sexual interaction patterns irrespective of Zn treatment (Class: sexual interaction; Subclass: Zn treatment). The online Galaxy application (version 1.0) was used with a threshold of 1.0 and a Wilcoxon P -value of 0.05 (<http://huttenhower.sph.harvard.edu/galaxy/>). A similarity

percentage (SIMPER) analysis was performed with the PRIMER 6 software to find differences between sexual interaction patterns and other treatments. The OTUs less than 1% of the relative abundance were discarded from the analysis according to Marasco et al. (2018). Correlation coefficients between the bacterial abundance at the phyla level and Zn amount were obtained with python (SciPy package). The heatmap of correlation coefficients was plotted with R (Heatmap package).

2.8. PICRUSt functional prediction

The *PICRUSt* (*phylogenetic investigation of communities by reconstruction of unobserved states*, v1.0.0) pipeline was used to predict the relative abundance of gene transcripts in bacteria. The *pick_closed_reference_otus.py* script in QIIME (similarity threshold, 0.97) was used to cluster sequences into OTUs. The OTUs were first normalized by the copy number by removing the copy number of the 16S marker gene. The Nearest Sequenced Taxon Index (NSTI) and KEGG Ortholog (KO) were obtained by the *predict_metagenomes.py* script. The KO level 3 within the pathway hierarchy of KEGG was collapsed using the *categorize_by_function.py* script (Langille et al., 2013; Hou et al., 2017). The predicted metagenomes were then annotated using the KEGG database, and the predicted relative abundances of gene transcripts for selected pathways associated with bacterial functions were analyzed and plotted using STAMP (Parks et al. 2014; Hou et al. 2017).

3. Results

3.1. Plant growth and Zn phytoextraction

Under control conditions (no excess Zn), males showed 21% and 48% higher CO₂ assimilation rate (A) and stomatal conductivity (g_s) respectively, than females under single-plant cultivation (Table 1). Females had lowest A and g_s under intersexual interaction (F/FM) under control conditions (Table 1). Compared with controls, A and g_s decreased by 29% and 18%, respectively, in females and by 19% and 22%, respectively, in males in single-sex cultivation under excess Zn (Table 1). Excess Zn decreased A by 18%, 11%, 12% and 16%, and g_s by 20%, 22%, 32% and 57%, in F/FM, M/FM (males in intersexual interaction), F/FF (females in intrasexual interaction) and M/MM plants (males in intrasexual interaction), respectively (Table 1). In addition, M/FM exhibited 10% and 55% increases in A and g_s compared to M/MM under excess Zn conditions (Table 1).

Excess Zn treatment decreased F_v/F_m , ETR and $Y (II)$ by 6%, 37% and 50%, respectively, in females and by 4%, 17% and 8%, respectively, in males under single-sex cultivation (Table 1). Sexual interactions did not affect F_v/F_m in either sex under control conditions (Table 1). Excess Zn did not affect F_v/F_m and ETR in males (M/MM) and females (F/FF) when compared with controls. However, F_v/F_m , ETR and $Y (II)$ were 4%, 37% and 54% higher, respectively, in M/FM plants than in F/FM plants under excess Zn, and $Y (II)$ of males was 13% higher when compared to females under

intrasexual interaction (Table 1). In addition, excess Zn treatment increased $Y(NPQ)$ by 71% in females, but not in males, under single-plant cultivation (Table 1) when compared to control conditions. Sexual interactions did not affect $Y(NPQ)$ in either sex under control conditions. However, excess Zn increased $Y(NPQ)$ by 32%, 58% and 14% in F/FM, F/FF and M/MM, respectively, when compared to controls (Table 1).

Excess Zn increased the Zn content in by 22.9- and 4.3-fold, respectively, in leaves, by 3.1- and 1.4-fold, respectively, in shoots, and by 2.7- and 3.2-fold, respectively, in roots of females and males under single-sex cultivation (Fig. 1). Under excess Zn conditions, Zn levels increased by 4.6-, 29.6-, 13.6- and 13.1-fold, respectively, in leaves, by 97%, 300%, 327% and 219%, respectively, in stems, and by 160%, 240%, % and 160%, respectively, in roots of F/FM, M/FM, F/FF and M/MM when compared to controls (Fig. 1). Under excess Zn, the Zn level of females was 37% and 39% higher in leaves and roots, respectively, but 82% lower in stems when compared to males in single-sex cultivation under excess Zn conditions. The leaf and stem Zn levels of males were lower than those of females under intersexual interaction; the contrary was true for intrasexual interaction under excess Zn (Fig. 1). In addition, there was no difference in root Zn concentrations between sexes under intersexual interaction, whereas the Zn level in roots of M/MM was 26% lower than that of F/FF plants.

Females had higher T_f (the ratio of Zn in the shoots to roots) than males under single-sex cultivation. T_f was higher in both sexes under single-sex cultivation than under

intersexual interactions (except for F/FF) when exposed to excess Zn (Fig. 1). F/FM had highest T_f and F/FF showed the lowest T_f among all excess Zn treatments (Fig. 1). Under excess Zn, M/MM showed 16% higher T_f than M/FM. In single-sex cultivations under excess Zn, females had 141% and 98% increases, respectively, in root BCF (the ratio of Zn in the roots to soil) and shoot BCF (the ratio of Zn in the shoots to soil) when compared to males. F/FF had highest root BCF among all excess Zn treatments. Root and shoot BCF values were 48% and 72% higher, respectively, in M/MM than in M/FM under excess Zn, while females had 1.2-fold higher shoot BCF than males under intersexual interaction with excess Zn (Fig. 1).

3.2. Dominant taxa of bacterial communities

The bacterial communities predominantly consisted of Proteobacteria (33%), Actinobacteria (17%), Acidobacteria (16%), Chloroflexi (14%), Planctomycetes (6%), Bacteroidetes (4%), Gemmatimonadetes (2%), Crenarchaeota (2%), Verrucomicrobia (1%), and Nitrospirae (1%). These dominant species were affected by plant interactions and excess Zn (Fig. 2). Excess Zn reduced the relative abundance of the Proteobacteria phylum in the rhizosphere of M/MM and FM, but did not affect Proteobacteria in the rhizosphere of F/FF. Excess Zn reduced the abundance of Actinobacteria and Planctomycetes phyla in both sexes under intersexual interactions. The relative abundance of Proteobacteria and Actinobacteria phyla were higher under the excess Zn treatment than under control conditions in both sexes when plants were cultivated alone,

while the contrary was true for the Chloroflexi phylum (Fig. 2).

3.3. Bacterial community diversity and structure

The alpha-diversities of bacterial communities (effective numbers of species) were compared in females and males under different plantation modes and excess Zn treatments. As shown in Fig. 3, intra- and intersexual interaction affected bacterial community structures, but the effects of these interaction patterns were greater under excess Zn. Permutational multivariate analysis of variance (PERMANOVA) further demonstrated that plantation modes were the largest source of variation (25.16%, $P < 0.001$; Table 2). The Zn levels were the second largest source of variation (5.00%, $P < 0.004$; Table 2).

3.4. Taxonomic composition of bacterial communities

The linear discriminant analysis (LDA) effect size analysis (LEfSe) was performed to compare the bacterial composition from phyla to genera between Zn treatments, as well as between sexes (Fig. 4). We found that bacterial compositions showed significant differences among sexual interaction patterns and Zn treatments. The orders Acidimicrobiales, Micromonosporaceae, RB40, Verrucomicrobiales, Thiotrichales, MND1, Piscirickettsiaceae and Pirellulaceae were predominant under excess Zn, while the genera *Pontibacter*, *Chryseobacterium*, *Lysobacter*, *Kaistobacter* and *Massilia*, and

the class Flavobacteria were enriched in plants without excess Zn under single cultivation (Fig. 4a). Moreover, the order Sva0725, the family A4b and the genus *Rubrivivax* were dominant in the rhizosphere of males, while other taxa were enriched in soil with females (Fig. 4b).

In sexual interaction experiments, the order Nitrososphaerales was generally more abundant in the soil of control plants (Fig. 4c). Under excess Zn, the class Saprospirae, the orders GCA004, Solirubrobacterales and AKYG1722, the families *AKIW874*, *Cellulomonadaceae*, *Mycobacteriaceae* and *Sporichthyaceae*, and the genera *Bacillus*, *Rhodoplanes* and *Mycobacterium* were predominant (Fig. 4d). Irrespective of the Zn treatment, the genera *Agromyces* and *Mycobacterium*, as well as the family *Mycobacteriaceae* were abundant in the soil of M/MM, whereas the order Methanobacteriales and the genus *Methanothermobacter* were more abundant in the soil of F/FF under intrasexual interaction (Fig. 4d). In contrast, orders Caldilineales and NB1-j were more prevalent in the soil of FM exposed to intersexual interaction (Fig. 4d).

3.5. Functional predictions of bacterial communities

The PICRUSt analysis was performed to predict the metagenome gene functions. As shown in Fig. 5, the PICRUSt analysis suggested that the predicted differential gene abundances between treatments were related to carbohydrates, amino acids, lipids and

secondary metabolism in the rhizosphere of females and males under single-plant cultivation. When compared to females under single-plant cultivation, males had a higher abundance of predicted genes involved in the citrate cycle and carotenoid biosynthesis in the rhizosphere in control conditions (Fig. 5a). Excess Zn stress reduced arachidonic acid metabolism, flavonoid biosynthesis and peptidoglycan, and increased the abundance of predicted genes related to glycolysis, citrate cycle and leucine synthesis in females under single-plant cultivation.

Under plant interactions, the differentially abundant genes in different treatments in the rhizospheres of both sexes were related to amino acid translation, and amino acid and lipolic acid metabolism under single-plant cultivation (Fig. 5b). Excess Zn reduced the abundance of the predicted genes related to cysteine, methionine and thiamine metabolism, especially in the rhizosphere of females exposed to intrasexual interaction. However, excess Zn elevated the abundance of predicted genes related to tyrosine metabolism in the rhizosphere of females, compared to males, both under inter- and intraspecific interaction (Fig. 5b).

3.6. Relative abundance of key taxa associated with Zn levels in plants

We examined the bacterial abundance in the rhizosphere at the genus level in relation to Zn levels in roots (Fig. 6). First, we examined the relative abundance of taxa positively associated with Zn levels. Under single-plant cultivation, females showed a

relatively higher abundance of families Streptomycetaceae and Rhodobacteraceae, and the phylum Chloroflexi than males under an increasing Zn supply in the rhizosphere. In contrast, the increased Zn level raised the relative abundance of the family Sphingomonadaceae in the rhizosphere of males compared to the female rhizosphere. Under intrasexual interaction, males had a higher relative abundance of the Actinomycetales order and Phyllobacteriaceae family when compared to intersexual interaction (Fig. 6). By contrast, the family Alphaproteobacteria was more abundant in the rhizosphere of males exposed to excess Zn under intersexual interaction than under intrasexual interaction. In females, the orders Myxococcales, Roseiflexales and Actinomycetales, and the class Betaproteobacteria were predominant in the rhizosphere exposed to intersexual interaction under excess Zn relative to intrasexual interaction (Fig. 6). The increased abundance of the order Rhizobiales under excess Zn was specific to females exposed to intrasexual interaction relative to intersexual interaction. Under intersexual interaction, the abundance of the order Actinomycetales was higher in males, while the order Rhizobiales was more abundant in the rhizosphere of females under excess Zn (Fig. 6).

Then, we examined the relative abundance of bacteria negatively associated with Zn levels in roots (Fig. 6b). We found that under excess Zn, the relative abundance of most bacterial classes, such as Planctomycetia, Betaproteobacteria and Acidobacteria-6, were lower in the rhizosphere of females than in that of males under single-sex conditions. When compared to females under single-sex conditions, males had a higher

abundance of the family Hyphomonadaceae and the order WD2101 irrespective of the Zn application. Under intrasexual interaction with excess Zn, the abundance of the class Gemm-5 in the rhizosphere of males, and the abundance of the genus *Planctomyces* in females and males were higher than under intersexual interaction (Fig. 6b). On the other hand, under intersexual interaction with excess Zn, the abundance of the RB40 family was higher but the abundance of the genus *Planctomyces* was lower in the rhizosphere of females than in males.

4. Discussion

4.1. Sexual interactions affect Zn phytoextraction in plants

Neighbor interactions of plants affect plant growth and community composition (Hodge and Fitter, 2013; Hawkins and Crawford, 2018). In turn, these interactions could be altered by abiotic stress, e.g. heavy metals (Chen et al., 2016, 2017). It has been shown that abiotic stress can alter competitive interactions (Chen et al., 2016, 2017). Selenium has been found to strongly influence plant-plant interactions and play a vital role in elemental allelopathy (El Mehdawi et al., 2011). In this study, under excess Zn stress, F/FM accumulated more Zn in their leaves and barks compared to F/FF, while males accumulated less under intersexual interaction than under intrasexual interaction (Table 1; Fig. 1). Responses of sexes to abiotic stresses depend mainly on the specific properties of stresses, the soil status and the exposure duration (Howard et al., 2000;

Tonneijck et al., 2004). Zn is an essential trace element for plants and, in our study, the leaf Zn levels did not reach the toxic threshold for poplars under any interaction (Fig. 1). Females and males exposed to inter- and intrasexual interaction showed strong tolerance to excess Zn (Table 1). Interestingly, we found that under intrasexual interaction both females and males accumulated more Zn in the roots, which largely inhibited Zn transportation to the shoots and leaves (Fig. 1). The reduced heavy metal accumulation in leaves facilitates heavy metal tolerance in woody plants (He et al. 2013a).

The tolerance of plants to heavy metals is also reflected in leaf photosynthesis (Chandra and Kang, 2016; Salisbury et al., 2018), which indicates the ability of plants to thrive in specific environments, including heavy metal stress (Simkin et al., 2019; Hu et al., 2020). The accumulation of heavy metal in leaves damages photosynthetic electron transport and reduces photosynthesis, which are signs of stress (Aggarwal et al., 2012). We found that females had higher Zn enrichment but lower Zn toxicity tolerance than males under Zn treatments. The inhibited photosynthesis and electron transmission efficiency were associated with Zn translocation into leaves (Fig. 1; Table 1). Females had higher root-to-shoot translocation under intrasexual than intersexual interaction, as visible as elevated root BCF but lower T_f under excess Zn (Fig. 1). However, males favored to accumulate more Zn in roots, showing higher root BCF but lower shoot BCF (Fig. 1). Moreover, females had a higher Zn translocation efficiency (T_f) under intersexual than intrasexual interaction, while the contrary was true for males (Fig. 1).

Moreover, the Zn translocation efficiency was higher in females than in males when subjected to excess Zn under intersexual interaction. In previous studies, female plants have showed higher competitive ability than males (Mercer and Eppley, 2010; Sánchez-Vilas et al., 2011; Chen et al., 2016). Consistently, we found that females had a stronger competitive ability under excess Zn even when excess Zn was toxic for plants. In a previous study, we found that females had a higher expression of genes, such as those from the ZIP family, which facilitate Cd uptake and root-to-shoot translocation in *P. cathayana* (Liu et al., 2020b). Importantly, the potential of females and males for extracting Zn also depends on underground processes that affect Zn availability (Fig. 6).

4.2. Soil microbial composition modified by plantation modes and Zn pollution

Heavy metals affect rhizosphere-driven microbial community structures (Hou et al., 2017). Soil microorganisms are also critical drivers in modifying community structures and plant-plant interactions (Hodge and Fitter, 2013; Hawkins and Crawford, 2018). The sexually differential trade-off between plant rewards and defense has been shown to influence the colonization of host-specific microbial communities (Bever et al., 2012; Varga et al., 2017). This finding was further demonstrated by our study. We discovered that the bacterial community diversity and richness in the rhizosphere of both sexes were not affected by plantation modes or excess Zn, but probably associated with the planting time and species (Siciliano et al., 2014; Tian and Gao, 2014; Fontana et al.,

2016). However, the bacterial community structure was strongly affected by plantation modes and Zn treatments (Fig. 3), which probably affected root Zn bioavailability.

Generally, heavy metals suppress the growth and abundance of low-resistance microbes but increase the prevalence of high-resistance bacteria (Pishchik et al., 2016; Wood et al., 2016; Hou et al., 2017). Consistently, the relative abundance of Cytophagales was significantly inhibited by excess Zn in both sexes, while the abundance of key phyla, such as Betaproteobacteria, Acidobacteria, Nitrospirales and Proteobacteria, increased under excess Zn in single-cultivated males (Fig. 4; Figs S1-S2). Interestingly, the abundance of Betaproteobacteria, Acidobacteria, Nitrospirales and Proteobacteria phyla were positively associated with root Zn levels (Fig. 6), which suggested that they are probably involved in excess Zn uptake. In addition, we found that when compared to untreated soil, Zn levels in excess Zn-treated soil were 3~7 -fold higher (Fig. S5), and the level was higher than the Zn toxicity risk screening value for soil (Huang, 2014; Carruthers, 2016). Our results support the view that phytoremediation may be an effective strategy to improve the soil quality by recruiting some beneficial microbes (Ancona et al., 2020). It is evident that if attempting to use microbial remediation of metals, it would be important to investigate further the responses of specific bacterial families.

Sexual interactions alter the structure of root systems and the secretion of root exudates in *P. cathayana* females and males, thus modifying the rhizosphere ecology (Ke and

Wang, 2020; Xia et al., 2020). The present study found that the Methanotheriales, Mycobacterium and Caldilineales phyla were enriched in the rhizosphere of F/FF, M/MM and FM, respectively (Fig. 4). Among these specific bacteria, the Actinobacteria and Streptomyces phyla have been identified as the most dominant taxa in soils with heavy metal contamination (Watve et al., 2001; Ellis et al., 2003; Alvarez et al., 2017), and they became more abundant under excess Zn in the rhizosphere of M/MM and M/FM (Fig. 4; Figs S3-S4). More importantly, the relative abundance of Actinobacteria showed a positive correlation with root Zn levels in M/MM and FM (Fig. 6). Actinobacteria produce siderophores for Cd acquisition and protect plants from the invasion of pathogenic bacteria (Viaene et al., 2016). The specific colonization of Actinobacteria in the rhizosphere of *Arabidopsis* confers a competitive advantage to these plants (Van der Meij et al., 2018). The increased abundance of Actinobacteria following the excess Zn treatment in the rhizosphere of FM was probably due to Zn chelation, which was likely the result of a long-term adaptation of sexes to excess Zn under intersexual interaction. *Streptomyces* is the largest genus producing antibiotics (Watve et al., 2001; Hong et al., 2009). It has been suggested that some strains belonging to *Streptomyces* promote metal solubility and auxin synthesis by stimulating siderophore synthesis (Zloch et al., 2016; Hou et al., 2017). Taking into account the higher Zn accumulation in females under single-sex and intersexual modes (Fig. 1), Streptomycetaceae probably plays a key role in the Zn accumulation in *P. cathayana* females under excess Zn.

We also found some sex-specific bacterial colonization in the *P. cathayana* rhizosphere under different interaction patterns. The excess Zn treatment increased the abundance of the phylum Euryarchaeota in both females and males with intrasexual interaction (Figs S3-S4). In addition, the abundances of the phyla Actinobacteria and Chloroflexi were elevated under excess Zn in the rhizosphere of FM and M/MM (Fig. 4; Figs S3-S4). Overall, our results suggest that most bacteria prefer to colonize the rhizosphere of both sexes in the case of intersexual interaction, irrespective of Zn levels, as well as in the rhizosphere of males exposed to intrasexual interaction.

4.3. Microbial functional prediction highlights the role of sexual interaction patterns

The PICRUSt analysis suggested that excess Zn and sexual interactions differentially regulate the abundance of predicted gene transcripts of bacteria in the rhizosphere. The excess Zn treatment increased the abundance of predicted gene transcripts related to amino acids and organic acids, such as valine, leucine and isoleucine biosynthesis, and the citrate cycle in both sexes under single-plant cultivation, which probably increased organic matter from dead roots, exudates and rhizodeposits (Hou et al., 2017, 2018). It is worth noting that the abundance of predicted gene transcripts related to carotenoid biosynthesis was higher in the rhizospheric bacteria of males than in those of females (Fig. 5a), which probably played an important role in heavy metal tolerance. Excess Zn increased the abundance of predicted gene transcripts related to tyrosine metabolism and secondary metabolites in males exposed to intersexual interaction and in females

under both intra- and intersexual interaction, suggesting that excess Zn differentially regulates the abundance of predicted gene transcripts. Further studies are needed to explore the relationship between heavy metal stress and gene expression among the bacteria in the rhizospheres of both sexes.

5. Summary

This study suggested that excess Zn and sexual interactions strongly affect Zn accumulation, and microbial abundance and diversity. Females have higher Zn accumulation and root-to-shoot translocation under intersexual than under intrasexual interaction combined with excess Zn; the contrary was true for males. Moreover, the plantation modes and excess Zn treatment altered the bacterial structure of the rhizosphere, which largely affected the Zn availability and uptake of roots. The Zn-polluted soil with males growing under different interactions promoted some key bacterial taxa related to metal activation and chelation in the rhizosphere, e.g., Actinobacteria and Streptomyces, while the excess Zn-treated soil with females had a lower bacterial abundance under intrasexual interaction than under intersexual interaction. To our knowledge, this is the first study that has showed a possible relationship of Zn availability with specific bacterial colonization in the rhizosphere of *P. cathayana* females and males under sexual interactions. Our study provides new

insight into the interactions among plant sex, plantation types, heavy metal stress and the composition of microbial communities.

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Author contributions Miao Liu had the main responsibility for data collection, analysis and writing, Yuting Wang and Xiucheng Liu contributed to data collection, 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.

Appendix A. Supplementary data Supplementary material related to this article can be found in the supplementary information.

References

- Aggarwal, A., Sharma, I., Tripathi, B.N., Munjal, A.K., Baunthiyal, M., Sharma, V., 2012. Metal toxicity and photosynthesis. In: *Photosynthesis: overviews on recent progress & future perspectives*. 1st ed. New Delhi: I K International Publishing House Pvt. Ltd; 229–236.
- Alvarez, A., Saez, J.M., Davila Costa, J.S., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C.S., Polti, M.A., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere* 166, 41–62.
- Ancona, V., Caracciolo, A.B., Campanale, C., Rascio, I., Grenni, P., Di Lenola, M., Bagnuolo, G., Uricchio, V.F., 2020. Heavy metal phytoremediation of a poplar clone in a contaminated soil in southern Italy. *J. Chem. Technol. Biot.* 95, 940–949.
- Arthur, E.L., Rice, P.J., Rice, P.J., Anderson, T.A., Baladi, S.M., Henderson, K.L., Coats, J.R., 2005. Phytoremediation - an overview. *Critical Rev. Plant Sci.* 24, 109–122.
- Ayangbenro, A.S., Babalola, O.O., 2017. A New strategy for heavy metal polluted environments: a review of microbial biosorbents. *Inter. J. Environ. Public Health* 14, 94.

649 Bever, J.D., Platt, T.G., Morton, E.R., 2012. Microbial population and community
650 dynamics on plant roots and their feedbacks on plant communities. *Annu. Rev.*
651 *Microbiol.* 66, 265–283.

652

653 Bi, J.W., Liu, X.C., Liu, S.R., Wang, Y.T., Liu, M., 2020. Microstructural and
654 physiological responses to cadmium stress under different nitrogen forms in two
655 contrasting *Populus* clones. *Environ. Exp. Bot.* 169, 103897.

656

657 Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., Lux, A., 2007. Zinc in
658 plants. *New Phytol.* 173, 677–702.

659

660 Burd, G.I., Dixon, D.G., Glick, B.R., 2000. Plant growth-promoting bacteria that
661 decrease heavy metal toxicity in plants. *Can. J. Microbiol.* 46, 237–245.

662

663 Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should
664 replace operational taxonomic units in marker-gene data analysis. *ISME J.* 11, 2639–
665 2643.

666

667 Carruthers, S., 2016. Zinc: deficiency and toxicity. *Practical Hydroponics and*
668 *Greenhouses* 172, 42.

669

670 Chandra, R., Kang, H., 2016. Mixed heavy metal stress on photosynthesis, transpiration

rate, and chlorophyll content in poplar hybrids. For. Sci. Technol. 12, 55–61.

Chen, L.H., Han, Y., Jiang, H., Korpelainen, H., Li, C.Y., 2011. Nitrogen nutrient status induces sexual differences in responses to cadmium in *Populus yunnanensis*. J. Exp. Bot. 62, 5037–5050.

Chen, J., Duan, B.L., Wang, M.L., Korpelainen, H., Li, C.Y., 2014. Intra-and inter-sexual competition of *Populus cathayana* under different watering regimes. Funct. Ecol. 28, 124–136.

Chen, J., Duan, B.L., Xu, G., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2016. Sexual competition affects biomass partitioning, carbon–nutrient balance, Cd allocation and ultrastructure of *Populus cathayana* females and males exposed to Cd stress. Tree Physiol. 36, 1353–1368.

Chen, J., Han, Q.Q., Duan, B.L., Korpelainen, H., Li, C.Y., 2017. Sex-specific competition differently regulates ecophysiological responses and phytoremediation of *Populus cathayana* under Pb stress. Plant Soil 421, 203–218.

Duruibe, J.O., Ogwuegbu, M.O.C., Egwurugwu, J.N., 2007. Heavy metal pollution and human biotoxic effects. Inter. J. Physical. Sci. 2, 112–118.

693 El Mehdawi, A.F., Quinn, C.F., Pilon-Smits, E.A., 2011. Effects of selenium
 694 hyperaccumulation on plant-plant interactions: evidence for elemental allelopathy?
 695 *New Phytol.* 191, 120–131.

696

697 Ellis, R.J., Morgan, P., Weightman, A.J., Fry, J.C., 2003. Cultivation-dependent and -
 698 independent approaches for determining bacterial diversity in heavy-metal-
 699 contaminated soil. *Appl. Environ. Microbiol.* 69, 3223–3230.

700

701 Fontana, A., Patrone, V., Puglisi, E., Morelli, L., Bassi, D., Garuti, M., Rossi, L., Cappa,
 702 F., 2016. Effects of geographic area, feedstock, temperature, and operating time on
 703 microbial communities of six full-scale biogas plants. *Bioresource Technol.* 218, 980–
 704 990.

705

706 Guo, Q.X., Yan L.J., Korpelainen, H., Niinemets, Ü., Li C.Y., 2019. Plant-plant
 707 interactions and N fertilization shape soil bacterial and fungal communities. *Soil Biol.*
 708 *Biochem.* 128, 127–138.

709

710 Hawkins, A.P., Crawford, K.M., 2018. Interactions between plants and soil microbes
 711 may alter the relative importance of intraspecific and interspecific plant competition in
 712 a changing climate. *AoB Plants* 10, ply039.

713

714 He, J.L., Li, H., Luo, J., Ma, C.F., Li, S.J., Qu, L., et al., 2013a. A transcriptomic

715 network underlies microstructural and physiological responses to cadmium in
 716 *Populus × canescens*. Plant Physiol. 162, 424–439.

717

718 He, J.L., Li, H., Ma, C.F., Zhang, Y.L., Polle, A., Rennenberg, H., Cheng, X.Q., Luo,
 719 Z.B., 2015a. Overexpression of bacterial γ -glutamylcysteine synthetase mediates
 720 changes in cadmium influx, allocation and detoxification in poplar. New Phytol. 205,
 721 240–254.

722

723 He, J., Ma, C., Ma, Y., Li, H., Kang, J., Liu, T., Polle, A., Peng, C.H., Luo, Z.B., 2013b.
 724 Cadmium tolerance in six poplar species. Environ. Sci. Pollut. R. 20, 163–174.

725

726 Hong, K., Gao, A.H., Xie, Q.Y., Gao, H.G., Zhuang, L., Lin, H.P., Yu, H.P., Li, J., Yao,
 727 X.S., Goodfellow, M., Ruan, J.S., 2009. Actinomycetes for marine drug discovery
 728 isolated from mangrove soils and plants in China. Mar. Drugs 7, 24–44.

729

730 Hou, D.D., Wang, K., Liu, T., Wang, H.X., Lin, Z., Qian, J., Lu, L.L., Tian, S.K., 2017.
 731 Unique rhizosphere micro-characteristics facilitate phytoextraction of multiple metals
 732 in soil by the hyperaccumulating plant *Sedum alfredii*. Environ. Sci. Technol. 51, 5675–
 733 5684.

734

735 Hou, D.D., Wang, R.Z., Gao, X.Y., Wang, K., Lin, Z., Ge, J., Liu, T., Wei, S., Chen,
 736 W.K., Xie, R.H., et al., 2018. Cultivar-specific response of bacterial community to

737 cadmium contamination in the rhizosphere of rice (*Oryza sativa* L.). Environ. Pollut.
738 241, 63–73.

739

740 Hodge, A., Fitter, A.H., 2013. Microbial mediation of plant competition and community
741 structure. Funct. Ecol. 27, 865–875.

742

743 Howard, R.J., Mendelsohn, I.A., 2000. Structure and composition of oligohaline
744 marsh plant communities exposed to salinity pulses. Aquatic Bot. 68, 143–164.

745

746 Hu, W., Lu, Z., Meng, F., Li, X., Cong, R., Ren, T., Sharkey, T.D., Lu, J., 2020. The
747 reduction in leaf area precedes that in photosynthesis under potassium deficiency: the
748 importance of leaf anatomy. New Phytol. <https://doi.org/10.1111/nph.16644>.

749

750 Huang, S.H., 2014. Fractional distribution and risk assessment of heavy metal
751 contaminated soil in vicinity of a lead/zinc mine. T. Nonferr. Metal. 24, 3324–3331.

752

753 Juvany, M., Munné-Bosch, S., 2015. Sex-related differences in stress tolerance in
754 dioecious plants: a critical appraisal in a physiological context. J. Exp. Bot. 66, 6083–
755 6092.

756

757 Ke, P.J., Wan, J., 2020. Effects of soil microbes on plant competition: a perspective
758 from modern coexistence theory. Ecol. Monogr. 90, 1391.

759

760 Koelbener, A., Ramseier, D., Suter, M., 2008. Competition alters plant species response
761 to nickel and zinc. *Plant Soil* 303, 241–251.

762

763 Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A.,
764 Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R., Beiko, R.G., Huttenhower,
765 C., 2013. Predictive functional profiling of microbial communities using 16S rRNA
766 marker gene sequences. *Nature Biotech.* 31, 814–821.

767

768 Li, X.Q., Meng, D.L., Li, J., Yin, H.Q., Liu, H.W., et al., 2017. Response of soil
769 microbial communities and microbial interactions to long-term heavy metal
770 contamination. *Environ. Pollut.* 231, 908–917.

771

772 Li, Y., Duan, B.L., Chen, J., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2016. Males
773 exhibit competitive advantages over females of *Populus deltoides* under salinity stress.
774 *Tree Physiol.* 36, 1573–1584.

775

776 Liu, M., Bi, J.W., Liu, X.C., Kang, J.Y., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2020a.
777 Microstructural and physiological responses to cadmium stress under different nitrogen
778 levels in *Populus cathayana* females and males. *Tree Physiol.* 40, 30–45.

779

780 Liu, M., Liu, X., Kang, J.Y., Korpelainen, H., Li, C.Y., 2020b. Are males and females

781 of *Populus cathayana* differentially sensitive to Cd stress? J. Hazard. Mater. 25, 122411.
782

783 Lu, L.L., Tian, S.K., Zhang, J., Yang, X.E., Labavitch, J.M., Webb, S.M., Latimer, M.,
784 Brown, P.H., 2013. Efficient xylem transport and phloem remobilization of Zn in the
785 hyperaccumulator plant species *Sedum alfredii*. New Phytol. 198, 721–731.
786

787 Marasco, R., Mosqueira, M.J., Fusi, M., Ramond, J.B., Merlino, G., Booth, J.M.,
788 Daffonchio, D., 2018. Rhizosheath microbial community assembly of sympatric desert
789 speargrasses is independent of the plant host. Microbiome 6, 215.
790

791 Mercer, C.A., Eppley, S.M., 2010. Inter-sexual competition in a dioecious grass.
792 Oecologia 164, 657–664.
793

794 Merloti, L.F., Mendes, L.W., Pedrinho, A., de Souza, L.F., Ferrari, B.M., Tsai, S.M.,
795 2019. Forest-to-agriculture conversion in Amazon drives soil microbial communities
796 and N-cycle. Soil Biol. Biochem. 137, 107567.
797

798 Mishra, J., Singh, R., Arora, N.K., 2017. Alleviation of heavy metal stress in plants and
799 remediation of soil by rhizosphere microorganisms. Front. Microbiol. 8, 1706.
800

801 Navarro-Noya, Y.E., Jan-Roblero, J., del Carmen González-Chávez, M., Hernández-
802 Gama, R., Hernández-Rodríguez, C., 2010. Bacterial communities associated with the

803 rhizosphere of pioneer plants (*Bahia xylopoda* and *Viguiera linearis*) growing on heavy
804 metals-contaminated soils. *Antonie Van Leeuwenhoek* 97, 335–349.

805

806 Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical
807 analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124.

808

809 Pilon-Smits, E.A., Freeman, J.L., 2006. Environmental clean up using plants:
810 biotechnological advances and ecological considerations. *Front. Ecol. Environ.* 4, 203–
811 210.

812

813 Pishchik, V.N., Vorob'ev, N.I., Provorov, N.A., Khomyakov, Y.V., 2016. Mechanisms
814 of plant and microbial adaptation to heavy metals in plant-microbial systems. *Microbiol.*
815 85, 257–271.

816

817 Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-
818 associated microbes in heavy metal phytoremediation. *Biotech. Adv.* 30, 1562–1574.

819

820 Rasmussen, L.D., Sørensen, S.J., Turner, R.R., Barkay, T., 2000. Application of a mer-
821 lux biosensor for estimating bioavailable mercury in soil. *Soil Biol. Biochem.* 32, 639–
822 646.

823

824 Salisbury, A.B., Gallagher F.J., Caplan J.S., Grabosky J.C., 2018. Maintenance of

825 photosynthesis by *Betula populifolia* in metal contaminated soils. *Sci. Total Environ.*
826 625, 1615–1627.

827

828 Sánchez-Vilas, J., Turner, A., Pannell, J.R., 2011. Sexual dimorphism in intra and
829 interspecific competitive ability of the dioecious herb *Mercurialis annua*. *Plant Biol.*
830 13, 218–222.

831

832 Shi, G.R., Liu, C.F., Cai, Q.S., Liu, Q.Q., Hou, C.P., 2010. Cadmium accumulation and
833 tolerance of two safflower cultivars in relation to photosynthesis and antioxidative
834 enzymes. *Bull Environ. Contam. Toxicol.* 85, 256–263.

835

836 Siciliano, S.D., Palmer, A.S., Winsley, T., Lamb, E., Bissett, A., Brown, M.V., van Dorst,
837 J., Ji, M., Ferrari, B.C., Grogan, P., Chu, H., 2014. Soil fertility is associated with fungal
838 and bacterial richness, whereas pH is associated with community composition in polar
839 soil microbial communities. *Soil Biol. Biochem.* 78, 10–20.

840

841 Simkin, A.J., López-Calcano, P.E., Raines, C.A., 2019. Feeding the world: improving
842 photosynthetic efficiency for sustainable crop production. *J Exp Bot.* 70, 1119–1140.

843

844 Tao, Q., Zhao, J., Li, J., Liu, Y., Luo, J., Yuan, S., Li, B., Li, Q., Xu, Q., Yu, X., Huang,
845 H. 2020. Unique root exudate tartaric acid enhanced cadmium mobilization and uptake
846 in Cd-hyperaccumulator *Sedum alfredii*. *J. Hazard. Mater.* 383, 121177.

847

848 Tian, Y., Gao, L., 2014. Bacterial diversity in the rhizosphere of cucumbers grown in
849 soils covering a wide range of cucumber cropping histories and environmental
850 conditions. *Microb. Ecol.* 68, 794–806.

851

852 Tokaloğlu, Ş., Çiçek, B., İnanç, N., Zararsız, G., Öztürk, A., 2018. Multivariate
853 statistical analysis of data and ICP-MS determination of heavy metals in different
854 brands of spices consumed in Kayseri, Turkey. *Food Anal. Method.* 11, 2407–2418.

855

856 Tonnabel, J., David, P., Pannell, J.R., 2017. Sex-specific strategies of resource
857 allocation in response to competition for light in a dioecious plant. *Oecologia* 185, 675–
858 686.

859

860 Tonneijck, A.E., Franzaring, J., Brouwer, G., Metselaar, K., Dueck, T.A., 2004. Does
861 interspecific interaction alter effects of early season ozone exposure on plants from wet
862 grasslands? Results of a three-year experiment in open-top chambers. *Environ. Pollut.*
863 31, 205–213.

864

865 Van Kooten, O., Snel, J.F., 1990. The use of chlorophyll fluorescence nomenclature in
866 plant stress physiology. *Photosyn. Res.* 25, 147–150.

867

868 Van der Meij, A., Willemse, J., Schneijderberg, M.A., Geurts, R., Raaijmakers, J.M.,

869 van Wezel, G.P., 2018. Inter-and intracellular colonization of Arabidopsis roots by
870 endophytic actinobacteria and the impact of plant hormones on their antimicrobial
871 activity. *Antonie van Leeuwenhoek* 111, 679–690.

872

873 Varga, S., Vega-Frutis, R., Kytöviita, M.M., 2017. Competitive interactions are
874 mediated in a sex-specific manner by arbuscular mycorrhiza in *Antennaria dioica*. *Plant*
875 *Biol.* 19, 217–226.

876

877 Viaene, T., Langendries, S., Beirinckx, S., Maes, M., Goormachtig, S., 2016.
878 *Streptomyces* as a plant's best friend? *FEMS Microbiol. Ecol.* 92, fiw119.

879

880 Watve, M.G., Tickoo, R., Jog, M.M., Bhole, B.D., 2001. How many antibiotics are
881 produced by the genus *Streptomyces*? *Arch. Microbiol.* 176, 386–390.

882

883 Weng, H.X., Ma X.W., Fu, F.X., Zhang, J.J., Liu, Z., Tian, L.X., Liu, C., 2014.
884 Transformation of heavy metal speciation during sludge drying: mechanistic insights.
885 *J. Hazard. Mater.* 265, 96–103.

886

887 Wood, J.L., Tang, C., Franks, A.E., 2016. Microbial associated plant growth and heavy
888 metal accumulation to improve phytoextraction of contaminated soils. *Soil Biol.*
889 *Biochem.* 103, 131–137.

890

891 Xia, Z.C., He, Y., Yu, L., Lv, R.B., Korpelainen, H., Li, C.Y., 2020. Sex-specific
892 strategies of phosphorus (P) acquisition in *Populus cathayana* as affected by soil P
893 availability and distribution. *New Phytol.* 225, 782–792.

894

895 Xie, Q., Gui, D., Liu, W., Wu, Y., 2020. Risk for Indo-Pacific humpback dolphins
896 (*Sousa chinensis*) and human health related to the heavy metal levels in fish from the
897 Pearl River Estuary, China. *Chemosphere* 240: 124844.

898

899 Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., Huang, Q., Bai, Y., Vivanco,
900 J.M., Kowalchuk, G.A., Berendsen, R.L., 2018. Root exudates drive the soil-borne
901 legacy of aboveground pathogen infection. *Microbiome* 6, 1-2.

902

903 Zhang, S., Tang, D.T., Korpelainen, H., Li, C.Y., 2019. Metabolic and physiological
904 analyses reveal that *Populus cathayana* males adopt an energy-saving strategy to cope
905 with phosphorus deficiency. *Tree Physiol.* 39, 1630–1645.

906

907 Zhao, H., Li, Y., Zhang, X.L., Korpelainen, H., Li, C.Y., 2012. Sex-related and stage-
908 dependent source- to-sink transition in *Populus cathayana* grown at elevated CO₂ and
909 elevated temperature. *Tree Physiol.* 32, 1325–1338.

910

911 Zhao, Z., Shi, H.J., Kang, X.J., Liu, C.Q., Chen, L.C., Liang, X.F., Jin, L., 2017. Inter-
912 and intra-specific competition of duckweed under multiple heavy metal contaminated

913 water. *Aquatic Toxicol.* 192, 216–223.

914

915 Zloch, M., Thiem, D., Gadzała-Kopciuch, R., Hryniewicz, K., 2016. Synthesis of

916 siderophores by plant-associated metallotolerant bacteria under exposure to Cd^{2+} .

917 *Chemosphere* 156, 312–325.

Figure legends

Fig 1. Zinc (Zn) accumulation in leaves (a), stems (b) and roots (c), translocation factor (T_f) (d), the root bio-concentration factor (BCF) (e) and shoot BCF (f) in *P. cathayana* females and males under different cultivation patterns and excess Zn. Values are expressed as means $\pm$ SD ($n = 4$). Different letters represent significant differences between treatments ($P < 0.05$). F, female; M, male; F/FF, female under intrasexual interaction; M/FM, male under intersexual interaction; M/MM, male under intrasexual interaction; F/FM, female under intersexual interaction.

Fig 2. Taxonomic compositions of bacterial communities in the rhizospheres of *P. cathayana* females and males at the phylum level with the relative abundance over 1% under excess zinc (Zn). Rhizospheric soil samples from plants under single-sex (a) and double-sex interactions (b). Average relative abundance of key phyla in the rhizospheric soil from plants under single-sex (c) and double-sex interactions (d); FF, female with intrasexual interaction; FF+Zn, FF with excess Zn; FM, female and male under intersexual interaction; FM+Zn, FM with excess Zn; MM, male with intrasexual interaction; MM+Zn, MM with excess Zn. A, Proteobacteria; B, Actinobacteria; C, Chloroflexi; D, Acidobacteria; E, Planctomycetes; F, Bacteroidetes; G, Gemmatimonadetes.

Fig 3. Box plots for alpha diversity (effective number of species) (a, b) and beta-

diversity (principal co-ordinates analysis) of bacteria from the rhizospheric soil of *P. cathayana* females and males under excess zinc (Zn) treatment. Alpha diversity of bacteria from the rhizospheric soil of plants under single-sex (a) and double-sex interaction (b). Principal coordinate analysis plots among bacterial communities in the rhizosphere of *P. cathayana* females and males under single-sex interactions (c) and double-sex interactions (d). F/FM, female under intersexual interaction; M/FM, male under intersexual interaction; FF, female under intersexual interaction; MM, male under intrasexual interaction.

Fig 4. LEfSe used to identify abundant taxa in the rhizospheric soil from *P. cathayana* females and males under excess zinc (Zn) treatment. A cladogram was generated by LEfSe indicating differences between bacteria at phylum, class, family and genus levels under single-plant cultivation between excess zinc (Zn) and control soil (no-Zn treatment), irrespective of sex and interaction (a), and between sexes, irrespective of Zn treatment (b). Another cladogram was generated by LEfSe indicating differences between bacteria at phylum, class, family and genus levels between excess Zn and control soil, irrespective of sex and interaction (c), and between interaction modes, irrespective of Zn treatment (d). The node colour indicates taxa enriched under different treatment and interaction patterns. Only taxa with LDA over 3 are shown. FF, female under intrasexual interaction; FM, female and male under intersexual interaction; MM, male under intrasexual interaction.

Fig 5. Predicted functions of the bacterial communities from the rhizospheric soil of *P. cathayana* females and males under excess zinc (Zn) treatment. Predicted functions of the bacterial communities from the rhizospheric soil of plants from single sex (a) and double-sexual interactions (b). F, female; F+Zn, F with excess Zn; M, male; M+Zn, M with excess Zn; F/FM, females under intersexual interaction; M/FM, male under intersexual interaction; FF, female under intrasexual interaction; FF+Zn, FF with excess Zn; MM, male under intrasexual interaction; MM+Zn, male under intrasexual interaction.

Fig 6. Heatmaps of the average relative abundance of key bacteria at the genus level positively (a) or negatively associated (b) with Zn levels in roots of *P. cathayana* females and males exposed to different interaction patterns ($P < 0.05$). F, female; M, male; FF, female exposed to intrasexual interaction; M/FM, male exposed to intersexual interaction; MM, male exposed to intrasexual interaction; F/FM, female exposed to intersexual interaction.

Table 1 Net photosynthesis rate (A), stomatal conductance (g_s), and fluorescence parameters F_v/F_m , ETR , $Y(II)$ and $Y(NPQ)$ of *P. cathayana* females and males exposed to different sexual interactions under control conditions or excess zinc (Zn). F_v/F_m , maximum quantum efficiency of *PS II* photochemistry; ETR , electron transport rate; $Y(NPQ)$, quantum yield of regulated non-photochemical energy loss in *PS II*; $Y(II)$, quantum yield of photochemical energy conversion in *PS II*.

Treatment	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	F_v/F_m	ETR	$Y(II)$	$Y(NPQ)$
Control						
F	19.21±0.35d	0.24±0.02f	0.79±0.01cd	51.48±0.52abc	0.45±0.04bc	0.28±0.01fg
M	23.31±1.41a	0.36±0.03b	0.81±0.00a	52.88±2.85ab	0.44±0.02cd	0.33±0.01de
F/FM	19.11±0.27d	0.29±0.00de	0.80±0.01bc	49.10±3.12cd	0.44±0.02cd	0.27±0.11fg
M/FM	22.49±0.26b	0.41±0.01a	0.80±0.01abc	54.4±2.20a	0.49±0.01a	0.26±0.01fg
F/FF	22.15±0.93ab	0.36±0.01b	0.80±0.01bc	51.93±1.86abc	0.48±0.02ab	0.28±0.02f
M/MM	21.64±0.48b	0.34±0.03bc	0.81±0.01ab	54.83±2.35a	0.50±0.01a	0.28±0.01fg
Zn treatment						
F	13.73±0.40f	0.20±0.01g	0.75±0.02f	32.23±2.21g	0.23±0.01i	0.49±0.02a
M	18.94±0.01e	0.28±0.03e	0.78±0.00d	43.78±1.12e	0.40±0.02e	0.35±0.01cd
F/FM	18.11±0.27e	0.23±0.02fg	0.76±0.01e	36.88±3.59f	0.27±0.04h	0.36±0.04c
M/FM	20.12±0.26c	0.32±0.02cd	0.80±0.01bcd	50.68±1.92bc	0.41±0.02de	0.25±0.03g
F/FF	19.77±0.86cd	0.25±0.04f	0.79±0.00d	44.33±1.36e	0.32±0.03g	0.45±0.04b
M/MM	18.15±0.10e	0.15±0.02h	0.78±0.01d	47.00±1.43de	0.36±0.01f	0.32±0.02e

F, female; M, male; FF, female exposed to intrasexual interaction; M/FM, male exposed to intersexual interaction; MM, male exposed to intrasexual interaction; F/FM, female exposed to intersexual interaction; Different letters represent significant differences between treatments ($P < 0.05$).

Values are means $\pm$ SE ($n = 4$).

Table 2 PERMANOVA results using Bray-Curtis as a distance metric.

Factor	% explained	<i>F</i>	R ²	<i>P</i>
Plantation modes	25	4.38	0.25	0.001***
Zn	5	3.49	0.05	0.004**
Sex	5	3.19	0.05	0.006**
Sex : Zn	2	1.28	0.02	0.227
Plantation modes : Zn	11	1.91	0.11	0.007**
Residuals	52		0.52	

Plantation modes: F, M, F/FF, FM, M/MM; sex: F, M; Zinc, excess Zn treatment. *

0.01 < *P* ≤ 0.05; ** 0.001 < *P* ≤ 0.01; *** *P* ≤ 0.001.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6