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Different sexual impacts of dioecious *Populus euphratica* on microbial communities and nitrogen cycle processes in natural forests

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Abstract Plant-soil microbe interactions are determined by plant characters. Sexual dimorphism in root development, nitrogen (N) assimilation and resource allocation have been studied in different environments. However, how dioecious plants affect soil microbial communities in natural forests, particularly in low precipitation regions, is still poorly known. In this study, natural *Populus euphratica* forests were investigated in three arid regions. We hypothesized that males and females impose sex-specific impacts on physiochemical traits of soil, microbial communities and N-cycling processes. We discovered only little sex effect on most physiochemical traits, and bacterial and fungal communities in top soil (0-20 cm) in the three studied forests. However, the sex effect was greater in deep soil. Compared with fungi, the structure and composition of bacterial communities were affected more by plant sex in the rhizosphere and bulk soil. Sex indirectly affected N-cycling processes through a negative impact on the soil water content. Expressions of AOA, AOB, *nifH*, *nirS* and *nirK* in the rhizosphere soil were significantly affected by sex, forest site and their interactions. *Proteobacteri*a, *Actinobacteria* and *Firmicutes* in the rhizosphere and bulk soils of *P. euphratica* males showed more significant effects on ammoxidation, N fixation, denitrification and protease activities when compared to females. The results suggest that sexual differences in shaping bacterial communities and affecting N-cycling processes are greater when the soil becomes drier. Thus, low precipitation causes intense sex differences in the nitrogen uptake and use efficiency, Our study

highlights the importance of sexual effects on shaping specific microbial communities and N-cycling processes.

Keywords: sexual dimorphism, plant-microbe interaction, water availability, nitrogen use efficiency

1. Introduction

Plants with divergent preference and demand of soil nutrients impose strong selective pressures on soil microbial communities by secreting variable but substantial amounts of photosynthesis-derived compounds, including sugars, secondary metabolites and organic acids (Xia et al., 2016; Zhalnina et al., 2018; Guo et al., 2019; Xiao et al. 2019). Specific roles of bacterial and fungal communities in nutrient cycling are reflected in their taxonomic composition, community structure, biotic interactions and gene functions (Bahram et al., 2018; Fahey et al., 2020). Many studies have demonstrated that plants can affect nitrogen (N) cycling directly by their uptake, use and loss of N, and by affecting soil decomposer activity and organic matter decomposition (Moreau et al., 2015; Smolander et al., 2019; Henneron et al., 2020; Mushinski et al., 2021).

Dioecious plants comprise one-half of all angiosperm families and are widely distributed worldwide (Heilbuth, 2000). Hultine (2016) has suggested that male and female plants differentially affect the structure and function of terrestrial ecosystems due to sexual dimorphism in morphology, nutrient uptake, defense chemicals or other physiological traits (Obeso, 2002; Song et al., 2019; Wu et al., 2021). Generally, females allocate more resources into chemical defense to protect photosynthetic foliage due to their higher reproduction cost compared to males (Chen et al., 2015; Hultine et al., 2016; Lei et al., 2017). However, males have higher photosynthetic ability and they

68 transport more carbon to maintain growth or to resist abiotic stresses, e.g. drought,
69 compared to females (Chen et al., 2014; Hultine et al., 2016). These differences in
70 carbon fixation and allocation would result in sex-specific effects on soil properties,
71 including microbial community structures and functions (Wu et al., 2019; Liu et al.,
72 2021a; Xia et al., 2021).
73
74 Nitrogen cycling processes, including N-fixation, nitrification and denitrification, are
75 controlled by specific microbial guilds and tightly connected with plant traits. Poplars
76 are dioecious tree species that play important roles in increasing biodiversity and
77 supplying other important ecosystem services worldwide (Rogers et al., 2020). Female
78 poplars demand more soil N than males to support growth or reproduction and they
79 capture more N with their higher root size and specific root length (root length per unit
80 of root biomass) (Hultine et al., 2016; Song et al., 2019; Xia et al., 2020). However,
81 male poplars can endure low soil nutrients better than females (Randriamanana et al.,
82 2014; Xia et al., 2020; Wu et al., 2021), because males have a higher rate of acid
83 phosphatase exudation in soil and a greater arbuscular mycorrhizal hyphal biomass to
84 explore soil phosphorus (Xia et al., 2020). The *nifH* gene is frequently used to study
85 diazotrophic microbes (Inoue et al., 2020). By encoding ammonia monooxygenase,
86 ammonia-oxidizing bacteria (AOB) and autotrophic ammonia-oxidizing archaea (AOA)
87 function as crucial microbial guilds in nitrification (Trivedi et al., 2019). The *nirK* and

nirS genes encode nitrite reductase to convert nitrite to nitric oxide during denitrification (Bowen et al., 2020).

Natural *Populus euphratica* Oliver forests occur in arid and semi-arid regions of the world (Liang et al., 2013; Yu et al., 2020). In many regions, they are known to play crucial roles in ecosystem functioning, sand fixation and in regulating oasis climate (Keyimu et al., 2018). Xia et al. (2021) have linked root phenolic metabolites of *P. euphratica* with sex-specific bacterial communities in artificial plantations of *P. euphratica*. Differences in foliar litter quality caused by foliar nutrient contents or defensive compounds between males and females (Randriamanana et al., 2014; Hultine et al., 2016; Xia et al., 2021) may differently impact soil properties after decomposition (Chen et al., 2021). In this study, we firstly hypothesized that *P. euphratica* males and females have sex-specific impacts on soil bacterial and fungal communities in natural *P. euphratica* forests. Many studies have demonstrated that a higher specific root length (associated with higher N acquisition) is positively related with nitrification rates but negatively with denitrification (Cantarel et al., 2015; Abalos et al., 2018). Thus, in the present study, we predicted a lower gene expression level related to denitrification but a higher expression related to nitrification and N-fixation in female rhizosphere and/or bulk soil compared to male soil.

2. Materials and methods

2.1. Field sampling

Populus euphratica forests are widely distributed in the Xinjiang province of China. At the beginning of April 2018 when *P. euphratica* began to flower, we searched for natural *P. euphratica* forests without human disturbance and having both male and female individuals. Three undisturbed *P. euphratica* forest communities with a similar understory species composition were selected in the following three places: Luntai (N 41°13′46″, E 84°12′22″, 930 m), Shaya (N 40°56′52″, E 83°4′39″, 950 m) and Yuli (N 41°11′18″, E 86°9′35″, 890 m). Dominant shrub species belong to *Tamarix* sp. and are sparsely distributed (Fig. S1). We identified and marked female and male individuals based on their distinct flower traits (Fig. S1). The study sites are characterized by strong winds and dryness with about 42 mm annual precipitation but with an annual evaporation capacity over 2,800 mm.

In the present study, sex-specific impacts on the properties of top soil (0-20 cm) were tested to reveal sex-related differences in plant litter. Soil sampling took place in mid-

August 2019 when *P. euphratica* is in a vigorous growing stage. On each site, five female and male individuals were selected. Pairwise distances between trees were 10-15 m. Firstly, three top soil samples (0-20 cm) were collected under the canopy and pooled into one soil sample. In natural conditions, the main and lateral root development of *P. euphratica* always occurs below 100 cm to allow the acquisition of deep soil water. No roots were found in the top soil layer (0-20 cm) in the three natural forests included in this study. After collecting the top soil samples, fine roots of targeted *P. euphratica* trees were collected at the depth of 100-130 cm. The rhizosphere soil samples were collected by carefully scratching soil from the root surface. At the edge of each selected tree canopy, bulk soil samples were collected at the same depth as rhizosphere soils.

2.2. Physicochemical parameters of soil

All samples were stored on ice and transported to the laboratory. Each sample was divided into two parts. One subsample was used to measure pH, organic matter (OM), total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-), total phosphorus (TP), available phosphorus (AP), Na^+, Mg^{2+}, Ca^{2+}, and protease and β-1,4-N-acetylglucosaminidase (NAG) activities. Another subsample was kept at -80 ºC until DNA extraction. The soil water content was measured by drying 5 g soil at 80 ºC for 72 h. A mixture of soil-water suspension (1:2.5 w/v) was used to determine soil pH. A potassium dichromate external

heating method was used to measure soil OM. Soil (10 g) was extracted with 50 ml 2M KCl, and the extractions were used to determine NH_4^+ and NO_3^-. After digesting 0.5 g soil sample in H_2SO_4, soil TN was determined by Kjeldahl method. Soil TP was determined by molybdenum blue colorimetry after digestion by H_2SO_4 and $HClO_4$. Soil AP was determined by a molybdenum-antimony anti-colorimetric method after 2.5 g soil was extracted with sodium bicarbonate. Soil Na^+, Mg^{2+} and Ca^{2+} were measured in a professional laboratory at the China National Rice Research Institute. Protease and NAG activities were measured by Elisa kits (Shanghai, China).

2.3. DNA extraction, PCR amplification and sequencing

Microbial DNA was extracted from 0.5 g fresh soil by E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocol. The final DNA concentration was determined by the NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and the DNA quality was checked by 1% agarose gel electrophoresis. The primers used for bacterial 16S (V3-V4) and fungal ITS rRNA gene amplifications were 338F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTWTCTAAT, and ITS3F: GCATCGATGAAGAACGCAGC and ITS4R: TCCTCCGCTTATTGATATGC, respectively. After PCR reactions, and quality control and purification processes, a library was constructed and all sequences were generated with paired-end sequencing (2 × 300) on an Illumina MiSeq platform

(Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with default settings. All bacterial and fungal sequences were assigned to operational taxonomic units (OTUs) using UPARSE pipeline at 97% similarities. The SILVA database and UNITE were used to classify bacterial and fungal taxa, respectively.

2.4. Real-time quantitative PCR (qPCR) for N-cycling genes

The DNA extractions were also used for the qPCR. The AOA, AOB, *nifH*, *nirS* and *nirK* genes were amplified using primers in Table S1. Each PCR was conducted in 20 µl total volume that contained 10 µl ChamQ SYBR Color qPCR Master Mix (Nanjing, China), 6.4 µl H_2O, 0.8 µl 5 µM forward primer, 0.8 µl 5 µM reverse primer and 2 µl DNA using ABI 7300 (Applied Biosystems, USA). For all these functional genes, an initial denaturation step was 95 ºC for 5 min, followed by forty cycles of 95 ºC for 5 s. The annealing temperatures were as follows: 58 ºC (AOA), 60 ºC (*nifH* and *nirK*) or 55 ºC (*nirS* and AOB). The final extension temperature was 72 ºC for 40 s. Serial dilutions of plasmids derived from cloned targets (10^{-2} - 10^{-8}) were used to generate standard curves per functional gene (Frey and Rieder, 2013).

2.5. Statistical analyses

193 Two-way ANOVA was used to check sex, forest site and their interactive effects on soil
194 physicochemical parameters, Chao1 richness, Shannon diversity index and evenness. If
195 an interactive effect was significant ($P < 0.05$), post hoc tests were conducted to
196 discover differences among treatments with Tukey's *b* tests. The relative abundance of
197 each phylum was calculated prior to beta diversity analyses. Principal coordinate
198 analyses (PCoA) and nonmetric multidimensional scaling (NMDS) were used to
199 visualize dissimilarities of beta diversity based on Bray-Curtis distances by using the R
200 package "Vegan" (https://www.r-project.org/). PERMANOVA test was used to find sex,
201 site and interactive effects on beta diversity of bacteria and fungi with the vegan
202 package in R. The linear discriminant analysis effect size (LEfSe) was used to reveal
203 taxon differences from phylum to genus as affected by sex and forest site (Segata et al.,
204 2011). To correlate various soil values and abundant bacterial taxa in bulk and
205 rhizosphere soil, Pearson's correlation analyses were performed. Mantel tests were used
206 to assess associations of soil physiochemical variables and abundant bacterial taxa with
207 the expression of N-cycling genes and protease activity. The results of the Mantel test
208 and Pearson's correlation analysis were visualized with ggocr package in R (Sunagawa
209 et al., 2015). Distance-based redundancy analysis (db-RDA) was applied before the
210 Mantel test with "vegan" package in R. Path analyses were performed to assess the
211 effects of site and sex on soil parameters and N-cycling processes with AMOS (version
212 20.0). Paths were included from forest site, sex, soil N condition, soil water and soil

salts to N-cycling processes. Soil N condition included TN, NH_4^+ and NO_3^-, and soil salts included Na^+, Mg^{2+} and Ca^{2+}.

3. Results

3.1. Physiochemical traits of soil

Sex had no significant impact on the physiochemical traits of top soil, except for TP in all three forests (Table S2), but it significantly affected soil water, TN and TP in bulk and rhizosphere soil (Table 1, S3).TN and Ca^{2+} of the rhizosphere soil were strongly affected by the interaction between sex and site. Sexual differences were found in most chemical traits in Luntai and Yuli. For example, TN of rhizosphere soil was higher in females than in males in Luntai and Yuli (Table 1).

3.2. Alpha- and beta-diversity of different soils

Sex affected bacterial Chao 1 of rhizosphere soil and fungal Chao 1 of bulk soil (Table S4, S5). Bacterial Shannon diversity index and evenness of bulk soil were affected by the sex and site interaction (Table S4, S5). In bacterial communities, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Chloroflexi* and *Bacteroidetes* were the most dominant

234 phyla in top, bulk and rhizosphere soils (Fig. 1). In bulk and rhizosphere soils collected
235 from natural forests in Luntai and Yuli, the relative abundance of *Proteobacteria* and
236 *Firmicutes* declined but that of *Actinobacteria* increased in males relative to females
237 (Fig. 1b, c). Ascomycota was the dominating fungal phylum in all soil samples (Fig. 1).
238 The abundance of many bacteria and fungi at the genus level varied among the three
239 forest sites and between *P. euphratica* males and females (Fig. S2).
240
241 Bacterial and fungal community structures in bulk and rhizosphere soils were
242 significantly affected by the interaction between sex and site (Table S6, Fig. 2, S3). The
243 PCoA and NMDS results for bulk and rhizosphere soils well separated the three natural
244 forests from each other. Relative to fungal communities, bacterial communities in bulk
245 and rhizosphere soils were much more affected by sex (Table S6).
246
247 *3.3. Effects of sex on taxonomic composition*
248
249 From phylum to genus, the three sites showed their specific bacterial and fungal
250 compositions (Fig. S4, S5). In addition, sex imposed specific impacts on bacterial and
251 fungal compositions (Fig. 3). In the bulk and rhizosphere soils, greater proportions of
252 bacteria and fungi preferred to inhabit female surroundings. However, most taxonomic
253 compositions were distinct between bulk and rhizosphere soils, expect for *Woeseia*,
254 *Woeselaceae* and *Parcubacteria* in female surroundings (Fig. 3b, c).

255

256 *3.4. Correlations between sex and N-cycling processes*

257

258 The effect of sex and its interactive effect with site significantly affected the NAG
259 activity in top and bulk soils (Fig. 4a, b). The effect of sex and its interactive effect with
260 site significantly affected the protease activity in bulk and rhizosphere soils (Fig. 4e, f).
261 The protease activity of bulk soil collected from female surroundings was higher than
262 that from male surroundings in Luntai and Yuli, but lower in Shaya (Fig. 4e).
263 Additionally, the female rhizosphere soil had a higher protease activity than that of
264 males in Luntai (Fig. 4f).

265

266 Sex and its interactive effect with site significantly affected gene expressions of AOA,
267 *nifH*, *nirS* and *nirK* in bulk and rhizosphere soils (Fig. 5, S6). AOA, *nifH*, *nirS* and *nirK*
268 of females had higher expression levels compared to males in bulk soil from Yuli (Fig.
269 S6). Expression levels of AOA and AOB were higher in the female rhizosphere soil in
270 Luntai and Yuli (Fig. 5a, b), and expression levels of *nifH* and *nirK* were higher in the
271 female rhizosphere soil in Luntai (Fig. 5c, e).

272

273 The soil water content, NO_3^-, total P and Mg^{2+} had large contributions to bacterial
274 communities in bulk and rhizosphere soil (Fig. S7). Overall, Mantel tests indicated that
275 the abundance of *Proteobacteria* and *Actinobacteria* were negatively related with each

other and they showed opposite relationships in all chemical traits of soil (Fig. 6). NH_4^+ and total N significantly correlated with ammoxidation, while organic matter and Mg^{2+} had significant effects on N fixation and denitrification, respectively, in the bulk soil of females (Fig. 6a). Compared to the results in the bulk soil of females, a wider range of chemical traits were significantly correlated with ammoxidation, N fixation, denitrification and particularly protease activity in the bulk soil of males (Fig. 6b). Total N, Mg^{2+} and *Proteobacteria* showed strongest correlations with protease activity. NO_3^- and available P significantly impacted denitrification and protease activity in the rhizosphere soil of females, respectively (Fig. 6c). The soil water content, total N, available P and Mg^{2+} affected functional genes (except for ammoxidation) or protease activity in the rhizosphere soil of males (Fig. 6d). Sex showed a significant negative effect on the soil water content, while the water content was positively related with N fixation and denitrification bacteria (Fig. S8).

4. Discussion

4.1. Sex imposes little differences in chemical composition and microbial communities of top soil

The quality and quantity of input litter is considered to be a main force in shaping chemical and microbial properties of top soil in different types of forests (Kooijman and Martinez-Hernandez., 2009; Legay et al., 2014; Bahram et al., 2018). Sexual differences in the composition of leaf elements and in physiological traits of males and females have been reported in different environments (Chen et al., 2014; Rogers et al., 2020; Song et al., 2019). In *P. euphratica*, our co-authors focusing on physiological traits have found that males tend to have higher leaf N concentrations compared to females (data unpublished). However, our results indicated that sex caused only small differences in chemicals (except for TP) and microbial communities of top soil (Table S2, S4, S5). A very small fraction of leaf litter is left in the top soil because of regular strong winds (Fig. S1). The decomposition of remaining litter is much slower in extreme climates, like those with a low precipitation combined with a high evaporation (Chen et al., 2021). Saprophytic *Basidiomycota* have evolved efficient ways to degrade lignin and soil organic complexes, as they are able to secrete a large variety of oxidoreductases (Ho et al., 2017; Kamimura et al., 2017; Bahram et al., 2020). The high abundance of copiotrophic *Ascomycota* implied that readily metabolizable and labile

310 resources of litter were captured, but the rest cannot be decomposed in the future
311 because of the much lower abundance of *Basidiomycota* (Fig. 1).
312
313 *4.2. Males and females harbor different microbial communities*
314
315 Besides the leaf litter discussed above, root exudates and input from dead roots are
316 crucial for shaping bacterial and fungal communities (Perez-Izquierdo et al., 2019;
317 Inoue et al., 2020). The sex-specific influence on soil properties increased in deeper
318 locations, where root proliferation begins. *P. euphratica* males and females harbor
319 specific compositions of microbial communities, and females possess more bacterial
320 species compared to males in both rhizosphere and bulk soils (Fig. 2, 3, S3). The family
321 *Sphingomonadaceae*, which prevails in the male rhizosphere, comprises many unique
322 strains capable of degrading certain lignin-derived compounds (Kamimura et al., 2017).
323 *Woeseia* is a chemoheterotrophic anaerobe closely connected with carbon degradation
324 (Zhang et al., 2020). It was more abundant in the female rhizosphere. These differences
325 implied that males and females harbor specific microbial species that use different root-
326 derived compounds.
327
328 Sex negatively impacts the soil water content that is another factor affecting microbial
329 communities in bulk and rhizosphere soils (Fig. S8). Our results indicated that the water
330 content of soil around *P. euphratica* males tended to be lower than that around females

(Table 1, S3). There is supporting evidence showing sexual differences in resistance or tolerance to stressful environments (Chen et al., 2014; Li et al., 2015; Rabska et al., 2021). The fungal phylum *Glomeromycota* contains all known arbuscular mycorrhizal fungi (Stuermer, 2012). In laboratory experiments, male *P. cathayana* roots with a higher colonization rate and hyphal biomass of arbuscular mycorrhizal fungi were found to enhance plants' ability to forage soil phosphorus or to resist water deficit (Li et al., 2015; Xia et al., 2020). However, the low abundance of *Glomeromycota* and lack of distinct fungal compositions in male and female rhizosphere soils suggested that the arbuscular mycorrhizal fungi imposed little influence on sexual dimorphism in natural *P. euphratica* forests.

β-1,4-N-acetylglucosaminidase (NAG) is related to chitin degradation and its activity is mainly driven and impacted by fungal communities (Chung et al., 2007). We found no sex effect on the NAG activity (Table S5, S6). By contrast, bacterial communities were affected by sex, since soil bacterial networks are less stable and more sensitive to water conditions compared to fungi (de Vries et al., 2018). The water content was positively related to the abundance of *Proteobacteria* but negatively to *Actinobacteria* (Fig. 1, 6). The relative abundance of *Actinobacteria* has been found to increase under decreasing soil water conditions (Xu et al., 2018; Castro et al., 2019).

4.3. Sexual dimorphism drives differentiation in N-cycling processes of soil

Bacterial communities drive soil N-cycling processes, i.e. nitrification, denitrification and N-fixation (Bardon et al., 2014; Trivedi et al., 2019). With a high specific root length and/or high root length density (indicating high rates of soil N acquisition), plants are positively related to nitrification but negatively related to denitrification (Cantarel et al., 2015; Moreau et al., 2015). Since females have a higher N demand, we expected higher gene expression levels for N-fixation (induced by free-living N-fixing bacteria) and nitrification (induced by AOA and AOB), but lower ones for denitrification (responsible for N loss). AOA, AOB and *nifH* tended to be higher in female surroundings in Luntai and Yuli. High and low soil NH_4^+ contents have been revealed to favor AOB and AOA communities during nitrification, respectively (Trivedi et al., 2019). However, the expressions of *nirS* and *nirK* tended to be higher in rhizosphere and bulk soils of females compared to males in Luntai and Yuli, which implied a sex differences in exudates. Denitrification can be inhibited by diverse secondary metabolites released by plant root; for example, procyanidins are key biological denitrification inhibitors (Bardon et al., 2014, 2016; Galland et al., 2019).

Many previous studies have demonstrated intensified sexual differences in poplars in response to a limited availability of soil nutrients and water deficit (Randriamanana et al., 2014; Chen et al., 2014, 2015; Song et al., 2019; Liu et al. 2021b; Wu et al., 2021). In fact, considerable sexual differences were found in correlations between soil traits

373 and N-cycling processes (Fig. 6). Based on the fact that there is little nitrogen payback
374 from leaf litter due to decomposition, *P. euphratica* was expected to acquire more N to
375 support growth and reproduction by affecting N-cycling processes. The present study
376 proved the presence of sex-specific impacts on mediating N-cycling functions through
377 negative effects on the water content of rhizosphere and bulk soils (Fig. S8). A lower
378 water availability limited nitrogen cycling by restraining extracellular enzymatic
379 activities (e.g. protease) and reducing N-cycling related gene expression levels (Castro
380 et al., 2019; Zuccarini et al., 2020). Protease activity is strongly coupled with plant N
381 uptake in the rhizosphere (Emmett et al., 2017). Higher soil moisture in female *P.*
382 *euphratica* rhizosphere promoted protease activity and N-cycling related bacteria to
383 meet higher N demand for reproduction and growth compared to males (Fig. 4, 5).
384 Plants with a higher N use efficiency recruit more abundant *Actinobacteria* in the
385 rhizosphere (Emmett et al., 2017). Male plants have been reported to have a higher N
386 use efficiency (lower N uptake) in limited soil water conditions (Chen et al., 2014; Wu
387 et al., 2021). In the present study, we found more abundant *Actinobacteria* in the
388 rhizosphere of *P. euphratica* males in Luntai and Yuli (Fig. 1). The results suggest that
389 sexual differences in shaping bacterial communities and affecting N-cycling processes
390 are greater in resource-limited conditions.
391
392 Plant growth strategies and/or root traits affect soil microbial communities and the
393 activity of N-cycling microbes. The present study illuminated that dioecious *P.*

euphratica possesses sex-specific soil microbial communities that impact N cycling processes. Significant differences in soil water between males and females, for example, in rhizosphere soils from Luntai and Yuli, strongly affected sex-related differences in N cycling. Increasingly frequent and severe regional droughts cause declines in microbial activities as well as remarkable changes in the abundance of different microbial taxa, with further effects on nitrogen cycling in arid and semi-arid regions. Our study suggested that more attention should be paid on the interactions between dioecious *P. euphratica* and soil microbial communities under the present climate change.

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Author contributions Qingxue Guo had the main responsibility for data collection, analysis and writing, Jiantong Liu and Lei Yu contributed to field work, 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.

Availability of data All bacterial and fungal data produced in this study are available at the National Center for Biotechnology Information under BioProject ID PRJNA670056.

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Table 1 Physiochemical traits of rhizosphere soil in *P. euphratica* males and females at three natural forest sites.

	Luntai		Shaya		Yuli		Two-way ANOVA test		
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	Sex	Site	Sex×site
pH	8.49±0.10^{b}	8.52±0.03^{b}	9.12±0.16^{a}	8.69±0.09^{ab}	8.89±0.13^{ab}	9.04±0.06^{a}	n.s	***	*
Water	10.18±2.44^{a}	3.35±1.11^{bc}	7.29±1.61^{ab}	10.63±2.38^{a}	4.94±1.37^{b}	2.71±0.21^{c}	*	**	*
OM	3.25±0.51	3.13±1.07	3.83±0.65	4.88±0.59	3.48±0.76	1.93±0.27	n.s	n.s	n.s
NH_4^+	0.59±0.09^{b}	1.47±0.38^{a}	0.70±0.08^{ab}	0.74±0.07^{ab}	0.81±0.19^{ab}	0.60±0.05^{b}	n.s	n.s	*
NO_3^-	19.70±1.41^{a}	16.82±0.93^{a}	0.76±0.14^{c}	3.44±1.26^{b}	0.65±0.18^{c}	0.72±0.09^{c}	*	***	*
TN	0.36±0.02^{a}	0.25±0.02^{b}	0.36±0.03^{a}	0.49±0.02^{a}	0.45±0.06^{a}	0.16±0.01^{c}	***	***	***
AP	1.72±0.61	1.44±0.29	1.97±0.75	2.52±0.60	0.69±0.18	0.25±0.11	n.s	**	n.s
TP	0.63±0.04	0.60±0.01	0.65±0.01	0.64±0.01	0.43±0.02	0.35±0.03	*	***	n.s
Na^+	2.54±0.59^{a}	1.38±0.18^{ab}	1.09±0.11^{bc}	1.56±0.18^{ab}	0.89±0.12^{bc}	0.64±0.04^{c}	n.s	***	**
Mg^{2+}	2.52±0.73	1.70±0.61	2.35±0.52	3.79±0.80	1.15±0.48	0.75±0.11	n.s	**	n.s
Ca^{2+}	21.27±1.24^{a}	18.17±1.05^{ab}	14.54±1.38^{b}	17.30±0.61^{ab}	10.22±0.79^{c}	4.83±0.19^{d}	***	***	***

OM ($g \cdot kg^{-1}$), soil organic matter concentration; NH_4^+ ($mg \cdot kg^{-1}$), ammonium concentration; NO_3^- ($mg \cdot kg^{-1}$), nitrate concentration; TN ($g \cdot kg^{-1}$), total nitrogen concentration; AP ($mg \cdot kg^{-1}$), available phosphorus concentration; TP ($g \cdot kg^{-1}$), total phosphorus concentration; Na^+ ($g \cdot kg^{-1}$), soil sodium concentration; Mg^{2+} ($g \cdot kg^{-1}$), soil magnesium concentration; Ca^{2+} ($g \cdot kg^{-1}$), soil calcium concentration. Two-way ANOVA analysis was performed to find sex, forest site and their interaction effects on soil physiochemical traits. If significant interactive effects were found, post hoc tests were conducted to discover differences among treatments with Tukey's *b* tests at $P < 0.05$. n.s, not significant; *, $0.05 \leq P < 0.01$; **, $0.01 \leq P < 0.001$; *** $P \leq 0.001$. Sex, sex effect; site, forest site effect; sex × site, sex and site interactive effect.

635 **Figure legends**

636 **Figure 1** Relative abundance of soil bacteria and fungi at phylum level in *P. euphratica*
637 males and females in three natural forests: Luntai, Shaya and Yuli. (a) and (d) top soil
638 (0-20 cm), (b) and (e) bulk soil, (c) and (f) rhizosphere soil.

639

640 **Figure 2** Principal coordinate analysis (PCoA) and nonmetric multidimensional scaling
641 (NMDS) of bacterial community structures in three natural *P. euphratica* forests. (a)
642 and (d) top soil (0-20 cm), (b) and (e) bulk soil, (c) and (f) rhizosphere soil.

643

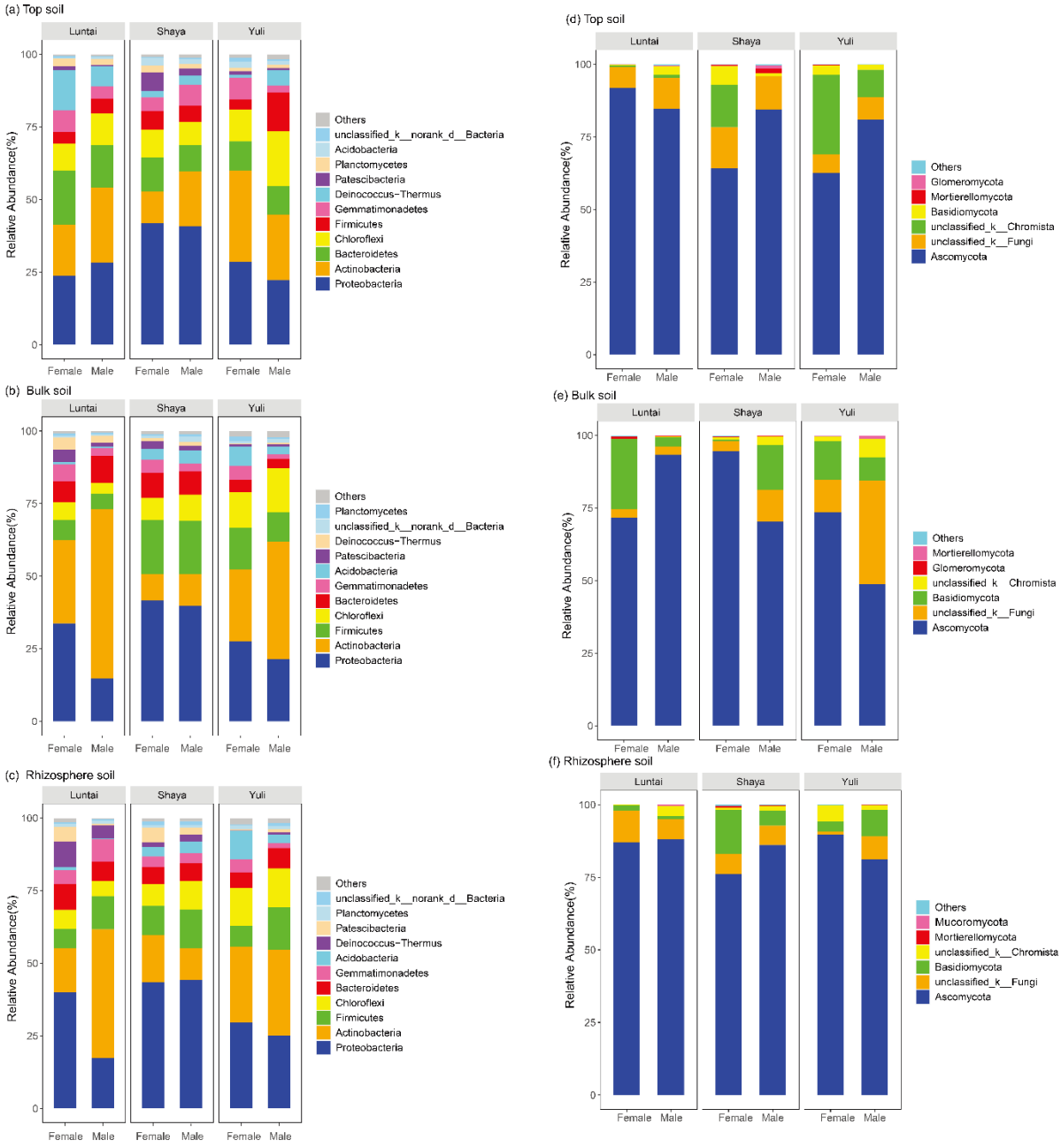
644 **Figure 3** Abundant bacterial and fungal taxa in males and females based on LEfSe
645 analysis. The taxa with absolute LDA ≥ 2 and *P* values less than 0.05 are shown. (a-c)
646 represent bacterial LEfSe results of top soil (0-20 cm), bulk soil and rhizosphere soil,
647 respectively. (d-f) represent fungal LEfSe results of top soil (0-20 cm), bulk soil and
648 rhizosphere soil, respectively.

649

650 **Figure 4** Protease and β-1,4-N-acetylglucosaminidase (NAG) activities in male and
651 female soil of *P. euphratica* in three natural forests: Luntai, Shaya and Yuli. (a) and (d)
652 top soil (0-20 cm), (b) and (e) bulk soil, (c) and (f) rhizosphere soil. Two-way ANOVA
653 analysis was performed to find sex, forest site and their interaction effects on enzyme
654 activities. If significant interactions were found, post hoc tests were conducted to
655 discover differences among treatments with Tukey's *b* tests at $P < 0.05$. *, $0.05 \leq P <$

656 0.01; **, $0.01 \leq P < 0.001$; *** $P \leq 0.001$. Sex, sex effect; site, forest site effect; sex ×
657 site, interaction between sex and site.
658
659 **Figure 5** Different expression of nitrogen cycling-related functional genes (AOA, AOB,
660 *nifH*, *nirS* and *nirK*) of rhizosphere soil between *P. euphratica* males and females in
661 three natural forests. Data were $\log_{10}$ transformed. Two-way ANOVA analysis was
662 performed to find sex, forest site and their interaction effects on gene expression. If
663 significant interactions were found, post hoc tests were conducted to discover
664 differences among treatments with Tukey's *b* tests at $P < 0.05$. *, $0.05 \leq P < 0.01$; **,
665 $0.01 \leq P < 0.001$; *** $P \leq 0.001$. Sex, sex effect; site, forest site effect; sex × site,
666 interaction effect between sex and site.
667
668 **Figure 6** Correlations among soil physiochemical traits, abundant bacterial taxa
669 (phylum level) and N-cycling processes. Pairwise comparisons of soil physiochemical
670 traits and abundant bacterial taxa with a color gradient denoting Pearson's correlation
671 coefficients. N-cycling processes were related to each soil physiochemical trait and
672 bacterial taxon by a Mantel test. (a) Correlations in bulk female soil, (b) correlations in
673 bulk male soil, (c) correlations in rhizosphere female soil, (d) correlations in
674 rhizosphere male soil. NH_4^+, ammonium; NO_3^-, nitrate; Na^+, soil sodium; Mg^{2+}, soil
675 magnesium; Ca^{2+}, soil calcium. Five abundant bacterial taxa were investigated based
676 on Fig. 1.
677

678 **Figure 1**



679

Figure 2

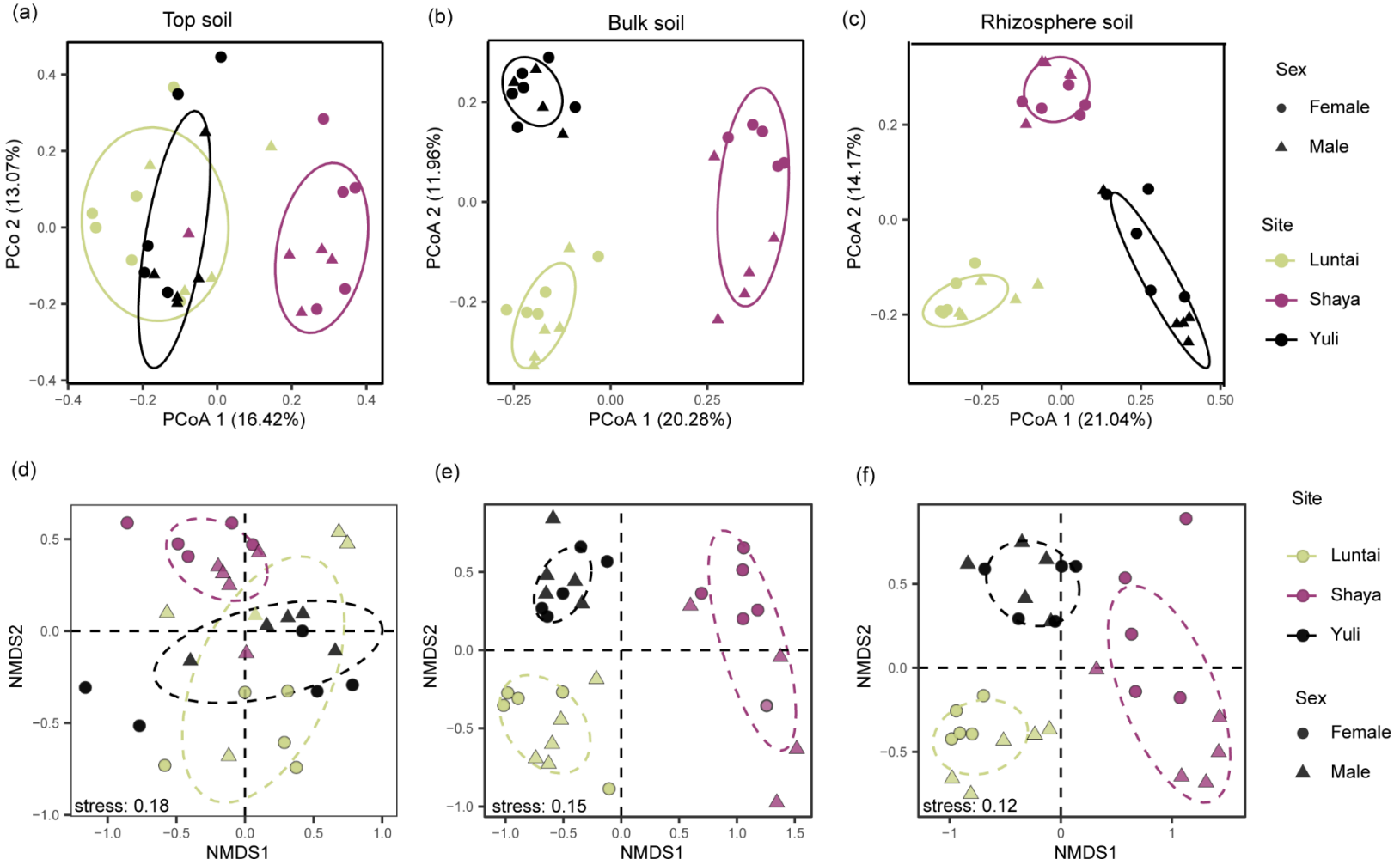
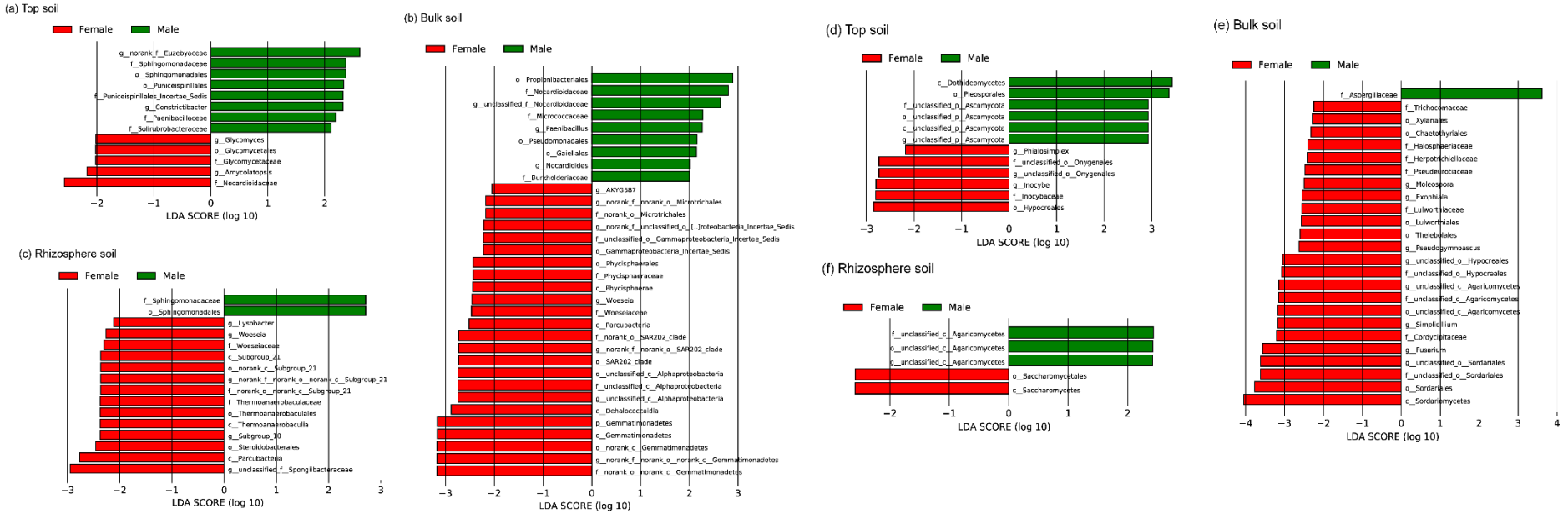
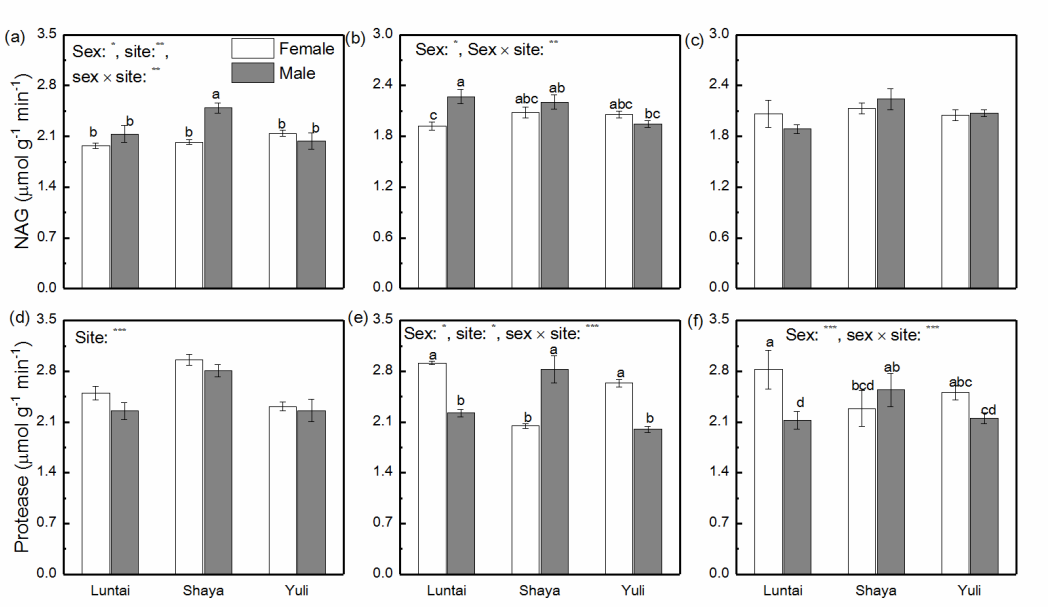

(a)
Top soil
PCo 2 (13.07%)
PCoA 1 (16.42%)
(b)
Bulk soil
PCoA 2 (11.96%)
PCoA 1 (20.28%)
(c)
Rhizosphere soil
PCoA 2 (14.17%)
PCoA 1 (21.04%)
Sex
Female
Male
Site
Luntai
Shaya
Yuli
(d)
NMDS2
NMDS1
stress: 0.18
(e)
stress: 0.15
(f)
stress: 0.12
Site
Luntai
Shaya
Yuli
Sex
Female
Male

Figure 3

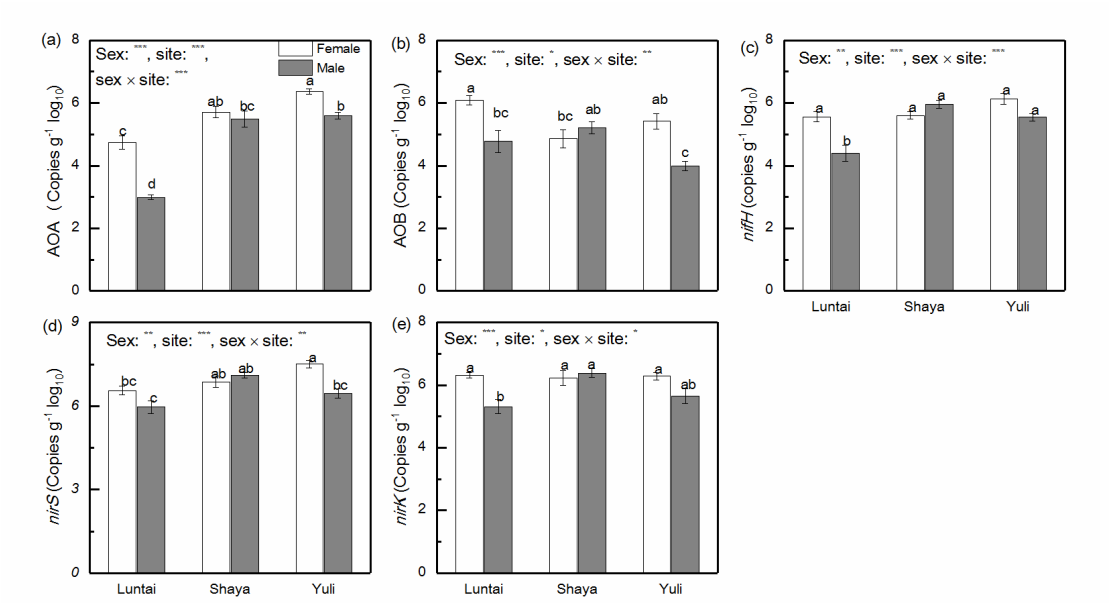

(a) Top soil
Female
Male
g__norank_f__Euzebyaceae
f__Sphingomonadaceae
o__Sphingomonadales
o__Puniceispirillales
f__Puniceispirillales_Incertae_Sedis
g__Constrictibacter
f__Paenibacillaceae
f__Solirubrobacteraceae
g__Glycomyces
o__Glycomycetales
f__Glycomycetaceae
g__Amycolatopsis
f__Nocardioidaceae
LDA SCORE (log 10)
(b) Bulk soil
Female
Male
o__Propionibacteriales
f__Nocardioidaceae
g__unclassified_f__Nocardioidaceae
f__Micrococcaceae
g__Paenibacillus
o__Pseudomonadales
o__Gaiellales
g__Nocardioides
f__Burkholderiaceae
g__AKYG587
g__norank_f__norank_o__Microtrichales
f__norank_o__Microtrichales
g__norank_f__unclassified_o__[..]roteobacteria_Incertae_Sedis
f__unclassified_o__Gammaproteobacteria_Incertae_Sedis
o__Gammaproteobacteria_Incertae_Sedis
o__Phycisphaerales
f__Phycisphaeraceae
c__Phycisphaerae
g__Woeseia
f__Woeseiaceae
c__Parcubacteria
f__norank_o__SAR202_clade
g__norank_f__norank_o__SAR202_clade
o__SAR202_clade
o__unclassified_c__Alphaproteobacteria
f__unclassified_c__Alphaproteobacteria
g__unclassified_c__Alphaproteobacteria
c__Dehalococcoidia
p__Gemmatimonadetes
c__Gemmatimonadetes
o__norank_c__Gemmatimonadetes
g__norank_f__norank_o__norank_c__Gemmatimonadetes
f__norank_o__norank_c__Gemmatimonadetes
(c) Rhizosphere soil
Female
Male
f__Sphingomonadaceae
o__Sphingomonadales
g__Lysobacter
g__Woeseia
f__Woeseiaceae
c__Subgroup_21
o__norank_c__Subgroup_21
g__norank_f__norank_o__norank_c__Subgroup_21
f__norank_o__norank_c__Subgroup_21
f__Thermoanaerobaculaceae
o__Thermoanaerobaculales
c__Thermoanaerobaculia
g__Subgroup_10
o__Steroidobacterales
c__Parcubacteria
g__unclassified_f__Spongibacteraceae
LDA SCORE (log 10)
(d) Top soil
Female
Male
c__Dothideomycetes
o__Pleosporales
f__unclassified_p__Ascomycota
o__unclassified_p__Ascomycota
c__unclassified_p__Ascomycota
g__unclassified_p__Ascomycota
g__Phialosimplex
f__unclassified_o__Onygenales
g__unclassified_o__Onygenales
g__Inocybe
f__Inocybaceae
o__Hypocreales
LDA SCORE (log 10)
(e) Bulk soil
Female
Male
f__Aspergillaceae
f__Trichocomaceae
o__Xylariales
o__Chaetothyriales
f__Halosphaeriaceae
f__Herpotrichiellaceae
f__Pseudeurotiaceae
g__Moleospora
g__Exophiala
f__Lulworthiaceae
o__Lulworthiales
o__Thelebolales
g__Pseudogymnoascus
g__unclassified_o__Hypocreales
f__unclassified_o__Hypocreales
g__unclassified_c__Agaricomycetes
f__unclassified_c__Agaricomycetes
o__unclassified_c__Agaricomycetes
g__Simplicillium
f__Cordycipitaceae
g__Fusarium
g__unclassified_o__Sordariales
f__unclassified_o__Sordariales
o__Sordariales
c__Sordariomycetes
LDA SCORE (log 10)
(f) Rhizosphere soil
Female
Male
f__unclassified_c__Agaricomycetes
o__unclassified_c__Agaricomycetes
g__unclassified_c__Agaricomycetes
o__Saccharomycetales
c__Saccharomycetes
LDA SCORE (log 10)
Bacterial community
Fungal community

684 **Figure 4**



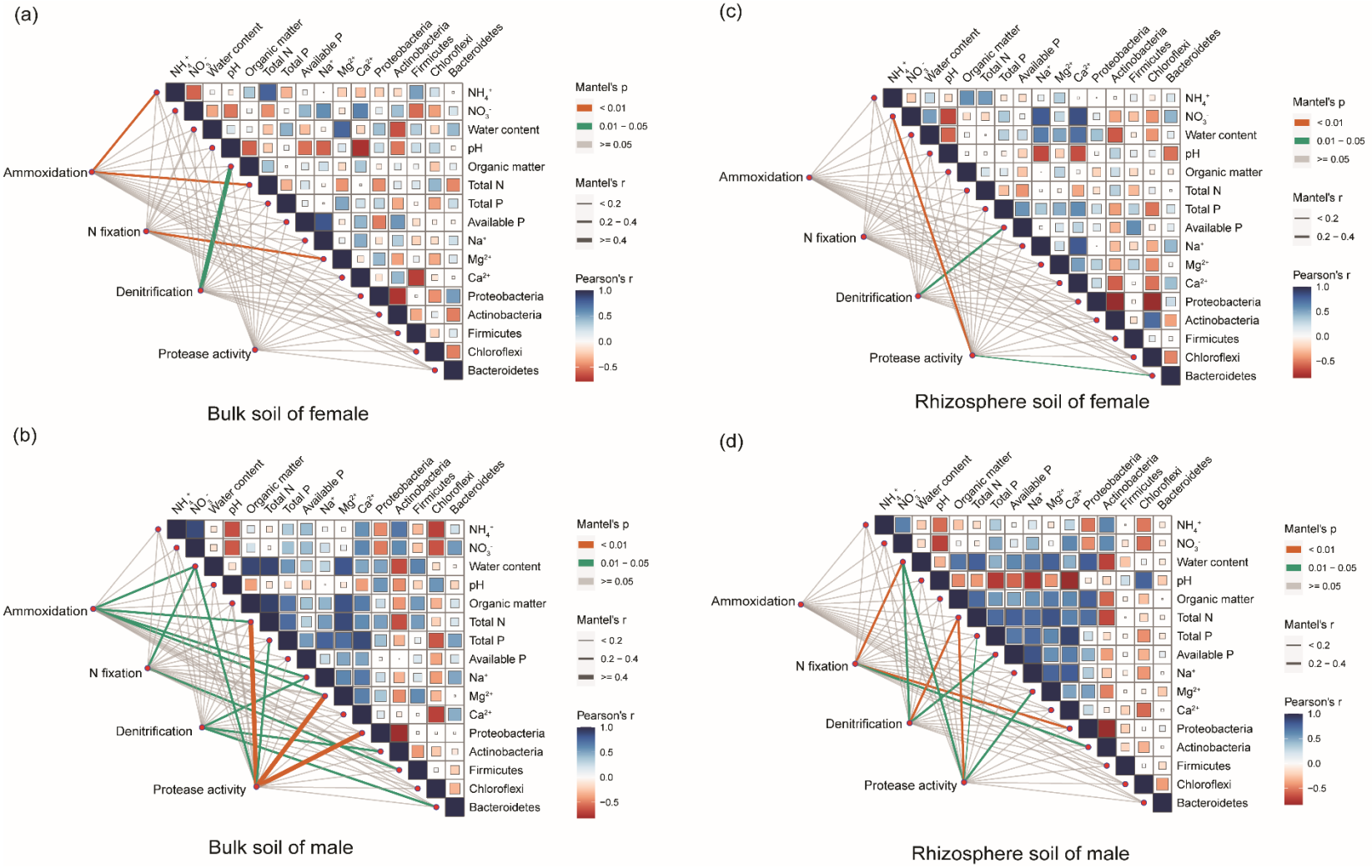
685

686 **Figure 5**



687

Figure 6


(a)
Ammoxidation
N fixation
Denitrification
Protease activity
Bulk soil of female
(b)
Bulk soil of male
(c)
Rhizosphere soil of female
(d)
Rhizosphere soil of male
Mantel's p
< 0.01
0.01 – 0.05
>= 0.05
Mantel's r
< 0.2
0.2 – 0.4
>= 0.4
Pearson's r
1.0
0.5
0.0
−0.5
Water content
pH
Organic matter
Total N
Total P
Available P
Proteobacteria
Actinobacteria
Firmicutes
Chloroflexi
Bacteroidetes