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RESEARCH ARTICLE

# Male, female, and mixed-sex poplar plantations support divergent soil microbial communities

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## Abstract

Males and females of dioecious plants have sex-specific adaptations to diverse habitats. The effects of inter- and intrasexual interactions in poplar plantations on composition, structure, and function of soil microbiota have not been explored in degraded areas. We conducted a series of greenhouse and field experiments to investigate how belowground competition, soil microbial communities, and seasonal variation nitrogen content differ among female, male, and mixed-sex *Populus cathayana* plantations. In the greenhouse experiment, female neighbors suppressed the growth of males under optimal nitrogen conditions. However, male neighbors enhanced stable isotope ratio of nitrogen ($\delta^{15}N$) of females under intersexual competition. In the field, the root length density, root area density, and biomass of fine roots were lower in female plantations than in male or mixed-sex plantations. Bacterial networks of female, male, and mixed-sex plantations were characterized by different composition of hub nodes, including connectors, modules, and network hubs. The sex composition of plantations altered bacterial and fungal community structures according to Bray-Curtis distances, with 44% and 65% of variance explained by the root biomass, respectively. The total soil nitrogen content of mixed-sex plantation was higher than that in female plantation in spring and summer. The mixed-sex plantation also had a higher β-1,4-*N*-acetyl-glucosaminidase activity in summer and a higher nitrification rate in autumn than the other two plantations. The seasonal soil N content, nitrification rate, and root distribution traits demonstrated spatiotemporal niche separation in the mixed-sex plantation. We argue that a strong female–female competition and limited nitrogen content could strongly impede plant growth and reduce the resistance of monosex plantations to climate change and the mixed-sex plantations constitutes a promising way to restore degraded land.

**KEYWORDS**
belowground competition, dioecious species, microbiota assembly, neighbor sexual identity, plant–microbe interactions, root distribution

  

## 1 | INTRODUCTION

Male and female plants of dioecious plants are characterized by sexual dimorphisms, for example, they have different suites of sexual and vegetative traits, reflecting sex-specific adaptations to different resource demands in diverse habitats (Hultine et al., 2016). A key dimorphic suite of traits is centered around nutrient uptake and use. Female individuals of many dioecious species require more nutrients during growth, especially at the reproductive stage (Liu et al., 2021; Montesinos et al., 2006; Obeso, 2002), partly because of a higher investment/cost in defense and reproduction (Hultine et al., 2016; Lei et al., 2017; Wu et al., 2021). Poplars are dioecious, and *Populus* males and females have clear sexual differences in nutrient uptake and use efficiency, adaptation to diverse environments, and impacts on soil nutrient availability (Guo et al., 2021, 2023; Yu, Huang, et al., 2022). A greater root system size and specific root length (SRL) in females in fertile soils suggest that young female poplars acquire more nitrogen (N) and phosphorus in comparison to males in such environments (Wu et al., 2021; Zhang et al., 2014).

Dioecious species can distinguish the sex identity of neighbor plants and might reduce the competition intensity or even facilitate one sex (Liu et al., 2023; Mercer & Eppley, 2014). In the dioecious grass *Distichlis spicata*, sex recognition occurs via root-derived compounds, and plant growth and carbon allocation are different under intrasexual and intersexual competitions (Mercer & Eppley, 2014). In the dioecious forb *Rumex hastatulus*, intrasexual competition is more severe than intersexual competition (Yu, Barrett, et al., 2022). Stronger intra- than intersexual competition of *Populus cathayana* for soil N has also been demonstrated (Chen et al., 2015). As the belowground resource availability decreased, the intensity of intersexual competition decreases, and male individuals display a beneficial role in supporting female growth compared with intrasexual competition (Chen et al., 2014, 2015).

Plants play decisive roles in regulating the composition and function of soil microbial communities (Henneron et al., 2020; Moreau et al., 2015), which crucially influence plant resistance to abiotic stressors (Chatterjee & Niinemets, 2022). Plants interacting with different neighbors have been shown to induce major changes in soil microbial communities (Guo et al., 2019). Recent studies found that males and females can recruit sex-specific soil microbial communities (Guo et al., 2021, 2022; Vega-Frutis & Guevara, 2009; Zhou et al., 2022; Zhu et al., 2024), which would in turn affect soil nutrient availability and plant growth (Guo et al., 2023). Alterations in belowground interactions between male and female plants or between the same sex plants might further cause important changes in the structure and function of bacterial and fungal communities. There are major efforts to unravel the secrets of sex determination and sexual dimorphisms (Xue et al., 2020), but the potential of sexual dimorphisms for establishing plantations that support a higher soil microbial richness and use more efficiently limiting resources has deserved little attention, particularly in degraded lands.

Planting of mixed species forest stands instead of monospecific stands has been recommended over the last decades due to a variety of reasons (Cagnoni et al., 2023; Pardos et al., 2021). There is evidence of differences in soil microbial communities between monocultures and mixed plantations (Chen et al., 2021), and an increase in nutrient utilization efficiency and availability in mixed plantations has also been reported (Chen et al., 2021; Oliveira et al., 2021). The beneficial interactions, such as resource partitioning, due to complementary traits in coexisting species in a mixed forest, potentially provide higher resistance to both biotic and abiotic disturbances when facing climate change (Anderegg et al., 2018; Jactel et al., 2017). Poplar species are widely used to establish monocultures and mixed species plantation forests around the world to protect environment, and to supply wood products or bioenergy materials (Pilipović et al., 2022; Thomas et al., 2022; Zheng et al., 2017). We argue that poplar plantations consisting of both male and female plants established based on sexual differences would be much more efficient in supporting key forest ecosystem services as such plantations have potentially a higher resistance to environmental stresses and a greater adaptive capacity to climate change than pure male or female poplar plantations.

To understand the plant interactions with same or opposite sex individuals, and their impacts on soil physicochemical and microbial characteristics, vertical distribution and architecture of roots in divergent plantations were investigated to reveal differences in belowground interactions among female–female, male–male and female–male plants. We postulated that the key root features, such as the root size and SRL, show pronounced sexual dimorphisms and that the inter- and intrasexual interactions lead to differences in composition, structure and function of soil microbiota. On the other hand, dimorphisms in the root system architecture were predicted to lead to a reduced niche overlap and enhanced whole stand growth in mixed-sex plantations. To verify this hypothesis, seasonal changes in soil microbial communities and N contents were investigated among poplar plantations with different sex compositions.

## 2 | MATERIALS AND METHODS

The study was conducted in Xuanhua County Plain (40°25′ N, 115°2′ E; elevation 600–1000 m), Hebei Province, China from 2019 to 2021. The site has a semiarid climate with an average annual mean precipitation of 400.3 mm, evaporation of 1862.5 mm, and air temperature of 7.6°C (Ren et al., 2021). This area has been severely destroyed by wind erosion and wind deposition, and currently, it has aeolian sandy soil. The soil has low fertility with a particularly low N content (Table S1). As one of the important ecological construction projects to prevent environmental deterioration and to restore the degraded land, poplar plantations, including those with *P. cathayana*, have been established by the state forest farms of Huang Yangtan. The current study was

conducted in *P. cathayana* plantations established in 1992. The *P. cathayana* individuals were evenly planted at 2 m × 3 m, and by the time of the study the trees were 8–10 m tall. To assess the sex distribution, the sex identity of each *P. cathayana* tree was marked based on flower traits during the flowering phase in April 2019. We observed that the number of male and female plants varied in different *P. cathayana* plantations. Plantation type contained >90% females or males was labeled as female plantation or male plantation, respectively. The third, 'mixed', stand contained a similar number of male and female individuals with a slight bias towards males (0.93–0.98; female:male ratio) (Xia et al., 2023).

## 2.1 | Experiment 1. Plant–plant competition under different N levels

To investigate the effects of the sex identity on the growth of neighboring plants, 1-year-old cuttings were sampled from male and female plantations and then cultivated in pots in a naturally lit greenhouse at the Hangzhou Normal University (30°17′ N, 120°0′ E). Thereafter, cultivated plants of a similar size were randomly chosen and transplanted in plastic pots (diameter 36 cm, height 27 cm). Each pot was filled with sand, vermiculite, and perlite (1:1:1). Each pot contained two rooted cuttings, either male–male, female–female, or male–female. Half of the plants were subject to an optimal N treatment (control) and half of the plants to a limited N treatment (N−). Each treatment had five replicates. The N levels were controlled with the Long Ashton solution (Supporting Information for detailed information of the Long Ashton solution). After growing for 14 weeks, the plants were harvested, separated between biomass fractions and dried at 75°C for 72 h, after which the dry mass of each fraction was measured. The root N content was measured by semimicro Kjeldahl method (Wu et al., 2021). The dried leaf samples ($n = 3$) were used to determine natural stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) with an isotope ratio mass spectrometer (DELTA V Advantage; Thermo Fisher Scientific, Inc., USA). The $\delta^{13}C$ can provide insights into plant long-term water use efficiency related to physiological trait, that is, the rate of carbon assimilation or stomatal conductance. The analysis of $\delta^{15}N$ values can reflect physiological mechanisms mainly N uptake, nitrate reduction, and N assimilation. The higher $\delta^{13}C$ and $\delta^{15}N$ values can indicate higher water and nitrogen use efficiency, respectively (Fuertes-Mendizábal et al., 2018; Huang et al., 2016). The Pee Dee Belemnite and $N_2$ were used as standards to calculate $\delta^{13}C$ and $\delta^{15}N$, respectively.

## 2.2 | Experiment 2. Variation in root features along soil vertical profile in different plantations

The root architecture and distribution along the soil profile was explored among the three plantation types in field. Twenty 10 m × 8 m plots were set up in the male and female plantations, and 17 plots in the mixed-sex plantation. A soil core sampler (diameter 70 mm) was used to sample soil and roots at soil depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, and 50–60 cm. The soil cores were sampled at a distance of 1 m from each plant. All soil samples were immediately put in plastic bags and taken to the lab. Roots in each sample were carefully separated from soil and washed by deionized water. Coarse roots (diameter ≥2 mm) of each sample were separated and dried at 75°C for 72 h to measure their biomass. Fine roots (diameter <2 mm) were immediately scanned, and the Win-Rhizo system (Régent Instrument, Inc., Québec, Canada) was used to measure the total fine root length. After scanning, fine roots of each sample were separated into two fractions based on the diameter 1–2 mm or <1 mm. Both root fractions were dried at 75°C for 72 h and their dry mass was estimated. Nitrogen contents of coarse and fine roots were measured by semimicro Kjeldahl method (Wu et al., 2021).

## 2.3 | Experiment 3. Seasonal dynamics in phospholipid fatty acids and N content

This experiment aimed to explore seasonal dynamics in the microbial biomass, extracellular enzyme activities, and net nitrification rate among the three plantations in field. Sampling was conducted in April, July, and October 2021 from the female, male, and mixed plantations. As the Experiment 2 demonstrated that the bulk of the root biomass was located in 0–10 and 10–20 cm soil layers (See Section 3.1), the samples were taken from these soil layers. From each stand, 10 replicate samples were collection.

The soil microbial biomass was assessed by phospholipid fatty acid (PLFA) analyses according to Schindlbacher et al. (2011). All measurements included a blank sample with the internal standard (peak 19:0, nonadecanoic fatty acid). The PLFA biomarkers including iso-, anteiso-(i13:0, i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0) and 10Me-(10Me17:0 and 10Me18:0) branched PLFAs were attributed to Gram-positive bacteria (G+ bacteria) (Luo et al., 2022); monounsaturated and cyclopropyl PLFAs including 17:1ω8c, 18:1ω5c, 18:1ω7c, cy17:0 and cy19:0 were attributed to Gram-negative bacteria (G− bacteria) (Luo et al., 2022); and 16:1ω5c, 18:1ω9c and 18:3ω6c were used for fungi (Joergensen, 2022; Willers et al., 2015). The PLFAs including 14:0, 16:0, 17:0, 18:0 and 20:0 were used as non-specific markers present in all microorganisms (Joergensen, 2022). The sum of all selected PLFAs was used to characterize the total microbial biomass. The sum of G+ and G− bacterial PLFAs was used to characterize the bacterial biomass. The G+:G− ratio and fungal:bacterial (F:B) ratio were separately calculated as the ratio of G+ PLFAs to G− PLFAs and the ratio of fungal PLFAs to bacterial PLFAs. The activities of three hydrolytic enzymes, including β-1,4-glucosidase (BG), β-1,4-*N*-acetyl-glucosaminidase (NAG), and acid phosphatase, were measured following the methods in Jing et al. (2020). The net nitrification rate was assessed by quantifying changes in $NO_3^-$ concentration before and after a 10-day aerobic incubation (Durán et al., 2014). Soil–water suspension (1:2.5 w/v) was used for pH determination.

### 2.4 | Experiment 4. Microbiota assembly in different sex plantations

At the beginning of August 2020, soil samples were taken from the depths 0–20 cm, 20–40 cm, and 40–60 cm to explore their chemical and microbial traits of different soil layers in field. Five plots (25 m × 15 m) were established in each plantation. In each plot, three soil samples were collected at each soil depth and pooled to form a composite sample. Five soil samples at each depth were also collected in the degraded area without any artificial management practices. A total of 45 samples were collected. After sampling, the soil samples were kept on ice before transportation to the laboratory, where a part was kept at −80°C until DNA extraction and a part was air-dried for chemical analyses, including contents of soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP) and available phosphorus (AP) contents. The remaining part of soil was kept at 4°C to measure the contents of $NH_4^+$, $NO_3^-$, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial carbon biomass (MBC), and microbial nitrogen biomass (MBN). SOM was determined by the potassium dichromate external heating method. Soil AP was determined by the molybdenum-antimony colorimetric method after extraction with sodium bicarbonate (Guo et al., 2021). TN was measured by the Kjeldahl method and TP by Ultraviolet spectrophotometer. Fresh soil (10 g) was extracted with 2 M KCl, and the extractions were used to determine $NH_4^+$ and $NO_3^-$ contents. The contents of DOC and DON were estimated according to Luo et al. (2022). MBC and MBN were determined by the chloroform fumigation extraction method (Vance et al., 1987).

Genomic DNA was extracted from the collected soil with a DNA extraction kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions and DNA was kept at −80°C. The paired-end amplicon sequencing of the 16S rRNA gene and the ITS region of fungal ribosomal DNA was investigated to study bacterial and fungal communities with Illumina MiSeq PE 300 platform (Illumina, San Diego, CA, USA). After determining the DNA concentration and quantifying the DNA quality, the 16S rRNA gene (V3–V4) was amplified using the primers, 338F: ACTCCTACGGGAGGCAGCAG and 806R: GGACTACHVGGGTWTCTAAT, while the ITS region was amplified using ITS3F: GCATCGATGAAGAACGCAGC and ITS4R: TCCTCCGCTTATTGATATGC (Guo et al., 2021). Detailed information on PCR reactions is shown in the Supporting Information. Raw data generated from sequencing were quality-filtered by Trimmomatic and merged by FLASH with default settings (https://ccb.jhu.edu/software/FLASH/index.shtml). All bacterial and fungal sequences were assigned to operational taxonomic units (OTUs) using the UPARSE pipeline at 97% similarities. The SILVA and UNITE databases were used to classify bacterial and fungal taxa, respectively. FUNGuild was used to categorize the fungal OTUs into functional groups, including arbuscular mycorrhiza and ectomycorrhiza (Nguyen et al., 2016). Only taxa with confidence ranking either "probable" or "highly probable" for guild assignment were used. The raw reads were deposited into the NCBI Sequence Read Archive database under the following accession codes: PRJNA1010679 (bacteria) and PRJNA1011010 (fungi).

### 2.5 | Data analyses

The statistical analyses were conducted with R version 4.2. The impact of treatment or plant sex on plant biomass, $\delta^{13}C$, $\delta^{15}N$, PLFAs, enzyme activity, root N content, and net nitrification rate were analyzed by the one-way ANOVA or Kruskal–Wallis test after checking data for normality and homogeneity of variances. The post hoc comparison was performed by either Tukey or Wilcoxon test. The relative abundance and the alpha-diversity of bacteria and fungi were visualized with R package 'ggplot2' (Wickham, 2009) and post hoc comparisons (Tukey test) were performed to compare differences in alpha-diversity among treatments. The beta-diversity of microbial communities was assessed with a Principle Coordinate Analysis based on Bray-Curtis distances, and PERMANOVA was utilized to test the effect of the plantation type using the "adonis" function in the 'vegan' package in R (Oksanen et al., 2017). The linear discriminant analysis effect size (LEfSe) with logarithmic LDA >2 (Wilcoxon $p < .05$) was applied to identify the biomarker taxa in each treatment (https://huttenhower.sph.harvard.edu/galaxy/) (Segata et al., 2011). A Venn analysis was used to visualize intersecting sets of OTUs in each treatment (Conway et al., 2017).

Differential abundances of bacterial and fungal OTUs between plantations and the unplanted site were determined using a negative binomial model in the 'DESeq2' package (Love et al., 2014). Package 'ggClusterNet' was used to construct the co-occurrence network after removing OTUs with a relative abundance <0.01% and package 'igraph' was used to visualize the network (Wen et al., 2022). Robust Pearson correlation scores ($|r| > .9$) and statistically significant correlations were tested ($p < .05$). The topological features, including degree, betweenness, and closeness centrality, were measured at the node level, and statistical differences were compared with the Wilcoxon test among networks. The degree represents the number of connections for a given node; betweenness centrality stands for the potential influence of a particular node on the connections of other nodes; and the closeness centrality of a node stands for the average distance of a node to any other node (Berry & Widder, 2014; Greenblum et al., 2012). High values of these topological features suggest a core position of a node in the network, while low values suggest a peripheral position. According to the topological features, the role of OTUs in the network was measured by their within-module connectivity (Zi) and among-module connectivity (Pi) (Guimerà & Amaral, 2005). The module hubs (Zi > 2.5, Pi < 0.62), connectors (Zi < 2.5, Pi > 0.62), network hubs (Zi > 2.5, Pi > 0.62), and peripherals (Zi < 2.5, Pi < 0.62) were investigated with 'ggClusterNet'. The Mantel test was employed to find connections between soil features and microbial traits (Huang et al., 2020). Structural equation modeling (SEM) was employed to explore the direct and indirect effects on the microbial structure and fungal guilds by using the

piecewise SEM package (Lefcheck, 2016). The specific hypothesized connections in the model are shown in Figure S1. The Bray-Curtis distances were used to indicate bacterial and fungal structure. The final test of *d*-separation (Fisher's $C = 22.655$, $p = .959$) showed a good model fit. The linear mixed effect models were used in the piecewise SEM.

## 3 | RESULTS

### 3.1 | Differences in intra- and intersexual competition

In the greenhouse experiment, the root and total biomass of males were strongly suppressed by the female neighbor under optimal N availability, whereas there was no difference between male and female under limited N treatment (Figure 1). The root N content of females were higher in intersexual competition than in intrasexual competition under optimal N availability (Figure 1C). The $\delta^{13}C$ and $\delta^{15}N$ values of females were higher in intersexual competition than in intrasexual competition under limited N treatment (Figure S2). In the field, the SRL and coarse root biomass showed no significant difference among the three plantation types with different sex compositions (Figure 2). The fine root biomass was lower in the female plantation than in the male plantation at 0–10 cm, 10–20 cm, 40–50 cm, and 50–60 cm depths, and in the mixed plantation at 10–20 cm, 20–30 cm, 40–50 cm, and 50–60 cm depths (Figure 2a). The root length density (RLD) and root area density (RAD) was lower in the female plantation than in the mixed plantation at 10–20 cm, 20–30 cm, 40–50 cm, and 50–60 cm depths (Figure 2c,d). The nitrogen content of fine roots was similar in all plantations at each soil depth (Table S2). The nitrogen content of coarse roots was lower in the female plantation than in the other two plantations at soil depths 10–20 cm and 20–30 cm (Table S2).

### 3.2 | Seasonal dynamics in microbial community composition and N content

The soil of the male plantation had a higher fungal, bacterial, and total biomass and a higher G+ and G− bacterial biomass at 0–10 cm soil depth in spring compared to the female plantation (Figure 3; Figure S3). In summer, the fungal, bacterial, and total biomass of the soil from the mixed plantation were higher than those in the male plantation (Figure 3). At 0–10 cm soil depth, the total soil N content in the female plantation was lower than that in the mixed-sex plantation in spring and summer, and lower than that in the male plantation in autumn (Table 1). The total soil N content was also lower in the female plantation than in the mixed-sex plantation at 10–20 cm depth in summer (Table 1).

At 0–10 cm soil depth, the net nitrification rate was higher in the mixed-sex plantation than in the female plantation in summer and autumn, and higher than that in the male plantation in autumn (Figure 4). At 10–20 cm soil depth, net nitrification rate in the male plantation was higher than that in the female plantation in autumn (Figure 4). The soil in the male plantation had higher BG and acid phosphatase activity compared to the other two plantations at 0–10 cm soil depth (Figure S4). The mixed-sex plantation showed a higher NAG activity than the other two plantations at 10–20 cm soil depth in summer (Figure S4).

### 3.3 | Effects of sex composition of poplar plantations on microbiota assembly

The relative abundance of Basidiomycota was higher in the three poplar plantations than in the control site at each soil depth (Figure S5). The mixed-sex plantation had a higher Shannon diversity than the control site and female plantation at 0–20 cm soil depth (Figure S6). The beta-diversity of soil bacterial and fungal

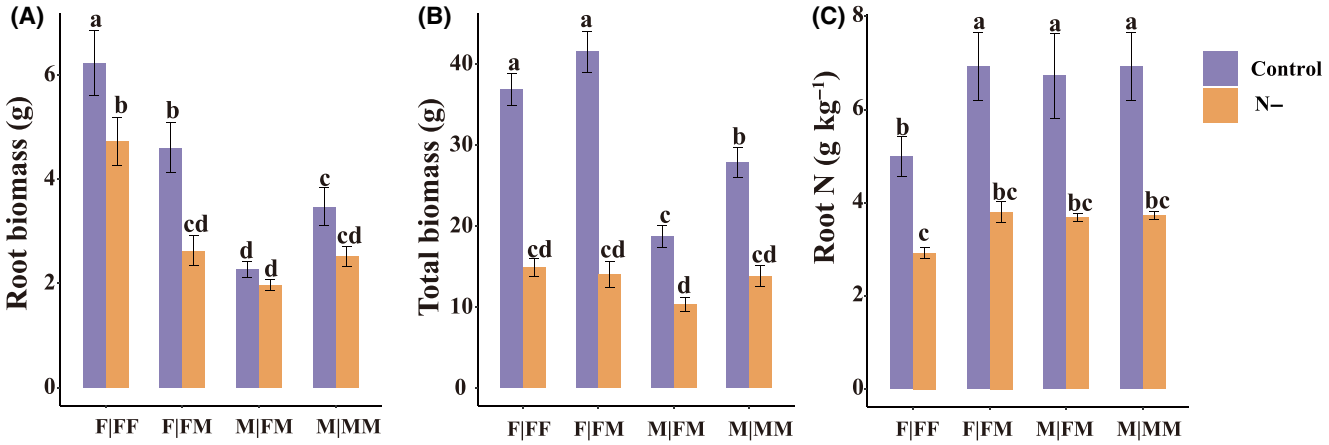


FIGURE 1 Plant biomass (A, B) and root N content (C) affected by plant–plant competition under different N availabilities, that is, optimal (control) versus limited (N−; $n = 5$ at each N level). Averages were compared by one-way ANOVA followed by post hoc Turkey tests. Different letters indicate statistically significant differences ($p < .05$). F|FF, female in female–female competition; F|FM, female in female–male competition; M|FM, male in female–male competition; M|MM, male in male–male competition.

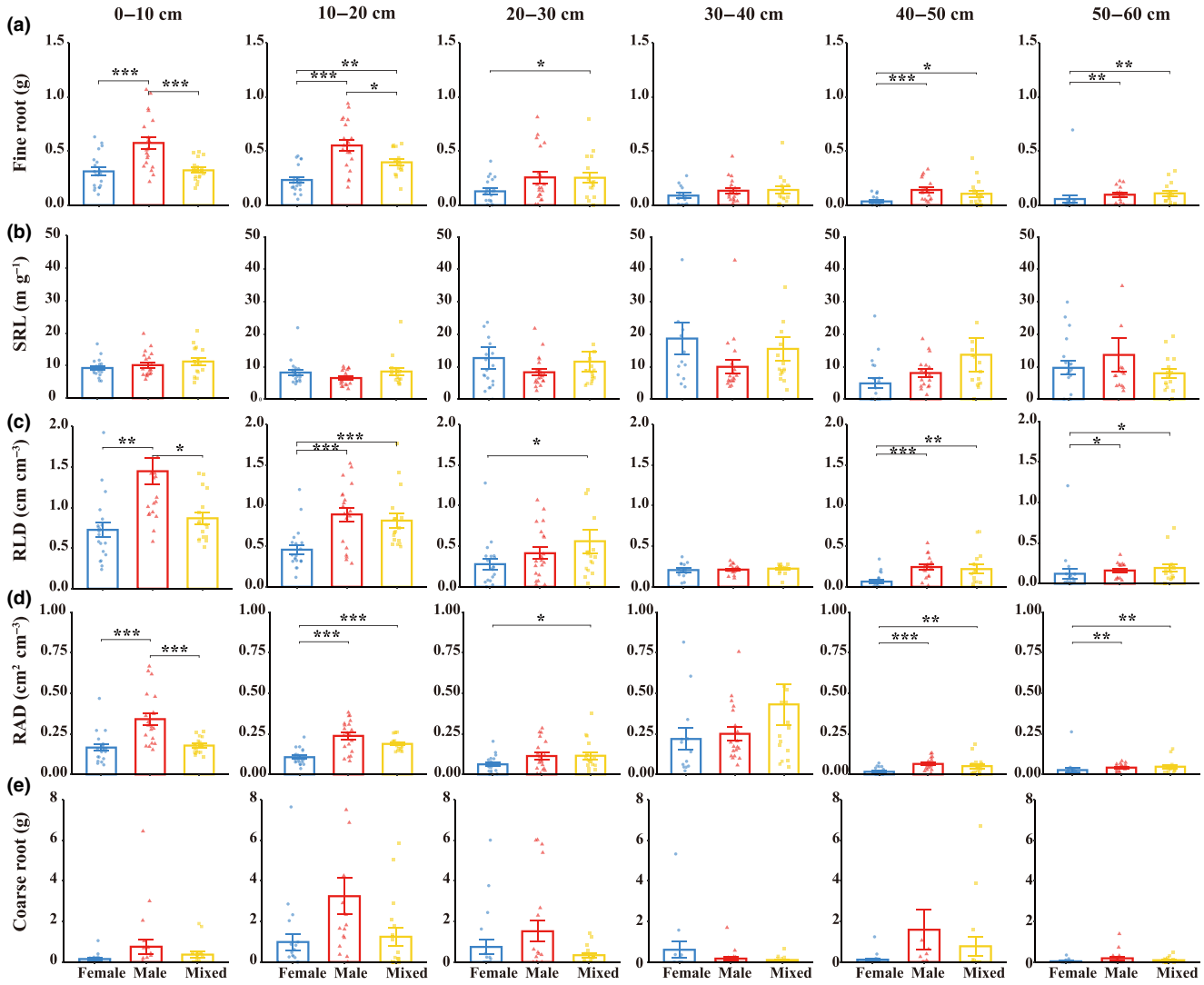


FIGURE 2 Root features in female ($n = 20$), male ($n = 20$), and mixed-sex plantations ($n = 17$) on different soil layers. Root length density (RLD) is the ratio between fine root length (diameter <2 mm) and sampled soil volume. Root area density (RAD) is the ratio between fine root area and soil volume. SRL, specific root length. (a) Fine root biomass, (b) SRL, (c) RLD, (d) RAD and (e) Coarse root biomass. Kruskal–Wallis and then Wilcoxon for pairwise comparisons were used. *$.01 \le p < .05$. **$.001 \le p < .01$. ***$p < .001$.

communities also differed among plantations of different sex composition based on the Bray-Curtis distance (Figure S7). The three plantations had a large number of shared and unique OTUs at each soil depth (Figure S8). In comparison with the control (bare land), all the three plantations had enriched OTUs from Inocybaceae and Thelephoraceae (Figures S9–S11). The OTU Xanthobacteraceae was enriched only in the mixed-sex plantation and the OTU Steroidobacteraceae was depleted only in the female plantation at the top soil (Figure S9). The bacterial and fungal biomarkers were different among the three plantations at each soil depth (Figures S12 and S13). For example, an unclassified OTU belonging to the family Dictyosporiaceae in the female plantation was a biomarker both on the first and second soil layer (Figure S13).

At 0–20 cm depth, the bacterial network from the female plantation had no connectors, while OTU 10562, OTU 10967, OTU 11151, OTU 4124, OTU 5430 and OTU 9412, and OTU 10127, OTU 10617, OTU 12776, OTU4155 and OTU 9679 within the bacterial network were connectors from mixed and male plantation, respectively (Figure 5A; Table S3). At 20–40 cm depth, OTU 2524 was the network hub of the bacterial network from mixed-sex plantation, while networks from other two plantations had no network hub (Figure 5A; Table S3). The taxon information that identified module hubs, connectors, and network hubs is listed in Table S3. The node degree of the bacterial networks from the male plantation was lower than in the other two plantations at 0–20 cm depth, while it was lower in the mixed-sex plantation than the other two plantations at 20–40 cm and 40–60 cm depth (Figure 5B). The node degree of fungal network from female plantation was much lower than that from the other two plantations at the 0–20 cm depth (Figure S14). The closeness centrality of the fungal network from the mixed-sex plantation was higher than that of other two plantations at 20–40 cm depth, while the trend was opposite at 40–60 cm depth (Figure S14).

For the 0–20 cm soil layer, the Mantel test revealed that SOM, TN, and $NO_3^-$ were significantly related to the bacterial and fungal

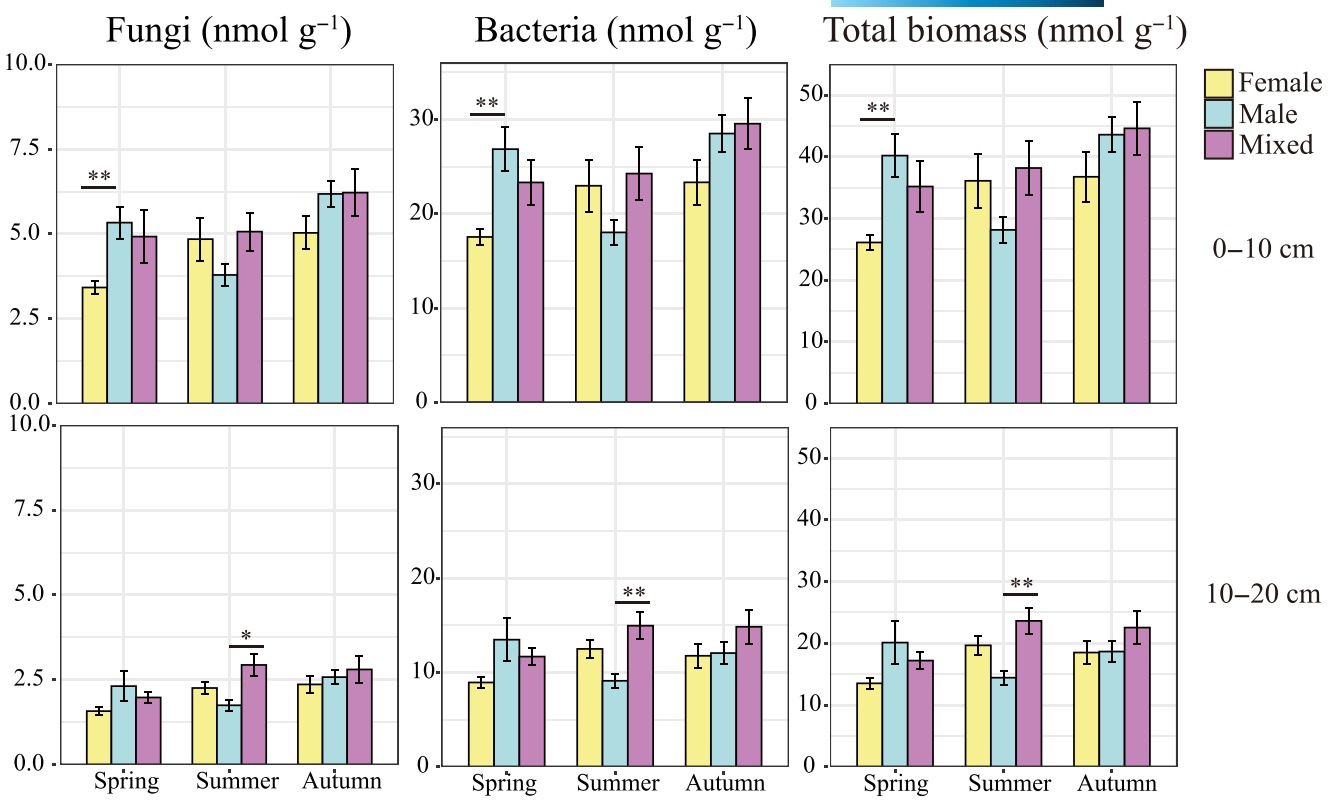


FIGURE 3 Seasonal dynamics in bacterial, fungal, and total microbial biomass in female, male, and mixed-sex plantations ($n = 10$). Soils were sampled at depths 0–10 cm and 10–20 cm where most of the roots were located (Figure 2). **.001 ≤ $p$ < .01.

TABLE 1 Seasonal variation in soil pH and total nitrogen content in female, male, and mixed-sex plantations ($n = 10$).

| | pH | | | Total N (g kg$^{-1}$) | | |
|---|---|---|---|---|---|---|
| Soil depth and stand | Spring | Summer | Autumn | Spring | Summer | Autumn |
| 0–10 cm | | | | | | |
| Female | 7.47 ± 0.03 ns | 7.72 ± 0.05 ns | 7.81 ± 0.05a | 0.60 ± 0.03b | 0.60 ± 0.06b | 0.77 ± 0.06b |
| Male | 7.49 ± 0.04 ns | 7.77 ± 0.06 ns | 7.53 ± 0.04b | 0.83 ± 0.05a | 0.80 ± 0.08ab | 1.02 ± 0.05a |
| Mixed | 7.49 ± 0.04 ns | 7.71 ± 0.05 ns | 7.65 ± 0.04b | 0.85 ± 0.19a | 0.98 ± 0.13a | 0.96 ± 0.14ab |
| 10–20 cm | | | | | | |
| Female | 7.47 ± 0.03 ns | 7.94 ± 0.02ab | 7.92 ± 0.04 ns | 0.38 ± 0.04 ns | 0.38 ± 0.02b | 0.53 ± 0.03 ns |
| Male | 7.57 ± 0.06 ns | 7.99 ± 0.03a | 7.86 ± 0.05 ns | 0.33 ± 0.05 ns | 0.45 ± 0.03ab | 0.53 ± 0.04 ns |
| Mixed | 7.47 ± 0.04 ns | 7.89 ± 0.03b | 7.91 ± 0.04 ns | 0.38 ± 0.02 ns | 0.47 ± 0.02a | 0.57 ± 0.07 ns |

*Note*: Soil samples were taken from two depths where most roots are located. One-way ANOVA followed by Tukey's test was used to compare the average pH and N contents among plantations at given season. Different letters indicate significant differences ($p < 0.05$), ns indicates nonsignificant difference.

communities (Figure 6a). The root biomass among different plantations was associated with the microbial structure and microbial carbon biomass (Figure 6a). The SEM discovered multiple direct and indirect associations between the root biomass, soil features, and structure of microbial communities and explained 44% and 65% of the bacterial and fungal structure (Figure 6b). The root biomass was directly positively related to the structure of bacterial and fungal communities. The root biomass was indirectly negatively related to the abundance of ectomycorrhizal fungi through the effect on soil $NO_3^-$ content (Figure 6c). Similarly, the root biomass was indirectly positively associated with the abundance of arbuscular mycorrhiza (Figure 6d).

## 4 | DISCUSSION

### 4.1 | Weaker intersexual competition than intrasexual competition in dioecious *P. cathayana*

The greenhouse experiment (Experiment 1) found a stronger intrasexual competition among females compared to intersexual competition under sufficient nutrients. The higher root N content and leaf $\delta^{15}N$ of females indicated a higher nitrogen uptake and use efficiency of females, as promoted by male neighbors under sufficient nutrient treatment (Figure 1; Figure S2). A recent study also found similar

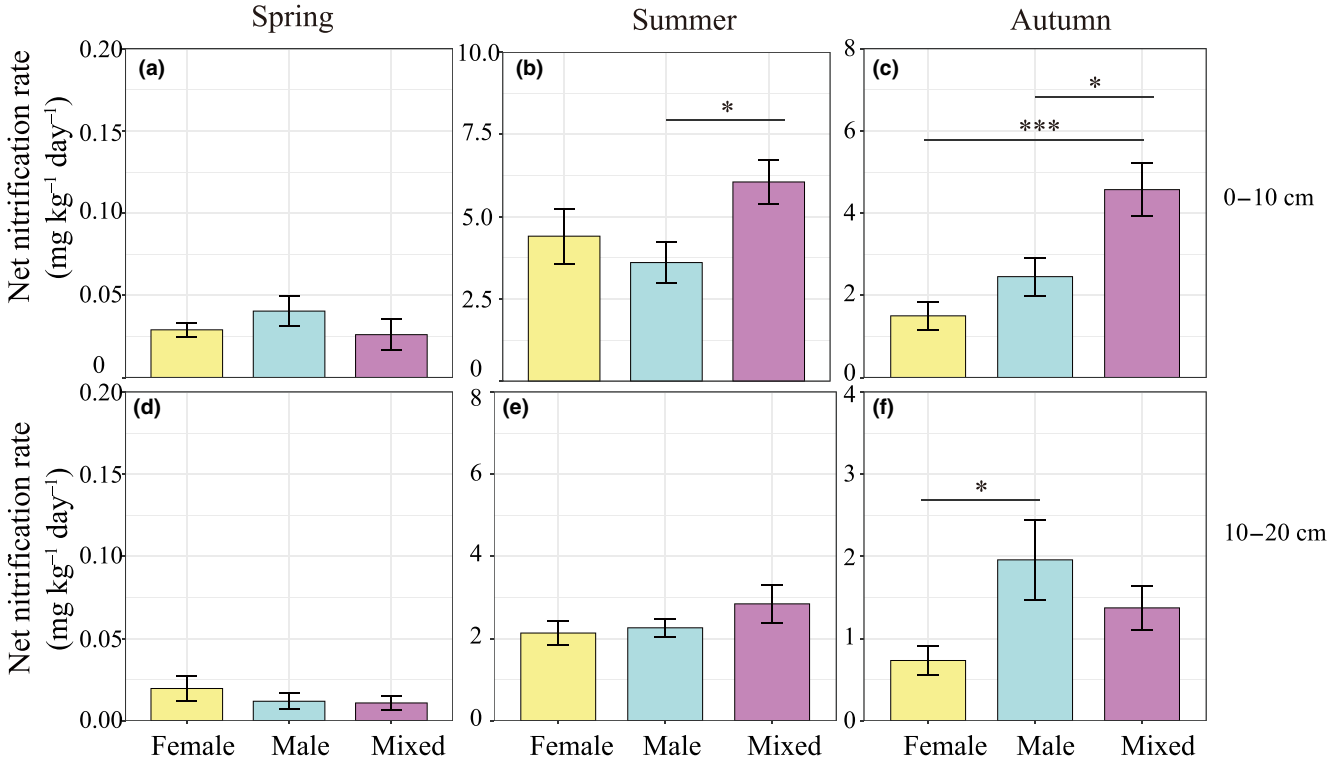


FIGURE 4 Seasonal dynamics in net nitrification rate in female, male, and mixed-sex plantations ($n = 10$). Soils were sampled at depths 0–10 cm (a–c) and 10–20 cm (d–f) where most of the roots were located (Figure 2). *$.01 \le p < .05$. ***$p < .001$.

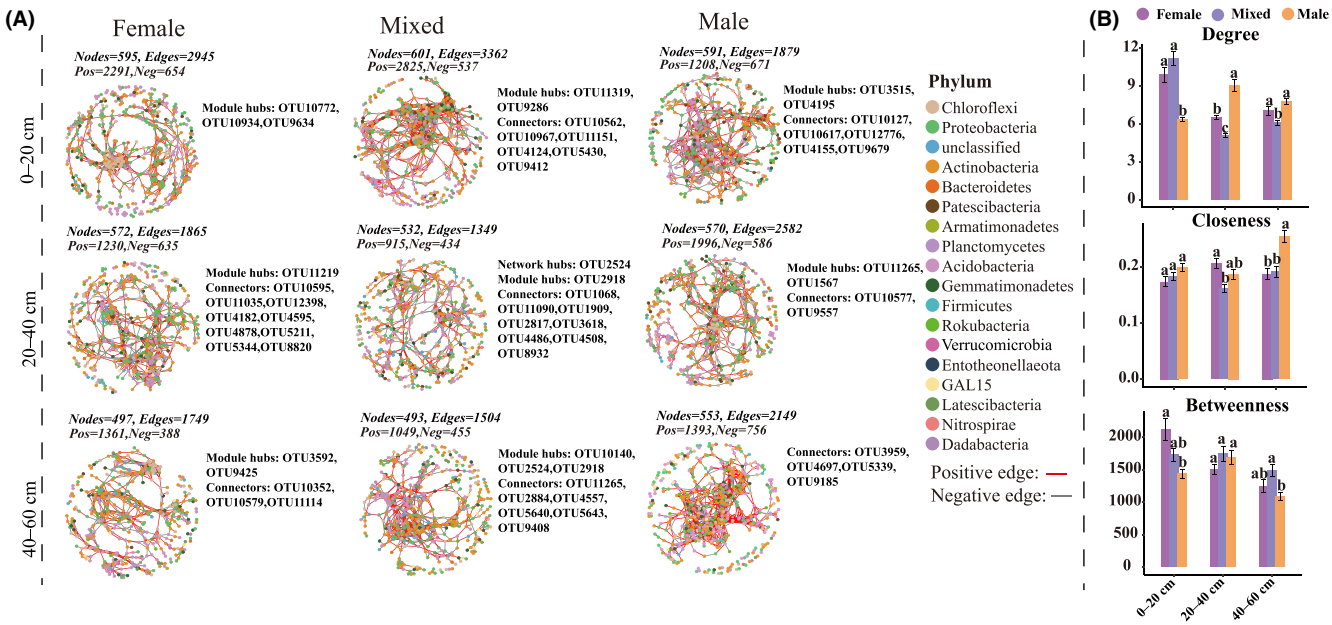


FIGURE 5 The co-occurrence networks and their topological features. (A) Co-occurrence networks of bacterial communities in female, male, and mixed-sex plantations along soil vertical profiles ($n = 5$). (B) The topological features of bacterial co-occurrence networks at the node level. The Kruskal–Wallis test was used to compare differences ($p < .05$). Different letters indicate significant difference ($p < .05$).

results showing that the intersexual competitive intensity was lower in *P. cathayana* females than the intrasexual competition under sufficient soil water (Liu et al., 2023). The superior competitive ability of *P. cathayana* females was due to their greater ability to explore soil N to meet their higher N demand in a habitat with sufficient nutrients (Chen et al., 2014). The superior competitive capacity of females has been postulated to be the key reason responsible for female-biased sex ratios in favorable environments (Hultine et al., 2016).

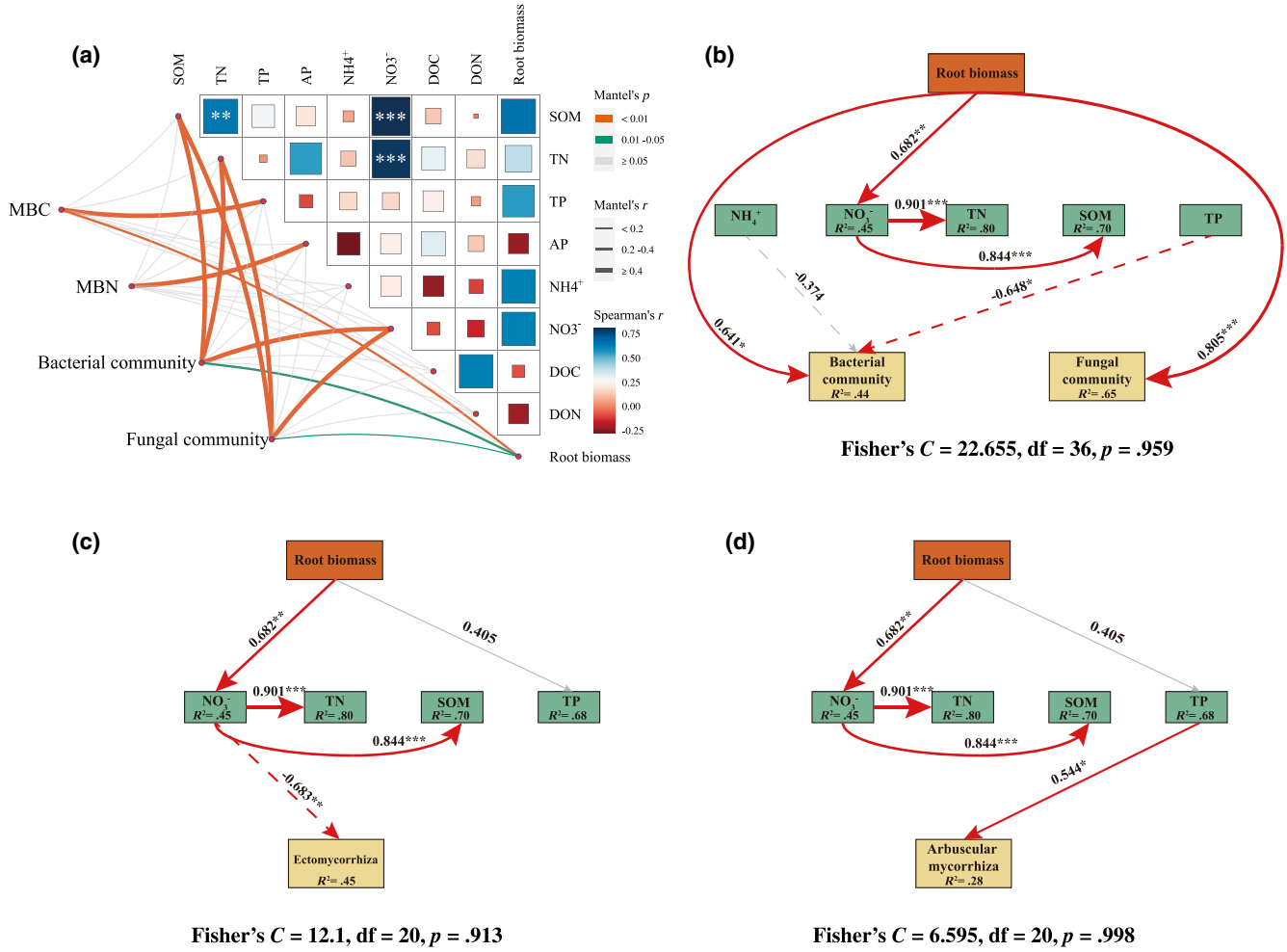


**FIGURE 6** Traits affecting bacterial and fungal communities of different plantations as revealed by Mantel tests and structural equation modeling (SEM). (a) Mantel test revealed the connections of soil chemical traits with microbial traits including microbial carbon and nitrogen contents and biomass, and the structure of microbial communities. Pearson correlation coefficients were used to test relationships among soil traits. (b–d) Results of SEM analyses linking plantation type to microbial community composition and functional guilds. The Bray-Curtis distances represent the structure of microbial communities. AP, available phosphorus; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial carbon biomass; MBN, microbial nitrogen biomass; SOP, soil organic matter; TN, total nitrogen; TP, total phosphorus. *.01 ≤ $p$ < .05. **.001 ≤ $p$ < .01. ***$p$ < .001.

Under declining nitrogen availability, the growth of females is suppressed more by a female neighbor than by a male neighbor, which has demonstrated the presence of a stronger intrasexual competition compared to intersexual competition (Chen et al., 2015). In the present study, the higher root biomass but no significant difference in the total biomass of females in intrasexual competition demonstrated the presence of a stronger intrasexual competition since plants allocate more photosynthetic carbon to root growth when compared to females in intersexual competition under N-limited conditions. Previously, Chen et al. (2015) found that *P. cathayana* males promote photosynthetic nitrogen use efficiency, and the present study also indicate a higher N use efficiency with an increased leaf $\delta^{15}N$ in females is promoted by male neighbor under limited N conditions (Figure S2). Thus, our greenhouse experiment demonstrated a stronger intrasexual competition in females compared to intersexual competition both at optimal and limited N conditions.

In general, the primary principle in establishing a mixed plantation is to use "ecological niches" of multiple genotypes, species, structures, and functions to increase the efficiency of resource utilization (Ammer, 2019; Oliveira et al., 2021). When belowground resources are limited, plants typically increase root foraging both in horizontal and vertical directions. However, under strong competition, there are limits to horizontal root spread. Niche partitioning may have evolved as a response to reduce competition between the sexes (Cox, 1981). Although the nutrient availability progressively decreases with soil depth, going deeper in the soil profile where soil resources are least exploited might be the only option under strong competition (Freschet et al., 2018). The fine root biomass, RLD, and RAD were lower in the female plantation than in the male or mixed-sex plantations, and many samples in the female plantation contained no roots when at soil depth below 40 cm (Figure 2). The smaller roots and shallower distribution indicated a lower ability to

explore and exploit soil nutrients compared to other two plantations. By contrast, plants in mixed-sex and male plantations increased their fine root biomass and distribution with preferential allocation to deeper soil. The deeper root distribution and larger fine root biomass in the mixed-sex plantation might indicate a lower competition between males and females, because of smaller niche overlap and better access to nutrients compared to the female plantation in infertile habitats.

### 4.2 | Root features drove the composition and structure of microbial communities in female, male, and mixed-sex plantations

Our results indicated that the plantations strongly affected the composition of fungal communities at each soil depth when compared with the bare land by increasing the relative abundance of Basidiomycota, such as Thelephoraceae, Inocybaceae, and Xenasmataceae (Figures S9–S11). Plant litter and the secretion of exudates from roots are the primary sources of carbon available to recruit diverse microbes (Glassman et al., 2018; Zhalnina et al., 2018). The ability to degrade lignin is mainly conserved in Basidiomycota (Baldrian, 2006), and carbon input from plantations drove these changes in the fungal communities when compared with bare land. Differences in the physical and chemical traits of the litter or the quality and quantity of root-deprived compound profiles can lead to divergent microbial communities (Palomino et al., 2023; Zhalnina et al., 2018). Males and females show differences in physical and chemical traits during leaf growth (Guo et al., 2022; Zhang et al., 2014; Zhou et al., 2022), and also different in nutrient resorption efficiency during leaf senescence (Yu, Huang, et al., 2022). The results implied that the leaf litter traits of males and females are different and they may influence microbial communities during the decomposition stage, which would be another interesting topic but not investigated in this study.

The present study found that female, male, and mixed-sex plantation have a different composition and structure of bacterial and fungal communities by recruiting a large number of unique OTUs, including biomarkers (LEfSe), and hub species (network), and affecting microbe–microbe interactions within networks at the same soil depth. The biomarkers and hub species are always proposed to be keystone species, which largely affect the structure and functions of bacterial or fungal communities (Herren & McMahon, 2018). For example, the module hub *Phaselicystis* (OTU11319) within the bacterial network of the mixed-sex plantation (0–20 cm) has capacity to degrade polysaccharides such as starch, chitin, and cellulose (Sharma et al., 2016). Root traits, including biomass, and root compounds and exudates as main carbon sources released into soil, impose selective pressure when different microbial communities are formed (Henneron et al., 2020; Pratama et al., 2020). The root biomass was closely associated with microbial the carbon biomass and community structure at soil depths harboring most roots (Figure 6a). Further SEM results provided strong evidence showing that the root biomass affected both the bacterial and fungal community structure. The lower fine root biomass in the female plantation indicated a lower root litter input compared to the other two plantations. The lower biomass of bacteria and fungi and total biomass in the female plantation compared with the male plantation in spring might be due to the lower among of carbon released from roots into the soil (Figure 3). Differences in root exudates have been demonstrated between males and females, which has been suggested as an important way to shape sex-specific microbial communities (Xia et al., 2023). Specifically, *P. cathayana* females released more diverse phenolic allelochemicals into the soil when interacting with females compared to interactions with male neighbors (Xia et al., 2023). Furthermore, the amount of carbon released from roots into the soil was positively related to the root size (Sun et al., 2021). This suggested that smaller amounts of root exudates were released into the soil in the female plantation than in the other two plantations.

This study also revealed indirect effects of root biomass on fungal functional guilds like ectomycorrhiza and arbuscular mycorrhiza fungi (AMF) (Figure 6). The germination of spores and the directional growth of AMF to the host depend on the release of diverse compounds from the roots. Zhu et al. (2024) found a strong sexual selective pressure in the construction of the AMF community in rhizosphere and root endosphere because of different root features between *P. cathayana* males and females. The sexual identity of neighboring plants influences soil fungal communities, which were demonstrated to be regulated by the root exudates that differ between intra- and intersexual plant interactions (Xia et al., 2023). The belowground mycelium can link neighboring plants, forming common mycelial networks, and they can modulate plant–plant interactions (Walder et al., 2012). Previous studies have demonstrated the beneficial role of *P. cathayana* males in alleviating negative effects of diverse unfavorable environments (Chen et al., 2014, 2015; Liu et al., 2023). The different effects of root biomass on ectomycorrhiza and arbuscular mycorrhiza might affect the mycelial network and finally in turn influence intra- and intersexual competition.

### 4.3 | The mixed-sex plantation increased seasonal N content

The extracellular enzymes, largely affected by root carbon deposits, are key driving factors in the biogeochemical cycles (Henneron et al., 2020; Martínez-García et al., 2021). The higher activity of BG and acid phosphatase in the male plantation might indicate a higher carbon and phosphorus cycling rate in spring (0–10 cm) (Chen et al., 2019). The NAG, a key glycosidase enzyme involved in the release of simple *N*-acetyl glucosamine units, is reported to be important in N cycling, and its activity is positively related to the total N in soil (Acosta-Martínez et al., 2007; Luo et al., 2017). We found a higher NAG activity in the mixed-sex plantation than in the other two plantations on the 0–10 cm layer in summer (Figure S4). The total N content of the soil in the mixed-sex plantation was also higher than that in the female plantation on the 0–10 cm soil layer, particularly

in spring and summer (Table 1). This could help the plants to sprout in spring and growth well in summer. Further evidence showed the occurrence of a relatively high net nitrification rate in the mixed-sex plantation, particularly in autumn was observed on the 0–10 cm layer (Figure 4). Nitrification is the microbial oxidation of ammonium to nitrate by ammonia-oxidizing organisms, nitrite-oxidizing bacteria, and complete ammonia-oxidizing bacteria (Prosser & Nicol, 2012). The seasonal changes in NAG, nitrification rate, total N in soil, and coarse roots demonstrated that the mixed-sex plantation may provide a higher N availability for plant growth by regulating N cycling processes and extracellular enzyme activities.

## 5 | CONCLUSIONS

Our findings have important implications for understanding a novel way of restoring degraded land by using dioecious poplars. The greenhouse experiment found that *P. cathayana* females suffered strong competition from female neighbors. In the field, the lower root size further restrained N acquisition in horizontal and longitudinal direction in the female plantation compared with the other two plantation types. By contrast, the larger and deeper roots of plants with a higher RLD and RAD in the mixed-sex plantation could explore N resources in deeper soil layers with higher N content. The different root features and seasonal soil N contents between female and mixed-sex plantations demonstrated a belowground partitioning and spatiotemporal niche separation, which thus reduced plant–plant competition by reducing niche overlap. As an important N source, a higher coarse root N content in male and mixed-sex plantations could mobilize more stored N to supply to different plant sinks during climate change. However, the higher female $\delta^{15}N$ under intersexual competition demonstrated that the N use efficiency enhanced by male neighbors. This suggests that the female plantation would be more negatively affected by poor soil conditions and climate change than the other two plantation types.

In this study, female, male, and mixed-sex plantations showed distinct bacterial and fungal communities. More phenolic compounds secreted by female roots in female–female competition (Xia et al., 2023) might deteriorate soil microbial communities and inhibit plant growth (Whitehead et al., 2021). The presence of a male neighbor improved the microhabitat for female growth by recruiting different microbes. The higher NAG activity and nitrification rate in the mixed-sex plantation compared to the female plantation suggested that different N cycling processes affected by microbes. The enhanced soil N content probably promoted the female growth in the mixed plantation. Overall, these findings help elucidate the associated plant–plant interactions controlling the microbiota assembly over periods of plant development. Further attention on how and to what extent the female–male interactions regulate soil microbiota to their own benefit, for example, leading to an increases soil N availability, is needed. The mixed-sex plantation is probably a novel way to restore more effectively degraded lands.

### AUTHOR CONTRIBUTIONS

**Qingxue Guo:** Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft. **Yuanjing Zhu:** Data curation; investigation. **Fangyuan Sun:** Data curation; investigation. **Helena Korpelainen:** Validation; writing – review and editing. **Ülo Niinemets:** Validation; writing – review and editing. **Chunyang Li:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; writing – review and editing.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Dryad at: https://doi.org/10.5061/dryad.t4b8gtj8d. The raw reads were deposited into the NCBI Sequence Read Archive database under the following accession codes: PRJNA1010679 (bacteria) and PRJNA1011010 (fungi).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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