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Research

Sexual dimorphism in root exudation mediates belowground neighbor recognition and shapes rhizosphere soil microbial assembly

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Summary

- Sexual dimorphism in physiological traits is a hallmark of dioecious plants, yet whether these differences extend belowground to mediate neighbor recognition and rhizosphere microbiome assembly remains largely unexplored.
- Using split-root experiments, ^{13}C-pulse labeling, and multi-omics with dioecious *Populus cathayana*, we dissected the interactions between plant sex, root exudation, and microbiome assembly.
- We demonstrated that male and female plants discriminate neighbor sex by modulating their root exudation. Females perceiving same-sex neighbors upregulated defense-related metabolites, whereas under inter-sexual interactions they shifted toward growth-promoting pathways. Males significantly increased the allocation of newly fixed photosynthetic carbon to the rhizosphere. Specifically, ^{13}C incorporation into bacterial and fungal phospholipid fatty acids (PLFAs) was markedly higher when males grew in female-conditioned soil compared to male-conditioned soil. This male-driven carbon investment fostered highly interconnected cross-kingdom microbial networks with a significantly higher proportion of positive associations. Consistently, females grown under inter-sexual interactions exhibited greater nitrogen contents and higher photosynthetic rates than those under intra-sexual interactions.
- Our findings demonstrated that sexual dimorphism in root exudation drives the assembly of more complex and positively connected microbial networks, providing a transformative framework for understanding how belowground sexual recognition enhances the ecological resilience and productivity of mixed-sex plant populations.

Introduction

Plant–soil interactions are fundamental drivers of terrestrial ecosystem processes and critical for the sustainability of forestry plantations. Central to these interactions is the chemical language of belowground root exudates, which orchestrate the dialogue between plants and the soil microbiome (Henneron *et al.*, 2020; Jing *et al.*, 2023). The chemical profile and release rate of root exudates are extremely sensitive, modulated by plant identity, environmental stressors, and neighbor presence (Kong *et al.*, 2018; Ulbrich *et al.*, 2022). This chemical plasticity actively shapes the structure and function of soil microbial communities, creating soil microbial legacies that can facilitate or inhibit the growth of subsequent plants (Hannula *et al.*, 2021; Nannipieri *et al.*, 2023). While previous research has extensively documented how such chemical language varies between plant species to drive these feedbacks, the potential for intraspecific diversification, specifically the effects of male and female interactions within dioecious plants, to alleviate or alter these belowground processes remains largely unexplored.

In dioecious angiosperms, which account for *c.* 5–6% of species, males and females frequently evolve divergent life-history strategies, particularly with respect to resource allocation to growth vs reproduction (Renner, 2014; Moutouama *et al.*, 2025). Females generally bear higher reproductive costs, which are often reflected as a greater belowground carbon investment and larger root systems compared to males, whereas males may prioritize rapid resource acquisition and maintain a more stable root carbon sink to sustain their microbial partners (Hultine *et al.*, 2016; Xia *et al.*, 2022; Guo *et al.*, 2024). These sex-specific resource allocation strategies directly affect the quantity and composition of rhizodeposits, providing a mechanistic basis for why male and female plants can cultivate distinct soil microbiomes (Xia *et al.*, 2022; Guo *et al.*, 2023; Mao *et al.*, 2024). Because root exudates serve as the primary chemical interface between plants and the soil microbiome, understanding how sexual

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dimorphism in exudation shapes microbial communities is essential for explaining sex-mediated plant–soil feedbacks.

Previous studies have shown that male and female plants can significantly influence one another's fitness when they coexist. For example, male plants have been shown to promote growth or improve the resistance abilities of female plants to unfavorable environments (Xia *et al.*, 2022; Liu *et al.*, 2024). By contrast, females often suffer severe negative impacts from female neighbors under unfavorable conditions (Guo *et al.*, 2024; Liu *et al.*, 2024). When a plant grows in soil conditioned by a predecessor, it responds to the existing microbial legacy while simultaneously remodeling that legacy through its own root exudates. The root exudates of a current plant growing in its own conditioned soil are probably different from those in soil conditioned by the opposite sex. These real-time modifications driven by the current plant's sex would contribute to the overall outcome of plant–soil interactions. However, a critical knowledge gap remains. Do plants actively perceive the sex of their neighbors and dynamically modulate their root exudation in response? How do these neighbor-induced chemical dialogues override or synergize with pre-existing soil microbial legacies? Such belowground sex discrimination could allow individuals to adjust their competitive or facilitative strategies depending on the sexual identity of their neighbors, with profound consequences for microbiome assembly and plant performance.

Poplars (*Populus* spp.) are globally distributed dioecious trees and widely used in forestry (Sloan & Sayer, 2015; Fang *et al.*, 2021). Here, using the dioecious tree *Populus cathayana* as a model, we conducted a series of integrated experiments to dissect this sex-specific dialogue from the mechanism to ecological consequences. Specifically, we aimed to answer the following three key questions: (1) Do male and female poplars generate distinct soil microbial legacies that differentially affect plant physiology? (2) Do these plants exhibit belowground sexual recognition, altering their root exudate profiles in response to same-sex vs opposite-sex neighbors? (3) Is this neighbor-induced chemical plasticity a direct driver of shifts in the soil microbial community? By addressing these questions, we sought to provide the first comprehensive evidence for a sophisticated, chemically mediated recognition capacity between sexes in plants and its cascading effects on the soil microbiome.

Materials and Methods

Study site and soil collection

The study utilized soils collected from the Xuanhua County Plain (40°25′N, 115°2′E) in Hebei Province, China. This region is characterized by a semiarid climate, with a mean annual precipitation of 400.3 mm, an evaporation rate of 1862.5 mm, and a mean annual temperature of 7.6°C (Ren *et al.*, 2021). The area is typified by aeolian sandy soil and has experienced significant land degradation due to wind erosion and deposition. To prevent further environmental deterioration and restore degraded lands, poplar plantations (*Populus cathayana* Rehder) have been widely established in this region. The plantations were originally established with a highly diverse pool of cuttings, with over 200 male and over 200 female parent trees sampled in the surrounding mountains. This ensured a broad genetic base in the mature plantation, from where we obtained our experimental plants. The traits of degraded soils are as follows: pH 8.82, soil organic matter 5.9 g kg^{-1}, dissolved organic carbon 31.59 mg kg^{-1}, total nitrogen 0.3 g kg^{-1}, total phosphorus 0.33 g kg^{-1}, and available phosphorus 5.80 mg kg^{-1}. For this study, degraded soils were collected from unplanted areas to serve as the initial substrate, and they were transported to the laboratory at Hangzhou Normal University for subsequent experiments.

Plant material and soil conditioning

The sexual identity of *P. cathayana* individuals was determined based on floral morphology during the spring flowering season. One-year-old cuttings of both sexes were collected from a local *P. cathayana* plantation (Guo *et al.*, 2024). To generate sex-specific soil legacies, a soil conditioning phase was conducted and each cutting was taken from a different mother tree. These cuttings were grown individually in plastic pots (33 cm height, 56 cm diameter) filled with homogenized degraded field soil for 2 yr. Following this 2-yr conditioning phase, the soils were designated as 'male-conditioned soil' and 'female-conditioned soil'.

To evaluate the response of *P. cathayana* to sex-specific soil microbial legacies, a microcosm experiment was conducted using window rhizoboxes (20 cm width × 40 cm height × 4 cm depth), as modified from Xia *et al.* (2023). Each rhizobox was filled with either male- or female-conditioned soil, and a 3-cm layer of sterilized sand was applied to the surface to minimize contamination from airborne microbes (Fig. 1a). To further ensure genetic independence, the experimental plants used for the microcosms were cuttings taken from different mother trees, randomly assigned to each treatment. A fully factorial design was employed with two soil history types and two plant sexes, resulting in four treatments: (1) male in home soil (male-conditioned); (2) male in foreign soil (female-conditioned); (3) female in home soil (female-conditioned); and (4) female in foreign soil (male-conditioned). Initially, 20 replicate rhizoboxes were established for each treatment. One month after sprouting, 15 replicates per treatment were selected based on uniform growth performance for the subsequent experiment. Plants were irrigated with sterile water throughout the growth period to maintain soil moisture.

After 3 months of growth, 10 replicates per treatment were selected for a ^{13}C-CO_2 pulse-labeling experiment. Five replicates were placed in a transparent plastic chamber for isotope labeling, while the other five replicates served as unlabeled controls placed in a separate chamber to account for background ^{13}C abundance. Inside the labeling chamber, $^{13}CO_2$ was generated by reacting barium carbonate ($Ba^{13}CO_3$, 99.9%) with 2 M HCl. To maintain a relatively stable CO_2 concentration (900–1000 ppm) during the labeling period, additional CO_2 was generated using sodium carbonate (Na_2CO_3) and 2 M HCl (Liu *et al.*, 2024). The unlabeled control chamber was maintained at the same CO_2 concentration (900–1000 ppm) using only Na_2CO_3. Fans were installed in both chambers to ensure uniform air circulation. The labeling was conducted from 8:30 to 12:30, after which the chambers were removed.

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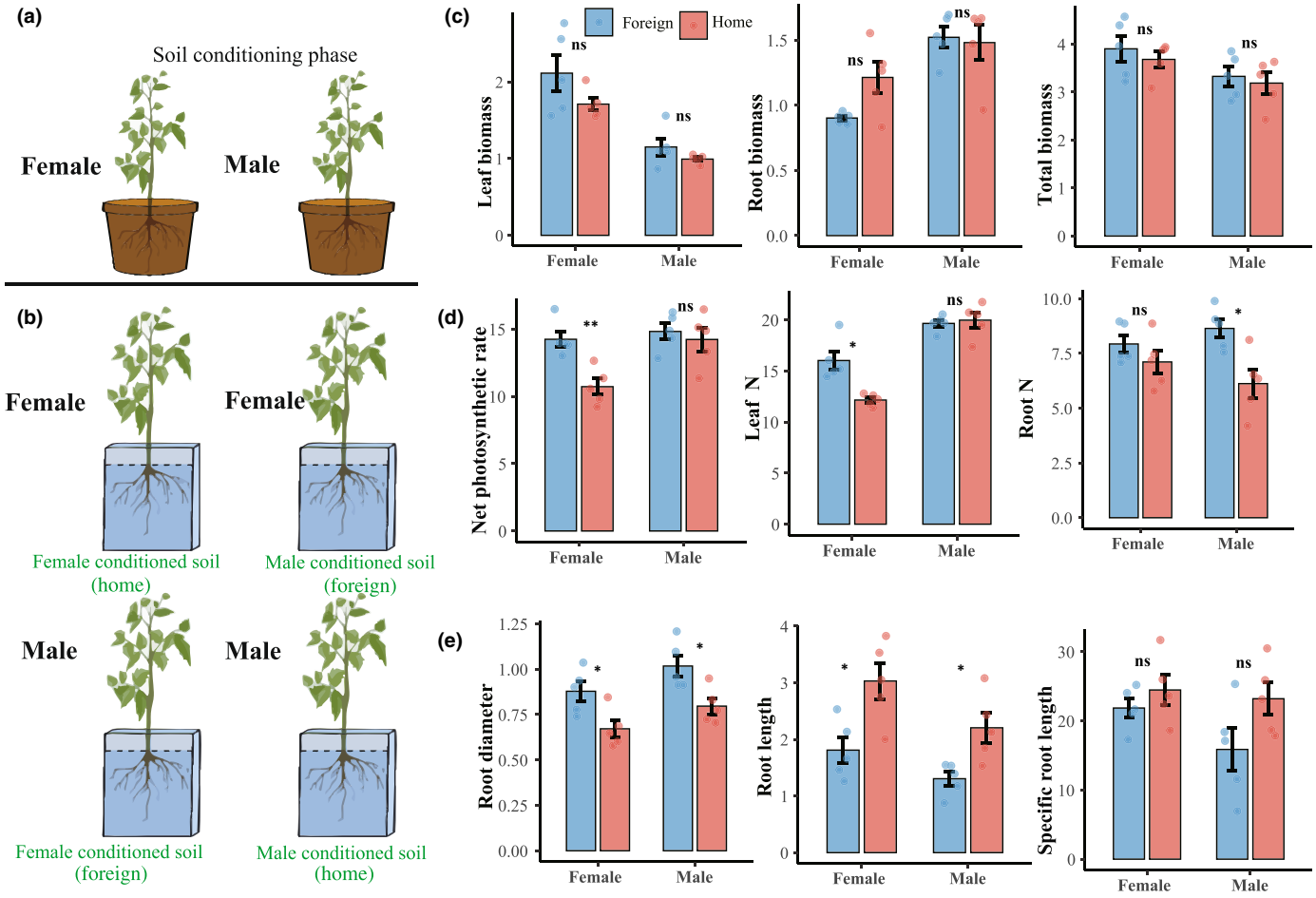


Fig. 1 Responses of *Populus cathayana* to sex-specific soil microbial legacies. (a) Schematic presentation of the soil conditioning phase generating female- and male-conditioned soils. (b) Experimental design involving window rhizoboxes with four treatment combinations: females grown in female-conditioned ('home') or male-conditioned ('foreign') soil, and males grown in female-conditioned ('foreign') or male-conditioned ('home') soil. (c–e) Comparison of plant biomass and root architecture between home (red bars) and foreign (blue bars) soil conditions. Biomass (g), net photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), N concentration ($mg \cdot g^{-1}$), root diameter (mm), root length (m), SRL, specific root length ($m \cdot g^{-1}$). Data are mean ± SE. Asterisks indicate significant differences according to Student's *t*-test (*ns*, not significant; *, $0.01 < P \leq 0.05$; **, $0.001 < P \leq 0.01$).

Rhizosphere soil was collected from both labeled ($n = 5$) and unlabeled ($n = 5$) plants 3 d after labeling. Before the destructive harvesting of the remaining five replicates, the net photosynthetic rate was measured. Plants were then harvested and separated into leaves, stems, and roots. Fresh roots were immediately scanned, and root architecture was analyzed using the WinRhizo system (Régent Instruments Inc., Québec, Canada). All plant tissues were oven-dried at 75°C to determine their biomass. Rhizosphere soil samples were divided into three subsamples: one portion was stored at −80°C for DNA extraction and ^{13}C-phospholipid fatty acid (PLFA) analysis, one portion was stored at −20°C for enzyme activity assays, and the final portion was air-dried for soil nitrogen (N) analysis.

Split-root soil choice

To further investigate the active foraging behavior and spatial root distribution of *P. cathayana* in response to sex-specific soil legacies, a root choice experiment was conducted. The same window rhizoboxes described above were utilized but modified to create a heterogeneous soil environment. Each rhizobox was divided into two distinct compartments using a central PVC barrier (37 cm length × 0.5 cm thickness). One compartment was filled with male-conditioned soil, while the adjacent compartment was filled with female-conditioned soil. As in the previous experiment, a 3-cm layer of sterilized sand was uniformly applied to the soil surface to minimize airborne microbial contamination. A single *P. cathayana* cutting (either male or female) was transplanted into the center of the rhizobox, positioned directly above the PVC divider. The male and female cuttings used in this experiment were collected from 10 distinct male and 10 distinct female mother trees, respectively. This arrangement allowed the developing root system to potentially access and forage in both soil types simultaneously. Ten replicate rhizoboxes were established for each plant sex. The ^{13}C-CO_2 pulse-labeling procedure was conducted following the protocol described above before harvest. Upon harvest, roots from the two separate compartments were carefully disentangled, washed, and immediately scanned to

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assess root architecture using the WinRhizo system (Régent Instruments Inc.). Rhizosphere soil samples were collected separately from each compartment for ^{13}C-PLFA analysis and soil N determination.

Hydroponic reciprocal transfer

To investigate the plasticity of root exudates in response to the perceived sexual identity of neighbors, we designed a hydroponic reciprocal transfer experiment in a glasshouse (Supporting Information Fig. S1). Male and female cuttings were propagated in individual opaque plastic containers filled with Long Ashton nutrient solution (Luo *et al.*, 2015). After a one-month establishment period, 15 uniform individuals of each sex were selected and organized into pairs to create three distinct interaction scenarios ($n = 5$ pairs per scenario): inter-sexual interactions (one male paired with one female) and intra-sexual interactions (two females paired together, or two males paired together). The growth conditions were set as follows: a 12 h : 12 h, light : dark photoperiod, relative humidity maintained at 60–65%, with temperature fluctuating between 27°C during the light period and 25°C during the dark period.

A continuous reciprocal transfer protocol was implemented to simulate neighbor recognition via root exudates. Specifically, each plant was grown in its individual container for 24 h to condition the solution with its own root exudates. Subsequently, the two plants within each pair were reciprocally transferred; that is, plant A was moved into the solution conditioned by Plant B, and vice versa for a 24-h perception phase. After this 48-h cycle, the nutrient solution was replaced with fresh medium, and plants were returned to their original containers to begin the next cycle. Plants were carefully moved between containers by gently lifting the entire root system while submerged in the solution. This procedure was repeated continuously for 50 d. Following this treatment period, plants were carefully removed, roots were rinsed, and each plant was transferred to a glass container filled with 800 ml of ultrapure water to collect root exudates. The containers were wrapped in black plastic bags to exclude light. After 12 h of incubation, the exudates were collected and immediately stored at −80°C. Before exudate collection, the net photosynthetic rate was measured, and plants were harvested and oven-dried to determine their biomass.

Effects of root exudates on microbiota assembly

To investigate the ecological consequences of sex-specific root exudate plasticity on the rhizosphere microbiota assembly, we conducted a controlled exudate-addition experiment (Fig. S2). We established a separate cohort of 'donor' plants (10 males and 10 females) in opaque plastic containers filled with Long Ashton solution. The 10 male donor cuttings were sampled from 5 distinct mother trees (2 cuttings per tree), and the 10 female donors were collected in the same way. Starting 1 month after planting, root exudates were collected weekly following the previously described water-incubation protocol. To create five independent samples, the exudates from the two cuttings derived from the same mother tree were combined and stored at −20°C until use. Simultaneously, a soil microcosm experiment was established using window rhizoboxes (35 cm width × 50 cm height × 5 cm depth). To isolate the effect of exudates on the home soil microbiome, male cuttings were planted in male-conditioned soil, and female cuttings were planted in female-conditioned soil. The experiment employed a factorial design with each plant–soil system receiving one of the three irrigation treatments: (1) sterile water (Control), (2) pooled male root exudates (M-exu), or (3) pooled female root exudates (Fe-exu). Solutions were applied daily to the root zone, with volumes adjusted from 20 to 50 ml during the course of the experiment. Each of the six treatment combinations (2 plant–soil systems × 3 exudate treatments) was replicated five times. After 3 months of growth, plants were harvested and oven-dried at 75°C for 72 h to measure biomass. Rhizosphere soil samples were collected immediately upon harvest and stored at −80°C for subsequent DNA extraction.

Leaf photosynthetic rates were measured using a portable photosynthesis system (LI-6800; Li-Cor Biosciences, Lincoln, NE, USA). The N content and soil enzyme assays of β-1,4-glucosidase (BG), acid phosphatase (AP), polyphenol oxidase (POX) and peroxidase (PER) were determined according to the protocol described by Jing *et al.* (2020). Soil PLFAs were extracted from 3.0 g of freeze-dried soil following a modified Bligh and Dyer method (Frostegård *et al.*, 1993).

The soil microbial biomass was estimated by PLFA analysis following established procedures (Schindlbacher *et al.*, 2011). Phospholipids were separated from neutral lipids and glycolipids via silicic acid chromatography and subsequently converted to fatty acid methyl esters by mild alkaline methanolysis (White *et al.*, 1979). The incorporation of ^{13}C into the PLFAs was calculated as in Zhang *et al.* (2019). Markers representing Gram-positive bacteria, Gram-negative bacteria and fungi were used (Willers *et al.*, 2015; Luo *et al.*, 2022). Non-specific PLFAs (14 : 0, 16 : 0, 17 : 0, 18 : 0, 20 : 0), present across a range of microorganisms, were also analyzed (Joergensen, 2022). Root exudate profiles were tested with LC-MS. Details of all these measurements are provided in Supporting Information.

Soil genomic DNA was extracted using the ALFA-SEQ Magnetic Soil DNA Kit. After assessing the DNA concentration and purity, the bacterial 16S rRNA gene and fungal ITS region were amplified with the primer pairs 799F/1193R (Beckers *et al.*, 2016) and ITS3-F/ITS4-R (McKay *et al.*, 1999), respectively. Detailed amplification conditions are provided in the Methods S1. Raw sequencing reads were quality-filtered, trimmed of primers, and merged. Amplicon Sequence Variants (ASVs) were generated by denoising the data, which included chimera removal. Taxonomic assignment for bacterial and fungal ASVs was performed against the SILVA and UNITE reference databases, respectively.

Statistical analysis

Independent Student's *t*-tests were employed to evaluate the effects of sex-specific soil legacies on plant growth, net photosynthetic rates, root architecture, soil enzyme activities, and

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^{13}C-PLFA profiles. Data were checked for normality and homogeneity of variance before analysis. Microbial community analysis was primarily conducted using the 'MICROECO' package (Liu *et al.*, 2021). To identify specific bacterial and fungal biomarkers associated with sex-specific soil legacies, Linear Discriminant Analysis Effect Size (LEfSe) was performed (LDA threshold > 2.5).

Metabolomic data were analyzed using Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) to discriminate root exudate profiles between treatments. Variable Importance in Projection (VIP) scores were calculated to rank the contribution of each metabolite to group separation. Differential metabolites were identified based on the following criteria: VIP > 1.0, $P < 0.05$ (Student's *t*-test), and $|\log_2 FC| > 1.5$. Functional annotation of these metabolites was performed using the KEGG database. Specific comparisons of phenolic and terpenoid compound concentrations between sexes were conducted using independent Student's *t*-tests.

In the exudate-addition experiment, the beta-diversity was based on Bray–Curtis dissimilarity, and pairwise comparisons were tested using the Wilcoxon rank-sum test. To identify taxa with significant differential abundance between treatments, we used the 'DESEQ2' package, visualizing results with 'GGTREEEXTRA' (Xu *et al.*, 2021). To decipher the mechanisms underlying microbial community assembly, the βNTI (Beta-Nearest Taxon Index) was computed using the 'PICANTE' package (Mikryukov, 2019). This metric was integrated with the 'RCbray' index to jointly infer the relative contributions of deterministic and stochastic processes in the soil microbial community assembly. To identify key microbial taxa closely associated with plant biomass, the Random Forest Analysis was implemented using the 'RANDOMFOREST' package, and the significance of the model and cross-validated R2 values were assessed with 5000 permutations of the response variable by using the 'A3' package (Jiao *et al.*, 2018).

Co-occurrence networks were constructed for three interaction scenarios: intra-sexual interactions (females and males receiving the same-sex exudates), inter-sexual interactions (females and males receiving opposite-sex exudates), and control (water-treated plants). Single-domain bacterial networks were inferred using SParse InversE Covariance Estimation for Ecological Association Inference (SPIECEASI) (Kurtz *et al.*, 2015). We utilized the 'glasso' method within the 'SPIECEASI' package, setting the lambda.min ratio to 0.05. Network topology was calculated and visualized using the 'IGRAPH' package, with node sizes proportional to relative abundances. Topological parameters were compared between networks using Wilcoxon tests. For cross-kingdom (bacteria–fungi) networks, ASVs were first filtered to include only those with > 60% occurrence frequency across samples. Networks were constructed using the 'GGCLUSTERNET' package via the SparCC method (Wen *et al.*, 2022), retaining only robust correlations ($r > 0.8$, $P < 0.001$). To define the 'core network' structure, all nodes lacking direct or indirect connections to the network's largest module were removed; the remaining interconnected structure was analyzed as the core network.

Results

Responses to sex-specific soil microbial legacies

To test how *P. cathayana* responds to sex-specific soil microbial legacies, we first conducted a plant–soil feedback experiment (Fig. 1a,b). Although the total biomass and root architecture were not significantly affected by the soil legacy of either sex, we observed significant physiological changes (Fig. 1c–e). Specifically, females grown in foreign (male-conditioned) soil exhibited a significantly higher net photosynthetic rate and leaf N content compared to those in home (female-conditioned) soil. Similarly, males in foreign (female-conditioned) soil showed a higher root N content than males in home soil (Fig. 1d).

Sex-specific carbon allocation and microbiome recruitment

The $^{13}CO_2$ pulse-labeling experiment revealed sex-specific differences in carbon allocation to the rhizosphere microbiome. In females, the assimilation rate of new photosynthetic carbon by soil bacteria and fungi was similar between home and foreign soils (Fig. 2). By contrast, in males grown in foreign soil, the associated microbiome assimilated significantly more new photosynthetic carbon compared to that in home soil (Fig. 2). In female-conditioned soil, female plants enriched for Actinobacteria, Bacilli, and Agaricomycetes, whereas male plants enriched for Gammaproteobacteria and Bacteroidia (Figs S3, S4). Male plants hosted a distinct and more diverse set of bacterial biomarkers in both soil types (Figs S3, S4). Functionally, females in foreign soil induced a higher AP activity, while males in foreign soil induced a higher β-1,4-glucosidase (BG) activity and increased soil N (Fig. S5).

Active foraging and carbon investment in a split-root system

To further explore belowground responses, we conducted a split-box experiment where plants could choose between male- and female-conditioned soils (Fig. 3a,b). While the traits of roots distributed into the home vs foreign compartments were not significantly different in either sex (Fig. 3c–e), $^{13}CO_2$ tracing revealed a strong preferential allocation of carbon. Female plants allocated significantly more new photosynthates to microbes in the home soil compartment (Fig. 3h,i,k). Conversely, male plants allocated significantly more new carbon to all microbial groups in the foreign soil compartment (Fig. 3h–k).

Sex recognition via root exudates

To test the hypothesis that plants can chemically perceive the sexual identity of conspecifics, we designed a reciprocal transfer experiment (Figs 4a, S1). Females had a higher biomass (Fig. 4b–e), higher leaf and root N contents, and photosynthetic rate under inter-sexual interactions (Fig. 4f,j,l) than under intra-sexual interactions. Both sexes produced distinct root exudate profiles depending on the neighbor identity (Fig. S6). In inter-sexual

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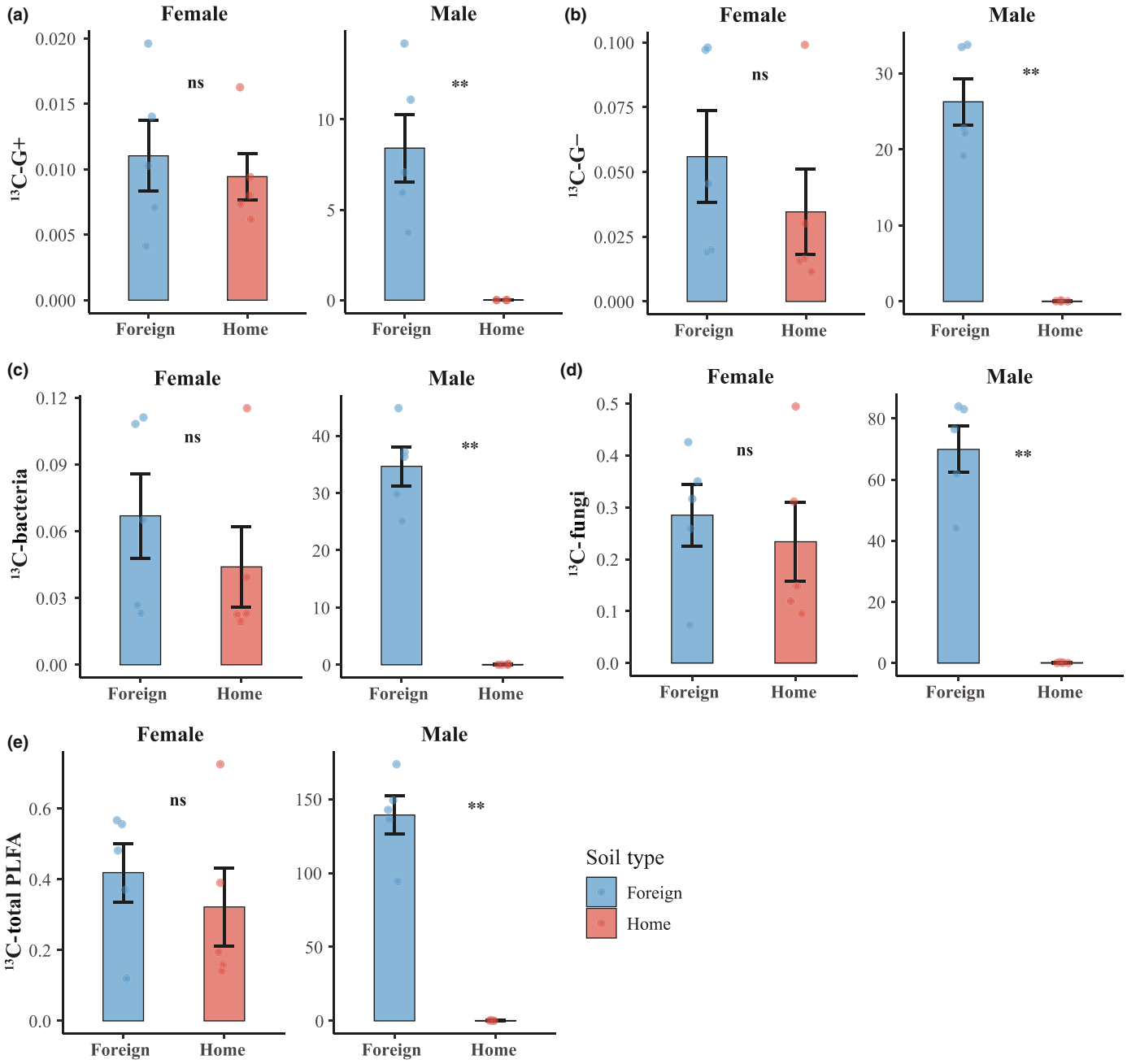


Fig. 2 Sex-specific carbon allocation to the rhizosphere microbiome revealed by $^{13}CO_2$ pulse-labeling. The relative abundance of ^{13}C assimilated by soil microbes is shown for female and male *Populus cathayana* plants grown in home vs foreign soils (n = 5, mean ± SE, ng·g^{-1}). The panels correspond to specific microbial fractions: (a) ^{13}C incorporation into Gram-positive (G+) bacteria, (b) ^{13}C incorporation into Gram-negative (G−) bacteria, (c) total ^{13}C assimilated by the whole bacterial community, (d) total ^{13}C assimilated by soil fungi, and (e) combined ^{13}C assimilation by both bacteria and fungi (total microbial uptake). PLFA, phospholipid fatty acid. Red bars represent home soil; blue bars represent foreign soil. Statistical significance was determined by Student's t-test. ns, not significant; **, 0.001 < P ≤ 0.01.

interactions, we identified 1025 differentially expressed metabolites (DEMs) upregulated in females and 1169 DEMs upregulated in males (Fig. 5e). By contrast, intra-sexual interactions yielded only 173 DEMs upregulated in females and 632 DEMs upregulated in males (Fig. 5f). The composition of these DEMs also differed markedly. Female exudates under inter-sexual conditions were enriched in certain flavonoids, whereas intra-sexual female exudates were characterized by defense-related compounds such as camalexin (Figs S7, S8). KEGG pathway analysis further confirmed distinct functional signatures. Inter-sexual interactions primarily activated pathways associated with growth-related processes, including ubiquinone biosynthesis and sphingolipid signaling. By contrast, intra-sexual interactions predominantly triggered stress-response pathways, such as the biosynthesis of secondary metabolites and arginine metabolism (Fig. 5g,h).

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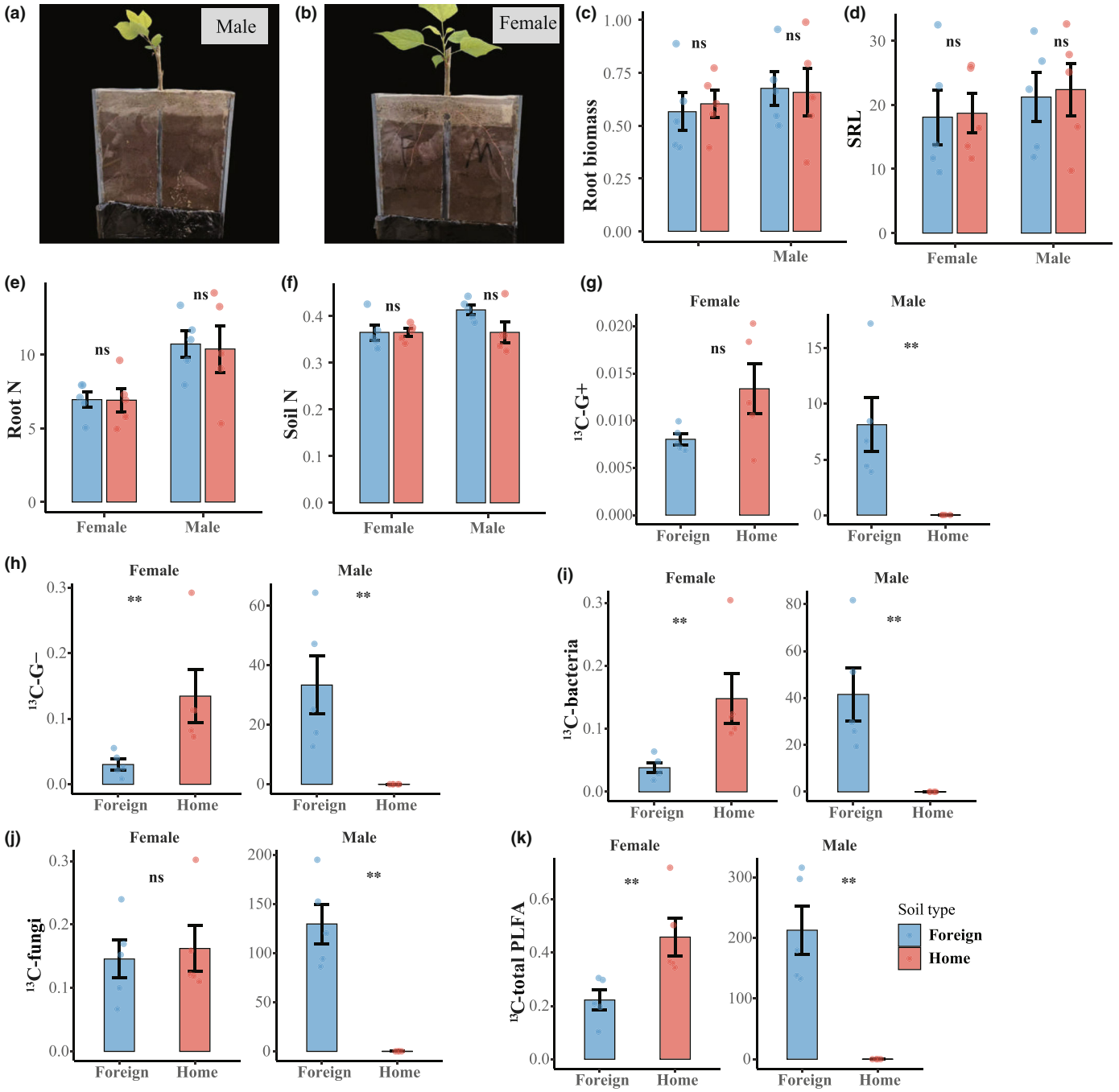


Fig. 3 Active foraging and preferential carbon investment in a split-root soil choice experiment (n = 5). (a, b) Schematic presentation of the split-root system where individual *Populus cathayana* plants had simultaneous access to both female- and male-conditioned soil compartments. (c–f) Root morphological traits and soil N in each soil compartment. Root biomass (g), SRL (m·g^{-1}), N concentration (mg·g^{-1}). (g–k) Allocation of ^{13}C-labeled photosynthates to microbial groups in each compartment (ng·g^{-1}). G+ (Gram-positive bacteria), G− (Gram-negative bacteria). ns, no significant difference; *, 0.01 < P ≤ 0.05; **, 0.001 < P ≤ 0.01; ***, P ≤ 0.001.

Exudate-driven assembly of rhizosphere microbiome

To determine if sex-specific root exudates directly drive microbial community assembly, we applied different exudate solutions to plants established in their home soils (Fig. S2). Plants of each sex were exposed to three treatments: sterile water (Control), male root exudates (M-exu), or female root exudates (F-exu). Proteobacteria and Actinobacteriota were the dominant bacterial phyla across all rhizosphere samples (Fig. S9A). The relative abundance of the Ascomycota class was lower in plants receiving opposite-sex exudates compared to other treatments (Fig. S9B). In the male rhizosphere, the bacterial community in the M-exu

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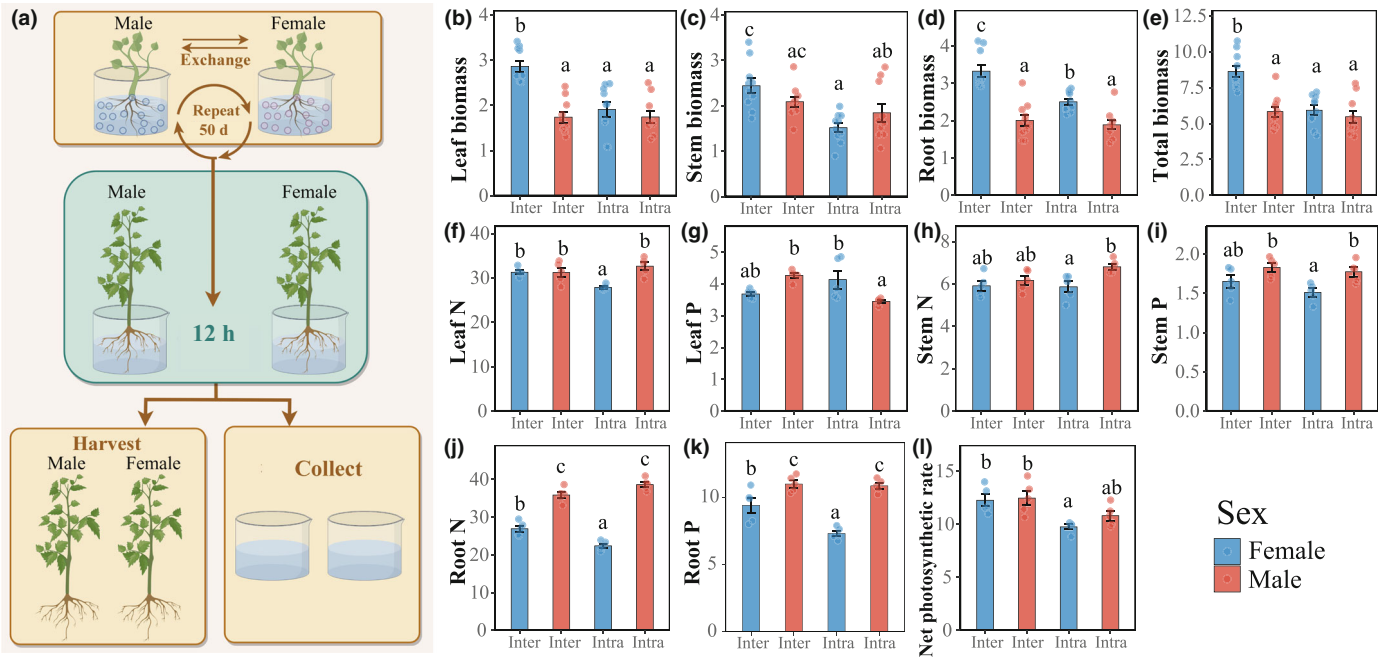


Fig. 4 Evidence of belowground sex recognition of *Populus cathayana* in a hydroponic reciprocal transfer system. (a) Schematic presentation of the experimental design. Plants were reciprocally transferred between hydroponic solutions conditioned by either same-sex or opposite-sex roots every 24 h (n = 5). (b–e) Biomass (g) of female and male plants in intra-sexual (female–female, male–male) vs inter-sexual (female–male) interactions. (f–l) Growth traits including net photosynthetic rate (P_n, μmol·m^{-2}·s^{-1}) and nutrient content (mg·g^{-1}). Data are mean ± SE. Tukey's test was used for multiple comparisons. Different letters indicate significant differences at $P \leq 0.05$.

treatment diverged significantly from both the Control and the F-exu treatments (Fig. S9C). In the female rhizosphere, however, both M-exu and F-exu treatments caused the bacterial community structure to shift significantly away from the Control group (Fig. S9D). In the male rhizosphere, both M-exu and F-exu treatments altered the fungal community structure compared to the Control (Fig. S9E), whereas the fungal community structure in the female rhizosphere showed no significant differences among the three treatments (Fig. S9F).

Community assembly processes and key taxa

In the female rhizosphere, β-Nearest Taxon Index (βNTI) values were similar across all treatments, suggesting consistent assembly processes (Fig. 6a). By contrast, in male plants, the βNTI value of the F-exu treatment was significantly higher than that in the other treatments, indicating a distinct shift in community assembly drivers (Fig. 6a). Compared to control, both same-sex and opposite-sex exudate applications led to a significant enrichment of distinct bacteria in both female and male rhizospheres (Fig. S10). A detailed analysis of these differentially enriched microbes revealed that exudate treatments induced highly treatment-specific ASV recruitment. In female plants, the F-exu treatment resulted in a distinct community profile, including many unique ASVs, compared to the M-exu treatment (Fig. 6b). Similarly, in male plants, a large number of ASVs were found exclusively in either the F-exu or M-exu treatment, highlighting sex-specific microbial filtering (Fig. 6b). The Random Forest Analysis (R^2 = 62.9%, P < 0.01) identified key taxa being significantly associated with plant growth. Taxa belonging to the phyla Actinobacteriota and Proteobacteria were highly prevalent (Fig. 6c).

Network complexity and cross-kingdom interactions

We grouped the exudate treatments into three distinct categories: intra-sexual interactions (females and males receiving same-sex exudates), inter-sexual interactions (females and males receiving opposite-sex exudates, respectively), and control (water-treated plants). The co-occurrence analysis revealed that these three interaction types generated distinct bacterial network topologies (Fig. S11). The network derived from inter-sexual interactions featured a greater number of key topological nodes (12 connectors and 1 module hub) compared to the other networks (Fig. S11). This increased complexity was associated with a significantly higher average node degree in the inter-sexual network, indicating greater overall connectivity (Fig. S11). We extended this analysis to core cross-kingdom networks (bacteria–fungi). The inter-sexual interaction network was significantly more complex, characterized by more nodes and edges (Fig. 7a). The composition of the taxa forming these networks was also distinct, and the phylogenetic diversity (PD) of these network members was significantly higher in the inter-sexual group (Fig. 7a–c). From control to intra-sexual to inter-sexual interactions, the relative abundance of Ascomycota decreased, while the relative abundance of Basidiomycota increased (Fig. 7d). Topologically, the

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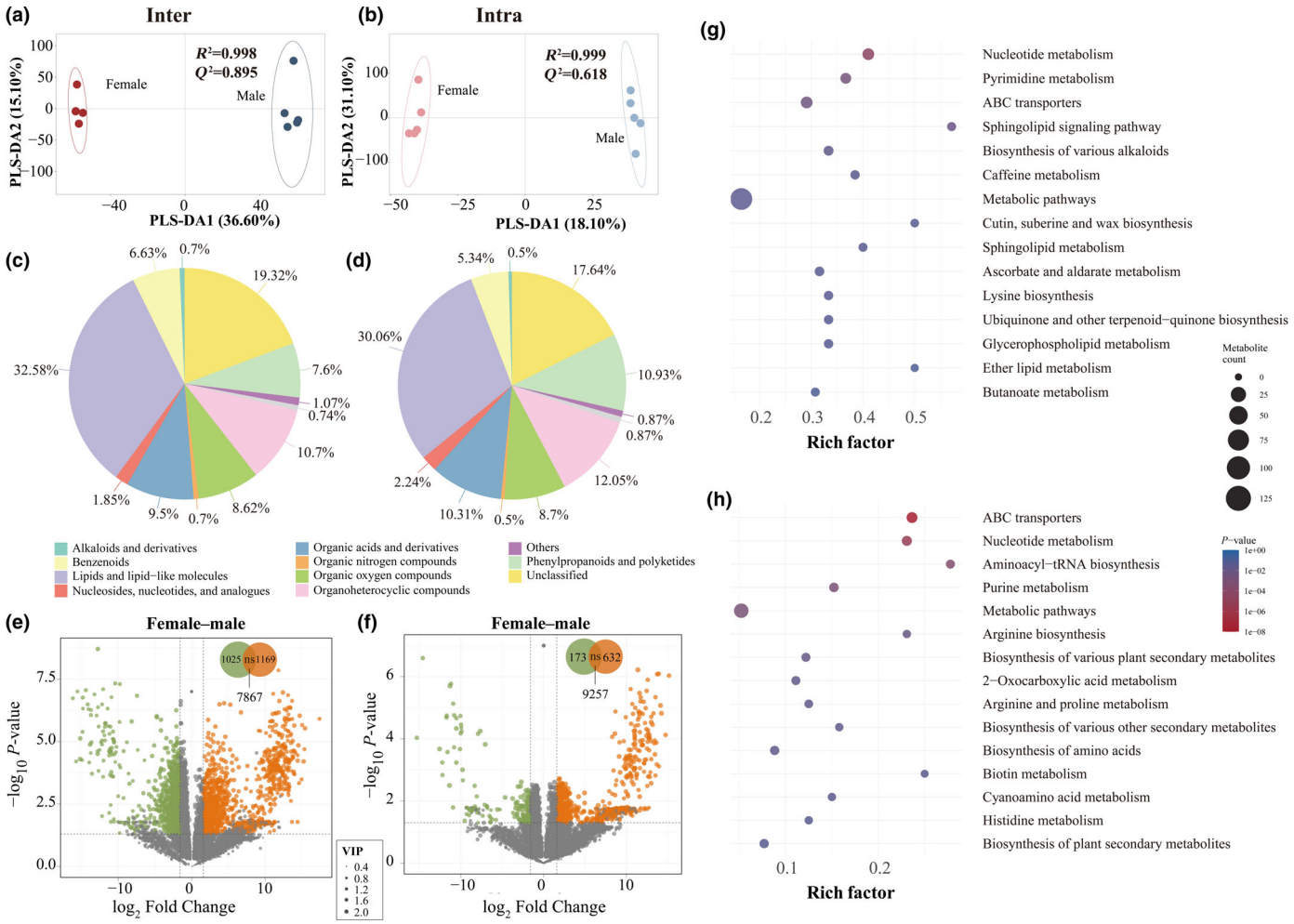


Fig. 5 Chemotaxonomic profiling and functional pathways of root exudates modulated by neighbor identity. (a, b) The Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) analysis of root exudates between males and females *Populus cathayana* under inter- and intra-sexual interactions, respectively. (c) The composition and proportion of root exudates under inter-sexual interactions. (d) The composition and proportion of root exudates under intra-sexual interactions. (e) The volcano plot of root exudate profiles from inter-sexual interactions. The green dots indicate differentially expressed metabolites (DEMs) enriched in males, while the orange dots indicate DEMs enriched in females. (f) The volcano plot of root exudate profiles from intra-sexual interactions. The green dots indicate DEMs enriched in males, while the orange dots indicate DEMs enriched in females. (g) KEGG functions of DEMs between females and males identified from inter-sexual interactions. (h) KEGG functions of DEMs between females and males identified from intra-sexual interactions. The size of circles shows the abundance of DEMs in each functional pathway. The color of each circle shows *P* values.

inter-sexual cross-kingdom network showed a significantly higher closeness centrality (Fig. 7e). Furthermore, its core network structure contained a significantly higher proportion of positive edges than the other two treatments (Fig. 7f).

Discussion

Sex-specific legacies and physiological priming

While soil microbial legacies are increasingly recognized as drivers of ecosystem productivity and as a contributing factor to the decline of continuous poplar monocultures, our results revealed that these legacies are sexually distinct. *Populus cathayana* females exhibited significantly higher net photosynthetic rates and leaf N contents when grown in male-conditioned soil compared to 'home' soil. This phenomenon likely represents a form of physiological priming, where plants living in favorable microbial environments prioritize nutrient acquisition and metabolic efficiency before allocating resources to structural growth. Sex-specific microbiota can be shaped by diverse dioecious species, which in turn affect their fitness in different environments (Vega-Frutis *et al.*, 2018; Guo *et al.*, 2022; Lin *et al.*, 2025). The underlying mechanism appears to be microbiome-mediated nutrient cycling. Previous research indicates that male and female poplars foster distinct microbial communities with divergent capacities for N cycling and phosphorus solubilization (Xia *et al.*, 2022; Guo *et al.*, 2024). Specifically, *P. euphratica* males typically maintain a higher abundance of phosphate-solubilizing bacteria (Xia *et al.*, 2022), and they show a higher net nitrification rate and BG activity compared to females (Guo *et al.*, 2024). Consequently, the microbial legacy in the male-conditioned soil appears to increase the bioavailability of limiting nutrients (e.g.

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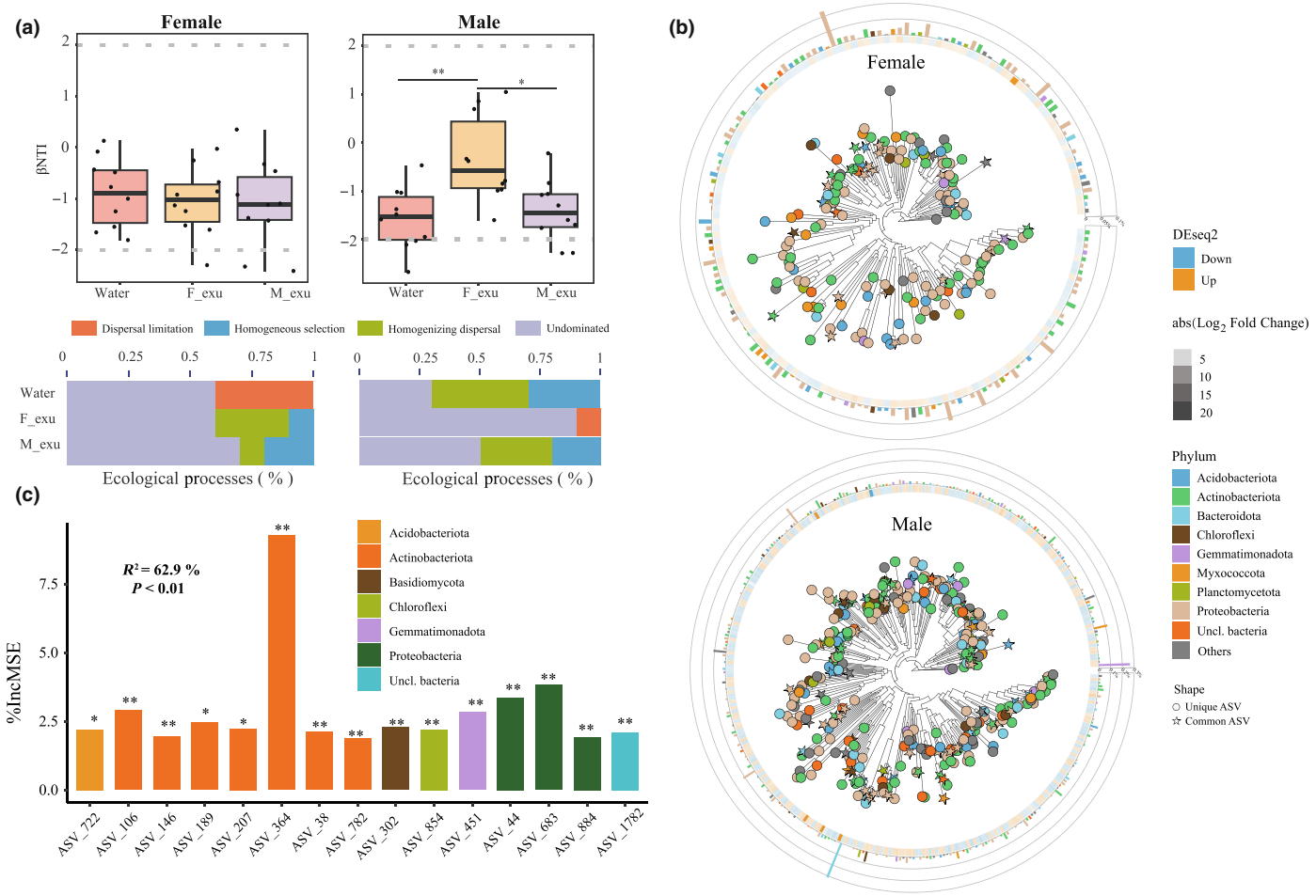


Fig. 6 Rhizosphere microbiome assembly driven by sex-specific root exudates of *Populus cathayana*. (a) Beta-Nearest Taxon Index (βNTI) indicating the relative importance of deterministic vs stochastic assembly processes. Box plots display the median (center line), interquartile range (box), and interquartile range (whiskers); individual data points are overlaid as dots (n = 5). (b) Phylogenetic tree of different enriched and depleted bacterial Amplicon Sequence Variants (ASVs). These ASVs were identified by DESeq2 analysis in Supporting Information Fig. S10. The blue and orange color within the first inner ring shows depleted and enriched ASVs, respectively, while the color gradients show Log_2 Fold Change. The circle and star shapes represent unique and shared ASVs, respectively. (c) Top 15 ASVs most relevant to plant biomass identified by Random Forest Analysis. *, 0.01 < $P \leq$ 0.05; **, 0.001 < $P \leq$ 0.01.

nitrogen), which in turn promotes enhanced N uptake and photosynthetic performance in subsequent female individuals (Fig. 1). This implies that in natural or mixed-sex plantation settings, male plants may act as 'soil conditioners' that alleviate intraspecific competition in females, a critical ecological phenomenon related to the higher reproductive costs typically present in females.

Divergent carbon investment strategies in response to soil legacies

The input of root exudates fuels the survival, growth, and reproduction of soil microbes that form a direct communication pathway between the host and rhizosphere microbes (Zhalnina *et al.*, 2018). The pattern of carbon input depends on whether a plant is exposed to home or foreign soil, with consequences for the microbial community composition and function (Guo *et al.*, 2024). Notably, ^{13}C-labeling revealed a clear sexual divergence in this response, with male plants allocating substantially more newly fixed photosynthates to soil bacteria and fungi in foreign soil compared to home soil, whereas females did not show such differential allocation (Figs 2, 3). This pattern aligns with known sexually dimorphic resource acquisition strategies in dioecious plants (Hultine *et al.*, 2016). Females, which typically bear high reproductive costs, may prioritize carbon conservation and exhibit more conservative belowground allocation under unfamiliar soil conditions (Xia *et al.*, 2022; Liu *et al.*, 2024). By contrast, males tend to maintain a stable root carbon sink to sustain their associated microbial partners and functions (Wu *et al.*, 2021; Xia *et al.*, 2022). By allocating significantly more new photosynthates to foreign soil, *P. cathayana* males were characterized by distinct microbial communities and elevated β-1,4-glucosidase activities (BG) (Figs S3–S5). BG catalyzes the final step in cellulose degradation, hydrolyzing cellobiose to glucose monomers that can be rapidly assimilated by microbial cells (Bakshi & Varma, 2010). This enzyme activity exhibits strong

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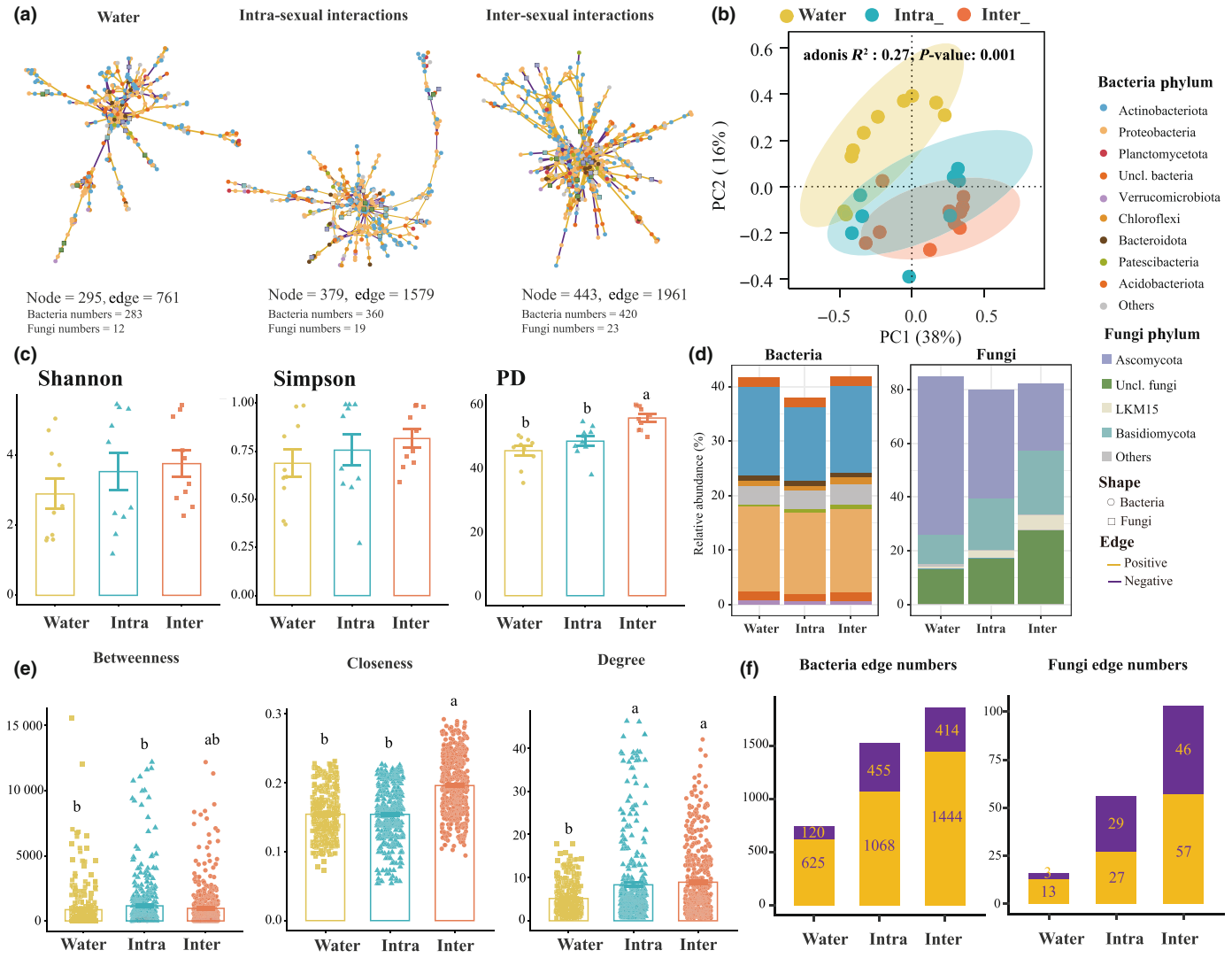


Fig. 7 Topology and composition of core cross-kingdom (bacterial–fungal) co-occurrence networks. (a) Topological properties of networks constructed under three interaction scenarios: intra-sexual (*Populus cathayana* plants receiving same-sex exudates, $n = 10$), inter-sexual (plants receiving opposite-sex exudates, $n = 10$), and control (water-treated plants, $n = 10$). Data are mean ± SE. In network diagrams, solid circles represent bacterial Amplicon Sequence Variants (ASVs), solid squares represent fungal ASVs, and node colors correspond to microbial phyla. (b) Principal Coordinate Analysis (PCoA) of bacterial and fungal communities across treatments. (c) Alpha diversity indices (Shannon, Simpson) and phylogenetic diversity (PD) of ASVs within the core networks. (d) Taxonomic composition and relative abundance of ASVs within each network. (e–f) Topological properties of networks. (e) Data are mean ± SE. The legend on the right applies to (a).

positive correlations with the actively growing microbial biomass, indicating that an elevated BG activity specifically marks microsites where rapid carbon turnover and microbial proliferation occur (Kuzyakov & Blagodatskaya, 2015). These findings suggest that males can remodel the microbial community of foreign soils through increased labile carbon inputs, underscoring their role not merely as resource competitors, but as active modulators of soil microbial functioning.

Root exudates as a sex-specific chemical language

Previous studies have suggested that the sexual identity of neighbors differentially affects the growth of dioecious plants (Mercer & Eppley, 2014; Chen *et al.*, 2015; He *et al.*, 2021). Generally, female plants experience intense intraspecific competition when growing near same-sex neighbors, whereas inter-sexual interactions often facilitate resource use efficiency (Zhang *et al.*, 2021; Guo *et al.*, 2024). Consistently, our results confirmed that *P. cathayana* females grew significantly better and exhibited higher N contents, photosynthetic rates, and biomass when exposed to male-conditioned solutions compared to female-conditioned solutions (Fig. 4). This distinct phenotypic plasticity demonstrates that plants possess a capability for belowground sex recognition, utilizing the chemical composition of root exudates as a reliable informative cue to distinguish neighbors.

We found that this recognition drives a dynamic metabolic shift, orchestrating distinct strategies for competition vs facilitation. In intra-sexual interactions (female–female), plants

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upregulated potent defense-related compounds, such as antiarol and camalexin (Fig. 5). Camalexin, a major phytoalexin typically synthesized in response to a pathogen attack or competitive stress, can reshape microbial interactions in the rhizosphere by restricting the growth of sensitive bacterial competitors (Koprivova *et al.*, 2019). Since the biosynthesis of such secondary metabolites is energetically costly, their accumulation suggests that females perceive same-sex neighbors as competitive threats, triggering an active, high-cost defensive posture. This chemical stress response likely explains the lower biomass accumulation observed in same-sex pairs (Fig. 4). Conversely, inter-sexual interactions triggered a cooperative chemical dialogue that promoted female growth. On one hand, females responding to males specifically upregulated flavonoids (e.g. kaempferol 3-neohesperidoside) and phenolics. Flavonoids function as modulators of auxin transport and natural iron chelators, thereby enhancing root plasticity and nutrient mobility (Peer & Murphy, 2007; Cesco *et al.*, 2010). On the other hand, male exudates were enriched in sorgolactone (a strigolactone analog) and gibberellin A59 (a gibberellin precursor; Fig. S7). Both strigolactones and gibberellins are known to function as plant growth regulators that can influence root development and nutrient acquisition (Kapulnik & Koltai, 2014; van Gelderen *et al.*, 2025). These compounds may therefore contribute to the enhanced root growth and N acquisition observed in females under inter-sexual interactions, mechanistically explaining their superior photosynthetic performance in mixed-sex settings.

While previous research attributed the benefits of mixed-sex stands to spatial differentiation, such as males exploring deeper soil layers for water and N (Guo *et al.*, 2024), our study revealed an additional 'chemical niche' dimension. In monocultures, females with high nutrient demands suffer from severe niche overlap, leading to intense competition and stress-induced metabolic shifts. In mixed stands, however, the divergent exudate profiles of males and females alleviate this competition. By shifting from defense (camalexin) to growth promotion (flavonoids/hormones), inter-sexual interactions optimize metabolic investments, driving the positive effects detected in this study. Although our experimental design did not directly test the causal effects of individual exudate compounds on microbial community shifts, the divergent metabolic profiles we observed under inter- vs intra-sexual interactions provide a mechanistic framework for future exudate-addition experiments for establishing direct chemical-microbiome linkages.

Sex-specific modulation of rhizosphere microbiome assembly

It is well-established that plant-mediated selective pressures, exerted through root exudation, determine the composition and function of the rhizosphere microbiota (Henneron *et al.*, 2020). In dioecious species, sexual dimorphism in exudate profiles is a crucial driver in shaping sex-specific microbial communities (He *et al.*, 2022; Guo *et al.*, 2024). Our findings confirm that the chemical plasticity of root exudates, triggered by neighbor identity, serves as a potent environmental filter that profoundly alters the rhizosphere microbiome, particularly the bacterial community (Figs 6, S9). We observed that male plants exhibited a stronger capacity to recruit unique bacterial ASVs and were more effective at modulating soil legacies than females (Figs 6b, S10). Specifically, the male rhizosphere assembly was characterized by a higher influence of homogeneous selection when exposed to male exudates, suggesting a more deterministic and specialized recruitment process. This superior modulating ability in males is supported by our ^{13}C-labeling experiment, which revealed that males allocated significantly more newly fixed photosynthetic carbon to soil microbes in foreign soils compared to females (Figs 2, 3). While female carbon flow to the rhizosphere often decreases under stress due to reduced photosynthetic capacity (He *et al.*, 2022; Liu *et al.*, 2024), males maintain a higher metabolic investment. This investment supports key taxa, such as Actinobacteriota, known for producing extracellular enzymes and antimicrobials, and Proteobacteria (particularly Gammaproteobacteria), which are metabolically versatile fast-growers linked to rapid nutrient cycling (Fig. 6; Silva *et al.*, 2022; Ai *et al.*, 2025). Linked to previously reported higher AP activities and stable phosphate-solubilizing communities reported in males (Xia *et al.*, 2024), our results underscore that male plants act as active modulators of soil legacies, capable of rapidly altering selective pressures to optimize the microbial environment.

Beyond altering the community composition, the presence of neighbor-specific exudates imposes selective pressures that reshape microbe–microbe interactions. The preferential uptake of divergent metabolites leads to distinct microbial assemblages (Ulbrich *et al.*, 2022; Liu *et al.*, 2024). We found that inter-sexual interactions fostered more complex bacterial networks, characterized by a higher density of connectors and module hubs, and an increased average node degree (Fig. S11). Such highly interconnected networks, particularly those with enriched hub taxa, are known to enhance the resilience of microbial communities to environmental perturbations (Gao *et al.*, 2022). Notably, inter-sexual interactions also shaped a more complex cross-kingdom network with a higher PD and a significantly greater proportion of positive edges compared to intra-sexual interactions (Fig. 7f). This shift suggests that inter-sexual dynamics facilitate cooperative microbial interactions rather than competition. The distinct exudate profiles of males and females under inter-sexual interactions likely create broader metabolic niches, reducing direct resource competition among microbial taxa and promoting niche complementarity. Males can improve female resistance to resource-limited environments by increasing water use-efficiency or nutrient uptake under inter-sexual interactions (Guo *et al.*, 2024; Liu *et al.*, 2024; Gao *et al.*, 2025). For example, the increased abundance of Basidiomycota in inter-sexual networks may reflect a shift in the fungal community composition including ectomycorrhizal taxa (Gao *et al.*, 2025), although a dedicated mycorrhizal assessment would be required to confirm this hypothesis (Gao *et al.*, 2025). In these mixed-sex settings, males may facilitate N transport to females via common mycelial networks, a hypothesis supported by the significantly higher N content and improved physiological resistance observed in females under inter-sexual interactions observed in this study

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(Fig. 4). Collectively, these results demonstrate that while same-sex neighbors trigger competitive stress, inter-sexual chemical dialogues cultivate a cooperative and resilient soil microbiome that alleviates resource limitations.

This study establishes that *P. cathayana* possesses a sophisticated capacity for belowground sex recognition, mediated by the metabolic plasticity of root exudates. Our findings reveal a distinct sexual dimorphism in response strategies to neighbor identity. Females upregulated energetically costly compounds to counter intraspecific competition from same-sex neighbors, while shifting toward growth-promoting pathways and enhanced nutrient acquisition when perceiving male-derived cues. Males act as active soil conditioners, investing more newly fixed photosynthetic carbon into the soil to reshape microbial communities in ways that facilitate subsequent nutrient uptake by females. This male-driven carbon investment fosters more complex cross-kingdom microbial networks, which mechanistically explains the improved nitrogen uptake and photosynthetic performance observed in females. From a silvicultural perspective, our results provide a mechanical basis for utilizing mixed-sex plantations to harness these natural sex-specific synergies, thereby enhancing ecosystem resilience and plantation productivity while mitigating the soil degradation common in single-sex monocultures.

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Competing interests

None declared.

Author contributions

QG contributed to conceptualization, data curation, formal analysis, investigation, methodology, funding acquisition, project administration, visualization, writing – original draft. XC and JD contributed to data curation, investigation. HK contributed to conceptualization, validation, and writing – review and editing. CL contributed to conceptualization, methodology, funding acquisition, project administration, resources, supervision, validation, writing – review and editing.

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Data availability

Data are available in Zenodo (https://zenodo.org/records/21222709). The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in the National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA037748 and CRA037747) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

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Supporting Information

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Fig. S1 The full experimental design of the hydroponic reciprocal transfer experiment.

Fig. S2 The experimental design to study the influence of belowground interactions between males and females *Populus cathayana* on the rhizosphere assembly.

Fig. S3 Influences of female and male plants on soil microbial legacy.

Fig. S4 Influences of female and male plants on soil microbial legacy.

Fig. S5 Soil enzyme activities, nitrogen content, and experimental setup in the soil microbial legacy experiment.

Fig. S6 Multivariate analysis of root exudate profiles.

Fig. S7 Differences in the phenolic and terpenoid compounds among DEMs between females and males *Populus cathayana* in inter-sexual interactions.

Fig. S8 Differences in the phenolic and terpenoid compounds among DEMs between females and males *Populus cathayana* in intra-sexual interactions.

Fig. S9 The influence of root exudates on soil bacterial and fungal communities.

Fig. S10 The volcano plot of different taxa with significant differential abundances in response to different exudations.

Fig. S11 Co-occurrence network traits affected by different belowground interactions.

Methods S1 Detailed information of DNA extraction and Illumina sequencing.

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