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Females face more positive plant-soil feedback and intersexual competition under adequate nitrogen conditions compared to males in *Populus cathayana*

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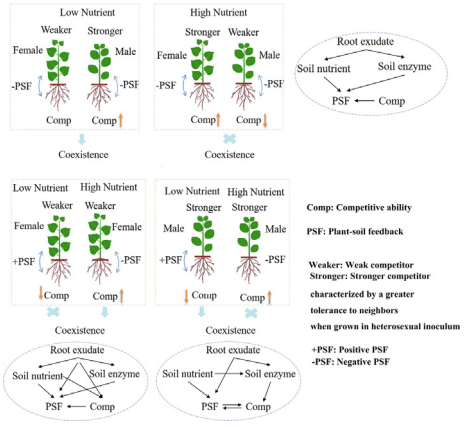
HIGHLIGHTS

- Cosexual soil inoculation can reduce the inequality in sexual competitive abilities.
- Low nutrient availability promotes intersexual interaction.
- Females face more positive plant-soil feedback and intersexual competition.
- Soil biota and root exudate can mitigate differences in sexual competitiveness.

GRAPHICAL ABSTRACT



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ABSTRACT

Plant-soil feedback (PSF) and competition influence plant performance, community structure and functions. However, how nutrient availability affects the interaction of PSF, sexual competition and coexistence in dioecious plants is poorly understood. In this study, the strengths of PSF and sexual competition, and their responses to nutrient availability were assessed in dioecious *Populus cathayana* using a garden experiment. We found that PSF reduced but did not eliminate the inequal sexual competition at low nitrogen (N) availability. Intersexual competition and nutrient limitation induced more negative PSF, which promoted sexual coexistence. PSF and competition were rather related to sexual dimorphism. Female plants experience more positive PSF and intersexual competition under adequate N conditions compared to males; the contrary was true with low N supply. Furthermore, the stability of root exudate networks and soil nutrient availability reflects the possibility of sexual coexistence regulated by PSF. Intersexual interaction promote more stable root exudate profiles and more saccharide secretion at low N supply. Meanwhile, the increased soil N and P mineralization in females with cultivated males explained the possible coexistence between females and males at low nutrient availability. Thus, these results indicate that soil biota can mitigate differences in sexual competitiveness and improve the stability of root exudate networks, consequently promoting sexual coexistence at low nutrient availability.

1. Introduction

Females and males in dioecious plant species experience different reproductive costs and selective pressures. Such differences cause sexual

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specialization and niche partitioning, reinforced by changing environments (Mercer and Eppley, 2014; Hultine et al., 2016; Simancas et al., 2018). Importantly, highly skewed sex ratios may cause plant population declines and even mass extinctions, with potential effects on the structure and stability of populations (Dawson and Geber, 1999; Petry et al., 2016). Therefore, it is urgent to understand the mechanism driving sexual spatial segregation and coexistence in dioecious plant species.

In natural systems, plants experience direct competition with neighboring species and simultaneously interact with their soil biota, which in turn, influence plant growth and coexistence, meaning plant-soil feedback (PSF) (Bever, 1994, 2003; Mangan et al., 2010a, 2010b; Lekberg et al., 2018). PSF has a negative effect on the buildup of soil-borne pathogens or depletion in nutrient availability, or a positive role in promoting soil mutualists and/or soil nutrient availability (Van der Putten et al., 2013). It has been suggested that the interactions of PSF and competition influence community segregation and coexistence (Teste et al., 2019; Heinen et al., 2022). Plants with positive PSFs gain competitive advantages over other species with fewer soil mutualists, promoting competitive dominance (Maron et al., 2016). Contrarily, negative PSF attenuates or eliminates competitive hierarchies and promotes plant species diversity (Bonanomi et al., 2005). Dioecious plants often exhibit spatial variability along a resource gradient, partly driven by the sex-specific selection for habitats and excluding the opposite sex from the habitats. In previous studies, sex-specific functional traits influence sexual competition and soil microbial community composition (Xia et al., 2022a), which in turn may affect the results of plant sexual competition. However, it is still not clear whether and how competition and coexistence are driven by PSF in dioecious woody species.

The PSF and plant-plant interactions are also associated with plant functional traits (Klinerová and Dostál, 2020). Plants can recognize kin and modify their functional traits in response to the genetic relatedness of neighbors (Dong et al., 2017; Semchenko et al., 2017). Such plasticity can have cascading effects on soil microbial activities, which in turn affect the PSF and competition (Semchenko et al., 2021). It has been reported that the neighboring plant species' identity can significantly modify the plant phenotypic plasticity, which can potentially change the intensity and direction of PSF, either eliminating or intensifying them (Casper and Castelli, 2007; Huangfu et al., 2022; Wandrag et al., 2023). The presence of genetic diversity in plant species could reduce negative PSF by decreasing soil pathogen accumulation (Nijjer et al., 2007). Conversely, some plant species with accumulating soil mutualists benefit more from conspecific than from heterospecific competition and, thus, produce positive PSF, which may reduce competition and maintain coexistence at broader spatial scales (Molofsky et al., 2001). Plant sexes optimize their performance and change competitive ability when confronted with the opposite individual, either the alter carbon allocation or root architecture (Chen et al., 2021; Liu et al., 2021). Thus, the functional trait plasticity by different neighboring sexes is likely to alter the results of PSF and sexual competition. However, how sexual dimorphism and the neighboring identity of each sex affect PSF and competition remains unclear.

PSF and competition are dynamic greatly depending on the response to nutrient availability (Wei et al., 2018; Ploughe et al., 2019; Zuñiga et al., 2019; Chen et al., 2022). The effects of increased nutrient availability on PSF and competition are associated with plant ecological optima concerning nutrients (Lemmermeyer et al., 2015; Klinerová and Dostál, 2020). It had been suggested that nutrient-demanding species faceless PSF and negative competition effects in a nutrient-rich environment (Lemmermeyer et al., 2015; de la Pena et al., 2016). More intense competition is expected in resource-rich environments through asymmetric competition for nutrients (DeMalach et al., 2016). A different view suggested plant species differing in the ecological optimal of nutrients are more coexistence in highly variable environments. Increased nutrient availability is assumed to drive more negative PSF via the increased possibility of producing more pathogens, or more positive PSF as the improved plant growth and defense against abiotic and biotic stressors (Lemmermeyer et al., 2015; Bennett et al., 2017). In poplar, females showed a greater competitive ability than males at adequate nutrient levels, but the opposite occurs in fertile conditions (Chen et al., 2014). The sex-specific utilization of resources may be decisive for the intensity and direction of competition and PSF in dioecious plants. However, how nutrient availability modifies the strength and direction of PSF and competition in dioecious plants is largely unknown.

Here, we employed *Populus cathayana* to assess the strength and direction of PSF, sexual competition and plant-plant and plant-microbial interactions under low and high nitrogen levels. We combined classic pairwise plant competition experiments with a PSF experiment in both sexes grown under intra- and intersexual soil inoculum. By doing so, we aimed to answer the following questions: (i) How does PSF drive sexual competition? (ii) How do sexual dimorphism drive PSF and sexual competition? (iii) How nutrient availability affects the interaction of PSF, sexual competition and coexistence in dioecious plants?

2. Materials and methods

2.1. Plant species and growth conditions

P. cathayana cuttings of each sex were collected from different individual parent trees in riparian and valley flat habitats (Datong, 35°56′N, 101°35′E) in Qinghai, China (Liu et al., 2020). Cuttings were grown in 0.5-l pots and randomly placed in a glasshouse at the Hangzhou Normal University (30.03′ N, 120.12′ E) in Zhejiang, China with 12–14 h photoperiod throughout the growth period, with the temperature of 21–25 °C during the day and 15–18 °C at night. After one month, healthy and uniform cuttings (about 20 cm in height) were selected and transplanted into plastic pots with 40 kg of sandy soils collected from the sampling sites at the Hangzhou Normal University. The soil had 2.82 g kg^{-1} organic matter, 0.28 g kg^{-1} total nitrogen (N), 2.62 mg kg^{-1} available P and 90.65 mg kg^{-1} available K, and pH 8.64.

2.2. Experimental design

The first experiment was the soil conditioning phase to obtain soil with sex-specific soil communities, which could then be used during the feedback phase (Fig. S1). In the conditioning phase, we mixed 6 % inoculum of specific soil from 5-yr-old monocultures or mixed cultures of each sex with 94 % sterilized sandy soil. Such a small proportion of natural soil inoculum was used to minimize the abiotic effects of different soil inoculum (Mangan et al., 2010a, 2010b). Uniform female and male cuttings grew for one month were transformed into 40-l pots filled with mixed soils (6 % specific inoculum and 94 % sandy soil). We created three plant interactive patterns with two cuttings in each pot, female-female interaction (FF) and male-male interaction (MM): 10 pots with each plant interactive type. Each plant interactive pattern was grown in its soil (FF pattern with female inoculum soil and MM pattern with male inoculum soil. The purpose was to create soils with strong PSF effects (Hendriks et al., 2015). All pots were randomly placed in the greenhouse and grown with regular weeding and watering during April–May 2018. After 3 years, the aboveground biomass was harvested. Soils from each sexual combination were harvested, pooled together, mixed and stored in the dark at 4 °C until use.

The second experiment was the feedback phase, during which we established three sexual groups (single cultivation without competition, intrasexual competition with two females or males, intersexual competition with one female and one male), two soil inoculum types (6 % soil from female group and male group), two nitrogen levels (normal N supply, low N supply) (Fig. S1). There were 20 treatment combinations, with four replications per treatment. Female and male cuttings were obtained as described above. After growing in background soils (with γ-irradiated sandy soils) for one month, healthy and uniform cuttings (about 20 cm in height) were selected and transplanted into plastic pots of 40 l with a different living soil inoculum from the conditioning phase (6 % of the pot volume; 94 % of the pot volume consisted of γ-irradiated soils). Cuttings were randomly placed in the experimental glasshouse and were maintained by weeding and watering regularly during April–May 2021. The experimental

condition was similar to the conditioning phase. In July 2021, half of the pots were fertilized by high N, while the other half of the pots were fertilized by low N. After three months, all cuttings were divided into roots, stems and leaves and harvested for further analysis.

2.3. Root trait measurements

After careful washing, one part of the root system was selected and scanned using the root scanner (Epson Expression 1600, Japan). The total root length and root volume were analyzed by the Win-RHIZO system (version 5.0, Regent Instruments Inc., Canada). After that, all harvested roots were dried at 75 °C for 72 h. The specific root length (SRL) and root length density (RLD) were calculated as the ratio of the root length to its dry mass and as the ratio of the total root length to its volume, respectively (Xia et al., 2022b).

2.4. Root exudate collection and analysis

The root exudate collection was performed according to previous method (Williams et al., 2021). Briefly, roots were washed thoroughly with deionized water and the plants were transferred into an aerated hydroponics solution with natural ambient light to let roots recover after washing for three days. After that, 100 ml of the root exudates were immediately collected and filtered through 0.22-μm syringes. The exudates were stored at −80 °C until use. About 6-ml samples were placed into 50-ml centrifuge tubes followed by freezing with liquid nitrogen. Then, the samples were freeze-dried with a vacuum freeze drier. The freeze-dried exudate pellets were re-suspended in 200 μl methanol internal standard extract and centrifuged with 12,000 r min^{-1} for 10 min at 4 °C. Thereafter, the supernatants were filtered through 0.22-μm syringes and stored in sample bottles until LC-MS/MS detection. The metabolomics data were acquired using ultra-performance lipid chromatography (UPLC, SHIMADZU extra X2) and Applied Biosystems 4500 QTAP. The analytical conditions were as follows: Agilent SB-C18 column (2.1 mm × 100 mm); the mobile phase: solvent A with 0.1 % pure water, and solvent B was acetonitrile with 0.1 % formic acid; the linear gradient of solvent B was 5 % at 0 min, 95 % within 9 min, 5 % within 1 min and kept at 5 % for 5 min. The flow rate was 0.35 ml min^{-1}, the column was 40 °C and the injection volume was 4 μl. The LIT and triple quadrupole scans were acquired on a triple quadrupole-linear ion trap mass spectrometer and AB4500Q TRAP UPLC/MC/MS system equipped with an ESI Turbo Ion-Spray interface, performing in negative and positive ion mode and controlled by Analyst 1.6.3 software (AB Sciex).

2.5. Measurement of soil physiochemical properties

Soil pH was assayed at a ratio of 1:2.5 soil to H_2O with glass electrodes connected to a PHBJ-260 pH meter (Shanghai Leici, China) (McLean, 1983). Soil cation exchange capacity (CEC) was measured with NaOAc saturation after extraction by NH_4OAc (Staff, 1954). Soil total C (TC) and N (TN) were assayed with a chemiluminescence detector (CLD-TOC) coupled with a C and N analyser (multi C/N 3100; Jena Analytics, Jena, Germany). Soil availability potassium (AK) was measured by plasma-atomic emission spectrometry (ICPS-7500, Shimadzu, Japan) after digestion with $HClO_4$ and H_2SO_4 (Zhang and Gong, 2012). Soil total P (TP) was measured by the molybdenum blue methods after digestion by H_2SO_4 and $HClO_4$ (Olsen et al., 1954). Soil availability phosphorus (AP) was determined with the extraction of $NaHCO_3$ and Mo-Sb colorimetric method by a spectrophotometer (UV-752, Shanghai Guangpu Technology Instrument Co., Ltd., Shanghai, China) (Thien and Myers, 1992). For the measurement of NO_3^--N and NH_4^+-N, the soil samples were extracted with 1 M and measured using a continuous flow analytical system (SEAL Analytical Auto Analyzer 3) (Sims and Jackson, 1971; Sahrawat and Prasad, 1975). Soil microbial C (MC) and N (MN) contents were assayed with the chloroform fumigation followed by extraction with 0.5 M K_2SO_4 (1:5 ratio of soil to solution) (Brookes et al., 1985; Vance et al., 1987). Soil organic matter content (OM) was assayed with an elemental analyser and the removal of inorganic C with 0.5 M HCl (Nelson and Sommers, 1982). Soil net nitrification rate (NNR) was measured following 2 M KCl extraction and incubated at 20 °C for 14 days in the dark (Zhang et al., 2019). Soil net ammonification rate (AMR) was assayed from the changed NH_4^+ after inoculating at 20 °C for 14 days in the dark followed by extraction of 2 M KCl (Zhang et al., 2019). The content of NO_3^- and NH_4^+ in extracts was analyzed colorimetrically as ammonium salicylate complex at 667 nm and nitro-salicylate at 410 nm, respectively (Kempers and Zweers, 1986; ISO 7890–3, 1988). Each treatment was replicated four times.

Soil urease (URE) activity was determined with the indophenol colorimetric method by the measurement of NH_4^+ from urea at 37 °C for 2 h (Kandeler and Gerber, 1988). Soil nitrate reductase (NR) activity was colorimetrically measured at 520 nm after incubation at 30 °C for 24 h (Nowak et al., 2002; Hu et al., 2018). Soil nitrite reductase (NIR) was measured with reduced nitrite to ammonium followed by the extraction of fresh soil samples with mixed 10 mM $NaNO_2$ and 5 mM methyl viologen (Krishna et al., 1988). Soil polyphenol oxidase (PPO) activity was measured after inoculation of the reactive solution of 1 g soil samples with 10 ml of 1 % pyrogallic acid and 4 ml of citric acid phosphate (buffered to pH 4.5) at 30 °C for 2 h (Yang et al., 2017). Soil catalase (CAT) activity was measured by titration with $KMnO_4$ after extraction of 5 g fresh soils with 5 ml sulfuric acid (1.5 mol l^{-1}) and 5 ml H_2O_2 (0.3 %) (Wu et al., 2016). Soil L-glutaminase (GLS) activity was assayed according to the methods of (Frankenberger and Tabatabai, 1991). Soil *N*-acetyl-β-D-glucosaminidase (NAG) activity was colorimetrically measured at 400 nm via the extraction of 1 g soils by the mixtures with 0.1 M boric acid, 0.1 M tri-hydroxymethyl aminomethane, 0.067 M citric acid monohydrate and 0.1 M tri-hydroxymethyl aminomethane followed by the addition of 0.05 M *p*-nitrophenyl-*N*-acetyl-β-d-glucosaminidine at pH 5.5 and inoculation for 1 h (Wang et al., 2015). Soil denitrification enzyme (RNR) activity was determined using a gas chromatograph equipped with an electron capture detector (Varian Chromatography Group, CA, USA) after extraction of 3 g soils with 1 mM KNO_3, 1 mM glucose and 1 g l^{-1} chloramphenicol. Soil β-glucosidase (GLU) activity was assayed colorimetrically measured at 410 nm via the inoculation of soil samples with 0.1 M Na-acetate buffers containing 1 ml of 10 mM pNPG at 30 °C for 3 h (Kotroczó et al., 2014). Soil acid phosphatase (APH) activity was colorimetrically measured at 420 nm via the inoculation of 1 g soil samples with para-nitrophenol phosphate (Tabatabai, 1994). Each treatment was replicated four times.

2.6. Statistical analysis

Effects of plant sex (S), cultivation condition (C), nitrogen level (N) and their interactions were analyzed with general linear models for competition and PSF with the SPSS software (version 22.0). Differences between means were analyzed by Duncan's tests at a significance level of $P < 0.05$ before the normality check of the data.

The calculation of biomass, root traits, root exudated and soil nutrients in this text was based on the plants harvested in September 2021. For each sex, we calculated the competition effect in terms of total, shoot and root biomass as the ratio of the natural log, $COMP = \log_e (B_{comp}/B_{alone})$, where B_{comp} is the mean biomass of plant sex in competition and B_{alone} is the mean biomass of plant sex in single cultivation. The competitive ability in heterosexual inoculum $COMP_{het}$ and cosexual inoculum $COMP_{co}$ were determined. The difference in the competitive ability was calculated as the competitive response of sexes grown in cosexual inoculation and heterosexual inoculation (i.e. of $\Delta COMP = COMP_{con}\text{-}COMP_{het}$) (Klinerová and Dostál, 2020). The intensity of PSF was calculated as the ratio of biomass of plants grown in cosexual and heterosexual inoculation. The overall competition effect equaled $COMP_{over} = \log_e (B_{comp,\ het,\ co}/B_{alone,\ het,\ co})$ and PSF effect was $PSF_{over} = \log e\ (B_{co,\ alone,\ comp}/B_{het,\ alone,\ comp})$ (Klinerová and Dostál, 2020), whereas $B_{comp,\ het,\ co}$ means the mean biomass of plants grown with competitors across pots with heterosexual and consexual soil inoculation, $B_{alone,\ het,\ co}$ means the biomass of plants alone across both inoculation treatments, $B_{co,\ alone,\ comp}$ refers to the mean biomass of plants

grown in cosexual soil inoculum across pots with and without neighbors, and $B_{het,\ alone,\ comp}$ refers to the mean biomass of plants grown in heterosexual soil inoculum across pots with and without neighbors. Heatmaps of the root exudate data were plotted using the "ggplot2" package (version 2.2.1).

Co-occurrence networks of root exudate profiles were constructed with "corr.test" function in the psych R package and visualized by the Gephi platform (version 0.9.2) based on the first 600 ASVs. The degree and modularity of microbial networks were extracted by the Gephi platform (version 0.9.2). Network nodes refer to individual microbial ASVs in the microbial network. Network edges refer to the significant Spearman correlations ($\rho > 0.7$, $P < 0.001$). Subsequently, we analyzed the network connectiveness with the degree function and network modules with the edge betweenness community function (De Vries et al., 2018).

Structural equation modeling (SEM) was constructed to evaluate the relationships among root exudate profiles, soil nutrient properties, soil enzymatic activities (Chen et al., 2019). We performed the principal component analysis (PCA) to develop multivariate functional indices considering the larger number of explanatories and collinearity. The first component (PC1) was considered as a new variable to represent the combined parameter to soil properties, soil enzymatic activities and secretion profiles to subsequent analysis. The soil enzymatic activities included NR, NIR, CAT, GLU, RNR, NAG, PPO, GLS, APH and URE. Soil properties included pH, TC, OM, TP, AP, NO_3^-, NH_4^+, CEC, NMR, AMR, MC, MN and AK. The SEM was constructed by AMOS 21.0 (Amos Development Corporation, Chicago, IL, USA). The fit of the suitable model was evaluated with the model χ^2 test, using RMSEA (RMSEA $\leq$ 0.05) and P value ($P > 0.05$).

3. Results

3.1. Total PSF and competition

The correlation between competitive ability (measured in heterosexual inoculum) and the change in competitive ability due to cosexual inoculum varied with soil N availability (Fig. 1). There were significant negative relationships between the change in competitive ability (ΔCOMP) and the competitive ability in heterosexual soils at low N supply, suggesting that the competitive ability of sexes was reduced in superior competitors but was improved in weaker competitors only at low N supply with cosexual inoculum (Fig. 1a, b).

Both sexes showed positive PSFs at high N availability and negative PSF at low N availability (Fig. 1c) at single cultivation and cosexual interaction. Contrary, both females and males displayed negative PSF under both N levels when grown with an intersexual neighboring plant. Females had more negative PSF than males with low N supply, whereas there was no significant difference in PSF at high N supply, when grown with intersexual interaction. The competitive ability of sexes varied with neighboring identity and N availability (Fig. 1d). Females would elevate competitive ability when grown in intersexual inoculum irrespective of N availability, while

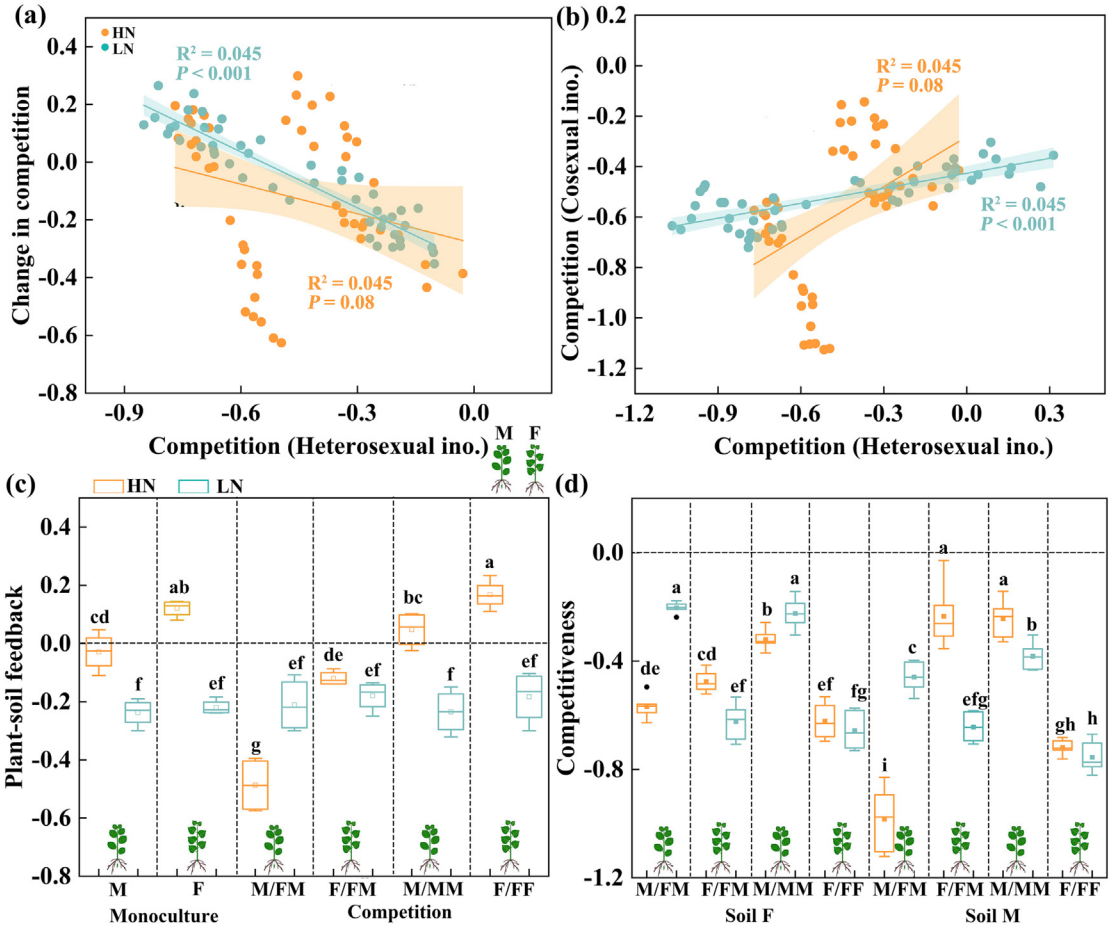

Fig. 1. Association between the competitive ability of *P. cathayana* grown with heterosexual and cosexual inocula, and influence of soil nutrient supply on plant-soil feedback (PSF) and sexual competition with cosexual and heterosexual inocula in terms of total biomass with high (HN) and low nitrogen addition (LN). (a) Association between difference in competitive ability of plant sexes in cosexual and heterosexual inoculation, and their competitive ability when cultivated in heterosexual inoculum. (b) Association between the competitive ability of plant sexes grown with cosexual and heterosexual inoculum with LN (in green) or HN (in red). PSF (c) and competitive ability (d) during the feedback experiment in soil inoculated with previously planted females and males.

the competitive ability of males would be decreased at high N supply but increased at low N supply, when grown with cosexual neighbor with intersexual inoculum (Fig. 1d). When grown with intersexual neighbor, intersexual biota strengthened male's competitive ability at both N supply and females' competitive ability only at high N supply, compared to cosexual inoculum. There was no significant difference in competitive ability for females between cosexual and intersexual soil inoculum at low N supply when grown with a intersexual neighbor.

3.2. The effect size of PSF and sexual competition

When pooled across all sexes and treatments, males faced less sensitivity to neighbor than females in terms of total biomass, shoot biomass and root biomass (Fig. 2). In contrast, females exhibited a strong competitive ability than males when grown with an intersexual neighbor than a cosexual neighbor irrespective of N level (Fig. 2a). Under low N supply, the female shoots grew better under intersexual competition than under intrasexual competition (Fig. 2b); the contrary was true in terms of root biomass (Fig. 2c). In addition, males grew better in cosexual soils than in other soils in terms of total and root biomass. Low N promoted the growth of both sexes in cosexual soils in terms of total and shoot biomass, except for the males in terms of total biomass. The female roots grew better than male roots when grown in cosexual soils vs other soils, but this was ameliorated by low N supply.

3.3. Soil physiochemical traits and enzymatic activities affected by soil inoculation

PCA was conducted to examine the relative contributions of soil physiochemical properties. PC1 and PC2 accounted for 33 % and 21 % of the variation, in soil physiochemical traits, for 25 % and 17 %, respectively, in soil enzymatic activities (Fig. 3a–b). Among soil physiochemical traits, the soil microbial C, microbial N, soil total phosphorus, available phosphorus and nitrate were the main contributors to PC1, whereas AMR was an important factor in PC2. Among soil enzymatic activities, NAG, PPO, GLS, NAG, APH and CAT were critical factors in PC1, whereas URE and GLU were the main contributors to PC2.

Then, we compared the effects of sexual competition on the differences in key factors among soil physiochemical traits and soil enzymatic activities between cosexual and intersexual soil inoculation (Fig. 3c). We found that soil biota had the largest effects on MC and MN in females under intrasexual competition only at high N supply, whereas differences in soil NO_3^-, total phosphorus, GLS and NAG between sexual soil inoculation were greatest in males under intrasexual competition with high N supply. When at low N supply, the greatest differences in soil NO_3^-, TP and AP were in the intersexual competition between cosexual and heterosexual inoculation. The differences in MC and MAG between soil inoculation types were greatest in females under intrasexual and intersexual competition with low N supply.

3.4. Root exudates

Soil inoculation patterns affected the root exudate profiles of males and females under intersexual and intrasexual competition (Fig. 4). PC1 and PC2 accounted for 29 % and 19 % of the variation, respectively, for high N availability, for 31 % and 9 %, respectively, at low N availability (Fig. 4a, b). Soil biota had a larger effect on root exudate profile in plants with intersexual cultivation than cosexual cultivation. Soil biota did not affect the root exudate profiles in females grown under intrasexual cultivation. Furthermore, the number of specific root exudates between two soil inoculation types were 38, 29 and 22 at high N levels, and 47, 29 and 21 at low N levels in females, males under intrasexual competition, and in intersexual competition (Fig. 4c). The ratios of root exudates with up-regulation to the competition-specific exudates were greater than those with down-regulation between F and M soil inoculation only under intersexual competition with high N supply (Fig. 4d). In males under intrasexual competition, the root exudates with intersexual inoculation included mainly phenolic acids at high N levels, while more nucleotides and phenolic acids were found in cosexual inoculation. Females at high N supply had more root exudates related to amino acid metabolism in heterosexual soils, and related to phenolic acids and saccharides in cosexual soils at low N levels. Contrarily, females induced more phenolic acid secretion with cosexual soil inoculum, and phenolic acids, nucleotides, organic acids, amino acids and lipids were secreted by females with heterosexual soil inoculation at low N levels.

3.5. Root exudate network stability

The root exudate network had the lowest stability in females under intrasexual competition, as showed by the highest network connectivity (degree) and lowest modularity (Fig. 5). Consistently, the root exudate networks had more positive correlations in females under intrasexual competition than in other treatments ($\chi^2 = 429$, $P < 0.001$). Contrarily, the root exudate networks were weakest under intersexual competition at low N levels, as visible as the lowest network degree, higher modularity, and least positive correlations between nodes relative to the remaining

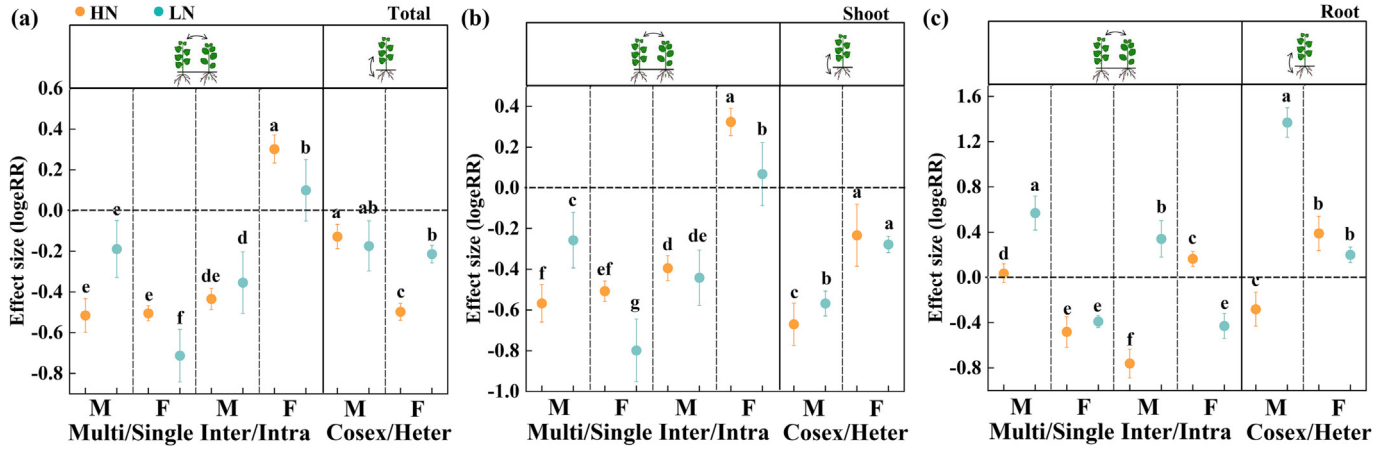

Fig. 2. Overall competition and plant-soil feedback (PSF) of pooled plants with low nitrogen (HN) and high nitrogen addition (LN) in *P. cathayana* females (F) and males (M) in terms of total biomass (a), shoot biomass (b) and root biomass (c). Multi, multiple plant interactions; single, single cultivation; inter, intersexual competition; intra, intrasexual competition; cosex, plant sex when grown in own soil; heter, plant sex when grown in the soil of the opposite sex. Bars represent mean ± SE ($n = 4$). The different lowercase letters above the columns denote significant differences ($P \leq 0.05$) according to ANOVA, followed by Tukey HSD tests.

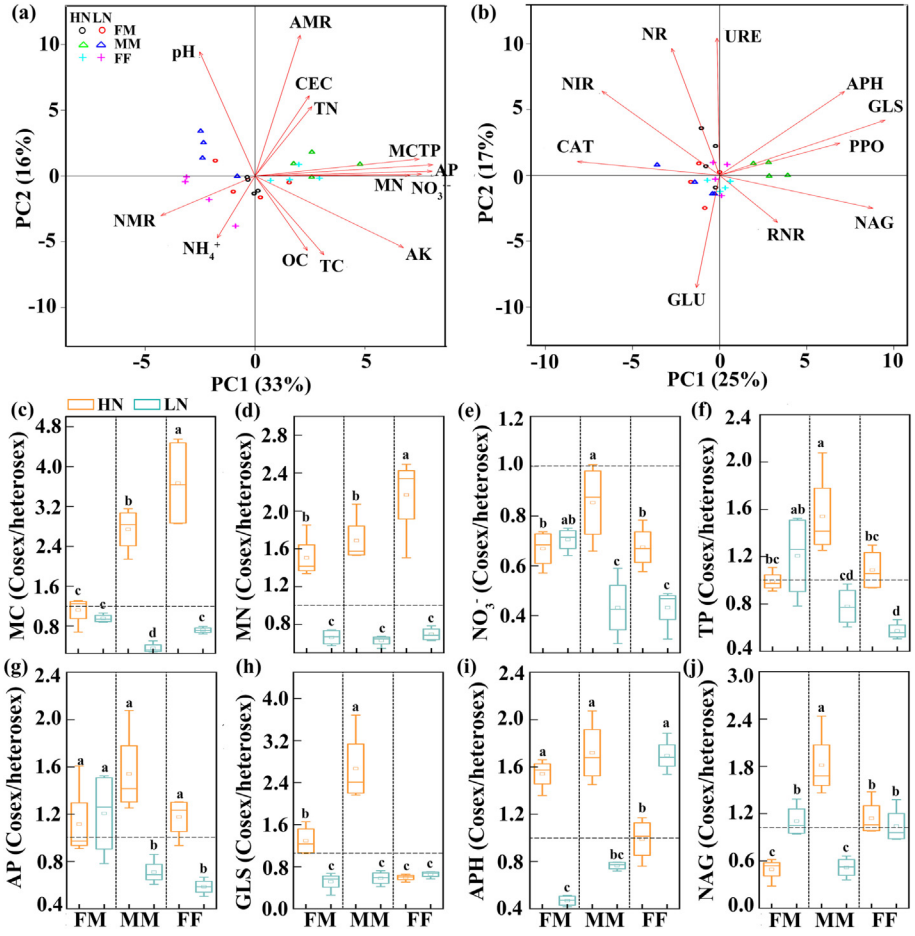

Fig. 3. Soil nutrients (a) and enzymatic activities (b), and the principal component analysis (PCA) and the ratio of soil nutrients in cosexual and heterosexual inoculation of *P. cathayana* females and males. (c) Soil nutritional traits and enzymatic activities were selected based on the importance according to (a) and (b). Bars represent mean ± SE ($n = 4$). The different lowercase letters above the columns denote significant differences ($P \leq 0.05$) according to ANOVA, followed by Tukey HSD tests. FM, a female grown with a male in a pot; MM, a male grown with a male in a pot; FF, a female grown with a female in a pot. NMR, soil net nitrification rate; AMR, soil ammonification rate; CEC, cation exchange capacity; TN, total nitrogen; TC, total carbon; TP, total phosphorus; OC, organic carbon; AK, availability of potassium; AP, availability of phosphorus; MC, microbial carbon; MN, microbial nitrogen; NO_3^-, nitrate ion; NH_4^+, ammonium ion; NR, nitrate reductase; NIR, nitrite reductase; RNR, denitrifying enzyme; NAG, *N*-acetyl-β-D-glucosaminidase; GLS, glutaminase; URE, urease; APH, acid phosphatase; GLU, glucosidase; CAT, peroxidase; PPO, polyphenol oxidase. HN, high nitrogen supply; LN, low nitrogen supply.

treatments (Fig. 5). In males under intrasexual competition, there was no significant difference in the root exudate network stability, as shown by the similar network degree and modularity. Under intrasexual competition, cosexual inoculation significantly stimulated root exudates in females with intrasexual competition at both N levels (Fig. 5). However, the greater amounts of root exudates released in males under intrasexual competition were stimulated by heterosexual inocula at high N levels but by cosexual inocula at low N levels. Under intersexual competition, males' inocula stimulated root exudate release more significantly at high N levels, but in females' inocula at low N levels.

3.6. Linking rhizosphere processes, PSF and competition

The structural equation modeling analysis revealed that root exudates and rhizospheric processes affected PSF and sexual competition in different sexual combinations. As shown in Fig. 6, under intrasexual competition, root exudates of females negatively regulated PSF and sexual competition via negatively affecting soil nutrient traits, whereas root exudates of males directly positively affected PSF, as well as competition via PSF. Moreover, there was a significant interaction between PSF and sexual competition in males under intrasexual competition. Under intersexual competition, root exudates had significant positive influences on soil nutritional traits and soil enzymatic activities. Soil nutritional traits had a positive role in PSF concerning intersexual competition.

4. Discussion

4.1. Low N induced negative PSF and reduced intersexual competition

This study found that stronger competitors (a greater tolerance to neighbors when grown with heterosexual inoculum) reduced competitive ability in self-trained soil, whereas weaker competitors became more tolerant to neighbors with cosexual inoculum only at low N availability, showing that PSF prevents any sex from dominating the plant cultivation, thus promoting coexistence at low nutrient availability. Cosexual inoculation reduces the inequality in the sexual competitive ability but it does not completely remove the differences, as has been shown for mutualistic symbiosis in nature and experimental treatments (Chen et al., 2014; Rogers and Eppley, 2012). Moreover, the intrasexual coexistence was promoted by low N in males, but by high N in females. Negative PSF reduces the potential for competitive exclusion and promotes coexistence in plant communities; the contrary was true for competitive exclusion (Lekberg et al., 2018). The negative PSF of both sexes under intersexual competition implies the possible coexistence of intersexual interaction.

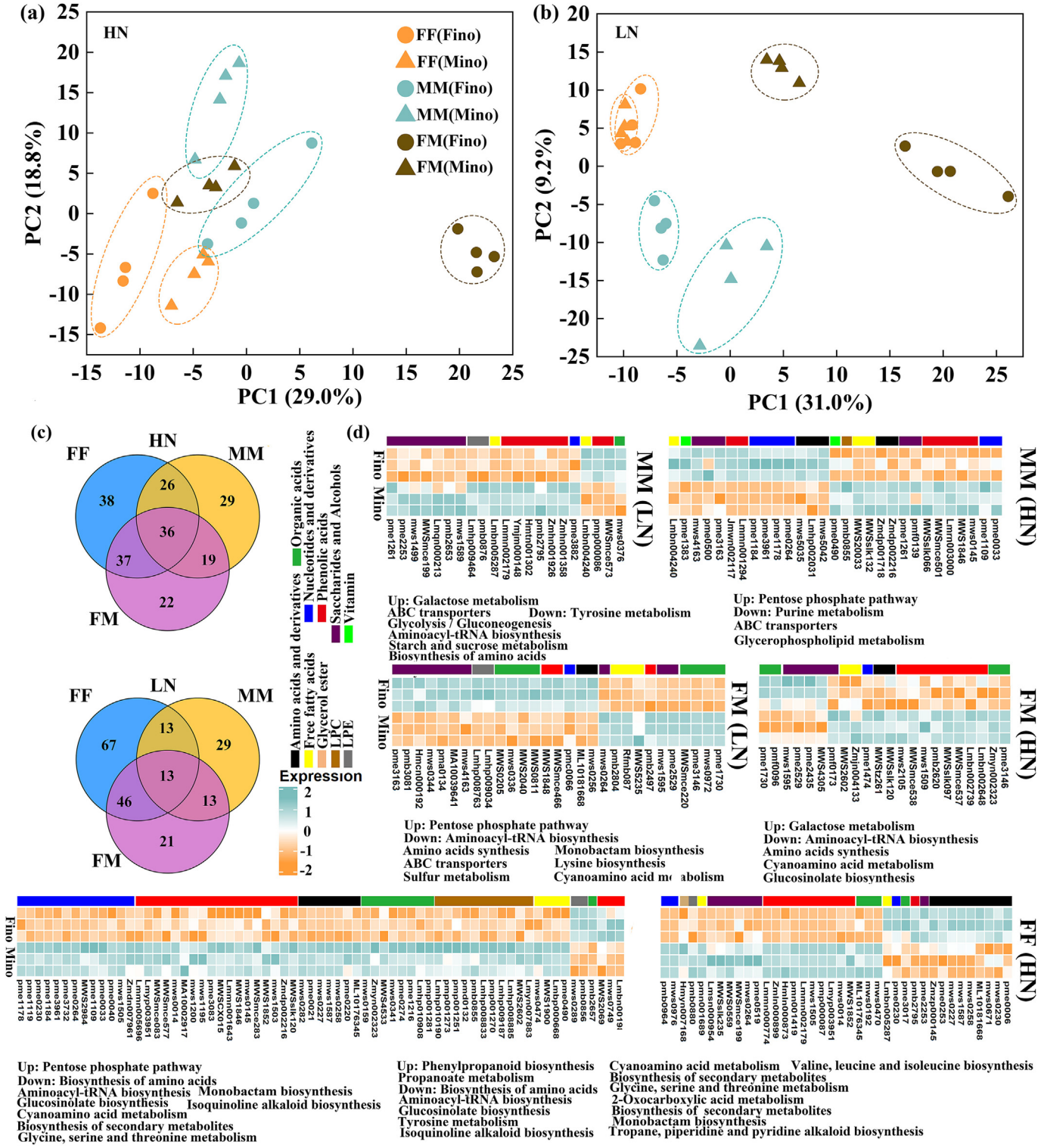

Fig. 4. Sex-specific root exudate outputs in *P. cathayana* females and males when grown with cosexual and heterosexual inoculum. Principal component analysis of the whole root exudate profile in *P. cathayana* samples at low (a, LN) and high (b, HN) nitrogen supply. (c) Venn diagrams show the distribution of differentially expressed root exudates identified among different interactions at LN or HN supply. (d) Heatmap analysis showing the differential amounts of root exudates between F soil inoculation and M soil inoculation in FF, FM or MM plantations. Color scale represents the root exudate fold-change (log_2). The right of heatmap was the annotation of root exudate. "Up" represents the predicted up-regulated metabolic process and "down" represents the predicted up-regulated metabolic process between F inoculum and M inoculum. Different color blocks represent different types of root exudates. FM, a female grown with a male in a pot; MM, a male grown with a male in a pot; FF, a female grown with a female in a pot. Fino, F soil inoculation; Mino, M soil inoculation.

However, the study found that males promoted coexistence with females, while females promoted the exclusion of males from the female's cultivation, especially at high N supply. Females generally had greater sex-related costs with higher resource uptake in high-resource habitats. Such sexual traits in females generally promoted their invasion to nutrients and thus female-biased sexual ratio in high-resource habitats (Hultine et al.,

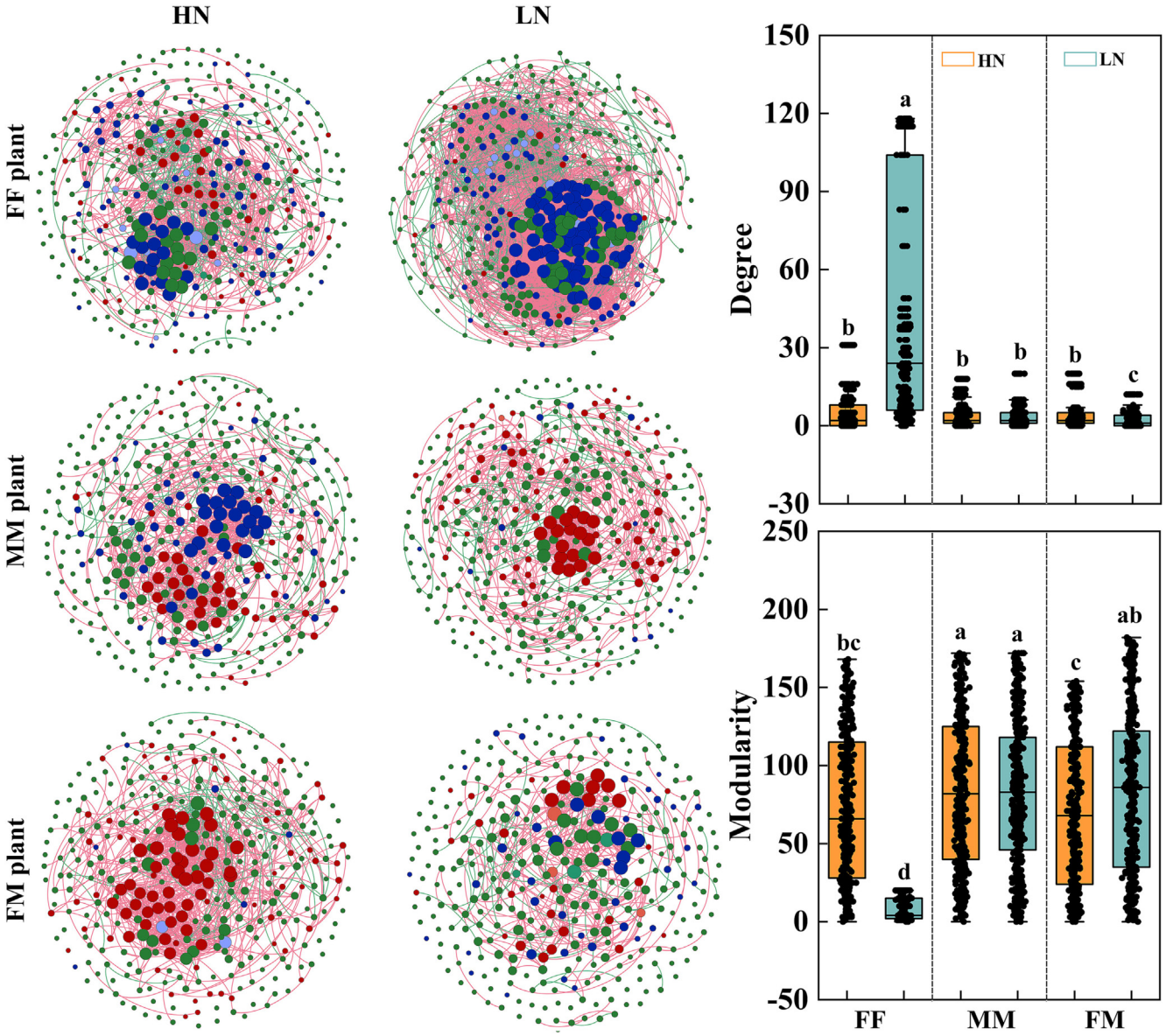

Fig. 5. Root exudate networks and their network properties in *P. cathayana* females and males affected by cosexual and heterosexual inoculation. The network edges indicate significant correlations ($r > 0.75$ and $P < 0.001$), where green lines show negative correlations and red lines represent positive correlations. Light red (relative abundance < 1 %) and dark red (relative abundance > 1 %) represent higher levels of root exudates induced by males' inocula, whereas light blue (relative abundance < 1 %) and dark blue (relative abundance > 1 %) represent higher levels of root exudates induced by females' inocula. Green nodes indicate nonsignificant differences in the relative abundance of root exudates between females' and males' inocula. Network node properties, including node connectedness (degree) and modularity. Different letters above the columns indicate statistically significant differences and asterisks indicate the statistical significance level (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). HN, high nitrogen; LN, low nitrogen. FF, females under intrasexual competition; MM, males under intrasexual competition; FM, females and males under intersexual competition.

2016). The greater resource use efficiency in males could maximize stress tolerance and survival in low-resource habitats (Hultine et al., 2016). Consistently, we found that Females were stronger competitors (characterized by a greater tolerance to neighbors when grown in heterosexual inoculum) relative to males at high N supply, while males had stronger competitive ability than females at low N supply, under intersexual competition, further supporting the above conclusion. At low N supply, the stronger competitors, males, would decrease their competitive ability, while the competitive ability of weaker competitors females was not affected by low N supply, when grown at their cosexual inoculum, meaning that soil inoculum decreased differences between sexes in their competitive ability. The reduced competitive ability of males and the constant competitive ability of females in self-trained soil would be the bases of intersexual coexistence in low-resource natural habitats.

Under intrasexual competition, N availability and PSFs affected sex-specific responses to the cosexual neighbors. Overall, males exhibited greater tolerance to neighbors than females under intrasexual competition, especially at low N. Males generally have a more conservative nutrient availability and greater resource use efficiency to maximize stress tolerance (Hultine et al., 2016). Plant species with lower resource requirements are more competitive (Grace and Tilman, 1990). For the stronger competitors, males, their competitive ability in cosexual inoculum was increased at high N supply, and reduced at low N supply, suggesting low N promoted intrasexual co-existence, while high N may promote spatial variation, for males with the same sexual neighbors. In contrast, females were weak competitors relative to males at both N supply, as their weaker competitive ability in cosexual inoculum when grown with the same sexual neighbor. Notably, the competitive ability of females would be increased at high N

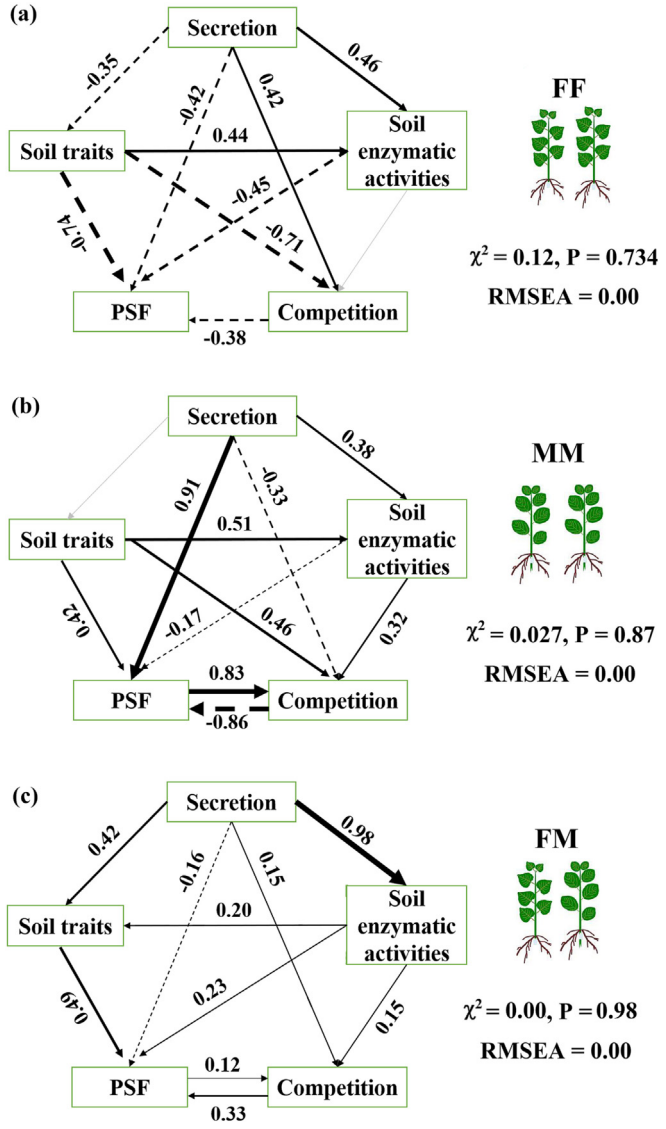

Fig. 6. Structural equation model of relationships among root exudates, soil traits, soil enzymatic activities, plant-soil feedback (PSF) and competition in FF (a), MM (b) or FM (c) plantation modes of *P. cathayana* grown with cosexual or heterosexual inoculation. The standardized coefficient is given alongside the arrow and the arrow weights are proportional to standardized coefficients. The dotted lines represent a negative correlation. Multiple-layer rectangles represent the first component from the principal component analysis, conducted for soil physicochemical properties (TC, pH, TC, TN, AK, TP, AK, MC, MN, NO_3^-, NH_4^+, CEC, NMR and AMR), C-related enzymatic activities (CAT, PPO and NAG) and N-related enzymatic activities (URE, NR, NiR and PRO). The arrow width is proportional to the strength of the relationship. Asterisks represent the statistical significance (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$). FF, females under intrasexual competition; MM, males under intrasexual competition; FM, females and males under intersexual competition.

supply but reduced at low N supply when grown at cosexual inoculum relative to heterosexual inoculum, suggesting females may coexist with the same sexes at high N supply. The nutrient-acquisitive, taller, and fast-growing plants are reported to have less sensitivity to competition in high-nutrient conditions and to be more common in native and invasive ranges (Dawson et al., 2012; Klinerová and Dostál, 2020). The highly competitive ability of females at high N supply may imply that females easily invade high-nutrient habitats previously occupied by males. In natural poplar communities, frequently observed female-biased sex ratios in habitats with good availability of resources may be a result of the superior competitive ability of females (Wade et al., 2003). This process may explain the spatial segregation of dioecious plants along environmental gradients in high-resource habitats for females and less favorable habitats for males of natural environments.

4.2. Sexual dimorphism regulated PSF and sexual competition at different nutrient supply

Plant-soil feedback and competition are dynamic, and can vary greatly depending on the response to environmental variation and nutrient availability (Ploughe et al., 2019; Zuñiga et al., 2019). The effects of increased nutrient availability on the interaction of PSF and competition are not similar across all study species but associated with their ecological optima with respect to nutrients. The plant-soil feedback and competition are related to the tradeoff of energy investing in the shoots and roots. In shoots, the PSF intensity and direction largely reflected the feedback pattern for the whole plant, probably associated with the greater ratio of shoot biomass in relation to the whole plant (Fig. S2a–l). Moreover, we found that the soil microbial community decreased differences among plant sexes in the competitive ability of both shoots and roots (Fig. S2f, j). However, the reduced inequality in sexual competitiveness by cosexual inoculation was true only for shoots at low N levels, and for roots, implying that the effect of soil biota on below-ground biomass does not cascade into below-ground effects, which was consistent with a previous report (Hendriks et al., 2015). This study proposed that sexually functional traits affected PSF and sexual competition, which become greater in response to low N supply.

For dioecious plant species, functional sex-related trait differences in dioecious plant species would be greater in stressful environments, as females often grow faster but are more sensitive to both abiotic and biotic stressors (Liu et al., 2022a, 2022b). Females and males could adjust the intensity and direction of PSF when confronting different neighbors. Plants recognize and respond to the presence of neighboring plants via selecting specific phenotypes, thus leading to genetic differentiation and local adaptation (Mercer and Eppley, 2014; Lipowsky et al., 2015). Poplar males have been reported to reduce their growth potential more than females under intersexual competition relative to intrasexual competition when sufficient nutrients are available (Chen et al., 2014). Indeed, the reduced inequality in sexual competitiveness by cosexual inoculation was true only for shoots at low N levels, and for roots, implying that the effect of soil biota on below-ground biomass does not cascade into below-ground effects (Fig. S2), which was consistent with a previous report (Hendriks et al., 2015). The greater competitive ability of males under intrasexual competition is likely associated with the escape of their roots from their soil patches, where they lose competition due to increased total root length irrespective of N availability when faced with females (Fig. S2). However, N availability changed the effects of PSF on SRL in both sexes under intersexual competition. Plants with greater SRL possess more absorptive roots, which increases nutritional capture at high nutrient levels (Hodge, 2004; Xia et al., 2022b). Hence, the greater SRL ratio in females with heterosexual inoculation suggested that females probably occupied patches occupied previously by males and increased the possibility for competitive exclusion during the continuous succession process at high nutrient levels. It has been documented that low nutrient levels decrease SRL after nutrient enrichment (Ostonen et al., 2007; Siebenkäs et al., 2015), which is associated with less negative PSF, as we detected in female roots. The lower SRL in females relative to males with heterosexual inoculation may indicate that male roots explore other soil patches, possibly allowing co-existence.

4.3. The stability of root exudate networks and soil nutrient properties reflects the possibility of sexual coexistence

Nutrient depletion typically causes negative PSF by limiting plant growth, and it can be amplified through declines in litter quality and consequent nutrient inputs into the soil (Fujii et al., 2018). Consistently, the negative PSF in both sexes at low N supply was associated with reduced soil

nutrients, such as microbial biomass, soil N and P levels and relative enzymatic activities (APH and NGA) in cosexual inoculum relative to heterosexual inoculum. The contrary was true for positive PSF at high N supply, consistent with previous studies (Smith-Ramesh and Reynolds, 2017). Specifically, at low N supply, the increased competitive ability in females was driven by the increased soil availability P levels and increased GLS activity. The increased soil APH activities in females cultivated with males also explained their greatly competitive ability in heterosexual soils. Indeed, a previous study suggested that males promote organic P mineralization via the promotion of APH in soils of poplar (Xia et al., 2022b). In contrast, the greater microbial biomass and NGA activity in females likely promoted females redistribution in high-resource habitats via the decomposition of organic C. For males, the positive PSF was also driven by the high soil nutrient levels and microbial biomass with cosexual inoculum at a high N supply. Most soil nutrient decrease also caused the decline in competitive ability for males grown with females at heterosexual inoculum, which may increase the possibility of exclusion of male from females. Moreover, the reduced ability of males when cultivated with females at low N supply with cosexual inoculum was likely explained by the microbial C, soil P and NAG-mediated organic C decomposition.

Next, the stability of root exudate networks also reflects the possibility of sexual coexistence, which is regulated by PSF. Root exudates are known to mediate root behavior and drive plant-plant interactions (Wang et al., 2021; Wang et al., 2022). Here, the networks of root exudate profiles have different properties and responses to PSF and competition, with PSF having a much stronger impact on females under intrasexual competition irrespective of nutrient availability. The coexistence networks of root exudates in females were characterized by low stability under soil inoculation, especially at low N supply, while males under intrasexual competition and plants with intersexual competition had properties that showed higher stability. A reduced degree of node connectivity and an increased network complexity are associated with increased network stability (Fan et al., 2018). Thus, sexual coexistence at low N levels may be associated with the network stability of root exudates. Under intersexual competition, the stimulation of PSF on root exudates may also be regulated by the demand of females for soil nutrients, taking into the roles of root exudate in activating soil nutrients and plant-plant recognition (Pierik et al., 2013; Chomel et al., 2016). Under high N conditions, females grow better and increase the root exudate release. Females are more sensitive to nutrient deficiency and competition (Chen et al., 2015; Hultine et al., 2016). The increased competitive ability of females under intersexual competition relative to intrasexual competition at low N levels may be associated with the stronger stimulation of root exudates in F soil inoculum, further demonstrating that PSF may regulate root exudate profiles and networks to influence intersexual co-existence. Interestingly, low N promote more phenolic acids in females with a cosexual neighbor. It had been reported that phenolic compounds are common allelochemicals and involved in self-inhibition of plant growth. The increased phenolic compounds in female with a cosexual neighbor may also decrease plant growth at low N supply, which need to be further study in the future. On the other hand, nutrient limitation-induced intersexual coexistence promotes better plant growth and, thus, induces root exudate release in F soil inoculum.

5. Conclusions

This study provides evidence of sexual co-existence at low nutrient levels in dioecious *P. cathayana*, as shown by more negative PSF at low N level, induced by intersexual competition. Importantly, we found that cosexual soil biota can reduce the inequality in sexual competitive abilities but it does not completely remove these differences at low N supply, which further implies that plant sexes may be coexist at low nutrient levels. The sex-specific intensity and direction of competition and PSF may be attributable to the root exudate profiles and their network stability, as well as to rhizosphere nutrient availability induced by soil biota. The stability of the root exudate network enhances intersexual co-existence regulated by PSF and competition.

CRediT authorship contribution statement

Miao Liu had the main responsibility for data collection, analysis and writing, Junhua Wang and Wenting Zhao contributed to data collection, Helena Korpelainen contributed to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162479.

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