



Tree root-mediated soil metabolome in agroforestry enhancing the growth and quality of *Panax notoginseng*

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Abstract

Purpose The ecological cultivation of *Panax notoginseng* under a forest canopy relies on the coupling of the *P. notoginseng* growth environment and the forest ecosystem

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Methods In this study, six tree species, such as *Platycladus orientalis* (L.) Franco, were chosen to research the effects of species interactions on the growth, quality, and disease occurrence under intercropping with *P. notoginseng*, with single *P. notoginseng* serving as the control.

Results Intercropping *P. notoginseng* with PO (*Platycladus orientalis*, a coniferous tree species) or with SW (*Schima wallichii* Choisy, a broad-leaved tree species) promoted the accumulation of *P. notoginseng* biomass, reduced the occurrence of root rot, improved the contents of nitrogen, phosphorus and potassium in *P. notoginseng*, and increased the saponin concentration. Then, 43 differentially abundant metabolites were screened in the *P. notoginseng*-tree intercropping system by soil metabolism analysis and compared with those in the monocropped system. Indole-3-carboxaldehyde showed a significant negative relationship with the occurrence of root rot disease and inhibited *Fusarium oxysporum*. In addition, 2-naphthalenesulfonic acid was significantly positively correlated with biomass and increased the dry weight in the underground part of *P. notoginseng* in the pot experiments.

Conclusions Thus, the coniferous tree species PO and the broad-leaved tree species SW are potentially good neighbours of *P. notoginseng*, and soil metabolic changes may be important mechanisms for the growth and disease resistance benefits observed in the understorey of *P. notoginseng*.

Keywords Agro-forestry intercropping system · Species interactions · Resistance to root rot disease · *P. notoginseng* nutrition and saponins · Soil metabolites

Introduction

Intercropping is a typical model of traditional agriculture and is a fundamental and prevalent practice in modern ecological cultivation (Zhang et al. 2023a and 2023b; Wu and Lin 2020). The intercropping system leverages the principle of species diversity to foster beneficial interactions between different plant species (Chen 2023; Peng, et al. 2020; Wen, et al. 2023a, 2022), resulting in notable productivity advantages. These benefits include increased yield (Finn et al. 2013; Guay et al. 2018; Li et al. 2020a, b), improved crop quality (Bélanger et al. 2014), reduced impacts from pests and diseases (Boudreau 2013; Gaba et al. 2015), soil conservation (Weyers et al. 2021), and carbon sequestration (Malézieux et al. 2009). Agroforestry, as a form of intercropping system, utilizes abundant forest resources and the ecological environment beneath the forest canopy, thus offering great potential for cultivating shade-loving plants. Chinese medicinal herbs such as *P. notoginseng* (Wang et al. 2021a; Xiong et al. 2022; Zhu et al. 2022), *Polygonatum kingianum* (Wang 2023) and *Bletilla striata* (Wan et al. 2018; Deng et al. 2023) are highly suitable for agroforestry intercropping under a forest canopy due to their extended cultivation period and compatibility with the native environment, additionally, these plants have significant economic value.

An innovative intercropping system, the cultivation of *P. notoginseng* in forests, has been developed and extensively tested in southwestern China. The implementation of this system has the potential to yield substantial social, economic, and environmental benefits (Ye et al. 2019). Moreover, this system can effectively facilitate the growth and enhance the quality of medicinal plants while concurrently mitigating pests and disease infestations (Wang et al. 2021a, b; Xiong et al. 2022). For instance, the quality of *P. notoginseng* divaricate cultivated in pine forests was slightly better than that cultivated in the field especially the content of saponins (Jia et al. 2022; Hei et al. 2023). The saponins were a critical component of *P. notoginseng*, exhibiting diverse pharmacological effects and health-promoting properties.

Furthermore, they served as a reliable indicator of the overall quality of *P. notoginseng*. The key medicinal constituent of *P. notoginseng* as saponins, specifically including Notoginsenoside R1, Ginsenoside Rb1, Ginsenoside Rd, Ginsenoside Re, and Ginsenoside Rg1 etc. (Chen et al. 2014; Liu et al. 2017). The forest environment provides optimal conditions for the growth of *P. notoginseng*, including suitable shade, appropriate temperature and moisture levels, as well as an abundance of essential nutrients, all of which contribute to its thriving development (Ye et al. 2019). The release of allelochemicals, including volatile organic compounds and leachate, by pine trees plays a pivotal role in enhancing the disease resistance of *P. notoginseng* (Ye et al. 2021; Li et al. 2023). However, there is currently no available information regarding direct root interactions between trees and *P. notoginseng* under field conditions.

Interspecific root interactions between intercropping species play pivotal roles in augmenting nutrient absorption and regulating soil-borne disease occurrence in agroforestry systems (Deng et al. 2023; Yang et al. 2022; Zhou et al. 2023a, b). Studies have reported that, compared with pepper monocultures, intercropped maize plants grown between pepper rows have reduced disease levels of pepper *Phytophthora* blight, which is related to the ability of maize plants to form a ‘root wall’ that restricts the movement of *Phytophthora capsici* across rows (Yang et al. 2014). Intercropping systems promote the development of a disease-suppressive rhizosphere microbiome, which confers protection to tomato plants against *Verticillium* wilt disease caused by the soil-borne pathogen *Verticillium dahliae* (Zhou et al. 2023b, a). The cultivation of *P. notoginseng* also faces significant challenges associated with root rot, which can result in compromised growth, disease susceptibility, diminished quality, reduced yield, or even complete crop failure (Cui et al. 2014; Yang et al. 2018). Therefore, assessing the interspecific interactions between *P. notoginseng* roots and trees is highly useful in agroforestry systems.

Root exudates play a pivotal role in mediating species-specific rhizosphere interactions, encompassing signal transmission, the activation of soil nutrient reservoirs, the modulation of rhizosphere microbial communities, and the induction of plant resistance (Hu et al. 2021; Li et al. 2020b; Dong et al. 2021). Numerous studies have demonstrated that various natural antimicrobial substances can be secreted by plant roots, creating defence networks in the rhizosphere (Zhu et al.

2021). This mechanism not only inhibits soil-borne pathogens in a sustainable manner but also prevents the development of drug resistance. Phenolic acids, terpenes and isothiocyanate secreted by nonhost plant roots significantly reduced *Phytophthora* blight in pepper and soybean plants (Zhang et al. 2019a, b; Li et al. 2022a, b). The intercropping of *Platycodon grandiflorum* and Welsh onion increased the content of benzothiazole and 2-methylthiobenzothiazole in the root exudates of *P. grandiflorum* and alleviated the continuous cropping obstacle of *P. grandiflorum* caused by *F. pythium* (Wang et al. 2019). Intercropping watermelon with wheat increased the release of coumaric acid in wheat roots and inhibited the growth of the watermelon pathogenic fungus *F. oxysporum* (Lv et al. 2018). Besides, as plant rhizosphere secretions, indole played an important role in regulating plant growth in intercropping systems (Xiao 2023). Indole was an aromatic heterocyclic compound, and its structural skeleton is widely found in many plants, such as jasmine, orange, daffodils, vanilla and robinia (Millan et al. 2022). Indole-3-carboxaldehyde (I3A) was found in a variety of plants and has been shown to have several biological effects, including effects on plant growth and development (Teng et al. 2018). Recent studies also suggested that I3A was involved in plant metabolism (Ge et al. 2024).

This study focused on the '*P. notoginseng*-tree species' intercropping mode. Hence, from the viewpoint of the interaction between trees and the rhizosphere of *P. notoginseng*, the present study delves into the influence and the underlying mechanisms that foster the enhanced growth, disease resistance, and quality augmentation of *P. notoginseng*. Overall, we aimed to address the following: (1) To investigate the effects of intercropping *P. notoginseng* with different tree species on growth, quality, and disease incidence; (2) to investigate the impact of intercropping *P. notoginseng* with various tree species in agroforestry on the rhizosphere soil metabolism of *P. notoginseng* using liquid chromatography-tandem mass spectrometry (LC-MS/MS); and (3) to verify the inhibitory effects of key compounds in *P. notoginseng* rhizosphere metabolites on root rot pathogens and their ability to promote the growth of *P. notoginseng*. Based on the above research, we hoped to find the key metabolites in the root interaction between trees and *P. notoginseng*, and thus select the suitable tree species to match with *P. notoginseng*. The purpose of this study was

to provide technical support for the selection of tree species for planting *P. notoginseng* in forestland and to provide practical recommendations for the biometric cultivation of *P. notoginseng* within forests.

Materials and methods

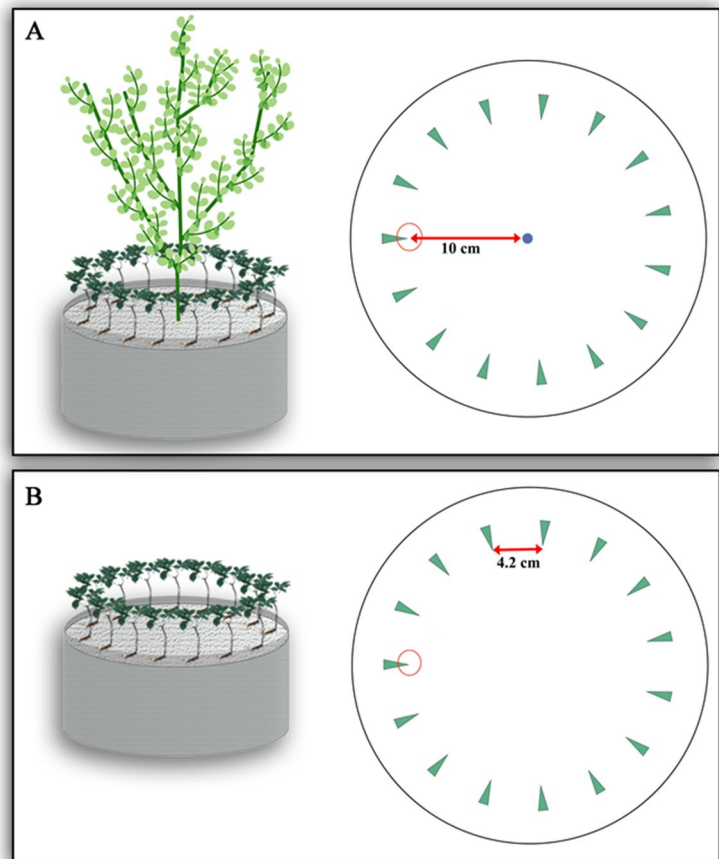
Experimental design and growth conditions

Experiments were conducted at the experimental station of Yunnan Agricultural University in Xundian County, Yunnan, China (103.286°E, 25.521°N; altitude of 1960 m). Seven different systems were used, namely, *P. notoginseng* monocropping (CK) and *P. notoginseng* respectively intercropped with *Eucalyptus robusta* Smith (ER), *Cunninghamia lanceolata* (CL), *Platycladus orientalis* (PO), *Schima wallichii* Choisy (SW), *Alnus cremastogyne* (AN), and *Pistacia weinmannifolia* J. Poisson ex Franch (PW). The reason for selecting these six tree species for experimentation was carefully considered as: firstly, these six tree species have large planting areas and widespread distribution in Yunnan Province, China, rendering them highly representative. Secondly, the spacing of trees in these forests is suitable for understory planting and field management. Additionally, the canopy architecture of these trees is conducive to intercepting appropriate sunlight, providing suitable shading for the healthy growth of *P. notoginseng* under the forest canopy. In this sense, six different trees with heights between 1.0 and 1.5 m were selected and planted in a greenhouse. Then, 15 *P. notoginseng* plants were planted under these trees 10 cm from the trunks of the trees (Fig. 1). All pots were arranged according to a completely randomized block design. The greenhouse was shaded with a polyethylene net that allowed 10% light transmission to simulate the natural conditions for *P. notoginseng* growth (Chen et al. 2014). The temperature was controlled at 18–30 °C. Throughout the experiment, consistent field management measures such as water and fertilizer management and pest control were implemented by intercropping modes.

Measuring the growth and disease status of *P. notoginseng* under different intercropping systems

To evaluate the growth of *P. notoginseng* in the seven intercropping systems, the germination and survival

Fig. 1 Pot experiment diagram, of the intercropping modes (A) and the *P. notoginseng* monoculture (B). Legend: In Fig. 1A, the arrowed straight line illustrates the direct distance between *P. notoginseng* and the tree species, while the circle denotes the soil sampling position of the interacting soil. At this position, the roots of *P. notoginseng* and the tree species have established an interactive relationship. In Fig. B, the circles represent the single-plot soil sample collection location, and the arrow distance indicates the position between *Panax notoginseng*, consistent with that in Fig. A. The circle signifies the sampling point of *P. notoginseng*, aligning with its position in Fig. 1A



rates of *P. notoginseng* were investigated in April and November, respectively, and calculated as follows:

$$\text{The seedling germination rates (\%)} = 100 \% \times \frac{\text{Germinated seedlings}}{\text{Total seedlings in each treatment}}$$

$$\text{The seedling survival rate (\%)} = 100 \% \times \frac{\text{Living seedlings}}{\text{Total seedlings in each treatment}}$$

To investigate the impact of intercropping *P. notoginseng* with different tree species on plant health, plants that displayed symptoms of root rot

were recorded. Once the *P. notoginseng* plants were harvested, the soil and debris on the root surface were rinsed off. The remaining soil attached to the roots was gently brushed away with a soft bristle brush. Subsequently, the incidence of root disease in *P. notoginseng* was calculated, and the severity of the root disease was assessed by classifying and quantifying the disease based on the severity of the disease (Table 1).

$$\text{The rate of root rot (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants investigated in each treatment}} \times 100\%$$

$$\text{Disease index} = \frac{\sum \text{Number of plants of each disease grade} \times \text{Representative value of each disease grade}}{\text{Total number of plants investigated} \times \text{Representative value of the highest disease grade}} \times 100\%$$

In August, *P. notoginseng* was collected and brought to the laboratory after the final stored seedlings were counted. The general samples were washed to remove the soil, dried to remove surface moisture, and weighed (fresh weight); then, the plants were dried to a constant weight at 60 °C in an oven, cooled to 20 °C, and

weighed again (dry weight). Sixty plants were tested in each treatment and divided into three replicates; each replicate contained 20 plants. The average weight of a single plant in each replicate was subsequently calculated. Finally, the yield per unit area and root-to-shoot ratio were calculated using the following formulas:

Table 1 Grading standards for the occurrence of plant disease

level	Pathogenic symptoms	Disease area (accounting for the taproot area)
0	No disease or a healthy plant	0
1	Mild necrosis on the surface of the main root	< 10%
2	Taproot decay	10–30%
3	Taproot decay	30–50%
4	Taproot decay	50–75%
5	Taproot decay	100%

$$\text{Production per unit area} = \frac{\text{Total production}}{\text{Total area}}$$

$$\text{Root to shoot ratio} = \frac{\text{Root dry weight}}{\text{Aboveground dry weight}}$$

Effect of seven intercropping modes on the nutritional status of *P. notoginseng* plants

The aboveground components and underground roots of the oven-dried plants were digested in a mixture of H₂SO₄ and H₂O₂, and the N, P, and K concentrations in the dry matter of the aboveground components and underground roots were measured via the micro-Kjeldahl procedure, vanadomolybdate method, and flame photometry, respectively (Du et al. 2024; Lu 2000).

Analysis of the saponin content

The extraction of saponins was conducted as previously described (Liu et al. 2021). The detailed process was as follows: firstly, the oven-dried roots of *P. notoginseng* were grinded into powder and sift through a 250-μm mesh sieve for uniform mixing. Then a total of 0.2 g of *P. notoginseng* root powder was accurately weighed, placed in a conical flask with a grinding mouth, added with 15 mL of 75% methanol, ultrasonically extracted at 40 °C for 40 min, taken out and allowed to stand for 40 min. After filtration, the filtrate was filtered with a microporous membrane (0.45 μm), and the content was determined by HPLC (Yang et al. 2022). The target compounds in minimal medium were identified by comparing their retention times to those of ginsenoside standards Ginsenoside Re (CAS number 51542–56-4), Ginsenoside Rg1 (CAS number 22427–39-0), Ginsenoside Rd (CAS number 52705–93-8), Ginsenoside Rb1 (CAS

number 41753–43-9), and Notoginsenoside R1 (CAS number 80418–24-2), which were purchased from Guizhou Dida Biological Technology Co (Guizhou, China). The content of the target compounds in minimal medium was quantified using standard curves that showed the linear relationships between the peak areas and the concentrations (Luo et al. 2021). Analysis were performed using an UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex Triple TOF 6600) in Shanghai Applied Protein Technology Co., Ltd. A chromatographic column (Waters, Ireland, 1.8 μm, C18, 100 Å, 100 mm×2.1 mm) was used. The initial injection volume was 2 μL, and the flow rate was 0.6 mL min⁻¹. The column temperature was maintained at 40 °C, and the detection wavelength was 203 nm. The mobile phase was a linear gradient elution of water (A)-acetonitrile (B) (V/V). The levels of R1, Rg1, Re, Rb1, and Rd saponins in the roots were measured using a Nexera X2 UPLC system (Shimadzu, Japan). The instrument parameters were set according to the methods of Liu (Liu et al. 2021).

Collection of soil samples and detection of soil physicochemical properties

Bulk soil samples were collected at locations where the root systems of *P. notoginseng* and the intercropping trees were intertwined. The samples were placed in an icebox and taken back to the laboratory, after which the soil was separated into two parts. One was packed in 2 ml centrifuge tubes and stored in a liquid nitrogen tank until frozen. The plants were then transported back to the laboratory for soil metabolite detection. The second subsample was dried and sieved for the determination of potential of hydrogen (pH) and electrical conductivity (EC). The soil pH and EC were determined using an Inolab pH/Cond 720 in a water suspension (water:soil, 2.5:1, v:w) following the method described by Abdalmoula (Abdalmoula et al. 2019).

Soil metabolite extraction and analysis

Analysis was performed by using an UHPLC (1290 Infinity LC, Agilent Technologies) instrument equipped with a 2.1 mm×100 mm ACQUITY UPLC BEH 1.7 μm column (Waters, Ireland) coupled to a quadrupole time-of-flight (AB Sciex Triple TOF 6600). The column oven was maintained at

25 °C, and the flow rate was 0.5 mL/min. In both the ESI-positive and ESI-negative modes, the mobile phase contained 25 mmol/L ammonium acetate and 25 mmol/L ammonium hydroxide in water (A) and acetonitrile (B). The gradient was 95% B for 0.5 min, linearly reduced to 65% in 7 min, decreased to 40% in 1 min, maintained for 1 min, and subsequently increased to 95% in 0.1 min, with a 3 min re-equilibration period. The MS experiment was performed with the ESI source under the following conditions: Ion Source Gas1 (Gas1) and Ion Source Gas2 (Gas2) were both set to 60 psi, the curtain gas (CUR) pressure was 30 psi, the source temperature was 600 °C, and the ion spray voltage floating (ISVF) was ± 5500 V. In MS-only acquisition, the mass range of the instrument was from m/z 60 to 1000 Da, and the accumulation time for the TOF MS scan was set at 0.20 s/spectra. Moreover, for auto MS/MS acquisition, the mass range was set to 25–1000 Da with 0.05 s/spectra of the accumulation time for the production scan. The product ion scan was acquired using information-dependent acquisition (IDA) with high-sensitivity mode selected. The parameters were set as follows: the collision energy (CE) was fixed at $35 \text{ V} \pm 15 \text{ eV}$; the declustering

potentials (DPs) were 60 V (+) and -60 V (–); and the number of isotopes within 4 Da and candidate ions were excluded for monitoring every cycle: 10. Refer to Chen's method for compound identification (Yang et al. 2023; Chen et al. 2022).

Effects of indole-3-carboxaldehyde on the growth of *Fusarium*

The effects of indole-3-carboxaldehyde on the growth of culturable *Fusarium* were measured following a published procedure with some modifications (Lv et al. 2018). Briefly, a mycelium block of *Fusarium solani* (main pathogen of root rot disease of *P. notoginseng*, isolated from the rot root of *P. notoginseng* and identified by ITS sequencing and Coch's rule, as detailed in Fig. S1, 6 mm diameter) was placed in the middle of Potato dextrose agar medium (PDA) media supplemented with indole-3-carboxaldehyde (at final concentrations of 0, 25, 50, 100, 200, and 400 mg/L). Six replicate plates were used for each concentration. The mycelial growth of the isolates was determined by measuring the colony semidiameter after dark incubation at 25 °C for 5 days. The growth inhibition rate was calculated as follows:

$$\text{Growth inhibition rate(\%)} = 100 \times \frac{\text{Radial growth of control} - \text{Radial growth of treated sample}}{\text{Radial growth of control}}$$

Effects of soil metabolites on plant biomass

To test the effect of indole-3-carboxaldehyde on plant growth, we evaluated the effect of plant biomass on *P. notoginseng* after irrigation with indole-3-carboxaldehyde. The *P. notoginseng* seeds were immersed in 1% sodium hypochlorite for 5 min and then washed three times with sterile water. Seeds were sown in a pot (15.5 cm $\times$ 11.5 cm $\times$ 13.5 cm) and cultivated for half a year for growth analysis. All the pots were placed in a greenhouse shaded with a polyethylene net that allowed 10% full sunlight transmission. After six months, the plants in all the pots were subjected to the growth-promoting effects of indole-3-carboxaldehyde. Fifty millilitres of indole-3-carboxaldehyde (at final concentrations of 0, 1, 3, and 5 mg/L) was added to the soil via irrigation once a week. Each treatment included six pots. Then,

2 months after treatment, the plant weights were recorded.

Statistical analyses

IBM SPSS Statistics version 27 (SPSS, Inc., Chicago, Illinois, USA) was used for general statistical analyses. One-way analysis of variance (ANOVA) and Turkey HSD corrections ($p < 0.05$) were used to analyse the mean separations among treatments. Box plots were drawn with GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA). Referring to Li's method, we performed soil metabolite analysis after appropriate improvement (Li and Jiang 2021). Partial least squares discriminant analysis (PLS-DA) was conducted to analyze and verify the differences and reliability of metabolites in the samples. The

metabolite content data were normalized by the range method, and the accumulation mode of metabolites among different samples was analyzed by heatmap cluster analysis (hierarchical cluster analysis, HCA) through using the OmicStudio tool (<https://www.omicstudio.cn/tool>). PLS-DA analysis was applied to calculate the corresponding variable importance in projection (VIP) value. PLS-DA, volcano plot and univariate statistical analysis t-test were used to screen the differential metabolites between groups. Metabolites with Fold Change > 2 or Fold Change < 0.5, VIP ≥ 1 and $P \leq 0.5$ were considered differentially expressed metabolite. Then, spearman correlation analysis was used to comprehensively analyzed the differential metabolites and field indicators to further determine the differential metabolites affecting the disease and growth of *P. notoginseng*.

Results

Effects of different intercropping modes on the growth and disease status of *P. notoginseng*

The seeding germination and survival rates of *P. notoginseng* were affected when *P. notoginseng* was intercropped with different tree species (Fig. 2A and B). The germination rates of *P. notoginseng* significantly increased when cultivated under PO (61.89%), CL (68.89%) and ER (69.49%) but decreased under PW (39.72%) (Fig. 2A). After intercropping, *P. notoginseng* cultivated under SW (34.07%), CL (45.31%), PO (42.89%), and ER (41.21%) had significantly greater seedling survival rates than that cultivated under the control treatment (20.32%) (Fig. 2B). Interestingly, when *P. notoginseng* was

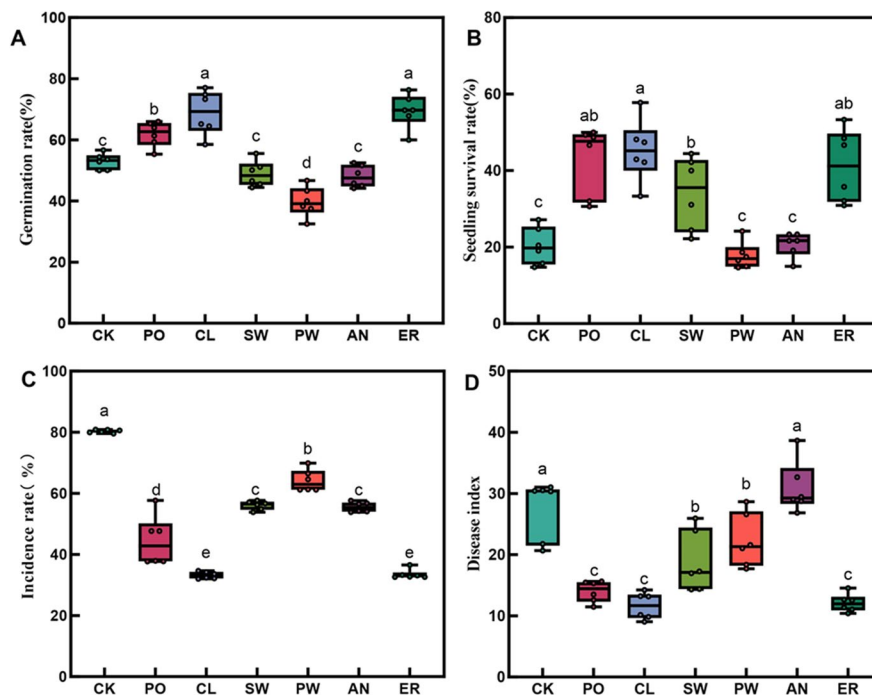


Fig. 2 Effects of seven intercropping systems on the growth and disease status of *P. notoginseng*. **A.** Germination rates ($F=33.524$, Degrees of Freedom=6, $p<0.01$). **B.** Seedling survival rates ($F=15.989$, Degrees of Freedom=6, $p<0.01$). **C.** Incidence rate of *P. notoginseng* root rot disease ($F=139.137$, Degrees of Freedom=6, $p<0.01$). **D.** Indices of *P. notoginseng* root rot disease ($F=25.602$, Degrees of Freedom=6, $p<0.01$). Each cycle in one box represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. Different lowercase let-

ters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p<0.05$, $n=6$ biologically independent samples). Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

planted with different tree species, the incidence of root rot was significantly lower (Fig. 2C). Furthermore, disease indices showed that, in comparison to the control treatment, all the intercropping modes, except for AN, significantly lowered the incidence of root rot disease in *P. notoginseng*, especially PO (14.01%), CL (11.61%), SW (18.81%), PW (22.33%) and ER (12.09) (Fig. 2D). These results indicate that the interactions of *P. notoginseng* with PO, CL and ER were more conducive to seedling growth and reduced root rot disease incidence.

Appropriate effects of intercropping modes were more conducive to increasing *P. notoginseng* biomass

To evaluate the real growth of *P. notoginseng* under the seven intercropping patterns, the yield per square metre was calculated according to the dry weight per plant and the seedling survival rate. In this study, the biomass of *P. notoginseng* was influenced when it was cultivated under the seven intercropping modes (Table 2). The dry weight per unit area of aboveground of *P. notoginseng*

Table 2 Analysis of *P. notoginseng* biomass and root/shoot ratio data

	<i>P. notoginseng</i> biomass (g/m ²)		Root/shoot ratio
	Aboveground	Underground	
CK	4.017 ± 0.305c	6.725 ± 0.245c	1.674 ± 0.148d
PO	5.968 ± 0.060a	11.895 ± 0.373a	1.993 ± 0.021c
CL	4.602 ± 0.188b	12.975 ± 0.491a	2.819 ± 0.029a
SW	3.683 ± 0.168 cd	8.123 ± 0.434b	2.206 ± 0.049b
PW	3.218 ± 0.130d	5.066 ± 0.152d	1.574 ± 0.094e
AN	1.901 ± 0.048e	6.601 ± 0.518c	3.472 ± 0.038a
ER	4.065 ± 0.229c	8.016 ± 0.417b	1.972 ± 0.067c

Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p < 0.05$, $n = 6$ biologically independent samples.) (Aboveground of *P. notoginseng* biomass: $F = 46.970$, Degrees of Freedom = 6, $p < 0.01$; Underground of *P. notoginseng* biomass: $F = 53.720$, Degrees of Freedom = 6, $p < 0.01$; Root/shoot ratio of *P. notoginseng*: $F = 47.716$, Degrees of Freedom = 6, $p < 0.01$). Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

was significantly greater in the PO and CL treatments than in the CK treatment. The underground dry weight per unit area of *P. notoginseng* was significantly greater in the PO, CL, SW, and ER treatments than in the CK treatment. A comparison of the root-to-shoot ratio of *P. notoginseng* revealed that the PW treatment had a significantly lower ratio than the CK treatment. Taken together, these results indicate that the intercropping of *P. notoginseng* with PO and CL resulted in greater aboveground dry weight and underground dry weight than did the monocropping of *P. notoginseng*, as well as, the underground dry weight of *P. notoginseng* of SW and ER were notably elevated compared to the control.

Effect of seven intercropping modes on the nutritional status of *P. notoginseng*

Significant differences in plant nutrition were observed among the seven intercropping systems. The total nitrogen content of aboveground of *P. notoginseng* in CL was significantly greater than that of CK (Fig. 3A); the underground total nitrogen content of *P. notoginseng* in PO and CL was significantly greater than that of CK (Fig. 3D); the aboveground total phosphorus in PO and CL was significantly greater than that of CK (Fig. 3B); the underground total phosphorus in PO, CL and SW was significantly greater than that of CK (Fig. 3E); and POP and SW were significantly greater in total potassium aboveground than CK (Fig. 3C). Similarly, the underground parts of CL, AN and ER contained significantly less total potassium than those of CK (Fig. 3F). These results suggested that the intercropping system promoted *P. notoginseng* nutrient uptake: PO and CL were more conducive to nitrogen uptake, PO was more conducive to aboveground phosphorus uptake and PO, SW and ER were more conducive to aboveground potassium uptake compared with single-cropped *P. notoginseng*.

Effect of seven intercropping modes on the saponin content of *P. notoginseng*

The saponins in the seven intercropping systems of *P. notoginseng* were determined. Initially, the amount of R1 saponins in the PO, CL, SW and PW intercropping systems was significantly greater than that in the CK treatment (Fig. 4A). Second, the

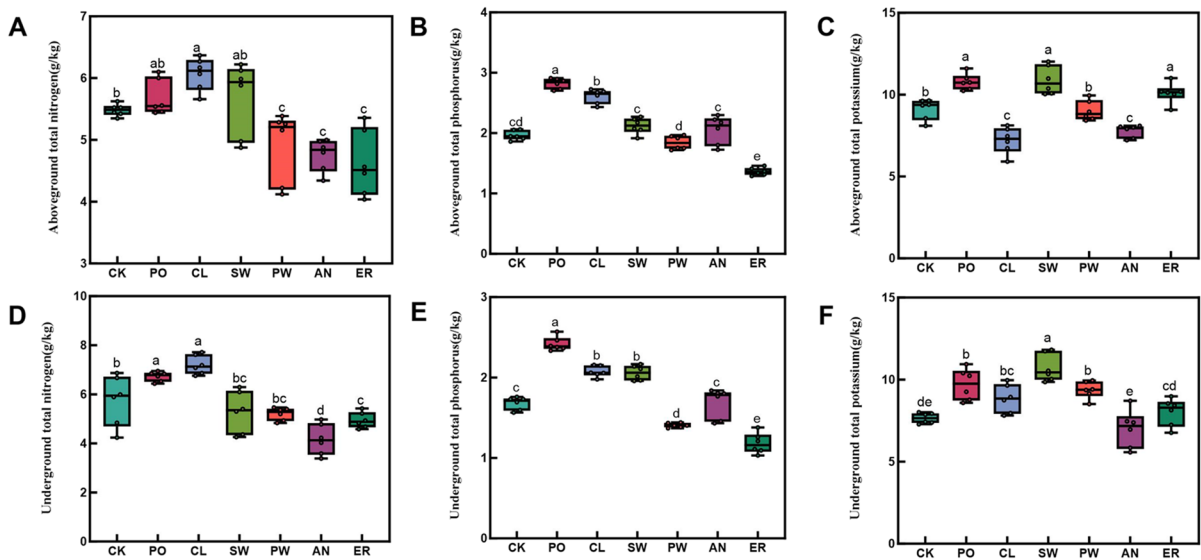


Fig. 3 Effects on characteristics of N, P, and K in *P. notoginseng* of different intercropping modes. **A** Aboveground total nitrogen ($F=10.617$, Degrees of Freedom=6, $p<0.01$). **B** Aboveground total phosphorus ($F=84.063$, Degrees of Freedom=6, $p<0.01$). **C** Aboveground total potassium ($F=28.804$, Degrees of Freedom=6, $p<0.01$). **D** Underground total nitrogen ($F=18.202$, Degrees of Freedom=6, $p<0.01$). **E** Underground total phosphorus ($F=96.526$, Degrees of Freedom=6, $p<0.01$). **F** Underground total potassium ($F=14.561$, Degrees of Freedom=6, $p<0.01$). Each cycle represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively.

Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p<0.05$, $n=6$ biologically independent samples). Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

amount of the Rg1 saponin in the PO, CL, SW, and PW intercropping systems was significantly greater than that in the CK treatment (Fig. 4B). In addition, the Re saponin content in the PO intercropping treatment was significantly greater than that in the CK treatment (Fig. 4C). Moreover, the abundance of the Rb1 saponin in the PO, SW, and PW intercropping systems was significantly greater than that in the CK treatment (Fig. 4D). Moreover, the Rd saponin content in the PO, SW and PW intercropping systems was significantly greater than that in the CK treatment (Fig. 4E). Finally, the total ginsenoside saponin content in the PO, SW and PW intercropping systems was significantly greater than that in the CK treatment (Fig. 4F). These results indicate that the intercropped *P. notoginseng* plants under different trees had greater amounts of root saponins than did the monocropped *P. notoginseng* plants, especially

R1, Rg1, Rb1, Rd and the five total ginsenosides in PO, SW and PW intercropping modes.

Effect of seven intercropping modes on the pH and EC of soil in the interaction regions

pH and EC were selected as indicators of changes in soil chemical properties, therefore, the pH and EC were measured in the seven intercropping systems and showed significant differences between the intercropped and CK soils (Fig. 5). The EC of soil in the interaction regions in the PO, CL, SW and ER treatments was significantly greater than that in the CK treatment (Fig. 5A). The pH values of soil in the interaction regions in SW, CL, PO, AN, and ER were significantly lower than that of CK (Fig. 5B). Taken together, these findings show that the root exudates of

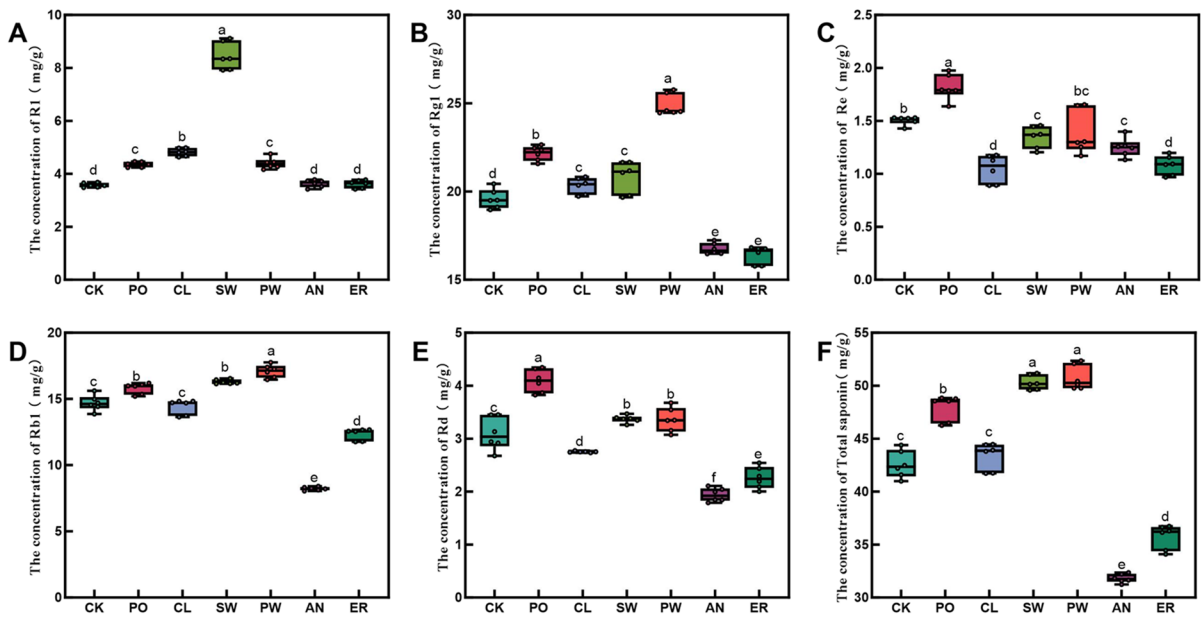


Fig. 4 Effect of seven treatments on the saponins content of *P. notoginseng* roots. **A** Notoginsenoside R1 (F=321.607, Degrees of Freedom=6, $p < 0.01$). **B** Ginsenoside Rg1 (F=168.925, Degrees of Freedom=6, $p < 0.01$). **C** Ginsenosides Re (F=28.182, Degrees of Freedom=6, $p < 0.01$). **D** Ginsenoside Rb1 (F=294.439, Degrees of Freedom=6, $p < 0.01$). **E** Ginsenosides Rd (F=85.595, Degrees of Freedom=6, $p < 0.01$). **F** Total ginsenosides (R1 + Rg1 + Re + Rb1 + Rd) (F=272.983, Degrees of Freedom=6, $p < 0.01$). Each cycle represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respec-

tively. Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p < 0.05$, $n = 6$ biologically independent samples). Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

trees may change the physical and chemical properties of soils in different intercropping systems.

Effect of seven intercropping modes on the soil metabolites of *P. notoginseng*

Soil metabolomics can correlate fingerprint type to environmental factors, soil nutrients, microbial diversity, and plant phenotypes. Thus, we performed a soil metabolomics study of the seven intercropping systems to explore root-mediated underground communication differences in soil metabolites under the different intercropping systems. A total of 630 metabolites, including amino acids, organic acids, carbohydrates, ketones, lipids, alcohols, etc., were detected and identified among all the soil samples. Lipids and lipid-like molecules were the most common compounds detected, accounting for 38% of all the metabolites, followed by organic

acids and their derivatives (24%) and benzenoids (20%) (Fig. S2). The PLS-DA analysis demonstrated that the metabolite profiles associated with the interaction of the six species differed significantly from those associated with the control group (Fig. 6A). To reveal the accumulation patterns of metabolites in the trees and CK, pairwise comparisons of PO vs CK, CL vs CK, SW vs CK, PW vs CK, AN vs CK, and ER vs CK were conducted. 10 significant metabolites were identified between PO and CK (6 increased and 4 decreased). In addition, 10 significant metabolites were identified in CL vs CK (1 increased and 9 decreased), and 17 significant metabolites were identified in SW vs CK (1 increased and 16 decreased). 35 significant metabolites were identified between PW and CK (28 increased and 7 decreased). In addition, 22 significant metabolites were identified in AN vs CK (20 increased and 2 decreased), and 29 significant metabolites were identified in ER vs CK (12

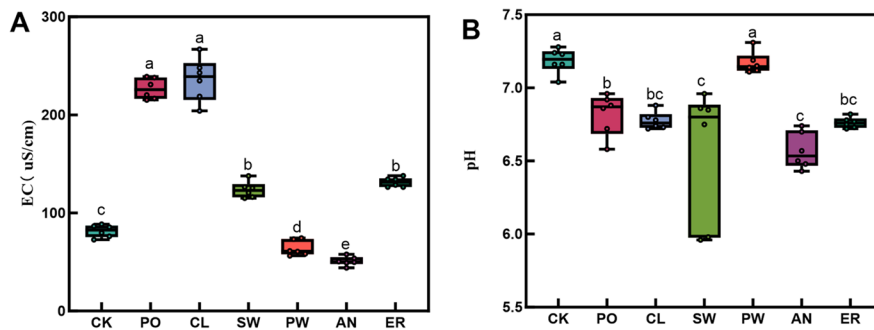


Fig. 5 The pH and EC analysis of soil in the interaction regions of different intercropping modes. **A** EC of soils ($F=285.711$, Degrees of Freedom=6, $p<0.01$). **B** The pH of soils ($F=10.213$, Degrees of Freedom=6, $p<0.01$). Each cycle represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted

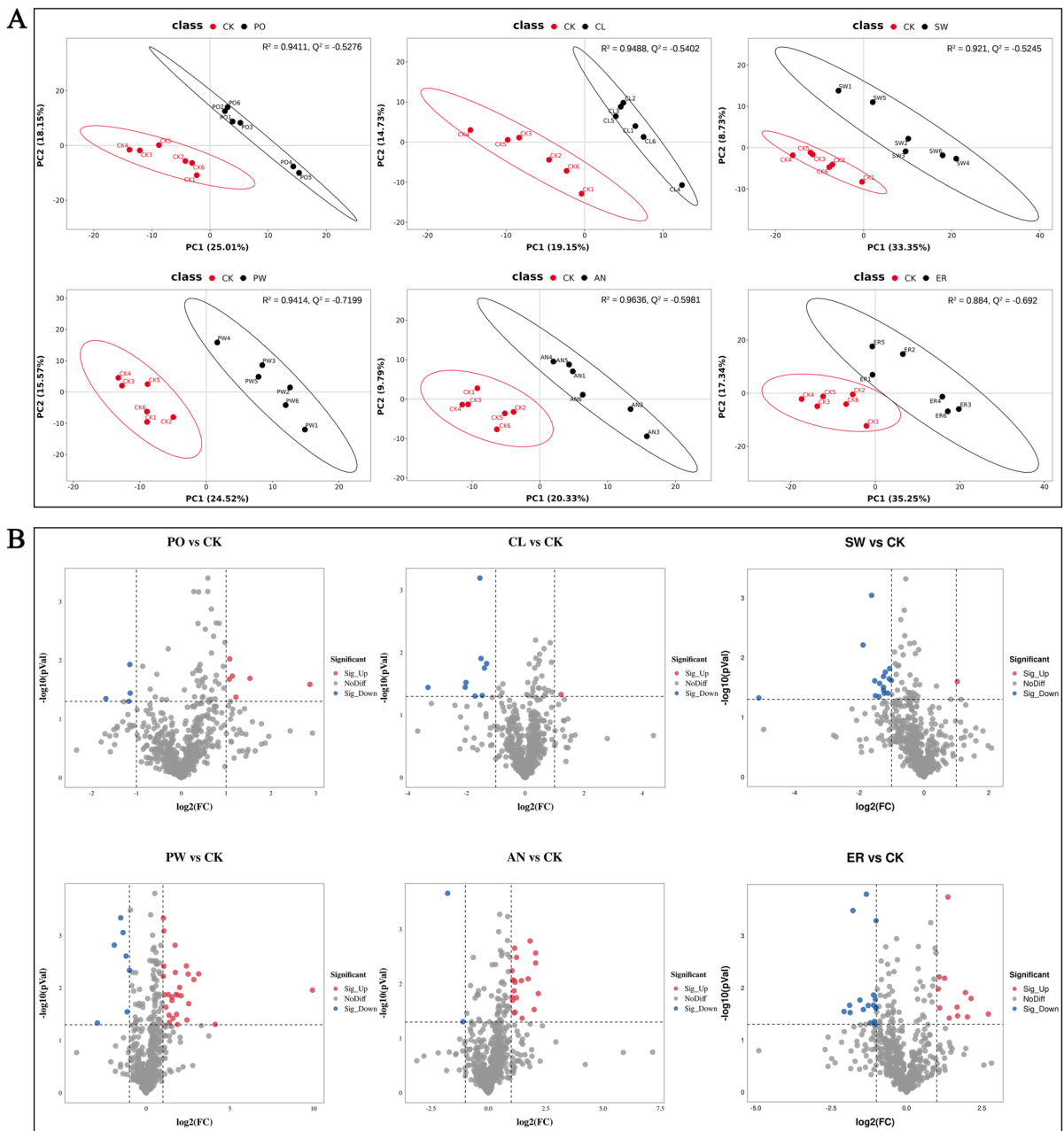
$p<0.05$, $n=6$ biologically independent samples). Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

increased and 17 decreased) (Fig. 6B). Overall, there were 77 metabolites, with 41 upregulated and 36 down-regulated. Based on the results of $FC>2$ or $FC<0.5$, $VIP>1$ and p -value <0.05 , 43 metabolites (23 increased and 20 decreased in CK) were then selected to validate our hypothesis that the soil metabolites assemble a metabolome that leads to promoting healthy growth of *P. notoginseng* (Fig. S3). The selected metabolites was increased in six intercropping modes including eplerenone hydroxy acid, thalsimine, zinniol, MG (18:2(9Z,12Z)/0:0/0:0) (rac), 1-palmitoylglycerol, octadecanoic acid, 1-stearoyl-sn-glycerol, 2-carboxybenzaldehyde, indole-3-carboxaldehyde, diethylene glycol, 2-deoxy-d-glucose, 4-imidazoleacetic acid, imidazole, 3-hydroxy-3',4',5'-trimethoxyflavone, imidazoleacetic acid, L-glutamic acid, thymidine, 5'-monophosphate, 4-hydroxybenzaldehyde, 3',5'-cyclic inosine monophosphate, ethylenediaminetetraacetic acid, cis,cis-muconic acid, 2,6-di-tert-butyl-4-methoxyphenol and ginsenoside Rf, while those significant decreased in intercropping modes including 2,4,6-tri-tert-butylaniline, spermidine, (r)-(+)-arachidonyl-1'-hydroxy-2'-propylamide, N-palmitoyl-d-sphingosine, N6-methyl-L-lysine, 2-naphthalenesulfonic acid, linoleic acid, 1-o-(9z-octadecenyl)-sn-glycero-2,3-cyclic-phosphate, benzenesulfonic acid, cis-9-palmitoleic acid, 1,2-dioleoyl-sn-glycero-3-phosphate, amastatin, gamma-Glu-Cys, andrastin a, 2-methylamino-1-phenylbutane, N-benzylacetamidine, dibutyl

phthalate, 3-cyclohexyl-1,1-dimethylurea, valerenic acid and confertifoline (Fig. S3). Among the 43 compounds, 25.58% of Lipids and lipid-like molecules, 18.60% of Benzenoids, 13.95% of Organic acids and derivatives, and 9.30% of Organoheterocyclic compounds were included (Fig. S4).

Inhibition test of metabolites against *F. solani*

Correlation analysis of 43 selected compounds with the incidence and disease indices of *P. notoginseng* root rot revealed a significant negative correlation between the indole-3-carboxaldehyde concentration with incidence rate ($R=-0.40$, $p<0.01$) and disease index ($R=-0.37$, $p<0.05$); however, the 2-naphthalenesulfonic acid concentration was significantly positively correlated with the incidence rate $R=0.48$, $p<0.01$) (Fig. 7A). Therefore, indole-3-carboxaldehyde and 2-naphthalenesulfonic acid were selected for validation. The mycelial growth of *F. solani* was significantly inhibited by indole-3-carboxaldehyde when the concentration of indole-3-carboxaldehyde reached 25 mg/L (inhibition rate=3.01, $p<0.05$) (Fig. 7B); as the concentration of indole-3-carboxaldehyde increased, the inhibitory ability increased ($R=0.51$, $p<0.01$) (Fig. 7B). The mycelial growth of *F. solani* was significantly promoted by 2-naphthalenesulfonic acid when the 2-naphthalenesulfonic acid concentration reached 25 mg/L (inhibition rate = -1.48, $p<0.05$)



(Fig. 7D), but the mycelial growth of *F. solani* was significantly inhibited by 2-naphthalenesulfonic acid when the 2-naphthalenesulfonic acid concentration reached 50 mg/L and the inhibition ability increased (inhibition rate=3.77, $R=0.51$, $p<0.05$) (Fig. 7D). Treatment of *P. notoginseng* with indole-3-carboxaldehyde at 0.1 mg/L significantly increased the survival rate, and the survival rate reached its maximum value at 0.25 mg/L (Fig. 7C).

However, there was no significant difference in the survival rate of seedlings treated with 2-naphthalenesulfonic acid compared to that of the control group, but the survival rate showed a downwards trend as the concentration increased (Fig. 7E). Specifically, the content of indole-3-carboxaldehyde in the CK treatment was significantly lower than that in the intercropped tree species, and the content of 2-naphthalenesulfonic acid in the CK

Fig. 6 Soil metabolite analysis of the seven intercropping systems and CK. **A** PLS-DA of the samples from seven treatments. The x-axis represents the first principal component, and the y-axis represents the second principal component. Each point represents a biologically independent sample in PLS-DA. Among them, R^2 represented the predictive ability of the model, and $R^2 > 0.7$ indicates that the model is excellent; Q^2 indicated the predictive ability of the model, the absolute value of Q^2 was greater than 0.5, and it indicated that the model was stable and reliable. **B** Volcano plot of differentially abundant metabolite clustering for the six trees vs CK comparison. The volcano plot map reflects the differences in metabolite expression levels in the two samples. The vertical coordinates represent $-\log_{10}(p\text{-value})$, and the horizontal coordinate values are the $\log_2(\text{fold changes})$. Each point in the graph represents a detected metabolite. The legend of the volcano plot indicates the number of significant Metabolites. Legend: CK: *P. notoginseng* monocropping; ER: *P. notoginseng* intercropped with *Eucalyptus robusta* Smith; CL: *P. notoginseng* intercropped with *Cunninghamia lanceolata*; PO: *P. notoginseng* intercropped with *Platycladus orientalis*; SW: *P. notoginseng* intercropped with *Schima wallichii* Choisy; AN: *P. notoginseng* intercropped with *Alnus cremastogyne*; PW: *P. notoginseng* intercropped with *Pistacia weinmannifolia* J. Poisson ex Franch

treatment was significantly greater than that in the intercropped tree species. These findings are consistent with the incidence of root rot in the field.

Effects of metabolites on dry matter accumulation in *P. notoginseng*

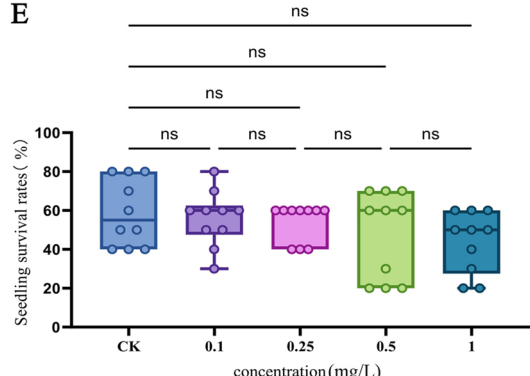
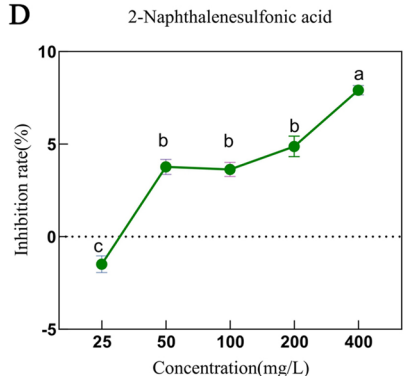
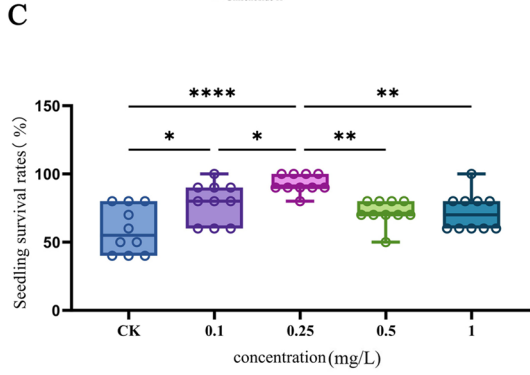
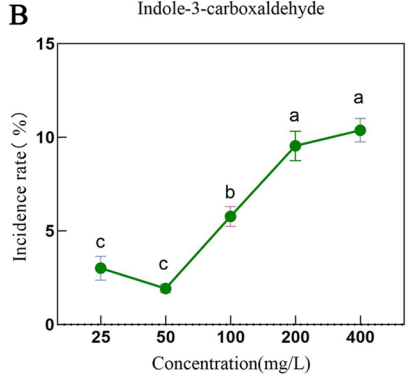
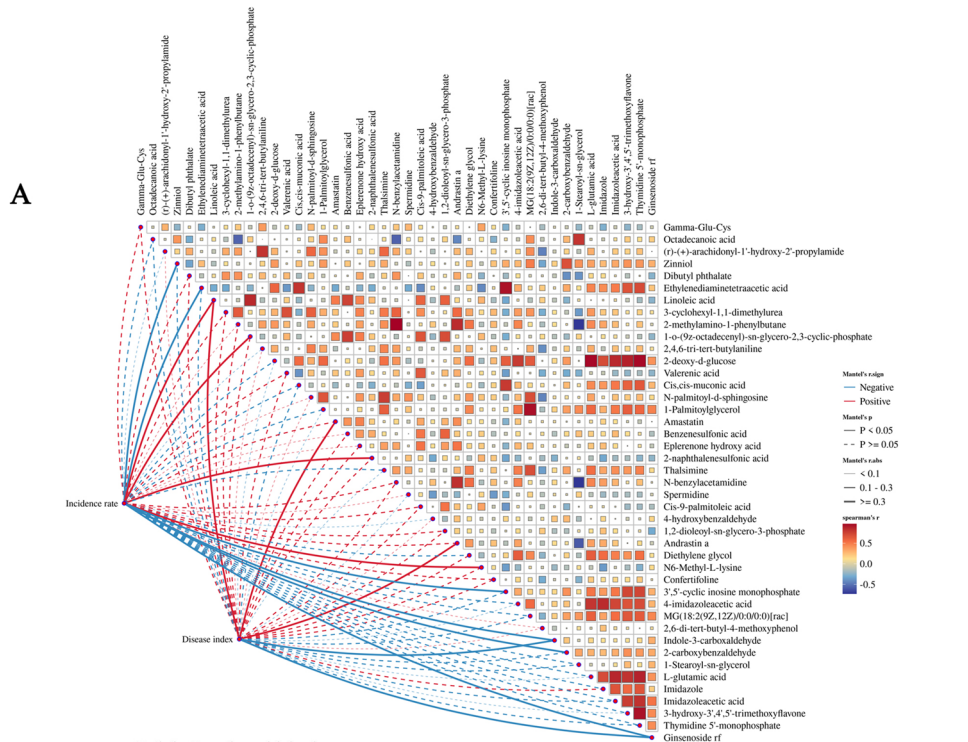
Correlation analysis between 43 metabolites and the aboveground and underground dry weights of *P. notoginseng* revealed significant relationships between metabolites and plant growth. Specifically, 2-naphthalenesulfonic acid was positively correlated with aboveground dry weight per unit area (ADWA, $R=0.54$, $p<0.05$) and underground dry weight per unit area (UDWA, $R=0.43$, $p<0.05$), while diethylene glycol was negatively correlated with ADWA ($R=-0.34$, $p<0.05$) and UDWA ($R=-0.39$, $p<0.05$), and 2-deoxy-d-glucose was also negatively correlated with ADWA ($R=-0.73$, $p<0.05$) and UDWA ($R=-0.58$, $p<0.05$) (Fig. 8A). 2-deoxy-d-glucose to examine the effects of these three metabolites on the growth of *P. notoginseng*, a pot experiment was conducted for 6 weeks. After six successive weeks of *P. notoginseng* treatment (Fig. 8B), the metabolites significantly influenced *P. notoginseng* plant biomass compared to that in the

CK treatment. As shown in the Fig. 8B, there was no significant difference in the biomass of plants treated with 2-naphthalenesulfonic acid or diethylene glycol compared to that of the control, while 2-deoxy-d-glucose reduced the biomass accumulation in the aerial parts of *P. notoginseng*. A study of the underground biomass of *P. notoginseng* revealed that 2-naphthalenesulfonic acid promoted underground biomass compared to that of the control; 2-deoxy-d-glucose did not significantly affect the biomass compared to that of the control, while diethylene glycol reduced the biomass accumulation of *P. notoginseng* (Fig. 8B). These results indicate that the underground plant growth-promoting effects could be attributed to 2-naphthalenesulfonic acid, while 2-deoxy-d-glucose reduced the aboveground biomass. The difference in the biomass of *P. notoginseng* among the interaction systems may be related to the differences in the levels of these metabolites in the soil. Specifically, the content of 2-deoxy-d-glucose in the CK pots was significantly lower than that in the intercropping pots, and the content of diethylene glycol in the CK pots was significantly lower than that in the intercropping tree species AN and PW; however, the content of 2-naphthalenesulfonic acid in the CK pots was significantly greater than that in the intercropping pots. These findings are consistent with the findings of the seven interaction experiments.

Discussion

Intercropping *P. notoginseng* with trees can contribute to its healthy growth.

In agroforestry systems, intercropping via interactions between different plants can mitigate disease and promote crop growth (Moore et al. 2022; Gong et al. 2019). In this study, interspecific interactions between six tree species and *P. notoginseng* significantly reduced the incidence of root rot and increased the survival rate of *P. notoginseng* (Fig. 2). Among these species, the coniferous tree species PO and the broad-leaf tree species ER demonstrated the most effective results. This finding suggested that the interaction between tree species and the root system of *P. notoginseng* may play a crucial role in reducing the incidence of root rot in forest environments.



◀**Fig. 7** Screening and validation of disease-related metabolites. **A** Heatmap of the correlation between differentially abundant metabolites and the incidence and disease indices of *P. notoginseng* root rot. Antagonistic test results of indole-3-carboxaldehyde (**B**) and 2-naphthalenesulfonic acid treatments (**D**) against *Fusarium solani*. Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p < 0.05$, $n = 6$ biologically independent samples, indole-3-carboxaldehyde treatment: $F = 41.126$, Degrees of Freedom = 4, $p < 0.01$; 2-naphthalenesulfonic acid treatment: $F = 65.327$, Degrees of Freedom = 4, $p < 0.01$). The survival rate of *P. notoginseng* seedlings after indole-3-carboxaldehyde treatment (**C**) and 2-naphthalenesulfonic acid (**E**). Each cycle represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p < 0.05$, $n = 6$ biologically independent samples, indole-3-carboxaldehyde treatment: $F = 9.198$, Degrees of Freedom = 4, $p < 0.01$; 2-naphthalenesulfonic acid treatment: $F = 1.376$, Degrees of Freedom = 4, $p > 0.05$)

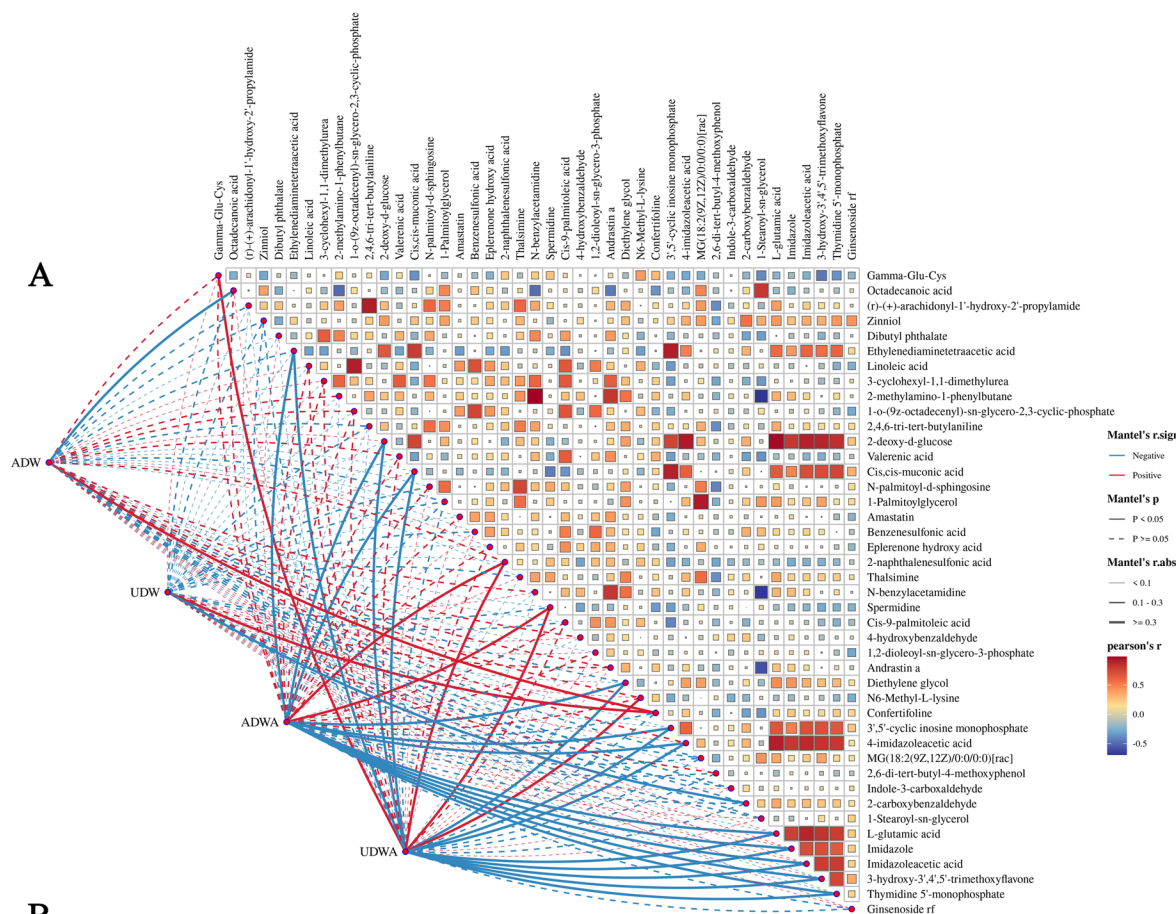
Similar benefits have been observed in other intercropping systems, e.g., intercropping ginseng in a forest reduced the occurrence of ginseng damping-off disease (Liu et al. 2023). Furthermore, in addition to disease inhibition, the interaction of roots between *P. notoginseng* and other tree species, especially PO and CL, which are coniferous forest species, also enhanced the germination rate and root biomass of *P. notoginseng*. Therefore, coniferous forest species may serve as better companions for *P. notoginseng* than broad-leaved forest species.

Intercropping in agroforestry systems has been proven to enhance crop growth and increase yields, as supported by several studies, such as an increase in the yield of *Ginkgo biloba* intercropped with *Triticum aestivum* (Cao et al. 2009) and *Malus spectabilis* trees intercropped with *Glycine max* (Wang et al. 2022; Zhou et al. 2023a, 2023b). In the present study, the yield of *P. notoginseng* significantly increased with the intercropping of PO and CL (Table 2). Similar results have also been reported for forest and mushroom intercropping systems; for example, *Lentinula edodes* cultivated under *Populus* L. and *Alnus japonica* exhibited a greater yield compared with cultivated land with ground cultivation (Tian et al. 2019). Additionally, Huang et al. (2019) and Gao et al. (2020) reported that *Bletilla striata* and *Polygonatum sibiricum* had

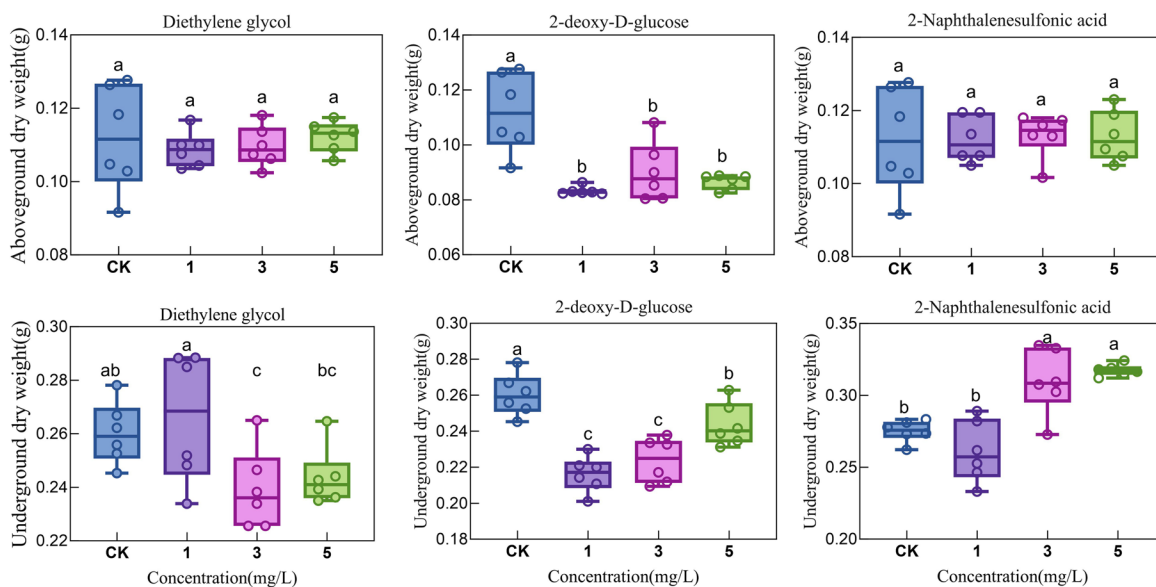
higher yields in a forest-medicine composite system than in a field. Therefore, interactions between species in forest ecosystems significantly contribute to the overall health and well-being of plants.

Improving the quality of *P. notoginseng* in agroforestry systems through interspecific interactions by intercropping trees

Relevant research has provided compelling evidence for the potential advantages of species intercropping in augmenting crop quality (Zhou et al., 2023a; Wei et al. 2019; Millan et al. 2022; Xu et al. 2022). In our study, we found that PO, CL and SW significantly improved the nutrient concentration of *P. notoginseng* (Fig. 3). Moreover, the content of the six saponins significantly increased with PO treatment. Similarly, the SW and PW treatments significantly enhanced the content of five saponins, especially saponin R1 (Fig. 4). Therefore, by combining biomass data, we found that PO and SW promoted the growth of *P. notoginseng* while also increasing its nitrogen, phosphorus, and saponin concentrations. This dual promotion led to a much greater increase in nutrient absorption and saponin accumulation per unit area of *P. notoginseng* (Parent et al. 2012). Ji et al. (2023) and Wang et al. (2023a, b) reported that nutrient uptake and the notoginsenoside content significantly improved in the cultivation of *P. notoginseng* in pine forests. Similar studies have also shown that the contents of 20 monomeric saponins, such as Rg1 and Re, significantly increased in *P. ginseng* cultivated in a *Pinus sylvestris* var. *mongolica* forest (Kim et al. 2017; Dai et al. 2020). Some research has suggested that a slight decrease in soil pH promotes the adsorption, dissolution, and release of fixed inorganic phosphorus in the soil and transforms it into available and soluble inorganic phosphorus available to plants (Tian et al. 2020; Long et al. 2016). pH was also the key environmental factor for the synthesis of Rb1 saponins (Moameri et al. 2024; Wang et al. 2023a, b). Our investigation revealed that cultivating *P. notoginseng* in the understorey led to a decrease in soil pH (Fig. 5), which may result in greater growth and improved quality of *P. notoginseng* within the forest. Based on the above results, coniferous forest PO and broad-leaved forest SW are also good neighbours of *P. notoginseng* and can be beneficial to the growth and quality of *P. notoginseng*.



B



◀**Fig. 8** Screening and validation of grown-related metabolites. **A** Heatmap of the correlation between differentially abundant metabolites and the growth indices. **B** Effects of soil metabolites on *P. notoginseng* of aboveground and underground dry weight (Aboveground dry weight of diethylene glycol treatment: $F=0.260$, Degrees of Freedom=3, $p>0.05$; Aboveground dry weight of 2-deoxy-d-glucose treatment: $F=12.065$, Degrees of Freedom=3, $p<0.01$; Aboveground dry weight of 2-naphthalenesulfonic acid treatment: $F=0.025$, Degrees of Freedom=3, $p>0.05$; Underground dry weight of diethylene glycol treatment: $F=3.755$, Degrees of Freedom=3, $p<0.05$; Underground dry weight of 2-deoxy-d-glucose treatment: $F=11.980$, Degrees of Freedom=3, $p<0.01$; Underground dry weight of 2-naphthalenesulfonic acid treatment: $F=20.321$, Degrees of Freedom=3, $p<0.01$). Each cycle represents a biologically independent sample. Horizontal bars within boxes represent the median. The tops and bottoms of boxes represent 75th and 25th quartiles, respectively. Different lowercase letters indicated significant differences among respective groups based on two-sided tests for multiple comparisons by Turkey HSD corrections (ANOVA, adjusted $p<0.05$, $n=6$ biologically independent samples). Legend: ADW: Aboveground dry weight per plant; UDW: Underground dry weight per plant; ADWA: Aboveground dry weight per unit area; UDWA: Underground dry weight per unit area

Regulation of soil metabolism changes by understorey planting promotes the health of *P. notoginseng*

Soil metabolism regulated by rhizosphere interactions between trees and crops has various functions in interspecies root interactions, except for changing the soil pH (Chen 2022; Yuan et al. 2021; Machiani et al. 2018). In our research, a noteworthy alteration in EC was observed (Fig. 5), this finding suggested that agroforestry with tree species and *P. notoginseng* has a substantial impact on the diversity and quantity of soil metabolites. Faced with changing environmental conditions, plant metabolism is usually disturbed, and metabolites must be adjusted to maintain basic metabolism, which helps plant hormones respond to stress conditions first (Kosmacz et al. 2020). All initial reactions involve synthesizing and degrading amino acids to produce primary metabolites, such as carbohydrates, proteins, and lipids (Singh et al. 2020). Primary metabolism provides the necessary energy and precursor substances for the biosynthesis of secondary metabolites in the final step (Liu et al. 2017, 2021). In the present study, we utilized HPLC–MS to analyse soil metabolism in a *P. notoginseng*–tree species interaction experiment and identified 43 differentially abundant metabolites between intercropped

P. notoginseng and monocropped *P. notoginseng* (Fig. 6). The differentially abundant metabolites included lipids, lipid-like molecules, organic acids, derivatives, benzenoids and organoheterocyclic compounds (Fig. S4). In this study, diethylene glycol and 2-deoxy-d-glucose had significant negative relationships with the biomass of *P. notoginseng*, yet 2-naphthalenesulfonic acid had a positive relationship with the biomass (Fig. 8A). Studies have shown that the use of 2-deoxyglucose as a glycolytic inhibitor renders fungi unusable (Zhou et al. 2011). Ji et al. (2024) found that the antibacterial agent diglycolate, which is the active ingredient, could inhibit the growth of wheat, but there are few reports on the use of 2-deoxyglucose and diethylene glycol for promoting production. Our pot experiment confirmed that increasing 2-naphthalenesulfonic acid concentrations or decreasing diethylene glycol and 2-deoxy-d-glucose concentrations is beneficial for the growth of *P. notoginseng* (Fig. 8B). Therefore, soil metabolic changes mediated by tree species roots may be an important mechanism for the growth advantages observed in *P. notoginseng* understorey plantings (Xu et al. 2020).

Studies have shown that the metabolites in the rhizosphere soil of *Panax quinquefolius*, such as lipids (Wu and Lin 2020), organic acids, sugars, etc., can provide carbon (Li et al. 2022a, b) and nitrogen (Zhou et al. 2023b, a) for soil microorganisms and promote root adaptation to soil environmental changes, affecting the healthy growth of plants (Guo et al. 2023). When pathogens invade plant roots, plant growth can be affected by inhibiting, promoting, and causing the chemotaxis of rhizosphere metabolites (Zhang et al. 2019a, b; Lei et al. 2018). Through correlation analysis with the growth indices of *P. notoginseng* and through plate confrontation and pot experiments, we determined that indole-3-carbaldehyde was beneficial for reducing the occurrence of root rot in *P. notoginseng*, while 2-naphthalenesulfonic acid was positively correlated with disease (Fig. 7). The synthesis of indole-3-carbaldehyde compounds derived from indoles has received significant attention in the medical field (Metwally et al. 2010). This is due to the broad-spectrum biological activities of indole-3-carbaldehyde, such as antibacterial activity (Xu et al. 2011) and anti-inflammatory activity (Rani et al. 2004). We also found that 2-naphthalenesulfonic acid is an important intermediate for the production of dyes, pesticides and drugs (Lin et al.

1988). However, limited research has been conducted on the inhibition of plant pathogens by indole-3-acyl compounds or 2-naphthalenesulfonic acid. Only substituted indole-3-carboxaldehyde compounds, which have attracted worldwide attention due to their unique chemical structure and extensive physiological and pharmacological activities, have been demonstrated to effectively inhibit *Exserohilum turcicum* and *Helminthosporium maydis* (Che et al. 2015). However, there is little direct research on the effects of this fungus on resistance to pathogenic bacteria or plant growth. Our pot interaction experiment confirmed these findings by revealing a greater relative abundance of indole-3-carboxaldehyde in the soil metabolism of PO and CL and the opposite trend for 2-naphthalenesulfonic acid. Therefore, these findings may help to elucidate the disease resistance mechanism of the interaction system of these three species with *P. notoginseng*.

Moreover, numerous studies had documented changes in the composition of rhizosphere metabolites in response to various neighbouring plants (Wen et al. 2023b, 2023c). This interaction contributed to the development of disease-suppressive soils by recruiting beneficial microbial populations such as *Bacillus subtilis* and *Pseudomonas* strains (Wen et al. 2021; Li and Jiang 2021; Haichar et al. 2014). Although the exact mechanisms remained elusive, these findings highlight the complex relationship between root metabolites and microorganisms. In the future, we aimed to conduct more detailed research to further elucidate the relationship between root metabolites and microorganisms, and their role in plant disease resistance.

Conclusion

The occurrence of root rot in *P. notoginseng* plants was significantly reduced when intercropped with PO and SW, resulting in enhanced growth, nutrient, and saponin concentrations. Additionally, indole-3-carboxaldehyde, a compound present in intercropping soil, was instrumental in mitigating the root rot of *P. notoginseng*. Simultaneously, compound such as 2-naphthalenesulfonic acid, also found in intercropping soil, was increased the development and quality of growth in *P. notoginseng*. These findings suggest that soil metabolic alterations may underlie the observed growth and disease resistance benefits in the understorey cultivation of *P. notoginseng*. Consequently,

Platyclusus orientalis (coniferous forests) and *Schima wallichii* Choisy (broad-leaved forests) emerged as potentially beneficial companions for *P. notoginseng*.

Authors' contributions All authors contributed to the study conception and design. Cuiying Wang, Guomin Mao, Yingbin Li, Wenjing Zi, Qingying Wang original researcher, introduction, methodologist, statistical analyst, writing- review and editing; Huichuan Huang, Min Yang, Fei Du, Xinyue Mei, Weiping Deng, Jian Lu methodologist, statistical analyst; Cuiying Wang sampling, methodologist; Shusheng Zhu, Chen Ye, Yixiang Liu, writing- review and editing. All authors read and approved the final manuscript.

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Data availability All Raw sequencing data have been submitted to the NCBI GenBank accession number(s) for your nucleotide sequence(s) under the accession number PP493919 and PP514759.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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