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Rain Shelter Cultivation Reduces Root Rot Incidence of *Panax notoginseng* by Altering Root Exudates and Bacterial Communities under Micro-Irrigation and Fertilization

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Abstract: *Panax notoginseng* is an important medicinal crop in China. The high incidence of root rot in *P. notoginseng* during the rainy season has restricted the development of the industry. It is believed that frequent rainfall and a warm soil environment are important factors that promote root rot incidence. However, there is still a significant knowledge gap in the relationship between rainfall and root rot incidence. To understand the effects of rainfall and fertilizer on root exudates, the soil bacterial structure, and root rot in *P. notoginseng*, four treatments were chosen for both field and pot experiments. These treatments included DW (rain shelter and no fertilizer), RW (no rain shelter and no fertilizer), DWF (rain shelter and fertilizer), and RWF (no rain shelter and fertilizer). The results showed that both factors (rain shelter and fertilizer) significantly affected root rot incidence and several other parameters. Among them, the effect of a rain shelter is more significant than that of fertilizer, and the combination of the two further improves the effect. DW and DWF treatments significantly reduced the soil moisture, phenolic acid, and root rot incidence, while significantly increasing the soil temperature and enzyme activities compared to RW or RWF. Seven phenolic acids secreted by *P. notoginseng* roots were all positively correlated with root rot incidence. Root rot was also positively correlated with *Planctomycetota*, *Acidobacteriota*, and *Gemmatimonadota* and negatively correlated with *Firmicutes*, *Proteobacteria*, *Patescibacteria*, and *Nitrospirota*. DWF treatment decreased the soil moisture and the concentration of p-hydroxybenzoic acid, syringic acid, phthalic acid, and vanillic acid and promoted the growth of *Firmicutes* and *Proteobacteria*, leading to the lowest incidence of root rot.

Keywords: *Panax notoginseng*; root exudates; root rot; soil moisture

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1. Introduction

Panax notoginseng, (Burk) F. H. Chen, is a traditional precious medicine in China. The root of *P. notoginseng* contains a high amount of saponins, which have a substantial therapeutic effect on cardiovascular diseases. Therefore, the root of *P. notoginseng* and its products are widely used in medicine, health supplements, food, etc. [1,2]. However, root rot has restricted the development of the *P. notoginseng* industry, which has led to a decrease in the production of *P. notoginseng* by more than 70% [3]. *P. notoginseng* planting requires strict constraints on the soil environment, and high-quality *P. notoginseng* roots are mainly grown in shade and humid environments [4]. Long-term *P. notoginseng* planting in a warm and humid environment, however, leads to the accumulation of allelochemicals and the imbalance of microbial communities, eventually leading to the frequent occurrence of *P. notoginseng* root rot [5]. At present, the prevention of root rot is mainly achieved by

spraying pesticides. However, the application of pesticides leads to pesticide residue in the root, reducing its medicinal value [6]. Therefore, green *P. notoginseng* root rot protection measures are of great significance to the development of the *P. notoginseng* planting industry.

Root rot is typically a soil-borne disease, and is greatly influenced by the activity and quantity of soil pathogens (*Fusarium oxysporum*, *Gemmatimonadota* et al.) [7]. Root exudates have been identified as major drivers of the rhizosphere soil microbial community dynamics, assembly, and functional capacity [8]. Root exudates play a significant role in the nutrient substance cycle and signal transduction between the root system, soil, and microorganisms. On one hand, plants can attract or inhibit some microorganisms by altering the pattern of released compounds, affecting the assembly of rhizosphere microbial communities and thus enhancing their ability to adapt to the environment [8]. On the other hand, changes in the soil environment and soil microorganisms can stimulate the plant root to secrete allelochemicals [9,10]. The content of organic acids secreted by roots and the diversity of microbial communities are not uniform or static and change with the environment [11–13]. Soil moisture and nutrients are two essential factors that affect plant growth and soil microbial communities [14]. Inappropriate water and fertilizer supply can lead to inhibited plant growth [15] and increased root exudates [13,16] and promote the growth of pathogenic microorganisms [16]. The primary growing area for *P. notoginseng* in China (Wenshan, Yunnan province) experiences frequent rainfall during the rainy season, which makes it more difficult to adjust the soil moisture accurately and results in frequent root rot occurrence. Studies have shown that proper rain shelter treatment during the rainy season can effectively reduce the adverse effects of natural rainfall on crops and attenuate some external factors of crop diseases [17,18]. However, the mechanism of reducing the incidence of the crop by rain shelter cultivation is unclear. Whether rain shelter cultivation affects the root exudates and/or root microorganisms of *P. notoginseng*, or reduces the root rot of *P. notoginseng* needs to be studied. Therefore, field experiments and pot experiments were conducted to explore the effects of rain shelter or no rain shelter cultivation on the soil's physicochemical properties, the response relationship among root exudates, microbial communities, and root rot incidence in *P. notoginseng*.

2. Materials and Methods

2.1. Experimental Site Descriptions

The field experiment was conducted in the *P. notoginseng* key technology research and demonstration base for water control, emission reductions, quality, and efficiency improvement (24°29′57″ N, 103°34′22″ E, with an altitude of 1790 m) in Luxi County, Yunnan Province, China. The area where the field experiment was conducted has a subtropical low-latitude plateau monsoon climate with an average annual temperature of 16.48 °C and an annual rainfall of 895.6 mm. In 2021, the rainfall in the whole year was 856 mm and the average annual temperature was 20.23 °C (SI: Figure S1). The pot experiment was conducted in the greenhouse of Kunming University of Science and Technology (24°50′40″ N, 102°51′49″ E, with an altitude of 1835 m). The soil used in the field experiment was red soil (Ferric Acrisols according to IUSS Working Group WRB, 2015), with a pH of 6.7, an alkali-hydrolyzed nitrogen of 29.12 $mg \cdot kg^{-1}$, an available phosphorus of 31.25 $mg \cdot kg^{-1}$, an available potassium of 168.35 $mg \cdot kg^{-1}$, a soil capacity of 1.18 g/cm^3, a field water holding capacity (FC) of 40.26%, a catalase activity of 8.13 $mg \cdot g^{-1}$ 24 h^{-1}, a sucrase activity of 11.32 $mg \cdot g^{-1}$ 24 h^{-1}, a urease activity of 0.23 $mg \cdot g^{-1}$ 24 h^{-1}, and a phosphatase activity of 0.15 $mg \cdot g^{-1}$ 24 h^{-1} in 0–20 cm soil.

The soil in the pot experiment was red soil (Ferric Acrisols according to IUSS Working Group WRB, 2015) with a pH of 8.04, an alkali-hydrolyzed nitrogen of 47.19 $mg \cdot kg^{-1}$, an available phosphorus of 85.91 $mg \cdot kg^{-1}$, an available potassium of 267.56 $mg \cdot kg^{-1}$, a soil capacity of 1.08 g/cm^3, a field water holding capacity of 49.82%, a catalase activity of 15.62 $mg \cdot g^{-1} \cdot 24\ h^{-1}$, a sucrase activity of 11.39 $mg \cdot g^{-1} \cdot 24\ h^{-1}$, a urease activity of 2.12 $mg \cdot g^{-1} \cdot 24\ h^{-1}$, and a phosphatase activity of 0.15 $mg \cdot g^{-1} \cdot 24\ h^{-1}$. Additionally, the P-hydroxybenzoic acid, para-coumaric acid, vanillin acid, syringic acid, ferric acid,

3-indoleacetic acid, and phthalic acid levels initially in the soil in the pot experiment were 1.84 $\mu g \cdot g^{-1}$, 3.53 $\mu g \cdot g^{-1}$, 2.21 $\mu g \cdot g^{-1}$, 3.90 $\mu g \cdot g^{-1}$, 1.96 $\mu g \cdot g^{-1}$, 1.04 $\mu g \cdot g^{-1}$, and 2.14 $\mu g \cdot g^{-1}$, respectively.

2.2. Experimental Design

The trial crops for the field and pot experiment were 5-year-old *P. notoginseng* crops. The field experiment was conducted from March 2021 to October 2021 and the pot experiment was conducted from March 2022 to October 2022. Stages of the experiments included the stage of seedling, rapid growth, and flowering of *P. notoginseng*. The field and pot experiments consisted of two experimental factors, rain shelter and fertilization, with a total of four treatments, which were DW (rain shelter and no fertilizer), RW (no rain shelter and no fertilizer), DWF (rain shelter and fertilizer), and RWF (no rain shelter and fertilizer) (Figure 1).

In the field experiment, each plot was cultivated by ridge farming and separated by a 0.3 m ditch. The area of each plot was 32 m^2 (length: 16 m and width: 2 m), with 1200 of *P. notoginsengs* planted in each plot. Each treatment was conducted in three replicate plots. Shade nets were used to protect all plots from sunlight, while transparent plastic film was used to protect the DWF and DW treatments from rainfall (Figure 1a,b). All plots were irrigated with an independent micro-sprinkler irrigation system, which consisted of a main pipe, a water valve, a water meter, and three micro-sprinkler irrigation nozzles. A water valve and a water meter installed on the main water pipe of each plot were used to control the amount of irrigation. The spray area of each nozzle was circular and the diameter of the spray area was about 1.5 m. The irrigation (Table 1) was deficit irrigation with an upper~lower limit (85~70%) θ_{FC}. Each treatment was replicated three times. The irrigation and fertilization schedule for the field experiment is shown in Table 1.

The pot experiment was designed based on Liao's study [4], with 800 g of soil placed in a PVC box with an upper and lower diameter of 18 cm and 13 cm and a height of 15 cm (Figure 1c,d). Each pot was planted with one *P. notoginseng*, and 20 pots were used for each treatment. Due to the pot experiment being conducted in greenhouse, a rainfall simulator was used to simulate rainfall in RWF and RW treatments. The rainfall simulation period was the rainy season (June to October 2022), according to the meteorological data in the field experiment (Figure S1). Artificial rainfall simulations were performed every afternoon for 1 h, and the rainfall intensity was set to 5 mm/h according to the rainfall records from the *P. notoginseng* key technology research and demonstration base. The rain pattern from the rainfall simulation was drop-shaped and the rainfall uniformity was greater than 80%. The pots were weighed daily to record the mass of water loss by evapotranspiration, and properly watered to keep the soil water constant. Each treatment was performed in twenty replicates. The irrigation and fertilization schedule for the field experiment is shown in Table 1.

A water-soluble organic compound fertilizer (21%N-21%P_2O_5_21%K_2O + 6% humic acid + trace elements) was used for fertilization in both the field and pot experiments. In the field experiment, MixRite2502 was selected as a comprehensive water and fertilizer equipment with a flow rate of 2.5 $m^3 \cdot h^{-1}$ and a fertilizer/water ratio of 1:500. In the pot experiment, the ratio of fertilizer to water was 1:500, which was manually added through a spray bottle. The amount of fertilizer in each treatment is shown in Table 1. A total of 25% of this fertilizer was applied in seedling stage, 30% in rapid growth stage, and 45% in flowering stage of *P. notoginseng*. No pesticides were applied throughout the crop growth period, and weeds were manually removed in all plots.

Table 1. The irrigation and fertilization gradient of the experiment schedule for *P. notoginseng*.

Irrigation Regimes		Irrigation (mm)	Fertilizer (kg ha^{-1})	Rainfall (mm)	Drainage (mm)	Total Water Volume (mm)
Field experiment	DW	512	0	0	0	512
	DWF	512	1680	0	0	512
	RW	424	0	856	768	512
	RWF	424	1680	856	768	512
Pot experiment	DW	672	0	0	0	672
	DWF	672	1680	0	0	672
	RW	432	0	856	613	672
	RWF	432	1680	856	613	672

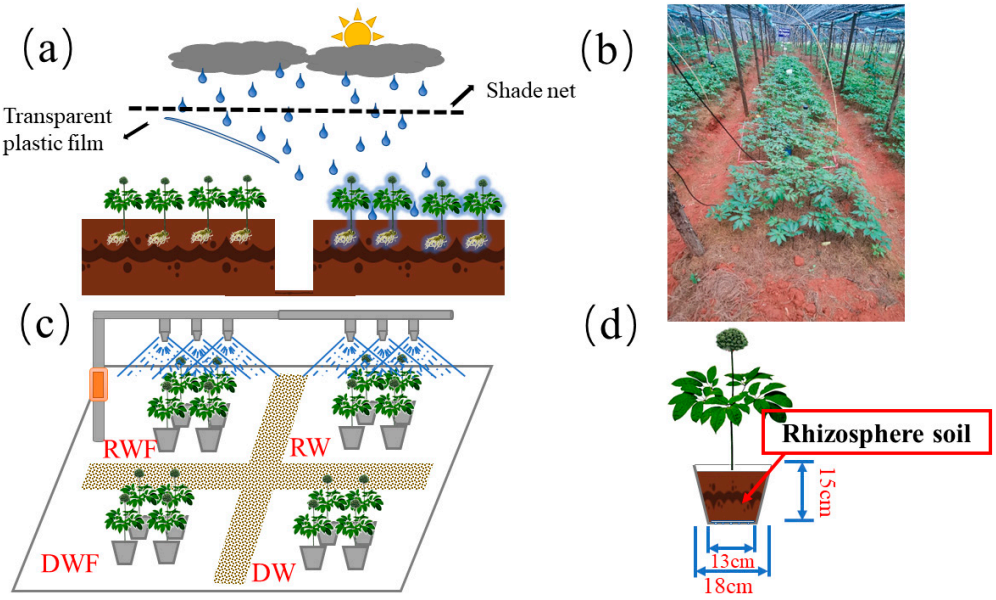

Figure 1. Schematic diagram of field experiment (**a**,**b**) and pot experiment (**c**,**d**).

2.3. Sampling and Determination

Soil samples were collected following the late flowering period, which was October 2021 in the field experiments and October 2022 in the pot experiment.

In the field experiment, a five-core composite soil sample was collected from each plot at 0–20 cm using a sample probe. Each fresh soil sample was approximately 250 g. Each soil sample was air-dried and then passed through a 100-mesh sieve for determining the alkali-hydrolyzed nitrogen, available phosphorus, available potassium, and soil pH. A portion of the soil that had passed through the 100-mesh sieve was then ground through a <60-mesh sieve for determination of catalase, phosphatase, urease, and sucrase activity.

In the pot experiment, three evenly growing and representative pots of each treatment were selected before using the root shaking method. The roots of *P. notoginseng* were carefully moved from the pot with the adhering soil. Additionally, the roots were then carefully shaken to remove the loosely adhering soil, and the remaining attached soil was carefully collected using sterile brushes and considered as rhizosphere soil (approximately 150 g). The soil sample was divided into two parts. After being refrigerated with liquid nitrogen, a portion of soil was quickly transported into the lab and stored in a −80 °C refrigerator for the determination of soil microorganisms; the rest of the soil samples were air-dried and the indicators mentioned above in the field experiment were determined. Additionally, to determine the organic acids, 5 g air-dried soil was taken and passed through a <60-mesh sieve. The soil sample treatment before soil microbial detection was as follows: three duplicate soil samples for each treatment were evenly mixed as one DNA sample of mixed soil to be tested.

The pH of the soil samples was measured using a pH electrode at a soil/water ratio of 1:2.5 [19]. The alkali-hydrolyzed nitrogen, available phosphorus, and available potassium were analyzed by the diffusion method [20], the Olsen method [21], and the ammonium acetate extraction flame photometry method [22], respectively. The soil enzyme activities of urease, catalase, phosphatase, and sucrase were determined by the indophenol-blue colorimetry method, the potassium permanganate titration method, the phosphate sodium method, and the nitrophenol colorimetry method, respectively [23]. The soil moisture was measured using the weight method [4]. The soil temperature was measured using a soil thermograph. Each sample was measured three independent times for all the above indicators.

2.4. DNA Extraction and Illumina MiSeq Sequencing

DNA was extracted from 0.3–0.4 g of the four experimental mixed soil samples from the pots and one control soil mixed sample (the soil before the experiment) using an EZNATM Soil DNA Kit (Omega, Norcross, GA, USA). The eluted DNA samples were analyzed by 1% (*m*/*v*) agarose gel electrophoresis, and the DNA concentration was determined using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3–V4 regions of the bacterial 16S rRNA gene were amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Amplification was performed with pre-denaturation for 3 min at 95 °C, followed by 26 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C and a final extension for 10 min at 72 °C. PCR amplicons were detected by electrophoresis on 2% (*w*/*v*) gels, and the Axygen® AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to recover the target fragments. The purified amplicons were sequenced on an Illumina MiSeq platform (Majorbio Bio-Pharm Technology Company, Shanghai, China). The measurement was repeated three times for each soil DNA sample to be tested.

2.5. Analysis of the Sequencing Data

The high-throughput data were preliminarily processed. USEARCH (v10.0.240, http://www.drive5.com/usearch/download.html, accessed on 1 November 2022) was used to align the overlapping regions with complementary base pairing. The barcode and primer sequences were excised, followed by quality control, filtering, and chimera removal. Unoise3 was used to separate the operational taxonomic units (OTUs) at a similarity threshold of 97%. A representative sequence for each OTU was randomly selected and compared against the SILVA database v1.3.2 (https://www.arb-silva.de/documentation/release-132/, accessed on 1 November 2022) using the RDP classifier with a similarity threshold of 0.8. A total of 740,055 high-quality sequences were obtained from five mixed soil samples after quality control and filtration, and 4749 OTUs were obtained after clustering.

2.6. Extraction and Determination of Soil Organic Acids

Soil phenolic acids were extracted according to the procedure described by Wang [3]. Briefly, 5 g of air-dried soil samples from 12 pot experimental soil samples and 3 control soil samples (the soil before the experiment) was suspended in 50 mL of 1 M NaOH and shaken on a rotary shaker at 150 rpm at 30 °C for 24 h. After centrifugation at 10,000 rpm for 10 min, the supernatant was acidified to 2.5 with 9 M HCl and then extracted three times with ethyl acetate. The resultant extracts were pooled and evaporated to dryness at 35 °C. The obtained residue was dissolved in 5 mL of 100% methanol, filtered (0.22 μm), and stored at 4 °C for further analyses.

Based on the results of our preliminary experiment, seven authentic phenolic acids (including p-hydroxybenzoic acid, vanillic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, and salicylic acid, purity ≥ 98%, Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China) were selected to establish the standard curves. The phenolic acids in the soil extracts were identified and quantified using HPLC (Milford, MA, USA) with a SunFire-C18 column (250 mm × 4.6 mm, 5 μm; Waters Technologies (China) Co., Ltd., Shanghai, China). The mobile phase consisted of methanol (A) and 0.16% acetic acid (B) with

gradient elution. The gradient flow program was set as follows: 0 min, 5% A and 95% B; 5 min, 20% A and 80% B; 10 min, 35% A and 65% B; 21 min, 45% A and 55% B; 30 min, 60% A and 40% B; and 33 min, 5% A and 95% B. The chromatogram was monitored at 280 nm. The injective volume was 10 μL and the column temperature was kept constant at 40 °C. Phenolic acids were identified by comparing their retention times with standard compounds, and the concentrations of phenolic acids were calculated based on their peak areas.

2.7. Statistics of the Incidence Rate of Root Rot

Diseased and wilted *P. notoginseng* plants were gently pulled out to record the number of root-rotted plants every 7 days. The incidence rate was calculated according to the following formula:

$$IR = DI/T \times 100\%$$

where IR represents the incidence rate, %; DI represents the number of diseased plants; and T represents the total number of plants per unit area.

2.8. Statistical Analysis

The effects of a rain shelter with irrigation and fertilization on the soil available nutrients, soil temperature, soil moisture, and root exudates were assessed using a 2-factor ANOVA with interactions. The means were separated using a Duncan's multiple range test at $p < 0.05$ in SPSS 25.0. A heatmap was used to analyze the effects of the rain shelter with irrigation and fertilization on the soil bacterial structure with the vegan package in R. A redundancy analysis (RDA) was used in Canoco 5.0 to analyze the effects of root exudates and soil physicochemical properties on soil bacterial microorganisms, as well as the effects of root exudates and bacterial microorganism on root rot. All other figures were drawn in Origin 2018 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Soil Physical and Chemical Properties

Both factors, rain shelter and fertilization, significantly affected multiple soil properties, with some interaction between the two factors. The highest alkaline hydrolyzed nitrogen, available phosphorus, and available potassium in both the field and pot experiments were observed in the DWF treatment ($p < 0.05$), and the lowest was observed in the RW treatment (Table 2). In the field experiment, the contents of available potassium, alkali hydrolyzed nitrogen, and available phosphorus in the soil of rain shelter treatments (DWF and DW) were 22.67–37.42%, 13.48–16.67%, and 5.52–20.30% higher than those in non-rain shelter treatments (RWF and RW), respectively. Similarly, the contents of available potassium, alkali hydrolyzed nitrogen, and available phosphorus in the soil of rain shelter treatments (DWF and DW) were 66.39–93.92%, 21.20–27.98%, and 18.00–21.90% higher thsn non rain shelter treatments (RWF and RW), respectively. This suggested that rainfall leads to a decrease in the available nutrients in 0–20 cm soil.

The soil pH in the field and pot experiments was significantly higher in DWF than all other treatments. In both the field and pot experiments, the soil temperature in rain shelter treatments (DW and DWF) was significantly higher than that in the corresponding non rain shelter treatments (RW and RWF), and the soil moisture was the opposite. This suggested that frequent rainfall indeed led to an increase in the soil moisture and a decrease in soil temperature.

Table 2. The physicochemical properties of *P. notoginseng* soil in (no) rain shelter and fertilizer treatments.

	Treatments	Soil pH	Available Potassium	Alkali-Hydrolyzed Nitrogen	Available Phosphorus	Soil Temperature	Soil Moisture
			mg·kg^{-1}	mg·kg^{-1}	mg·kg^{-1}	°C	%
Field experiment	DW	6.61 ± 0.1 bb	158.64 ± 16.11 ab	20.2 ± 0.26 b	25.25 ± 0.88 b	26.01 ± 0.12 b	24.16 ± 0.35 b
	DWF	6.83 ± 0.05 a	186.29 ± 21.32 a	22.4 ± 0.23 a	29.45 ± 0.56 a	26.28 ± 0.1 a	23.6 ± 0.22 b
	RW	6.49 ± 0.05 c	129.32 ± 8.88 b	17.8 ± 0.25 c	23.93 ± 0.79 c	24.19 ± 0.11 d	30.25 ± 0.33 a
	RWF	6.67 ± 0.06 b	135.56 ± 10.84 b	19.2 ± 0.31 b	24.48 ± 1.12 b	24.69 ± 0.13 c	30.41 ± 0.26 a
Pot experiment	DW	7.86 ± 0.1 B	198.36 ± 15.16 B	30.41 ± 3.56 C	59.07 ± 2.22 C	24.86 ± 0.29 A	33.15 ± 0.22 B
	DWF	8.02 ± 0.09 A	287.34 ± 21.32 A	52.74 ± 1.56 A	81.32 ± 3.56 A	25.3 ± 0.26 A	32.10 ± 0.11 C
	RW	7.76 ± 0.12 B	119.26 ± 8.68 D	25.09 ± 1.23 D	50.4 ± 1.79 D	23.74 ± 0.33 B	42.76 ± 0.56 A
	RWF	7.72 ± 0.07 B	148.92 ± 10.13 C	41.21 ± 2.12 B	66.71 ± 2.12 B	23.65 ± 0.11 B	42.43 ± 0.63 A

DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer. Different lowercase letters in the same column indicate a significant difference in the field experiment at $p < 0.05$ using ANOVA with a Duncan's test. Different capital letters in the same column indicate a significant difference in the pot experiment at $p < 0.05$ using ANOVA with a Duncan's test. The soil temperature and soil moisture present the average soil temperature and moisture during the rainy season (June–October), which were detected every 7 days.

3.2. Soil Enzyme Activities

The activities of catalase, phosphatase, urease, and sucrase in the field were in the range of 7.8–12.4 mg·g^{-1}·24 h^{-1}, 0.09–0.29 mg·g^{-1}·24 h^{-1}, 0.1–0.19 mg·g^{-1}·24 h^{-1}, and 8.23–18.26 mg·g^{-1}·24 h^{-1}, respectively, and in the pot experiment were in 16.63–17.72 mg·g^{-1}·24 h^{-1}, 0.11–0.43 mg·g^{-1}·24 h^{-1}, 0.85–1.61 mg·g^{-1}·24 h^{-1}, and 8.05–25.11 mg·g^{-1}·24 h^{-1}, respectively. Except for the phosphatase activities in DW, the enzyme activities in the pot experiment were higher than those in the field experiment. The four detected enzyme activities in the pot experiment were significantly affected by rain shelter, fertilizer, and their interaction ($p < 0.05$; SI: Table S1). However, in the field, only catalase was significantly affected by rain shelter, fertilizer, and their interaction ($p < 0.05$; SI: Table S1). The rest of the enzymes detected were mainly affected by rain shelter or fertilizer ($p < 0.05$; SI: Table S1). At the same fertilizer level, the four detected enzyme activities in rain shelters (DW or DWF) were higher than those in corresponding non rain shelter treatments (RW or RWF) (Figure 2). In particular, the catalase activities in DWF and DW in the field experiment were 15.38–34.78% higher than those in RWF or RW in the field experiment, sucrase was 35.36–50.43% higher, phosphatase was 87.58–100% higher, and urease was 45.03–50% higher. In the pot experiment, the catalase activities in DWF and DW in the field experiment were 3.99–5.91% higher than those in RWF or RW in the field experiment, sucrase was 84.43–86.22% higher, phosphatase was 73.56–204% higher, and urease was 15.19–38.67% higher.

3.3. Root Exudates

Seven phenolic acids, including p-hydroxybenzoic acid, vanillin acid, syringic acid, para-coumaric acid, ferulic acid, 3-indoleacetic acid, and phthalic acid, were detected and saponins were not detected in *P. notoginseng* root soil in the pot experiment (Figure 3). The concentrations of p-hydroxybenzoic acid were in the range of 2.22–3.03 μg·g−1, vanillin acid concentrations were 4.13–5.22 μg·g^{-1}, syringic acid concentrations were 2.45–3.38 μg·g^{-1}, para-coumaric acid concentrations were 5.48–9.69 μg·g^{-1}, ferulic acid concentrations were 2.03–2.61 μg·g^{-1}, 3-indoleacetic acid concentrations were 1.55–2.39 μg·g^{-1}, and phthalic acid concentrations were 2.39–2.58 μg·g^{-1}. The concentrations of para-coumaric acid and vanillin acid in root soil were significantly higher than the other detected phenolic acids, suggesting that para-coumaric acid and vanillin acid were the main phenolic acids secreted by the roots of *P. notoginseng*. Of these phenolic acids (Table S2), p-hydroxybenzoic acid, vanillin acid, syringic acid, and para-coumaric acid were significantly affected by rain shelter, fertilizer, and their interaction ($p < 0.05$). Ferulic acid and phthalic acid were mainly significantly affected by rain shelter or fertilizer ($p < 0.05$),

and not significantly affected by their interaction ($p > 0.05$). 3-indoleacetic acid was only significantly affected by fertilizer ($p < 0.05$).

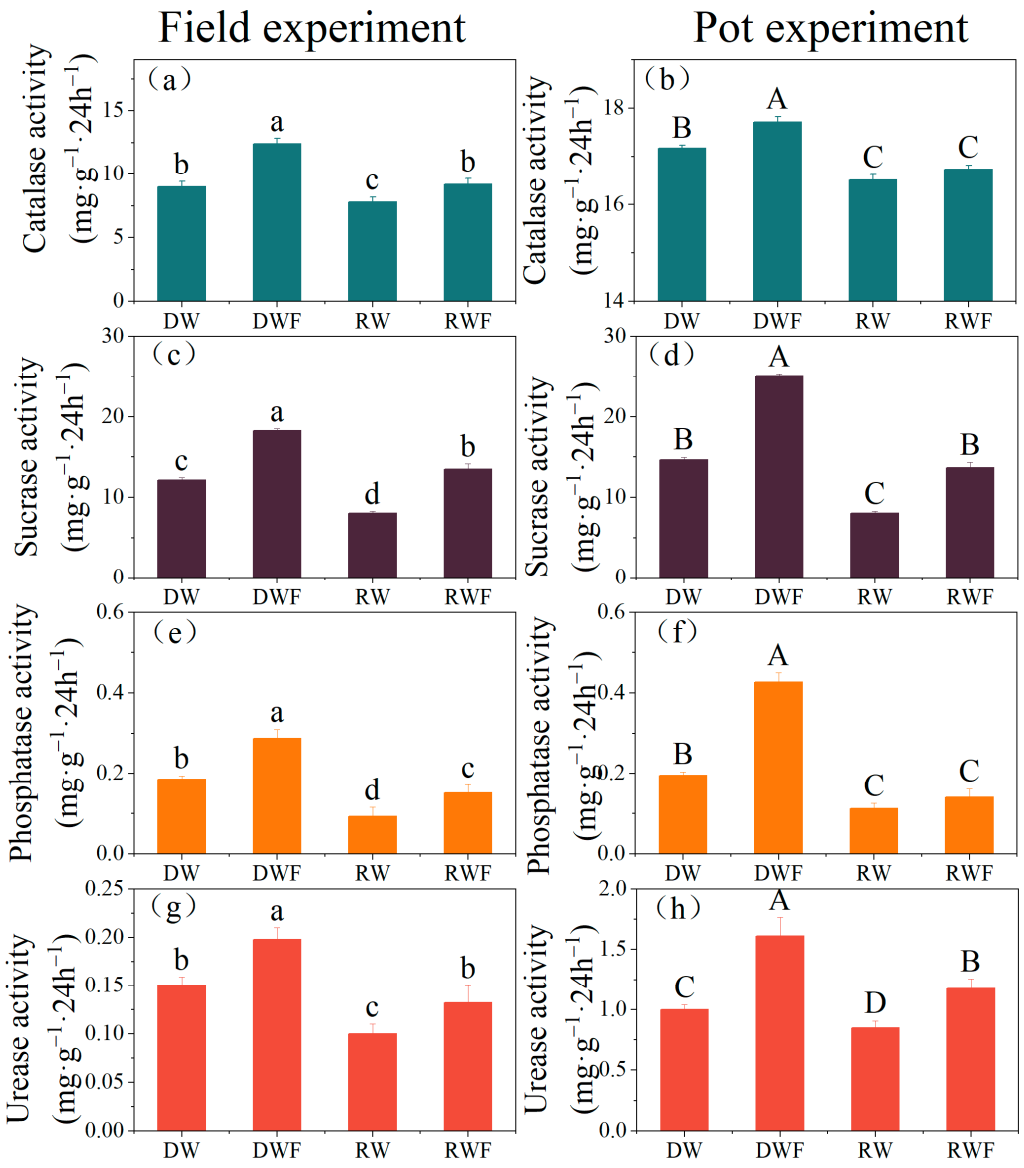

Figure 2. Effects of (no) rain shelter and fertilizer on soil enzyme activity: catalase (**a**,**b**), sucrase (**c**,**d**), phosphatase (**e**,**f**), and urease (**g**,**h**). DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer. Different lowercase letters in the same enzyme activity indicate a significant difference in the field experiment at $p < 0.05$ using ANOVA with a Duncan's test. Different capital letters in the same enzyme activity indicate a significant difference in the pot experiment at $p < 0.05$ using ANOVA with a Duncan's test.

The concentration of seven phenolic acids in the root soil from field and pot experiments significantly increased in each treatment, compared with that in the initial soil before the experiments (p-hydroxybenzoic acid increased by 21.01–64.49%, vanillin acid by 17.15–47.79%, syringic acid by 12.60–53.18%, para-coumaric acid by 40.69–148.45%, ferulic acid by 3.83–32.82%, 3-indoleacetic acid by 49.87–130.34%, and phthalic acid by 9.82–20.51%). Of the treatments, the RWF treatment resulted in the highest concentration of ferulic acid, syringic, para-coumaric acid, vanillin acid, and p-hydroxybenzoic acid. The ferulic acid, para-coumaric acid, and 3-indoleacetic acid concentrations in DWF and RWF were significantly higher than those in DW or RW. This suggested the fertilizer promotes the secretion of these three phenolic acids from the roots of *P. notoginseng*. Interestingly,

in rain shelter treatments, the concentrations of syringic acid and vanillin acid in the DW treatment was higher than in DWF; however, in non-rain shelter, those in RWF were higher than RW. This suggested that rainfall causes differences in the response of plant roots to excrete syringic acid and vanillin acid.

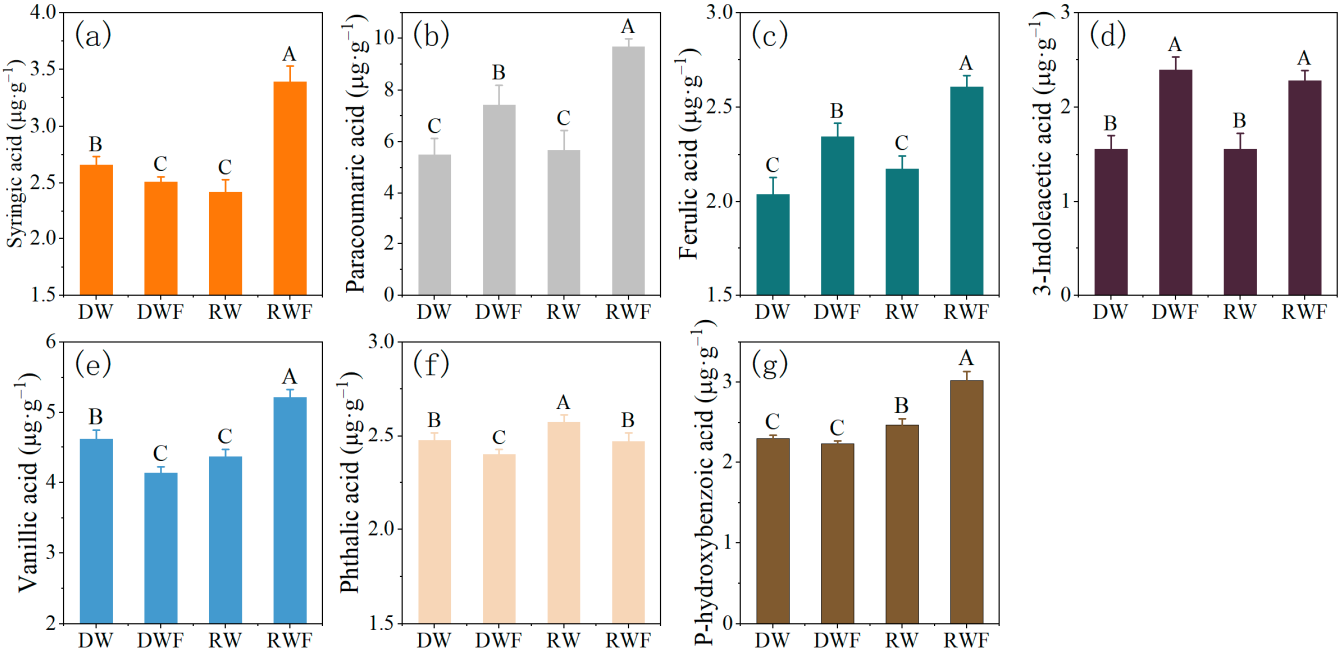

Figure 3. Effects of (no) rain shelter and fertilizer on syringic acid (**a**), para-coumaric acid (**b**), ferulic acid (**c**), 3-indoleacetic acid (**d**), vanillin acid (**e**), phthalic acid (**f**), and p-hydroxybenzoic acid (**g**) secreted from roots of *P. notoginseng*. DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer. Different capital letters in the same phenolic acid indicate a significant difference in the pot experiment at $p < 0.05$ using ANOVA with a Duncan's test.

3.4. Root Rot Incidence of P. notoginseng

The incidence of *P. notoginseng* root rot was in the range of 11.52–22.12% in the field experiment and 6.81–15.35% in the pot experiment (Figure 4). The incidence of root rot in both field and pot experiments was mainly affected by rain shelter ($p < 0.01$, SI: Table S3). Fertilizer also significantly affected the incidence of root rot ($p < 0.05$), while the interaction of fertilizer and rain shelter did not exert a significant effect on the incidence. Both the field and pot experiments showed that RW treatment (22.12% in the field and 15.35% in the pot experiment) resulted in the highest incidence of root rot, followed by RWF (21.15% in the field and 14.78% in the pot experiment). The incidence of root rot in these two treatments was significantly higher than that in DW and DWF, at 83.59~92.01% higher in the field and 87.88~117.03% higher in the pot experiment. Additionally, the incidence of root rot in DWF treatment was 13.06–24.36% lower than that in DW treatment.

3.5. Bacterial Community in Rhizosphere Soil of P. notoginseng

A total of 2714 OTUs of bacteria were determined in DW, 2577 OTUs in DWF, 2605 OTUs in RW, and 2145 in RWF; among which, the detected OTUs in DW, DWF, and RW were higher than the OTUs before *P. notoginseng* was transplanted (2510 OTUs in control). The bacteria were mainly distributed in *Actinobacteriota* (18.70–44.98%), *Proteobacteria* (15.91–35.62%), *Chloroflexi* (8.98–22.98%), *Acidobacteriota* (3.81–29.39%), and *Bacteroidota* (2.37–4.65%). The Shannon indexes of DW (6.50), DWF (6.35), and RWF (6.23) were significantly higher than that of the control (6.09), while the Shannon indexes of RW (5.96) were lower than that of the control. The Chao 1 indexes of DW (3459) and DWF (3284) were also significantly higher than that of the control (3036). However, the Chao 1 index of RW (3296)

was higher than that of the control and RWF (2709) was lower than the control (Table 3). These data suggested that rain shelter treatment promotes the diversity and quantity of bacteria.

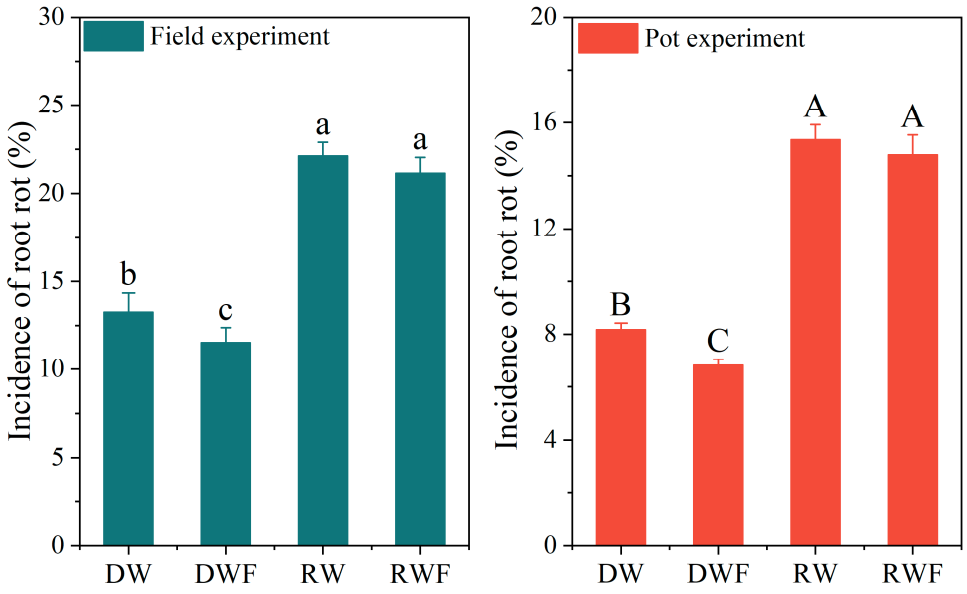

Figure 4. Effects of moisture management and fertilizer on root rot incidence of *P. notoginseng*. DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer. Different lowercase letters indicate a significant difference in root rot incidence in the field experiment at $p < 0.05$ using ANOVA with a Duncan's test. Different capital letters indicate a significant difference in root rot incidence in the pot experiment at $p < 0.05$ using ANOVA with a Duncan's test.

Table 3. Effects of moisture management and fertilizer on the diversity of the bacterial community in *P. notoginseng* soil.

Treatments	Shannon	Chao1
Control	6.09 ± 0.02 C	3036 ± 50 C
DW	6.50 ± 0.09 A	3459 ± 70 A
DWF	6.35 ± 0.03 B	3284 ± 85 B
RW	5.96 ± 0.04 D	3296 ± 77 B
RWF	6.23 ± 0.06 B	2709 ± 62 D

Different capital letters in the same column indicate significant differences in the pot experiment at $p < 0.05$ using ANOVA with a Duncan's test. DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer; control: the soil before the experiment.

A cluster analysis of bacterial phyla among the rain shelter or fertilizer and control showed the bacterial community structure under DW conditions was similar to RW, and DWF was similar to the control (Figure 5a). The bacterial community structure under RWF conditions was largely different from the control and the other treatments. Specifically, the relative abundance of *Actinobacteria* in RWF was significantly (15.11–19.80%) lower than that of other treatments. The relative abundance of *Acidobacteriota*, however, was 19.1–25.58% higher than that of other treatments. Additionally, RWF conditions also resulted in a significant increase in the relative abundance of *Planctomycetota* and *Verrucomicrobiota*, both of which exceeded 1%. The heat map further revealed the classification of each treatment (Figure 5b). RWF and control were located on both sides. RWF conditions significantly promoted the relative abundance of *Firmicutes*, *Bacteroidota*, *Planctomycetota*, *Verrucomicrobiota*, and *Acidobacteriota* and suppressed the relative abundance of *Actinobacteriota* and *Patescibacteria*. DWF conditions promoted the relative abundance of *Nitrospirota*, *Proteobacteria*, *Cyanobacteria*, *Methylomirabilota*, and *Bacteroidota*.

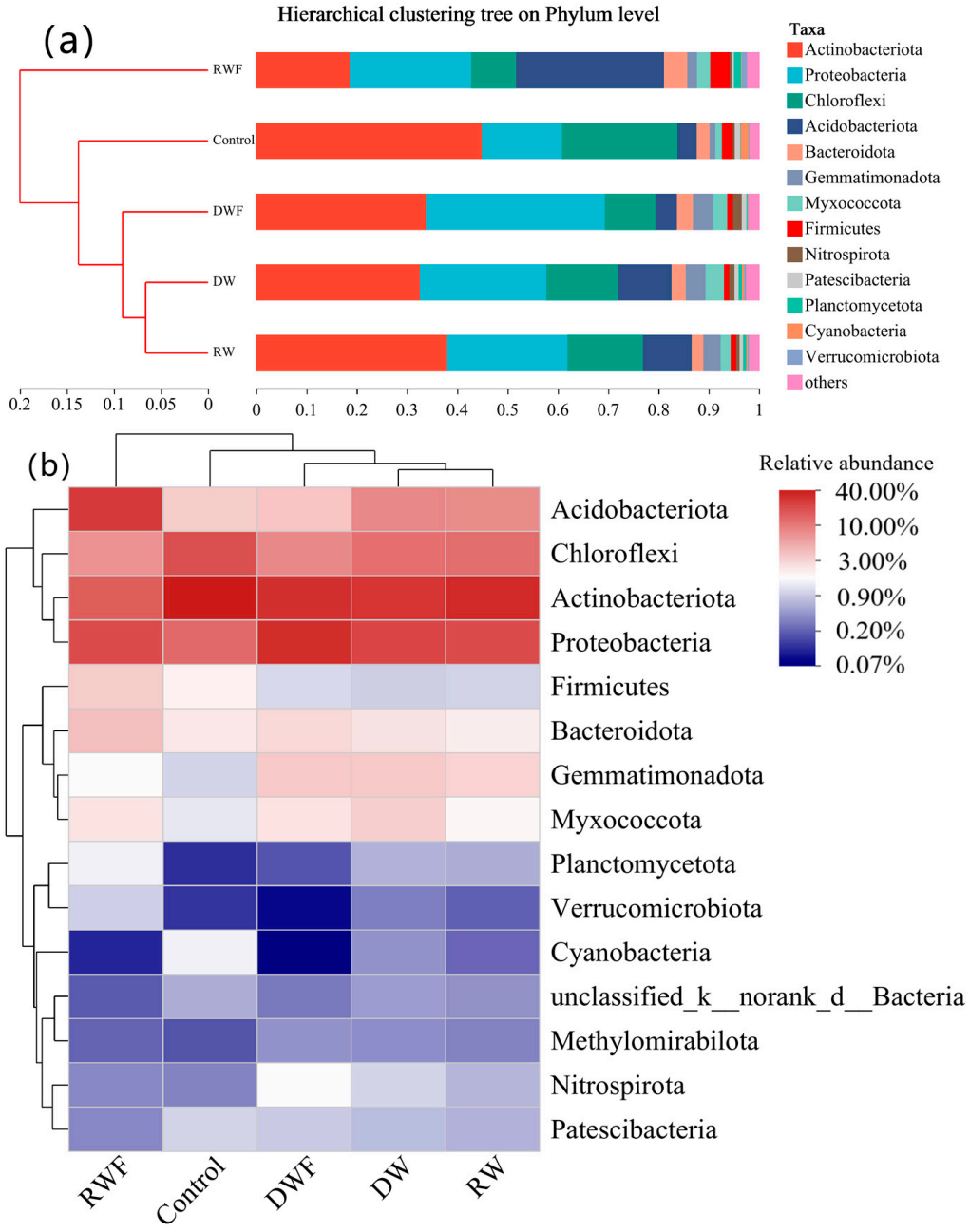

Figure 5. Effects of rain shelter fertilization on the microbial community of *P. notoginseng*: (**a**) sample level cluster analysis + portal accumulation of dominant bacteria in phylum (relative abundance > 1%, clustering algorithm is Weighted_UniFrac); (**b**) heatmap of rhizosphere soil bacteria drawn using the R vegan package. Legend represents relative richness. DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer; control: the soil before the experiment.

Additionally, an OTU abundance co-occurrence network analysis (Figure 6) showed that the root exudates in the soil significantly affected the network connectivity (L: 125 in RW, 129 in DW, 187 in RWF, and 174 in DWF), while the network size did not change much (node number). The ratio of positive correlation to negative correlation in RW (5.58) and RWF (6.79) was higher than that in DW (1.11) and DWF (1.56).

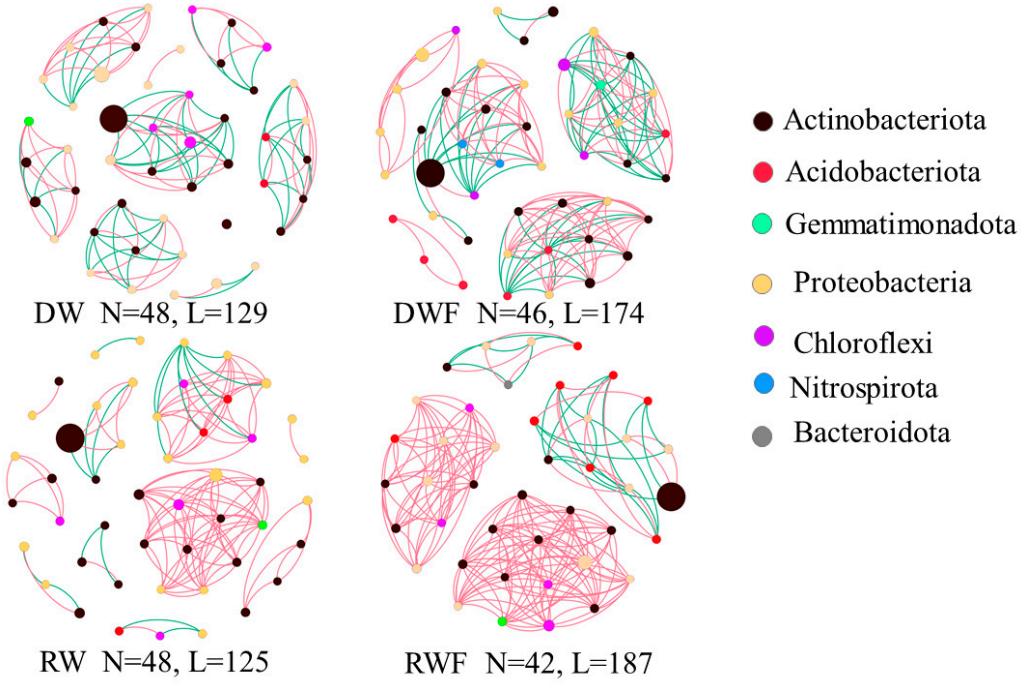

Figure 6. OTU abundance co-occurrence network analysis (top 50 relative abundance of our data). The circle size represents the relative abundance size. The color represents the nodes at the level of the gate. N, node. L, side. The edges represent significant Spearman correlations ($\varrho > |0.8|$, $p < 0.01$). Light blue lines represent negative correlations and light red lines represent positive correlations.

3.6. Correlation Studies

The relative abundance of bacteria and phenolic acid were used for a redundancy analysis (RDA). The RDA1 and RDA2 could explain 67.19% of differences in the bacteria structure among all the samples. Seven detected phenolic acids were negatively correlated with the relative abundance of *Patescibacteria*, *Cyanobacteria*, *Actinobacteria*, and *Chloroflexi* (Figure 7a). Of those phenolic acids, phthalic acid and 3-indoleacetic acid were positively correlated with *Gemmatimonadetes* and vanillin acid, ferulic acid, syringic acid, p-hydroxybenzoic acid, and para-coumaric acid were positively correlated with *Firmicutes*, *Verrucomicrobiota*, *Bacteroidota*, *Acidobacteriota*, *Myxococcota*, *Planctomycetota*, and the other bacteria. Additionally, para-coumaric acid was also negatively correlated with *Proteobacteria* and *Nitrospirota*.

The bacterial community structure was also affected by the physiochemical properties of the soil. As shown in Figure 7b, the RDA1 and RDA2 could explain 88.97% of differences in the bacteria structure among all the samples affected by the detected soil enzyme activities and soil physiochemical properties. *Chloroflexi*, *Actinobacteria*, *Firmicutes*, and *Patescibacteria* were positively correlated with urease activity, available phosphorus, soil pH, available potassium, soil temperature, and alkali-hydrolyzed nitrogen and negatively correlated with soil moisture. Interestingly, the remaining bacteria were negatively correlated with urease activity, available phosphorus, soil pH, available potassium, soil temperature, and alkali-hydrolyzed N and positively correlated with soil moisture.

The root rot incidence and phenolic acid content were used for a redundancy analysis (Figure 8a). RDA1 and RDA2 could explain the structural differences in 89.00% of phenolic acids in all samples. Seven detected phenolic acids were negatively correlated with the incidence rate of root rot. Among these phenolic acids, the effects of ferric acid, symmetric acid, and p-hydroxybenzoic acid on root rot disease were more significant.

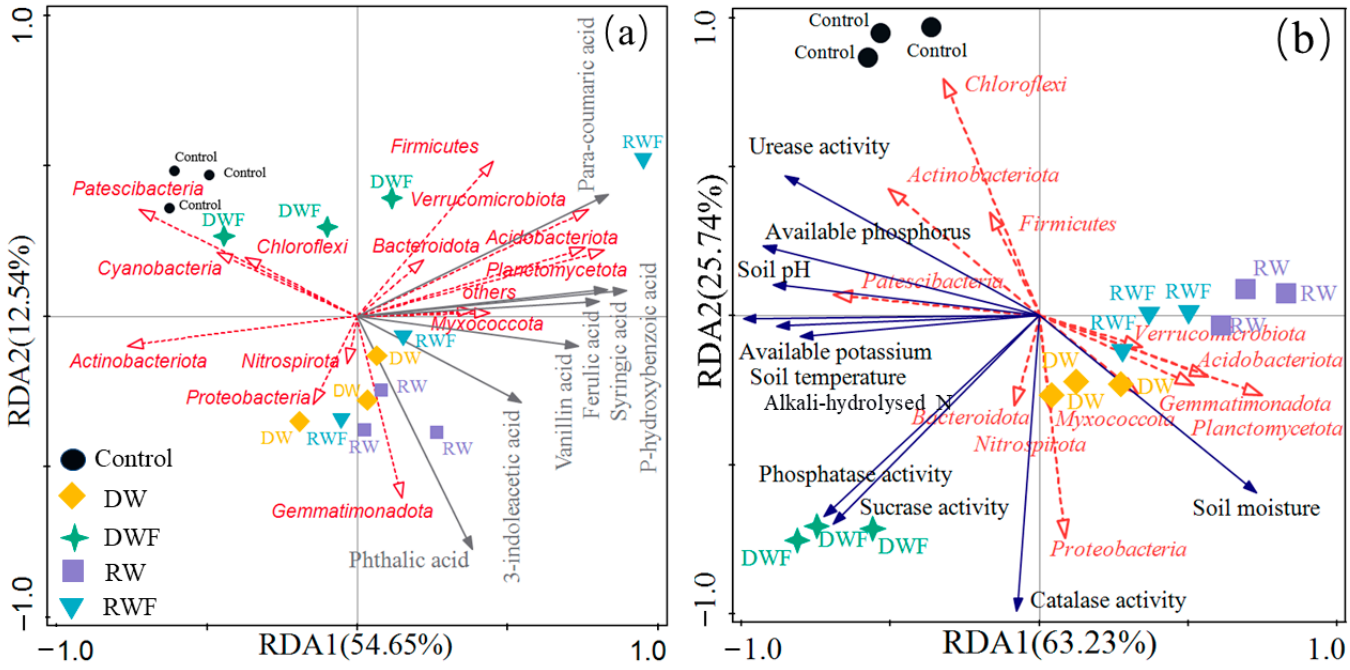

Figure 7. Relationship between organic acids secreted by roots and the rhizosphere microbial community (**a**). Relationship between the rhizosphere soil environment and the microbial community (**b**). The red arrows in (**a**,**b**) represent the phylum of bacteria; the light gray arrows in Figure 6a represent the secretion of organic acids by the root system; the black arrows in (**b**) represent the detected environmental factors of the soil (including nutrients, enzyme activity, soil temperature, and moisture). DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer; control: the soil before the experiment.

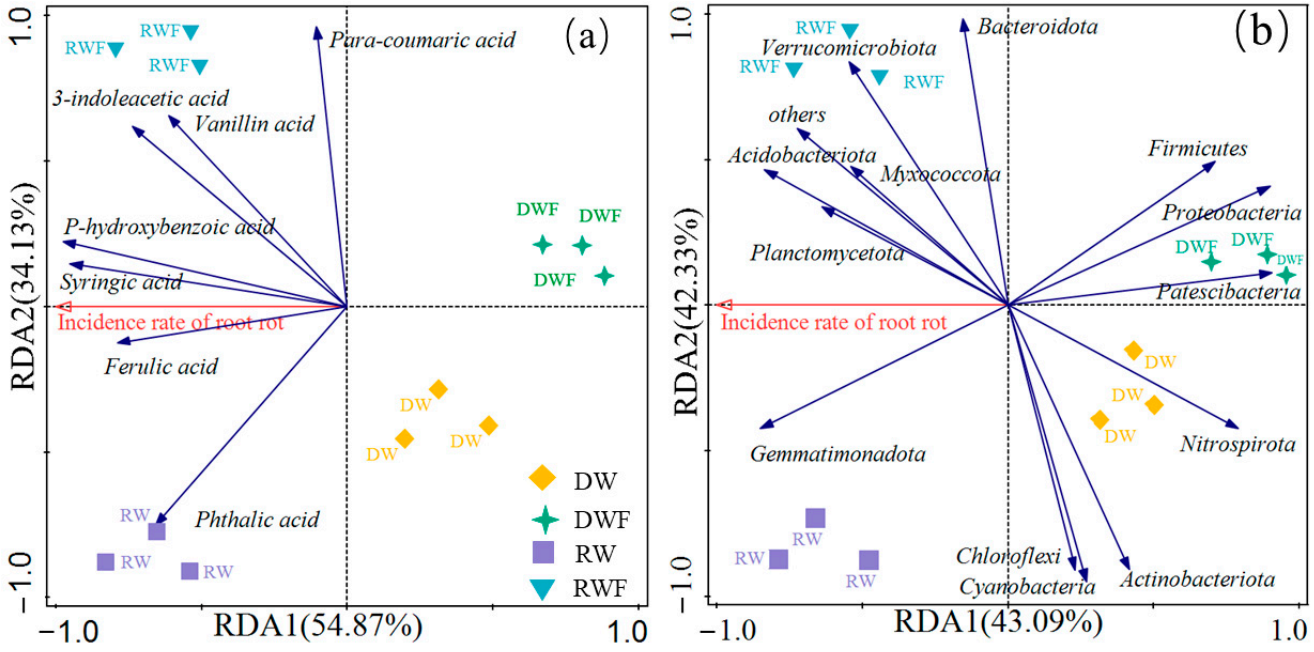

Figure 8. Relationship between organic acids secreted by roots and incidence of root rot (**a**). Relationship between the rhizosphere microbial community and root rot incidence (**b**). The red arrows in (**a**,**b**) represent the incidence rate of root rot; the blue arrows in (**a**) represent the secretion of organic acids by the root system; the black arrows in (**b**) represent the phylum of bacteria. DW: rain shelter and no fertilizer; RW: no rain shelter and no fertilizer; DWF: rain shelter and fertilizer; RWF: no rain shelter and fertilizer.

The bacteria and the root rot incidence were used for a redundancy analysis (Figure 8b). The RDA1 and RDA2 explained 85.42% of the differences in the root rot incidence among all the samples. The root rot incidence of *P. notoginseng* was correlated with *Planctomycetota, Acidobacteriota, Gemmatimonadota, Acidobacteriota, Myxococcota,* and *Verrucomicrobiota* and negatively correlated with *Firmicutes, Proteobacteria, Patescibacteria, Nitrospirota, Actinobacteria, Chloroflexi,* and *Cyanobacteria.* Of these bacteria, *Planctomy-cetota, Acidobacteriota, Gemmatimonadota, Firmicutes, Proteobacteria, Patescibacteria*, and *Nitrospirota* had more significant effects on root rot.

4. Discussion

4.1. Effects of Root Exudates and Soil Physical and Chemical Properties on the Microbial Community of P. notoginseng

Root exudates have been identified as a major driver of rhizosphere soil microbial community dynamics or assembly and functional capacity in multiple existing lines of evidence [24–26]. Some bioactive molecules specifically attract or inhibit microorganisms in soil communities, forming host-specific rhizosphere microbial communities [7,8,25]. Those biologically active molecules will also serve as signaling molecules for some specific microorganisms and play a key role in plant–microbe interactions [12]. Long-chain organic acids are the main bioactive components of *P. notoginseng* plants, which can be secreted into the rhizosphere soil through the roots to exert autotoxicity or promote the growth of pathogens [8]. Studies have shown that metabolite conditioning weakens the function of autotoxin degradation undertaken by *Bradyrhizobium*, *Variovorax*, *Pseudomonas*, and *Sphingomonas* in the *Proteobacteria* phylum, and *Streptomyces* in the *Acticnobacteriota* phylum, while promoting the metabolism of small-molecule acids initiated from *Anaeromyxobacter* (which belong to *Myxococcota*), *Flavobacterium* (which belong *Bacteroidota*), and *Gemmatimonas* (which belong *Gemmatimonadota*) [8,27]. This was supported by our data. An RDA showed that vanillin acid, ferric acid, systolic acid, p-hydroxybenzoic acid, and para-coumaric acid promote the growth of pathogenic bacteria such as *Myxococcota* and *Gemmatimonadota* (Figure 7a).

The bacterial community structure was also affected by the physiochemical properties of the soil. Soil moisture is an important driving factor for microbial activity [28]. The excessive soil moisture will fill the soil pores, affecting the soil redox environment and causing changes in the soil microbial community. Studies have shown that a high soil moisture promotes the relative abundance of *Planctomytota*, *Myxococcota*, *Verrumicrobiota*, and *Acidobacteria* and restrains the relative abundance of *Actinobacteriota*, *Firmicutes*, *Patescibactera*, and *Chloroflexi* [29,30]. Interestingly, the urease activity was negatively correlated with *Planctomytota*, *Myxococcota*, *Verrumicrobiota*, and *Acidobacteria* and positively with *Actinobacteriota*, *Firmicutes*, *Patescibactera*, and *Chloroflexi*. Studies have pointed out that soil enzymes are mainly secreted by soil microorganisms, animals, plants, and their residues, of which bacteria were one of the main sources of soil enzymes [31]. This might be the reason that a high soil moisture leads to changes in the soil microbial community and the concentration of soil alkali-hydrolyzed N, limiting the soil urease activity. Studies have shown that the increases in the activities of catalase activity, phosphatase activity, and sucrase activity reduce the relative abundance of *Acidobacteria and Actinobacteria* [14] and increase the relative abundance of proteobacteria [32]. However, our data showed that only catalase activity was negatively correlated with *Actinobacteria* and positively correlated with *Proteobacteria.* The phosphatase activity and sucrase activity were not significantly correlated with soil bacterial phylum. Soil enzymes were central to soil nutrient cycling, which was caused by the synergistic effect of multiple factors. This might be because the sucrase is related to carbon cycling, promoting the growth of fungi [33], and phosphatase activity is affected by various environmental factors [34]. The soil pH was significantly negatively correlated with *Acidobacteriota*, which might be caused by the phenolic acid secreted from the root of *P. notoginseng*, decreasing the soil pH to promote the growth of *Acidobacteriota* [30,35].

4.2. Effects of Bacterial Communities on Root Rot in P. notoginseng

The diversity of the microbial community is key to maintaining soil and plant health and quality [36]. The abundance of microorganisms in soils with a high prevalence of *P. notoginseng* root rot is much lower than that in healthy soils [3]. In this study, the Shannon indexes of bacteria in the non rain shelter conditions (RW and RWF) were lower than those in the corresponding rain shelter conditions (DW and DWF, Table 3). This led to significantly lower root rot incidences in *P. notoginseng* in DW- and DWF-treated fields or pots than those in RW and RWF conditions (Figure 4). However, the soil microbial diversity in the fertilization treatment (DWF) was slightly lower than that in the non-fertilization treatment (DW) under the rain shelter. This may be because the microbial community in the soil is more consistent under a long-term fertilization environment, resulting in decreases in diversity and abundance [37].

Additionally, the structure of the microbial community was also one of the important factors that determined the health of the plants. Studies have shown that the imbalance in the soil microbial community structure caused by long-term cultivation was an important reason for the high incidence of soil-borne diseases of *P. notoginseng* [38]. Our data showed that fertilization and the rain shelter had significant effects on the bacterial structure (Figure 5), which resulted in different root rot incidences. Of those bacteria, *Planctomycetota*, *Acidobacteriota*, *Gemmatimonadota*, *Firmicutes*, *Proteobacteria*, *Patescibacteria*, and *Nitrospirota* had more significant effects on root rot (Figure 8b). A study has shown that soil contains a large number of *Proteobacteria* in healthy plant roots and a large number of *Bacteroidetes* and *Verrucomicrobia* in root rot plants [39]. *Proteobacteria* and *Firmicutes* can produce high levels of anti-pathogen secondary metabolites, such as chitinase and protease, to induce plant defenses against plant fungal diseases [40–42]. The high relative abundance of *Proteobacteria* and *Firmicutes* leads to a decrease in root rot [43]. Additionally, *Nitrospirota* has been associated with the suppression of various soil-borne diseases such as *Fusarium oxysporum* in strawberries, bananas, and Panax ginseng, as well as bacterial wilt in Panax ginseng [44,45]. On the contrary, the increase in the relative abundance of *Bacteroidetes* may cause a decrease in soil environmental resistance and increase the incidence of plant diseases [46].

4.3. Rain Shelter with Irrigation and Fertilization Could Effectively Reduce the Secretion of Organic Acids of P. notoginseng and Reduce the Incidence of Root Rot

The soil moisture was an important factor affecting soil microbial communities and physicochemical properties. As the soil moisture increases, soil pores become filled with water and anaerobic, leading to a decrease in the activities of soil enzymes and microbiome diversity [30]. This was supported by our data. Frequent rainfall during the rainy season led to a significantly higher soil moisture and lower soil temperature in RW and RWF compared to DW or DWF (Table 2). This further led to significantly lower activities of catalase, phosphatase, urease, and sucrase and a lower microbial community diversity index in RW compared to DW and in RWF compared to DWF (Figure 2 and Table 3). The decline in soil enzyme activity hinders the absorption of nutrients by *P. notoginseng*, which intensifyies the accumulation of autotoxic substances in the soil and induce root rot in *P. notoginseng* [47]. Additionally, studies have shown that a high soil moisture promotes the growth of *Planctomytota*, *Myxococcota*, and *Verrumicrobiota* [30], which were positively correlated with root rot incidence of *P. notoginseng* (Figure 8b).

Studies have pointed out that the pathogenesis of *P. notoginseng* root rot caused allelochemical substances in the root exudates, leading to imbalance in the microbial community [48], among which the decrease in fungal-to-bacterial ratios was an important indicator [49]. The concentrations of ferulic acid, syringic acid, and p-hydroxybenzoic acid, which were more significant contributing factors to root rot (Figure 8a), in RWF were significantly higher than in DWF. Studies have shown that plants' roots might increase their inputs to the ground to alleviate soil nutrient availability or environmental stress [50]. Our data showed that the available N, P, and K in non-rain shelter treatments (RW and

RWF) was lower than that in corresponding rain shelter treatments (DW or DWF). This might be one of the reasons why the phenolic acid in no rain-shelter treatments was higher than in rain shelter treatments. Moreover, it can be seen from the symbiotic relationship of the microbial network (Figure 6) that local aggregation occurred between RWF- and RW-treated microorganisms, while there were more associations between DW- and DWF-treated microorganisms. This suggested the rain shelter treatments (DW and DWF) were conducive to the stability of the bacterial community, while the non rain shelter conditions were not conducive to the stable development of the community [51]. Therefore, reducing the root rot of *P. notoginseng* could be achieved through a rain shelter with irrigation.

5. Conclusions

Our results from field and pot experiments showed that DW and DWF treatments significantly reduced the soil moisture and root rot incidence, while significantly increasing the soil temperature and enzyme activities compared to RW or RWF conditions. Additionally, RWF led to significantly higher p-hydroxybenzoic acid, syringic acid, para-coumaric acid, ferulic acid, 3-indoleacetic acid, and vanillic acid concentrations compared to the other treatments. The root rot incidence was positively correlated with all seven phenolic acids secreted by *P. notoginseng* roots, as well as *Planctomycetota*, *Acidobacteriota*, and *Gemmatimonadota*, and negatively correlated with *Firmicutes*, *Proteobacteria*, *Patescibacteria*, and *Nitrospirota*. Considering the lowest soil moisture, the lowest incidence of root rot, and the highest soil nutrients, as well as an appropriate soil phenolic acid concentration and bacterial community structure, DWF conditions were recommended for *P. notoginseng* planting.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051257/s1, Figure S1: The cumulative rainfall and air temperature in field experiments (2021); Table S1: Effects of rain shelters and fertilizer on soil enzyme activity; Table S2: Effects of rain shelters and fertilizer on syringic acid (a), para-coumaric acid (b), ferulic acid (c), 3-indoleacetic acid (d), vanillin acid (e), phthalic acid (f), and p-hydroxybenzoic acid (g) secreted from roots of *P. notoginseng*; Table S3: Effects of rain shelters and fertilizer on incidence of root rot.

Author Contributions: Conceptualization, Y.Z. and Z.T.; methodology, Y.Z., Z.T., J.L. and Q.Y.; formal analysis, Y.Z. and Z.T.; investigation, Y.Z.; resources, J.L.; data curation, Y.Z., Z.T. and Q.Y.; writing—original draft preparation, Y.Z.; writing—review and editing, Y.Z., Z.T., J.L. and Q.Y.; visualization, Y.Z.; supervision, Z.T.; project administration, Q.Y. and Z.T.; funding acquisition, Q.Y. and Z.T. All authors have read and agreed to the published version of the manuscript.

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