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Comparison of rhizosphere microbial community of *Panax notoginseng* under different habitat

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This study compared and analyzed the diversity differences of soil microbial communities in the rhizosphere of *Panax notoginseng* under different habitats (under forest and field) and their correlation with environmental factors. Illumina MiSeq high-throughput sequencing revealed that the diversity index of rhizosphere bacterial communities in *P. notoginseng* under forest (Ace, Chao1, Simpson, Shannon) was significantly higher than in field. In comparison to the microbial networks observed in the field (*Nodes*=186, *Edges*=3407), the microbial networks found in the underforest (*Nodes*=266, *Edges*=4549) demonstrate a more intricate and sophisticated correlation among microorganisms. The results revealed that organic carbon emerged as the primary main influencing the assembly of rhizosphere bacterial communities in *P. notoginseng* under forest, accounting for 84.23% of the observed community variation. However, in fungi, the pH (75.10 %) value assumes significance. Moreover, the total saponin content of *P. notoginseng* in the forest was significantly higher compared to that in the field, particularly with regards to saponin R1, which exhibited a significant association with the microbial community present under forest conditions.

Panax notoginseng (Burk.) F. H. Chen (PN) is a perennial herbaceous medicinal plant of the family *Araliaceae* in the order *Apiales*¹. PN has an important influence in the traditional Chinese medicine industry in China. It is a unique and precious Chinese medicinal herb, and mainly growing in some shady and cool areas of Yunnan and Guangxi provinces in China. Its artificial cultivation history has been more than 400 years¹. Mature PN rhizomes contain abundant active substances such as saponins (Rg1, Re, and Rb1), polysaccharides, and flavonoids, etc. which possess diverse effects including anti-inflammatory¹, hypoglycemic², antibacterial³, hepatoprotective⁴, antioxidant⁵, blood-activating and stasis-resolving abilities⁶, as well as swelling-reducing and pain-relieving characteristics⁶. It is commonly employed for treating coronary heart disease and cardiovascular diseases⁷⁻⁸. Moreover, it can also be used as a health supplement to lower blood lipids, reduce blood pressure, improve sleep quality, enhance immune function, and prevent cardiovascular diseases⁷⁻⁸. Therefore, PN has great research value and application prospects.

With the development of modern traditional Chinese medicine, the market demand for PN is constantly increasing, and the cultivation areas is also expansion. However, due to its unique growth environment and single variety, issues such as soil nutrient imbalance, disruption of soil microbial communities, and continuous cropping obstacles (such as root rot disease and epidemic diseases) arise during cultivation. These factors seriously affects the yield and quality of PN, thereby impeding

its sustainable development in the industry. Consequently, exploring novel cultivation models has become the focus of current research on PN cultivation. In recent years, under forest planting techniques have made significant progress in simulating wild habitats for medicinal herbs like *P. ginseng*, *P. notoginseng*, *Polygonatum flavum*, *poria cocos*, *bupleurum*, *gastrodia elata* etc. Planting traditional Chinese herbs in under forest has become a crucial component of the understory economic industry, it play a pivotal role in promoting their development⁹. Under forest planting can not only make full use of forest land, and reduce contradiction of competing for land between traditional Chinese herbs planting and field crop, but also "return medicine to the forest" through semi-wild cultivation methods, and further improve the quality of traditional Chinese herbs⁹⁻¹⁰. Moreover, under forest medicinal plant cultivation is an integral part of ecological agriculture for traditional Chinese herbs, and research has shown that natural forest ecosystems have high microbial community diversity. By applying biodiversity principles such as "mutual promotion and restraint" within under forest planting for PN, which is beneficial for ecological prevention and control of diseases, thereby reducing pesticide use and achieving ecological planting of PN¹¹.

Plant rhizosphere soil microorganisms are an important component of plant rhizosphere microecology, it playing a crucial role in plant growth and development, nutrient absorption, enhancing pathogen defense capabilities, and improving stress resistance. They not only participate in nutrient decomposition and transportation (such as nitrogen fixation in the atmosphere), Meanwhile, they also maintain soil microbial ecological balance¹². Various physical and chemical properties in soil, including mineral-organic colloid content, soil moisture, temperature, soil structure, nutritional composition, pH etc., these deeply affect the types, diversity, and metabolic functions of soil microbial populations¹³⁻¹⁴. Dombrowski¹⁵ studied the composition of rhizosphere microbial communities in perennial yellow flowered mustard under natural and controlled conditions, and found that soil properties have a more significant impact on rhizosphere microbial communities. Different soil types and nutrient components are among the important factors affecting the structure of rhizosphere microbial communities. Tan¹⁶ studied the correlation between root-soil microbial diversity and environmental factors in PN, it was demonstrated that the root-soil microorganisms of healthy PN are mainly *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, and *Acidobacteria* in the continuous cropping field. Meanwhile, the effective P, pH, and organic matter in the soil have the greatest impact on the composition of the rhizosphere bacterial community of PN. Research by Zhang¹⁷ has shown that adding appropriate amount quicklime to PN soil can effectively increase soil pH, and leading to a significant enhancement in bacterial community diversity. Furthermore, this treatment resulted in an increase relative abundance of *Ascomycota* and *Proteobacteria* at the phylum level, as well as *Massilia* and *Sphingomonas* at the genus level. Zhao¹⁸ found that the soil moisture content in PN has a significant impact on the abundance of microbial communities. The relative abundance of the key pathogenic bacterial group *Fusarium* is the highest at soil moisture levels of 10% and 20%. However, when the moisture levels increased to 30%, there was a notable decrease in the relative abundance of *Fusarium*, while the beneficial fungal genus *Trichoderma* exhibited a substantial increase. Zhao¹⁹ added an appropriate quantity rice husk biochar to PN soil and found that the content of NO₃⁻-N and available potassium significantly increased, while NH₄⁺-N content notably decreased. Additionally, it was found that the abundance of beneficial bacterium *Mortierella* notably increased in fungi, while the abundance of pathogenic bacterium *Fusarium oxysporum* significantly decreased. It can be seen that changing the soil environmental factors of PN will have a profound impact on relative abundance of microbial diversity, pathogenic bacteria and

87 probiotics in the soil.

88 Currently, a plethora of studies have extensively investigated the diversity of rhizosphere
89 microbial communities in PN, primarily focusing on field planting patterns; however, there is a
90 paucity of research regarding under forest planting patterns. In this study, we used Illumina-MiSeq
91 high-throughput sequencing technology to analyze the 16S/ITS rRNA of rhizosphere soil
92 microorganisms (bacteria and fungi) in three-year-old healthy PN in both under forest and field
93 planting modes. Our objective was to elucidate the influence of soil environmental factors on
94 rhizosphere microbial community diversity in PN and identify the key factors that affect them under
95 different planting modes. These studies provide valuable insights into the correlation between
96 rhizosphere microorganisms and soil environmental factors in PN forests, as well as contribute to the
97 sustainable development of PN.

98

99 **Materials and Methods**

100 **Materials**

101 3-year-old healthy soil samples of PN were collected come from four different regions of Wenshan
102 Prefecture from September to October 2023 (Table 1), include under forest and field (Figure 1). 15
103 plants were collected from each planting area, every 5 plants is 1 replicate, each sample has 3
104 replicates. Extract the entire plant and dislodge the adhered soil through agitation, and soil adhered to
105 the root surface were removed by using a sterile brush and place it in a 50 mL sterile centrifuge tube,
106 Store it in -80°C refrigerator by liquid nitrogen for future use, The first portion was allocated for
107 microbiological detection, the second portion was designated for soil nutrient analysis, finally PN
108 plants were utilized to determine saponin content.

109

110

label	collection sites	soil type	altitude (m)	longitude	latitude	Planting land type
WA	Pingba Town, Wenshan County	Brick red loam soil	1260 ~ 1300	104°24'~ 104°278'	23°22'~23°36'	Pine forest
WB	Weimo Town, Yanshan County	Brick red loam soil	1540 ~ 1709	103°35'~104°35'	23°19'~23°59'	Pine forest
WC	Lianhuatang Town, Xichou County	Brick red loam soil	1102 ~ 1575	104°22'~104°58'	23°06'~23°37'	field
WD	Jiahanjing Town, Maguan County	Brick red loam soil	1553 ~ 1700	103°52'~104°39'	22°42'~23°15'	field

111

Table 1. Information of collect sites.

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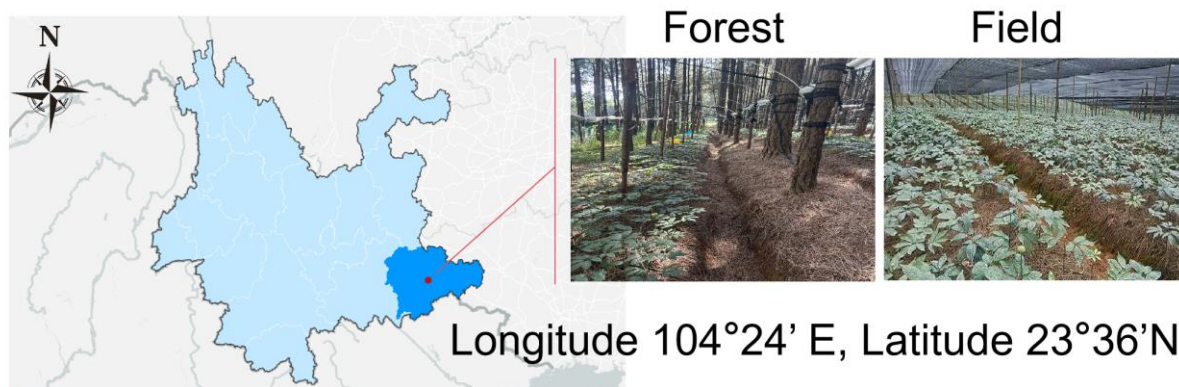


Figure 1. Map of the *Panax notoginseng* (PN) soil sampling site in Wenshan State.

Reagents and instruments

Reagents: Acetonitrile and methanol were purchased from Merck Co., Ltd. The standard substance of total saponins for *P. notoginseng* was purchased from Academy of Food and Drug Identification in China (Batch Number: 110870-202105). E.Z.N.ATM Mag-Bind Soil DNA Kit (OMEGA), Qubit dsDNA HS Analysis Kit (ThermoFisher), 2×Hieff[®] Robust PCR Master Mix (Yeasten) and Hieff NGSTTM DNA Selection Beads (Yeasten) were purchased from Beijing Dongsheng Innovation Biotechnology Co., Ltd.

Instruments: High performance liquid chromatography (Agilent 1260) and ultrasonic cleaner (SB-5200DTD type) were purchased from Ningbo Xinzhi Biotechnology Co., Ltd. Electronic analytical balance (SECURA125-1CN), Desktop centrifuge Pico-21 (Thermo Fisher), Electrophoresis instrument power supply (DYY-6C), and Electrophoresis tank (DYCZ-21) were purchased from Beijing Liuyi Instrument Factory. Qubit[®] 4.0 Fluorescence meter (Q33238) and PCR instrument ETC 811 purchased from Beijing Dongsheng Innovation Biotechnology Co., Ltd.

Experimental methods

The detection of soil pH, soil available N, P, K, and soil organic carbon content follows the method of Mohsen et al.²⁰. The content of total saponins in *P. notoginseng* was determined by using HPLC method²¹. Soil samples DNA extraction reference E Z. N.ATM Mag Bind Soil DNA Kit instruction manual. Genomic DNA was accurately quantified by utilizing Qubit[®] 4.0 DNA Detection Kit and using it as a template was amplified, the primer information were shown in Supplement Table 1. First round PCR reaction system (Total volume 30 μL): 2×Hieff[®] Robust PCR Master Mix 15 μL, Bar-PCR primer F 1 μL, Primer R 1 μL, PCR products 10~20 ng, Deionized water 9-12 μL. PCR reaction conditions: 94°C for 3 min; 5× (94°C for 30 sec; 45°C for 20 sec; 65°C for 30 sec) ; 20× (94°C for 20 sec; 55°C for 20 sec; 72°C for 30 sec) ; 72°C for 5 min. Second PCR reaction system (Total volume 30 μL): Illumina bridge PCR compatible primers was introduced, 2×Hieff[®] Robust PCR Master Mix 15 μL, Primer F 1 μL, Index-PCR Primer R 1 μL, PCR products 20~30 ng, deionized water 9~12 μL. PCR reaction conditions: 95°C for 3 min; 5× (94°C for 20 sec; 55°C for 20 sec; 72°C for 30 sec); 72°C for 5 min. Library construction and high-throughput sequencing (Illumina NextSeq2000, PE300) were completed by Sheng Gong Biotechnology (Shanghai) Co., Ltd.

Data statistical and analysis

The raw data obtained from high-throughput sequencing was concatenated and filtered, low-quality sequences were removed by using Cutadapt (v1.9.1). valid sequences based on 97% sequence similarity cluster into operational taxonomic units (OTUs) by using software Usearch. Then based on bacterial taxonomy database Silva (<http://www.arb-silva.de/>) and fungal Taxonomy Database Unite (<http://unite.ut.ee/index.php>), the representative sequences of OTUs were annotated by species classification using software RDP Classifier (2.12), and the community composition of each sample was statistically analyzed by different species classification levels.

Sample diversity were analyzed, Chao1, ACE, Shannon, and Simpson indices of samples were calculated by using software Qiime (1.9.1). The diversity index Chao1 and ACE mainly focus on abundance information of species, the larger numerical value, the higher species richness. The Shannon and Simpson index comprehensively reflect richness and evenness of species, the larger numerical value, the higher species diversity. Principal component analysis (PCA) was conducted using the pca function in the R language platform Ape to examine variations in microorganism community composition among different samples, based on a distance matrix derived from Bray-Curtis distance. The Linear discriminant analysis Effect Size, LefSe 1.0 (<http://huttenhower.sph.harvard.edu/galaxy/root/index>), was used to identify statistically significant biomarkers distinguishing between different groups. In R, utilize the cca and envfit functions from the vegan package for Canonical Correspondence Analysis and Envfit test respectively. Employ Gephi for network analysis. Perform Mantel test analysis using the Mantel test function from the cultevo package in R.

Results

PN sample soil Microorganisms community sequencing data quality control and diversity analysis

In this experiment, 12 samples were collected and through high-throughput sequencing and optimization, as shown in Supplement Table 2, the effective sequence lengths for bacteria range from 64, 957 to 107, 313 bp, with base numbers range from 26, 838, 766 to 44, 532, 027 bp and average lengths range from 421.61 to 414.97 bp. For fungi samples, the effective sequence lengths range from 43, 788 to 120,758 bp, with base numbers range from 11, 622, 714 to 78, 086, 186 bp and average lengths range from 220.51 to 265.43 bp. Rarefaction curves were constructed for Operational Taxonomic Units (OTUs) based on a similarity threshold of 97 %, as shown in Supplement Figures 1, it can be observed that as the flattening depth increases, the curve tends to flatten, and further increase in sequencing depth cannot detect unknown new OTUs, this indicates that the sequencing results can basically cover all species in sample, and the sequencing data is reasonable and can be used for subsequent analysis.

As shown in Figure 2A, the maximum number of OTUs was 2307 to WA treatment in bacteria, and significantly (P -value<0.05) higher than WB treatment was 1298, WC treatment was 701, and WD treatment was 1012. For fungi (Figure 2B), the highest number of OTUs was 290 in WC treatment, followed by was 282 in WB treatment, 234 in WA treatment, and lowest was 169 in WD treatment. Comparison of the number of unique OTUs in bacterial communities, the number of unique OTUs in under forest was significantly higher than that in field (Figure 2C). But the two groups are no distinctness in fungi communities (Figure 2D). In the comparison of overall community structure at the phylum classification level, the top 5 abundance of bacterial groups from high to low were Proteobacteria (29.52% ~ 43.41%), Acidobacteria (12.09% ~ 22.61%),

Actinobacteria (5.74% ~ 19.62%), Bacteroidetes (2.96% ~ 8.43%), and Verrucomimicrobia (3.26%~5.69%). The Proteobacteria abundance of rhizosphere soil samples WA and WB of PN under forest were 29.52% and 35.70%, respectively, Significantly (P -value<0.05) lower than 43.41 % and 42.13 % of WC and WD in the field. In addition, the unclassified_Bacteria abundance of rhizosphere soil samples WA and WB of *Panax notoginseng* under forest were 11.32% and 9.34%, respectively, significantly (P -value<0.05) higher than the 5.20% and 6.81% of WC and WD in the field (Figure 2E). As shown in Figure 2F, The top 5 abundance of fungi groups from high to low were Ascomycota (65.76%~88.60%), Mortierellomycota (2.75%~26.20%), Basidiomycota (2.82%~5.44%), Rozellomycota (1.89%~4.65%), and Glomeromycota (0.02%~1.09%). The Ascomycota abundance of rhizosphere soil samples WA and WB of *Panax notoginseng* under forest were 65.76% and 72.14%, respectively, Significantly (P -value<0.05) lower than 88.60% and 88.20% of WC and WD in the field. In addition, the Mortierellomycota abundance of rhizosphere soil samples WA and WB of *Panax notoginseng* under forest were 26.20% and 14.21%, respectively, significantly (P -value<0.05) higher than the 2.75% and 4.70% of WC and WD in the field.

In alpha diversity comparison, the community diversity of bacterial and fungal in rhizosphere soil show significant differences. For bacteria (Figure 3A~D), it can be seen that the indices of Chao1 and Ace was highest in WA treatment. WA and WB treatments (under forest) have not significant difference, but both were significantly higher than WC and WD (field). For fungi (Figure 3E~H), there was no significant difference among four samples. In PCA analysis, the distance between sample points indicates difference size of microbial community structure between samples, the greater the distance, hence the difference. For bacteria (Figure 4A), the community structure exist significant differences among different sample treatments (Adonis: $R^2=0.650$, P -value=0.001), the first two coordinate axes co-explain 52.11% of bacterial community vary. For fungi (Figure 4B), the microbial community structure also showed significant differences among different sample treatments (Adonis: $R^2=0.533$, P -value=0.001), the first two coordinate axes co-explain 51.33 % of fungal community vary. In LEfSe analyse (LDA scores greater than 4 were selected as significant differences microbial communities), As shown in Figure 4C, the significantly enrichment bacterial communities in sample WA include *Micromonosporae*, *Gp4* etc. The significant enrichment in WB contain *Gp1*, *Alphaprotobacter* etc. The significant enrichment in WC include *Xanthomonadaceae*, *Burkholderia*, *Bradyrizobium* etc. The significant enrichment in WD contain *Chloroplast*, *Streptophyta*, *Flavobacteriae*, *Enterbacteriae*, *Buttiauxella*, *Comamonadaceae*, *Sphingobium* etc. As shown in Figure 4D, for fungi, the significantly enrichment fungal communities in sample WA include *Mortierella*, *Tetracladium*, *Setophaeosphaeria*, *Penicillium*, *Latorua* etc. The significantly enrichment in WB have *Fusarium*, *Herotrichiellaceae* etc. The significantly enrichment in WC include *Plectophaerella*, *Xenochalara*, *Pyrenochaetopsis* etc. The significantly enrichment in sample WD include *Pyxidiophorales*, *Ionia*, *Candida*, *Microcera* etc.

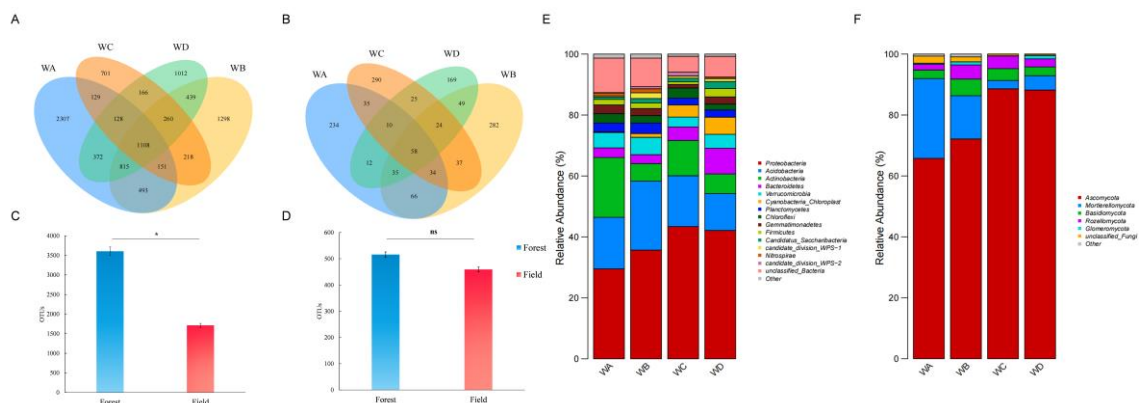


Figure 2. Sample microorganism community structure analysis in natural forest and field.

A: Venn chart of bacterial OTUs; B: Venn chart of fungal OTUs; C: Comparison of the number of unique OTUs in bacterial communities; D: Comparison of the number of unique OTUs in fungal communities; E: Bar chart of bacterial community under Phylum class; F: Bar chart of fungal community under Phylum class. * indicates a significant difference (P -value<0.05), ns indicates a no significant difference.

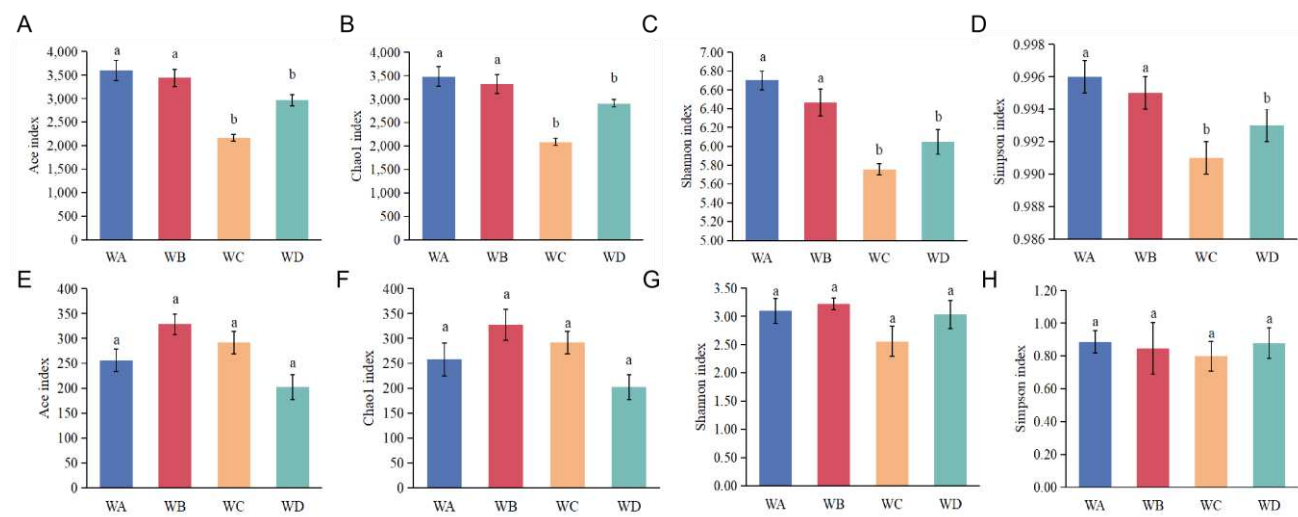


Figure 3. Microorganism community Alpha diversity analysis in natural forest and field.

A~D: Alpha diversity index(Ace、Chao1、Simpson、Shannon) in bacterial community; E~H: Alpha diversity index(Ace、Chao1、Simpson、Shannon) in fungal community. Mean values were compared with a one-way ANOVA, and the letters on the boxes indicate significant differences among all experimental treatments based on Tukey's HSD (P -value<0.05).

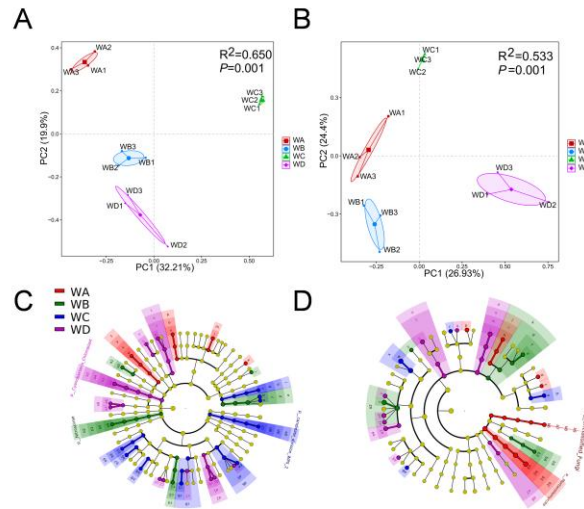


Figure 4. Microorganism communities difference analysis.

A: Bacterial community PCA analysis(Adonis: $R^2=0.650$, $P\text{-value}=0.001$); B: Fungi community PCA analysis(Adonis: $R^2=0.533$, $P\text{-value}=0.001$); C: Bacterial community LefSe analysis(Significant enrichment $LDA>4$); D: Fungal community LefSe analysis(Significant enrichment $LDA>4$).

Soil environmental factors and microorganism community correlation analysis among different PN sample

Soil environmental factors have significant differences in different samples (Table 2). The effective phosphorus(P) content in understory samples WA and WB (0.971 and 0.840 g/kg) was significantly lower than that of WC and WD (1.378 and 1.454 g/kg) in the field, similarly, the effective potassium(K) content of samples WA and WB (47.786 and 53.981 g/kg) was significantly lower than that of WC and WD (95.532 and 79.299 g/kg). The effective nitrogen content of samples WA and WD (1.824 and 1.660 g/kg) was significantly higher than that of WB and WC (1.451 and 1.320 g/kg). For organic carbon (TOC), the samples WA (15.458 g/kg) and WB (13.166 g/kg) in under forest were significantly higher than those of WC (7.848 g/kg) and WD (10.879 g/kg) in the field. The soil pH values of samples WA (5.640) and WB (6.007) in under forest were significantly higher than those of WC (4.933) and WD (5.107) in the field. In summary, the soil P and K content of under forest samples were significantly lower than those of field samples, but the TOC content and pH value were significantly higher than those of field samples.

CCA(Canonical Correspondence Analysis) reflects the correlation between sample communities and environment. As shown in Figure 5A, in the CCA analysis of bacteria, the first two axes together explain a total of 75.42% (CCA1 accounts for 47.34%, and CCA2 accounts for 28.08%). TOC was the main environmental factor that significantly affects the bacterial community structure of understory samples, its explanatory contribution rate was 84.23% (envfit analysis, $r^2=0.8423$, $P\text{-value}<0.01$, Supplement Table 3). The next was available nitrogen, which contribution rate for 61.66%(envfit analysis, $r^2=0.6166$, $P\text{-value}<0.05$, Supplement Table 3). In addition, available potassium is the main environmental factor that significantly affects the structure of bacterial communities in the field, its explanatory contribution was 83.10% (envfit analysis, $r^2=0.8310$, $P\text{-value}<0.01$, Supplement Table 3).

According to Figure 5B, in the fungal CCA analysis, the first two axes together explained a total of 68.97% (CCA1 accounted for 38.60%, and CCA2 accounted for 30.37%). Effective phosphorus is the main environmental factor that significantly affects the structure of fungal communities in the

field, its explanatory contribution was 63.55% (envfit analysis, $r^2=0.6355$, $P\text{-value}<0.01$, Supplement Table 3). Secondly, the explanatory contribution ratio of pH value on the variation of fungal community structure is 75.1%(envfit analysis, $r^2=0.751$, $P\text{-value}<0.01$, Supplement Table 3)

Sample	Effective Phosphorus Content(g/kg)	Effective Potassium Content(g/kg)	Effective nitrogen Content(g/kg)	organic carbon content(%)	pH
WA	0.971±0.110 b	47.786±2.845 d	1.824±0.079 a	15.458±1.083 a	5.640±0.168 b
WB	0.840±0.072 b	53.981±2.528 c	1.451±0.094 c	13.166±1.582 b	6.007±0.211 a
WC	1.378±0.028 a	95.532±4.206 a	1.320±0.037 c	7.848±0.901 d	4.933±0.120 c
WD	1.454±0.118 a	79.299±2.774 b	1.660±0.072 b	10.879±0.535 c	5.107±0.110 c

Table 2. Soil environmental factors.
Note: Mean values were compared with a one-way ANOVA, and the letters indicate significant differences among all experimental treatments based on Tukey’ s HSD ($P\text{-value}<0.05$).

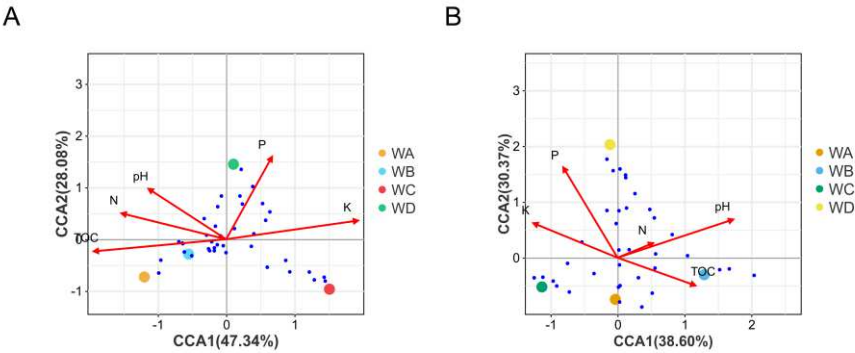


Figure 5. Microbial community and soil environmental factors Correlation analysis.
A: bacterial community; B: fungal community.
Note: N indicates effective nitrogen, P indicates effective phosphorus, K indicates effective potassium, TOC indicates total organic carbon. The arrows represent various environmental factors, and the length of each arrow corresponds to the magnitude of its impact on community structure. The projection point of a sample onto an environmental factor vector indicates a positive correlation when it lies above the direction of the arrow, and a negative correlation when it lies below.

Network analysis of rhizospheric microorganisms in PN under natural forest and field conditions

Network analysis was used to study the potential interactions between microbial communities (bacteria and fungi) in the rhizosphere of PN under forests and in fields. The network of rhizosphere microorganisms of PN in under forest consists of 266 nodes and 4549 edges (Figure 6A), the network of rhizosphere microorganisms of PN in the field consists of 186 nodes and 3407 edges (Figure 6B). The number of nodes and edges included in the network parameters is higher rhizosphere soil samples in under forest than rhizosphere soil samples in the field, and the lines are also more dense, this indicates that the microbial communities of rhizosphere soil in under forest more closely and complex than those rhizosphere soil in the field. However, the soil microbial community in the field shows relatively single and fragile (the association between microorganisms is only limited to certain microorganisms). These indicators are utilized to identify microbial species that exert significant influence within the network, its maybe play a pivotal role in information

transmission or control of the microbial community structure. We had identified microbial species that have significant influence within the network. In rhizosphere soil of under forest, *Cylindrocarpon*, *Paraglomus*, *WPS-2_genera_incertae_sedis* etc. were key groups and may play an important role in constructing under forest rhizosphere microbial communities (Figure 6A). In rhizosphere soil of the field, *Knufia*, *Rhizomicrobium*, *Thielavia* etc. were key groups and may play an important role in constructing rhizosphere microbial communities in the field (Figure 6B).

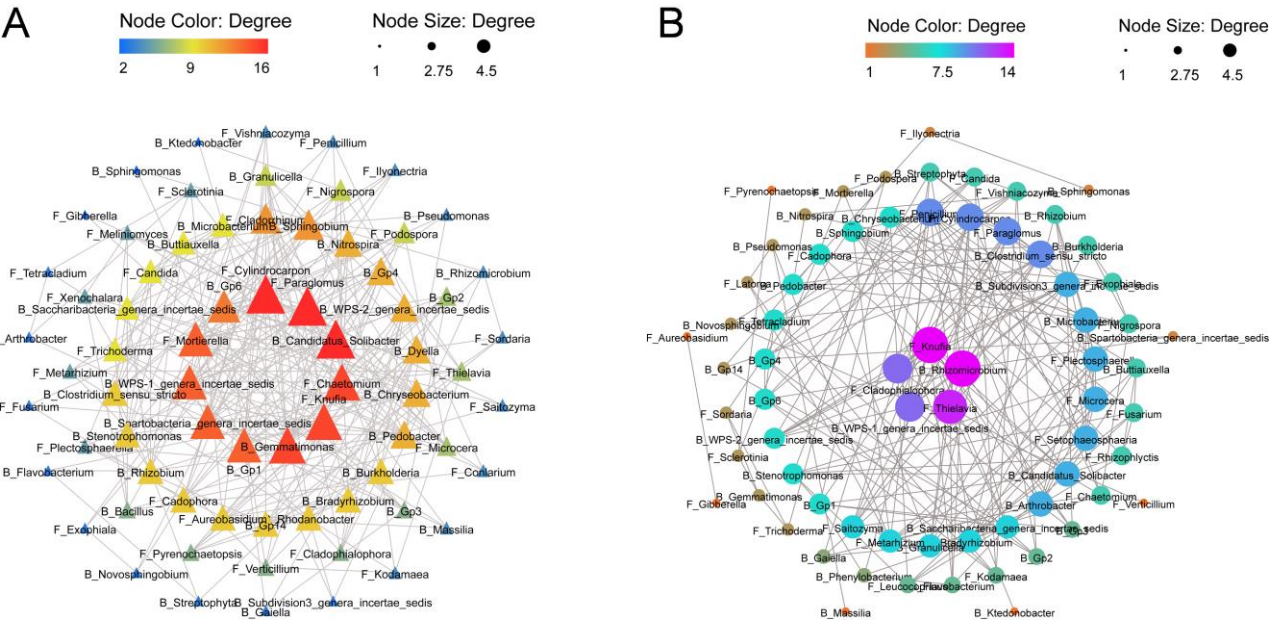


Figure 6. Network analysis of soil microorganisms (bacteria and fungi) in the rhizosphere of PN plants under forest and field conditions (at the genus level, Pearson correlation, $\rho > |0.8|$, P -value < 0.01). A: Network of soil microorganisms in the rhizosphere of *PN* plants under forest conditions (Nodes=266, Edges=4549); B: Network of soil microorganisms in the rhizosphere of *PN* plants under field conditions (Nodes=186, Edges=3407). B_ indicate Bacteria; F_ indicate Fungi.

Content of the saponins in PN and microorganism community correlation analysis among different treatments

As shown in Table 3, the R1, Rg1, Re, Rb1, Rd, and total saponins of *PN* in different samples have significant differences. For four treatments, the R1 content of WA and WB in understory samples was 1.25% and 1.17%, respectively, were significantly higher than that of WC and WD (0.77% and 0.78%) in field samples. The content of Rg1 in WA (5.63%) was the highest, was significantly higher than other treatments. The content of Re in WC was the lowest, was only 0.59%, while there was no significant difference in other treatments. The content of Rb1 was the highest in WB, was 4.22%, was significantly higher than other treatments, and the lowest was WD at only 2.77%. The content of Rd was the highest in WB, was 1.00%, was significantly higher than other treatments, and the lowest was WD at only 0.65%. Finally, the total saponin content of WA (12.07%) was the highest, followed by WB (11.66%), but both are significantly higher than WC (9.71%) and WD (8.41%) in field samples.

Additionally, as shown in Figure 7A, the correlation between the rhizosphere microbial community (bacteria and fungi) of *PN* in under forest were significant with saponin R1

(*P*-value<0.05), while there was no significant correlation between the rhizosphere microbial community of PN in the field and different saponins. In summary, the content of R1 and total saponins in PN under forest were significantly higher than that in the field, the rhizosphere microbial community of PN in under forest exist significant correlation with saponin R1. Conduct Pearson correlation analysis to investigate the relationship between saponins in PN roots and diverse microbial communities (Figure 7B). For bacteria, the groups related to saponin content were mainly concentrated in *Streptophyta*, *Sphingomonas*, *Flavobacteria*, *Rhizomicrobium*, *Nitrospira*, *Phenobacteria*, *Massilia*, etc. For fungi, the groups related to saponin content were mainly concentrated in *Ilyonectria*, *Sclerotinia*, *Penicillium*, *Latorua*, *Verticillium* etc. Among them, Bacteria contain *Sphingomonas* and *Nitrospira* were positively correlated with the content of saponin R1, while negatively correlated bacteria include *Streptophyta*, *Flavobacteria*, *Phenobacteria*, and *Massilia*. The presence of *Mortierella*, *Penicillium*, and *Latorua* fungi exhibited a positive correlation with saponin R1, whereas the fungus *Sclerotinia* showed a negative correlation.

Table 3. Compare of saponins content in PN.

Sample	R1	Rg1	Re	Rb1	Rd	Total content
WA	1.25±0.01 a	5.63±0.03 a	0.70±0.01 ab	3.73±0.02 b	0.78±0.02 b	12.07±0.07 a
WB	1.17±0.12 a	4.47±0.28 b	0.78±0.08 a	4.22±0.04 a	1.00±0.03 a	11.66±0.14 a
WC	0.77±0.18 b	4.39±1.00 b	0.59±0.21 b	3.21±0.59 b	0.75±0.15 b	9.71±0.12 b
WD	0.78±0.11 b	3.43±0.94 c	0.78±0.06 a	2.77±0.84 c	0.65±0.15 b	8.41±0.29 b

Table 3. Compare of saponins content in PN.

Note: Mean values were compared with a one-way ANOVA, and the letters indicate significant differences among all experimental treatments based on Tukey’ s HSD (*P*-value<0.05).

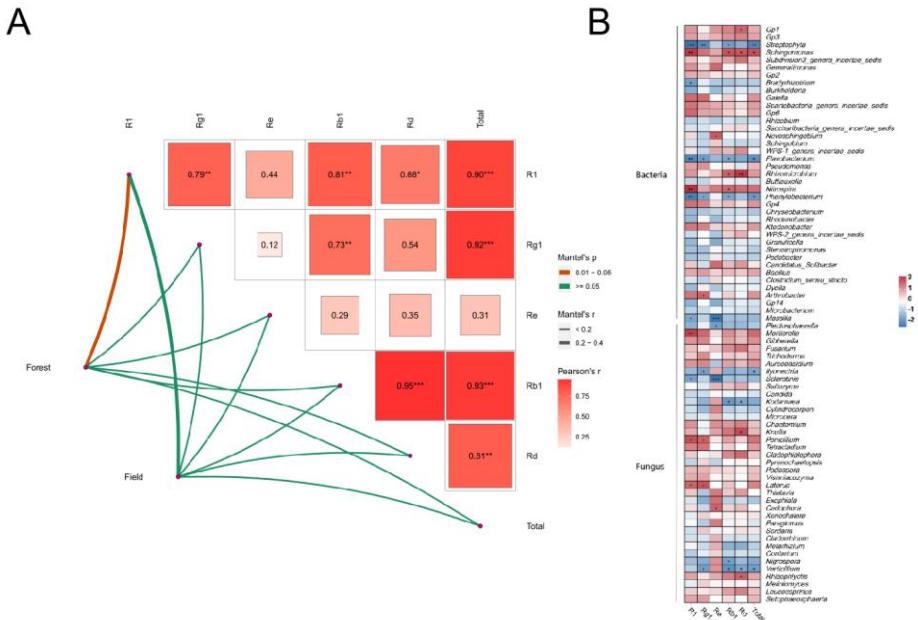


Figure 7. Analysis of the correlation between microbial communities in the rhizosphere of Panax notoginseng and saponin content.

A: Microorganism community(forest and field)to saponins composition influence analyse by Mantel test. Pairwise

comparisons of environmental factors are shown, with a color gradient denoting Spearman's correlation coefficients. Microorganism community (forest and field) to saponins composition influence analysis by Mantel test. Edge width corresponds to the Mantel's statistic for the corresponding distance correlations, and edge color denotes the statistical significance based on P -value < 0.05. ** and *** represented significant correlation (P -value < 0.01) and extremely significant correlation (P -value < 0.001), respectively. B: Analysis of the correlation between saponins and rhizosphere microorganisms (at the genus level, Top30).

Discussion

Soil not only serves as a microenvironment for the growth and reproduction of microbial communities, but its physicochemical properties also exert indirect influence on the composition of soil microbial community structure. Soil microorganisms actively participate in carbon and nitrogen nutrient cycling and energy flow within the soil, as well as contribute to the formation of soil humus and improvement of soil structure²². Soil plays a crucial role in soil microecology and significantly influences plant growth and quality formation. Consequently, the diversity of soil microbial communities emerges as a pivotal factor impacting both soil quality and the robust development of plants, with notable variations observed in microbial community composition across diverse habitats²³. We found that compared with the field, the abundance of *Proteobacteria* and *Ascomycota* were significantly decreased in the under forest PN soil, while the abundance of more unknown classified bacteria and *Mortierellomycota* were significantly increased. *Mortierellomycota* is an oil-producing filamentous fungus, whose mycelium exerts influence on the nutrient absorption of other microbial groups during organic degradation. Additionally, well-developed hyphae can also modify the soil microbial environment by impacting soil aggregates²⁴⁻²⁵. *Mortierella alpina* can produce a wide range of polyunsaturated fatty acids, such as arachidonic acid, γ Linoleic acid and eicosapentaenoic acid, these fatty acids contain abundant carbon sources and may alter the habitat of soil microorganisms²⁵. The previous study suggested that the application of *Aspergillus altissima* and *Aspergillus longisporum* fungi enhances nutrient availability and promotes the activity of carbon, nitrogen, and phosphorus-related enzymes in red soil and sandy ginger black soil, effectively mitigating red soil acidification. This finding may contribute to creating a favorable microecological environment for PN growth under forest²⁶.

The Chao1, Ace, Simpson, and Shannon indexes of rhizosphere soil bacteria in PN under forest samples WA and WB were significantly higher than those in PN field samples WC and WD, indicating a significantly greater diversity of rhizosphere soil bacteria in understory PN compared to field PN. There was no significant difference observed in fungi among the 4 treatments, which is consistent with the findings of Wang et al.²⁷, suggested that the rhizosphere soil bacterial community of under forest PN exhibited higher diversity. The presence of pine forests, characterized by abundant species, high biodiversity, and a diverse range of carbon sources in root exudates, contributes to an overall higher richness of soil organic carbon compared to that found in fields. Additionally, the decomposition of various organic matter serves as a nutrient source for the growth and reproduction of different types of microorganisms²⁸. In addition, the long-term interference of human factors such as cultivation, fertilization, and pesticides in the field soil environment has led to soil compaction and degradation²⁹⁻³⁰. Meanwhile, the extended cultivation period of PN (typically exceeding 3 years for harvesting) contributes to a significant accumulation of same single carbon sources from root secretions in the soil, thereby serving as a prominent factor leading to reduced microbial diversity in field soils compared to under forest³¹.

The rhizosphere soil microorganisms establish a intricate network of microorganisms, which have gained increasing recognition for their structural role in upholding the stability of plant-soil microbial communities. Community network stability refers to the capacity of communities to withstand and recover from both biotic and abiotic disturbances³²⁻³³. By networks analyzed, We found that the network topological parameters, such as nodes and edges, of the soil microbial community in PN under forest were higher than those in PN field. The connectivity among microbial communities in the rhizosphere of understory plants surpasses that observed in the field rhizosphere, implying a more intricate and sophisticated association among microorganisms. This enhanced association contributes to heightened ecological niche stability and resistance. This is consistent with the research results of Tang³⁴, the complexity and stability of microbial networks have decreased by long-term application of chemical fertilizers in soil. The high degree and betweenness centrality of nodes and points in the network indicate that the microorganisms represented by these nodes play a more important role in building communities and are closely related to most microorganisms. The key taxonomic groups of rhizosphere co-occurrence network were *Cylindrocarpon*, *Paraglomus*, *WPS-2_genera_incertae_sedis* etc. in under forest PN, the key taxonomic groups of rhizosphere co-occurrence network were *Knufia*, *Rhizomicrobium*, *Thielavia* etc. in the field, this implied that these taxonomic groups play an important role in constructing overall microbial community. Research has shown that compound infections such as *Cyclocarpon spp.* and *Fusarium spp.* can lead to the occurrence of root rot disease, and it have become the main factor to limit the development of ginseng, American ginseng, and PN cultivation industry³⁵. However, in our test materials, we found that under forest PN did not develop root rot disease, which may be due to the fact that most microorganisms in network have antagonistic effects with pathogenic bacteria such as *Cyclocarpon spp.* and *Fusarium spp.*, and to limit large-scale reproduction of pathogenic bacteria. The rhizosphere growth promoting bacteria (PGPR) in plant roots can not only secrete growth hormones to directly promote plant growth and development, improve soil nutrients, and regulate the active ingredients of medicinal plants, but also produce antagonistic substances (such as antibiotics, hydrogen cyanide (HCN), siderophores, etc.) to inhibit pathogenic microorganisms and alleviate medicinal plant diseases³⁶⁻³⁸. Chandra³⁹ isolated a strain of *Pseudomonasaeruginosa* from rhizosphere of medicinal plant *Valeriana Wallichii*, and found that it had an antagonistic effect of 56.2% against *Fusarium oxysporum* and 51.02% against *Alternaria alternata*. The maximum inhibition rates were 69.2%, 46.4%, and 15%, respectively, for radial growth of *Alternaria alternata*, *Alternaria solani*, and *Aspergillus flavus*. Zhong⁴⁰ found that Cucurbitaceae plants can synthesize a group of bitter triterpenes include cucurbitacin B (CuB) and E (CuE), which in CuB was released in the rhizosphere and may endow plants with resistance to *Fusarium spp.* by enhancing colonization of *Bacillus* and *Enterobacter* in the melon rhizosphere. These microorganisms can induce plant systemic resistance to protect plants from pathogenic attacks by competing for niches and substrates, fungal parasitism, and antibiotic metabolites⁴¹.

The assembly results of rhizosphere microbial communities were influenced not only by plants but also by soil environments, including effective N, P, K, and pH. By compared soil chemical properties, the survey results revealed significant differences in the rhizosphere soil of under forest PN compared to field samples. Specifically, the effective P and K content in the rhizosphere soil of under forest PN were significantly lower than that of field samples, while the organic carbon content and pH were significantly higher. Environmental factor correlation analysis indicated that TOC was the primary driving factor affecting the assembly of rhizosphere soil bacterial communities in under

forest PN with an explanatory power of 84.23% for community variation. Dissolved TOC played a crucial role in regulating microbial community structure, with plant inputs (litter fall and roots) serving as important sources of soil TOC⁴². The rich biodiversity and higher carbon input associated with under forest may be one of the main factors influencing the assembly of rhizosphere soil bacterial communities in under forest PN. Additionally, for fungal communities, pH emerged as a significant environmental factor impacting overall fungal community structure with an explanatory power of 75.1% for community variation. This finding aligns with Zhang's conclusion⁴³ that soil pH significantly influences fungal abundance and composition. Moreover, changes in soil pH can alter both the number and structure of soil microbial communities by affecting nutrient utilization rates⁴⁴. The study revealed a significant presence of pathogenic fungi, including *Mortierella*, *Fusarium*, and *Aspergillus* etc., in the continuous field soil that can induce root rot disease in PN. Moreover, the abundance of these fungi exhibits a negative correlation with both soil pH value and the survival rate of PN⁴⁵. In this experiment, the pH value of the soil under forest was significantly higher than that in the field, potentially contributing to the suppression of pathogenic bacteria reproduction and a reduction in the occurrence of root rot disease in PN.

By conducting saponin content analysis of PN in both under forest and field environments, it was observed that the levels of saponin R1 and total saponin content were significantly higher in the under forest compared to the field. Notably, saponin R1 emerged as a key determinant contributing to the elevated total saponin content specifically in the under forest PN samples. Furthermore, through an investigation into microbial communities, we discovered a significant correlation between rhizosphere microbial community composition and saponin R1 levels in under forest PN. The top 35 groups in terms of taxonomic abundance of bacteria and fungi were selected and Pearson analysis with saponins. It indicates that the groups in bacteria related to saponin content are mainly concentrated in *Streptophyta*, *Sphingomonas*, *Flavobacteria*, *Rhizomicrobium*, *Nitrospira*, *Phenobacteria*, *Massilia*, etc. which in *Sphingomonas* and *Nitrospira* are significantly positive correlation with PN saponin R1, and the relative abundance of under forest PN rhizosphere soil samples is significantly higher than field. *Sphingomonas* is mainly participate in the degradation and carbon cycling of aromatic compounds and their derivatives in soil, and it has significant degradation ability towards chloroacetanilide herbicides⁴⁶. In the study of citrus black spot pathogen infection and rice bacterial ear blight pathogen, *Sphingomonas* was produced significantly antagonistic effects to pathogenic bacteria and can endow crops with disease resistance, and can present beneficial genomic characteristics for plants⁴⁷⁻⁴⁸. Deng's⁴⁹ study showed that *Sphingomonas* significantly enriched in the rhizosphere of tomato plants inoculated with bacterial wilt, which can inhibit the invasion of bacterial wilt. Wang⁵⁰ found that with the continuous growth of PN, phenolic acids such as coumaric acid, ferulic acid, and benzoic acid gradually accumulate in the rhizosphere soil. The insufficient metabolic and decomposition ability of PN rhizosphere microorganisms to specific allelopathic and self-toxic substances is one of the reasons for the occurrence of continuous cropping obstacles in PN. *Nitrospira* is a type of Gram negative bacteria, it as nitrifying bacteria and can oxidize nitrite into nitrate. However, when nitrite in soil is converted into nitrate, it is easy to form nitrate, which it become some nutrients that can be absorbed and utilized by plants⁵¹. This may provide an available nutrient source for the growth of under forest PN to promote the transportation and accumulation of PN saponins. The groups in fungi related to saponin content are mainly concentrated in *Ilyonectria*, *Sclerotinia*, *Penicillium*, *Mortierella*, *Latorua*, *Verticillium*, etc. which in *Ilyonectria*, *Sclerotinia*, and *Verticium* are significantly negative correlation with content of different

types saponins. Research reports that these fungi belong to pathogenic bacteria and are closely related to plant diseases, such as *Ilyonectria* and *Sclerotinia*, which can lead to the occurrence of PN root rot⁵²⁻⁵³. *Verticillium* can infect plants, leads to symptoms such as plant wilt, leaf yellow and cell death, growth retardation, and plant premature aging⁵⁴. The excessive enrichment of these fungi can lead to decrease in yield and quality of PN, which may cause decrease in saponin content. At the same time, some fungi are significantly positive correlation with saponin content, such as *Penicillium*, *Mortierella*, *Latorua*, etc. which in the relative abundance of *Mortierella* in rhizosphere soil of under forest PN is significantly higher than that in field. Research by Li⁵⁵ shows that rare fungus *Mortierella* was inoculated during the growth process of maize, and it was found that maize biomass, chlorophyll, and gibberellin have significantly increased, the genes of encoding chitinase, peroxidase, lipid transfer protein, sugar efflux transporter protein, and various transcription factor proteins in the maize root system were significantly upregulated, and the composition of the maize rhizosphere bacterial community was significantly altered, it is believed that *Mortierella* indirectly promotes plant growth through interactions with bacteria. Therefore, we speculate that there are various beneficial/harmful microorganisms in rhizosphere soil of under forest PN, which together construct a complex microbial network of PN and play an important role in it, and then forming a dynamic equilibrium. In rhizosphere soil of PN in field, due to long-term monoculture and extensive use of fertilizers and pesticides, which lead to accumulation of equal allelopathic substances and decrease of beneficial microorganisms abundance in soil (Especially those microorganisms that have the ability to decompose and metabolize self-toxic substances and antagonize pathogenic bacteria), as a result, it causes a large number of pathogenic bacteria to reproduce, produce diseases and lead to decline in the quality of PN.

Conclusion

There are notable disparities in the diversity of rhizosphere microbial communities (bacteria and fungi) between understory cultivation mode and field cultivation mode for PN. The diversity of bacterial communities in the rhizosphere of under forest PN is significantly higher than that in the field, while no significant difference was observed for fungal communities. In comparison to the rhizosphere soil of field PN, microorganism associations in the rhizosphere soil of under forest PN exhibit closer proximity, resulting in a more intricate and stable microbial community network. Moreover, organic carbon emerges as a primary influencing factor driving assembly of bacterial communities in under forest PN rhizosphere soil, whereas pH value plays a predominant role in shaping fungal community composition.

The total saponin content in the under forest PN is significantly higher compared to that in the field, particularly for saponin R1, which exhibits a significant elevation in the under forest. Additionally, a noteworthy positive correlation exists between saponin R1 and the microbial community within the under forest. Certain beneficial microorganisms (*Mortierellomycota*, *Sphingomonas*, *Nitrospira*, *Mortierella*), known for their ability to decompose toxic substances, antagonize pathogens, and facilitate soil element cycling, demonstrate a significantly positive association with saponin content. These microorganisms may confer disease resistance to PN while playing an indispensable role in maintaining overall community stability.

References

1. Liu, J. C. *et al.* Anti-Inflammatory Activity of *Panax notoginseng* Flower Saponins Quantified Using LC/MS/MS. *Molecules*. **28**(5), 2416. <https://doi.org/10.3390/molecules28052416> (2023).
2. Li, X., Liu, H., Yang, H. R. & Zeng, Y. J. Hypoglycemic effects of novel *Panax notoginseng* polysaccharide in mice with diet-induced obesity. *Foods*. **11**(19), 3101. <https://doi.org/10.3390/foods11193101> (2022).
3. Wajima, T., Kinugawa, R., Yamada, T., Ikoshi, H. & Noguchi, N. *Panax notoginseng* extract possesses significant antibacterial activity against pathogenic streptococci. *Pharmacology*. **103**(5), 221-227. <https://doi.org/10.1159/000496830> (2019).
4. Xu, Y. *et al.* Gut-liver axis modulation of *Panax notoginseng* saponins in nonalcoholic fatty liver disease. *Hepatol Int*, **15**(2), 350-365. <https://doi.org/10.1007/s12072-021-10138-1> (2021).
5. Huo, N. L. *et al.* Preparation, characterization, structural analysis and antioxidant activities of phosphorylated polysaccharide from Sanchi (*Panax notoginseng*) flower. *J Food Sci Technol*. **59**(12), 4603-4614. <https://doi.org/10.1007/s13197-022-05539-5> (2022).
6. Zhang, S. Q., Chen, C., Lu, W. X. & Wei, L. Phytochemistry, pharmacology, and clinical use of *Panax notoginseng* flowers buds. *Phytother Res*. **32**(11), 2155-2163. <https://doi.org/10.1002/ptr.6167> (2018).
7. Guo, H. B., Cui, X. M., An, N. & Cai, G. P. Sanchi ginseng (*Panax notoginseng* (Burkill) FH Chen) in China: distribution, cultivation and variations. *Genetic Resources and Crop Evolution*. **57**(3), 453-460. <https://doi.org/10.1007/s10722-010-9531-2> (2010).
8. Fan, Z. Y. *et al.* Diversity, distribution, and antagonistic activities of rhizobacteria of *Panax notoginseng*. *Journal of ginseng research*. **40**(2), 97-104. <https://doi.org/10.1016/j.jgr.2015.05.003> (2016).
9. Zhan, P., Wei, M. T., Xu, B. & Han, R. L. Research on the development status and countermeasures of China's *Panax notoginseng* industry based on the industrial Chain. *Northern Horticulture*. **20**(14), 158-164. <https://doi.org/10.1051/e3sconf/202123503059> (2021).
10. Liang, J. Y., Liang, Y. & Ma, J. M. Research progress of forest medicine planting pattern. *Journal of Guangxi Normal University* (Natural Science Edition). **40**(5), 366-375. <https://doi.org/10.16088/j.issn.1001-6600.2021041801> (2022).
11. Tian, F. M. *et al.* Research Progress in Planting and Application of Understory Chinese Medicine. *Chinese Wild Plant Resources*. **41**(6), 64-71. <https://doi.org/10.3389/fpls.2021.691838> (2022).
12. Zhou, N. *et al.* Research progress of rhizosphere microorganisms in *Fritillaria* L. medicinal plants. *Frontiers in Bioengineering and Biotechnology*. **7**(10), 1054757. <https://doi.org/10.3389/fbioe.20221054757> (2022).
13. Liu, X. Q. *et al.* Interlinkages between soil properties and keystone taxa under different tillage practices on the North China Plain. *Applied Soil Ecology*. **178**(2), 104551. <https://doi.org/10.1016/j.apsoil.2022.104551> (2022).
14. Sun, F., Ou, Y. Y., Ou, Q. J. & Zeng, L. D. The invasive potential of a hybrid species: insights from soil chemical properties and soil microbial communities. *Journal of Plant Ecology*. **13**(1), 20-26. <https://doi.org/10.1093/jpe/rtz050> (2020).
15. Dombrowski, N. *et al.* Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *The ISME journal*. **11**(1), 43-55. <https://doi.org/10.1038/ismej.2016.109> (2017).
16. Tan, Y. *et al.* Diversity and composition of rhizospheric soil and root endogenous bacteria in

- 585 *Panax notoginseng* during continuous cropping practices. *Journal of Basic Microbiology*. **57**(4),
 586 337-344. <https://doi.org/10.1002/jobm.201600464> (2017).
- 587 17. Zhang, Y. J. *et al.* Modification of quicklime on acid soil under forest and their effect on the
 588 growth of *Panax notoginseng*. *Chinese Journal of Applied Ecology*. **33**(4), 972-980.
 589 <https://doi.org/10.13287/j.1001-9332.202204.081> (2022).
- 590 18. Zhao, L. Y. *et al.* Effects of Soil Moisture on the Microbial Community Under Continuous
 591 Cropping of *Panax notoginseng*. *Biotechnology Bulletin*. **38**(7), 215-223.
 592 <https://doi.org/10.13560/j.cnki.biotech.bull.1985.2021-1287> (2022).
- 593 19. Zhao, L. Y. *et al.* Effects of applying biochar on the rhizosphere fungal community and survival
 594 rate of *Panax notoginseng* under continuous cropping. *Biotechnology Bulletin*. **39**(7), 219-227.
 595 <https://doi.org/10.13560/j.cnki.biotech.bull.1985.2022-1358> (2023).
- 596 20. Idrees, M., Anjum, M. A., Mirza, J. I. & Ahmad, I. Potassium humate amendment regulates soil
 597 NPK supply and growth parameters of potato (*Solanum tuberosum* L.) in a calcareous soil.
 598 *Pakistan Journal of Botany*. **52**(5), 1647-1653. <https://doi.org/10.30848/pjb2020-525> (2020)
- 599 21. National Pharmacopoeia Committee. People's Republic of China (PRC) Pharmacopoeia, one
 600 department. *China Medical Science and Technology Press*. 12-13. (2020).
- 601 22. Jing, H., Wang, H. I., Wang, G. L., Liu, G. B. & Cheng, Y. Hierarchical traits of rhizosphere soil
 602 microbial community and carbon metabolites of different diameter roots of *Pinus tabulaeformis*
 603 under nitrogen addition. *Carbon Research*. **2**(1), 1-17.
 604 <https://doi.org/10.1007/s44246-023-00081-1> (2023).
- 605 23. Strunnikova, O. K. *et al.* The influence of soils with different textures on development,
 606 colonization capacity and interactions between *Fusarium culmorum* and *Pseudomonas*
 607 *fluorescens* in soil and on barley roots. *Plant and Soil*. **389**(2), 131-144. <https://doi.org/10.1007/s11104-014-2351-y> (2015).
- 609 24. Zhang, H. D., Cui, Q. & Song, X. J. Research advances on arachidonic acid production by
 610 fermentation and genetic modification of *Mortierella alpina*. *World Journal of Microbiology and*
 611 *Biotechnology*. **37**(1), 1-9. <https://doi.org/10.1007/s11274-020-02984-2> (2021).
- 612 25. Li, L. F. & Jin, N. Effect of nitrogen concentration on the growth and fatty acid content of
 613 *Mortierella alpina*. *International Journal of Agriculture and Biology*. **24**(4), 838-848.
 614 <https://doi.org/10.17957/ljab/15.1507> (2020).
- 615 26. Ning, Q. *et al.* Effects of mortierella on nutrient availability and straw decomposition in soil.
 616 *Acta Pedologica Sinica*. **59**(1), 206-217. <https://doi.org/10.11766/trxb202006020213> (2022).
- 617 27. Wang, H. J. *et al.* Comparison of rhizosphere microbial community of *Panax notoginseng* with
 618 root rot under natural forest and in the field. *Microbiology China*. **50**(5), 1988-2001.
 619 <https://doi.org/10.13344/j.microbiol.china.220723> (2023).
- 620 28. Velmurugan, A., Swarnam, T. P., Jaisankar, I., Swain, S. & Subramani, T.
 621 Vegetation–soil–microbial diversity influences ecosystem multifunctionality across different
 622 tropical coastal ecosystem types. *Tropical Ecology*. **63**(3), 273-285. <https://doi.org/10.1007/s42965-021-00209-7> (2022).
- 624 29. Momoh, D., Eze, C. L. & Ogbonna, D. N. Impact of Drifts Resulting from Pesticide Application
 625 on Soil Microorganisms around Waste Receptacles in Port Harcourt City, Nigeria. *Biotechnology*
 626 *Journal International*. **24**(6), 1-8. <https://doi.org/10.9734/bji/2020/v24i630118> (2020).
- 627 30. Liu, E. K. *et al.* Biological properties and enzymatic activity of arable soils affected by
 628 long-term different fertilization systems. *Journal of Plant Ecology*. **32**(1), 176-182.

- 629 <https://doi.org/10.3773/jlssn.1005-264x.2008.01020> (2008).
- 630 31. Xiang, W., Chen, J. H., Zhang, F. Y., Huang, R. S. & Li, L. B. Autotoxicity in *Panax*
631 *notoginseng* of root exudates and their allelochemicals. *Frontiers in Plant Science*. **20**(13),
632 1020626. <https://doi.org/10.3389/fpls.2022.1020626> (2022).
- 633 32. Zandt, D., Kolarikova, Z., Cajthaml, T. & Munzbergova, Z. Plant community stability is
634 associated with a decoupling of prokaryote and fungal soil networks. *Nature Communications*.
635 **14**(1), 3736. <https://doi.org/10.1038/s41467-023-39464-8> (2023).
- 636 33. Anggrainy, E. D. *et al.* Shifting of microbial biodiversity and soil health in rhizomicrobiome of
637 natural forest and agricultural soil. *Open Agriculture*. **5**(1), 936-942.
638 <https://doi.org/10.1515/opag-2020-0090> (2020).
- 639 34. Tang, S. *et al.* Microbial community succession in soil is mainly driven by carbon and nitrogen
640 contents rather than phosphorus and sulphur contents. *Soil Biology and Biochemistry*. **180**(1),
641 109019. <https://doi.org/10.1016/j.soilbio.2023.109019> (2023).
- 642 35. Wang, P. P. *et al.* Structure and function of rhizosphere soil and root endophytic microbial
643 communities associated with root rot of *Panax notoginseng*. *Frontiers in Plant Science*. **5**(12),
644 752683. <https://doi.org/10.3389/fpls.2021.752683> (2022).
- 645 36. Khaledian, N., Weisany, W. & Schenk, P. M. Arbuscular mycorrhizae and rhizobacteria
646 improve growth, nutritional status and essential oil production in *Ocimum basilicum* and
647 *Satureja hortensis*. *Industrial Crops and Products*. **160**(1), 113163.
648 <https://doi.org/10.1016/j.indcrop.2020.113163> (2021).
- 649 37. Zhao, Q. *et al.* Improved growth and metabolite accumulation in *Codonopsis pilosula* (Franch.)
650 Nannf. by inoculation of *Bacillus amyloliquefaciens* GB03. *Journal of agricultural and food*
651 *chemistry*. **64**(43), 8103-8108. <https://doi.org/10.1021/acs.jafc.6b03390> (2016).
- 652 38. Pace, L. *et al.* Plant growth-promoting rhizobacteria for *in vitro* and *ex vitro* performance
653 enhancement of Apennines' Genepi (*Artemisia umbelliformis subsp. eriantha*), an endangered
654 phytotherapeutic plant. *In Vitro Cellular & Developmental Biology-Plant*. **56**, 134-142.
655 <https://doi.org/10.1007/s11627-019-10035-1> (2020).
- 656 39. Chandra, H., Kumari, P., Bisht, R., Prasad, R. & Yadav, S. Plant growth promoting *Pseudomonas*
657 *aeruginosa* from *Valeriana wallichii* displays antagonistic potential against three
658 phytopathogenic fungi. *Molecular Biology Reports*. **47**(8), 6015-6026.
659 <https://doi.org/10.1007/s11033-020-05676-0> (2020).
- 660 40. Zhong, Y. *et al.* Root-secreted bitter triterpene modulates the rhizosphere microbiota to improve
661 plant fitness. *Nature Plants*. **8**(8), 887-896. <https://doi.org/10.1038/s41477-022-01201-2> (2022).
- 662 41. Huang, X. Q., Zhou, X., Zhang, J. B. & Cai, Z. C. Highly connected taxa located in the
663 microbial network are prevalent in the rhizosphere soil of healthy plant. *Biology and Fertility of*
664 *Soils*. **55**(3), 299-312. <https://doi.org/10.1007/s00374-019-01350-1> (2019).
- 665 42. Feng, J. G., He, K. Y., Zhang, Q. F., Han, M. G. & Zhu, B. Changes in plant inputs alter soil
666 carbon and microbial communities in forest ecosystems. *Global Change Biology*. **28**(10),
667 3426-3440. <https://doi.org/10.1111/gcb.16107> (2022).
- 668 43. Zhang, Y. *et al.* pH-induced changes in fungal abundance and composition affects soil
669 heterotrophic nitrification after 30 days of artificial pH manipulation. *Geoderma*. **366**(4), 114255.
670 <https://doi.org/10.1016/j.geoderma.2020.114255> (2020).
- 671 44. Zhang, Y. J. *et al.* Soil Acidification caused by excessive application of nitrogen fertilizer
672 aggravates soil-borne diseases: Evidence from literature review and field trials. *Agriculture*,

- ecosystems & environment*. **340**(1), 108176. <https://doi.org/10.1016/j.agee.2022.108176> (2022).
45. Effects of Biochar on the Soil Properties and Fungal Community Structure under Continuous Cropping of *Panax notoginseng*. *Biotechnology bulletin*. **39**(2), 221-231. <https://doi.org/10.13560/j.cnki.biotech.bull.1985.2022-0583> (2023).
 46. Zhang, Y., Yang, Y., Wei, Q. H. & Zhao, M. Research progress in characteristic and application of *Sphingomonas*. *Chemistry & Bioengineering*. **38**(3), 6-13. <https://doi.org/10.3969/j.issn.1672-5425.2021.03.002> (2021).
 47. Matsumoto, H. *et al.* Bacterial seed endophyte shapes disease resistance in rice. *Nature plants*. **7**(1), 60-72. <https://doi.org/10.1038/s41477-020-00826-5> (2021).
 48. Li, P. D. *et al.* The phyllosphere microbiome shifts toward combating melanose pathogen. *Microbiome*. **10**(1), 56-56. <https://doi.org/10.1186/s40168-022-01234-x> (2022).
 49. Deng, X. H. *et al.* Bio-organic soil amendment promotes the suppression of *Ralstonia solanacearum* by inducing changes in the functionality and composition of rhizosphere bacterial communities. *New Phytologist*. **235**(4), 1558-1574. <https://doi.org/10.1111/nph.18221> (2022).
 50. Wang, B. Y. *et al.* Root rot-infected Sanqi ginseng rhizosphere harbors dynamically pathogenic microbiotas driven by the shift of phenolic acids. *Plant and Soil*. **465**(3), 385-402. <https://doi.org/10.1007/s11104-021-05034-4> (2021).
 51. Ayiti, O. E., Ayangbenro, A. S. & Babalola, O. O. 16S Amplicon Sequencing of Nitrifying Bacteria and Archaea Inhabiting Maize Rhizosphere and the Influencing Environmental Factors. *Agriculture*. **12**(9), 1328. <https://doi.org/10.3390/agriculture12091328> (2022).
 52. Deng, J. *et al.* Dirigent gene family is involved in the molecular interaction between *Panax notoginseng* and root rot pathogen *Fusarium solani*. *Industrial Crops and Products*. **178**(1), 114544. <https://doi.org/10.1016/j.indcrop.2022.114544> (2022).
 53. Xu, Y. G. *et al.* Autotoxin Rg1 induces degradation of root cell walls and aggravates root rot by modifying the rhizospheric microbiome. *Microbiology Spectrum*. **9**(3), e01679-21. <https://doi.org/10.1128/spectrum.01679-21> (2021).
 54. Zhang, Y. *et al.* Verticillium dahliae secretory effector PevD1 induces leaf senescence by promoting ORE₁-mediated ethylene biosynthesis. *Molecular plant*. **14**(11), 1901-1917. <https://doi.org/10.1016/j.molp.2021.07.014> (2021).
 55. Li, F. *et al.* Rare fungus, Mortierella capitata, promotes crop growth by stimulating primary metabolisms related genes and reshaping rhizosphere bacterial community. *Soil Biology and Biochemistry*. **151**, 108017. <https://doi.org/10.1016/j.soilbio.2020.108017> (2020).

Author contributions

All authours were involved in the conception and design of the work. C.W. and H.W.S. wrote and reviewed the manuscript. Y.L.Y. and C.X.P. collected the samples. Y.H.T. and Q.S.Y. provided analysis ideas and reviewed the manuscript. D.J.C. and Y.H.Q. performed its design, conducted the sampling. L.L. and D.W.Z. reviewed the manuscript and prepared the illustrations. Y.H.T. and Q.Y. supervised the work. All authors read and approved the final manuscript.

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719 **Competing Interest**

720 The authors declare that they have not competing financial interests or personal relationships.

721 **Additional information**

722 Supplementary Information Raw data material available at Related files.

723 **Correspondence** and requests for materials should be addressed to Y.H.T.

724 **Data availability**

725 The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive
726 in china National center for Bioinformation(CNCB), and BIG Submission accession number is
727 PRJCA026678(<https://ngdc.cncb.ac.cn/bioproject/>)

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