

Analysis of bacterial and fungal community structure in *Panax notoginseng* rhizosphere samples from different locations

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Research Article

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Abstract

Panax notoginseng is a well-known Chinese herb that is used worldwide. The goal of this work was to explore the diversity and structure of rhizosphere microbial communities of *Panax notoginseng*. To do this, *Panax notoginseng* rhizosphere soil samples were collected from ten production areas in China, and the 16SrRNA and internal transcribed spacer (ITS1) sequences were analyzed by Illumina high-throughput sequencing technology. The results revealed similar species composition of fungal and bacterial communities in the different producing areas, but significant variation in the abundances of some dominant flora. Redundancy analysis showed that environmental factors explained 41.3% of the fungal community and 45.7% of the bacterial community. We detected significant enrichment of some root rot pathogens, including *Ilyonectria*, *Fusarium*, and *Pseudomonas*, in samples from Wenshan City and Yunnan Province. In summary, the results reveal differences in the structure of rhizosphere soil microbial community of *Panax notoginseng* in different production areas. The beneficial fungus *Chaetomium* was the most abundant, with an average abundance of 19.65%. The results can guide strategies to improve *Panax notoginseng* quality and yield and for biological control of root rot in *Panax notoginseng*.

Introduction

Sanqi [*Panax notoginseng* (Burkill) F. H. Chen] is an important Chinese herb unique to Southwest China that is used to treat cardiovascular diseases, inflammation, body pain, trauma, and internal and external bleeding due to injury[1, 2]. The global demand for *Panax notoginseng* has been increasing in recent years. With sensitivity to sunlight, heat and humidity, *Panax notoginseng* has a narrow distribution range and there are few wild resources, so to meet market demand, it is cultivated in southwest China[3]. Currently, planting of *Panax notoginseng* has expanded from Wenshan city in Yunnan Province to Jingxi city in Guangxi Province and Panzhou city in Guizhou Province. With its requirement for a specific growth environment and long growth cycle, *Panax notoginseng* is susceptible to various diseases, and large-scale intensive planting increases the risk of persistent soil diseases, which can seriously affect plant quality[4, 5]. In particular, root rot, a soil-borne disease, causes serious damage to *Panax notoginseng* in cultivated areas in China[6]. The field symptoms of the disease are often manifested as improper leaf color, leaf wilting, leaves turning yellow and falling off, and decay of the underground part of the part, causing poor plant growth and yield reduction. Root rot significantly limits the industrialization of this precious herb, with infection potentially leading to a 5–70% reduction in the yield of *Panax notoginseng*, or even crop failure [7].

The plant root system is responsible for the absorption of nutrients such as water, carbon dioxide, and inorganic salts from the soil and transportation of these nutrients to the above-ground parts of the plant[8]. Root rot can affect plant growth of both root and tuber plants[9], such as potatoes[10], hemlock[11], aconite[12], and ginger[13]. Previous reports have shown regulation of root rot by inter-root microorganisms [14], with accumulation of harmful soil-borne pathogens an important factor leading to root rot[15]. A study by Lixia Tian et al. [16] found that increased relative abundance of pathogenic fungi *Gibellulopsis* and *Gibberella* was associated with the onset of root rot of American ginseng. Root rot can affect inter-root microbial communities and functions. For example, root rot was shown to disrupt the *P. cyrtoneura* rootstock microbial community and reduce diversity[17].

Inter-root microorganisms can have important effects on plant growth, root structure, and nutrient uptake[18]. Increased inter-root microbial abundance has been associated with suppression of soil-borne pathogens, and changes in microbial diversity and composition can affect ecosystem function, balance, and health[19]. The inter-rhizosphere of medicinal plants is a special micro-ecosystem of plant-soil-microbe interactions[20]. By secreting various root exudates, including sugar, protein, organic acid, and various secondary metabolites, plant roots provide nutrients required for growth of rhizosphere microorganisms, and thus can affect the composition and diversity of the associated microbial community[21, 22]. The composition and diversity of rhizosphere microbial communities are essential to maintain soil productivity and plant health [23].

There are many microorganisms present in rhizosphere soil, including beneficial, harmful, and neutral microorganisms, and these microorganisms may interact with the roots to affect plant growth and health [24]. The effective components and quality of medicinal materials cultivated in different regions can vary, and this difference is closely related to rhizosphere microorganisms [25]. Microbial characteristics, such as inter-root microbial abundance, composition, and diversity, are potentially valuable indicators of soil quality[26]. Root rot disease has become a bottleneck for the development of the *Panax notoginseng*, so the objectives of this study were to compare the composition and diversity of the inter-root microbial communities of *Panax notoginseng* in ten

cultivation areas. Characterization of the pathogenic and probiotic flora of these different areas can guide microbial approaches to control root rot and improve the quality and yield of *Panax notoginseng* for improved industrial production.

Materials And Methods

Sampling locations

Yunnan Province is the major growing area of *Panax notoginseng* in China, so inter-root soil samples were collected from eight separate cultivation areas in Yunnan Province, as well as one each from cultivation areas in Guangxi Province and Guizhou Province, for a total of 10 locations (Table 1). The *Panax notoginseng* cultivated in these areas were all the same variety, and all plants had been cultivated for three years using the same cultivation methods. The longitude and latitude data of these producing areas were obtained from the "National geographic information resource directory service system" website and ArcGis 2.0 software was used to obtain the climate factor data for these sites, as listed in Table 1.

Table 1
Basic information of *P. notoginseng* growing areas

Number	Producing area	Environmental Factor							
		bio1	bio2	bio3	bio4	bio5	bio6	bio7	bio8
BS	Laojiezi Village, Pupiao Town, Longyang District, Baoshan City, Yunnan Province	14.9	23.1	48	11.2	75	99.19	25.54	1388
GX	Liangbiao Village, Xinjing Town, Jingxi City, Guangxi Province	19.9	22.2	36	8.2	84	106.43	23.09	720
GZ	Tiechang Village, Danxia Town, Pangzhou City, Guizhou Province	14.8	23.8	39	9.4	82	104.55	25.64	1783
KM	Changhu Town, Shilin County, Kunming City, Yunnan Province	15.2	22.5	46	10.4	84	103.24	24.42	1905
LX	Xiaosama Village, Xiangyang Township, Luxi County, Honghe Prefecture, Yunnan Province	17.6	23.2	45	10.6	79	103.88	24.33	2078
QJ	Dawulong Villager Group, Wulong Village, Caiyun Town, Shizong County, Qujing City, Yunnan Province	14.3	23.1	43	10.1	84	103.98	25.6	1868
WS	Baishapo Village, Kaihua Town, Wenshan City, Wenshan Prefecture, Yunnan Province	17.4	20.7	42	8.7	78	104.13	23.41	1390
XC	Lin Anchong Village, Wangjiatang Village Committee, Xichou County, Wenshan Prefecture, Yunnan Province	17.0	20.7	40	8.4	82	104.49	23.32	1073
XSBN	Xing Volcano, Mengman Town, Menghai County, Xishuangbanna Prefecture, Yunnan Province	19.4	23.3	53	12.4	84	100.1	21.56	1805
YS	Huilong Base, Huilong Community, Pingyuan Town, Yanshan County, Wenshan Prefecture, Yunnan Province	17.2	21.7	45	9.9	80	103.7	23.75	1476
bio1: annual mean temperature (°C), bio2: annual difference in temperature (°C), bio3: isothermality (%), bio4: daily difference in mean temperature (°C), bio5: seasonal variation of precipitation (%), bio6: longitude (°E), bio7: latitude (°N), bio8: altitude (m)									

Sample Collection

Soil samples were randomly collected from multiple locations at each site and then mixed/homogenized. Healthy and disease-free plants with uniform growth were selected, and soil was collected 0-0.5 cm away from the root/ rhizome as the rhizosphere soil sample. The soil samples of *Panax notoginseng* from each area were collected and put into self-sealing bags and then stored in the freezer at - 20 °C in the laboratory before sample processing.

Dna Extraction And Sequencing

According to the manufacturer's instructions, the MN NucleoSpin 96 Soil kit was used to extract DNA from samples. The soil fungal ITS1 regions were amplified with primers of 5'-CTTGGTCA TTTAGAGGAAGTAA-3' and 5'-GCGCGTTCTT-CATCGATGC-3', and the soil bacteria V3-V4 regions of the 16S rRNA gene were amplified using primers 5'-CTTGGTCA TTTAGAGGAAGTAA-3' and 5'-GCGCGTTCTTCATCGATGC-3'. Sequencing was completed by Beijing Biomarker Technologies Co., Ltd. using the Illumina MiSeq sequencing platform.

Data analysis

The sequencing data were analyzed using the cloud platform of Biomark Technology. Briefly, analysis included effective tag acquisition, OTU clustering, alpha diversity analysis, beta diversity analysis, species richness analysis, and redundancy of correlation analysis. Using Flash V1.2.7, the software performs overlapping PCR assembly on the reading length of each sample to obtain the original label. Trimmomatic V0.33 software was used to filter the assembled original labels to obtain high-quality clean labels, Uchime V4.2 software was used to identify and remove chimeras, and uclust in QIIME V1.8.0 software was used to cluster the effective tags with 97% similarity and then classify and label the operational classification units (OTUs). The representative sequences of OTUs were compared with the microbial reference database to obtain the taxonomic information of species corresponding to each OTU. Next, the community composition of each origin sample was determined at multiple levels (phylum, class, genus), and species abundance tables at different taxonomic levels were generated using QIIME software. Histograms of species distribution of samples at each taxonomic level were plotted using R language tools to determine the similarity of species abundance among samples. Using Mothur V1.3.0 software, the species richness index (Chao1) and the species diversity index (Shannon) were determined for bacterial and fungal communities of the different samples. Additionally, the species diversity of the inter-root microbial community of *Panax notoginseng* of a single origin was studied by alpha diversity analysis. Using QIIME software, principal coordinate analysis (PCoA) was carried out according to Bray Curtis distance, and principal component analysis (PCoA) plots were drawn using R language tools to compare the magnitude of differences in species diversity (community composition and structure) among the different groups through beta diversity analysis. In addition, linear discriminant analysis (LDA) effect size (LEfSe) was used to detect significantly different taxa with differential abundances (LDA scores greater than 3.0, $P < 0.01$). Microbial community with statistically significant differences between groups were identified by between-group difference significance analysis. One-way analysis of variance (ANOVA) was performed using SPSS (20.0) software to test for significant differences in the relative abundances of taxonomic taxa, and a value of $P < 0.05$ was considered statistically significant. Redundancy analysis (RDA) was used to assess the effects of environmental factors on the composition of inter-rooted microorganisms, to distinguish potential influences of temperature, precipitation, and latitude and longitude.

Results

To investigate potential microbial variation in different cultivation sites, inter-root soil samples were collected from eight separate *Panax notoginseng* cultivation areas in Yunnan Province, as well as one sample each from cultivation areas in Guangxi Province and Guizhou Province, for a total of 10 sampling locations (Table 1).

Driving factors of the *Panax notoginseng* rhizosphere microbial community

Redundancy analysis (RDA) showed that there was a correlation between rhizosphere soil microorganisms and environmental factors of climate, longitude, latitude and altitude. The RDA results are presented in Fig. 1. Solid arrow rays represent different environmental factors. The longer the ray, the greater the influence degree of the factor. A blue dashed arrow ray indicates the affected species. The relationship between rays is represented by the angle, where an acute angle represents a positive correlation and an obtuse angle represents a negative correlation. Rda1 and rda2 axes represent the two variables with the largest degree of interpretation, and the abscissa and ordinate values respectively represent the degree of interpretation of the two ranking axes to the environment. The greater the sum of the two, the greater the ability to explain the environmental community structure and species distribution.

Ten genera of fungi were related to environmental factors: *Chaetomium*, *Cladosporium*, *Fusarium*, *Ilyonectria*, *Mortierella*, *Plectosphaerella*, *Penicillium*, *Pseudonymnoascus*, *Tetracladium*, and *Trichoderma*. RDA showed that the isotherm of bio3 and the daily range of average temperature of bio4 had the greatest impact on the rhizosphere fungal community. *Mortierella*, *Trichoderma*, and *Ilyonectria* were positively correlated with bio3 and bio4, and the other seven genera were negatively correlated with these two factors. The first axis could explain 23.8% of all information, the second axis could explain 17.5%, for a cumulative amount of interpretation information of 41.3% (Fig. 1a).

Ten genera of bacteria were related to environmental factors, including *Sphingomonas*, *Pseudomonas*, *Novosphingobium*, *Rhodanobacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Sphingobium*, *Lelliottia*, *Arthrobacter*, and *Streptomyces*. RDA showed that the annual average temperature of bio1 and the latitude of bio7 had the greatest impacts on the rhizosphere bacterial community. *Sphingomonas* was negatively correlated with bio1 and positively correlated with bio7, and the other nine genera were positively correlated with bio1 and negatively correlated with bio7. The first axis can explain 26.9% of all information and the second axis can explain 18.8%, for a cumulative amount of interpretation information of 45.7% (Fig. 1b). Therefore, the first two axes well reflect the relationship between diseases and soil factors.

Fungal Community Composition

We sequenced the fungal ITS1 region in the 30 soil samples (three samples from each of the ten production areas) and obtained a total of 9,259,728 pairs of reads. After paired end comparison, mass filtration, and chimera deletion, about 250,000 effective tags were generated for each sample, and 431 fungal OTUs were identified, with a sequence similarity of 97%. The number of fungal OTUs detected for BS, GX, GZ, KM, LX, QJ, WS, XC, XSBN and YS were 158, 205, 292, 201, 314, 164, 284, 259, 302 and 158, respectively. The distributions of fungal OTUs were evaluated at different classification levels.

The sequences revealed three main fungal phyla, Ascomycota (52.56% ~ 97.75%), Mortierellomycota (0.13%~ 39.72%), and Basidiomycota (0.32%~4.98%), accounting for more than 75%. However, the ten samples differed in the relative abundances of these major fungal species. Ascomycota was the highest content category in the ten groups, and its relative abundance Xsbn was significantly lower than that in other groups (ANOVA, $P < 0.05$). The relative abundance of Mortierellomycota was significantly higher in QJ than that in other groups (ANOVA, $P < 0.05$). There were significant differences in the relative abundances of Basidiomycota in GZ and other groups (ANOVA, $P < 0.05$) (Fig. 2a). At the class level, the highest average relative abundances were Sordariomycetes (69.90%), Mortierellomycetes (10.16%), and Leotiomycetes (6.09%), with Sordariomycetes the dominant class in all ten groups (Fig. 2b). At the genus level, *Chaetomium* was the most abundant genus in the ten groups, but exhibited a different distribution in each group, with significantly higher relative abundance of *Chaetomium* in GX compared to that in other groups (ANOVA, $P < 0.05$). There were other differences, with *Plectosphaerella* significantly higher in XC than in other groups (ANOVA, $P < 0.05$), *Mortierella* significantly higher in QJ (ANOVA, $P < 0.05$), and *Ilyonectria* and *Fusarium* more abundant in WS (ANOVA, $P < 0.05$) (Fig. 2c).

Bacterial Community Composition

We sequenced the V3-V4 region of bacterial 16SrRNA in the 30 soil samples (three samples from each of the ten production areas) and obtained a total of 5,300,226 reads. After assembly and filtration, paired end alignment, mass filtration, and deletion of chimeras, an average of 120,000 effective tags were generated for each sample. A total of 1570 bacterial OTUs were identified, with a sequence similarity of 97%. The numbers of bacterial OTUs detected were 1400, 1397, 1418, 1376, 1346, 1409, 1359, 1302, 1210 and 1217 for BS, GX, GZ, KM, LX, QJ, WS, XC, XSBN and YS, respectively. The distributions of bacterial OTUs were analyzed at different classification levels.

The sequencing revealed ten main bacterial phyla, accounting for 99% of the whole bacterial community. Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant, accounting for respectively 37.81% ~ 85.42%, 2.44% ~ 40.21%, and 1.33% ~ 21.57% of the total sequences of the groups. Actinobacteria exhibited the highest abundance in GX, with a significantly higher relative abundance in GX and a significantly lower abundance in LX (ANOVA, $P < 0.05$). Of the other groups, proteobacteria is the most abundant, and was highest in LX ($P < 0.05$) (Fig. 2d). At the class level, Gammaproteobacteria (28.35%), Alphaproteobacteria (25.88%), Actinobacteria (13.90%), and Acidobacteria (6.97%) were the most abundant (Fig. 2e). *Arthrobacter* was identified as the most abundant genus for the ten sites, but the distribution varied for the different locations. The abundance of

Arthrobacter was significantly higher in GX than in other locations (ANOVA, $P < 0.05$) and that of Sphingomonas was significantly higher in KM than in other locations (ANOVA, $P < 0.05$). The relative abundance of Burkholderia-caballeronia-paraburkholderia was significantly higher in LX than that in other groups (ANOVA, $P < 0.05$). The relative abundance of Sphingobium was significantly higher in XC than that in other groups (ANOVA, $P < 0.05$) and that of Pseudomonas was significantly higher in WS than that in other groups (ANOVA, $P < 0.05$) (Fig. 2f).

Diversity Analysis Of Fungi And Bacteria

To investigate the diversity at the different sites, the species richness (Chao1 index) and diversity (Shannon index) of soil rhizosphere microorganisms were calculated for the ten cultivation areas, as shown in Fig. 3. The difference in the Chao1 index and Shannon index results may be due to the uneven distribution of species. The Chao1 index measures species abundance, or the number of species, and the Shannon index measures species diversity. The fungi species richness was relatively high for GZ, LX, and XSBN and relatively low for BS, QJ, and YS. The diversity of fungi in the rhizosphere soil samples significantly varied for the different producing areas. Compared with other groups, the species diversity level of XSBN was the highest and that of GX was the lowest (ANOVA, $P < 0.05$). Between BS and YS α , there was no significant difference in diversity (ANOVA, $P > 0.05$) (Fig. 3a). For bacteria, the species richness of YS was significantly lower than that of other regions, and the diversity level of QJ was significantly higher than that of other regions (ANOVA, $P < 0.05$) (Fig. 3b). Overall, the Chao1 and Shannon index values were significantly higher for bacteria than for fungi (ANOVA, $P < 0.01$).

The differences in fungi and bacteria from different producing areas were compared by PCoA analysis of Bray Curtis distance matrix. According to the PCoA diagram, the ten sites were roughly divided into four quadrants according to the diversity of fungi, with obvious separation. YS is the only member of its group; BS, XC, WS, LX, and GZ formed a group; GX and KM formed a group; and XSBN and QJ were grouped. About 51.45% of the observed changes can be explained by the first two principal coordinates (Fig. 4a). The PCoA diagram of bacteria is presented in Fig. 4b. The 10 locations were roughly divided into four groups according to bacterial diversity, with obvious separation between the groups. Among them, BS, QJ, GX, and KM formed a group; XSBN and YS clustered as a group; GZ, WS, and XC formed a group; and LX is the sole member of a separate group. About 53.04% of the observed changes can be explained by the first two principal coordinates (Fig. 4b). In conclusion, the PCoA diagrams of fungi and bacteria allow the grouping of samples from the ten producing areas, indicating differences in the rhizosphere microbial community in the different producing areas.

Linear Discriminant Analysis (Lda) Effect Size (Lefse) Analysis Of Microbial Community

To obtain more information about the variation in rhizosphere bacterial and fungal communities, we used LEfSe (Linear discriminant analysis effect size) to identify differential abundance taxa with LDA scores higher than 3.0 or 4.0 in the ten locations. The circle in the evolutionary cladistic diagram represents the classification level from phylum to species, moving from inside to outside. The diameter size of the small circles is proportional to the relative abundance size, different colors indicate different subgroups, and different colored nodes indicate microbial groups that play an important role in the subgroup represented by that color.

LEfSe analysis of rhizosphere fungi with LDA scores higher than 3.0 showed 190 significantly different abundant taxa for the ten sites. Among them, 58 groups have different abundances in XSBN, especially Archaeorhizomycetaceae, Desmazierella, Nigrospora, Sporobolomyces, and Saitozyma. The most abundant fungal groups in GX were Chaetomium, Endophora, and Apiotrichum; Hannaela and Papiliotrema were obviously enriched in KM; Staphylotrichum-coccosporum was significantly enriched in LX; Mortierella-samyensis and Mortierella-alpina were obviously enriched in QJ; Barnettozyma was obviously enriched in WS; Plectosphaerella, Pseudoeurotiaceae, Tetracladium, and Cladosporium were enriched in XC; and Mucor, Guehomyces, and Minimedusa were obviously enriched in YS (Fig. 5a).

LEfSe analysis of rhizosphere bacteria with LDA scores higher than 4.0 identified 163 significantly different abundant taxa for samples from the ten sites. Among them, 32 groups were present at different abundances in YS, including Elsterales and Micropepsales, Acidobacteriales, uncultured-bacterium-c-TK10, uncultured-bacterium-c-AD3, and uncultured-bacterium-p-WPS-2. The most abundant bacterial taxa in BS, GX, LX, WS, and XSBN, respectively, were Gaiellales, Micrococcaceae, Burkholderia-

caballeronia-paraburkholderia, Xanthomonadaceae, and Subgroup-2. Streptomycetales, Bacillales and Lelliottia were significantly enriched in GZ; Caulobacterales and Sphingomonas were significantly enriched in KM; Gemmatimonadales, Ktedonobacterales and uncultured-bacterium-c-subgroup-6 were significantly enriched in QJ; Rhizobiaceae and Flavobacteria were significantly enriched in XC (Fig. 5b).

Pathogenic And Beneficial Fungal And Bacterial Abundances

We further evaluated the pathogenic fungal genera (Plectosphaerella, Ilyonectria, Fusarium, Penicillium), pathogenic bacterial genera (Sphingomonas, Sphingobium, Pseudomonas, Lelliottia), and beneficial fungal genera (Chaetomium, Mortierella, Trichoderma, Pseudogymnoascus) of the inter-rhizosphere soil samples from the ten sites. We also identified the beneficial fungal genera (Chaetomium, Mortierella, Trichoderma, Pseudogymnoascus), and beneficial bacterial genera (Arthrobacter, Burkholderia-Caballeronia-Paraburkholderia) in terms of relative abundances (Table 2). The most abundant fungus was Plectosphaerella (mean abundance 16.81%), and the abundances of pathogenic bacteria Sphingomonas, Sphingobium, Pseudomonas, and Lelliottia did not vary significantly (ANOVA, $P > 0.05$). Ilyonectria, Fusarium, and Pseudomonas were significantly higher in abundance in WS than the other sites (ANOVA, $P < 0.05$). The average abundance of beneficial fungus Chaetomium was as 19.65%, and abundances of Chaetomium and Arthrobacter were significantly enriched in GX (ANOVA, $P < 0.05$). At YS, the presence of five pathogenic bacteria was almost negligible (ANOVA, $P \leq 0.05\%$), but the abundances of beneficial genera were relatively high.

Table 2
Relative abundance of pathogenic and beneficial bacteria in ten origins(%)

Producing area		BS	GX	GZ	KM	LX	QJ	WS	XC	XSBN	YS
Pathogenic fungal genera	Plectosphaerella	19.18	7.99	16.98	22.42	38.60	0.21	11.89	46.20	4.60	0.02
	Ilyonectria	0.01	0.83	7.22	0.36	3.67	0.53	15.70*	8.77	8.30	0.31
	Fusarium	4.46	0.78	5.58	0.10	1.66	0.50	24.24*	0.62	0.22	0.02
	Penicillium	0.54	2.03	1.75	0.27	0.69	0.75	0.81	0.63	1.41	2.77
Pathogenic bacterial genera	Sphingomonas	7.43	4.12	1.88	12.95	4.48	5.73	0.97	2.45	3.17	1.94
	Sphingobium	0.66	6.14	2.00	3.79	8.66	0.27	3.40	9.37	1.77	0.01
	Pseudomonas	0.16	0.79	8.29	0.92	9.53	0.23	11.78*	3.02	0.46	0.05
	Lelliottia	0.02	0.11	12.45	0.21	7.20	0.03	7.45	2.25	0.04	0.01
Beneficial fungal genera	Chaetomium	0.16	69.40*	0.50	49.07	0.05	22.00	0.20	0.45	0.03	54.68
	Mortierella	0.37	0.54	0.31	8.29	4.76	39.72	0.13	1.10	17.81	28.53
	Trichoderma	4.14	0.08	9.77	0.06	8.84	0.42	1.59	0.09	8.99	4.84
	Pseudogymnoascus	4.99	0.18	0.46	0.04	0.03	4.70	0.12	10.34	0.01	0.01
	Arthrobacter	2.84	31.91*	9.16	1.38	0.32	0.57	6.40	2.91	0.25	0.23
Beneficial bacterial genera	Burkholderia-Caballeronia-Paraburkholderia	0.35	0.49	3.01	0.62	12.53	0.18	1.14	3.76	11.50	4.27
*Significant at the 0.05 probability level.											

Discussion

Variation in rhizosphere microbial community structure for different cultivation areas

Analysis of the fungal and bacterial community composition of rhizosphere samples from ten *Panax notoginseng*-producing areas revealed similar species, but there were differences abundance of some dominant flora. These findings suggest that different habitats affect the composition of the rhizosphere microbial community. For example, *Arthrobacter* was enriched in GX and *Pseudomonas* was enriched in WS. Fu et al. also identified[27] *Pseudomonas* and *Arthrobacter* as dominant flora in the rhizosphere soil of *Panax notoginseng*. Interestingly, the fungus *Tetracladium* was significantly enriched in XC, and has been shown to be beneficial to the host[28]. The probiotics *Pseudourotiaceae* and *Cladosporium* were also significantly enriched in XC. The results reveal significant differences in the rhizosphere soil microorganisms of different *Panax notoginseng* production areas. Microbial diversity can be affected by many factors, such as plant varieties[29], planting years[30], and environmental factors[31]. In this study, all soil samples were collected from areas where the same variety of *Panax notoginseng* was cultivated for three years, so the influence of plant varieties and planting years can be excluded. Therefore, the observed rhizosphere microbial diversity may be explained by environmental factors. Yao et al. also found that microbial diversity was affected by local environmental conditions[32]. RDA showed that more than 40% of the overall changes in fungal and bacterial community composition were explained by environmental factors. The seasonal variation of precipitation exhibited little impact on the microbial community structure, consistent with little variation in this parameter for the test locations. In addition, the number of bacterial OTUs was higher than that of fungi for all producing areas, with higher Chao1 and Shannon index values for bacteria than those for fungi. Compared with fungal communities, bacterial communities may be more resistant and resilient to environmental interference [33]. There are multiple explanations for this phenomenon. One is that bacteria metabolize a series of compounds, indicating that the bacterial community is relatively stable [34]. Additionally, the growth rate of bacteria is usually faster than that of fungi, which may also lead to relatively stable bacterial colonies [35].

Rhizosphere microorganisms related to root rot of *Panax notoginseng*

Microbial diversity and richness play key roles in the sustainable development of soil health, ecosystem function, and plant growth [36]. Changes in the inter-root microbial community are thought to be the main cause of the high mortality of *Panax notoginseng* in continuous cropping systems[37]. Typically, a richer microbial community composition has a greater ability to suppress pathogens[38]. Highly diverse microbial communities have stronger functional redundancy and transboundary associations[39, 40]. Reduced microbial diversity was found in the inter-root soil of diseased *P. cytonema*[41] and banana[42], and higher microbial diversity was found in healthy sugar beets[43] and peppers[44]. Reduced diversity also contributes to the invasion of potentially pathogenic bacteria and fungi into soil- or plant-associated microbial communities[45].

In this study, we detected fungi and bacteria including root rot pathogens and beneficial microorganisms that control disease and promote nutrient supply. This information is valuable for the development of sustainable disease management strategies, such as those using biocontrol agents[46]. The rhizomes are used for many medicinal plants, and 70% of these plants are limited by continuous cropping [47]. WS is a main production area for *Panax notoginseng*, with a cultivation history of at least 400 years[48], with a growth cycle that is generally at least three years. A large amount of chemical fertilizers and pesticides are applied during cultivation, and this can cause continuous cropping soil diseases, such as an imbalance of the microbial community, allelopathy, and self-toxicity[49]. Long-term continuous cropping can also change soil microbial diversity[50, 51]. Our test results confirmed significantly higher abundances of root rot pathogens *Ilyonectria*, *Fusarium*, and *Pseudomonas* in WS than those in other producing areas, which suggests that the long-term cultivation of WS has increased the risk of disease. Wen[52] and Wang et al.[53] pointed out that *Fusarium* is the main pathogen causing *Panax notoginseng* root rot, acting to infect the vascular system, destroy transport tissues, and cause wilting and death to reduce yield and quality. We also detected beneficial microorganisms *Chaetomium* and *Arthrobacter*, which can promote the healthy growth of *Panax notoginseng*. Li et al. found that the enrichment of *Arthrobacter* in soils with high yield may contribute to the increase of *Panax notoginseng* yield[54]. Therefore, the regulation of rhizosphere microbial community may help overcome the obstacles of continuous cropping and can improve the yield of medicinal plants by improving the soil environment. Beneficial microorganisms do not directly act on pathogenic bacteria to reduce the occurrence of root rot, but increase the number of other beneficial microbial flora in the soil to antagonize pathogenic microorganisms and reduce disease[55]. Currently, there is no effective means to control continuous cropping obstacles, but some measures can be taken to reduce the occurrence of *Panax notoginseng* root rot. In particular, the application of antagonistic microbial agents may be part of a safer and environmentally friendly biological control strategy[56].

Conclusion

The results of this study show that there are differences in the structure of rhizosphere soil microbial community of *Panax notoginseng* in different producing areas. These differences in the composition and diversity of the rhizosphere microbial community may affect the quality of *Panax notoginseng*. Additionally, some rhizosphere microbes may be related to rhizosphere rot. The results of this work should be beneficial to the agricultural development of *Panax notoginseng* and can provide a theoretical basis for the control of diseases and pests during cultivation of this important plant. Although chemical pesticide applications can be used to treat root rot, these applications may lead to pesticide residue problems that reduce the safety of herbal medicines. Future research should study the structure and function of rhizosphere soil microorganisms to determine strategies to regulate the crop rhizosphere habitat to ensure high yield and high quality for continued sustainable development of *Panax notoginseng*.

Declarations

Author Contributions: Yang Lou wrote the main manuscript text. Zilong Zhang directed the design of experiments and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest: The authors declare no competing interests.

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Figures

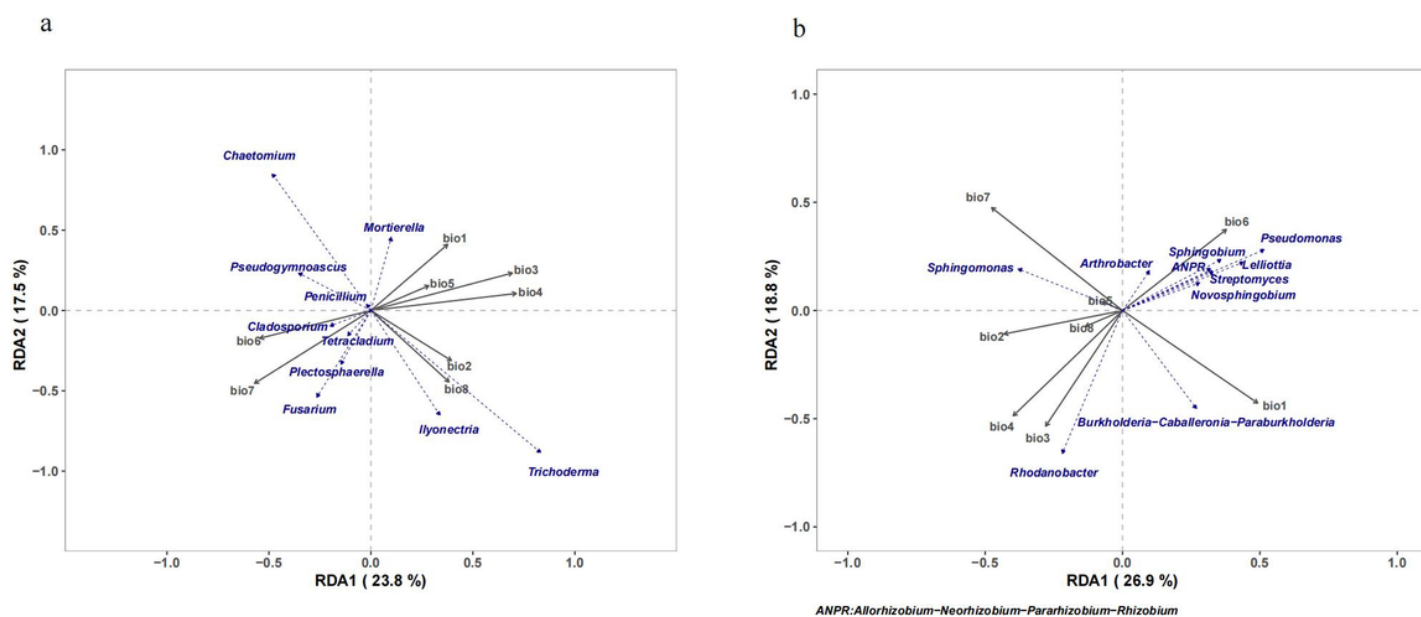


Figure 1

RDA diagram of inter-root microorganisms and environmental factors. (a) and (b) represent the fungal and bacterial communities, respectively

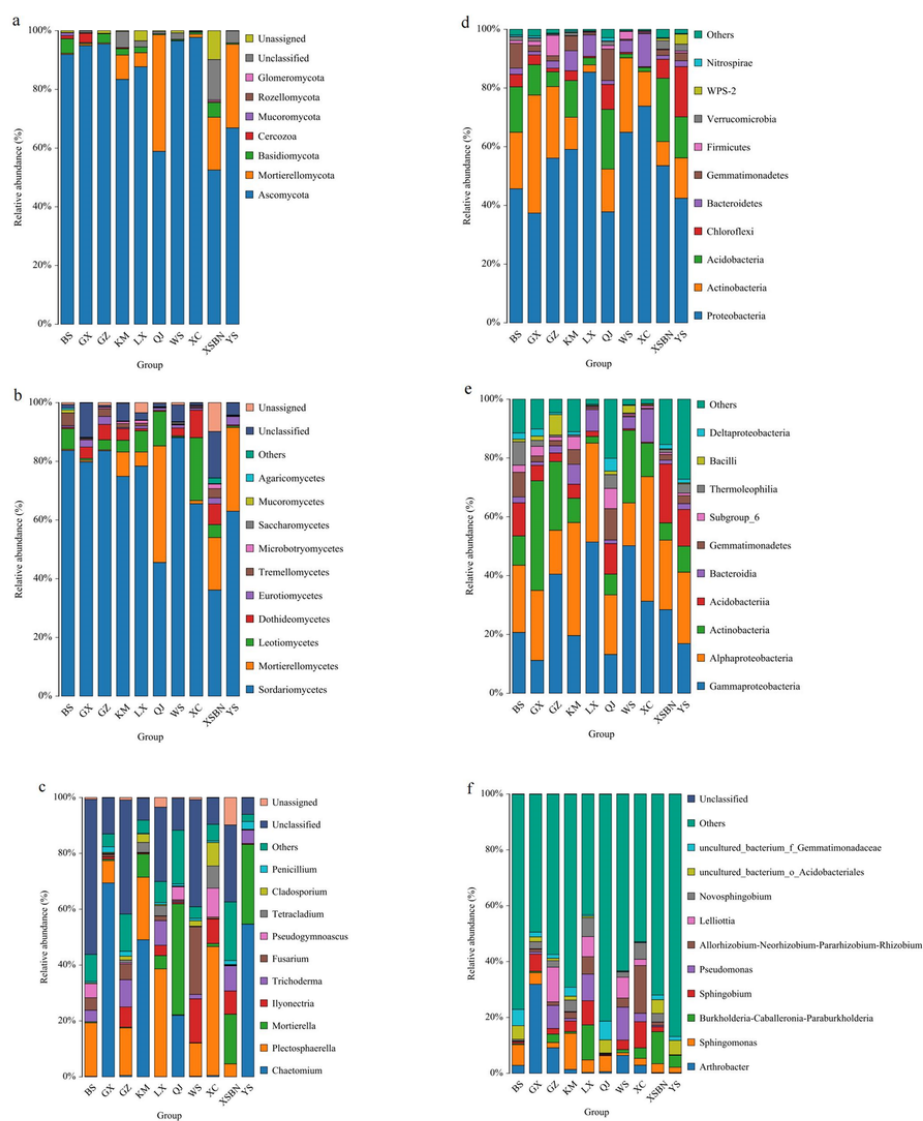


Figure 2

Composition and structure of fungal and bacterial communities in ten *P. notoginseng* origins. (a), (b) and (c) represent phylum, class and genus level of fungi, respectively. (d), (e) and (f) represent phylum, class and genus level of bacteria, respectively. The top 10 relative abundances are shown, and the remaining abundances are indicated as 'Others'

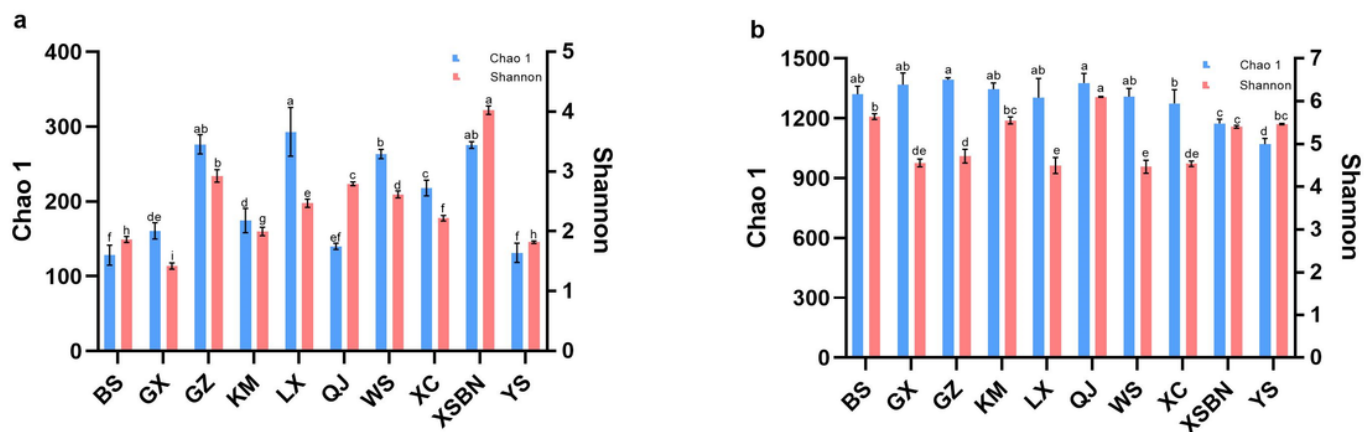


Figure 3

The Chao 1 (species richness) and Shannon (species diversity) index values of fungal (a) and bacterial (b) communities for the ten *P. notoginseng* sites. Different lower case letters in the figure indicate significant differences ($P < 0.05$) in the numbers of sequences between origins.

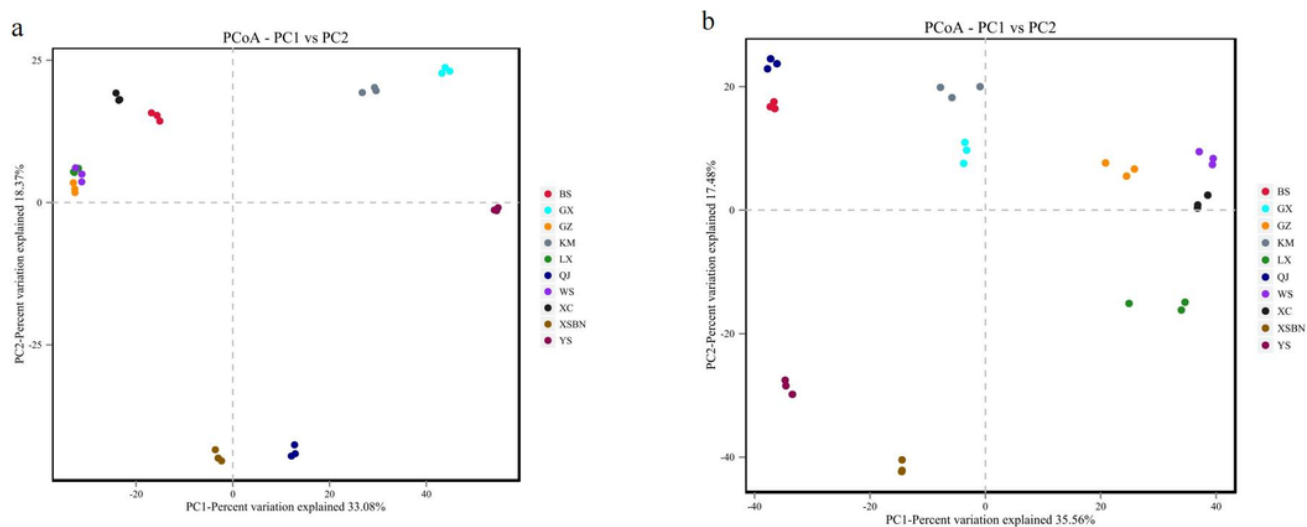


Figure 4

PCoA diagram of fungi (a) and bacteria (b) from different origins

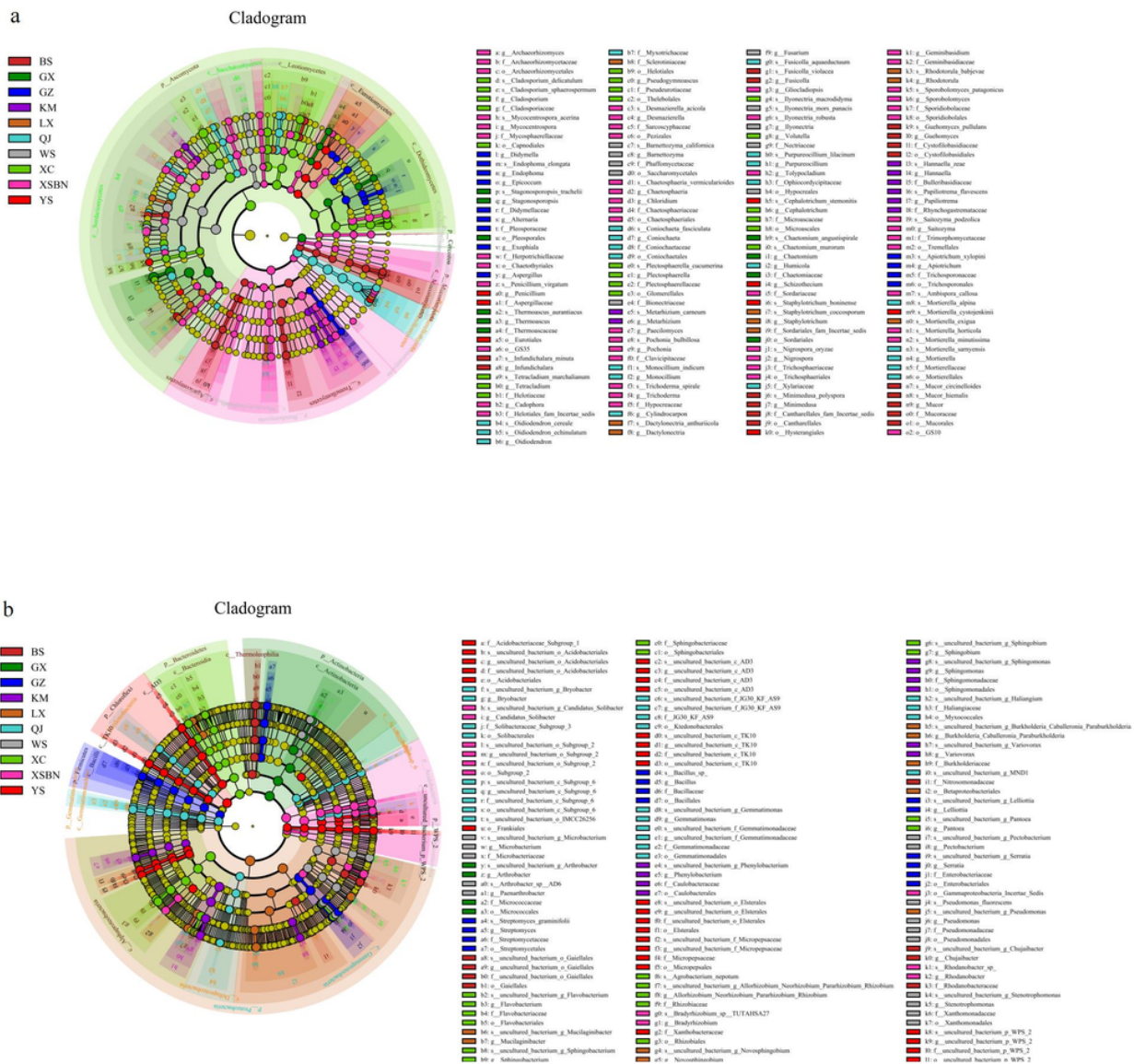


Figure 5

LEfSe analysis showing the different taxon among *P. notoginseng* origins rhizospheres for fungi (a) and bacteria (b)