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DIFFERENCES IN PRODUCTION AREA AFFECT BACTERIAL AND FUNGAL COMMUNITY STRUCTURE IN *PANAX NOTOGINSENG* RHIZOSPHERE

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Abstract: *Panax notoginseng* is a well-known Chinese herb that is used worldwide. Studies on the factors affecting the quality of *Panax notoginseng* have mainly focused on the physical and chemical properties of the soil, with little reference to the differences in the microbial structure of different production areas. 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. Among all samples, the beneficial fungus Chaetomium was the most abundant with an average abundance of 19.65%. 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 results of this study show that there are differences in the structure of rhizosphere soil microbial community of *Panax notoginseng* in different producing areas and 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.
Keywords: *Panax notoginseng*; Microbial community; Rhizosphere; Root rot; Regional differences

1 INTRODUCTION

Sanqi [*Panax notoginseng* (Burkill) F. H. Chen] is a valuable Chinese herbal medicine endemic to Southwest China, which can be used for the treatment of cardiovascular diseases, inflammation, various kinds of body pains, internal and external bleeding caused by wounds and injuries [1], and is popular within the global market. Due to the special growing environment of Panax ginseng, there are fewer wild resources, and it mainly relies on cultivation to meet the market demand. Currently, the cultivation area of Panax pseudoginseng has been expanded from Wenshan City, Yunnan Province, a localized production area, to ten regions, including Jingxi City, Guangxi Province, and Panzhou City, Guizhou Province. In the process of cultivation, it was found that long-term large-scale intensive cultivation of *Panax notoginseng* made the continuous soil diseases increasingly serious, which seriously affected the quality of *Panax notoginseng* [2,3]. Among them, the soil-borne disease root rot has caused the most serious damage to panax pseudoginseng in cultivated areas in China. Root rot prevents plant roots from absorbing and transporting nutrients such as water, CO_2 and inorganic salts in the soil, thus affecting the healthy growth of plants. The field symptoms of the disease often manifest in the early stage of the aboveground part of the plant with incorrect leaf color, wilting of leaves, yellowing and shedding of leaves and rotting of the underground part of the plant, which can lead to a 5-70% reduction in yield or even extinction of the harvest. Therefore this also hinders the industrialization of this valuable medicinal herb[4].

Inter-root of medicinal plants is a special micro-ecosystem of plant-soil-microbe interaction[5]. The plant root system provides nutrients for the inter-root microorganisms to meet their growth needs by secreting secretions such as sugars, proteins, organic acids, and a variety of secondary metabolites, which in turn affects the composition and diversity of the inter-root microbial community. At the same time, inter-root microorganisms can also affect plant growth by synthesizing various hormones and compounds or improving the soil environment[6,7].. Some studies have shown that the accumulation of harmful soil-borne pathogens is an important factor contributing to root rot. the study by Lixia Tian et al. found that increased relative abundance of the pathogenic fungi Gibellulopsis and Gibberella was associated with the onset of root rot in western ginseng. Meanwhile, a study reported that root rot disrupted the P. cyrtonema rhizosphere microbial community and reduced diversity [8], suggesting that root rot affects the inter-root microbial community and function. There are a variety of microorganisms in the inter-root soil, including beneficial, harmful and

neutral microorganisms, which may interact with Panax pseudoginseng roots and thus affect the growth and health of *Panax pseudoginseng* [9]. Root rot disease has become a bottleneck for the development of the *Panax notoginseng.* The effective components and quality of medicinal materials cultivated in different regions can vary, and this difference is closely related to rhizosphere microorganisms [10]. At present, the factors affecting the quality of *Panax notoginseng* mainly focus on climatic factors and soil factors, in which the physical and chemical properties of soil are the main research factors, and the research on soil microbial diversity is less. The effective components and quality of medicinal materials cultivated in different regions can vary, and this difference is closely related to rhizosphere microorganisms [10]. Microbial characteristics, such as inter-root microbial abundance, composition, and diversity, are potentially valuable indicators of soil quality [11]. Therefore, 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.

2 MATERIALS AND METHODS

2.1 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 physico-chemical properties of the soil from different origins are shown in Table 2. 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 (℃), bio2: annual difference in temperature (℃), bio3: isothermality (%), bio4: daily difference in mean temperature (℃),bio5: seasonal variation of precipitation (%), bio6: longitude (°E), bio7: latitude (°N), bio8: altitude (m)

Table 2 Physico-Chemical Properties of Soils of Different Origins

Number	pH	Organic matter (g/kg)	Alkaline-hydrolyzed N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
BS	6.59	25.63	139.42	15.76	212.94
GX	4.86	43.3	123.41	48.81	203.70
GZ	5.98	52.01	125.23	13.80	51.82
KM	5.50	33	158.20	34.20	110.97
LX	6.34	14.33	325.10	11.60	316.80
QJ	6.57	38.83	123.29	34.67	220.25
WS	5.49	16.19	58.27	94.87	129.95
XC	6.59	31.77	150.99	28.50	84.42
XSBN	5.51	48.43	71.21	2.88	189.47
YS	5.46	16.6	92.51	36.87	181.62

2.2 Sample Collection

Soil samples were collected in October 2019 using a five-point sampling method at each site of 1-2 acres in size 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℃ in the laboratory before sample processing.

2.3 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.

2.4 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. Using the species-sample data to do DCA analysis, the first axis data of Lengths of gradient in the analysis result is less than 3.0, and the RDA is selected 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.

3 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).

3.1 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 Figure 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, Tetraclaudium, 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% (Figure 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% (Figure 1b). Therefore, the first two axes well reflect the relationship between diseases and soil factors.

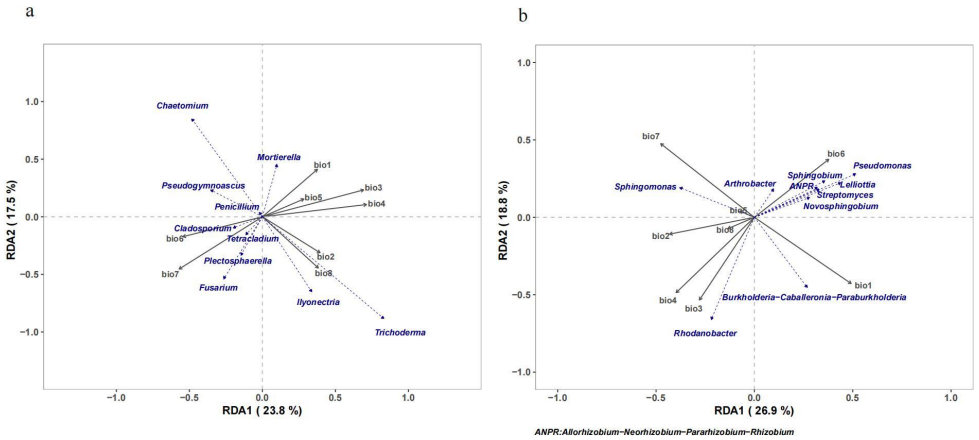

Figure 1 RDA Diagram of Inter-Root Microorganisms and Environmental Factors. (a) and (b) Represent the Fungal and Bacterial Communities, Respectively

3.2 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 diff ered 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 grou ps (ANOVA, $P < 0.05$).There were significant differences in the relative abundances of Basidiomycota in GZ and other groups (ANOVA, $P < 0.05$) (Figure 2a). At the class level, the highest average relative abundances were Sordariomycetes (69.90%), Mortierellomycetes (10.16%), and Leotiomycetes (6.09%), with Sordariomycete s the dominant class inall ten groups (Figure 2b). At the genus level, Chaetomium was the most abundant ge nus in the ten groups, but exhibited a different distribution in each group, with significantly higher relative ab undance of Chaetomium in GX compared to that in other groups (ANOVA, $P < 0.05$). There were other diffe rences, 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, $P < 0.05$) (Figure 2c).

3.3 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 ($P < 0.05$) (Figure 2d). At the class level, Gammaproteobacteria (28.35%), Alphaproteobacteria (25.88%), Actinobacteria (13.90%), and Acidobacteria (6.97%) were the most abundant (Figure 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, $P < 0.05$) (Figure 2f).

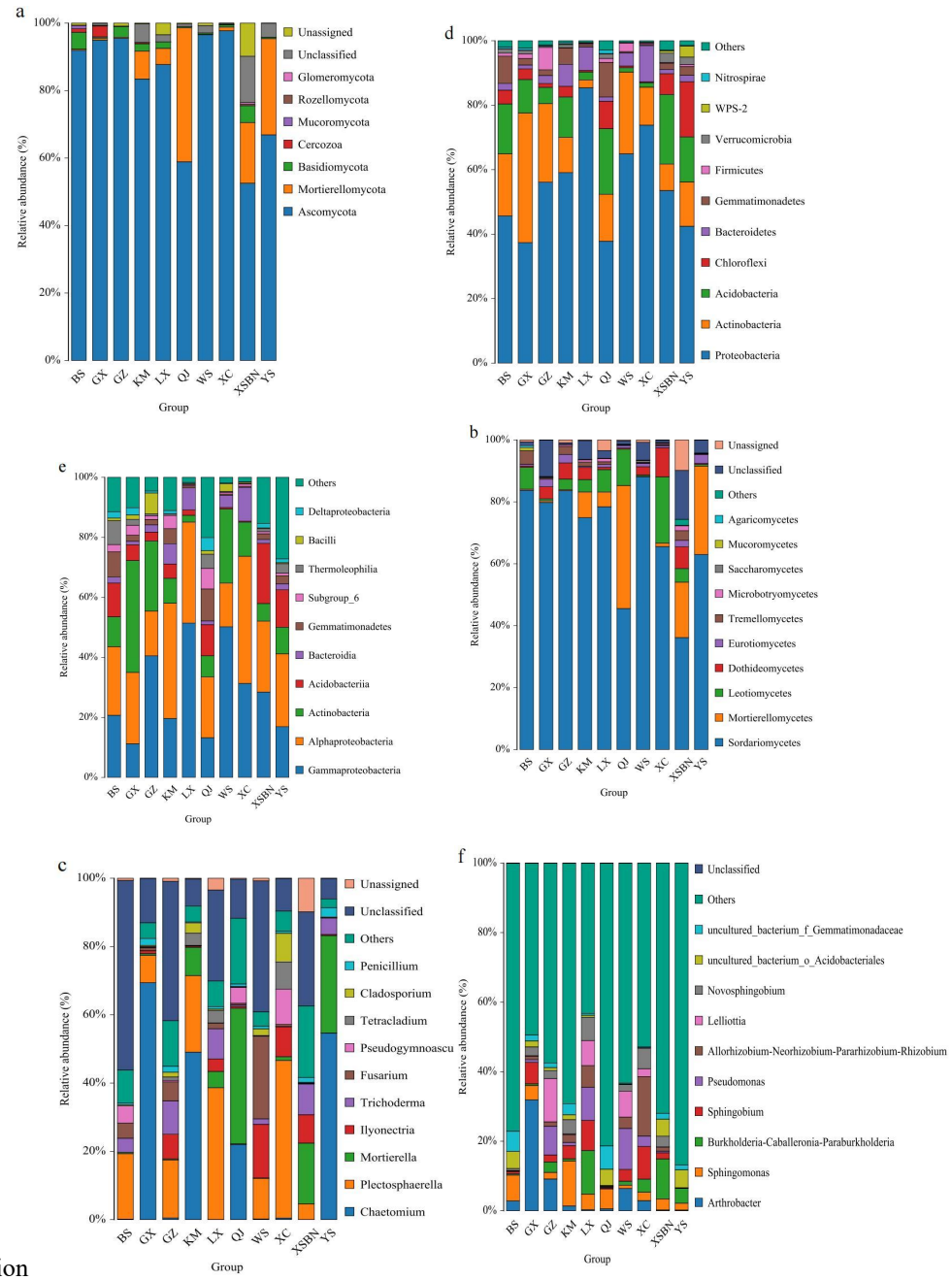

Figure 2 Composition and Structure of Fungal and Bacterial Communities in ten P. Notoginseng Origins. (a), (b) and (c) Represent Phylum, Class and Genus Level Of Fungi, Respectively. (d), (e) and (f) Represent Phylum, Classr and Genus Level of Bacteria, Respectively. The Top 10 Relative Abundances are Shown, and the Remaining Abundances are Indicated as 'Others'

3.4 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 Figure 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, $P > 0.05$) (Figure 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, $P < 0.05$) (Figure 3b). Overall, the Chao1 and Shannon index values were significantly higher for bacteria than for fungi (ANOVA, $P < 0.01$).

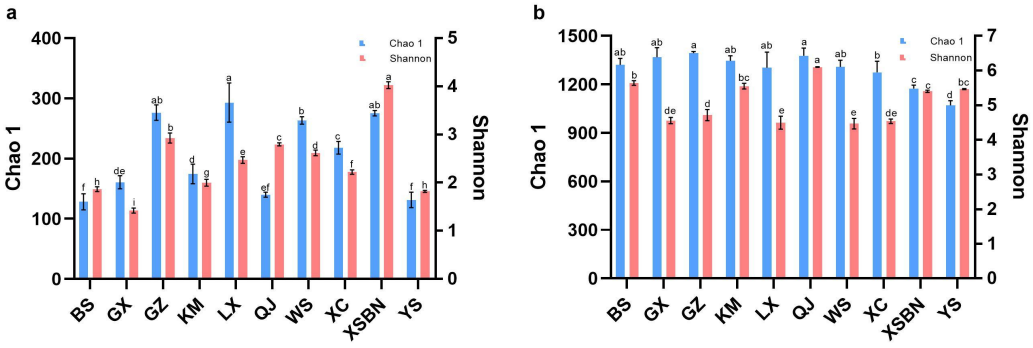

Figure 3 The Chao 1 (Species Richness) and Shannon (Species Diversity) Index Values of Fungal (a) and Bacterial (b) Communities for the Ten P. Notoginseng Sites. Different Lower Case Letters in the Figure Indicate Significant Differences (P <0.05) in the Numbers of Sequences between Origins

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 (Figure 4a). The PCoA diagram of bacteria is presented in Figure 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 (Figure 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.

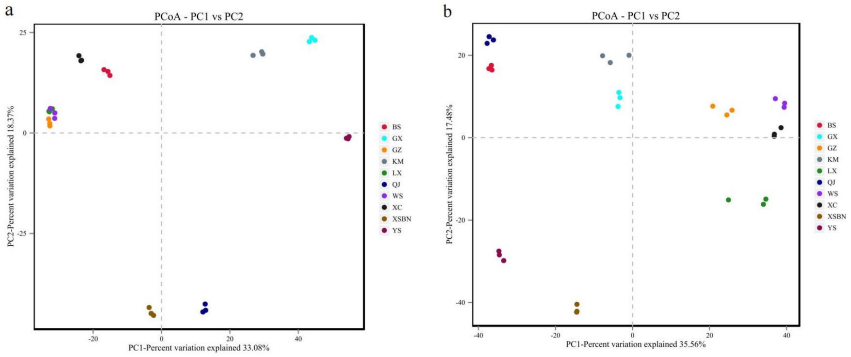

Figure 4 PCoA Diagram of Fungi (a) and Bacteria (b) from Different Origins

3.5 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; Staphylotrichun-coccosporum was significantly enriched in LX; Mortierella-samyensis and Mortierella-alpina were obviously enriched in QJ; Barnettozyma was obviously enriched in WS; Plectosphaerella, Pseudoeurotiaceae, Tetraclaudium, and Cladosporium were enriched in XC; and Mucor, Guehomyces, and Minimedusa were obviously enriched in YS (Figure 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-bac-terium-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. Streptomycethales, Bacillales and Leliottia 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 Falvobacteria were significantly enriched in XC (Figure 5b).

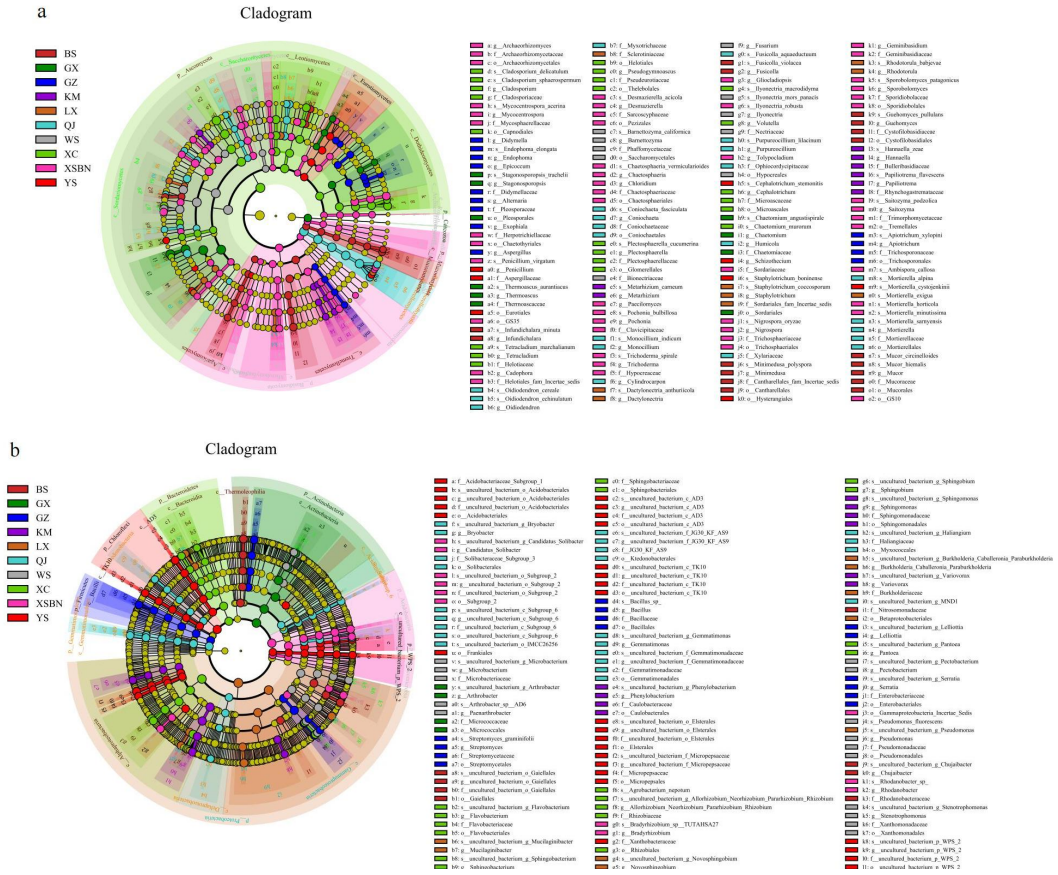

Figure 5 LEfSe Analysis Showing the Diferent Taxon among P. Notoginseng Origins Rhizospheres for Fungi (a) and Bacteria (b)

3.6 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 3). 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 3 Relative Abundance of Pathogenic and Beneficial Bacteria in Ten Origins(%)

Producing area	BS	GX	GZ	KM	LX	QJ	WS	XC	XSBN	YS

	Plectosphaerella	19.18	7.99	16.98	22.42	38.60	0.21	11.89	46.20	4.60	0.02
Pathogenic fungal genera	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
	Sphingomonas	7.43	4.12	1.88	12.95	4.48	5.73	0.97	2.45	3.17	1.94
Pathogenic bacterial genera	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
	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
Beneficial fungal genera	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

*Signifcant at the 0.05 probability level.

4 DISCUSSION

4.1 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. Lina et al. also identified Pseudomonas and Arthrobacter as dominant flora in the rhizosphere soil of Panax notoginseng [12]. Interestingly, the fungus Tetraclaudium was significantly enriched in XC, and has been shown to be beneficial to the host [13]. 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 [14], planting years [15], and environmental factors [16]. 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. 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. As a direct living environment for microorganisms, the influence of soil on the distribution of microbial communities is obvious, and this study found that climatic factors, latitude and longitude, and altitude can also explain the differences in the distribution of microbial communities in Panax pseudoginseng well. The inter-root microorganisms were closely related to the soil and plant growth conditions, while the altitude, latitude and longitude had an effect on the physicochemical properties of the soil, the regional climate as well as the plant growth, which further drove the microbial distribution, therefore, although latitude, longitude and altitude could not directly act on the microorganisms, their effects on the microorganisms were multifaceted [17,18]. 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 [19].

4.2 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 [20]. 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 [21]. Typically, a richer microbial community composition has a greater ability to suppress pathogens [22]. Highly diverse microbial communities have stronger functional redundancy and transboundary associations [21,23]. Reduced microbial diversity was found in the inter-root soil of diseased

cyrtonema [24] and banana [25], and higher microbial diversity was found in healthy sugar beets [26] and peppers [27]. 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 [28]. The rhizomes are used for many medicinal plants, and 70% of these plants are limited by continuous cropping [29]. WS is a main production area for Panax notoginseng, with a cultivation history of at least 400 years, 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 [30]. 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. Li et al. found that the enrichment of Arthrobacter in soils with high yield may contribute to the increase of *Panax notoginseng* yield [31]. In the present study we found that Chaetomium and Arthrobacter are abundant in the origin and they promote healthy growth of *Panax notoginseng*.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 [32]. Currently, there is no effective means to control continuous cropping obstacles, the application of chemical pesticides can reduce the incidence of root rot of Panax pseudoginseng, but it leads to the problem of pesticide residues. Li et al. suggest that the application of antagonistic microbial agents may be part of a safer and more environmentally friendly biocontrol strategy [33]. Therefor, 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*.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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