Grape cultivar features differentiate the grape rhizosphere of microbiota

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Abstract

Background: Despite its importance to crop health and plant quality, the structure and diversity of the grape rhizosphere remains poorly described. In this study, the microflora community structures of grapes in northern China were compared using high-throughput sequencing. Results: We found that richness, diversity of bacterial and fungi community networking in the root compartments were significantly influenced by the grape variety. The bacterial linear discriminant analysis showed that Pseudomonas , Rhizobium which considered as potential plant-growth-promoting bacteria were more enriched in Pinot noir, and potential ammonia-oxidizing Nitrosospira was enriched in Gem. The fungal linear discriminant analysis showed that Fusarium was more enriched in Longan, Sporormiella more enriched in Merlot while Gibberella and Pseudallescheria were more enriched in Gem, and Mortierella was more abundant in Cabernet Sauvignon. Conclusions: The 16S rRNA functional prediction indicated that no significance differentiate among the varieties. The results showed that may be we can extinguish the varieties according to these characteristic bacterial and fungal genus.

Introduction

The rhizosphere is the soil compartment influenced by plant roots [1]. It is one of the most complex environments influenced by plant roots and microbes and is very important for plant functioning because it provides nutrients for plants and offers protection against pathogens, influencing plant health, development and productivity [2]. Host plants can adjust environmental factors such as pH and soil nutrients to shape their rhizosphere microbiome [3]. Additionally, plant genotype may also greatly influence the structure of rhizosphere microbial communities [4, 5]. In many cases, diseases are caused by the microbial community, while other microbes may antagonize phytopathogens, supply nutrients for different host plants and modulate plant growth [6]. Rhizosphere microbes were characterized based on the extraction of community DNA to illustrate the relationship between plants and their microbes. It has been reported that the roots of maize, wheat and rape carry various microbes as a consequence of the assimilation of root exudates [7].

The grapevine rhizosphere contains a large community of microorganisms that interact with plant organs, these microorganisms may be delivered to the winery and affect wine quality [8]. Recently, molecular biology studies have suggested that the grape microbiome is linked with vineyard location, climatic, and other environmental factors [2]. Using a traditional cultivable method as well as T-RFLP, Martins et al. expounded the epiphytic bacterial communities on the grapes, leaves, bark, and soil of Merlot [9]. Applying sequencing analysis, Zarraonaindia et al. showed that the Suffolk County Merlot soil microbiome influences the grapevine-associated microbiota [10].

Microbiological studies in the soil environment are performed at a small scale because the majority of soil bacteria cannot yet be cultured [2]. The Shacheng district is located in northern China and grows most varieties of wine grapes. Its cinnamon soil with a sandy texture and its hilly and mountainous areas make

it very suitable for the growth of grapes. However, little is known about the microbial communities in the rhizosphere of the varieties of wine grapes in this district, which grows the most wine grape varieties in China. In this study, we used high-throughput sequencing to illustrate the microbial communities in northern China. The distribution of the microflora was investigated, and the results showed divergence among varieties of grapevine.

Results

Richness and diversity of microbiome communities associated with the root systems of different grape varieties

The rarefaction curves decreased slowly, indicating that the sequences were of suitable quality and could be further analyzed (Supplementary Fig. 1). On average, for bacterial communities in the grape rhizosphere samples, the sequences ranged from 52,256 to 85,644, and 3117 OTUs were obtained from different varieties (Supplementary Table 1). A bipartite network consisting of 1384 nodes and 6973 edges was generated for the rhizosphere of the nine different varieties of wine grape, and the modularity index was 0.073 (Supplementary Table 2). Zin had the highest number of OTUs, followed by Cab, and Pin had the lowest (Figure 1a). For fungal communities, the sequences ranged from 222,674 to 282,519, and 320 OTUs were obtained from the rhizosphere. A bipartite network consisting of 1020 nodes and 4038 links was generated for the rhizosphere of the nine different varieties of wine grape, and the modularity index was 0.151(Supplementary Table 2). Lon had the highest number of OTUs, followed by Cab, and Mer had the lowest number (Fig.1b). The results showed that no matter bacteria or fungi, both have complex microbial communities structures.

Fig.1 Bipartite network analysis of grape rhizosphere bacterial and fungal communities. a, bacterial OTU interactions in the samples; b, fungal OTU interactions in the samples.

The coverage indexes for bacterial communities were higher than 97% (Table 1), and for fungal communities, they were higher than 99% (Table 2). For the bacterial communities, Cha showed the highest richness in the ACE and Chao 1 index results, while Syr showed the highest diversity in the Shannon and Simpson index results (Table 1). However, there were no significant differences among these samples. For the fungal communities, in both of richness and diversity, Cha had much higher values than the other varieties (Table 2). The ACE and Chao1 diversity estimates among the nine wine grapes were extremely significantly different ($P<0.01$).

Table 1 The α diversity of bacterial communities

Note: Letters indicate the results of Tukey's 'honestly significant different' test.

Table 2 The α diversity indices of fungi communities

	Coverage	ACE (10^3)	Chao 1 (10^3)	Simpson (10^{-2})	Shannon
Zin	0.82±0.06a	8.83±0.61a	6.67±0.68a	0.18±0. 01a	7.43±0.07a
Cab	0.83±0.05a	9.11±0.65a	6.78±0.55a	0. 17±0.02a	7.44±0.14a
Cha	0.85±0.03a	8.44±0.55a	6.81±0.36a	0.21±0.04a	7.43±0.12a
Syr	0.87±0.04a	8.39±0.65a	7.08±0.45a	0.24±0.09a	7.37±0.15a
Mer	0.81±0.02a	9.29±0.30a	6.63±0.26a	0.19±0.03a	7.42±0.09a
Gem	0.82±0.04a	8.35±0.61a	6.11±0.59a	0.24±0.02a	7.32±0.18a
Rie	0.86±0.03a	8.29±0.79a	6.40±0.32a	0.25±0.06a	7.27±0.10a
Pin	0.91±0.02a	8.15±0.55a	6.86±0.22a	0.39±0.00a	7.20±0.26a

	Coverage (10^{-1})	ACE (10^3)	Chao 1(10^3)	Simpson	Shannon
Zin	9.90±0.02a	2.21±0.30a	1.74±0.29af	0.10±0.04a	3.47±0.29a
Cab	9.86±0.02a	2.17±0.45abc	1.66±0.33ag	0.08±0.02a	3.69±0.16a
Cha	9.90±0.03a	2.73±0.17b	2.17±0.16a	0.09±0.04a	3.56±0.35a
Syr	9.92±0.01a	2.78±0.11a	2.14±0.11b	0.15±0.06a	3.09±0.34a
Mer	9.87±0.03a	2.33±0.31abc	1.81±0.29ae	0.08±0.03a	3.58±0.31a
Gem	9.89±0.03a	2.55±0.30a	1.96±0.27acd	0.07±0.01a	3.69±0.20a
Rie	9.87±0.06a	2.17±0.43a	1.66±0.41bh	0.13±0.04a	3.18±0.24a
Pin	9.90±0.02a	2.47±0.21abc	1.90±0.21ad	0.08±0.004a	3.57±0.11a
Lon	9.91±0.01a	1.81±0.32c	1.41±0.28abi	0.08±0.01a	3.43±0.09a

Note: Letters indicate results of Tukey's 'honestly significant different' test.

Grape microbiome distribution in the rhizosphere of different varieties of wine grape

To compare the differences in total bacteria and fungi among different varieties of grape rhizosphere, pairwise dissimilarities within the communities were visualized by PCoA. Dimensional scaling of the Bray-Curtis dissimilarity matrix to two dimensions revealed separation of the communities among the varieties in different samples, including the bacterial communities and fungi communities. PerMANOVA analysis also revealed that varieties were weak significantly linked to rhizosphere bacterial and fungi community composition ($P < 0.05$) (Fig.2), from the Fig.2a, it showed that Mer and Pin grouped together, while Zin, Cab, Cha, Syr, Rie grouped together, Gem separately with other varieties. This showed that Mer and Pin was closer to each other at the communities composition, Zin, Cab, Cha, Syr, Rie may have relatively similar communities composition. From the Fig.2b, Pin and Rie was closer to each other, Lon was separately from others, while Zin, Cab, Cha, Syr, Mer have similar fungi composition.

Comparing the bacterial communities among the rhizopsphere samples from different grape varieties showed that the amounts of the major phyla and genera were different (Fig.3). The dominant 9 bacterial phyla in the rhizospheres of Mer, Syr, Zin, Cha, Gem, Pin, Rie, Lon, and Cab were Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Gemmatimonadetes, Acidobacteria, Chloroflexi, Nitrospirae, and Verrucomicrobia. Among them, the most abundant bacterial phylum was *Actinobacteria* in Cab (44.24%), and the lowest abundance of Actinobacteria, in Pin, was also high, at 38.25%. The 10 dominant bacterial genera in the grape rhizosphere were *Arthrobacter*, *Blastococcus*, *Bacillus*, *Nocardioides*, *Sphingomonas*, *Gaiella*, *Turicibacter*, *Pseudomonas*, *Streptomyces*, and *Kocuria*. Among them, *Arthrobacter* was the most abundant bacterial genus, and the varieties with the highest abundance Rie (7.19%), Cab (7.11%), and Mer (6.53%).

Comparing the fungal communities in the rhizospheres of different varieties of grape, showed that many of the phyla and genera had varying abundances (Fig.3). The dominant three fungal phyla in these varieties were Ascomycota, Basidiomycota and Zygomycota, which composed approximately 99% of the total phyla. Among them, the most abundant fungal phylum was Ascomycota in Syr (79.48%), and the lowest abundance of Ascomycota in Rie was also high at 70.30%. The ten dominant fungal genera in these varieties were *Guehomyces*, *Alternaria*, *Cladosporium*, *Phoma*, *Acremonium*, *Chaetopyrena*, *Mycocentrospora*, *Monographella*, *Emericella*, and *Tetracladium*, which composed approximately 50% of the total sequences. Among them, the most abundant fungal genus was *Guehomyces* in Lon (23.66%), and the lowest abundance of *Guehomyces* in Pin was 14.28%.

Eighteen bacterial core OTUs were obtained and found to constitute more than 60% of the total sequences (Supplementary Table 3). When these OTUs were blasted against the NCBI database, the relative abundance of *Blastococcus* sp. was found to be higher than those of the others. The relative abundance of *Blastococcus* sp. (OTU2257) was higher than 27.41%. *Arthrobacter* sp. contained the most OTUs (OTU130, OTU2425, OTU2536, OTU3419, OTU3574), and the relative abundance of *Arthrobacter* sp. (OTU130, OTU2425, OTU2536, OTU3419, OTU3574) was between 7.71% and 18.20%. The phylogenetic tree of core bacterial communities showed that these genera were included in bacterial phyla. The amount of each taxonomic group differed among the samples. Actinobacteria accounted for 13.75%, 14.68%, 14.30%, 12.92%, 15.10%, 11.84%, 14.68% and 12.78% in Zin, Cab, Cha, Syr, Mer, Gem, Rie and Pin, respectively. Firmicutes accounted for 1.85%, 1.45%, 2.24%, 2.17%, 2.16%, 1.08%, 2.15% and 3.55% in Zin, Cab, Cha, Syr, Mer, Gem, Rie and Pin, respectively. Proteobacteria accounted for 0.74%, 0.62%, 0.68%, 0.52%, 0.60%, 0.77%, 0.62% and 0.63% in Zin, Cab, Cha, Syr, Mer, Gem, Rie and Pin, respectively (Fig.4). The results obtained above showed that most varieties of grape rhizospheres were similarity in the constitute of bacterial core OTUs, and the most of the OTUs were *Arthrobacter* sp., which abundant in the soil with high genetic adaptability [11, 12] and has the potential for bioremediation [13, 14]

Thirty-six fungal core OTUs were obtained and found to constitute more than 60% of the total sequences (Supplementary Table 4). When these OTUs were blasted against the NCBI database, it was found that among all the fungal core OTUs, the relative abundance of uncultured fungi was higher than those of the other OTUs. The relative abundance of uncultured fungi (OTU1795) was higher than 54.78%. *Alternaria*

sp. contained the most OTUs (OTU765, OTU896, OTU1019, OTU1683, OTU1859), and the relative abundance of *Alternaria* sp. was between 5.63% and 18.20%. The phylogenetic tree of core fungal communities showed that the fungal genera were included in three fungal phyla. The amount of each taxonomic group differed among the samples. Ascomycota accounted for 25.62%, 29.31%, 41.20%, 36.12%, 51.63%, 31.77%, 26.41%, 42.34%, and 46.04% in Lon, Zin, Cab, Cha, Syr, Mer, Gem, Rie and Pin, respectively. Basidiomycota accounted for 6.44%, 12.62%, 4.25%, 9.88%, 6.91%, 7.17%, 5.14%, 15.96% and 4.06% in Lon, Zin, Cab, Cha, Syr, Mer, Gem, Rie and Pin, respectively. Mucoromycota accounted for 0.03%, 3.99%, 0.41%, 0.71%, 0.29%, 2.03%, 0.56%, 0.82% and 0.38% in Lon, Zin, Cab, Cha, Syr, Mer, Rie and Pin, respectively (Fig.5). From the fungi core OTUs analysis, it was found that most of the grape rhizosphere also have similarity constitution, with the abundant genera of *Alternaria* sp. and *Fusarium* sp..

Differences in bacterial and fungal diversity among varieties

We used LEFSe to identify discriminative bacteria taxon among the different grape varieties. The LEFSe results for all species showed 30 bacterial taxa with significant differences. Cab and Syr were extinguished from others at the phylum level, with the significant enriched in Proteobacteria and Actinobacteria, respectively; Pin, Cha, Gem, Mer were extinguished from each other at the order level, with the significant enriched in *Enterobacteriales*, *Micromonsporales*, *Rubrobacterales*, *Micrococcale*s or *Ardenticatenales* (Fig.6a). After analyzing differences among varieties from phylum to genus, a closer look was taken into the differences in bacterial community structure at the genus level occurring over the different varieties. The rhizosphere bacterial taxa with the greatest differences over the eight wine grape varieties are displayed in Fig.6b. It was showed that Zin, Cab, Cha, Syr, Mer, Gem, Rie, Pin could all be clearly distinguished at the genus level, respectively. The number of enriched genera in Pin and Gem were higher than in Cab, Syr, Rie, Mer, Cha and Zin, while Lon was nearly no significant genus. In addition, *Pseudomonas*, *Rhizobium*, which considered as potential plant-growth-promoting bacteria were more enriched in Pin, and potential ammonia-oxidizing bacteria *Nitrosospira* was enriched in Gem.

Similarly, we used LEFSe to identify distinctive fungi taxa among the different grape varieties. The LEFSe results for all species showed 34 fungal taxa with significant differences. At the phylum level, Zygomycota was significantly enriched in Cha, and others of them were extinguished even at the order level (Fig.7a). In order to analysis the differences among varieties from phylum to genus, a closer look was taken into the differences in fungal community structure at the genus level occurring over the different varieties. The rhizosphere fungal taxa with the greatest differences over the nine wine grape varieties are displayed in Figure 7b. It was showed that Lon, Cab, Cha, Syr, Mer, Gem, Pin could all be clearly distinguished at the genus level, respectively. The number of enriched genera in Pin were higher than in other six grape varieties, followed by Mer, while Rie and Zin were nearly no significant genus. In addition, *Fusarium* more enriched in Lon, *Sporormiella* was more enriched in Mer, *Gibberella* and *Pseudallescheria* were more enriched in Gem, and *Mortierella* which can produce polyunsaturated fatty acids was more enriched in Cab [15].

To elucidate the differences in bacterial and fungal composition among the different grape varieties, Bray-Curtis and unweighted-Unifrac dissimilarity were adopted to dissect varieties relationships. We calculated the taxonomic metric (calculated from OTUs), and tests showed that community structure varied among different varieties, exerting a significant impact on bacterial Bray-Curtis (R_{adonis} = 0.319, $P <$ 0.01; adonis, permutational multivariate analysis of variance; R_{anosim} = 0.317, $P <$ 0.01; aonsim, analysis of similarities) and unweighted Unifrac (R_{adonis} = 0.215, $P <$ 0.01, R_{anosim} = 0.245, $P <$ 0.01) dissimilarity (Table 3).

Table 3 The taxonomic metric (calculated from OTU) for bacterial diversity patterns

		Bray-Curtis				unweighted-Unifrac			
		ADONIS		ANOSIM		ADONIS		ANOSIM	
Group	Factor	R^2	P	R	P	R^2	P	R	P
Nine	Variety	0.319	0.001	0.317	0.001	0.215	0.001	0.245	0.001

For the fungal composition, tests showed that community structure varied among different varieties and showed a significant impact on fungal Bray-Curtis (R_{adonis} = 0.373, $P <$ 0.01, R_{anosim} = 0.295, $P <$ 0.01) and unweighted-Unifrac (R_{adonis} = 0.442, $P <$ 0.01, R_{anosim} = 0.401, $P <$ 0.01) dissimilarity (Table 4). These results revealed that grape variety plays a significant role in shaping the bacterial and fungal communities, and varieties can be identified by the proportions of several key bacterial and fungal taxa.

Table 4 The taxonomic metric (calculated from OTUs) for fungal diversity patterns

		Bray-Curtis				unweighted-Unifrac			
		ADONIS		ANOSIM		ADONIS		ANOSIM	
Group	Factor	R^2	P	R	P	R^2	P	R	P
Nine	Variety	0.373	0.001	0.295	0.001	0.442	0.001	0.401	0.001

Functional genomics of grapevine rhizosphere community

A functional characterization of amplicons was performed to learn about the physiological capabilities of the microbial communities and to link taxonomic shifts with functional. The differential abundances of KEGG orthologs (KOs) identified the key genotypic features of the grapevine rhizosphere microorganisms. In total, 193 different KOs were detected and organized into 39 small metabolic pathways at KEGG level 2 and 239 metabolic subsystems at KEGG level 3. These metabolic pathways were included in six basic metabolic systems at KEGG level 1: metabolism, genetic information processing, environmental information processing, human diseases, cellular processes, and organismal systems. The main

metabolic pathways were considered those with OTU sequences representing more than 5% of all the sequences in the grape rhizosphere samples for all the varieties. As shown in Supplementary Table S8, 24 main metabolic pathways at level 2 were found; the top three were membrane transport, amino acid metabolism, and carbohydrate metabolism, with total relative abundances of 97.79%, 91.11% and 86.79%, respectively. A total of 53 main metabolic pathways at level 3 were found; the highest three were transporters, ABC transporters, and general function prediction only, with total relative abundances of 49.96%, 30.68% and 27.43%, respectively (Supplementary Table S9). Microbial enzyme catalysis determines the functions of rhizosphere communities. Therefore, most KOs were related to enzyme commission number. The most common of 32 enzymes found were drawn in a heatmap (standardized and normalized by Z-score) to further visualize the functional variation in the rhizosphere microbial communities (Supplementary Fig. S1). From the heatmap, we can see that enoyl-CoA hydratase (4.2.17), error-prone DNA polymerase (2.7.7.7) and aldehyde dehydrogenase (NAD+) (2.3.19) were the top three enzymes, and more enriched in all of the varieties.

Discussion

Micro flora exist in complicated associations with crops and have key roles in shaping soil quality and improving crop health as well as its productivity. The origin of the microbes involved in wine fermentation is still poorly understood; they are commonly assumed to come from the grapes themselves. Many bacterial and fungi actively colonize the rhizosphere have metabolic activities that modulate plant health or suppress disease-causing pathogens. Previous studies have attempted to determine the factors that significantly influence the rhizosphere microbiome. Some of these studies suggest that geographical location influence on community composition [16-18], some studies suggest that agricultural management practices play key roles in community structure [19, 20], accompany with studies exposed that seasonal changes are strongly correlated with shifts in the microsystems [21], while other studies suggest that host plant species is the primary factor driving the community [16, 22]. Despite the host plant improving our knowledge, more species observed in the rhizosphere stills unexplained. Our study covered nine varieties of wine grapes in north China to identify the variety factor that potentially play roles in the population, diversity, taxonomic composition. The results indicated that the composition of community structures was influenced by the variety, whereas some of varieties did not appear to have features differentiate on the community.

Grape varieties were strongly correlating factor to populations, diversity and taxonomic composition of the microbiome community, including the bacterial and fungal community. Bokulish et al. [16] found that Chardonnay, Zinfandel, Cabernet exert some effect on community structure across the regions and vintages. Zarraonaindia et al. [10] reported the structure of soil and other parts of grapevine, indicated the importance of soil for plant organ-associated bacterial taxa. In our study, the OTU distribution showed the bacterial and fungal structures respective clustering, and combined together, indicate their all have same origin and no differences in structure but populations (Fig. 1). The alpha-diversity (OTU diversity, richness and evenness) of nine varieties was weak or no significant differentiate from each other (P > 0.05). These

microorganisms identified on the varieties come from the same vineyard, and most of the grapes were planted also in 1979, cleft grafting on the same rootstock, long-term equal environment make less difference in diversity among the varieties. These bacteria and fungi may migrate from the surrounding soil and airborne environment [10, 23, 24]. Bokulish et al. [16] stressed the importance of *terroir* through comparing regional microbial biodiversity in *Napa* and *Sonoma*, though our study choose just only one place, which also can be consider as a part of *terroir*, the bacteria and fungi communities have no diversity differentiate, which may proved the significant role of *terroir* indirectly. As for composition of communities, the rhizosphere samples from varieties showed that the major phyla and genera were similar though different abundances (Fig.3). Michele et al. [25] found that *Acetobacter* existed on all grapevine plants. *Alternaria* produces alternariol, alternariol monomethyl, altenuene, altertoxin, and tenuazonic acid, these metabolites exhibit some degree of toxicity to mammalian and bacterial cells as well as to higher plants [26]. *Fusarium* sp. are involved in wine making and can produce pectinase, raising juice yields during the process of wine making [27, 28]. In our study, all of these two potential functional microorganisms were existed in nine varieties of wine grapes.

Though similarity, several differences were found in terms of beta-diversity, population, and interactions within rhizosphere bacteria and fungi among nine varieties. Due to variety difference, the PCoA and PerMANOVA analysis showed the variety change was the most important factor affecting the change of the community structure of total bacterial and fungi communities. As for the bacterial and fungal communities' population and composition, some phyla and genera showed significant differences. At the phylum level, Actinobacteria was significantly enriched in Syr compared with other eight varieties, while Proteobacteria was significantly enriched in Cab, which was in accordance with previous studies in Cab [16]. At the genus level, the number of enriched genera in Pin and Gem were higher than in Cab, Syr, Rie, Mer, Cha and Zin, while Lon was nearly no significant genus. Lon was unique grape in China, which can be considered as both wine grape and table grape, may be the features of brewing and table lead to it not extinguished from other varieteis. *Pseudomonas*, *Rhizobium* were more enriched in Pin. *Nitrosospira* was enriched in Gem. *Pseudonmonas* can grow under extremely conditions [29, 30], studies found *Pseudonmonas* was the most prevalent on vine leaves, micro flora are closely interconnected to plants, plants transfer carbon to bacteria and fungi, while micro flora improve phosphate and nitrogen accessibility as well as performing other nutrient acquisition for the plants [31, 32], literatures also investigated the relationships among soils and other parts of the grapevine [9, 33]. Cultivar differ in growth habit, and this invasion could explain how *Vitis* manages its grape-surface susceptibilities to disease pressures in different environments [34]. The late maturing characteristics increase the survival probability of this genus, which could also explain why *Pseudonmonas* enriched in Pin.

Zhang et al. found that the microflora on leaves and grapes mainly originated from the soil, and these microflora ultimately form part of the juice and participate in fermentation [33]. Zarraonaindia et al. [10] found that the Mer soil and root were dominated by *Proteobacteria* spp., *Acidobacteria* spp., *Bacteroidetes* spp., and *Verrucomicrobia* spp; the reason for this difference may be the climate. Bokulich also reported the importance of climate [16]. In our study, though not comparing the influence of climate,

such as seasons, growth periods, all of the varieties were collected in the same time, same growth period, which controlling other factors indirectly. Marzano observed *Amnibacterium*, *Methylobacterium*, *Hymenobacter*, *Sphingomonas* and *Thermomonas* in Cab; consistent with the theory mentioned above, most of these genera come from the soil, and therefore, we can infer that the Cab microflora in that case was significantly richer in *Amnibacterium*, *Methylobacterium*, *Hymenobacter*, *Sphingomonas* and *Thermomonas* [35]. However, in our study, *Propionibacteriales*, *Solirubrobacterales*, *Gemmatimonadales*, *Gemmatimonadetes*, *Caulobacterales*, and *Proteobacteria* were significantly enriched in Cab. Of course, these results also indirectly proved the key role of environmental conditions. In addition, we compared the differences among varieties using statistical methods and found that the grape variety significantly influenced the taxa in the grape microbial community, this results were consistent with the literature [36].

Root exudate compositions are considered to explain the plant-specific microbial communities associated with the rhizosphere among varieties. Functional redundancy is crucial for maintaining the balance of a functioning ecosystem [37]. Marasco et al., showed that cultivars grafted onto the rootstocks represent the soil and root endosphere partially have no potential function [20]. In our study, PICRUSt functional predictions showed there were some similarities in utilize root secretions, and no significance function differentiate. How the microorganisms in different varieties of influence the root function is deserved to be further research.

Materials And Methods

Site description and sample collection

This study was performed in a grape-growing region in Huailai County (40°4'-40°35'N, 115°16'-115°58'E). The winery was built in 1979, and most of the grapes were planted also in 1979, the average tree age is 28 years. The vineyard contains as many as 16 cultivars, including Zinfandel (Zin), Cabernet Sauvignon (Cab), Syrah(Syr), Merlot (Mer), Gem (Gem), Pinot noir (Pin), Riesling (Rie), Longan (Lon), Chardonnay (Cha), also have other varieties, such as Midknight Beauty, Traminer, Chenin blanc. The grapevine region about 75 hectare, most of the grapes were Rie, which can brew dry white wine, and other varieties accounted for almost the same proportion except Midknight Beauty, Traminer, Chenin blanc. All of the varieties growing in the same soil type. No bactericide or insecticide was applied in the vineyard and no chemical fertilizer was applied (Supplementary Table 5, Supplementary Table 6). All of the selected grapes were cultivated in the same vineyard field, which was characterized by a clay-rich soil. We chose the widespread nine varieties of Zin, Cab, Syr, Mer, Gem, Pin, Rie, Lon as well as Cha which abound in the vineyard, with the rootstock of all varieties same and differ in many characteristics, including bunch and berry traits. In order to minimize cross contamination, such as wind, rain wash, and roadside traffic activities, all the samples were taken in the middle of the corresponding vineyard. Samples of the nine varieties of grapes were collected at veraison. It corresponds to stage 35 in the modified E-L system for identifying major and intermediate grapevine growth stages. At the time of sampling, the outside temperature was as follows: the maximum temperature was 29°C, and the minimum temperature was 16 °C, all the day was cloudy, wind power smaller than three level, and no rain in the week before sampling

(Supplementary Table S7). Collecting fresh soil at about 10-15 cm depth close to the stem as soon as possible, but not damaged the stem. The fresh soil was passed through a 1-mm sieve to remove plant residues and stones [10, 38]. Considering the heterogeneity of the tested rhizosphere, the soil samples were collected from at least 5 plants to form a composite sample, and five composite samples of rhizosphere were collected for each variety of grape. In total, 45 grape samples were collected.

DNA extraction and sequencing

For each soil sample, 0.5 g was weighed for DNA extraction. Genomic DNA was extracted from all the samples above using the FastDNA SPIN Kit for Soil (MP, USA), as described by the manufacturer's handbook [2]. The bacterial 16S rRNA gene V5-V7 region primers 799F (5'-AAC MGG ATT AGA TAC CCK G-3') [39] as well as 1193R (5'-ACG TCA TCC CCA CCT TCC-3') were used, and the fungal rRNA internal transcribed spacer ITS1 was amplified with the primers (5'- CTT GGT CAT TTA GAG GAA GTAA -3') and (5'-GCT GCG TTC TTC ATC GATGC–3') [40]. The reverse primer modified to contain a barcode [41]. PCR contained 5 μL 10× Pyrobest Buffer (Takara, Japan), 1 μL DNA template, 2 μL of each primer (10 μmol/L), 4 μL dNTPs (2.5 μmol/L), 0.3 μL Pyrobest DNA Polymerase (2.5 U/μL, Takara, Japan), and water up to 50μL. The PCR procedure was 94°C for 3 min, 94°C for 45 s, 50°C for 60 s and 72°C for 90 s for 35 cycles, and a final extension of 72°C for 10 min. The reaction products were purified using an Ultra Clean PCR clean-up kit (Mobio, USA). Then divided the purified reaction products into equal two parts, one for bacterial sequencing, the other for fungal sequencing, a total of 90 sequencing samples. After the amplicons subjected to library preparation, the products were sequenced using an Illumina HiSeq PE250 paired-end (HiSeq 2500, PE250). One of the bacteria samples (Lon) was not sequenced successfully. The sequencing data were deposited in the NCBI database with the accession number SRP216299 and fungi under number SRP216297.

Data and statistical analysis

The software package Mothur (version 1.36.1) was applied for sequence analysis following the standard operating procedure outlined on http://www.mothur.org/wiki/Schloss_SOP [42]. All raw fastq files were quality-filtered using Trimmomatic [43], and assigned to their respective samples according to the unique nucleotide barcodes. Chimeric sequences were identified and removed using UCHIME. After removal of barcodes and primers, pair-ended sequences were merged using FLASH [44]. The sequences were clustered into operational taxonomic units (OTUs) with a sequence threshold of 97% similarity and putative chimeric sequences were removed using UPARSE algorithms in Usearch 7.0 at the 0.03 level [45] and representative sequences of OTUs were picked up simultaneously. The singletons and chimeras were filtered during the UPARSE procedure. For fungi, any sequence classified as nonfungal Eukarya was removed from further analysis.

The Majorbio cloud platform was applied to define OTU rarefaction curves. Species richness and diversity indices (ACE, Chao 1, Shannon and Simpson) were also calculated by the majorbio. Community similarities based OTU using a principal coordinates analysis (PCoA) based on Bray-Curtis distance

matrices as well as PerMANOVA analysis. A linear discriminant analysis effect size (LEFSe) was applied to the OTU table (non-parametric factorial Kruskal-Wallis (KW) sum-rank test $P < 0.05$, LDA > 3.0; http://huttenhower.sph.harvard.edu/galaxy/, LDA, linear discriminant analysis) to identify the discriminant bacterial and fungi clade [46]. A co-occurrence network analysis was performed for each microbiome associated with the rhizosphere to expound the significant relations among the OTUs [47], to build the network, we filtered out the OTUs with frequencies less than 0.05, and with the spearman correlation coefficients higher than 0.85 [48] and visualized using Gephi 0.9.2. The OTUs accounted for more than 5% of the total sequences were considered as core OTUs. The phylogenetic tree of the core OTUs was generated with Mega 6. Using the 16S rRNA gene data, the funtion of the rhizosphere communities was predicted by PICRUSt [49]. The KEGG database was selected and used to predicted molecular functions, and the relatives of enzyme larger than 3.0% were drew in heatmap. The statistical significance was analyzed by one-way ANOVA followed by Tukey's test ($P < 0.01$) calculated with SPSS 21.0 software.

Abbreviations

Zin: Zinfandel; Cab: Cabernet Sauvignon; Syr: Syrah; Mer: Merlot; Gem: Gem; Pin: Pinot noir; Rie: Riesling; Lon: Longan; Cha: Chardonnay.

Declarations

Acknowledgements

Author contributions

Z.B. designed and directed the experiments. S.Z., Z.Y., L.B. and B.S. performed the experiments and analyzed the data. S.Z. and Z.Y. wrote the main manuscript text. Z.B., Y.W and Z.L. improved the manuscript.

Ethics approval and consent to participate

All the samples in this study gave permission to conduct the study on these sites. No specific permissions were required for these locations, with no endangered or protected species in these areas, and this study neither involve endangered nor protected species.

Consent for publication

Not applicable.

Funding

Availability of data and materials

All the sequencing data were deposited at the NCBI database with the accession number SRP216299 and fungi under number SRP216297.

Competing interests

The authors declare that they have no competing interests.

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Additional File Legends

Additional file 1: Table S1. Number of sequences, total OTUs in bacteria and fungi. Table S2. Indices of co-networks. Table S3. Bacterial sequences aligned using BLAST from the NCBI nucleotide database. Table S4. Fungal sequences aligned using BLAST from the NCBI nucleotide database. Table S5. Analysis of soil physical and chemical indexes of the rhizosphere soils. Table S6. Physico-chemical characteristics of the soil samples. Table S7. Air condition in the sampling site. Table S8. KEGG orthologs (KOs) identified the key genotypic features. Relative abundance pathways in KEGG level 2 in the grapevine rhizosphere. Table S9. KEGG orthologs (KOs) identified the key genotypic features. Relative abundance pathways in KEGG level 3 in the grapevine rhizosphere. Fig. S1. KEGG orthologs (KOs) identified the key genotypic features. The 32 most prevalent ECs, standardized by Z-score across all data sets, and the stars represents the relatives of enzyme larger than 3.0%.

Figures

Figure 1

Bipartite network analysis of grape rhizosphere bacterial and fungal communities. a, bacterial OTU interactions in the samples; b, fungal OTU interactions in the samples.
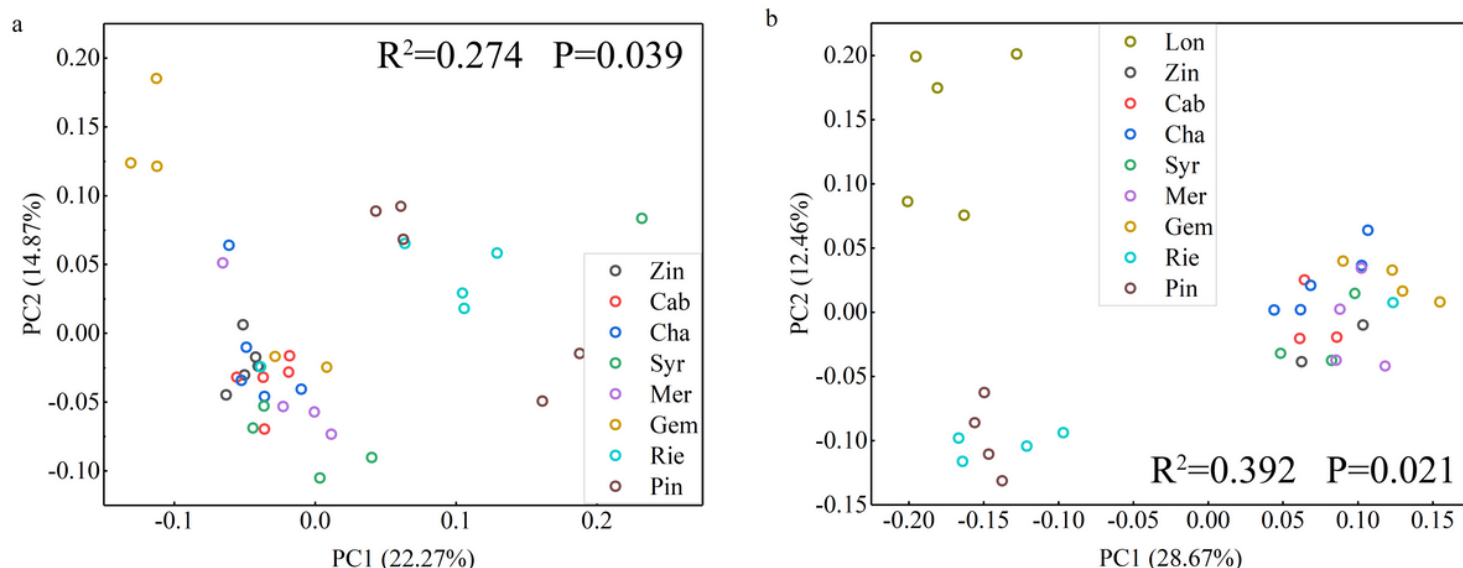
Figure 3

Principal coordinates analysis (PCoA) of the variation in bacteria (a), and fungi (b) community structures of nine types of wine grape rhizosphere.
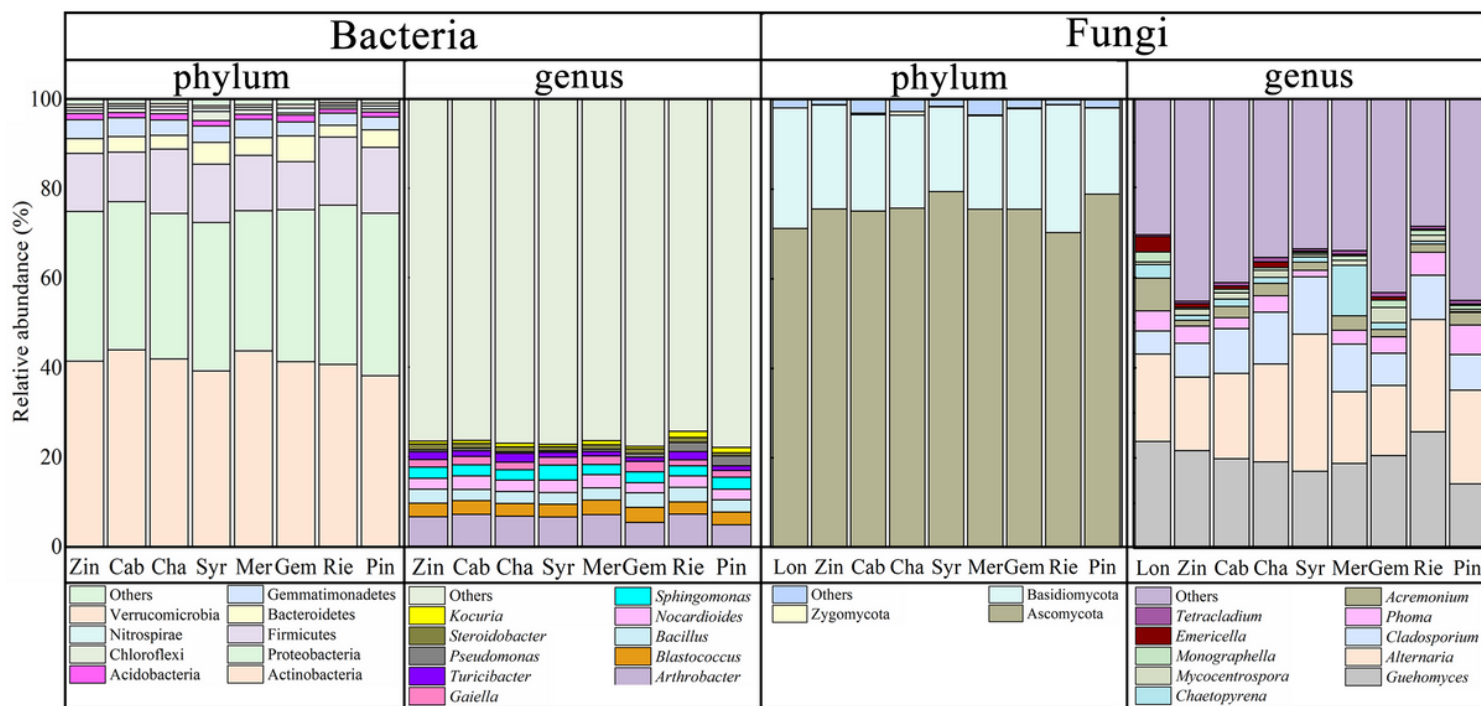

Figure 5

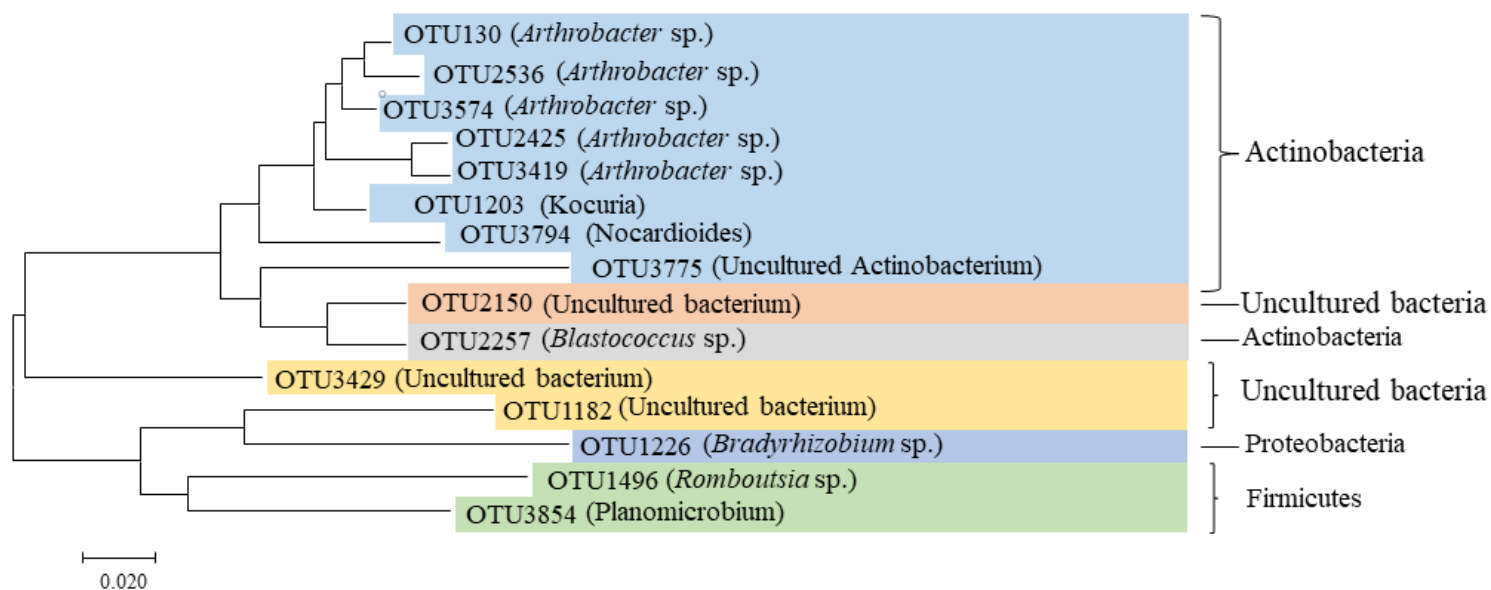Relative abundance of bacteria and fungi associated with phylum and genus.

Figure 7

Phylogenetic tree of the core bacterial communities in the rhizospheres of nine grape cultivars.
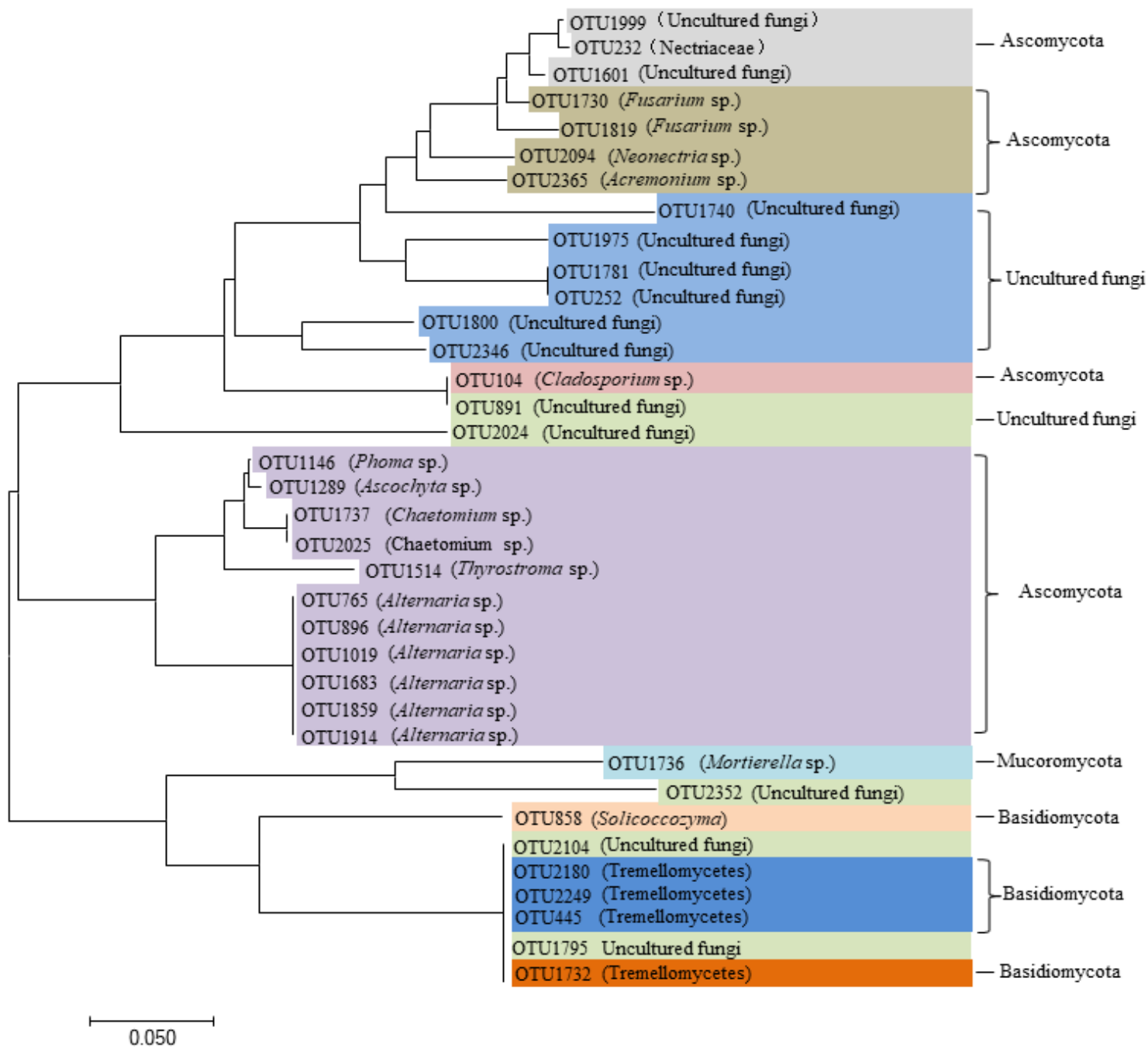
Figure 9

Phylogenetic tree of the core fungal communities in the rhizospheres of nine cultivars of grapes.
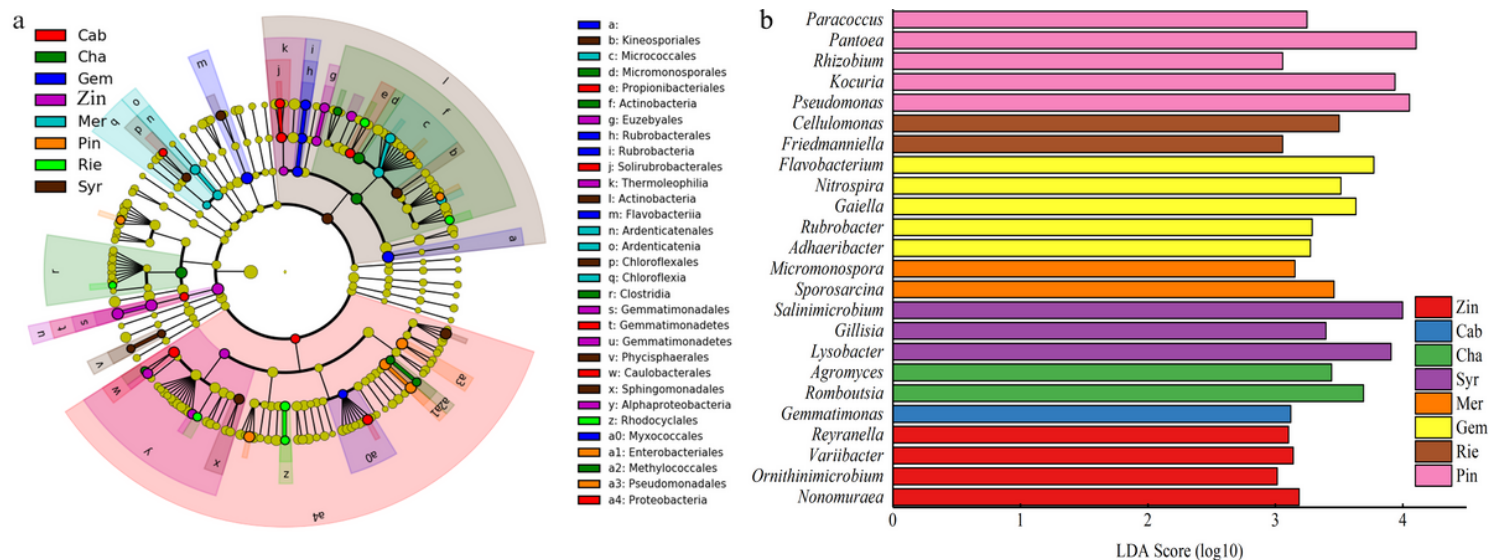
Figure 11

Cladograms suggesting the polygenetic distribution of bacterial in the rhizosphere of nine grape varieties as determined by linear discriminant analysis (LDA) effect size (LEFSe).
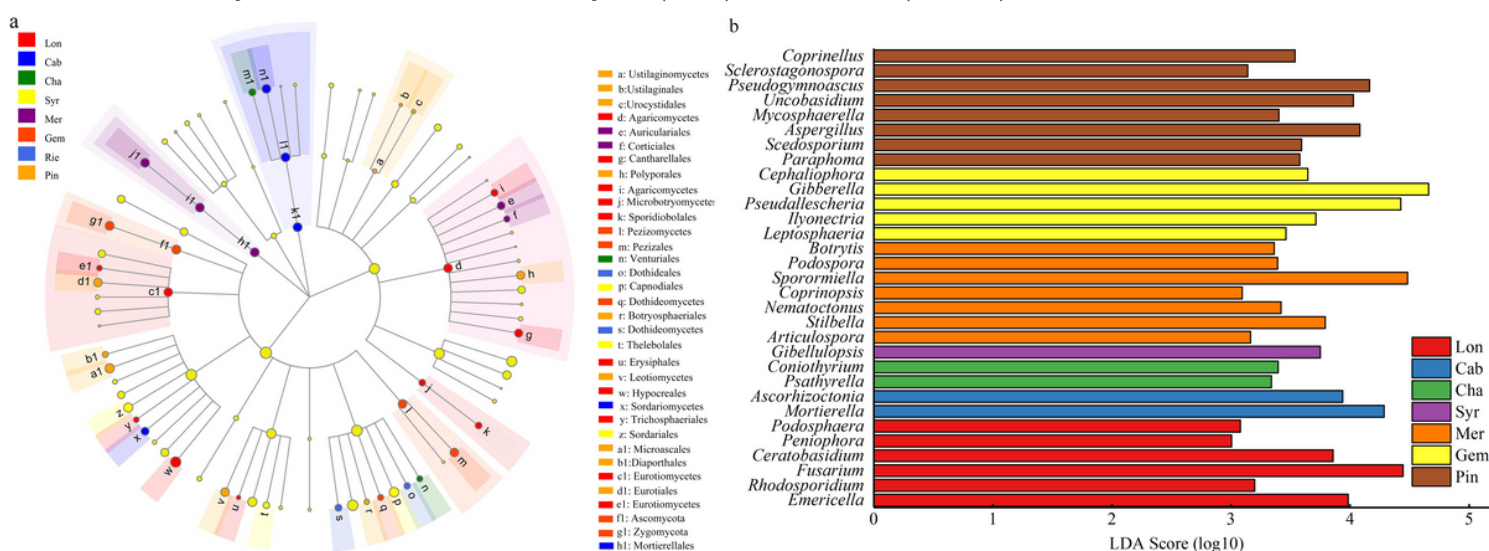

Figure 13

Cladograms suggesting the polygenetic distribution of fungal in the rhizosphere of nine grape varieties as determined by linear discriminant analysis (LDA) effect size (LEfSe).

Supplementary Files

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