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Variation of Bacterial and Fungal Communities in Soils from Three Major Apple-Pear (*Pyrus bretschneideri Rehd.*) Orchards

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Abstract: The microbial communities are closely related to the overall health and quality of soil, but studies on microbial ecology in apple-pear orchard soils are limited. In the current study, 28 soil samples were collected from three apple-pear orchards in northeastern China, and the composition and structure of fungal and bacterial communities were investigated by high-throughput sequencing. Molecular ecological network showed that the keystone taxa of bacterial communities were *Actinobacteria*, *Proteobacteria*, *Gemmatimonadetes*, *Acidobacteria*, *Nitrospirae*, and *Chloroflexi*, and the keystone taxa of fungal communities was *Ascomycota*. Mantel tests showed that soil texture and pH were important factors shaping soil bacterial and fungal communities, and soil water soluble organic carbon (WSOC) and nitrate nitrogen (NO_3^--N) were also closely related to soil bacterial communities. Canonical correspondence analysis (CCA) and variation partition analysis (VPA) analysis revealed that geographic distance, soil texture, pH, and other soil properties could explain 10.55%, 13.5%, and 19.03% of the overall variation of bacterial communities, and they explained 11.61%, 13.03%, and 20.26% of the overall variation of fungal communities, respectively. The key stone taxa of bacterial and fungal communities in apple-pear orchard soils and their strong correlation with soil properties could provide useful clues toward sustainable management of orchards.

Keywords: bacterial community; fungal community; network analysis; soil; structure equation model; sustainable management

1. Introduction

Yanbian Korean Autonomous Prefecture of Jilin Province is the largest apple-pear producer in China, known as the "Hometown of Apple-Pears". Apple-pears have great nutritional value and are favored by many people, because they are rich in vitamin C, vitamins B_1, B_2, calcium, iron, phosphorus, and other nutrients and trace elements, which promote appetite and improve digestion [1]. A previous research has shown that bacterial communities in apple orchard soils were significantly correlated with the overall quality and productivity of the orchard, e.g. size of the apple and the soluble solids concentration in apples [2]. Investigations have shown that the composition of microbial community was closely related with the plant productivity [3]. Similarly, it is of practical significance to investigate the soil microbial communities in apple-pear orchard soils.

Soils contain many microbes (around 1×10^8 cells/g for arable soils), which play an important role in soil ecological functions [4]. Microorganisms in orchard soils are conducive to the decomposition of organic matter and the release of minerals [5]. They participate in the nutrient cycle and energy flow, which may determine the overall quality of soils [6,7]. The soil microbes have symbiotic and parasitic relationships with plants and can affect the growth of plants [8]. Soil microbial ecology may be affected by both historical factors and environmental factors. Historical factors refer to parameters

such as physical barriers, species diffusion and environmental heterogeneity over the course of history, while environmental factors refer to determinants such as pH, soil texture, organic carbon, moisture, and nutrient content. Both soil managerial historical and environmental factors may be critical in shaping the soil microbial communities [9–13]. Many studies have shown that soil pH is considered to be a key factor influencing the compositions and diversity of soil bacterial communities [14,15]. Fungi are more adaptable to changes in pH than bacteria. Fungi usually have a wider pH growth range [16], but some studies have shown that soil pH could affect soil fungal populations and fungal diversity [17,18]. In most soils, clay particles can adsorb organic carbon and nutrients, and at the same time, provide a better living environment for bacteria and fungi [19,20]. Dissolved organic carbon and dissolved nitrogen are necessary carbon and nitrogen sources for microbial growth, so they are usually significantly related to soil microbial communities [21]. Previous studies focused more on the microbial ecology of major fruits producing soil [22], while study of minor fruit producing farm is limited, e.g. apple-pear growing soils in the current study.

It is well accepted that with the increase of geographic distance, community similarities decrease [23]. The distribution of microorganisms is zonal and regional, which shows that geographic distance within a local spatial scale is an important factor in microbial communities and diversity changes [24,25]. Therefore, this study tried to identify the key factors affecting the soil microbial community in orchards by correlating community structure with both environmental factors and geographic distance.

Soil bacterial and fungal communities account for the vast majority of total soil biomass and they play an important role in maintaining soil functions including plant supporting, genetic resource bank, and pollution purification. Soil microbial communities in combination with soil physicochemical properties would enable people to evaluate the overall health, quality, and fertility of soils. Therefore, in this study, the sequencing data, soil properties, and geographic distance were combined, and the research objectives were to: (i) understand the compositions of bacterial and fungal communities, (ii) investigate keystone taxa in the network structure of bacterial and fungal co-occurrence networks, (iii) obtain the relationship among microbial community, geographic distance, and environmental factors, (iv) discuss potential implications for sustainable management of orchards.

2. Materials and Methods

2.1. Soil Sample Collection and Soil Physiochemical Properties Determination

A total of 28 soil samples were collected from three apple-pear orchards in Jilin province in northeast China (Figure S1), 8 were from Yanji (YJ, N42°55′, E129°33′), 11 were from Longjing (LJ, N42°30′, E129°43′), and 9 were from Helong (HL, N42°42′, E129°8′) in Spring (early May). All orchards were subjected to the similar agricultural management strategies, i.e. the soils were mainly supplemented with organic fertilizer (animal feces) plus minor chemical fertilizer (about 20%). Soil samples were collected with a sterile stainless steel shovel. Each sample was composed of 3 separate soil cores at a distance of 5 meters. All soil samples were transported on ice to the laboratory within the same day, and sieved to 2 mm to remove stones and plant residues. Two subsamples were then made, one subsample was air dried for soil physical and chemical properties determination as well as annual mean temperature and precipitation (Table S1), and the other subsample was stored in the refrigerator under -80 °C until DNA extraction. Soil pH was measured with a glass electrode at a soil-water ratio of 1:2.5. Electrical conductivity salinity (EC, soil to water ratio, 1:2.5) was measured by a conductivity meter. Soil water soluble organic carbon (WSOC, mg/kg) was determined by UV absorbance at 254 nm [26]. Soil particle-size distribution (clay, silt, and sand contents in g/100g) was measured with a laser particle size analyzer (Bettersize 2000, Dandong, China), soil total soluble nitrogen (TN, mg/kg) was determined by potassium persulfate oxidation spectrophotometry (MapData, Shanghai, China), total dissolved phosphorus (TDP, mg/kg) was determined by antimony molybdenum colorimetry (MapData, Shanghai, China), ammonium nitrogen (NH_4^+-N, mg/kg) was

determined by Nessler's reagent colorimetry, nitrate nitrogen (NO_3^--N, mg/kg) was measured by dual-wavelength UV spectrophotometry.

2.2. Soil DNA Extraction, Sequencing, and Data Analysis

Total DNA was extracted from the soil by DNA isolation kit (Omega, Norcross, GA, USA), and DNA extraction quality was determined by 0.8% agarose gel electrophoresis. The quality and concentration of soil DNA were evaluated by NanoDrop NC2000 spectrophotometer (Thermo Scientific, Waltham, MA USA). According to the bacterial 16S rRNA amplicon library construction, the primers 338F: ACTCCTACGGGAGGCAGCA and 806R: GGACTACHVGGGTWTCTAAT were used to amplify the V3-V4 region of the 16S rRNA gene. In terms of fungi, the ITS1 gene was amplified using the primers ITS5F: GGAAGTAAAAGTCGTAACAAGG and ITS1R: GCTGCGTTCTTCATCGATGC [27]. The PCR amplification products were detected on 2% agarose gel electrophoresis, and the target fragments were cut and recovered. With reference to the preliminary quantitative results of electrophoresis, the PCR amplification products were quantified by fluorescence. High-throughput sequencing was performed on the Illumina Miseq platform (Illumina, San Diego, CA, USA) [28] at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The QIIME software (Quantitative Insights Into Microbial Ecology, v1.8.0, http://qiime.org/) [29] was used to identify interrogative sequences. Then QIIME software was used to call USEARCH (v5.2.236) in order to check and remove chimera sequences. QIIME software was then applied using UCLUST sequence comparison tool [30], which could merge and divide OTU sequences previously obtained according to 97% sequence similarity based on Greengenes database (Release 13.8) for bacteria and UNITE database (release 115) for fungi. The sequencing data have been deposited with links to BioProject under accession number PRJNA640242 for bacterial community and PRJNA640241 for fungal community in the NCBI BioProject database.

2.3. Statistical Methods

Graphics were constructed with OriginPro9.0 (OriginLab, Northampton, MA, USA), histograms and boxplots described the compositions and differences of bacterial and fungal communities. Pearson correlation was calculated using SPSS 22 (IBM, Armonk, NY, USA). Principal coordinates analysis (PCoA) and dissimilarity analysis were used to analyze the differences in community compositions at the three sampling points. Principal coordinates of neighbor matrices (PCNM) was used to convert latitude and longitude coordinates into geographic distance. Partial Mantel test and canonical correspondence analysis (CCA) were used to study the relationship between community structure and soil physiochemical parameters. Variation partition analysis (VPA) was used to quantify the contribution of physical and chemical properties which explain the composition of bacterial and fungal communities. PCoA, CCA, VPA, and PCNM statistical analyses were calculated using R 3.5.2.

2.4. Network Construction and Statistical Analysis

Combined with high-throughput sequencing data, molecular ecological networks (MENs) were constructed based on random matrix theory (RMT) methods, providing a way for understanding network interactions in microbial communities and their responses to environmental changes [31,32]. MENs of bacteria and fungi were constructed using a network method based on random matrix theory (RMT), network construction and acquisition of network properties parameters were completed on the Molecular Ecological Network Analyses Pipeline (MENA, http://ieg4.rccc.ou.edu/mena) website [31,33,34]. MENA can be divided into two stages [31]: the first stage is the network construction, including uploading high-throughput sequencing data, standardizing relative abundance, calculating the Pearson correlation of any two OTUs, and converting the correlation matrix into similarities based on the pairwise correlation coefficient matrix, which uses a specific threshold based on random matrix theory to convert the similarity matrix into an adjacency matrix [35]. The second stage is the network analysis. The network property parameters

are obtained through analysis and calculation. Network property parameters include similarity threshold (st), network size (the number of OTUs), R^2 of power law, link, average connectivity (avgK), average path (GD), average clustering coefficient (avgCC), and modularity. The module has a high degree of internal connectivity and relatively few external connections. The role of each node is determined according to two attributes: within-module connectivity (Zi), which is to quantify the degree of connection between a node and other nodes in the module; among module connectivity (Pi), which is to quantify the degree of node connection to different modules [36,37]. The role of OTU in the network is characterized by Zi and Pi, which divide all categories into four subcategories: peripherals, connectors, module hubs, and network hubs [38]. Generally, all nodes with $Zi \geq 2.5$ or $Pi \geq 0.62$ were designated as keystone taxa [31].

3. Results

3.1. Bacterial and Fungal Community Composition

The relative abundance of bacteria and fungi at phylum and class levels were shown in Figure 1. The main phyla of the bacterial communities (with relative abundance greater than 1%) in all samples were *Actinobacteria* (41.81%), *Proteobacteria* (24.07%), *Chloroflexi* (8.74%), *Gemmatimonadetes* (5.40%), *Acidobacteria* (10.44%), *Bacteroidetes* (2.03%), *Firmicutes* (3.02%), *Saccharibacteria* (0.21%), *Nitrospirae* (0.75%), and these dominant phyla accounted for 90% of the bacterial compositions. *Proteobacteria* were mainly composed of *Alphaproteobacteria* (14.08%), *Betaproteobacteria* (4.33%), *Gammaproteobacteria* (2.66%), and *Deltaproteobacteria* (2.99%) (Figure 1b).
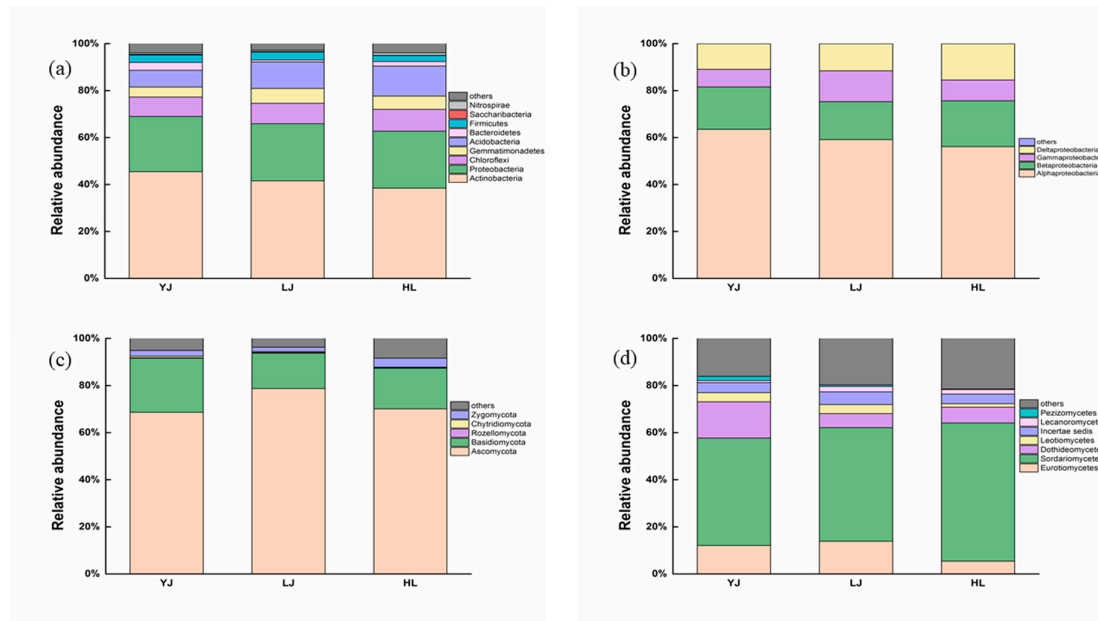

Figure 1. Stacked diagrams of bacterial and fungal communities' composition, (**a**) bacterial phyla; (**b**) the main classes of *Proteobacteria*; (**c**) fungal phyla; (**d**) the main classes of *Ascomycota*. YJ, LJ, and HL indicated soil samples from Yanji, Longjing, and Helong, respectively.

The main phyla of fungal communities (with relative abundance greater than 0.1%) were *Ascomycota* (72.49%), *Basidiomycota* (18.43%), *Rozellomycota* (0.20%), *Chytridiomycota* (0.43%), and *Zygomycota* (2.69%), and more than 80% of the fungi composition belonged to these phyla (Figure 1c). The relative abundance of *Ascomycota* in the three orchards was YJ (68.6%), LJ (78.8%), and HL (70.1%), respectively. Figure 1d showed the relative abundance of the main class *Ascomycota*, including *Eurotiomycetes* (10.43%), *Sordariomycetes* (50.89%), *Dothideomycetes* (9.34%), *Leotiomycetes* (3.06%), *Incertaesedis* (4.61%), *Lecanoromycetes* (1.69%), and *Pezizomycetes* (0.88%).

Differences existed in bacterial community composition among the three orchards were presented in Figure 2. Relative abundance of both *Acidobacteria* and *Bacteroidetes* demonstrated significant difference between any two orchards ($p<0.05$, Figure 2a, b). Relative abundance of *Actinobacteria* was found significantly different only between HL and YJ ($p<0.05$, Figure 2c), relative abundance of *Nitrospira* in HL was significantly higher than those in LJ and HL ($p<0.05$, Figure 2d).
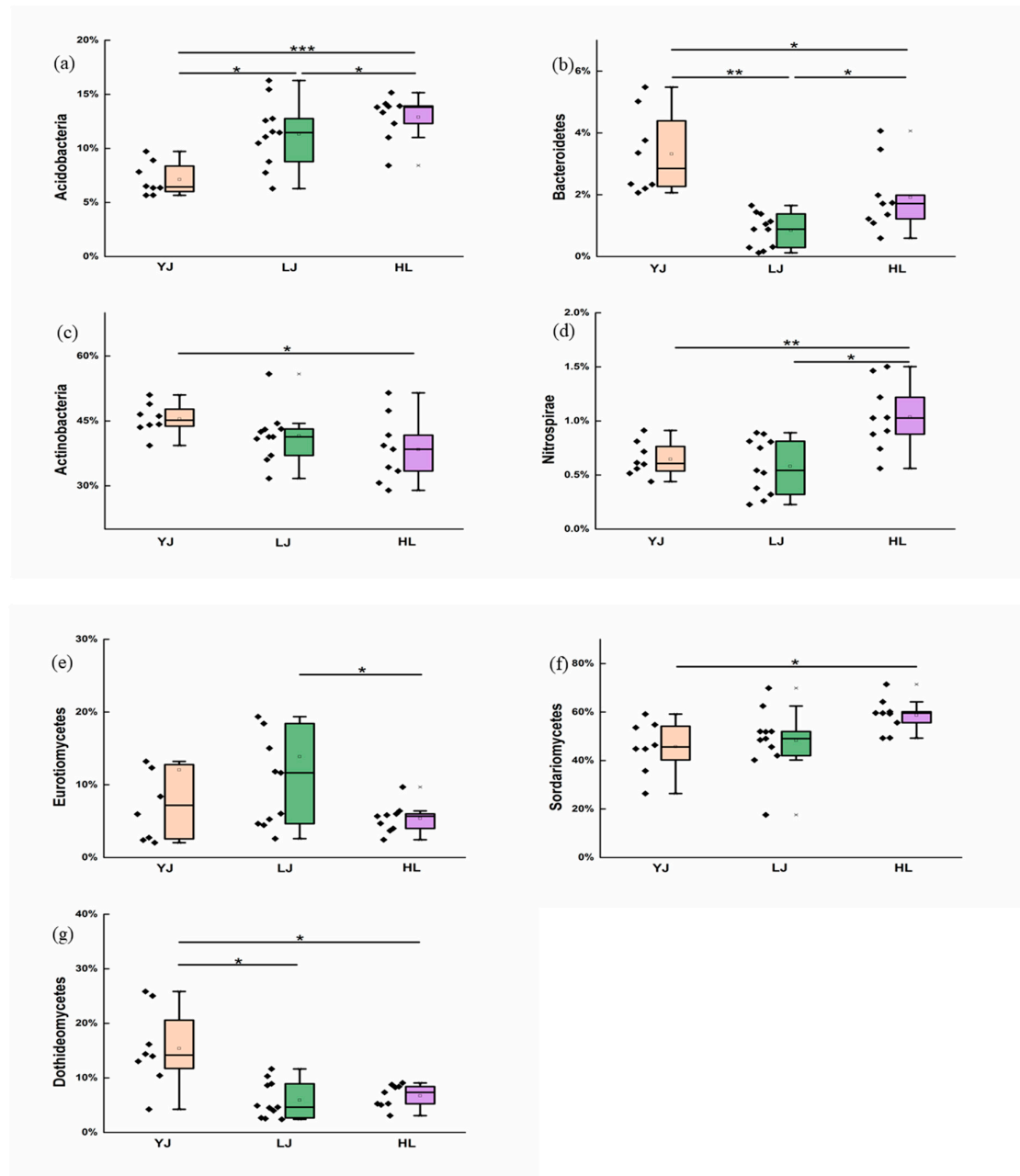

Figure 2. Difference analysis of percent relative abundance of bacterial and fungal communities in three orchard soils. (**a**) to (**d**) were the bacterial phyla, and (**e**)-(**g**) were the classes of the *Ascomycota* of fungi. YJ, LJ, and HL indicated soil samples from Yanji, Longjing, and Helong, respectively. *, **, and *** indicated *p* values were significant at 0.05, 0.01, and 0.001 level, respectively.

For fungal communitiy, no difference was found on the relative abundance of phylum *Ascomycota* in all three orchards (data not shown), thus further analysis at class level of phylum *Ascomycota* was performed. It was found that the relative abundances of *Eurotiomycetes* in LJ is higher than that in HL ($p<0.05$, Figure 2e), and the relative abundance of *Sordariomycetes* in YJ was less than

that in HL (*p*<0.05, Figure 2f). In YJ soils, the relative abundance of *Dothideomycetes* was greater than those in HL and LJ (*p*<0.05, Figure 2g).

The bacterial and fungal community composition of all soil samples were visualized via principal coordinates analysis (PCoA). Figure 3a and 3b revealed that the three sites YJ, LJ, and HL could be well separated by PCo1 and PCo2. A combination of different dissimilarity analysis methods including mrpp, adonis, and anosim of the three sites using bray, horn, gower, jaccard, and kulczynski distance indicated that the structure and composition of bacterial and fungal communities were significantly different (*p*<0.01, Table 1).
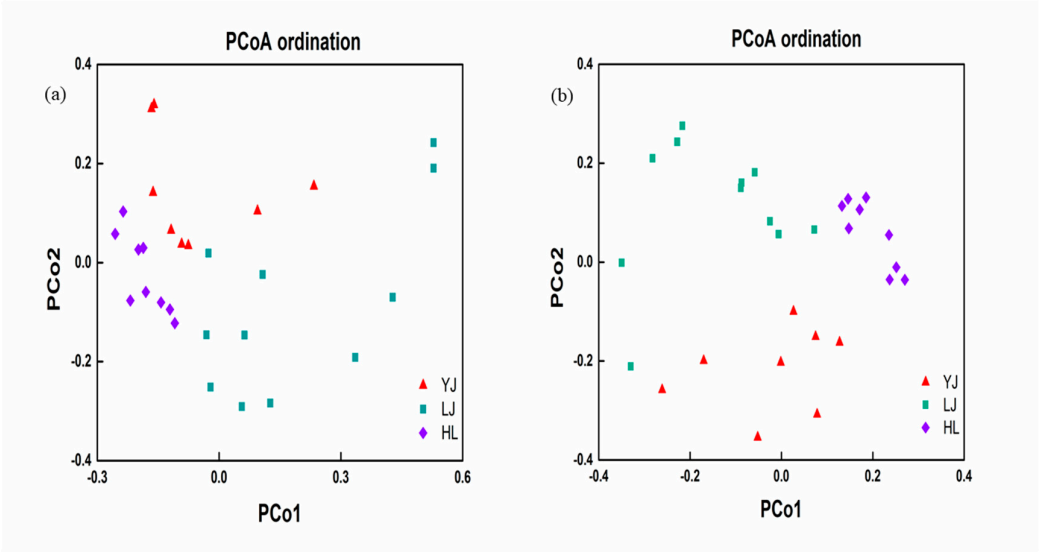

Figure 3. Principal coordinates analysis (PCoA) of bacterial (**a**) and fungal (**b**) communities in orchards. Red triangles, green squares and purple diamonds represented soil samples from Yanji (YJ), Longjing (LJ) and Helong (HL), respectively.

Table 1. Dissimilarity analysis based on Bray-Curtis distance of bacteria and fungi in three orchards. Three distance indices were mrpp (*δ*), Adonis (*F*), and Anosim (*R*). MRPP, Multi Response Permutation Procedure; Anosim, Analysis of similarities.

		mrpp		Adonis		Anosim	
		δ	*p*	*F*	*p*	*R*	*p*
	Bray	0.684	0.001	2.193	0.002	0.407	0.001
	Horn	0.542	0.001	2.609	0.017	0.337	0.001
bacteria	Gower	0.120	0.001	1.914	0.001	0.324	0.001
	Jaccard	0.808	0.001	1.808	0.002	0.407	0.001
	Kulczynski	0.683	0.001	2.198	0.003	0.409	0.001
	Bray	0.656	0.001	2.789	0.001	0.369	0.001
	Horn	0.559	0.001	3.010	0.001	0.273	0.001
fungi	Gower	0.118	0.001	2.091	0.001	0.223	0.001
	Jaccard	0.785	0.001	2.224	0.001	0.369	0.001
	Kulczynski	0.656	0.001	2.789	0.001	0.369	0.001

3.2. Network Analysis

Specifically, in the analysis of bacterial community, only the operational taxonomic units (OTUs) appearing in >90% of the total samples were used for network calculations. The overall topological indices (Table 2) showed that all the curves of the network connectivity distribution completely fit with the power-law model (R^2 values from 0.80 to 0.93). All the modularity values were from 0.70 to 0.88, GD values were from 5.65 to 9.86, and avgCC values were from 0.19 to 0.28, which were significantly higher than the three values of the randomized network, respectively. From Table 2, with the combination of molecular ecological network (Figure 4a, b and c), it could be seen that LJ's nodes and edges were the least among the three orchards, YJ's nodes were less than HL's, but YJ's edges were higher than HL's, and HL's modularity was the largest of the three.

In the analysis of fungal communitiey, only the operable taxonomic units (OTUs) appearing in >60% of the total samples were involved in the network calculation (Figure 4d, e and f), and all the curves of the network connectivity distribution were fully consistent with the power law model (R^2 values from 0.84 to 0.89, Table 2). All modular values were from 0.74 to 0.86, GD values were from 5.62 to 6.73, and avgCC values were between 0.13 and 0.24, which were significantly higher than those three values of the Random network, LJ had the least nodes and edges in the three orchards. The numbers of nodes of HL and YJ are similar, but the edges of HL were higher than those of YJ. YJ's network was the most modular in the three network diagrams.

According to within-module connectivity (Zi) and among-module connectivity (Pi), the majority (more than 95%) of the bacteria's OTUs were in peripheral (Figure 5a). The number of OTU links in peripheral was small and connected to the nodes in their own module. A total of 8 nodes in the three orchards were in connectors (four nodes in YJ, four nodes in LJ), and eight nodes were in module hubs (three nodes in YJ, three nodes in LJ, and two nodes in HL). The nodes in connectors and module hubs were as following: *Actinobacteria*, *Alphaproteobacteria*, *Gammproteobacteria*, *Gemmatimonadetes* in YJ soils; *Actinobacteria*, *Deltaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, *Nitrospirae* in LJ soils; *Alphaproteobacteria* and *Chloroflexi* in HL soils.

In the fungal network diagram, most (>95%) of the OTUs were in peripheral (Figure 5b). A total of 10 nodes in the three orchards were in the connectors (three nodes in LJ and seven nodes in HL), and a total of 8 module hubs (one node in YJ, three nodes in LJ, and four nodes in HL). All nodes in connectors and module hubs were exclusively affiliated with the specific *Ascomycota* phylum. Unidentified class in Yanji; *Eurotiomycetes*, *Sordariomycetes*, *Lecanoromycetes* in LJ; *Sordariomycetes*, *Dothideomycetes*, *Eurotiomycetes*, and unidentified in HL soils.
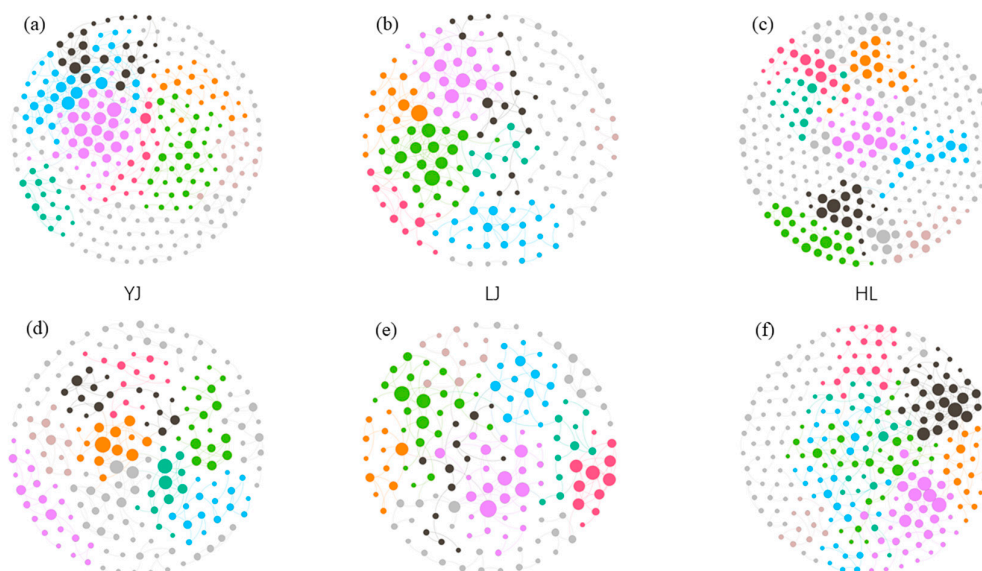

Figure 4. Molecular ecological network diagram visualizing interactions between bacterial communities (**abc**) and fungal communities (**def**). YJ, LJ, and HL indicated soil samples from Yanji,

Longjing, and Helong, respectively. The bigger the size of each circle indicated that the OTU had more links with other OTUs. The circles shared the same color indicated that those circles belonged to the same module.

Table 2. Topological properties of empirical molecular ecological networks (MENs) of bacterial and fungal communities and their related random MENs.

		Bacterial community			Fungal community		
		YJ	LJ	HL	YJ	LJ	HL
	st	0.88	0.86	0.87	0.9	0.85	0.9
	Network size	275	177	324	201	157	253
	link	491	287	383	252	216	403
Empirical	avgK	3.57	3.24	2.36	2.51	2.75	3.19
networks	GD	7.33	5.65	9.86	6.73	6.23	5.62
	avgCC	0.28	0.2	0.19	0.24	0.13	0.14
	Modularity	0.71	0.7	0.88	0.86	0.78	0.74
	R^2	0.86	0.93	0.8	0.87	0.84	0.89
	GD	4.23	4.07	6.60	5.40	4.80	4.45
	±SD	±0.06	±0.08	±0.18	±0.16	±0.14	±0.08
Random	avgCC	0.019	0.023	0.005	0.009	0.014	0.016
networks	±SD	±0.006	±0.008	±0.003	±0.006	±0.008	±0.006
	Modularity±SD	0.54	0.55	0.74	0.69	0.63	0.58
		±0.01	±0.01	±0.01	±0.01	±0.01	±0.01

st, similarity threshold; network size, the nodes in a network; avgK, average connectivity; GD, average path; avgCC, average clustering coefficient. YJ, LJ, and HL indicate soil samples from Yanji, Longjing, and Helong, respectively.

3.3. Relationship of Microbial Communities to Physicochemical Properties and Geographic Distance

Mantel and partial Mantel tests were used to investigate the relationship between the environmental attributes and the microbial community structure (Table 3). Mantel tests based on Bray-Curtis and Euclid distances showed that soil texture (clay, silt, and sand content), pH, WSOC, NO_3^--N were significantly correlated with bacterial communitiy ($p<0.05$). Clay and pH were significantly correlated with fungal communities ($p<0.05$). Results of Pearson correlation (Table S2) between bacterial communities and physicochemical properties showed that sand and silt contents were positively correlated with PCo1 ($p<0.05$), pH had a negative correlation with PCo1 ($p<0.01$), WSOC and NO_3^--N were negatively correlated with PCo2 ($p<0.01$). According to the Pearson correlation between fungal communities and soil properties (Table S3), pH had a positive correlation with PCo1 ($p<0.01$) and a negative correlation with PCo2 ($p<0.05$).
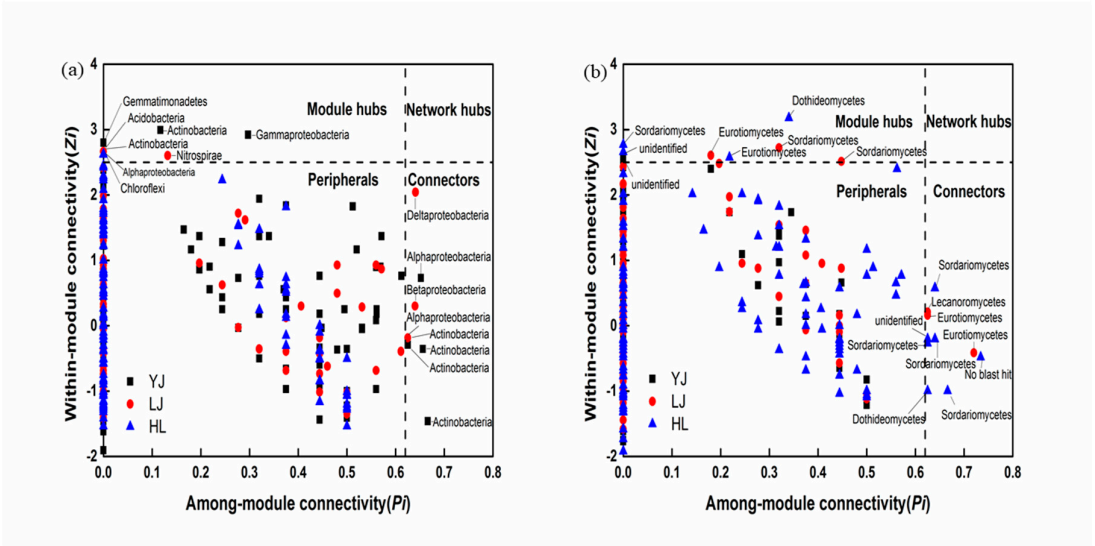
Figure 5. *Zi-Pi* diagram showed the topological distribution of OTUs based on bacterial (**a**) and fungal networks (**b**). The classification thresholds of *Zi* and *Pi* of OTU were 2.5 and 0.62, respectively. YJ, LJ, and HL indicated soil samples from Yanji, Longjing, and Helong, respectively.

Table 3. Mantel and Partial Mantel tests assessed the importance of environmental factors in the entire network of bacterial and fungal communities.

	Bacterial community				Fungal community			
	Mantel		Partial		Mantel		Partial	
clay	0.232**	0.009	0.117	0.090	0.172*	0.049	0.152	0.072
silt	0.352***	0.001	0.284*	0.011	0.158	0.113	0.140	0.152
sand	0.335**	0.007	0.236*	0.019	0.177	0.087	0.159	0.072
pH	0.555***	0.001	0.573***	0.001	0.277*	0.019	0.277*	0.031
WSOC	0.236**	0.008	0.174*	0.022	0.082	0.207	0.063	0.216
NO_3^--N	0.263*	0.033	0.042	0.310	0.054	0.275	-0.020	0.537

WSOC, water soluble organic carbon; NO_3^--N, nitrate nitrogen. *, **, and *** indicated *p* values were significant at 0.05, 0.01, and 0.001 level, respectively.

Canonical correlation analysis (CCA) and variation partition analysis (VPA) were used to quantify the relationship among the environmental attributes, geographic distance and the microbial community structure. The relationship among soil parameters, geographic distance and soil bacterial community structure were shown in Figure 6a and c. These variables explained nearly 47.23% of overall variations of the bacterial communities, with geographic distance, soil texture and pH (including clay, sand, and pH), and other physical and chemical properties (WSOC, TN, NH_4^+-N, NO_3^--N, TDP) being explained 10.55%, 13.35%, and 19.03%, respectively. For fungal communities (Figure 6b and d), geographic distance, soil texture and pH (clay, sand, and pH), and other soil properties could explain 11.61%, 13.03%, and 20.26%, respectively.
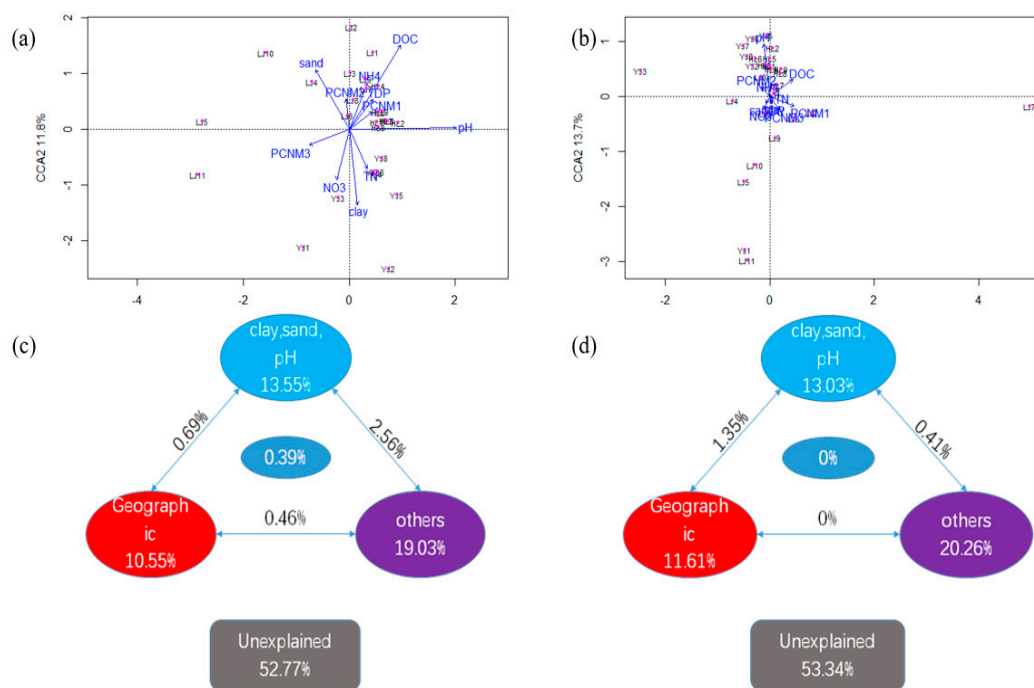
Figure 6. Canonical correspondence analysis (CCA) and variation partition analysis (VPA) explored the relationship among soil properties, geographic distance, and bacterial communities (**ac**) or fungal communities (**bd**).

4. Discussion

4.1. Soil Microbial Composition and Keystone Taxa

The composition and structure of soil bacterial and fungal community in apple-pear orchards were characterized by high-throughput sequencing and the keystone taxa were identified by molecular ecological network analysis based on within-module connectivity and among-module connectivity. Among the bacterial communities, *Actinobacteria* and *Proteobacteria* accounted for the highest proportion. *Alphaproteobacteria* and *Betaproteobacteria* accounted for the highest proportion of *Proteobacteria*, which was consistent with a previous study on bacterial community composition of apple orchards [2]. Manuel's research on major global bacterial systems found that *Proteobacteria* and *Actinobacteria* were the two bacterial phyla with the highest relative abundances [3], which was consistent with the results of our study showing that *Actinobacteria* and *Proteobacteria* were also the keystone taxa in the apple-pear orchards' bacterial communities. Therefore, *Actinobacteria* and *Proteobacteria* were both dominant phyla and keystone taxa, indicating that they might play an essential role in the ecological functioning of microbial communities. Similar findings were also found for rhizospheric and bulk soils, subtropical, and low temperate soils [21,39]. In addition to *Actinobacteria* and *Proteobacteria*, the keystone taxa of the three orchards included *Acidobacteria*, *Nitrospirae*, and *Chloroflexi*. *Acidobacteria* in the soil was usually considered to be negatively correlated with pH, so this may be the reason why *Acidobacteria* became keystone taxa in LJ soils with relatively lower pH. *Nitrospirae* were keystone taxa in the bacterial communities, and they could convert ammonia nitrogen into nitrate nitrogen. This conversion directly affected the ratio of nitrate nitrogen to ammonia nitrogen in the soil, thus played an important role of nitrogen cycling in apple-pear orchard soils. *Chloroflexi* were bacteria that produced energy through photosynthesis, and had facultative anaerobic characteristics [40]. Light intensity might be the main reason why *Chloroflexi* become keystone taxa of bacterial community. For the fungal community, *Ascomycota* and *Basidiomycota* were the dominant phyla, and all keystone taxa identified belonged to *Ascomycota*

according to the *Zi-Pi* diagram. Studies have shown that *Ascomycota* were dominant in soil fungal communities [41]. *Ascomycota* were mostly saprophytes and played an important role in degrading soil organic matters. *Basidiomycota* were saprophytic or parasitic. In soils with higher moisture, they could decompose lignocellulose. Previous research had shown that *Ascomycota* and *Basidiomycota* were the main decomposers in soils [42,43].

The keystone taxa generally played an important role in structuring and functioning of the microbial community and such role might not change due to their abundance [44]. Moreover, the keystone taxa position in the microbial community were very special, and their extinction would bring huge changes to the structures and functions of the microbial communities [44]. The importance of keystone taxa might be related to the complexity of biological processes, e.g. nitrogen fixation or organic carbon turnover. When the processes were carried out by some special microbes, the impact of keystone taxa might be more direct and obvious [45]. The keystone taxa might affect the microbial communities in different ways: 1) they might act via indirect groups, which could selectively adjust their abundance to regulate community structure and function by producing metabolites, bacteriocins or toxins to change community composition and structure [46,47]; 2) they might excrete antibiotics to selectively alter the composition and structure of the microbial communities; 3) they might also establish synergies and change the number of their partners, which might have an major impact on microbial community structure, composition, as well as their ecological functioning [44].

4.2. Effects of Soil Properties on Communities

Results of Mantel and Partial Mantel tests showed that soil texture, pH, WSOC and NO_3^--N could significantly affect the composition of bacterial communities, while soil texture and pH could significantly influence the composition of fungal communities. Obviously, soil texture was an important factor in shaping both bacterial and fungal communities in apple-pear orchards. Clay could serve as a binding site for microorganisms and provide nutrients, adjust the soil pH range, absorb harmful metabolites in the environment, as well as protect microorganisms from external adversity and predators [48]. Soil texture had a stronger impact on bacterial communities than on fungal communities (Table 3). This was because bacteria mainly exist in soil particles, while fungi mainly lived in soil aggregates and most could not survive in micropores as they were larger than bacteria [49,50]. This study found that sand showed a significantly positive relation to PCoA1, which could be explained by the spatial isolation hypothesis [51]. This hypothesis proposed that when the sand content was relatively large, the soil pores appeared to be larger, and water formed a water film in the pores, so many hydrated microhabitats appeared in the soil, which helped to increase the bacterial diversity [51]. While most fungi lived in the soil aggregates, so the soil texture had a less impact on fungi than on bacteria. pH was also an important factor affecting soil bacterial and fungal communities, which was consistent with previous research results [52]. When the soil pH was too high or too low, it would be a direct stress to the soil microbial communities as an external adversity, and pH could also directly determine the speciation and bioavailability of the mineral nutrients. The current research also showed that soil pH had a significant effect on the bacteria *Deltaproteobacteria*, *Saccharibacteria*, and *Rozellomycota* of the fungi. Previous studies have found that the relationship between fungi and pH was weaker than that between bacteria and pH [53], which was roughly consistent with our results. The composition of the bacterial community was also affected by WSOC and NO_3^--N. According to the results of mantel and partial mantel, the impact of WSOC on the bacterial community could be explained from two perspectives, one was that WSOC directly served as the carbon source of bacteria, and the other was that WSOC indirectly influence bacterial communities by changing soil properties, e.g. the average size of soil aggregates [54]. It was also found that NO_3^--N affected the bacterial community by affecting other soil properties, and might not directly affect the bacterial community, this results were somewhat in contrast to those from other studies [55,56], the potential reason might lie in the amount and history of nitrate-containing fertilizer applied into the apple-pear orchards soils.

4.3. Interactions between Bacterial Taxa as Revealed by Co-Occurrence Networks

MENs were widely used in ecological studies, such as comparing networks under different yields and different management modes, comparing networks of bacteria, fungi, and archaea, as well as symbiotic and trophic interactions between species within the network [57–60]. An ecological network reflected complex biological interactions in the ecosystem, with nodes representing species and edges representing interactions between species [55,61]. Since nodes in the network represented species in the community, the topological role of different nodes could be described as the basis for determining keystone taxa. The keystone taxa were highly related taxa, and they would have a considerable impact on the structure and function of the microbiome, therefore, if they were removed, rapid changes would occur in the structure and function of the microbiome [44]. Our data provided some useful insights into a better understanding of the soil microbial community structure of the apple-pear orchards and the keystone taxa in the bacterial and fungal communities. Such information would be helpful in design of managerial strategies for apple-pear orchard soils.

4.4. Effects of Geographic Distance on Microbial Communities

It could be seen from CCA and VPA that geographic distance could significantly affect bacterial and fungal communities, as it could explain 10.55% of bacterial community differences and 11.61% of fungal community differences. Environmental selection and geographic distance had usually been considered as the two main ecological processes affecting the overall variation in microbial communities [11]. The current study showed that some of the variation in orchard soil bacterial and fungal communities were due to geographic distance, which was consistent with previous research results [55]. It was a common phenomenon that the similarity of community structure decreased with the increase of geographic distance. The main driving force of this biogeographic pattern was the diffusion limitation [23].

The geographic distance of the three orchards samples was relatively close, but the microbial communities' differences were significant (Table 1). This might be due to the major different in regional microclimate, especially the mean annual precipitation which is much higher in HL soils (Table S1). In this study, although soil characteristics (such as soil texture, pH, WSOC) and geographic distance could explain about 50% of overall variation of bacterial and fungal communities, leaving another 50% unexplained, which might be attributed to the unmeasured soil parameters, including soil moisture, redox potential, soil virus, protozoa, as well as soil animal communities. Obviously, additional work was needed to a better understanding the factors shaping the apple-pear orchard soil microbial communities.

In the current work, soil physical, chemical and biological factors, as well as geographic distance were found to be important parameters in shaping the composition and structure of both bacterial and fungal communities in apple-pear orchards soils. However, all of those factors only explained less than 70% of overall variation of microbial communities across all samples. The possible reasons might lie in the unmeasured soil properties, including but not limited to soil minerals, trace metals, available P and K, organic N, soil animals, protists and viruses. The local climate should also be taken into account. Previous studies revealed that local anthropogenic activities and climate change might exert a major influence in soil quality as indicated by soil enzymatic activities [22,62].

5. Conclusions

The current study, high throughput sequencing in combination with bioinformatics analysis techniques were applied to probe the composition and structure of bacterial and fungal communities in apple-pear orchard soils. The keystone taxa were identified by topological properties of molecular ecological networks. We also found that soil properties and geographic distances significantly affected bacterial and fungal community compositions. Soil texture and pH significantly affected the compositions of bacterial and fungal communities, and bacterial communities were also affected by WSOC and NO_3^--N. The results of our study provided useful background information for apple-pear orchard soil management in order to improve the overall productivity and quality of apple-pear.

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