Community Structure and Co-Occurrence Network Analysis of Bacteria and Fungi in Wheat Fields vs Fruit Orchards

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Abstract

Soil microorganisms play a vital role in biogeochemical processes and nutrient turnover in agricultural ecosystems. However, the information on how the structure and co-occurrence patterns of microbial communities respond to the change of planting methods is still limited. In this study, a total of 34 soil samples were collected from 17 different fields of two planting types (wheat and orchards) along the Taige Canal in Yangtze River Delta. The distribution and diversity of bacterial and fungal communities in soil were determined using amplicon sequencing targeting the 16S rRNA gene and ITS gene, respectively. The dominated bacteria were Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, Bacteroidota, and Firmicutes. The relative abundance of Actinobacteriota and Firmicutes was higher in the orchards, while Chloroflexi and Nitrospirota were more abundant in wheat fields. Ascomycota, Mortierellomycota, and Basidiomycota were the predominant fungi in both types of soils. The diversity of bacterial and fungal communities was greater in the wheat fields than in the orchards. The statistical analyses showed that pH was the main factor shaping the community structure. Moreover, high co-occurrence patterns of bacteria and fungi were confirmed in both wheat fields and orchards. Network analyses showed that both the wheat fields and orchards occurred modular structure, which mainly contained nodes of Acidobacteriota, Chloroflexi, Gemmatimonadota, Nitrospirota and Ascomycota. In summary, our work showed the co-occurrence network and the convergence/divergence of microbial community structure in wheat fields and orchards, giving a comprehensive understanding of the microbe-microbe interaction during planting methods changes.

Highlights

- The diversity of both bacteria and fungi were higher in the wheat fields than in orchards.
- pH was the main factor affected the soil bacterial in wheat fields, while WC, TOC and TN all had great influences on bacterial community in the orchards.
- Co-occurrence of bacteria and fungi were both investigated in wheat and orchard soils.

1. Introduction

Soil microorganisms, as an active participant of soil ecosystem, are the drivers for transformation and cycling of nutrient elements, such as carbon, nitrogen, phosphorus, sulfur, and so on. They are also involved in metabolism processes, directly affecting the earth's biochemical cycle (Gao et al., 2020). Microbial-biogeography studies have focused greatly on community structure, and on how their diversity and composition respond to local abiotic and biotic factors of soil (Fierer, 2017; Gao et al., 2020). It is reported that the soil microbial community structure and diversity are impacted by environmental changes or human disturbance, such as soil ages (Shanmugam & Kingery, 2018), environmental stress (Sinha et al., 2009; Jiang et al., 2020; Zhang et al., 2020), natural succession process (Zhang et al., 2016), allelopathic plant effect (Hortal et al., 2015; Kong et al., 2008), continuous cropping (Ying et al., 2012), long-term crop rotation (D'Acunto et al., 2018), and fertilization (Zhang et al., 2019). For a long time, impacts from planting types have been considered as one of the most important factors that change the diversity of soil microbial communities (Deng et al., 2019; Guo et al., 2020).

In addition to environmental factors, interspecies interactions also have a strong impact on microbial communities. Microorganisms, such as bacteria and fungi, always coexist and interact with each other in various habitats and positively or negatively interact with each other (D'Acunto et al., 2018), contributing significantly to biodiversity and biomass, and affecting essential soil processes and function (Bahram et al., 2018). For example, bacteria act as plant rhizobacteria which are beneficial to their hosts with nutrients, and fungi known as plant symbionts are helpful for plant health (Fisher et al., 2012). Additionally, molecular communications between bacterial and fungal communities are highly relevant for sustainable soil management (Lemanceau et al., 2016). For example, fungi usually play a key role in the decomposition of complex organic compounds, producing small molecules, which are then further decomposed by bacteria in the same habitat. Moreover, many fungal groups can secrete large amounts of antibacterial compounds, causing bacterial antibiotic resistance (Bahram et al., 2018). Bacteria may contribute to nutrient provision for plants, e.g., performing important steps of the nitrogen cycle, including nitrogen fixation, nitrification, and denitrification (Nelson & Sadowsky, 2015; Meng et al., 2017). The interactions between bacteria and fungi, such as the binding of soil bacterial and fungal spores, the injection of molecules into fungal spores by bacteria, the production of volatiles by bacteria, and the degradation of fungal cell walls, have been summarized by a previous study (Miransari, 2011). Therefore, it is important to investigate both bacteria and fungi at the same biotopes.

Co-occurrence network analysis provides new insight into the interaction of microbial taxa in the complex community by employing a more standard suite of analytical approaches. In general, the inter association between taxa may help to reveal the niche shared by community members (such as bacteria, archaea and fungi), or may help to reveal the more direct symbiotic relationship between community members (Barberati et al., 2012). But reliable network analysis is based on deep exploring patterns in large and complex datasets, which are more difficult to detect using the standard alpha/beta diversity metrics widely used in microbial ecology (Proulx et al., 2005). Fortunately, due to the advances in barcoded pyrosequencing technique (Feng et al., 2014; de Vries et al., 2018; Wang et al., 2020), it is now possible to generate microbial datasets using network analysis approaches in highly diverse communities, like those found in soils, to explore co-occurrence patterns. Exploring co-occurrence patterns between soil microorganisms can help to decipher inter- or intra-phyla interactions related to biotic factors, habitat affinities or shared common physiology to guide more focused research.

The Taige Canal is located in the economically developed and densely populated Taihu Basin. The Taihu Basin has sufficient water, light and heat resources, which greatly support the development of agriculture. The major planting methods operated by farmers in this area are rice-wheat rotation (Zhou et al., 2012). It is a typical region of the Yangtze River Delta, which is the most developed region in Chinese agriculture ecosystem. With the adjustment of the industrial structure and the renewal of farmers' ideas, some wheat fields have been replaced with orchards. The area of farmland has decreased, and the planting area of various orchards has increased year by year in the Yangtze River Delta (Ji et al., 2008; Min et al., 2020). Due to changes in planting methods, the amount of fertilization, plant density, and the content of elements such as N and P in the soil, the structure and diversity of microbial communities have changed. The co-occurrence networks help to determine associations between microbial groups. However, few studies focus on the co-occurrence network of microbial communities in response to changes in planting patterns. Therefore, we addressed the following questions: 1) Do soil microorganisms tend to co-occurrence networks of microbe-microbe intra-reaction patterns in soils? 2)

Which taxa are generalists and how these ecological categories (niche differentiation) shape network structure?

2. Material And Methods

2.1 Soil sample collection and processing

A total of 17 sites including wheat fields (10 sites) and orchards (7 sites) were selected to collect samples asides the Taige canal in April 2018 (Fig. S1), where is a typical agriculture mode in the Yangtze River Delta, China. At each site, 5 replicates of soil samples at depths of 0-5 cm and 5-10 cm were collected, put into a sterilized sampling bag, and stored with ice bags. All the samples were sent to the laboratory immediately after sampling. The soil samples were thoroughly mixed and divided into two parts. One part was sieved by 2 mm mesh for determining the physical-chemical properties of soil, and the other part was stored at -20°C for molecular analysis.

2.2 Determination of soil physic-chemical properties

The pH value was measured by 1:2.5 soil-water mixture with a pH meter (PB-10/C, Sartorius, Germany) (Bao 2005). The water content (WC) was calculated by weighing the sample before and after drying at 105°C for 12 hours. TN was determined by alkaline potassium persulfate digestion and UV spectrophotometry (CNS-HJ 636-2012). The concentrations of ammonium (NH_4^+-N) and nitrate (NO_3^--N) were measured using AutoAnalyzer3 (SEAL, Germany) water quality flow analyzer after extraction by 1M KCl. The concentration of total organic carbon (TOC) was measured using a TOC analysis meter (Multi N/C 2100S, Analytikjena, Germany).

2.3 DNA extraction, PCR amplification, and sequencing

Soil genomic DNA was extracted by DNeasy PowerSoil Kit (QIAGEN, USA) according to the manufacture guidance, followed by measuring the DNA quantity and quality with the NanoDrop™ 2000 spectrophotometer (Thermo Science, USA). DNA extracts were stored at −20°C for further molecular experiments.

The primer sets of 341F (5’-CCTAYGGGRBGCASCAG-3’) and 806R (5’-GGACTACNNGGGTATCTAAT-3’) were used to amplify the bacterial 16S rRNA gene against the V3-V4 region (Luisa et al., 2014), and the primers of ITS5-1737F (5’-GGAAGTAAAAGTCGTAACAAGG-3’) and ITS2-2043R (5’-GCTGCGTTCTTCATCGATGC-3’) were applied to amplify the ITS1 regions of fungi (Taylor et al., 2016). In order to distinguish different samples, a 12 bp barcode was fused into the forward primer for each sample. PCR was performed in a 50 μL reaction mixture system, including 25 μL premixed EX Taq PCR enzyme (2×) (Takara, Shanghai, China), 1 μL primer pair (Shenggong, Shanghai, China), 1 μL template DNA and sterilized water. Polymerase chain reaction (PCR) of bacterial 16S rRNA gene was conducted using the following procedures: initial denaturation at 95°C for 3 min, then 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 45 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 10 min. Amplification of the fungal ITS gene was operated with the following steps: initial denaturation at 95°C for 3 min, 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 35 s were carried out, and finally extended at 72°C for 10 min. Negative controls were set up to confirm that there was no contamination in the process. The PCR products were detected by agarose gel electrophoresis, and purified with FastPure Gel DNA Extraction Mini Kit (Vazyme, Nanjing, China). A paired-end strategy (2×250 bp) was employed for shotgun sequencing on Illumina Novo seq platform sequencing (Novo gene, Beijing, China).

Microbiome bioinformatics was performed with QIIME 2 (version 2019.7) (Bolyen et al., 2018). All the clean reads were paired-end merged and followed by denoising with DADA2 (Callahan et al., 2016) (via q2-dada2). The similarity of 97% was set as the same operational taxonomic units (OTUs) without singletons. Alpha-diversity metrics of Chao1 (Chao, 1984), Shannon index (Shannon & Weaver, 1962), Simpson's index (Simpson, 1949) and Pielou's evenness (Pielou, 1966) were analyzed, as well as the beta diversity metrics like weighted UniFrac (Lozupone et al., 2007), unweighted UniFrac (Lozupone & Knight, 2005), Jaccard distance, and Bray-Curtis dissimilarity. The representative sequence of each 16S rRNA gene OTU was aligned to the SILVA database (v138) (Christian et al., 2013), and endowed with taxonomic information. While taxonomy of fungal ITS gene was assigned to OTUs using the q2-feature-classifier (Bokulich et al., 2018) classify sklearn naïve Bayes taxonomy classifier against the UNITE 97% OTUs reference sequences (Nilsson et al., 2019). Finally, the OTU table of bacterial and fungal sequences was subsampled for downstream statistical analyses.

High-throughput sequencing data have been deposited in NODE (The National Omics Data Encyclopedia) under the accession number OEP002256.

2.4. Statistical analysis

SPSS (v22.0) (IBM Corp., Armonk, NY, USA) was used to compare the differences in soil physical-chemical parameters using two-tailed Mann-Whitney U test. Spearman's rank correlation was used to analyze the relationship between soil characteristics and the relative abundance of each taxon (order level), as well as the relationship between soil characteristics and the microbial α-diversity index. The Kruskal-Wallis test (Wallace, 1959) results generated were used to compare the dissimilarity of α-diversity between the wheat fields and the orchards. The non-metric multidimensional scaling (NMDS) analysis based on the Bray-Curtis distance was performed to analyze the β-diversity pattern of the bacterial and fungal communities. The analysis was performed in R (Team, 2013) using the "vegan" package (Oksanen et al., 2015).

The co-occurrence network was calculated with the R package “psych” (Revelle, 2013). Based on the two planting modes (wheat fields and orchards), two integrated networks were constructed individually for samples from wheat fields and orchards. In order to reduce false positive predictions and improve the reliability of network analysis, the OTU with a relative abundance < 0.02% and OTU presented in less than <1/2 of the sample were removed (Jiao et al., 2020). In addition, interspecies network analysis only accepted strong (|r|>0.60) and statistically significant (p value <0.01) correlations. Subsequently, these constructed networks were visualized in Gephi (v0.9.2) (Bastian et al., 2009).

3. Results And Discussion

3.1 Soil characteristics of physical-chemical parameters

The soil physical and chemical characteristics were investigated for the 34 soil samples, including 20 samples from wheat fields and 14 samples from orchards, respectively (Table 1). The distribution (interquartile range) of soil physical and chemical properties in the two cropping systems (the wheat fields and orchards) was summarized in Table S1. The pH value of orchards was lower than that of wheat fields, although the difference was not significant. The pH of soil ranged from 4.93 to 7.60 in the wheat fields, which could be classified as very strongly acidic to slightly alkaline according to USDA Soil Survey Manual (USDA, 2020), while the soil pH

in orchards samples varied from 4.15 to 7.07 that were defined as extremely acidic to neutral acidic. The water content of the orchards was significantly lower than that of the wheat fields ($p < 0.01$). Water content varied from 19.0–45.1% in the wheat fields, while it ranged from 9.0–27.4% in the orchards. Similarly, TOC ($p = 0.001$) and TN ($p < 0.05$) content were also significantly lower in the orchards relative to the wheat fields. TOC contents were 12.34~26.07 and 6.05~19.94 g/kg for the wheat fields and the orchards, respectively. TN contents in the wheat fields were 1.33~2.45 g/kg while they were 0.62~3.15 g/kg in the orchards. On the other hand, the average concentrations of NH_4^+-N and NO_3^--N in the orchards were higher than those in the wheat fields. The physical and chemical properties compared to published data showed our samples along Taige Canal are typical planting pattern (wheat fields and orchards) in the Yangtze River Delta (Zhao et al., 2009). The amount and types of chemical fertilizers used under two planting methods, as well as farming management, might contribute to the difference in soil characteristics of the wheat fields and orchards.

Table 1
Physical and chemical properties of soil samples.

Planting methods	Sample ID*	pH	Water content (%)	TOC (g/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (g/kg)
Wheat fields	W1_S	4.93	25.8±1.16%	14.1±0.012	15.23±1.144	20.55±0.001	1.45±0.063
	W1_D	5.28	26.7±0.31%	12.34±0.003	10.82±1.109	13.85±0.001	2.14±0.024
	W2_S	5.83	45.1±0.24%	22.26±0.002	3.03±0.759	28.27±0	2.4±0.112
	W2_D	5.67	41.1±0.95%	15.44±0.009	8.1±0.806	19.53±0.001	2.45±0.278
	W3_S	5.8	19.5±1.27%	16.33±0.013	0.87±0.419	29.15±0.001	1.41±0
	W3_D	5.67	29.7±2%	16±0.02	3.69±0.042	17.53±0.003	2.44±0.116
	W4_S	5.74	30.8±0.98%	16.14±0.01	18.2±0.748	30.6±0.004	1.78±0.108
	W4_D	5.99	30.1±2.92%	14.23±0.029	9.36±0.28	7.77±0.001	1.86±0.061
	W5_S	5.4	25.8±1.04%	15.42±0.01	0.2±0.141	37.15±0.003	1.92±0.113
	W5_D	5.66	26.2±0.79%	18.26±0.008	4.31±0.826	8.55±0.001	2.64±0.141
	W6_S	5.01	22±0.37%	15.8±0.004	6.17±0.236	31.97±0.002	2.04±0.144
	W6_D	5.42	24.7±0.15%	20.04±0.001	5.79±0.316	6.93±0.001	1.33±0.15
	W7_S	6.3	30.8±0.64%	21.23±0.006	10.23±0.621	37.15±0.002	2.24±0.068
	W7_D	6.23	30.9±0.56%	23.12±0.006	3.45±0.159	11.03±0.003	1.63±0.131
	W8_S	7.6	22.8±1.59%	26.07±0.016	n.a.	11.05±0.001	1.88±0.069
	W8_D	7.58	21.6±2.23%	20.91±0.022	3.25±0.082	5.77±0.001	1.42±0.021
	W9_S	6.16	19.8±3.18%	22.13±0.032	15.88±0.828	28.53±0.004	2.08±0.031
	W9_D	6.57	19±2.34%	23.52±0.023	5.06±1.402	8.6±0.004	1.4±0.127
	W10_S	5.81	41.6±0.9%	18.04±0.009	10.92±0.828	17.4±0.002	2.04±0.05
	W10_D	5.63	38.5±0.42%	24.53±0.004	3.37±0.297	5.13±0.001	1.64±0.087
Orchards	O1_S	4.61	18.7±1.39%	13.59±0.014	91.8±0.7	52±0.002	1.93±0.019
	O1_D	4.53	22.4±0.41%	12.93±0.004	92.27±1.416	10.3±0.003	2.34±0.031
	O2_S	7.07	9±0.89%	7.94±0.009	7.33±0.478	9.55±0.002	0.62±0.031
	O2_D	6.55	15.3±0.3%	6.05±0.003	4.85±0.186	3.13±0.001	2.34±0.079
	O3_S	4.42	23.9±0.68%	19.94±0.007	77.35±2.25	177.87±0.006	2.72±0.056
	O3_D	4.15	27.4±1.24%	19.74±0.012	37.68±0.73	134.57±0.008	3.15±0.039

*Sample ID is made up of W(wheat)/O(orchards)+site+S/D, S indicates the samples from 0-5 cm and D means 5-10 cm; n.a. suggest no data collected.

Planting methods	Sample ID*	pH	Water content (%)	TOC (g/kg)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (g/kg)
	O4_S	4.46	27.1±2.08%	14.15±0.021	12.63±0.464	10.67±0.004	1.75±0.044
	O4_D	4.7	24±0.29%	16.27±0.003	6.1±0.771	5.87±0.003	2.21±0.008
	O5_S	6.07	18.6±1.91%	15.04±0.019	3.5±0.07	17.9±0.002	1.57±0.101
	O5_D	5.66	19.9±0.56%	16.54±0.006	3.51±0.128	5.5±0	1.21±0.055
	O6_S	6.53	23.1±0.4%	12.62±0.004	30.73±0.704	16.57±0.002	1.27±0.061
	O6_D	5.08	24.3±0.28%	12.81±0.003	13.97±1.332	10.13±0.001	0.92±0.082
	O7_S	5.69	15.1±0.5%	10.87±0.005	6.09±0.39	9.15±0.005	0.94±0.016
	O7_D	6.37	17.5±0.36%	10.89±0.004	3.67±0.19	2.97±0	0.93±0.025

*Sample ID is made up of W(wheat)/O(orchards)+site+S/D, S indicates the samples from 0-5 cm and D means 5-10 cm; n.a. suggest no data collected.

3.2 Community structure of bacteria and fungi in soil

After a series of quality control and sequence filtering, a total of 1,524,387 sequences of bacteria and 2,519,645 sequences of fungi were obtained for community analysis. Taxonomic composition on the phylum and order levels of each sample was shown in heatmap (Fig. 1). A total of 479 orders (61 phyla) of bacteria were detected in all soil samples. Specifically, the top 10 phyla (accounting for 92% of the total sequences) of bacteria were Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, Bacteroidota, Firmicutes, Gemmatimonadota, Nitrospirota, Myxococcota, Verrucomicrobiota and Patescibacteria. The most abundant order was Acidobacteriales from Acidobacteriota (phylum level) ranging from 0.13-27.59%, followed by Burkholderiales (1.85-15.61%) and Rhizobiales (1.93-10.31%) from Proteobacteria, Ktedonobacterales and Anaerolineales from Chloroflexi (0.01-18.49% and 0-13.24%, respectively), Chitinophagales from Bacteroidota (0.46-12.38%), and Gemmatimonadales from Gemmatimonadota (0.47-8.16%) (Fig. 1a). The difference between two cropping types were obvious. For instance, the relative abundance of Actinobacteriota and Firmicutes was higher in the orchards than in the wheat fields, while Chloroflexi and Nitrospirota were more abundant in the wheat fields. Actinobacteria, which are able to form hyphae and available of phytic acid as source for the seeds, have been abundantly observed in fruit orchards in the previous study such as our research (Konietzny & Greiner, 2002). And Actinobacteria also have been described as mainly ecological “K-strategists” (stable community, strong ability to resist predators) that maintain the environment stable (Pascault et al., 2013). Firmicutes have been described as mainly copiotrophs (Fierer, 2017), which are able to survive in adverse environmental conditions due to their ability to produce endospores (MandicMulec and Prosser 2011). Proteobacteria have also been known as fast growing copiotrophs preferring C-rich environments (Fierer, 2017). Here, physical and chemical properties showed that TOC content in the wheat fields was significantly higher than that in orchards. As can be seen from Fig. 1, the relative abundance of Burkholderiales and Rhizobiales, which belong to Proteobacteria, were higher in the wheat fields than that in the orchards. Acidobacteria, have been described as mainly oligotrophs (K-strategists), which utilize complex carbon substrates that are more likely to be present in the native SOM (Fierer, 2017).

A total of 131 orders (belonging to 17 phyla) of fungi were detected in all soil samples. Members of Ascomycota were more frequently identified than other phyla. Among them, the Hypocreales ranged from 5.11–50.58% were the most numerous in fungi, followed by Sordariales of Ascomycota (1.66-53.23%), Mortierellales of Mortierellomycota (1.96-57.94%), Pleosporales of Ascomycota (0.59-47.21%), and Microascales of Ascomycota (0.18-12.91%) (Fig. 2b). In the fungal community of the wheat fields, the most abundant fungal order was Hypocreales, with the relative abundance ranging from 5.11-32.19%, followed by Sordariales (2.48-46.48%), Pleosporales of Ascomycota (0.63-47.21%), and Mortierellales (1.96-19.83%). In the fungal community of the orchards, the most abundant fungal order was Hypocreales with the relative abundance ranging from 8.37-50.58%, followed by Mortierellales (2.25-57.94%) and Sordariales (2.25-57.94%). The dominance of these taxa were consistent with previous studies of soil fungi in the fruit orchard (Zheng et al., 2021). Basidiomycota in our results, are consistent with de Boer et al.'s (2005) description that had a higher relative abundance in fruit orchards than wheat fields (harvested), playing key role in lignin degradation. Chytridiomycota which have been shown to be able to recover from drying and high temperature events, were more likely to occur under the wheat fields than under fruit orchards.

3.3 Diversity of bacteria and fungi

The pattern of soil microbial community varied with soil conditions, and it can be seen that there were obvious differences in the community structure between different planting patterns (Thomson et al., 2015). Chao1, Shannon, Simpson and Pielou's evenness indexes were evaluated for alpha diversity in both bacterial and fungal communities (Fig. 2). The alpha diversity of bacteria was higher than that of fungi in both wheat fields and orchards. In details, the evenness of bacteria was significantly different in the two planting methods, which was higher in wheat fields than in the orchards (Fig. 1a). There was no significant difference ($p > 0.05$) in Chao1, Shannon and Simpson's indexes for bacterial community. However, Chao1 and Simpson's indexes in alpha diversity of fungal community were significantly higher in the wheat fields than in the orchards (Fig. 1b).

The beta-diversity of bacteria and fungi were illustrated by the nonmetric multidimensional scaling (NMDS) map based on Bray-Curtis distances (Fig. 3). NMDS is a data analysis method that simplifies the research object from multi-dimensional to low-dimensional space for position analysis and classification while retaining the original data relationship between groups (Wagner et al., 2007; Xu et al., 2020). All the 34 soil samples were widely distributed on the NMDS map due to the wide variation in soil properties. According to Fig. 3, these samples were aggregated based on soil planting type, indicating the significant community difference of β-diversity both in bacterial (stress = 0.0737, $p<0.01$) and fungal (stress = 0.0954, $p<0.01$) communities (wheat field soil vs. orchards), the stress values were both lower than 0.2, indicating our results are appropriate.

According to the analysis of community properties, the bacterial and fungal communities in the two types of soil showed great dissimilarity both in diversity and community structure, which supports the consensus on the heterogeneous distribution of microbial diversity among different habitat types (Jangid et al., 2011). There were significant differences in the relative abundance of dominant taxa and the overall community structure based on Bray-Curtis distance between the wheat fields and orchards, which in accordance with our expected results. For the soil samples of the two planting types, the bacterial community diversity in the wheat fields was higher than that in the orchards, reflecting the impact of changed planting method on the microbial community.

3.4 Co-occurrence network structure and composition of bacterial and fungal communities

Co-occurrence network analyses were conducted to reveal the complexity of the interactions among members of the bacterial and fungal communities in the two types of soils (Fig. 4). The dominated fungi and bacteria were distributed evenly in the wheat fields, while the bacterial population in the orchard accounted for a larger proportion with more core node (OTU) numbers (Table S2). Most of the nodes in the wheat fields and orchards network belonged to 15 dominant bacterial phyla and 6 dominant fungal phyla. Two networks mainly contained nodes of Acidobacteriota, Chloroflexi, Gemmatimonadota, Nitrospirota and Ascomycota, but the relative abundance of Ascomycota in wheat field was much higher than others. Ascomycetes was the dominant fungal phylum and played a key role in the wheat field microbial network, mainly because Ascomycetes can quickly decompose and utilize the original organic matter in the environment (Xiao et al., 2020), and are more competitive than other fungal phyla such as Basidiomycetes. The phyla of Chloroflexi and Gemmatimonadota had the ability to adapt well to new environments as the planting mode changed from wheat field to orchards. In addition, the network of wheat fields and orchards connected nearly 257 nodes, and only 16 nodes were not connected, indicating the two types of soil shared most co-occurrence taxa. Network analyses showed that both the wheat fields and orchards occurred modular structure in some degree and divided into modules (> 0.4), indicating the important network characteristics of complex ecosystems (Newman, 2006). The modularity of the wheat fields and orchards was 0.784 and 1.089, respectively. Since the percentage of positive connections was higher than 70% (Table. S3), the two networks were all characterized by co-occurrence. Average degree and graphic density were used to reveal network complexity. According to Fig. 4, the average degree and graphic density of the orchards network (11.366 and 0.043) were higher than the wheat fields network (10.274 and 0.039), indicating that the orchards network was more complex than the wheat fields. The average clustering coefficient reflects the clustering characteristics of the network, which is related to the stability of the network structure and the response of the microbial network to environmental interference (Jiao et al., 2020). Compared with the wheat field network, the clustering coefficient of the orchards network was much lower, indicating that the network of orchards was more unstable than the wheat fields.

3.5 Correlation between microbial taxa and soil characteristics

The correlation of dominant bacterial/fungal taxa with environmental factors were analyzed to explore the relationship between them (Fig. 5). According to the figure, strong species-environment relationship of bacteria and fungi were found in both orchards and the wheat fields. The effects of soil physical-chemical parameters on the relative abundance of bacterial communities were as follows: pH was significantly positively correlated with the abundance of Vicinamibacterales in both wheat fields and orchards (Fig. 5a, 5b). Vicinamibacterales belongs to Vicinamibacteria in the phylum of Acidobacteriota, is tolerant to a wide range of pH values (4.7–9.0) (Huber et al., 2016). From our results, they were abundant in acidic conditions, as well as in neutral and slightly alkaline environments. There was a strong negative correlation between Vicinamibacterales and TN in the orchards, and a negative (though not significant) correlation between Vicinamibacterales and TOC, probably because Vicinamibacterales has an oligotrophic lifestyle. According to the correlation coefficients, it was found that pH was the main factor that affected the structure of soil bacterial community in wheat fields, while parameters of pH, WC, TOC and TN all had great influences on bacterial community in the orchards.

The results of the fungal community and soil physical-chemical factors were shown in Fig. 5c-d. The correlation between environmental properties and fungi of wheat fields did not meet the requirements of statistical significance, but we can see that pH had positive correlations with Eurotiales and Helotiales and negative correlations with Sordariales, Phacidiales and Diaporthales, although not significant (Fig. 5c). It can be seen that different orders of Ascomycetes responded differently to soil pH levels. Therefore, not all members of the same phylum have the same behavior pattern, as reported in previous studies (Zhang et al., 2016). In the fungal community of orchard, pH positively affected Pleosporales, while TOC had a negative effect on Pleosporales (Fig. 5d). Parameters of pH, WC, TOC, NH_4^+-N and TN all had great influences on the orchard fungal community though no statistical significance. These environmental factors may alter the taxonomic composition of the fungal community by affecting the growth of many fungal species in the soil. Geographic location soil physical and chemical properties (pH, NO_3^--N, organic nitrogen, NO_2^--N and organic carbon) are important geochemical factors that affect the composition of soil fungal communities (Grau et al., 2017; Zhang et al., 2020).

Soil microorganisms are recognized as a key element in the development of agriculture (Gabriele et al., 2016; Gabriele et al., 2017), as they play a vital role in ecosystem functions such as nutrient cycling. In this study, the community structure of bacteria was strongly related to most soil properties, which helps explain the large differences observed between soil communities with different cultivation methods. This is not surprising, because many soil conditions are interrelated, and numerous studies have revealed the relationship between the structure of different microbial populations distributed around the world and the soil properties of different soils (Suleiman et al., 2013). Franciska et al. studied that environmental factor promotes the unstable characteristics of soil bacteria (rather than fungi) symbiosis, causing changes in bacterial community links more strongly to soil diversity and function during the restoration process than do changes in fungal communities (de Vries et al., 2018). It is verified that fungi have lower diversity than bacteria. Additionally, in our study, the correlation between community (both bacteria and fungi) and environmental factors was stronger in the wheat fields than that in the orchards, confirming the conclusion that co-occurrence network was more reliable and stable in the wheat fields than in the orchards.

4. Conclusions

In this study, the community structure and diversity of bacteria and fungi in two typical planting patterns (wheat fields and orchards) in the Taige region were determined using high-throughput sequencing. According to molecular analysis, the dominated bacteria in soils were Proteobacteria, Acidobacteriota, Actinobacteriota, Chloroflexi, Bacteroidota, Firmicutes, Gemmatimonadota, Nitrospirota, Myxococcota, Verrucomicrobiota and Patescibacteria. Specifically, the relative abundance of Actinobacteriota and Firmicutes was higher in the orchards than that in the wheat fields, while Chloroflexi and Nitrospirota were more prevalent in the wheat fields. To fungi, Ascomycota, Mortierellomycota, and Basidiomycota were the predominant phyla in the two types of soil. Compared to orchards samples, the diversity of both bacteria and fungi were higher in the wheat fields. Statistical analyses further showed that pH was the main factor that significantly impacted the community structure. Network analyses indicated the possibility of bacteria and fungi co-occurrence with modular structure in both wheat fields and orchards. Moreover, more complex and unstable community interaction was found in the orchards due to change of wheat planting pattern.

Declarations

Data availability

Nucleotide sequence data of this study are deposited in NODE (The National Omics Data Encyclopedia) public available database for full access.

Code availability

Not applicable

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Authors' contributions

Xinyu Cui: Sampling, methodology, data analysis, writing - original draft. Huan He: Data analysis, supervision, writing - review & editing. Fengxiao Zhu: Supervision, review & editing. Xiaobo Liu: Writing & review. You Ma: Sampling, methodology. Han Meng: Conceptualization, supervision, methodology, investigation, writing - review & editing. Limin Zhang: Supervision, review & editing.

Ethics declarations

Ethics approval

Not applicable

Consent for publication and participation

Not applicable

Conflict of interest

The authors declare no competing interests.

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Figures

Figure 1

Heatmap of soil bacterial and fungal community composition along the Taige Canal on the order level. a, bacterial community; b, fungal community.

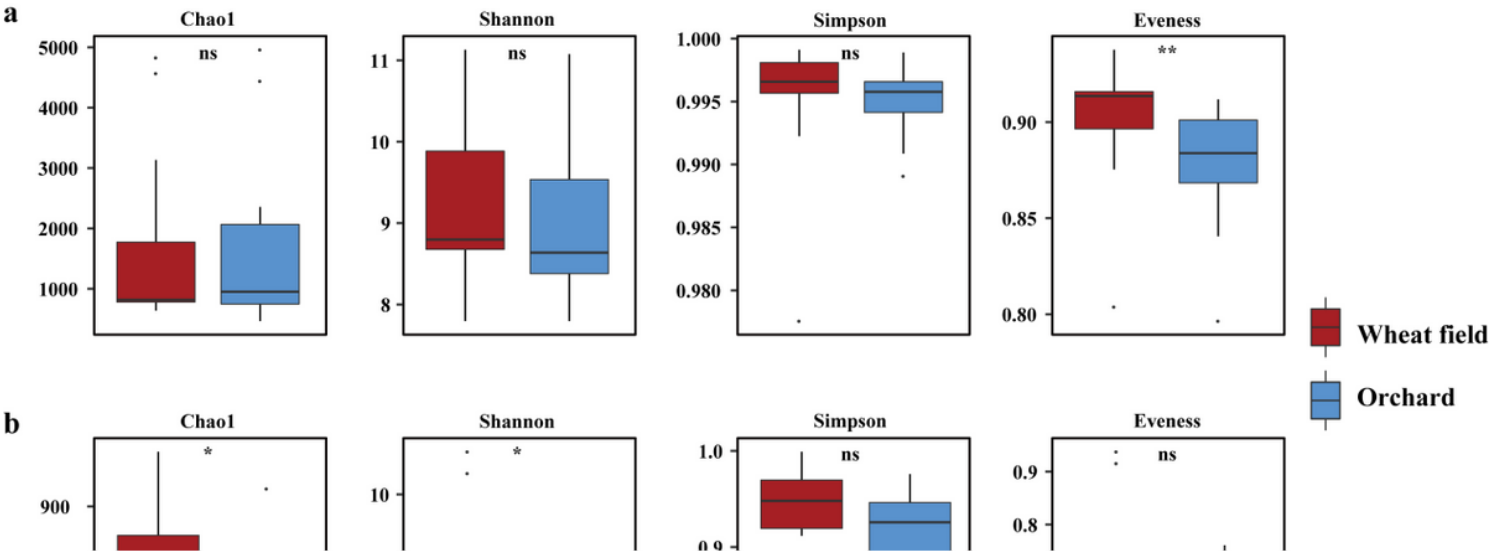

Figure 2

α-diversity of bacteria (a) and fungi (b) in the wheat fields (blue) and orchards (red). Different symbols above the boxes denote statistically significant dissimilarity (p<0.001, ***; 0.001<p<0.01, **; 0.01<p<0.05, *; p>0.05, ns), one-way nonparametric test with Kruskal-Wallis test.

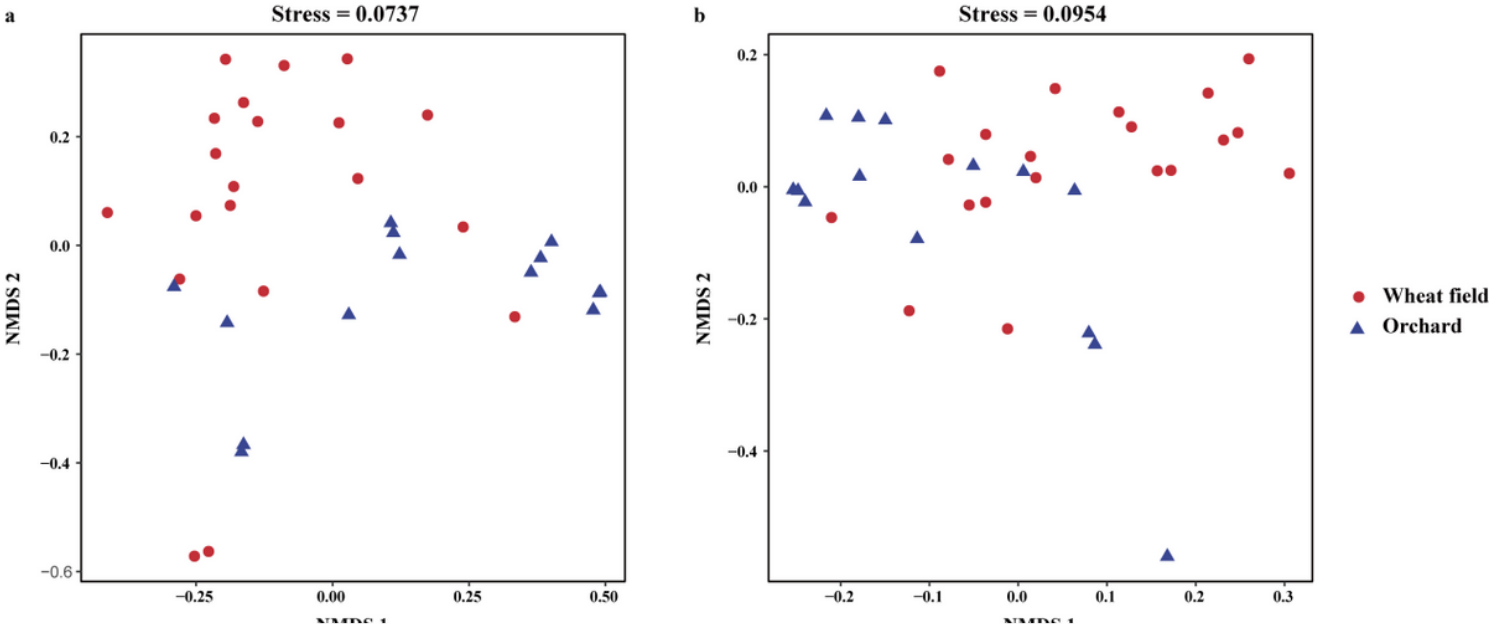

Figure 3

NMDS map of soil bacterial (a) and fungal (b) community structures along the Taige Canal (OTU level). Bray-Curtis distance was used to rank the two-dimensional NMDS.

Figure 4

Microbial species-species network structures in soils along the Taige Canal: (a) the wheat fields, and (b) the orchards. The networks are visualized with group attributes layout based on phylum. Each node denotes a bacterial OTU (defined at a 97% similarity level); each edge linking two nodes represents a positive (pink line) or negative (black line) relationship. OTUs are colored by different phylum. The size of each node is proportional to the number of connections. A connection between two nodes is a statistically significant (p<0.01) and strong (|r|>0.60) correlation. The percentage of positive links in every network: a: 90.08%, b: 73.87%.

Figure 5

Spearman's correlation between soil microbial community and environmental factors along the Taige Canal (Order level). Different symbols above the boxes denote statistically significant dissimilarity (p<0.001, ***; 0.001<p<0.01, **; 0.01<p<0.05, *; p>0.05, ns). Samples contained in the figure are bacterial community of wheat fields (a) and orchards (b), as well as fungal community of the wheat fields (c) and orchards (d).

Supplementary Files

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