Differences in the soil bacterial community under organic farming and conventional farming modes revealed by 16S rDNA sequencing

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Abstract

Background The bacterial communities is complex and different in various agricultural ecosystem. In this study, high-throughput Illumina MiSeq sequencing was conducted to assess the differences in soil bacterial communities between organic farming and conventional farming modes.

Results A total of 3,919 operational taxonomic units were identified and classified as 26 phyla, 42 classes, 78 orders, 120 families, 281 genera. The dominant genera were *Lysobacter*, *Pseudomonas*, *Massilia*, *Pseudarthrobacter*, *Ferruginibacter*, *Flavobacterium*, *Flavisolibacter*, *Brevundimonas*, *Haliangium* and *Sphingomonas*. Analysis of soil bacterial diversity showed that the soil under the organic farming had a greater bacterial diversity than that under the conventional farming. Linear discriminant analysis effect size analysis showed that the major bacterial groups identified in the soil sample CK1 (2015.4) and CK6 (2017.10) under conventional farming mode were largely different from those in the soil sample O6 (2017.10) under organic farming mode. Redundancy analysis demonstrated that available nitrogen was the most important factor regulating bacterial composition under the organic farming mode, followed by soil rapidly available phosphorous and potassium. *Massilia*, *Pseudomonas*, *Lysobacter* and *Pseudarthrobacter* abundances showed a strong positive correlation with the content of available nitrogen.

Conclusions Organic farming could improve soil organic matter, available nitrogen, rapidly available phosphorus and retard soil acidification. This modification of soil can directly or indirectly relate to the bacterial communities in soil. The shifts of bacterial communities were complicated and dynamic with respect to all sorts of the measures of cultivation and management under organic farming mode.

Introduction

Organic farming is a system intended to produce food with minimal damage to animals, humans and ecosystems [1]. In recent years, with the improvement of people's living standards and health awareness, the development of organic agriculture has become a trend [2]. Moreover, organic farming has been considered more sustainable than conventional farming and is increasingly used as an alternative to conventional farming because of its positive effects on agroecosystem health [3, 4]. As a matter of fact, compared with conventional agriculture, organic agriculture is environmentally friendly, and delivers equally or more nutritious foods with few pesticide residues although its yields is lower in most cases [5, 6]. However, under certain conditions, such as good management measures, particular crop types and planting levels, organic agriculture may nearly match conventional agriculture in yields [1]. Therefore, it is significant for organic agriculture to constantly improve and optimize the organic farming management levels.

Because chemical pesticides and fertilizers are completely banned in organic agriculture, applying organic fertilizers has become one of the most important measures for maintaining and improving soil fertility in organic agriculture [7]. Organic fertilizers have vital impacts on the physical and chemical

properties of soil. At first, long-term manure application increases the contents of soil available phosphorus (AP), soil available potassium (AK), organic carbon and prolong nitrogen availability [8–11]. Secondly, manure application increases soil organic matter and thus the soil pH buffering capacity and eventually reduces soil acidification [12]. Additionally, long-term manure application could also influence the microbial abundance and activity in the soil [13, 14].

It is known that soil microorganisms are important decomposers of organic matter in the soil. They can promote the circulation of soil nutrients and improve soil structure, soil fertility and crop nutrient supply. Therefore, the diversity of soil microorganisms is an important indicator for evaluating the biological traits of soil [15, 16]. Currently, high-throughput sequencing technology provides an effective method for studying soil microbial community changes under different conditions. Most studies using high-throughput sequencing demonstrate that soil microbial community diversity and structure composition are influenced by all kinds of cultivation measures, including fertilization management, no-till, cover crops, crop rotation and so on [17–21]. In organic farming, soil microbiological activity is of primary importance as nutrient supply is mainly dependent on the degradation of soil organic matter by soil microorganisms [22]. For instance, organically managed tea gardens could stimulate higher phosphate solubilizing microbial load, and the percent of mineral phosphate solubilisation capacity reached as high as 64% while it is only 22% in conventional tea gardens [23]. Soils in organic agriculture contained specific microbial communities, where certain taxa were richer than in conventional agriculture [24]. A meta-analysis and meta-regression confirmed that organic farming also enhanced soil microbial abundance and activity [25]. In addition, the soil bacterial community compositions were different in paddy fields under organic and conventional farming conditions [26]. In short, the diversity of soil microbial communities (bacterial communities) is complex and different in various agricultural ecosystem.

In order to find out the difference of soil bacterial communities between organic farming mode and conventional farming mode, the study on the effects of organic farming and conventional farming on soil bacteria in soybean-leguminous green manure rotated fields was conducted in the agricultural ecological garden of Swan Island, Shishou city, China during 2015.4-2018.4. Our results may provide certain theoretical support for improving the development of organic farming levels.

Results

Effects of the different farming modes on the chemical properties of soil

The different farming modes had particular effects on the chemical properties of soil. Compared with those under the conventional farming mode, the changes in soil pH under the organic farming mode were smaller during 2015-2017 (Fig. 1A). The content of soil organic matter (SOM) was higher under the organic farming mode than that under the conventional farming mode in 2016 and 2017 (Fig. 1B), while the amount of readily oxidized organic carbon (ROOC) under the organic farming mode was lower than that under the conventional farming mode in 2016 and 2017 (Fig. 1C). The amounts of total nitrogen

(TN) total phosphorus (TP) and total potassium (TK) were not significantly different between the organic farming mode and the conventional farming mode in 2015, 2016 and 2017 (Fig. 1D, E and F). The contents of available nitrogen (AN) and rapidly available phosphorus (AP) under the organic farming mode were lower than those under the conventional farming mode in 2014 and 2015, while they were higher under the organic farming mode than those under the conventional farming mode in 2016 and 2017. The rapidly available potassium (AK) under the organic farming mode was higher than or nearly equal to that under the conventional farming mode in 2015-2017 (Fig. 1G, H and I).

Reads and OTU statistics

According to sequencing analysis of the V4 regions of the 16S rRNA gene for bacteria in the soil samples under the different farming modes, we obtained paired sequences with barcode and primer sequences. After removing the short reads and trimming the low-quality regions, 1, 339, 599 optimized sequences (clean reads) were identified from the 42 soil samples with an average length of 412-413 bp (Fig. S1, Table S1). The coverage index was used to reflect the coverage scale of the sample libraries. The coverages of all 42 samples were larger than or equal to 0.97, indicating that sequencing depths could meet the experimental requirements (Table 1). The rarefaction curve was flat when the number of sequences was between 30, 000-40, 000, suggesting that the sequencing data amount was reasonable to obtain the real richness of the community of soil bacteria (Fig. 2A). Based on the DNA sequence similarity (97%), a total of 3, 919 OTUs were identified by aligning the sequence to the Greengenes database (v13_5).

Soil bacteria identification and Venn diagram analysis

Analysis of the taxonomic distributions of bacterial communities at different levels of classification showed that 3, 919 operational taxonomic units (OTUs) belonged to 26 phyla, 42 classes, 78 orders, 120 families, 281 genera, and 340 known species (Table S2). The distributions of common and unique OTU subsets of soil bacteria under the organic farming mode and the conventional farming mode were analyzed through a Venn diagram in April 2015 and October 2017 (Fig. 2B). A total of 1, 507 bacterial OTUs were identified as common to all the soil samples. O6 had the highest number of unique OTUs (177), followed by O1 (169), CK1 (145), and CK6 (115). In addition, There were 2, 225 common OTUs between CK1 and O1 in April 2015, but there were 1, 926 common OTUs between CK6 and O6 in October 2017. Furthermore, there were 1,889 common OTUs between CK1 and O6 in October 2017. Hence, on the basis of OTUs, different farming mode led to the difference of common and unique OTU subsets of soil bacteria.

Community structure of bacteria

Species accumulation curves are widely used to evaluate the sampling adequacy and the estimates of species richness in biodiversity and community surveys. In this study, the species accumulation curve first rose sharply and then rose slowly, indicating that the sampling was sufficient and that many species were present (Fig. 3A). Based on the shared OTUs of the sample and the species represented by the OTUs,

the analysis of the core bacteria showed that the shared OTU number of all the soil samples was more than 300 (Fig. 3B). The relative abundances of the dominant phyla were similar among the 42 soil samples under the different farming modes. Proteobacteria, Acidobacteria, Bacteroidetes, Gemmatimonadetes, Chloroflexi, Actinobacteria, Planctomycetes and Nitrospirae were the most abundant phyla in the 42 soil samples (Fig. 4A). The dominant genera were *Lysobacter*, *Pseudomonas*, *Massilia*, *Pseudarthrobacter*, *Ferruginibacter*, *Flavobacterium*, *Flavisolibacter*, *Brevundimonas*, *Haliangium* and *Sphingomonas*. The histogram of the microbial species distribution at genera levels showed that *Massilia* and *Lysobacter* increased quickly in soil under the organic farming mode in comparison with their performance under the conventional farming mode (Fig. 4B, Fig. 5A, B). To evaluate differences in the composition of the soil sample bacteria under different farming modes during 2015-2018, principal component analysis (PCA) was conducted. The results showed significant clustering of bacterial communities (Fig. 6). PC1 and PC2 explained 47.57% and 24.57% of the global variation in the communities, respectively. O4, O5, O6 and O7 were clearly distinguished from CK1 and O1, indicating the significant influence of different farming modes on soil bacteria.

Analysis of bacterial diversity

OTUs with 97% similarity were used to calculate Shannon, Simpson, Chao and Ace values under different farming modes by the *mothur* and R (3.4.1) language tools. We found that the Ace and Chao index first increased, then decreased and increased under different farming modes (Fig. 7A, B), indicating that different farming modes had different effects on soil bacterial biodiversity. The Shannon index first decreased and increased under the different farming modes, but the conventional farming mode had larger effects on soil bacterial diversity than the organic farming mode (Fig. 7C). The Simpson index first increased under different farming modes during 2015-2016, but decreased under the conventional farming mode and did not change under the organic farming mode during 2017 (Fig. 7D).

LEfSE of the bacterial community

LEfSE (linear discriminant analysis effect size) is a valid statistical method for high-efficiency biomarker discovery that enables the comprehensive identification of richness characteristics by describing potential differential taxa between different biological samples. A linear discriminant analysis (LDA) score higher than 2 was employed to distinguish only the bacterial groups that were clearly different among the four samples (CK1, CK6, O1, O6). A cladogram depicted all the taxa with LDA values higher than 2.5 by treatment (Fig. 8). The major bacterial groups identified in CK1 were *Proteobacteria*, *Gammaproteobacteria*, *Oxalobacteraceae* and *Massilia*. CK6 had the highest abundance of *Deltaproteobacteria*. O1 had a higher level of *Acidobacteria* and *Actinobacteria*, while O6 had a higher abundance of *Gemmatimonadales*, *Chloroflexi*, *Gemmatimonadaceae*, and *Gemmatimonadetes*.

Correlation between soil chemical features and Bacterial Communities

The relationships between environmental factors and bacterial community at the phylum level were elucidated using redundancy analysis (RDA). The 5 most dominant genera were used for the RDA (Fig. 9).

The results showed that the first two axes explained approximately 96.73% of the variation in the bacterial composition, with 89.31% explained by the first axis and 7.42% by the second axis. AN was the most important factor mediating the bacterial community under the organic farming mode ($p < 0.05$), followed by soil AP and AK content. The *Massilia*, *Pseudomonas*, *Lysobacter* and *Pseudarthrobacter* displayed a strong positive correlation with the AN content. A strong negative correlation between AN and *Haliangum* was found. A significant separation among the four treatments, which were almost divided into three groups, was found along the first two axes. Additionally, the long distances between conventional farming and organic farming in 2017 further suggested that organic farming and conventional farming significantly affected soil bacterial communities and that there were significant differences between them.

Discussion

Chemical properties of soil under different farming modes

Compared with conventional agriculture, organic agriculture is an environmentally friendly agricultural system that emphasizes crop diversification, crop rotation, natural pest management and manure application [1]. Therefore, the soil features under organic farming mode are different from that under conventional farming mode. In this study, the results on chemical properties of soil showed organic farming could retard soil acidification, improve soil organic matter, available nitrogen, rapidly available phosphorus, but the amount of total nitrogen, [total phosphorus](#) and total potassium were not significantly different between organic farming mode and conventional farming mode. On the one hand, these results confirmed that the rational application of manure could have the same effect as chemical fertilizer, but the effects of manure were slower than those of chemical fertilizer [27, 28]. In addition, in the long term, the organic farming system was more effective than the conventional farming system in reducing soil erosion and in maintaining soil productivity [5, 29, 30].

Community composition of soil bacteria under different farming modes

In farming systems, the soil is related to many biological processes that are mediated by microbes, including residue decomposition, biological nitrogen fixation, nutrient cycling and denitrification [31]. Therefore, soil microbes must play pivotal roles in organic farming. In our study, O6 had the highest number of unique OTUs (177), followed by O1 (169), CK1 (145), and CK6 (115), suggesting that the organic farming mode improved the community structure of the soil bacteria more than the conventional farming mode. Under organic farming and conventional farming, the community structure of the soil bacteria at the phylum level was similar among the 42 samples. Acidobacteria and Proteobacteria occupied the top two phyla, as found in recent reports [21, 32]. These results indicate that organic farming and conventional farming did not change the taxonomic structure at the phylum level. However, some studies reported that organic farming and conventional farming could modified the taxonomic structure at the phylum level [6, 33]. This difference may be related to the duration of organic cultivation. The histogram of bacterial species distribution at the genera level showed that *Massilia* increased more

quickly in soil under the organic farming mode in comparison with the conventional farming mode. *Massilia* bacteria can not only synthesize a variety of secondary metabolites and enzymes but also have a variety of functions, such as phosphorus dissolution, phenanthrene degradation and heavy metal tolerance [34]. These results suggested that *Massilia* could play an important role in organic farming.

Diversity of soil bacteria under different farming modes

Various strategies and management practices have been suggested to preserve and improve soil functioning, including organic farming. However, how soil-improving cropping systems affect a range of soil functions is still unclear [35]. In this study, the changes in biodiversity indexes (Ace, Chao, Shannon and Simpson) under organic farming were different from those under conventional farming at different times, which indicated that there were significant differences between organic farming and conventional farming at different times. Furthermore, the major bacterial groups in the soils under organic farming and conventional farming were significantly different in 2015 and 2017, which may be relative to the change of the bacterial diversity to certain extent.

The relationship between soil bacteria and soil chemical features

Redundancy analysis also showed that AN was the most significant factor regulating the bacterial composition under the organic farming mode, followed by soil AP and AK content. *Massilia*, *Pseudomonas*, *Lysobacter* and *Pseudarthrobacter* showed a strong positive correlation with the AN content. The long distances between conventional farming and organic farming shown through RDA further suggested that organic farming and conventional farming affected soil bacterial communities and chemical features, and there were significant differences between organic farming and conventional farming. These results also demonstrated that the soil bacteria were closely related to soil chemical features [36].

Conclusions

This research discovered differences in the soil bacterial community under organic farming and conventional farming modes. The results confirmed that organic farming may optimize the soil biochemical feature and microbial communities better than conventional farming. In brief, the results on chemical properties of soil showed organic farming could improve soil organic matter, available nitrogen, rapidly available phosphorus and retard soil acidification. This soil modification can directly or indirectly relate to the soil bacterial communities. The changes of bacterial communities characterized in this study were complex and dynamic with respect to all sorts of the measures of cultivation and management under organic farming mode. Therefore, further studies are required to estimate the molecular and genetic modulations of the relative functional bacteria under all kinds of organic farming modes.

Materials And Methods

Field treatment

The experimental field is located in the agricultural ecological garden of Swan Island, Shishou city, China, which belonged to the old Yangtze River area with typical geographical and climatic characteristics. Field trials were conducted from April, 2015 to April, 2018. Two treatments including organic farming (OF) and conventional farming (CK) were set up. Only organic fertilizers were used in the organic farming treatment and chemical fertilizers and pesticides were completely forbidden. In the organic fertilizer, the organic matter content is more than 35%, and a total nutrient amount ($N+P_2O_5+K_2O$) is more than 6.0 %. The amount of organic fertilizer applied was 10 t /ha in 2015, 5 t /ha in 2016 and 2.5 t /ha in 2017. Biological pesticides (such as *Bacillus thuringiensis* against lepidoptera pests, *Trichoderma hartz* against *Verticillium dahliae*) are only allowed, and all weeds are removed by hand. In the conventional farming treatment (CK), chemical fertilizer was applied every year according to local production practices and recommended dosages. The amount of compound fertilizer used was 555 kg /ha and the number of urea and topdressing fertilizer were 150 kg /ha respectively. In addition, weeding (glyphosate), insecticide (methomyl, imidacloprid) application, etc. were performed in the conventional farming treatment. Other cultivation and management practices were identical in the organic and traditional agriculture treatments.

Soil sample collection

The soil samples were collected using the "S" shape method after crops were harvested in the test fields in April and October each year (2015.4-2018.4). There were 7 conventional farming soil samples (CK1: 2015.4; CK2: 2015.10; CK3: 2016.4; CK4: 2016.10; CK5: 2017.4; CK6: 2017.10; CK7: 2018.4) and 7 organic farming soil samples (O1: 2015.4; O2: 2015.10; O3: 2016.4; O4: 2016.10; O5: 2017.4; O6: 2017.10; O7: 2018.4). There were 3 replications at each time point. In total, there were 42 soil samples. Each soil sample was taken from the soil plow layer (0-15 cm), and the rocks and crop residues in the samples were removed. After the soil samples were mixed well, some fresh soil samples were stored in a 4°C refrigerator for the determination of soil characteristics. The other fresh soil samples were put into sterile bags, quickly placed in ice boxes, and then stored in a -80°C refrigerator for analysis of the soil bacterial community.

Measurement of soil chemical properties

The soil pH value was measured by the water extraction-potentiometric method [37]. Soil organic matter (SOM) was determined using the potassium dichromate external thermal method [38]. Soil readily oxidized organic carbon was measured by spectrophotometer. Soil total nitrogen (TN) was obtained by the semitrace Kelvin method [39]. The total phosphorus (TP) in soil was measured by the sulfuric acid-perchloric acid boiling method [40]. The total potassium in the soil was determined by the perchloric acid-sulfuric acid boiling method. The alkali-diffusion method was used to measure available nitrogen in soil. Soil available phosphorus was obtained by sodium bicarbonate extraction and the molybdenum-antimony anticolorimetric method. Available potassium (AP) in soil was analyzed by an ammonium acetate-flame photometer.

Sequence and bioinformatics analysis

High-throughput sequencing of the soil samples was conducted using MiSeq sequencing by Shanghai Personal Biotechnology co. Ltd. after high-quality DNA was obtained from the soil samples. The highly variable V4 region of the bacterial 16S rRNA gene with a length of approximately 250 bp was selected for sequencing, and the specific primers (520F and 802R) of the bacterial 16S rRNA V4 region were selected for PCR amplification. The sequencing library was prepared using the TruSeq Nano DNA LT Library Prep Kit. DNA terminal repair was carried out first, and then fragment selection and purification of the library were performed through gel electrophoresis. After quality inspection and quantification of the relative libraries, high-throughput sequencing was carried out. The original sequencing data were screened for quality, and the cleaned reads were divided according to the OTU method. The specific components of samples at different classification levels were analyzed. A rarefaction curve was constructed for all soil samples. Principal component analysis (PCA) was conducted to evaluate differences in the composition of the soil sample bacteria. OTUs with 97% similarity were used to calculate Shannon, Simpson, Chao and Ace values by the mothur and R (3.4.1) language tools [41] (Schloss et al. 2009). Principal coordinate analysis (PCoA) based on the Bray–Curtis distances was used to visualize the differences in bacterial community among the different groups. Heat maps were used to show correlations between dominant phyla/genera and soil properties. LEfSe (LDA effect size) was used to find the bacteria that made a significant difference in sample classification. RDA was used to evaluate the correlations between bacterial communities and environmental factors. The sequencing data were deposited in the NCBI Short Read Archive database with the accession number XXXXX **(The data is uploading)**.

Statistical analysis

The relative data (including phyla/genera, diversity indexes, and soil chemical properties) were analyzed using SPSS 20.0 software (SPSS Inc., USA). The difference of the chemical properties of soil under the organic farming mode and the conventional farming mode was statistically analyzed with t test for independent samples. Two significance levels (*, $P < 0.05$ and **, $P < 0.01$) were used.

Abbreviations

16S rRNA: 16S ribosomal RNA; SOM: Soil organic matter; ROOC: Readily oxidized organic carbon; TN: Total nitrogen; TP: [Total phosphorus](#); TK: Total potassium; AN: Rapidly available nitrogen; AP: Rapidly available phosphorus; AK: Rapidly available potassium; LEfSE: Linear discriminant analysis effect size; RDA: Redundancy analysis; OTU: Operational taxonomic unit;

Declarations

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Authors' contributions

YYZ and ZJQ designed experimental detail and have gone through with the total study, FMK participated in experimental design and assisted to finish all research work, WMY, ZHS and LHZ participated in test design and data analysis. All authors approved of their contents.

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Availability of data and materials

The datasets in this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All the work was not involved in ethics

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Statistics of sample sequencing data

Farming mode	Sample	Date	OTU number	OTU sequence number	Total base number of OTU sequence	Coverage
Conventional farming	CK1_1	4/2015	2152	30386	12546633	0.98
	CK1_2	4/2015	2016	28674	11838682	0.98
	CK1_3	4/2015	2133	32887	13578256	0.98
	CK2_1	10/2015	2018	32376	13347826	0.98
	CK2_2	10/2015	1925	31270	12893753	0.98
	CK2_3	10/2015	2059	33388	13769627	0.98
	CK3_1	4/2016	2241	33852	13960497	0.98
	CK3_2	4/2016	2265	31198	12874674	0.98
	CK3_3	4/2016	1885	28650	11809160	0.98
	CK4_1	10/2016	1595	36807	15191973	0.99
	CK4_2	10/2016	1619	32836	13559079	0.98
	CK4_3	10/2016	1558	30205	12471320	0.98
	CK5_1	4/2017	1808	36803	15188216	0.98
	CK5_2	4/2017	1626	20755	8564909	0.97
	CK5_3	4/2017	1941	36245	14958579	0.98
	CK6_1	10/2017	1887	36333	14996784	0.98
	CK6_2	10/2017	1903	34537	14257918	0.98
	CK6_3	10/2017	1911	35519	14662221	0.98
	CK7_1	4/2018	2040	35802	14767143	0.98
	CK7_2	4/2018	1866	30127	12420034	0.98
	CK7_3	4/2018	2061	34860	14373806	0.98
	total				282031090	
Organic farming	O1_1	4/2015	2001	29149	12032425	0.98
	O1_2	4/2015	1932	28246	11660849	0.98
	O1_3	4/2015	2080	31751	13104572	0.98
	O2_1	10/2015	2109	30709	12660653	0.98
	O2_2	10/2015	2023	28794	11869225	0.98
	O2_3	10/2015	1993	29092	11997426	0.98
	O3_1	4/2016	2356	32100	13245938	0.98
	O3_2	4/2016	2503	33806	13946524	0.98
	O3_3	4/2016	2484	30741	12681590	0.98
	O4_1	10/2016	1650	38283	15793691	0.99
	O4_2	10/2016	1563	28869	11913231	0.98
	O4_3	10/2016	1568	28223	11644956	0.98
	O5_1	4/2017	1349	31003	12781240	0.98
	O5_2	4/2017	1217	24352	10035522	0.98
	O5_3	4/2017	1368	32387	13348661	0.98
	O6_1	10/2017	1689	30404	12536853	0.98
	O6_2	10/2017	1670	27670	11409420	0.98
	O6_3	10/2017	1883	35143	14488275	0.98
	O7_1	4/2018	1750	36009	14839877	0.98
	O7_2	4/2018	1811	37053	15270487	0.98
	O7_3	4/2018	1705	32305	13311319	0.98
	total		total		270572734	

Supplementary Information

Supplementary information accompanies this paper at

Additional file 1: Figure S1. Distribution of optimized sequence length.

Additional file 2: Table S1. Analysis on the sequence data of soil bacteria under different planting mode.

Additional file 3: Table S2. Summary of species based OTU.

Figures

Figure 1

Effects of different farming modes on the chemical properties of soil. A. pH, B. SOM: soil organic matter, C. ROOC: readily oxidized organic carbon, D. TN: total nitrogen, E. TP: total phosphorus, F. TK: total potassium, G. AN: available nitrogen, H. AP: rapidly available phosphorus, I. AK: rapidly available potassium.

Figure 2

Analysis of sequencing data for soil bacteria under different farming modes. A. Rarefaction curve chart. B. Venn diagram based on operational taxonomic units (OTUs) of bacteria. Soil sample: CK1, 2015.4; CK2, 2015.10; CK3, 2016.4; CK4, 2016.10; CK5, 2017.4; CK6, 2017.10; CK7, 2018.4; O1: 2015.4; O2, 2015.10; O3, 2016.4; O4, 2016.10; O5, 2017.4; O6: 2017.10; O7: 2018.4. Each sample had three replications.

Figure 3

Analysis on sample volume adequacy and core bacteria covering 100% of samples. A. Analysis on sample volume adequacy using sample-based species accumulation curves. Purple represents different

species, while blue is a statistical analysis of species B. Analysis on the core bacteria covering 100% of samples.

Figure 4

Analysis of microbial communities under different farming modes. A. Phylum level, B. Genus level. Soil sample: CK1, 2015.4; CK2, 2015.10; CK3, 2016.4; CK4, 2016.10; CK5, 2017.4; CK6, 2017.10; CK7, 2018.4; O1: 2015.4; O2, 2015.10; O3, 2016.4; O4, 2016.10; O5, 2017.4; O6: 2017.10; O7: 2018.4. Each sample had three replications.

Figure 5

The abundance of bacterial communities under different farming mode at genera levels. A. Heatmap of bacterial communities at the genus level. B. Star analysis on the abundance of the species from the first 10 genera. Soil sample: CK1, 2015.4; CK2, 2015.10; CK3, 2016.4; CK4, 2016.10; CK5, 2017.4; CK6, 2017.10; CK7, 2018.4; O1: 2015.4; O2, 2015.10; O3, 2016.4; O4, 2016.10; O5, 2017.4; O6: 2017.10; O7: 2018.4. Each sample had three replications.

Figure 6

Principal component analysis (PCA) between different timepoints under the two farming modes. A Soil sample: CK1, 2015.4; CK2, 2015.10; CK3, 2016.4; CK4, 2016.10; CK5, 2017.4; CK6, 2017.10; CK7, 2018.4; O1: 2015.4; O2, 2015.10; O3, 2016.4; O4, 2016.10; O5, 2017.4; O6: 2017.10; O7: 2018.4.

Figure 7

Comparison of the four alpha diversity indexes under different farming modes. A. Chao1 index, B. Shannon index, C. Ace index, D. Simpson index, Soil sample: CK1, 2015.4; CK2, 2015.10; CK4, 2016.10; CK6, 2017.10; O1: 2015.4; O2, 2015.10; O4, 2016.10; O6; 2017.10.

Figure 8

Linear discriminant analysis effect size (LEfSE) cladogram showing differences of bacteria community structure between different farming mode. The black circles from center to outer represent phylum, class, order, family, genus, and species. Red, green, blue and purple circles show taxa that were abundant in the CK1, CK6, O1 and O6, respective. Soil sample: CK1, 2015.4; CK6, 2017.10; O1: 2015.4; O6, 2017.10.

Figure 9

RDA (redundancy analysis) of environmental factors and bacterial composition. SOM: soil organic matter; ROOC: readily oxidized organic carbon; TK: total potassium; AN: available nitrogen. Soil sample: CK1, 2015.4; CK6, 2017.10; O1: 2015. 4; O6; 2017.10.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xls](#)
- [Fig.S1.jpg](#)
- [TableS2.xls](#)