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The Influence of Organic and Conventional Cultivation Patterns on Physicochemical Property, Enzyme Activity and Microbial Community Characteristics of Paddy Soil

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Abstract: Cultivation patterns can cause soil structure alteration. However, few studies have clarified the influence of cultivation pattern and soil depth on soil. The purpose of this experiment was to study the community characteristics of soil microorganisms in the 0–10 cm and 10–20 cm layers beneath paddy fields under organic and conventional cultivation patterns, and reveal the response mechanism of microbial community to cultivation patterns through the correlation analysis of soil nutrient content, enzyme activity and microbial dominant phyla, so as to provide a theoretical basis for high-yield rice cultivation from the perspective of microorganisms. In this study, four types of soil organic cultivation topsoil (OF_S), organic cultivation undersoil (OF_X), conventional cultivation topsoil (CF_S) and conventional cultivation undersoil (CF_X) in paddy fields were collected for nutrient and enzyme activity determination, and composition spectrum analysis of soil microbial community diversity was performed using a high-throughput sequencing platform. The results revealed that organic cultivation increased the contents of alkali-hydrolyzable nitrogen, available phosphorus, available potassium and organic substances in both topsoil and undersoil as well as sucrase and urease activity in the undersoil. α diversity indicated that bacterial abundance in both topsoil and undersoil was organic > conventional cultivation; the microbial diversity index in the undersoil under organic cultivation technique was greater than that of conventional cultivation. A Venn diagram revealed that there was considerable difference in species between topsoil and undersoil under organic and conventional cultivation patterns. The composition of the community structure indicated that *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Bacteroidetes* were the dominant phyla of bacterial communities in paddy fields. *Ascomycota* and *Basidiomycota* were the dominant phyla of the fungal community. Cluster analysis results indicated that soil depth of both patterns produced apparent clustering effects on microorganisms. Correlation analysis revealed that contents of various soil nutrients and enzyme activities affected the relative abundance of the dominant bacteria and fungi in varying degrees. Alkali-hydrolyzable nitrogen, available potassium and organic matters were significant factors affecting the dominant phyla of soil. The present study demonstrated that compared with conventional cultivation, organic cultivation improved soil physicochemical property, enhanced soil enzyme activity, and altered soil microbial diversity and bacterial abundance. Soil nutrients, enzyme activity and the microbial community of paddy fields interacted with each other and affected the soil structure together.

Keywords: organic matters; soil depth; soil nutrients; soil enzyme; high-throughput sequencing; soil microorganisms

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1. Introduction

As one of the vital models for sustainable development of agriculture, organic farming can not only improve the soil physicochemical property, but also diversify soil microorganisms and maintain the stability of the ecosystem [1]. Organic cultivation improves soil quality, and soil microorganisms, as a typical strain resource bank of the ecosystem, can reflect the sensitivity of soil quality [2]. The exploration on the differences in soil microbial community characteristics between organic and conventional cultivation patterns can provide a theoretical basis for sustainable paddy farming.

As the high-throughput sequencing technology advances, numerous studies have been conducted on changes in soil microorganisms in paddy fields under organic cultivation pattern at home and abroad. The current analysis of soil microorganisms can be classified into two categories, one is diversity analysis, and the other is species composition and variance analysis. Hartmann et al. [3] have found that organic cultivation can increase the abundance of soil microorganisms using a ribosome labeling method, and the impact of organic fertilizer on the structure of the microbial community is greater than that of pesticides. Wang et al. [4] have studied three types of crops grown in the middle and lower reaches of Yangtze River and reported that organic cultivation significantly increased the abundance of certain nutrient-associated bacteria and reduced the abundance of partial acid and alkali-tolerant bacteria. The study of Hideki et al. [5] has pointed out that the bacterial population in soil of organic cultivation is more diverse than that of traditional cultivation and the vigorous and stable bacterial population contributes to controlling outbreak of rice diseases. The previously described research has confirmed the advantages of organic cultivation through the analysis of the soil microbiome of paddy fields. Moreover, owing to the high heterogeneity of soil, different soil environments will affect the abundance of microorganisms [6], and it has been widely recognized that microbial biomass carbon decreases significantly with the increase of soil depth [7–9]. Some studies have revealed that with the increase of soil depth, the interaction network between microorganisms turns more intricate, and the interaction between bacteria and fungi communities converts from cooperation into competition [10,11]. Others believe that the soil microbial community in paddy fields has a high adaptive capacity to the gradients of soil depth, and the interaction between microbial communities is stable [12].

The 0–20 layer is the depth of direct contact between rice root systems and soil, and previous studies mostly collected only 0–20 cm soil and mixed evenly for determination. However, the microbial community structure was probably different between the surface and subsurface soils, which was closely related to the change of the total carbon and carbon to nitrogen ratios. Although there are many reports on the comparative study of organic cultivation and conventional cultivation on soil microorganisms, few studies have been reported on the effects of organic cultivation on soil microorganisms at different gradients of soil depth. The current experiment was carried out in the rice cropping regions in Huaiyang, the lower reach of Yangtze River. Through a 9-year long-term positioning experiment, this trial combined previous organic and conventional cultivation fields and compared the topsoil and undersoil fields. The purpose of the study was to (1) explore the diversity and composition differences of microbial communities under the coupling effect of cultivation patterns and soil depth; (2) determine the contents of soil nutrients and enzyme activity using two cultivation patterns, and analyze the correlation of soil physiochemical property, enzyme activity and microbial dominant phyla; (3) reveal the response mechanism of microbial communities in paddy fields to cultivation patterns at soil depths of 0–10 cm and 10–20 cm; and (4) from the perspective of microorganisms, provide some inspiration for the research on high yield and high quality of organic rice, and offer theoretical guidance for rice yield.

2. Materials and Methods

2.1. Plot Location and Test Materials

The experiment was carried out in the experimental plot of Mapengwan Ecological Agriculture Technology Co., Ltd., Gaoyou, China, from 2019 to 2020. The plot sits at Mapengwan, Gaoyou City, Jiangsu Province in China (119°25′ east longitude, 32°47′ northern latitude). It belongs to the northern subtropical monsoon climate zone. The annual average temperature is around 16.2 °C, the annual precipitation is approximate 1341.5 mm, and the annual sunshine hours are 2100 h, and the frost-free season lasts for around 221 d. The experimental plot was scheduled under long-term organic cultivation research since 2012. The soil property is stable and the texture is clay loam. The soil sample collected in May 2019 was identified containing 25.48 $g \cdot kg^{-1}$ organic matters and 108.23 $mg \cdot kg^{-1}$ alkali-hydrolyzable nitrogen, available phosphorus 7.36 $mg \cdot kg^{-1}$, available potassium 65.43 $mg \cdot kg^{-1}$ and total nitrogen 1.31 $g \cdot kg^{-1}$ at the pH value of 8.72. The rice variety tested was Nanjing 46. The whole growth period of the rice variety is 165 days (Figure 1).

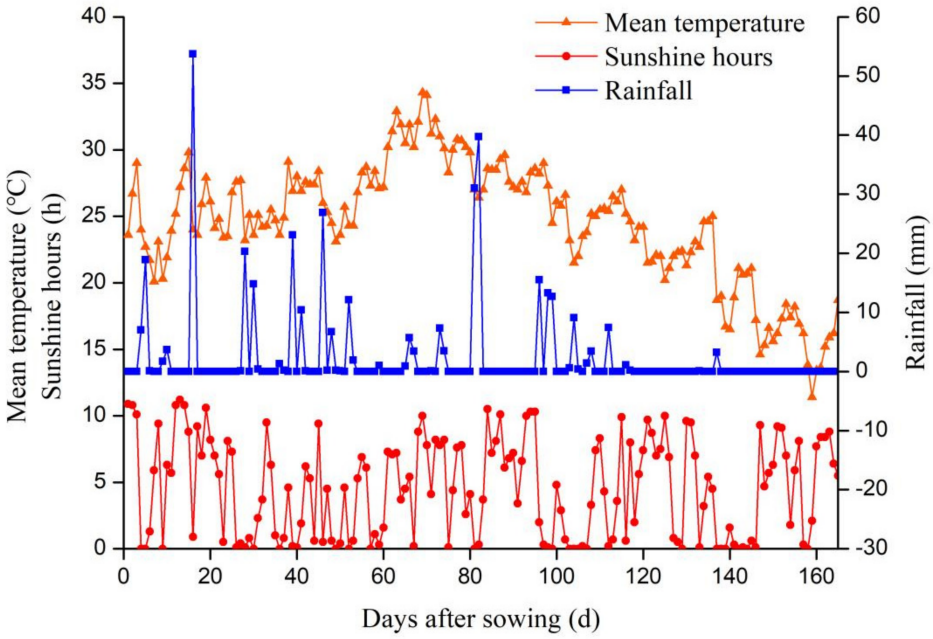

Figure 1. Daily mean temperature, sunshine hours, and rainfall during the rice growing season in 2019, Yangzhou City, Jiangsu Province, China.

2.2. Experimental Design

This experiment was divided by cultivation methods, which was designed using patterns of organic cultivation (OF) and conventional cultivation (CF). Each treatment was repeated 3 times, and each treatment was arranged in random blocks at an area of 105 m^2 per plot. The test rice was sown on 22 May 2019, and rice transplantation by hand was carried out on June 10, with a transplanting row spacing of 30.0 cm × 12.5 cm, and 3 seedlings were planted per hole.

Organic cultivation was managed as per the standards of Organic products—Requirements for production (GB/T19630.1), and the milk vetch–rice planting model was adopted. Milk vetch (containing 0.33% N, 0.08% P_2O_5 and 0.23% K_2O) was applied as a base fertilizer in one turn at plowing two weeks prior to rice transplanting, with an amount of 12,000 $kg \cdot hm^{-2}$. Rapeseed cake (containing 5.25% N, 0.80% P_2O_5, 1.04% K_2O, 0.80% Ca, 0.48% Mg and various trace elements) and San'an bio-organic fertilizer (containing 3.35% N, 1.87% P_2O_5, 2.28% K_2O, several organic acids, peptides and rich nutrients containing 53% organic matter [13]) were applied as base fertilizer 2 days prior

to rice transplanting, and the application amount was 1200 kg·hm^{-2}. Organic fertilizer from San'an Biology was applied again as earing fertilizer in mid-July, and the application amount was 450 kg·hm^{-2}.

Conventional cultivation was managed based on the local production ways in Gaoyou region. The base fertilizers included application of nitrogen fertilizer 108 kg·hm^{-2}, P_2O_5 150 kg·hm^{-2}, and K_2O 150 kg·hm^{-2}. When topdressing nitrogen fertilizer, the tillering fertilizer was applied at quantities of 27 kg·hm^{-2} and 54 kg·hm^{-2} on the 7th and 21st days, respectively, after plantation, and earing fertilizer was applied to the inverse fourth and secondary leaves at 40.5 kg·hm^{-2}, respectively.

2.3. Sample Collection

Based on a five-point sampling method, on 4 September 2019, the plowed layer soil at the depth of 0–20 cm in each plot was collected using a soil extractor, and the collected soil was divided into topsoil (0–10 cm) and undersoil (10–20 cm), marked as S and X, respectively. Each treatment was repeated three times. Four samples were ultimately obtained including conventional cultivation topsoil (CF_S), organic cultivation topsoil (OF_S), conventional cultivation undersoil (CF_X) and organic cultivation undersoil (OF_X). The collected soil samples were evenly mixed with the removal of sundries like rice root residues and stones. Some soil samples were air-dried and ground, and subsequently passed through 20-mesh and 100-mesh sieves to determine soil nutrients and enzyme activities. The other part was sealed in a bag and stored in a refrigerator at −70 °C for subsequent analysis of soil microorganisms.

2.4. Determination of Sample Nutrients and Enzyme Activity

The contents of each soil nutrient were determined with reference to the conventional analysis method [14]. Alkali-hydrolyzable nitrogen in soil was measured by the alkaline hydrolysis nitrogen diffusion method; total nitrogen in soil was determined using the H_2SO_4-mixed accelerator distillation method; soil organic matter was measured using the $K_2Cr_2O_7$-H_2SO_4 external heating method; soil available phosphorus was determined using $NaHCO_3$ extraction spectrophotometry; available potassium in soil was determined using CH_3COONH_4 extraction flame spectrophotometry; and the soil pH value was measured using the COMBI 5000 handheld measuring meter based on operation manual.

Soil sucrase was determined using the 3,5-dinitrosalicylic acid colorimetric method [15], and the enzyme activity was expressed in mg of glucose produced in 1 g soil after 24 h; soil urease was determined using the indophenol blue colorimetry method [15]; and the enzyme activity was expressed in μg of NH_3-N produced in 1 g soil sample after 24 h.

2.5. DNA Extraction, PCR Amplification and High-Throughput Sequencing

Each soil sample obtained 0.5 g and extracted DNA of the microorganisms using Omega soil DNA kit. The DNA quality was subsequently detected by 1.2% agarose gel electrophoresis. The bacterial target DNA sequence used was 16S_V4V5 region. The PCR amplification of 16S rDNA applied forward primer 515F (5′-GTGCCAGCMGCCCGCCGCGTAA-3′) and reverse primer 907R (5′-CCCGTCATCAATTTTCMTTTRAGTTT-3′). The fungal sequencing region was ITS_V1, and forward primer was ITS5F (5′-GGAGTAAGTCGTACAGG-3′) and reverse primer ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) for PCR amplification. The amplification system (25 μL) was: 5 × reaction buffer 5 μL, 5 × GC buffer 5 μL, dNTP (2.5 mM) 2 μL, forward primer (10 μM) 1 μL, reverse primer (10 uM) 1 μL, DNA Template 2 μL, ddH_2O 8.75 μL, and Q5 DNA polymerase 0.25 μL. The amplification parameters included: pre-denaturation at 98 °C for 2 min; denaturation at 98 °C for 15 s; annealing at 55 °C for 30 s; extension at 72 °C for 30 s; and final extension at 72 °C for 5 min and kept at 10 °C, 25–30 cycles in total. Following the amplification, gel electrophoresis was performed, and 2% agarose was freshly prepared for detecting the effect of PCR amplification products. Illumina Mi Seq sequencing was performed as per QIIME2 dada2 analysis process for

quality control, de-noising, splicing and de-chimerism. High-throughput sequencing of the soil was consigned to Shanghai Personalbio Technology Co., Ltd., Shanghai, China.

2.6. Data Analysis

Microsoft Excel 2019 and SPSS 23.0 software were adopted for data sorting and analysis, and Origin 8.5 was applied for plotting. Univariate analysis was performed for data of soil nutrients and enzyme activity and LSD was for comparison of data differences; the relationship between nutrients and enzyme activity factors and the phyla was analyzed using Person correlation. The microbial community characteristics were mapped using the Genescloud platform (https://www.genescloud.cn, accessed on 24 June 2020). Based on ASV/OTU distribution in different treatments, the Alpha diversity was calculated for each treatment. A Venn diagram was plotted, which reflected overlapped sample OTUs. A community abundance composition map was plotted to compare the proportion of each bacterial community. A hierarchical clustering hierarchical tree diagram was made to reflect the similarity among samples.

3. Results

3.1. The Impact of Organic and Conventional Cultivation Patterns on the Physiochemical Property of Topsoil and Undersoil

Table 1 presented that the nutrient contents of the topsoil was always greater than that of the undersoil, regardless of organic cultivation or conventional cultivation. In topsoil, the contents of AN, AP, AK and SOM in the soil of organic cultivation were increased by 3.40%, 23.05%, 8.28% and 10.57%, respectively, compared with conventional cultivation, whereas in the undersoil, the three described nutrients increased at the proportions of 2.31%, 15.7%, 3.37% and 31.10%, respectively. In contrast, the TN content in organic cultivation soil was lower than that of conventional cultivation, which was 6.22% lower in the topsoil and 10.14% lower in the undersoil. The soil pH value under organic cultivation technique was lower than that under conventional cultivation, and the pH value of the topsoil was lower than that of the undersoil.

Table 1. Effects of cultivation patterns on soil physicochemical properties in different soil layers.

Treatment	AN ($mg \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)	AK ($mg \cdot kg^{-1}$)	SOM ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	pH
CF_S	164.50 ± 0.40^{Bb}	6.55 ± 0.05^{Bb}	96.42 ± 0.57^{Bb}	38.69 ± 0.26^{Bb}	2.25 ± 0.01^{Aa}	8.40 ± 0.01^{Aab}
OF_S	170.10 ± 0.57^{Aa}	8.06 ± 0.05^{Aa}	104.40 ± 0.58^{Aa}	42.78 ± 0.27^{Aa}	2.11 ± 0.01^{Bb}	8.31 ± 0.02^{Ab}
CF_X	91.00 ± 0.48^{Cd}	4.72 ± 0.11^{Dd}	88.44 ± 0.57^{Cd}	20.48 ± 0.38^{Dd}	1.38 ± 0.01^{Cc}	8.69 ± 0.01^{Aa}
OF_X	93.10 ± 0.37^{Cc}	5.46 ± 0.08^{Cc}	91.42 ± 0.58^{Cc}	26.85 ± 0.19^{Cc}	1.24 ± 0.02^{Dd}	8.53 ± 0.01^{Aab}

Data are presented by mean value ± SD; Different lower case letters in the same column indicate significant differences among the four treatments at $p < 0.05$, different upper case letters in the same column indicate very significant differences among the four treatments at $p < 0.01$. AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; SOM, soil organic matter; TN, total nitrogen. The same below.

3.2. The Effect of Organic and Conventional Cultivation Patterns on the Enzyme Activities of the Topsoil and Undersoil

As shown in Figure 2, activities of sucrase and urease in the topsoil were always better than those of the undersoil using both cultivation techniques. In the left part of Figure 2, the sucrase activity in the topsoil of organic cultivation was 4.31% lower than that of conventional cultivation, but the difference was not significant; however, in the undersoil, the sucrase activity using organic cultivation was 107.69% higher than that of conventional cultivation, and the activity of sucrase under conventional cultivation was significantly reduced. In the right part of Figure 2, in the topsoil and the undersoil, urease activity of organic cultivation was increased by 14.92% and 40.90%, respectively, than that of conventional cultivation, and the difference in the latter was extremely significant.

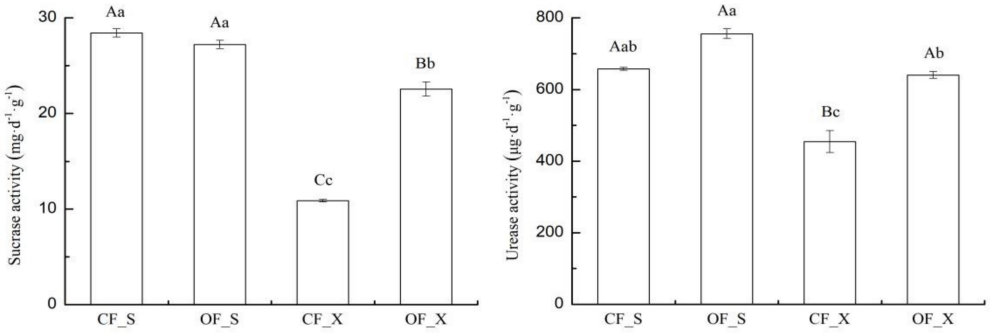

Figure 2. Effects of cultivation patterns on soil enzyme activities in different soil layers. Different lower case letters indicate significant differences among the four treatments at $p < 0.05$, different upper case letters indicate very significant differences among the four treatments at $p < 0.01$.

3.3. The Influence of Organic and Conventional Cultivation Patterns on the Microbial Community Characteristics in the Topsoil and Undersoil

3.3.1. α Diversity Analysis

Coverage was used to assess the variety and quantity of microorganisms in soil samples. As shown in Table 2, the coverage of bacteria and fungi samples was greater than 96%, indicating that the sequencing results could widely reflect the authenticity of the varieties and quantities of bacteria and fungi. As an ecological index for OTUs number evaluation, Chao1 can be applied to characterize abundance. In the bacterial community, Chao1 indexes in the topsoil and undersoil under organic cultivation were 15.34% and 27.10% higher than those under conventional cultivation, indicating that organic cultivation could increase the abundance of bacteria in both topsoil and undersoil. Regardless of bacteria or fungi, the abundance decreased while the soil depth increased. Compared with the topsoil, Chao1 index of the bacterial community in the undersoil decreased by 11.97% under organic cultivation and 20.11% under conventional cultivation; Chao1 index of the fungal community decreased by 22.53% under organic cultivation and 40.49% under conventional cultivation. The findings indicated that the topsoil of paddy fields had a higher quantity of microorganisms under both cultivation methods, and the decrease of the microbial abundance of the organic cultivation was smaller with the increase of soil depth.

Table 2. Alpha diversity of microorganisms in upper and lower soil layers under two cultivation patterns.

Treatment		Chao1	Coverage	Evenness	Shannon	Simpson
Bacteria	OF_S	5403.12	0.965715	0.826086	10.0863	0.991817
	OF_X	4756.5	0.978876	0.889906	10.7828	0.998627
	CF_S	4684.5	0.982093	0.892017	10.8153	0.998645
	CF_X	3742.45	0.984714	0.876004	10.3057	0.997565
Fungi	OF_S	720.282	0.999896	0.732942	6.95595	0.965398
	OF_X	557.984	0.999977	0.763078	6.96222	0.975632
	CF_S	850.795	0.999777	0.782208	7.60819	0.985116
	CF_X	506.321	0.999864	0.733932	6.58871	0.970523

Evenness was used to measure the relative abundance of species in a community. The evenness index of bacteria was higher than that of fungi, indicating that the evenness of individual distribution in the bacterial community was higher than that of the fungal community. The Shannon index combined the number and abundance of species, and the Simpson index (Gini Simpson index) integrated the abundance and evenness of species, both of which could be applied to estimate the diversity of microorganisms in the samples. This study revealed that the Shannon index of bacteria and fungi in the soil was consistent with the Simpson index, both were organic topsoil < conventional topsoil, organic undersoil

> conventional undersoil, indicating that the organic topsoil had less microbial diversity than the conventional topsoil, whereas organic undersoil had greater microbial diversity than conventional undersoil.

3.3.2. Venn Diagram Analysis Based on OTUs

To identify the number of overlapped OTUs and specific OTUs in topsoil and undersoil under organic and conventional cultivation patterns, the following Venn diagram (Figure 3) was used for community analysis. The results revealed that the abundance of soil bacterial community under organic cultivation was higher than that under conventional cultivation, and there was a big difference in species between organic and conventional cultivations. As for fungi, the abundance of soil bacterial community under organic cultivation was similar, and there was a big difference in species. Regardless of bacteria or fungi, the microorganism groups in the topsoil were always more abundant than those in the undersoil, and the species differences between the topsoil and undersoil were more apparent.

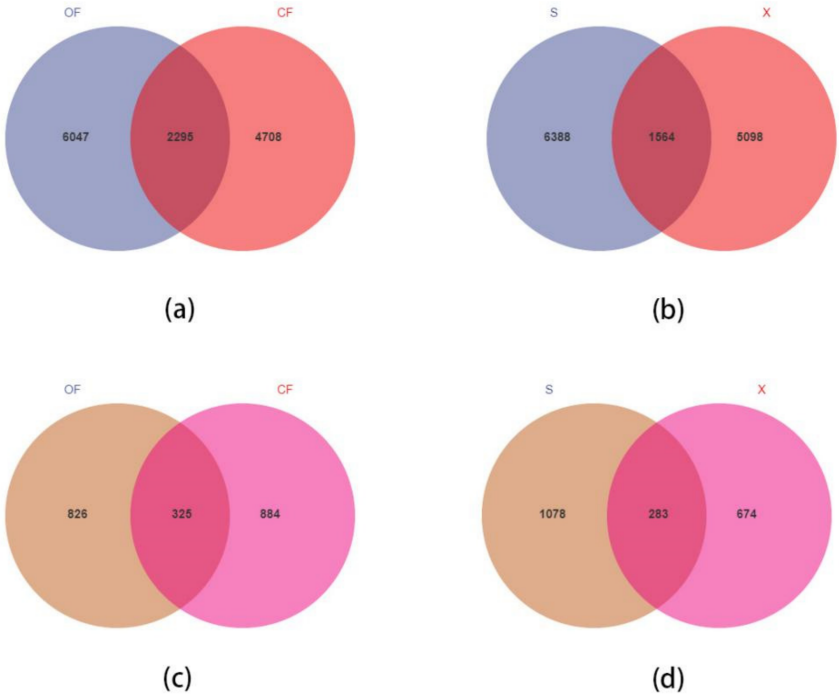

Figure 3. Venn Diagram of soil microorganisms: (**a**) OTU number of bacteria under different cultivation patterns; (**b**) OTU number of bacteria in different soil layers; (**c**) OTU number of fungi under different cultivation patterns; (**d**) OTU number of fungi in different soil layers.

3.3.3. Analysis of Community Structure Composition

Figure 4 presented the relative abundance statistics of soil bacteria at phylum and order levels. After high-throughput sequencing, 10 species with the highest relative abundance were presented at the bacterial community phylum level, and the relative abundances of the remaining species were combined and classified as others. The 10 species included *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Bacteroidetes*, *Thaumarchaeota*, *Nitrospirae*, *Planctomycetes*, *Gemmatimonadet*, *Latescibacteria* and *Rokubacteria*. Among them, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Bacteroidetes* were the four dominant bacterial community, falling into the ratio intervals of 65.94–82.65%. In the topsoil, the relative abundance under organic cultivation was reduced compared with conventional cultivation of *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Bacteroidetes*, which decreased by 18.83%, 18.99%, 11.76% and 45.65%, respectively. In the undersoil, the relative abundance under organic cultivation was higher than that of conventional cultivation, increased by 1.29% and 34.33%, respectively, whereas

the relative abundance of *Acidobacteria* and *Bacteroidetes* reduced, decreasing by 8.51% and 37.92%, respectively. It is worth noting that the relative abundance of *Thaumarchaeota* in the topsoil under organic cultivation had the largest proportion at 20.81% compared with the other three treatments. The 10 species with the highest relative abundance at the order level of bacteria consisted of Beta*Proteobacteria*les, Subgroup_6, *Anaerolineales*, *Nitrososphaerales*, MBNT15, *Rhizobiales*, Subgroup_17, *Myxococcales*, Chitinophagales, and *Latescibacteria*. Among them, Beta*Proteobacteria*les, Subgroup_6, and *Anaerolineales* were three of the higher relatively abundance at order level, at the range of 28.75–39.01%. In the topsoil, the relative abundance of Beta*Proteobacteria*les, Subgroup_6, and *Anaerolineales* under organic cultivation was reduced by 17.66%, 21.83% and 10.63%, respectively, compared with conventional cultivation. In the undersoil, the relative abundance of Beta*Proteobacteria*les and *Anaerolineales* under organic cultivation was higher than that of conventional cultivation, increasing by 10.62% and 55.80%, while the relative abundance of Subgroup_6 decreased by 10.53%. The relative abundance of *Nitrososphaerales* in the topsoil of organic cultivation was 20.77%, and the ratio was greater than the other three treatments.

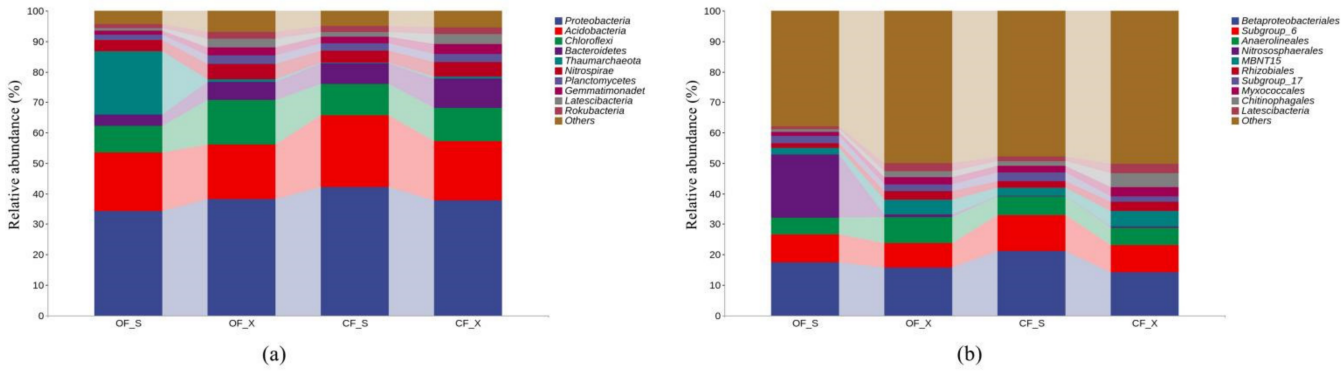

Figure 4. Relative abundance of bacteria at (**a**) phylum level and (**b**) order level.

Figure 5 presented the relative abundance statistics of soil fungi at phylum and order levels. As there were only six species with the highest number of units in the fungal community at phylum level after high-throughput sequencing, the relative abundances of the remaining species excluding the described six species were combined and classified as Others. The six fungal species at phylum level included *Ascomycota*, *Basidiomycota*, *Mucoromycota*, *Chytridiomycota*, *Olpadiomycota* and *Zoopagomycota*. *Ascomycota* and *Basidiomycota* were the most abundant in the fungal community at phylum level, accounting for 68.38% of the conventional undersoil samples and about 30% of the remaining three samples. In the topsoil, the relative abundance of *Ascomycota* under organic cultivation was only 0.33% lower than that of conventional cultivation, and the change was not significant, whereas the relative abundance of *Basidiomycota* increased by 48.34%; In the undersoil, the relative abundance of *Ascomycota* and *Basidiomycota* under organic cultivation was lower than that of conventional cultivation, decreasing by 49.26% and 45.42%, respectively. The 10 dominant species obtained through sequencing at the fungal order level were *Hypocreales*, *Trichosphaeriales*, *Eurotiales*, *Pleosporales*, *Cystofilobasidiales*, *Sordariales*, *Mortierellales*, *Saccharomycetales*, *Agaricales*, and *Helotiales*. *Hypocreales* and *Trichosphaeriales* ranked as the top two, but the proportion was not apparent compared with the rest of the fungal orders. In the topsoil, the relative abundance of *Hypocreales* under organic cultivation was significantly higher than that of conventional cultivation, with an increase of 147.34%, whereas the relative abundance of *Trichosphaeriales* decreased by 49.48%. In the undersoil, the relative abundance of *Hypocreales* and *Trichosphaeriales* under organic cultivation was lower than that of conventional cultivation, decreasing by 51.27% and 11.51%, respectively. The relative abundance of *Eurotiales* in the undersoil samples under conventional cultivation was significantly increased, with the proportion of 16.48%. The relative abundance of *Pleosporales* in the undersoil of organic samples was significantly increased, and its proportion was 7.94%.

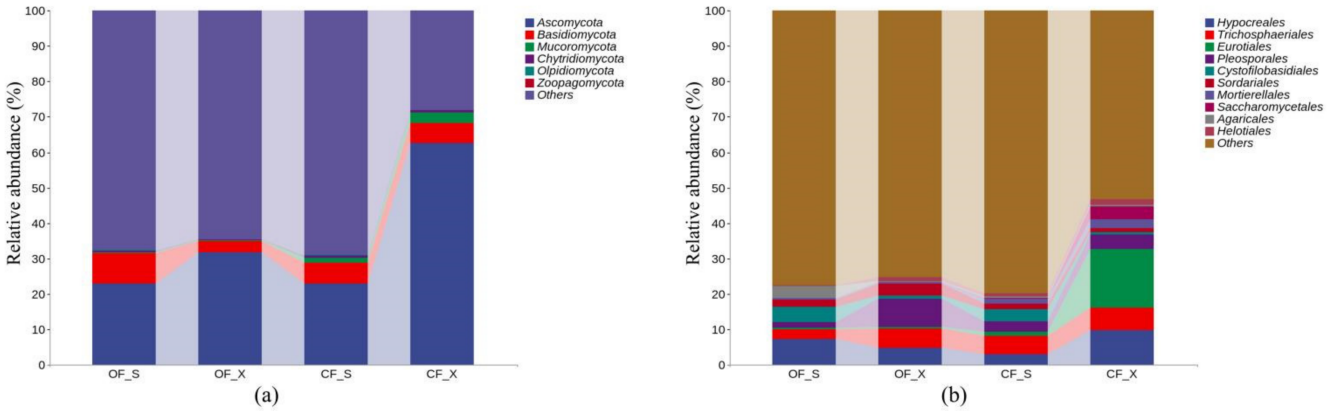

Figure 5. Relative abundance of fungi at (**a**) phylum level and (**b**) order level.

3.3.4. Hierarchical Cluster Analysis

Figure 6 presented the cluster analysis of microorganisms at genus level. The figure revealed that in the bacterial community, clusters of CF_S branch and OF_S branch were similar, and clusters of CF_X branch and OF_X branch were similar. It indicated that the bacteria in the topsoil and the undersoil had a big difference. In the fungal community, the composition of CF_S and OF_S was close to each other, and the composition of OF_X differed greatly from the two. The difference between CF_X and OF_X was great, and there was a greater difference between CF_S and OF_S. It suggested that cultivation methods and soil depth affected the composition of fungi.

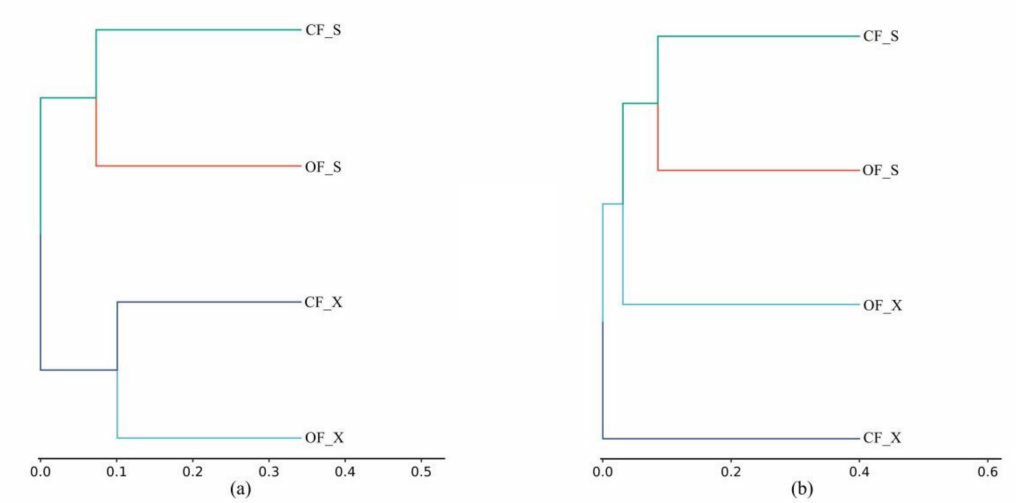

Figure 6. Hierarchical clustering analysis at genus level in (**a**) the bacterial community and (**b**) the fungal community.

3.4. Correlation Analysis of Physiochemical Property and Enzyme Activity with Microorganisms in Soil

The correlation between the dominant bacteria phyla in the composition of bacterial and fungal communities was compared with the soil physiochemical properties and enzyme activities. The results were shown in Table 3. In the bacterial phylum community, the relative abundance of *Acidobacteria* was significantly positively correlated with AP and TN. However, the relative abundances of *Chloroflexi* and *Bacteroidetes* were negatively correlated with AN, AK and SOM, and that of *Thaumarchaeota* was the opposite. TN was negatively correlated with *Chloroflexi*, whereas the pH value was positively correlated with *Bacteroidetes*. In the fungal phylum community, the pH value was significantly positively correlated with *Ascomycota*, whereas AN, AK, SOM and TN were significantly negatively

correlated with *Ascomycota*, but significantly positively correlated with *Basidiomycota*. The relative abundance of *Mucoromycota* was significantly negatively correlated with AK and SOM, and significantly positively correlated with pH. *Chytridiomycota* had a significant positive correlation with AP. *Olpidiomycota* also had a significant positive correlation with AN, AP, SOM and TN.

Table 3. Correlation analysis of soil physicochemical properties, enzyme activities and dominant phylum.

	Phylum	AN	AP	AK	SOM	TN	pH	Sucrase	Urease
Bacteria	*Proteobacteria*	−0.011	0.554	−0.416	−0.107	0.140	0.128	0.131	−0.248
	Acidobacteria	0.573	0.891 **	0.142	0.401	0.720 **	−0.229	0.377	0.050
	Chloroflexi	−0.762 **	−0.559	−0.641 *	−0.606 *	−0.804 **	0.388	−0.234	−0.247
	Bacteroidetes	−0.644 *	−0.053	−0.863 **	−0.813 **	−0.475	0.739 **	−0.813 *	−0.961 **
	Thaumarchaeota	0.608 *	−0.036	0.869 **	0.675 *	0.469	−0.564	0.406	0.674
Fungi	*Ascomycota*	−0.756 **	−0.418	−0.755 **	−0.878 **	−0.660 *	0.769 **	−0.991 **	−0.935 **
	Basidiomycota	0.739 **	0.333	0.776 **	0.654 *	0.712 **	−0.463	0.268	0.403
	Mucoromycota	−0.402	0.066	−0.619 *	−0.623 *	−0.231	0.620 *	−0.790 *	−0.888 **
	Chytridiomycota	0.392	0.683 *	0.035	0.141	0.560	0.028	−0.102	−0.298
	Olpidiomycota	0.852 **	0.905 **	0.523	0.732 **	0.933 **	−0.518	0.651	0.411

The significant correlations are denoted by asterisks (* $p < 0.05$; ** $p < 0.01$).

Sucrase and urease were affected by *Bacteroidetes* at bacteriophyta, which had a significantly negative correlation. Similarly, at fungal phyla, both were affected by *Ascomycota* and *Mucoromycota*, and had a significantly negative correlation.

4. Discussion

4.1. The Effects of Both Cultivation Patterns on the Physiochemical Properties and Enzyme Activities of the Topsoil and Undersoil

Organic cultivation could effectively improve the physiochemical and biological properties of the soil, and coordinate the contradiction between high yield and high environmental risk [16]. As soil depth increased, soil nutrients and physiochemical properties changed significantly [17]. Regardless of organic cultivation or conventional cultivation, the physiochemical properties of the topsoil were better than those of the undersoil in the study. The contents of alkali-hydrolyzable nitrogen, available phosphorus, available potassium and organic matter in the soil under organic cultivation were higher than those of conventional cultivation. The results were consistent with previous studies [18,19]. The main reason was that the high efficiency of nutrients under organic cultivation provided a feasible growth environment for microorganisms, the carbon and nitrogen sources in organic fertilizers were fully absorbed, decomposed and released by soil microorganisms, thereby increasing the contents of multiple nutrients in the soil. Conversely, the single fertilizer application under conventional cultivation resulted in its poor absorption by microorganisms and thereby lacking a continuous supply of nutrients. Total nitrogen content of the soil under organic cultivation in this study was lower than conventional cultivation, which differed from previous studies [20]. There might be two reasons: One was that the inorganic nitrogen directly used by plants only accounted for approximately 1% of total nitrogen. And organic nitrogen could be mineralized into inorganic nitrogen only under the action of microorganisms [21,22], and there was more non-available nitrogen in the soil under conventional cultivation. The other was because the influence of fertilizers on total nitrogen content of the soil was affected by climate, soil quality and farming, while the effect of organic cultivation on the content of total nitrogen had not been observed yet. Actually, compared with total nitrogen, the content of alkali-hydrolyzable nitrogen could reflect the recent supply of nitrogen in the soil and could be used as a scientific basis for fertilization. The present study revealed that the acidity of topsoil (0–10 cm) was stronger than the lower layer (10–20 cm) and the cause might be that the surface layer had a higher

level of microorganisms and nitrogen. On the one hand, the soil tended to be acidified after nitrification by nitrifying bacteria [23]. On the other hand, nitrogen addition reduced the acid buffering capacity by minimizing exchangeable cations and inhibiting effective cation exchange capacity [24].

In terms of enzyme activity, as soil depth increased, the activities of sucrase and urease decreased, which was consistent with the conclusion proposed by Taylor et al. [25]. As the topsoil contained more oxygen and nutrients, and the microbial activity was vigorous, the biochemical reactions were more active. Multiple studies have revealed that, compared with conventional fertilizers, soil amendments (farm manure, compost and green manure) significantly increase the activity of various enzymes such as urease in the soil and improve the biochemical quality of soil [26,27]. This study assumes that the increase in enzyme activity after organic cultivation mainly occurred in the soil layer at 10–20 cm depth, while in the 0–10 cm soil layer, both cultivation methods had no significant effect on sucrase. The research results revealed that there were advantages in the undersoil using the organic cultivation pattern.

4.2. The Impact of Both Cultivation Patterns on the Microbial Diversity of the Topsoil and Undersoil

Soil microbial diversity was closely related to the functions of soil ecosystems [28]. Xun et al. [18] have found that long-term application of organic fertilizers or combined application of organic and inorganic fertilizers can maintain the diversity of soil bacteria, whereas long-term application of inorganic fertilizers significantly reduces the diversity of soil bacteria. Unfortunately, Innerebner et al. [29] had pointed out that neither organic fertilizer nor farming methods can change soil microbial diversity. This study subdivided soil layers according to the heterogeneous characteristics of soil, and explored the difference of soil layers between 0–10 cm and 10–20 cm in depth. Soil microorganisms under organic cultivation presented higher abundance, and as soil depth increased, the number of microorganisms in soil decreased more slowly under organic cultivation. It revealed that sufficient carbon and nitrogen sources were provided under organic cultivation with a more feasible growth environment, which contributed more to microbial reproduction, and there was a better advantage in the undersoil. Organic cultivation also improved the microbial diversity of soil at 10–20 cm depth. However, in the 0–10 cm soil layer, the microbial diversity index under organic cultivation was lower than conventional cultivation. This might be because the excessive application of organic fertilizers added some heavy metals into the soil, which was enriched in the topsoil thereby reducing soil microbial diversity to a certain extent [29]. Wang Xiaoling et al. [30] have reported a similar conclusion on fungi that the application of organic fertilizer under no-tillage mulch measures can increase AM fungi abundance index (Chao1 index and ACE index) in soil, whereas the diversity of AM fungi (Shannon index, Shannon index, The Simpson index) in soil declines. Generally, the effects of soil abundance and diversity in organic cultivation were not limited by a single factor, and they might also correlate with plot management, farming years and to some extent, climate change.

4.3. The Influence of Both Cultivation Patterns on the Composition of Microbial Communities in the Topsoil and Undersoil

The composition of bacterial community in soil acts as a biological evaluation index of soil quality and affects the evolution of biological environmental systems [31]. Previous studies have revealed that *Proteobacteria*, *Acidobacteria* and *Chloroflexi* are the main dominant populations in paddy soil under long-term fertilization [32–34]. The current experiment indicated that the top four dominant bacterial phyla in soil were *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Bacteroidetes*. The abundance of the four previously described bacteria in the topsoil under organic cultivation was lower than conventional cultivation. In the undersoil, only *Proteobacteria* and *Chloroflexi* had higher abundance under organic cultivation. The reason might be the increase in the number of various microorganisms under organic cultivation, and the enhancement of competitive inhibition between microorganisms (e.g., the

apparent competitive advantage of *Thaumarchaeota* in the organic topsoil). Consequently, the relative abundance of several dominant bacterial phyla decreased under organic cultivation. The research of Zhou et al. [35] has revealed that the application of inorganic fertilizer can result in the proliferation of a-Proteus and g-Proteus. During the present experiment, this effect might also occur in the upper layer of soil. Additionally, Wang et al. [36] have revealed that the increase in diversity index in soil links to the reproduction of *Acidobacteria*. Luckily, the present study clarified that the diversity index in soil of paddy fields was related to *Proteobacteria* and *Chloroflexi*.

Fungi in soil can decompose plant residues and it serves as an important indicator for evaluating soil changes under nature or natural interference [37]. In this study, *Ascomycota* and *Basidiomycota* were identified as the main dominant phyla of fungi in the paddy field ecosystem, which was basically in agreement with the results revealed by Choudhary et al. [38]. Based on the research of Ding et al. [39], who have conducted long-term positioning fertilization in the black soil region in Northeast China for 35 years, it has indicated that the application of organic fertilizer can significantly elevate the relative abundance of the dominant fungus *Ascomycota*, whereas its abundance decreases under the treatment of inorganic fertilizer. However, the results of this study were not in agreement with their discovery. The cause might be that the sensitivity of fungi to nutrient conditions in the 9-year test was lower than that of bacteria [40], leading to unapparent advantages of the main bacterial phyla under organic cultivation.

4.4. Interrelationship of Nutrient Content, Enzyme Activity and Main Microbial Phyla in the Soil

Multiple environmental factors can affect the microbial community structure [41]. There has been numerous research on the correlation or redundancy of nutrients and microorganisms at home and abroad. This study did not reveal a strong connection between *Proteobacteria* and contents of each nutrient. *Proteobacteria*, as absolute dominant bacteria in soil at phylum level classification, presented a high degree of stability in the ecosystem of paddy fields, which was not substantially affected by the application of fertilizers. Wang et al. [36] have indicated that *Acidobacteria* may exert a vital role in improving organic matter, available phosphorus, alkali-hydrolyzable nitrogen content in soil as well as soil development. In the present study, *Acidobacteria* was significantly positively correlated with available phosphorus and total nitrogen, which linked to Wang's conclusion to a certain extent. *Acidobacteria* represents anaerobic bacteria, the main nutrients of which originate from plant residues. During the process when *Acidobacteria* degrades plant residues into organic nutrients, the effective nutrient content of the soil increases. Li Ruixia et al. [42] argue that as the reduction proportion of nitrogen fertilizer increases, the abundance of *Ascomycota* in the underground node roots increases significantly, whereas that of *Basidiomycota* decreases greatly. The present study revealed a similar conclusion: alkali-hydrolyzable nitrogen, available potassium, organic matter and total nitrogen were significantly negatively correlated with *Ascomycota*, whereas significantly positively correlated with *Basidiomycota*. This might link to the denitrification of low abundance *Ascomycota* [43], while *Basidiomycota* accelerated the absorption of nutrients due to ectotrophic mycorrhiza formation with plants [44]. The present work demonstrated that alkali-hydrolyzable nitrogen, available potassium and organic matter acted as both the significant influencing factors of the dominant phyla of soil bacteria, and the significant influencing factors of the dominant phyla of soil fungi. The change of soil microbial community structure is a complex process, and the interrelationship between its community characteristics and nutrients needs further investigation.

A significant correlation has been revealed between soil enzyme activity and soil properties including carbon, nitrogen, phosphorus, organic matter contents, pH values and water content in soil. It is also sensitive to changes in environmental factors [45–47]. The correlation analysis of this experiment indicated that the response of sucrase and urease to the dominant bacteria phyla was basically in agreement with the response of soil nutrient factors to the dominant bacteria phyla. The findings demonstrated that soil

enzymes directly reflect the nutrient contents in soil, the soil nutrient contents would affect the number of microorganisms, and consequently, enzymes secreted by microorganisms would also be reflected at the level of soil enzymes. The three described factors were intimately connected, with response and feedback to each.

The physicochemical properties of soils are affected by many factors, such as various kinds of nutrients, bulk density, texture, moisture content and micro-elements. However, the present study conducted only nutrient determination. Some properties of soil are affected by alterations in the soil structures caused by cultivation. Therefore, future studies still need multifaceted determination of soil properties to analyze the community structure of soil microorganisms.

5. Conclusions

Compared with conventional cultivation, organic cultivation could increase nutrient contents (alkali-hydrolyzable nitrogen, available phosphorus, available potassium and organic matter) in soil at 0–10 cm and 10–20 cm depth, and significantly increase the activities of soil enzymes (sucrase and urease) at 10–20 cm depth. Organic cultivation could improve the capacity of fertility retention in soil. The microbial diversity at 10–20 cm depth was higher than conventional cultivation, which improved the soil fertility level to a certain extent. As competitive inhibition of bacteria at 0–10 cm in soil layer under organic cultivation, the relative abundance of the dominant bacteria phyla may be reduced, and the relative abundance of rare bacteria phyla may be increased, while the decline of the dominant fungal phyla under organic cultivation occurs at 10–20 cm soil layer. Furthermore, soil nutrient contents were intimately correlated to the soil microbial community. Alkali-hydrolyzable nitrogen, available potassium and organic matter were significant influencing factors of the dominant phyla of microorganisms in paddy field soil. The present study revealed the microbial community characteristics and change rules in the soil at 0–10 cm and 10–20 cm depth under organic cultivation and conventional cultivation patterns. It also explored the changes of soil microorganisms in paddy fields and provided theoretical reference for the development of organic agriculture. In the future, further in-depth research remains necessary to investigate changes of soil microorganisms under organic cultivation through long-term location experiments.

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References

1. Sugden, A.M. AGRICULTURAL ECOLOGY: Organic Farming. *Science* **2001**, *293*, 17. [CrossRef]
2. Pankhurst, C.E.; Ophel-Keller, K.; Doube, B.M.; Gupta, V.V.S.R. Biodiversity of soil microbial communities in agricultural systems. *Biodivers. Conserv.* **1996**, *5*, 197–209. [CrossRef]
3. Hartmann, M.; Frey, B.; Mayer, J.; Maeder, P.; Widmer, F. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* **2015**, *9*, 1177–1194. [CrossRef]
4. Wang, W.; Wang, H.; Feng, Y.; Wang, L.; Xiao, X.; Xi, Y.; Luo, X.; Sun, R.; Ye, X.; Huang, Y.; et al. Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River. *Sci. Rep.-UK* **2016**, *6*, 35046. [CrossRef] [PubMed]
5. Takahashi, H.; Matsushita, Y.; Ito, T.; Nakai, Y.; Nanzyo, M.; Kobayashi, T.; Iwaishi, S.; Hashimoto, T.; Miyashita, S.; Morikawa, T.; et al. Comparative analysis of microbial diversity and bacterial seedling disease-suppressive activity in organic-farmed and standardized commercial conventional soils for rice nursery cultivation. *J. Phytopathol.* **2018**, *166*, 249–264. [CrossRef]
6. Bickel, S.; Or, D. Soil bacterial diversity mediated by microscale aqueous-phase processes across biomes. *Nat. Commun.* **2020**, *11*, 116. [CrossRef] [PubMed]
7. Xu, X.; Thornton, P.E.; Post, W.M. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecol. Biogeogr.* **2013**, *22*, 737–749. [CrossRef]
8. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [CrossRef]
9. Blume, E.; Bischoff, M.; Reichert, J.M.; Moorman, T.; Konopka, A.; Turco, R.F. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Appl. Soil. Ecol.* **2002**, *20*, 171–181. [CrossRef]
10. Deveau, A.; Bonito, G.; Uehling, J.; Paoletti, M.; Becker, M.; Bindschedler, S.; Hacquard, S.; Herve, V.; Labbe, J.; Lastovetsky, O.A.; et al. Bacterial-fungal interactions: Ecology, mechanisms and challenges. *Fems. Microbiol. Rev.* **2018**, *42*, 335–352. [CrossRef]
11. Gu, Y.; Wang, Y.; Lu, S.; Xiang, Q.; Yu, X.; Zhao, K.; Zou, L.; Chen, Q.; Tu, S.; Zhang, X. Long-term Fertilization Structures Bacterial and Archaeal Communities along Soil Depth Gradient in a Paddy Soil. *Front. Microbiol.* **2017**, *8*, 1516. [CrossRef]
12. Yu, H.; Xue, D.; Wang, Y.; Zheng, W.; Zhang, G.; Wang, Z. Molecular ecological network analysis of the response of soil microbial communities to depth gradients in farmland soils. *Microbiologyopen* **2020**, *9*, e983. [CrossRef]
13. Ling, Q.; Zhang, H.; Ju, Z.; Dai, Q.; Huo, Z. Effects of different application rates of San'an bio-organic fertilizer on Yield, Quality and Nitrogen Absorption and Utilization of Organic Rice. *China Rice* **2010**, *16*, 17–22.
14. Bao, S. *Analysis Method of Soil and Agricultural Chemistry*, 3rd ed.; China Agricultural Press: Beijing, China, 2000.
15. Guan, S. *Soil Enzyme and its Study Method*; China Agriculture Press: Beijing, China, 1986.
16. Li, R.; Tao, R.; Ling, N.; Chu, G. Chemical, organic and bio-fertilizer management practices effect on soil physicochemical property and antagonistic bacteria abundance of a cotton field: Implications for soil biological quality. *Soil Till. Res.* **2017**, *167*, 30–38. [CrossRef]
17. Peng, X.; Wang, W. Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. *Soil Biol. Biochem.* **2016**, *98*, 74–84. [CrossRef]
18. Xun, W.; Zhao, J.; Xue, C.; Zhang, G.; Ran, W.; Wang, B.; Shen, Q.; Zhang, R. Significant alteration of soil bacterial communities and organic carbon decomposition by different long-term fertilization management conditions of extremely low-productivity arable soil in South China. *Environ. Microbiol.* **2016**, *18*, 1907–1917. [CrossRef]
19. Bending, G.D.; Turner, M.K.; Rayns, F.; Marx, M.C.; Wood, M. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biol. Biochem.* **2004**, *36*, 1785–1792. [CrossRef]
20. Li, F.; Chen, L.; Zhang, J.; Yin, J.; Huang, S. Bacterial Community Structure after Long-term Organic and Inorganic Fertilization Reveals Important Associations between Soil Nutrients and Specific Taxa Involved in Nutrient Transformations. *Front. Microbiol.* **2017**, *8*, 187. [CrossRef] [PubMed]
21. Das, A.K.; Boral, L.; Tripathi, R.S.; Pandey, H.N. Nitrogen mineralization and microbial biomass-N in a subtropical humid forest of Meghalaya, India. *Soil Biol. Biochem.* **1997**, *29*, 1609–1612. [CrossRef]
22. Bai, J.; Deng, W.; Wang, Q.; Cui, B.; Ding, Q. Spatial distribution of inorganic nitrogen contents of marsh soils in a river floodplain with different flood frequencies from soil-defrozen period. *Environ. Monit. Assess.* **2007**, *134*, 421–428. [CrossRef]
23. Gao, W.; Yang, H.; Kou, L.; Li, S. Effects of nitrogen deposition and fertilization on N transformations in forest soils: A review (vol 15, pg 863, 2015). *J. Soil Sediment.* **2015**, *15*, 1233–1234. [CrossRef]
24. Cai, J.; Luo, W.; Liu, H.; Feng, X.; Zhang, Y.; Wang, R.; Xu, Z.; Zhang, Y.; Jiang, Y. Precipitation-mediated responses of soil acid buffering capacity to long-term nitrogen addition in a semi-arid grassland. *Atmos. Environ.* **2017**, *170*, 312–318. [CrossRef]
25. Taylor, J.P.; Wilson, B.; Mills, M.S.; Burns, R.G. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil. Biol Biochem.* **2002**, *34*, 387–401. [CrossRef]
26. Lazcano, C.; Gomez-Brandon, M.; Revilla, P.; Dominguez, J. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biol. Fert. Soils* **2013**, *49*, 723–733. [CrossRef]
27. Liang, Q.; Chen, H.; Gong, Y.; Yang, H.; Fan, M.; Kuzyakov, Y. Effects of 15 years of manure and mineral fertilizers on enzyme activities in particle-size fractions in a North China Plain soil. *Eur. J. Soil Biol.* **2014**, *60*, 112–119. [CrossRef]
28. Kennedy, A.C.; Smith, K.L. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* **1995**, *170*, 78–86. [CrossRef]

29. Innerebner, G.; Knapp, B.; Vasara, T.; Romantschuk, M.; Insam, H. Traceability of ammonia-oxidizing bacteria in compost-treated soils. *Soil Biol. Biochem.* **2006**, *38*, 1092–1100. [CrossRef]
30. Wang, X.; Ma, K.; Wang, Z.; Li, Y.; Wei, C. Effects of no-tillage, mulching and organic fertiliation on soil microbial composition in winter wheat field. *Chin. J. Eco-Agric.* **2019**, *27*, 267–276. [CrossRef]
31. Delgado-Baquerizo, M.; Oliverio, A.M.; Brewer, T.E.; Benavent-Gonzalez, A.; Eldridge, D.J.; Bardgett, R.D.; Maestre, F.T.; Singh, B.K.; Fierer, N. A global atlas of the dominant bacteria found in soil. *Science* **2018**, *359*, 320. [CrossRef]
32. Li, X.; Wang, H.; Li, X.; Li, X.; Zhang, H. Shifts in bacterial community composition increase with depth in three soil types from paddy fields in China. *Pedobiologia* **2019**, *77*, 77. [CrossRef]
33. Osman, J.R.; Fernandes, G.; DuBow, M.S. Bacterial diversity of the rhizosphere and nearby surface soil of rice (Oryza sativa) growing in the Camargue (France). *RHIZOSPHERE* **2017**, *3*, 112–122. [CrossRef]
34. Chen, D.; Yuan, L.; Liu, Y.; Ji, J.; Hou, H. Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties. *Eur. J. Agron.* **2017**, *90*, 34–42. [CrossRef]
35. Zhou, J.; Jiang, X.; Zhou, B.; Zhao, B.; Ma, M.; Guan, D.; Li, J.; Chen, S.; Cao, F.; Shen, D.; et al. Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biol. Biochem.* **2016**, *95*, 135–143. [CrossRef]
36. Wang, H.; Wang, S.; Wang, R.; Wang, X.; Li, J. Conservation tillage increased soil bacterial diversity and improved soil nutrient status on the Loess Plateau in China. *Arch. Agron. Soil Sci.* **2020**, *66*, 1509–1519. [CrossRef]
37. Tedersoo, L.; Bahram, M.; Polme, S.; Koljalg, U.; Yorou, N.S.; Wijesundera, R.; Villarreal Ruiz, L.; Vasco-Palacios, A.M.; Pham, Q.T.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1078. [CrossRef]
38. Choudhary, M.; Sharma, P.C.; Jat, H.S.; McDonald, A.; Jat, M.L.; Choudhary, S.; Garg, N. Soil biological properties and fungal diversity under conservation agriculture in Indo-Gangetic Plains of India. *J. Soil Sci. Plant Nutr.* **2018**, *18*, 1142–1156. [CrossRef]
39. Ding, J.; Jiang, X.; Guan, D.; Zhao, B.; Ma, M.; Zhou, B.; Cao, F.; Yang, X.; Li, L.; Li, J. Influence of inorganic fertilizer and organic manure application on fungal communities in a long-term field experiment of Chinese Mollisols. *Appl. Soil Ecol.* **2017**, *111*, 114–122. [CrossRef]
40. Wang, J.; Song, Y.; Ma, T.; Raza, W.; Li, J.; Howland, J.G.; Huang, Q.; Shen, Q. Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. *Appl. Soil Ecol.* **2017**, *112*, 42–50. [CrossRef]
41. Jiang, Y.; Liang, Y.; Li, C.; Wang, F.; Sui, Y.; Suvannang, N.; Zhou, J.; Sun, B. Crop rotations alter bacterial and fungal diversity in paddy soils across East Asia. *Soil Biol. Biochem.* **2016**, *95*, 250–261. [CrossRef]
42. Li, R.; Huo, Y.; Li, H.; Wang, W.; Zhang, A.; Yang, Z. Effect of nitrogen fertilizer reduction on endophytic fungal community composition of summer maize in north China. *Trans. Chin. Soc. Agric. Mach.* **2018**, *49*, 312–318.
43. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an ecological classification of soil bacteria. *Ecology* **2007**, *88*, 1354–1364. [CrossRef]
44. Magill, A.H.; Aber, J.D. Variation in soil net mineralization rates with dissolved organic carbon additions. *Soil Biol. Biochem.* **2000**, *32*, 597–601. [CrossRef]
45. Zantua, M.I.; Dumenil, L.C.; Bremner, J.M. Relationships between soil urease activity and other soil properties. *Soil Sci. Soc. Am. J.* **1977**, *41*, 350–352. [CrossRef]
46. Steinweg, J.M.; Dukes, J.S.; Paul, E.A.; Wallenstein, M.D. Microbial responses to multi-factor climate change: Effects on soil enzymes. *Front Microbiol.* **2013**, *4*, 146. [CrossRef] [PubMed]
47. Nahidan, S.; Nourbakhsh, F.; Mosaddeghi, M.R. Variation of soil microbial biomass C and hydrolytic enzyme activities in a rangeland ecosystem: Are slope aspect and position effective? *Arch. Agron. Soil Sci.* **2015**, *61*, 797–811. [CrossRef]