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Effect of Soybean and Maize Rotation on Soil Microbial Community Structure

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Abstract: Examining the soil microbiome structure has a great significance in exploring the mechanism behind plant growth changes due to maize (*Zea mays L.*) and soybean (*Glycine max Merr.*) crop rotation. This study explored the effects of soil microbial community structure after soybean and maize crop rotation by designing nine treatments combining three crop rotations (continuous cropping maize or soybean; and maize after soybean) with three fertility treatments (organic compound fertilizer, chemical fertilizer, or without fertilizer). Soil was sampled to 30 cm depth the second year at approximately the middle of the growing season, and was analyzed for physical, chemical, and phospholipid fatty acid (PLFA) profiles. Bacteria was found to be the predominant component of soil microorganisms, which mainly contain the PLFAs i15:0, 16:1 ω 7c, 16:0, 10Me16:0, and 18:1 ω 7c. The concentration of soil gram-negative bacteria from the soybean and maize rotation was less than in soybean continuous cropping when organic fertilizer was applied to both. Crop rotation reduced the percentage of fungi in the soil, among which the effect of organic compound fertilizer application was significantly reduced 24%. The combined crop rotation with organic fertilizer can reduce maximum the percentage of fungi/bacteria. In addition, the content of soil aggregate and organic matter had great influence on gram-positive bacteria and actinomyces, and soil pH had a greater impact on other fungi.

Keywords: Crop rotation; Fertilization; Maize; Microbial community structure

1. Introduction

Soil is one of the major components of the environment and it is necessary for field crops' survival, so the quality of soil is one of the important factors affecting crop growth. Maize is the world's largest cash crop with high economic value. Driven by this economic value, the continuous planting of maize leads to the lack of soil nutrient uniformity and intensifies the occurrence and transmission of soil diseases in the same plot. In order to relieve the land pressure, knowledge of the effects of crop rotation patterns of maize and other crops on soil properties is important. Owing to the long-term economic and ecological benefits of Leguminosae crops and Gramineae crops, crop rotation patterns between the two plant families has long been considered the optimal system for maintaining the soil nutrient cycle. A great deal of research has been done on inserting exotic Leguminosae into various crop rotation patterns. For example, the three-course cropping of ancient Greece and Rome, the rotation model of Norfolk in Britain, and the six years rotation model mentioned in 1794 were all examples of the rotation of legumes and non-legumes in the United States; the British Norfolk 4-year rotation model in 1730 and the America 6-year rotation model in 1794 were both examples of legume and non-legume crop rotation [1]. However, there were few studies on the

ecological effects of crop rotation, most of which focus on crop yield and the effect on soil physical and chemical properties. Furthermore, detection and analysis of the change of soil microbial community structure before and after crop rotation was not common.

Microorganisms are an important component of the material cycle and energy transformation in a soil ecosystem. Due to the effect of fertilization, soil microorganisms not only affect the physical and chemical properties of soil, but also affect the effectiveness of fertilizers on plants [2]. Fertilization mainly affects soil microorganisms by changing soil physical and chemical properties and nutrient contents. On the other hand, fertilization can also affect the population and yield of above-ground vegetation, thus affecting soil microorganisms through the input of roots and plant residue [3]. Rhizosphere is the microenvironment in which plants come into contact with soil. The soil microbial community composition is an important limiting factor of soil processes, and the composition and activity of microbial community largely determine biogeochemical cycles, metabolic processes of soil organic matter, soil fertility and quality [4,5]. In addition, soil microorganisms are closely related to the stability and health of the soil ecosystem. Soil microorganisms are more sensitive to changes of external conditions, such as land use change, management measures and cultivation than other soil physical and chemical indexes. Therefore, soil microbial biomass, community composition and diversity are often used as indicators of soil quality changes [6, 7].

Phospholipid fatty acid (PLFA) spectrogram technology was used to analyze biological community structures in the 1980s [8, 9]. This method of analysis relies on fatty acid spectrograms to quantify the entire microbial community without the need for soil enrichment or cultivation, and therefore is quicker and more reliable than traditional approaches [10]. Although this method cannot identify the specific microbial species at the strain level, PLFA doesn't depend on the influence of the plant culture system, but can directly provide information and quantitatively describe the whole microbial community. This method also has the advantages of objective and reliable test results, simple operation of test conditions and multiple test functions and has been used widely in the field of cycle microbiology. In order to clarify the effects of crop rotation and fertilization on soil microbial community structure, PLFA was used to analyze the microbial community composition of soil samples.

2. Materials and Methods

2.1 Study survey and design

The experiments were conducted at Chifeng Academy of Agricultural and Animal Husbandry, Inner Mongolia Autonomous Region, northeastern China (42°15'N, 118°72') in 2016 and 2017. The climate type of the study area belongs to temperate semi-arid continental climate, with an average annual temperature of 6.5 °C and an average annual precipitation of 380 mm, the precipitation mainly concentrated in July and August. Meanwhile, sunshine time was more than 2800 to 3200 hours. The main crops grown in the area are corn, buckwheat and millet.

Randomized block design was used in this experiment with 3 replications. There were nine cropping designs for the study including (1) Continuous cropping of maize with organic compound fertilizer (M+M), (2) Continuous cropping of maize with chemical fertilizer (M+NP), (3) Continuous cropping maize without fertilizer (M+0), (4) Maize after soybean with organic compound fertilizer (MS+M), (5) Maize after soybean with inorganic fertilizer (MS+NP), (6) Maize after soybean with no

fertilizer (MS+0), (7) Soybean continuous cropping with organic compound fertilizer (S+M), (8) continuous cropping soybean with inorganic fertilizer (S+NP), and (9) continuous cropping soybean without fertilizer (S+0). The annual planting date was May 18th, and the amount of fertilizer applied per year was consistent. According to the local recommendation, 300 kg/hm² or 150 kg/hm² of chemical (NP) fertilizer diammonium phosphate and 900kg/hm² of organic compound fertilizer were applied for maize and soybean, respectively when sowing. The rotation area was planted soybeans in 2016 and maize in 2017. The annual planting date was May 18th. The field management measures are similar to local management measures.

Table 1. Sowing rate of soybean and maize rotation

plants	Varieties	Sowing rate	Plant spacing	Row spacing
		plant /hm ²	cm	cm
maize	Fengdan189	67500	33	45
soybean	Red bean 3	210000	12	40

2.2 Samples collection

The soil samples of the study area were obtained during the vigorous growth of crops on August 8, 2017. In each plot, three points 5cm from the root were selected from the rhizosphere of maize and soybean randomly. Soil samples were mixed into two samples, which were picked up at the soil depth of 0-30 cm. One sample was dried to analyze the soil physical structure and chemical properties, the other sample was stored in a freezer at - 20 °C for the determination of soil microbial community structure.

2.3 Soil physical and chemical properties

The content of soil macroaggregates was measured by mechanical sieving method. Soil pH in water was measured at a soil/water ratio of 2: 5 (w: v) after 10 min and 2 h in suspension for water. Soil available nitrogen (AN) was determined using the alkaline diffusion method; soil available phosphorus and soil organic matter (SOM) was measured by NaHCO₃ leaching molybdenum-antimony anti-absorption spectrophotometry, and potassium dichromate volumetric method, respectively [11-13].

2.4 Determination of soil microbial community structure

Soil microorganisms PLFAs were extracted by Bligh-Dyer modified method and esterified C19:0 was used as internal standard [14]. The processes of extraction, purification and analysis briefly consisted of measuring 2 g freeze-dried soil, then 20 ml chloroform-methanol-citric acid buffer (1:2:0.8, v/v/v) was added to extract total PLFAs of samples. The extracted PLFAs were subsequently separated by silica gel column (SPE-SI), and consisted of neutral fatty acids, sugar fatty acids, and phosphatidic acid. Phospholipid acid is dissolved in methanol/toluene (1:1, v/v) solution, then added 0.2 mol/L KOH, esterified at 37 °C for 15 minutes, then separated by GC-MS (Gas Chromatograph-Mass Spectrometry) analyzer, and then separated by Bacterial Fatty Acid standards and commercial

MIDI system (Microbial Identification System) to identify and quantify phospholipid fatty acids. Soil microbial phospholipid fatty acid profiles were obtained by analyzing the corresponding microbial communities, and the structural diversity of soil microbial communities could be judged by statistical analysis [15]. Phospholipid fatty acids are based on Frostegard et al. [16]: (i/a/cy/br/10Me) X:Y ω Z (OH/cis/t), where X represents the total number of C atoms of fatty acid molecule, Y indicates the number of unsaturated olefin bonds, ω represents the position of the olefinic bond from the carboxyl group, Z represents the position of the olefin bond or cyclopropane chain. Prefixes "i" (iso) represents the isomeric methyl branched chain (the third carbon atom from the methyl end), "a" (anteiso) represents the pre-isomeric methyl branched chain (the third carbon atom from the methyl end), "cy" represents the cyclopropyl group, and "br" represents the unknown position of the methyl chain.

The suffixes "cis" and "trans" represent cis and trans isomers, respectively, and the number before "OH" denotes the position of hydroxyl groups (counted from the carboxyl end, the second carbon is alpha, and the third carbon is beta). Characterization of microbial PLFA is shown in Table 2 [17-23]:

Table 2. PLFA characterization of microorganisms.

Microbial type	Phospholipid fatty acid labelled
Bacteria in general (B)	i14:0, i15:1, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1 ω 7cis, 16:1 ω 9cis, 17:1 ω 7cis, 17:1 ω 8cis, 18:1 ω 7cis, 18:1 ω 5cis, cy17:0, cy19:0, 16:12 OH, 16:0, 18:0
Gram-positive bacteria (G+)	i14:0, i15:1, i15:0, a15:0, i16:0, i17:0, a17:0
Gram-negative bacteria (G-)	16:1 ω 7cis, 16:1 ω 9cis, 17:1 ω 7cis, 17:1 ω 8cis, 1:1 ω 7cis, 18:1 ω 7cis, 18:1 ω 5cis, cy17:0, cy19:0, 16:12 OH
Actinomycetes(A)	10Me16:0, 10Me17:0, 10Me18:0
Fungi (F)	16:1 ω 5cis, 18:1 ω 9cis, 18:2 ω 6cis, 18:2 ω 9cis, 18:3 ω 6cis

2.5 Data analysis

The data in this paper were analyzed by variance analysis, principal component analysis (PCA) and nonlinear dimensionality reduction analysis (RDA) in Excel, SPSS 25.0, SAS 9.0 and Canoco 4.5. To reduce the error of the study data, when a sample percentage of fatty acids was less than 1%, the sample was reanalyzed repeatedly.

3. Results

3.1 Effects of each treatment on the composition and content of phospholipid fatty acids

From Table 3, we can see that 20 kinds of phospholipid fatty acids were mainly detected in this study, in which i15:0(Gram-positive bacteria), 16:1 ω 7c(Gram-negative bacteria), 16:0 (Actinomycetes), 10Me16:0(Actinomycetes), 18:1 ω 7c (Gram-negative bacteria), were the primary ones, accounting for 7.2%-8.2%, 6.6%-7.2%, 11.6-12.7%, 8.4-9.0%, 7.2-8.7% of the total phospholipid fatty acid content, respectively. These five kinds of phospholipid fatty acids accounted for 41-45% of the total phospholipid fatty acids.

Table 3. Effects of each treatment on the percentage of microbial phospholipid fatty acids in soil (%). Values are the average ± 1 standard error.

	Treatments								
	M+M	M+NP	M+0	MS+M	MS+NP	MS+0	S+M	S+NP	S+0
i15:0	†8.2±0.4a	†7.5±0.4b	†7.7±0.1ab	†8.1±0.5a	†7.9±0.4ab	†8.2±0.2a	†7.9±0.2ab	†8.2±0.1a	†7.7±0.4ab
a15:0	4.4±0.2cd	4.4±0.1d	4.4±0.0bcd	4.7±0.1abc	4.6±0.3abcd	4.7±0.3ab	4.6±0.2abcd	4.8±0.1a	4.6±0.1abcd
15:0	1.4±0.0ab	1.5±0.1ab	1.4±0.1ab	1.4±0.0ab	1.5±0.2ab	1.3±0.0ab	1.3±0.1b	1.4±0.2ab	1.5±0.2a
i16:0	3.9±0.3a	3.6±0.1b	3.7±0.0ab	3.8±0.1ab	3.9±0.1a	3.8±0.1ab	3.6±0.1b	3.8±0.1ab	3.8±0.1ab
16:1 ω 9c	1.2±0.1a	1.1±0.1a	1.1±0.1a	1.2±0.1a	1.1±0.1a	1.2±0.0a	1.1±0.0a	1.2±0.0a	1.2±0.1a
16:1 ω 7c	7.1±0.2a	7.2±0.2a	6.6±0.4b	6.8±0.3ab	6.8±0.3ab	6.8±0.3ab	7.0±0.4ab	7.0±0.1ab	7.0±0.1ab
16:1 ω 5c	3.9±0.1a	3.9±0.1a	3.9±0.2a	3.9±0.1a	3.7±0.1a	3.7±0.2a	3.2±0.2b	3.1±0.2b	3.1±0.1b
16:0	11.7±1.0b	11.6±0.3b	11.8±0.5b	11.6±0.4b	11.7±0.2b	12.0±0.4ab	12.7±0.6a	12.7±0.1a	12.1±0.4ab
10Me16:0	8.9±0.4a	8.5±0.9a	8.7±0.2a	8.7±0.3a	8.5±0.6a	8.9±0.3a	8.5±0.5a	9.0±0.3a	8.5±0.6a
i17:0	2.2±0.2ab	2.1±0.2b	2.2±0.1ab	2.2±0.1ab	2.2±0.0ab	2.3±0.1a	2.1±0.1ab	2.2±0.1ab	2.2±0.1ab
a17:0	2.1±0.2a	2.0±0.1a	2.1±0.1a	2.1±0.1a	2.1±0.0a	2.2±0.1a	2.0±0.1a	2.1±0.0a	2.1±0.1a
17:1 ω 8c	1.5±0.1ab	1.6±0.2ab	1.6±0.1ab	1.5±0.1ab	1.5±0.1ab	1.4±0.1ab	1.5±0.2ab	1.5±0.2b	1.7±0.2a
cy17:0 ω 7c	2.9±0.2a	2.8±0.1ab	2.7±0.0b	2.7±0.1ab	2.8±0.1ab	2.8±0.1ab	2.7±0.1b	2.6±0.1b	2.7±0.1b
17:1 ω 7c	2.1±0.3ab	2.0±0.3ab	2.2±0.4a	1.9±0.2ab	1.9±0.1ab	1.9±0.1ab	1.8±0.1b	2.0±0.1ab	1.8±0.2b
18:2 ω 6c	2.9±0.5a	2.9±1.1a	2.4±0.4a	2.1±0.1a	2.4±0.2a	2.1±0.3a	2.8±0.6a	2.1±0.2a	2.3±0.4a
18:1 ω 9c	6.3±0.6a	5.9±0.3ab	5.9±0.3ab	5.9±0.2ab	5.8±0.4ab	5.7±0.3b	5.7±0.1b	5.8±0.2ab	5.8±0.2ab
18:1 ω 7c	8.3±0.8a	8.0±0.4a	7.4±0.4a	7.3±0.3a	7.4±0.3a	7.2±0.6a	8.7±1.8a	7.4±0.7a	8.0±1.4a
18:0	2.1±0.2c	2.1±0.1c	2.2±0.2bc	2.3±0.2abc	2.3±0.1abc	2.2±0.2bc	2.5±0.1a	2.5±0.1a	2.5±0.1ab
10Me18:0	1.9±0.2bc	1.8±0.1c	1.9±0.1bc	2.2±0.2a	1.9±0.1bc	2.1±0.4ab	1.8±0.1bc	1.8±0.1c	1.8±0.1bc
cy19:0 ω 7c	3.0±0.4a	2.7±0.1ab	2.6±0.2b	2.7±0.0ab	2.7±0.1ab	2.8±0.1ab	2.6±0.2b	2.7±0.1ab	2.7±0.1ab

† Means within a column and growth stage followed by the same letter are not significantly different at $P \leq 0.05$, and the different letter are significantly different at $P \leq 0.05$.

For i15:0, one of the PLFAs displaying a higher percentage in the soil, the treatment of S+NP significantly increased this compound over the levels from the M+NP treatment, and for 16:1 ω 7c, treatments of M+M and M+NP significantly increased this PLFA over those found in the M+0 treatment. Maize continuous cropping (M) significantly decreased the percentage of a15:0 (gram-positive bacteria), and fertilizer (NP) treatment decreased a15:0 the most, in contrast to soybean continuous cropping (S), that increased the percentage of cy17:0 ω 7c. For maize continuous cropping, the percentage of 16:0 was significantly increased by applying organic compound fertilizer compared to the inorganic fertilizer treatment. Soybean and corn crop rotation play a certain role in maintaining the stability of soil microbial community.

Table 4 shows that bacteria were the main component of soil microorganisms, which accounts for 69% to 71% of the total amount of soil microorganisms. While fungi only accounts for about 3% of the total amount of microorganisms, other components include actinomycetes and some protozoa. For maize continuous cropping (M) and soybean maize rotation (MS), there was no obviously difference in application of fertilizer that affected the content of gram-positive bacteria. When grown with inorganic fertilizer, soybean maize rotation increased the content of gram-positive bacteria compared to fields grown with continuous maize (M), but soybean continuous cropping had the

highest percentage. The application of organic compound fertilizer on the gram-negative bacterial content in soybean continuous cropping had an increasing effect. The organic compound fertilizer (M) applied in soybean continuous cropping significantly increased the percentage of gram-negative bacteria compared to the inorganic fertilizer. Additionally, either fertilization method in maize continuous cropping increased the gram-negative bacteria over unfertilized soil. Rotation significantly reduced the percentage of fungi in the soil with the application of organic fertilizer, as well as the fungi/bacteria ratio.

Table 4. Influence of each treatment on soil microbial community structure (%). Values are the average \pm 1 standard error.

treatment	Gram-positive bacteria	Gram-negative bacteria	Fungi	Actinomycetes	Other Bacteria	Fungus/Bacteria
	G+	G-				
M+M	†37.3 \pm 0.5bc	†33.2 \pm 0.3abc	†3.4 \pm 0.3a	†17.5 \pm 0.8a	†8.3 \pm 1.3ab	†0.049a
M+NP	36.2 \pm 0.6c	33.6 \pm 0.4ab	3.4 \pm 0.5a	17.0 \pm 1.5a	9.7 \pm 0.4a	0.050a
M+0	37.0 \pm 0.4bc	32.1 \pm 0.6c	3.0 \pm 0.5ab	18.1 \pm 0.3a	9.9 \pm 0.5a	0.043abc
MS+M	38.1 \pm 0.8ab	32.4 \pm 0.8bc	2.6 \pm 0.1b	17.7 \pm 0.5a	9.2 \pm 0.6ab	0.036c
MS+NP	37.5 \pm 0.8ab	32.7 \pm 0.5bc	2.9 \pm 0.3ab	17.1 \pm 0.6a	9.7 \pm 1.0a	0.041abc
MS+0	38.0 \pm 0.9ab	32.3 \pm 0.8bc	2.6 \pm 0.3b	18.1 \pm 0.8a	9.0 \pm 0.6ab	0.037c
S+M	37.0 \pm 0.7bc	34.4 \pm 0.3a	3.4 \pm 0.8a	16.9 \pm 0.8a	8.3 \pm 0.6ab	0.048ab
S+NP	38.5 \pm 0.7a	32.7 \pm 1.6bc	2.6 \pm 0.2b	18.1 \pm 0.5a	8.1 \pm 0.4b	0.037c
S+0	37.8 \pm 0.7ab	33.6 \pm 1.5ab	2.8 \pm 0.5ab	17.3 \pm 1.0a	8.5 \pm 0.4ab	0.039bc

† Means within a column and growth stage followed by the same letter are not significantly different at $P \leq 0.05$, and the different letter are significantly different at $P \leq 0.05$.

3.2 Principal component analysis of microbial fatty acids in soil by each treatment

The results of principal component analysis showed that the first principal component could explain 34.76% of variation and the second principal component could explain 28.98% of variation (Fig. 1). The first principal component and the main phospholipid fatty acids were i17:0, a17:0, and i15:0, which all belong to gram-positive bacteria. The lowest scores of the first main component were 18:2 ω 6c, 17:1 ω 8c and 18:1 ω 7c. The treatments along the direction of the second principal component significantly affected the percentage content of 18:1 ω 9c, cy17:0 ω 7c, and 16:1 ω 5c, mainly affecting the percentage content of fungi. The second principal component has the least influence on 18:0, 16:0 and a15:0.

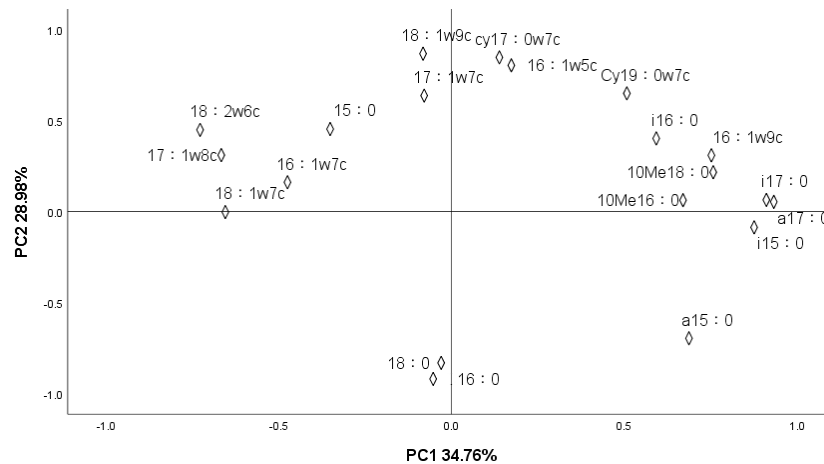


Fig. 2. Principal component analysis of microbial fatty acids in soil. This figure is a principal component analysis based on the phospholipid fatty acid structure nomenclature.

3.3 Relationship between soil microbial community structure and environmental factors

In the Fig. 3, the results of the RDA sorting analysis between soil microorganisms and soil environment shows that the first two sorting axes explained 89.74% of soil microbial information and 45.75% of soil microbes and environment (Fig. 3). The Monte Carlo test results show that the environmental variables corresponding to all sorting axes contributed to the interpretation of the response variables ($P < 0.05$). The content of soil agglomerates (SA) was negatively correlated with the order axis 1, and the soil pH was positively correlated with the sequence axis 2. Among them, soil available phosphorus (OP), soil macroaggregate content (SA) and the two axes have a large correlation coefficient.

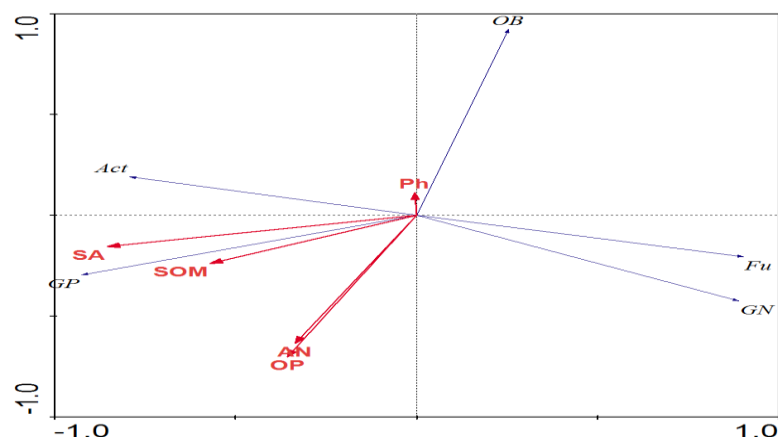


Fig. 3. Effects of environmental factors on soil microbial community structure. Gram-positive bacteria (GP), Gram-negative bacteria (GN), Fungi (Fu), Actinomycetes (Act), Other Bacteria (OB). Soil macroaggregate content (SA), Available phosphorus (OP), Soil available nitrogen (AN), Soil organic matter (SOM), pH (Ph).

The correlation of PLFA among the microorganisms of gram-positive bacteria and actinomycetes was greater, meanwhile, gram-negative bacteria and fungi were more related. Among the soil environmental factors, the contents of soil macroaggregates and organic matter (SOM) were significantly affected by gram-positive bacteria and actinomycetes. Soil pH has great influence on other fungi. Our results show that the surrounding habitat of microbial communities was changed by providing available carbon and nitrogen and suitable soil pH.

4. Discussion

Soil microbes are greatly affected by cultivation and management, which include the measures of tillage and fertilization. Although the studies of natural ecosystems have generally presented that increased nitrogen (N) fertilization reduces microbial biomass, but microorganisms in soils often benefit from mineral fertilizer supplementation under dryland crops. Due to the application of mineral fertilizers, increases in microbial biomass have been found more significantly in rice-growing systems than in dry-land systems. That experiment showed that fertilization could not always select specific microbial populations (e.g. Gram-positive or Gram-negative bacteria, fungi, actinomycetes) in rice systems. However, it affects the composition of microbial community by changing the soil properties [24]. In this experiment, the amount of Gram-positive bacteria in soil increased by the continuous cropping soybean with inorganic fertilizer (S+NP).

The land use pattern of farmland has a significant effect on microbial activity and community structure. Studies have shown that the proportion of bacterial PLFA and fungal PLFA follows the order of Paddy-Upland rotation (PU) with the most, followed by upland land (US) soils and paddy fields (PS; however, the proportion of Gram-positive bacteria PLFA ((G+) PLFA) and Gram-negative bacteria PLFA ((G-) PLFA) in PU is the highest [25]. Complex crop rotation can improve soil quality and crop productivity including perennial plants [26]. Bacteria account for a large proportion of soil microbial community, and continuous cropping can increase the content of fungi in soil, which leads to the intensification of soil-borne diseases. Others' study found that crop rotation could effectively reduce soil fungi content, and previous studies present that increasing mulch crops could increase the bacterial content in PLFA especially the content of Gram-positive bacteria such as oat/radish/vetch [27]. Soybean maize rotation with organic compound fertilizer reduced Gram-negative bacteria compared to soybean continuous cropping, and increased Gram-negative bacteria with inorganic fertilizer (NP).

The total abundance of PLFAs, fungal biomass, bacterial biomass, fungal biomass/bacterial biomass (F/B), monounsaturated fatty acid/saturated fatty acid (MUFA/STFA) and microbial stress (PLFA) were measured by phospholipid fatty acid method. The ratio of MUFA to STFA reflects the soil ventilation, and the higher ratio of MUFA to STFA, the better the soil ventilation conditions. In addition, the ratio of cy19:0 to 18:1 ω 7c is considered to be an indicator of physiological or nutritional stress in microbial communities [28]. Some studies suggest that the ratio of (cy17:0 + cy19:0) to (16:1 ω 7c + 18:1 ω 7c) is related to water stress in the environment [29-30]. This experiment did not study water status; further study would be needed to determine physiological or other environmental effects.

The relationship between environmental factors and soil microbial community structure is rather complicated. In recent years, the newly developed soil aggregate quality fractal dimension

(Dm) [31-32] and S index [33] can also better reflect the quality of soil structure. The interaction between environment and soil microbial community are of great significance to agricultural production and sustainable use of soil.

5. Conclusions

In this experiment, bacteria are the main components of soil microbial community, accounting for 69 to 71% of the total soil microbial community. Among them i15: 0 (Gram-positive bacteria), 16: 1 ω 7c (Gram-negative bacteria), 16:0 (Bacteria), 10 Me16:0 (Actinomycetes) and 18: 1 ω 7c (Gram-negative bacteria) accounted for 41% -45% of total phospholipid fatty acids. For gram-positive bacteria, there was significant difference between application of organic compound fertilizer and inorganic fertilizer treatments in soybean continuous cropping. Besides, soybean continuous cropping applied inorganic fertilizer was greater than maize continuous cropping which also applied inorganic fertilizer. For gram-negative bacteria, continuous maize with the application of inorganic fertilizer treatment had a significant increase than no fertilizer treatment. But in soybean continuous cropping, applied organic compound fertilizer was greater than inorganic fertilizer. The greater influence on the percentage of gram-positive bacteria in the soil of the test site was i15:0, meanwhile, the most significant effect on fungal contents were 18:1 ω 9c, cy17: 0 ω 7c, 16: 1 ω 5c.

The correlation between gram-positive bacteria and actinomycetes was greater than that between gram-negative bacteria and fungi. Among the soil environmental factors, the content of soil macroaggregates and organic matter had a great effect on gram-positive bacteria and actinomycetes. Soil pH has a great effect on other fungi. The results showed that the physical and chemical properties of soil could be changed by crop rotation, such as improving soil structure, increasing soil carbon and nitrogen content, adjusting soil pH and so on, thus affecting the structure and function of soil microbial community. The application of organic compound fertilizer in combination with crop rotation is helpful to the maintenance of soil ecological environment and to the sustainable utilization of cultivated land.

Author Contributions: Peng Zhang, Ji-ying Sun and Li-jun Li conceived and designed the experiments; Xin-xin Wang, Xiao-ting Li and Jia-hui Qu performed the experiments; Yi Bu performed the statistical analysis; Peng-zhang and Li-jun Li wrote the paper.

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References

1. Olsen, R. J.; Hensler, R. F.; Attoe, O. J.; Witzel, S. A.; Peterso, L. A. Fertilizer nitrogen and crop rotation in relation to movement of nitrate nitrogen through soil profiles. *Soil Science Society of America Journal* 1970, 34, 448-452. DOI:10.2136/sssaj1970.036159950034
2. Jenkinson, D. S.; Ladd, J. N. Microbial Biomass in Soil: Measurement and Turnover. *Soil Biochemistry*: 1981, 5, 415-471.
3. Wu Qifeng; Lu Kouping; Mao Xiali; Qin Hua; Wang Hailong. Responses of Soil Nutrients and Microbial Biomass and Community Composition to Long-term Fertilization in Cultivated Land. *Chinese Agricultural Science Bulletin*. 2015, 31 (05), 150-156. ISSN: 1000-6850.

4. Cavigelli, M. A.; Robertson, G. P. The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*. 2000, 81 (5), 1402-1414. DOI:10.1890/0012-9658(2000)081[1402:tfsoedc]2.0.co;2
5. Zelles, L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology & Fertility of Soils*. 1999, 29 (2), 111-129. DOI:10.1007/s003740050533
6. Miller, M.; Dick, R. P., Dynamics of soil C and microbial biomass in whole soil and aggregates in two cropping systems. *Applied soil ecology*. 1995, 1995 v.2 no.4 (no. 4), pp. 253-261. DOI:10.1016/0929-1393(95)00060-6
7. Bucher, A. E.; Lanyon, L. E. Evaluating soil management with microbial community-level physiological profiles. *Applied Soil Ecology*. 2005, 29 (1), 59-71. DOI:10.1016/j.apsoil.2004.09.007
8. White, D. C.; Davis, W. M.; Nickels, J. S.; King, J. D.; Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*. 1979, 40 (1), 51-62. DOI: 10.1007/BF00388810
9. Tunlid, A.; H. Baird, B.; B. Trexler, M.; Olsson, S.; Findlay, R.; Odham, G.; C. White, D. Determination of phospholipid ester-linked fatty acids and poly β -hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* (L.). *Canadian Journal of Microbiology*. 1985, 31(12), 1113-1119. DOI:10.1139/m85-210
10. Roslev, P.; Iversen, N.; Henriksen, K. Direct fingerprinting of metabolically active bacteria in environmental samples by substrate specific radiolabelling and lipid analysis. *Journal of Microbiological Methods*. 1998, 31 (3), 99-111. DOI:https://doi.org/10.1016/S0167-7012(97)00094-8
11. ISSCAS, Physical and chemical analysis methods of soils. Institute of Soil Sciences, Chinese Academy of Sciences. Shanghai Science and Technology Press, Shanghai, 1978, pp 7-59 (in Chinese)
12. Hong, S. B.; Piao, S. L.; Chen, A. P.; Liu, Y. W.; Liu, L. L.; Peng, S. S.; Sardans, J.; Sun, Y.; Penuelas, J.; Zeng, H., Afforestation neutralizes soil pH. *Nat. Commun.* 2018, 9, 7. DOI:10.1038/s41467-018-02970-1
13. Chen, X. H.; Duan, Z. H. Changes in soil physical and chemical properties during reversal of desertification in Yanchi County of Ningxia Hui autonomous region, China. *Environ. Geol.* 2009, 57 (5), 975-985. DOI:10.1007/s00254-008-1382-1
14. Bossio, D. A.; Scow, K. M. Impacts of Carbon and Flooding on Soil Microbial Communities: Phospholipid Fatty Acid Profiles and Substrate Utilization Patterns. *Microbial ecology*. 1998, 35 (3), 265-278. DOI: 10.1007/s002489900082
15. Wu Jianjun; Jiang, Yanmei; Wu Yuping; Xu Jianming. Effect of complex heavy metal pollution on biomass and community structure of soil microbes in paddy soil. *Acta Pedologica Sinica*. 2008, 45 (06), 1102-1109. DOI:10.3321/j.issn:0564-3929.2008.06.013
16. Frostegård, Å.; Bååth, E.; Tunlio, A. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*. 1993, 25 (6), 723-730. DOI:https://doi.org/10.1016/0038-0717(93)90113-P
17. Federle T W. Microbial distribution in the soil-new techniques. In: Megusar, F., Gantar M. (Eds) *Perspectives in microbial ecology*. International Symposium of Microbial Ecology IV, Slovene Society for Microbiology, Ljubljana, Slovenia, pp. 493 - 498.
18. Hamel, C.; Vujanovic, V.; Jeannotte, R.; Nakano-Hylander, A.; St-Arnaud, M. Negative feedback on a perennial crop: Fusarium crown and root rot of asparagus is related to changes in soil microbial community structure. *Plant and Soil*. 2005, 268 (1), 75-87. DOI:10.1007/s11104-004-0228-1
19. Tunlid; A.; Hoitink; J., H. A.; Low; C.; White; C., D. Characterization of bacteria that suppress rhizoctonia damping-off in bark compost media by analysis of Fatty Acid biomarkers. *Applied and environmental microbiology*, 1989, 55(6), 1368-1374. URL:https://aem.asm.org/content/aem/55/6/1368.full.pdf
20. Zogg, G. P., Zak, D. R., Ringelberg, D. B., White, D. C., MacDonald, N. W., Pregitzer, K. S. Compositional and Functional Shifts in Microbial Communities Due to Soil Warming. *Soil Science Society of America Journal*. 1997, 61(2), 475. DOI:10.2136/sssaj1997.0361599500610
21. 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. *Applied Soil Ecology*. 2002, 20 (3), 171-181. DOI: 10.1016/S0929-1393(02)00025-2
22. Brant, J. B.; Myrold, D. D.; Sulzman, E. W. Root controls on soil microbial community structure in forest soils. *Oecologia*. 2006, 148 (4), 650-659. DOI:10.1007/s00442-006-0402-7
23. Xu Youping; Cai Xinzong; Zhu Xiaoxiang. Comparative analysis of microbial community structures in soils from rice-upland crop rotation fields by PLFA profile technique. *Acta Agriculturae Zhejiangensis* 2013, 25 (05), 1056-1061. DOI: 10.3969/j.issn.1004-1524.2013.05.26

24. Geisseler, D.; Linguist, B. A.; Lazicki, P. A. Effect of fertilization on soil microorganisms in paddy rice systems - A meta-analysis. *Soil Biology & Biochemistry*. 2017, 115, 452-460. DOI:10.1016/j.soilbio.2017.09.018
25. Chen, X.-J.; Wu, X.-H.; Liu, S.-L.; Yuan, H.-Z.; Li, M.-M.; Zhu, H.-H.; Ge, T.-D.; Tong, C.-L.; Wu, J.-S. Microbial activity and community structure analysis under the different land use patterns in farmland soils: based on the methods PLFA and MicroResp. *Huan Jing Ke Xue= Huanjing Kexue*. 2013, 34 (6), 2375-2382. ISSN: 0250-3301
26. Kiani, M.; Hernandez-Ramirez, G.; Quideau, S.; Smith, E.; Janzen, H.; Larney, F. J.; Puurveen, D. Quantifying sensitive soil quality indicators across contrasting long-term land management systems: Crop rotations and nutrient regimes. *Agr Ecosyst Environ*. 2017, 248, 123-135. DOI:10.1016/j.agee.2017.07.018
27. Chavarría, D. N.; Verdenelli, R. A.; Serri, D. L.; Restovich, S. B.; Andriulo, A. E.; Meriles, J. M.; Vargas-Gil, S. Effect of cover crops on microbial community structure and related enzyme activities and macronutrient availability. *European Journal of Soil Biology*. 2016, 76, 74-82. DOI:10.1016/j.ejsobi.2016.07.002
28. Guo, L.-J.; Zhang, Z.-S.; Wang, D.-D.; Li, C.-F.; Cao, C.-G. Effects of short-term conservation management practices on soil organic carbon fractions and microbial community composition under a rice-wheat rotation system. *Biology & Fertility of Soils*. 2015, 51 (1), 65-75. DOI:10.1007/s00374-014-0951-6
29. Jackson, L. E.; Calderon, F. J.; Steenwerth, K. L.; Scow, K. M.; Rolston, D. E. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. *Geoderma*. 2003, 114 (3/4), 305. DOI:10.1016/S0016-7061(03)00046-6
30. Moore-Kucera, J.; Dick, R. P. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Microbial Ecology*. 2008, 55 (3), 500-511. DOI:10.1007/s00248-007-9295-1
31. Gülser, C. Effect of forage cropping treatments on soil structure and relationships with fractal dimensions. *Geoderma*. 2006, 131 (1/2), 33-44. DOI:10.1016/j.geoderma.2005.03.004
32. Hebb, C.; Schoderbek, D.; Hernandez-Ramirez, G.; Hewins, D.; Carlyle, C. N.; Bork, E. Soil physical quality varies among contrasting land uses in Northern Prairie regions. *Agriculture, Ecosystems & Environment*. 2017, 240, 14-23. DOI:10.1016/j.agee.2017.02.008
33. Naderi-Boldaji, M.; Keller, T. Degree of soil compactness is highly correlated with the soil physical quality index S. *Soil & Tillage Research*. 2016, 159, 41-46. DOI:10.1016/j.still.2016.01.010