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The Preliminary Research on Shifts in Maize Rhizosphere Soil Microbial Communities and Symbiotic Networks under Different Fertilizer Sources

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Abstract: The use of chemical fertilizer along with organic fertilizer is an important agricultural practice that improves crop yield but also affects soil biogeochemical cycles. In this study, a maize field experiment was conducted to investigate the effects of NPK fertilizer (NPK), organic fertilizer (OF), and their combination (NPK+OF) on soil chemical properties, bacterial and fungal community structures, and diversity compared the control (CK, without any fertilizer). The results showed that the application of OF and NPK-combined OF increased soil organic matter (OM), total N, total P, available N, available P, and available K levels. For alpha diversity analyses, the application of fertilizers led to decreases in soil bacterial and fungal Shannon indices (except for NPK in fungi). Compared with CK, NPK, OF, and NPK+OF fertilization treatments significantly increased the abundances of Acidobacteriota, Gemmatimonadota, and Basidiomycota. Network analysis showed that fertilization produced fewer connections among microbial taxa, especially in the combination of NPK and OF. A redundancy analysis combined with Mantel test further found that the soil OM, available N and P were the main soil-fertility factors driving microbial community variations. Therefore, using organic fertilizer or biological fertilizer combined with chemical fertilizer to improve the status of soil C, N, and P is a promising method to maintain the balance of soil microorganisms in maize field.

Keywords: organic fertilizer; soil properties; soil quality; microbial community; co-occurrence network

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1. Introduction

In recent decades, the maize yield per unit area significantly increased, and was mainly driven by the large amount of chemical fertilizer input [1,2]. However, long-term and excessive chemical fertilizer input, especially inorganic fertilizer, had a negative impact on soil quality [3], leading to a series of environmental problems, such as water eutrophication, soil erosion and biodiversity loss [3–5]. The ministry of agriculture and rural affairs of China came up with a plan in 2015 characterized by the policy of "zero growth of chemical fertilizer in 2020" to solve the environmental problems caused by the excessive application of chemical fertilizer [6], which stressed the need to improve fertilization management in crop production. In short, sustainable management practices are essential to reduce the negative impact of agriculture on the environment.

Although there are still some disputes on organic agriculture, it is generally believed that organic fertilizer is superior to chemical fertilizer in improving soil biological fertility, such as animal manure [7]. The use of organic fertilizer combined with chemical fertilizer can not only reduce the use of synthetic fertilizers, but also improve soil fertility and nutrient circulation, further achieving the long-term stability of crop production by reshaping more active microbial soil–plant interactions [8,9]. However, as a typical organic fertilizer,

manure is rich in nutrients and binders; thus, it is widely used to improve soil structure and function [10,11], such as improving soil nutrient availability loss and phosphorus fixation for crop utilization by reducing nitrogen [12]. Additionally, since the alkaline substances released from manure can also lead to an increase in soil pH, they are closely related to various soil physicochemical and biological processes determining crop productivity [13,14]. Moreover, animal manure also contains fresh organic matter, and its application to the soil will cause a significant increase in the soil organic carbon [15,16].

Soil microorganisms are critical to the function and sustainability of agroecosystems [17,18], not only playing an important role in driving soil–plant rhizosphere interactions, but also directly affecting soil properties and crop yields [19]. In addition, soil microorganisms not only play a key role in nutrient cycling and organic matter decomposition, but can also metabolize and transform organic matter by secreting specific extracellular enzymes [20]. However, soil ecological processes are mainly driven by soil microorganisms, and microbial metabolic activity and functional diversity are closely related to biogeochemical cycles and are considered more important indicators for assessing soil ecological functions and productivity [5,21]. Rhizosphere microorganisms grow together with plant roots and are critical for plant productivity because they play a key role in circulating soil nutrients [22], as well as in abiotic stress tolerance [23] and soil-borne pathogen inhibition [24]. Previous studies have provided comprehensive evidence that plant rhizosphere bacterial communities can comprise many characteristics, including soil physicochemical characteristics and nutrient availability [25], plant root exudates and other metabolites [26], and plant genotypes [27]. Ding et al. [28] also found that, compared with chemical fertilizer, organic fertilizer increased microbial functional diversity in paddy soil and changed bacterial community composition. Previous studies showed that the long-term application of organic fertilizer changed the activity of functional microorganisms related to carbon fixation and decomposition, increased the abundance of soil carbon fixation and refractory C compound degradation genes [29], and reduced the abundance of the degradation of unstable C compounds. It has been reported that manure application can significantly increase the number of key microorganisms related to nitrogen immobilization and mineralization, ammonification and nitrification [30]. The combined application of organic and inorganic fertilizers can accelerate the growth of microorganisms, change the structure of the soil's microbial community and improve enzyme activity [31,32]. The application of organic fertilizer will have a positive impact on bacterial and fungal diversity, regardless of whether chemical fertilizer is applied [33]. Although the impact of fertilization on soil microorganisms has been revealed in many studies, research on the impact mechanism of different fertilizer sources on the maize soil microbial community and the main factors affecting them is relatively weak. Further research would be helpful to evaluate the fertility and health status of maize soil and further promote the sustainable development of the maize industry and environment.

In addition, the ecological network of microbial communities is now a priority area in soil ecology research. In recent years, it has been widely used to study the interaction between soil organisms in different ecological environments, in which species (i.e., nodes) are connected by pairwise interactions (links) [34,35]. Based on this analysis, we can not only determine the symbiotic patterns of characteristic microbial species at different taxonomic levels [36,37], but also identify the key species affecting the stability of the microbial community [38,39]. However, to date, the establishment of the soil's microbial community network in maize fields under different fertilizer sources is still in the primary stage; thus, it is of great significance to further establish the network of the soil's microbial community to determine the interaction between microorganisms. Moreover, the effects of fertilizer sources on maize soil microbial communities and their symbiotic networks in arid irrigated areas of Ningxia have not been revealed. Here, a field trial was carried out to investigate the impacts of fertilizer sources on rhizosphere soil bacterial and fungal communities in maize field using amplicon sequencing and network analysis. The objectives were to: (i) investigate the effects of the use of chemical fertilizer/organic fertilizer/chemical

fertilizer combined with organic fertilizer on the soil chemical properties; (ii) compare the differences in the effects of different fertilizer sources on the soil bacterial and fungal community abundances; and (iii) explore the soil fertility factors driving soil microbial community changes.

2. Materials and Methods

2.1. Study Site and Field Experiment Design

The field experiment site is located in Jinmaoyuan family farm, Huangquqiao Town, Yellow River Diversion Irrigation Area in the north of Yinchuan, Ningxia (38°30′ N, 106°18′ E) (Figure S1). The altitude and average annual temperature are about 1090 m and 8.8 °C, and there is a large temperature difference between day and night. In addition, the average annual precipitation is about 200 mm, and the evaporation is high. The experimental region is low-lying and the east–west trend is slightly undulating. The soil pH in the area is alkaline (alkalinity 18.67%), soil type is irrigation silted soil, and the soil texture is silty clay loam (according to international classification standards), which belongs to chloride sulfate saline soil and is irrigated by the Yellow River water.

The experiment was carried out with a completely randomized block design in 2019, which was being repeated for the third year. The maize variety was Xianyu No. 987. The wide and narrow rows (70 cm wide and 50 cm narrow) were used for planting, and the hole spacing was 18–20 cm. The roller seeder was used for sowing. The maize was irrigated twice every year at the jointing stage (mid-June) and tasseling stage (mid-July), and the single irrigation amount was 1500 m^3 ha^{-1}. The four fertilization treatments included (1) CK: with none fertilizer (control); (2) NPK: with chemical NPK fertilizer (360 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1}, and 45 kg K_2O ha^{-1}); (3) OF: with organic fertilizer 9000 kg ha^{-1}; (4) NPK+OF: mineral NPK fertilizer with organic fertilizer (360 kg CH_4N_2O ha^{-1}, 120 kg P_2O_5 ha^{-1}, 45 kg K_2O ha^{-1}, and 9000 kg ha^{-1} organic fertilizer). Each treatment was replicated three times. The organic fertilizer adopted for this study was fermented by chicken manure, which contained 1.95% of total TN, 0.92% of total TP, 0.85% of total K, and 26.25% of organic C. The chemical fertilizers, including N (urea, 46% N), P (superphosphate, 12% P_2O_5), and K (potassium sulfate, 50% K_2O) fertilizers, were applied as a basic fertilizer before sowing, and organic fertilizer was applied once at the beginning of April every year. Additionally, regular weed and pest management was performed during maize growth stages.

2.2. Soil Sampling

At the harvest stage of maize in October, 2019, the rhizosphere soil samples were collected using a 5 cm diameter auger according to the five-point sampling method. Five fresh samples were taken from each plot and mixed thoroughly as one composite sample for further study. After the obtained roots were removed, all soil samples were passed through a 2 mm sieve, and each sample was separated into two subsamples. One subsample was used for the measurement of chemical properties, while the other was immediately stored at −80 °C for later DNA extraction.

2.3. Soil Chemical Analysis

The soil chemical properties included soil total nitrogen (TN), alkali-hydro nitrogen (AN), total phosphorus (TP), available P (AP), total potassium (TK), available K (AK), and organic matter (OM). After being air-dried, soil chemical properties were determined based on the methods described in Ji et al. [40]. Soil OM was determined by the potassium dichromate volumetric method. Soil TN, TP, and TK were determined by the Kjeldahl method, Vanado–Molybdate phosphoric yellow colorimetric procedure, and atomic absorption spectrophotometry, respectively. Soil AN, AP, and AK were determined by the alkali hydrolysis diffusion method, molybdenum antimony colorimetric method, and flame photometry, respectively.

2.4. DNA Extraction, PCR Amplification and Pyrosequencing

The Qiagen DNA Isolation Kit (Shanghai Tongyuan Biotechnology Co. Ltd, Shanghai, China) was used to extract soil genomic DNA according to the manufacturer's instructions. Then, DNA purity was detected by 1% agarose gel electrophoresis. For bacteria, the V4 region of the 16S rRNA gene was amplified using the primer set 515F/806R [41], and ITS region of fungi was amplified using the primer set ITS1/ITS2 [42]. Amplifications were performed using the KAPA 2G Robust Hot Start Ready Mix (KAPA, UK). The obtained products were purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Bria, California, USA). High-throughput sequencing was performed on the Illumina Miseq PE300 platform at Allwegene Technology Co., Ltd. (Beijing, China). The raw sequence data were deposited in the NCBI small read archive dataset under the study number PRJNA880499 and PRJNA880503.

2.5. Data Analysis

After removing the barcode and primer sequences, the raw sequences were assembled for each sample according to a unique barcode. The paired-end sequences for each sample were merged using the FLASH v1.2.0 tool. Quality filtering of the raw tags was performed under specific filtering conditions to obtain high-quality clean tags according to the quality control process of QIIME v1.8.0 (http://qiime.org). The effective tags were obtained after removing the chimeric sequences obtained. The sequence analysis was conducted using the UPARSE v7.0.1001 software, and the sequences with a similarity of ≥97% were assigned to the same operational taxonomic unit (OTU) to generate rarefaction curves (Figure S2) and calculate the richness and diversity indices. For each representative sequence, the RDP (bacteria) and UNITE (fungi) databases were used to annotate taxonomic information. Principal component analysis (PCA) was used to examine the similarity and differences among all soil samples based on the OTUs level.

Network analysis was performed to explore the ecological connections within and between bacterial and fungal taxa at the genus level in the soil microbial communities. Bacterial and fungal genera with the relative abundances greater than 0.1% were used to construct the networks in all treatments. The networks were visualized by the Gephi software (v0.9.2) combined with the 'psych' package in R (V4.0.3).

2.6. Statistical Analysis

All analysis results were reported as the means standard deviation (SD) for the three replications. A two-way analysis of variance (ANOVA) was performed to compare the data, including soil chemical properties, the α-diversity indices, and the relative abundances of soil microbial taxa among four treatments using the IBM SPSS v22.0 (IMB Corp., Armonk, USA). Three diversity indices (observed OTUs species, Shannon, and Chao1) were used to assess the diversity of microbial communities. The differences in microbial community compositions among the four fertilization treatments were analyzed with the Kruskal–Wallis in R, and p-values < 0.05 were considered significant. The differential abundant taxa were identified by the linear discriminant analysis (LDA) effect size (LEfSe) method. The LEfSe algorithm used the nonparametric factorial Kruskal–Wallis test ($\alpha = 0.05$) to analyze the differential taxa among the four fertilization treatments (LDA score > 3). The redundancy analysis (RDA) was performed using the vegan package in R with 999 permutations to identify the major factors driving microbial distribution in maize field with the four fertilization treatments. Spearman correlation analysis was performed using the IBM SPSS Statistics (v 25.0) (SPSS, Chicago, IL, USA).

3. Results

3.1. Soil Chemical Properties

The influences of different fertilizer sources on soil chemical properties are shown in Table 1. Except for total potassium (TK), significant differences ($p < 0.05$) in the soil organic matter (OM), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN),

available phosphorus (AP), and available potassium (AK) were observed among the four fertilization treatments. Compared with the CK, NPK, OF, and NPK+OF significantly increased soil OM, TN, AN, and AP. The NPK+OF was a better fertilization method than the CK, increasing the soil OM, TP, AN, and AP by 58.03%, 8.22%, 55.22%, and 162.70%, respectively, while the highest values of TN and AK occurred in OF treatments. TK was decreased by all fertilization treatments, but there were no significant differences among the four fertilization treatments ($p > 0.05$).

Table 1. Soil chemical properties analysis.

Treatments	OM (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
CK	11.8 ± 0.21 d	0.75 ± 0.04 c	0.73 ± 0.04 ab	19.3 ± 0.92 a	32.5 ± 0.12 c	10.7 ± 0.06 c	243 ± 7 b
NPK	15.8 ± 0.35 c	0.86 ± 0.01 bc	0.71 ± 0.02 b	18.7 ± 0.61 a	39.5 ± 0.40 b	14.1 ± 2.70 b	222 ± 7 c
OF	17.9 ± 0.40 b	1.04 ± 0.13 a	0.74 ± 0.03 ab	18.7 ± 0.06 a	49.9 ± 0.70 a	26.2 ± 0.32 a	304 ± 9 a
NPK+OF	18.6 ± 0.36 a	0.98 ± 0.09 ab	0.79 ± 0.05 a	18.9 ± 0.40 a	50.4 ± 1.25 a	28.0 ± 0.12 a	253 ± 3 b
Source of variance							
NPK	142 ***	0.27	0.79	0.29	76.8 ***	11.2 *	87.2 ***
OF	529 ***	20.1 ***	4.06	0.54	1074 ***	351 ***	139 ***
NPK+OF	72.4 ***	3.37	2.34	1.28	57.8 ***	0.95	15.5 ***

Values are mean ± standard deviation (n = 3). Different letters in the same column represent significant differences at the $p = 0.05$ level. * $p < 0.05$ and *** $p < 0.001$. OM, organic matter; TN, total N; TP, total P; TK, total K; AN, alkali-hydrolyzed N; AP, available P; AK, available K.

3.2. Impacts of Fertilization on Bacterial and Fungal Alpha and Beta Diversity

Similar to the soil chemical properties, bacterial and fungal alpha diversity indices were also affected by the the four fertilizer sources. The observed species, Shannon, and Chao 1 indices showed that the changes in soil bacterial and fungal alpha diversity observed in the various fertilization treatments were significant (Figure S3 and Table 2). The observed species, Shannon, and Chao 1 indices showed that fertilizer sources markedly decreased soil bacterial diversity, and the lowest value of bacteria occurred in NPK+OF treatments. Regarding soil fungal alpha diversity indices, NPK and NPK+OF fertilization treatments significantly decreased the observed species and Chao1 indices, while NPK significantly increased fungal Shannon index, which was significantly decreased by OF and NPK+OF treatments. Spearman correlation analysis suggested that some bacterial diversity indices were significantly negatively correlated with soil OM, N, and P, while fungal diversity indices were only closely negatively correlated with soil OM and P (Table 3). In addition, the numbers for the shared bacterial and fungal OTUs in all four fertilization treatments were 359 and 18, respectively (Figure S4).

Table 2. Bacterial and fungal alpha-diversity.

Treatments	Bacteria			Fungi		
	Observed_Species	Shannon	Chao1	Observed_Species	Shannon	Chao1
CK	4639 ± 155 a	10.4 ± 0.09 a	6388 ± 150 a	687 ± 46 a	5.19 ± 0.38 ab	977 ± 98.3 a
NPK	4114 ± 111 b	10.1 ± 0.19 ab	6132 ± 83 b	589 ± 50 b	5.64 ± 0.28 a	792 ± 16.49 b
OF	4074 ± 86 b	10.2 ± 0.09 bc	5968 ± 91 bc	685 ± 7 a	4.99 ± 0.36 bc	922 ± 1.14 a
NPK+OF	3870 ± 42 c	9.98 ± 0.06 c	5867 ± 87 c	451 ± 10 c	4.46 ± 0.22 c	622 ± 7.86 c
Source of variance						
NPK		14.15 ***	8.43 *	69.8 ***	0.05	70.8 ***
OF	19.18 ***	6.33 *	31.2 ***	12.43 ***	14.49 ***	15.29 ***
NPK+OF	0.17	0.02	1.60	11.72 ***	7.32 *	3.90

Values are mean ± standard deviation (n = 3). Different letters in the same column represent significant differences at the $p = 0.05$ level. * $p < 0.05$ and *** $p < 0.001$.

Table 3. Correlation analysis between bacterial and fungal diversity indexes and soil chemical properties.

	Indices	OM	TN	TP	TK	AN	AP	AK
Bacteria	Observed species	−0.81 **	−0.60 *	−0.54	0.19	−0.71 *	−0.88 **	−0.27
	Shannon	−0.63 *	−0.39	−0.40	0.26	−0.52	−0.76 **	−0.10
	Chao 1	−0.90 *	−0.75 *	−0.61 *	0.02	−0.81 **	−0.93 **	−0.47
Fungi	Observed species	−0.43	−0.11	−0.35	0.21	−0.27	−0.48	0.22
	Shannon	−0.69 *	−0.55	−0.71 *	−0.22	−0.49	−0.62 *	−0.53
	Chao 1	−0.59 *	−0.37	−0.28	0.22	−0.51	−0.65 *	0.15

* $p < 0.05$, ** $p < 0.01$. OM, organic matter; TN, total N; TP, total P; TK, total K; AN, alkali-hydrolyzed N; AP, available P; AK, available K.

To evaluate the differences in bacterial and fungal beta diversity, principal component analysis (PCA) was performed based on the OTU level. The first two main coordinates of PCA (PC1 = 25.89% and PC2 = 14.54%) explained 40.43% of the variation in bacterial beta diversity (Figure 1a), similarly, which also explained 34.57% of the variation in fungal beta diversity (Figure 1b). This suggested that the influences of fertilization on bacterial beta diversity were larger than that of fungi. Additionally, the bacterial and fungal communities in the three fertilization treatments (NPK, OF, and NPK+OF) were separated from the CK (Anosim analysis, $p = 0.001$) (Table S1), indicating that the fertilization treatments had significant effects on the bacterial and fungal communities.

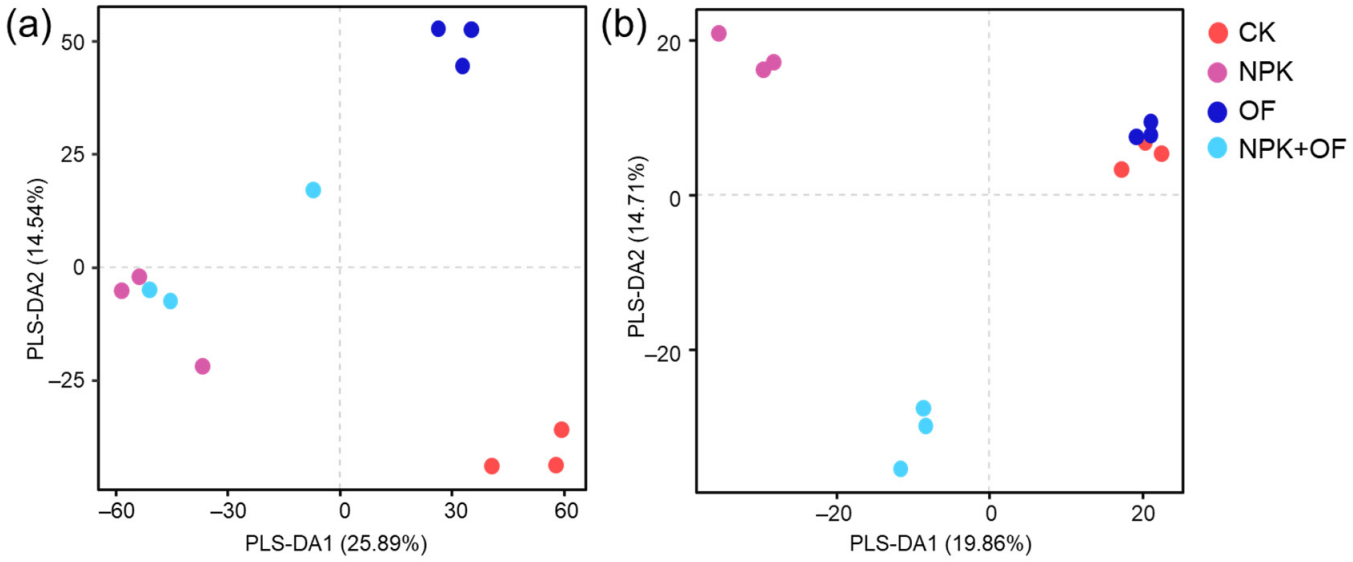

Figure 1. Principal component analysis (PCA) of bacterial (**a**) and fungal (**b**) communities in soils of four treatments.

3.3. Relative Abundance of Major Bacterial and Fungal Taxa

A total of 59 bacterial phyla and 848 genera were detected in all soil samples, and the average relative abundance of 11 phyla and 10 genera exceeded 1%. Among the top bacterial community at the phylum level, Proteobacteria (23.15%) were clearly dominant, followed by Acidobacteriota (13.15%), Gemmatimonadota (10.14%), Actinobacteriota (9.72%), Chloroflexi (9.25%), Planctomycetota (7.74%), Bacteroidota (6.74%), Myxococcota (4.58%), Crenarchaeota (3.09%), and Desulfobacterota (3.07%) (Figure 2a and Table S2). There were significant differences in the relative abundances of Proteobacteria, Acidobacteriota, Gemmatimonadota, Bacteroidota and Desulfobacterota. The relative abundances of Acidobacteriota and Gemmatimonadota were significantly increased by all fertilization treatments, while those of Proteobacteria and Desulfobacterota were markedly de-

creased. The top 10 dominant genera included *MND1* (3.09%), *RB41* (3.09%), *Pseudomonas* (3.09%), *Haliangium* (3.09%), *Citrifermentans* (3.09%), *metagenome* (3.09%), *Subgroup_10* (3.09%), *Lysobacter* (3.09%), *Sphingomonas* (3.09%) and *Defluviicoccus* (3.09%) (Figure 2b and Table S2). These genera belong to five phyla (Proteobacteria, Acidobacteriota, Myxococcota, Desulfobacterota, and Actinobacteriota). All fertilization treatments significantly decreased the relative abundances of *MND1*, *Citrifermentans*, and *Lysobacter*, and increased that of *RB41*. The relative abundance of genus *metagenome* was significantly reduced only in NPK+OF treatment, while that of *Sphingomonas* was significantly reduced by NPK and NPK+OF treatments.

Regarding soil fungal community, a total of 12 phyla and 347 genera were detected in all soil samples, and the average relative abundance of 5 phyla and 5 genera exceeded 1%. All these samples were dominated at the phylum level by Ascomycota, which comprised 57.45% of the total sequences on average. Only NPK+OF caused an increase in the relative abundance of Ascomycota. The other dominant fungal phyla were Basidiomycota (6.84%), Mortierellomycota (5.52%), Glomeromycota (1.77%) and Chytridiomycota (1.55%). In addition, among the top 10 dominant fungal phyla, only the relative abundance of Basidiomycota was significantly different in all treatments, fertilization treatment significantly increased its relative abundance, and the maximum abundance appeared in NPK treatment (Figure 2c and Table S3). At the genus level, *Botryotrichum* (19.66%), *Mortierella* (5.51%), *Fusarium* (3.10%), *Acremonium* (2.66%), *Chaetomium* (2.06%), *Clitocybe* (0.93%), *Neopaxillus* (0.90%), *Stachybotrys* (0.86%), *Lophotrichus* (0.71%), and *Plectosphaerella* (0.65%), detected in all samples, were the top 10 dominant fungal genera (Figure 2d and Table S3). These genera belong to three phyla (Ascomycota, Mortierellomycota, and Basidiomycota). Among the fungal genera, compared to the CK, the NPK treatment caused a remarkable increase in the relative abundances of *Fusarium*, *Neopaxillus*, and *Stachybotrys*, while a similar result occurred in the OF treatment regarding the relative abundance of *Clitocybe*. Furthermore, the relative abundances of *Acremonium* and *Plectosphaerella* markedly decreased following all fertilization treatments relative to the CK. These results indicated that fertilizer sources can cause significant changes in the composition of the soil microbial community.

Additionally, linear discriminant biomarker analysis (LEfSe) was performed to determine the classified bacterial and fungal taxa with significant abundance differences among the fertilization and control groups (Figures S5 and S6). The biomarkers differ depending on the fertilizer sources. Specifically, the number of bacterial biomarkers in the NPK, OF, and NPK+OF treatment groups was 10, 2, and 7, respectively. Similarly, the number of fungal biomarkers in the NPK, OF, and NPK+OF treatment groups was 39, 8, and 8, respectively. Bacterial biomarkers were mostly distributed in Acidobacteria, Thaumarchaeota, Nitrospirae, Gemmatimonadetes, Bacteroidetes, Proteobacteria, Actinobacteria, and Chloroflexi, and fungal biomarkers were mostly distributed in Ascomycota, Basidiomycota, Mortierellomycota, and Glomeromycota. In addition, although there were no common bacterial biomarkers between the three fertilization treatments at the genus level, the common fungal biomarkers mostly included genera *Lecanicillium*, *Mortierella*, *Filobasidium*, *Vishniacozyma*, *Cladosporium*, *Hannaella*, *Mycosphaerella*, *Coprinellus*, and *Gliomastix*.

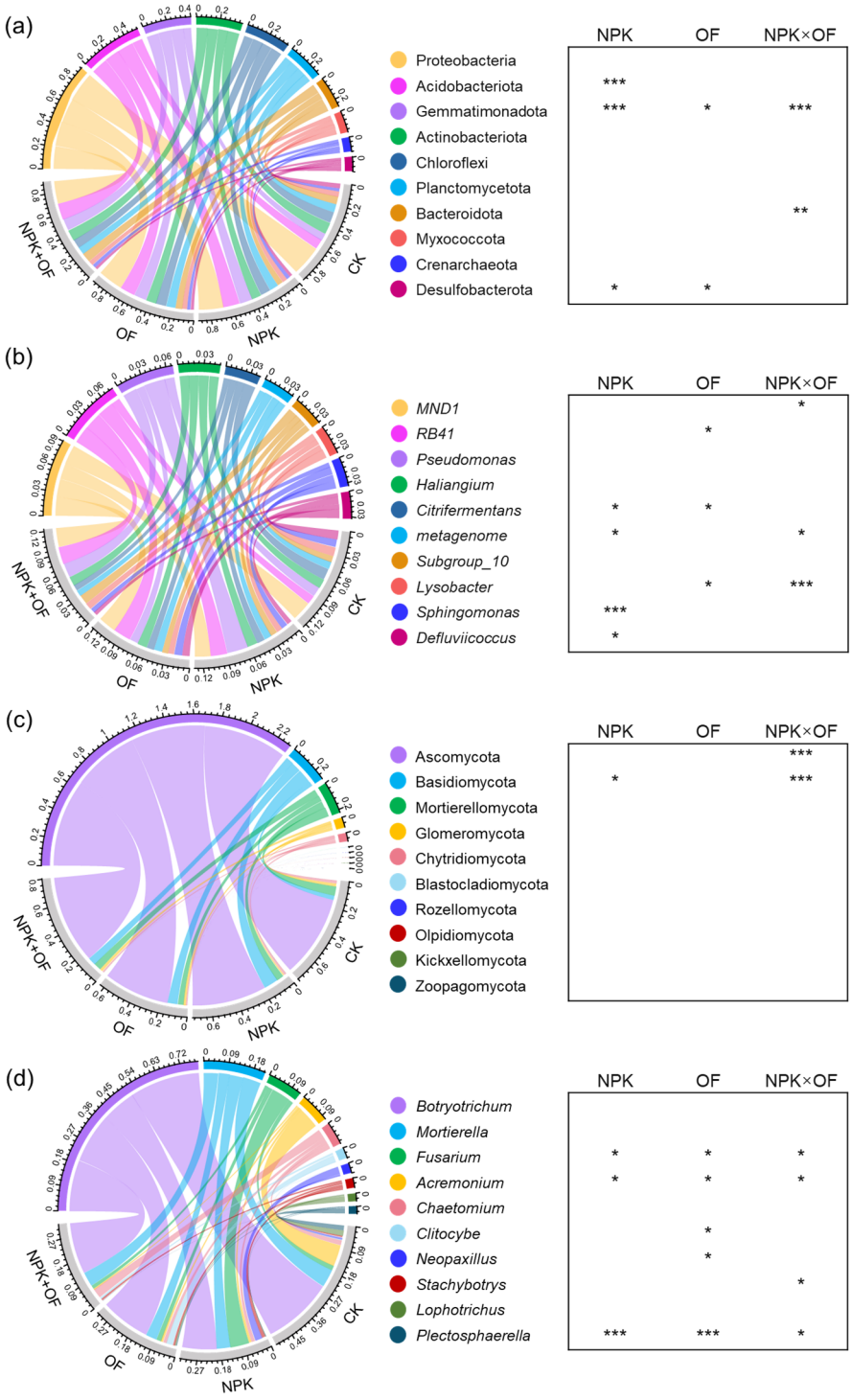

Figure 2. Relative abundances of the top 10 bacterial (**a**) and fungal (**c**) phyla, and bacterial (**b**) and fungal (**d**) genera in corn soils under four fertilization treatments. CK = no fertilization, NPK = chemical fertilizer only, OF = organic fertilizer only, and NPK+OF = chemical plus organic fertilizer. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3.4. The Influences of Fertilization Treatment on the Complexity of the Microbial Co-Occurrence Network

Microorganisms usually form complex networks to deal with the interference of foreign substances. The co-occurrence networks of soil bacterial and fungal communities were constructed to explore the co-occurrence patterns of microbes under fertilization treatments (Figure 3). The soil microbial network of each treatment showed a different

co-occurrence pattern. Network topology parameters (node number and edge number) are used to evaluate the complexity of the soil microbial network. The results showed that there were significant differences in the topological structure of co-occurrence networks between the control and the fertilization treatment groups. In general, fertilizer sources interfered with the network complexity of microbial communities. The numbers of nodes and edges showed that the fertilization treatments significantly reduced the network complexity of microbial communities, and network density showed that fertilization treatments significantly increased the density of microbes compared with the CK. In addition, the clustering coefficients suggested that the three fertilization treatments did not change the tightness of microbial community connections, and the average degree in NPK+OF treatment was also significantly lower than the control, indicating that NPK+OF weakened the relationships between bacterial fungal taxa. The complexity of the soil microbial community networks proved that fertilizer sources can interfere with the complexity of the soil microbial community co-occurrence networks, further reducing microbial relationships.

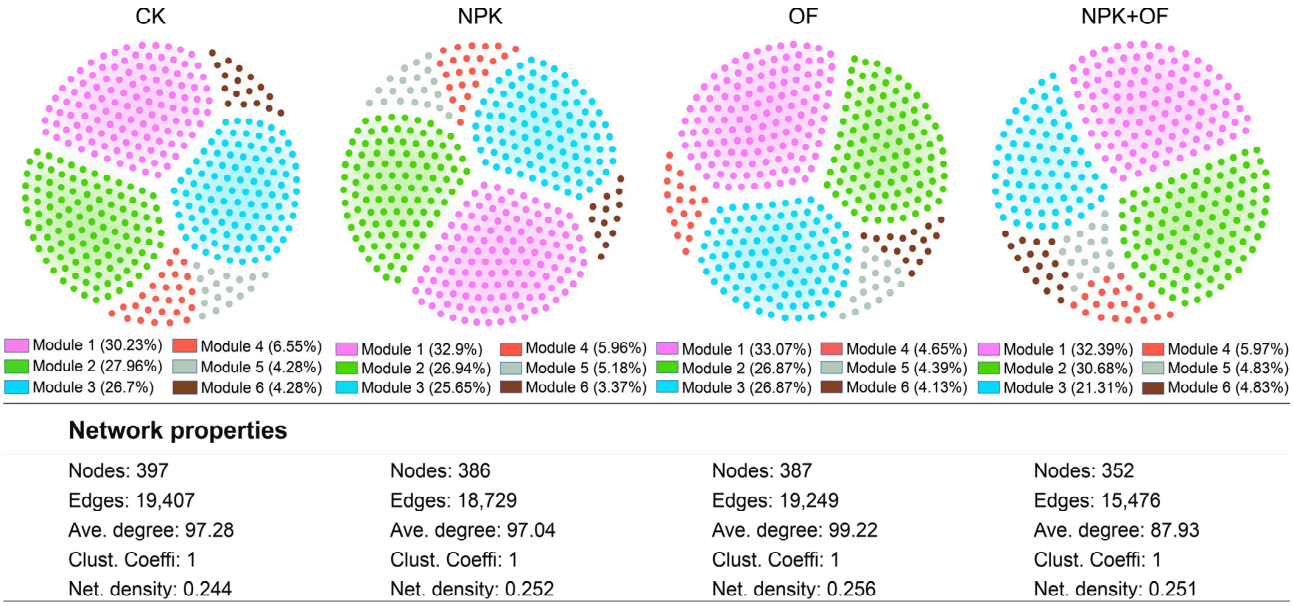

Figure 3. Effect of different fertilization regimes on the co-occurrence patterns of soil bacterial and fungal community. Networks were constructed at the genera level. The size of nodes is scaled to the degree of nodes, and the nodes are colored according to modules. Edges indicate correlations among nodes ($r > 0.9$, $p < 0.01$). Ave. degree: average degree; Clust. coeff.: clustering coefficient; Net. density: network density.

3.5. Driving Soil Fertility Factors of Microbial Community Variations

The relationships among soil chemical variables and microbial communities were found using a redundancy analysis (RDA) (Figure 4). Similar to beta diversity, RDA results also revealed clear separations among all fertilization treatments. The environmental characteristics associated with the bacterial and fungal communities showed that the selected soil fertility factors explained 53.15% of the bacterial community structure variation and 69.09% of the fungal community structure variation (Figure 4a,b), and permutation tests showed a significant correlation between soil OM, TN, AN, AP, and AK and bacterial community structure, and soil OM, AN, AP, AK and fungal community structure (Table S4). Additionally, the Mantel test revealed that the soil OM, AN, and AP were significantly correlated with bacterial community composition, while soil OM, TP, AN, and AP were remarkably correlated with fungal community composition (Figure 4c). These results indicated that soil OM, and available N and P, were the main soil fertility factors driving microbial community variations.

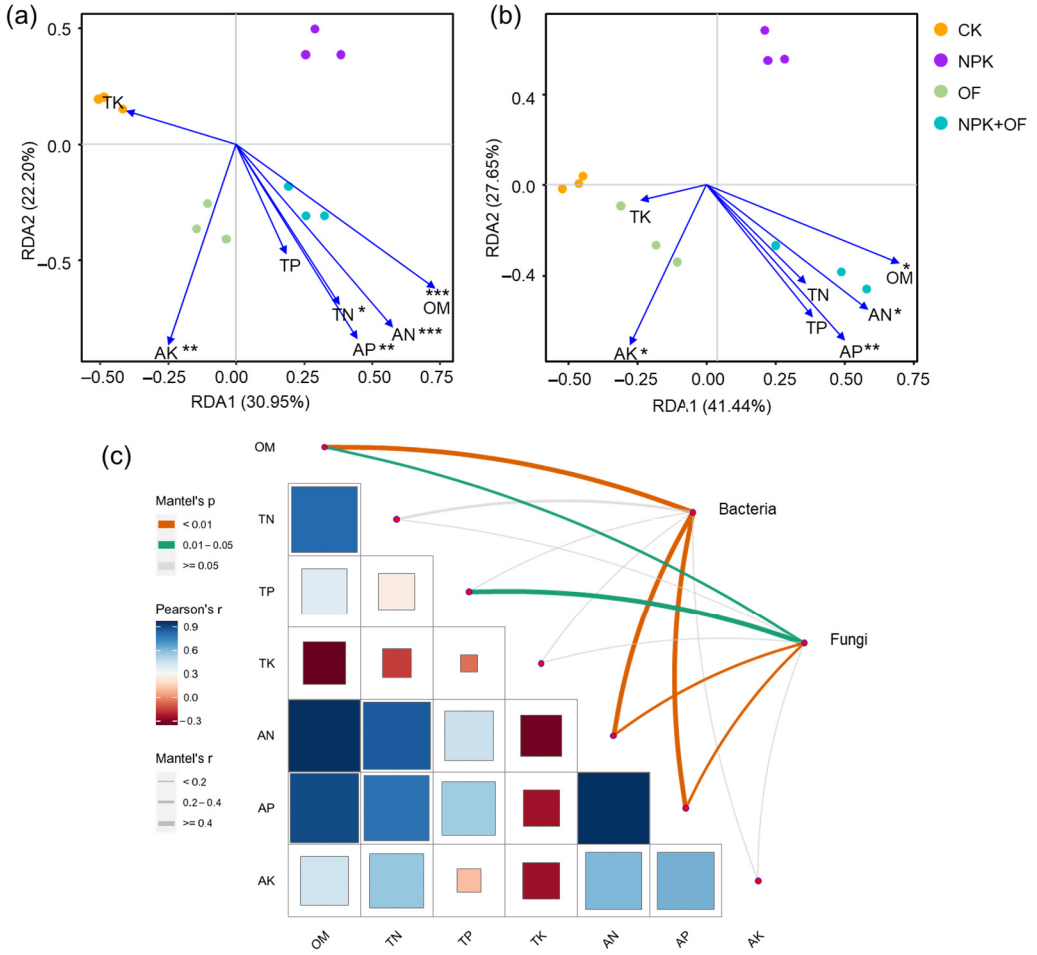

Figure 4. Environmental drivers of soil microbial community compositions. Redundancy analysis (RDA) of the bacterial (**a**) and fungal (**b**) communities and soil properties for individual samples. The direction of the arrows indicates correlations with the first two canonical axes, and the length of the arrows represents the strength of the correlations. Pairwise comparisons of soil fertility factors with soil bacterial and fungal communities with color gradient denoting Spearman's correlation coefficients (**c**). Edge width corresponds to Mantel's r statistic for the corresponding distance correlations, and edge color denotes the statistical significance based on 9999 permutations. Soil properties included soil organic matter (OM), total N (TN), total P (TP), total K (TK), dissolved organic C (AN), available P (AP), available K (AK). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

4. Discussion

Many studies have explored the responses of the soil microbial community to different fertilization systems and fertilizer sources. However, we studied the changes in bacterial and fungal communities in maize field under different fertilizer sources after irrigation. PCA analysis showed significant differences in microbial community compositions among different fertilization treatments. A large number of studies suggested that microbes showed different biogeographic patterns, which were continuously and permanently affected by the local climate, crop diversification, tillage, fertilization type, and other environmental factors [43], while our results showed that the application of organic and inorganic fertilizers could cause significant changes in microbial communities in maize fields after irrigation.

Soil physicochemical properties are not only considered to be the key to soil quality, but are also generally defined as an important indicator to maintain soil environmental quality and improve crop productivity [44,45]. Previous studies have confirmed that fertilization may interfere with the soil's physicochemical properties, mainly due to the

soil type, and fertilization dosage [7]. Other studies have shown that the application of chemical fertilizers can lead to soil acidification; however, adding the organic improvers can not only alleviate soil acidification, but can increase organic matter input, which is helpful to promote soil nutrients' transformation and cycling [46]. In the present study, we found that many soil nutrients were significantly altered by the chemical and organic fertilizer sources. In addition, the application of an organic fertilizer can also lead to improvements in nutrient utilization efficiency and further reduce losses, such as N and P [47]. Similarly, our study showed that the application of organic fertilizer and its combination with NPK led to increases in the soil OM, TN, TP, AN, AP, and AK, further improving soil fertility, which also confirmed the results of many studies [31,48]. Other research also found that organic and N fertilizer significantly increased the topsoil total N, PON, MBN, DON, and NO_3^- [49], mainly due to the nature of the organic fertilizer containing certain nutrients, and can improve soil fertility and crop yield [50]. Moreover, the combination of chemical fertilizer and organic fertilizer not only improves the soil nutrients [17], but also facilitates the growth of crop roots and improves crop yield [51,52].

Microorganisms are not only an important indicator of soil health [53], but their activities regulate the accumulation of soil organic carbon and the nutrient cycles. Some studies have shown that long-term chemical fertilization will reduce the diversity of the soil bacterial community, but the use of an organic fertilizer caused it to increase [54]. Additionally, it has also been found that long-term NPK fertilization lead to a decrease in soil bacterial community diversity, while the input of organic fertilizer significantly stabilized bacterial diversity, further restoring it to the level of unfertilized soil [17]. Our results showed that the soil bacterial and fungal diversity in maize farmland were significantly reduced with different fertilization treatments, which was inconsistent with many research results. For example, most studies found that the diversity of soil bacteria was not significantly increased by fertilization [11,46,55], but other studies found that the application of a bio-fertilizer lead to a decrease in soil fungal diversity [46,56]. In addition, the useo f NPK combined with organic fertilizer can not only increase the diversity and richness of soil bacterial population, but also can promote the root exudates [57]. More interestingly, soil organic matter and other nutrients were significantly correlated with soil diversity indices, and the same results were found in other studies [46,58], which are basically consistent with previous studies showing that the fertilization system leads to changes in soil microbial communities by changing soil chemistry [55,59].

Additionally, soil health depends on not only the diversity and richness of soil microbes, but also the community compositions [60]. Firstly, bacteria are the most important decomposers in soil, and long-term organic fertilization leads to the enrichment of specific bacteria that can effectively utilize nutrients [31]. The dominant bacterial phyla detected in the kiwifruit orchard soil were Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria [46,61]. Some researchers also found that the dominant bacteria after long-term fertilization were Actinobacteria, Proteobacteria, Chloroflexi, and Acidobacteria [62]. Actinobacteria are a group of co-trophic organisms suitable to enhance plant growth in high-C environments. Biological manure promoted the proliferation of Actinomycetes, because they can create an environment that is rich in nutrients and carbon, and their abundances significantly increase under the treatment of biological manure [63]. Although Proteobacteria, Acidobacteriota, Gemmatimonadota, and Actinobacteriota were detected to be the dominant bacterial phyla in our study, the relative abundance of Actinobacteriota decreased under organic fertilizer treatment, which may be caused by the difference between manure and biological manure. The relative abundance of Acidobacteriota significantly increased while that of Proteobacteria significantly decreased, but no significant differences were observed among the three fertilization treatments. However, in other studies, the results were the opposite [46], which may be the reason that Acidobacteriota belongs to a class of oligotrophs (k-strategists), which can degrade relatively stable carbon, grows slowly, dominates the microbial community in a dystrophic environment, and is negatively correlated with C level [17]. At the same time, studies found that high C/N and N limitations

caused by long-term organic or inorganic fertilization affected the structure of soil microbial communities and their dominant SOC decomposition [64].

Followed by bacteria, the dominant fungal phyla were also detected in the soil samples in our study, such as Ascomycota, Basidiomycota, and Mortierellomycota, which is consistent with the findings in a kiwifruit orchard soil under organic and inorganic fertilization, but the results were different from the findings in soybean rotation system under organic and inorganic fertilization [65]. Moreover, Ascomycota is a kind of saprophytic fungi, which can thrive in an arid environment, has strong environmental adaptability, degrades organic matter, and is the main decomposer of soil organic matter containing cellulose, lignin, and pectin [66]. Mortierellomycota are saprophytic and widely exist, which can dissolve P, increase crop yield, and form symbiotic relationships with plants [67]. In the present study, the abundances of Ascomycota and Mortierellomycota decreased in NPK and OF fertilization treatments, while in other studies, the opposite results were observed [62]. Importantly, the relative abundance of *Fusarium*, a soil-borne plant pathogenic fungus that can induce crop *Fusarium wilt*, was significantly reduced after the use of OF combined with NPK fertilizer [46,68], which showed that the application of organic fertilizer was beneficial to inhibit the growth of plant pathogenic fungi. Therefore, fungal communities are closely related to soil fertilizer sources.

The co-occurrence networks are not only an important manifestation of microbial community stability in response to external disturbances [69], but they also have the ability to maintain the relationships between soil microbial diversity and ecosystem multifunctionality [70]. At present, network analysis has been widely used in microbial ecology. For example, some studies indicated that the complexity of soil microbial networks was affected by environmental changes, such as agricultural management and the addition of other foreign substances [71]. The use of a bio-organic fertilizer enhanced stable network of soil microbial communities [46]. A high altitude not only reduced the diversity of microbial communities, the complexity of co-occurrence networks, and the versatility of ecosystems, but also revealed that the impacts of microbial community diversity on versatility was indirectly driven by the complexity of microbial networks [70]. Another study suggested that the molecular ecological network became more stable under warming conditions [72]. Organic fertilization drives changes in the complexity of microbial communities, and key groups increase the resistance of microbial-mediated functions to biodiversity loss [73]. Similarly, our study on the complexity of soil microbial community networks under different fertilization treatments showed that fertilizer sources interfered with the complexity of the co-occurrence networks in soil microbial communities, and reduced the interactions among them. Therefore, both organic and inorganic fertilizers will reveal its response to external interference by affecting the ability of the soil microbial co-occurrence pattern, and during this process, some synergistic microbial co-occurrence patterns may be generated to resist soil microbes.

Additionally, soil properties after fertilization can explain the changes in microbial communities in this experiment [74]. Among them, OM, AN, and AP were the main factors driving the changes in bacterial and fungal communities. Some studies have shown that most microbial parameters are mainly related to the soil OM [74]. Previous studies also showed that pH and SOC were the main indirect factors regulating the crop yield by regulating the structure and diversity of bacterial communities and even the abundances of potential functional genes of microorganisms [11]. In addition, soil organic carbon was not only closely related to nutrient availability, but was another key factor affecting soil microbial community composition and diversity [75]. In summary, our research showed that fertilizer sources (chemical fertilizer, organic fertilizer and their combination) changed the abundances, diversity and compositions of soil microbes by changing the soil chemical properties.

5. Conclusions

In this work, a field trial was carried out to investigate the impacts of different fertilizer sources on the rhizosphere soil bacterial and fungal communities in maize field by using amplicon sequencing and network analysis. Our results showed that different fertilizer sources significantly improved the soil chemical properties, changed the soil bacterial and fungal communities, and improved the soil microenvironment in maize field after irrigation. Network analysis also suggested that the fertilization treatments reduced interactions among bacterial and fungal taxa in the microbial community, especially the combination of NPK and OF, indicating that treatment can decrease microbial competition among microbes by improving soil fertility, etc. Moreover, the redundancy analysis combined with Mantel test further revealed that soil OM, available N and P were the main soil fertility factors driving microbial community variations. Above all, the results indicated that the application of NPK combined with OF is the most appropriate method for planting, which is beneficial for regulating soil biogeochemical cycles through significantly affecting the soil C, N, and P, as well as microbial communities in maize rhizosphere soil. This study can provide important methods and data support for further research on improvements in soil quality and yield in the future, as well as the green development of agriculture.

Supplementary Materials: The following Supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13082111/s1. Table S1. The differences in the bacterial and fungal community Structures among Samples; Table S2. The relative abundance of phyla and genera (top 10) for bacteria; Table S3. The relative abundance of phyla and genera (top 10) for fungi; Table S4. The results of Monte Carlo permutation test in the RDA in the vegan package of R; Figure S1. The geographical location of the experimental field; Figure S2. Rarefaction curves of bacteria (a) and fungi (b); Figure S3. Comparison of microbial diversity indices among all four treatments; Figure S4. Shared and unique OTUs in all treatments for bacteria (a) and fungi (b); Figure S5. Results of LEfSe analysis Showing bacterial taxa that Significantly differed in the four treatments (a). Cladogram plotted from LEfSe analysis Showing the Significant differences ($p < 0.05$) in the relative abundance of bacterial taxon among four treatments (b); Figure S6. Results of LEfSe analysis Showing fungal taxa that Significantly differed in the four treatments (a). Cladogram plotted from LEfSe analysis Showing the Significant differences ($p < 0.05$) in the relative abundance of fungal taxon among four treatments (b).

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