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Effects of Returning Different Organic Materials in Combination with Inorganic Fertilizers on the Diversity of Eukaryotic Microorganisms in Semi-Arid Northern China

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Abstract: Soil eukaryotic microorganisms are important to biodiversity, and returning different kinds of organic materials to the field could improve the biodiversity of soil eukaryotic microorganisms. However, no detailed research has been conducted in the northern China semi-arid area in returning the different organic materials to the field and determining the status of eukaryotic microorganisms. Therefore, we explored the effects of various organic materials returning to the soil in combination with inorganic fertilizer on the diversity and community structure of eukaryotic microorganisms in Shanxi province, China. Soil samples were collected from five different fertilization treatments: chemical fertilizer (F), chemical fertilizer + cattle manure (FM), chemical fertilizer + straw (FS), chemical fertilizer + pig manure (FC) and control without fertilizers (CK). High-throughput sequencing was applied to analyze the eukaryotic diversity and community structure. Results showed that the dominant eukaryotic microorganisms among soil samples were Fungi, *Viridiplantae*, *Metazoa* and *Protist*. Although α-diversity was not significantly different among the five treatments, principal coordinate analysis and permutational multivariate analysis of variance illustrated significant differences ($p < 0.001$) in β-diversity of eukaryotic microorganism under treatments with different organic materials. Redundancy analysis showed that the soil properties, including total potassium, available nitrogen, available potassium and organic matter were the main factors attributed to eukaryotic microorganisms' community structure in this region.

Keywords: soil fertility; nutrients availability; Cabon sequestration

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1. Introduction

Soil microorganisms are important indicators in the characterization of soil fertility, and they play a vital role in soil material transformation and energy flow [1–3]. As an important part of soil microorganisms, eukaryotic microorganisms including fungi, protists, metazoan, etc., are diverse in size, form and function and play an important role in organic decomposition [4,5], nutrient cycling and soil structural formation of the ecosystem as primary producers, consumers and decomposers [6–9]. Previous studies have shown that soil eukaryotic microorganisms are highly sensitive to changes of soil properties, including pH, organic matter, moisture content, the presence of mineral substances, etc. [10,11]. Furthermore, studies have shown that the physical and chemical properties of soil could be largely influenced by fertilization management practices [12,13].

It is well known that fertilization is an important measure in agricultural production, which can improve soil nutrients and increase crop yield [14,15]. However, soil problems including nutrients imbalance, organic matter reduction, etc. caused by the excessive chemical fertilizer put into farmland are becoming more and more serious [12,16]. Returning organic amendments to the field can improve the soil components by increasing soil organic matter and improving soil fertility to a certain extent. It may be because organic materials themselves contain a lot of organic matter and functional microorganisms which

can effectively transform the nutrients in the soil, but many microorganisms may not be culturable. Crop straw and animal manure are the largest agricultural renewable resources and organic amendments in north China. With the promotion of ecological agriculture in China, returning crop straws, livestock and poultry manure to the field has become an important way of recycling agricultural resources [17]. These organic materials can directly or indirectly affect the soil microbial community by changing soil physical and chemical properties. Previous research on soil microbial changes under different organic modifications focused on bacteria [18–20] but neglected eukaryotic microorganisms. Thus, it is necessary to investigate the soil's eukaryotic microorganisms' community structure with different organic materials returning. Traditional identification and description of microeukaryotes is based on their morphological characteristics, but this method is only applicable to the study of specific groups and cannot reveal a relatively comprehensive diversity of eukaryotes [21,22]. The rise of molecular biology techniques has greatly improved our understanding of microbial ecology. For example, the biggest advantage of high-throughput sequencing technology is that it is culture independent, which can make up for the deficiency of traditional methods. It can generate tremendous amounts of sequence data at a fraction of the cost of earlier methods and obtain more comprehensive and reliable results [22–25], which is gradually being applied in many fields, including soil, sediments, water, etc. [26–28].

This study explored the effects of returning different organic materials to the field on the diversity of eukaryotic microorganisms in the semi-arid area of north China with high-throughput sequencing technology. The objectives of this study were as follows: (1) identify the composition and richness of the current soil eukaryotic microorganisms in north China; (2) elucidate the diversity and community structure differences of soil eukaryotic microbes with different organic materials returning; (3) and assess the drivers of environmental factors on soil eukaryotic microorganism variation in this semi-arid region.

2. Materials and Methods

2.1. Field Description and Experimental Design

This study was conducted in Jingshang Village, Shouyang County, Shanxi Province (37°45′ N, 113°12′ E, altitude 1080 m). This region belongs to the temperate continental monsoon climate zone, with an annual average temperature 8.1 °C, ≥10 °C accumulated temperature 3200 °C, frost-free period 130 days, and precipitation 474.2 mm. The experimental site has a sandy loam cinnamon soil, classified as a Calcarie–Fluvie Cambisols in the World Reference Base for Soil Rescoures (WRB) [29,30]. The soil layer is deep and thick, and the terrain is flat without groundwater supplement. The fertilization experiment started in April 2018 and has lasted for 3 years. The selected chemical properties of the basic soil tested in spring of 2018 were shown in Table 1.

Table 1. The selected chemical properties of the basic soil.

Soil Layer (cm)	pH	Organic Matter ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Total K ($g \cdot kg^{-1}$)	Alkali-Hydrolyzable N ($mg \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Available K ($mg \cdot kg^{-1}$)
0–20	8.49	11.92	1.07	0.67	23.55	88.65	8.09	104.98
20–40	8.54	7.48	0.81	0.50	24.22	54.16	6.36	75.2

Maize (*Zea mays* L.) is planted in spring around May 1st every year with a density of 72,000 plants/hm^2. Five fertilization treatments were designed: chemical fertilizer (F), chemical fertilizer + cattle manure (FM), chemical fertilizer + maize straw (FS), chemical fertilizer + pig manure (FC) and control without fertilizers (CK). The plot area was 60 m^2 (10 m × 6 m) and randomly arranged. Each treatment was repeated three times. The nitrogen was applied in the form of urea (N 46%), phosphorus was applied as calcium superphosphate (P_2O_5 12%) and potassium was in the form of potassium chloride (K_2O 60%). The nutrient contents of different organic materials were as follows: cattle manure, N 8.2 g/kg, P_2O_5 5.3 g/kg, K_2O 17.1 g/kg; maize straw, N 7.5 g/kg, P_2O_5 4.2 g/kg, K_2O

13.3 g/kg and pig manure, N 8.8 g/kg, P_2O_5 15.3 g/kg, K_2O 12.4 g/kg. All fertilizers were evenly distributed over the plots before sowing, and the specific amount of nutrients input was shown in Table 2.

Table 2. Experimental treatments and fertilization with different organic materials.

Treatment	Chemical Fertilizer (kg/hm^2)			Organic Fertilizer (kg/hm^2)			Total Nutrient (kg/hm^2)		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
F	225	75	75	0	0	0	225	75	75
FM	225	75	75	70	45	145	295	120	220
FS	225	75	75	45	25	80	270	100	155
FC	225	75	75	60	105	85	285	180	160
CK	0	0	0	0	0	00	0	0	0

Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

2.2. Soil Sampling

Five bulk soil samples of 0~20 cm between maize plants were collected from each subplot by "S" method with a soil auger and mixed into one on 11 July 2021, so a total of fifteen soil samples were obtained. After screening through 2 mm sieves to remove stones and debris, samples were put in sterilized bags and transported to the laboratory with an icebox. One part was temporarily stored in a −80 °C refrigerator for DNA extraction and further sequencing. The other part was screened through 1 mm and 0.15 mm sieves respectively after natural air drying for soil properties analysis.

2.3. Soil Physicochemical Analytical Procedures

Generally, soil samples screened over 1 mm were used for detecting pH and available nutrients, while soil samples screened over 0.15 mm were used for detecting organic matter and total nutrients. The detection methods are described as follows:

pH was measured with a pH meter and the soil-water ratio was 1:2.5. Organic matter (OM) was extracted with $K_2Cr_2O_7$ and determined by titration. Total nitrogen (TN) was analyzed by semi-trace Kjeldahl method. Available nitrogen (AN) was determined by alkali-hydrolytic diffusion method. Total phosphorus (TP) and available phosphorus (AP) were extracted by NaOH and $NaHCO_3$, respectively, and determined by molybdenum-antimony resistance colorimetry. Total potassium (TK) and available potassium (AK) were extracted by HNO_3-HClO and NH_4COOCH_3, respectively, and determined by flame spectrophotometer. All of the specific procedures can be found in the reference [31].

2.4. Soil DNA Extraction and High-Throughput Sequencing

A soil sample of 0.5 g was accurately weighed, and soil genomic DNA was extracted using a Fast DNA SPIN Isolation Kit (MP Biomedicals, Santa Ana, CA, USA) according to the kit manufacturer's instructions. Upstream primer 547F (5′-CCAGCASCYGCGGTAATTCC-3′) and downstream primer 952R (5′-ACTTTCGTTCTTGATYRA-3′) [32,33] were used to amplify the V4 region of eukaryotic 18S rRNA with a TransFast TaqDNA Polymerase Kit (TransGen, Beijing, China). After accessing the quantity and quality with a Nanodrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA) and 0.75% agarose gel electrophoresis, the DNA was used to conduct the PCR. The PCR reaction system contained: 5 × reaction buffer 5 µL, 5 × GC buffer 5 µL, 2.5 mmol/L dNTPs 2 µL, 10 µmol/L primers 1 µL, respectively, DNA template 2 µL, 5 U/µL Taq enzyme 0.25 µL, ddH_2O 8.75 µL and total volume 25 µL. PCR reaction conditions was: 98 °C for 2 min; 98 °C 15 s, 55 °C 30 s, 72 °C 30 s, for 30 cycles; and 72 °C for 5 min. After purification with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA), the target DNA fragment was stored in a refrigerator at −20 °C and used for high-throughput sequencing based on Illumina Miseq platform at Per-

sonalbio Technology Co., LTD (Shanghai, China). Raw sequence data files were deposited in the NCBI Sequence Read Archive database (Accession numbers: PRJNA827283).

2.5. Statistical Analysis

2.5.1. Bioinformatics Analysis

The operational taxonomic unit (OTU) table for subsequent analysis was obtained after quality control, merger and redundancy removal of the original data. Sequence data analyses were mainly performed using QIIME2 dada2 analysis process and R (version 3.1.2, vegan package) [34]. Alpha-diversity metrics including Chao1, Observed_species, and Shannon and Simpson indices were calculated based on the OTU table using QIIME2. The formulas were as follows:

$chao1 = S_{obs} + \frac{F_1(F_1-1)}{2(F_2+1)}$, where F_1 and F_2 were the count of singletons and doubletons, respectively;

$H = -\sum_{i=1}^{s} (p_i \log_2 p_i)$, where s was the number of OTUs, and p_i was the proportion of the community represented by OTU i;

Simpson's index = $1 - \sum pi^2$ where p_i was the proportion of the community represented by OTU i.

"VennDiagram" and "grid" packages of R were used to draw Venn diagrams for different treatments of eukaryotic microorganisms OTU. The "ggplot2", "vegan" and "plyr" program packages were used to conduct principal coordinate analysis (PCoA) of OTU among different treatments [35], and permutation multiple variance analysis (PERMANOVA) was used to evaluate the significance of differences in OTU composition between groups [36]. The "barplot" function of R was used to draw species composition of eukaryotic microorganisms at phylum and genus level. Redundancy analysis (RDA) was performed to identify relationships between phylum-level eukaryotic microorganisms and soil physicochemical parameters using the CANOCO 4.5 software.

2.5.2. Significance Analysis

The data of soil chemical factors, α-diversity and species abundance among different treatments collected had been tested for normality and homogeneity of variance using the "T-test". The differences were evaluated by a one-way analysis of variance (ANOVA) using Software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) method was used to analyze the significance of differences among data at 95% confidence level.

3. Results

3.1. Soil Physicochemical Characteristics

At the beginning of the experiment, the soil organic matter content was 11.82 g/kg, which was at a low level (Table 1). Fertilization for three consecutive years generally improved the soil fertility and reduced the soil pH (Table 3). Among them, OM, TP and AP were increased in the treatments FM, FS and FC with organic materials returning to the field, and the effect was better than that of F treatment with single application of chemical fertilizer.

Table 3. Soil chemical properties with different organic materials.

Treatment	pH	Organic Matter ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Total K ($g \cdot kg^{-1}$)	Alkali-Hydrolyzable N ($mg \cdot kg^{-1}$)	Available P ($mg \cdot kg^{-1}$)	Available K ($mg \cdot kg^{-1}$)
F	8.28 ± 0.01 a	10.56 ± 0.99 d	1.02 ± 0.01 a	0.71 ± 0.01 b	21.48 ± 1.28 c	91.33 ± 7.95 c	9.62 ± 1.11 d	75.88 ± 2.23 e
FM	8.10 ± 0.02 b	13.03 ± 1.02 a	1.08 ± 0.01 a	0.83 ± 0.01 ab	23.07 ± 1.40 a	103.57 ± 9.91 a	21.18 ± 2.14 b	156.63 ± 9.92 a
FS	8.25 ± 0.01 a	11.17 ± 1.23 c	0.98 ± 0.01 a	0.86 ± 0.02 a	22.49 ± 1.22 b	78.75 ± 1.19 e	11.25 ± 1.82 c	83.94 ± 8.00 c
FC	8.27 ± 0.03 a	12.17 ± 0.90 b	1.08 ± 0.02 a	0.72 ± 0.02 b	20.76 ± 1.99 d	97.20 ± 5.81 b	31.92 ± 2.18 a	113.02 ± 5.98 b
CK	8.31 ± 0.01 a	11.13 ± 0.75 c	0.95 ± 0.01 a	0.58 ± 0.01 c	18.00 ± 1.20 e	87.83 ± 3.27 d	7.87 ± 1.90 e	82.53 ± 2.59 d

Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers. The different lowercase letters after numbers mean significant differences between treatments ($p < 0.05$).

Compared with CK, fertilization generally increased the contents of soil nutrients. However, OM and AK in treatment F were significantly lower than in that of CK. Apart from that, AN in treatment FS was significantly lower than in that of CK. Compared with F, organic material returning to the field significantly increased the contents of OM, AP and AK by 5.8%~23.4%, 16.9%~231.8% and 10.6%~106.4%, respectively. In the three treatments with organic materials, OM, TK, AN and AK were significantly higher in treatment FM than the other three treatments. On the contrary, pH was significantly lower in FM treatment. AP was the highest in treatment FC.

3.2. Alpha-Diversity of Eukaryotic Microorganisms

A total number of 939,615 high quality sequences were generated with an average length of 380 bp and 17,198 OTU were identified. In order to comprehensively assess the alpha-diversity of eukaryotic microorganisms, Chao1 and Observed Species indices were used in this process to characterize the richness, and diversity was characterized by Shannon and Simpson indices. As shown in Table 4, indices of Chao1 and Observed_species were the highest in treatment F and indices of Shannon and Simpson were the highest in treatment FC while the lowest in treatment FS. However, no significant differences were observed among the five treatments, which showed that returning different organic materials to the field had little effect on α-diversity of eukaryotic microorganisms.

Table 4. Eukaryotic microorganisms α-diversity under different treatments.

Treatment	Chao1	Observed_Species	Shannon	Simpson
F	1302.99 ± 93.29 a	1215.77 ± 97.41 a	7.0417 ± 0.8380 a	0.9610 ± 0.0315 a
FM	1227.52 ± 176.91 a	1167.53 ± 147.06 a	6.8935 ± 0.6906 a	0.9418 ± 0.0451 a
FS	1031.93 ± 195.72 a	968.43 ± 162.85 a	6.3081 ± 0.4664 a	0.9420 ± 0.0171 a
FC	1275.53 ± 177.55 a	1211.47 ± 167.38 a	7.2898 ± 0.5858 a	0.9715 ± 0.0148 a
CK	1218.75 ± 21.89 a	1161.00 ± 6.88 a	7.2049 ± 0.1519 a	0.9708 ± 0.0110 a

Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.The different lowercase letters after numbers mean signficant differences betwee treatments ($p < 0.05$).

3.3. Composition of Eukaryotic Microbial Community under Different Treatments

The number of shared and specific OTUs were showed in Figure 1. There were 384 shared OTUs among the five different treatments, accounting for 5.89% of the total. The specific OTU numbers of treatments F, FM, FS, FC and CK were 1045, 983, 629, 1105 and 842, respectively, which were significantly different. Compared with CK, fertilization generally increased the number of specific OTUs except the treatment FS. The number of specific OTUs in FC treatment was increased by 5.7% compared with treatment F. On the contrary, the numbers of specific OTUs in FM and FS treatments were decreased compared with treatment F by 5.9% and 4.0%, respectively. These results indicated that there were quite significant differences in the sequence alignment of eukaryotic microorganisms in soil with different organic materials returning.

3.4. Taxonomic Composition Analysis at the Phylum and Genus Level

The top ten eukaryotic microorganisms at phylum level belonged to four kingdoms: Fungi (*Ascomycota*, *Mucoromycota*, *Basidiomycota*, *Chytridiomycota*), *Viridiplantae* (*Streptophyta*, *Chlorophyta*), *Metazoa* (*Nematoda* and *Arthropoda*) and *Protist* (*Apicomplexa* and *Dinophyceae*), accounting for 75.5%~90.8% of the total phyla (Figure 2). Thus, fungi comprised the main group of eukaryotic microorganisms in soil of this region. *Ascomycota* was significantly higher in treatment FS than in treatments FM, FC and CK by 90.3%, 204.6% and 159.4%, respectively. *Basidiomycota* was significantly higher in treatment F than in treatments FM, FS, FC and CK by 1537.1%, 6.6%, 31.1% and 99.3%, respectively. *Streptophyta* was significantly higher in treatment CK than in treatments F and FM by 425.6% and 881.4%, respectively.

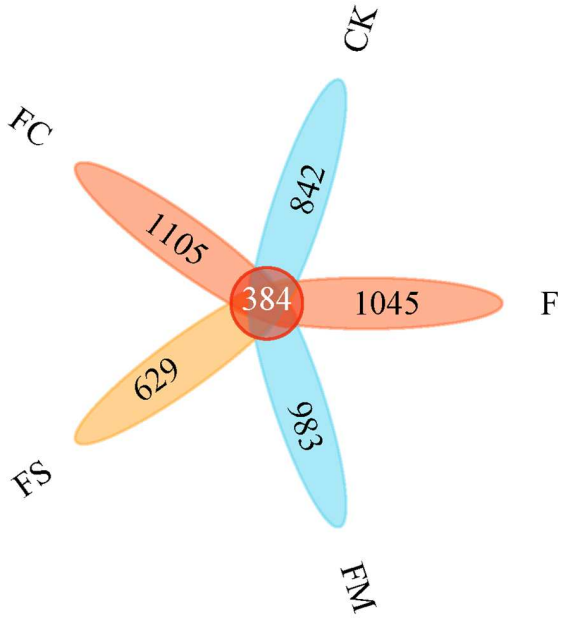

Figure 1. Venn digram of eukaryotic microorganisms OTU under different treatments. Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

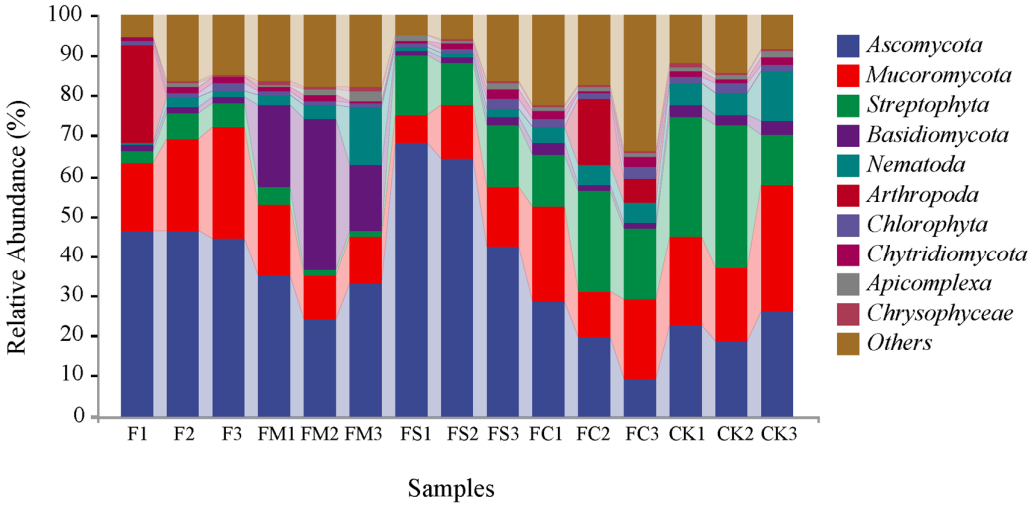

Figure 2. Relative abundance of eukaryotic microorganisms at phylum level in different samples. Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

The top ten eukaryotic microorganisms at genus level were *Mortierella*, *Fusarium*, *Gamsiella*, *Pratylenchus*, *Atopochthonius*, *Glomus*, *Septoglomus*, *Rhogostoma*, *Rhizophagus* and *Oxyrrhis*, averagely accounting for 24.3% of the total (Figure 3). Among which, *Glomus*, *Septoglomus* and *Rhizophagus* belonged to AM fungus. Compared with chemical fertilizer alone (F treatment), organic materials returning (FM, FS, FC treatments) and no fertilizer (CK treatment), both reduced the relative abundances of these three genera, but the differences were not significant between groups.

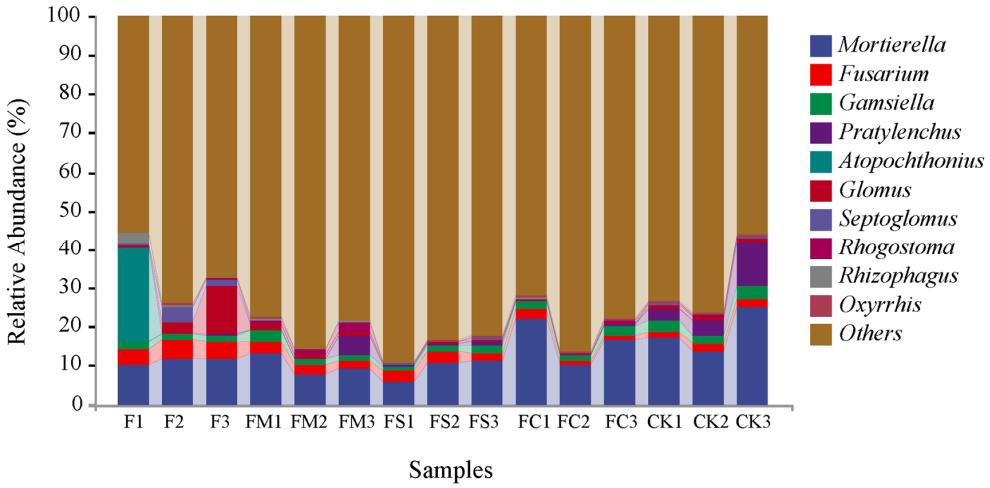

Figure 3. Relative abundance of eukaryotic microorganisms at genus level in different samples. Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

3.5. Beta-Diversity of Eukaryotic Microorganisms

PCoA was carried out to analyze the difference of β-diversity of eukaryotic microorganisms among different treatments. The two main coordinates together explained 45.2% of the variance, with the first axis explaining 25.8% and the second explaining 19.4% (Figure 4). The three samples of the same treatment clustered together, indicating good parallelism. The obvious boundary between samples of different treatments indicated that different organic materials had an effect on the eukaryotic microorganisms' community. PERMANOVA further showed that the difference was significant ($p < 0.001$) (Figure 5).

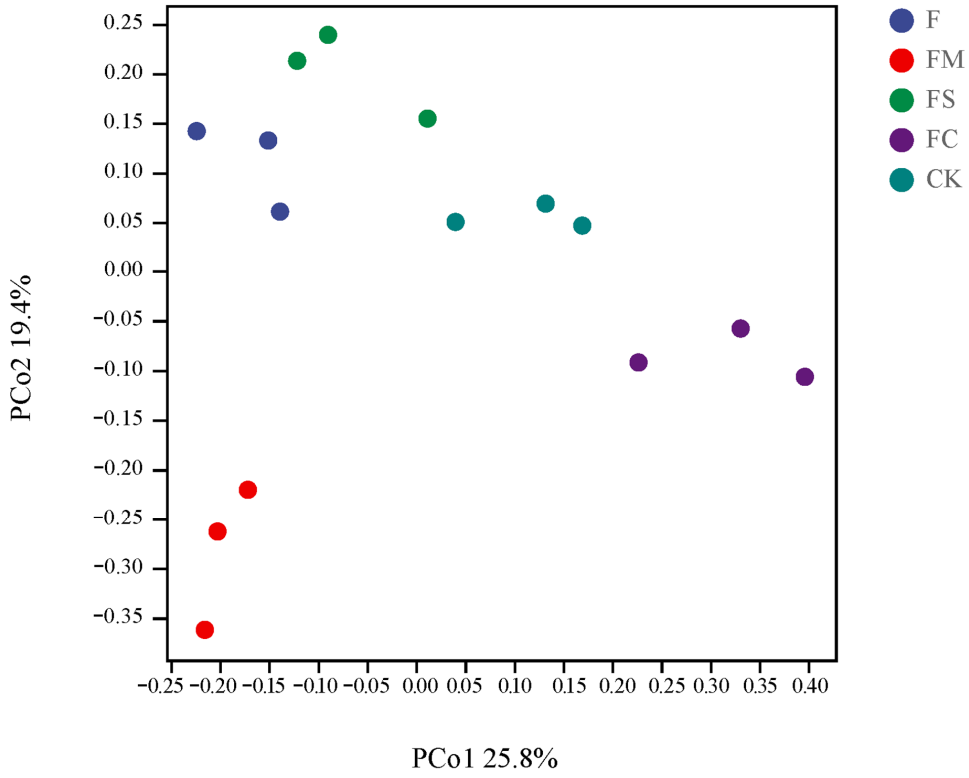

Figure 4. PCoA of eukaryotic microorganisms among different treatments. Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

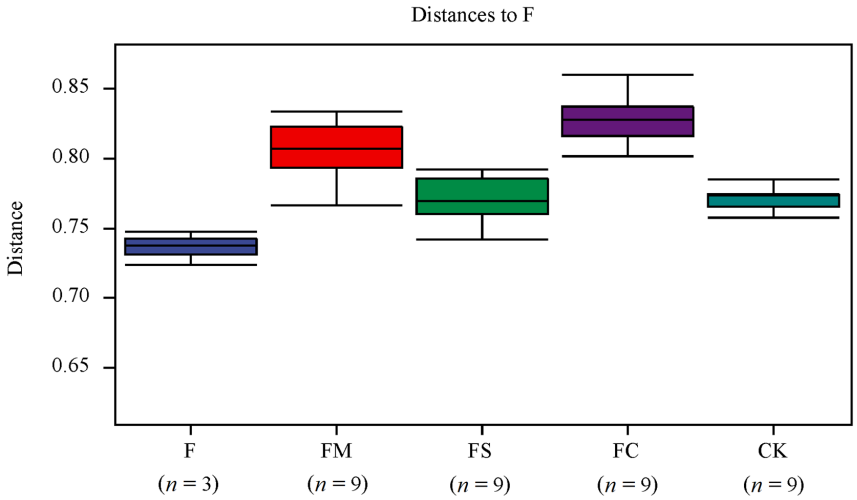

Figure 5. PERMANOVA between groups. Note: F: chemical fertilizer; FM: chemical fertilizer + cattle manure; FS: chemical fertilizer + maize straw; FC: chemical fertilizer + pig manure; CK: control without fertilizers.

3.6. Correlations

RDA was conducted to analyze the relationship between the top ten phylum-level eukaryotic microorganisms and soil properties. As shown in Figure 6, the first two axes together explained 60.3% of the total variation in eukaryotic microorganisms' composition, 36.5% for the first axis and 23.8% for the second axis. TK (F = 4.33, p = 0.016), AN (F = 4.188, p = 0.012), AK (F = 3.848, p = 0.012) and OM (F = 3.518, p = 0.018) were statistically significant physicochemical parameters that were associated with eukaryotic microorganisms' community composition (based on 499 Monte Carlo permutations). The contribution rate

of eight environmental factors to eukaryotic microorganism variation was in the following order: TK > AN > AK > OM > TP > pH > AP > TN.

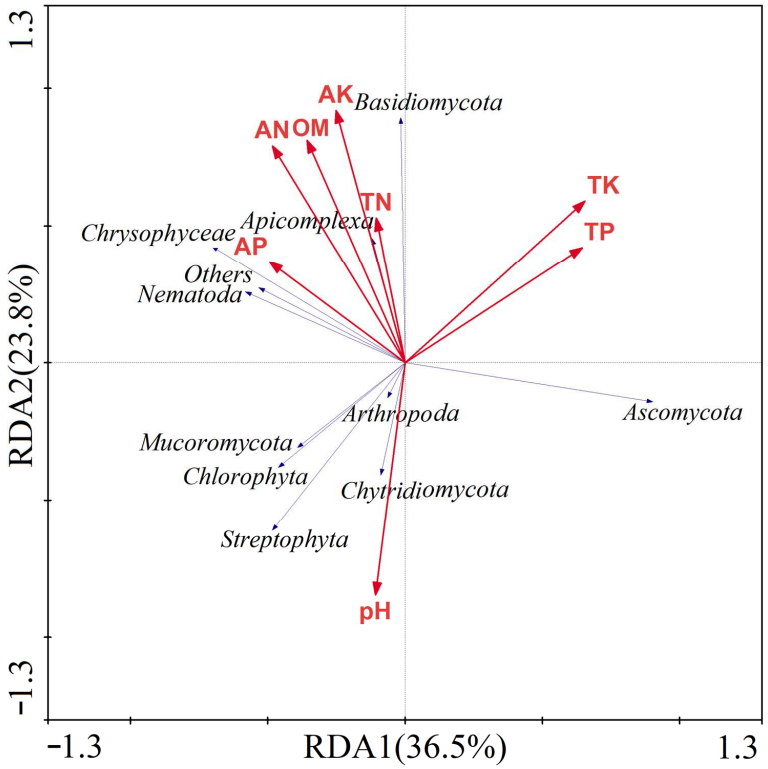

Figure 6. RDA between the dominant eukaryotic microorganisms and soil properties.

4. Discussion

4.1. Effects of Treatments on the Soil Physicochemical Properties

In the present study, fertilization improved most of the soil nutrition contents including TN, TP, TK and AP compared with treatment CK but lowered soil pH, which might be caused by hydrolyzed nitrate-releasing H^+ of the amide nitrogen fertilizer urea applied to the soil [37,38]. Compared with treatment F (the chemical fertilizer), returning organic amendments to the field also decreased soil pH, which might be due to the increase of organic acids during the decomposition process of organic matter [39]. No significant differences of pH were observed in these treatments apart from treatment FM, which might be due to the fact that calcareous soils contain higher carbonates to buffer soil pH [40].

Fertilization generally increased soil nutrients, but compared with CK, F treatment with fertilizer significantly reduced soil OM and AK, which might because chemical fertilizer input increased crop yields, consuming more organic matter and removing more potassium. Moreover, AN in treatment FS was significantly lower than in that of CK. This might be caused by the degradation of straw requiring the consumption of nitrogen in the soil. AP was the highest in treatment FC because pig manure contains the most phosphorus.

4.2. Effects of Different Treatments on the Eukaryotic Microorganisms Diversity and Community Composition

A straw composting experiment showed that the dominant eukaryotic microorganisms were *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Algae* [41]. The main eukaryotic microorganisms in a compost made from cow manure and straw were *Ascomycota* (40.2%), *Zygomycota* (20.3%), *Algae* (2.3%), *Basidiomycota* (1.5%) and *Protist* (0.4%) [42]. In this study, Fungi, *Viridiplantae*, *Metazoa* and *Protist* were the dominant soil eukaryotic microbial groups, which showed that the diversity of eukaryotic microorganisms was affected by different habitats. Fungi including *Ascomycota*, *Mucoromycota*, *Basidiomycota*, *Chytridiomycota*, etc.

had the highest relative abundance, and were widely found in terrestrial and aquatic ecosystems [43,44]. *Glomus*, *Septoglomus* and *Rhizophagus* belong to *Glomeromycota* and are classified as arbuscular mycorrhizal fungi (AMF), which could provide abundant nutrients for crops and promote their growth. *Nematoda* and *Arthropoda* dominated the *Metazoa*, which was similar to previous studies [45–47]. Previous research showed that the SAR supergroup and *Metazoa* were the dominant soil eukaryotic groups in the forest soil habitat [43], indicating that the soil eukaryotic microbes were closely related to different soil habitat types. Although sequencing results have elucidated that the dominant compositions of eukaryotic microorganisms among soil samples under different treatments were roughly similar, PCoA and PERMANOVA illustrated significant differences ($p < 0.001$) in β-diversity of eukaryotic microorganisms' community structure under treatments with different organic materials to the field. This is consistent with the results of Wang et al., who documented a clear separation of soil fungi under different fertilization modes [48].

Fertilization directly affected the soil's eukaryotic microorganisms' community structure by changing nutrients in the soil [49,50]. Additionally, the change of soil pH caused by fertilization had an indirect effect on the eukaryotic microorganisms' community structure as many eukaryotic groups exhibited distinct preferences for specific edaphic pH, or soil pH affected the host of some eukaryotic microbes [51–53].

4.3. Relationship between Soil Eukaryotic Microorganisms and Environmental Factors

The composition of the eukaryotic microorganisms' community was influenced by its microenvironment, including soil properties. In this study, we found that TK, AN, AK and OM were the main factors attributed to eukaryotic microorganisms' community structure in this region through the RDA analysis. This might be due to eukaryotic microorganisms mainly participating in the decomposition of organic materials, which contained a lot of potassium. Previous studies have also found that available potassium and total potassium content played an important role in regulating the soil fungal community structure [48,54]. In addition, carbon, nitrogen and their proportions were requisite factors and major nutrients supporting eukaryotic microorganisms' growth and reproduction [52].

5. Conclusions

Returning organic materials to the field generally improved soil fertility, and then significantly affected the β-diversity of eukaryotic microorganisms ($p < 0.001$). The dominant eukaryotic microorganisms at phylum level were *Ascomycota*, *Mucoromycota*, *Streptophyta*, *Basidiomycota*, *Nematoda*, *Arthropoda*, *Chlorophyta*, *Chytridiomycota*, *Apicomplexa* and *Dinophyceae*. RDA indicated that soil potassium, carbon and nitrogen were main environmental factors attributed to eukaryotic microorganisms' community structure. As an important agricultural resource, eukaryotic microorganisms play an important role in soil carbon cycling and plant nutrition. The input of organic fertilizers contributes to the maintenance of eukaryotic microbial diversity and the improvement of soil fertility. Treatment FC with pig manure returning had the highest Shannon and Simpson diversity indices and most unique OTU number, which showed that pig manure was the best organic material to improve soil eukaryotic microbial diversity.

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