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Effects of Continuous Manure Application on the Microbial Community and Labile Organic Carbon Fractions

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Abstract: The application of organic materials contributes to the sustainable development of agriculture. Increased manure inputs have a fundamental effect on the composition and dynamics of soil organic carbon (SOC). In this study, we conducted a 10-year field experiment in Changchun, Jilin, Northeast China, to investigate the effects of manure addition on soil organic carbon components and soil microorganisms. Specifically, we established four treatments: (i) chemical fertilizer or no addition of manure (CK), (ii) pig manure with chemical fertilizer (ZF), (iii) cow manure with chemical fertilizer (NF), and (iv) chicken manure with chemical fertilizer (JF). The results showed that the JF treatment significantly increased the soil organic carbon (SOC), dissolved organic carbon (DOC), and readily oxidized organic carbon (ROC) content by 20.36%, 105.9%, and 61.32%, respectively, relative to CK. The microbial biomass carbon (MBC) content in JF, ZF, and NF treatments were significantly higher than that of CK, which increased by 107.24%, 116.45%, and 96.71%, respectively. The particulate organic carbon (POC) content in NF and JF treatments differed significantly, increasing by 25.61% and 19.01%, respectively, relative to CK. Redundancy analysis showed that continuous manure application had a positive effect on soil microbial community diversity and abundance, which was favorable for the accumulation of soil carbon. We also found that soil fungi were more sensitive than bacteria to changes in soil carbon composition following manure application. In conclusion, adding different organic materials can better support biodiversity conservation and realize ecosystem services of surface carbon storage and soil conservation. Our results reveal the importance of microbial fixation in soil carbon dynamics according to the different distribution of active organic carbon pools, which will help enhance our understanding of the carbon cycle.

Keywords: Mollisols; manure; organic carbon; soil microbial diversity

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1. Introduction

Soil is the largest reservoir of organic carbon in terrestrial systems and plays a crucial role in the carbon cycle. Soils contain a rich diversity of taxa and is an important biological treasure trove [1]. Soil organic carbon (SOC) is the predominant component of the organic carbon pool in terrestrial ecosystems and has significant impacts on improving soil fertility, moderating climate change, and strengthening ecosystem sustainability [2,3]. SOC in croplands is essential for soil fertility, ensuring crop production and food security [4–6]. The net SOC stock is determined by the balance between organic carbon inputs and carbon effluxes through microbial decomposition [7]. Agricultural practices enormously affect cropland SOC, particularly in the topsoil, by directly altering organic carbon inputs and indirectly modifying the environmental conditions for microbes [8–10].

Increasing organic material inputs to enhance soil organic carbon (SOC) sequestration in farmland is an important means of maintaining and enhancing soil fertility, regulating ecosystem carbon cycling, and mitigating global climate change [11,12]. Soil stores a large amount of carbon, and enhancing soil carbon sequestration capacities can provide a viable answer for retarding global climate [13]. Organic carbon in soils mainly comes from plants and microorganisms, and the stabilization and preservation of soil organic carbon are affected by different sources due to the varying retention times of the two in the soil [14].

The addition of manure can increase soil organic carbon content. However, the effects of added manure on the native organic carbon in the soil and the driving mechanisms behind these effects are not well understood [15,16]. Therefore, this experiment aims to explore the abiotic and biotic driving mechanisms of soil organic carbon processes through the application of different manure fertilizers. The conversion of organic material inputs into stable soil organic carbon is a pivotal ecological process, but little is known about it. This process affects soil carbon stocks, nutrient effectiveness, net primary productivity, and ecosystem sensitivity to global change [17–19]. The largest and slowest cycling organic carbon pools are predominantly microbial products stabilized by binding to soil minerals, suggesting that microbial production mediates the transfer of organic material inputs to mineral-associated organic carbon pools [20]. In a previous study, it was revealed that long-term chemical and organic fertilization had distinct influences on the microbial functional traits responsible for soil carbon and nitrogen cycling in the Mollisols of Northeast China [21].

The synergistic relationship between soil organic matter accumulation and the evolution of soil structure and biological communities is a novel area of focus in soil science today. To elucidate the effects of long-term application of different manures on the characteristics of organic carbon accumulation and changes in microbial dynamics in Mollisols, we established a long-term field experiment on black soils in Northern China. We compared the 10-year cyclic application of manure with a single application of chemical fertilizer under different environmental conditions, aiming to demonstrate the key processes and mechanisms of manure return to the field to promote organic matter accumulation in black soils. We compared and evaluated manure-induced differences from a microscopic point of view to provide a theoretical basis for the diagnostic application of manure and to determine the differences in long-term soil fertilization with different manures. We also investigated the relationship between bacterial and fungal taxa and labile organic carbon fractions. In this investigation, we sought to answer two research questions: (1) How do continuous applications of different manures differ in their effects on soil SOC? (2) Which bacterial and fungal taxa are closely associated with active carbon?

2. Materials and Methods

2.1. Site Description

This study was conducted at a long-term fertilizer experiment station (43°82′ N, 125°41′ E) established in 2010 in Changchun, Northeast China. The experimental site has a temperate continental monsoon climate, with a mean annual air temperature of 4.6 °C and a mean annual precipitation of 600 mm. Approximately 60% of the precipitation occurs during the months from June to August. The frost-free period lasts about 145 days, and the temperature difference between day and night is large. Autumn is short, spring is long, summer is warm, and winter is cold. The growing season is from early May to mid-October, while the dormant season is from mid-October to early May.

The soils at this test site are classified as Mollisols according to the U.S. [22] Soil Classification (also known as black soil in Chinese Soil Classification). Table 1 shows the basic physical and chemical properties of the initial soil before the test was established. Soybean (*Glycine max*) is the crop grown, and fertilizers are applied annually to the soil surface of the field plots before sowing (end of April). The organic material is applied at the bottom of the furrows of the ridges, covered with a thin layer of compacted soil, and then planted in May of each year.

Table 1. Basic physical and chemical properties of soil. Note that values (± standard deviation) were averaged over three replicates that were the same as below.

Soil	pH	SOC ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)	Sand (%)	Silt (%)	Clay (%)
Mollisol	6.76 ± 0.19	19.37 ± 0.43	1.13 ± 0.08	33.91 ± 1.12	93.12 ± 1.84	16.27 ± 0.05	68.19 ± 0.92	15.54 ± 0.32

2.2. In-Situ Experiment

The experimental plots were 25 m^2 in size, with eight rows per plot, with a spacing of 0.65 m and a length of 5 m. Each treatment was provided with three replications in a randomized block arrangement. To reduce potential edge effects, a buffer zone was placed around each plot. Four manure treatments with three replications were selected in this study for practical comparison: (i) control with chemical fertilizers alone (CK); (ii) pig manure + chemical fertilizers (ZF); and (iii) cow dung + chemical fertilizers (NF); (iv) chicken manure + chemical fertilizers (JF). The application rate of the fertilizers in the plot was 120 kg hm^{-2} of diammonium phosphate and 90 kg hm^{-2} of potassium chloride. Organic materials were applied in successive years according to the principle of equal carbon, including chicken manure (4931 kg hm^{-2}), pig manure (4402 kg hm^{-2}), and cow manure (3916 kg hm^{-2}). The pig manure and cow manure used in this experiment were obtained from the farm of College of Animal Science and Technology, Jilin Agricultural University; and the chicken manure was obtained from Liaoyuan Xinrong Bio-fertilizer Technology Co. Chicken manure, pig manure, and cow manure were all rotted manure. The nutrient contents of the organic materials are shown in Table 2.

Table 2. The major properties of the organic materials.

Material	pH	Organic Carbon ($g{\cdot}kg^{-1}$)	Total N ($g{\cdot}kg^{-1}$)	Total P ($g{\cdot}kg^{-1}$)	Total K ($g{\cdot}kg^{-1}$)	C/N
pig manure	7.04 ± 0.12	267.93 ± 0.48	21.14 ± 0.14	7.05 ± 0.18	8.32 ± 0.13	12.67 ± 0.21
cow manure	7.25 ± 0.08	175.41 ± 0.43	13.89 ± 0.14	3.62 ± 0.12	8.37 ± 0.09	12.63 ± 0.23
chicken manure	8.06 ± 0.31	242.45 ± 0.51	17.05 ± 0.19	8.81 ± 0.23	14.11 ± 0.22	14.21 ± 0.13

2.3. Soil Sampling

The test soil samples for this experiment were collected after crop harvest (September–October) in 2009 (initial sample), 2014, and 2019; and the topsoil layer of 0–20 cm from different treatments was sampled in sterile bags using the S-shaped five-point method. Fresh soil samples were transported to the laboratory in ice packs as soon as possible, visible animal and plant debris and stones were removed, one part of the collected soil samples was stored at −80 °C for DNA extraction for high-throughput sequencing, and the other part of the soil samples used for soil physicochemical property analysis was divided into two parts: one part of the soil samples was naturally air-dried at room temperature for the soil reactive organic carbon analysis, and the other part of the soil samples was kept wet at 4 °C for an analysis of the microbiological carbon (MBC). Historical soil samples were collected in 2009, 2014, and 2019, using the same methods described above.

2.4. Soil Properties

The composite electrode measured the soil pH using a soil/water ratio of 2.5. Total nitrogen (TN) was determined with the Kjeldahl digestion method. Soil organic carbon (SOC) was measured using the $K_2Cr_2O_7$-volumetric method [23]. Total labile organic carbon (LOC) was composed of dissolved organic carbon (DOC), soil microbial biomass carbon (MBC), particulate organic carbon (POC) and readily oxidizable organic carbon (ROC). Dissolved organic carbon (DOC) was obtained via 0.5 mol L^{-1} K_2SO_4 [24]. The soil microbial biomass carbon (MBC) was measured with chloroform fumigation [25].

Particulate organic carbon (POC) was determined by $(NaPO_3)_6$ [26], and readily oxidizable carbon (ROC) was measured with 333 mol L^{-1} $KMnO_4$ [27].

2.5. Microbial Community Structure and Diversity

Soil DNA was extracted from 0.5 g of the fresh soil sample using a Fast DNA SPIN Kit (Irvine, CA, USA) for soils, according to the manufacturer's instructions. The DNA was quantified on a NanoDrop spectrometer and was stored at −80 °C before use. To amplify the V3-V4 region of the bacterial 16S rDNA gene and the internal transcribed spacer 1 (ITS1) region of fungal genes, forward and reverse primers, respectively, were selected. PCR products were collected with an AxyPrep DNA Gel Extraction Kit and quantified with a Quanti FluorTM-ST Fluorometer and then equivalently pooled together for IIumina Miseq sequencing. The 16S rDNA and ITS1 gene fragments were sequenced using the Miseq sequencing platform. The QIIME 2 (qiime2.org.com, accessed on 20 Match 2022) package was used for α and β diversity analysis.

2.6. Statistical Analysis

SPSS 26.0 (IBM Statistics 26.0) was used for data statistical analysis. Graphs were compiled using origin 2022 software (OriginPro 2022, OriginLab Corporation, Northampton, MA, USA). The significant differences of the dependent variables were determined with a one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test, with statistically significant difference defined as $p < 0.05$. Redundancy analysis (RDA) was performed using CANOCO 5.0 (Canoco 5 Application, Microcomputer Power Inc., Ithaca, NY, USA) to analyze the relationships between activated carbon fraction and bacterial taxa.

3. Results

3.1. Changes in Total Soil Organic Carbon

At year 0, the soil organic carbon (SOC) content did not show significant differences among the four treatments. However, from year 5 to 10, there were significant differences observed in SOC content among the treatments (Figure 1). All livestock manure treatments showed an increase in organic carbon content compared to the control treatment (CK) during each period, with the chicken manure treatment (JF) consistently showing the highest values, significantly higher than CK. Over time, the difference in SOC content between the CK treatment and the others became significant by the tenth year, while it was not significant by the fifth year.

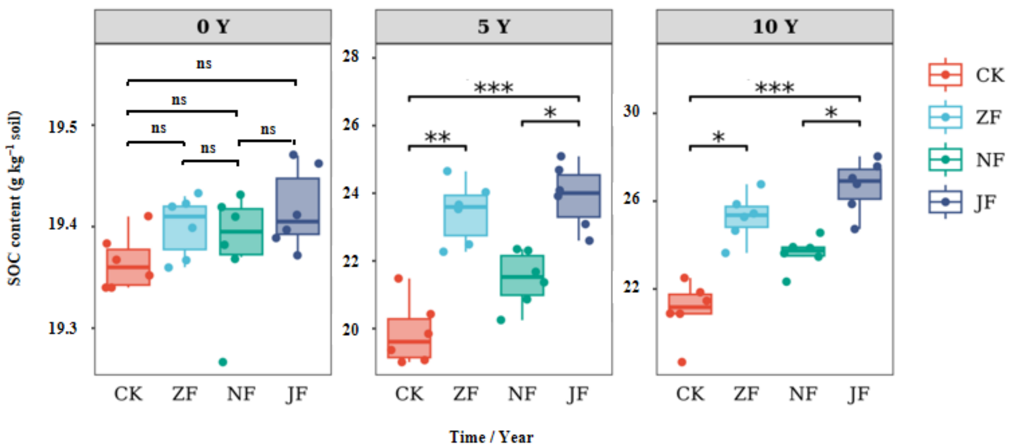


Figure 1. Soil organic carbon content of the four different treatments during the 10-year experimental period. The p value between two variables is indicated by the symbol on the figure. The number of "*" indicates the degree of significance. For example, "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$, and "ns" means no significance. CK, blank control treatment; ZF, pig manure + chemical fertilizers; NF, cow dung + chemical fertilizers JF, chicken manure + chemical fertilizers.

After 5 years of application, the SOC content in the JF, ZF, and NF treatments were significantly higher than that of CK, with increases of 20.36%, 17.97%, and 8.07%, respectively. After 10 years of continuous application, the JF and ZF treatments showed significant increases of 26.79% and 20.14%, respectively, compared to CK. The SOC content in the JF treatment remained the highest, significantly higher than that of ZF and NF treatments. Specifically, the organic carbon content in the JF treatment increased by 5.54%, 12.89%, and 26.79% compared to ZF, NF, and CK, respectively. The differences between each livestock and poultry manure treatment and the CK treatment were found to be significant.

3.2. Changes in Labile Organic Carbon Fractions

3.2.1. Changes in Dissolved Organic Carbon

In the 10-year field experiment, the dissolved organic carbon (DOC) content in the CK, ZF, NF, and JF treatments showed a similar changing trend (Figure 2). From year 0 to 10, the DOC content of each treatment increased. After 5 years of application, the DOC content in the JF, ZF, and NF treatments was significantly higher than that of CK, with increases of 105.90%, 65.28%, and 85.07%, respectively. After 10 years of continuous application, the JF treatment had the highest DOC content, and the difference in DOC content between the ZF and NF treatments and the JF treatment was significant. The DOC content in the CK treatment showed an increasing and then decreasing trend with the prolongation of experimental time.

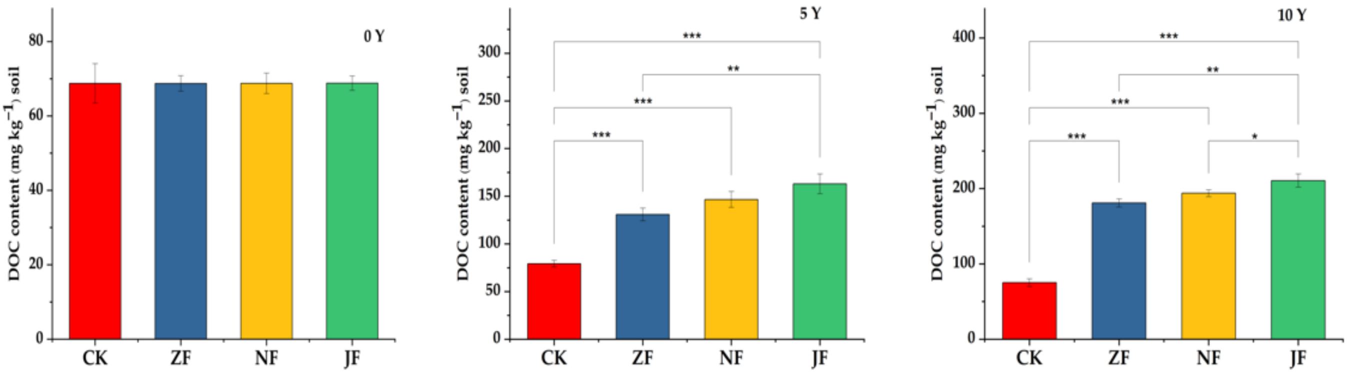


Figure 2. Effects of CK, ZF, NF, and JF on soil dissolved organic carbon (DOC) concentrations in the 0–20 cm soil depth. Each bar represents the mean ± standard deviation in the figure (n = 3). "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$.

3.2.2. Changes in Readily Oxidizable Organic Carbon

Between year 0 and 10, the recalcitrant organic carbon (ROC) content of all the treatments showed an increasing trend (Figure 3). After 5 years of application, the ROC content in the JF, ZF, and NF treatments was significantly higher than that of CK, with increases of 61.32%, 41.24%, and 52.70%, respectively, and the differences between the JF and ZF and NF treatments were highly significant. After 10 years of continuous application, the JF treatment had the highest ROC content, with significant differences among treatments. With the prolongation of the experimental time, the ROC content in the CK treatment showed a tendency to increase and then decrease.

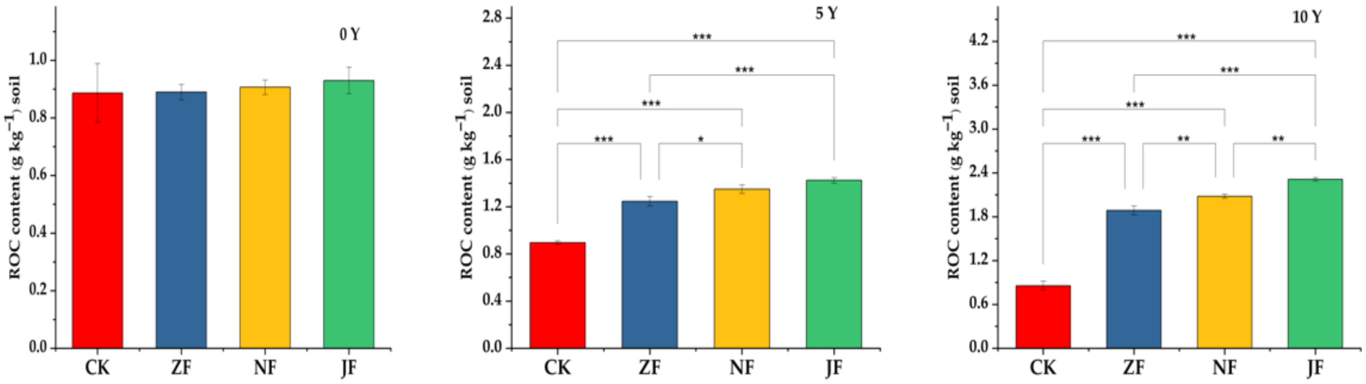


Figure 3. Effects of CK, ZF, NF, and JF on soil readily oxidizable carbon (ROC) concentrations in the 0–20 cm soil depth. "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$.

3.2.3. Changes in Microbial Biomass Carbon

In the 10-year field experiment, the microbial biomass carbon (MBC) content in the CK, ZF, NF, and JF treatments showed a similar changing trend (Figure 4). After 5 years of application, the MBC content in the JF, ZF, and NF treatments was significantly higher than that of CK, with increases of 107.24%, 116.45%, and 96.71%, respectively. After 10 years of continuous application, the order of the MBC content of treatments was ZF > JF > NF, with significant differences between treatments. The MBC content of the CK treatment showed a decreasing and then increasing trend with the prolongation of experimental time.

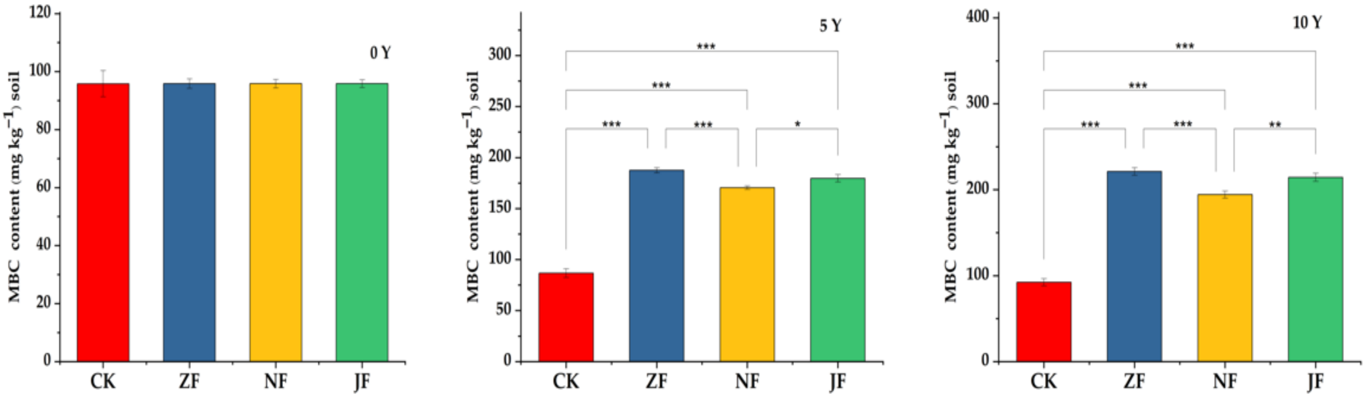


Figure 4. Effects of CK, ZF, NF, and JF on soil microbial biomass carbon (MBC) concentrations in the 0–20 cm soil depth. "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$.

3.2.4. Changes in Particulate Organic Carbon

The addition of organic matter increased the particulate organic carbon (POC) content in the soil, and it increased over time (Figure 5). After 5 years of application, the POC content in the NF and JF treatments differed significantly and increased by 25.61% and 19.01%, respectively, relative to CK. After 10 years of continuous application, the POC content in the JF, NF, and ZF treatments was higher than that of CK, with increases of 81.87%, 84.58%, and 63.00% from the initial contents, respectively, and the difference between the ZF treatment and the JF and NF treatments was significant.

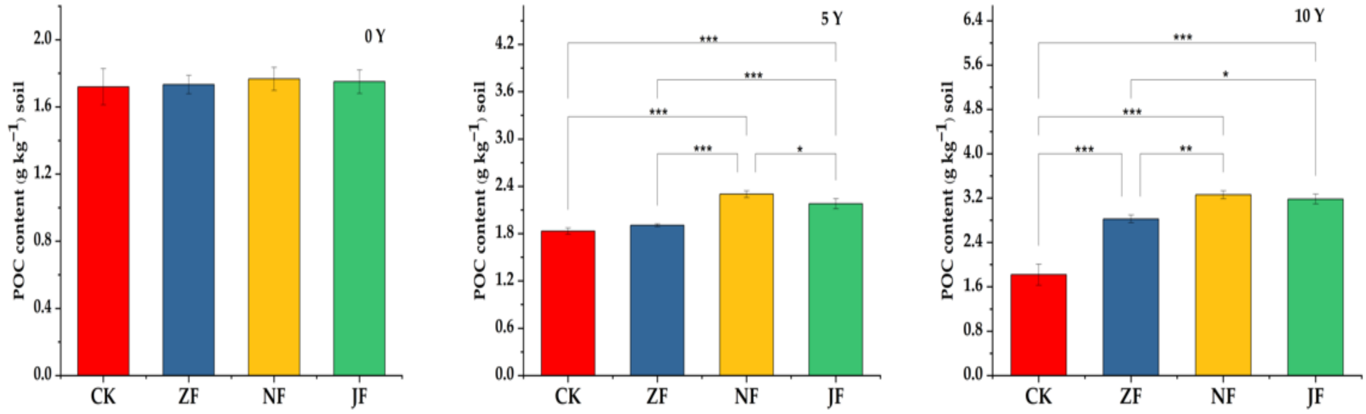


Figure 5. Effects of CK, ZF, NF, and JF on soil particulate organic carbon (POC) concentrations in the 0–20 cm soil depth. "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$.

3.3. Soil Microbial Community Diversity

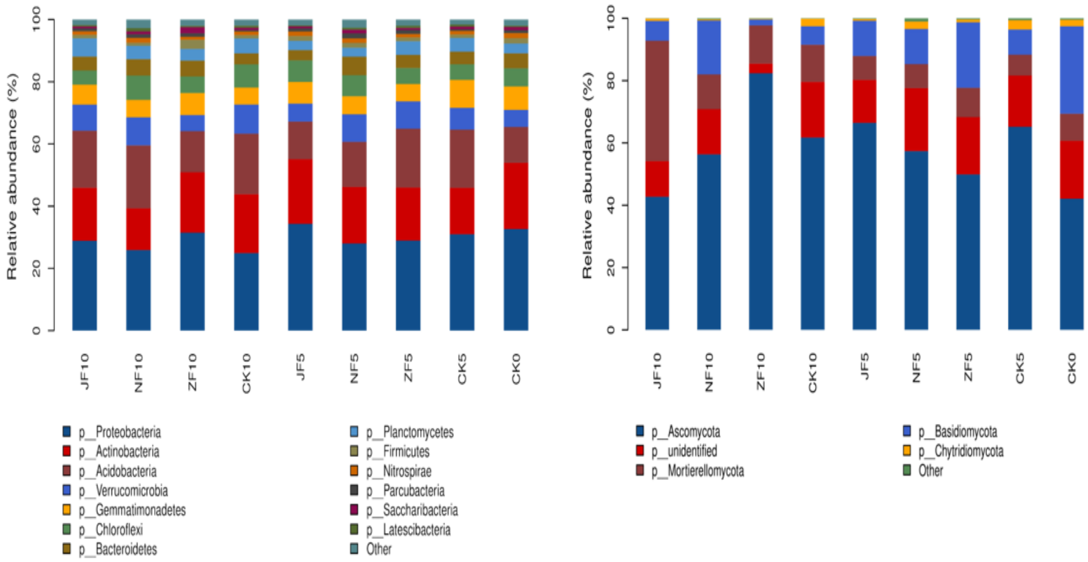
In all the treatments, the diversity and richness of bacteria and fungi in the soils at the 10th year were greater than those at the 5th year (Table 3). In terms of bacterial and fungal communities, the Chao1 and Shannon indices of the CK treatment tended to increase with time. In the bacterial community, the highest Chao1 and Shannon indices were exhibited by NF and JF at the 5th and 10th year, respectively, compared to the CK treatment. In the fungal community, the highest Chao1 and Shannon indices were exhibited by JF at the 5th year and 10th year, respectively, compared to the CK treatment.

Table 3. The diversities of bacterial and fungal communities in the different manures and years.

Time (year)	Treatments	Bacterial Community		Fungal Community	
		Chao1 Index	Shannon Index	Chao1 Index	Shannon Index
0	CK	4647.02 c	9.67 b	916.15 c	3.56 b
5	CK	4704.96 c	9.91 b	980.02 c	5.21 d
	ZF	4716.14 c	9.92 b	1030.53 b	5.60 c
	NF	4969.69 a	10.14 a	1079.36 b	6.12 b
	JF	4890.91 b	9.97 ab	1119.78 a	6.32 a
10	CK	4893.20 c	9.96 c	1019.69 c	5.43 c
	ZF	5014.52 b	10.00 bc	1109.31 b	6.11 b
	NF	5055.35 a	10.22 a	1122.65 b	6.17 b
	JF	5055.70 a	10.04 b	1286.15 a	6.39 a

Different lowercase letters within the same column indicate significant differences at $p < 0.05$.

Regarding the bacterial phyla, *Proteobacteria* (24.9–34.3%), *Actinobacteria* (13.4–21.3%), *Acidobacteria* (11.5–20.2%), *Verrucomicrobia* (5.2–9.4%), and *Gemmatimonadetes* (5.4–9.0%) were the dominant bacterial phyla in each treatment (Figure 6a). Manure application greatly increased the abundance of *Actinobacteria* and *Proteobacteria* in the 5th year and 10th year. Compared with CK, *Proteobacteria* and *Actinobacteria* were more abundant under JF in the 5th year and increased greatly under NF and ZF. The *Proteobacteria* were more abundant under ZF compared to CK and other manure treatments in the 10th year. Concerning the fungal phyla, *Ascomycota* (42.1–82.4%), *Mortierellomycota* (6.7–38.6%), *Basidiomycota* (1.9–28.1%), and *Chytridiomycota* (0.2–3.0%) were the dominant fungal phyla in each treatment (Figure 6b). Manure application greatly increased the abundance of *Ascomycota*, *Mortierellomycota*, and *Basidiomycota* in the 5th year and 10th year. Compared with CK, *Basidiomycota* were more abundant under ZF in the 5th year and increased greatly under NF and JF. The *Ascomycota* were more abundant under ZF, the *Mortierellomycota* were more abundant under JF, and the *Basidiomycota* were more abundant under NF.

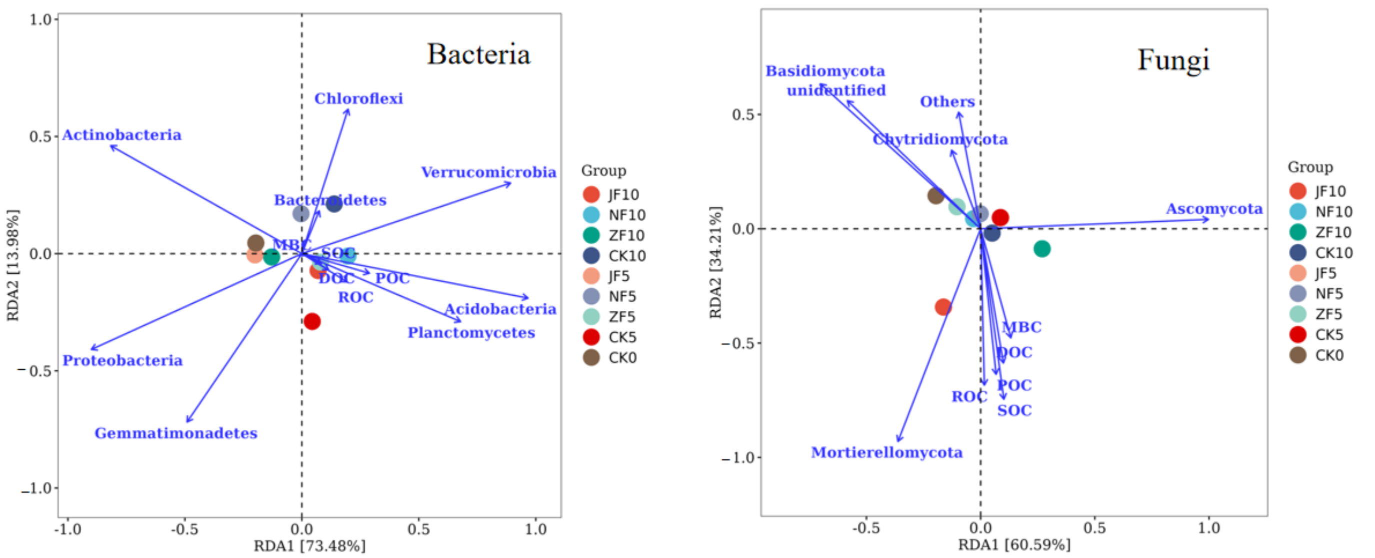


(**a**) (**b**)

Figure 6. (**a**) Relative abundance of soil bacterial phyla at different manures and years (0, 5th, and 10th year). (**b**) Relative abundance of soil fungal phyla at different manures and years (0, 5th, and 10th year).

3.4. Relationships between Labile Organic Carbon Fractions and the Soil Microbial Community

RDA was employed to assess the effects of varying manure applications on soil bacterial populations in relation to soil carbon pools (Figure 7a) and fungal phyla (Figure 7b) under different years. Among the bacterial communities, the dominant *phyla-Proteobacteria*, were positively correlated with the JF5 and ZF5 treatments and *Actinobacteria* and negatively correlated with the other two (*Acidobacteria* and *Verrucomicrobia*) dominant microbial phyla. Meanwhile, JF10 and NF10 treatments were positively correlated with the *Acidobacteria*, organic carbon (SOC), dissolved organic carbon (DOC), readily oxidizable carbon (ROC), and particulate organic carbon (POC). *Acidobacteria* and *Planctomycetes* were also positively correlated with SOC, DOC, ROC, and POC. In addition, the microbial biomass carbon (MBC) was positively correlated with *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi*. Among the fungal communities, the dominant *phyla-Ascomycota* and *Mortierellomycota* were positively correlated with SOC, DOC, ROC, POC, and MBC, while *Ascomycota* were negatively correlated with *Mortierellomycota*. *Mortierellomycota* were positively correlated with JF10; *Ascomycota* and ZF10 followed the same pattern. *Mortierellomycota* were positively correlated with JF10, *Ascomycota* and ZF10 had the same results, and *Basidiomycota* were positively correlated with NF10. Moreover, *Basidiomycota* and *Chytridiomycota* were negatively correlated with SOC, DOC, ROC, POC, and MBC.



(**a**) (**b**)

Figure 7. Redundancy analysis (RDA) of soil microbial communities and SOC fractions at different manures and years (0, 5th, and 10th year).

4. Discussion

4.1. Effects of Continuous Manure Application on SOC Accumulation

Soil organic carbon is an important indicator of soil fertility, which provides a large amount of nutrients for plants [28–30]. Many studies have shown that soil organic carbon can be affected by planting years and fertilizer types [31–33], and the application of organic materials is the most direct method of improving soil organic carbon. In our study, continuous manure application significantly increased the soil organic carbon content (SOC) in the topsoil. Related results were reported by Cui [34]. Continuous manure application can significantly increase the level of soil organic carbon, and many studies have demonstrated the role of manure in improving the soil environment and enhancing soil organic carbon stocks. The effect of different types of manure on soil organic carbon varies [35].

4.2. Effects of Continuous Manure Application on Labile SOC Fractions

Soil dissolved organic carbon (DOC) is relatively unstable in nature, highly active, and easily occurs under certain conditions. This part of the carbon comes from the soil itself and the input of exogenous organic materials, such as crop residues, litter, poultry manure, straw returning to the field, and other ways to enter the soil. Some studies have shown that the combined application of corn straw, cattle manure, and chemical fertilizer can significantly increase soil DOC content [36]. This was consistent with the results of Wang et al. [37]. After 5–10 years of continuous application, the DOC content of livestock and poultry manure treatment gradually increased with time, and the chicken manure treatment effect was better. The application of manure in the early stage improved microbial activity, which was absorbed and utilized by animals and plants, and the microorganisms improved the mineralization rate of organic carbon with time [38–41].

Microbial biomass carbon plays an important role in soil fertility and plant nutrition [42]. The results of this study showed that after 5–10 years of continuous application, the MBC content of treatments gradually increased, showing ZF > JF > NF. In their study, Mi et al. [43] observed that the content of MBC in soil increased significantly after the application of organic materials. The reason is that the organic matter in livestock manure can improve the soil microbial abundance, improve the trend of the decrease in soybean richness with time, and promote the accumulation of microbial biomass carbon content [44].

Particulate organic carbon (POC) accounts for about 30–60% of soil organic carbon and plays an important role in the soil carbon cycle [45]. Émilie et al. [46] also proved that the POC content of organic fertilizer treatment increased by 92.6% compared with that of no organic fertilizer treatment. Li [47] reported that the change trend of POC and SOC in soil with organic materials was the same, which indicated that the change in POC content was the decisive factor for the change in SOC content. The increase in soil ROC content after adding livestock manure was due to improvement in microbial activity by applying livestock manure and the increase in active organic carbon components by newly decomposed organic materials and decomposed substances. Haoan et al. [48] also reached a similar conclusion. The application of livestock manure increased the density of organic carbon, and the organic carbon content of organic materials decomposed in soil with time, mainly increasing the active organic carbon of each component, while the application of organic fertilizer increased the soil ROC content.

4.3. Effects of Soil Microbial Community on Labile SOC Fractions at Different Times

The microbiological characteristics of soil were also significantly improved, and the effect was obviously better than that of a single application of chemical fertilizer. This is consistent with the research results of Marcote et al. [49] and Fugen et al. [50], who observed an effective improvement in soil microbiological characteristics and soil nutrient status by applying organic materials. On the one hand, organic materials improve the microbial environment in soil, and the improvement in soil microbial environment is beneficial to improving the vitality of roots, promoting the migration and absorption of nutrients, thus increasing the yield. On the other hand, organic materials can reduce the activity of soybean root exudates, reduce the incidence degree and frequency of soil-borne diseases, and thus reduce the loss of yield [51]. Organic materials can improve the soil microbial environment of continuous cropping soybean, which is beneficial to improving soil microbial environment quality and promoting the increase in yield. It could be seen that organic materials play a virtuous circle role in the continuous planting of soybean [52].

Continuous manure application increased the abundance of bacterial phyla-Proteobacteria, Bacteroidetes, and fungal phyla-*Mortierellomycota* [53]. The redundancy analysis (Figure 7) showed that the soil labile organic carbon fractions had effects on soil microbial communities. Several studies have demonstrated an increase in the diversity and abundance of soil microorganisms following the application of livestock manure, leading to significant alterations in the overall microbial community composition [54]. Our results showed that *Acidobacteria* and *Planctomycetes* were positively correlated with SOC, DOC, ROC, and POC. *Proteobacteria* thrive in environments rich in nutrients and capable of utilizing unstable forms of carbon for their growth and metabolic processes [55]. Microbial biomass carbon (MBC) was positively correlated with *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi.* RDA showed that the dominant phyla-*Ascomycota* and *Mortierellomycota* were positively correlated with SOC, DOC, ROC, POC, and MBC, which can be evidenced through the strong accumulation effects *Mortierellomycota* have on soil organic carbon [56]. The lower occurrence of *Ascomycota* in livestock manure treatments may be due to the poor adaptation of *Ascomycota* to changes in the soil environment. Therefore, the return of manure to the farmland alters the soil conditions and affects the adaptability of *Ascomycota* [57]. In this study, *Basidiomycota* and *Chytridiomycota* were negatively correlated with SOC, DOC, ROC, POC, and MBC. This is mainly due to different manure and soil environments, seasonality, management practices, and other factors that affect the composition of the soil fungal community [58]. Manure would be an important source of energy for *Basidiomycota*, thus increasing microbial activity, reproduction rate, and abundance [59].

5. Conclusions

The continuous application of different manures has a significant impact on soil functional processes, and the key to these processes is soil microorganisms, which will affect the feedback mechanism of carbon cycle mediated by microorganisms in soil. The input of exogenous organic matter significantly improves the development of soil organic carbon and promotes the accumulation and long-term sequestration of soil organic matter. The results were consistent with our hypothesis that continuous manure application would significantly increase the soil organic carbon (SOC), dissolved organic carbon (DOC), and readily oxidized organic carbon (ROC) content. Our study also provides evidence that manure application greatly increased the abundance of Actinobacteria and Proteobacteria. In a word, adding different organic materials can better support biodiversity conservation and realize ecosystem services of surface carbon storage, carbon neutralization, and soil conservation. Our results reveal the importance of microbial fixation in soil carbon dynamics according to the different distribution of active organic carbon pools, which will help enhance our understanding of the carbon cycle.

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