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Effects of Different Conditioners on Soil Microbial Community and Labile Organic Carbon Fractions under the Combined Application of Swine Manure and Straw in Black Soil

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Abstract: The return of straw and manure to agricultural fields can impact soil organic carbon (SOC) and biological properties. However, there is a lack of research on how using swine manure, maize straw, and various conditioners together affects soil bacterial and fungal populations. This study aimed to investigate six treatments, namely, only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ). The results showed that after the two-year return, all treatments increased the SOC content in 2023, which was 12.55–26.89% higher than S00, and the SCZ treatment significantly increased the soil organic carbon (SOC), dissolved organic carbon (DOC), easily oxidizable carbon (EOC), particulate organic carbon (POC), and microbial biomass carbon (MBC) content by 26.89%, 25.44%, 56.88%, 16.08%, and 43.54%, compared to S00. A redundancy analysis (RDA) showed that the continuous application of manure, maize straw, and conditioners has a positive impact on the diversity and abundance of soil microbial communities, enhancing the accumulation of soil carbon. Furthermore, our research revealed that soil fungi exhibited higher sensitivity in soil carbon composition following the addition of manure, straw, and conditioners to agricultural fields than bacteria. In conclusion, the addition of different conditioners to the fields is beneficial to biodiversity conservation from the perspective of achieving soil carbon storage and soil protection. Our findings suggested that the combination of maize straw, biochar, and swine manure has been proven to be the most effective treatment for increasing labile organic carbon fractions and enhancing the microbial community.

Keywords: manure; straw strip returning to field; different conditioners; soil organic carbon components; microbial community

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1. Introduction

Soil organic carbon (SOC) plays a significant role in the global carbon cycle [1,2], contributing to both soil quality enhancement and the whole world's climate change [3]. Labile organic carbon (LOC) is a significant component of SOC and is an important indicator of soil quality [4]. Its components include dissolved organic carbon (DOC), easily oxidized carbon (EOC), microbial biomass carbon (MBC), and particulate organic carbon (POC) [5,6].

In agricultural ecosystems, C input mainly depends on biomass decomposition, root deposition, etc. [7], while exogenous C input can alter soil microbial communities and functions [8]. Microorganisms in soil play a crucial role in regulating the organic carbon present. They have the ability to modify the composition of soil carbon through various mechanisms associated with carbon sequestration, ultimately influencing the carbon cycle within the soil [9–12]. Studies have shown that the return of organic matter to the field has been proven to increase SOC storage [13–17] and promote the stability of the soil carbon pool [18]. Straw return has been found to facilitate the accumulation of labile organic carbon components [19]. Similarly, returning organic fertilizer has also been proven to greatly enhance the levels of SOC and EOC [20,21], resulting in an improvement in the rate of soil humification and the humification index [22,23]. Biochar can promote the accumulation of unstable organic C components and is not easily decomposed [24,25]. The synergistic effect of biochar and other amendments has been found to have a positive impact on the formation of a stable soil environment rich in organic matter [26]. Adding microbial agents can significantly improve the straw decomposition rate and diverse forms of soil organic carbon content within a brief time period [27]. Boron slag, as an acidic inorganic conditioner, contains essential elements for crop growth and various trace elements, which can alter microbial activity and increase corn yield [28]. Straw return can influence the composition and variety of soil microbial communities [29–31], regulate soil biological processes and nutrient supply [32,33], and thereby increase the activity of soil urease and phosphatase [34]. The mixed application of both straw and green manure has been proven to increase SOM as well as influence the soil microbial community [35]. The combined application of straw and manure can enhance the levels of organic carbon in labile soil, leading to enhancements in the structure and variety of microbial communities within the soil [36,37]. Biochar return to the field has been demonstrated to improve the quality of soil and enhance microbial activity [38], inducing microbial community succession [19]. The above research results all indicate that soil microorganisms can mineralize and fix soil organic matter, alter dominant species in microbial communities [39], and thereby affect the turnover of SOC [40], while the biomass and activity of soil microorganisms tend to rise as the SOC content increases [41]. Therefore, elucidating the composition of soil microbial communities is crucial for the storage of carbon in agricultural land.

At present, straw return has been widely promoted and applied, while under the traditional strip-returning method, the slow decomposition rate of straw brings many unfavorable factors to agricultural production. Therefore, organic materials and straw were generally used for returning to the field of agriculture. However, the conversion efficiency of straw and organic materials into soil organic matter after returning to the field is low, and the humification coefficient is difficult to improve. Therefore, more studies have focused on exogenous conditioners as an effective alternative to sequestering soil C and promoting microbial growth. However, there are few studies on the interaction mechanism between soil carbon components and microbial communities in the combined application of different conditioners in the field of returning manure and straw strips. Therefore, our study used high-throughput sequencing techniques of 16S rRNA and ITS rRNA genes to investigate the soil's active carbon composition and microbial community after mixed application of straw strips and returning to the field. We assume that the incorporation of straw combined with swine manure and different conditioners greatly increases the content of labile organic components and alters the soil microbial community. Specifically, certain bacterial and fungal phyla are thought to be responsible for the alteration in carbon components within the soil. The objectives of our study are to (i) assess variations in labile carbon components within the soil, (ii) investigate the impact on the soil microbial community, and (iii) examine the correlation between the microbial community and labile carbon components in the soil.

2. Materials and Methods

2.1. Study Cite

Soil was collected from the experimental field at Yushu County (44°26′59″ N, 125°21′37″ E), in Jilin Province, China, spanning from 2021 to 2023. The research site displays a temperate continental monsoon climate, with an average annual temperature of 4.6 °C and an average annual precipitation of 331.9 mm. The soil identified at this location was classified as Mollisols in accordance with the U.S. soil classification system. The key soil properties measured include 13.17 g·kg^{-1} of soil organic carbon, 2.31 g·kg^{-1} of total nitrogen, 0.57 g·kg^{-1} of total phosphorus, 2.35 g·kg^{-1} of total potassium, an electrical conductivity (EC) of 55.03 µs·cm^{-1}, and pH of 6.43. The biochar was made of waste, and it was commercially produced by JiLin Mingtai Enterprise, under oxygen-limited conditions at 450 °C in a muffle furnace. The bio-organic fertilizer was chicken manure, the liquid biological agent was a mixture of crop straw decomposition agents developed by Jilin Jiabo Biotechnology company, and the effective bacteria were Bacillus subtilis and Trichoderma harzianum. The boron slag was an inorganic material. Maize straw was generated onsite at the experimental location, and Table 1 displays the essential characteristics of the organic materials.

Table 1. Basic properties of organic materials.

Materials	Total Organic Carbon ($g\cdot kg^{-1}$)	Total Nitrogen ($g\cdot kg^{-1}$)	Total Phosphorous ($g\cdot kg^{-1}$)	Total Potassium ($g\cdot kg^{-1}$)	pH	EC ($\mu s\cdot cm^{-1}$)
Maize straw	424.03	8.46	1.18	10.27	7.02	1.7
Biochar	330.87	8.9	15.96	2.18	7.07	1.3
Swine manure	280.52	20.04	0.9	22.15	7.04	3.89
Biological-organic fertilizer	255.63	17.08	7.82	14.11	8.06	3.7
Biological agent	446.15	8.37	4.27	12.32	6.51	----
Boron slag	----	----	----	----	3.66	----

2.2. Experimental Design

The two-year field experiment consisted of six treatments that were replicated three times in a randomized complete block design with each plot measuring 100 m^2. The crop varieties and agronomic practices remained consistent except for fertilization. The six treatments included only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ). In May 2021 and 2022, the straw strip composting technique was employed to return straw by incorporating it with swine manure in the field. The experimental area utilizes a straw strip combined application technique for covering and returning to the field. Straw strip composting was employed for straw incorporation, where furrows were first plowed to a depth of 20 cm, adding swine manure and straw into the furrows and then covering it with soil. All materials were applied in their entirety. Application rates per plot (100 m^2) were 100 kg for straw, 15 kg for biochar, 1.5 kg for boron slag, 20 kg for swine manure, 20 kg for bio-organic fertilizer, and 1 kg for biological agent. The application was carried out in May 2021. The annual application rate of fertilizer is N: 225 kg/ha, P_2O_5: 120 kg/ha, K_2O: 60 kg/ha. The research focused on a cropping system involving maize, with the crop being planted in May and harvested in October.

2.3. Soil Sampling and Analysis

Three soil samples were randomly collected for each treatment from a depth of 0–20 cm using the S-shaped five-point method without disturbance. After the removal of any visible organic debris and stones, all samples were placed in sterile PET resin bags, stored in iceboxes, and transferred to the lab for additional processing. Based on the required analysis, a portion of the soil samples was promptly frozen at −80 °C for DNA extraction, whereas the remaining portion was refrigerated at 4 °C for MBC and DOC analysis [42].

2.4. Analysis Methods

The $K_2Cr_2O_7$-volumetric method [43] was utilized to measure SOC. The methods outlined by Blair and Jiang were followed to determine EOC, POC, and DOC [44]. MBC determination was the measurement of carbon content in the extraction solution of soil after fumigation with chloroform. The soil microbial carbon content was calculated based on the variation in total carbon between fumigated and non-fumigated soil [45].

The extraction and amplification of DNA were performed using the CTAB method. The DNA sample that had been washed was diluted to a concentration of 50 ng μL^{-1}, and then the purity and concentration of the DNA were confirmed using 1% agarose gel electrophoresis. The universal primers (338 F, 5′-CAAGCTGCAGGACGAGACTC-3′; 806 R, 5′-TTACTTGCCTGTCCGACAG-3′) were used to amplify the bacterial 16S rRNA gene region (approximately 420 bp) targeting the V3–V4 hypervariable region. As for fungal genes, the ITS1 region was amplified using the ITS1F primer, 5′-TGGAGCTGTTGGTGTCGCTG-3′, and ITS2 primer, 5′-AGCTGCGTGTCATCGCGTG-3′. An analysis of bacterial 16S rRNA and fungal ITS gene sequences was conducted utilizing the high-throughput sequencing platform Illumina Miseq (Biomarker Technologies, Rohnert Park, CA, USA). The software QIIME 2 was employed for Beta diversity analysis in order to assess the species diversity similarity across various samples.

2.5. Statistical Analysis

The impacts of different straw return methods on soil parameters were assessed using statistical analyses performed using IBM Statistics 21.0 (SPSS 21.0). To determine differences in mean values among straw return strategies, significance testing was conducted utilizing the least significant difference test ($p < 0.05$). Spectral graphs were created using ggplot 2 in R language (4.2.3) and Origin 2021 software.

3. Results

3.1. SOC Content

Changes in soil organic carbon (SOC) content within a field experiment of two years (from 2022 to 2023) were illustrated in Figure 1. The application of manure resulted in higher organic carbon levels compared to the control treatment (S00) throughout the study, with biochar conditioners (SCZ) consistently exhibiting the highest values, significantly surpassing S00. In the field experiment conducted from 2022 to 2023, Figure 1 depicts the variations in SOC content. It was observed that the application of manure led to increased levels of organic carbon compared to the control treatment (S00) over the duration of the study. Furthermore, the application of biochar conditioners (SCZ) consistently showed the highest values of organic carbon, significantly outperforming the S00 treatment. Despite varying yearly conditions, each treatment displayed similar trends in SOC content, with levels in 2023 exceeding those in 2022. The S0Z, SCZ, and SBZ treatments saw respective increases of 10.64%, 15.69%, and 8.18% compared to S00, while the SJZ and SFZ treatments experienced declines of 3.20% and 8.39% in 2022. Nevertheless, all treatments demonstrated an increase in SOC in 2023, ranging from 12.55% to 26.89% higher than S00, with SCZ exhibiting the greatest increase of 26.89%. The results indicate that the addition of biochar in soil improves the level of organic carbon more so than other conditioners.

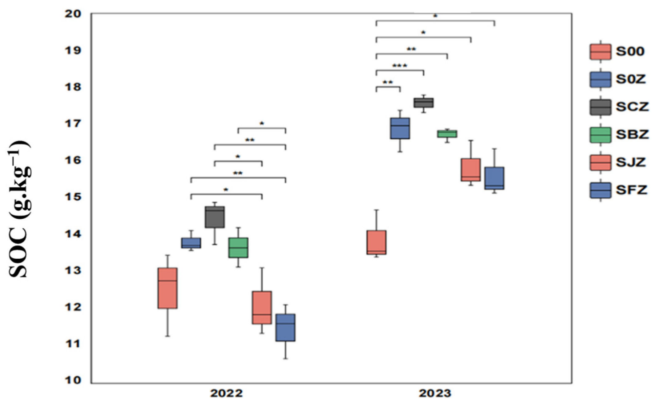


Figure 1. Effects of the combined application of swine manure, maize straw, and different conditioners on SOC. Note: The experiment treatments included only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ). For example, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$.

3.2. LOC Fractions

Regarding the two-year field experiment, the DOC contents under different treatments are shown in Figure 2. Within two years, the DOC content of each treatment increased. After one year of application, the DOC content in the S0Z, SCZ, and SBZ treatments was significantly higher than that of S00, with increases of 13.20%, 24.03%, and 4.48%, respectively. Following two years of consecutive application, the SCZ treatment had the highest DOC content, with a notable discrepancy in DOC content between this treatment and the other treatments.

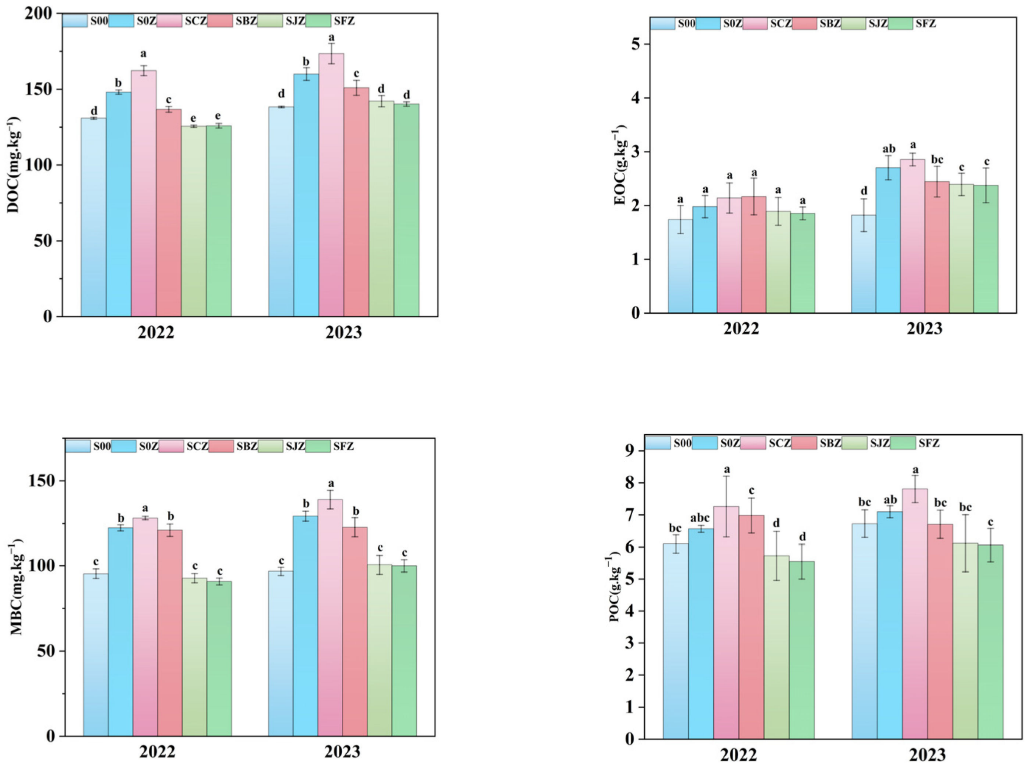


Figure 2. Effects of the combined application of manure and straw and different conditioners on labile organic carbon. Note: The experiment treatments included only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ). The lowercase letters represent significant differences among different treatments ($p < 0.05$).

During the 2 years of straw return, the EOC content of each treatment changed differently. The EOC content for all treatments after two years was significantly higher than that after one year after returning to the fields. Compared with S00 over two years, the EOC content of each treatment in 2022 was 1.74–2.17 g·kg^{-1}, and the EOC content of each treatment in 2023 was 1.82–2.85 g·kg^{-1}. After two years of consistent application, it was found that the SCZ treatment resulted in the highest EOC content, showing significant differences between the various treatments. This implied that the addition of biochar conditioners had a greater impact than other conditioners in terms of increasing EOC content.

The MBC content of soil under various treatments in the two-year experiment exhibited a consistent trend, as illustrated in Figure 2. Compared with S00, in 2022, the MBC content of S0Z, SCZ, and SBZ treatments increased by 29.93%, 35.86%, and 27.20%, respectively, while the MBC content of SJZ and SFZ treatments decreased by 1.46% and 3.51%, respectively. In 2023, all treatments increased the content of MBC, with the SCZ treatment showing the highest increase of 43.54%. This indicated that the addition of different conditioners was beneficial for increasing the MBC content, with biochar having the most significant effect, and MBC content also increased with the increase in the year.

After two consecutive years of straw return, the changes in POC content varied among different treatments. The POC content of each treatment in different years showed similar patterns. Compared to 2022, the POC content of all treatments has increased in 2023. In 2022, the soil POC content for each treatment ranged from 5.54 to 7.26 $g \cdot kg^{-1}$, while in 2023, it varied from 6.06 to 7.81 $g \cdot kg^{-1}$. Following two years of consistent treatment, the POC content within the SCZ treatment exceeded that of the remaining treatments.

3.3. Soil Microbial Community Structure

During the two-year experiment, in 2023, the abundance of soil bacteria and fungi was greater compared to 2022 across all treatments (Table 2). The Chao1 and Simpson index for bacterial and fungal communities in the S00 treatment showed a steady increase over time. The SBZ and SCZ treatments exhibited the highest Chao1 and Simpson index in bacterial communities in 2023, while the SJZ and SCZ treatments demonstrated the highest Chao1 index in fungal communities for the same year. The results indicate a notable increase in the number of OTUs in both soil bacteria and fungi two years after the field was returned, compared to just one year after (Figure 3). Specifically, in the bacterial community, there were 477 OTUs shared across all the treatments in 2023, with each sample having its unique set. Notably, the number of OTUs in SBZ and SCZ treatments was the highest at 2827 and 2526, respectively, while the SFZ treatment had the lowest at 1797. In the fungal community, there were 91 OTUs shared across different treatments after 2 years, with the SFZ and SCZ treatments having the highest numbers at 621 and 590, respectively. Conversely, the S00 treatment had the smallest number of OTUs at 501.

Table 2. The diversities of soil bacterial and fungal diversity among different treatments.

Time (Year)	Treatments	Bacterial Community		Fungi Community	
		Simpson Index	Chao1 Index	Simpson Index	Chao1 Index
2022	S00	0.994 abcd	697 c	0.945 a	298 c
	S0Z	0.994 cd	680 c	0.982 a	314 b
	SCZ	0.991 d	600 c	0.982 a	278 c
	SBZ	0.993 abc	583 c	0.8 a	245 c
	SJZ	0.993 bcd	696 c	0.984 b	413 c
	SFZ	0.993 abcd	626 c	0.967 a	412 b
2023	S00	0.994 abcd	1775 ab	0.982 a	462 b
	S0Z	0.996 ab	1881 ab	0.978 a	505 ab
	SCZ	0.995 abc	1978 ab	0.965 a	531 a
	SBZ	0.996 a	2061 a	0.962 a	494 a
	SJZ	0.996 abc	1838 ab	0.887 ab	537 a
	SFZ	0.996 abc	1612 b	0.959 a	479 ab

Note: The experiment treatments included only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ). The lowercase letters represent significant differences among different treatments ($p < 0.05$).

The diversity of bacteria in soil samples from various treatments was similar, while the proportion of each treatment was different. The bacterial community abundance at the phylum level can be observed in Figure 4a. Among the entire soil samples, the predominant bacterial phyla included Proteobacteria (33.76–45.66%), Acidobacteria (19.26–30.96%), Gemmatimonadota (8.54–11.48%), and Actinobacteriota (5.46–10.24%). After the combined application of swine manure and straw, the abundance of Proteobacteria and Gemmatimonadota (8.54–11.48%) significantly increased. Compared to the S00 treatment, the abundance of Proteobacteria and Acidobacteria was higher one year after S0Z and SFZ treatment, with Acidobacteria being more abundant two years after SCZ and SFZ treatment. The relative bacterial community abundance at the genus level after two years was further analyzed

(Figure 5a) among which the dominant genera for all soil samples were Sphingomonas, Gemmatimonadaceate, Acidobacterials, and Gemmatimonas. The relative abundance of Sphingomonas was larger in the SJZ treatment than in other treatments. In the SCZ treatment, Gemmatimonadaceate was the most abundant genus. Regarding the Fungi phylum, the dominant fungal phyla in all treatments were Ascomycota (34.43–58.63%), Basidiomycota (17.71–54.77%), Mortierellomycota (2.53–8.66%), and Chytridiomycota (1.28–14.46%) (Figure 4b). The relative abundance of Ascomycota after the combined application of manure straw strips was lower than that of the S00 treatment. Compared with the S00 treatment, the relative abundance of Basidiomycota in the SJZ treatment was highest one year after returning to the field. After maize straw returned to the field for 2 years, the abundance of Mortierellomycota was more abundant in the SCZ treatment, while the proportion of Chytidiomycota in the SBZ treatment was significantly greater than in other treatments. The dominant fungal genera consisted of Tausonia, Mortierlla, Conocybe, and Agrocybe. Nevertheless, the SJZ treatment had the highest relative abundance of Tausonia. The relative abundance of Conocybe was larger in the SBZ treatment than in the other treatment (Figure 5b).

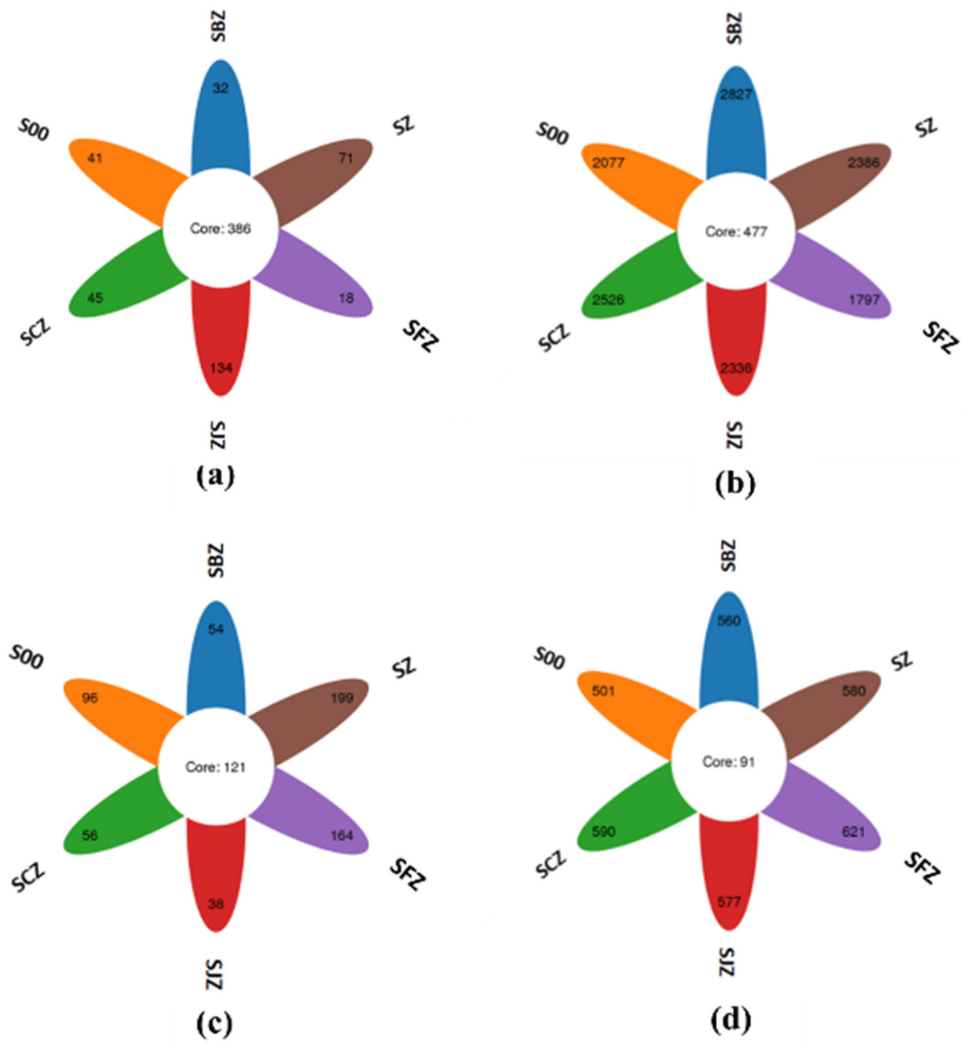
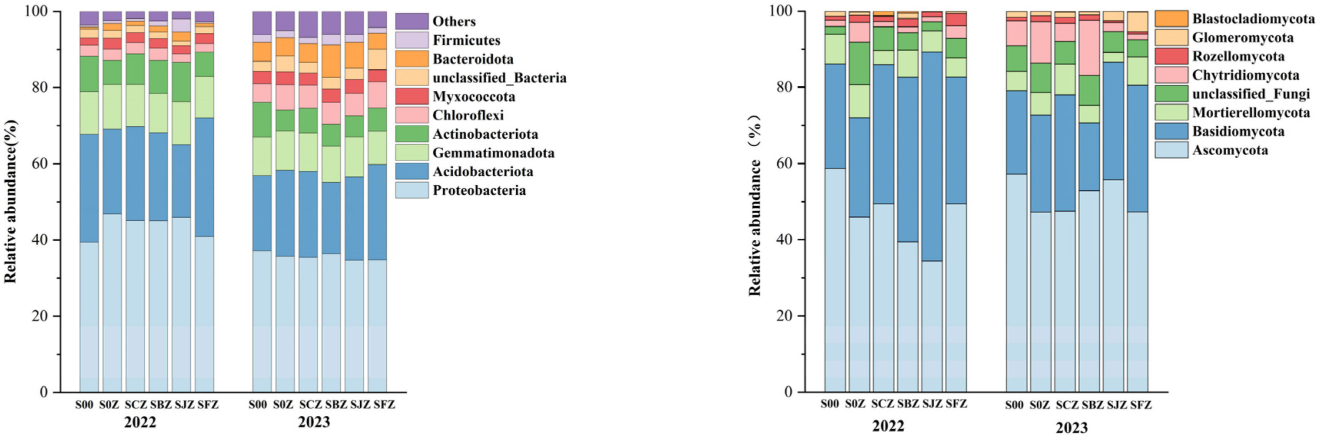
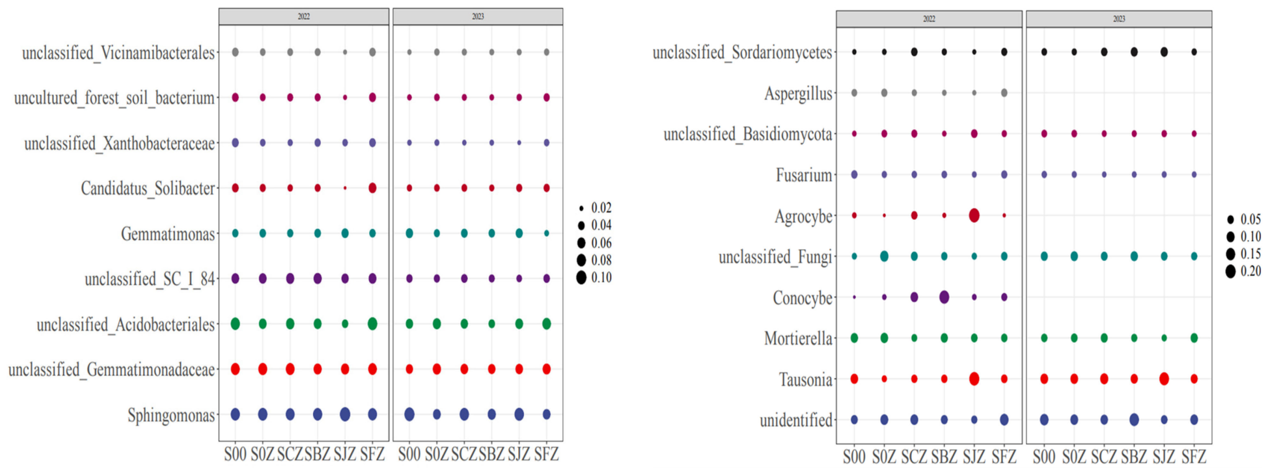


Figure 3. The OTU number of soil bacteria in (**a**) 2022 and (**b**) 2023 and soil fungal in (**c**) 2022 and (**d**) 2023.



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

Figure 4. Relative abundance of soil bacteria at phylum among different treatments (**a**); relative abundance of soil fungi at phylum among different treatments (**b**). Note: The experiment treatments included only maize straw (S00), maize straw combined with swine manure (S0Z), maize straw combined with biochar and swine manure (SCZ), maize straw combined with boron slag and swine manure (SBZ), maize straw combined with biological agent and swine manure (SJZ), and maize straw combined with bio-organic fertilizer and swine manure (SFZ).



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

Figure 5. Bacterial abundance of soil bacteria at genus among different treatments (**a**); relative abundance of soil fungi at genus among different treatments (**b**).

3.4. Relationships between Microbial Community and Organic Carbon Fractions

Redundancy analysis was conducted to examine the associations between the soil microbial community at the phylum level and the variability in various soil carbon pools, including DOC, EOC, POC, and MBC after a period of 2 years. The composition of SOC accounted for 90.83% of the variance at the phylum level (Figure 6a). In the analysis, the first axis (RDA1) accounted for 69.92% of the variance, with the second axis (RDA2) explaining 20.914%. SOC and EOC were identified as the main influencers on the composition of soil bacterial communities. In the bacterial community, Bacteroidota exhibited positive associations with POC, MBC, SOC, DOC, and EOC, whereas Proteobacteria showed strong positive correlations with POC. Regarding fungal communities, the primary and secondary axes of the redundancy analysis accounted for 61.23% and 32.73% of the variation in soil fungal communities, respectively, with soil organic carbon constituents explaining 93.96% of the variation (Figure 6b). DOC and MBC were found to be the major influencers of the soil fungal community. Ascomycota displayed significant negative correlations with SOC and MBC levels ($p < 0.05$), while Basidiomycota exhibited negative associations with POC levels.

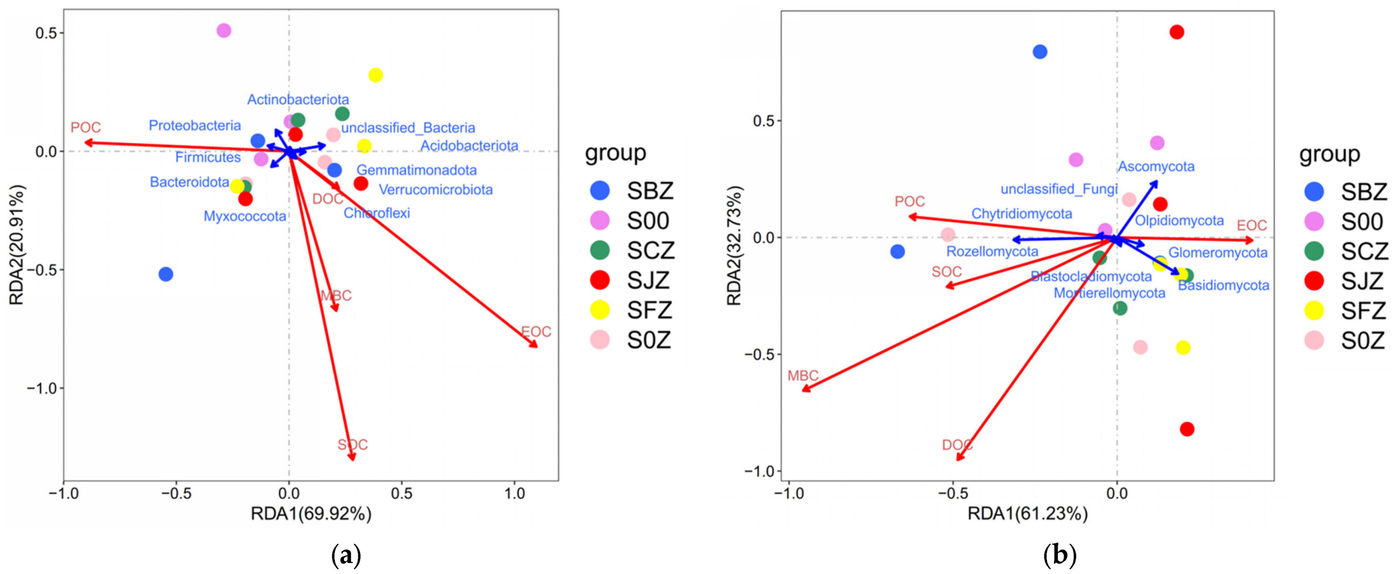


Figure 6. Redundancy analysis (RDA) of SOC fractions and soil bacterial (**a**) and fungal communities (**b**). (SOC, soil organic carbon; DOC, dissolved organic carbon; EOC, easily oxidizable carbon; MBC, microbial biomass carbon).

4. Discussion

4.1. Effects of the Application of Manure and Straw on SOC Fractions

Numerous research findings indicate that manure return benefits soil organic carbon storage, providing a carbon source for soil microorganisms and affecting the soil microbial environment [46]. In our study, the SOC content of each treatment in 2023 was higher than that in 2022, which was attributed to the continuous application of animal manure and materials leading to more residual organic matter in the soil, which promotes soil organic carbon accumulation. The combination of maize straw with swine manure resulted in a significantly higher SOC content compared to straw alone. Swine manure has more available forms of carbon and higher SOC protection mechanisms [47]. Additionally, the combination of straw and swine manure has the potential to reduce the decomposition of indigenous SOC, promote interaction between organic matter and minerals, stimulate the formation and durability of aggregates, and elevate the level of soil organic matter [48]. Among all the conditioners, the biochar conditioner had a notably higher SOC content compared to other conditioners, aligning with the findings of Novak et al. [49]. It may be that the biochar could have stable organic carbon that is hard to degrade and large porosity, providing an activity space and nutrient source for microorganisms [50], accelerating the decomposition of straw and converting it into humus and nutrients, which is conducive to the accumulation of SOC [51], reducing carbon mineralization [52], and thus increasing soil carbon storage [53]. Furthermore, biochar can promote the formation of soil aggregates [54], inhibit the rapid decomposition of soil particulate organic carbon by microorganisms, and reduce the mineralization rate of SOC [55]. In addition, the application of biochar to soil will cause partial degradation naturally, providing new carbon sources for microorganisms, thereby improving microbial activity [56], promoting the conversion of biochar into soil carbon components, and increasing the content of organic carbon in soil carbon components, which can enhance the soil fertility [57]. A recent study demonstrated that the application of biochar and swine manure could significantly increase various ecosystem functions, including crop productivity, soil nutrient storage, soil enzyme activities, and microbial abundance. These improvements contributed to an overall increase in ecosystem multifunctionality. [58]. The combination of biochar and manure can have complementary or synergistic effects, enhancing microbial nutrient fixation, extending the release period of fertilizers [59], reducing nutrient leaching [60], and increasing nitrogen fixation [61], thereby increasing soil active carbon and nitrogen content [62].

Dissolved organic carbon (DOC) was produced by the decomposition of plant residues by humic fungi and animals, catalyzed by biological and extracellular enzymes to convert large molecular components [7], and the higher the SOC content, the greater the amount of DOC generated [63]. In our study, the DOC content was the lowest in the single application of straw treatment due to the complex aromatic structure of straw, which was difficult for microorganisms to decompose and utilize [64], and the higher content of lignin and polyphenols with less soluble substances, resulting in less accumulation of soil DOC [65]. MBC was the most active component of soil organic carbon [66], and the number of microorganisms was responsible for soil organic matter mineralization and nutrient turnover [67]. This study found that the combined application of manure and straw return significantly increased the soil MBC content, which was consistent with the results of Chakraborty et al. [66,68]. This indicated that animal manure as a carbon source input stimulated microbial activity [69], thereby promoting the accumulation of MBC in the soil. EOC was an important energy source for soil microbial activity, which easily oxidized and decomposed organic carbon in soil and can reflect early changes in soil organic carbon [70]. Lützow et al. showed that the content of EOC was closely related to MBC [71], and therefore the content of EOC was highly related to the microbial activity in the soil. In our study, compared with the application of straw alone, the combined application of manure and straw return significantly increased the content of EOC, especially in the SCZ and S0Z treatments. It may be that the addition of swine manure and biochar increased the microbial activity and increased the content of soil labile organic carbon components, consistent with the study by Hao et al. [72]. In addition, the addition of biochar conditioner also significantly increased the soil POC content in this study, aligning with the findings of Shi et al. [73]. It is possible that biochar increases soil POC content through the promotion of root development and secretion, and the porous structure of biochar itself can inhibit the rapid decomposition of soil POC.

4.2. Effects of the Application of Manure and Straw on Soil Microbial Diversity

Studies have shown that returning organic materials to the field increases soil microbial diversity and richness and alters microbial community composition [29,36]. In this study, the continuous application of manure and different conditioners significantly increased the relative abundance of Proteobacteria, with the highest relative abundance observed in S0Z and SCZ treatments. It is possible that swine manure and biochar contain more available carbon, while Proteobacteria can utilize unstable forms of carbon for growth and metabolism, thus changing the soil microbial community structure [25], accelerating the decomposition of straw return, and promoting the increase in labile organic carbon content such as EOC and MBC in the soil, thereby promoting the increase in the content of Proteobacteria. Compared with the straw application treatment alone, the relative abundance of Acidobacteria in the SFZ treatment was higher. This may be due to the decrease in soil pH after adding bio-organic fertilizer to the field, and the number of Acidobacteria was negatively correlated with the soil pH [74]. Compared with the S0Z treatment, the abundance of Bacteroidota was higher in the SBZ treatment because the addition of boron fertilizer increases the content of Bacteroides, which can synthesize and secrete various enzymes for polysaccharide decomposition. After catalytic action, it can produce polysaccharides and other substances that are more conducive to plant absorption. Our research indicated that the ratio of Firmicutes was low, and compared with the S00 treatment, the other treatments increased the abundance of Firmicutes, and it was the swine manure that had a high abundance of Firmicutes [75]. Our study revealed that Sphingomonas was the predominant genus across all the treatments. Given Sphingomonas' significant metabolic capability for biodegrading aromatic compounds, the presence of aromatic substances in maize and swine manure likely serves as favorable substrates for Sphingomonas. For the fungal communities, the dominant phyla include Ascomycota and Basidiomycota. Ascomycota and Basidiomycota are mainly saprophytic and are the main decomposers of recalcitrant organic matter such as plant cellulose and lignin and can play an important role in soil nutrient cycling [76,77]. Moreover, Basidiomycota can counteract the effects of root pathogens on plant growth, providing a healthy soil microbial ecological environment for plant growth [78]. However, the combined application of manure and straw return for two consecutive years significantly reduced the relative abundance of Ascomycota while increasing the relative abundance of Basidiomycota. This was possibly caused by the poor adaptability of Ascomycota to soil environmental changes, and fertilization changed soil conditions and affected the adaptability of Ascomycota. On the other hand, Basidiomycota relies more on the decomposition of woody components to obtain sufficient carbon sources to form mycorrhizal [79]. In this study, compared to the S0Z treatment, the SJZ treatment showed the highest relative abundance of Ascomycota and Basidiomycota, possibly due to the biological agent promoting the rapid degradation of lignin and cellulose by laccase in anaerobic environments [80], thereby increasing microbial activity, the reproduction rate, and abundance [81]. After one year of returning to the field, the abundance of Mortierellomycota in the SCZ treatment was low, while the abundance of Mortierellomycota increased significantly after two years of return to the field with the addition of biochar. This may be due to Mortierella being a potential biocontrol bacterium that can promote plant root absorption of nutrients and inhibit pathogens [82,83]. The relative abundance of the genus Conocybe was relatively low in the treatment after 2 years of returning to the field, indicating that the application of manure can affect the genus Conocybe, which is known for causing crop viruses.

4.3. Relationship between Soil Microbial Communities and Soil Organic Carbon Components

Due to the variation of microorganisms with ecological sites and substrates, the soil environmental changes caused by different fertilization treatments significantly affect the composition of microbial communities [84]. The changes in soil microbial communities explain the changes in soil carbon content, thus proving that soil microbial communities participate in the dynamic changes of soil carbon [85]. RDA analysis showed that soil microbial communities have a certain impact on the composition of soil labile carbon (Figure 6). In our study, Acidobacteria was positively correlated with DOC, EOC, SOC, and MBC, and the POC was positively correlated with Actinobacteria and Proteobacteria. Proteobacteria are rhizosphere plant-promoting bacteria that affect carbon accumulation [86–88] and can preferentially utilize labile components in soil organic carbon for growth and metabolism [89]. Therefore, higher Proteobacteria abundance can accelerate the accumulation of DOC, thereby increasing soil C reserves.

RDA showed that Chytidiomycota was positively correlated with SOC, DOC, POC, and MBC, indicating that Chytidiomycota has a strong accumulation effect on soil organic carbon. This may be due to the fact that Chytidiomycota can accelerate the degradation of lignocellulose and other components in exogenous organic materials and produce organic binders, which can enhance the formation of aggregates in soil [90]. Basidiomycota was positively correlated with DOC and EOC, mainly due to factors such as different conditioners, soil environments, and management measures affecting the composition of soil fungal communities. The decomposition of straw and organic materials increased the proportion of carbon and nitrogen sources and increased the relative abundance of part fungal groups, thereby changing the composition of the fungal community [85].

5. Conclusions

In a two-year study, the continuous application of various conditioners combined with maize straw and manure had a significant impact on soil functional processes. The key players in these processes were soil microorganisms, which in turn influence the feedback mechanism of the carbon cycle mediated by soil microorganisms. The addition of exogenous conditioners notably enhanced the labile fractions and the composition and diversity of the soil microbial community. Specifically, the SCZ treatment led to significant improvements in SOC, DOC, EOC, MBC, and POC contents, as well as an increase in the relative abundance of Basidiomycota and Proteobacteria. The study also demonstrated that the addition of conditioners to fields increased the abundance of Proteobacteria and Basidiomycetes. Furthermore, DOC showed a positive correlation with Actinobacteria and Proteobacteria, while Basidiomycota exhibited positive correlations with DOC, SOC, and EOC. As these were short-term effects after the addition of the amendments, the amount of SOC will not increase at this rate over the years. Overall, the incorporation of different conditioners can enhance biodiversity conservation efforts and contribute to ecosystem protection.

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