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Combined Application of Chemical and Organic Fertilizers Promoted Soil Carbon Sequestration and Bacterial Community Diversity in Dryland Wheat Fields

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Abstract: The use of fertilizers is mainly adopted in arid regions to improve the soil carbon (C) pool and crop productivity. However, the mechanisms underlying improvements in dryland wheat field soils related to microbial metabolic activity and community structure remain poorly understood. Therefore, a field experiment with four fertilization treatments and no fertilizer as the control (CK) was conducted for 10 years in a semi-arid region of China. The results revealed that the combined application of chemical and organic fertilizers (fermented chicken manure) clearly increased the levels of soil organic carbon (SOC), dissolved organic carbon (DOC), and light-fraction organic carbon (LFOC) by 13.54–16.72%, 6.96–9.01%, and 11.00–13.51%, respectively, compared to the sole use of chemical fertilizers (FP treatment). Moreover, the combined treatment not only enhanced the metabolic activity of microorganisms concerning carbon source utilization but also increased the diversity of the bacterial community. This caused noticeable changes in the composition of the bacterial community. A Mantel test analysis revealed that Bacteroidetes and Mortierellomycota significantly enhanced the metabolic activity associated with carbohydrate, amino acid, and carboxylic acid C sources. Actinobacteria, Bacteroidetes, and Mortierellomycota facilitated the accumulation of active C and particulate organic carbon (POC), whereas Mortierellomycota specifically promoted the accumulation of heavy-fraction organic carbon (HFOC), thereby collectively influencing the SOC content. The combined application of chemical and organic fertilizers increased the abundance of Bacteroidetes and Mortierellomycota. This enhancement improved the metabolic utilization of carbohydrates, amino acids, and carboxylic acids, resulting in alterations in the types and quantities of soil metabolites. Consequently, these alterations ultimately affect the composition and quantity of the SOC pool in arid agroecosystems. In conclusion, the combined application of balanced NPK fertilizers and organic fertilizers has a strong positive effect in improving soil microbial activity and the soil C pool.

Keywords: long-term fertilization; dryland wheat fields; soil organic carbon fraction; Biolog Eco-Plates; soil microbial community

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1. Introduction

Soil organic carbon (SOC) plays a key role in regulating soil fertility and is essential for the sustainable development of agroecosystems [1,2]. However, distinguishing short-term and long-term variances in the soil C pool is difficult because SOC is a heterogeneous

mixture of organic substances [3]. DOC, easily oxidizable carbon (EOC), and LFOC are active organic carbon fractions that reflect the nutrient availability in the soil. Conversely, POC and HFOC are passive and recalcitrant C fractions. Therefore, these fractions are considered initial and final indicators of changes in SOC [4,5]. As the primary source of essential nutrients and energy, SOC influences the activity of soil microorganisms. Concurrently, soil microorganisms are the key regulators of SOC formation, turnover, and decomposition. Fertilization measures can affect the metabolic activity of the soil microbial community by changing the type, quantity, and quality of the C substrate entering the soil [6,7]. Moreover, differences in soil microbial community composition are predicted to occur under long-term different fertilization treatments because microbes frequently display a preference for specific substrates [8,9], and changes in microbial metabolic activity and community, in turn, affect SOC accumulation and cycling. Accordingly, the mechanisms by which fertilization practices affect SOC and microbiome changes should be determined to facilitate sustainable crop development and agricultural practices.

The application of chemical fertilizers can influence SOC content by boosting biomass production [10–12], which may lead to positive [13,14], neutral [15], or negative effects on the SOC content [16]. The application of organic fertilizers and combinations of chemical and organic fertilizers is generally believed to enhance SOC content because organic fertilizers enrich the C sources and accessible nutrients [17,18]. Overall, the sequestration of SOC depends on the equilibrium between C inputs and outputs, provided that other nutrients are present at concentrations that do not limit crop growth. Labile C fractions exhibit rapid turnover rates and act as energy sources for soil food webs, thereby enhancing nutrient cycling. Conversely, recalcitrant fractions are highly resistant to microbial decomposition, playing a crucial role in long-term C sequestration. Rudrappa et al. [19] observed that the application of NPK fertilizers at rates ranging from 50 to 150% of the recommended dosage significantly increased POC and EOC in a semi-arid, subtropical region of India. Wei et al. [20] reported that returning granulated straw significantly increased the contents of SOC and EOC in the topsoil layer (0–20 cm). Additionally, it enhanced the levels of DOC, SOC, and LFOC in the subsoil layer (20–40 cm) within dryland farming systems. Benbi et al. [21] also reported that the application of farmyard manure in a rice–wheat cropping system under semi-arid subtropical conditions resulted in the build-up of not only the labile but also the recalcitrant pool of SOC. Thus, changes in the quantities of both active and inert C pools following different fertilization practices can effectively indicate the mechanisms of C storage in soils.

Previous studies have commonly focused on the impact of fertilization on specific aspects of the soil microbial community, such as metabolic activity or structural composition [6,22]. The Biolog-Eco method detects carbon metabolic activity by analyzing the utilization of 31 carbon sources among microorganisms, swiftly characterizing the ecological status and functional diversity of microbial communities. The application of manure typically enhances the ability of soil microorganisms to utilize total carbon sources and improves the utilization rate of carboxylic acids, amino acids, and amines to a greater extent in semi-arid areas [23], and it may change the soil microbial community [24,25]. The long-term application of chemical fertilizers may have a positive, neutral, or negative influence on soil microbial activity and biomass [26], and the impact of such practices on the structure of the microbial community remains a subject of ongoing debate [27,28]. In agroecosystems, soil microorganisms facilitate the conversion of organic matter into SOC via mineralization and immobilization [29]. The microbial carbon pump (MCP) facilitates the release of C to the atmosphere through its catabolic activities, and it promotes C storage by stabilizing C into a form that is resistant to being decomposed [30]. The net effect of these microorganism-mediated processes on C sequestration or C loss is related to soil environmental conditions. Therefore, microbial involvement has a significant impact on the magnitude of the organic C reservoir in soil. However, the interaction between soil microbial metabolism activity, community composition, and C sequestration under different fertilization conditions remains largely unknown. Currently, there is a global focus on the

C cycle, with soil C sequestration in agricultural lands playing a vital role in mitigating the global greenhouse effect. This study aimed to preliminarily explore the mechanisms of soil C sequestration, with the hope of contributing to a better understanding of the global C cycle.

The arid and semi-arid regions of the Loess Plateau are the most important winter wheat cultivation areas in China. The region in question has a low content of soil organic matter (SOM); however, it possesses a high potential for carbon sequestration [31,32]. Thus, fertilization is the primary method for enhancing soil characteristics and fertility. In this study, a 10-year field study was set up with four typical fertilization modes. We explored the effects of different fertilizer inputs on SOC fractions, the metabolic activity and community composition of soil microorganisms, and the interactions among these factors. We hypothesized that the combined application of chemical and (bio)organic fertilizers would better promote the metabolic activity of microorganisms compared to traditional fertilizers used by local farmers (NP) or balanced chemical fertilizers (NPK). This is because organic fertilizer is a rich source of C and accessible nutrients and thus may alter microbial community composition, stimulate the degradation of organic matter by soil microorganisms, promote the production of more active organic carbon, and ultimately increase the SOC content.

2. Materials and Methods

2.1. Study Area

The field experiment was established in 2013 at Dong liang Village, Liujiayuan Town, Hongtong city, Shanxi Province, China (36°22′ N, 111°35′ E), which is situated on the Loess Plateau. The study site has a typical semi-arid climate (see Figure S1 in the Supplemental Material). The mean annual temperature is 12.6 °C, the effective accumulated temperature is 3326.9 °C, the annual average precipitation is 493.3 mm (concentrated in July, August, and September), and the average number of frost-free days is 180–210. The soil is classified as Chromic Cambisols based on the FAO Soil Map classification system, which includes sand, silt, and clay percentages of 67.7%, 28.7%, and 3.6%, respectively, with a sandy clay loam texture (based on the International System of Soil Texture Classification). The physicochemical properties of the initial experimental soil were as follows: topsoil pH = 7.66 (0–20 cm depth and measured in distilled water following the Chinese national standards HJ962-2018), soil bulk density = 1.20 g·cm^{-3}, organic matter = 14.10 g·kg^{-1}, total N = 0.79 g·kg^{-1}, available P = 12.18 mg·kg^{-1}, available K = 198.19 mg·kg^{-1}, and NO_3^-–N = 8.64 mg·kg^{-1}.

2.2. Experimental Design

This study consisted of five treatments: (1) no fertilization (CK); (2) traditional fertilization used by local farmers (FP), with only N and P elements but not K because the local soil is relatively rich in K elements; (3) optimized fertilization pattern (OF); (4) optimized fertilizer + organic fertilizer pattern (OFM); and (5) optimized fertilizer + biological organic fertilizer pattern (OFB). Optimized fertilization, also known as balanced fertilization, includes the combined application of N, P, and K at a scientific ratio based on the crop and soil conditions. The N, P, and K application rates were determined from the yield of the preceding crop, as well as the nitrate-nitrogen content of the 0–100 cm soil layer and the quick-acting potassium and quick-acting phosphorus content of the 0–40 cm soil layer prior to sowing wheat. OFM was used to replace 30% of chemical fertilizer N with organic fertilizer (chicken manure) based on the OF treatment. OFB was used to replace part of chemical fertilizer with bio-organic fertilizer based on the OF treatment, and the bio-organic fertilizer was a mixed bacterial solution consisting of Lahn-type bacteria and *Pseudomonas* 1 and 2 (effective strains were Pseudomonas and Lahn-type bacteria, effective viable bacteria count $\geq 0.5 \times 10^8$ CFU·g^{-1}, organic matter $\geq$ 40%), blended with rotted chicken manure at a ratio of 1:9. The organic and bio-organic fertilizers used the same carrier, which is chicken manure. The N, P, and K contents of these fertilizers are detailed

in Table S1. The fertilizers included urea (46.0% N), calcium superphosphate (12% P_2O_5), and potassium chloride (60% K_2O). In treatments 3 to 5, equal nutrient quantities of N, P, and K were applied. All fertilizers are commercially manufactured products supplied by Shanxi Jinnongkang Biotechnology Co., Ltd. (Hongtong, China). Table 1 provides the 10-year cumulative fertilizer applications and C inputs for each treatment. The C inputs include four components, namely, straw, root system, root secretion inputs, and C inputs from organic materials.

Table 1. Cumulative fertilizer application for each treatment.

Treatment	Cumulative Fertilizer Application (kg/ha)			Cumulative Organic Fertilizer Application (kg/ha)	Cumulative Input of Exogenous C (Mg/ha)
	N	P	K		
CK	0	0	0	0	21.63
FP	1500.0	261.0	0.0	0.0	31.10
OF	899.3	284.6	236.1	0.0	34.10
OFM	567.8	158.6	32.0	19,991	42.39
OFB	567.8	158.6	32.0	19,991	41.06

A completely randomized block design was used, and each plot was 120 m^2. The seeding process included utilizing machinery to cover the ridges with mulch and sowing the furrows between the mulch. All fertilizers were evenly applied to the respective plots once per growing season prior to sowing. Sowing was conducted annually in late September at a rate of 150 kg/ha. Crops were harvested in early June of the subsequent year, with a fallow period from mid-June to mid-September. During the experimental period, irrigation was not performed.

2.3. Sample Collection and Determination

Following the wheat harvest in June 2023, topsoil samples were collected from five sites within three replicate plots for each treatment. The soil samples were mixed evenly, and impurities were removed. Then, the mixed sample was divided into three portions, with one stored at −80 °C for high-throughput DNA sequencing, one stored at 4 °C, and one dried naturally.

2.3.1. Soil Sample Determination

Soil nitrate-nitrogen was measured using 1.0 mol/L KCL extraction and analyzed with an ultraviolet spectrophotometer [33]; quick-acting potassium was measured using 1.0 mol/L NH_4OAC extraction, followed by an analysis with a flame spectrophotometer [33]; and quick-acting phosphorus was measured using 0.5 mol/L $NaHCO_3$ extraction, followed by molybdenum–antimony resistance colorimetry with a spectrophotometer [33]. SOC was measured using the $K_2Cr_2O_7$ volumetric method [33]. DOC was extracted using 0.5 mol·L^{-1} K_2SO_4 [5]. POC was determined using $(NaPO_3)_6$ [5], and EOC was measured using 333 mol·L^{-1} $KMnO_4$ [2]. LFOC and HFOC were measured using the specific gravity separation approach and the $K_2Cr_2O_7$ volumetric method [5].

2.3.2. Determination of Metabolic Activity for Soil Microbial Carbon Sources

The metabolic activity for soil microbial C sources was assessed using the biological microplate technique [23]. Weighed fresh soil samples, equivalent to 10 g of dry mass, were added to 90 mL of a sterilized 0.85% NaCl solution. The bottle was shaken at 200 rpm for 30 min, the suspension was filtered, and 1 mL of the supernatant was added to a 9 mL NaCl solution and thoroughly mixed. Next, 1 mL of the diluted supernatant was added to a 9 mL NaCl solution. After dilution, this soil solution was finally diluted to 10^{-3} and prepared for immediate reaction. On an ultra-clean workbench, the diluent was applied to an ecological test plate (Biolog Inc., Hayward, CA, USA), with each well receiving 150 µL of diluent and being incubated at 25 °C. A Biolog microplate reader (Tecan Austria GmbH, China Tecan (Shanghai) laboratory equipment Co., Ltd., Shanghai, China) was used to

measure the absorbance at 590 nm (OD590) for each well after incubation periods of 6, 12, 24, 48, 72, 96, 120, 144, 168, and 192 h.

Average well-color development (AWCD) represents the carbon-source-specific microbial activity on the EcoPlates [23]. The formula for calculating AWCD according to the absorbance values of the solution in the hole of the Biolog EcoPlate is as follows (1):

$$\mathrm{AWCD} = \sum (\mathrm{C_i} - \mathrm{R})/\mathrm{n} \quad (1)$$

where C_i denotes the absorbance of each well, R represents the absorbance of the control well, and n represents the number of carbon sources in the culture medium (n = 31).

The Shannon–Wiener diversity index (H) was calculated using Equation (2):

$$\mathrm{H} = -\sum(\mathrm{P_i} \ln \mathrm{P_i}) \quad (2)$$

where $P_i = (C_i - R)/\sum(C_i - R)$, which represents the ratio of the absorbance value in the ith non-control well to the sum of the absorbance values of all non-control wells.

The Pielou index (J) quantifies the evenness of the species distribution within a community and is calculated using Formula (3):

$$\mathrm{J} = \mathrm{H}/\ln\mathrm{S} \quad (3)$$

where S denotes the average number of substrates utilized, which represents the carbon source on the ECO plate (AWCD > 0.25). Additionally, the richness index (R) is expressed as the mean number of these substrates and serves as a reflection of the soil microbial carbon source utilization and metabolic activity.

2.3.3. Extraction of Soil Microbial DNA and High-Throughput Sequencing

DNA from the soil samples was extracted using the E.Z.N.A.® soil DNA kit (Omega Bio-Tek, Norcross, GA, USA). DNA concentration and purity were determined using a NanoDrop2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). PCR of the V3–V4 variable region of the 16 S rRNA gene was performed using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The V4 variable region of the 16S rRNA gene was amplified by PCR. ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the hypervariable ITS region of fungi. The PCR products were recovered on a 2% agarose gel and purified using the DNA Gel Recovery and Purification Kit (PCR Clean-Up Kit, NEB, Ipswich, MA, USA). The recovered products were quantified using Qubit 4.0 (Thermo Fisher Scientific, USA) with the assistance of Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) [6,34].

2.4. Data Analysis

The data were summarized and calculated using Microsoft Office 2021(Microsoft, Redmond, WA, USA). Differences in soil carbon fractions, the capacity of soil microorganisms to utilize different carbon sources, microbial functional diversity indices, and OUT-based alpha-diversity indices among treatments were statistically compared using one-way analysis of variance (ANOVA) in SPSS 24.0 (IBM, Armonk, NY, USA). Subsequently, Duncan's test was applied to ascertain significant differences ($p < 0.05$) between the control group and each of the treatment groups. The AWCD values after a 96 h incubation period from the Biolog Eco Plate wells were extracted and subjected to principal component analysis (PCA) using SPSS 24.0 software. Variables with absolute loading values (>0.6) were incorporated to discern the main types of carbon sources utilized by microorganisms across different fertilization treatments. A principal component analysis (PCA) ordination plot was generated, where the distances between sample points signify the degree of dissimilarity among the samples. The variations in carbon source utilization patterns across different fertilization treatments can be inferred from these distances. Graphical representations were generated

using Origin 2022 (OriginLab, Northampton, PA, USA). Alpha diversity was calculated by QIIME 2.0. Species abundance data derived from high-throughput sequencing were utilized for Principal Coordinate Analysis (PCoA). Additionally, replacement multiple analysis of variance (PERMANOVA) based on Bray–Curtis distances was employed to randomly permute samples to generate an F-value, from which a *p*-value was derived. All of these analyses were performed using the "vegan" package in R, aimed at evaluating microbial community variability. The relationships between SOC components and bacterial (fungal) abundance and microbial carbon source utilization were determined based on Spearman's correlation and the Mantel test (999 permutations) using the "ggcor" package in R (R-4.4.1 for Windows).

3. Results

3.1. Effects of Fertilization on SOC and Its Fractions

Compared to the FP treatment, the OF treatment did not show significant differences in the various SOC fractions, although the SOC content was significantly increased by 11.79% ($p < 0.05$), as shown in Figure 1. The combined application of inorganic and (bio-)organic fertilizers (OFB and OFM) significantly increased the contents of SOC, DOC, and LFOC fractions compared with the FP treatment, with increases of 13.54–16.72% ($p < 0.05$), 6.96–9.01% ($p < 0.05$), and 11.00–13.51% ($p < 0.05$), respectively. Among them, the OFB treatment exhibited the greatest increase and highest SOC content of 11.38 g·kg^{-1}.

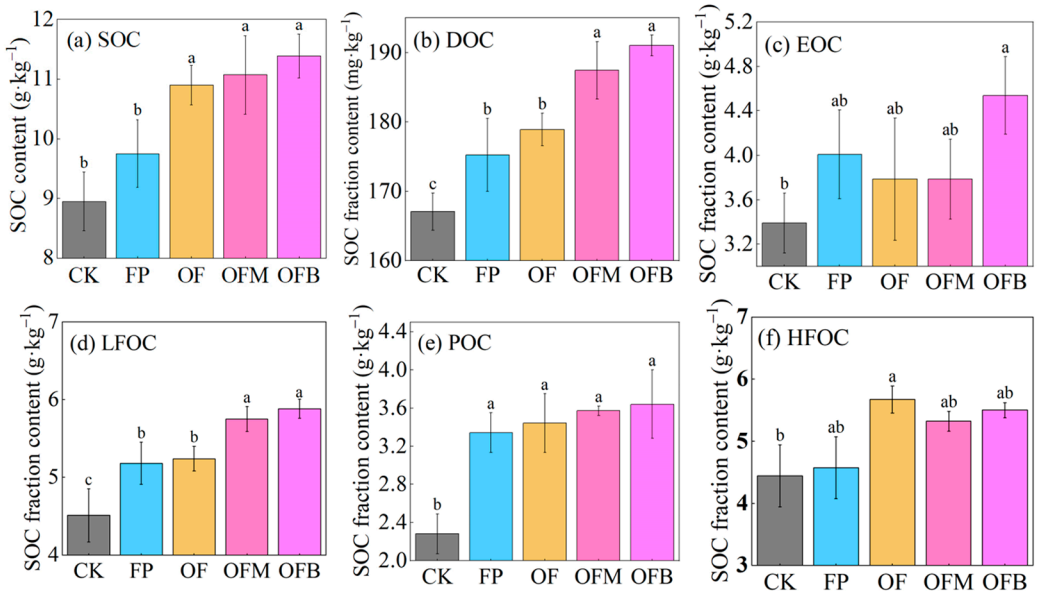

Figure 1. The contents of SOC and its components under different fertilization treatments. (**a**) SOC; (**b**) DOC; (**c**) EOC; (**d**) LFOC; (**e**) POC; (**f**) HFOC. Different lowercase letters indicate significant differences among treatments ($p < 0.05$). CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

3.2. Metabolic Functions of Soil Microbial Carbon Sources

3.2.1. Metabolic Capacity of Soil Microbial Carbon Sources

The AWCD of the carbon sources used by the soil microorganisms increased rapidly after 12 h and slowly after 168 h, reaching an asymptote after 168 h of incubation (Figure 2). The AWCD of OFB was always the highest, indicating that the metabolic activity of soil microorganisms under the OFB treatment was the strongest. The AWCD of OFB, OFM, and OF was significantly higher than that of FP from 48 to 144 h ($p < 0.05$).

Significant differences ($p < 0.05$) in the utilization capacity of soil microorganisms were observed in the different fertilization treatments for the six types of carbon sources

according to AWCD at 96 h (Figure 3). At this time point, AWCD rapidly increased. In the OFM and OFB treatments, the carbon source utilization abilities of soil microorganisms were ordered carbohydrates > amino acids > carboxylic acids > polymers > amines > phenolic acids. In the FP, these abilities were ordered polymers > carbohydrates > carboxylic acids. In the OF treatment, these abilities were ordered carbohydrates > carboxylic acids > polymers. Phenolic acid had the lowest utilization in all fertilization treatments. The carbon source utilization ability of carbohydrates, amino acids, and carboxylic acids was significantly improved under the OFM and OFB treatments relative to the FP treatment ($p < 0.05$). The preference of soil microorganisms for carbon source utilization changed to a certain extent under the different fertilization treatments, which changed the metabolic function of the soil microbial community.

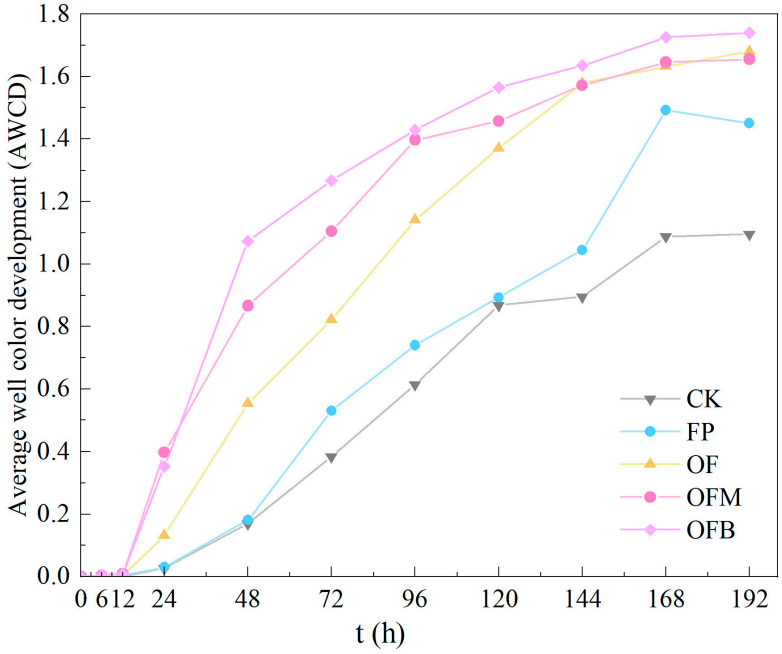

Figure 2. AWCD variation in carbon utilization by microorganisms under different fertilization treatments. CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

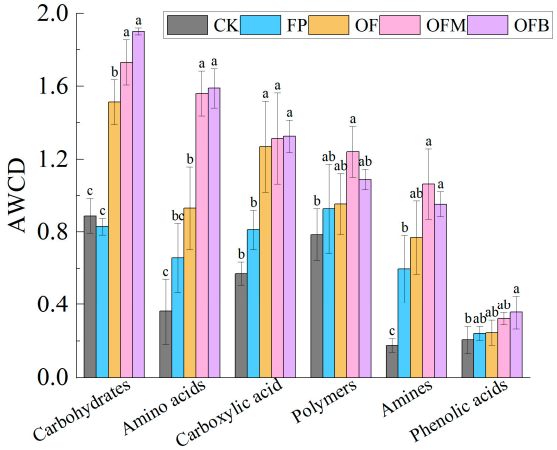

Figure 3. Microbial utilization of six types of C sources under different fertilization treatments. Different lowercase letters indicate significant differences among treatments for each type of C source. CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

3.2.2. Metabolic Diversity of Soil Microbial Carbon Sources

Table 2 shows the carbon-source-utilization-based functional diversity index results under the different fertilization treatments. The Shannon (H) and richness (R) indices in the OFB, OFM, and OF treatments were significantly increased relative to those in the FP treatment by 5.85–14.76% ($p < 0.05$) and 24.95–68.17% ($p < 0.05$), respectively. The Pielou index (J) was highest in the FP and CK treatments but showed a downward trend in the OFM and OFB treatments. The value in the OFB treatment was the lowest. However, the three diversity indices did not significantly differ between the OFM and OFB.

Table 2. Metabolic function indices of soil microbial communities among fertilization treatments.

Treatment	Shannon Diversity Index (H)	Richness Index (R)	Pielou Evenness Index (J)
CK	3.69 ± 0.09 d	12.33 ± 0.58 c	1.47 ± 0.01 a
FP	3.93 ± 0.09 c	14.67 ± 2.31 c	1.47 ± 0.06 a
OF	4.16 ± 0.10 b	18.33 ± 1.53 b	1.43 ± 0.04 ab
OFM	4.37 ± 1.12 a	22 ± 2.65 a	1.42 ± 0.03 ab
OFB	4.51 ± 0.08 a	24.67 ± 1.15 a	1.41 ± 0.07 b

Note: Data are means ± standard errors, and different lowercase letters indicate significant differences among treatments ($p < 0.05$). CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

3.2.3. Principal Component Analysis (PCA) of Carbon Sources

Table 3 lists the loading values of 31 carbon sources on the PC1, PC2, and PC3 axes. A carbon source with an absolute loading value greater than 0.6 was regarded as the main utilization type of the soil microbial community. Nineteen types of carbon sources contributed greatly to PC1, including eight types of carbohydrates (D-cellobiose, β-methyl-D-glucoside, D-xylose, D-mannitol, N-acetyl-D-glu-cosamine, Glucose-1-phosphate, L-α-glycerol phosphate, and D-galactonic acid-γ-lactone), four types of amino acids (L-asparagine, L-serine, L-threonine, and Glycyl-L-glutamic acid), three types of carboxylic acids (D-glucosaminic acid, D-galacturonic acid, and γ-hydroxybutyric acid), three types of polymers (Tween 40, Tween 80, and Glycogen), and one type of amine (Putrescine). Five carbon sources contributed significantly to PC2 and PC3, including one carbohydrate, one amino acid, two carboxylic acids, and one phenolic acid. This indicated that carbohydrates, amino acids, and carboxylic acids were the main carbon sources that led to microbial community changes in the soil.

Table 3. Loading factors of 31 carbon sources on PC1, PC2, and PC3.

Type of Carbon Source	Substrates	PC1	PC2	PC3
Polymers	Tween 40	0.670	−0.087	0.274
	Tween 80	0.793	0.275	−0.204
	α-Cyclodextrin	0.098	0.390	0.392
	Glycogen	−0.631	−0.365	0.295
Carbohydrates	D-cellobiose	0.833	−0.342	−0.157
	α-D-lactose	0.004	0.684	−0.313
	β-Methyl-D-glucoside	0.786	−0.369	−0.050
	D-xylose	0.671	0.229	−0.126
	I-erythritol	0.580	−0.215	−0.323
	D-mannitol	0.910	−0.278	0.142
	N-acetyl-D-glu-cosamine	0.878	−0.120	−0.046
	Glucose-1-phosphate	0.716	−0.275	0.338
	L-α-glycerol phosphate	0.895	0.131	−0.109
	D-galactonic acid-γ-lactone	0.780	−0.155	0.191
Phenolic acids	2-Hydroxybenzoic acid	−0.217	−0.552	0.042
	4-Hydroxybenzoic acid	−0.039	−0.568	0.610

Table 3. *Cont.*

Type of Carbon Source	Substrates	PC1	PC2	PC3
Carboxylic acid	Pyruvic acid methyl ester	0.215	0.236	0.717
	D-glucosaminic acid	0.607	−0.366	0.577
	D-galacturonic acid	0.878	−0.098	−0.211
	γ-Hydroxybutyric acid	0.868	0.024	0.150
	Itaconic acid	0.057	0.470	0.730
	α-Ketobutyric acid	−0.418	0.337	−0.066
	D-malic acid	0.310	0.528	0.355
Amino acids	L-arginine	0.558	0.590	0.147
	L-asparagine	0.714	0.011	−0.140
	L-phenylalanine	0.300	0.647	0.151
	L-serine	0.955	−0.135	−0.010
	L-threonine	0.602	−0.066	−0.522
	Glycyl-L-glutamic acid	0.608	0.114	−0.491
Amines	Phenylethylamine	0.148	0.341	−0.167
	Putrescine	0.780	0.302	0.269

PCA was performed using the absorbance values of 31 carbon sources incubated for 96 h. The variance contribution rates of PC1 and PC2 were 40.5% and 12.4%, respectively. Figure 4 shows the approximate distribution ranges of the five fertilization treatments. Both OFM and OFB were in the second quadrant, and the distance between them was small, indicating that they had similar carbon source utilization efficiencies. FP, OF, OFM, and OFB were distributed in different quadrants, and there were noticeable spatial differences in terms of carbon source utilization, indicating that the soil microorganisms of different fertilization treatments had different carbon source utilization patterns.

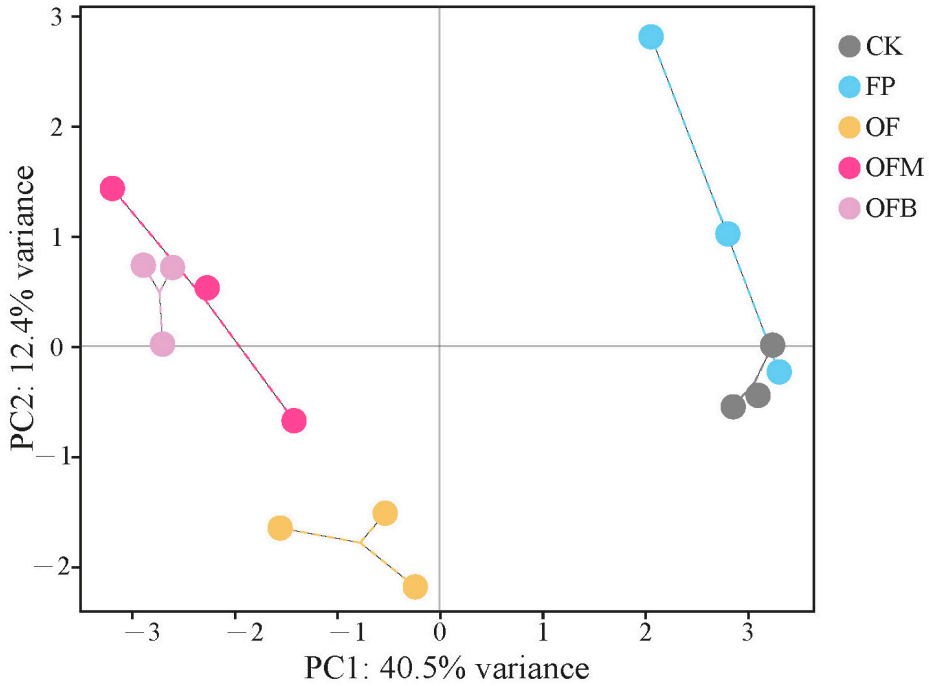

Figure 4. PCA for carbon source utilization of soil microbial communities under different fertilization treatments. CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

3.3. Effects of Fertilization on Soil Microbial Communities

3.3.1. Diversity Analysis of Soil Microbial Community

The total number of bacterial and fungal sequences across the 15 samples was 1,014,735 and 1,141,274, respectively. The sequences obtained were subjected to random sampling, followed by OTU clustering at a 97% similarity level. The observed total number of OTUs for bacteria and fungi was 1544 and 1911, respectively. To reduce the impact of the sequencing depth on the subsequent analyses of alpha and beta diversity, the number of sequences for bacterial and fungal samples was adjusted to 27,641 and 62,080, respectively, based on the sample with the fewest sequences. The coverage rates of all samples remained high, at 96.52% for bacteria and 99.85% for fungi.

The richness and diversity of the microbial communities were evaluated using the Chao1 and Shannon indices for alpha diversity. Compared with the application of chemical fertilizers alone (FP and OF treatments), both the OFM and OFB treatments significantly elevated the Chao1 index of bacteria by 9.59–18.25% ($p < 0.05$) (Figure 5). Additionally, under the OFB treatment, the Shannon index was enhanced by 2.31% ($p < 0.05$) and 2.0% ($p < 0.05$) compared with the FP and OF treatments, respectively. However, the application of chemical fertilizers alone did not have a distinct influence on the diversity of soil bacterial communities compared to the control (CK). Furthermore, the Chao1 and Shannon indices of soil fungi were not distinctly influenced by the different fertilization treatments.

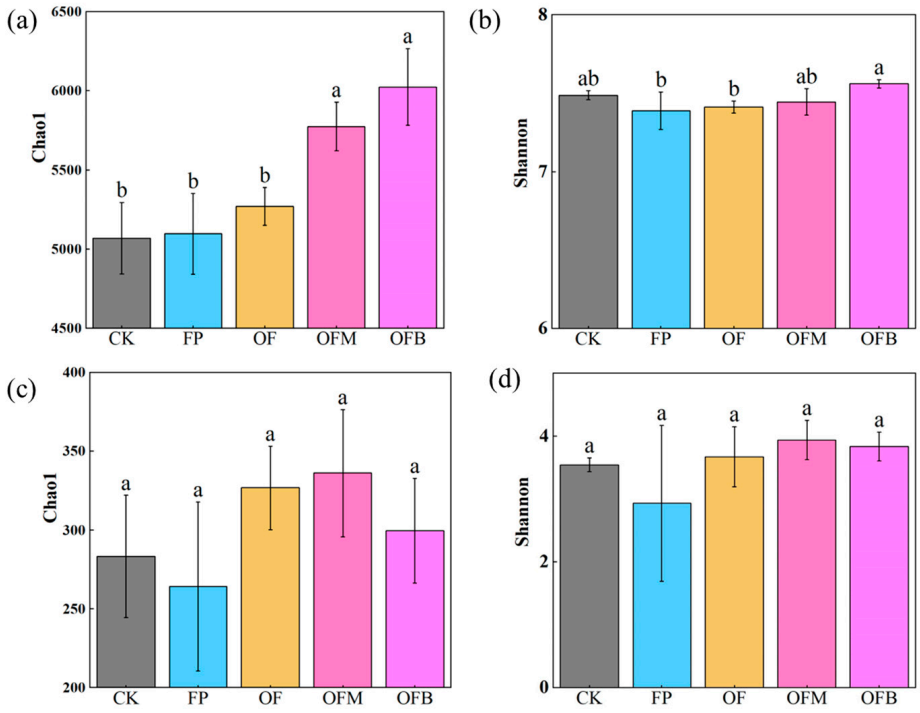

Figure 5. Differences in OUT-based species alpha diversity under different fertilization treatments. Chao1 index and Shannon diversity index of bacteria (**a**,**b**); Chao1 index and Shannon diversity index of fungi (**c**,**d**). Different lowercase letters indicate significant differences among treatments ($p < 0.05$). CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

According to the PERMANOVA ($F = 1.224$, $p < 0.01$), statistically significant differences were observed in the soil bacterial community among the four fertilization treatments. The bacterial communities in the single-fertilizer treatments (OF and FP) and the organic fertilizer combined with inorganic fertilizer treatments (OFM and OFB) exhibited significant differences compared to CK (Figure 6a). The PCoA results of the fungal community showed that the community structure in the fertilizer treatments was different from that in CK, but

the points were closer together, indicating that the difference may not be as significant as that in the bacterial community (Figure 6b).

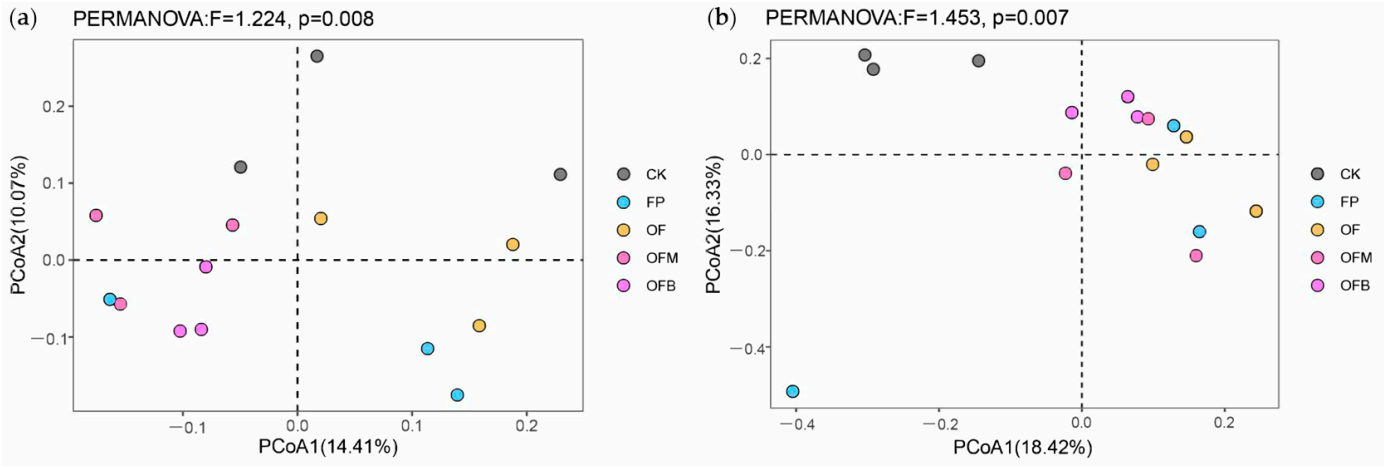

Figure 6. The beta diversity of soil bacteria (**a**) and fungi (**b**) under different fertilization treatments. CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

3.3.2. Composition of Soil Microbial Community

An analysis of the microbial community components of the different fertilization treatments revealed that Proteobacteria (33.84%), Actinobacteria (22.07%), Acidobacteria (16.56%), Chloroflexi (7.08%), Firmicutes (6.28%), and Bacteroidetes (5.28%) were the dominant phyla (Figure 7a). The OFM and FP treatments had the highest and lowest relative abundances of Proteobacteria (37.64% and 30.03%, respectively). For Acidobacteria and Firmicutes, the FP treatment had the highest relative abundances (19.57% and 7.62%, respectively), while OFM and OF showed decreases in abundance of 27.58–37.60% and 24.92–38.43%, respectively. OFM had the highest relative abundance of Actinobacteria (24.74%), while OFB had the highest relative abundance of Bacteroidetes (6.44%). However, the relative abundance of these two phyla decreased by 20.12% and 31.05% in the FP treatment.

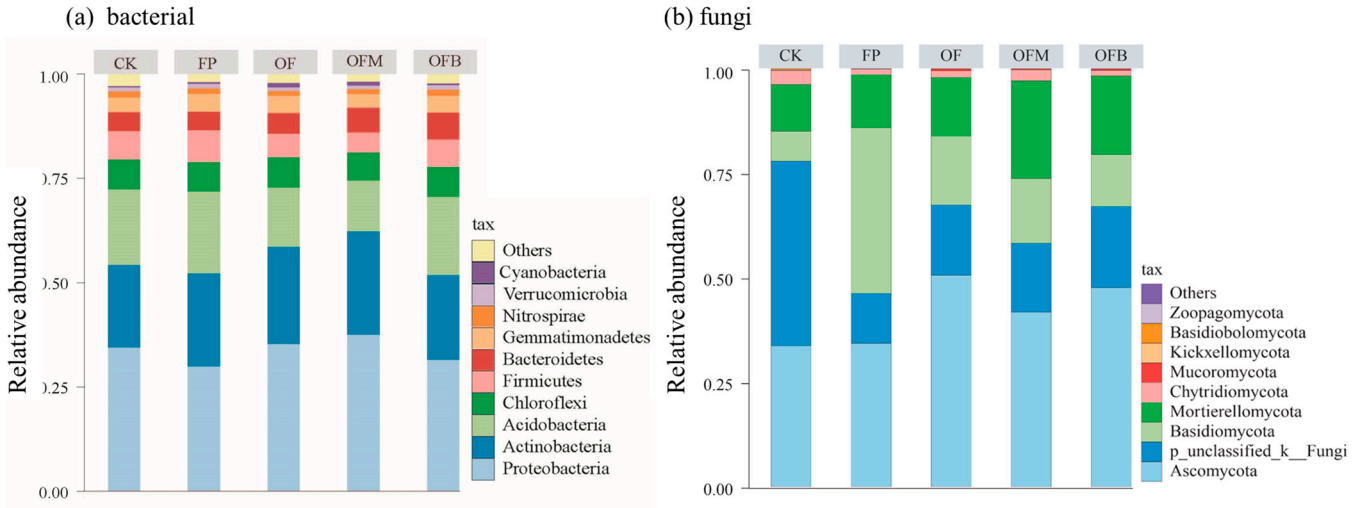

Figure 7. Microbial community composition at the phylum level under different treatments: (**a**) bacterial, (**b**) fungi. CK (no fertilization), FP (farmer pattern), OF (optimized fertilizer pattern), OFM (optimized fertilizer + organic fertilizer pattern), OFB (optimized fertilizer + biological organic fertilizer pattern).

Ascomycota (41.62%), Basidiomycota (18.23%), and Mortierellomycota (16.02%) were the dominant fungi in the fungal community (Figure 7b). The CK treatment had the lowest relative abundance of Ascomycota (33.71%), while the fertilizer treatments showed increases in abundance of 1.76–50.14%. OFM had the highest abundance of Mortierellomycota (26.76%), followed by OFB (19.92%) and CK (12.75%). Notably, the FP treatment had the highest abundance of Basidiomycota (39.62%), while that in CK was only 7.16%.

3.4. Correlation between SOC Fractions and Soil Microbial Community

The Mantel test was used to determine the interaction between SOC fractions, bacterial or fungal abundance, and carbon source utilization (carbohydrates, amino acids, and carboxylic acids) (Figure 8). The utilization of carbohydrates, amino acids, and carboxylic acids by microorganisms was positively correlated with the abundance of Bacteroidetes (r = 0.67–0.83, $p < 0.001$) and Mortierellomycota (r = 0.47–0.69, $p < 0.001$) but negatively correlated with that of Firmicutes (r = −0.53–−0.56, $p < 0.05$). Moreover, the heightened metabolic functions enhanced the content of SOC components. Labile organic carbon fractions (DOC, EOC, and LFOC) exhibited a significant positive correlation with Actinobacteria, Bacteroidetes, and Mortierellomycota. POC was notably correlated with Actinobacteria, while HFOC was positively correlated with Mortierellomycota. These correlations suggest that Actinobacteria, Bacteroidetes, and Mortierellomycota enriched the labile carbon components and POC by enhancing carbon source metabolism. Additionally, Mortierellomycota increased the HFOC content by enhancing metabolic processes.

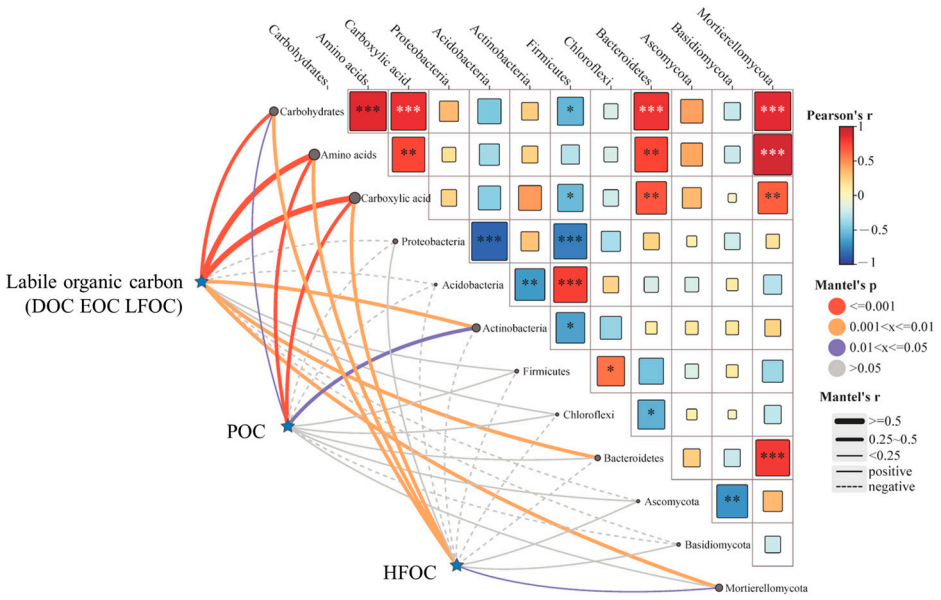

Figure 8. A mantel test analysis of SOC fractions, dominant bacterial or fungal abundance, and soil microorganisms' utilization of carbon sources (carbohydrates, amino acids, and carboxylic acids). The pairwise correlation of bacterial or fungal abundance and the soil microorganism's utilization of a C source is shown with a color gradient based on the Spearman correlation coefficient. The relationships between labile organic carbon or POC or HFOC and soil microbiome-related indices were determined using the Mantel test. The width of edges corresponds to the Mantel r-value, and the color of the edges indicates statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4. Discussion

4.1. Effect of Long-Term Fertilizer Application on SOC Components in Soil

Fertilizer application is an important agricultural measure to improve the soil C pool [1,2,35,36]. Moreover, the addition of an organic fertilizer along with a chemical fertilizer to poor soil can improve SOC sequestration and soil fertility [37]. In this study, compared to the traditional fertilization treatment (FP), 10-year applications of chemical

and organic fertilizers (OFM and OFB) significantly increased the contents of SOC and labile organic carbon fractions (DOC and LFOC) in the topsoil (Figure 1a,b,d). This result was associated with the enrichment of organic C sources and additional nutrients present in organic fertilizers. Furthermore, compared with the FP treatment, the OFM and OFB treatments could indirectly contribute to soil C inputs in the form of higher plant biomass, such as wheat residues, litter fall, and root systems [38]. Consistent with our results, several long-term studies have reported a positive correlation between biomass C input and the SOC content [3,39,40] (Table 1). Moreover, research conducted within this experimental field showed that the application of organic fertilizers improved water-stable soil aggregates through the bonding of primary soil particles [41], which strengthened the physical protection of SOC and prevented its decomposition, resulting in higher SOC content. Notably, the OF treatment also showed a pronounced C accumulation effect compared with the FP treatment. This is expected because the application of inorganic fertilizers can affect soil C accumulation by increasing crop and biomass productivity. This is supported by the greater crop and biomass productivity of the OF treatment compared with the FP treatment.

Active SOC fractions can be derived from the decomposition of organic matter, such as plant litter and root excretion, as well as from intermediate compounds of microbial metabolism or the death of microorganisms [10,42]. Inorganic and organic fertilizers provided nutrients to both plants and microbes and stimulated microbial community growth, enhanced enzyme activity, and eventually increased plant debris, root exudates, and microbial residue accumulation in the soil [30,43]. These components represented major sources of active C fractions. Nevertheless, POC and HFOC did not appear to vary between the OF and FP treatments and OFM and OFB treatments (Figure 1e,f). This could be because applying organic fertilizer along with chemical fertilizers, especially in the bio-organic fertilizer treatment, provided new inputs of fresh C. These inputs could activate microbial groups that were dormant or inactive and lead to the synthesis of a wide variety of enzymes capable of decomposing recalcitrant SOC. Thus, a "priming effect" of POC and HFOC may occur [44]. These may offset some of the positive influences of high C inputs on POC and HFOC contents in the OFM and OFB treatments.

4.2. Effects of Long-Term Fertilization on Functional Diversity of Soil Microorganisms

AWCD serves to quantify microbial carbon metabolic activity. The highest AWCD values of the soil microorganisms were consistently observed in the OFB treatment throughout the 192 h culture period (Figure 2); conversely, the lowest values were observed in the FP treatment. These findings may be attributed to the SOC content. The activity and biomass of microorganisms were positively correlated with the organic matter content [45]. Section 4.1 revealed that the SOC content was higher in the treatment combining chemical and organic fertilizers than in the FP treatment. This differentiation in SOC content among treatments is likely a factor underlying the metabolic activity variations among microbial carbon sources. As emphasized by Sabir et al. [37], organic fertilizers enhanced C input, which strongly promoted the growth of heterotrophic microorganisms and increased the overall microbial number and activity in the soil. According to the indices of carbon-source-utilization-based metabolic diversity (Table 2), the functional richness (R) and Shannon diversity index (H) of soil microbial communities in the OFM and OFB treatments were significantly higher than those in the FP and OF treatments. However, the Pielou evenness indices (J) in the OFM and OFB treatments were lower than in the FP and OF treatments, suggesting that the combined application of organic and chemical fertilizers could not only increase the species richness and overall diversity but also potentially attenuate the dominance of any single species within the community [46]. In the FP treatment, the observed indices' variations suggested that the long-term application of inorganic fertilizers could decrease topsoil species diversity relative to the combined application of organic fertilizers. Eo et al. [47] also found that continuous imbalanced fertilization could enhance the growth of certain microorganisms that are well adapted to the environment, thereby reducing microbial diversity. This shift could account for the lower overall metabolic activity of the microbial

community under the FP treatment. As expected, the functional richness (R) and Shannon diversity index (H) were significantly higher after the OF treatment than after the FP treatment. This phenomenon may be explained by several potential mechanisms. For example, Wei et al. [48] demonstrated that the balanced application of NPK was generally more beneficial to the improvement of soil fertility and microbial activity than the application of N or NP. In addition, Li et al. [49] showed that balanced fertilization (e.g., OF treatment) was favorable for the synthesis of the cellular components of microorganisms, thereby increasing their metabolic activity.

The PCA showed that the FP, OF, OFM, and OFB treatments were plotted in different quadrants (Figure 4), indicating that they had different patterns of carbon utilization. The analysis of factor loading values, as presented in Table 3, revealed that the variations in carbon metabolism across different fertilization treatments were predominantly associated with eight carbohydrates, four amino acids, and three carboxylic acids. Differences in organic matter composition and substrate availability in the soil under different fertilization treatments may be the main reasons for the differences in the carbon sources used in this study [50]. This was attributed to the substrate preferences exhibited by microbes; e.g., bacteria generally demonstrate a higher adaptability to environments with abundant, readily available C and rich nutrients. In contrast, fungi are more adept at exploiting recalcitrant C sources [48], ultimately affecting the diversity of microbial functions [51]. This finding indicated that the activity of soil microbial carbon metabolism had been significantly altered by various fertilization treatments over the decade-long study period. It is worth noting that the culture environment of inoculated microplates differs significantly from the natural conditions of soil samples. Thus, the results may not fully reflect the behavior of soil microorganisms under natural conditions. Moreover, the general incubation period for microplates is relatively short, which may not capture information on microorganisms that utilize carbon sources at a slower rate [23].

4.3. Effects of Fertilization on Soil Microbial Community and Its Interactive Relationship with C Pool

The OFB treatment increased the α-diversity of bacteria in the topsoil. In contrast, neither the OF nor FP treatment had a distinct influence on the bacterial diversity (Figure 5). This finding was not entirely consistent with prior studies, in which the application of inorganic and manure fertilizer was shown to increase the richness and diversity of bacteria, while inorganic fertilizer alone decreased the α-diversity of soil bacteria [28,52]. This discrepancy could be attributed to variations in soil fertility and associated soil properties. Soil microorganisms are typically constrained by the soil's labile C substrate supply. The repeated organic and inorganic fertilizer input during the 10-year experiment increased the accumulation of aboveground plant litter, root exudates, and organic residues from fertilizers in the soil. Researchers have shown that plant roots can release exudates including carbohydrates, N-free carbonic acids, and amino acids, which are the main stable sources of soil C [53,54]. Moreover, litter inputs resulted in an increase in the levels of soil metabolites, including amino acids, cellulose, phenolic acids, and lignin [55]. These ample and stable C sources stimulated the growth and abundance of symbiotic microorganisms [56]. In contrast, the long-term application of a chemical fertilizer alone, especially with imbalanced fertilization (e.g., FP treatment), primarily supplied available nutrients but maintained relatively low C inputs. Meanwhile, high nitrogen inputs could decrease the "carbon–nitrogen ratio" in the soil, accelerate the decomposition of organic matter to a certain extent, and thereby reduce substrate availability for microorganisms [9]. Such changes may alter the abundance of oligotrophic bacteria and those highly sensitive to C sources, such as Acidobacteria and Firmicutes [47]. Nutrient-poor soils with recalcitrant C substrates could increase the abundance of Acidobacteria and enhance the activity of Firmicutes because these phyla are more active in nutrient-deficient environments [57]. Moreover, soils with high C content may promote the growth of Actinobacteria, Bacteroidetes, and Proteobacteria [58,59] while reducing the abundance of Acidobacteria. Thus, the higher

α-diversity in OFB compared with FP and OF suggested that less specialized microbial communities occurred under C-rich conditions with the continuous addition of organic fertilizers [60]. Conversely, the application of chemical fertilizers alone may induce nutrient imbalances that could adversely affect microbial diversity.

The PCoA of bacterial communities revealed significant differences in their composition compared to that of fungi (Figure 6). Our results echoed the viewpoint of De Vries et al. [61], who found that changes in bacterial communities are linked more strongly to soil functioning during recovery than changes in fungal communities. Moreover, bacteria are considered more sensitive to environmental changes in the soil due to their much shorter turnover time compared to fungi. The abundance of the phyla Actinobacteria and Bacteroidetes increased in the OFM treatment compared with the FP treatment. Members of these phyla are generally classified as copiotrophic bacteria, which prefer C-rich environments and may exhibit rapid growth rates in the presence of abundant nutrients [59]. We found that the abundance of Mortierellomycota increased with the application of both organic and inorganic fertilizers, while the lowest percentage was for no fertilizer. These dynamics are related to the capacity of these organisms to decompose plant residues and produce raw materials for humus synthesis, including aromatic compounds and N-containing compounds like amino acids and peptides [62,63]. Furthermore, this phylum can produce antibacterial compounds and chemically recalcitrant melanin to delay soil organic carbon turnover [62], thereby contributing to HFOC accumulation in the soil.

The Mantel test (Figure 8) showed that SOC fractions were significantly correlated with microbial carbon source utilization. This trend indicates that the quantity and quality of SOC are ultimately related to microbial growth and metabolic activity [30], which, in turn, affects alterations in the microbial community structure. Hall et al. [64] reported that SOC consists of microbial necromass and low-molecular-weight decomposition products, such as carbohydrates, proteins, and organic acids. These biomolecules are essential to the C cycle, thus connecting microbial activity with SOC sequestration.

5. Conclusions

The results of this study showed that the 10-year application of chemical and organic fertilizers significantly increased the SOC and labile C fraction contents (DOC and LFOC) in the topsoil, promoted microbial metabolic activity, and caused noticeable changes in the bacterial community compared to the traditional fertilization treatment (FP). This is primarily because the combined treatment increases the abundances of Bacteroidetes and Mortierellomycota and improves the utilization of carbohydrates, amino acids, and carboxylic acids. The input of exogenous organic nutrients can enhance microbial activity and help to preserve a larger microbial biomass, leading to alterations in the types and quantities of soil metabolites. This, in turn, ultimately affects the composition and quantity of the SOC pool in arid agroecosystems. Thus, the use of organic fertilizers in conjunction with balanced NPK fertilizers is recommended to enhance soil microbial activity and sustain soil fertility.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/land13081296/s1, Figure S1: Location of the test area (a), and average monthly precipitation (b), monthly average temperature (c) of the test station from 2013 to 2023; Table S1: Annual fertilization amount of each treatment in the tested area from 2013 to 2023; Table S2: Cumulative C input under different fertilization treatments from 2013 to 2023. References [65–67] are cited in Supplementary Materials.

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Conflicts of Interest: The authors declare no conflicts of interest.

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