

Article

Organic Amendments Drive Soil Organic Carbon Sequestration and Crop Growth via Microorganisms and Aggregates

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Abstract

Exogenous carbon addition is widely regarded as an effective soil management strategy for rapidly increasing soil organic carbon, improving soil structure and function. However, a systematic comparison of the effects of diverse organic amendments on key soil attributes and processes is needed to inform their targeted application. We evaluated the impacts of seven organic amendments (biochar, organic fertilizer, corn straw, soybean straw, rapeseed straw, green manure, and carbon material) on a purple soil (Luvic Xerosols) in a pot experiment. The results showed that organic fertilizer and carbon material performed best in enhancing soil nutrient availability and promoting soil organic carbon content. Straw amendments promoted the formation of macro-aggregates. Green manure and straws enhanced carbon transformation-related β -glucosidase and cellobiohydrolase activities. Random Forest and structural equation modeling indicated that the organic amendments enhanced maize carbon sequestration capacity and biomass by improving aggregate stability and regulating the fungal community and by increasing nutrients and enhancing active carbon fractions. Green manure and organic fertilizer demonstrated the most significant agronomic effects. These findings provide guidelines for targeted organic amendment selection in purple soil regions.

Keywords: aggregate stability; exogenous carbon; organic carbon fractions; purple soil; microbial community



Academic Editor: Hailin Zhang

Received: 18 November 2025

Revised: 11 December 2025

Accepted: 16 December 2025

Published: 18 December 2025

Citation: Zong, D.; Quan, Y.; Penttinen, P.; Qi, L.; Wang, J.; Tang, X.; Xu, K.; Chen, Y. Organic Amendments Drive Soil Organic Carbon Sequestration and Crop Growth via Microorganisms and Aggregates. *Agronomy* **2025**, *15*, 2919. <https://doi.org/10.3390/agronomy15122919>

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1. Introduction

China's terrestrial ecosystems play a significant role in the global terrestrial carbon sink. Although China accounts for only about 6.5% of the world's land area, it contributes 10–31% of the global terrestrial carbon sink [1]. As one of the three major terrestrial systems, cropland ecosystems cover 38.5% of the terrestrial ecosystem area and represent one of the most active carbon pools in the carbon cycle process. However, their carbon sink and carbon sequestration capacity have often been underestimated or even overlooked in previous studies [2]. Cropland ecosystems can function as either a carbon source or a carbon sink. Crop growth and soil respiration in croplands release CO₂ into the atmosphere. Globally, agriculture accounts for 21–25% of anthropogenic greenhouse gas emissions, making it a major source of greenhouse gases [3]. The soil carbon pool is closely linked to atmospheric carbon, and changes in soil carbon can strongly influence atmospheric CO₂

levels [4]. Therefore, enhancing soil organic carbon reserves in croplands and achieving effective carbon sequestration capacity in agricultural systems have become critical strategic measures for addressing global climate change, ensuring food security, and promoting sustainable agricultural development [5]. However, globally, long-term unsustainable land use and farming practices—such as insufficient inputs of organic amendments and excessive tillage—have led to widespread and severe challenges across large areas of cropland soils [5]. These challenges include persistent depletion of soil organic matter, saturation or exhaustion of carbon storage capacity, degradation of soil structure, reduced aggregate stability, and diminished erosion resistance. Concurrently, issues such as imbalances in soil biological community structure, inefficient nutrient cycling, and declining ecosystem services are also commonly observed [6]. Against this background, exploring effective approaches to synergistically enhance soil carbon sequestration capacity and land productivity in croplands is not only of significant ecological and environmental importance but also an urgent necessity for ensuring food security.

Exogenous carbon input, as a direct and efficient soil management practice, is widely regarded as a strategic approach for rapidly increasing soil organic carbon stocks, improving soil structure and function, and enhancing the carbon sink capacity of cropland ecosystems [7]. The core principle involves supplementing depleted carbon pools by adding carbon-rich organic materials to the soil, thereby stimulating internal biogeochemical cycles and fostering a healthier, more stable, and productive soil ecosystem. In recent years, numerous in-depth studies have been conducted on the effects of various exogenous carbon amendments on carbon sequestration [8–10]. Among these amendments, biochar—produced from biomass pyrolysis under anaerobic conditions—exhibits a highly aromatic structure and abundant porosity, conferring strong chemical and microbial stability. This allows it to persist in soils for long periods, even on a centennial scale, forming a recalcitrant carbon pool [11]. Additionally, biochar can improve soil water retention and nutrient-holding capacity, while providing unique habitats for microorganisms, thereby indirectly influencing carbon transformation and stabilization. Within appropriate application ranges, organic fertilizers are renowned for their rich content of readily available nutrients and labile organic matter, enabling rapid improvement of soil fertility, stimulation of microbial activity, and promotion of soil aggregate formation. They contribute to soil organic carbon accumulation primarily through microbial transformation pathways, although certain carbon components may exhibit relatively fast turnover rates [12]. Crop straw incorporation not only directly supplements soil organic carbon but also effectively promotes the formation and stabilization of macro-aggregates through cementing substances produced during decomposition. This physically protects organic carbon from microbial decomposition, facilitating long-term carbon sequestration [13,14]. Furthermore, cultivating and incorporating green manure offers unique advantages in nitrogen fixation, supplying fresh organic matter, and improving the soil micro-ecological environment [15]. With advancements in materials science, carbon materials are also emerging as soil amendments. These materials may exert unprecedented influences on nutrient adsorption, microbial metabolism, and even carbon cycling pathways due to their specific surface properties and catalytic effects. However, most studies have focused on one or a few traditional organic amendments, lacking a systematic, comprehensive, and equitable comparative analysis of the carbon sequestration effects of different types of organic amendments within a unified experimental framework.

Based on this, we compared seven representative organic amendments under equal carbon addition conditions: biochar (BC), organic fertilizer (OF), corn straw (CS), soybean straw (SS), rapeseed straw (RS), green manure (GM), and a novel carbon-based fertilizer (CM). We hypothesized that (1) different organic amendments, due to their distinct chemical composition and structural properties, will induce specific improvements in the purple

soil ecosystem; (2) the shaping effects of organic amendments on soil bacterial and fungal communities differ significantly, and the response of specific microbial functional groups is key to organic amendments input and soil carbon transformation; and (3) the promoting effect of organic amendments on crop growth is not achieved through a single pathway but rather through the synergistic interaction of two core pathways: the “physical structure-biology” pathway and the “chemical property-nutrient” pathway. Therefore, the primary objective of this study was to elucidate and compare the distinct mechanisms by which different organic amendments drive soil organic carbon sequestration and crop growth in purple soil.

2. Materials and Methods

2.1. Research Site and Experimental Design

An outdoor pot experiment was conducted at the Ya'an campus of Sichuan Agricultural University, China (29°58'55" N, 102°59'20" E). The site is situated within a subtropical monsoon climate zone. During the maize growing season, the average rainfall is approximately 6.5 mm, with peak levels reaching up to 114.5 mm, while temperatures range from 10 to 38 °C. (Figure S1). Pots were filled with purple sandy soil, characterized by low nutrient content and a high sand fraction (Table S1). Soil was collected from the plough layer (0–20 cm depth) in Longxi, Chongqing, China, air-dried and passed through a 5 mm sieve. The basic physicochemical properties of the soil are in Table S1. The maize (*Zea mays*) cultivar used was ‘Zhongyu 3’, purchased from an agricultural supply store in Ya'an. Biochar (BC) produced from maize straw was purchased from Nanjing Fengqin Straw Technology Co., Ltd. (Nanjing, China). Organic fertilizer (OF) was purchased from Sichuan Tianbao Bio-technology Co., Ltd. (Chengdu, China). It belongs to the category of bio-organic fertilizer, with its raw materials mainly composed of chicken manure and microbial inoculants. Corn straw (CS), soybean straw (SS), and rapeseed straw (RS) were collected from Shuangmiao, Guangyuan, Sichuan, oven-dried, and crushed into approximately 1–2 cm long pieces. Green manure (GM) consisted of common vetch (*Vicia sativa*) collected at the initial flowering stage from the Chongzhou Base of Sichuan Agricultural University. The aboveground parts were oven-dried and crushed into approximately 1–2 cm long pieces. Carbon material (CM), prepared via hydrothermal carbonization method using pig manure as the raw material, was provided by Southwest University [16]. The nutrient contents of these amendments are presented in Table 1 (see below for details).

Table 1. Nutrient contents and application rates of organic amendments.

Treatment	TOC (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	C/N	Application Rate (g/pot)
Corn straw (CS)	408.70	8.20	0.24	1.04	49.85	195.77
Soybean straw (SS)	368.60	6.20	0.18	0.90	59.45	217.07
Rape straw (RS)	309.10	7.70	0.27	1.58	40.14	258.85
Green manure (GM)	298.40	20.20	0.32	1.38	14.77	268.13
Organic fertilizer (OF)	158.50	26.40	1.44	0.91	6.00	504.78
Biochar (BC)	342.10	11.00	0.19	0.88	31.10	233.88
Carbon Material (CM)	143.80	11.90	0.61	1.71	12.08	556.30

TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium

2.2. Organic Amendment Application Rates

The organic amendments were applied to achieve a target carbon input equivalent to 1% of the soil mass (soil-to-organic carbon ratio of 100:1). Pots (inner diameter: 26 cm, height: 14 cm) were filled with 8 kg of soil and supplemented with 80 g of carbon. The application rate per pot for each amendment was calculated according to its specific carbon content (Table 1). The amendments were thoroughly mixed with the soil before potting.

The seven carbon amendment treatments and a not amended control were performed with four replicates. Soil moisture was adjusted to 60–70% of the field water holding capacity. On 20 April 2023, four to five maize seeds were sown per pot, and seedlings were thinned to one plant per pot at the four-leaf stage. Fertilization was conducted according to the recommended practices for maize cultivation in southwestern China, with application rates equivalent to 180 kg N ha⁻¹, 75 kg P₂O₅ ha⁻¹, and 105 kg K₂O ha⁻¹. These rates were scaled down to the pot level, corresponding to 0.828 g of urea, 1.340 g of calcium superphosphate, and 0.373 g of potassium chloride per pot. The total nitrogen was supplied in two equal splits as topdressing on 12 May and 31 May (0.414 g urea per application). The full amounts of phosphorus and potassium fertilizers were applied basally in a single application at the first fertilization event.

2.3. Soil and Plant Sampling

Soil and plant samples were collected at the maize silking stage on 3 August 2024. The aboveground portion of the maize plant was carefully harvested from each pot. For soil sampling, approximately 2 kg of undisturbed soil was collected from each pot after plant removal. Visible plant residues and stones were manually removed. The soil was then gently broken apart along natural fracture lines, homogenized, air-dried, and passed through a 2 mm sieve. The processed soil was divided into two aliquots: one aliquot was air-dried in a ventilated, shaded area for the determination of soil physicochemical properties, and the other aliquot was stored at −80 °C for subsequent DNA extraction. The harvested plant shoots were first placed in an oven at 105 °C for 30 min for enzyme deactivation, and then dried at 75 °C to constant weight to determine the aboveground dry biomass. The dried samples were weighed, ground, and passed through a 0.25 mm sieve for further analysis.

2.4. Aggregate Fractionation

Water-stable aggregate distribution was determined using the wet sieving method described by Elliott [17]. Air dried soil samples were first gently crushed and passed through a 2 mm sieve. The sieved soil was then sequentially dry sieved through 0.25 mm and 0.053 mm sieves. The material retained on each sieve was collected and weighed to calculate the proportional distribution of the initial aggregate size fractions. Subsequently, a 70 g subsample was taken for wet sieving, proportionally representing the aggregate distribution obtained from the initial dry sieving. The subsample was immersed in deionized water on the top of a 2 mm sieve for 5 min to allow slaking. The nest of sieves (2 mm, 0.25 mm, and 0.053 mm) was then mechanically oscillated vertically in water for 25 min. The material retained on each sieve was carefully washed into pre weighed containers and oven dried at 50 °C to constant weight. The following aggregate size classes were defined based on the material collected from the wet-sieving: large macroaggregates (>2 mm), small macroaggregates (0.25–2 mm), microaggregates (0.053–0.25 mm), and the silt plus clay fraction (<0.053 mm). The weight of each fraction was measured to determine their mass percentages. Key indices of aggregate stability, including the mean weight diameter (MWD, mm), geometric mean diameter (GMD, mm), and fractal dimension (D), were calculated according to established methods [17]. The formulas used for the calculations are as follows:

$$\text{MWD} = \sum_{i=1}^n x_i w_i$$

$$\text{GMD} = \exp(\sum_{i=1}^n m_i \times \ln x_i / \sum_{i=1}^n m_i)$$

$$D = 3 - \frac{\log[m(r < R_i)/m_T]}{\log(R_i/R_{\max})}$$

In the formula, x_i is the mean diameter (mm) of a given aggregate size class, w_i is the corresponding percentage content (%) of that aggregate size class, m_i is the corresponding mass (g) of that aggregate size class, R_i is the mean diameter (mm) of the i -th aggregate class, $m(r < R_i)$ is the cumulative mass (g) of aggregates with a diameter smaller than R_i , m_T is the total mass (g) of the aggregates, and $R_{\max}$ is the maximum aggregate diameter (mm).

2.5. Chemical and Biological Analyses

Soil basic physicochemical properties were determined using standard methods: Soil pH was measured potentiometrically in a 1:2.5 (w/v) soil–water suspension [18]. Total nitrogen (TN) was determined using the semi-micro Kjeldahl method [19]. Alkali-hydrolyzable nitrogen (AN) was assessed using the alkaline diffusion method [20]. Available phosphorus (AP) was extracted with 0.5 M NaHCO_3 (pH 8.5) and measured using the molybdenum-antimony colorimetric method [20]. Available potassium (AK) was extracted with 1 M ammonium acetate (NH_4OAc , pH 7.0) and determined using flame photometry [21]. Soil organic carbon (SOC) was quantified using the potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) external heating-volumetric method [22]. Microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method with 0.5 M K_2SO_4 as the extractant [23]. Easily oxidizable organic carbon (EOC) was measured using colorimetric analysis after oxidation with 333 mM KMnO_4 [24]. Recalcitrant organic carbon (ROC) was calculated as the difference between SOC and EOC. β -Glucosidase (BG) activity was determined using the nitrophenol method with p -nitrophenyl β -D-glucopyranoside as substrate. Cellobiohydrolase (CBH) activity was measured using the 3,5-dinitrosalicylic acid method with carboxymethyl cellulose as substrate. Polyphenol oxidase (PPO) activity was assessed using the pyrogallol method. Peroxidase (PER) activity was measured using the pyrogallol-hydrogen peroxide method [25]. Based on the measured carbon fractions, the soil carbon pool index (CPI) was calculated as described by Blair [26], using a reference soil as the control: Carbon Pool Index (CPI) = (SOC content of treated sample)/(SOC content of reference soil). The net primary productivity of crop carbon (CNPP), representing the total carbon fixed by the crop in grams per pot, was calculated using the formula

$$C_{\text{NPP}} = CW$$

where W is the aboveground plant biomass (g/pot), and C is the carbon concentration in the aboveground plant biomass (g C/g biomass).

2.6. Soil DNA Extraction and Microbial Sequencing

Soil DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method [27]. The lysis buffer contained 2% (w/v) CTAB, 1% polyvinylpyrrolidone (PVP), and 100 mM β -mercaptoethanol to enhance cell lysis and remove inhibitors. The integrity of the extracted DNA was verified by 1% agarose gel electrophoresis (120 V, 30 min) with ethidium bromide staining, and its concentration was quantified spectrophotometrically using a NanoDrop 2000c system (Thermo Fisher Scientific, Waltham, MA, USA). Polymerase chain reaction (PCR) amplification of the target regions was performed using barcoded primers: the V3–V4 hypervariable region of the prokaryotic 16S rRNA gene was amplified using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The fungal ITS1 region was amplified using primers ITS1F_KYO2 (5'-TAGAGGAAGTAAAAGTCGTA-3') and ITS2R_KYO2 (5'-TTYRCTRCGTTCTTCATC-3'). Amplification was performed in triplicate 25 μL PCRs, containing 12.5 μL KAPA HiFi HotStart ReadyMix (Roche), 0.2 μM of each primer, and 10 ng of

template DNA. The amplification products were checked by 2% agarose gel electrophoresis, purified using a GeneJET Gel Extraction Kit (Thermo Fisher, USA), and quantified with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA). Equimolar amounts of the purified amplicons from all samples were pooled together. Sequencing libraries were constructed using a TruSeq Nano DNA LT Library Preparation Kit, and the final library quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing (2×250 bp) was performed on an Illumina NovaSeq 6000 platform (Illumina, Shenzhen, China).

2.7. Statistical Analysis

The effects of different organic amendments treatments on soil physicochemical properties, enzyme activities, carbon fractions, aggregate stability, microbial diversity, plant biomass, and carbon sequestration were assessed using one-way analysis of variance (ANOVA). Multivariate analyses of microbial community structure were performed based on Bray–Curtis distances. Principal coordinate analysis (PCoA), implemented with the “vegan” package in R 4.5.1, was used to visualize differences in microbial community composition among treatments [28]. The linear discriminant analysis effect size (LEfSe) method was employed to identify taxonomic groups that were significantly enriched and served as biomarkers in different treatments, using a linear discriminant analysis (LDA) score threshold of >5 [29]. The relationships between microbial community structure and environmental variables were examined using canonical correspondence analysis (CCA) via the “vegan” package [30]. Furthermore, Mantel tests and heatmap analysis were conducted to explore the correlations between soil factors and both microbial diversity indices and the relative abundance of dominant bacterial phyla. Random forest analysis, performed using the “randomforest” package in R, was applied to determine the relative importance of various predictor variables in explaining the variance in maize yield and carbon sequestration. A partial least squares path modeling (PLS-PM) approach was implemented using the “plsppm” package in R to construct a linear statistical model [31]. This model was developed to predict the complex causal relationships among the following latent constructs: organic amendments input, soil carbon fractions, the activity of carbon transformation-related enzymes, microbial diversity, and maize productivity.

3. Result

3.1. Effects of Different Organic Amendments Additions on Soil Organic Carbon Fractions and Carbon Transforming Enzyme Activities

The organic amendments influenced soil pH, nutrient contents, and carbon pool indicators (Figure 1a–j). In the OF treatment, the values of all the measured variables were among the highest and each was higher than in the not amended control; in the CM treatment, the values of all the measured variables except pH were higher than in the control. In addition, pH was lower in the CS, RS, SS, and BC treatments than in the control. Compared to the control, AK content was higher in all the treatments except BC. In all the treatments, TN content was higher than in the control, yet AN content was higher only in RS, GM, OF and CM treatments. Compared to the control, MBC content was higher in the RS, SS, GM, OF and CM treatments; EOC content was higher in the RS, GM, OF and CM treatments. In all the treatments, ROC and SOC contents and CPI values were higher than in the control.

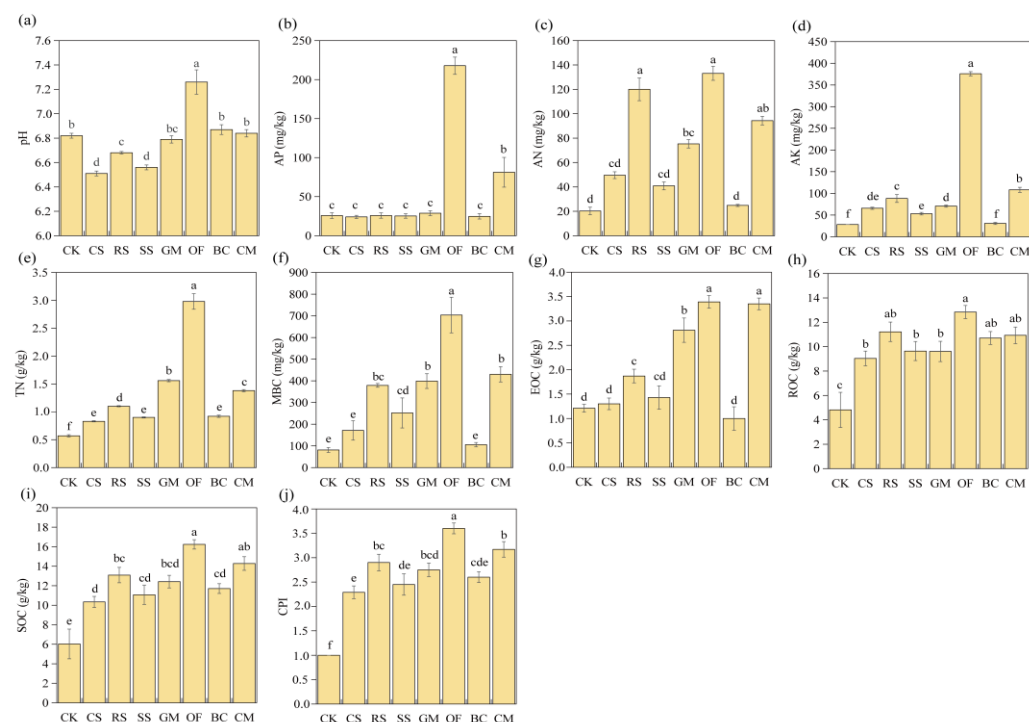


Figure 1. Effects of different organic amendments additions on soil physicochemical properties and organic carbon fractions (a–j). AP: available phosphorus; AN: alkali-hydrolyzable nitrogen; AK: available potassium; TN: total nitrogen; MBC: microbial biomass carbon; EOC: easily oxidizable carbon; ROC: recalcitrant organic carbon; SOC: organic carbon; CPI: carbon pool index. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. MBC on fresh weight basis; all others on dry weight basis. Different lowercase letters indicate significant differences among treatments at $p < 0.05$.

The addition of different organic materials also significantly enhanced the activities of soil carbon-transforming enzymes (Figure 2a–d). Regarding the enzyme activities involved in lignin degradation, the OF treatment resulted in the highest polyphenol oxidase (PPO) activity. Furthermore, OF, BC, and the CM collectively maintained the highest peroxidase (PER) activity, suggesting that these materials may promote the decomposition of recalcitrant carbon components in the soil. In terms of enzyme activities associated with cellulose degradation, the GM treatment demonstrated a distinct advantage, exhibiting the highest β -glucosidase (BG) and cellobiohydrolase (CBH) activities among all treatments. This indicates that GM strongly stimulated microbial decomposition of cellulose. Additionally, RS also showed relatively high CBH and BG activities, while CS and SS provided a moderate increase in BG activity. Regression analysis further revealed intrinsic relationships between soil carbon fractions and enzyme activities. Both SOC and ROC showed significant positive correlations with peroxidase (PER) activity (Figure 2e,f). These results highlight the dual role of organic amendments additions in enhancing soil biochemical interactions, thereby improving soil fertility and promoting carbon stabilization.

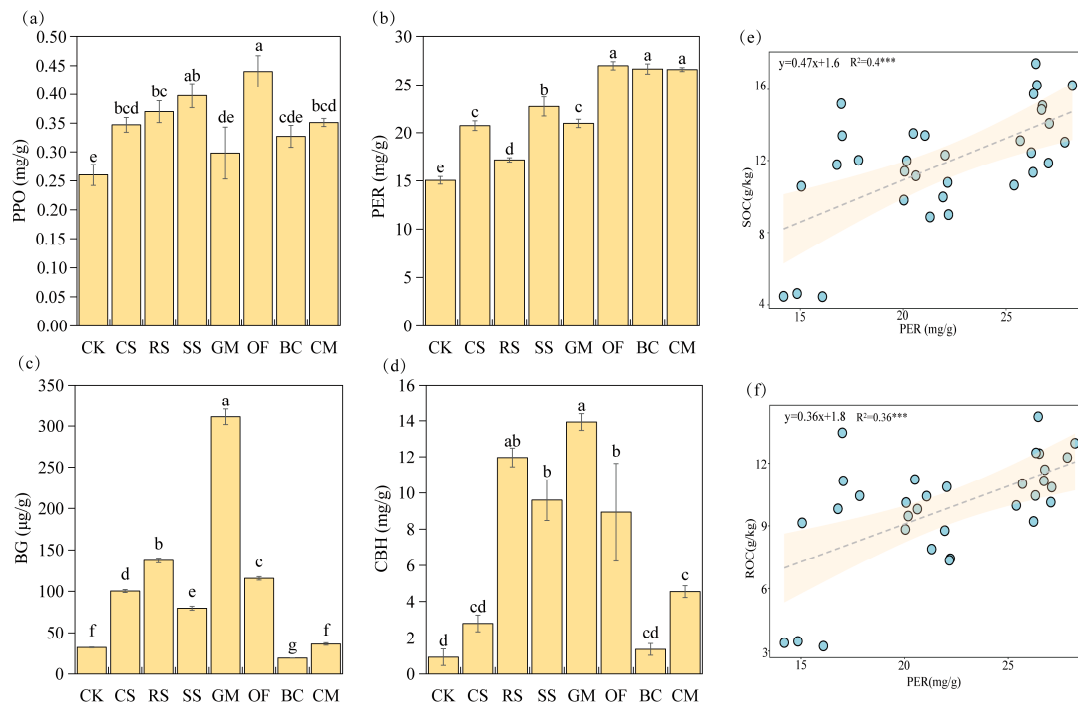


Figure 2. Activities of PPO (a), PER (b), BG (c), and CBBH (d) under different organic amendments treatments, along with regression analysis of peroxidase activity with SOC and ROC (e,f). BG: β -glucosidase; CBH: cellobiohydrolase; PER: peroxidase; PPO: polyphenol oxidase. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. Different lowercase letters indicate significant differences among treatments at $p < 0.05$, and *** denotes $p < 0.001$.

3.2. Effects of Different Organic Amendments Additions on Aggregate Stability and Organic Carbon Content

The addition of different organic materials significantly altered the aggregate structure and organic carbon distribution patterns in the purple soil (Figure 3). Straw-based materials (CS, RS, SS) were particularly effective in promoting the formation of macroaggregates (>2 mm), achieving proportions of 54.12%, 53.52%, and 50.37%, respectively, while simultaneously significantly reducing the silt-plus-clay content (Figure 3a). Among these, the CS treatment performed best. Regarding aggregate stability, CS and RS resulted in the largest mean weight diameter (MWD) and geometric mean diameter (GMD), indicating the formation of the most stable macroaggregates (Figure 3b,c). Although the OF treatment had a slightly lower MWD, its lowest fractal dimension (D) value suggested a more uniform and compact aggregate structure (Figure 3d). The CM uniquely increased the proportion of intermediate-sized aggregates to 37.57%, whereas BC and GM showed limited effects on aggregate improvement.

Analysis of organic carbon distribution revealed that OF and CM exhibited comprehensive and balanced carbon sequestration capabilities (Figure 3e–h). The OF treatment maintained the highest SOC content across all aggregate size classes, particularly in microaggregates and the silt-plus-clay fraction, reaching 16.15 and 15.98 g/kg, respectively. The SOC content in the CM treatment was second only to OF in macroaggregates and the silt-plus-clay fraction, and it achieved the highest content within the intermediate-sized aggregates. In contrast, the carbon sequestration effects of straw materials and GM were primarily concentrated in macroaggregates, while BC only significantly increased the SOC content within macroaggregates. In summary, straw-based materials primarily optimized soil structure by promoting the formation of large and stable aggregates. In contrast, OF and CM excelled in comprehensively enhancing carbon sequestration across

all aggregate size classes. Specifically, OF tended to build a uniform and compact aggregate structure, whereas CM uniquely enriched the intermediate-sized aggregates, providing diverse pathways for improving the structure of purple soil.

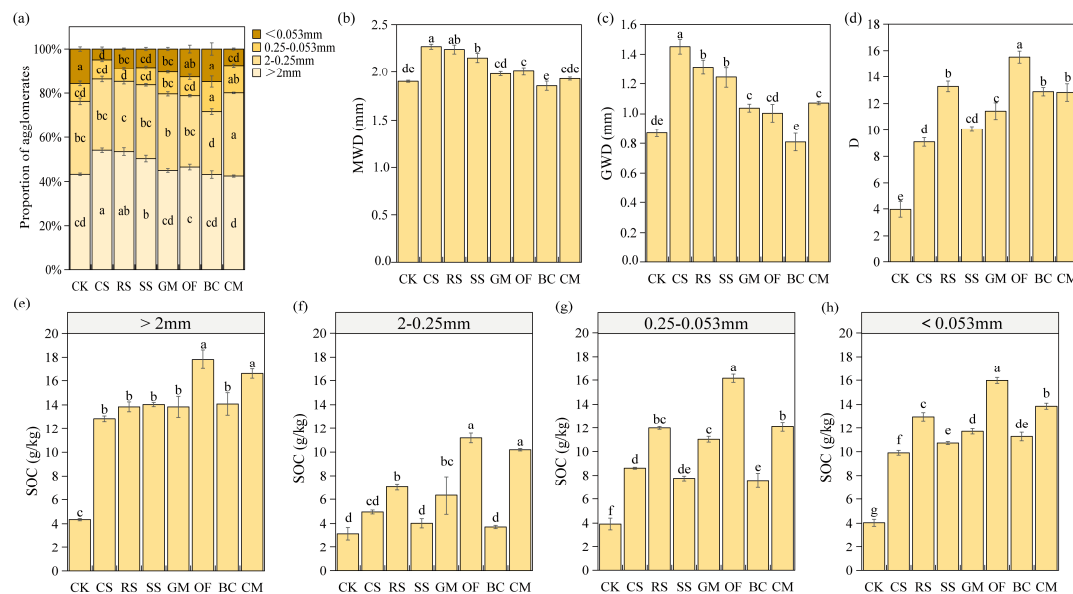


Figure 3. Aggregate size distribution (a), aggregate stability indices (b–d), and organic carbon content within different aggregate size fractions (e–h) under different organic amendments treatments. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. Different lowercase letters indicate significant differences among treatments at $p < 0.05$.

3.3. Effects of Different Organic Amendments Additions on Soil Microbial Diversity

The addition of different organic materials significantly altered the diversity and structure of the soil microbial community in the purple soil, with bacterial and fungal communities exhibiting distinct response patterns (Figure 4). The impact on bacterial Shannon diversity was relatively limited (Figure 4a), with only the RS treatment causing a significant decrease. In contrast, fungal diversity was generally suppressed (Figure 4b). The Shannon index for the RS, SS, and GM treatments dropped to a range of 1.39–1.80, significantly lower than that of the CK. BC was the only treatment that maintained relatively high fungal diversity. Regarding the bacterial community (PCoA explained variance: 66.6%), the community structures of the CK and CS treatments were similar (Figure 4c). The RS and SS samples formed a distinct cluster, indicating that rapeseed and soybean straw had analogous effects on the bacterial community. For the fungal community (PCoA explained variance: 86.8%), the GM, BC, and CM samples formed unique community types, clearly separated from other treatments (Figure 4d). The fungal compositions of the CK and CS samples were relatively similar. In summary, the addition of organic materials significantly modified soil microbial community structure, with fungal communities demonstrating greater sensitivity. BC was particularly effective in preserving fungal diversity, whereas CS had minimal impact on the overall microbial community structure.

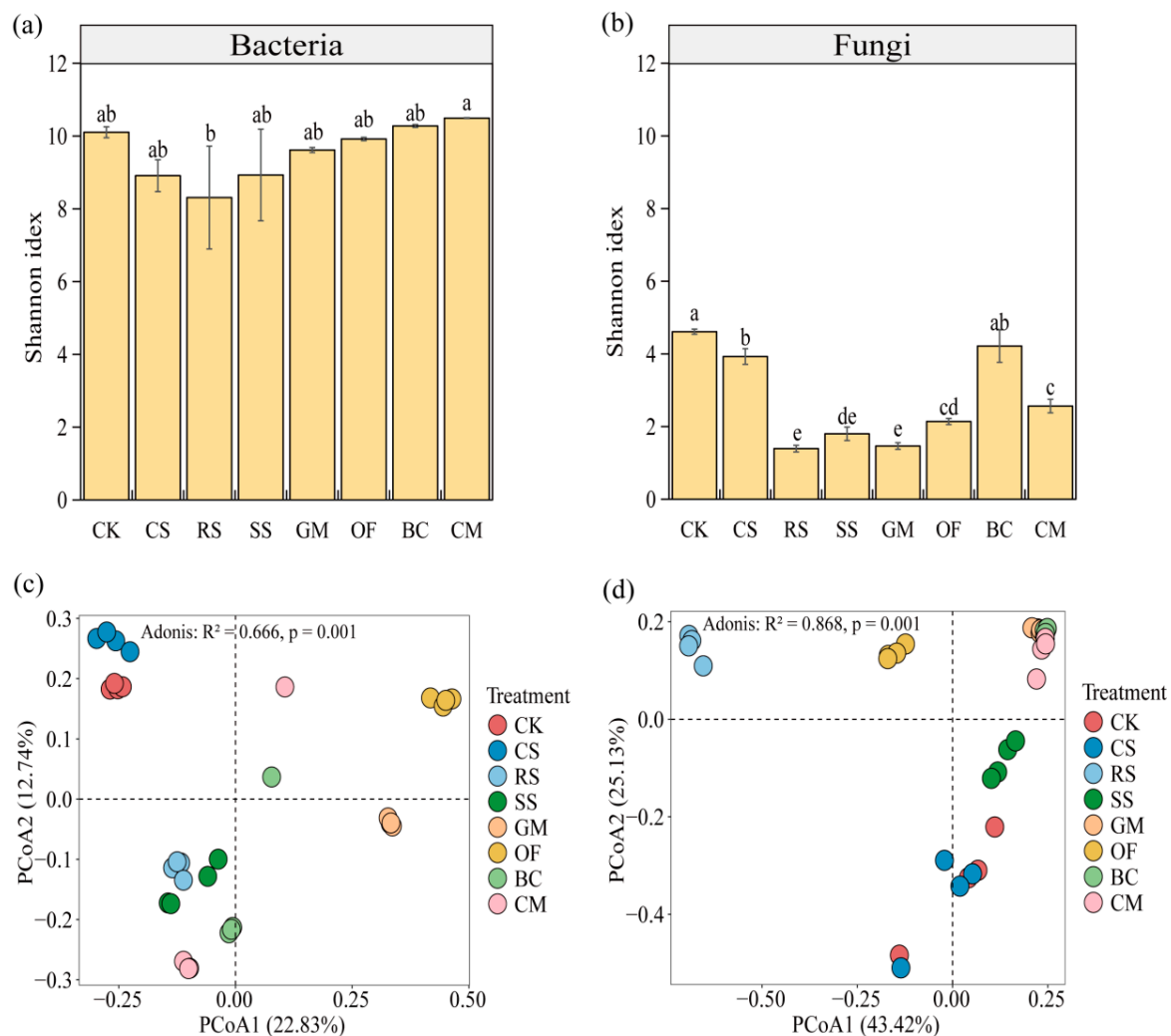


Figure 4. Shannon index of bacteria (a) and fungi (b), and Principal Coordinate Analysis (PCoA) of bacterial (c) and fungal (d) communities under different organic amendments treatments. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. Different lowercase letters indicate significant differences among treatments at $p < 0.05$.

3.4. Effects of Different Organic Amendments Additions on Soil Microbial Community Structure and Biomarkers

The addition of different organic materials significantly altered the structure of the soil microbial community in the purple soil, eliciting specific responses at the phylum level and in biomarker distributions (Figures 5 and 6). In the bacterial community, Proteobacteria, Actinobacteria, and Acidobacteria were the three dominant phyla (Figure 5a). RS and GM treatments significantly enriched Proteobacteria, with relative abundances of 61.74% and 52.76%, respectively. CS and SS notably increased the proportion of Actinobacteria, while the CM treatment resulted in the highest abundance of Acidobacteria at 16.98%. BC demonstrated the best performance in maintaining a balanced distribution of phyla such as Chloroflexi and Gemmatimonadetes. Changes in the fungal community were more pronounced (Figure 5b). Most organic amendments treatments led to the absolute dominance of the Ascomycota phylum, with its abundance exceeding 93% in the RS, SS, GM, and OF treatments. In contrast, the abundances of Chytridiomycota and Mortierellomycota were significantly reduced. The BC treatment again exhibited a unique effect, maintaining relatively high abundances of Chytridiomycota (26.39%), Mortierellomycota (3.51%), and Glomeromycota (1.12%), thereby preserving the most balanced fungal community structure.

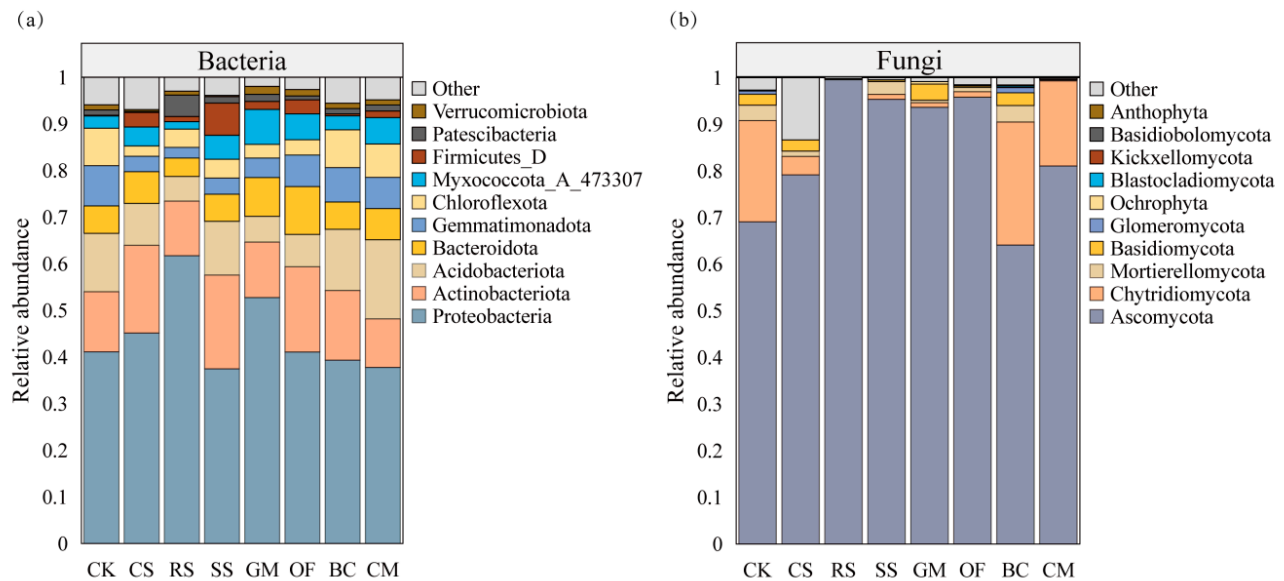


Figure 5. Relative abundance of bacterial (a) and fungal (b) communities at the phylum level under different organic amendments treatments. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material.

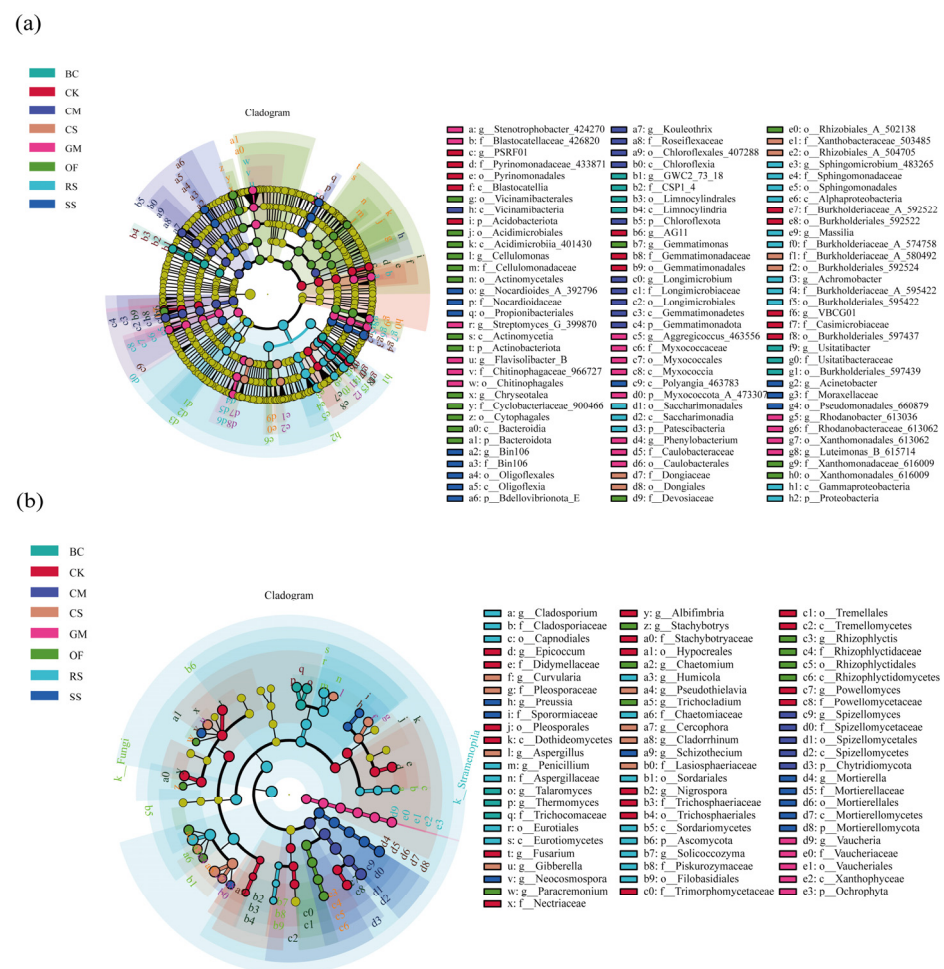


Figure 6. LEfSe analysis showing discriminant bacterial (a) and fungal (b) taxa under different organic amendments treatments. CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material.

LEfSe analysis further verified the specific regulatory effects of different organic materials (Figure 6). A total of 99 bacterial biomarkers were identified, primarily belonging to the phyla Bdellovibrionota, Patescibacteria, Actinobacteriota, Bacteroidota, Myxococcota, Gemmatimonadota, Chloroflexota, and Acidobacteriota (Figure S2). Among these, the OF and GM treatments yielded the highest number of biomarkers (18), whereas the CS treatment showed the lowest number (6). For fungi, 72 biomarkers were detected, mainly affiliated with the phyla Mortierellomycota, Ascomycota, and Chytridiomycota. The CK and RS treatments contained the highest numbers of fungal biomarkers (17 and 16, respectively), while the BC treatment contained the fewest (only 3) (Figure S3). The distribution patterns of these biomarkers indicate that different organic materials achieve specific regulation of the soil microbial community by selectively enriching particular microbial taxa. Among them, BC demonstrates a distinct value in maintaining microbial diversity.

3.5. Effects of Different Organic Amendments Additions on Drivers of Soil Microbial Communities

Canonical Correspondence Analysis (CCA) and Mantel tests revealed distinct driving mechanisms of environmental factors on microbial community structure (Figure 7). CCA indicated that bacterial community structure was primarily and strongly influenced by basic soil physicochemical properties (Figure 7a): pH ($R^2 = 0.87$, $p < 0.01$), AP ($R^2 = 0.72$, $p < 0.01$), AK ($R^2 = 0.68$, $p < 0.01$), and TN ($R^2 = 0.64$, $p < 0.01$). In contrast, fungal community structure was most strongly driven by the activity of BG ($R^2 = 0.82$, $p < 0.01$), with significant additional influences from pH ($R^2 = 0.64$, $p < 0.01$) and AP ($R^2 = 0.37$, $p < 0.01$) (Figure 7b). Mantel analysis further confirmed that bacterial Shannon diversity showed no significant correlation with any of the measured environmental factors (Figure 7c). Conversely, fungal Shannon diversity was significantly positively correlated with CBH activity, BG activity, and several carbon fraction indicators.

Specific associations between dominant phyla and environmental factors further elucidated the microbial response mechanisms (Figure 7d). At the bacterial phylum level, the abundance of Acidobacteria showed a significant negative correlation with pH and AP, whereas Firmicutes and Eisenbacteria were significantly positively correlated with pH. The abundance of Proteobacteria was significantly positively correlated with AN and AK. Fungal responses were more complex: the abundances of Ascomycota and Glomeromycota were significantly negatively correlated with pH and nitrogen/phosphorus availability, whereas Mortierellomycota abundance was significantly positively correlated with nitrogen and potassium levels. Regarding enzyme activities, Chytridiomycota abundance was significantly negatively correlated with BG activity, while Mucoromycota abundance was significantly positively correlated with PER activity. Collectively, these results demonstrate that the addition of organic materials specifically shapes microbial community structure by altering soil physicochemical properties and enzyme activity profiles. Bacterial communities are primarily driven by fundamental chemical properties, whereas fungal community structure and diversity are closely linked to carbon fractions and carbon transforming enzyme activities, highlighting the pivotal role of fungi in the soil carbon cycle.

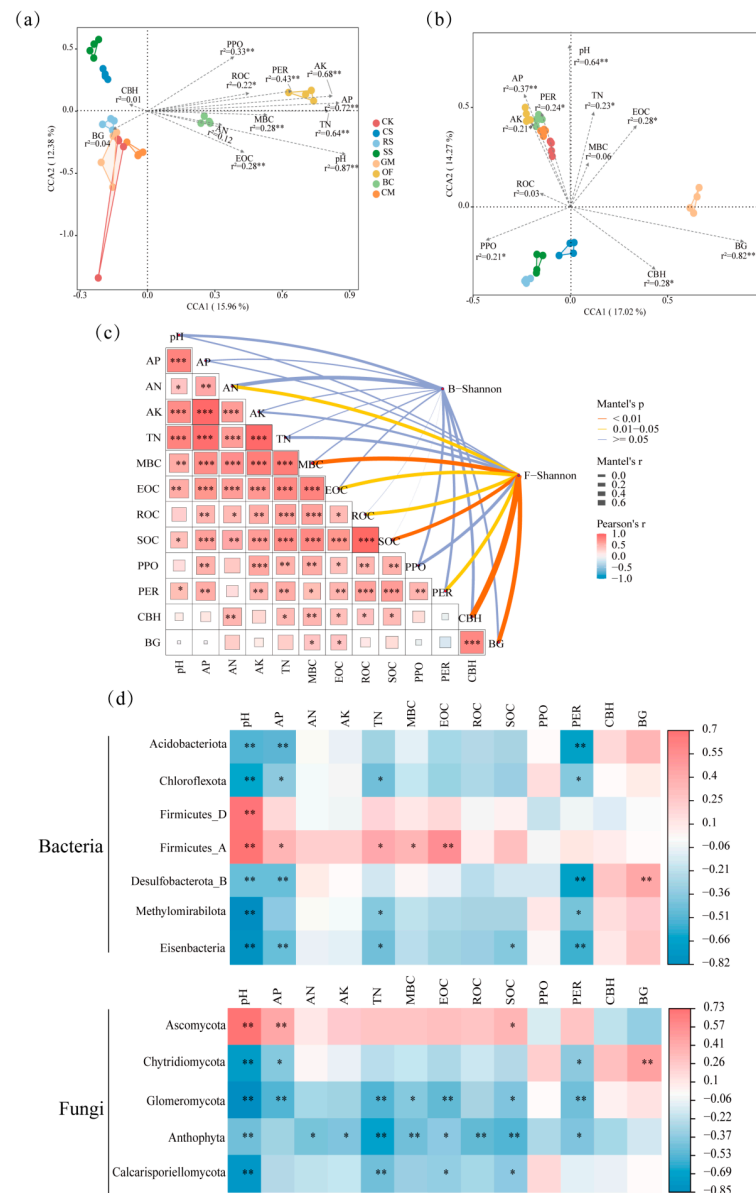


Figure 7. Canonical Correspondence Analysis (CCA) of bacterial (a) and fungal (b) community structures with environmental factors, and correlation heatmap based on Mantel tests between microbial diversity and environmental factors (c,d). AP: available phosphorus; AK: available potassium; AN: alkali-hydrolyzable nitrogen; TN: total nitrogen; SOC: organic carbon; MBC: microbial biomass carbon; EOC: easily oxidizable carbon; ROC: recalcitrant organic carbon; BG: β -glucosidase; CBH: cellobiohydrolase; PER: peroxidase; PPO: polyphenol oxidase; CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. The asterisks represent the following significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.6. Effects of Different Organic Amendments Additions on Maize Biomass and Carbon Sequestration Capacity

The organic amendments influenced maize biomass and carbon sequestration capacity (Figure 8). The amount of carbon sequestered was greater in the GM treatment than in all the other treatments except the OF treatment ($p < 0.05$) (Figure 8a). Plant biomass was higher in the GM and OF treatments than in the control ($p < 0.05$) (Figure 8b). In the RS and CM treatments, biomass was lower than in the control ($p < 0.05$) (Figure 8b).

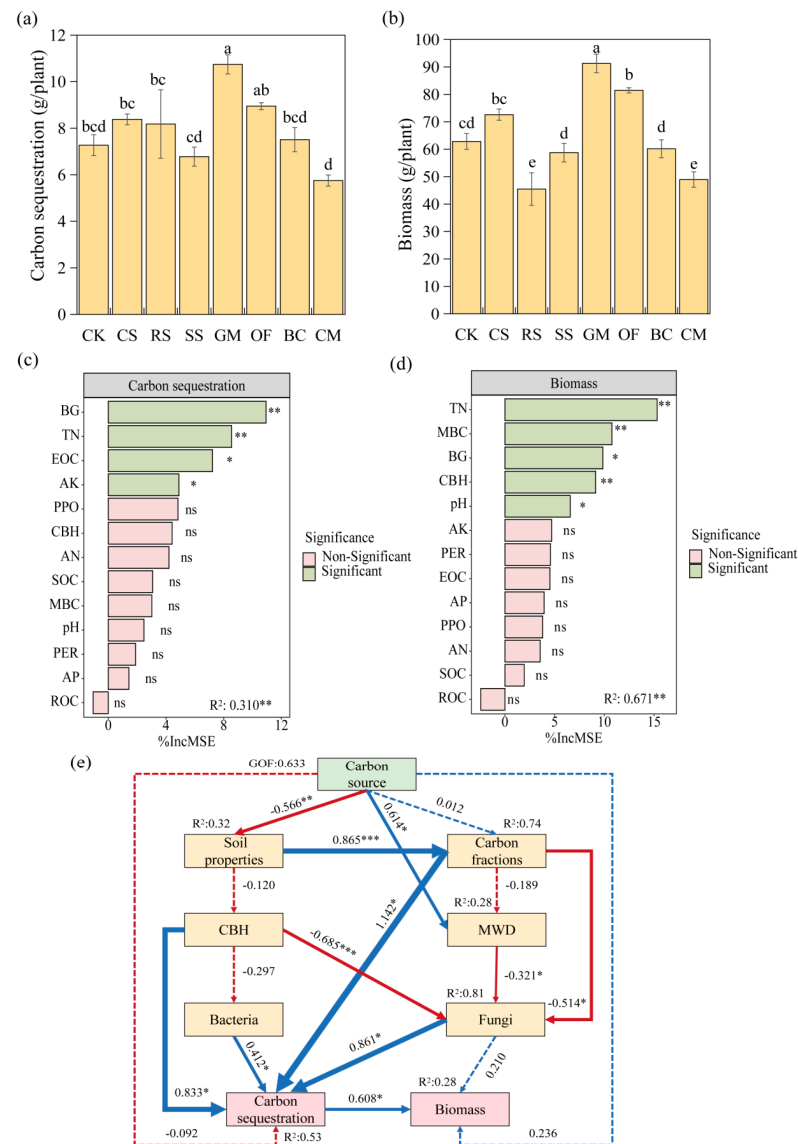


Figure 8. Effects of different organic amendments treatments on maize plant biomass and carbon sequestration (a,b), variable importance from random forest analysis for predicting carbon sequestration (c) and biomass (d), and the partial least squares path model (PLS-PM) illustrating the relationships among management practices, soil properties, microbial communities, and maize performance (e). AP: available phosphorus; AK: available potassium; AN: alkali-hydrolyzable nitrogen; TN: total nitrogen; SOC: organic carbon; MBC: microbial biomass carbon; EOC: easily oxidizable carbon; ROC: recalcitrant organic carbon; BG: β -glucosidase; CBH: cellobiohydrolase; PER: peroxidase; PPO: polyphenol oxidase; MWD: mean weight diameter; CK: control treatment; CS: corn straw; SS: soybean straw; RS: rapeseed straw; GM: green manure; OF: organic fertilizer; BC: biochar; CM: carbon material. Note: Solid and dashed arrows in (e) represent significant and non-significant paths, respectively. Arrow width and color (blue for positive, red for negative) reflect the strength and direction of the path coefficients. Different lowercase letters indicate significant differences among treatments at $p < 0.05$; The asterisks represent the following significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

By integrating Random Forest analysis and Partial Least Squares Path Modeling (PLS-PM), we elucidated the possible variables through which organic amendments affect maize growth and carbon sequestration. Maize carbon sequestration was associated with BG, TN, EOC, and AK (Figure 8c), and biomass was associated with TN, MBC, BG, CBH, and pH (Figure 8d). Based on the PLS-PM analysis, organic amendments could enhance

carbon sequestration by improving aggregate stability, which may subsequently influence the fungal community and further on carbon sequestration capacity. In addition, by altering soil physicochemical properties, the amendments may drive changes in soil carbon fractions which may directly enhance carbon sequestration capacity and act indirectly by suppressing the fungal community.

4. Discussion

4.1. The Role of Organic Amendments in Enhancing Soil Nutrients

Organic fertilizer and carbon material demonstrated the most prominent effects in enhancing soil fertility, as the contents of TN, AN, AK, and AP were consistently high in those treatments. This efficacy is closely related to their intrinsic properties. Organic fertilizer is a comprehensive nutrient source, capable of continuously releasing mineral nutrients such as nitrogen, phosphorus, and potassium during its mineralization process [32,33]. The carbon material used in this study, produced from pig manure via hydrothermal carbonization technology, is an organic fertilizer analogue. This hydrothermal carbonization retains most of the raw material's nutrients while potentially generating some stable organic components, endowing it with both rapid nutrient supply and potential slow-release functions [34,35]. Consequently, it exhibited soil improvement effects comparable to high-quality organic fertilizer. The differential effects of various organic materials on soil nutrients are not only related to their chemical composition and structural properties, but are fundamentally regulated by their carbon—to—nitrogen ratio as a key stoichiometric attribute. Therefore, it is generally believed that exogenous substrates with a C/N ratio of 15–25 are more conducive to the development of soil microorganisms [36]. This explains why materials with high C/N ratios (e.g., crop straw) and those with low C/N ratios exhibit distinctly different behaviors and functions in soil. First, the C/N ratio of organic materials directly regulates their decomposition rate and nutrient release patterns in soil [37]. In this study, organic manure ($C/N \approx 6$) and carbon—based materials ($C/N \approx 12$) possess relatively low C/N ratios, implying that their carbon units are associated with relatively abundant nitrogen, which is more readily available for rapid microbial utilization, thereby accelerating their own mineralization and decomposition. This leads to rapid nutrient release, manifested as significant increases in soil available nitrogen, phosphorus, potassium, and total nitrogen content, meeting the immediate needs of crop growth. In contrast, after incorporation of high C/N ratio materials such as corn, soybean, and rapeseed straw (C/N ranging from 41 to 61), the initial decomposition phase triggers strong microbial immobilization of nitrogen, which may temporarily limit the supply of available nitrogen in the short term. However, their continuous carbon input is more conducive to promoting soil aggregate formation and fungal—dominated metabolic pathways, thereby leading to more stable carbon sequestration [38,39].

In terms of soil carbon pool dynamics, distinct patterns were observed among different amendment treatments. The organic fertilizer (OF) and carbon material (CM) treatments maintained higher contents of soil organic carbon (SOC), easily oxidizable carbon (EOC), and microbial biomass carbon (MBC), indicating effective retention and stabilization of a portion of the added carbon. This aligns with previous findings on the ability of such amendments to enhance active carbon pools [40,41]. In contrast, while the biochar (BC) treatment showed elevated SOC content, its EOC and MBC levels were comparable to those of the control group. This characteristic underscores biochar's primary role in increasing recalcitrant carbon, enhancing carbon storage capacity, and improving long-term carbon stability [42,43]. Soil enzymes are key agents catalyzing the decomposition and synthesis processes of soil organic carbon [44], with different enzyme classes catalyzing specific biochemical reactions against particular substrates [45]. For instance, cellobiohydrolase ac-

tivity is influenced by exogenous organic matter inputs [46], and β -glucosidase participates in soil metabolism and the carbon cycle [47]. A central finding was that the carbon loss from the amendments in these treatments ranged from nearly 0% to 60%. This phenomenon is closely related to the decomposability of the amendments and the microbial responses they induce. Materials with high cellulose content and a high C/N ratio significantly stimulated the activity of key cellulolytic enzymes [48]. The enhanced activity of these rate-limiting enzymes indicates a phase of intense microbial decomposition, which not only mineralizes the added labile carbon but may also accelerate the mineralization of a portion of the native SOC through a positive priming effect. Therefore, changes in the soil carbon pool are governed by the balance between new carbon inputs and their mineralization losses, which is directly regulated by the properties of the amendments. These differential effects on carbon pool dynamics, nutrient availability, and enzymatic activity confirm our first hypothesis: the properties of the amendments determine their functional pathways in soil.

4.2. Differential Shaping of Aggregate Structure and Organic Carbon Distribution by Organic Amendments INPUTS

Soil aggregates represent a critical spatial scale for the functional expression of the soil microbiome in agricultural ecosystems, regulating resource availability in habitats and driving community dynamics and biological interactions [49]. Our results showed that straw-based materials were the most effective organic amendments for promoting the formation of macroaggregates. This is possibly attributed to their richness in cellulose and hemicellulose, which provide ample energy sources for soil microorganisms, stimulating fungal hyphal extension and bacterial secretion of cementing agents like extracellular polysaccharides. These processes play a key “biological bridging” role in aggregate formation [50]. The higher mean weight diameter and geometric mean diameter observed in the straw treatments further indicate that the macroaggregates they promoted were not only more numerous but also structurally more stable, consistent with findings of [51]. Although the organic fertilizer treatment resulted in a lower proportion of macroaggregates compared to the straw treatments, its highest fractal dimension value reflected a more uniform and compact aggregate architecture. This likely originates from the complexity of organic fertilizer components: the readily decomposable fractions stimulate microbial cementation processes, while the highly humified components can interact with clay particles to form stable organo-mineral complexes. This optimizes pore structure and arrangement at a more microscopic scale, constructing more stable “granular structures” [52,53].

Aggregates are not only physical units of soil structure but also key sites for the differentiation and storage of organic carbon [54]. In this study, the SOC content was distributed evenly across all aggregate size classes under the organic fertilizer and the carbon material treatments, suggesting a widespread distribution of their input organic carbon within the soil. It is noteworthy that the SOC content in macroaggregates was significantly higher than in other size classes, aligning with results from Okolo [55]. This indicates that macroaggregates are more responsive to exogenous organic inputs. On one hand, macroaggregates can physically protect organic materials by encasing them, limiting microbial access and decomposition [56]. On the other hand, humic acids and polysaccharides produced during organic matter decomposition act as natural cementing agents, facilitating the further cementation of microaggregates into macroaggregates [52,53]. This process not only increases the proportion of macroaggregates but also potentially reduces oxidative loss of organic carbon by occluding pores [57]. In contrast, the carbon sequestration effects of straw-based amendments and green manure were primarily concentrated within macroaggregates, indicating that these types of organic amendments are more readily intercepted and stabilized rapidly by macroaggregates in the short term [58,59].

4.3. Specific Shaping of Microbial Community Structure and Functional Groups by Organic Amendments

Microorganisms are core drivers of the soil ecosystem, and their response to exogenous carbon inputs directly regulates key ecological processes such as soil nutrient cycling, aggregate formation, and organic carbon dynamics [60,61]. A key finding of this study is that the fungal community responded more sensitively and strongly to organic amendments inputs than the bacterial community. Specifically, with the exception of biochar, all organic amendments reduced the Shannon diversity of the fungal community. Fungi are key participants in soil carbon cycling and organic matter transformation [62,63], not only driving the deposition of organic carbon but also indirectly enhancing the physical protection of soil carbon by promoting the formation and stability of aggregates [64,65]. When organic materials rich in readily utilizable substrates like soluble sugars and proteins, such as straws and green manure, enter the soil, specific fungal taxa capable of rapidly utilizing these resources—such as the significantly increased Ascomycota observed in this study—can proliferate rapidly, gaining a competitive advantage and leading to simplified community structure and reduced diversity. In contrast, the biochar treatment uniquely maintained fungal diversity, possibly attributable to its porous structure providing physical refuge for microorganisms and alleviating resource competition pressure [66,67].

At the bacterial phylum level, different carbon sources drove specific phylogenetic selection. Straw-based carbon sources significantly enriched Proteobacteria, which comprises a large number of r-strategists characterized by rapid growth capacity and potential for degrading simple organic compounds [68]. These taxa are capable of quickly utilizing soluble organic matter released during the initial decomposition of straw. In contrast, the carbon material treatment significantly increased the relative abundance of Acidobacteriota, which are generally considered oligotrophic K-strategists that may be better adapted to the relatively stable micro-environment created by carbon material [69]. The response pattern of the fungal community was more complex and functionally explicit. The phylum Ascomycota was significantly enriched across all carbon source treatments, particularly under straw and green manure treatments. This finding strongly suggests that cellulose-degrading taxa within Ascomycota are key functional groups driving the decomposition of cellulose components in straw and green manure treatments. LEfSe analysis further identified several biomarker taxa under Ascomycota in the green manure and rapeseed straw treatments, providing robust support for this inference. In contrast, the biochar treatment uniquely maintained higher abundances of Chytridiomycota and Mortierellomycota. These taxa may play distinct ecological roles in decomposing complex organic compounds adsorbed by biochar or participating in nutrient cycling. Linking microbial responses to environmental factors via CCA revealed that bacterial community structure was associated with basic chemical factors like pH, AP, AK, and TN. This reflects that bacteria, as primary agents of biochemical processes like nitrification and phosphorus solubilization, have their composition significantly constrained by soil chemical properties. In contrast, the fungal community was more strongly associated with carbon-related indicator β -glucosidase activity, highlighting the dominant role of fungi in the transformation of complex organic carbon [44]. In summary, this study confirms that the influence of organic amendments inputs on soil bacterial and fungal community structures is significantly different, and that the response of specific microbial groups is a key link connecting exogenous carbon input with soil carbon transformation processes, in line with our second hypothesis.

4.4. Comprehensive Analysis of the Growth-Promoting Effects of Organic Amendments

Integrating random forest and structural equation modeling suggested exogenous organic materials promote maize growth and carbon sequestration via two paths. First, the

random forest model indicated that TN, MBC, BG and AK are the most critical explanatory variables for predicting crop growth response. In the structural equation model, the first path is a “physical structure–biology” path, primarily manifested where straw-based organic amendments enhance soil aggregate stability, which indirectly influences fungal community structure, thereby augmenting the crop’s carbon sequestration capacity. The second is a “chemical property–nutrient” pathway, in which organic fertilizer and carbon material increase the contents of mineral nutrients such as TN and AK, as well as active carbon fractions EOC and MBC, thereby creating a favorable rhizosphere growth environment for crop roots [36,37]. Notably, green manure amendment affects maize growth and carbon sequestration efficiently by acting via both paths simultaneously. Collectively, these results are in line with the third hypothesis of this study: the promotion of crop growth by organic materials is a synergistic effect achieved through multiple mechanisms.

The findings of this study provide clear guidance for agricultural management in the purple soil region. The selection of organic amendments should be based on specific production and ecological objectives. For rapid yield increase and soil fertility enhancement, green manure or organic fertilizer should be prioritized because they improve soil fertility rapidly and comprehensively, boosting crop yield directly. For improving soil physical structure and preventing soil erosion, straw-based materials are suitable choices as they effectively promote the formation of macroaggregates and improve soil permeability. For fostering a healthy and resilient soil microbial community, biochar demonstrated advantages; although its direct contribution to yield increase in the short term may be limited, it holds potential value for maintaining soil biodiversity and enhancing the long-term stability and resilience of the soil ecosystem. Curiously, the carbon material improved fertility but not yield, necessitating further research. In addition, further research is needed on the other amendments as well: the findings need validation over multiple growing seasons in field experiments where complex climatic, hydrological, and management factors may alter the effects of organic amendments.

5. Conclusions

We provide insights into the mechanisms through which organic amendments affect crop growth and soil carbon accumulation. Organic fertilizer and carbon material were effective in enhancing soil nutrients and promoting a more uniform distribution and increase in organic carbon across different aggregate fractions. Straw amendments were proficient in promoting the formation of macroaggregates. Green manure and straw amendments were associated with an efficient carbon-transforming microbial functionality, as indicated by the enhanced activities of β -glucosidase and cellobiohydrolase. Structural equation modeling revealed that the organic amendments enhanced maize carbon accumulation and biomass primarily by improving aggregate stability, regulating the fungal community, and increasing nutrient availability alongside active carbon fractions. Among the treatments, green manure and organic fertilizer exhibited the most prominent short-term agronomic effects. Therefore, in practical applications, green manure or organic fertilizer could be prioritized if the goal is rapid soil fertility enhancement and yield increase. Straw-based materials can be selected when the focus is on improving soil physical structure. The carbon material showed potential for enhancing overall soil carbon storage and fertility.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15122919/s1>, Table S1: Basic physicochemical properties of the experimental soil; Figure S1: Daily average temperature and precipitation during the maize growing season of 2024 at the experimental site in Ya’an, Sichuan, China; Figure S2: Differential bacterial taxa at various taxonomic levels under different organic materials; Figure S3: Differential taxa of fungi at various taxonomic levels under different organic materials.

Author Contributions: Conceptualization, D.Z. and Y.Q.; Methodology, D.Z. and Y.Q.; Software, J.W., P.P. and Y.Q.; Validation, L.Q., J.W., Y.C., X.T. and K.X.; Formal analysis, J.W. and D.Z.; Data curation, Y.Q.; Writing—original draft preparation, P.P. and L.Q.; Writing—review and editing, K.X., X.T. and Y.C.; visualization, K.X., D.Z. and P.P.; Supervision, Y.C. and K.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program of China [Grant number: 2022YFD1901402]; the Science and Technology Program of Sichuan Province [Grant number: 2025YFHZ0115]; and the Key Research and Development Program in Chengdu, Sichuan Province [Grant number: 2024YF050 2138SN].

Data Availability Statement: The raw sequencing reads from this study have been deposited in the NCBI Sequence Read Archive (SRA) as part of BioProject PRJNA1356673.

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

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