Effects of inorganic-organic fertilization schemes on the soil carbon fixation microbial communities and organic carbon accumulation

Chenchen Kong[1], Huichun Ye[2], Shiwen Zhang[3*], Shan Wang[3]

(1. *School of Mechanical & Electrical Engineering, Huainan Normal University, Huainan 232038, Anhui, China*;
2. *Key Laboratory of Digital Earth Science, Aerospace Information Research Institute, Chinese Academy of Science, Beijing 100094, China*;
3. *School of Earth and Environment, Anhui University of Science and Technology, Huainan 232001, Anhui, China*)

Abstract: This study aimed to elucidate the effects of different fertilization on the accumulation of soil organic carbon (SOC) and the community structure of carbon fixation bacteria in farmland, as well as their carbon sink mechanisms. Four field-based treatments were set up: no fertilizer (CK), organic fertilizer (OF), inorganic fertilizer (CF), and organic-inorganic compound fertilizer (OCF). Differences in soil *cbbL* bacterial communities, SOC and its component contents, and carbon pool management indices were analyzed using methods such as NMDS, Anosim, and metagenomeSeq tests. The aim was to identify the key differential species under different fertilization treatments, analyze the response relationship between *cbbL* bacteria communities and SOC and its component content, and clarify the important carbon fixation functional genes. The main findings were as follows: 1) CF and OCF significantly reduced soil *cbbL* bacterial diversity (p<0.05). Significant differences were observed among *cbbL* bacterial communities under different fertilization treatments (p=0.001). CK and OF treatments had higher numbers of unique OTUs and similar species composition. The six bacterial orders with significant differences among different fertilization treatments predominantly belonged to Pseudomonadota and Actinomycetota. 2) The CF treatment had the lowest content of SOC and its components, and carbon pool management indices. OF and OCF were beneficial to the improvement of SOC and its components, but there was no significant difference between the groups (p>0.05). Short-term fertilization differences had no significant effect on the carbon pool management index (p>0.05). 3) Compared with *cbbL* bacterial diversity, the differentially abundant species had a higher contribution to SOC accumulation (83.90%). Among them, Chromatiales significantly affected the active components of SOC and the carbon pool management index (p=0.04). *Thiodictyon* was a major functional genus under this order that had a significant positive effect on SOC (p=0.034), with the application of organic fertilizer exhibiting a targeted effect, promoting its abundance. The research results have revealed the key pathways for regulating microbial carbon fixation through fertilization, providing a novel theoretical basis and practical targets for carbon fixation and emission reduction in farmland ecosystems.

Keywords: fertilization type, soil organic carbon, *cbbL* bacterial community, response relationship, carbon fixation functional genes

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1 Introduction

Soil organic carbon (SOC) constitutes a crucial component of soil. Its quantity and quality not only determine the fertility of farmland soil but are also crucial for the sustainable development of agriculture and the carbon balance of global terrestrial ecosystems[1]. The "soil microbial carbon pump theory" states that microorganisms are important contributors to SOC, with the assimilation of CO_2 by autotrophic microorganisms being a key process in the carbon cycle. Such microorganisms not only decompose and mineralize organic matter but also convert CO_2 into SOC, thereby regulating the CO_2 concentration in the atmosphere and improving the carbon fixation capacity of soil[2,3]. According to statistics, autotrophic microorganisms in global terrestrial soil can capture 0.5% to 4.1% of atmospheric CO_2 annually[4]. The Calvin cycle is a major pathway for photoautotrophic and chemoautotrophic organisms, driving CO_2 fixation and assimilation. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is a key enzyme that regulates the activity and turnover rate of this cycle. Among them, the Form I RubisCO large subunit encoded by the *cbbL* gene has the highest abundance among the four types of RubisCO proteins and is highly conserved. It has been used as a biomarker in studies of carbon fixation microorganisms[5,6].

Fertilization, as a fundamental field management practice, increasing yield and productivity, can alter the carbon fixation capacity of microbial communities by influencing their structure and relative population abundance. Studies have shown that fertilization can lead to significant differences in the population structure of soil carbon-assimilating autotrophic bacteria, with corresponding changes in the dominant carbon-fixating bacteria[7]. For example, research on black wheat soil in Germany by Selesi et al.[8] revealed that fertilization can increase the diversity of autotrophic microorganisms in wheat soil. Yuan et al.[7]

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Biographies: Chenchen Kong, PhD, research interest: soil carbon sequestration for sinks and ecological value enhancement, Email: cckong0123@126.com; **Huichun Ye**, Associate researcher, research interest: quantitative remote sensing of vegetation and application, Email: yehc@aircas.ac.cn; **Shan Wang**, MS candidate, research interest: soil environmental assessment, Email: 1303367079@qq.com.

***Corresponding author: Zhang Shiwen**, Professor, Doctoral supervisor, research interest: soil environment and ecological restoration, Anhui University of Science and Technology, Huainan 232001, Anhui, China, Email: shwzhang@aust.edu.cn.

demonstrated that fertilization increased the abundance of *cbbL* genes and RubisCO activities in paddy soil. It has also been acknowledged that carbon fixation bacteria involved in the soil carbon cycle exhibit distinct responses to different fertilizers[9,10]. Organic fertilizers can improve the physical environment of the soil, increase bacterial growth rate and activity, enrich community structure, and promote SOC accumulation[11]. Combined application of organic and inorganic fertilizers can change the abundance and community structure of soil functional microorganisms (including methanotrophs and methanogens), providing an adequate supply of nutrients and organic carbon required by crops, and has shown great potential in mitigating greenhouse gas emissions from farmland[9,12]. The application of chemical fertilizers alone frequently results in soil nutrient leaching, acidification, reduced carbon storage, and reduced microbial biomass and diversity[13]. Certain studies have indicated that the application of organic fertilizers did not significantly increase or decrease SOC[14]. Other studies have found that in poor soils with low fertility, despite the low abundance of autotrophic microorganisms, the carbon assimilation rate in the soil remained elevated[15]. Therefore, the current understanding of how fertilization management influences SOC accumulation through alterations in soil carbon fixation microorganisms remains insufficient. This knowledge gap hinders the development of optimized management strategies and fails to provide specific regulatory targets for precisely enhancing regional farmland soil carbon pools. From the perspective of material and energy flow in farmland ecosystems, more information and research on the impact of fertilization on carbon fixation bacteria communities and SOC accumulation is needed to better understand the cascade effect of fertilization - carbon fixation bacteria - SOC.

This study employed field fertilization experiments to determine the content of SOC components and the abundance of carbon-fixating bacteria carrying the *cbbL* gene (referred to as "*cbbL* bacteria" in this article). It aimed to investigate how different fertilizers (organic fertilizer, inorganic fertilizer, organic-inorganic compound fertilizer, and no fertilizer) affect 1) the abundance, diversity, community structure, and species composition of soil *cbbL* bacteria; 2) the contents of SOC and its components and the carbon pool management index; and to analyze 3) the key carbon-fixating bacteria causing SOC changes and their dynamic responses to SOC under different fertilization conditions. The objective was to stimulate the activity of carbon fixation functional bacteria by optimizing fertilization, thereby providing a theoretical basis towards a deeper understanding of the soil carbon sequestration process as affected by different fertilization management strategies.

2 Materials and methods

2.1 Site description and sample collection

To explore the effects of different fertilizers on SOC content and soil carbon fixation bacteria and their interaction relationships, field fertilization experiments were set up at the experimental base in Miyun District, Beijing (40°17′47″N, 116°47′10″E). The annual cultivation scheme in the experimental area primarily involves the rotation of summer maize and winter wheat. Before the experiment, soil organic matter measured 12 g/kg, total nitrogen was 0.71 g/kg, available phosphorus was 16.3 mg/kg, available potassium was 62 mg/kg, SOC was 12.00 g/kg, easily oxidized organic carbon (EOC) was 1.31 mg/kg, dissolved organic carbon (DOC) was 0.06 g/kg, light fraction of organic carbon (LFOC) was 1.95 g/kg, and heavy fraction organic carbon (HFOC) was 10.05 g/kg. Three prevalent fertilizer types used in the local agricultural production process [organic fertilizer (OF), inorganic fertilizer (CF), and organic-inorganic compound fertilizer (OCF)] were selected as the experimental design variables, while no fertilizer treatment (CK) was used as the control. The fertilization standard was measured in nitrogen. The nitrogen application was determined according to the soil background fertility, local crop target yield, and fertilizer nutrient ratio (194.40 N kg/hm^2 and 262.50 N kg/hm^2 for maize and wheat growing seasons, respectively). The fertilizer type and application rate of each treatment are detailed in Table 1. The experimental layout was completed in June 2022. Each treatment was repeated 3 times (in 14 m×6 m plots ×3). A 2 m isolation zone was left around each treatment. The experimental layout is shown in Figure 1. During the experiment, except for fertilization, other farmland management practices were consistent with local crop production.

Table 1 Fertilizer types and application rates for different experimental treatments

Treatment	Fertilizer application rate per plot/kg		Fertilizer type
	Maize	Wheat	
CK	0.00	0.00	-
OF	108.86	147.00	Organic matter≥45%, N≥1.5%, moisture content≤ 30%, Beijing Woshengjie Planting Soil Co., Ltd.
CF	9.07	12.25	N:P_2O_5:K_2O=18:19:5, total nutrients≥42%, Beijing Green Deli Industry & Trade Co., Ltd.
OCF	7.42	10.02	N:P_2O_5:K_2O=22:10:10, organic matter≥15%, Genliduo Biotechnology Co., Ltd.

Note: CK, OF, CF, and OCF are no fertilizer, organic fertilizer, inorganic fertilizer, and organic-inorganic compound fertilizer, respectively. The same applies below.

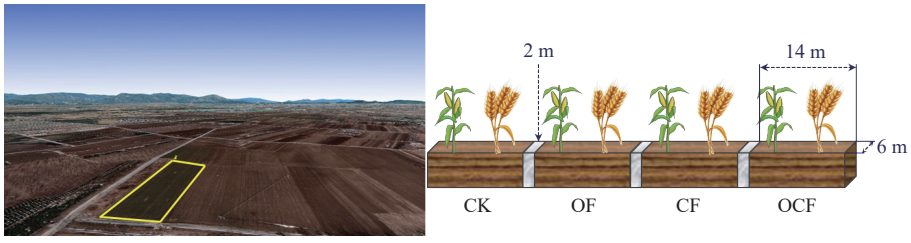


Figure 1 Layout of fertilization experiment

Soil samples were collected from three replicated plots corresponding to each treatment before the wheat harvest in June 2023. A 5 cm diameter soil drill was used to collect surface soil samples at a depth of 0-20 cm between crop plants in each plot using the five-point method (far from the roots of the crops). Three bulked soil samples were obtained for each treatment. After removing visible impurities such as stones and plant roots, the soil was divided into three parts and bagged for labeling. One was stored at –80°C for soil DNA extraction, one was stored in a 4°C refrigerator for DOC determination, and the third part was naturally air-dried in the laboratory for the determination of other SOC components.

2.2 Determination of soil properties and microbial communities

2.2.1 Quantification of soil *cbbL* genes and Illumina MiSeq sequencing

The total genomic DNA of soil samples was extracted using the QIAGEN DNeasy Power Soil Kit (50). The DNA was quantified using Nanodrop ONE (Thermo Scientific), and the quality of the extracted DNA was assessed using 1.2% agarose gel electrophoresis. *Pfu* high-fidelity DNA polymerase was used for PCR amplification (TransGen Biotech Co., LTD). The sequences of the upstream primer K_2F and the downstream primer V_2R of the *cbbL* gene were 5′-GCACCTAAYTGGGYDTAAAGNG-3′ and 5′-TACNVGGGTATCTAATCC-3′, respectively. Magnetic beads (Vazyme VAHTSTM DNA Clean Beads), at a 0.8-fold greater volume, were added to 25 μL of PCR products. After thorough agitation to achieve complete suspension, they were adsorbed on the magnetic rack for 5 min, and the supernatant was removed with a pipette. 200 μL of 80% ethanol was added, and samples were placed in the opposite orientation on the magnetic rack, to achieve adsorption on the other side of the PCR tube with magnetic beads. After full adsorption, the supernatant was removed and samples were let to stand at room temperature for 5 min until the alcohol was completely evaporated and cracks appeared in the magnetic beads. 25 μL of Elution Buffer was added for elution. Then the PCR tubes were placed on the adsorption rack for 5 min for full adsorption, and subsequently, the supernatant was removed and stored in a 1.5 mL centrifuge tube. The recovered products from PCR amplification were quantified by fluorescence. The fluorescence reagent was Quant-iT PicoGreen dsDNA Assay Kit, and the quantitative instrument was a Microplate reader (BioTek, FLx800). The sequencing library was prepared using the TruSeq Nano DNA LT Library Prep Kit of Illumina, and then double-ended sequencing was performed using the MiSeq sequencer, with the corresponding reagent being MiSeq Reagent Kit V3 (600 cycles). Cutadapt (v2.3) was used to remove the primer fragments of the sequence and discard the sequences with unmatched primers. Vsearch (v2.13.4_linux_x86_64) was used to assemble sequences, perform quality control, and remove duplicate sequences. Chimeras in the deduplicated sequences cluster were removed based on a 98% similarity threshold. The chimeras in the quality-controlled sequences were filtered to obtain high-quality sequences. Representative sequences and operational taxonomic units (OTUs) were determined from the high-quality sequences, based on a 97% similarity level[16–18].

2.2.2 Determination of soil organic carbon components

SOC was determined by the potassium dichromate volumetric method—external heating method, DOC was determined by the potassium dichromate-sulfuric acid oxidation method, EOC was determined by ultraviolet spectrophotometry, and LFOC and HFOF were determined by the relative density grouping method[19].

2.3 Data processing and analysis

The CK soil was used as a reference to calculate the carbon pool management index. The calculation formulas of its related indicators are as follows[20]:

$$CPMI = CPI \times CPAI \times 100\%$$

$$CPI = SOC_S / SOC_R$$

$$CPAI = L_S / L_R$$

$$L = LOC / ROC$$

where, CPMI denotes the carbon pool management index,%; CPI denotes the carbon pool index; CPAI denotes the carbon pool activity index; SOC_S denotes the organic carbon content of the test soil, g/kg; SOC_R denotes the organic carbon content of the reference soil, g/kg; L_S denotes the carbon pool activity of the test soil; L_R denotes the carbon pool activity of the reference soil; LOC denotes the soil active organic carbon content, g/kg; and ROC denotes the soil inert organic carbon content, g/kg.

IBM SPSS 27 was used to perform basic statistical and multiple comparison analysis of the data (Duncan test, p<0.05). The differences in the abundance, diversity, and community composition of *cbbL* bacteria under different fertilizers were examined by non-metric multidimensional scaling (NMDS) and Anosim. The differentially abundant bacterial orders were identified based on metagenomeSeq. The contributions of *cbbL* bacterial diversity, differentially abundant bacterial orders, and genera to SOC were assessed by redundancy analysis (RDA), and the key carbon fixation functional genes were identified. Moreover, the dynamic responses of carbon fixation functional genes and SOC under different fertilization treatments were analyzed by canonical correlation analysis (CCA). Origin 2022 and R 4.2.2 were used for chart plotting.

3 Results and analysis

3.1 Diversity and species composition of soil *cbbL* bacteria under different fertilization treatments

Under different fertilization treatments, the α diversity index of soil *cbbL* bacteria in CF and OCF treatments was relatively low (Table 2). The Chao1 index was significantly lower than that of the CK treatment, while the Shannon and Pielou's evenness indices were significantly lower than those of the CK and OF treatments (p<0.05). CF and OCF treatments had similar bacterial community structures but were significantly different from those of the CK and OF treatments (Figure 2a). The Anosim test revealed (Figure 2b) that the inter-group differences in the *cbbL* bacterial community structure under different fertilization types were greater than the intra-group differences under the same fertilization type (R=0.600), and the inter-group differences reached a significant level (p= 0.001), indicating that different fertilization schemes could significantly change the soil carbon fixation bacterial community.

Table 2 Alpha diversity of *cbbL* bacteria

Fertilization type	Chao1	Shannon	Pielou's evenness	Goods_coverage
CK	3726±226^a	8.49±0.08^a	0.746±0.006^a	0.973±0.002^b
OF	3222±290^b	8.35±0.54^a	0.743±0.040^a	0.978±0.002^a
CF	2873±208^b	7.42±0.10^b	0.677±0.004^b	0.980±0.002^a
OCF	3002±160^b	7.66±0.28^b	0.691±0.020^b	0.979±0.001^a

Note: different lowercase letters indicate significant differences between the indicators under different types of fertilizer application (p<0.05). The same applies below.

OTU quantification under different fertilization treatments (Figure 3a) indicated that the number of unique OUTs was higher in the CK and OF treatments, and lower in the CF and OCF treatments. Under different fertilization schemes, the relative abundance of Pseudomonadota in soil *cbbL* bacteria was predominantly high (85.25%-91.48%), followed by Actinomycetota (7.14%-12.02%), while the abundance of other bacterial phyla remained comparatively low (Figure 3b). The *cbbL* bacteria with a relative abundance greater than 0.1% at the genus level were further quantified (Figure 3c); *Mesorhizobium* had the highest relative abundance in soil samples across the different fertilization treatments (22.30%-35.12%), followed by *Bradyrhizobium* (18.47%-26.00%).

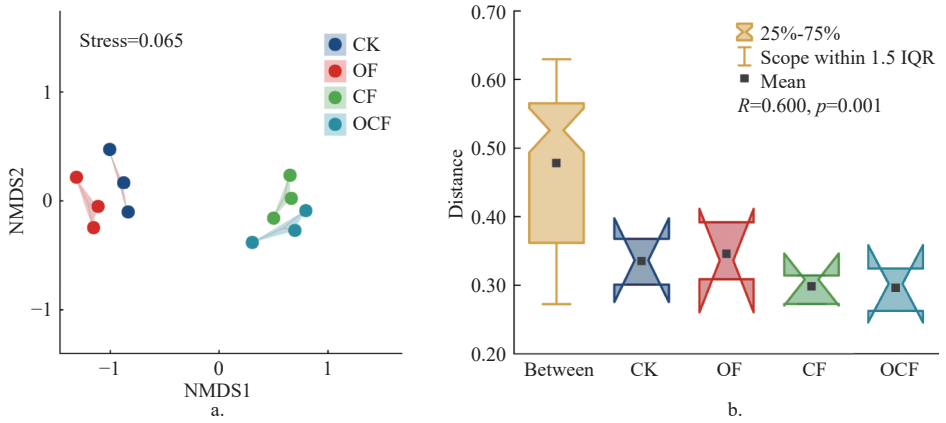


Figure 2 NMDS analysis (a) and Anosim-based community difference test (b) of *cbbL* bacterial communities

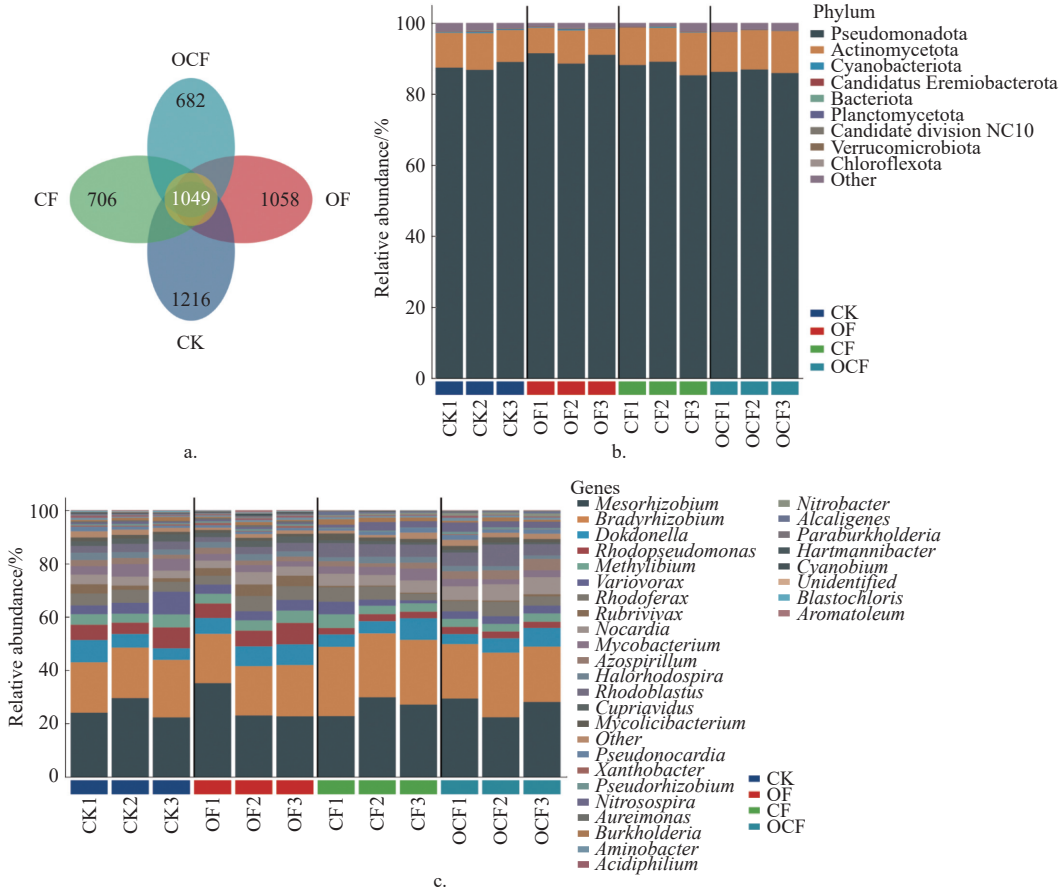


Figure 3 OTU abundance composition (a), and relative abundance at the phylum (b) and genus (c) levels of *cbbL* bacteria under different fertilization types

The *cbbL* bacterial OTUs with a relative abundance greater than 0.1% at the genus level were clustered, revealing that the species composition of the treatments without inorganic fertilizer application (CK and OF) was more similar. On the other hand, the CF and OCF treatments, which included inorganic fertilizer application, demonstrated a higher degree of similarity, which was consistent with the characteristics of bacterial community structure (Figure 4).

3.2 Differences in *cbbL* bacterial marker species in soils under different fertilization treatments

Considering that the bacteria responsible for SOC changes should primarily correspond to species with significant differences under different fertilization treatments, metagenomeSeq was used to compare the different treatments, screen the OTUs with differences between treatment groups, and analyze their enrichment trends (Figure 5). The OTUs with significant differences under different fertilization treatments were classified within Hyphomicrobiales, Xanthomonadales, Rhodospirillales, Burkholderiales, Pseudonocardiales, Mycobacteriales, Nitrosomonadales, Eubacteriales, Chromatiales, and Unassigned, mainly belonging to Pseudomonadota and Actinomycetota. Compared with no fertilizer treatment, application of organic fertilizer suppressed the differential OTUs of Hyphomicrobiales and Burkholderiales (Figure 5a); application of inorganic fertilizers reduced the abundance of Mycobacteriales, Pseudonocardiales, and Nitrosomonadales, and enhanced the abundance of Rhodospirillales and Burkholderiales (Figure 5b); finally, application of organic-inorganic compound fertilizer suppressed Mycobacteriales,

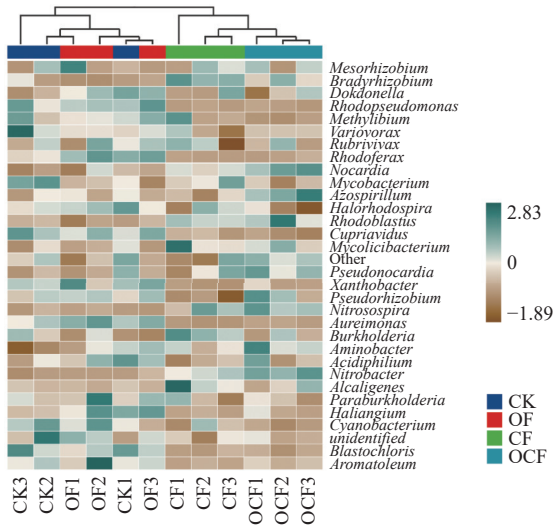


Figure 4 Clustered heat map of *cbbL* bacterial composition at genes level under different types of fertilizer application

Nitrosomonadales, and Rhodospirillales, and promoted the abundance of Eubacteriales, Burkholderiales, and Xanthomonadales (Figure 5c). Compared with the application of organic fertilizer, inorganic fertilizer and organic-inorganic compound fertilizer application suppressed the differential OTUs of Mycobacteriales, Pseudonocardiales, and Chromatiales (Figure 5d and 5e). Compared with inorganic fertilizer, the application of organic-inorganic compound fertilizer had a stimulating effect on the OTUs of Hyphomicrobiales and Xanthomonadales, and had a certain inhibitory effect on those of Burkholderiales (Figure 5f).

3.3 Characteristics of SOC and carbon pool management index under different fertilization treatments

The highest content of SOC and its components across different fertilization treatments was observed in the OF treatment (Table 3). The CF treatment had the lowest EOC and DOC contents, which were significantly lower than those in the CK, OF, and OCF treatments ($p<0.05$). There were no significant differences in the contents of SOC and its components among the CK, OF, and OCF treatments ($p>0.05$). The soil carbon pool management indices, L_S, CPAI, CPI, and CPMI, were similarly the lowest in the CF treatment (Table 4), but no significant differences were observed ($p>0.05$). Overall, the short-term application of inorganic fertilizers was not conducive to the increase of SOC and its component contents, nor to the healthy development of the carbon pool.

Table 3 SOC and its component contents under different fertilization types

Fertilization type	SOC/ g·kg^{-1}	EOC/ mg·kg^{-1}	DOC/ g·kg^{-1}	LFOC/ g·kg^{-1}	HFOC/ g·kg^{-1}
CK	12.00±1.33^{a}	1.68±0.08^{a}	0.07±0.017^{a}	1.86±0.40^{a}	10.14±1.72^{a}
OF	13.22±1.70^{a}	1.97±0.05^{a}	0.08±0.01^{a}	1.97±0.63^{a}	11.25±1.07^{a}
CF	11.25±1.89^{a}	1.28±0.28^{b}	0.04±0.01^{b}	1.59±0.37^{a}	9.65±1.92^{a}
OCF	12.49±1.36^{a}	1.90±0.22^{a}	0.07±0.01^{a}	2.06±0.43^{a}	10.40±1.25^{a}

Table 4 Soil carbon pool management index under different fertilization types

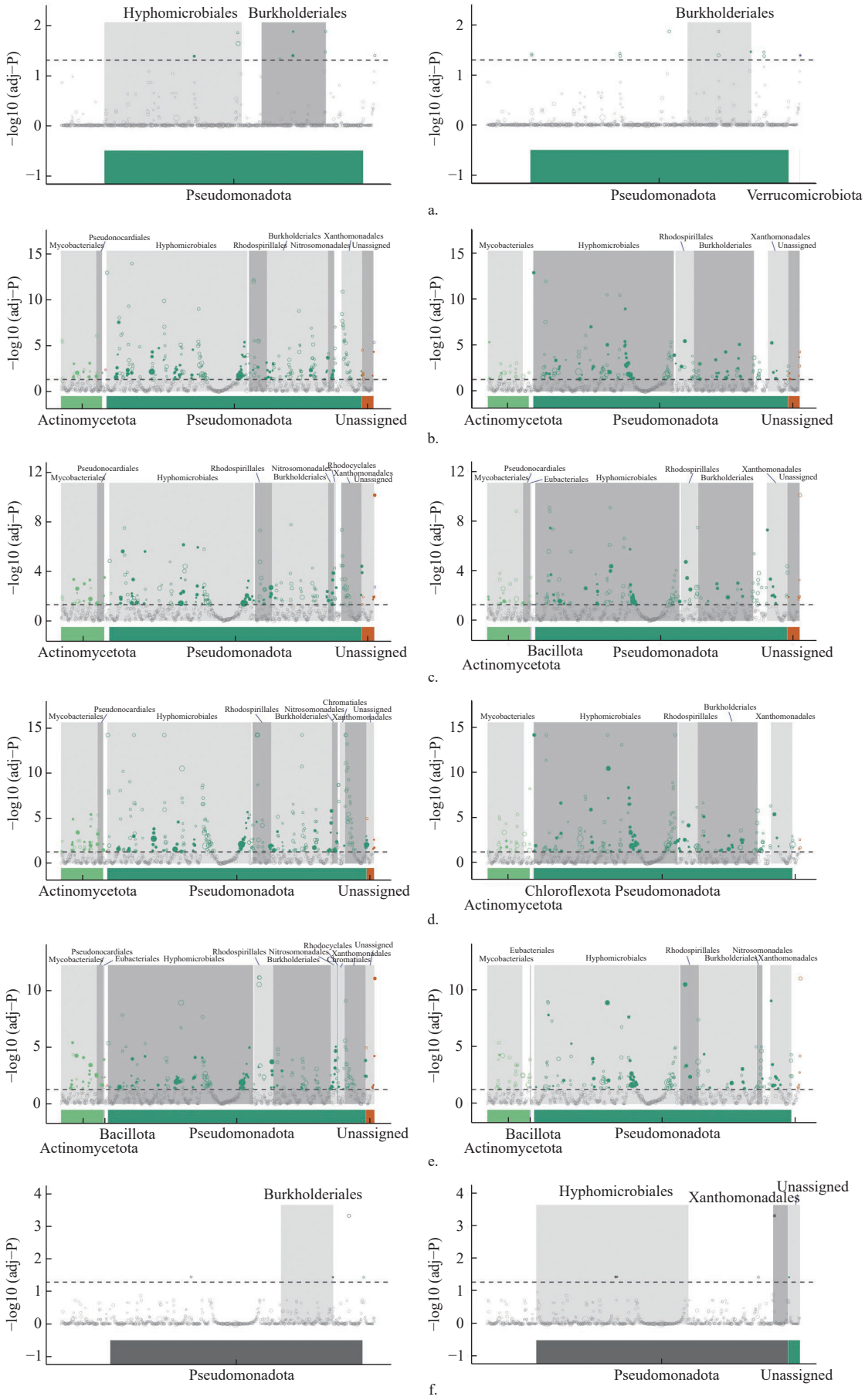
Fertilization type	L_S	CPAI	CPI	CPMI/%
CK	0.19±0.08^{a}	0.98±0.37^{a}	1.00±0.11^{a}	95.54±26.56^{a}
OF	0.17±0.05^{a}	0.89±0.22^{a}	1.10±0.14^{a}	100.15±34.53^{a}
CF	0.17±0.05^{a}	0.88±0.26^{a}	0.94±0.16^{a}	80.53±21.47^{a}
OCF	0.20±0.04^{a}	1.02±0.23^{a}	1.04±0.11^{a}	106.42±25.45^{a}

3.4 Associations between soil *cbbL* bacteria and SOC

The α diversity index was used to characterize the diversity of *cbbL* bacterial communities, and the key differentially abundant species were identified in the differential bacterial orders between groups. RDA was used to explore the effects on SOC of the diversity and differentially abundant species in *cbbL* bacterial communities. The first two axes explained 89.92% of the variance, indicating that the diversity and differential species in soil *cbbL* bacterial communities can explain most of the changes in SOC (Figure 6a). Among them, SOC, EOC, DOC, HFOC, and CPI were positively correlated with Burkholderiales and Rhodospirillales, while the Shannon index, LFOC, L_S, CPAI, and CPMI were positively correlated with Rhodospirillales, Eubacteriales, Hyphomicrobiales, Chao1, and Xanthomonadales. On the other hand, Chromatiales and Nitrosomonadales had negative effects on SOC and its components and carbon pool management index. In terms of relative contribution (Figure 6b), the *cbbL* bacterial community variability accounted for merely 16.10% ($p>0.05$) of the overall change in soil organic carbon (SOC), while differential bacterial orders contributed a substantial 83.90%. Chromatiales, Mycobacteriales, and Unassigned had a relatively high contribution, at 21.70%, 17.30%, and 10.60%, respectively, among which Chromatiales reached a significant level (pseudo- F=3.2, p=0.04).

The contribution levels of six genera in the Chromatiales order were analyzed, and the key carbon fixation functional genes that exhibited a significant impact on SOC and its components as well as the carbon pool management index were screened. The genera explained 40.83% of the changes in SOC-related indicators. *Thiodictyon* was positively correlated with SOC components and carbon pool management indices, while *Halorhodospira* and *Nitrococcus* exhibited a negative correlation (Figure 7a). Among them, *Thiodictyon* explained the highest degree of variation in SOC and its components and in carbon pool management indices (53.10%) and reached a significant level (pseudo- F=2.90, p=0.034). It can be concluded that *Thiodictyon* is an important functional genus that has a significant effect on SOC under different fertilization management strategies (Figure 7b).

The dynamic response relationship between each component of SOC and the carbon pool management indices and the key functional genera of *cbbL* bacteria under different fertilization was further elucidated through CCA, and the specific *cbbL* bacteria associated with SOC changes caused by fertilization were explored. Certain similarities were observed between the *cbbL* bacterial functional genera involved in carbon cycling in the OCF treatments and those in OF, as shown in Figure 8a. Each genus had a strong influence on SOC and its components and carbon pool management indices, indicating that the important functional genes that play a core role in SOC mainly exist in the soil applied with organic fertilizer and organic-inorganic compound fertilizer. An overlap was observed in the distribution of soil samples collected from OF and OCF, as shown in Figure 8b. *Thiodictyon* and *Thiohalospira* were the major functional genera that had a greater impact on SOC and its components and carbon pool management index in OF and OCF treatments. Overall, the key carbon-fixation functional bacteria exhibited variability due to the differences in fertilization types. This study revealed strong correlations between the major carbon fixation functional genera of *cbbL* bacteria and the components of SOC and the carbon pool management indices when organic fertilizers and organic-inorganic compound fertilizers were applied.



Note: a–f denote pairwise comparisons (both directions included) of differentially enriched *cbbL* bacteria; a, CK vs OF; b, CK vs CF; c, CK vs OCF; d, OF vs CF; e, OF vs OCF; f, CF vs OCF.

Figure 5 Manhattan plot of differential OTUs analyzed by metagenomeSeq

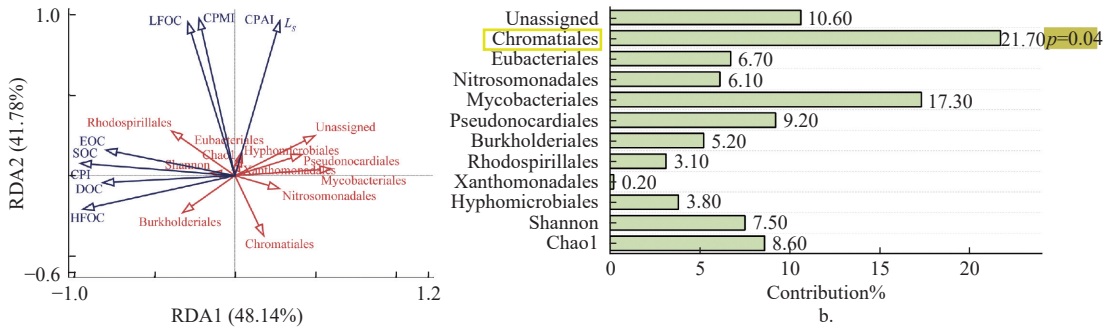


Figure 6 RDA of *cbbL* bacterial diversity and differential orders with SOC, its components and carbon pool management indices (a) and relative contribution of *cbbL* bacterial diversity and differential orders (b)

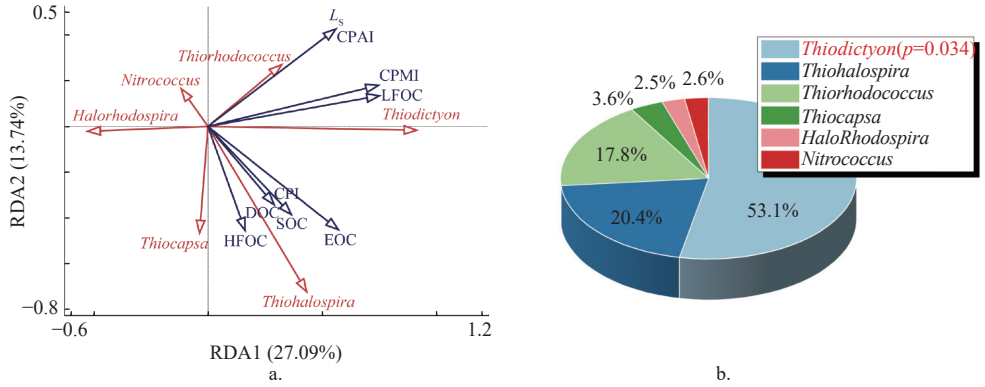
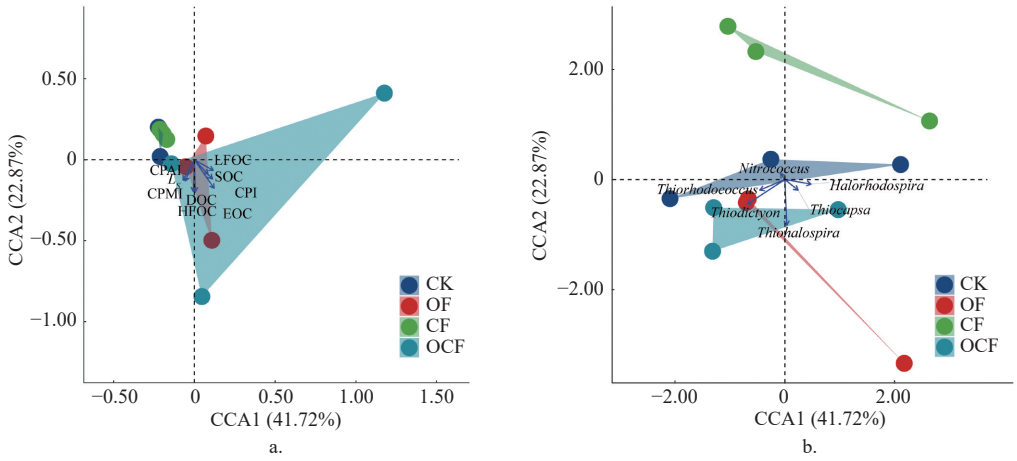


Figure 7 RDA of *cbbL* bacterial key functional genes with SOC, its components and carbon pool management indices (a) and relative contribution of *cbbL* bacterial key functional genes (b)



Note: a. is the *cbbL* bacterial key functional genes as explanatory variables, and the SOC components and the carbon pool management index as response variables; b. is the SOC components and the carbon pool management index as explanatory variables, and the *cbbL* bacterial key functional genes as response variables.

Figure 8 CCA of *cbbL* bacterial key functional genes with SOC, its components and carbon pool management indices under different types of fertilizer application

4 Discussion

The addition of exogenous nutrients can disrupt the original ecological balance of the soil. The structure and number of microbial communities in the soil can change significantly due to fertilization, thereby changing its CO_2 fixation capacity[21]. Liu et al[22] believed that the soil bacterial Shannon index for inorganic fertilizer alone and for the combination of organic and inorganic fertilizers was lower than that of no fertilizer. The results of Wei et al.[23] also showed that the application of inorganic fertilizer and the combination of organic and inorganic fertilizers would significantly reduce the diversity of soil bacterial communities. This aligns with this study's results which indicated that the Chao1 index of soil *cbbL* bacteria in the CF and OCF treatments was significantly lower than that in the CK treatment, and the Shannon and Pielou's evenness indices were significantly lower than those in the CK and OF treatments (p<0.05). Inorganic fertilizers may stimulate the proliferation of certain bacterial groups with specific resistance effects, causing a decrease in bacterial diversity[24]. The inorganic fertilizer components in compound fertilizers can lead to soil acidification. Although their organic components can prevent and control soil acidification, the pH range for the optimal growth of bacteria is very narrow. Therefore, to a certain extent, inorganic fertilizers are still not conducive to maintaining bacterial diversity.

Organic fertilizer can provide rich substrates for bacterial growth and reproduction, improve soil micro-environment, and optimize community structure and function[25]. Differences in nutrient types can alter the bacterial community structure and species composition. In this study, the soil *cbbL* bacterial community structure and species composition of CF and OCF treatments were similar, and CK and OF treatments had high similarity (p=0.001), which is consistent with the findings of Liu et al.[22]. However, the differences in soil bacterial communities and species composition under different fertilization schemes do not imply that all species exhibit substantial changes. Typically, only a few of the components differ. Diverse environmental conditions or excessive temporal and spatial distribution differences among bacteria may result in variations at the phylum and class levels. In this experiment, the temporal and spatial distribution was highly similar, while the soil environment also exhibited high similarity. The OTUs with significant differences between different fertilization treatments at the order level mainly belonged to the Pseudomonadota and Actinomycetota phyla, which is consistent with the research results of fertilization experiments on the Yunnan-Guizhou Plateau by Yu et al.[26]. Research shows[27] that as the dominant bacterial phyla in soil, Pseudomonadota and Actinobacteria play a vital role in the nutrient cycle, carbon and cellulose degradation, and humus formation in soil. Among them, Pseudomonadota has a strong ability to degrade organic matter, can adapt to various complex environments, and promotes the carbon cycle in the soil ecological environment system[28].

In this study, the response of farmland SOC to different fertilization treatments was different. The contents of SOC and its components were higher in the OF treatment, while they were the lowest in the CF treatment. This is mainly because organic fertilizer can add a large amount of organic matter to the soil, providing sufficient carbon source and energy for microorganisms, thereby increasing their metabolic rate, and accelerating SOC turnover and decomposition to release more active organic carbon. At the same time, it can also improve soil structure and promote the physical protection of soil for organic carbon[29]. Meanwhile, inorganic fertilizer can lead to a decrease in soil C/N, accelerate the decomposition and mineralization of organic carbon in the soil, and reduce SOC storage. Long-term application can also cause acidification and hardening, and change the living environment of microorganisms. Studies have shown that[30] organic carbon in the soil where inorganic fertilizer was applied alone for 30 consecutive years decreased by 0.58 g/kg every 10 years. Overall, the application of organic and inorganic fertilizers can improve the physiochemical properties of soil, increase the number and diversity of soil microorganisms, and thus increase the contents of SOC and its components. Wei et al.[31] pointed out that while the inorganic constituents in compound fertilizers accelerate acidification, the organic components therein are beneficial to maintain or increase the SOC content to a certain extent and prevent soil degradation. This can explain the relatively high contents of SOC and its components in the OCF treatment in this experiment. In addition, the CF fertilization treatment had significantly lower EOC and DOC contents than other fertilization treatments (p<0.05). This may be because the active components of SOC comprise monosaccharides, polysaccharides, and lignin, which are easily decomposed and utilized by microorganisms and have solubility and instability[32]. Among them, EOC is the most easily oxidized active organic carbon and is easily affected by microorganisms, while DOC originates from soil microorganisms and can also be decomposed and utilized by microorganisms. Zhang et al.[33] found a strong correlation between soil bacteria and DOC. Li et al.[34] also pointed out that DOC is the main source of unstable carbon and energy for microorganisms, and the proportion of its components that can be directly decomposed and utilized by microorganisms is as high as 10% to 40%. Therefore, due to different compound compositions and decomposition rates, EOC and DOC are more sensitive to field management measures. Generally, compared with the application of inorganic fertilizer alone, the applications of organic fertilizer or organic and inorganic fertilizer significantly improved the soil carbon pool management indices; however, they did not attain a significant difference level (p>0.05). Zhao et al.[35] pointed out in a field positioning test study that continuous fertilization for more than three years is usually required to significantly affect the soil carbon pool management index. Therefore, the effects of different fertilization treatments on each component of SOC and the soil carbon pool management index still need to be continuously monitored over the long term to enhance the scientific validity of the results.

In this study, the effect of *cbbL* bacterial diversity on the overall change of SOC was not statistically significant (p>0.05), and the relative contribution was only 16.10%. On the other hand, the relative contribution of key differential abundant genera was as high as 83.90%, among which Chromatiales significantly affected the active components of SOC and the carbon pool management indices (pseudo- F=3.2, p=0.04). It is speculated that the differences in the species composition of *cbbL* bacteria may explain the changes in soil carbon fixation capacity better than their diversity. Liao et al.[36] showed that there was no significant difference in the diversity of *cbbL* bacteria in different fertilization treatments. Zhao et al.[15] also found no significant correlation between soil carbon fixation capacity and *cbbL* bacterial diversity, but different autotrophic bacteria in the soil showed different carbon assimilation potentials and metabolic strategies[37]. This suggests that, in comparison to diversity, the species variations of *cbbL* bacteria with distinct metabolic strategies and activities may significantly influence SOC. The RDA results indicated that six bacterial genera belonging to the Chromatiales could explain 40.83% of the changes in SOC and its components as well as changes in the carbon pool management indices, among which *Thiodictyon* most significantly affected SOC (pseudo- F=2.90, p=0.034) and was positively correlated with SOC and its components as well as the carbon pool management indices. Chromatiales is a type of anaerobic bacteria that performs photosynthesis and can produce acid through the reduction of hydrogen sulfide. The sulfur cycle facilitates the absorption and utilization of carbon by crops, while the carbon cycle supplies the essential energy and material foundation for sulfur transformation. Therefore, Chromatiales can modify the soil micro-environment by regulating soil pH and redox potential and indirectly play a role in the cycle and transformation of SOC[38]. It is worth noting that although the relative abundance of Chromatiales was low (2.23%), it exerted a significant effect on the various components of SOC and the carbon pool management indices (p<0.05). In particular, *Thiodictyon* is only a genus belonging to this order, and its effect on SOC still reached a significant level (p<0.05). This shows that rare bacterial populations with low abundance should not be ignored, and they may also play a vital role in the soil carbon cycle process.

The effects of each key functional bacterial genera on SOC and its components as well as the carbon pool management index were relatively strong in the OF and OCF treatments. Specifically, *Thiodictyon* and *Thiohalospira* were identified as key functional

genera exerting considerable influence on SOC components and carbon pool management indices under OF and OCF treatments. The conclusion that Thiodictyon was positively correlated with each component of SOC and the carbon pool management indices, and was a significant functional genus influencing SOC, suggests that application of organic fertilizer may have a stimulating effect on some key functional genera that play a key role in the SOC cycle process. This stimulation subsequently promotes the increase in SOC and its component contents, as well as the healthy development of the carbon pool. These findings substantiate that in the future, the activity of soil carbon fixation bacteria can be targeted and enhanced through improved fertilization techniques. The concept of promoting carbon fixation in farmland soil is feasible. In addition, bacteria are bidirectional in regulating the soil carbon cycle. The trade-off between decomposition and assimilation determines the storage of organic carbon in soil[39]. Therefore, long-term monitoring of the inherent relationship between SOC and carbon fixation functional microorganisms is still needed to provide a scientific basis for investigating the dual regulation of soil carbon pools by microorganisms.

5 Conclusions

This study confirmed the importance of fertilization types for the growth and reproduction of *cbbL* bacteria and their fixation of SOC, thereby expanding the understanding of the response relationship between SOC and carbon-sequestering functional bacteria mediated by fertilization management.

1) The richness and diversity of soil *cbbL* bacteria were significantly reduced in soils amended with inorganic fertilizers (CF and OCF) (p<0.05). The OTUs with significant differences among different fertilization treatments were categorized within Hyphomicrobiales, Xanthomonadales, Rhodospirillales, Burkholderiales, Pseudonocardiales, Mycobacteriales, Nitrosomonadales, Eubacteriales, Chromatiales, and Unassigned.

2) The SOC and its component contents and carbon pool management indices reached their lowest values under the sole application of inorganic fertilizer (CF). In contrast, the application of organic fertilizer (OF) and organic-inorganic compound fertilizer (OCF) stimulated key *cbbL* SOC-sequestering bacteria (*Thiodictyon*), thereby promoting SOC accumulation (p=0.034). Additionally, low-abundance rare bacteria order (Chromatiales) also played a significant role, explaining 21.70% of the SOC variance.

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