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Combined metabolomic and microbial community analyses reveal that biochar and organic manure alter soil C-N metabolism and greenhouse gas emissions

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ABSTRACT

The use of biochar to reduce the gas emissions from paddy soils is a promising approach. However, the manner in which biochar and soil microbial communities interact to affect CO_2, CH_4, and N_2O emissions is not clearly understood, particularly when compared with other amendments. In this study, high-throughput sequencing, soil metabolomics, and quantitative real-time PCR were utilized to compare the effects of biochar (BC) and organic manure (OM) on soil microbial community structure, metabolomic profiles and functional genes, and ultimately CO_2, CH_4, and N_2O emissions. Results indicated that BC and OM had opposite effects on soil CO_2 and N_2O emissions, with BC resulting in lower emissions and OM resulting in higher emissions, whereas BC, OM, and their combined amendments increased cumulative CH_4 emissions by 19.5 %, 31.6 %, and 49.1 %, respectively. BC amendment increased the abundance of methanogens (*Methanobacterium* and *Methanocella*) and denitrifying bacteria (*Anaerolinea* and *Gemmatimonas*), resulting in an increase in the abundance of *mcrA*, *amoA*, *amoB*, and *nosZ* genes and the secretion of a flavonoid (chrysosplenetin), which caused the generation of CH_4 and the reduction of N_2O to N_2, thereby accelerating CH_4 emissions while reducing N_2O emissions. Simultaneously, OM amendment increased the abundance of the methanogen *Caldicoprobacter* and denitrifying *Acinetobacter*, resulting in increased abundance of *mcrA*, *amoA*, *amoB*, *nirK*, and *nirS* genes and the catabolism of carbohydrates [maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose], thereby enhancing CH_4 and N_2O emissions. Moreover, puerarin produced by *Bacillus* metabolism may contribute to the reduction in CO_2 emissions by BC amendment, but increase in CO_2 emissions by OM amendment. These findings reveal how BC and OM affect greenhouse gas emissions by modulating soil microbial communities, functional genes, and metabolomic profiles.

1. Introduction

In recent decades, climate change has attracted considerable attention worldwide, posing threats to the environment and human health (Zhu et al., 2019). Global climate change is primarily caused by greenhouse gas emissions, including those of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Elbasiouny et al., 2021; Franco-Luesma et al., 2020). Approximately 76 % of the greenhouse effect is attributed to CO_2 (Walkiewicz et al., 2020). Although CH_4 and N_2O are present at considerably lower concentrations in the atmosphere than CO_2, they have global warming potentials 28 and 298 times greater than that of CO_2, respectively (Yang et al., 2019). Soil is one of the largest sources of greenhouse gases, accounting for 35 %, 47 %, and 53 % of total annual emissions of CO_2, CH_4, and N_2O, respectively (Oertel et al., 2016). Therefore, efforts to reduce soil greenhouse gas emissions are crucial for mitigating global climate change (Liu et al., 2021; Walkiewicz et al., 2020).

The metabolism of soil carbon (C) and nitrogen (N) regulates

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greenhouse gas emissions (Qin et al., 2023). The transformation of soil C, particularly the decomposition of organic matter into CO_2, can affect soil organic C sequestration and contribute to CO_2 emissions (Huang et al., 2021). CH_4 is produced as a byproduct of the anaerobic degradation of organic matter by methanogens and is eventually oxidized by methanotrophs to CO_2 (Cheng et al., 2021). The abundances of methanogens and methanotrophs control the production and emission of CH_4 in the soil. In addition, soil nitrification and denitrification are essential for the biogeochemical cycling of N, resulting in the production of N_2O in the soil (Shi et al., 2022). The denitrification process reduces NO_3^- to N_2O and N_2, with N_2O being released as an intermediate product (Shi et al., 2022). It is estimated that 40 % to 85 % of global nitrous oxide (N_2O) emissions are caused by microbial denitrification in soil ecosystems (Perez-Garcia et al., 2017). Microbial metabolism regulates the cycling of C and N, which is closely related to the emissions of CO_2, CH_4, and N_2O (Ding et al., 2022; Poeplau et al., 2016).

The application of organic manure (OM) in agricultural production has a significant impact on greenhouse gas emissions (Ravindran et al., 2022). Guenet et al. (2021) found that OM significantly increases N_2O emissions by improving soil organic matter and facilitating soil denitrification. He et al. (2023) reported that OM increases CH_4 and CO_2 emissions, contributing to global warming. OM provides metabolic substrates and energy sources for methanogenic bacteria, which may exacerbate CH_4 emissions (He et al., 2023). Organic C pools increase with OM application, which, in turn, enhances soil respiration and ultimately increases CO_2 emissions (Alam et al., 2023). Due to the negative effects of OM on greenhouse gas emissions, it is imperative to find alternative soil amendments for C sequestration and soil fertilization.

Biochar (BC) produced by biomass pyrolysis under anaerobic conditions exhibits a well-developed porous structure and a large surface area (Ravindran et al., 2022). Owing to its recalcitrant nature and ability to sorb native organic matter, BC is a viable soil amendment for C sequestration in soils (Elzobair et al., 2016). Several studies have demonstrated that BC amendments can improve C sequestration capacity and mitigate emissions of greenhouse gases (Chung et al., 2021; Wang et al., 2024a; Wang et al., 2020). BC inhibits the mineralization of labile organic matter through sorption, thereby stabilizing soil C and reducing CO_2 emissions (Kravchenko et al., 2023). Moreover, BC amendments may decrease N_2O emissions by suppressing anaerobic denitrification (Ameloot et al., 2013). Additionally, Yang et al. (2019) reported that BC amendment increases the ratio of methanotrophic to methanogenic bacteria, resulting in reduced CH_4 emissions. Nevertheless, other studies have reported conflicting results. Zhang et al. (2023b) found that BC amendment increased substrates for methanogens, thereby increasing the emission of CH_4. Edwards et al. (2018) found that BC promoted nitrification-derived N_2O emissions in soils with high NH_4^+ concentrations. Various factors may contribute to the inconsistent outcomes of BC amendment, such as soil C and N availability, soil moisture and temperature, and BC type and application rate (Chung et al., 2021; Duan et al., 2019; Liu et al., 2021). BC has been extensively studied; yet, little consensus exists on how BC and various C and N cycling processes interact to affect CO_2, CH_4, and N_2O emissions and the mechanisms by which these emissions are impacted. Moreover, previous studies have primarily examined the mechanism underlying the effect of BC on greenhouse gas emissions from the perspective of the composition and function of soil microbial communities, without focusing on metabolomic profiles. Until now, no comprehensive comparisons have been conducted on the effects of BC and other amendments on soil microbial community composition, function, and metabolomic profiles, thereby affecting soil C-N metabolism and greenhouse gas emissions. Therefore, a comprehensive study linking greenhouse gas emissions with soil C and N cycling processes, as well as microbial community structure, function, and metabolomic profiles, is necessary.

In this study, high-throughput sequencing, metabolomics, and quantitative real-time PCR (RT-qPCR) were used to address the following aims: (1) assess the influence of BC and OM on soil microbial community structure, metabolomic profiles and functional genes (*mcrA*, *pmoA*, *nirK*, *nirS*, *nosZ*, *AOA*, and *AOB*); (2) compare the effects of BC and OM on soil C/N-cycling and greenhouse gas emissions (CO_2, CH_4, and N_2O); and (3) investigate how soil microbial community structure, metabolism, and function interact to affect soil C/N cycling processes, and, ultimately, the emissions of CO_2, CH_4, and N_2O. The results of our study provide insights into how BC and OM affect the soil C/N cycling processes that regulate greenhouse gas emissions.

2. Materials and methods

2.1. Soil, BC, and OM preparation

We collected clay loam soil (19.3 % sand, 36.9 % silt, and 43.8 % clay) from a traditional cropland in Hangzhou, Zhejiang Province, China. The soil sample was air-dried, homogenized, and sieved through a 2-mm mesh, and its basic properties were determined before further processing. BC was produced from rice straw and pyrolyzed at 600 °C for 5 h in an oxygen-limited muffle furnace. OM was prepared from composted, sterilized, and air-dried pig manure. In Section 1 of the Supplementary Material, the soil, BC, and OM are characterized, and Tables S1 and S2 summarize the main physicochemical characteristics of the soil, BC, and OM.

2.2. Experimental design

The present study was conducted to determine the effects of BC and OM on soil C-N metabolism and the emission of greenhouse gases, including CO_2, CH_4, and N_2O. This study used a randomized complete block design, with four treatments (control, BC, OM, and BC+OM) and three replicates. BC and OM were applied at a rate of 1 % by weight. Static chambers were constructed to collect gas samples. A stainless-steel bottom base and a polyvinyl chloride pipe chamber (diameter: 20 cm; height: 30 cm) comprised the static chamber. Above the chamber, a vent hole sealed with a three-way valve was installed to collect the gases. The soil samples were destructively collected after 60 days of incubation, a subsample was stored at −80 °C before DNA extraction, and the remainder of soil samples were air dried and screened through a 100-mesh sieve for chemical analysis.

2.3. Gas sampling and analysis

Throughout the soil incubation period, gas samples (30 mL) were collected using a syringe and placed in gas sampling bags at 1, 3, 7, 14, 30, and 60 days. Analyses of CO_2, CH_4, and N_2O concentrations were performed using a gas chromatograph (Agilent 6820A, Agilent, USA). The specific gas measurement methods are described in Section 2 of the Supplementary Material.

We calculated the flux (F) of greenhouse gases (CO_2, CH_4, and N_2O) using the following equation (Wang et al., 2020; Zhang et al., 2023b):

$$F = \frac{M \times h}{V_0} \times \frac{273.15}{273.15 + T} \times \frac{dc}{dt} \tag{1}$$

where F (mg $m^{-2}h^{-1}$ for CO_2 and CH_4; μg $m^{-2}h^{-1}$ for N_2O) represents the flux of a greenhouse gas; M (g mol^{-1}) represents the molar mass of CO_2 (44 g mol^{-1}), CH_4 (16 g mol^{-1}), or N_2O (44 g mol^{-1}); V_0 (22.4 L mol^{-1}) represents the molar volume of gas; h represents the height of the air layer in the static chamber; T represents the temperature of the incubation chamber (25 °C); 273.15 represents the absolute temperature under standard conditions; and $\frac{dc}{dt}$ represents the rate of change in the concentration of the greenhouse gas (mg $m^{-3}h^{-1}$).

Cumulative emissions (CE) of CO_2, CH_4, or N_2O were calculated using the following equation (Ding et al., 2022):

$$CE = \frac{\sum_{i=1}^{n}(F_i + F_{i+1})}{2} \times (t_{i+1} - t_i) \times 24 \tag{2}$$

where i represents the ith measurement, n represents the total number of measurements, F represents the flux of greenhouse gases, $\frac{(F_i+F_{i+1})}{2}$ represents the mean flux over two consecutive days of sampling, (t_{i+1}-t_i) represents the interval between two adjacent sampling days, and 24 represents the number of hours per day.

2.4. *Soil microbial community analysis*

Soil DNA was isolated using the HiPure Stool DNA Kit (Magen, China). The V3-V4 regions of the bacterial 16 S rRNA gene were amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806 R (5′-GGACTACHVGGGTATCTAAT −3′). Prior to sequencing, the PCR products were purified and quantified. The 16 S rRNA genes were analyzed using high-throughput sequencing. Amplicons were paired-end sequenced on an Illumina platform at GeneDenovo Biotechnology Co., Ltd. (Guangzhou, China). Principal coordinate analysis (PCoA) was used to compare similarities between treatments. Circos was used to graph the circular representations of species abundance. The ecological functional profiles of bacteria were predicted using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database. A detailed description of the method for analyzing soil microbial communities is presented in Section 3 of the Supplementary Material.

2.5. *Soil functional genes*

The abundances of seven genes encoding ammonia-oxidizing archaea (*AOA*), ammonia-oxidizing bacteria (*AOB*), denitrifying bacteria (*nirK*, *nirS*, and *nosZ*), methanogens (*mcrA*), and methanotrophs (*pmoA*) were analyzed using a RT-qPCR system (Bio-Rad iQ5, USA). The 20 μL reaction volume contained 1 μL DNA-diluted template, 0.2 μL of each primer, 10 μL of iTaq™ Universal SYBR Green Supermix (Bio-Rad, USA), and 8.6 μL sterile water. A summary of the primer sequences and thermal conditions used for RT-qPCR is shown in Supplementary Table S3.

2.6. *Soil metabolite analysis*

The soil sample (1 g) was transferred to a 5 mL Eppendorf tube, to which 1 mL of extract solution (acetonitrile: methanol = 1:1) was added, containing 10 μL of adonitol (0.5 mg mL^{-1}) as an internal standard, and vortexed for 30 s, sonicated in ice water for 10 min, and incubated for 1 h at −40 °C for protein precipitation. The supernatant was collected after centrifugation at 12000 rpm for 15 min at 4 °C and analyzed using ultra-high performance liquid chromatography Q-exactive mass spectrometry (UHPLC-QE-MS). The detailed determination of metabolites is presented in Section 4 of the Supplementary Material. Significant difference analysis of all metabolites was conducted using partial least squares discriminant analysis (PLS-DA), with a variable importance (VIP) value > 1 and a p value < 0.05. Metabolic enrichment and pathway analyses were used to summarize the differential metabolites and map them to their corresponding biochemical pathways.

2.7. *Statistical analyses*

The data are expressed as the mean ± standard deviation of three replicates. To evaluate differences between treatments, the least significant difference test was used at a level of $p < 0.05$. Data were analyzed using SPSS v24.0.

3. Results

3.1. *Effects of amendments on soil properties*

Soil properties were influenced by the soil amendment treatments (Table 1). After 60 days of incubation, the soil pH of the OM, BC, and BC+OM treatments increased by 0.25, 0.54, and 0.85 units, respectively, in comparison with that of CK, which had a soil pH just below 6. The OM-containing treatments, including OM alone and BC+OM, showed an increase in soil dissolved organic carbon (DOC) content of 40.45 % to 43.4 % compared to the CK, whereas the BC amendment led to a slight but non-significant increase. The concentrations of soil NH_4^+-N for treatments with BC and/or OM amendments were decreased by 28.9–42.8 % relative to the CK. The BC and OM amendments resulted in a significant ($p > 0.05$) increase in soil NO_3^--N contents (12.8–32.7 %).

Table 1
Soil pH, DOC, NH_4^+-N, and NO_3^--N contents after incubation with different treatments. Data represent the means ± standard deviations of three replications.

Treatment	pH	DOC (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
CK	5.98 ± 0.12b	1.66 ± 0.23b	31.8 ± 2.4 a	77.6 ± 3.4c
BC	6.52 ± 0.14 a	1.78 ± 0.17b	22.6 ± 1.1b	103.5 ± 5.6 a
OM	6.23 ± 0.06b	2.33 ± 0.26 a	18.2 ± 1.2c	88.2 ± 4.7b
BC-OM	6.83 ± 0.21 a	2.38 ± 0.19 a	19.5 ± 1.5 bc	87.5 ± 3.9b

Different letters in each column indicate significant differences between the treatments ($p < 0.05$).

3.2. *Effects of amendments on emission dynamics of CO_2, CH_4, and N_2O from soil*

The CO_2 emissions from the soil for all treatments initially increased and then decreased gradually, with the highest values observed on day 3 (Fig. 1a). BC and OM had opposite effects on soil CO_2 emissions, with BC resulting in lower emissions and OM resulting in higher emissions. After 60 days of incubation, the flux of CO_2 for the BC treatment was 566.2 mg m^{-2}h^{-1}, which was 20.5 % lower than that of the CK, whereas the flux for the OM treatment was 828.9 mg m^{-2}h^{-1}, which was 16.4 % higher than that of the CK. The cumulative CO_2 emissions for the BC treatment during 60 days of incubation were 913.3 g m^{-2}, a reduction of 19.9 % compared with CK (Fig. 1b). In contrast, cumulative CO_2 emissions for the OM and BC+OM treatments were 1255.7–1372.4 g m^{-2}, which were 10.1–20.3 % higher than that of the CK.

With increasing incubation time, all treatments consistently exhibited increasing and then decreasing trends in CH_4 emissions from the soil (Fig. 1c). Amendment with BC and/or OM negatively affected the CH_4 emissions. Following 60 days of incubation, CH_4 fluxes for the BC alone and OM alone treatments were 4.15–4.78 mg m^{-2}h^{-1}, which were 13.7–19.2 % higher than that of the CK. The combined application of BC and OM had a cumulative effect on soil CH_4 flux, as evidenced by the highest CH_4 flux (30.9 %) among all treatments. The cumulative emissions of CH_4 from soil during 60 days of incubation were 19.5–49.1 % higher after adding BC, OM, or their mixture (Fig. 1d).

A similar trend was observed for N_2O and CO_2 emissions. The BC treatment showed the lowest N_2O flux of 145μg m^{-2}h^{-1}, which was 24.4 % lower than that of the CK after 60 days (Fig. 1e). In contrast, the OM amendment significantly accelerated N_2O emissions, with an 18.2–32.7 % increase in flux values. However, this negative effect of OM can be mitigated by amendment with BC. The N_2O flux for the BC+OM treatment was 10.5 % lower after 60 days of incubation that that of the OM alone application. During 60 days of incubation, the cumulative emissions of N_2O from soil under different treatments ranged from 237.3 to 387.0 mg m^{-2} (Fig. 1f). Compared with the CK, the addition of BC reduced soil cumulative N_2O emissions by 23.3 %, whereas the addition of OM and BC+OM increased soil cumulative N_2O emissions by 13.6–25.1 %.

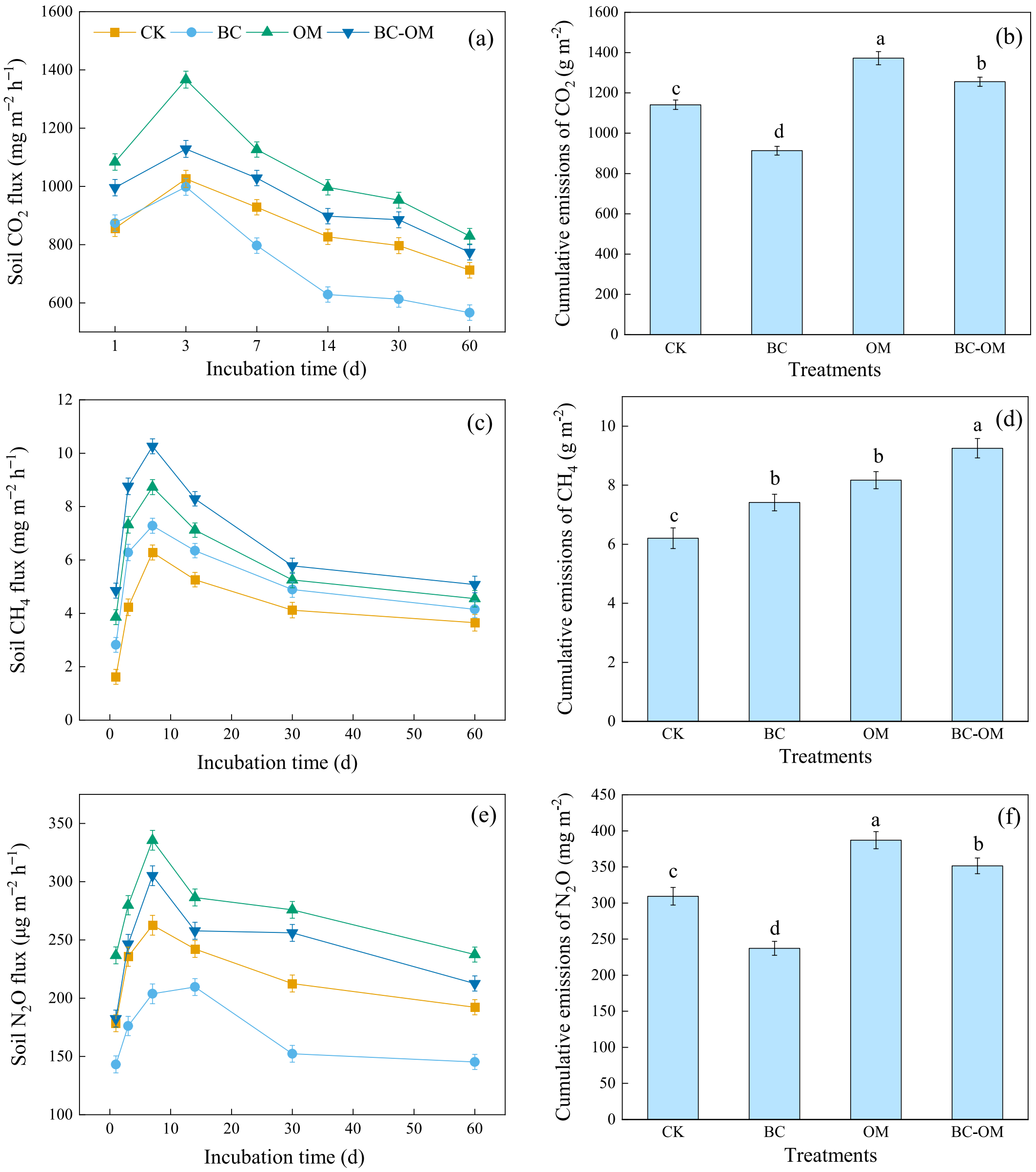

Fig. 1. The fluxes and cumulative emissions of CO_2 (a and b), CH_4 (c and d), and N_2O (e and f) from soils under different treatments. The error bars represent the standard deviation of the means (n = 3) and a probability of 0.05. Significant differences between treatments are indicated by different lowercase letters above each bar ($p < 0.05$).

3.3. *Shift of microbial community composition following amendments*

The α-diversity refers to the degree of microbial diversity as measured by the Shannon and Simpson indices, and the level of microbial richness as measured by the Chao1 and ACE indices (Table 2). The Shannon and Simpson indices exhibited opposite trends. The Simpson index decreased, whereas the Shannon index increased in the soils amended with BC and/or OM. As greater diversity is associated with higher Shannon index values and lower Simpson index values (Mao et al., 2018), this would indicate that both BC and OM amendments increased bacterial community diversity. The Chao1 and ACE values showed consistent variation trends and the microbial richness of the BC+OM-amended soils was the highest, followed by that of the OM-amended soil. As a result, the microbial diversity and richness were greater in the BC and/or OM amended soils than in the control soils.

Table 2

A comparison of microbial community diversity among different treatments. Data represent the means ± standard deviations of three replications.

Treatment	Shannon	Simpson	Chao1	ACE
CK	8.75 ± 0.32	0.993 ± 0.065	1526.8 ± 26.3	1675.3 ± 33.5
BC	9.23 ± 0.36	0.968 ± 0.073	1728.9 ± 29.6	1789.6 ± 28.9
OM	9.46 ± 0.43	0.889 ± 0.065	1839.6 ± 33.2	1869.5 ± 35.2
BC-OM	9.87 ± 0.52	0.853 ± 0.058	1922.5 ± 35.7	1945.3 ± 40.5

Significant changes in the soil microbial community structure were observed following amendment with BC and OM (Fig. 2). PCoA based on Bray–Curtis dissimilarity showed that BC and OM amendments had distinct effects on soil microbial communities (Fig. 2a). PCo1 contributed 48.03 % of the variation, which clearly distinguished treatments with and without OM, indicating that OM amendment significantly shaped the microbial community. Furthermore, PCo2 revealed a distinct separation between treatments with BC (BC and BC+OM) and without BC (CK and OM), indicating that the structure of the microbial communities was also affected by BC amendment.

Additionally, a Venn diagram was used to visualize the specificity and commonality of microorganisms among the treatments (Fig. 2b).

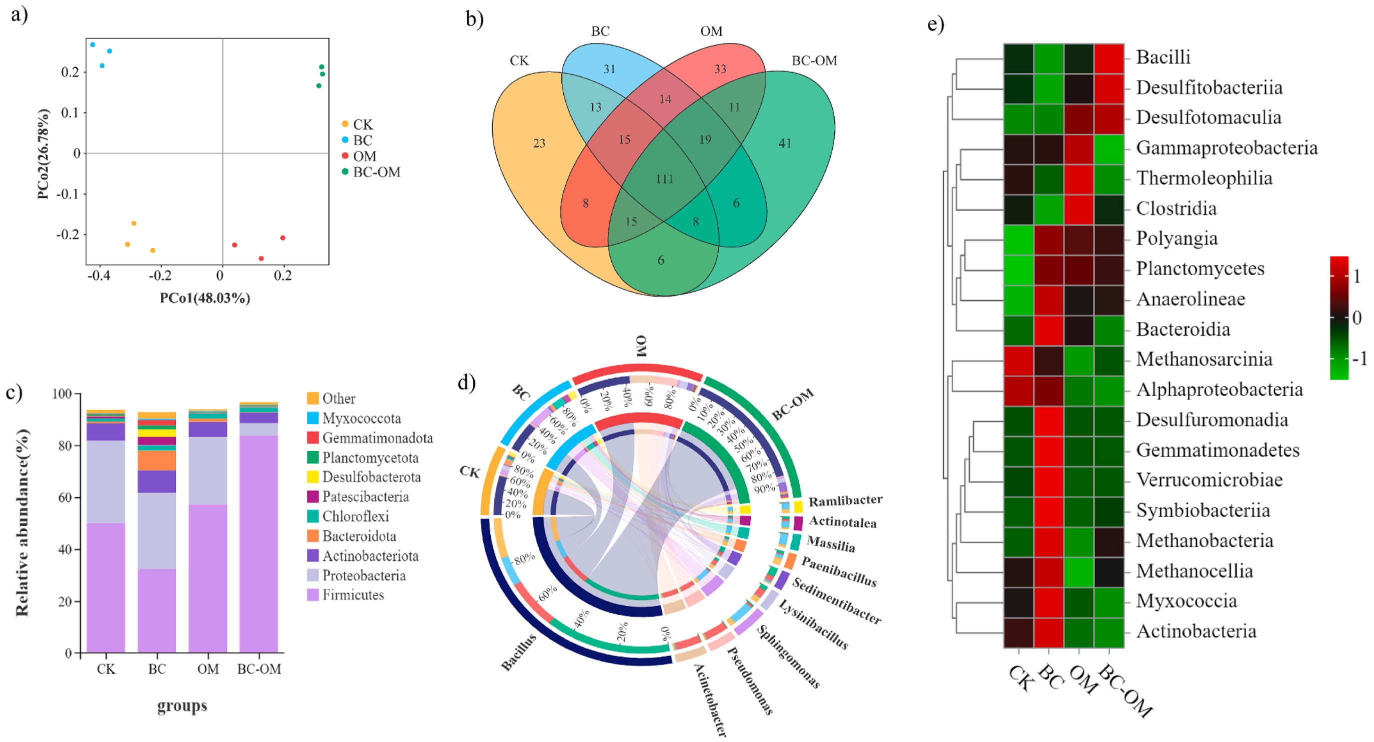

Fig. 2. The response of microbial communities to biochar (BC) and organic manure (OM) amendments. a) Principal component analysis; b) a Venn diagram showing shared and unique OTUs; c) Relative abundances (%) of the major bacterial phyla; d) Circos diagram illustrating the correlation between treatments and abundance of different bacterial groups at the genus level; e) a Hierarchically clustered heatmap representing the dominant classes across all treatments.

The four treatments shared 111 OTUs, which accounted for 31.4 % of the total number of OTUs (354). A higher abundance of distinctive OTUs was observed in soils with BC and/or OM amendments than those without, which is consistent with the microbial diversity results shown in Table 2.

A distribution histogram was generated to identify the dominant bacterial species at the phylum level (Fig. 2c). The most abundant phyla were *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Chloroflexi*, *Patescibacteria*, *Desulfobacterota*, *Planctomycetota*, *Gemmatimonadota*, and *Myxococcota*. A considerable decrease in *Firmicutes* relative abundance observed following BC addition (35.3 %); however, the relative abundance increased dramatically after BC+OM amendment, reaching 83.6 %. In addition, BC amendment significantly increased the relative abundances of *Actinobacteriota*, *Bacteroidota*, *Desulfobacterota*, and *Gemmatimonadota*. At lower taxonomic levels, such as genera, three groups of bacteria were significantly affected by the OM amendments: *Bacillus* (phylum *Firmicutes*), *Pseudomonas* (phylum *Proteobacteria*), and *Acinetobacter* (phylum *Proteobacteria*) (Fig. 2d). Furthermore, *Sphingomonas*, *Massilia*, and *Ramlibacter*, belonging to the phylum *Proteobacteria*, were significantly increased by the BC amendment (Fig. 2d).

A heatmap was constructed to visualize the changes in microbial communities associated with the emissions of CO_2, CH_4, and N_2O under the impact of BC and OM (Fig. 2e). The analysis of the 20 most abundant microbial classes revealed that most were strictly or facultatively anaerobic. As illustrated in Fig. 2e, the BC amendments significantly increased the abundance of several microbial classes, including *Bacteroidia*, *Anaerolineae*, *Polyangia*, *Desulfuromonadia*, *Gemmatimonadetes*, *Verrucomicrobiae*, *Symbiobacteriia*, *Methanobacteria*, *Methanocellia*, *Myxococcia*, and *Acinetobacter*. Among the microorganisms stimulated by BC amendment, *Methanobacteria* and *Methanocellia* are typical methanogens implicated in methanogenesis. *Gammaproteobacteria*, *Thermoleophilia*, and *Clostridia* grouped together and were significantly more abundant following OM amendment. Furthermore, *Bacilli*, *Desulfitobacteriia*, *Desulfotomaculia*, the major classes of the *Firmicutes* phylum, clustered together and were significantly more abundant in the BC+OM combination treatment (Fig. 2e).

FAPROTAX was used to annotate and decipher the critical ecological functions of microbial communities involved in the emissions of CO_2, CH_4, and N_2O. As shown in Fig. S1, methanogenesis-related functions, such as methanogenesis, methanogenesis by the reduction of methyl compounds with H_2, methanogenesis by CO_2 reduction with H_2, and hydrogenotrophic methanogenesis, were enhanced by BC and OM amendment, whereas methanotrophy-related functions were inhibited by BC and OM amendment, resulting in higher CH_4 emissions for both BC- and OM-amended treatments. Additionally, ammonia oxidation-related functions (aerobic ammonia oxidation and nitrification) and denitrification-related functions (denitrification, nitrate denitrification, nitrite denitrification, nitrate respiration, nitrite respiration, and nitrogen respiration) related to N_2O emissions were enhanced by OM amendment, thereby contributing to an increase in N_2O emissions for OM-amended treatments.

3.4. Effects of amendments on microbial functional gene abundances

RT-qPCR detected seven functional microbial genes related to CH_4 and N_2O emissions, including genes involved in methanogenesis (*mcrA*), methanotrophy (*pmoA*), ammonia oxidation (*amoA* and *amoB*), and denitrification (*nirK*, *nirS*, and *nosZ*). The abundances of these functional genes varied significantly between treatments (Fig. 3). The addition of BC, OM, or their combination significantly affected the abundance of functional genes associated with the production and consumption of CH_4 (Fig. 3a). Soil samples from the BC, OM, and BC+OM treatments had significantly higher abundances of *mcrA*, a functional methanogenesis gene, than soil samples from the CK. In contrast to the functional genes associated with CH_4 production, the methanotrophic gene (*pmoA*) associated with CH_4 consumption was lower in the BC, OM, and BC+OM treatments than in the CK. In particular, OM-amended soils exhibited the highest *mcrA* gene abundance and lowest *pmoA* gene abundance.

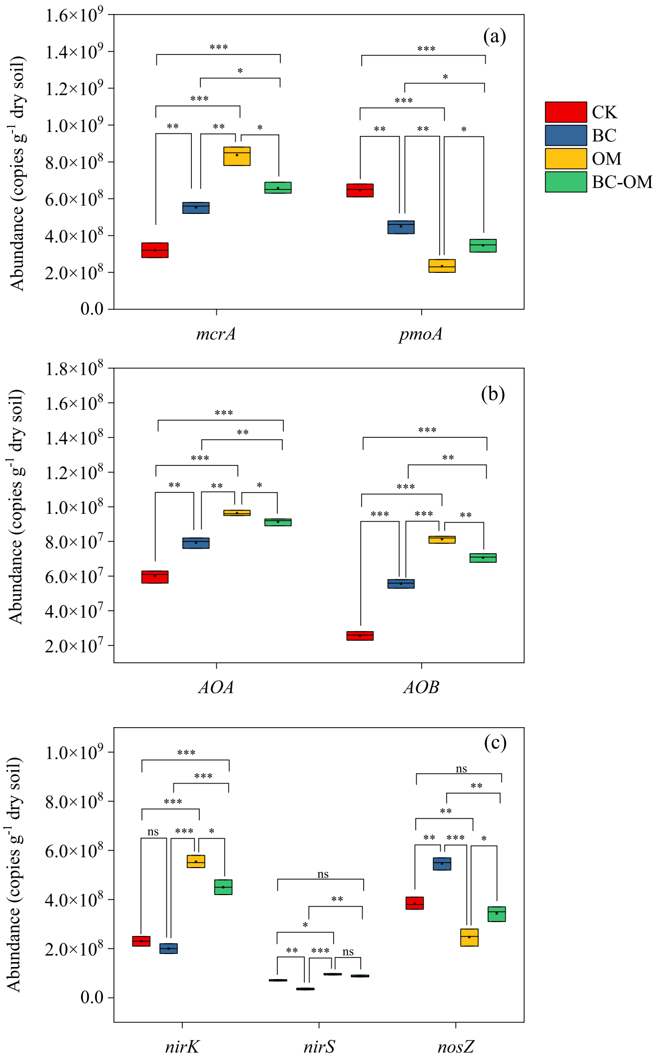

Fig. 3. The abundance of microbial functional genes involved in CH_4 and N_2O emissions from soil, including *mcrA* and *pmoA* (a), *AOA* and *AOB* (b), and *nirS*, *nirK*, and *nosZ* (c). The significance of differences was determined using the paired *t*-test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and ns: no significance.

In addition, the gene abundances of ammonia oxidation genes (*amoA* and *amoB*) and denitrification genes (*nirK*, *nirS*, and *nosZ*), which are related to N_2O emissions, also differed significantly among treatments (Fig. 3b and c). The *amoA* and *amoB* abundances increased following soil treatment with BC, OM, or their combination (Fig. 3b), resulting in a decrease in the NH_4^+ content and an increase in the NO_3^- content (Table 1). In terms of denitrification function genes, *nirK* and *nosZ* were detected at considerably higher levels (1.8×10^8 to 7.1×10^8 copies g^{-1} dry soil) than ammonia oxidation genes *amoA* and *amoB* (2.3×10^7 to 9.8×10^7 copies g^{-1} dry soil). The BC amendment led to a 4.8–21.7 % and 47.3–52.9 % reduction in the abundances of the *nirK* and *nirS* genes, respectively, but caused a significant 39.1–44.7 % increase in the abundance of the *nosZ* gene (Fig. 3b). In contrast, the OM amendment resulted in a 132.0–152.4 % and 33.8–36.8 % increase in the abundances of the *nirK* and *nirS* genes, respectively, but led to a 26.3–48.8 % decrease in the abundance of the *nosZ* gene. The combined BC+OM amendment did not significantly affect the abundances of *nirS* and *nosZ*, but stimulated the abundance of *nirK*.

3.5. Effects of amendments on soil metabolites

This study investigated the metabolites and metabolic pathways activated by BC and/or OM amendments using non-targeted metabolomics. A total of 471 metabolites were detected in all soil samples. Classification of the differential metabolites and their percentages are presented in Table S4. Lipids and lipid-like molecules comprised 29.3 % of all metabolites, followed by organic acids and derivatives (20.4 %), organic oxygen compounds (17.4 %), organoheterocyclic compounds (10.4 %), benzenoids (8.7 %), phenylpropanoids and polyketides (5.9 %), organic nitrogen compounds (2.5 %), nucleosides, nucleotides, and analogs (1.3 %), and others. The detected soil metabolites were assumed to be derived from microbial metabolites and soil organic matter decomposition (Song et al., 2020).

Based on the PLS-DA score plot, the metabolites were significantly different among the BC, OM, and BC+OM treatment groups (Fig. 4a). Furthermore, Fig. 4b shows the significant upregulation and downregulation of metabolites within the differentially treated groups, thereby revealing the specific metabolomic changes caused by the addition of BC and/or OM. For example, in the CK vs. BC comparison, 88 metabolites were identified as significantly different, of which 24 were upregulated and 64 were downregulated. However, the CK vs. OM comparison identified 227 significantly different metabolites, 141 upregulated and 86 downregulated. The CK vs. BC+OM comparison identified 255 significantly different metabolites, including 197 upregulated and 58 downregulated metabolites. Furthermore, 198 significantly different metabolites were identified in the BC vs. OM comparison, with 165 and 33 upregulated and downregulated metabolites (Fig. 4b).

A VIP value > 1.0 in the PLS-DA model and significance level of $p < 0.05$ in the *t*-test were considered as indicative of differentially expressed metabolites between the treatments (Phulpoto et al., 2024). Fig. 5a-f presents the top 20 distinguishing metabolites, each of which is highlighted by VIP>1 and $p < 0.05$ across all treatment groups. By utilizing both the VIP bubble plots and the abundance heatmap, the patterns of differential metabolites and their expression levels were clearly depicted. In the CK vs. BC comparison, only three of the top 20 distinguishing metabolites (dibutyl phthalate, dihydro-4-mercapto-3 (2H)-furanone, and 8-isoprostaglandin E2) were upregulated, whereas the rest were downregulated (Fig. 5a). Table S5 shows that the downregulated metabolites were predominantly classified as organo-oxygen compounds and fatty acyls, whereas the upregulated metabolites were classified as benzene and dihydrofurans. In addition, the CK vs. OM and CK vs. BC+OM comparisons revealed that nearly 50 % of metabolites were upregulated and 50 % were downregulated (Fig. 5b-c). The upregulated metabolites were primarily classified as prenol lipids, organic sulfonic acids, and fatty acids, whereas the downregulated metabolites were predominantly organo-oxygen compounds, dihydrofurans, and non-metal organides (Table S6-7).

Moreover, the two metabolites, trehalose and sucrose, contributed the most to the differences in the CK vs. BC, CK vs. OM, and CK vs. BC+OM comparisons (Fig. 5a-c). Trehalose and sucrose are both carbohydrate compounds that belong to the class of organo-oxygen compounds. Owing to the significant increase in the metabolism of trehalose and sucrose in the BC- and OM-amended treatments, these two metabolites were significantly reduced in the BC, OM, and BC+OM treatments. 3-Methyl-1-butylamine was the most significant contributor to the difference between the BC vs. OM and BC vs. BC+OM comparisons, and dibutyl phthalate contributed the most to the difference between the OM and BC+OM amendments (Fig. 5d-f).

An analysis of metabolic pathways was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The KEGG analysis of metabolic pathways showed that the significant metabolic differences between the various treatments were related to carbohydrate metabolism, amino acid metabolism, xenobiotic degradation and metabolism, and secondary metabolite synthesis (Fig. S2). Furthermore, we investigated the differences in the metabolites of the main metabolic pathways following BC and/or OM amendment. The BC amendment significantly altered the metabolites of dioxin degradation, aromatic

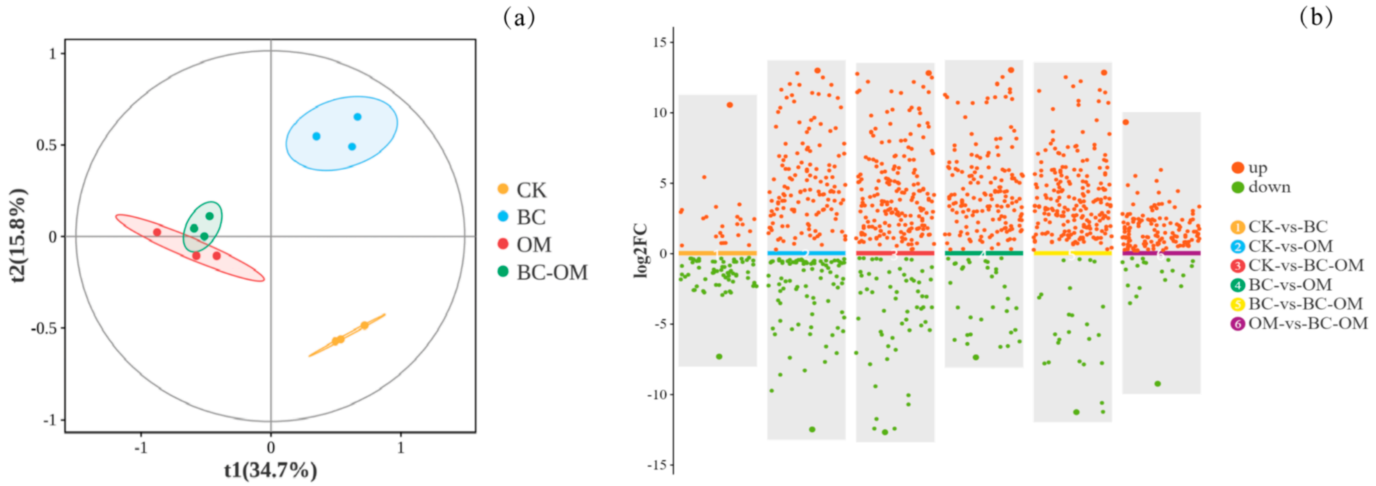

Fig. 4. (a) The partial least squares discriminant analysis (PLS-DA) score plot displays a clear separation among samples from different treatments; (b) The differential scatter plot illustrates the differentially expressed metabolites between groups of treatments based on pair-wise comparisons. Red dots represent upregulated metabolites and blue dots represent downregulated metabolites.

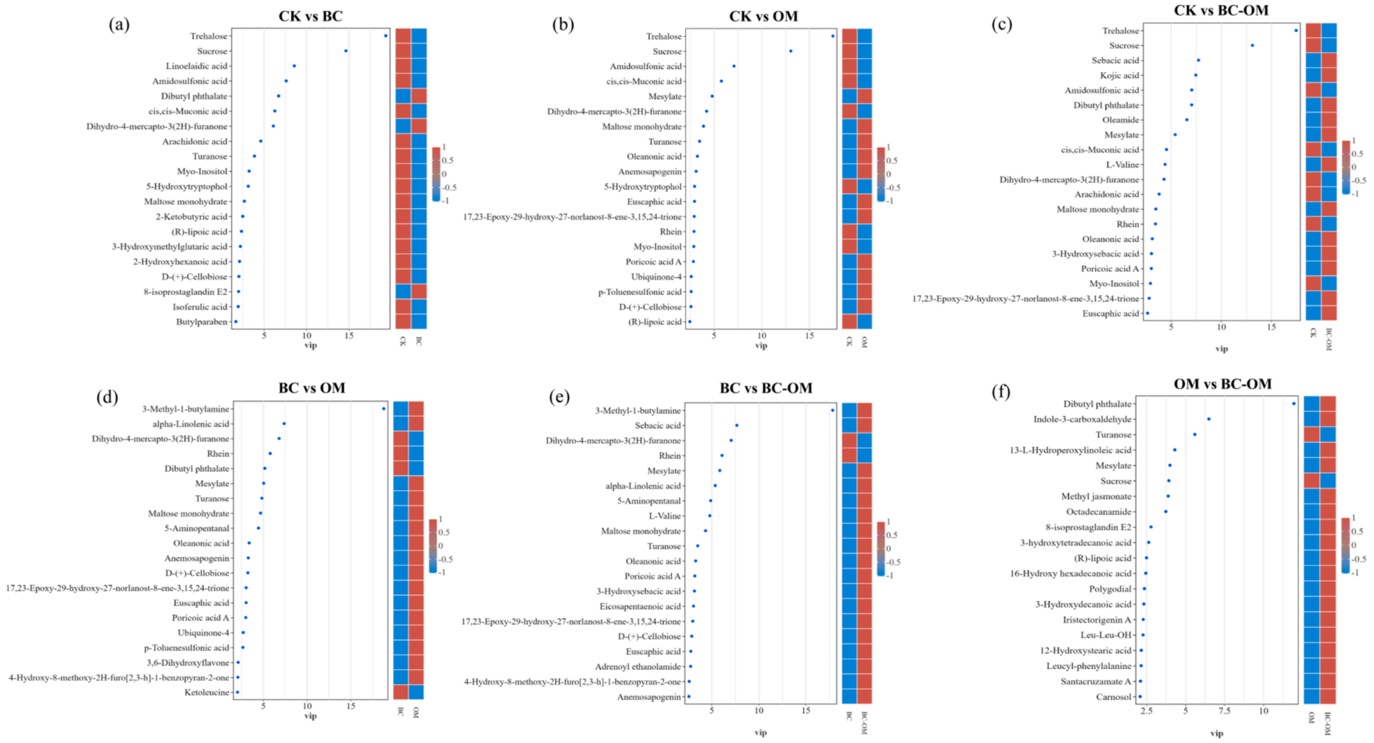

Fig. 5. The bubble plots display the discriminating metabolites with a VIP value > 1.0 and significance level of $p < 0.05$; CK vs. BC (a), CK vs. OM (b), CK vs. BC-OM (c), BC vs. OM (d), BC vs. BC-OM (e), and OM vs. BC-OM (f). VIP indicates how much each metabolite contributed to the difference between the two groups.

compound degradation, starch and sucrose metabolism, galactose metabolism, and C5-branched dibasic acid metabolism, whereas the OM amendment significantly altered carbohydrate digestion and absorption, arginine and proline metabolism, and chloroalkane and chloroalkene degradation (Fig. S3). The top five pathways associated with the differentially expressed metabolites between the BC and OM treatments were valine, leucine, and isoleucine biosynthesis; aminobenzoate degradation; tropane, piperidine, and pyridine alkaloid biosynthesis; 2-oxocarboxylic acid metabolism; and sulfur metabolism (Fig. S3).

3.6. *Correlation of greenhouse gas emissions and soil microbial communities, functional genes, and metabolomics*

Using a Pearson correlation heatmap, we explored the correlation between gas emissions and soil microbial communities, including *Bacillus*, *Methanobacterium*, *Methanocella*, *Caldicoprobacter*, *Methylocystis*, *Anaerolinea*, *Gemmatimonas* and *Acinetobacter*, and their associated functional genes (*mcrA*, *pmoA*, *amoA*, *amoB*, *nirK*, *nirS*, and *nosZ*) (Fig. 6a). It has been shown that CO_2 emissions were strongly correlated with the *Bacillus* genus, with a Pearson correlation coefficient of 0.81. CH_4 emissions were significantly positively correlated with the methanogens *Methanobacterium*, *Methanocella*, and *Caldicoprobacter*, with Pearson correlation coefficients of 0.78, 0.89, and 0.96, respectively;

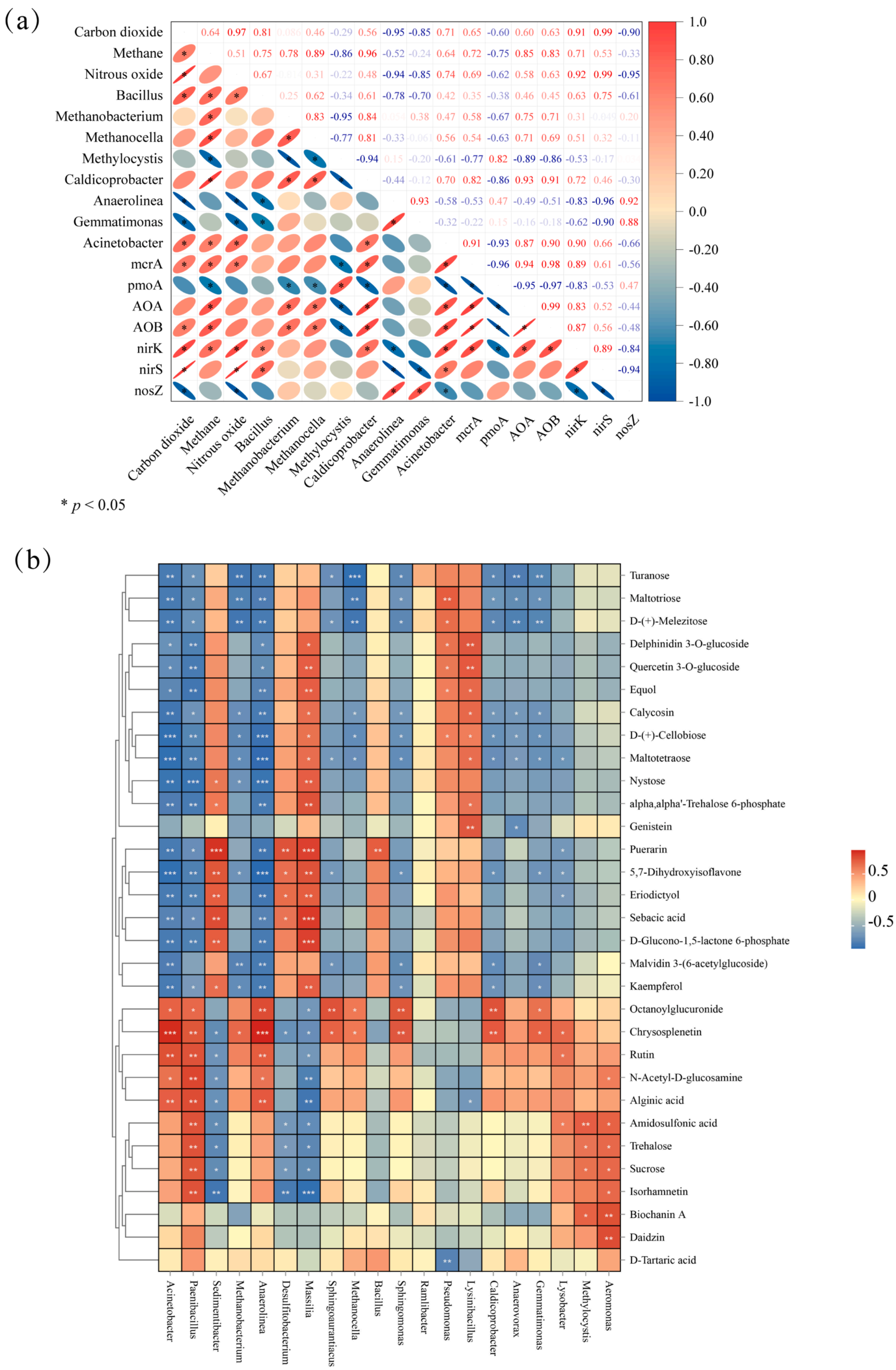

Fig. 6. (a) Pearson correlation heatmap between greenhouse gas emissions and abundance of microorganisms and functional genes; (b) Correlation heatmap illustrating the relationship between metabolites and bacterial species; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

however, the Pearson correlation coefficient for the methanotroph *Methylocystis* was negative (−0.86). Accordingly, CH_4 emissions were significantly positively correlated to the functional gene for methanogenesis (*mcrA*) but negatively correlated to the functional gene for methanotrophy (*pmoA*). Moreover, N_2O emissions were positively correlated with the denitrifying bacteria *Acinetobacter* and denitrification genes (*nirK* and *nirS*), but negatively correlated with the denitrifying bacteria *Anaerolinea* and *Gemmatimonas* and the denitrification gene (*nosZ*).

Furthermore, we examined the relationship between the significantly impacted bacterial taxa and specific metabolites. In this study, different metabolites were positively or negatively associated with different types of bacteria (Fig. 6b). Puerarin showed a significant positive correlation with the *Bacillus* genus ($p < 0.01$). Notably, the metabolites turanose, maltotriose, D-(+)-melezitose, malvidin 3-(6-acetylglucoside), calycosin, D-(+)-cellobiose, and maltotetraose exhibited strong negative associations with bacteria associated with CH_4 and N_2O emissions, including methanogens (*Methanobacterium*, *Methanocella*, and *Caldicoprobacter*) and denitrifying bacteria (*Anaerolinea*, *Acinetobacter* and *Gemmatimonas*). In contrast, chrysosplenetin exhibited strong positive associations with methanogens (*Methanobacterium*, *Methanocella*, and *Caldicoprobacter*) and denitrifying bacteria (*Anaerolinea*, *Acinetobacter* and *Gemmatimonas*). Trehalose and sucrose were positively associated with the common methanotroph *Methylocystis*.

4. Discussion

4.1. Effects of amendments on soil physicochemical characteristics

Application of BC and OM can alter soil properties. OM and BC are naturally alkaline because of the presence of calcium, magnesium, and potassium, which serve as buffers (Zhang et al., 2023a). Therefore, they can increase pH of acidic soils by neutralizing acidity (Zhang et al., 2017). Soil DOC provides a carbon source for microorganisms and plays a vital role in the global carbon cycle (Liu et al., 2023). The substantial increase in DOC in the BC- and/or OM-amended soils can be attributed to the direct addition of carbon from OM and the indirect addition of carbon from the decomposition of organic matter stimulated by soil microbes (Iqbal et al., 2023). Additionally, BC and/or OM amendments resulted in a 28.9–42.8 % reduction in soil NH_4^+ content and a substantial increase (12.8–32.7 %) in soil NO_3^- content (Table 1), which indicates an enhanced nitrification process. Sánchez-García et al. (2015) also observed a reduction in NH_4^+ content and an increase in NO_3^- content with BC amendment, which was attributed to a better nitrifying bacterial environment.

4.2. Effects of amendments on soil microbial communities and subsequent CO_2 emission

CO_2 emissions from soil were found to decrease in the BC treatment but increased in OM-amended treatments, including OM alone and BC+OM (Fig. 1a and b). Similarly, Zhang et al. (2023b) reported a significant decrease in CO_2 emissions following BC amendment. Soil CO_2 is produced and emitted via soil respiration, which is largely determined by soil microbial metabolism (Ling et al., 2022). Consequently, soil CO_2 emissions are influenced by factors that affect soil microbial metabolic activity. Because of its stability, BC rarely decomposes in soil, which may result in decreased microbial metabolic activity (Duan et al., 2019). Conversely, adding OM to the soil can increase DOC, provide nutrients to microorganisms, and enhance their metabolic activity (You et al., 2022).

Firmicutes represented the most dominant phylum and accounted for 32.3–83.6 % of the bacterial communities (Fig. 2c). *Firmicutes* have previously been shown to secrete extracellular enzymes to decompose organic matter, including carbohydrates and lignocellulose (Lu et al., 2023; Mao et al., 2019; Xu et al., 2021). *Bacillus*, *Lysinibacillus*, *Sedimentibacter*, and *Paenibacillus* were the dominant genera in phylum *Firmicutes* (Fig. 2d). Among these genera, *Bacillus* was the most abundant, and its relative abundance decreased under the BC amendment, but increased under the OM-amended treatments (Fig. 2d). This trend corresponds to the changes in CO_2 emissions under the BC and OM amendments, which were reduced under the BC amendment but increased under the OM amendment (Fig. 1a and b). Accordingly, we hypothesize that the decline in CO_2 emissions with the BC amendment may be due to a decrease in *Bacillus* abundance, whereas the acceleration of CO_2 emissions with the OM amendment may be due to an increase in *Bacillus* abundance. This hypothesis is supported by the strong positive correlation between CO_2 emissions and the *Bacillus* genus (Fig. 6a). Several other studies have reported the contribution of *Bacillus* spp. to CO_2 emissions. For example, Ding et al. (2023) found that *Bacillus* can influence soil organic carbon transformation and increase the release of CO_2. Wang et al. (2024b) demonstrated that *Bacillus* can metabolize glucose and xylan by secreting glycoside hydrolases and esterases, thereby affecting the amount of CO_2 released.

4.3. Effects of amendments on soil methanogenic bacterial communities and CH_4 emission

The amount of CH_4 emitted from the soil is determined by the balance between CH_4 production by methanogens and CH_4 oxidation by methanotrophs (Zhang et al., 2023b). The addition of BC and/or OM to soils can alter soil properties (Table 1), resulting in a shift in the balance between the production and oxidation of CH_4. The addition of BC and/or OM to soils resulted in enhanced CH_4 emissions (Fig. 1c and d). Several previous studies have reported similar results, in which a single amendment with BC or organic fertilizer increased soil emissions of CH_4 (Yang et al., 2019; You et al., 2022). However, other studies have reported a significant reduction in soil CH_4 emissions following BC amendment (Mao et al., 2019; Yin et al., 2021). The contradictory results between studies may be attributed to the different types of soil and amendments, which lead to varying effects on methanogen and methanotroph abundance and activity.

BC, OM, and their combination increased soil pH values to a range between 6.52 and 6.83 (Table 1), which was favorable to methanogen activity (Zhang et al., 2023b), resulting in an increase in CH_4 production. Several types of methanogens were identified in this study, including *Methanobacteria* and *Methanocellia*, which are implicated in methanogenesis and are stimulated by BC amendment (Fig. 2e). This may be the reason for the higher CH_4 emissions from the BC treatment compared with those of CK. Moreover, *Caldicoprobacter*, a methanogen found under anaerobic conditions that belongs to the phylum *Firmicutes*, which was promoted by BC and/or OM amendment (Mulat et al., 2018). These results are consistent with our findings that BC and OM improved the abundance of *mcrA* (Fig. 3). In contrast, *Methylocystis*, a common methanotroph, was identified in this study, and its abundance was reduced by BC and OM amendments, in agreement with the observation that BC- and OM-amended soils had a lower abundance of *pmoA* than the control soil (Fig. 3). By combining the correlation results between CH_4 emissions and methanogens and methanotrophs displayed in Fig. 6a, the increase in methanogens and the decrease in methanotrophs appear to be the primary reasons for the increase in CH_4 emissions under BC and/or OM amendments.

Desulfitobacteriia and *Desulfotomaculia* are typical sulfate-reducing bacteria commonly associated with methanogenic archaea (Misson et al., 2021). Sulfate-reducing bacteria can stimulate the conversion of propionate into acetate, providing an alternative metabolic pathway for CH_4 production (Zan and Hao, 2020). Based on the results of this study, *Desulfitobacteria* and *Desulfotomaculia* were promoted by OM-amended treatments (OM and BC+OM), which could be partially responsible for the higher CH_4 emissions from OM-amended treatments compared to those without OM (CK and BC).

4.4. Effects of amendments on soil nitrifying and denitrifying bacterial communities and N_2O emission

N_2O production in the soil is primarily governed by microbial-mediated nitrification and denitrification (Ottani et al., 2023). Amendment of soil with BC and/or OM can alter the composition of the microbial community and the abundance of genes associated with nitrification and denitrification, thereby affecting the emission of N_2O (Shi et al., 2022). Based on our findings, BC amendment suppressed cumulative N_2O emissions, whereas OM amendment increased cumulative soil N_2O emissions compared to CK (Fig. 1e and f).

The relative abundances of functional genes involved in ammonia oxidation (*amoA* and *amoB*) and denitrification (*nirK*, *nirS*, and *nosZ*) were examined (Fig. 3). Nitrification involves the conversion of NH_4^+ to NO_3^-, which is controlled by the ammonia oxidation genes *amoA* and *amoB* (Liu et al., 2023). The *amoA* and *amoB* gene abundances were higher in the BC-and/or OM-amended treatments, suggesting that both BC- and OM-amended treatments stimulated soil nitrification, as indicated by the lower NH_4^+ and higher NO_3^- contents (Table 1). The nitrite reductase genes *nirS* and *nirK* facilitate the conversion of NO_2^- to NO during denitrification (Liu et al., 2023). In addition, the *nosZ* gene regulates the production of N_2O reductase, an enzyme critical for converting N_2O to N_2 (Liu et al., 2023). This can explain why N_2O emissions were significantly positively correlated with the *nirK* and *nirS* genes but negatively correlated with *nosZ* (Fig. 6a). BC and OM treatments had distinct effects on soil denitrification genes (*nirK*, *nirS*, and *nosZ*) (Fig. 3c). Specifically, BC reduced the levels of *nirS* and *nirK* but significantly increased the levels of *nosZ*, whereas OM treatment induced an elevation in *nirS* and *nirK* levels but decreased the levels of *nosZ*. Therefore, the mechanism by which OM accelerates soil N_2O emissions is assumed to be the higher abundances of *nirS* and *nirK* genes, along with decreased abundance of *nosZ*, which stimulates nitrite reduction but inhibits N_2O conversion. Conversely, BC minimized soil N_2O emissions, possibly because of the increased abundance of the *nosZ* gene, which causes complete denitrification reactions, resulting in the reduction of N_2O to N_2.

OM amendment significantly promoted the growth of *Gammaproteobacteria*. In this study, *Acinetobacter* was the major genus in the *Gammaproteobacteria* class. As a denitrifying bacterium, *Acinetobacter* may be responsible for the highest N_2O emissions observed in the OM treatment (Fig. 1f). Ye et al. (2022) demonstrated that *Acinetobacter* was capable of denitrification and emitted N_2O into the atmosphere. Furthermore, Read-Daily et al. (2022) reported that denitrifying bacteria contribute to the high generation and emission of N_2O.

Gemmatimonadetes, another class of denitrifying bacteria, is related to *nosZ* (Ding et al., 2022), which reduces N_2O to N_2, thereby resulting in a reduction in N_2O emissions. Compared to the other treatments, the BC treatment resulted in a higher abundance of *Gemmatimonadetes*, which was in accordance with the high level of abundance of the *nosZ* gene, which contributed to the reduction of N_2O emissions in the BC treatment. In contrast, OM amendment increased the abundances of ammonia oxidation genes (*amoA* and *amoB*) and denitrification genes (*nirK* and *nirS*), both of which contribute to nitrification and denitrification, and ultimately to the production of N_2O.

4.5. Relationship of greenhouse gases with microbial communities and metabolites

Microbial communities are crucial in shaping the distribution of metabolites in the soil environment (Phulpoto et al., 2024). Carbohydrate metabolism, amino acid metabolism, xenobiotic degradation and metabolism, and secondary metabolite synthesis were the main differential metabolic pathways between treatments, providing energy and carbon sources for microorganism growth and metabolism (Dai et al., 2023). Metabolites and microbes may be negatively correlated due to metabolic consumption, whereas microbes and metabolites may be positively correlated because of the metabolites secreted by microbes (Ding et al., 2022).

Carbohydrate degradation can provide nutrients and energy to bacteria involved in the production of CO_2, CH_4, and N_2O (Phulpoto et al., 2024). Among the metabolites negatively associated with methanogens and denitrifying bacteria, maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose were carbohydrate compounds (Fig. 6b). These results suggest that methanogens and denitrifying bacteria may grow by consuming the carbohydrates maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose. An increased abundance of methanogens may result in a higher conversion of carbohydrate compounds to acetate and propionate, which can be used by methanogens to generate more CH_4 (Ding et al., 2022). Carbohydrates [e.g., maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose] may also be metabolized by denitrifying bacteria in the soil. Ding et al. (2022) observed that carbohydrates promote the growth of denitrifying bacteria, which in turn cause denitrification and the emission of N_2O.

Chrysosplenetin, a flavonoid was positively associated with methanogens and denitrifying bacteria (Fig. 6b). This suggests that methanogens and denitrifying bacteria produce chrysosplenetin metabolites through the consumption of carbohydrates. Song et al. (2020) also observed a significant positive correlation between flavonoids and *mcrA* gene abundance and a negative correlation between flavonoids and *pmoA* gene abundance, which resulted in increased CH_4 emissions. *Methylocystis*, a methanotroph, is positively associated with trehalose and sucrose, the major differential metabolites involved in starch and sucrose metabolism. The presence of starch and sucrose could provide abundant substrates for *Methylocystis* (Song et al., 2020), however, the contents of these compounds were lower in the BC- and/or OM-amended soils than in the CK (Fig. 5), which contributed to the low abundance of *Methylocystis*. The decrease in *Methylocystis* caused a decrease in CH_4 oxidation and an increase in CH_4 emissions. Furthermore, Song et al. (2020) found that secondary metabolites such as flavonoids and isoflavonoids are related to the *nosZ* gene, which causes the reduction of N_2O to N_2, thereby reducing N_2O emissions.

Additionally, puerarin content was positively correlated with the *Bacillus* genus. As reported by Wan et al. (2022), puerarin stimulates both ethanol dehydrogenase and acetaldehyde dehydrogenase activities. Ethanol dehydrogenase is responsible for dehydrogenating ethanol and oxidizing it to acetaldehyde, which is then converted into acetic acid or CO_2 by acetaldehyde dehydrogenase. Thus, it is hypothesized that *Bacillus* produce puerarin, which in turn promotes ethanol dehydrogenase and acetaldehyde dehydrogenase activities and contributes to CO_2 emissions.

4.6. The mechanisms by which BC and/or OM affect greenhouse gas emissions

A possible explanation for the variation in greenhouse gas emissions under BC and/or OM amendment is proposed based on the above results (Fig. 7). First, BC amendment resulted in increased levels of methanogens (*Methanobacterium* and *Methanocella*) and denitrifying bacteria (*Anaerolinea* and *Gemmatimonas*), and decreased levels of *Bacillus* and a methanotroph (*Methylocystis*) (Fig. 2). Simultaneously, OM amendment increased the levels of *Bacillus* and the methanogen *Caldicoprobacter* and denitrifying bacteria *Acinetobacter*, while decreasing the level of the methanotroph *Methylocystis*. Secondly, a shift in the composition of the microbial community affected the abundance of genes related to CH_4 and N_2O emissions, including methanogenesis (*mcrA*), methanotrophy (*pmoA*), ammonia oxidation (*amoA* and *amoB*), and denitrification (*nirK*, *nirS*, and *nosZ*). Both BC and OM amendments enhanced the abundance of *mcrA*, *amoA* and *amoB*, and decreased the abundance of *pmoA* (Fig. 3). Notably, BC decreased the abundance of *nirK* and *nirS* while increasing the abundance of *nosZ*, which caused complete denitrification reactions and resulted in the reduction of N_2O to N_2, whereas OM increased the abundance of *nirK* and *nirS* while reducing the abundance of *nosZ*, which

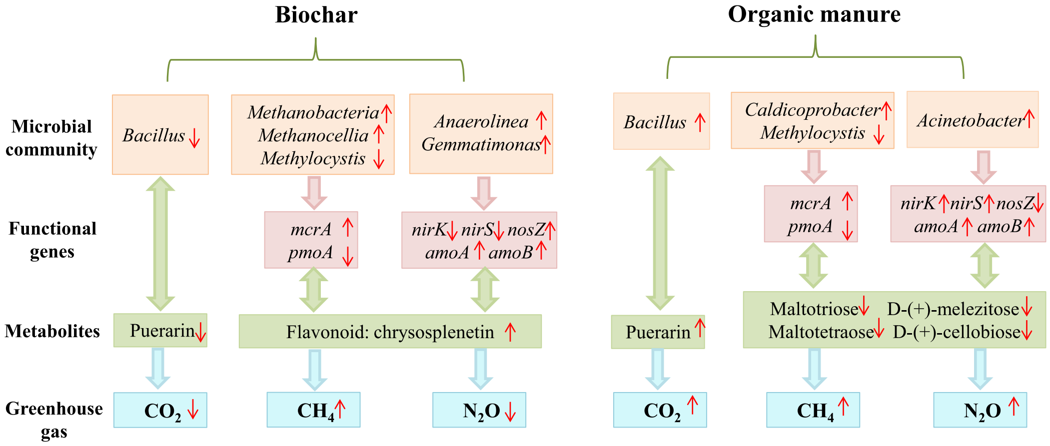

Fig. 7. A possible mechanism for greenhouse gas emission regulation by biochar (BC) and organic manure (OM) through affecting microbial communities, functional genes, and metabolites.

stimulated nitrite reduction but inhibited N_2O conversion. Finally, the metabolites and metabolic pathways affected by BC and/or OM amendments differed. Puerarin produced by *Bacillus* metabolism could stimulate the transformation of soil organic carbon and increase CO_2 release, for which CO_2 emission was reduced under BC amendment but increased under OM amendment. Furthermore, it is hypothesized that *Methanobacterium*, *Methanocella*, *Anaerolinea*, and *Gemmatimonas* may secrete chrysosplenetin, which could result in CH_4 generation and N_2O reduction to N_2; thus, BC amendment accelerated CH_4 emission while inhibiting N_2O emission from soil. Simultaneously, the addition of OM increased the metabolism of soil carbohydrates [e.g., maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose] by the methanogen *Caldicoprobacter* and the denitrifying bacteria *Acinetobacter*, which in turn enhanced the emission of CH_4 and N_2O.

In conclusion, puerarin produced by *Bacillus* metabolism may contribute to a reduction in CO_2 emissions by BC amendment and an increase in CO_2 emissions by OM amendment. The methanogen *Caldicoprobacter* and denitrifying bacteria *Acinetobacter* can metabolize carbohydrates [maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose] from soil amended with OM, thereby enhancing the emissions of CH_4 and N_2O. A secondary metabolite (flavonoid chrysosplenetin) secreted by methanogens (*Methanobacterium* and *Methanocella*) and denitrifying bacteria (*Anaerolinea* and *Gemmatimonas*) caused the generation of CH_4 and the reduction of N_2O to N_2, which resulted in an acceleration of CH_4 emissions but a reduction of N_2O emissions in soils amended with BC. Although we investigated keystone bacteria involved in C/N-cycling and greenhouse gas emissions, conducting metagenomics studies involving field experiments and laboratory incubation would allow for a more comprehensive understanding of the role of the microbial community in greenhouse gas emissions. Therefore, further field experiments are needed to verify the effects of BC and OM on gas emissions following actual application in the field. Additionally, soil incubation experiments will be conducted through metagenomics to determine the contribution of soil functional microbial communities to greenhouse gas emissions of CO_2, CH_4 and N_2O by adding exogenous bacteria such as *Bacillus*, *Methanobacterium*, *Methanocella*, *Anaerolinea* and *Gemmatimonas* to the soil.

5. Conclusions

The results indicated that BC and OM caused opposite effects on soil CO_2 and N_2O emissions, with BC resulting in lower emissions and OM resulting in higher emissions, while cumulative CH_4 emissions were increased by BC, OM, and their combined amendments. BC amendment led to an increase in the abundance of methanogens (*Methanobacterium* and *Methanocella*) and denitrifying bacteria (*Anaerolinea* and *Gemmatimonas*), which, in turn, increased *mcrA*, *amoA*, *amoB*, and *nosZ* gene abundance and flavonoid (chrysosplenetin) production, generating CH_4 and reducing N_2O emissions. In contrast, OM amendment resulted in a higher abundance of the methanogen *Caldicoprobacter* and denitrifying bacteria *Acinetobacter*, thereby enhancing the abundances of the genes *mcrA*, *amoA*, *amoB*, *nirK*, and *nirS* and the catabolism of carbohydrates [maltotriose, D-(+)-melezitose, D-(+)-cellobiose, and maltotetraose], which enhanced CH_4 and N_2O emissions. Additionally, puerarin produced by *Bacillus* metabolism may be responsible for reducing CO_2 emissions with BC amendment, while increasing CO_2 emissions with OM amendment. Investigations into the effects of BC and OM on greenhouse gas emissions and the related microbial communities, functional genes, and metabolomic profiles are necessary to reduce gas emissions and promote sustainable agriculture.

Author contributions

Wendan Xiao: Conceptualization, Methodology, Software, Investigation, Writing – Original Draft.

Qi Zhang: Data curation, Validation, Formal analysis.

Shouping Zhao: Methodology, Resources, Software.

De Chen: Resources, Formal analysis.

Zhen Zhao: Validation, Formal analysis.

Na Gao: Investigation, Validation.

Miaojie Huang: Investigation, Validation.

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Wendan Xiao: Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Qi Zhang:** Validation, Formal analysis, Data curation. **Shouping Zhao:** Software, Resources, Methodology. **De Chen:** Resources, Formal analysis. **Zhen Zhao:** Validation, Formal analysis. **Na Gao:** Validation, Investigation. **Miaojie Huang:** Validation, Investigation. **Xuezhu Ye:** Writing – review & editing, Supervision, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Appendix C. Supplementary data

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