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Synergistic effects of biochar and nitrogen fertilizer enhance soil carbon emissions and microbial diversity in acidic orchard soils

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To explore the synergistic effects of biochar and nitrogen fertilizer on soil carbon emission and microbial diversity in acidic orchards were studied. A 300-day pot experiment was conducted, including control (CK), nitrogen fertilizer (N), 1% biochar (B1), 3% biochar (B3), nitrogen fertilizer with 1% biochar (NB1), and nitrogen fertilizer with 3% biochar application (NB3). After biochar and nitrogen fertilizer treatments, soil pH increased from 4.73 to 6.75 unit, soil organic carbon (SOC), mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) contents increased 17.88%–41.14%, 31.95%–73.44% and 15.50%–48.90%, respectively, Dissolved organic carbon (DOC) content decreased by 33.56%–55.35%. The release of CO_2–C increased by 0.73%–232.43%, with the synergistic effect of NB3 being the most significant. NB1 and B1 reduced VOCs-C release, while NB3 and B3 increased VOCs-C release. B1 and B3 significantly enhanced the abundance of *Bradyrhizobium*, while decreasing the abundance of *Streptomyces* and *Streptacidiphilus*, NB3 exhibited opposite trends. Compared with CK, B1 and B3 increased the abundance of *acyl-CoA dehydrogenase* (*acdA*), and NB1 and NB3 reduced the abundance of *β-galactosidase* (*β-gaL*) and *glucosidase* (*GA*). Correlation analysis showed that the release of CO_2-C was significantly positively correlated with MAOC and negatively correlated with DOC, while VOCs-C was significantly negatively correlated with DOC. This synergistic effect of biochar and nitrogen fertilizer has positive implications for improving soil health and represents a viable strategy for sustainable agricultural practices.

Keywords Acidic soil, Biochar, Carbon emissions, Mineral-associated organic carbon, *β-galactosidase*

Soil is the largest carbon reservoir in terrestrial ecosystems[1]. The soil carbon cycle regulates the dynamics of soil organic matter and gas exchange between the soil and the atmosphere[2]. The soil carbon cycle involves processes such as carbon degradation, fixation, and methane fermentation, driven by soil microbial activity[3]. These processes influence carbon storage in soils, ultimately impacting greenhouse gas emissions and global climate change[4]. Soil organic carbon (SOC) can be readily assimilated by microorganisms, while mineral-associated organic carbon (MAOC) is often regarded as a chemical barrier that hinders decomposition and has a longer residence time[5]. In contrast, particulate organic carbon (POC) exhibits a higher turnover rate. However, studies have found that microbial activity in the rhizosphere environment may alter the interactions between MAOC and minerals, thereby influencing its decomposition rate[6]. Soil microbial respiration decomposes organic carbon, releasing CO_2 and CH_4[7,8]. Furthermore, SOC can be converted into VOCs, with the production depending on oxygen levels. Under aerobic conditions, microorganisms preferentially utilize organic carbon sources for growth, resulting in the production of CO_2, with only a small amount used for VOCs production[9]. According to statistics, atmospheric CO_2 and CH_4 concentrations have reached 1889 ppm and 333 ppb, respectively, representing 262% and 123% of pre-industrial levels[10]. Agricultural soils contribute about 70% of CO_2 emissions, 40% of CH_4 emissions and 14% of VOC emissions[11,12]. Therefore, there is a pressing need for effective strategies to enhance carbon storage and mitigate losses.

Soil microorganisms act as key mediators in biogeochemical cycles, facilitating carbon sequestration, converting inorganic carbon to organic carbon, and decomposing organic matter[13,14]. Increased microbial

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abundance accelerates organic matter degradation and carbon emissions, leading to adverse environmental impacts[15,16]. Changes in the abundance of functional genes in soil microorganisms significantly impact soil carbon dynamics, suggesting that these genes can serve as indicators for assessing carbon sequestration potential[17]. Microbial functional genes related to soil carbon cycling can be classified into three main categories: genes associated with carbon degradation (e.g., *amyA*, *nplT*, *xylA*, *CDH*, and *glx*), genes related to carbon fixation (e.g., *PCC*, *accA*, *aclB*, *acsA*, and *rbcL*), and genes involved in methane metabolism (e.g., *mcrA*, *mrtA*, *pmoA*, and *mmoX*)[18].

Orchards account for approximately 5% of global agricultural land, with the total production of citrus fruits reaching about 152.45 million tons[19]. To meet the high nutrient demands of fruit trees, excessive nitrogen fertilizers are frequently applied, leading to soil acidification and increased carbon loss. Soil acidity affects microbial community structure, influencing soil carbon transformation. Research indicates a close relationship between plants, soil microbial communities, and functional microorganisms, with variations in these communities linked to changes in carbon and nutrient concentrations[20,21]. The soil carbon pool is crucial for soil fertility, productivity, and biodiversity. The recalcitrant carbon pool, which is characterized by high chemical stability or is tightly bound to soil minerals, helps maintain soil structure and promote carbon sequestration[22]. However, degradation rates of unstable and recalcitrant carbon vary based on acidic soil conditions, fertilizer inputs, crop types, and management practices[23,24].

Biochar is applied to soils due to its carbon-rich porous structure and large surface area[1,25]. Studies show that biochar application can reduce CO_2 emissions by 18–41% and CH_4 emissions by 20–132%, while some studies indicate a 22% increase in CO_2 emissions and no significant impact on CH_4 emissions[26,27]. Biochar can adsorb VOCs such as dichloromethane (54.9 mg g^{-1}) and toluene (308 mg g^{-1})[28]. Nitrogen fertilizers are commonly used to enhance carbon fixation capacity and promote plant photosynthesis. However, soil carbon dynamics respond variably to nitrogen inputs. Wang et al.[29] found that nitrogen addition affects different carbon components, such as POC and MAOC, differently in terms of their formation and stability. The co-application of biochar and inorganic fertilizer may be more beneficial for soil carbon sequestration than the single application of biochar[30]. The combined use of biochar and nitrogen fertilizer has been shown to be more effective for increasing POC[31]. These controversial findings are believed to depend on whether nitrogen addition leads to soil acidification and the subsequent changes in microbial processing of organic carbon, as well as the chemical processes dominating the formation of organic-mineral associations.

This study aims to investigate the effects of biochar and nitrogen fertilizer applications on soil carbon emissions and microbial diversity in acidic orchard soils. Therefore, this research evaluates the impacts of different treatment combinations on soil carbon components, gas emissions, and microbial characteristics involved in the carbon cycle through a pot experiment. We hypothesize that (1) biochar and nitrogen fertilizer application would significantly increase or decrease soil organic carbon content; (2) higher application rates of biochar and nitrogen fertilizer result in more pronounced effects on carbon release; and (3) biochar and nitrogen fertilizer may alter soil pH and influence the abundance of carbon transformation functional genes, such as *amyA*, *aclB*, and *pmoA*.

Materials and methods

Test materials

The test soil, which belongs to the siliceous red soil type with a clay loam texture, was collected from an orchard in Pinghe County, Fujian Province. Field soil was collected from three layers (0–20 cm, 20–40 cm, 40–60 cm), and transported back to the laboratory, where animal and plant residues, as well as gravel were removed. The soil was then sieved through an 8 mm mesh and then thoroughly mixed for each layer. Simultaneously, the 40–60 cm and 20–40 cm layers were backfilled in sequence into a 60 cm × 60 cm × 60 cm cement frame. The 0–20 cm soil layer was blended with varying proportions of biochar and then filled into the cement frame. Subsequently, one-year-old honey pomelo seedlings were planted within the prepared substrate. Biochar was purchased from Jiangsu Huafeng Agricultural Bioengineering Co., Ltd. (Xinyang Road, Yangzhong City, Zhenjiang, Jiangsu Province, China). It was produced at 600 °C via a slow pyrolysis of rice straw. Meanwhile, the soil gas collection mask base was buried for gas collection. Each layer of soil was then compacted, and water was applied regularly to equilibrate the soil for two months. The soil chemical properties of the equilibrated pot soil before the experiment started: pH 4.6, organic matter content 19.8 g kg^{-1}, and total nitrogen 1.02 g kg^{-1}. The biochar utilized in the trial had a pH of 9.3, an organic carbon content of 28.6 g kg^{-1}, a total nitrogen of 0.46%, and a specific surface area (SSA) of 54.0 m^2 g^{-1}.

Experimental design and sample collection

Considering the feasibility of operation in honey pomelo orchards, preliminary tests revealed that biochar application rates of 10 and 30 g kg^{-1} significantly improved the properties of acidic soil. This experiment was conducted using indoor pot trials, with a total of 6 treatments and 4 replicates: CK (without urea and biochar), N (with 0.22 g kg^{-1} urea), B1 (with 10 g kg^{-1} biochar), B3 (with 30 g kg^{-1} biochar), NB1 (with 0.22 g kg^{-1} urea and 10 g kg^{-1} biochar), and NB3 (with 0.22 g kg^{-1} urea and 30 g kg^{-1} biochar). In order to ensure the growth of grapefruit seedlings, all treatments were applied: phosphate (potassium dihydrogen phosphate) and potassium (potassium sulfate), an application rate of 13.21 kg P_2O_5 and 11.38 kg K_2O per mu was implemented. In accordance with the conventional management mode of local pomelo gardens, fertilization was conducted four times in July, September, January and March, with the fertilization ratio of 20%, 25%, 30% and 25%, respectively, for a period of 10 months. Soil and gas samples were collected during the experiment.

Soil samples were collected using a soil auger and the five-point sampling method in the middle and end of the experiment. The soil was thoroughly mixed and divided into three portions: one portion was air-dried for the analysis of soil pH, total carbon (TC), total nitrogen (TN), MAOC and POC, another portion was stored at

4 °C for assessing exchangeable aluminum, microbial biomass carbon (MBC), dissolved organic carbon (DOC), while the third portion was stored at − 80 °C for the detection of soil carbon-functional microorganisms.

CO_2 and CH_4 were collected using a static box-gas chromatography method, while VOCs-C were collected and analyzed using a dynamic box-pre-concentration GC–MS instrument. The sampling cuvette consists of two parts: the base and the sampling hood, both constructed from Polytetrafluoroethylene (PTFE). The base is equipped with a 3 cm deep water tank, which is used to seal the sampling device. A metal probe and a small electric fan are installed on the sampling hood, which are used to monitor the air temperature inside the chamber and pre–mix the air before formal sampling, respectively.. During formal sampling, the sampling hood is placed on base, and water is used to seal the groove, ensuring airtightness. The total duration of the experiment was 300 days. After the soil reached equilibrium, the first fertilization was conducted in July. Gas samples were collected at specific time intervals (1, 3, 5, 10, 20, 30, 45, 60, 75 days) following the initial fertilization. Subsequently, a second round of fertilization was applied, and gas samples were collected at specific time intervals (3, 5, 10, 20, 30, 45, 60, 75 days) to capture the changes in gas emissions during the early stages after fertilization. A third round of fertilization was then conducted, and gas sampling was repeated at the same time intervals until the experiment concluded. as collection involved using a 60 mL syringe at 0 and 30-min marks, with collected gas stored in an aluminum foil bag for the analysis of CO_2–C and CH_4–C. Additionally, gas samples were collected for the analysis of VOCs-C on the 150th and 300th days of the experiment, zero air, which had been pretreated to remove the majority of VOCs using an adsorption column (silica gel–activated carbon–potassium iodide), was introduced into a sealed collection device. After 10 min, 1000 mL of gas was drawn into a Tedlar bag and analysis. All samplings were carried out between 9:00 AM and 11:00 AM.

Measurement indicators and methods

The concentrations of CH_4–C and CO_2–C gases were analyzed via gas chromatography (Agilent GC-2010Pro) and VOCs-C were examined using a pre-concentration GC–MS instrument[32,33]. Soil pH was determined in a 1:2.5 soil-to-water ratio using a pH meter. Soil exchangeable acidity was measured by using a potassium chloride exchange-neutralization titration method. Soil organic carbon and total nitrogen were determined by an elemental analyzer (TruMax CNS)[34]. Soil microbial carbon was determined by a chloroform fumigation method (with a conversion factor of 0.45), and soluble organic carbon was estimated by a potassium chloride leaching and total organic carbon analyzer method[35]. To separate soil size fractionation, 10 g of air-dried and sieved (< 2 mm) soil was dispersed in 50 mL of 0.5% sodium hexametaphosphate solution and shaken for 18 h. The dispersion was then sieved through a 53 μm sieve, with organic material passing through (< 0.053 mm) was identified as mineral-associated organic matter (MAOM) and the remainder (0.053–2 mm) as particulate organic matter (POM)[36]. Particulate organic carbon and mineral combined organic carbon were analyzed by elemental analyzer methods[35].

Carbon functional microorganisms were studied using metagenomic microbial sequencing. DNA extraction was performed following the instructions of Power Soil DNA Isolation Kit (MoBio Laboratories, Inc., CA). The extracted DNA samples was sheared to 300 bp with the Covaris ultrasonic crusher. And to prepare sequencing library, the fragments were treated by end repair, A tailing, and ligation of Illumin a compatible adapters. DNA sequencing libraries were deep sequenced on Illumina Novaseq PE150 platform at Allwegene Technology Co., Ltd. (Beijing, China)[37]. The KEGG annotation was conducted using Diamond[38] (http://www.diamondsearch.org/index.php, version 0.8.35) against the Kyoto Encyclopedia of Genes and Genomes database (http://www.genome.jp/keeg/, version 94.2) with an e-value cutoff of 1e-5. The corresponding KEGG functions of the genes were obtained, and the abundance of each function was calculated by summing the abundances of the genes associated with that function.

Data processing and statistical analysis

$$F = \frac{\rho \times V \times \Delta c \times 273}{A \times \Delta t \times (273 + T)} \times \alpha \tag{1}$$

where F represents the emission rate of CO_2–C and CH_4–C (mg m^{-2} h^{-1}); ρ denotes the gas density (kg m^{-3}); V stands for the volume of the sampling box (m^3); A is the surface area (m^2); ΔC refers to the change in gas concentration (mg kg^{-3}); Δt is the time interval (0.5 h); T suggests the environmental temperature (℃) and α represents the conversion factor of CO_2 and CH_4 into carbon[39].

$$F = \frac{\Delta c \times f \times 273}{A \times V_m \times (273 + T)} \times \alpha \tag{2}$$

where F signifies the emission rate of VOCs-C (pmol m^{-2} s^{-1});Δc represents the change in gas concentration (pmol mol^{-1}); f is the volume of the sampling chamber (L s^{-1}); A refers to the surface area (m^2); Vm is the molar volume under standard conditions (22.4 L mol^{-1}); T is the ambient temperature (℃), while α is the conversion factor for converting various VOCs to carbon[40].

$$C = \frac{\sum_{i=1}^{n}(F_{i+1} + F_i)}{2} \times (t_{i+1} - t_i) \times 24 \times \mathrm{k} \tag{3}$$

where *C* represents the cumulative emission of CO_2–C and CH_4–C (kg m^{-2} or g m^{-2}); F represents the emission rate of CO_2–C and CH_4–C; i represents the sampling time; t represents the sampling time; n represents the total

Treatment	SOC (g kg^{-1})	MBC (mg kg^{-1})	DOC (mg kg^{-1})	POC (g kg^{-1})	MAOC (g kg^{-1})
CK	11.52 ± 0.58d	26.64 ± 4.16d	41.48 ± 4.34a	2.41 ± 0.18b	8.84 ± 0.40bc
B1	13.58 ± 0.53c	34.97 ± 1.84bc	27.56 ± 1.45bc	3.18 ± 0.58ab	10.21 ± 0.45b
B3	15.40 ± 0.53b	32.09 ± 3.55cd	21.71 ± 2.06cd	3.58 ± 0.57a	12.01 ± 1.11a
N	11.57 ± 0.48d	30.17 ± 5.75cd	42.31 ± 2.87a	2.51 ± 0.32b	8.16 ± 0.76c
NB1	13.81 ± 0.51c	38.85 ± 3.32ab	33.47 ± 6.39b	3.40 ± 0.33a	9.95 ± 0.85b
NB3	16.33 ± 0.25a	42.04 ± 2.37a	18.89 ± 2.57d	3.43 ± 0.59a	12.15 ± 1.07a

Table 1. Effects of biochar and nitrogen fertilizer application on soil organic carbon components. SOC, soil organic carbon; MBC, microbial biomass carbon; DOC, dissolved organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon. The data in the table represent mean values ± standard error; with n = 4. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Treatment	pH	Exchangeable acidity (cmol kg^{-1})	TN (g kg^{-1})	C:N
CK	4.73 ± 0.10d	2.70 ± 0.23a	1.18 ± 0.04cd	9.74 ± 0.14d
B1	5.55 ± 0.29c	0.97 ± 0.32b	1.20 ± 0.00bcd	11.25 ± 0.42b
B3	7.11 ± 0.11a	0.21 ± 0.02c	1.14 ± 0.03d	13.48 ± 0.26a
N	4.48 ± 0.25d	2.71 ± 0.30a	1.26 ± 0.08ab	9.14 ± 0.26e
NB1	5.36 ± 0.11c	1.37 ± 0.24b	1.30 ± 0.03a	10.56 ± 0.19c
NB3	6.75 ± 0.11b	0.40 ± 0.18c	1.24 ± 0.02abc	13.14 ± 0.09a

Table 2. Effects of biochar and nitrogen fertilizer application in soil properties. The data in the table represent mean values ± standard error, with n = 4. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

number of samples; t_{i+1}-t_i is the number of sampling interval days. (d); k is the unit conversion coefficient, where k is 10^{-5} for calculating CH_4–C and 10^{-2} for calculating CO_2–C[41].

Data are presented as mean ± standard error (SE, n = 4). Significant differences among treatments ($P < 0.05$) were determined using Analysis of Variance (ANOVA) followed by the LSD post hoc test. ANOVA was performed using SPSS version 26.0. Prior to analysis, the normality of the data was assessed using the Shapiro–Wilk test, and the homogeneity of variance was evaluated using Levene's test. To assess the relationships between variables, correlation analysis was conducted, and Pearson's correlation coefficient (or Spearman's correlation coefficient, depending on the data distribution) was used to quantify the correlations. The results of the correlation analysis were visualized using a heatmap, which was generated using Origin Lab 8.0 software. Additionally, redundancy analysis (RDA) was performed using Canoco 5.0 software to explore the relationships between environmental variables and microbial quantities, and the significance of the RDA was assessed using a permutation test. All graphs were plotted using Origin Lab 8.0.

Results

Organic carbon fractions and soil properties

The results (Table 1) indicated variations in the impacts of different application modes of biochar and nitrogen fertilizer on soil carbon components presented. Compared with CK, SOC, POC and MAOC contents of B1 and B3 were significantly increased by 2.06 and 3.88 g kg^{-1}, 0.77 and 1.77 g kg^{-1}, 1.37 and 3.17 g kg^{-1}, respectively, showing an increasing trend with higher application rates ($P < 0.05$). DOC contents were significantly decreased by B1 and B3 ($P < 0.05$). Additionally, N treatment had no significant effect on soil organic carbon components. Compared to N, NB1 and NB3 treatments not only significantly increased SOC, POC, and MAOC contents but also significantly increased MBC content, and significantly decreased DOC content ($P < 0.05$).

As shown in Table 2, the combined application of biochar and nitrogen fertilizer increased soil pH and C:N ratio, while reducing exchangeable acidity. Compared with CK, B1 and B3 treatments increased soil pH by 0.82 and 2.38 unit, increased the C:N ratio by 1.51 and 3.74, and reduced exchangeable acidity by 64.07% and 92.22%, respectively ($P < 0.05$). N treatment significantly increased total nitrogen content ($P < 0.05$). Based on the nitrogen fertilizer application, increasing rates of biochar and nitrogen fertilizer significantly increased pH and C:N ratio, and significantly decreased exchangeable acidity ($P < 0.05$). NB1 and NB3 treatments increased pH by 0.88 and 2.27 units, increased the C:N ratio by 1.42 and 4.00, and reduced exchangeable acidity by 49.45% and 85.24%, respectively.

Soil carbon emissions

Throughout the duration of the experiment, all fertilizer applications affected the CO_2–C emissions from the soil. The emission rate peaked between the 5th and 10th day after each application and gradually leveled off by the 30th day (Fig. 1a). Compared to the control group (CK), N, B1 and B3 treatments increased the CO_2–C emission rate. The increases ranged from 0.73% to 122.47%, 4.57% to 232.43%, and 6.81% to 58.50%, respectively. Compared with N, NB1 and NB3 also increased the CO_2–C emission rate, with an increase ranging from 0.24%

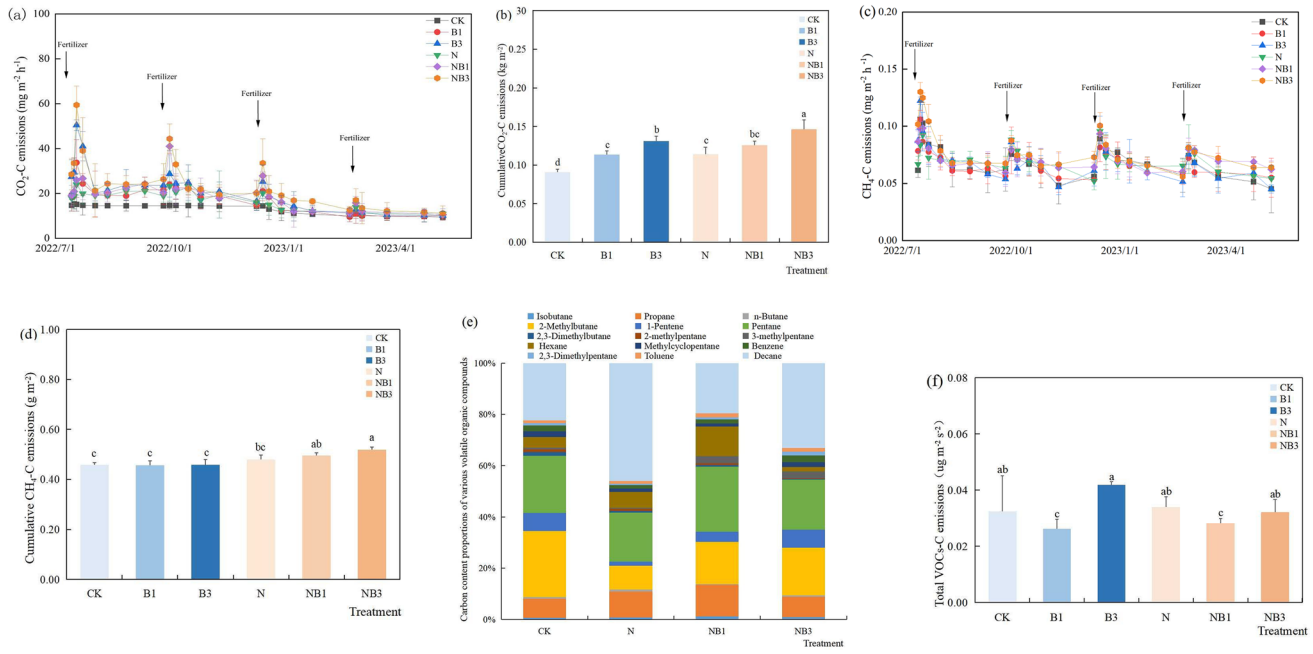

Fig. 1. Carbon release characteristics after application of biochar and nitrogen fertilizer. Note: (**a**) CO_2–C emission flux, (**b**) CO_2–C cumulative emission, (**c**) CH_4–C emission flux, (**d**) CH_4–C cumulative emission, (**e**) proportion of VOCs-C substance, and (**f**) total VOCs-C emission rate.

to 75.92% and 0.21% to 147.46%, respectively. Furthermore, both biochar and nitrogen fertilizer applications significantly increased the cumulative CO_2–C emissions ($P<0.05$) compared to CK, with increases of 25.41%, 44.28%, and 25.99%, respectively (Fig. 1b). The addition of biochar to nitrogen fertilizer also increased the cumulative CO_2–C emissions, with NB3 reaching significant levels ($P<0.05$) and a cumulative emission of 0.15 kg m^{-2}.

The application of nitrogen fertilizer and biochar has a significant impact on CH_4–C emissions ($P<0.05$). The CH_4–C emission rate peaks on the third day after each fertilizer application and gradually decreases thereafter (Fig. 1b). Compared to CK, either biochar or nitrogen fertilizer application alone increased the CH_4–C emission rate. The percentage increase ranged from 0.19% to 27.61%, 1.58% to 42.10%, and 1.99% to 35.16% for B1, B3, and N, respectively. When biochar was applied on top of nitrogen fertilizer, it further increased the CH_4–C emission rate, with an increase ranging from 0.01% to 29.63% for B1 and 1.37% to 55.68% for B3. In comparison to the treatment with nitrogen fertilizer alone, the addition of biochar increased the cumulative CH_4–C emissions, with NB3 reaching a significant level. The cumulative emission for NB3 was 0.52 g m^{-2} (Fig. 1c).

At the end of the experiment, the emission rate of volatile organic compounds (VOCs-C) was measured (Fig. 1d). The results indicated that the B1 treatment significantly reduced the VOCs-C emission rate compared to the CK, resulting in a decrease of 0.06 μg m^{-2} s^{-1} ($P<0.05$). However, the B3 treatment increased the VOCs-C emission rate. When biochar was applied in combination with nitrogen fertilizer, NB3 treatment significantly reduced the VOCs-C emission rate, resulting in a decreasing of 0.02 μg m^{-2} s^{-1} ($P<0.05$). Further analysis of the composition of VOCs-C (Fig. 1e) revealed that a significant proportion, ranging from 86.44% to 96.09%, of the volatile organic compounds released from the soil were released in the form of alkanes. Additionally, 1.47% to 7.04% were released as alkenes, and 2.44% to 8.70% were released as benzene. Among these compounds, the majority of alkanes were released in the form of n-hexane carbon into the atmosphere.

Soil carbon cycling functional microorganisms

Sixty-one functional genes related to carbon cycling were identified through soil sample analysis, with their abundance quantified using TPM (Transcripts Per Million) values. Experimental treatments influenced four processes related to soil carbon degradation, carbon fixation, fermentation, and methane oxidation (Fig. 2). Biochar and nitrogen fertilizer applications notably impacted carbon degradation and fermentation processes, with functional microorganisms contributing up to 79.94% and 20.65%, respectively, based on the calculation of TPM-relative contribution. The relative contribution of microorganisms involved in carbon degradation decreased by 0.63% to 1.69% with increasing biochar application compared to the control treatment, while those contributing to fermentation increased by 3.35% to 8.28%. Nitrogen fertilizer alone enhanced the abundance of microorganisms associated with carbon degradation but reduced those linked to fermentation. Increasing biochar alongside nitrogen fertilizer decreased carbon degradation by 0.41% to 0.56% and boosted fermentation-related microorganisms by 2.17% to 7.18%.

The microbial community composition in soil carbon degradation and fermentation processes was analyzed at the genus level (Fig. 3). The results indicated that the main bacterial genera in the soil carbon degradation process were *Bradyrhizobium*, *Streptomyces*, and *Streptacidiphilus*. The application of biochar alone increased

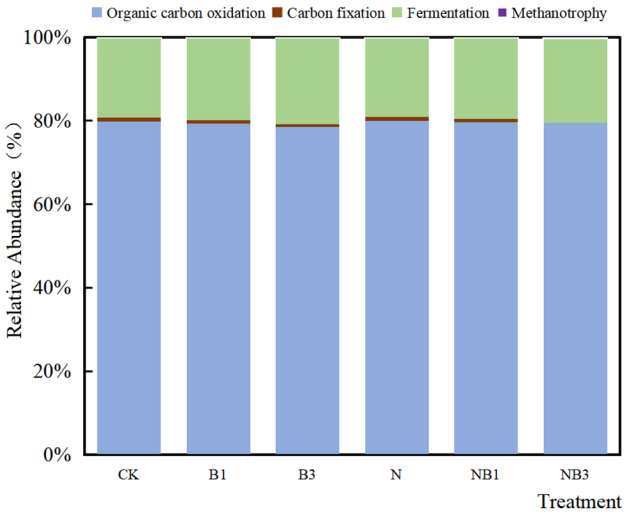

Fig. 2. Processes related to the soil carbon cycle.

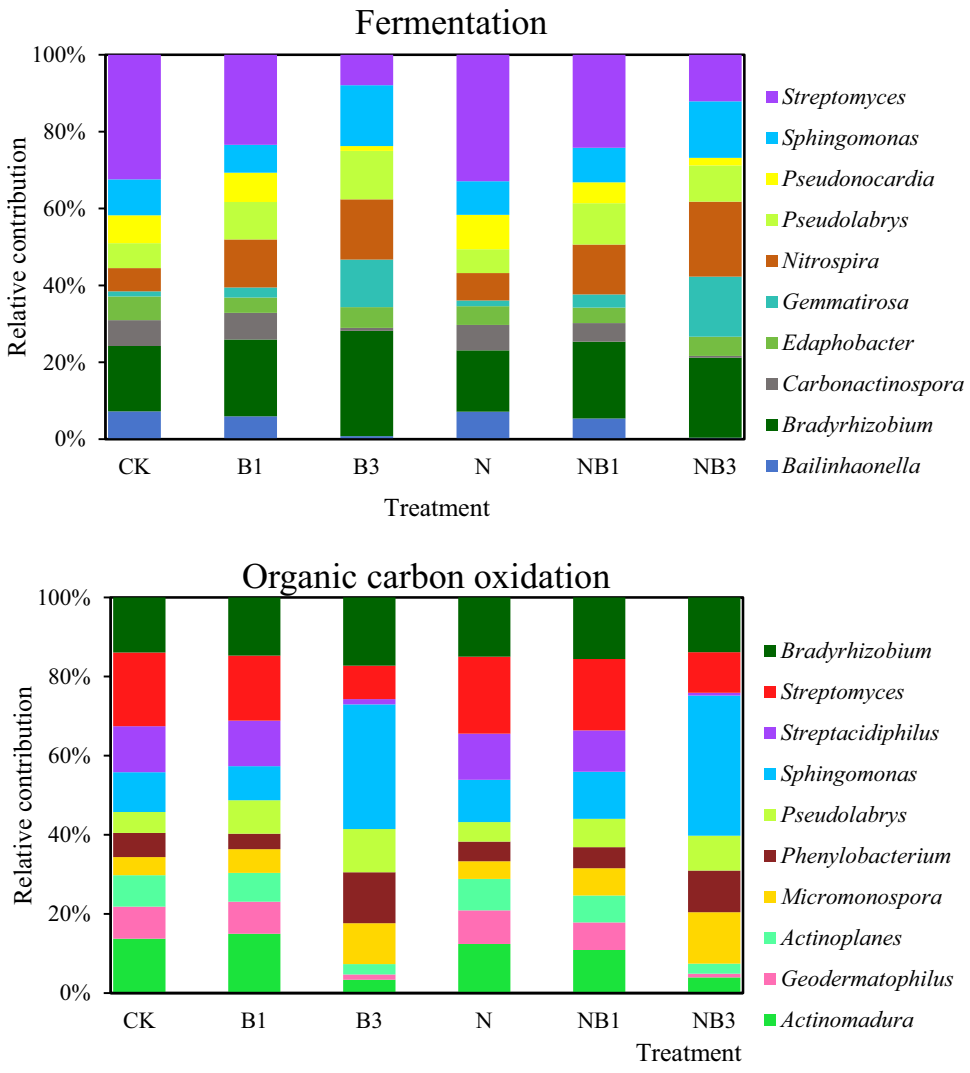

Fig. 3. Species contribution to genes at the genus level.

Bradyrhizobium by 0.81% to 3.33%, but decreased *Streptomyces* and *Streptacidiphilus* by 0.17% to 10.33% and 2.19% to 10.16%, respectively. With the application of nitrogen fertilizer, *Bradyrhizobium* increased by 0.58% under the NB1 treatment, but decreased by 1.14% under the NB3 treatment. Both *Streptomyces* and *Streptacidiphilus* exhibited a decreasing trend with increasing biochar application rates, with reductions ranging from 1.37% to 9.39% and 1.26% to 10.96%, respectively. In the analysis of soil carbon fermentation, the main bacterial genera were *Streptomyces*, *Sphingomonas*, and *Bradyrhizobium*. Compared to the control (CK) treatment, biochar application alone increased *Bradyrhizobium* by 2.97% to 10.38%, decreased *Streptomyces* by 8.96% to 24.48%, and *Sphingomonas* increased by 6.44% only in the B3 treatment. With increasing biochar application rates, *Streptomyces* decreased by 8.69% to 20.79%, while *Sphingomonas* and *Bradyrhizobium* increased by 0.23% to 5.97% and 4.06% to 4.90%, respectively.

Further analysis revealed distinct changes in soil carbon cycle functional genes resulting from different applications of biochar and nitrogen fertilizer, whether individually or in combination (Fig. 4). Compared to the CK treatment, the sole application of biochar significantly increased the abundance of the maxF, *α-amY*, *acdA* genes, with increases of 1631.73% to 1731.86%, 7.97% to 31.36%, and 48.02% to 70.89% respectively. It also significantly decreased the abundance of the *β-gaL* and *GA* genes, with decreases of 7.54% to 39.53% and 12.68% to 41.46%, respectively. Conversely, the sole application of nitrogen fertilizer significantly increased *mxaF* gene abundance by 751.26% and decreased the abundance of the *GA*, *adh*, *cooS* genes by 0.24%, 1.59% and 50.73% respectively. When compared to the N treatment, both NB1 and NB3 significantly increased the abundance of the *fdhA* and *acD* genes, with increases of 2.26% to 138.23% and 8.66% to 10.95% respectively. They also significantly decreased the abundance of the *β-gaL* and *GA* genes by 12.21% to 41.52% and 10.00% to 53.59% respectively.

Correlation analysis of soil carbon components and properties with carbon function microorganisms

The results of the inter-group correlation heatmap analysis indicate that CO_2–C release is significantly positively correlated with SOC, pH, MAOC, and C/N, while it is significantly negatively correlated with DOC and exchangeable acidity. Specifically, CH_4–C release is significantly positively correlated with SOC, with a correlation coefficient of 0.42, and significantly negatively correlated with DOC, with a coefficient of − 0.28. VOC_S-C release is significantly positively correlated with C/N, with a coefficient of 0.33, and significantly negatively correlated with DOC, with a coefficient of − 0.22 (Fig. 5).

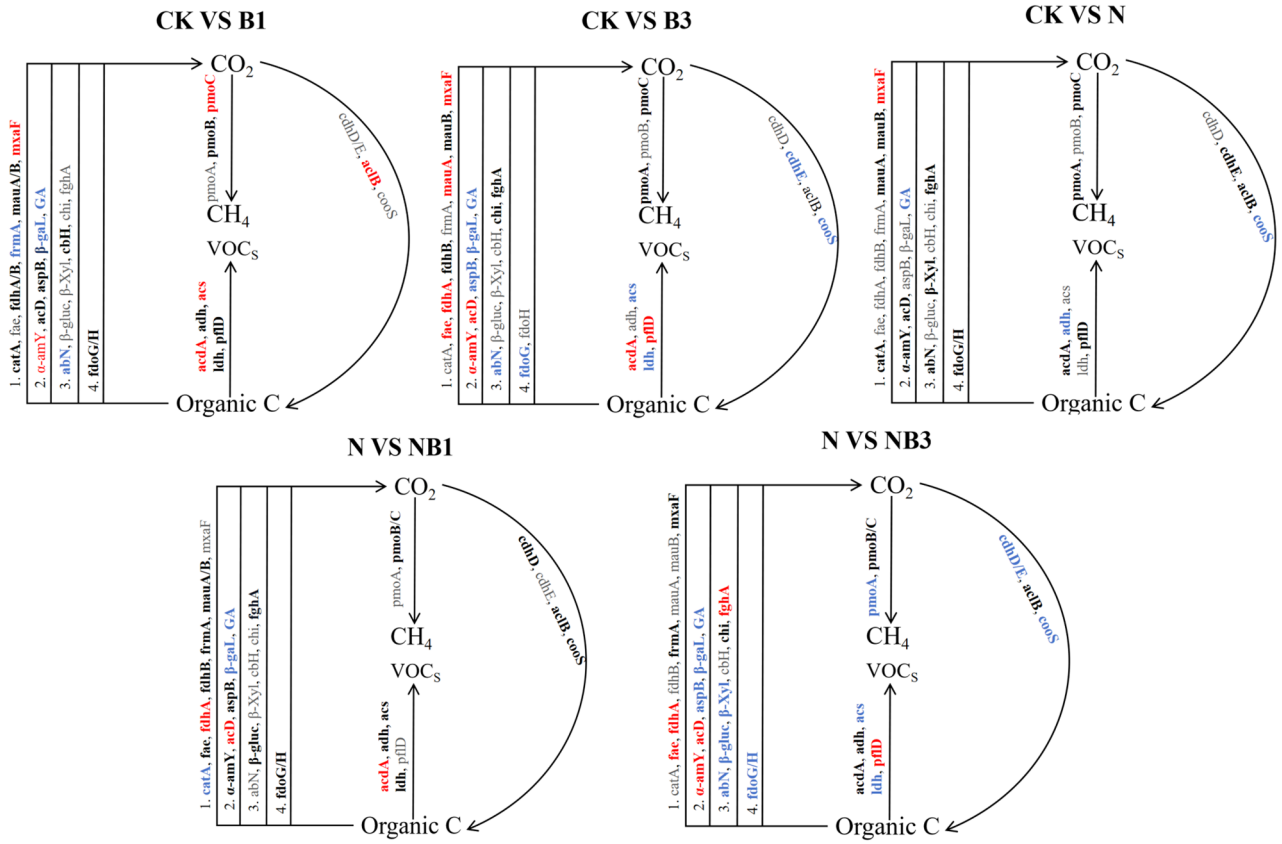

Fig. 4. Comparative analysis of carbon cycle functional genes under different treatments. Note: Compared with the previous treatment, red indicates a significant increase after treatment; blue indicates a significant decrease after treatment; deep black indicates an increase but not significant; light gray indicates a reduction but not significant.

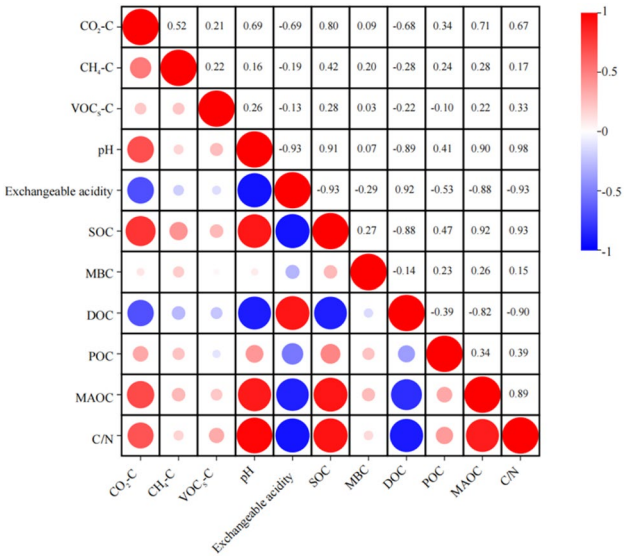

Fig. 5. Heatmap analysis of inter-group correlation of soil carbon release, composition, and properties.

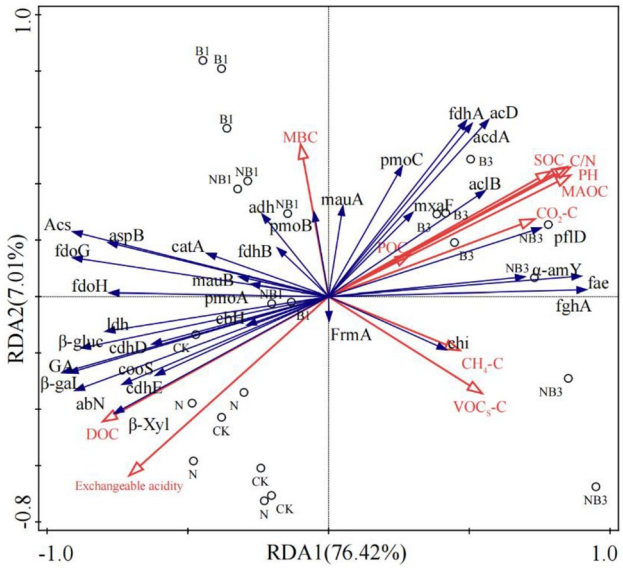

Fig. 6. Redundancy analysis of soil carbon forms, properties, and carbon functional genes.

Redundancy analysis between soil carbon components, soil properties and soil carbon cycling functional genes showed that the first and second axes accounted for 74.54% and 6.50% of the variation, respectively, with a cumulative explanation of 81.04%. Positive correlations were observed between soil SOC, MAOC, C/N, pH, CO_2–C cumulative emission, and microbial functional genes *aclB*, *fghA*, *pflD*, *acdA*, *acD*, *fghA* and *fae*, which are involved in soil carbon transformation processes. Conversely, negative correlations were found between soil *ldh*, *cooS*, *cdhD*, *cdhE*, *fdoH*, *aspB*, *acs*, *fdoG*, *β-Xyl*, *β-gluc*, *abN*, *GA*, *β-gaL*, and soil carbon components ($P < 0.05$). However, soil DOC and exchangeable acid content showed opposite results. The release rate of VOCs-C exhibited significant positive correlation ($P < 0.05$) with functional genes *fae* and *fdhA*, while showing significant negative correlation with *fdoH*, *aspB*, and *acs* ($P < 0.05$). CH_4–C showed significant positive correlations with *fae* and *α-amY*, and significant negative correlations with *aspB*, *acs*, and *fdoG* ($P < 0.05$). Soil MAOC, SOC, and pH were identified as the primary factors influencing microbial carbon transformation in the soil carbon cycling process, explaining 49.00%, 8.30% and 9.30% of the variation in functional microbial communities, respectively (Fig. 6).

Discussion

Soil carbon components response to biochar and nitrogen fertilizer application

This study has identified that application of biochar and nitrogen fertilizer significantly increase the MAOC in soil. This finding aligns with previous studies on the biochar enhancement mechanism in acidic soil systems.

Biochar, characterized by high porosity and a large specific surface area, enhances the stability of unstable organic carbon through adsorption, ultimately increasing the overall soil carbon content[42,43]. However, in sandy soils with low clay content, Yang et al.[44] reported that in sandy soils with low clay content, the enhancing effect of biochar on MAOC is diminished, suggesting that the regulation of MAOC by biochar is influenced by soil matrix properties. Additionally, biochar interacts with nitrogen fertilizers to form stable organic-mineral complexes, further promoting soil carbon stability[45]. During this process, organic improvers stabilized in the mineral-bound state undergo significant microbial transformation[46]. This microbial activity further elucidates the observed increase in MAOC content when biochar and nitrogen fertilizer were applied either separately or in combination[45]. Studies have found that in alkaline soils, the high pH environment inhibits the ligand exchange between iron oxides and organic matter, resulting in reduced efficiency of complex formation compared to acidic soils[47]. This explains the more pronounced carbon increase effect observed in acidic orchard soils in this study. The beneficial effects of biochar are further amplified when combined with nitrogen fertilizer, as evidenced by the increased organic carbon content observed in this study. Therefore, the application of biochar not only increases soil carbon content but also promotes carbon accumulation, thereby enhancing the fertility of acidic orchard soils.

An interesting finding of this study is that the application of biochar and nitrogen resulted in a reduction of soil DOC content. Similar results were reported by Jia et al.[48], who observed a decrease in soil DOC levels following biochar application. Biochar, with its large specific surface area and porous structure, can adsorb organic carbon dissolved in soil water, transforming the carbon from a dissolved state into a more stable solid form, thereby reducing the concentration of DOC in the soil solution[49]. It is possible that some of the adsorbed carbon is converted into MAOC through microbial processes, achieving a dynamic equilibrium between DOC and MAOC. This study revealed that the organic carbon in the soil was mainly MAOC (Table 1). The application of nitrogen fertilizer provides an abundant nitrogen source for soil microorganisms, significantly promoting their growth and activity. These activated microorganisms accelerate the decomposition of various organic matter in the soil, including DOC[50]. Furthermore, the combined application of biochar and nitrogen fertilizer alters soil properties, such as pH and aeration. Changes in soil pH directly affect the dissociation and forms of organic carbon, while modifications in aeration influence microbial respiration and redox conditions, thereby impacting the stability of DOC. For instance, under conditions of higher pH and improved aeration, some dissolved organic carbon may undergo polymerization or bind with metal ions in the soil, forming more stable macromolecular organic compounds. These compounds may be slowly decomposed and utilized by microorganisms, becoming part of the MAOC pool. These comprehensive changes in soil properties collectively influence the solubility and stability of dissolved organic carbon, ultimately leading to a reduction in DOC content[51].

Soil gaseous carbon emission response to biochar and nitrogen fertilizer application

The application of both biochar and nitrogen fertilizer has been shown to increase CO_2–C emissions. When biochar and nitrogen fertilizer are combined, a synergistic effect on gaseous carbon emission is observed, as reported by Jia et al.[52]. Several factors contribute to the observed increase in gaseous carbon emissions. Firstly, the effective carbon compounds present in biochar, such as volatile compounds and carbonates, as well as the addition of biochar to acid soils, may promote the decomposition and conversion of native soil organic carbon into CO_2–C[53,54]. Secondly, biochar can enhance microbial activity and soil respiration intensity by modifying soil properties, including an increase in soil pH and improved soil aeration[55]. This study found that biochar application indeed raised soil pH, which was positively correlated with CO_2–C release (Table 1). Thirdly, biochar is characterized by its high carbon content, which can directly increase the soil carbon pool after being incorporated into the soil. The easily decomposable organic carbon components in biochar are rapidly utilized by microorganisms, promoting the priming effect, which may lead to soil SOC loss and increased CO_2 emissions in the short term. However, in the long term, the stable carbon components in biochar may gradually accumulate, forming a more persistent carbon pool and thereby offsetting some of the carbon loss caused by the priming effect[56]. Fourthly, physical interactions between plant roots and biochar can lead to increased mineralization of SOC[57]. In this experiment, well-developed citrus roots were selected. At the end of the experiment, it was observed that the taproots were significantly longer, the number of lateral roots increased, and their density was higher, forming an extensive network structure. Additionally, some biochar particles were found attached to the surface of the fibrous roots, and some biochar aggregates were split open, resulting in an intertwined structure of roots and biochar. Future studies should further explore how to optimize biochar and nitrogen fertilizer application strategies to maximize their environmental benefits.

An interesting finding is that the co-application of biochar with nitrogen fertilizer increases CH_4–C emissions, whereas the application of either alone does not significantly affect CH_4–C emissions. Several factors may contribute to this phenomenon: Firstly, the combined application of nitrogen fertilizer enhances the activity of methanogenic bacteria or suppress the activity of methane-oxidizing bacteria more effectively than biochar alone, leading to increased CH_4–C emissions (Fig. 3). Secondly, the application of nitrogen fertilizer can alter soil acidity, while biochar may buffer soil pH. Variations in soil pH can affect the activity of methanogenic bacteria and their role in methane emissions[58,59]. Thirdly, the use of both biochar and nitrogen fertilizer can locally influence the anaerobic microenvironment of the soil[60], driving mechanisms induced by biochar that lead to increased CH_4–C emissions.

This study found that the application of 1% biochar significantly reduced the emission rate of VOCs-C, and when combined with nitrogen fertilizer, the reduction in VOCs-C emissions was even more pronounced. Several possible reasons are as follows: during the co-application of biochar and nitrogen fertilizer, nitrogen groups and oxygen-containing functional groups contribute to the formation of a layered porous structure with the biochar. This structural modification creates active sites that enhance the absorption capacity for VOCs-C. The strong electron absorption capacity of biochar further facilitates its interaction with and adsorption of VOCs-C[61–63].

Additionally, previous studies have indicated that biochar exhibits a synergistic effect with nitrogen doping, leading to an increased adsorption capacity for VOCs-C[28]. This enhanced adsorption can play a significant role in reducing the overall emissions of VOCs-C from soil. Furthermore, it is important to note that the roots of citrus plants can also release VOCs-C into surrounding soil environment. Microorganisms present in the rhizosphere can modify the emission of these root-derived VOCs-C by secreting enzymes and other metabolic products, which can further influence the dynamics of VOCs-C emission. However, there are some limitations to this study. Notably, the correlation between soil VOCs-C and soil carbon components is found to be low. When combined with findings from other studies, it becomes apparent that plants and their roots systems significantly impact on soil VOCs-C emissions. In future studies, it would be explore the relationship between soil VOCs-C and plants and roots dynamics, Understanding these interactions could pave the way for more effective management strategies aimed at mitigating VOCs-C emissions in agricultural systems.

Soil carbon function microorganisms' response to biochar and nitrogen fertilizer application

The results of this study indicate that the application of biochar and nitrogen fertilizer significantly impacts the degradation processes of organic carbon, particularly affecting *Bradyrhizobium* and *Streptomyces*. *Bradyrhizobium*, which belongs to the *Proteobacteria* phylum, is an important genus involved in intracellular carbon decomposition and plays a crucial role in the degradation of unstable carbon in soil[64]. Notably, NB3 led to increased levels of *Bradyrhizobium* and enhanced carbon release (Figs. 1 and 4). This effect may be attributed to the essential nutrients provided by nitrogen fertilizer, which stimulate soil microbial activity, thereby enhancing the oxidation and transformation of organic carbon and influencing *Bradyrhizobium*[65]. Additionally, *Streptomyces*, which belongs to the *Actinobacteria* phylum, is known for its ability to decompose plant residues and soil humus, playing a significant role in the decomposition of recalcitrant carbon in soil. Studies have shown that different microbial species exhibit varying adaptability to pH levels[66]. Some acidophilic microorganisms can maintain high activity under low pH conditions, while certain alkaliphilic microorganisms thrive in alkaline environments. When soil pH changes, microorganisms must adjust their metabolic and physiological mechanisms to adapt to the altered environment. This process may affect their ability to decompose and transform organic carbon in soil. Research by Cao et al.[67] demonstrated that increased applications of biochar and nitrogen fertilizer resulted in higher soil organic carbon content, increased pH values, and a greater relative abundance of *Actinobacteria*. Furthermore, pH levels can influence the characteristics of microbial membrane proteins and their surface charge, which in turn affect nutrient absorption and survival rates of these microorganisms[68]. The addition of biochar and nitrogen fertilizer provides essential nutrients to soil microorganisms, optimizing the microbial composition related to soil carbon and, consequently, improving soil health and quality[65].

In the experimental treatments, it was observed that the application of biochar and nitrogen fertilizer increased the abundance of *acdA* functional genes, and decreased the abundance of *β-gaL* and *GA* functional genes. These findings align with those of other studies[69,70]. The observed functional gene abundance changes may be attributed to the interactions between biochar and nitrogen substrates, or the inhibitory effect of small molecules released by biochar, such as phenols and polyphenols. In the context of the carbon cycle, *β-gaL* functional gene is involved in the catabolism of galactoside-containing compounds, enabling the carbon within these substances to enter microbial metabolic pathways and subsequently participate in the ecosystem's carbon cycling processes. Biochar provides a substantial carbon source, and microorganisms preferentially utilize the carbon from biochar, reducing their reliance on galactoside-containing compounds metabolized by the *β-gaL* functional gene. This leads to decreased expression and abundance of theβ-galactosidase genes[71,72], Additionally, the high C:N rates and aromatic carbon content in biochar alter the soil environment[73]. such as by increasing soil pH. The expression of the *β-gaL* functional gene is sensitive to pH, and higher pH levels may inhibit the growth of related microorganisms and the expression of the *β-gaL* functional gene. This is further supported by the significant negative correlation between the abundance of *β-gaL* functional gene and soil pH (Fig. 6).

Microbial communities tend to balance their elemental requirements through selective degradation under varying environmental conditions[2]. Changes in the soil environment may enhance or inhibit the activity of specific microorganisms, leading to shifts in soil microbial community structure. In conclusion, the combined application of biochar and nitrogen fertilizer can also alter the dynamics of soil microbial communities, ultimately influencing the decomposition rate and storage of soil carbon.

Conclusion

This study demonstrates that biochar and nitrogen fertilizer application not only improved soil properties and nutrient retention capacity, but also enhances the activity of beneficial microorganisms engaged in carbon cycle. Specifically, biochar application increases soil pH and soil organic carbon content. The synergistic effect of biochar and nitrogen fertilizer leads to an increase in carbon emissions, particularly a 0.73% to 232.43% rise in CO_2–C. This is attributed to the stimulation of microbial activity, which accelerates the degradation of organic carbon. Following the application of biochar and nitrogen fertilizer, the dominant soil functional microorganisms are primarily those involved in carbon degradation. Among these, *Bradyrhizobium* and Streptomyces are identified as key genera promoting carbon decomposition. Conversely, the activities of *β-gal* and *GA* functional microorganisms are inhibited, which in turn affects the soil carbon decomposition rate. In summary, the combined application of biochar and nitrogen fertilizer can improve soil health by increasing soil pH, enhancing microbial activity, and alleviating soil acidification. However, there are also drawbacks, such as increased carbon emissions and the inhibition of certain microbial activities. Additionally, since this study was conducted through pot experiments, there are inherent differences compared to actual field conditions, and the practical application effects may vary. Therefore, when promoting and implementing this technology, these factors must be carefully considered. Future research should also focus on evaluating the application effects in different ecological environments to ensure broader applicability and effectiveness.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

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