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Effects of biochar and nitrogen fertilizer on microbial communities, CO_2 emissions, and organic carbon content in soil

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This study examined the effects of biochar and nitrogen fertilizer application on CO_2 emissions, microbial communities, and soil organic carbon (SOC) in irrigated wheat fields through a 3-year field experiment. Eight treatment groups were established for this study: (1) CK, without fertilizer or biochar, (2) N1 group, with nitrogen fertilizer application (300 kg/ha), (3) B group, with biochar application (20 t/ha), (4) BN1 group, with nitrogen fertilizer and biochar application, (5) N2, with a 15% reduction in nitrogen fertilizer (255 kg/ha), (6) BN2, with a 15% reduction in nitrogen fertilizer + biochar. (7) N3, with a 30% reduction in nitrogen fertilizer (210 kg/ha); and (8) BN3, with a 30% reduction in nitrogen fertilizer + biochar. The results revealed an increase in active organic carbon (AOC) and SOC contents of soil after the addition of biochar and N fertilizer, particularly with their combined application. In the BN2 treatment, SOC and AOC contents reached 27.48 g/kg and 1.47 g/kg, representing increases of 3.04% and 30.91%, respectively, compared to N1. In comparison to CK, cumulative CO_2 emissions increased by 9–48% with the addition of both biochar and nitrogen fertilizer, possibly due to biochar's influence on the composition and functional diversity of soil microbial communities. The functional diversity of soil microbes in the BN1 group differed significantly from that in CK ($p < 0.01$). In the B group, soil microbial attributes were lower than those in BN1, BN2, and BN3 groups. Furthermore, the bulk density of biochar-amended soil was 0.19 g/cm^3 lower than that of untreated soil in CK. Overall, the combination of biochar application and a nitrogen dose of 255 kg/ha emerged as the most effective strategy for irrigated wheat fields in northern Xinjiang, enhancing SOC content while reducing carbon emissions. However, further research is required to assess the long-term effects of this approach on soil health and sustainability.

Keywords Biochar, Soil organic carbon, CO_2 emission, Nitrogen

Fertilizers are introduced into the soil to provide nutrients for plant growth. Chemical fertilizers are widely used due to their efficiency and convenience, significantly increasing crop yields and ensuring stable agricultural production[1]. However, extensive application of chemical fertilizers causes a nutrient imbalance in agricultural soil, leading to a lower content of organic matter[2]. These changes negatively affect the physiochemical and biological characteristics of soil, resulting in lower soil fertility[3]. Thus, continuous application of chemical fertilizers affects crop yield by degrading soil quality[4]. Biochar application to soil is considered a crucial strategy to resolve the environmental issues associated with current agronomic practices, including the overuse of chemical fertilizers[5]. Various research studies recommend biochar application as an effective approach to improving soil quality and enhancing crop production[6,7]. Biochar not only promotes the sequestration of carbon but also enhances the soil fertility. Studies have reported that the combined application of biochar and nitrogen fertilizer or a nitrogen-containing nutrient can improve crop productivity and enhance nitrogen-use efficiency[7–9]. Biochar contributes to carbon stabilization by influencing soil biological properties, including enzymatic activities, microbial communities, and respiration[10], which significantly influence the dynamics of soil organic carbon (SOC). Biochar application may also alter the chemical characteristics of soil, such as pH[11] and cation exchange capacity (CEC), thereby enhancing nutrient retention[12–14]. However, soil microbes, including fungi and bacteria, are highly sensitive to environmental changes caused by biochar[15], making it essential to assess its impact on microbial communities due to their crucial role in soil processes[16]. For instance, soil microbes are required

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for complete decomposition of organic matter in soil[17]. The role of biochar in regulating soil carbon storage, including microbial biomass carbon (MBC) and soil organic carbon (SOC), along with its influence on CO_2 emissions, remains a topic of debate. Moreover, determining its effectiveness in enhancing SOC sequestration is essential. However, previous studies have demonstrated both adverse and positive impacts of biochar on SOC storage[18,19]. Therefore, a thorough understanding of its influence on SOC mineralization is essential before considering its widespread use as a soil additive.

Based on the findings derived from previous studies, a 3-year field experiment was carried out in northern Xinjiang, China, to assess the effects of biochar and nitrogen fertilizer on soil dynamics. The study aimed to (1) investigate the changes in soil respiration rate after biochar application with varying concentrations of nitrogen fertilizers; and (2) determine the effect of the interaction between nitrogen fertilizer and biochar on microbial community, SOC content, and soil characteristics. Further, CO_2 emission levels were determined at different application rates of biochar and nitrogen fertilizer. This study hypothesized that the combined application of biochar and nitrogen fertilizers alters soil microbial dynamics and respiration processes, with the extent of these effects depending on the application rates. If biochar application is found to increase CO_2 emissions while simultaneously enhancing SOC levels, its potential as a soil management strategy could be more significant than previously anticipated. The results of this study could contribute to the development of effective agronomic measures to reduce CO_2 emissions while improving the N consumption efficiency in irrigated croplands.

Material and methodology

Experimental site

The field experiment was conducted at the Qitai Wheat Test Station in Xinjiang (89° 13′ to 91° 22′ E; 42° 25′ to 45° 29′ N) (Fig. 1). This region has a continental temperate climate, with an annual average temperature of 5.5 °C. In July and January, the average temperatures are 22.6 °C and − 18.9 °C, respectively, while the yearly maximum can reach approximately 39 °C. The region has a mean annual relative humidity of 60% and an average of 153 frost-free days, ranging from late April to early October. The annual average rainfall is 269.4 mm. The test site has sandy loam soil, with 15.15 g/kg organic matter in the 0–20 cm soil layer. The basic properties of soil were as follows: pH 8.2; SOC content of 8.79 g/kg; total nitrogen content of 0.93 g/kg; available phosphorus content of 7.10 mg/kg; and available potassium content of 35.1 mg/kg.

Experiment and treatment groups

A total of 24 plots, each measuring 35 m^2, were established at the research station following a completely randomized factorial design. The experiment consisted of 8 treatment groups: (1) CK, control group without fertilizer or biochar; (2) N1 group, with nitrogen fertilizer application (300 kg/ha); (3) B group, with biochar application (20 t/ha); (4) BN1 group, with nitrogen fertilizer and biochar application; (5) N2, with 15% reduction in nitrogen fertilizer (255 kg/ha), (6) BN2, with biochar + 15% less nitrogen fertilizer; (7) N3, with 30% reduction in nitrogen fertilizer (210 kg/ha and (8) BN3, with biochar + 30% less nitrogen fertilizer. Each group had three replicates. Urea served as the nitrogen fertilizer, with a recommended application rate of 300 kg N/ha for the region. Initially, biochar was manually spread over the designated plots in a single application before sowing. It was then thoroughly integrated into the 0–30 cm soil layer using a rotary cultivator to ensure uniform

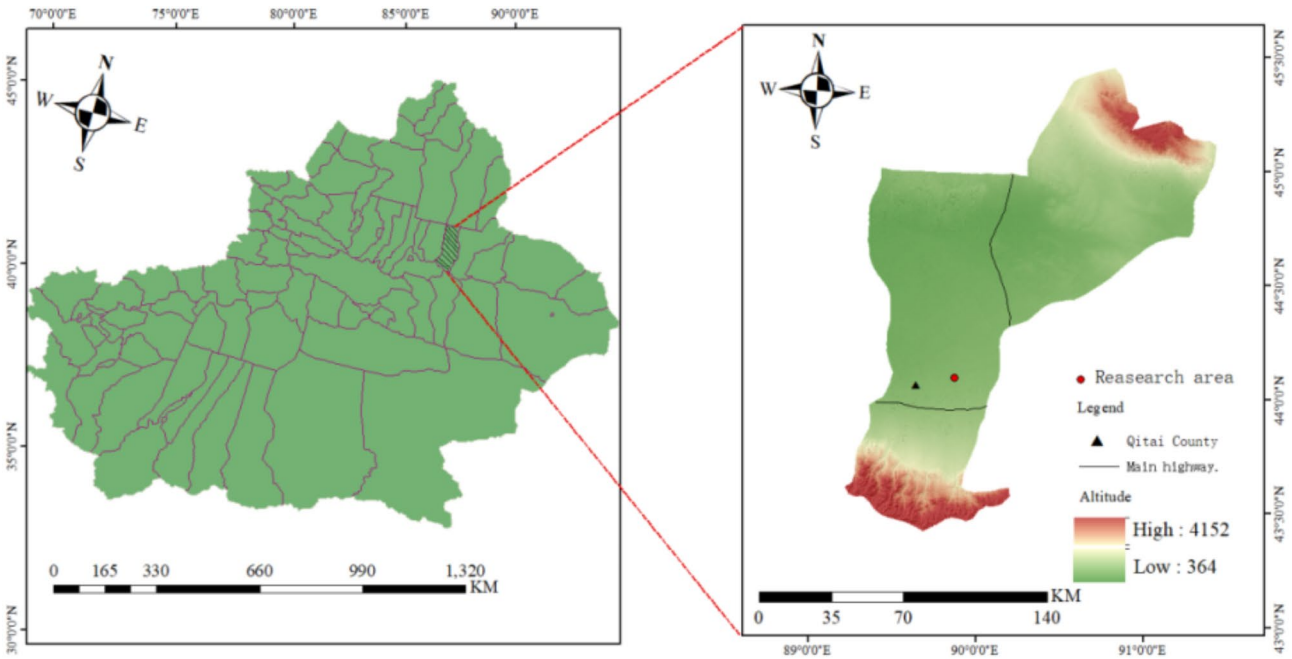

Fig. 1. Study site, with a green area representing the Xinjiang Uygur Autonomous Region of China.

distribution. Biochar was applied only once at the start of the experiment and was not reapplied in the next 2 years. Urea application (46% pure nitrogen) was performed once in the designated plots. Strip sowing was carried out at a density of 4.5 million plants per hectare, with a row spacing of 20 cm. All other agricultural practices followed the standard protocols for high-yield farmland in the region.

Biochar application to the soil was performed by Jinhefu Shenyang Agricultural Technology Development Corporation, China. The biochar used in this study was prepared through the pyrolysis of corn straw for 4 h at 450 °C, in the absence of oxygen. Its key properties included a surface area of 0.8 m^2 g^{-1}; mean particle diameter of 0.004–3.7 mm; ash content of 45%; total carbon content of 59.84%; C/N ratio of 38.59; and pH of 9.3. Before application, biochar was sieved using a 2-mm sieve.

Soil sampling

After harvesting in July, five soil cores were randomly collected from each plot to a depth of 30 cm. The samples were immediately placed in a cooler and stored at 4 °C until further processing. Subsampling of moist soil was performed for all assessments. The bulk density of the soil was calculated as the dry mass per unit volume of the cylinder. Since the soil contained no stones, the total bulk density is reported in this study[20]. The pH of the soil samples was measured using a 2:1 water-to-soil mixture[21]. CEC was determined by using ammonium acetate extract, while the sum of cations was expressed as the base saturation[22]. A field kit was employed to determine the active organic carbon (AOC) content in soil using a modified permanganate method[23,24]. Hach colorimeter (Hach Company, Boulder, CO) was used to measure the absorbance of samples at 550 nm. The decrease in the purple permanganate color reflected the oxidizable carbon content in the soil samples, with greater color loss indicating a higher oxidizable carbon concentration[25]. The total SOC content was measured following the method described by Jones and Willett[26]. Briefly, soil samples were extracted with distilled water (soil-to-water ratio: 1:5) for 30 min under agitation at 230 rpm. The mixture was centrifuged at 4000 rpm, filtered through a 0.45-µm membrane, and analyzed using a multi-N/C analyzer (Analytik Jena 3100, Germany) to quantify the total SOC content[27].

Bacterial and fungal isolation and cultivation

The drop plate process was employed to determine the bacterial colony forming units (CFUs)[28]. The soil suspensions were diluted tenfold to prepare three samples, and five drops (10 µL each) were placed on TSB agar plates (3 g/L). Two plates were prepared for each soil sample, dried, inverted, and incubated at 15 °C. After 36 h and 48 h of incubation, bacterial colonies were counted by Leica MZ6 modular stereomicroscope while the viable fungi were quantified by inoculating soil suspensions on wort agar plates, supplemented with streptomycin[29,30].

Analysis of the functional diversity of soil microbes

To assess the functional diversity of microbes in soil samples, BIOLOG ECO well plates (Hayward, USA) were used[31]. Soil (2.0 g) was added to 18 mL of disinfected NaCl solution (0.87% w/v) and allowed to shake for 20 min. Aliquots (150 µL) of this solution were then introduced into the wells of a BIOLOG ECO plate and incubated at 15 °C in the absence of light. Absorbance in each well was measured at 590 nm using a plate reader (EL808, BioTek) over 8 days at regular intervals (18, 44, 68, 89, 115, 138, 182, and 206 h).

Determination of CO_2 emissions from the soil

Three days before the first emission measurement, three polyvinyl chloride (PVC) collars (10 cm diameter, 5 cm height) were vertically inserted 5 cm into the soil between plant rows in each plot. To prevent the leakage of gas, the soil around each collar was pressed tightly. CO_2 emissions from the PVC collars were measured weekly between 9:00 and 11:00 a.m. using an automated CO_2 flux system (LI-8100, LI-COR Inc., USA) throughout the wheat growing season. During CO_2 flux measurements, stem thermometers were placed near the PVC collars to measure the temperature of soil at a depth of 5 cm.

The total CO_2 emissions over the growth period were obtained by integrating daily fluxes. For days without measurements, fluxes were interpolated using the mean of the two nearest measurement days and scaled by the respective time duration. The yield-wise CO_2 emissions were determined by dividing the cumulative CO_2 emissions by the yield of crop[32].

Calculations

The average well color development (AWCD) of BIOLOG ECO plates represents the microbial capacity to metabolize carbon sources, serving as a key indicator of functional diversity and activity in the soil. AWCD values were calculated as follows:

$$AWCD = \sum (C - R) / 31 \tag{1}$$

where C represents the absorbance of soil suspension in each well of plate, while R denotes the absorbance observed for the control well.

AWCD values reflect the microbial activity in the soil samples, while diversity indices describe microbial community composition, species distribution, and functional diversity. The commonly used diversity indices include the Simpson, McIntosh, and Shannon indices.

Mclntosh index (U), which reflects the homogeneity of the microbial community, was calculated as follows:

$$\mathrm{U} = \sqrt{\sum \mathrm{n}_\mathrm{i}^2} \tag{2}$$

Simpson index (D) reflects the dominant species in the microbial community which can be calculated by using the following formula:

$$D = 1 - \sum p_i^2 \tag{3}$$

Shannon index (H) represents the richness of species in a microbial community and can be calculated as follows:

$$H = -\sum p_i \ln p \tag{4}$$

where P is the ratio of absorbance in i well to the total absorbance across all wells.

Statistical analysis

Statistical analyses of data were performed using SPSS 21.0 (SPSS Incorporation, USA). Data were expressed as means ± standard error (SE) based on triplicate measurements for each treatment group. The differences between the measured parameters of treatment groups were assessed by one-way analysis of variance (ANOVA). The significance of variations was examined through least significant difference (LSD) analysis at a confidence level of 95%.

Results

Soil respiration and SOC content

Biochar application influenced the soil respiration rate, with most treatments demonstrating a sharp decrease in CO_2 release during the first 7 days, followed by a stabilized release (Fig. 2b). Biochar and nitrogen fertilizer application significantly affected the cumulative C mineralization ($p < 0.01$). Cumulative emissions were higher in BN1 and N3 upon comparison with CK and B groups (Fig. 2a). The combined application of biochar and nitrogen fertilizer increased cumulative CO_2 emissions by 9–48% relative to CK (Fig. 2a). Similarly, the effect size distribution for AOC and SOC showed a consistent pattern across all observations. The changes in SOC

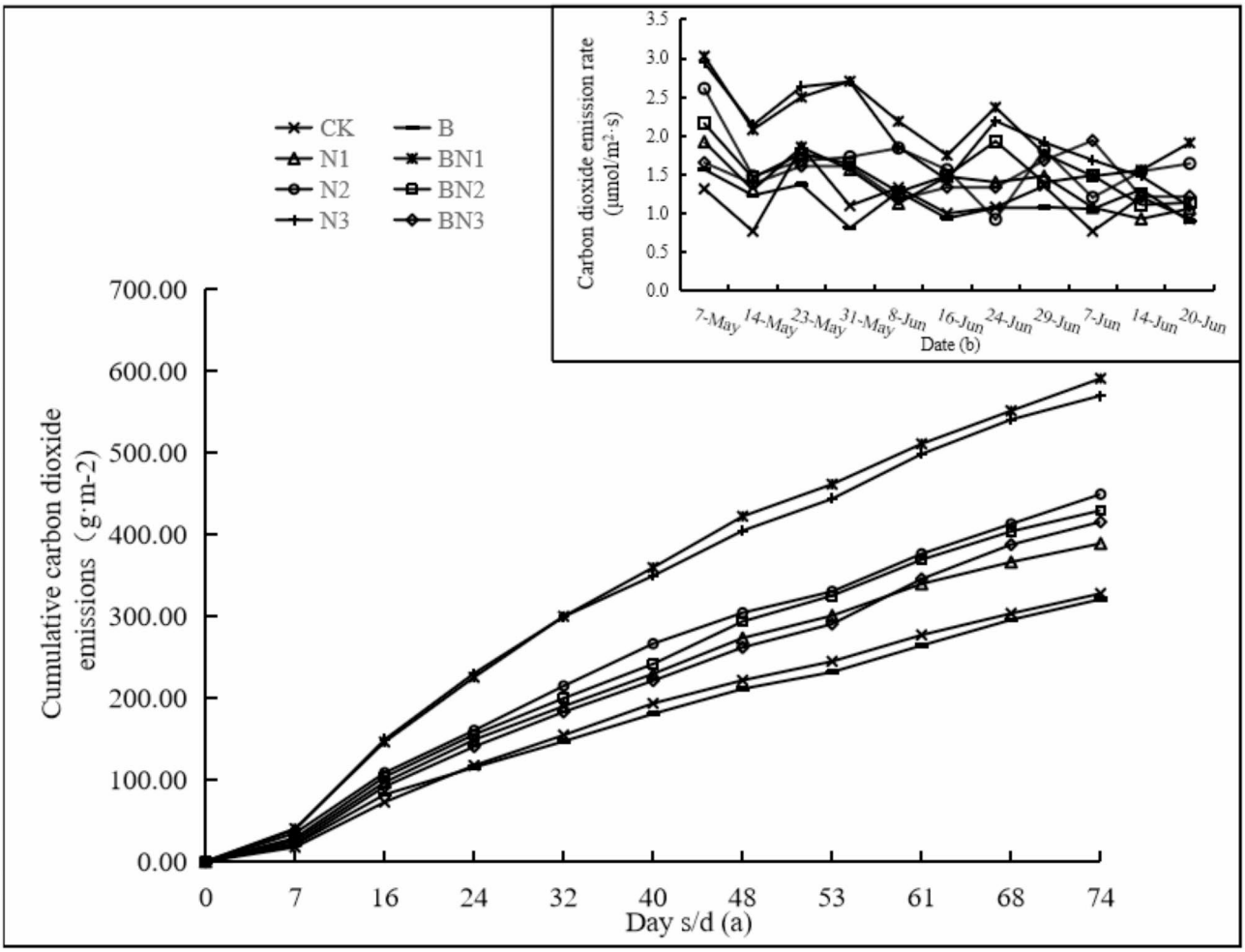

Fig. 2. Changes in cumulative CO_2 emissions (**a**) and soil respiration rate (**b**).

content were consistent, showing a significant increase after biochar application (Fig. 3). The combined influence of nitrogen fertilizer and biochar application was more pronounced for SOC and AOC ($p < 0.01$).

Composition of the microbial community in soil

The application of biochar significantly influenced microbial functional diversity (Fig. 4). Compared to CK, BN1 showed higher AWCD values ($p < 0.01$), followed by BN3, BN2 and B groups. These differences became more pronounced in the later stages of incubation (after 72 h). Furthermore, Shannon and McIntosh indices were significantly affected by the application of biochar ($p < 0.01$) (Table 1). The interaction between nitrogen fertilizer and biochar played a key role in determining bacterial abundance and the bacteria-to-fungi ratio in the soil microbial community (Fig. 5). Compared to the groups with combined application of biochar and nitrogen fertilizer, microbial community attributes were lower when only biochar was applied (Fig. 4, Table 1).

Effects of treatments on the soil properties

Electrical conductivity, bulk density, and pH of soil under various treatments are presented in Table 2. Biochar application reduced soil bulk density, with a consistent decrease of 0.19 g/cm^3 compared to CK[3]. Soil bulk density increased when biochar was applied alongside nitrogen fertilization (BN1, BN2, BN3). Similar to bulk density, soil electrical conductivity also showed an increasing trend. Moreover, biochar application led to a slight change in soil pH compared to CK.

Discussion

Soil CO_2 emission and SOC content were substantially influenced by the combined application of N fertilizer and biochar (Figs. 2 and 3). The increase in CO_2 emissions following biochar application may be due to the expansion of variable SOC pools and the mineralization of indigenous SOC, leading to enhanced production of CO_2[33–35]. The decrease in soil CO_2 emissions after biochar application may be due to biochar adsorbing rhizodeposits and enzymes, which inhibited the activity of carbon-degrading microbes and reduced the priming effect on native SOC[36–38]. Maucieri et al.[39] suggested that the decrease in CO_2 emissions observed after biochar application may be associated with the decreased available N in soil due to microbial biomass and immobilization. According to Xiao et al.[40], variations in CO_2 emissions following biochar and nitrogen fertilizer application depend on the nitrogen fertilizer application rate. The observed decrease in CO_2 emissions following

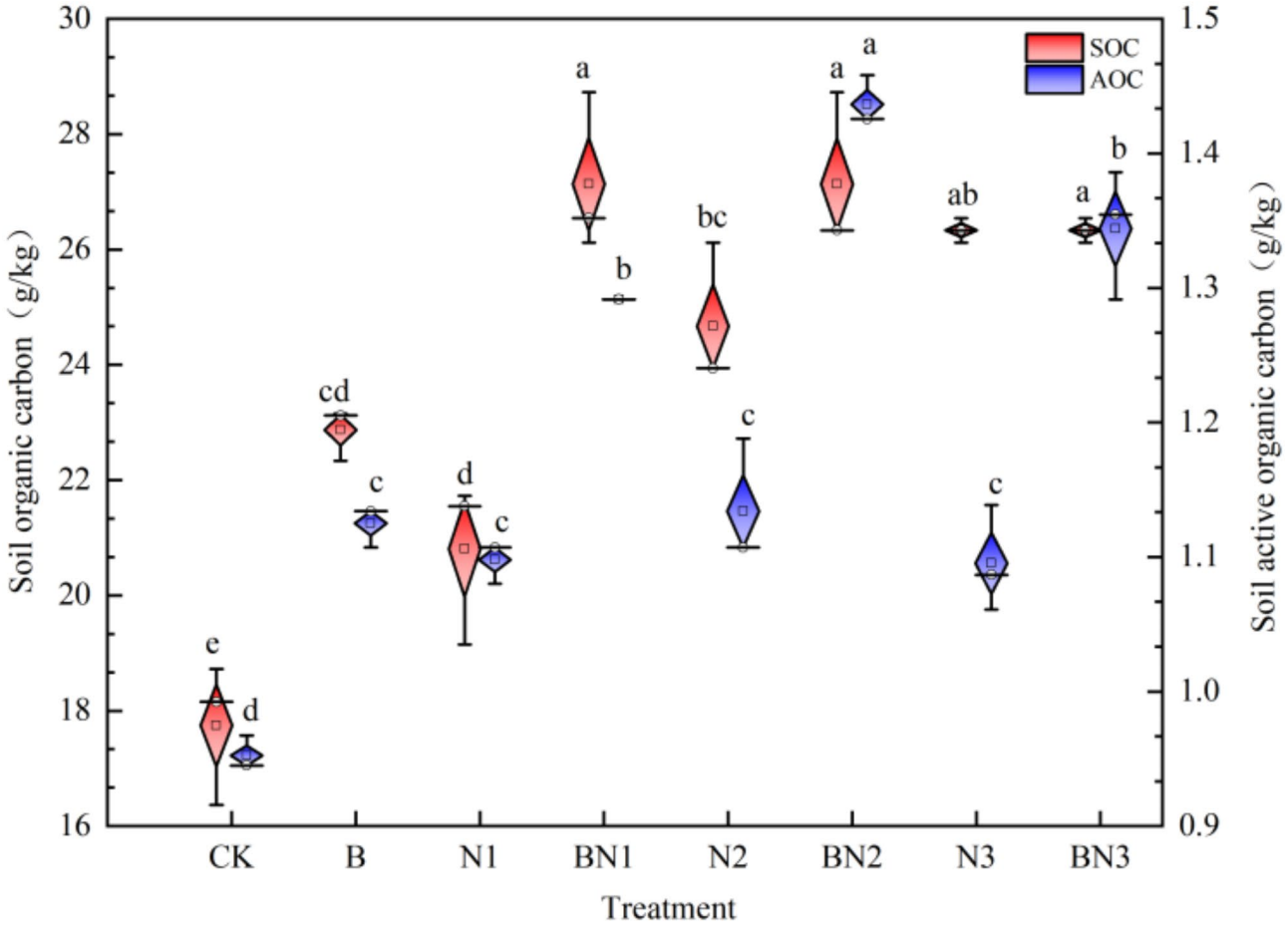

Fig. 3. Impacts of biochar and N fertilization on SOC and AOC contents.

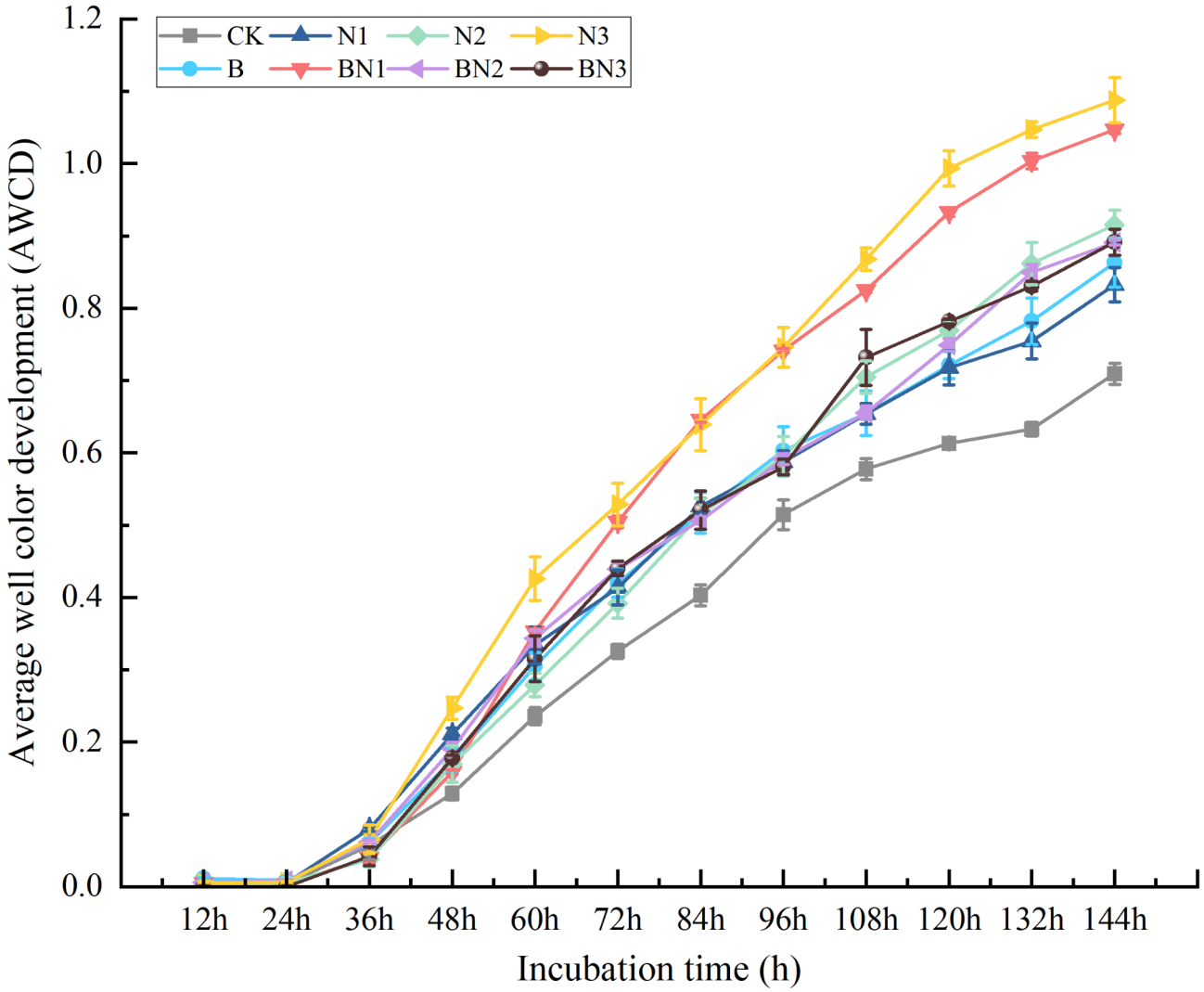

Fig. 4. Changes in the AWCD values indicating the functional diversity of soil microbes.

Treatments	Simpson index	Shannon index	McIntosh index
CK	0.94 f	2.95 d	4.80 f
B	0.94 cd	3.02 c	5.32 e
N1	0.94 e	2.96 d	5.45 e
BN1	0.95 a	3.11 a	6.54 b
N2	0.94 de	3.00 c	5.86 c
BN2	0.95 bc	3.03 bc	5.41 e
N3	0.95 ab	3.07 b	7.08 a
BN3	0.94 c	3.03 bc	5.72 d
F			
B	1.00	0.30	0.83
N	1.63	0.94	0.83
B × N	25.38**	18.36**	282.01**

Table 1. Functional diversity indexes of the microbial communities in soil across different groups. Different lowercase letters within a column indicate significant differences between groups ($p < 0.05$), with * and **indicating significant levels of 0.05 and 0.01.

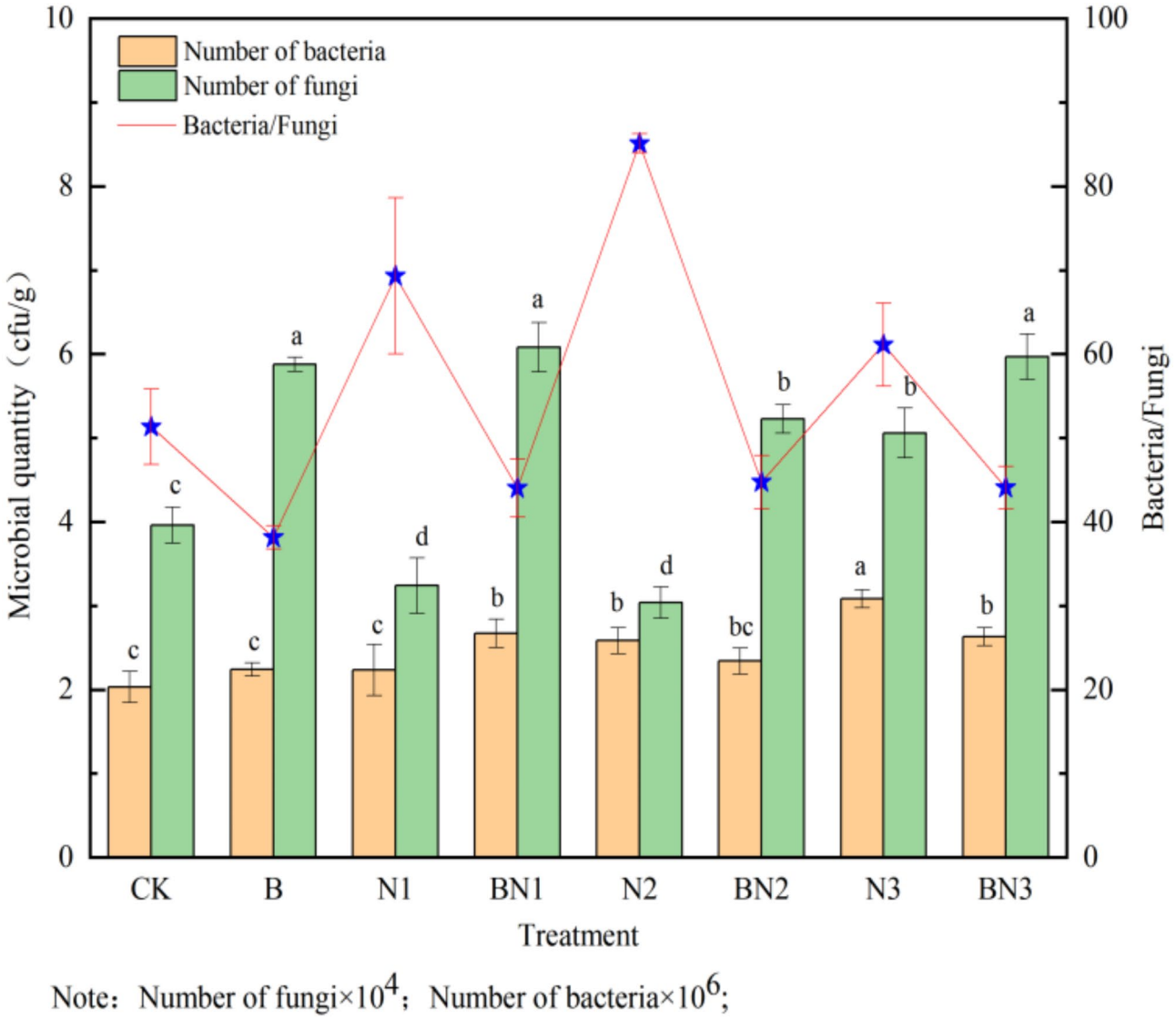

Fig. 5. Impacts of biochar and N fertilization on the number of bacteria and fungi in soil. Different lowercase letters indicate significant differences among groups ($p < 0.05$).

biochar application suggested SOC immobilization and decreased microbial activity due to nutrient adsorption on the biochar surface. Buss et al.[41] also reported the decrease in CO_2 emissions attributing it to the adsorption of DOC on the biochar surface or the formation of soil aggregates that protect SOM from decomposition[41]. Other possible reasons include the mineralization and immobilization of N, and variation in soil microbial populations. Furthermore, the increase in CO_2 emissions induced by biochar application is likely associated with a reduction in soil bulk density and enhanced microbial activity. Microorganisms play a critical role in the cycling of materials and energy flow in the soil. In addition to influencing biochemical cycles[42], they also contribute to biochar stabilization in the soil[43]. Biochar can directly promote microbial growth and activity by providing favorable conditions[15] and indirectly by enhancing soil aeration, moisture retention[44], and chemical characteristics[45]. Moreover, biochar influences microbial biomass and activity, modifies the fungal-to-bacterial ratio, alters enzyme activity, and restructures the soil microbial community. Due to its aromatic structure, biochar is resistant to microbial decomposition, though certain biochar types can serve as a carbon source for microbial growth. Biochar is generally recalcitrant to microbial degradation due to higher C/N compared to the feedstock. The limited nitrogen availability further restricts the microbial decomposition of biochar. Therefore, biochar application contributed to an increase in the SOC pool and enhanced soil carbon sequestration. Bacteria and fungi prefer different carbon sources and exhibit varying levels of tolerance for changes in environmental conditions, such as pH and moisture content. Compared to bacteria, fungi can colonize larger soil aggregates, with higher C/N and total N, providing a survival advantage for fungi. Under similar environmental conditions, biochar application favored fungal growth by promoting the formation of larger soil aggregates. The observed changes in the fungal-to-bacterial ratio in this study may be associated with alterations in soil C/N following biochar addition or the initial C/N ratio of untreated soil. These findings suggested that nutrients within biochar,

Treatments	Electrical conductivity (μS/cm)	pH	Bulk density (g/cm³)
CK	3.19×10^2e	8.2 b	1.44 ab
B	3.14 × 102f	8.3 b	1.25 d
N1	2.97×10^2g	8.3 ab	1.47 ab
BN1	4.03×10^2c	8.2 b	1.41 bc
N2	4.89×10^2a	8.3 ab	1.41 bc
BN2	3.99×10^2d	8.3 ab	1.36 c
N3	2.85×10^2h	8.4 a	1.48 a
BN3	4.16×10^2b	8.3 b	1.41 bc
F			
B	2.46	0.74	18.92*
N	1.79	5.36	11.01*
B × N	12,418.17**	3.95*	0.34

Table 2. Bulk density, pH, and electrical conductivity of soil in different groups. Different lowercase letters within a column indicate significant differences between groups ($p < 0.05$), with * and **indicating significant levels of 0.05 and 0.01, respectively.

including both inherent nutrients and those absorbed from the soil, support microbial growth and activity. Further, the fungal hyphae and bacterial cells colonized into the inner and outer pores of biochar contribute to spatial heterogeneity. With the increase of biochar aging, the decline in its pH may enhance fungal adaptation and colonization, influencing microbial community composition in the soil. Soil bulk density influences its structural stability, water and nutrient transport, and aeration[46,47]. Several studies have revealed the decline in the bulk density of soil is associated with biochar application[48–50]. Niu[51] and Kang[52] observed a decrease in the bulk density of upland soil after biochar application. The findings of this study align with those of Zhang[53], who reported a significant reduction in soil bulk density, ranging from 0.02 to 0.17 t/m^3 under conventional fertilization and 0.03–0.20 t/m^3 under balanced fertilization, following biochar application at rates of 0, 20, and 40 t/ha in a maize field. Azeem et al.[54] reported that biochar addition (0, 5, and 10 t/ha) decreased the soil bulk density in a wheat-mash bean crop system, with no significant influence on fertilization.

CEC and pH are crucial soil factors affecting nutrient availability and retention. Studies have shown that biochar application increases soil pH and CEC, enhancing nutrient availability and supporting stable crop productivity[55–57]. Major et al.[58] reported higher soil pH after biochar treatment with an application rate of 20 t/ha at 30 cm depth, as compared to 0 and 8 t/ha rates. Hailegnaw et al.[59] and Zhang et al.[60] also reported an increase in soil pH with the increase in the application rate of biochar. Due to its inherently high pH, biochar application generally increases soil pH. However, the extent of this change varied depending on field conditions, such as soil properties and seasonal variations. Wu et al.[61] reported no significant change in CO_2 emission in the alkaline soil after the application of olive biochar, while acidic soil was significantly influenced. These trends were associated with the presence of labile compounds in olive biochar, which undergo rapid mineralization in acidic soil. While biochar had a minimal effect on soil pH, it significantly increased CEC, likely due to its high specific surface area[62,63].

When combined with external fertilizers, biochar enhances carbon fixation and limits carbon transfer, contributing to greater soil carbon retention[64]. The combined application of biochar and fertilizers (e.g., organic or P/N fertilizers) increased nutrient availability in the soil, thereby enhancing plant growth[65]. Lou et al.[66] reported that combining biochar with soil fertilization not only enhanced carbon sequestration and reduced carbon emissions but also promoted crop growth by increasing soluble organic matter production and nitrogen mineralization. Meanwhile, biochar affects the soil microbes, which are key regulators of organic matter conversion and nutrient cycling in soil[67]. Biochar influences microbial communities both directly and indirectly by modifying abiotic factors such as C availability and pH of soil[68,69]. Furthermore, it alters soil pore size distribution, which in turn affects microbial composition and habitat conditions. Elbasiouny et al.[70] reported that biochar application gradually released nitrogen and carbon due to its high content of recalcitrant organic carbon, with the release rate influenced by soil conditions regulating microbial biomass. Therefore, optimizing biochar application rates based on environmental factors is essential for enhancing soil fertility and crop productivity.

Conclusions

In this study, a 3-year field trial was performed to study the effects of applying biochar to soil, in conjunction with N fertilizer. The combined application of biochar and nitrogen fertilizer enhanced SOC content, while in the irrigated wheat fields of North Xinjiang, applying biochar (20 t/ha) with a lower nitrogen dose effectively increased SOC levels and decreased the emissions of CO_2. These results provide valuable insights regarding sustainable agricultural practices, particularly the benefits of biochar application.

Data availability

Data is provided within the Related files. All data generated or analysed during this study are included in this published article.

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Author contributions

The present report was conducted by ZLY, WZ under the guidance and supervision of YWJ. The studies were

carefully selected, and the data was subjected to analysis by YWJ, ZLY, WZ, and ZJS. YWJ, ZLY drafted the report and subsequently evaluated and reviewed it by YWJ, LPY, and SLL. All the authors approved the final version of this study.

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Declarations

Competing interests
The authors declare no competing interests.

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