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Biochar promotes soil aggregate stability and associated organic carbon sequestration, and regulates microbial community structures in Mollisols from Northeast China

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Abstract: Since the 1950s, heavy plowing of Mollisols, combined with a lack of organic matter intake, has resulted in severe soil degradation in Northeast China. The use of biochar in combination with fertilizer is a sustainable method of improving soil quality. In this paper, we conducted field experiments to explore the response of the stability mechanism of the soil aggregates, the dynamic properties of organic carbon, and changes in the microbial community structure to biochar. The biochar input levels were C1, C2, and C3 (9.8, 19.6, and 29.4 Mg·ha^{-1}, respectively), while the nitrogen (N) fertilizer rates were N1/2 (300 kg·ha^{-1}) and N (600 kg·ha^{-1}). The field test showed that the C2N treatment increased the aggregate contents of the > 2 mm and 0.25–2 mm fractions by 56.59 and 23.41%, respectively. The mean weight diameter increased by 41.53%, while the geometric mean diameter increased by 21.62%. The organic carbon content of large aggregates shows a greater increase, with an average of 28.14%. The phospholipid fatty acids analysis revealed that bacteria (B) were the most prevalent organisms in the soil, followed by fungi (F). The C3N

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treatment increased the F/B ratio by 36.46%, whereas the C3 treatment increased the gram-positive (Gm^+)/gram-negative (Gm^-) ratio by 19.67%. We concluded that the response of Mollisols to biochar is primarily determined by the interplay of aggregates, organic carbon, and microorganisms. Based on the sequestration of SOC and the sustainability and stability of the ecosystem, we selected the optimal ratio for biochar and N fertilizer application and provide a scientific basis for the sustainable utilization of Mollisols resources.

Keywords biochar · nitrogen fertilizer · aggregate stability · organic carbon · microbial community · Mollisols

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1 Introduction

Mollisols, considered the world's high-yield soils, are typically found in the northern and southern hemispheres in mid-latitudes and constitute about 7% of the world's soil resource base ((Zhang et al. 2018; Eswaran et al., 2011). However, Mollisols have been significantly degraded as a result of intensive, continuous cultivation and soil erosion, which leads to the destruction of the soil ecosystem as well as a vicious cycle of increased poor, with profound implications for global climate change (He et al. 2021; Antonello et al. 2019). Mollisols in China are mainly distributed in Heilongjiang and Jilin provinces, as one of the world's four major black soil regions, which has always been China's most important food production base (Mei et al. 2021; Zhang et al. 2018). The organic matter content of the Mollisols in Northeast China decreased by 30–50% from 1980 to 2011, which directly threatened the stability of the regional grain yields (Li et al. 2016). The principal manifestations of the decline in soil fertility and quality deterioration were a significant loss of soil organic carbon (SOC), a decrease in soil aggregation (Zhang et al. 2018), and degradation of soil structure (Luo et al. 2020; Zhang et al. 2019). The climate (Bottinelli et al. 2017), tillage (Xue et al. 2019), microbial activities (Zhang et al. 2021), and SOC content, all affect the size, number, and composition of soil aggregates (Yin et al. 2018). The SOC can promote the formation of large aggregates in soils, and soil agglomeration can increase SOC storage. The interaction between carbon sequestration and aggregates stability can reduce soil nutrient loss, improve effective water holding capacity, increase crop yields, and mitigate global warming through lengthy soil carbon sequestration (He et al. 2021; Scow et al. 2017). It is critical to identify effective strategies to manage the soil in order to enhance its structure, increae its SOC content (Oksana et al. 2022; Plaza et al.

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2016). Straw return has been demonstrated to be an effective approach for promoting SOC stabilization, improving soil aggregation, and influencing the structure of microbial communities by using organic amendment to promote (Xiu et al. 2019). However, direct straw return frequently causes problems, such as creating an adverse soil environment for crop sowing and root penetration (Li et al. 2019) and increasing the number of disease-causing pests and weeds (Wang et al. 2011) during the subsequent growing season. This is especially likely in high-latitude Chinese Mollisols, where straw decomposition time is very limited. Therefore, developing proper straw returns that can increase soil productivity has been a major challenge in this context.

Biochar is produced by pyrolyzing biomass at 400–700 °C in an oxygen-depleted environment (Xiu et al. 2019; Kung et al. 2015). The method has been promoted as a win-win technology for recycling straw while also potentially improving agricultural soils (Islam et al. 2021). Biochar can enhance SOC storage, soil granular structure, cation exchange capacity, and crop yield. For example, Wang et al. (2019) discovered that biochar improved the structural stability of Latosols in southern China. The aggregate mean weight diameter (MWD) and geometric mean diameter (GMD) were improved by 36.3 and 28.3%, respectively. Furthermore, Xiu et al. (2019) investigated the effect of corn stalk biochar application dose on Albic soils in northern. They discovered that a high biochar application level reduced the bulk density of Albic soils by 9.93% while increasing the pH value. Biochar was also found to significantly improve soil granular structure and organic carbon aggregation (Li et al. 2022). Thus, biochar had a favorable influence on soil quality and aggregation in these acidic soils, which could be attributed to the liming activity of biochar treatments on those acidic soils and the neutralization of the soil pH, which consequently had a significant effect on soil

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aggregation (Islam et al. 2021). Although the effect of biochar on soil agglomeration in neutral or alkaline soils has yet to be verified, some researchers believe there is no significant effect (Zhang et al. 2015). Furthermore, due to the low quantity of biochar minerals and inorganic nitrogen, several studies have indicated that only combination application with other fertilizers can improve soil fertility (Song et al. 2020). Chen et al. (2018) proposed that an 8-year manure amendment could recover soil nitrogen supplying capacity of lightly eroded Mollisols to natural levels. Therefore, biochar combined with an organic/inorganic fertilizer has the potential to improve soil fertility (Li et al. 2020), promote plant growth (Aneseyee et al. 2021; Mete et al. 2015), and carbon storage potential (Wang et al. 2019). Fungo et al. (2017) conducted a two-year field trial in the impoverished Ultisol of western Kenya and found that biochar combined with urea increased MWD by 13%, whereas biochar alone was less effective.

Principal ecological activities including organic matter formation and breakdown, nutrient cycling, and soil aggregate size redistribution are all controlled by soil microbial populations (Chen et al. 2022; Trivedi et al. 2017). Phospholipid fatty acid (PLFAs) are the main components of living cell membranes, which play an important role in maintaining cellular fluids, nutrient transportation, elimination of metabolites, etc. Changes in their components can more accurately express the response of soil microbial biomass and community structure to environmental disturbances (Zhang et al. 2013). The structure of the microbial community is closely related to the change of soil function (E.-L et al. 2014). The higher the ratio of soil fungal to bacterial fatty acids, the more sustainable and stable the soil ecosystem (Wang et al. 2017). High Gm^+/Gm^- bacterial ratios facilitate soil organic carbon accumulation. Soil total nitrogen (TN) content is the main driver of

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variations in the community composition (Zhang et al. 2021). Wang et al. (2021) discovered that after using biochar in rice fields, the abundance of bacteria (B) and fungi (F) increased by 102 and 178%, respectively, which was likely related to an increase in soil total organic carbon (TOC), TN, and rice biomass. According to the study of Chen et al. (2018), the improvement of microbial community structure by biochar was clearly determined by the ratio of gram-positive (Gm^+)/gram-negative (Gm^-) and F/B in the paddy soil of central-southern China. In addition, Tian et al. (2016) investigated the mechanism of interaction between biochar and mineral fertilizer addition on microbial community and soil organic matter cycling in heavy loam soil. It was found that the addition of biochar alone did not significantly improve microbial community structure and that its effect on microbial community structure was dependent on fertilization. The ability of biochar and nitrogen fertilizer to stimulate microbial activity is regulated by the soil conditions and application rates (Palansooriya et al. 2019).

Soil organic carbon sequestration and microbial activity are critical for soil health and quality regulation. However, the beneficial effects of biochar on soil aggregates, associated SOC, and microbial activity have been observed primarily in nutrient-poor acidic soils (e.g. Ultisol and Albic soils), and relevant studies on Mollisols in Northeast China have been limited. Furthermore, studies on the combined application of biochar and nitrogen fertilizer are insufficient, limiting the scope of production practice and theory. Therefore, this study using the northeast Mollisols as a pilot, the objectives are to (1) explore the effects of three biochar gradients combined with N fertilizer on the size, proportion, stability, and carbon content of Mollisols aggregates; (2) explore the influence mechanism of biochar on microbial population structure and identify the major determinants for

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microbial community composition changes; (3) develop scientific and effective field management measures for Mollisols by improving the structure of soil aggregates and microbial communities.

2 Materials and methods

2.1 Site description

The field experimental site was located at the test base of the Northeast Institute of Geography and Agroecology, Jilin Province (43° 59' 51" N, 125° 24' 5" E). The annual average temperature is 4.6 °C, the precipitation is 600–700 mm, and the frost-free period during the whole year is 140–150 d. For many years, continuous maize cropping has been carried out in conventional tillage patterns. The soil of the field was classified as Mollisols (Mei et al. 2021). The experimental surface soil pH was approximately 6.06, TN was 1.26 g·kg^{-1}, available phosphorus was 26.78 mg·kg^{-1}, available potassium was 133.54 mg·kg^{-1}, and organic matter was 26.72 g·kg^{-1}. The biochar was created by pyrolyzing corn straw at 400–500 °C for 4 h under anaerobic conditions. The biochar had a mean particle diameter of 0.003–3.5 mm, a surface area per volume of 0.7 m^{2}g^{-1}, and an ash concentration of 45% (Biochar particles need to pass through a 2 mm sieve before application). Also, the biochar had a pH of 9.16, the total carbon content was 62.64%, and the C/N was 39.08. The fertilizer was high-quality urea that was produced by Erdos Yi Ding Ecological Agriculture Development Co. Ltd., the TN was ≥ 46%, and the particle size range was 1.18–3.35 mm.

2.2 Field experimental design

A split zone design was adopted for the field experiment and three biochar input levels were set: 9.8 Mg·ha^{-1} (C1), 19.6 Mg·ha^{-1} (C2), and 29.4 Mg·ha^{-1} (C3). Nitrogen was applied as a basal

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fertilizer at rates of 300·kg·N·ha^{-1} (N1/2) and 600 kg·N·ha^{-1} (N). The CK treatment was used as a control. In total, ten treatments were studied: CK, C1, C2, C3, C1N1/2, C2N1/2, C3N1/2, C1N, C2N, and C3N. Each treatment was performed on a plot with the dimensions 3.9 × 6.5 m, and each treatment plot had a 1 m buffering zone. A randomized block design was used to conduct the three replicate plots. Biochar with N fertilizer was applied to the soil in April 2013 and 2021, and corn was sown in May 2013 and 2021.

2.3 Soil bulk density and water content

On October 29, 2021, after the corn harvest was complete, soil samples were obtained from each plot using the five-point sampling method, which involved taking 1 kg of soil samples from each plot. Undisturbed soil columns were collected using a soil drill and were placed into ziplocked bags after the removal of plant and animal residues. Some of the soil was promptly refrigerated at 4 °C for PLFA measurement. A 5 mm mesh screen was used to remove the water-stable soil aggregates from the rest of the sample, which was then allowed to dry naturally. For the determination of the bulk TOC, subsamples of 2 mm soil particles were passed through a 0.15 mm filter after being air-dried. The TOC in the aggregate fractions was determined by $K_2Cr_2O_7$ titration (Chen et al. 2018). Next, the surface (0-10 cm) and bottom (10-20 cm, 20-40 cm) soils were sampled with a cutting ring (V = 100 cm^3) and dried at 105 °C for 24 h to measure the soil bulk density and water content using the following formulae:

$$X = \frac{m_2 - m_1}{m} \times 100\% \quad (1)$$

$$\rho_b = \frac{m}{V} \quad (2)$$

where X is the field water holding capacity (%), ρ_b is the soil bulk density (g·cm^{-3}), m is the

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dry soil weight (g), *v* is the cutting ring volume (cm^3), m_2 is the total weight of the cutting ring and soil after 2 h on dry sand, and m_1 is the total weight of the cutting ring and soil after drying.

2.4 Soil water-stable aggregate analysis and calculation

In this experiment, the soil aggregates were fractionated utilizing a modified version of the wet sieving method which was given by Zhang et al. (2018). The dry soil sample (100 g) was uniformly coated on automatic vibrating sleeve screens of 2, 0.25, and 0.053 mm in diameter.

The formula for calculating the mass fraction of the water-stable aggregates is as follows:

$$W_\mathrm{t} = M_\mathrm{i} M_\mathrm{t} \times 100\% \quad (3)$$

where W_t is the percentage of the component weight of the *i*th sized aggregate.

The MWD and GMD represent the size distribution of the soil aggregates. The larger the value, the higher the agglomeration degree and the stronger the stability. The formulas are as follows:

$$\mathrm{MWD} = \sum X_\mathrm{j} W_\mathrm{j} \quad (4)$$

$$\mathrm{GMD} = \mathrm{Exp}\left[\frac{\sum_{i=1}^{n}(M_\mathrm{i}\, ln\, X_\mathrm{i})}{\sum_{i=1}^{n} M_\mathrm{i}}\right] \quad (5)$$

where *j* is the aggregate size, X_j is the average diameter of the particle size, W_j is the ratio of the aggregate sample weight of each particle size on the screen, X_i is the average diameter of a size *i* aggregate, M_i is the weight of a size *i* aggregate, and M_t is the total weight of all the aggregates. The aggregate content was determined as follows:

$$R_{0.25} = \frac{M_{\mathrm{r}>0.25}}{M_\mathrm{T}} \quad (6)$$

where $R_{0.25}$ is the aggregate content (%) with an aggregate size of > 0.25 mm, $M_{\mathrm{r}>0.25}$ is the weight of the soil aggregates that are > 0.25 mm, and M_T is the total weight of all the aggregate fractions.

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The formula for the soil carbon contribution rate of each aggregate grain size is as follows:

$$C_C = \frac{w_i \times C_i}{C_s} \times 100\% \quad (7)$$

where C_C represents the contribution rate of each particle size aggregate to the carbon level in the soil sample, w_i is the weight percent (%) of the *i*-sized aggregate component, C_i is the organic carbon content of the soil aggregates at size *i,* and C_s represents the soil TOC content.

2.5 Phospholipid fatty acid analyses

The PLFA analysis is a crucial technique for identifying microbes and analyzing the community structure. It may be more responsive to changes in the relevant microbial ecology when compared to other approaches (Antonietti et al. 2009). The PLFA extraction method used in this study was described by Luo et al. (2017). The nonadecanoic acid methyl ester (19:0) was employed as an endogenous control. The identified PLFAs were classified into specific microbiota: bacteria (i15:0, a15:0, 15:0, i16:0, 16:1 ω5, 16:1 ω9, i17:0, 17:0, a17:0, cy17:0, and cy19:0); fungi (18:2ω6c and 18:3ω6c); actinomycetes (16:1ω7c, 17:1ω8c, and 18:1ω7c); Gm^+ bacteria (i14:0, a15:0, i15:0, i16:0, a17:0, and i17:0); and Gm^- bacteria (16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c, and cy19:0) (Luo et al. 2017).

The concentration of the target PLFAs in the sample was calculated as follows:

$$C_{PLFA} = \frac{F_{FLFA}}{F_{IS}} \times \frac{C_{IS}}{M_{FLFA}} \times \frac{V}{m} \quad (8)$$

where C_{PLFA} is the concentration of the target PLFA (nmol·g^{-1}), F_{PLFA} is the peak area for the PLFAs, F_{IS} is the area of the internal standard peak, C_{IS} is the internal standard concentration (25 ng·μl^{-1}), M_{PLFA} is the molecular weight of the target PLFA, V is the sample dissolution volume (120 μl), and m is the soil weight (4 g).

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2.6 Statistical analyses

IBM Statistics SPSS 22.0 software was used to test the data normality and homogeneity and conduct a principal component analysis (PCA). An analysis of variance (ANOVA) was performed to determine the significant differences between the treatments in R ($P < 0.05$). If the data did not meet the criteria, a nonparametric Kruskal-Wallis test was performed to determine the statistical significance. Canoco 5 (Windows Release 5.02 trial version) software was used for redundancy analysis (RDA), and fitting and mapping were conducted using Origin Pro 9.0.

3 Results

3.1 Soil physical properties

The biochar had a substantial impact on the soil (0–10 cm) bulk density ($P < 0.05$; Fig. 1), but its coupling effect with N fertilizer was not significant. Also, soil bulk density showed distinct regularities in all profiles and increased with soil depth. The C2N1/2 treatment had the greatest improvement effect of all treatments, and the soil bulk densities of the 0–10, 10–20, and 20–40 cm layers decreased by 13, 8, and 3%, respectively. The surface soil (0–10 cm) had the highest moisture content in the original profiled soil, while the 10–20 cm soil had the lowest water content. Additionally, there was a substantial positive relationship between biochar application amount and the soil water content in the profile ($P < 0.01$; Fig. 1), with the C3 treatment improving the most when compared to the CK. Furthermore, the soil moisture content increased by 15–35%. The two-factor ANOVA (Table S1) showed that biochar significantly improved soil water content ($P < 0.01$) and that the biochar contributed significantly to soil bulk density and water content.

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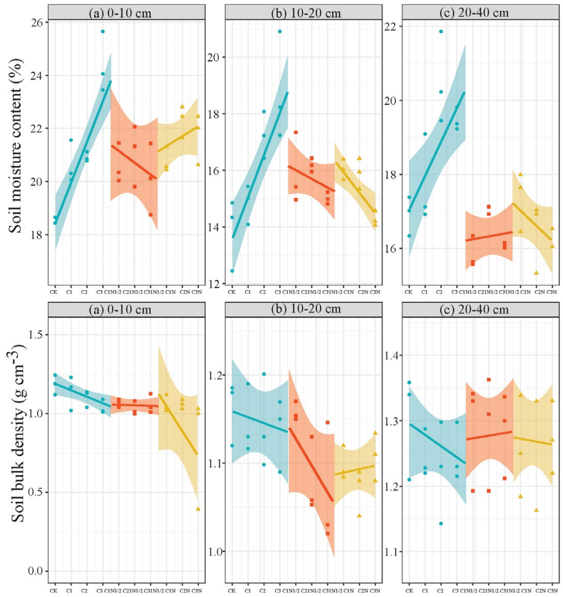

Figure 1 The effects of biochar and nitrogen fertilizer on the soil bulk density and soil moisture content in the soil profile.

3.2 Soil aggregation

The proportions of soil aggregates in descending order were as follows: microaggregates (0.053–0.25 mm), small aggregates (0.25–2 mm), silt and clay (< 0.053 mm), and large aggregates (> 2 mm; Fig. 2). First, the number of macroaggregate components was lower in the bottom soil (10–40 cm) than in the surface soil. Second, the biochar considerably increased the percentage of large aggregates (11.59–50.40%) while decreasing the percentage of < 0.053 mm aggregates (5.12–38.66%). Third, the combined application had a synergistic effect, and the proportion of macroaggregates continued to increase (38.98–56.59%) before stabilizing.

According to the interactive analyses, N fertilizer had a greater effect on the fraction of macroaggregates in the profile (Table S2). The C2N treatment increased the > 2 and 0.25–2 mm

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fractions of soil aggregates by 56.59 and 23.41%, respectively. Furthermore, the proportions of aggregates 0.053–0.025 and < 0.053 mm decreased by 4.09 and 43.64%, respectively. The C2N treatment had the highest growth rate of large aggregates within the 0-10 cm layer, which was 3.66 and 20.16% higher than that of the C2N1/2 and C2 treatments, respectively. The quantity of soil aggregates with each profile showed the same trend (Fig. 2b and c). Furthermore, as soil depth increased, the water-stable aggregates were gradually replaced with 0.053–0.25 mm sized aggregates (35.95–46.42%).

The MWD, GMD, and $R_{0.25}$ values increased significantly as the biochar addition ratios increased (Fig. 3). The increasing trend in the stability index was more noticeable after the application of biochar together with fertilizer. Additionally, the $R_{0.25}$ values of the 0–10, 10–20, and 20–40 cm soil layers increased by 30.33, 57.90, and 17.70%, respectively, and the MWD increased by 28.22, 50.37, and 46.01%, respectively in this treatment. The GMD then increased by 18.32, 29.43, and 17.71%, respectively.

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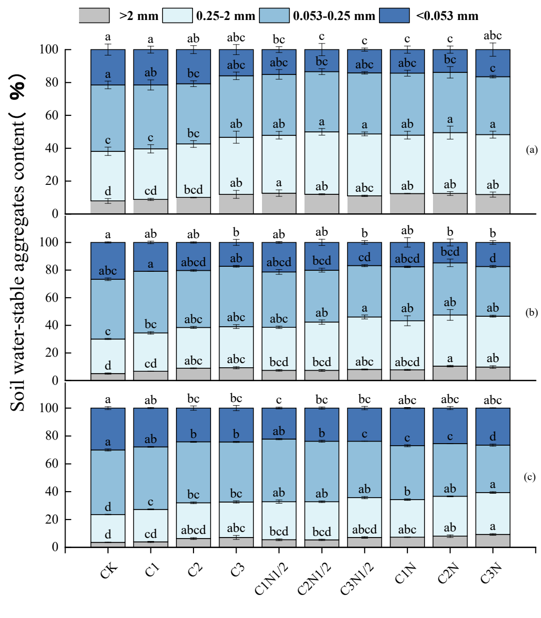

Figure 2 The size distribution of the soil aggregates at 0–10 cm (a), 10–20 cm (b), and 20–40 cm (c). The letters indicate significant differences among various treatments ($P < 0.05$). The bars indicate the standard error.

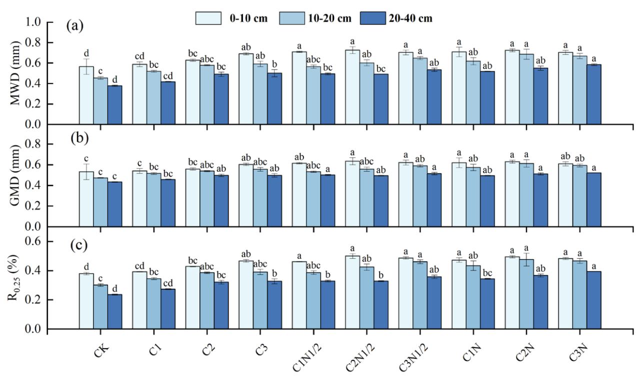

Figure 3 The aggregate content with an aggregate size of > 0.25 mm ($R_{0.25}$), mean weight diameter (MWD), and geometric mean diameter (GMD) of the soil aggregates under different treatments. The letters indicate significant differences between the various treatments ($P < 0.05$). The bars indicate the standard error.

3.3 Total organic carbon distribution in the bulk soil and aggregate fractions

The average TOC content of the surface layer was 20.26% higher than that of the 20–40 cm

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soil layer (Fig 4). The TOC content was significantly correlated with the application rates of the biochar and nitrogen fertilizer (P < 0.01). Among all the treatments, the C3N treatment in comparison to the CK resulted in the greatest increase in organic carbon content, and the TOC increased by 35.59, 30.62, and 29.53% in the soil profile from top to bottom.

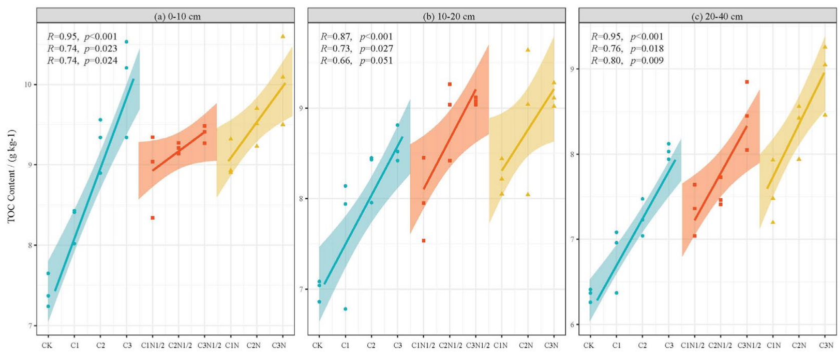

Figure 4 The total organic carbon (TOC) of the soil profile under different treatments.

The TOC was significantly associated with aggregate fractions of > 2 mm and 0.25–2 mm but inversely associated with fractions of 0.25–0.053 mm and 0.053 mm aggregates (Fig. 5). We also compared the TOC of the particle size components of the various aggregates under different biochar treatments (Fig. 6 a, b, and c) and found that large aggregates had higher carbon content than microaggregates. The C3+N1/2 treatment increased the TOC content in the > 2 mm, 2–0.25 mm, 0.25–0.053 mm, and < 0.053 mm fractions by 36.89, 20.39, 15.41, and 16.14% respectively (P < 0.05). Furthermore, the 0.25–2 mm aggregate fractions contributed the most to TOC, followed by the > 2 mm fractions (Fig. 6 d, e, and f). The contribution rate of the C+N treatment to the TOC did not change significantly when compared to the C+N1/2 treatment.

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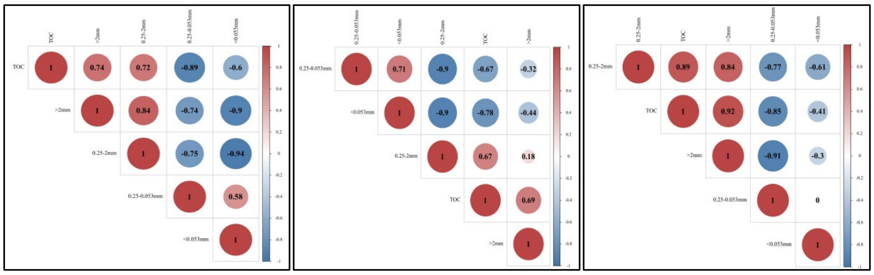

Figure 5 The correlation between the total organic carbon (TOC) and the aggregate contents of the different particle sizes in the soil profile (from left to right: 0–10 cm, 10–20 cm, and 20–40 cm).

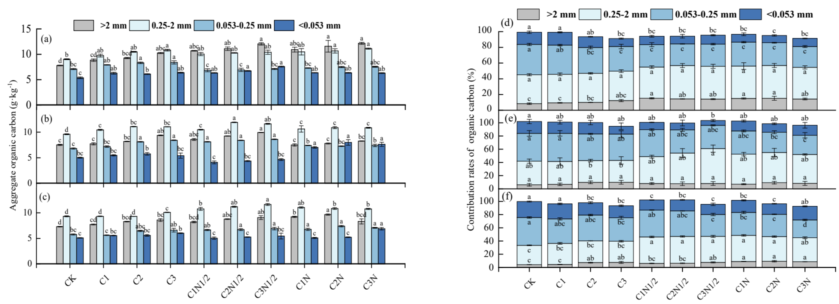

Figure 6 The total organic carbon (TOC) levels of the four aggregate fractions: (a) 0–10 cm, (b) 10–20 cm, and (c) 20–40 cm; the contribution rates of the aggregate fractions to the TOC: (d) 0–10 cm, (e) 10–20 cm, and (f) 20–40 cm. The letters indicate significant differences among various treatments ($P < 0.05$) for a given aggregate fraction. The bars indicate the standard error.

3.4 Microbial community structure

The PLFAs of microorganisms (i.e., bacteria, fungi, actinomycetes, Gm^{+} bacteria, and Gm^{-} bacteria) in the soil were identified (Fig. 7). The biochar treatment resulted in the highest increases in F/B and Gm^{+}/Gm^{-} proportions of 28.17 and 7.91%, respectively (Fig. 7 g and h). Also, the two-factor ANOVA (Table S3) showed that N fertilizer effectively altered the abundance of microorganisms, with the exception of fungi and Gm^{-} bacteria ($P < 0.05$). The abundances of the

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bacteria, fungi, actinomycetes, Gm^{+}, and Gm^{-} in the C3N1/2 treatment increased by 36.10, 72.35, 100.72, 14.91, and 12.72%, respectively. The total PLFAs increased by 56.12%.

The RDA was performed to determine the relationship between soil environmental change and the PLFA response variables (Fig. 8). The two RDA axes were significant, accounted for 94.12% of the overall variation in the soil microbial characteristics. The first axis explained 85.83 % of the total variation in microbial community composition, while the second axis explained 8.29%. Soil bulk density was the most significant variable, accounting for 62.61% of the microbial community characteristics, followed by MWD, soil moisture, TOC, $R_{0.25}$, and GMD, all of which were significantly correlated with the microbial community composition and explained 15.90, 13.42, 4.01, 2.83, and 1.28% of the various rates of microbial PLFAs, respectively.

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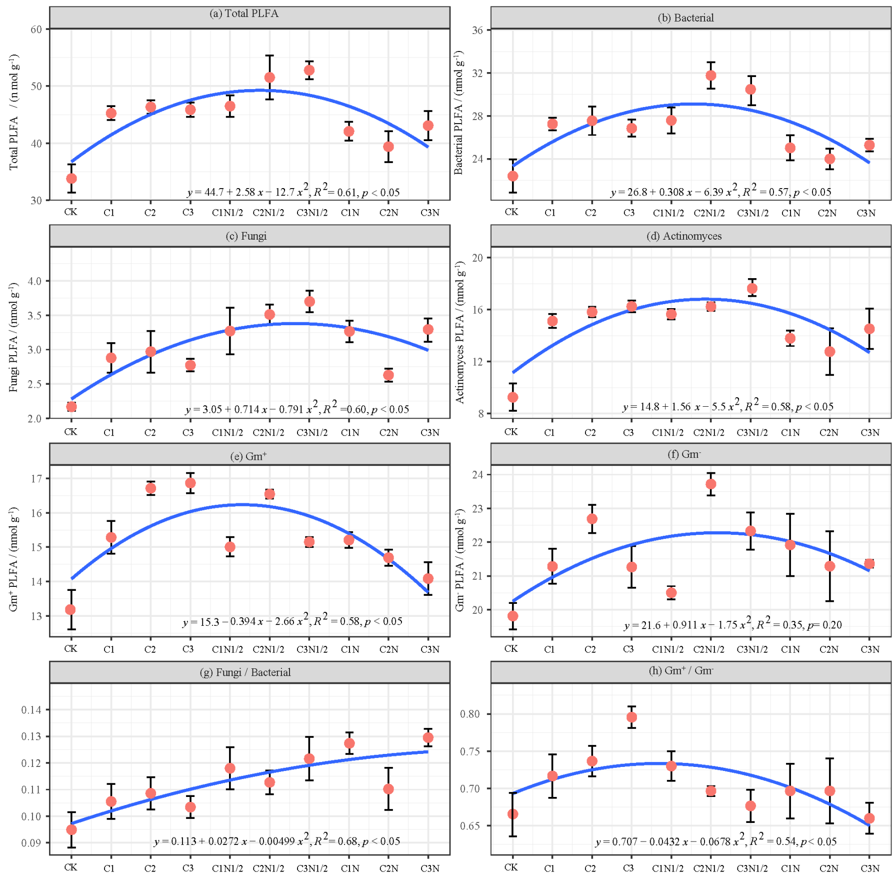

Figure 7 The concentration of the (a) total phospholipid fatty acids (PLFAs; nmol·g^{-1}), (b) bacteria PLFAs, (c) fungi PLFAs, (d) actinomycetes PLFAs, (e) gram-positive bacteria (Gm$^+$) PLFAs, (f) gram-negative bacteria (Gm$^-$) PLFAs, (g) ratio of the bacteria PLFAs/fungi PLFAs (F/B), and (h) ratio of the Gm$^+$ to Gm$^-$ bacteria of the microbial community in the soils under the treatments.

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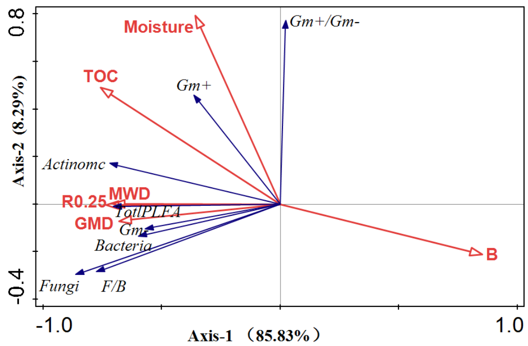

Figure 8 A redundancy analysis was used to clarify the relationship between the soil parameter variables and microbial communities. The red arrows represent the explanatory variables (soil physicochemical properties), and the blue vectors represent the response variables (phospholipid fatty acid biomass).

The PCA was used to evaluate the effects of various treatments on the soil traits in Northeast China (Table 1, Table S4). The results showed that the cumulative variance contribution rate was 90.13%, which adequately explained the variation. The higher the F value, the better the improvement effect, and the C2N1/2 treatment was optimal.

The expression of the principal component is as follows:

$$F1 = 0.27X1 + 0.31X2 + 0.31X3 + 0.30X4 + 0.23X5 + 0.23X6 + 0.27X7 + 0.08X8 + 0.31X9 + 0.33X10 + 0.32X11 + 0.31X12 - 0.35X13 + 0.20X14 \quad (9)$$

$$F2 = 0.25X1 - 0.09X2 + 0.22X3 + 0.22X4 - 0.38X5 + 0.45X6 + 0.16X7 + 0.46X8 - 0.05X9 - 0.24X10 - 0.25X11 - 0.27X12 + 0.15X13 + 0.16X14 \quad (10)$$

$$F3 = 0.34X1 + 0.35X2 + 0.20X3 + 0.29X4 + 0.14X5 - 0.09X6 + 0.21X7 - 0.36X8 - 0.28X9 - 0.13X10 - 0.16X11 - 0.13X12 + 0.19X13 - 0.52X14 \quad (11)$$

$$F = (56.52\%/90.13\%) \times F1 + (18.41\%/90.13\%) \times F2 + (15.20\%/90.13\%) \times F3 \quad (12)$$

where X1–X14 represent the bacteria PLFAs, fungi PLFAs, actinomycetes PLFAs, total PLFAs,

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F/B, Gm^+, Gm^-, Gm^+/Gm^-, TOC, $R_{0.25}$, MWD, GMD, B, and moisture, respectively.

Table 1 The principal component evaluation values and comprehensive evaluation values.

Treatments	F1	F2	F3	F	Rank
CK	-7.03	-0.53	0.32	-4.46	10
C1	-2.45	1.78	1.04	-1.00	9
C2	-0.17	2.11	0.42	0.39	4
C3	1.52	2.12	-2.74	0.92	3
C1N1/2	0.55	-0.36	0.12	0.29	5
C2N1/2	3.47	0.68	0.85	2.46	1
C3N1/2	2.59	-0.44	2.35	1.93	2
C1N	0.61	-1.50	-0.06	0.06	7
C2N	-0.13	-1.48	-1.98	-0.72	8
C3N	1.06	-2.36	-0.32	0.13	6

4 Discussion

4.1 The effects of the biochar and nitrogen fertilizer treatments on soil physical properties

The soil quality can be determined by its bulk density. This study found that the poor condition of the original soil was altered by the addition of biochar. As a result, with a microporous and carbon-rich structure for preventing oxidative degradation, the bulk density of the surface was dramatically reduced, but not in the bottom soil (Xiu et al. 2019). The biochar had a slow and gradual effect on the soil improvement. According to Chaganti et al. (2015), the biochar in the soil will gradually migrate to the lower soil over time due to natural factors and human activities. Also, Luo et al. (2020) concluded that biochar was often applied to the surface layer, resulting in a greater decline in the bulk density of the surface soil than the underlying soil. This suggests that biochar has a great benefit in ameliorating soil compaction problems in modern agriculture. Our study also found a considerably strong correlation between the soil water content of the Mollisols and the amount of

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biochar applied, particularly in the topsoil. An et al. (2022) discovered through CT scanning, that after the addition of biochar, soil porosity decreased, pore size decreased, and water retention increased, implying that water was stored in smaller pores in the soil, and drainage was delayed. One possible explanation is that the porosity, hydrophilic domains, and huge specific surface area of biochar may aid in water retention. However, some studies contradicted this study, and found either reduced water retention capacity (Madari et al. 2017) or no effect (Baiamonte et al. 2015) after biochar application. The variation in the actions may be attributed to biochar properties, soil texture type, climate change, and experimental design and duration.

4.2 The effects of biochar and nitrogen fertilizer on soil aggregate distribution and stability

Soil aggregation is essential for the performance of soil functions and is primarily responsible for the formation of the soil structure (Zhang et al. 2018). In this study, biochar increased the formation of macroaggregates (>0.25 mm), especially small macroaggregates (0.25–2 mm), but decreased the number of microaggregates in Mollisols. Grunwald et al. (2016) also confirmed this point by treating Haplic Phaeozem and Gleyic Luvisol with biochar in field experiments. Our findings also showed that when biochar was combined with N fertilizer, the fraction of macroaggregates steadily increased while the content of the microaggregates and clay particles decreased (Fig. 2). Field studies revealed a favorable influence on soil aggregation in sandy loam to clayey soils (Du et al. 2017). Therefore, the surface hydrophobic-hydrophilic interactions between clay minerals and biochar particles, as well as the biochar ability to integrate with the soil biota, and labile carbon, may all contribute to soil aggregation (Joseph et al. 2010). Furthermore, surface area,

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microporous structure, and O/C ratio are key biochar features for binding to organo-mineral complexes, an initial stage in aggregate formation and stability (Du et al. 2017).

Long-term field trials appear to have improved the effect of on soil aggregation (Dong et al. 2016). According to the findings of this study, the soil aggregate stability increased by 10.9–23.49%, which is consistent with the findings of a meta-analysis (Peng et al. 2015). The initial TOC level (26.72 $g \cdot kg^{-1}$) and protracted field experiments (8 years) with large effects could explain this. In a laboratory incubation experiment, the Albic soil of Northeast China had the lowest (0.7–4.4%) soil aggregation stability (Xiu et al. 2019). Our data showed that biochar improves the agglomeration of Mollisols better than Albic soil. This could be due to the lower initial SOC and shorter biochar application time (2 years) in our study, which is consistent with Demisie et al. (2014). According to the MWD (Fig. 3), increased TOC and microbial biomass (Fig. 7) were responsible for the significant increase in aggregation caused by biochar addition. This was also found to be the case in other studies, which found that biochar served as a cementing material, assisting more microaggregates, silt, and clay components to cement together into larger soil aggregates (Xu et al. 2019). Biochar improved water-stable soil aggregation, as evidenced by increases in soil TOC in large and small macroaggregates (Fig. 5). Thus, biochar application has a longer-term favorable influence on aggregate stability, prevents the humus layer from becoming thinner, and provides a theoretical basis for future surface runoff and soil erodibility reduction. Our findings were in contrast with those of Zhou et al. (2019), who discovered neutral or even antagonistic effects on soil aggregate formation and stabilization due to fewer binding agents produced during the decomposition of recalcitrant biochar. Therefore, there were variations in the soil aggregations in

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response to biochar due to the initial SOC, clay content, biochar attributes, application rate, and other factors (Peng et al. 2015). As a result, the evaluation results should be thoroughly examined, taking into account these factors as well as the effect of time in the field.

Biochar and N fertilizer had a synergistic effect on soil aggregate stability according to the two-factor ANOVA (Table 2). This could be because biochar combined with N fertilizer promotes crop root growth, improves crop root fungi reproductive capacity, and promotes crop roots and mycelia in the soil (Islam et al. 2021). The improved aggregates stability is due to a combination of increased root activity and biochar's significant role as a soil particle binding agent (Wang et al. 2019).

4.3 The effects of biochar combined with nitrogen fertilizer on the total organic carbon

In this investigation, the TOC level of the Mollisols increased significantly following biochar application, which is consistent with the results of Dong et al. (2016). More recently, Shi et al. (2020) proposed that the combined application of biochar and nitrogen fertilizer was conducive to soil carbon sequestration, with the cumulative mineralization rate of TOC decreasing by 0.6–1.1% when compared to the CK treatment. These findings can be interpreted in three ways. First, the use of biochar increased soil microbial activity (Fig. 7) and crop yields, thereby promoting further degradation and transformation of the plant residues, increasing SOC (Lin et al. 2020). Second, when added to the soil, biochar with a high organic carbon concentration (34.9%) directly improved the soil organic matter content. Xiu et al. (2019) found similar results in Albic soil. Third, the enrichment degree of the organic carbon occluded within the macroaggregates (Fig.5, 6) was higher than that in the microaggregates, which promoted carbon fixation in the soil aggregates (Zhang et

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al. 2018). The fourth explanation is that biochar has a high inert carbon content, which increased the Gm^{+}/Gm^{-} (Fig. 7) in the decomposition of persistent and complex substrates, indicating that carbon accumulation was greater than carbon decomposition (Dong et al. 2020). Thus, biochar effectively prevented the bulk TOC in the Mollisols from decreasing.

In this study, the TOC concentration was positively correlated with the proportion of large aggregate size (Fig. 5), which is consistent with the aggregate hierarchy model proposed by Tisdall (1982). Figure 6 shows that the > 0.053 mm fractions had a much higher carbon content than silt and clay, especially in the 0.25–2 mm fraction. Our findings confirmed those of Du et al. (2017) and Dong et al. (2016).

These results showed that the C+N1/2 treatment was more economically efficient. Under the C+N1/2 treatment, the carbon of the < 0.053 mm aggregates in the 0–20 and 20–40 cm soil layers decreased significantly, which could be explained by the finding of Ying (2018) that N fertilization promoted the mineralization rates of primary organic carbon by affecting the soil microbial community. Overall, the C+N treatment had no advantage over the C+N1/2 treatment in terms of increasing the organic carbon content of soil aggregates. This could be due to the high N content, which caused an imbalance in the soil C/N ratio, affecting the breakdown and turnover of soil organic matter (Kimetu et al. 2010).

4.4 The effects of biochar combined with nitrogen fertilizer on microbial community biomass and structure

Biochar can alleviate the negative effects of soil structure and function degradation on soil microbial activities, particularly when applied in conjunction with nitrogen fertilizer (Oksana et al.

2022). According published research biochar addition alone did not change the microbial community structure in spring maize fields or rice paddy fields, but when combined with fertilizer, the structure was changed (Luo et al. 2017; Tian et al. 2016). These findings are consistent with our experimental results. Soil F/B and total PLFA contents were significantly increased following biochar and N fertilizer treatments, which may be accompanied by increased SOC and N cycling and mineralization rates (Khadem et al. 2021). The higher the ratio of PLFA of soil fungi to bacteria, the more stable the soil ecosystem (Thiet et al. 2006). Compared to Gm^- bacteria, Gm^+ bacteria generally possess a greater proportion of peptidoglycan, which is a relatively decay-resistant soil organic matter (Zhang et al. 2013). The high $Gm^+/Gm^−$ bacteria ratio means that SOC accumulation is higher than mineralization (Wang et al. 2017). Therefore, the effect of biochar and organic fertilizer application on microbial community structure may be more inclined to the retention of easily decomposed organic carbon in northeast Mollisols (Jiang et al. 2016).

The RDA showed that the number of fungi, bacteria, actinomycetes, Gm^+ bacteria, and $Gm^−$ bacteria was positively related to the fraction of large aggregates and negatively linked to the soil bulk density. The RDA showed that the number of fungi, bacteria, actinomycetes, Gm^+ bacteria, and $Gm^−$ bacteria was positively related to the fraction of large aggregates and negatively linked to the soil bulk density. Also, Yuan et al. (2015) and Zheng et al. (2020) found that mycelial growth and mycelial products secretion by fungi can help stabilize soil aggregates. Consequently, increased fungal abundance has been proposed as an important biological factor in soil aggregate formation.

Previous research has shown that aggregates stability and the SOC are the most important components in microbial communities (Zhang et al. 2021). In addition, our results showed that the mutual effects of biochar and half-N fertilizer could effectively affect the abundance of microorganisms, which is attributed to the increased soil C/N content as a result of the applied N fertilizer providing more N sources for microbial decomposition and organic matter utilization (Jia et al. 2020). These findings were consistent with those of Zhang et al. (2021), who discovered that combining biochar with fertilizer significantly increased microbial abundance in the soil sample, implying that the addition of inorganic fertilizer reduced crop N limitation and microbial N immobilization. Furthermore, the TOC and C/N affected the fungal community composition, most likely because fungi were the primary decomposers of TOC (Chen et al. 2013). This conclusion is further confirmed by Sekaran et al. (2019), who found that the amount of soil microbial PLFAs and the ratio of soil carbon to nitrogen were strongly and positively correlated, but biochar and a full dose of N fertilizer had little effect. Based on the sequestration of SOC and the sustainability and stability of the ecosystem, we selected the most reasonable biochar ratio (C3N1/2).

5 Conclusion

The field experiments showed that the porous structure of biochar and its carbon source can effectively improve soil structure and carbon storage. Biochar significantly increased the proportion of large soil aggregates and the stability of soil aggregates. The combined application of biochar and nitrogen fertilizer provided an abundance of living space and nutrients for soil microorganisms, but microbial activity and abundance were limited by carbon input and soil nitrogen availability. The effect of excessive N application was unsatisfactory, which affects the further improvement of soil microbial abundance. The PCA showed that the C2N1/2 treatment provided the best fertilizer application rate in this experimental area. Thus, the combination of biochar and nitrogen fertilizer reduction is the optimal strategy for improving Mollisols fertility and promoting the sustainable development of the agroecosystem. Further research is needed to explore the cumulative effect of the combined application on the soil physical and chemical properties, as well as crop yield.

Credit authorship contribution statement

Jing Sun: Investigation, Experimentation, Data collection and analysis, manuscript writing. Xinrui Lu and Guoshuang Chen: Revise the manuscript. Xiujun Li: Concept and design, Project administration, Funding acquisition. Nana Luo and Qilin Zhang: Investigation, Material preparation, and Experimentation. The final manuscript was read and approved by all of the authors.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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