Ding *et al. Biochar* (2023) 5:28
https://doi.org/10.1007/s42773-023-00226-w

Biochar

ORIGINAL RESEARCH

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Biochar application significantly increases soil organic carbon under conservation tillage: an 11-year field experiment

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Abstract

Biochar application and conservation tillage are significant for long-term organic carbon (OC) sequestration in soil and enhancing crop yields, however, their effects on native soil organic carbon (native SOC) without biochar carbon sequestration in situ remain largely unknown. Here, an 11-year field experiment was carried out to examine different biochar application rates (0, 30, 60, and 90 Mg ha^{-1}) on native SOC pools (native labile SOC pool I and II, and native recalcitrant SOC) and microbial activities in calcareous soil across an entire winter wheat–maize rotation. The proportions of C_3 and C_4-derived native SOC mineralization were quantified using soil basal respiration (SBR) combined with ^{13}C natural isotope abundance measurements. The results showed that 39–51% of the biochar remained in the top 30 cm after 11 years. Biochar application rates significantly increased native SOC and native recalcitrant SOC contents but decreased the proportion of native labile SOC [native labile SOC pool I and II, dissolved organic carbon (DOC), and microbial biomass carbon (MBC)]. Biochar application tended to increase the indicators of microbial activities associated with SOC degradation, such as SBR, fluorescein diacetate hydrolysis activity, and metabolic quotient (qCO_2). Meanwhile, higher biochar application rates (B60 and B90) significantly increased the C_4-derived CO_2 proportion of the SBR and enhanced C_4-derived native SOC mineralization. The effect of the biochar application rate on the content and proportion of native SOC fractions occurred in the 0–15 cm layer, however, there were no significant differences at 15–30 cm. Soil depth also significantly increased native labile SOC pool I and II contents and decreased qCO_2. In conclusion, the biochar application rate significantly increased native SOC accumulation in calcareous soil by enhancing the proportion of native recalcitrant SOC, and biochar application and soil depth collectively influenced the seasonal turnover of native SOC fractions, which has important implications for long-term agricultural soil organic carbon sequestration.

Highlights

- Biochar application combined with conservation tillage significantly increased native SOC contents and its recalcitrant proportion.
- Higher biochar application rates significantly enhanced C_4-derived SOC mineralization and in the growing season, biochar application decreased qMBC and increased qCO_2.
- After 11 years, 39–51% of biochar remained in the top 30 cm.

Handling Editor: Xiaoyuan Yan

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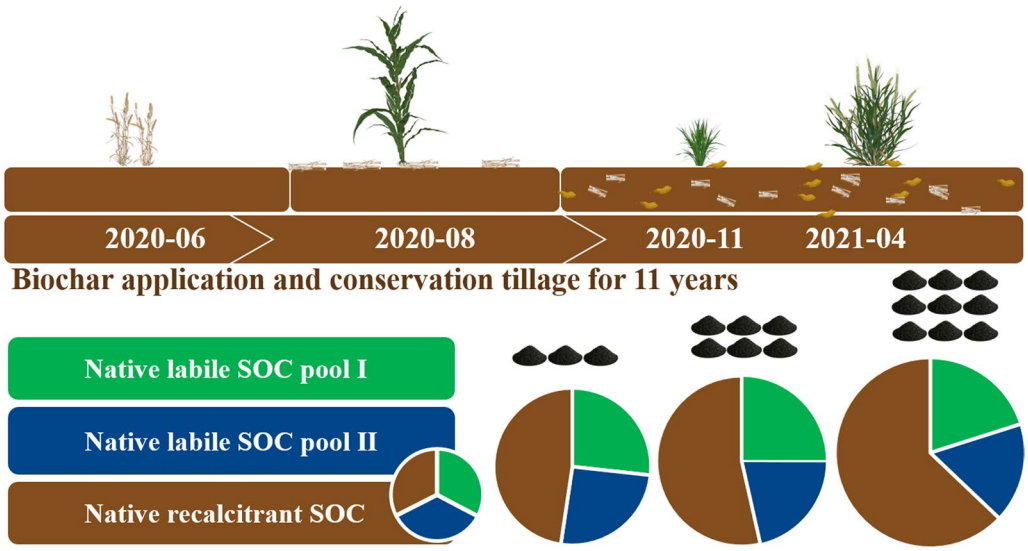
Keywords Biochar, Long-term field experiments, Soil organic carbon, Organic carbon components, Conservation tillage, ^{13}C natural isotope

Graphical Abstract



1 Introduction

Biochar application and conservation tillage are common agricultural strategies for enhancing the organic carbon (OC) content in the soil-biochar mixture (Cheng et al. 2017; Das et al. 2022) and crop yields (Zhang et al. 2020), which have multiple environmental and economic benefits for agricultural systems (Marris 2006; Page et al. 2020). The combined application of crop residue and biochar reduced the turnover rate of soil organic matter by 2–5 times compared to the control no-biochar soil (Cheng et al. 2017) and OC increased with biochar addition (Gross et al. 2021). Meanwhile, Dong et al. (2018) found that incorporating biochar application and conservation tillage significantly decreased the native organic carbon (native SOC) content of the soil without biochar-carbon by 11–30% after 5 years compared with control. However, the mechanisms underlying the long-term effects of biochar application on native SOC sequestration in the field remain largely unclear.

Native SOC influences microbial growth and diversity by affecting the chemical, physical and biological properties of the soil (Crystal-Ornelas et al. 2021). Meanwhile, biochar directly or indirectly changes the physicochemical properties of soil by reducing soil bulk density, decreasing nutrient leaching, and improving soil water-holding capacity and cation exchange capacity (Alghamdi et al. 2020; Blanco-Canqui 2017; Chen et al. 2021). However, biochar application in the long term might change the physicochemical properties and distribution of aged biochar in the field; for instance, aged biochar had a limited impact on soil water-holding capacity in a 6-year field experiment (Wang et al. 2019). At the beginning of biochar application, an apparent priming effect was observed, as the labile organic carbon of biochar contributed to microbial activation and increased CO_2 from soils of different pH (Luo et al. 2011; Maestrini et al. 2014). Activated microbes, K-strategist microbes, and biochar-induced bacterial and fungal diversity and competition (Chen et al. 2019, 2021; Ling et al. 2022) cause real priming effects (both positive and negative) (Cross and Sohi 2011; Ling et al. 2022; Maestrini et al. 2014; Wang et al. 2016), with a duration of a month to 2 years. However, labile organic carbon accounts for only 3–4% of biochar carbon (Maestrini et al. 2014; Wang et al. 2016) and is depleted within 2 years (Kuzyakov et al. 2014; Wang et al. 2016). Currently, there are no consistent conclusions regarding the effect of biochar application rate on native SOC accumulation in the field over the long term.

Biochar application affects both native labile and recalcitrant SOC fractions (Dong et al. 2018; Lin et al. 2022). For instance, Dong et al. (2016) found that biochar application significantly increases the OC of soil macro-aggregates (increased by 44–242%) by improving soil aggregate

stability and increasing OC in the light fraction after 3 years. The light fraction was also the major OC component of the macro-aggregates, which mainly consisted of mycelia, spores, monosaccharides, polysaccharides, and animal and plant residues. Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are important components of labile OC, and biochar addition increases them in the short term (Cheng et al. 2017; Chen et al. 2021). Meanwhile, biochar addition promotes DOC (such as condensed aromatics and tannin) mineralization by shifting the microbial community structure, particularly the bacterial communities (Ling et al. 2022). Biochar application also significantly increased the recalcitrant OC in paddy soils by reducing the abundance of key functional genera involved in OC degradation (Lin et al. 2022). The biochar application rate had no significant effect on native recalcitrant SOC content in the winter wheat–maize system (Dong et al. 2018), but the proportion of recalcitrant SOC increased with biochar addition. Although the short-term effects of biochar on SOC fractions have been well studied, understanding the impact of biochar application rate on native SOC fractions and transformation requires long-term investigation.

Biochar application also influences C_3/C_4-derived SOC decomposition (Chen et al. 2021; Dong et al. 2018; Wynn et al. 2020). In the wheat–maize system, the relative contribution of C_3-derived SOC (wheat residues) was higher than that of C_4-derived SOC because of the relatively faster decomposition rate of C_3 residue (Liu et al. 2020; Wang et al. 2015) and C_4-derived SOC (Dong et al. 2020; Wynn and Bird 2007) regardless of tillage practices and soil amendments. Dong et al. (2018) conjectured that biochar application increased C_3-derived SOC degradation; thus, the biochar application decreased C_3-derived SOC, and native SOC storage and enhanced the proportion of C_4-derived SOC. However, the effect of long-term biochar application on C_3/C_4-derived native SOC mineralization requires further study. In addition, Ekblad et al. (2002) reported that the addition of different carbon sources had a similar effect on soil respiration, and ^{13}C-discrimination of microbial respiration was minor, both in the laboratory and in the field by adding C_4-sucrose and C_3-glucose to the surface soil, indicating that $^{13}CO_2$ of soil basal respiration (SBR) provided valuable information on the decomposition of SOC sources (Chen et al. 2021; Ekblad et al. 2002). Nevertheless, the turnover rate of labile SOC was faster than that of total SOC, and the SBR results of this study only represent the native labile SOC mineralization, and the sources of native recalcitrant SOC remain unclear.

Therefore, an 11-year biochar field experiment was conducted to examine the native SOC pool distribution, stabilization, and transformation across the entire winter wheat–maize rotation with conservation tillage in calcareous soil. The OC, biochar carbon (BC), native SOC, native SOC fractions, DOC, MBC, SBR, $\delta^{13}C$ of SBR, and fluorescein diacetate (FDA) hydrolysis activity of typical crop growing periods of winter wheat–maize rotation were determined. The following hypotheses were tested: (H1) after 11 years, a higher biochar application rate increased SOC content and its recalcitrant component; (H2) biochar application rate influenced active organic carbon pools (DOC and MBC) and microbial activities at different depths across an entire winter-wheat and maize rotation; (H3) biochar application promoted C_4-derived native SOC mineralization.

2 Materials and methods

2.1 Experimental site and experimental design

The long-term biochar application experiment was initiated in 2009 at the Shangzhuang Experimental Station of the China Agricultural University, Beijing, China (40°08′21″N, 116°10′52″E). Shangzhuang has a typical continental monsoon climate, with 400 mm annual precipitation and 11.6 °C annual air temperature. The soil was alluvial (Fluvisol, FAO), with a texture of 28% sand, 52% silt, and 20% clay. Before the experiment, the native SOC content was 4.32 g kg^{-1} (Fig. 1a) and the pH of the soil was 8.0. Biochar was obtained by pyrolyzing a mixture of cottonseed husk (30%) and rice husk (70%) at 400℃ for 4 h. The OC content of the biochar was 491 g kg^{-1} and the pH of the biochar was 10.64. The basic properties of the soil and biochar before the experiment are presented in Additional file 1: Table S1.

Briefly, 0, 30, 60, and 90 Mg ha^{-1} biochar (B0, B30, B60, and B90) was applied to the 0–20 cm soil layer of each plot (11 × 10 m) in June 2009. Biochar was applied uniformly on the soil surface and incorporated into the soil using a rotavator, and each treatment had three replications (plots). In this winter wheat-maize rotation, wheat was sown in October and harvested in June, after which the crop residue was mechanically chopped (2–3 cm) and mulched onto the soil surface. Maize was then sown and harvested in October and the crop residue was chopped (1–2 cm) and plowed into the 0–20 cm layer, together with wheat straw. The fertilization rate and time of winter wheat and maize were the same, which were 112.5 kg ha^{-1} of N, 112.5 kg ha^{-1} of P_2O_5, and 112.5 kg ha^{-1} of K_2O, respectively, at sowing time.

Soil samples were collected four times, in June, August, and November of 2020, and in April of 2021, at two depths: 0–15 cm and 15–30 cm soil layers, which were the wheat maturity stage, maize flowering stage, wheat seedling stage, and wheat booting stage, respectively.

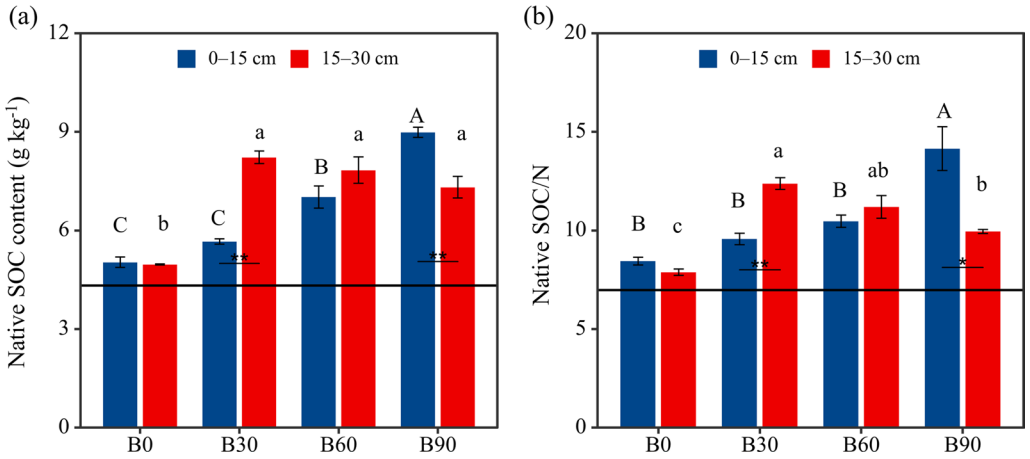


Fig. 1 Native soil organic carbon (native SOC) content (**a**) and native SOC/N (**b**) under four treatments: 0 (B0), 30 (B30), 60 (B60), and 90 (B90) Mg ha^{-1} biochar application in the 0–15 cm and 15–30 cm (n = 3; different capital letters and small letters denote significant differences among treatments in the same depth, $p < 0.05$; and * indicates significant differences between the two depths in the same treatment, *$p < 0.05$, **$p < 0.01$)

In each plot, three soil cores (3.5 cm diameter) of each sampling depth were randomly collected, combined and sieved (2 mm) to create a composite sample. The native SOC and acid-hydrolysis SOC fractions were measured with the air-dried soil samples that were collected the first time and DOC and MBC were measured with fresh soil in all four-period samples, and SBR, FDA hydrolysis activity, and $\delta^{13}C$ of SBR were measured in the two periods of plant growth, April and August.

2.2 Native SOC content

Soil samples were treated separately in a 1:10 (w/v) ratio with 1 mol L^{-1} HCl to remove carbonates. The total OC content of soil-biochar mixtures (TOC) and OC content within pure biochar particles separated from the soil ($OC_{biochar}$) were measured using an elemental analyzer after removing carbonates (Vario PYRO cube, Elementar, Germany). The biochar particles (> 0.5 mm) were all separated by hand, washed thoroughly in distilled water (w/v = 1:10) four times to remove soil particles, then dried at 60 °C (Dong et al. 2018). The loss on ignition method (LOI) was utilized to measure the biochar content based on the difference in thermal stability of pure soil and biochar (Dong et al. 2018; Raya-Moreno et al. 2017). Samples of pure soil (B0), pure biochar (collected from B30, B60, and B90 by hand), and biochar-soil mixture (B30, B60, and B90) were heated to 550 ℃ for 4 h in a muffle furnace and the mass of samples was weighed before and after heating. The LOI results of the samples were used to estimate biochar content. Briefly, the amount of biochar in the biochar-soil mixture was calculated using the following equation:

$$\text{Biochar amount (g biochar kg}^{-1}\text{soil)} = \frac{L_{mix} - L_{soil}}{L_{biochar} - L_{soil}} \times 100, \quad (1)$$

where the L_{mix}, L_{soil}, and $L_{biochar}$ are the loss rates of the biochar-soil mixture, soil, and biochar after heating, respectively. Where, $L_{soil} = 3.01 \pm 0.05\%$ and $L_{biochar} = 54.01 \pm 0.04\%$. The BC content (g kg^{-1} soil) in the soil was calculated as the amount of biochar multiplied by the $OC_{biochar}$. The native SOC-to-nitrogen ratio was calculated based on the contents of native SOC and total nitrogen (native SOC/N), and the native SOC content was calculated by subtracting the BC from TOC in the soil-biochar mixture using the following equation:

$$\text{Native SOC (g C kg}^{-1}\text{soil)} = 1000 \times \frac{\text{TOC} - \text{BC}}{1000 - \text{biochar amount}}. \quad (2)$$

2.3 Fractionation of native SOC

The fractions of the soil-biochar mixture and 11-year-aged biochar samples were measured using the acid hydrolysis method (Rovira and Vallejo 2002). The first hydrolysate (labile OC pool I) of the samples was separated in a 1:40 (w/v) ratio of sample and 2.5 mol L^{-1} H_2SO_4 for 30 min at 105 ℃, which comprised non-cellulosic polysaccharides from microbes and plants. The second hydrolysate (labile OC pool II, the major constituent of cellulose) of the samples used the residue of pool I. The residue was dried at 60 ℃ and hydrolyzed with 13 mol L^{-1} H_2SO_4 (w/v = 1: 4) for 8 h at 25 ℃ under continuous shaking, and then diluted to 1 mol L^{-1} by water and hydrolyzed for 3 h at 105 ℃. The

OC contents of labile pool I and II were analyzed using a TOC analyzer (Liqui TOCII, Elementar, Germany). The native labile SOC pools were calculated after accounting for biochar-labile OC pools within the soil-biochar mixture (Eq. 2). The native recalcitrant SOC pool was the native SOC minus the sum of labile SOC pool I and II, which are resistant to acid hydrolysis, including lignin, fats, waxes, resins, and suberin (Rovira and Vallejo, 2002).

2.4 DOC and MBC

DOC content was determined using 0.5 mol L^{-1} K_2SO_4 (soil: extractant = 1:4), filtered using 0.45 μm polytetrafluoroethylene filters, and MBC was estimated by the chloroform fumigation extraction method. Both DOC and MBC concentrations were analyzed using a TOC analyzer (Liqui TOCII, Elementar, Germany). MBC content was employed by the following equation: $MBC = E_C/K_{EC}$, where E_C is the C extracted by 0.5 mol L^{-1} K_2SO_4 (soil: extractant = 1:4) from fumigated soil minus that from non-fumigated soil, and K_{EC} is 0.45 (Wu et al. 1990). *q*MBC reflects the efficiency of native SOC conversion into MBC and was calculated using the following equation: *q*MBC = MBC/native SOC (%), where MBC and native SOC represent the contents of MBC and native SOC after biochar application for 11 years.

2.5 Microbial activities and their sources

Soil samples from winter wheat and maize growing seasons were incubated at 25 ℃ to measure CO_2 emissions (SBR) for 24 h in the dark using a gas chromatograph (Agilent 7890A, Agilent Ltd., Shanghai, China). Metabolic quotient (qCO_2) = SBR/MBC (d^{-1}). The total microbial activity of the soil samples was measured by the FDA hydrolysis method (Adam and Duncan 2001). Briefly, 2 g fresh soil, 15 mL potassium phosphate (pH = 7.6), and 0.2 mL FDA (1000 μg mL^{-1}) were mixed and incubated for 20 min at 30 ℃ and 200 rpm, and 15 mL of chloroform/methanol (2:1) was used to terminate the reaction. The supernatant of the above solution (centrifuged at 2000 rpm for 3 min) was filtered and measured at 490 nm using a spectrophotometer (TU1900, Persee, China).$\delta^{13}CO_2$ was measured using an isotope ratio mass spectrometer (MAT253, Thermo Fisher, USA). The ^{13}C natural isotope abundance of native SOC-derived CO_2 ($\delta^{13}CO_{2\ native\ SOC\text{-}derived}$) was calculated using the following equation (Ekblad et al. 2002):

$$\delta^{13}CO_{2native\ SOC\ derived} = \frac{\delta^{13}CO_{2(t)} \times c_t - \delta^{13}CO_{2(0)} \times c_0}{c_t - c_0}, \quad (3)$$

where $\delta^{13}CO_{2\ (t)}$ represents the $\delta^{13}C$ of CO_2 after incubation for *t* h, $\delta^{13}CO_{2\ (0)}$ represents the $\delta^{13}C$ of the atmosphere (7.6 ‰), and c_t and c_0 are the gas concentrations at *t* and 0 h of incubation, respectively.

The labile OC of biochar was decomposed within 2 years (Maestrini et al. 2014; Wang et al. 2016), so we considered that there were only two carbon sources of CO_2, making it possible to separate the CO_2 sources. The CO_2 derived from the C_3 source material (winter wheat) was calculated using the following equation:

$$CO_{2C_{3\text{-}derived}} = \frac{(c_t - c_0) \times (\delta^{13}CO_{2\ native\ SOC\ derived} - \delta^{13}C_4)}{\delta^{13}C_3 - \delta^{13}C_4}, \quad (4)$$

where $\delta^{13}C_3$ represents the $\delta^{13}C$ of wheat (−27.47 ‰), $\delta^{13}C_4$ represents the $\delta^{13}C$ of maize (−13.58 ‰), and c_t and c_0 are the gas concentrations of *t* and 0 h of incubation, respectively.

2.6 Statistical analysis

Statistical analyses included paired t-test and one- or two-way ANOVA of native SOC, native SOC/N, native SOC fractions, DOC, DOC/native SOC, MBC, *q*MBC, SBR, qCO_2, and FDA hydrolysis activity with Bonferroni's multiple comparison test using R software (R 4.2). Pearson's correlations were performed between the biochar application rates and SOC fractions. Structural equation modeling (SEM) was used to evaluate the direct and indirect effects of biochar application, soil depth, and soil water content on native SOC and its fractions. Data are expressed as the mean ± SD and $p < 0.05$ was assigned as statistically significant.

3 Results

3.1 Biochar content in the field

After 11 years, 39–51% of the biochar remained in the 0–30 cm soil layer and the aged BC content increased with biochar addition (Table 1). At 0–15 cm, biochar erosion significantly increased with biochar addition, and 52%, 33%, and 27% of biochar addition remained in the soil, which accounted for 20–39% of total biochar addition under B30, B60, and B90 treatments (Table 1). At 15–30 cm, the biochar residue showed no significant difference, but an increasing tendency with biochar application, accounting for 48%, 61%, and 74% of biochar application in B30, B60, and B90, respectively.

3.2 Native SOC, native SOC/N, and native SOC fractions

The native SOC content at 0–30 cm significantly increased after 11 years of biochar application ($p < 0.05$, Fig. 1a), and the increase was dependent on biochar application rates (Fig. 1). Compared with B0, the mean native SOC content increased by 39%, 49%, and 63% in B30, B60, and B90, respectively. Soil depth influenced the effect of the biochar application rate on native SOC. The native SOC content significantly increased with biochar

Table 1 Loss on ignition (LOI), biochar addition rate, and biochar residue rate in the soil under biochar applied treatments: 30 (B30), 60 (B60), and 90 (B90) Mg ha^{-1} in the 0–15 cm and 15–30 cm

Soil depth	Treatments	Biochar addition rate %	LOI %	Biochar residue rate %	Biochar carbon content g kg^{-1}	Residual biochar/total biochar addition %
0–15 cm	B30	1.11	3.31 ± 0.01c	0.57 ± 0.02	2.97 ± 0.1c	38.65 ± 1.34a
	B60	2.22	3.39 ± 0.01b	0.73 ± 0.02	3.75 ± 0.1b	24.55 ± 0.66b
	B90	3.33	3.47 ± 0.01a	0.89 ± 0.03	4.55 ± 0.13a	19.99 ± 0.59c
15–30 cm	B30	0.37	3.09 ± 0.02c	0.18 ± 0.04	0.92 ± 0.2c	11.96 ± 2.65a
	B60	0.74	3.23 ± 0.06b	0.45 ± 0.12	2.33 ± 0.64b	15.27 ± 4.19a
	B90	1.11	3.42 ± 0.02a	0.82 ± 0.05	4.22 ± 0.23a	18.55 ± 1.03a

Different letters denote significant differences among treatments in the same depth ($p < 0.05$)

addition at 0–15 cm; however, there were no differences at 15–30 cm (other than B0), which resulted in an increase in the mean native SOC content with biochar addition at 0–30 cm.

Biochar application significantly increased the native SOC/N ($p < 0.05$, Fig. 1b). At 0–30 cm, the native SOC/N increased by 34%, 33%, and 48% in B30, B60, and B90, respectively, compared with the control (B0). Meanwhile, the native SOC/N had significant negative and positive correlations with the biochar application rate at 0–15 cm and 15–30 cm, respectively ($p < 0.05$, other than B0).

Biochar application rates and soil depth significantly affected the native SOC fractions, including labile pools I and II and recalcitrant SOC. In the two-way ANOVA, biochar application rate, soil depth, and their interactions had significant effects on the three native SOC fractions ($p < 0.05$), other than the effect of soil depth on native recalcitrant SOC. Biochar application significantly decreased the proportions of native labile SOC pools I and II compared to B0. The proportion of native labile SOC pools decreased with biochar addition at 0–15 cm and did not differ at 15–30 cm (Fig. 2). The biochar application significantly increased the mean native recalcitrant SOC content at 0–30 cm. The proportion of native recalcitrant SOC significantly increased with biochar addition at 0–15 cm but did not differ at 15–30 cm ($p < 0.05$, Fig. 2). In addition, in the lower biochar applied treatment (B30), all native SOC fractions increased with soil depth, particularly the recalcitrant SOC (increased by 72%), which led to a significant increase in total native SOC in the 15–30 cm layer compared to that in the 0–15 cm soil layer.

3.3 DOC and MBC contents and their proportions of native SOC

Across the entire winter wheat–maize rotation, the mean rotational DOC content had a significant Pearson correlation with biochar addition ($p < 0.05$), and biochar addition increased the mean rotational DOC content by 3.4%, 12.2%, and 15.5% in B30, B60, and B90, respectively, compared with the control at 0–30 cm. Although the mean rotational DOC content did not differ significantly with

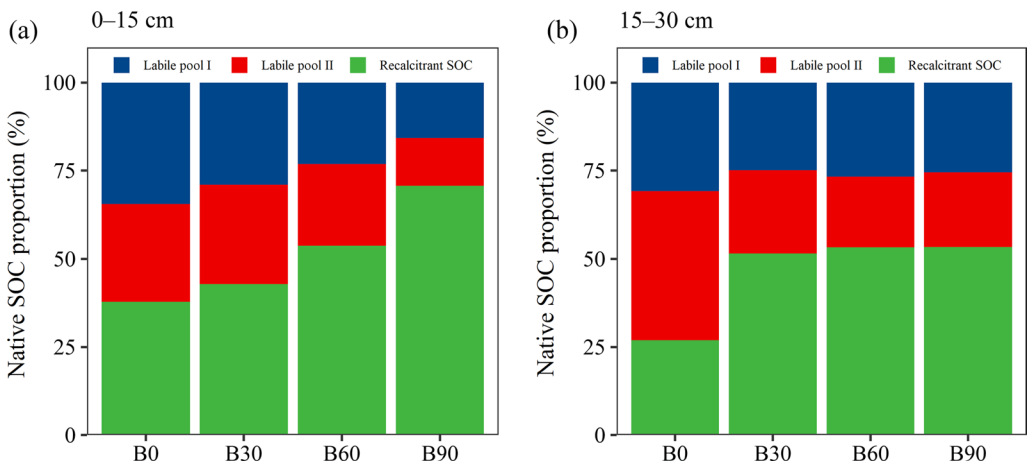


Fig. 2 The proportions of native SOC fractions (native labile SOC pool I, native labile SOC pool II, and native recalcitrant SOC, g kg^{-1}) in the 0–15 cm (**a**) and 15–30 cm (**b**) under four treatments: 0 (B0), 30 (B30), 60 (B60), and 90 (B90) Mg ha^{-1} biochar application

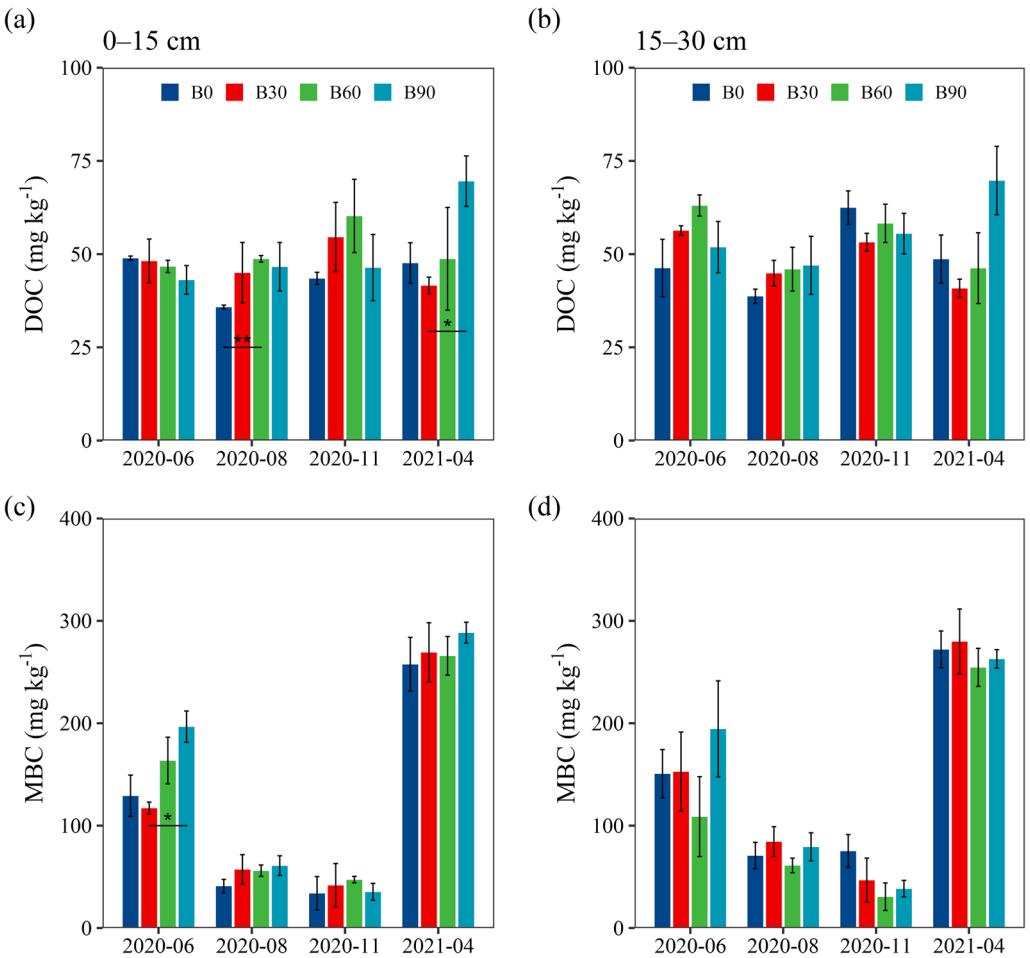


Fig. 3 Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) contents in four stages of the winter wheat–maize rotation system under four treatments: 0 (B0), 30 (B30), 60 (B60), and 90 (B90) Mg ha^{-1} biochar application in 0–15 cm (**a** and **c**) and 15–30 cm (**b** and **d**, n = 3, significance = *$p < 0.05$, **$p < 0.01$)

soil depth, there was an upward trend with depth, and the DOC content was only significantly lower in June. In each growing season, the effect of biochar application rate on DOC was different (Fig. 3a, b); for instance, B90 only decreased DOC in June at 0–15 cm and November at 15–30 cm; in addition to the above results, B30 also decreased DOC in April B30 at both depths. In addition, in the 0–15 cm layer, the biochar application rate significantly increased the DOC content in August and April ($p < 0.01$).

Biochar application rate had no significant effect on MBC content during winter wheat–maize rotation. MBC content was lower at 0–15 cm than at 15–30 cm among sampling times and was significantly different in August ($p < 0.05$). In the 0–15 cm layer, biochar application increased MBC content by 5%, 15%, and 26% in B30, B60, and B90, respectively, compared with B0, and was only significant in June. Furthermore, the MBC content significantly decreased over the months ($MBC_{Apr.} > MBC_{Jun.} > MBC_{Aug.}$ and $MBC_{Nov.}$, $p < 0.05$, Fig. 3c, d).

Biochar application significantly decreased the DOC and MBC proportions of native SOC (DOC/native SOC and *q*MBC; Fig. 4). DOC/native SOC decreased by 23%, 22%, and 28% in B30, B60, and B90, respectively, compared with B0, and did not differ between 0–15 cm and 15–30 cm. Biochar application decreased *q*MBC by 25%, 33%, and 30% in B30, B60, and B90, respectively, compared with the control, and *q*MBC was positively correlated with MBC ($p < 0.05$), which was significantly different among sampled periods but did not differ among treatments ($p > 0.05$, other than April). Although insignificant, the *q*MBC was lower at 0–15 cm than at 15–30 cm during winter wheat–maize rotation. Furthermore, the *q*MBC had significant negative correlations with native SOC, native recalcitrant SOC, and native SOC/N ratio, and a positive correlation with DOC/native SOC ($p < 0.05$).

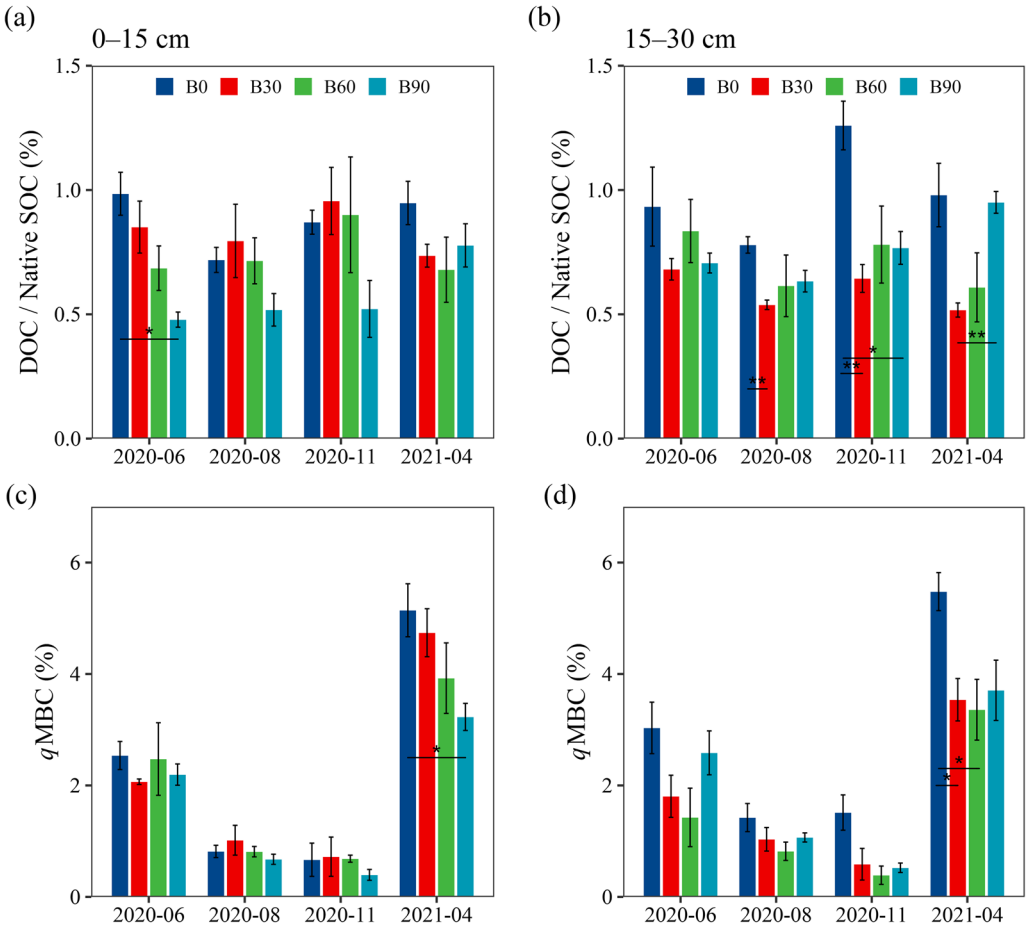


Fig. 4 The proportions of DOC and MBC in native SOC (DOC/native SOC and microbial carbon quotient (*q*MBC)) in four stages of the winter wheat–maize rotation system under four treatments: 0 (B0), 30 (B30), 60 (B60), and 90 (B90) Mg ha^{-1} biochar application in the 0–15 cm (a and c) and 15–30 cm (b and d, n = 3, significance = *$p < 0.05$, **$p < 0.01$)

3.4 Microbial activities and their sources

Biochar application increased SBR and FDA hydrolysis activities during the growing season of winter wheat and maize. Neither SBR nor FDA hydrolysis activity was affected by treatment ($p > 0.05$) or depth; however, they were significantly different in the growing season of winter wheat and maize (Fig. 5a, b). The SBR of the maize growing season was 1.9 times higher than that of the wheat growing season (Fig. 5a). In contrast to SBR, the mean FDA hydrolysis activity was lower during the maize season.

Biochar application increased qCO_2 in two depths, but not at 0–15 cm of maize season. The mean qCO_2 was 7.4 times higher in the maize season than in the wheat season (Fig. 5C, $p < 0.01$) and did not differ among biochar application rates. qCO_2 significantly decreased with soil depth ($p < 0.01$) in wheat and maize growth seasons, and qCO_2 in biochar application treatments (B30, B60, and B90) was higher than that in the control.

The $\delta^{13}C$ of the SBR was significantly positively correlated with the biochar application rate and native recalcitrant SOC ($p < 0.05$). The $\delta^{13}C$ of SBR showed no significant difference between the 0–15 cm and 15–30 cm depths and was enriched in high biochar addition treatments (B60 and B90, $\delta^{13}C$ ranging from −13.2 to −11.6 ‰), compared with B0 (−16.5 to −15.5 ‰) and B30 (−17.5 to −16.2 ‰). The proportions of C_4 sources in the SBR were 35%, 19%, 78%, and 86% for B0, B30, B60, and B90, respectively. Higher biochar application rates (B60 and B90) resulted in more C_4-derived native SOC respired at the two depths (Fig. 5d).

The structural equation model (SEM) included 11 factors and the model was established ($p > 0.05$, Fig. 6). The SEM results showed that soil depth and aged biochar content were the two key factors affecting native SOC

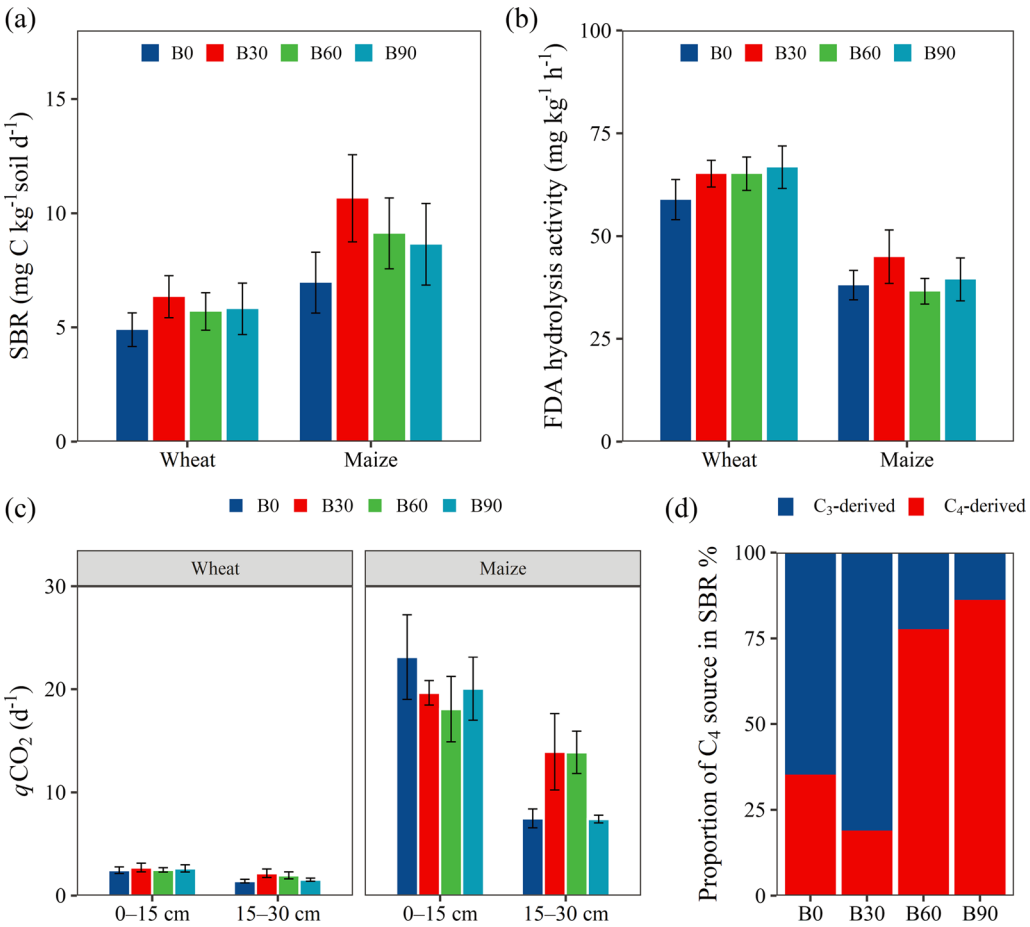


Fig. 5 Soil basal respiration (SBR, a), fluorescein diacetate (FDA) hydrolysis activity (b), metabolic quotient (qCO_2, c), and proportion of C_4 source in SBR (d) during two growing periods under four treatments: 0 (B0), 30 (B30), 60 (B60), and 90 (B90) Mg ha.$^{-1}$ biochar applied at 0–30 cm (**a**, **b**, and **d**: n = 6, depth = 0–30 cm; **c**: n = 3, depth = 0–15 cm and 15–30 cm)

cycling (Fig. 6). Biochar application had a positive influence on TN content but was negatively correlated with native SOC and its labile pool II content. Soil depth was positively correlated with TN and native SOC contents. The SOC content was also indirectly improved by the native recalcitrant SOC and native labile SOC pool II contents. In addition, native SOC had a negative effect on DOC/native SOC. Soil depth and soil water content (SWC) had impacts on CO_2 emissions at all four sampling intervals, while sample time and SWC had impacts on MBC content.

4 Discussion

4.1 Biochar transport in the soil

After 11 years of biochar addition, 39–51% of the biochar remained in the soil (Table 1). This result was in line with those of Dong et al. (2017) and Obia et al. (2017), who showed that biochar was recovered for 45–66% in the field. The results were due to biochar loss in the soil, which consisted of microbial breakdown, DOC leaching, and lateral or vertical movement (Wang et al. 2016). Previous studies have shown that 3–4% of biochar is labile OC and can be decomposed within 2 years (Kuzyakov et al. 2014; Maestrini et al. 2014; Wang et al. 2016), and 97% of biochar is recalcitrant for 556 ± 483 years (Wang et al. 2016). Therefore, the loss of biochar was due to vertical and lateral transfer, particularly at 0–15 cm, and its mineralization was minor.

The total biochar recovery rate decreased with biochar addition. Nevertheless, the biochar content still increased with biochar addition and was higher in the top 15 cm than at 15–30 cm (Table 1). Dong et al. (2017) and Obia et al. (2017) found that 10–20% of biochar moved to 10–20 cm deeper soil, which was in line with the results here that biochar recovery increased in the 15–30 cm soil layer. Furthermore, the loss of biochar in this study was 49–61%, indicating that more biochar was laterally transferred through erosion with annual plowing and irrigation.

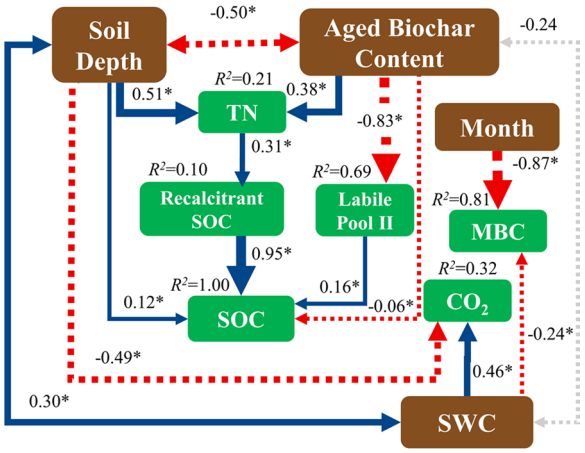


Fig. 6 Structural equation model (SEM) showing the multivariate effects of native soil carbon cycling (the blue lines indicate significant positive relationships and orange lines indicate significantly negative relationships, while the grey lines mean no significantly different relationships. The width of the arrows indicates the strength of standardized path coefficients)

The biochar residue rate of higher biochar applications was significantly lower than that of the lower application at 0–15 cm and was not significantly different at 15–30 cm. This might be due to biochar particle movement in the surface soil by tillage (0–20 cm) for 11 years, such as the Brazil nut effect (BNE), which separates large particles from small ones under vibration. Biochar application could increase the stability of soil aggregates (Dong et al. 2016) and biochar-soil particles might become larger and heavier than common soil particles. The biochar-soil particles might have been brought to the surface soil and eroded with annual irrigation before winter. However, there are few studies on the movement of aged biochar (newly formed small pores and smoother surfaces (Dong et al. 2017)) in the soil (amount or speed), and we cannot explain this result clearly. Additionally, the biochar application rate had no significant correlation with SWC (Fig. 6), and the results matched the water retention capacity loss of aged biochar in the field (Wang et al. 2019).

4.2 Effects of biochar application rates on native SOC pools

Biochar application rates significantly increased the native SOC content in the 0–30 cm layer. The native SOC increased by 39%, 49%, and 63% in B30, B60, and B90 in 2020, respectively (Fig. 1), which is in line with the results of a meta-analysis in the field (Gross et al. 2021; Singh et al. 2022). Chen et al. (2019) found that biochar application had a negative priming effect on SOC by increasing bacterial and fungal diversity and generating competitive microbial interactions. However, our results differed from those of an earlier study in the same field (Dong et al. 2018), which found that native SOC was negatively correlated with biochar application rate, with similar organic material input every year (Liang et al. 2014). Despite the positive correlation with biochar addition, the SEM results showed the same significant negative effect of BC on native SOC (Fig. 6, standardized coefficients = −0.06, $p < 0.01$), which is in line with the results of Dong et al. (2018).

We provided insights into the effect of biochar application rate on native labile SOC pools (DOC, MBC, and native labile SOC pool I and II). The results showed that biochar application significantly decreased the proportions of native labile SOC pools (Figs. 2, 4). The native labile SOC pool I content was similar to the results of Dong et al. (2018), who found that biochar addition significantly increased the degradation of non-cellulosic polysaccharides (labile pool I). DOC and MBC contents are important indicators of native labile SOC pool I and biochar application rates did not significantly affect them during the study periods, which was in line with the results of biochar addition for 6 years (Luis Moreno et al. 2022) and opposite to the incubation results, which found that biochar application significantly increased MBC compared with no-biochar soil (Cheng et al. 2017; Chen et al. 2021). In this study, after winter wheat was harvested, residues were retained on the surface, and residues of maize and wheat were chopped into pieces and incorporated into the soil. During the slow decomposition of crop residues, the MBC content might be controlled by the labile residual substrate content and soil temperature (Jat et al. 2018), resulting in seasonal variations in MBC content. The *q*MBC reflects the efficiency of native SOC conversion into MBC (Zhou et al. 2018) and in our study, less native SOC was allocated to MBC in biochar addition treatments. Jat et al. (2018) found that *q*MBC ranged from 1.1% to 2.6% in conservation tillage, which was similar to the results here from June to November. *q*MBC and MBC were significantly correlated across the rotation (other than in the surface soil of April), indicating that the fast turnover proportion of SOC was significantly affected by the degradation of the easy metabolism component of residues. Simultaneously, the SEM results showed that native labile SOC pool II content, which consists of cellulose (Rovira and Vallejo 2002), was significantly negatively correlated with BC content (Fig. 6, $p < 0.05$), and the result was similar to that of Dong et al. (2018). Overall, the decrease in the fast turnover proportion (native labile SOC pools) suggested that native SOC stability increased with biochar addition.

Biochar application also significantly improved native recalcitrant SOC content and proportion at 0–30 cm (Fig. 2) after biochar application for 11 years, particularly in B90 (increased by three times compared with B0). Dong et al. (2018) found that the native recalcitrant SOC proportion had an increasing tendency with biochar addition for 5 years; however, the native recalcitrant SOC content was not significantly different. Considering all native SOC pools together, biochar application promoted the transfer of native SOC from native labile pools to the recalcitrant pool, and native recalcitrant SOC components and native SOC accumulation increased with biochar addition; however, the transfer rate of native SOC pools was very slow. SEM results showed that native recalcitrant SOC had a significant positive correlation with TN (Fig. 6) and indirectly increased with BC, which increased TN. Nitrogen is mostly present in soil organic matter (Tan et al. 2015; Gao et al. 2019) and N might transfer with SOC from labile pools to recalcitrant pools and generate new unhydrolyzable N in humification processes (Rovira and Vallejo 2002). Therefore, further research is needed to explore the effects of long-term biochar application on organic nitrogen turnover.

Biochar application had positive effects on SBR and FDA hydrolysis activity (Fig. 5), representing an increase in total microbial activity (Adam and Duncan 2001; Das and Adhya 2014). Although biochar application increased bacterial and fungal competitiveness and diversity in the field and decreased carbohydrate catabolism (Chen et al. 2019; Whitman et al. 2016), the results of SBR and FDA showed opposite trends, suggesting that biochar application did not limit the carbohydrate catabolic function of microbial communities after the long-term application. Biochar application has been reported to increase, have no effect on, or decrease SOC mineralization, depending on carbon sources, the quality of soil organic matter, and soil properties (Cross and Sohi 2011; Maestrini et al. 2014; Wang et al. 2020). Previous studies have shown that biochar application increases SBR by increasing fungal biomass in the field (Luis Moreno et al. 2022) and increases CO_2 emission by biochar mineralization (Cheng et al. 2017), which decreases native SOC mineralization (Cross and Sohi 2011; Maestrini et al. 2014). Since labile biochar C was nearly non-existent after 1 year (Wang et al. 2016), the SBR increase may have been caused by variations in native labile SOC pools (DOC, MBC, necromass) (Pang et al. 2021), which were controlled by biochar application rates. Biochar application increased qCO_2 (except for the surface soil of the growing season of maize); however, there was no significant difference among treatments (Fig. 5). Spohn and Chodak (2015) found that qCO_2 increased with increasing soil C/N and C concentrations; however, the qCO_2 had no response to them ($p > 0.05$), which indicated that soil C/N ratios and SOC content might not be limiting factors for qCO_2 in this agricultural system. In the wheat growing season (April), qCO_2 was significantly lower than that in the maize growing season (August, Fig. 5c) and showed efficient microbial respiration in the maize season.

Furthermore, in the relatively higher biochar addition treatments (B60 and B90), the native SOC released more CO_2 from C_4-derived SOC than the lower biochar addition treatments (B30 and B0, Fig. 5), and the proportions of C_4-derived CO_2 were 35%, 19%, 78%, and 86% of SBR in B0, B30, B60, and B90, respectively. The results were in line with those of Dong et al. (2018), who found that different biochar application rates increased the proportion of C_4-derived SOC accumulation from 26 to 38%. In winter wheat–maize rotation, the decomposition of C_4-derived SOC was faster and led to a lower relative contribution of C_4-derived SOC (26–45%), regardless of tillage practices and soil amendments (Dong et al. 2020; Liu et al. 2020; Wang et al. 2015). Considering that the newly added labile SOC and the proportion of C_4-derived active SOC decomposed faster than the total SOC (Wynn and Bird 2007; Wynn et al. 2020), the result of C_4-derived CO_2 indicated that C_4-derived SOC might be relatively higher in the labile pool under higher biochar addition (B60 and B90), despite the potential overestimation. On the other hand, the biochar application significantly altered SOC fractions (Fig. 2) and might shift the structure and functions of microbial communities (Six et al. 2006; Zhang et al. 2022), which led to the selective loss of C_4-derived SOC. Furthermore, the effect of the biochar application on SOC turnover in different fractions requires further study.

4.3 Effects of soil depth on native SOC pools

Soil depth not only influenced the native SOC pool content and proportion but also changed the effect of biochar application rate on native pools. Labile native SOC pool I was significantly lower at 0–15 cm than at 15–30 cm, and the other native SOC pools were influenced by biochar application rate, soil depth, and their interactions, other than B0. Because of the annual plough before wheat sowing, the native SOC of B0 did not differ between 0–15 cm and 15–30 cm, which is similar to the homogenous SOC results of the plough horizon (Hobley et al. 2018).

The biochar application significantly increased the content of native SOC and native recalcitrant SOC, and significantly decreased the proportion of native labile SOC pool I and II in the 0–15 cm layer; however, it had no significant effect on them at 15–30 cm. Gross et al. (2021) found that biochar increased SOC at 0–15 cm compared to deeper soil, which was only similar to the result of B90 and conversed to B30 and B60. The apparent native SOC

content, which was significantly lower in surface soil at the relatively low biochar addition (B30 and B60), was controlled by the different effects of biochar application rate on native SOC pools, in particular the native recalcitrant SOC. Additionally, DOC and MBC increased with depth. However, the underlying mechanisms of biochar application rate and soil depth effects require further study.

Soil depth also influenced microbial activities and significantly increased qCO_2, although depth did not affect SBR and FDA hydrolysis activities. Previous studies found that soil C/N and C concentrations controlled qMBC and qCO_2 (Malik et al. 2019; Spohn and Chodak 2015; Zhou et al. 2018), which indicated that carbon was partitioned between growth and respiration. The native SOC/N increased with biochar addition at 0–15 cm and decreased at 15–30 cm, with significant differences in B30 and B90. Zhou et al. (2018) found that qMBC was negatively correlated with C/N ratios, which was similar to the results of our study, showing efficient microbial growth in the lowest SOC/N resources (B0). Soil depth also significantly increased SWC at 0–30 cm and indirectly led to higher MBC and qMBC values (Fig. 6). Meanwhile, depth significantly increased the mean qCO_2, indicating that labile SOC was a key factor of qCO_2 at two depths.

5 Conclusion

This study examined the long-term effects of adding biochar at different application rates (0, 30, 60, and 90 Mg ha^{-1}) on the native SOC pool distribution, microbial activities, and their sources. In conclusion, biochar application significantly enhanced native SOC and promoted the transfer of native SOC fractions from labile pools to the recalcitrance pool in wheat and maize residues retention fields after 11 years. Compared with B0 at 0–30 cm, the mean native SOC increased by 39%, 49%, and 63% in B30, B60, and B90, respectively, and the biochar application significantly decreased the proportions of native labile SOC pool I and II and increased the native recalcitrant SOC content. The effect of biochar application on the contents and proportions of native acid-hydrolyzed SOC fractions occurred in the surface soil. The biochar application rates decreased the proportions of DOC (DOC/native SOC) and MBC (qMBC). Meanwhile, biochar application increased SBR, FDA hydrolysis activity, and qCO_2, indicating that biochar application promoted microbial activities. Furthermore, the higher biochar application rates (B60, B90) increased the C_4-derived SOC mineralization and the proportion of C_4 source in the SBR increased from 19–35% (B0 and B30) to 77–86% (B60 and B90). Collectively, biochar application combined with conservation tillage has a great potential to enhance native SOC sequestration in calcareous soils over the long term.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-023-00226-w.

Additional file 1: **Table S1** Properties of thesoil (0–20 cm layer) and biochar before its application in the field experiment(Dong et al. 2017).

Acknowledgements

There is great appreciation from all authors for the technical staff of Shang-Zhuang Experimental Station who worked in the long-term biochar applied experiment.

Author contributions

QL, GL and XZ contributed to the long-term experiment conception and design, and GL and XW contributed to this research conception and design. DX performed the material preparation, data collection, and analysis. The first draft of the manuscript was written by DX and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This research received funding from the National Natural Science Foundation of China (No. 61511012).

Availability of data and materials

All data generated or analyzed during this research are included in this published manuscript and its Additional files.

Declarations

Competing interests

The authors have no financial, competing interests, affiliations with or involvement in any organization or proprietary interests in any material discussed in this manuscript.

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Received: 25 November 2022 Revised: 6 April 2023 Accepted: 10 April 2023
Published online: 19 May 2023

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