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Biochar application constrained native soil organic carbon accumulation from wheat residue inputs in a long-term wheat-maize cropping system

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ABSTRACT

An understanding of the influence of biochar on soil organic carbon (SOC) formed from different carbon (C) sources, other than biochar, at field scale is required to accurately assess and predict the C sequestration potential of biochar. For this study, we set up a field experiment in 2009, including four treatments (*i.e.* B0, B30, B60, and B90, where the biochar application rates were 0, 30, 60, and 90 t ha^{-1}, respectively). We then assessed the impact of biochar after five years (*i.e.* in 2014) on native SOC derived from C_3 (wheat) and C_4 (maize) crop residues, and also changes in relatively labile and stable SOC fractions. After five years, the content of native SOC derived from crop residues increased by 81% (from 4.32 to 7.84 g kg^{-1}) in the B0 treatment, while the increases of native SOC were relatively lower in the B30 (61%), B60 (43%), and B90 (26%) treatments. Thus biochar decreased the content of native SOC compared to the B0. Additionally, biochar decreased "labile pool I" (first-step, weak acid hydrolysable) of native SOC by 11.2–47.7%, compared to the B0, but did not influence "labile pool II" (second-step, strong acid hydolysable) and "recalcitrant pool" (acid non-hydolysable). Using the natural abundance ^{13}C, our results showed that 62–74% of the native SOC was derived from wheat across all the treatments. Biochar application decreased the contribution of wheat-derived C to native SOC by 14.7, 29.0, and 41.5% in the B30, B60, and B90 treatments, respectively, while the content of maize-derived native SOC did not change, relative to the B0. In conclusion, although wheat-derived native SOC was higher than maize-derived native SOC, biochar application decreased the contribution of wheat residue to native SOC, possibly by enhancing its degradation, thus decreasing wheat-derived native SOC storage in an agricultural system.

1. Introduction

Biochar, which is the carbon (C)-rich product of pyrolysis of biomass materials, produced at a high temperature in an oxygen-limited condition, has commonly been promoted for long-term sequestration of organic C in soils, while abating climate change (Lehmann et al., 2006; Woolf et al., 2010). Biochar is mostly rich in condensed aromatic compounds and has a relatively high resistance to biodegradation (Baldock and Smernik, 2002; Knicker et al., 2013). Thus, biochar can persist in soil for centuries or millennia (Cheng et al., 2008; Kuzyakov et al., 2009; Singh et al., 2012; Fang et al., 2014a). In addition to directly increasing total organic carbon (TOC) contents in soil (Lehmann et al., 2006; Liu et al., 2014; Dong et al., 2016), biochar application has also been shown to affect the mineralization rates of soil organic carbon (SOC), such as derived from plant residue inputs, through the process of positive (Keith et al., 2011; Luo et al., 2011; Singh and Cowie, 2014) or negative priming effects (Kuzyakov et al., 2000). Therefore, the C sequestration potential of biochar in soil may be altered depending on the magnitude and direction of priming effects of biochar on native SOC (Fang et al., 2015; Maestrini et al., 2015; Weng et al., 2015). However, the influence of biochar on native SOC derived from different types of organic materials (such as maize and wheat residues) over a long term under a realistic field condition and the related mechanisms is not yet clear (Whitman et al., 2014; Dharmakeerthi et al., 2015; Weng et al., 2015).

There are a few key priming mechanisms through which biochar can influence the mineralization rates of native SOC. First, biochar provides some labile components and a suitable environment for microorganism growth and survival to simultaneously increase mineralization of other organic C forms in soil *via* co-metabolism (Luo et al., 2011; Zimmerman et al., 2011; Singh and Cowie, 2014). Second, biochar may enhance microbial nutrient mining of soil organic matter, particularly when nutrient availability is suppressed by biochar (Whitman et al., 2014). In contrast, biochar can promote formation of organo-mineral complexes

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(Keith et al., 2011; Weng et al., 2015) and sorb labile organic C (Pignatello et al., 2006), thus suppressing native SOC mineralization rates.

In recent years, studies on the effect of biochar on native SOC gains or losses has progressed from the initial laboratory-incubation experiments with biochar and soil mixtures (Kuzyakov et al., 2009; Zimmerman et al., 2011; Singh and Cowie, 2014) to the mixing of biochar-treated soil with exogenous organic matter additions (Keith et al., 2011; Dharmakeerthi et al., 2015). Further, there have been a few glasshouse- or field-simulated experiments with crop planting (Whitman et al., 2014; Keith et al., 2015; Weng et al., 2015). However, up to now, studies continue to be limited under realistic field conditions (Singh et al., 2015; Ventura et al., 2015; Weng et al., 2015, 2017) that involve stochastic variations of environment and repeated residue-C inputs, relative to controlled laboratory or glasshouse studies (Fierer and Schimel, 2002; Feng et al., 2007). Hence there is a lack of accurate assessment of the long-term impact of biochar on native SOC storage in agroecosystems. In a meta-analysis study, Maestrini et al. (2015) also emphasized the need for future studies at field scale to enhance our understanding of long-term interactions between biochar and native SOC, as well as to accurately assess the C sequestration potential of biochar in agricultural systems.

In the North China Plain, the wheat–maize rotation is a common practice in cropping systems. Recent studies have shown that C_3-derived plant residues were more effective at promoting the accumulation of native SOC than the residues of C_4 plants, possibly due to differences in their decomposition dynamics (Wang et al., 2015). Further, Wynn and Bird (2007) found that C_4-derived SOC decomposed faster than its C_3 counterpart in soils containing C_3/C_4-derived SOC. However, there have been no field-based studies on how biochar and its application rates influence the contributions of C_3 and C_4 plant residues to the formation of SOC, such as in a wheat-maize rotation cropping system.

Further, isolation of SOC into fractions with different stabilities may provide insights into the forms of SOC that may be impacted by biochar. For example, sequential hydrolysis of SOC with increasing concentration of H_2SO_4 is a common chemical fractionation procedure to obtain “labile pool I” (relatively labile C), “labile pool II” (less labile C) and “recalcitrant C pool” (Rovira and Ramón Vallejo, 2007; Rovira and Vallejo 2002). However, only limited research has been done on assessing changes in native SOC fractions, derived from constant inputs of different types of crop residues over a longer-term, caused by biochar and its application rates, particularly at field scale.

In our field experiment that began in 2009, we assessed the response of native SOC changes five years after biochar application at normal to very high rates (0, 30, 60, and 90 t ha^{-1}). These application rates of biochar are consistent with other studies (Chan et al., 2007; Jones et al., 2012; Singh et al., 2015). In our study, combined with the annual incorporation of wheat (C_3) and maize (C_4) straw residues, we aimed to investigate: 1) the impacts of the application rates of biochar on native SOC content (*i.e.* without biochar C) in a field condition after 5 years; and 2) the impacts of biochar application on native SOC fractions (labile, less labile or recalcitrant). We hypothesized that 1) a continuous return of crop residues to a soil system will increase native SOC content; however biochar presence will constrain native SOC storage derived from the residues, *i.e.*, the native SOC gains will decrease with increasing application rates of biochar; 2) biochar will have a greater negative influence on relatively labile than stable native SOC fractions because labile native SOC can respond easily to changes in soil environment and properties caused by biochar; and 3) biochar presence will constrain the gains of native SOC derived from maize residues (C_4 plant), which is more labile than wheat residues (C_3 plant).

Table 1
Properties of the soil (0–20 cm layer) and applied biochar from the field experiment.

Properties	Soil	Biochar
Sand (> 0.05 mm), g kg^{-1}	280 ± 14	–
Silt (0.05-0.002 mm), g kg^{-1}	520 ± 26	–
Clay (< 0.002 mm), g kg^{-1}	200 ± 10	–
Bulk density, g cm^{-3}	1.63 ± 0.05	–
Total organic carbon, g kg^{-1}	4.32 ± 0.08	491 ± 3
Total nitrogen, g kg^{-1}	0.62 ± 0.02	12.2 ± 0.8
C/N	6.97	40.2
$CaCO_3$, g kg^{-1}	17.3 ± 0.2	25.0 ± 0.2
Ash, g kg^{-1}	–	360 ± 3
pH	8.02 ± 0.02	10.64 ± 0.01
EC, mS cm^{-1}	0.19 ± 0.01	1.02 ± 0.05
CEC, cmol(+) kg^{-1}	10.0 ± 0.1	12.5 ± 0.1
Particle size	–	0.5–5 mm
Surface area (m^2 g^{-1})	–	15.68

2. Materials and methods

2.1. Experimental site conditions and design

This field-based experiment was performed at the Shang-zhuang Experimental Station of the China Agricultural University in Haidian District, Beijing, China (40°08′21″N, 116°10′52″E) (Liang et al., 2014). The field site was within an alluvial plain with an elevation of 51 m and a shallow underground water table at 1.0–1.5 m depth. This location experiences a typical continental monsoon climate, with a recent average annual air temperature of 11.6 °C and average annual precipitation of 400 mm. The highest and lowest air temperatures occur in July and January, respectively. Rainfall mainly occurs between July and August. The soil of the experimental field is classified as Fluvisol according to the FAO system. The properties of the 0–20 cm soil layer are provided in Table 1. Soil particle distribution was measured following the methods of Stemmer et al. (1998), and TOC and total nitrogen (TN) were analyzed using an elemental analyzer (see Section 2.2). Soil pH was determined in soil suspension at a soil-to-water ratio of 1-to-5 using a glass electrode. Electrical conductivity (EC) was measured in 1-to-5 soil-to-water extracts using an electrical conductivity meter (Fang et al., 2014b). Soil carbonate was measured by potentiometric titration (Loeppert and Suarez, 1996). Cation Exchange Capacity (CEC) was measured through sodium acetate extraction followed by flame-photometry (Rhoades, 1986). Soil bulk density was assessed by core (5 cm diameter) sampling and calculated as the ratio of oven-dried mass to its volume (Andreev et al., 2016).

The field experiment was started in June of 2009, as a set-up of four treatments with three replicate plots for each treatment. Plots were 11 × 10 m (110 m^2), and randomly arranged throughout the experimental field. The four treatments included no application of biochar and biochar application rates of 30, 60, and 90 t ha^{-1}, all abbreviated as B0, B30, B60, and B90, respectively. The biochar used in the experiment was produced from organic waste substrates used for mushroom production by slow pyrolysis at 400 °C for 4 h in a sealed oven. Organic wastes were a mixture of rice husks (70%) and cotton seed hulls (30%). The conversion efficiency of the pyrolysis was about 35%. As a commercial biochar-producing system, pyrolysis conditions between each oven were almost the same. In the experiment, the biochar was a uniform mixture of a single production of the batch. The measured properties of the biochar are shown in Table 1. The analytical procedures for most biochar properties (organic C, TN, pH, EC, CEC) were the same as used for the soil properties. The biochar surface area was analyzed by following a Brunauer–Emmett–Teller (BET) method (Dai et al., 2013). Ash contents was measured by heating biochar at 550 °C in a muffle furnace for 4 h. The biochar particles (> 90% were within the 0.5–5 mm range) were evenly spread by hand on the surface of the plots and then mixed well with the 0–20 cm soil layer using a rotary cultivator.

In the experimental field, winter wheat (C_3 plant) was planted in October and harvested in June. Then summer maize (C_4 plant) was planted in June and harvested in October, which follows the typical local cropping system. Winter wheat was flood irrigated annually in early December and mid-May with 900 m^3 ha^{-1} of water each time. Summer maize was not irrigated during the growing season. The 0–20 cm soil layer was tilled during the harvest of summer maize in preparation for sowing of winter wheat in early October. Fertilization of both maize and wheat occurred at sowing time at the same fertilization rates of 112.5 N, 112.5 P_2O_5, and 112.5 K_2O kg ha^{-1}. The fertilizer was a compound fertilizer including 15% content each for N, P_2O_5, and K_2O. Before 2009, wheat and maize straw residues were not returned to the site after harvest and were baled and removed for a decade or more. From 2009 onwards, the crop residue management was also consistent with one of the local practices, that is, the crop straw was mechanically chopped and returned to the site after harvest. Wheat straw was chopped into 2–3 cm lengths and mulched on the soil surface in early June. Maize straw was chopped into 1–2 cm lengths in early October, and then both maize straw and wheat residue (i.e. after the 4-month mulching period) were plowed into the 0–20 cm soil layer straightway.

Before the harvest of wheat in June of 2014, *ca.* 2 kg soil samples from the 0–20 cm soil layer were collected. Sampling was conducted in all three plots of each treatment at 10 randomly selected sites within in each plot. Soil samples were collected with a soil auger and then mixed well. After air-drying and manually removing visible matter (plant roots, soil fauna, etc.), soil or soil–biochar samples were gently sieved through a 2 mm sieve for various measurements (see below).

2.2. *Total organic carbon (TOC) content*

To remove carbonates, samples of soil, soil-biochar mixtures or biochar particles were soaked separately in a 1:10 (w/v) ratio of sample and 1 M HCl. The TOC content of the HCl-treated samples was then measured using an elemental analyzer (vario EL III, CHNOS Elemental Analyzer, Elementar, Germany). In the soil-biochar mixtures, TOC means native SOC plus biochar C content.

2.3. *Separation of aged biochar particles*

Biochar particles (> 0.5 mm) from the B30, B60, and B90 treatments were separated by hand until no visible biochar particles were present in soil samples (Koide et al., 2011). Biochar particles were then suspended into distilled water at a ratio of 1:10 (w/v), and shaken vigorously to dislodge the soil particles. The biochar was then rinsed four times with distilled water, and dried at 60 °C (Koide et al., 2011).

2.4. *Biochar carbon amount in soils*

The biochar C content in the soil was quantified following the loss on ignition method of Koide et al. (2011). This method involved separately drying 3.0 g representative subsamples of soil without biochar (B0) and with biochar (B30, B60 and B90) and also aged biochar particles (collected by hand), at 105 °C for 24 h. Samples were then weighed and placed into a 550 °C muffle furnace to react for 4 h, before they were weighed to determine the residual mass of each sample. The residual masses were used to calculate the loss rates for each sample type (*i.e.*, L_{soil}, $L_{biochar}$, and $L_{mixture}$).

The amount of biochar in a soil sample was calculated as follows:

$$Biochar\ \ amount(g\ biochar\ kg^{-1}\ soil\ sample) = \frac{L_{mixture} - L_{soil}}{L_{biochar} - L_{soil}} \times 1000 \tag{1}$$

Where, $L_{soil} = 3.55\% \pm 0.05\%$, $L_{biochar} = 64.20\% \pm 0.06\%$.

$$\text{Biochar C amount}(\text{g C kg}^{-1}\text{soil sample}) = \text{Biochar amount} \times TOC_{biochar} \tag{2}$$

$TOC_{biochar}$ is the total organic C content within biochar, and was measured by an elemental analyzer.

2.5. *Native soil organic carbon (SOC) content*

The native SOC content that excludes biochar C, is calculated by subtracting the biochar C amount from the TOC amount in a soil-biochar mixture.

$$\text{Native SOC}(\text{g C kg}^{-1}\text{soil}) = \frac{\text{TOC} - \text{biochar carbon amount}}{1000\text{–biochar amount}} \times 1000 \tag{3}$$

2.6. *Fractions of native SOC*

The fractions of native SOC were measured following acid hydrolysis of soil according to the methods of Rovira and Vallejo (2002). Firstly, about 500 mg of B0, B30, B60 and B90 samples (< 0.15 mm) were weighed and then hydrolyzed with 20 ml of 2.5 *M* H_2SO_4 for 30 min at 105 °C in sealed Pyrex tubes. The hydrolysate was recovered by centrifugation and decantation. The residue was washed with 20 ml of water and the washing added to the hydrolysate. This hydrolysate was taken as labile pool I (first step, weak acid hydrolysable C). The residue was dried at 60 °C. Secondly, the residue was hydrolyzed with 2 ml of 13 *M* H_2SO_4 overnight at room temperature, under continuous shaking. Thereafter, water was added to dilute the acid to 1 *M* and the sample was hydrolyzed for 3 h at 105 °C with occasional shaking. The hydrolysate was recovered by centrifugation and decantation. The residue was washed with 20 ml of water, and the washing added to the hydrolysate. This hydrolysate was taken as labile pool II (second-step, strong acid hydolysable C). Labile pool I and labile pool II were measured using TOC/TN analyzer (Elementar Analysensysteme GmbH, Germany). The difference between native SOC and sum of labile pool I and labile pool II was defined as "recalcitrant C pool" (Rovira and Vallejo, 2002). These C fractions in the 5-year aged biochar (about 50 mg) across the treatments were analysed using the same process, as for the soil-biochar mixture. The contents of native SOC (without biochar) in labile pool I and labile pool II were calculated after accounting for aged biochar C labile pool I (8.33 ± 0.25 g kg^{-1}) and labile pool II (7.30 ± 0.18 g kg^{-1}) within the soil-biochar mixtures of each treatment.

2.7. *$\delta^{13}C$*

The $\delta^{13}C$ of soil or soil-biochar mixture ($\delta^{13}C_{TOC}$), straw residues, freshly applied and aged biochar (recovered from soil in 2014) were measured using an isotope ratio mass spectrometer (IsoPrime IRMS, GV Instruments, Manchester, UK). The mean and standard deviation (SD) $\delta^{13}C$ values of wheat straw (collected in 2012 and 2014) and maize straw (collected in 2012 and 2013) were −27.47 ± 0.67‰ and −13.58 ± 0.39‰, respectively; see Table 2 for the mean and SD $\delta^{13}C$ values of other organic materials.

2.8. *Native SOC sources*

The amounts (g C kg^{-1} soil) of TOC, native SOC and biochar C, and the $\delta^{13}C$ values of TOC ($\delta^{13}C_{TOC}$) and biochar ($\delta^{13}C_{biochar}$) in the soil-biochar mixture (see Sections 2.4 and 2.7, respectively) were used to calculate $\delta^{13}C$ of native SOC ($\delta^{13}C_{soc}$) by the following equation:

$$\delta^{13}C_{SOC} = \frac{(\delta^{13}C_{TOC} \times \text{TOC} - \delta^{13}C_{biochar} \times \text{biochar C})}{Native\ SOC} \tag{4}$$

The $\delta^{13}C_{soc}$ values were shown in Fig. 1.

Table 2
Mean $\delta^{13}C$ (± standard deviation) values of freshly applied (2009) and 5-year aged biochar (recovered from soil in 2014) and soil-biochar mixture.

	Biochar	TOC
2009	−26.79 ± 0.26	−21.38 ± 0.23
B0	–	−23.83 ± 0.32
B30	−26.09 ± 0.53	−24.39 ± 0.31
B60	−26.50 ± 0.01	−24.93 ± 0.24
B90	−26.50 ± 0.28	−25.54 ± 0.25

B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

The proportion of native SOC attributed by wheat-(f_1) and maize-derived (f_2) organic C was calculated using the isotope mixture model:

$$-27.47\text{‰} \times f_1 + -13.58\text{‰} \times f_2 = \delta^{13}C_{SOC} \quad (5)$$

$$f_1 + f_2 = 1 \quad (6)$$

where, $\delta^{13}C_{soc}$ is the $\delta^{13}C$ value of native SOC, and f_1 and f_2 are the proportional contributions of wheat and maize to the native SOC.

2.9. Statistical analyses

Significant differences in TOC, native SOC, SOC fraction and residue-C source contents among the biochar treatment were tested with a one-way ANOVA using R software (R i386 3.02). Duncan's multiple range test was used to compare the treatment mean values at 5% level of significance. Pearson's correlations were performed between biochar application rates and TOC, native SOC, SOC fraction or proportional residue-C contributions to native SOC.

3. Results

3.1. TOC and native SOC contents

The inclusion of a biochar produced from rice husks and cotton seed hulls increased ($p < 0.05$) the TOC content of the soil after 5 years; the effect was dependent on the dose of biochar applied (Fig. 2) The TOC content of the soil was 4.32 g kg^{-1} in 2009, which increased to 7.84 g kg^{-1} in 2014 in the B0 treatment. The TOC contents in the B30, B60, and B90 treatments were increased by 32.3%, 67.4%, and 104.7%, respectively, compared to the B0 treatment (Fig. 2). Correlations between biochar application rates and TOC contents were significant ($p < 0.05$) and positive, and applying 1 t ha^{-1} of biochar increased the TOC content by 0.09 g kg^{-1} after five years.

The amount of residue left after loss on ignition was influenced by increasing biochar application rates. Calculations using Eqs. (1) and (2) showed that the relative amount of biochar (% of total soil-biochar amount) in the B30, B60, and B90 treatments were 0.67%, 1.37%, and 2.09%, respectively (Table 3). Biochar application significantly decreased native SOC ($p < 0.05$), and with increasing biochar application rates, the content of native SOC decreased by 11.4%, 21.4%, and 30.4%, respectively, relative to the B0 treatment (Fig. 2). There was a significant negative correlation between the biochar application rate and the native SOC content ($p < 0.05$), implying that five years after application of 1 t ha^{-1} of biochar, the native SOC content could be decreased by 0.35–0.37%.

3.2. Native SOC fractions

With increasing biochar application rates, the labile pool I in the soil significantly decreased ($p < 0.05$) but not the labile pool II. Five years after biochar application, the labile pool I content in the B0 treatment was 2.41 g kg^{-1} soil, which decreased by 11.2, 33.2, and 47.7% in the B30, B60, and B90 treatments, respectively (Table 4). The labile pool I content correlated significantly ($p < 0.05$, $n = 4$) and negatively with the quantity of biochar applied at −0.013 g kg^{-1} per ton of biochar application per hectare.

3.3. Native SOC sources

Maize residue-derived C contributed 26.2% of the native SOC in the B0 treatment (Fig. 3), which increased to 29.0%, 33.4%, and 38.1%, respectively, in the B30, B60, and B90 treatments. The contribution of wheat residue-derived C to native SOC in the B0 treatment was 73.8%, which decreased by 2.8%, 7.2%, and 11.9% in the B30, B60, and B90 treatments, respectively. The quantity of applied biochar correlated significantly and positively with the proportional contribution of maize C to native SOC ($p < 0.01$, $n = 4$), but negatively with the proportional contribution of wheat C to native SOC ($p < 0.01$, $n = 4$).

The native SOC content derived from maize residues in the B0 treatment was 2.05 g kg^{-1}. In the B30, B60, and B90 treatment plots, maize contributed 2.02, 2.06, and 2.08 g kg^{-1} of organic C, respectively, with no significant differences among the biochar treatments. The native SOC content derived from wheat residues in the B0 treatment was 5.78 g kg^{-1}, and it decreased by 14.7%, 29.1%, and 41.5% in the B30, B60, and B90 treatments, respectively.

4. Discussion

To our knowledge, this study reports for the first time the influence of biochar and its application rates on native SOC derived from

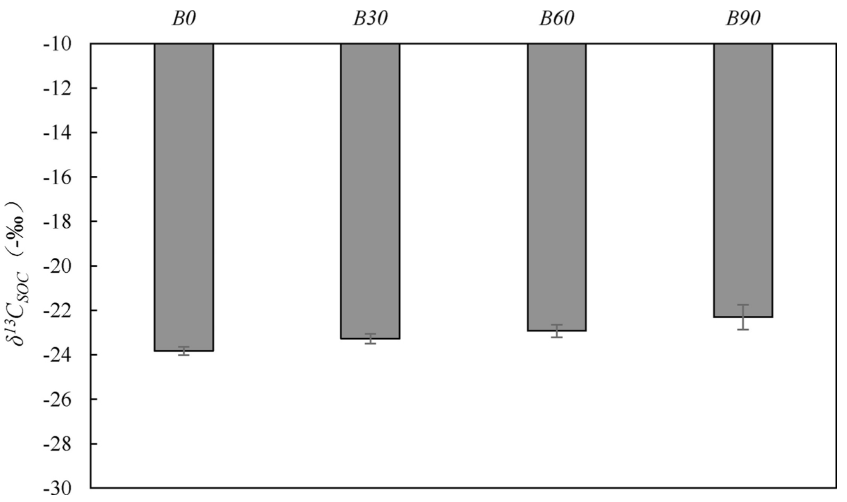

Fig. 1. The $\delta^{13}C$ of soil native organic carbon ($\delta^{13}C_{SOC}$) across different biochar treatments. B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

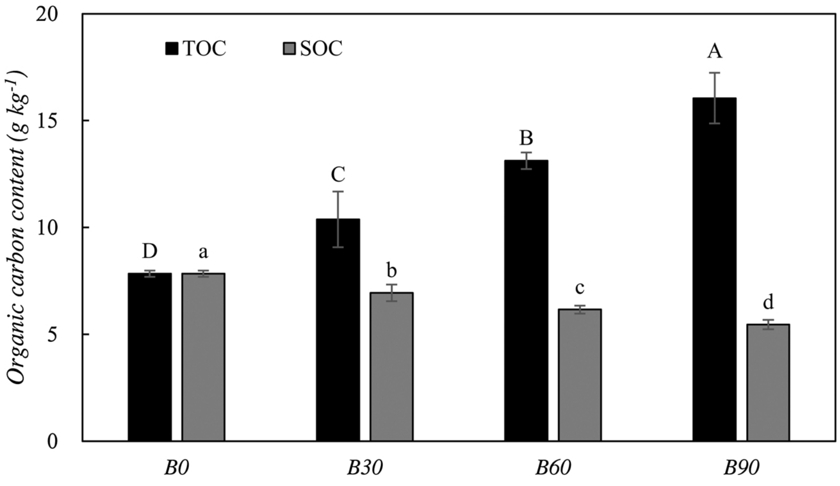

Fig. 2. The total organic carbon (TOC) and native soil organic carbon (SOC) contents in soils across different biochar treated plots. Different letters demarcate significant differences between treatments ($p < 0.05$). B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively. The red line means the SOC content (4.32 g kg^{-1}) in 2009. TOC includes biochar carbon; native SOC excludes biochar carbon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Loss on ignition and biochar amount in different biochar treatments.

Treatments	Loss on ignition (%)	Biochar amount (g kg^{-1})	Biochar C amount (g kg^{-1})
B0	3.55 ± 0.05 d	0	0
B30	3.96 ± 0.07 c	6.70 ± 1.16 c	3.45 ± 0.56 c
B60	4.38 ± 0.03 b	13.67 ± 0.58 b	7.05 ± 0.28 b
B90	4.82 ± 0.09 a	20.90 ± 1.40 a	10.78 ± 0.72 a

Different letters mean the difference between the treatments is significant ($p < 0.05$). B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

Table 4
SOC fractions content (g kg^{-1}) in SOC in different treatments.

Treatments	Labile pool I	Labile pool II	Recalcitrant
B0	2.41 ± 0.23 a	1.65 ± 0.53 a	3.78 ± 0.26 a
B30	2.14 ± 0.10 b	1.30 ± 0.06 a	3.50 ± 0.31 a
B60	1.61 ± 0.08 c	1.33 ± 0.26 a	3.22 ± 0.48 a
B90	1.26 ± 0.05 d	1.24 ± 0.17 a	2.96 ± 0.34 a

Different letters demarcate significant differences between treatments ($p < 0.05$). B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

repeated returning of wheat (C_3 plant) and maize (C_4 plant) crop residues (straw- and root-derived) after five years, with biochar serving as a third C source, in a two-crop rotation system at field scale. It is indeed hard or impossible to separate native SOC derived from two C sources by using only one tracer (^{13}C), while a third C source is also present (Tian et al., 2016). However, some studies have shown that by using two tracers (natural ^{13}C and labelled ^{14}C), one can successfully separate three C sources in soil (Kuzyakov and Cheng, 2001; Tian et al., 2016). Even though Kuzyakov and Bol (2004) distinguished three soil C sources using the natural ^{13}C differences through a complex experimental design, it had a few shortcomings including accuracy, background variations of natural ^{13}C in soil organic matter pools, and the correspondence of δ^{13}C signatures from two C sources to calculate the third C source contributions to SOC. Hence, the only useful approach to distinguish three C sources by using one ^{13}C tracer was to firstly derive C content and δ^{13}C signature of one of three C sources (such as biochar in our study). We then elucidated the interaction of the biochar with the other two organic C sources (*i.e.* C_3 wheat and C_4 maize-derived) and their contributions to native SOC using a two-pool isotopic mixing model.

4.1. Impact of biochar on the content of native soil organic carbon

The 81% increase in native SOC content in the B0 treatment observed in this long-term field-based study, *i.e.* from 4.32 g kg^{-1} in 2009 to 7.84 g kg^{-1} in 2014, was likely due to the constant returning of crop

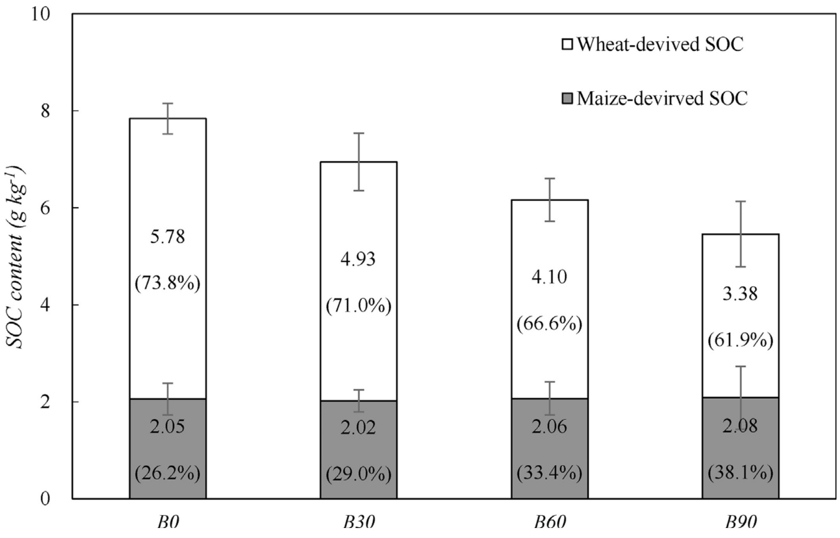

Fig. 3. Contributions of maize- and wheat-derived residues to soil organic carbon (SOC) content. The B0, B30, B60, and B90 are treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively. The upper numbers represent the SOC content derived from wheat or maize, while the lower numbers in parentheses are the percentage of SOC derived from wheat or maize residues.

straw after each harvest since 2009. However, we found that the gains in native SOC from the input of crop residues at the field site were constrained in the presence of biochar (B30, B60 or B90), relative to the B0 treatment. Clearly, biochar application contributed to the decrease of native SOC content (Fig. 2 and 3), possibly *via* enhancing decomposition of organic C derived from the crop residues over the five years in this study. This argument is further supported by no significant differences in the annual crop biomass yield (for both wheat and maize) among the biochar treatments during the first four years (Liang et al., 2014) and also in the fifth year (4.3–5.0 t ha^{-1} for wheat and 7.9–8.7 t ha^{-1} for maize). These results of lower native SOC content in the presence *vs.* absence of biochar are consistent with the observed positive priming effects of biochar on native SOC, reported in short- or long-term incubation experiments (Luo et al., 2011; Singh and Cowie, 2014). Thus, the reduced incorporation of crop residue-derived C into native SOC after biochar application may be explained by certain mechanisms that would enhance positive priming of native SOC mineralization by biochar. These mechanisms could be: (a) increased soil microbial activity due to some labile C and nutrient inputs from biochar, and with biochar being serving as a habitat for microorganisms (Lehmann et al., 2011); and (b) enhanced soil aeration and water holding capacity favored by high porous structure and surface area of biochar (Zimmerman et al., 2011; Liang et al., 2014; Watzinger et al., 2014; Whitman et al., 2014). These mechanisms, if occurred, seem to be supported by the increased application rates of the biochar.

4.2. Impacts of biochar on native SOC fractions

In our study, we also provided insights into the influence of biochar on relatively labile and recalcitrant native SOC fractions, which cannot be distinguished by measuring CO_2 released from mineralization of native SOC (Keith et al., 2011; Weng et al., 2015). We found that the increasing rates of biochar application significantly decreased labile pool I of native SOC (Table 4), which may have comprised of ‘relatively labile’ non-cellulosic carbohydrates and polyphenolics (Rovira and Vallejo, 2002). However, biochar and its application rates did not affect the labile pool II and recalcitrant C pool of native SOC, which may predominantly comprise of ‘less labile’ cellulosic carbohydrates and polyphenolics (labile pool II) or aliphatic and aromatics (recalcitrant pool) (Rovira and Vallejo, 2002). Our results are of greater degradation (positive priming) responses of labile *vs*. stable native SOC fractions to biochar-induced changes in soil properties, such as aeration, available water and nutrients, and microbial activity, are in agreement with other studies (Lentz and Ippolito, 2012; Novak et al., 2012; Mukherjee et al., 2014; Laghari et al., 2015; Ma et al., 2016). Cheng et al. (2016) also found that biochar presence can stimulate decomposition of relatively simple organic materials (glucose and amino acids) added to a sandy loam soil. Many other studies have also reported a greater response of labile *vs*. stable native SOC fractions to changes in management practices in agro-ecosystems (Neff et al., 2002; Bhattacharyya et al., 2011).

4.3. Impact of biochar on native SOC derived from C_3-C_4 residue inputs

During the experiment, the five-year average biomass yield of summer maize (~9.2 t ha^{-1}) was almost two times that of winter wheat (~5.3 t ha^{-1}) across the treatments, however, their contributions to native SOC were opposite (Fig. 3), and these results were consistent with the findings of other studies (Fuentes et al., 2010; Tang et al., 2012; Wang et al., 2015). The differences between wheat and maize contributions to native SOC could be due to a relatively faster decomposition rate for C_4-derived organic matter (maize straw) compared to C_3-derived wheat straw (Buyanovsky and Wagner, 1996; Wynn and Bird, 2007). In general, maize residues have lower lignin contents and smaller C:N ratios, implying a greater decomposition rate relative to wheat residues (Zhang et al., 2008; Talbot and Treseder, 2012; Wang et al., 2015). Also, maize-derived root residues would have faster decomposition rates, likely due to high soil temperatures during summer, compared to the winter wheat-derived root residues, thus resulting in overall lower contribution of maize *vs.* wheat residues to native SOC (Wang et al., 2015). Furthermore, as maize straw was incorporated into the soil soon after harvest, while wheat straw was mulched until the next crop (maize) harvest, this would have increased decomposition of maize straw and decreased its contribution to native SOC (*cf.* wheat residue-derived SOC).

It is known that, under realistic field conditions, there could be complex interactions among biochar (where applied), fresh straw and root inputs, and semi-decomposed plant residues (Duong et al., 2009; Nguyen et al., 2016). Further, there are stochastic variations in temperatures and soil wetting-drying cycles in a field environment (Fierer and Schimel, 2002). Thus, it is quite challenging to assess the influence of biochar on native SOC storage or mineralization from different residue-derived C sources under planted field settings (Singh et al., 2015; Ventura et al., 2015; Weng et al., 2015, 2017). Using the novel natural ^{13}C isotopic approach, our results clearly suggest that biochar application would have accelerated the decomposition of wheat-derived residues, so its relative contribution to native SOC decreased with increasing biochar application rates (Fig. 3).

Our findings of decreased storage of wheat- *vs.* maize-derived native SOC after five years in the presence of biochar did not support the third hypothesis, which was based on previous published results on maize-derived native SOC being more labile and responsive to environmental changes than wheat-derived native SOC (Wynn and Bird, 2007; Wang et al., 2015). There could be a few reasons for these contradictory results. First, as wheat-derived (*cf.* maize-derived) SOC was higher, the labile fraction of wheat-derived SOC may also be higher and hence may be more responsive to changes to management practices (e.g. biochar *vs.* non-biochar) than maize-derived SOC (Neff et al., 2002; Bhattacharyya et al., 2011). Second, previous studies have shown biochar can provide labile substrates and an environment to support growth of both bacterial and fungal biomass, with a shifting to the dominance of fungal biomass in an aged biochar soil system (Gul et al., 2015). Further, fungal biomass can enhance degradation of organic residues with a high C:N ratio and lignin content, such as derived from wheat residues (Hu et al., 2014; Rex et al., 2015) and hence may enhance their humification efficiency (Six et al., 2006; Rousk and Bååth, 2007).

5. Conclusions

This is the first field-based study that investigated the long-term impact of biochar applied at different rates on native SOC derived from C_3 and C_4-derived crop residues. The results demonstrated that biochar application constrained the accumulation of native SOC derived from wheat residues during the first five years. Our novel isotopic approach, i.e. firstly separating biochar as a third C source, and then isolating contributions of two residue-derived C sources (C_3 vs. C_4) to native SOC using the two-pool isotopic model, further showed that biochar presence decreased wheat residue contribution to native SOC. Further, a labile C fraction (first-step, weak acid hydrolysable C) decreased with increasing biochar application rates to the soil over the long-term, while the stable C fractions did not respond to the biochar treatments. In general, our findings suggest that the decreased storage of wheat-derived C in response to biochar application would decrease the C sequestration potential of biochar in agro-ecosystems receiving wheat and maize-derived residues. Future research is needed to evaluate long-term responses of C_3–C_4 crop residues (e.g. their degradation dynamics and humification efficiency) to changes in microbial community abundance and composition in the presence of biochar.

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