Biochar altered native soil organic carbon by changing soil aggregate size distribution and native SOC in aggregates based on an 8-year field experiment

Zhencai Sun, Zhengcheng Zhang, Kun Zhu, Zhimin Wang, Xiaorong Zhao,
Qimei Lin, Guitong Li

PII: S0048-9697(19)34821-1
DOI: https://doi.org/10.1016/j.scitotenv.2019.134829
Reference: STOTEN 134829

To appear in: *Science of the Total Environment*

Received Date: 18 July 2019
Revised Date: 21 September 2019
Accepted Date: 3 October 2019

Please cite this article as: Z. Sun, Z. Zhang, K. Zhu, Z. Wang, X. Zhao, Q. Lin, G. Li, Biochar altered native soil organic carbon by changing soil aggregate size distribution and native SOC in aggregates based on an 8-year field experiment, *Science of the Total Environment* (2019), doi: https://doi.org/10.1016/j.scitotenv.2019.134829

Biochar altered native soil organic carbon by changing soil aggregate size distribution and native SOC in aggregates based on an 8-year field experiment

Zhencai SUN[a], Zhengcheng ZHANG[b*], Kun ZHU[b], Zhimin WANG[a], Xiaorong ZHAO[b], Qimei LIN[b], Guitong LI[b†]

a College of Agronomy, China Agricultural University

b College of Resources and Environment, China Agricultural University

Address: No. 2. West Road of Yuan-Ming-Yuan, Haidian District, Beijing, China, 100193

*Contribution is the same as the first author

†Corresponding author: lgtong@cau.edu.cn

Biochar altered native soil organic carbon by changing soil aggregate size distribution and native SOC in aggregates based on an 8-year field experiment

Zhencai SUN[a], Zhengcheng ZHANG[b*], Kun ZHU[b], Zhimin WANG[a], Xiaorong ZHAO[b], Qimei LIN[b], Guitong LI[b†]

a College of Agronomy, China Agricultural University

b College of Resources and Environment, China Agricultural University

Address: No. 2. West Road of Yuan-Ming-Yuan, Haidian District, Beijing, China, 100193

*Contribution is the same as the first author

†Corresponding author: lgtong@cau.edu.cn

Abstract

Soil aggregates play an important function in soil carbon sequestration because larger aggregates have higher soil organic carbon contents. A field experiment was set up in 2009 that included four treatments, i.e., B0, B30, B60, and B90 representing biochar application rates of 0, 30, 60, and 90 t ha^{-1}, respectively. In 2017, we investigated the soil aggregate distribution, biochar and n-SOC contents in soil and different aggregate sizes using the ignition method, as well as the contribution of wheat and maize residues to n-SOC content in each aggregate by isotopic analysis. The results showed that, relative to B0, the n-SOC content presented an 14.0% decrease in B30, compared with an 18.8% and 8.2% increase in B60 and B90 ($p<0.05$), respectively. Furthermore, the decreased n-SOC content in B30 was due to the decreased proportions of < 53 μm and 1000-250 μm aggregates. The increased n-SOC content in B60 was due to the significantly enhanced proportion of 2000-1000 μm and 1000-250 μm aggregates because the n-SOC contents of these two aggregates size classes were not changed by biochar. However, in B90, the increased n-SOC content was ascribed to the enhanced proportions of 2000-1000 μm and < 53 μm aggregates, although the n-SOC content in 2000-1000 μm aggregate was significantly decreased by biochar. Further analysis showed that the decreased n-SOC content in 2000-1000 μm aggregates was associated with decreased wheat-derived n-SOC content. In synthesis, our study showed a long-term effect of biochar on the n-SOC content by mainly changing soil aggregation and native organic carbon derived from wheat residue, and this effect was dependent on the applied amount. The biochar rate of 60 t ha^{-1} is recommended for carbon sequestration in terms of the more pronounced negative priming of native SOC, while the feasible combination between other biochars and soils needs further clarification.

Key words: long-term field experiment, biochar rates, aggregate distribution, native SOC, wheat-maize cropping system.

1. Introduction

Biochar is made by the pyrolysis of organic materials such as agricultural and forestry residues under low oxygen or anaerobic conditions (Lehmann and Joseph, 2015). Due to the richness of aromatic carbon, biochar is recalcitrant and therefore has considerable potential for increasing soil carbon sequestration (Lehmann et al., 2006; Smith, 2016). Studies have reported that biochar can significantly increase the total soil organic carbon content (including biochar itself) after its application to soils in several agroecosystems (Krull et al., 2006; Novak et al., 2009; Van Zwieten et al., 2010; Wang et al., 2018). In addition, biochar has been reported to alter soil physico-chemical properties in terms of increased soil pH (Jeffery et al., 2011; Wang et al., 2018), enhanced soil moisture (Abel et al., 2013; Sun et al., 2013; Hardie et al., 2014; Sun et al., 2015), and the increased microbiological activities (Lehmann et al., 2011; Duan et al., 2017; Senbayram et al., 2019). However, more and more recent evidence has shown that biochar has a strong interaction with native soil organic carbon (n-SOC) (Herath et al., 2014; Lu et al., 2014; Singh and Cowie, 2014; Tian et al., 2016; Weng et al., 2017; Dong et al., 2018). For instance, it was found that biochar suppressed the decomposition of n-SOC within 1 year (Lu et al., 2014; Herath et al., 2014) while other studies reported that biochar stimulated the decomposition of n-SOC after 3.5-5 years application (Singh and Cowie, 2014; Dong et al., 2018). With respect to potential mechanisms, Zimmerman et al. (2011) proposed that at the early stage (first 90 d) of biochar application, the decomposition of labile-C in biochar was stimulated due to cometabolism with soil organic carbon, whereas later, biochar suppressed n-SOC mineralization due to n-SOC sorption to biochar based on a one-year incubation trial. However, results from the incubation experiments may not be representative of field condition in terms of the input of residues, variations in moisture and temperature, and other management options that determine the soil carbon turnover. For instance, there were stochastic variations in temperatures and soil wetting-drying cycles in a field environment (Fierer and Schimel, 2002). In addition, there are complex interactions among roots, semi-decomposed plant residues and the applied fresh straw (Duong et al., 2009; Nguyen et al., 2016). A certain amount of biochar may be lost due to run-off and leaching in the field (Koide et al., 2011; Dong et al., 2018), which in turn may affect the total soil organic carbon by potentially changing interactions with n-SOC. In addition, although the potential impact of biochar on agricultural ecosystems has been thoroughly investigated for many years (Lehmann et al., 2006), most of the current reported results on n-SOC are still limited to a relatively short-term period, and, to date, there is limited evidence on the effect of biochar on n-SOC content more than 5 years after its application. This, to a certain extent, hinders the understanding of how biochar affects soil carbon sequestration potential or soil fertility over the long term. Weng et al., (2017) conducted a more than 9-year field study on a rhodic ferralsol of subtropical grass land with 10 t ha^{-1} biochar and found that biochar decreased n-SOC degradation through accelerating the formation of micro-aggregates having a protection effect on n-SOC. However, the long-term effect of biochar on n-SOC content in other soil types and in other regions remains unknown. In addition, the applied amounts of biochar largely ranged from less than 10 t ha^{-1} to more than 100 t ha^{-1} (Chan et al., 2008; Herath et al., 2014; Sun et al., 2015; Dong et al., 2018; Baiamonte et al., 2019), and it is still unclear about the response of n-SOC content to the applied biochar amounts over the long term.

Soil aggregates play a central role in soil carbon and nutrient turnover. The formation and stability of soil aggregates are affected by many factors, such as plant roots, microorganisms, and soil organic matter content, and their interactions (Six et al. 2006). For instance, SOC is an important cementitious substance that enhances the agglomeration of soil particles and promotes the formation of aggregate structures (Six et al., 2006). Larger soil aggregates have higher soil organic carbon contents (Gupta and Germida, 1988). Simultaneously, agglomeration can protect the internal organic carbon from the decomposition of microorganisms and can increase the stability of soil organic carbon. Therefore, there is a close relationship between soil aggregates and organic carbon. Previous studies have shown the effect of biochar application on the formation and stability of soil aggregates (Sun and Lu, 2014; Blanco-Canqui et al., 2017; Weng et al., 2017; Zhang et al., 2017; Baiamonte et al., 2019). For instance, Sun and Lu (2014) found that 6% rice husk biochar application not only significantly increased the proportion of macro-aggregates (2000-5000 μm and 250-500 μm) in a clayey soil, but also reduced the micro-aggregates proportion (<

250 μm). A similar observation was reported for a Mollisol in Northeast China (Zhang et al., 2019). Zhang et al. (2017) reported that biochar application at a rate of 16 t ha^{-1} increased the proportion of soil macro-aggregates (> 2000 μm) on the Loess Plateau of China, while no biochar application effect was observed at a rate of 8 t ha^{-1}, indicating dependence on the amount of biochar applied. In addition, it has been indicated that biochar can increase wet aggregate stability more in sandy than in silty clay and clayey soils (Ouyang et al., 2013; Burrell et al., 2016). A long-term field trial showed biochar aging due to physical, chemical, and biological effects on biochar, which may in turn influence the effects of biochar on soil quality, such as carbon sequestration (Blanco-Canqui, 2017). In addition, the considerable loss of biochar in a field trial of North China Plain (Dong et al., 2016) highlights the need for assessing the long-term biochar effects on soil aggregation and soil carbon sequestration, as well as the underlying mechanisms in terms of the contributors to n-SOC in each aggregate size. The long-term biochar as well as the biochar amount effect on soil aggregation and n-SOC content in different aggregate size classes is still unknown and needs clarification, this would facilitate the understanding on how the use of biochar influence n-SOC at a scale of aggregates.

Based on a field experiment established on the North China Plain in 2009, we assessed the interaction between biochar and n-SOC in different aggregate size classes at various biochar rates (0, 30, 60, and 90 t ha^{-1}) 8 years after application. The aim of this study was to investigate: under field conditions, (1) the total n-SOC content change 8 years after application of different rates of biochar, (2) change in soil aggregates size distribution and n-SOC content of each aggregates at different rates of biochar, and their contribution to total n-SOC content, and (3) the difference between contributions of wheat residue and maize residue to n-SOC content of each aggregate at different rates of biochar, and the association with changed n-SOC content. We hypothesized that biochar may change both soil aggregates size distribution and n-SOC content of aggregates with certain size class, and this scenario should depend on the applied biochar rates.

2. Materials and methods

2.1. Experimental site conditions and design

This experimental field was located at the Shangzhuang Experimental Station of China Agricultural University, Beijing, China (40°08′21″N, 116°10′52″E) (Liang et al., 2014). The field site has a typical continental monsoon climate, with an average annual air temperature of 11.6°C and an average annual precipitation of 400 mm. The highest and lowest air temperatures occur in July and January, respectively. Annual rainfall mainly occurs from July to August. The soil at the experimental field site is classified as a Fluvisol according to the FAO system. The soil particle distribution was measured following the method of Stemmer et al. (1998). Total organic carbon (TOC) and total nitrogen (TN) were analyzed using an elemental analyzer (vario EL III, CHNOS Elemental Analyzer, Elementar, Germany). Prior to the TOC and TN measurement, carbonates of all aggregates and biochar were removed by potentiometric titration (Loeppert and Suarez, 1996). In the soil-biochar mixtures, TOC represents n-SOC content plus the biochar C content. Soil pH was determined based on a soil-to-water ratio of 1:5 (*w/v*); the same ratio was used for electrical conductivity (EC) measurement (Fang et al., 2014). Cation exchange capacity (CEC) was measured by flame photometry (Rhoades, 1986). The soil bulk density was measured using a core (5 cm diameter × 5 cm length). The soil had SOC of 4.32 g kg^{-1}, TN of 0.62 g kg^{-1}, pH of 8.02, EC of 0.19 mS cm^{-1}, and CEC of 10.00 cmol(+)

kg^{-1}.

The field experiment was established in June 2009. Four treatments with three replicate plots for each treatment were set up following a completely random design. Each plot measured 11 m × 10 m (110 m^2). The four treatments consisted of biochar application rates of 0, 30, 60, and 90 t ha^{-1}, abbreviated as B0, B30, B60, and B90, respectively. The applied biochar rates refer to the results of our previous incubation up to 60 t ha^{-1} and the study of Chan et al., (2008). The biochar used was from a mixture of rice husks (70%) and cotton seed hulls (30%) used for mushroom production via a slow pyrolysis of 400°C for 4 h in a sealed oven. The conversion efficiency of the pyrolysis was approximately 35%. As a commercial biochar-producing system, the pyrolysis conditions between each oven were almost the same. The analytical procedures for most biochar properties, i.e., organic C, TN, pH, EC, and CEC, were the same as those used for the soil properties. The biochar surface area was analyzed following the Brunauer–Emmett–Teller (BET) method (Dai et al., 2013). The ash content was measured by heating biochar at 550°C in a muffle furnace for 4 h. More than 90% of the biochar particles were within the 0.5-5.0 mm range. In addition, the biochar had an SOC of 491.30 g kg^{-1}, TN of 12.20 g kg^{-1}, pH of 10.64, EC of 1.02 mS cm^{-1}, CEC of 12.51 cmol(+) kg^{-1}, and surface area of 15.68 m^2 g^{-1}.

At the experimental field site, all the treatments followed the traditional cultivation mode of winter wheat-summer maize rotation in the North China Plain, in which the winter wheat was planted in October and harvested in June, and the summer maize was planted in June and harvested in October. Prior to the experiment, the wheat and maize straw residues were removed from the field after harvest. The field management during the experiment was identical for all the treatments. Briefly, winter wheat was flood-irrigated annually in early December and in the middle of the following May at a rate of 900 m^3 ha^{-1} each time, and summer maize did not receive irrigation during the growing season. The different amount of biochar was applied once to the field at the beginning of the experiment. Briefly, the biochar was 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. The 0-20 cm soil layer was tilled after harvesting summer maize in early October. Both maize and wheat were fertilized once at sowing, i.e., 112.5 kg N ha^{-1}, 112.5 kg P_2O_5 ha^{-1}, and 112.5 kg K_2O ha^{-1}. The fertilizer was a compound fertilizer that included 15% N, P_2O_5, and K_2O. The crop residue was mechanically chopped after harvest and then returned to the field site. The chopped wheat straw was 2-3 cm in length and was mulched on the soil surface from June to October, and the chopped maize straw was 1-2 cm in length, and was plowed into the 0-20 cm soil layer.

The soil sampling was conducted before the wheat harvest in June 2017. Soil was collected from all three plots of each treatment by randomly selecting 5 sites, the 0-20 cm soil layer in each plot was sampled and the soil samples were pooled together, resulting in approximately 2 kg for each plot. After 2 weeks of air-drying, visible stones, plant roots, and soil fauna were manually removed, and all the samples were sieved through a 2 mm mesh for the various measurements mentioned below.

2.2 Soil water-stable aggregates, contents of biochar carbon and n-SOC in aggregates

The wet-sieving procedure proposed by Elliott (1986) was followed to separate soil water-stable aggregates. The sample in each plot of one treatment was used to separate the aggregates into four size classes, i.e., 2000-1000 μm, 1000-250 μm, 250-53 μm, and < 53 μm.

To measure the organic carbon content of pure biochar, the pure biochar (> 0.5 mm) from the B30, B60, and B90 treatments was picked out by hand until no visible biochar particles in the soil samples (Koide et al., 2011). Then, biochar particles were suspended in distilled water at a ratio of 1:10 (*w/v*), shaken vigorously to dislodge the soil particles, and dried at 60°C after rinsing the biochar four times with distilled water (Koide et al., 2011). The biochar C and n-SOC contents in aggregates of different sizes were measured following the ignition method of Koide et al. (2011). Briefly, 3.0 g subsamples of soil or each aggregate without biochar (B0) and with biochar (B30, B60 and B90) as well the above-mentioned picked pure biochar were weighed and placed into a 550°C muffle furnace for 4 h and were then weighed again to calculate the loss rate of the soil (L_{soil}), biochar ($L_{biochar}$) and soil-biochar mixture ($L_{mixture}$). The amounts of biochar and biochar C in aggregates were calculated as follows:

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

where $L_{biochar}$ = 64.20% ± 0.08%. L_{soil} indicates the aggregates of different sizes or the whole soil sample.

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

where $TOC_{biochar}$ is the total organic C content of biochar.

The n-SOC content was calculated by subtracting the biochar C amount from the TOC content in the soil-biochar mixture.

$$\text{n-SOC (g C kg}^{-1}\text{soil)} = \frac{TOC - \text{biochar C amount}}{1000 - \text{biochar amount}} \times 1000 \qquad \text{(Eq. 3)}$$

2.3 $\delta^{13}C$ of n-SOC and the contribution from wheat and maize

The δ^{13}C values of the soil or soil-biochar mixture, straw residues, and pure biochar were measured using an isotope ratio mass spectrometer (IsoPrime IRMS, GV Instruments, Manchester, UK). The δ^{13}C values of wheat straw (collected in 2016 and 2017) and maize straw (collected in 2016 and 2017) were -27.47 ± 0.54‰ and -13.58 ± 0.45‰ (n=3), respectively. The value for biochar collected in 2017 was -26.50‰ ± 0.38‰ (n=3). The contents (g C kg^{-1} soil) of TOC, native SOC and biochar C, and the δ^{13}C values of TOC ($\delta^{13}C_{TOC}$) and biochar ($\delta^{13}C_{biochar}$) in the soil or each aggregate were used to calculate the δ^{13}C value of n-SOC ($\delta^{13}C_{soc}$) using the following equation:

$$\delta^{13}C_{SOC} = \frac{\delta^{13}C_{TOC} \times TOC - \delta^{13}C_{biochar} \times biocharC}{\text{native SOC}} \qquad \text{(Eq. 4)}$$

The contribution proportion of wheat straw and maize straw to native SOC was calculated by the following equations:

$$-27.47‰ \times f1 + -13.58‰ \times f2 = \delta^{13}C_{SOC} \qquad \text{(Eq. 5)}$$

$$f1 + f2 = 1 \qquad \text{(Eq. 6)}$$

where $\delta^{13}C_{soc}$ is the $\delta^{13}C$ value of native SOC, and $f1$ and $f2$ are the respective contribution proportions of wheat straw and maize straw to the native SOC in each aggregate.

2.4 Statistical analysis

Significant differences in TOC, n-SOC contents of total soil and aggregates of different sizes, the biochar and biochar-C amounts of aggregates of different sizes, and the contribution of wheat or maize straw to n-SOC of aggregates of different sizes among the treatments B0, B30, B60, B90 were assessed. First, the parameters were checked to determine whether they followed a normal distribution and homogeneity of variance, if so, two-way analysis of variance with Tukey's test was used to conduct the comparison. For data that did not follow a normal distribution, a non-parametric test (Kruskal-Wallis test) was conducted to compare the treatment differences. The statistical analysis was carried out using SPSS 22.0 version (IBM Inc., Chicago, IL, USA).

3 Results

3.1 Effect of biochar on soil aggregation

Among the treatments, the proportion of different aggregate sizes followed the order of < 53 μm > 250-53 μm > 1000-250 μm > 2000-1000 μm, except for the B30 treatment (Table 1). The B0 and B60 treatments had similar proportions of 250-53 μm and < 53 μm aggregates based on the statistical analysis (result not shown here). In contrast, the B30 treatment had a significantly higher proportion of 250-53 μm than that of < 53 μm aggregates, while the opposite occurred in the B90 treatment. In the presence of biochar, the proportion of 2000-1000 μm aggregates was significantly higher relative to the treatment without biochar ($p<0.05$), i.e., the B30, B60, and B90 treatment values were 1.81, 2.70, 2.96 times that of the B0 treatment, respectively. Furthermore, the difference between each of two biochar treatments reached significant level of $p<0.05$. The proportion of 1000-250 μm aggregates in the B60 treatment was significantly higher than that in the B0, B30, and B90 treatments ($p<0.05$), but there was no difference between the later three treatments. The proportion of 250-53 μm aggregates was significantly higher in the B30 treatment than in the other three treatments ($p<0.05$) and that in the B60 and B90 treatments was significantly lower than that in the B0 treatment ($p<0.05$). The proportion of the < 53 μm aggregates in the B60 and B90 treatments was not significantly different from that in the B0 treatment. The B30 treatment had a significantly lower proportion of < 53 μm aggregates compared with the B0, B60, and B90 treatments ($p<0.05$).

3.2 Biochar content in aggregates of different sizes

The result from the ignition method showed that the content of biochar and biochar C were higher in aggregates with a large size relative to smaller aggregates. In each aggregate, the biochar content consistently increased following an increased biochar rate. For instance, in aggregates of 2000-1000 μm, the biochar content in the B90 treatment was 4.30 times and 3.21 times that of the B60 and B30 treatments, respectively ($p<0.05$) (Table 1). Similarly, the biochar content in the 1000-250 μm aggregates of the B90 treatment was 5.41 times and 3.04 times that of the B60 and B30 treatments, respectively ($p<0.05$) (Table 1). In aggregates of 250-53 μm, the biochar content presented an increasing trend following an increasing biochar rate, while there was no significant difference between each of the two biochar rates. There was no biochar in aggregates of < 53 μm.

3.3 TOC and n-SOC contents of total soil and aggregates of different sizes

TOC content had an increasing trend following increased biochar application rate, and was significantly higher at high biochar rates than at lower biochar rates ($p<0.05$) (Fig. 1). In contrast, the n-SOC content in the treatments followed the order: B60 > B90 > B0 > B30. The n-SOC content in the B60 treatment was 18.8%, 38.2%, and 9.8% higher than that in the B0, B30, and B90 treatments ($p<0.05$), respectively (Fig. 1). The n-SOC content was significantly higher in the B90 treatment than in the B0 treatment, but was significantly lower in the B30 treatment than in the B0 treatment ($p<0.05$).

The TOC content consistently decreased following a decrease in the aggregate size from 2000-1000 μm to < 53 μm (Fig. 2). In each aggregate size class, the TOC content increased following an increase in the biochar application rate except for the < 53 μm aggregates (Fig. 2). The TOC content in 2000-1000 μm, 1000-250 μm, and 250-53 μm aggregates were 29.2, 21.9, and 4.16 g kg^{-1}, respectively, in the B0 treatment, compared with increase of 9.24% (11.2%), 10.6% (6.49%), and 5.29% (35.3%) in the B30 (B60) treatment and of 25.7%, 35.8%, and 80.3% in the B90 treatment. However, only the TOC content of the B90 treatment was significantly higher than that in the other treatments ($p<0.05$).

In contrast to the increased TOC content following the increase in the biochar application rate, the applied biochar had contrasting effects on the n-SOC content in aggregates of different sizes, e.g., biochar decreased the content of n-SOC in macro-aggregates (> 250 μm) but increased the n-SOC content of 250-53 μm micro-aggregates. Briefly, in the 2000-1000 μm aggregates, the n-SOC content was 28.61, 27.89, and 22.38 g kg^{-1} in the B30, B60, and B90 treatments, respectively; these values were 2.03%, 4.49%, and 23.4% lower than the value (29.20 g kg^{-1}) measured in the B0 treatment (Fig. 2). There was a significant difference between the B90 treatment and the B0 treatment ($p<0.05$). Similarly, with respect to the 1000-250 μm aggregates, the B30, B60, and B90 treatments decreased the n-SOC content by 0.06%, 3.73% and 9.97%, respectively, compared with the B0 treatment. The B90 treatment had a significantly lower n-SOC content than the B0 and B30 treatments ($p<0.05$). Conversely, with respect to the 250-53 μm aggregates, the presence of biochar increased the n-SOC content by 5.83% (B30), 32.6% (B60) and 43.3% (B90) compared with the B0 treatment. The B60 and B90 treatments had significantly higher n-SOC contents compared with the B30 and B0 treatments ($p<0.05$). Across the treatments, the TOC and n-SOC contents in < 53 μm aggregates were not significantly different.

3.4 Contribution of wheat and maize straw residues to n-SOC in aggregates

The contribution of wheat and maize straw to the n-SOC in each aggregate size class is presented in Fig. 3. The wheat residue-derived C contributed 71.0% of the n-SOC in the 2000-1000 μm aggregates in the B0 treatment (Fig. 3), which decreased to 68.5%, 65.3%, and 60.9%, respectively, in the B30, B60, and B90 treatments. Regarding the contribution, in contrast to the B0 treatment, the B30, B60, and B90 treatments decreased the contribution of wheat residue-C by 5.5%, 8.6%, and 34.3%, respectively. The difference between each of the two treatments was significant ($p<0.05$). The same trend was observed in the 1000-250 μm aggregates, and the n-SOC content of the 1000-250 μm aggregates derived from wheat residue decreased by 4.9% (B30), 12.3% (B60) and 22.7% (B90) compared with the B0 treatment. The contribution of wheat residue to n-SOC was significantly lower in the B60 and B90 treatments than in the B0 treatment ($p<0.05$). Furthermore, the contribution of wheat residue to n-SOC between the B60 and B90 treatments was also significantly different ($p<0.05$). However, for the 250-53 μm aggregates, the n-SOC content derived from wheat residue in the B30, B60, and B90 treatments increased by 12.4%, 47.9% and 70.7%, respectively, compared with the B0 treatment. The contribution amounts of wheat residue to n-SOC in the B60 and B90 treatments were significantly higher than those in the B0 and B30 treatments ($p<0.05$). Based on the correlation analysis, following the increase in the biochar application rate, the content of n-SOC was significantly and negatively correlated with the contribution of wheat C to n-SOC in the macro-aggregates (2000-1000 μm and 1000-250 μm) ($p<0.01$) (Fig. 3) but was positively correlated with the contribution of wheat C to n-SOC in the micro-aggregates (250-53 μm) ($p<0.01$) (Fig. 3). In addition, the n-SOC content of all sizes of aggregate derived from maize residue had an increasing trend following the increase in the biochar application rate, but no significant difference was observed among the treatments.

4 Discussion

4.1 *Effect of straw return and biochar on native SOC*

Prior to the experiment, the wheat and maize residuals were removed after harvesting. Following 8-year crop residue return, the native SOC content increased from 4.32 g kg^{-1} to 7.87 g kg^{-1}, illustrating the promising potential of crop residue for soil carbon sequestration. This scenario is in agreement with other findings (Lu et al., 2009; Wang et al., 2015). Following biochar addition, it is difficult to clarify the biochar effect on native SOC under field conditions; ^{14}C can be used under laboratory control but is rarely recommended for application at a field site due to its radioactive nature. Furthermore, controlled laboratory conditions may create bias in the results compared with more complicated field conditions. One of the differences is the physical loss of biochar in the field due to run off or leaching, which has been shown to be significant (Dong et al., 2017). In this study, based on the ignition method (Koide et al., 2011), n-SOC content was significantly affected by the presence of biochar 8 years after application. We found that there was a notable effect of the amount of biochar on n-SOC content, e.g., a rate of 30 t ha^{-1} decreased n-SOC content while higher rates of 60 t ha^{-1} and 90 t ha^{-1} increased n-SOC content. Previous studies on the biochar effect on n-SOC were generally conducted over short-term periods or under laboratory control, ranging from less than 1 year to 2 years (Smith et al., 2010; Zimmerman et al., 2011; Rittl et al., 2015; Plaza et al., 2016; Zheng et al., 2018). For instance, Smith et al. (2010), using ^{13}C natural abundance, confirmed that biochar, but not soil organic carbon, was mineralized 6 days after its application, which was more related to the labile carbon in biochar promoting the microbial activity. Singh and Cowie (2014) conducted an incubation experiment and reported that the applied biochar caused an initial positive priming effect of n-SOC, and this effect diminished after a 5-year experiment due to the depletion of labile SOC or stabilization of SOC caused by biochar-induced organo-mineral interactions. In this field study with 8-year duration, the results provided evidence of the long-term effect of biochar on n-SOC, and this effect was dependent on the amount applied. Wardle et al. (2008) reported that fire-derived charcoal promoted the decomposition of organic carbon in forest soils 10 years after occurring since the enhancement of soil microbial activity. However, the amount of charcoal in the soil was not quantified. In this study, the variation in n-SOC content following the different biochar application rates indicated the changes in related processes.

4.2 Effect of biochar on soil aggregation and SOC content of each aggregate size class

Soil aggregate size distribution plays an important role in turnover of soil organic carbon content (Tisdall and Oades, 1982). Larger soil aggregates have higher soil organic carbon contents (Gupta and Germida, 1988). Following long-term biochar application, it is still unclear how biochar affects the soil aggregate distribution and n-SOC content in aggregates of different size classes, as well as the potential interaction between biochar and n-SOC. In this study, the results showed that the proportion of 2000-1000 μm aggregates was enhanced in the presence of biochar, and this enhancement was positively correlated with the biochar application rate. This finding is in accordance with a previous study conducted by Dong et al. (2016), who reported a similar phenomenon 5 years after biochar application. The highest biochar content was measured in the macro-aggregates (2000-1000 μm). Previous studies reported that biochar can form agglomerates with soil particles and organic-inorganic complexes (Brodowski, 2006; Zheng et al., 2018). Following biochar application, however, the enhanced 2000-1000 μm aggregate was not due to the direct effect of the applied biochar because internal biochar was less than 3.0% (Table 1), which cannot explain the 80.7%-196.4% increase in 2000-1000 μm soil aggregates relative to the treatment without biochar. The results therefore indicated an indirect effect of biochar on increased macro-aggregates. Ouyang et al., (2013) addressed that biochar can enhance the formation of macro-aggregates of sandy loam, similar soil texture to that in our study; the underlying mechanism could be ascribed to the intimate physic-chemical interaction between soil minerals and biochar particles by the recent study of Zheng et al. (2018) on aggregates of 2000-53 μm in a coastal soil. In addition, Zheng et al. (2018) reported that biochar induced obvious shift of the bacteria taxa responsible for stabilizing soil aggregates. A widely accepted theory of soil aggregation formation is the phase theory proposed by Tisdall and Oades (1982), which stated that soil micro-aggregates are generated from soil mineral particles through binding of multivalent ions and soil organic matter, and then the soil micro-aggregates are held together by plant roots and fungal hyphae to gradually form larger aggregates. Fungi, through the spread of hyphae between aggregates and into pores, may increase fungal binding to enmesh fine particles into macro-aggregates (Tisdall et al., 1997). It has been shown that the interaction between the oxidized carboxylic acid groups of biochar and minerals (Glaser et al., 2002; Zheng et al., 2018) or sorption of soil organic matter on biochar can bind soil particles (Brodowski et al., 2006; Joseph et al., 2010) to improve soil aggregation. Nevertheless, studies reported that the high C/N of biochar is favorable for the growth of fungi (Ouyang et al., 2013; Zheng et al., 2018), playing an important role in aggregate formation (De Gryze et al., 2006; Zheng et al., 2018). In addition, as proposed by Six et al. (2000), soil aggregate turnover may occur due to the breakage of macro-aggregates, which are scattered by tillage mechanical forces to form micro-aggregates, or particulate organic matter in macro-aggregates are degraded by microorganisms to form new micro-aggregates, producing free micro-aggregates from the originally bounded micro-aggregates, or the above-mentioned micro-aggregates are transformed back into macro-aggregates following the addition of organic residues. Thus, following biochar application, the enhancement in the proportion of 2000-1000 μm aggregates may also be derived from aggregates breakdown or reorganization. The proportion of micro-aggregates (< 250 μm) were not changed following biochar application at rates of 30 and 90 t ha^{-1}, which is in accordance with Herath et al. (2014), who used a similar size biochar (> 500 μm) as in our study and found that soil micro-aggregates (< 250 μm) were not changed after 295-day biochar application at rates of ranging from 10.0 to 17.3 t ha^{-1}. However, when dividing micro-aggregates into 250-53 μm and < 53 μm size classes, the results showed that 30 t ha^{-1} biochar significantly enhanced the proportion of 250-53 μm aggregates and decreased the proportion of < 53 μm aggregates, while the trend was opposite for biochar with 90 t ha^{-1} biochar. This finding indicated that biochar application has already interacted with soil aggregate sizes smaller than 250 μm. Dong et al., (2016) inferred that small amounts of biochar particle (e.g., 30 t ha^{-1}) could cause contact with < 53 μm particles cemented into 250-53 μm and then lead a decrease of < 53 μm; while a large amount of biochar (90 t ha^{-1}) would interact with all aggregates which can reorganize the aggregates distribution related to the reported aggregate turnover theories as mentioned before (Tisdall and Oades, 1982; Tisdall et al., 1997; Six et al., 2000). In addition, 60 t ha^{-1} biochar decreased the proportions of both 250-53 μm and < 53 μm aggregates, which is similar to the findings of Dong et al. (2016) based on a 5-year experiment which illustrated that the medium amount of biochar (e.g., 60 t ha^{-1}) could interact with both 250-53μm and < 53 μm to form larger aggregates. Overall, these results indicated that the amount of biochar applied dramatically affected the soil aggregation, which may in turn have influenced the associated functions in terms of carbon cycling in each aggregate.

Although the variation in different soil aggregate proportions was not correlated with the amount of biochar applied, the n-SOC content in each aggregate size class was linearly correlated with the biochar application amount. Interestingly, the n-SOC content in aggregates greater than 250 μm was significantly and negatively correlated with the amount of biochar applied ($p<0.01$), while that in 250-53 μm aggregates was significantly and positively correlated with the applied biochar amount ($p<0.01$). To our knowledge, this is first reported information on the quantified n-SOC content of different aggregates size classes following biochar application. For the underlying mechanisms, the reorganization of aggregates followed biochar application could cause the change in the n-SOC of reorganized aggregates size classes since the n-SOC is high in macro-aggregate than smaller size classes (Gupta and Germida, 1988). For instance, the increased macro-aggregates (2000-1000 μm) part could from binding of smaller aggregates, this would directly decrease n-SOC content because of the low n-SOC content in smaller aggregates. This assumption needs further clarification by putting emphasis on how the aggregates are reorganized. In addition, Gupta and Germida (1988) proposed the microbial biomass plays an important role in the formation of macro-aggregates and is the primary resource of labile organic carbon and nutrients. Relative to the macro-aggregates (> 250 μm), the micro-aggregates (< 250 μm) contained lower organic carbon, microbial biomass, fungal biomass, and respiratory activity, with a slow turnover rate of organic carbon (Gupta and Germida, 1988). Following biochar application, previous studies with a relatively short time scale from 4 months (Steinbeiss et al., 2009) to 140 days (Ye et al., 2017) reported that high application rates of biochar (≥ 5%) significantly increased the soil fungi/bacterial ratio with preferential stimulation of soil fungi. Soil hyphae also increased following biochar application (Lehmann et al., 2011). Additionally, Duan et al., (2017) found that biochar aged for 5 years after application stimulated the fungal-*nirK* gene abundance relative to fresh biochar. Mueller et al. (2014) reported a pronounced increase in bioavailability of SOC after aggregates disruption. The recent study of Zheng et al. (2018) on coastal soil found that biochar significantly increased microbial biomass C of two aggregates size classes (2000-53 μm and < 53 μm), and also changed the bacterial community. The presence of biochar may change the microbial biomass or diversity of soil in this study, which in turn changes the n-SOC turnover. Therefore, following biochar application, the reorganization of different aggregates size classes as well as the potential change in microbial activity in different aggregates size classes could all contribute to the n-SOC contents of different aggregates size classes, while these two potential mechanisms need further clarification.

4.3 The variation of contribution origins to SOC in different aggregate size classes

The presence of biochar has altered the proportions of n-SOC origins. For instance, in micro-aggregates, the wheat residue contribution to n-SOC in 250-53 μm aggregates, increased from 2.33 g kg^{-1} in the treatment without biochar to 3.97 g kg^{-1} in the B90 treatment. In this study, maize straw was incorporated into the soil immediately after harvest, while wheat straw is mulched until the next maize harvest. Based on the isotopic analysis, we found that the contribution of wheat residue to n-SOC in aggregates with different sizes was significantly changed by the applied biochar and was correlated with the change in the n-SOC of soil aggregates (Fig. 3). It has been reported that wheat residue contributions to n-SOC was higher than those of maize residue (Buyanovsky and Wagner, 1996; Wynn and Bird, 2007) because maize residue have lower lignin contents and C:N ratios but higher decomposition rates relative to wheat residue (Zhang et al., 2008; Talbot and Treseder, 2012). Recent studies have reported that wheat residue with more effective at promoting the accumulation of native SOC than maize residue is due to their different decomposition dynamics (Wang et al., 2015). Other studies reported that the labile fraction of wheat-derived SOC were be higher and therefore more responsive to changed management practices than maize-derived SOC (Neff et al., 2002; Bhattacharyya et al., 2011). The result of the B0 treatment showed that the contribution of wheat to n-SOC notably decreased from aggregates of > 250 μm to aggregates of < 250 μm, and this illustrates different aggregates size classes have different contributions of wheat or maize to n-SOC. In this study, the input of biomass from wheat or maize was not different among the treatments (data not shown here), illustrating that the carbon input into soil was not different. Following biochar application, the varied wheat-C contribution to n-SOC may be due to the reorganization of different aggregates because smaller aggregates (< 250 μm) owning low wheat-C contribution (Fig. 3). For instance, the decreased wheat-C contribution to n-SOC of macro-aggregates (2000-1000 μm) may be related to the increased macro-aggregates from binding of smaller aggregates. Considering a potential fractionation effect of n-SOC, advanced model is needed such as on the basis of two-end-member mixing model proposed by Balesdent et al. (1987). On the other hand, following increasing biochar rates, the N content (excluding biochar-N) in aggregates of above 250 μm showed an inverse relationship to the contents of n-SOC and wheat-derived n-SOC (Table A1, Fig. 3). This may be related to the enhanced wheat-derived SOC mineralization following increasing biochar rates causing more C loss and N left from wheat residue in these aggregates size classes. The aggregate size determines the microbial composition with micro-aggregates having higher bacterial biomass and lower fungal biomass and hyphal length relative to the macro-aggregates (Gupta and Germida, 1988). A study has found that biochar can provide labile substrates through the biochar itself or through the sorption of soil labile substrates to support the growth of both bacterial and fungal biomass, with a shift to the dominance of fungal biomass in an aged biochar soil system (Gul et al., 2015). Other studies also reported the changed microbial communities and activity following biochar application (Lehmann et al., 2011; Zhou et al., 2017; Chen et al., 2019; Senbayram et al., 2019). However, up to date, whether and how the use of biochar can trigger the change of the microbe biomass and composition in different aggregates is rarely known. In addition, based on a 5-year biochar application, Dong et al. (2017) found that the biochar had significantly aged, with a higher specific surface area and more formed small pores compared with the treatment without biochar. Nevertheless, whether the biochar in different aggregate sizes varies due to the known different physico-chemical and biological micro-conditions remains as an open question for the further investigation.

In synthesis, following the biochar application, the change in the total n-SOC content was due to the changed soil aggregate proportion and the varied n-SOC content in different aggregates size classes (Fig. 1; Fig. 4). Relative to the B0 treatment, n-SOC content in the B30 treatment was decreased, indicating a positive priming effect, and this scenario was also reported by Singh and Cowie (2014), who found a positive priming effect of biochar at a low rate of 8.17 g kg^{-1} (oven-dry basis) on a clayey soil based on a 5-year monitoring period. Here, the positive priming effect was due to the decreased n-SOC content in 1000-250 μm and < 53 μm aggregates, mainly due to the latter being significantly decreased and these aggregates being the predominant component of the investigated soil. Although the same increasing trend was observed in n-SOC content in the 60 and 90 t ha^{-1} treatments relative to the B0 treatment, the reasons were contrasted, the B60 treatment increased total n-SOC content due to the increased n-SOC contents in 2000-1000 μm and 1000-250 μm aggregates, while the B90 treatment increased the n-SOC content due to the increased n-SOC contents in 2000-1000 μm and <250 μm aggregates (Fig.4).

5. Conclusion

Based on an 8-year field-based study, the biochar rate of 30 t ha^{-1} caused a positive priming effect on n-SOC, while higher rates of 60 t ha^{-1} and 90 t ha^{-1} had a similar negative effect priming effect, indicating biochar rate lower than 30 t ha^{-1} may be not feasible on the investigated wheat-maize rotation system. Further, we found that, following biochar application, the altered soil aggregate distribution along with the change in n-SOC content in aggregates with different size classes leaded to the change in total n-SOC content. Moreover, following biochar application, in aggregates with different size classes (no biochar in aggregates of < 53 μm), the alteration of wheat-derived n-SOC was more pronounced compared with maize-derived n-SOC, which predominately accounted for the alteration of n-SOC. For the deeper mechanisms, how aggregates are reorganized and the change in microbial activity following different biochar rates could be potential keys, which needs further clarification. In addition, in the perspective of alleviating n-SOC degradation and increasing carbon sequestration, the feasible amount of applied biochar on other soil ecosystems should be investigated.

6. Acknowledgments

This work was supported by the National Key Research Projects (Grant numbers: 2017YFD0200801-02), the National Natural Science Foundation of China (No. 41171211), the National Key Research Projects (Grant numbers: 2018YFD0300505), and the National Wheat Industry Technology System (CARS301). All authors showed their great appreciation to the technical staff working for this long-term field experiment.

7. References

Abel, S., Peters, A., Trinks, S., Schonsky, H., Facklam, M., Wessolek, G, 2013. Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. Geoderma 202, 183-191.

Baiamonte, G., Crescimanno, G., Parrino, F., De Pasquale, C., 2019. Effect of biochar on the physical and structural properties of a desert sandy soil. Catena 175, 294-303.

Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics.Soil Biol. Biochem. 19, 25-30.

Bhattacharyya, R., Kundu, S., Srivastva, A.K., Gupta, H.S., Prakash, V., Bhatt, J.C., 2011. Long term fertilization effects on soil organic carbon pools in a sandy loam soil of the Indian sub-himalayas. Plant Soil 341, 109-124.

Blanco-Canqui, H., 2017. Biochar and soil physical properties. Soil Sci. Soc. Am. J. 81, 687-711.

Brodowski, S., John, B., Flessa, H., Amelung, W., 2006. Aggregate-occluded black carbon in soil. Eur. J. Soil Sci. 57, 539-546.

Burrell, L.D., Zehetner, F., Rampazzo, N., Wimmer, B., Soja, G., 2016. Long-term effects of biochar on soil physical properties. Geoderma 282, 96-102.

Buyanovsky, G., Wagner, G., 1996. Crop residue input to soil organic matter on Sanborn Field. In: Paul, E.A., Paustian, K., Elliot, E.T., Cole, C.V. (Eds.), Soil Organic Matter in Temperate

Agroecoystems: Long-Term Experiments in North America. CRPC Press Inc., Boca Raton, FL, pp. 73-84.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Agronomic values of greenwaste biochar as a soil amendment. Soil Res. 45, 629-634.

Chen, L.J., Jiang, Y.J., Liang, C., Luo, Y., Xu, Q., Han, C. Zhao Q.G., Sun, B., 2019. Competitive interaction with keystone taxa induced negative priming under biochar amendments. Microbiome 7, 77.

Dai, Z., Meng, J., Muhammad, N., Liu, X., Wang, H., He, Y., Brookes, P.C., Xu, J., 2013. The potential feasibility for soil improvement, based on the properties of biochars pyrolyzed from different feedstocks. J. Soil. Sediment. 13, 989-1000.

De Gryze, S., Six, J., Merckx, R., 2006. Quantifying water‐stable soil aggregate turnover and its implication for soil organic matter dynamics in a model study. Eur. J. of Soil Sci. 57, 693-707.

Dong, X.L, Guan, T.Y., Li, G.T., Lin, Q.M., Zhao, X.R., 2016. Long-term effects of biochar amount on the content and composition of organic matter in soil aggregates under field conditions. J. Soil. Sediment. 16, 1481-1497.

Dong, X.L., Li, G.T., Lin, Q.M., Zhao, X.R., 2017. Quantity and quality changes of biochar aged for 5 years in soil under field conditions. Catena 159, 136-143.

Dong, X.L., Singh, B.P., Li, G., Lin, Q.M., Zhao, X.R., 2018. Biochar application constrained native soil organic carbon accumulation from wheat residue inputs in a long-term wheat-maize cropping system. Agr. Ecosyst. Environ. 252, 200-207.

Duan, M.L., Li, H.C., Gu, J., Tuo, X.X., Sun, W., Qian, X., Wang, X.J., 2017. Effects of biochar on reducing the abundance of oxytetracycline, antibiotic resistance genes, and human pathogenic bacteria in soil and lettuce. Environ. Pollut. 224, 787-795.

Duong, T.T.T., Baumann, K., Marschner, P., 2009. Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. Soil Biol. Biochem. 41, 1475-1482.

Elliott E. T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627-633.

Fang, Y., Singh, B., Singh, B.P., Krull, E., 2014. Biochar carbon stability in four contrasting soils. Eur. J. Soil Sci. 65, 60-71.

Fierer, N., Schimel, J.P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biol. Biochem. 34, 777-787.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal–a review. Biol. Fert. Soils. 35, 219-230.

Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agric. Ecosyst. Environ. 20, 46-59.

Gupta, V.V.S.R., Germida, J.J., 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. Soil Biol. Biochem. 20, 777-786.

Hardie, M., Clothier, B., Bound, S., Oliver, G., Close, D., 2014. Does biochar influence soil physical properties and soil water availability?. Plant Soil 376, 347-361.

Herath, H.M.S.K., Camps-Arbestain, M., Hedley, M., Van Hale, R., Kaal, J., 2014. Fate of biochar in chemically-and physically-defined soil organic carbon pools. Org. Geochem. 73, 35-46.

Hu, L., Cao, L., Zhang, R., 2014. Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. World J. Microbiol. Biotechnol. 30, 1085-1092.

Jeffery, S., Meinders, M.B., Stoof, C.R., Bezemer, T.M., van de Voorde, T.F., Mommer, L., van Groenigen, J.W., 2015. Biochar application does not improve the soil hydrological function of a sandy soil. Geoderma 251, 47-54.

Joseph, S.D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P., Lehmann, J., Foidl, N., Smernik, R.J., Amonette, J.E., 2010. An investigation into the reactions of biochar in soil. Soil Res. 48, 501-515.

Koide, R.T., Petprakob, K., Peoples, M., 2011. Quantitative analysis of biochar in field soil. Soil Biol. Biochem. 43, 1563-1568.

Krull, E.S., Swanston, C.W., Skjemstad, J.O., McGowan, J.A., 2006. Importance of charcoal in determining the age and chemistry of organic carbon in surface soils. J. Geophys. Res-Biogeo. 111, G4.

Lehmann, J., Joseph, S., 2015. Biochar for environmental management: science, technology and implementation. Lehmann, J., Joseph, S., Eds., Routledge: Abingdon, UK, pp. 1-12.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems–a review. Mitig. Adapt. Strat. Gl. 11, 403-427.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota–a review. Soil Biol. Biochem. 43, 1812-1836.

Liang, F., Li, G.T., Lin, Q.M., Zhao, X.R., 2014. Crop yield and soil properties in the first 3 years after biochar application to a calcareous soil. J. Integr. Agric. 13, 525-532.

Loeppert, R., Suarez, L., 1996. Carbonate and gypsum. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America, Madison,WI, pp. 437-474.

Lu, F., Wang, X.K., Han, B., Ouyang, Z.Y., Duan, X.N., Zheng, H., Miao, H., 2009. Soil carbon sequestrations by nitrogen fertilizer application, straw return and no‐tillage in China's cropland. Global Change Biol. 15, 281-305.

Lu, W.W., Ding, W.X., Zhang, J.H., Li, Y., Luo, J.F., Bolan, N., Xie, Z.B., 2014. Biochar suppressed the decomposition of organic carbon in a cultivated sandy loam soil: a negative priming effect. Soil Biol. Biochem. 76, 12-21.

Mueller, C.W., Gutsch, M., Kothieringer, K., Leifeld, J., Rethemeyer, J., Brueggemann, N., Kögel-Knabner, I., 2014. Bioavailability and isotopic composition of CO_2 released from incubated soil organic matter fractions. Soil Biol. Biochem. 69, 168-178.

Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915-917.

Nguyen, T.T., Cavagnaro, T.R., Thanh Ngo, H.T., Marschner, P., 2016. Soil respiration, microbial biomass and nutrient availability in soil amended with high and low C/N residue–influence of interval between residue additions. Soil Biol. Biochem. 95, 189-197.

Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., Niandou, M.A.S., 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Sci. 174, 105-112.

Ouyang, L., Wang, F., Tang, J., Yu, L., Zhang, R., 2013. Effects of biochar amendment on soil aggregates and hydraulic properties. J Soil Sci. Plant Nut. 13, 991-1002.

Plaza, C., Giannetta, B., Fernández, J.M., López-de-Sá, E.G., Polo, A., Gascó, G., .Méndez, A., Zaccone, C., 2016. Response of different soil organic matter pools to biochar and organic fertilizers. Agr. Ecosyst. Environ. 225, 150-159.

Rhoades, J.D., 1986. Cation exchange capacity. In: Page, A. L., et al., editor. Methods of soil analysis, part Ⅱ. Chemical and microbiological properties, 2nd ed. ASA and SSSA, Madison, WI: Agron. Monogr, 1986. pp. 149-157.

Rex, D., Schimmelpfennig, S., Jansen-Willems, A., Moser, G., Kammann, C., Müller, C., 2015. Microbial community shifts 2.6 years after top dressing of Miscanthus biochar: hydrochar and feedstock on a temperate grassland site. Plant Soil 397, 261-271.

Rittl, T.F., Novotny, E.H., Balieiro, F.C., Hoffland, E., Alves, B.J.R, Kuyper, T.W., 2015. Negative priming of native soil organic carbon mineralization by oilseed biochars of contrasting quality. Eur. J. Soil Sci. 66, 714-721.

Senbayram, M., Saygan, E.P., Chen, R.R, Aydemir, S., Kaya, C., Wu, D., Bladogatskaya, E., 2019. Effect of biochar origin and soil type on the greenhouse gas emission and the bacterial community structure in N fertilised acidic sandy and alkaline clay soil. Sci. Total Environ. 660, 69-79.

Singh, B.P., Cowie, A.L., 2014. Long-term influence of biochar on native organic carbon mineralisation in a low-carbon clayey soil. Sci. Rep-UK. 4, 3687.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555-569.

Six, J., Paustian, K., Elliott, E., Combrink, C., 2000. Soil structure and organic matter I. distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64, 681-689.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biol. Biochem. 42, 2345-2347.

Smith, P., 2016. Soil carbon sequestration and biochar as negative emission technologies. Global Change Biol., 22, 1315-1324.

Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41, 1301-1310.

Stemmer, M., Gerzabek, M.H., Kandeler, E., 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biol. Biochem. 30, 9-17.

Sun, F.F., Lu, S.G., 2014. Biochars improve aggregate stability, water retention, and pore-space properties of clayey soil. J. Plant Nutr. Soil Sc. 177, 26-33.

Sun, Z.C., Moldrup, P., Elsgaard, L., Arthur, E., Bruun, E.W., Hauggaard-Nielsen, H., de Jonge, L.W., 2013. Direct and indirect short-term effects of biochar on physical characteristics of an arable sandy loam. Soil Sci. 178, 465-473.

Sun, Z.C., Arthur, E., de Jonge, L.W., Elsgaard, L., Moldrup, P., 2015. Pore structure characteristics after 2 years of biochar application to a sandy loam field. Soil Sci. 180, 41-46.

Talbot, J.M., Treseder, K.K., 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. Ecology 93, 345-354.

Tian, J., Wang, J.Y., Dippold, M., Gao, Y., Blagodatskaya, E., Kuzyakov, Y., 2016. Biochar affects soil organic matter cycling and microbial functions but does not alter microbial community structure in a paddy soil. Sci. Total Environ. 556, 89-97.

Tisdall, J., Oades J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141-163.

Tisdall, J.M., Smith, S.E., Rengasamy, P., 1997. Aggregation of soil by fungal hyphae. Soil Res. 35, 55-60.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S., Cowie, A., 2010. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327, 235-246.

Wang, C., Liu, J.Y., Shen, J.L., Chen, D., Li, Y., Jiang, B.S., Wu, J.S., 2018. Effects of biochar amendment on net greenhouse gas emissions and soil fertility in a double rice cropping system: A

4-year field experiment. Agr. Ecosyst. Environ. 262, 83-96.

Wang, J.Z., Wang, X.J., Xu, M.G., Feng, G., Zhang, W.J, Yang, X.Y., Huang, S.M., 2015. Contributions of wheat and maize residues to soil organic carbon under long-term rotation in north China. Sci. Rep-UK. 5 11409.

Wardle, D.A. , Nilsson, M.C. , Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science 320, 629.

Weng, Z. H., Van Zwieten, L., Singh, B.P., Tavakkoli, E., Joseph, S., Macdonald, L.M., Rose, T.J., Kimber, S.W.L., Morris, S., Cozzolino, D., Araujo, J.R., Archanjo, B.S., Cowie, A., 2017. Biochar built soil carbon over a decade by stabilizing rhizodeposits. Nat. Clim. Change, 7, 371-376.

Wynn, J.G., Bird, M.I., 2007. C4-derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. Global Change Biol. 13, 2206-2217.

Ye, J., Joseph, S.D., Ji, M., Nielsen, S., Mitchell, D.R.G., Donne, S., Horvat, J., Wang, J.L., Munroe, P., Thomas, T., 2017. Chemolithotrophic processes in the bacterial communities on the surface of mineral-enriched biochars. ISME J. 11, 1087-1101.

Zhang, M., Cheng, G., Feng, H., Sun, B.H., Zhao, Y., Chen, H.X., Chen J., Dyck, M., Wang, X.D., Zhang, A.F., 2017. Effects of straw and biochar amendments on aggregate stability, soil organic carbon, and enzyme activities in the Loess Plateau, China. Environ. Sci. Pollut. R. 24, 10108-10120.

Zhang, D., Hui, D., Luo, Y., Zhou, G., 2008. Rates of litter decomposition in terrestrial ecosystems:

global patterns and controlling factors. J. Plant Ecol. 1, 85-93.

Zhang, J.J., Wei, Y.X., Liu, J.Z., Yuan, J.C., Liang, Y., Ren, J, Cai, H.G., 2019. Effects of maize straw and its biochar application on organic and humic carbon in water-stable aggregates of a Mollisol in Northeast China: A five-year field experiment. Soil Till. Res. 190, 1-9.

Zheng, H., Wang, X., Luo, X.X., Wang, Z.Y., Xing, B.S., 2018. Biochar-induced negative carbon mineralization priming effects in a coastal wetland soil: Roles of soil aggregation and microbial modulation. Sci. Total Environ. 610, 951-960.

Zhou, H.M., Zhang, D.X., Wang, P., Liu, X.Y., Cheng, K., Li, L.Q., Zheng, J.W., Zhang, X.H., Zheng, J.F., Crowley, D., van Zwieten, L., Pan, G.X., 2017. Changes in microbial biomass and the metabolic quotient with biochar addition to agricultural soils: A Meta-analysis. Agr. Ecosyst. Environ. 239, 80-89.

Zimmerman, A.R., Gao, B., Ahn, M.Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 43, 1169-1179.

Figure captions

Fig. 1. Total organic carbon (TOC) and native soil organic carbon (n-SOC) contents of soil in the B0, B30, B60, and B90 treatments, representing biochar application rate of 0, 30, 60 and 90 t ha^{-1}, respectively. Data are shown as the mean ± standard error (n=3). Different letters indicate the significant differences in TOC or n-SOC content between the treatments ($p<0.05$)

Fig. 2. Total organic carbon (TOC) and native soil organic carbon (n-SOC) contents in different sized aggregates in the treatments (B0, B30, B60, B90, representing biochar application rate of 0, 30, 60 and 90 t ha^{-1}, respectively). Data are shown as the mean ± standard error (n=3). Different letters indicate the significant differences in TOC or n-SOC content between the treatments ($p<0.05$).

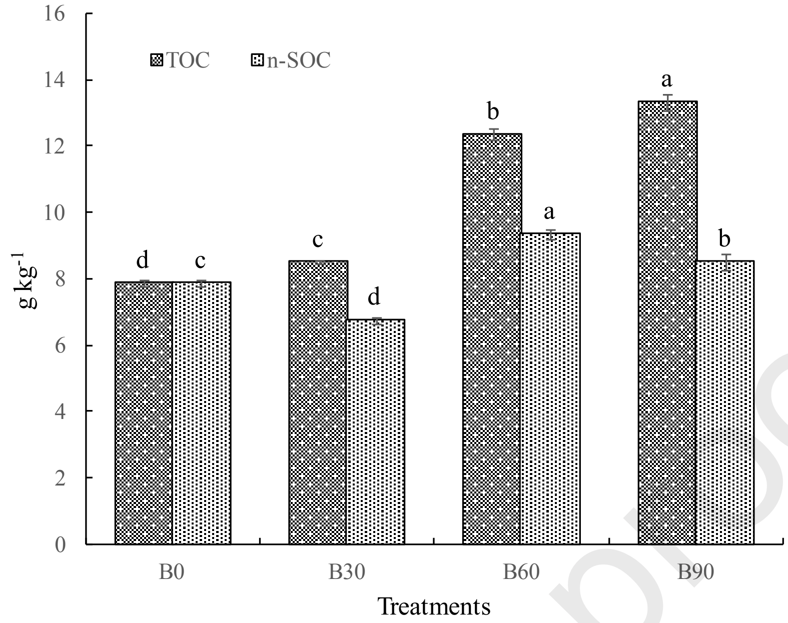
Fig. 3. The contribution of wheat and maize straw to native soil organic carbon (n-SOC) in aggregates of different sizes in the treatments with different biochar rates (B0, B30, B60, B90, representing biochar application rate of 0, 30, 60 and 90 t ha^{-1}, respectively) and the relation between the n-SOC derived from wheat straw and n-SOC in aggregates of different sizes following increased biochar rates shown by the head of the dashed arrow. Data are shown as the mean ± standard error (n=3). Different letters indicate the significant differences in wheat-derived SOC between the treatments ($p<0.05$).

Fig. 4. The proportion of native soil organic carbon (n-SOC) in each aggregate size class to total soil (10^{-2} g kg^{-1}) in different treatments (B0, B30, B60, and B90, representing biochar application rate of 0, 30, 60 and 90 t ha^{-1}, respectively). Data are shown as the mean ± standard error (n=3). Different letters indicate the significant difference in the proportion of n-SOC in each aggregate

size class to total soil between the different biochar rates ($p<0.05$).

- 30 t ha^{-1} of ~~Biochar~~ biochar decreased~~rates variedly altered~~ native ~~soil organic carbon~~SOC ~~after 8-year application~~, but inverse in 60 t ha^{-1} and 90 t ha^{-1}
- Biochar ~~concentration~~ content was higher in aggregates with larger size
- Increase in biochar rate increased proportion of aggregates of 2000-1000 μm
    - ~~Biochar of 90 t ha^{-1} significantly altered native SOC in aggregates above 53 μm~~
    - ~~Biochar altered native soil organic carbon by changing aggregation and inside SOC~~
- ~~The c~~Changed~~d~~ wheat-derived ~~organic carbon~~SOC ~~mainly~~ accounted for ~~the~~ native SOC content variation in aggregates

812 **Figure 1**



813

Figure 2

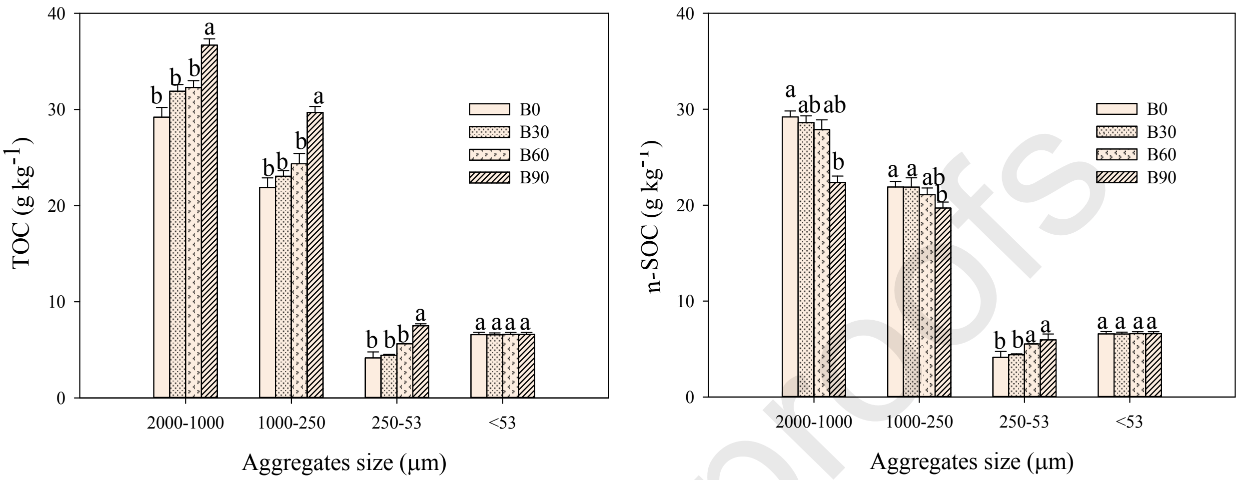

TOC (g kg^{-1})
0
10
20
30
40
b b b a
b b b a
b b b a
a a a a
B0
B30
B60
B90
2000-1000
1000-250
250-53
<53
Aggregates size (μm)
n-SOC (g kg^{-1})
0
10
20
30
40
a ab ab b
a a ab b
b b a a
a a a a
B0
B30
B60
B90
2000-1000
1000-250
250-53
<53
Aggregates size (μm)

Figure 3

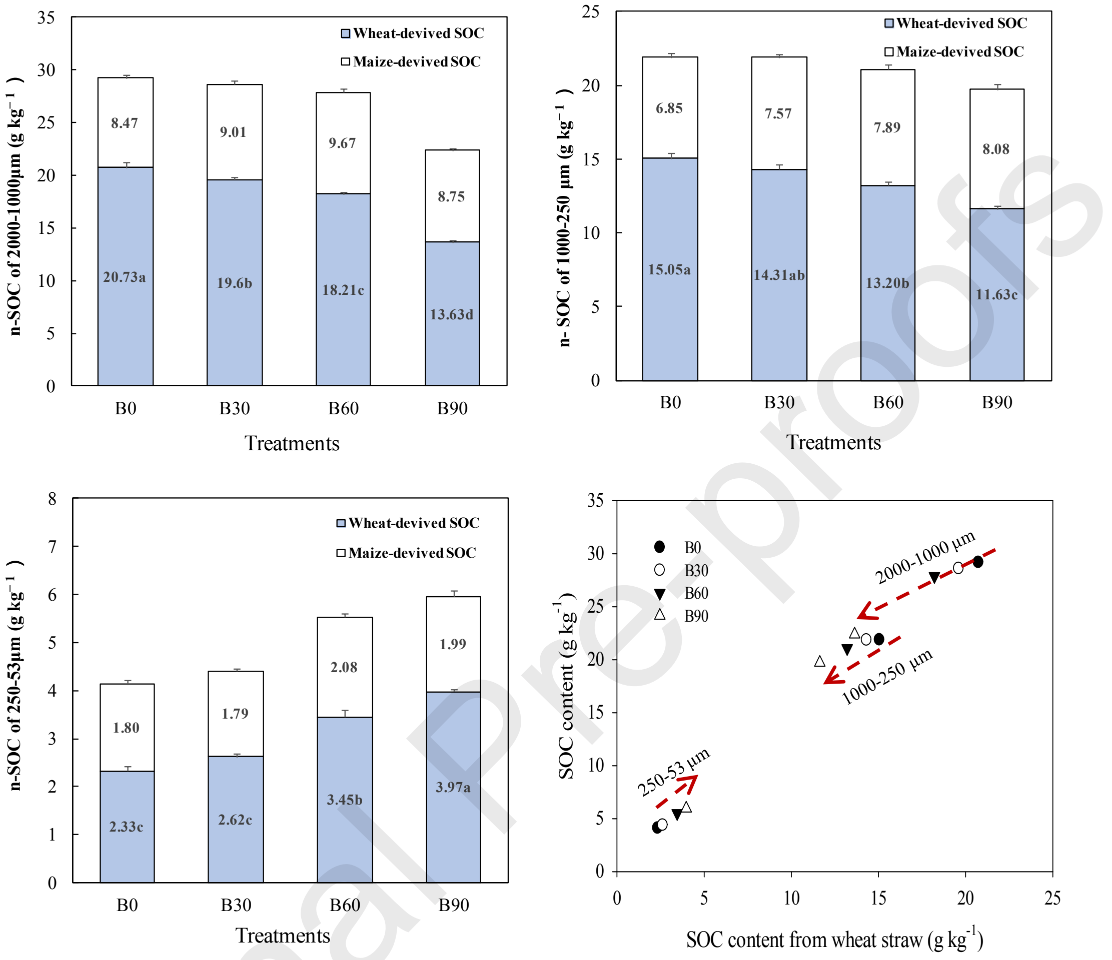
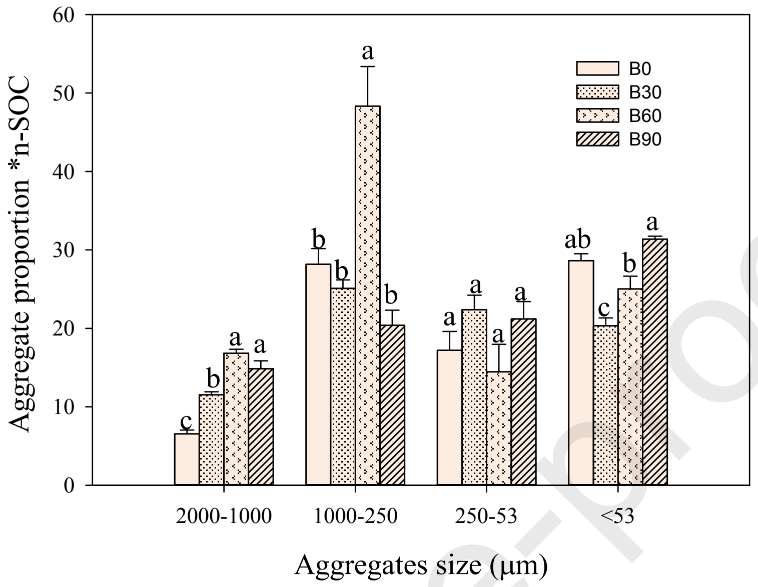

n-SOC of 2000-1000μm (g kg⁻¹)
35
30
25
20
15
10
5
0
Wheat-devived SOC
Maize-devived SOC
8.47
9.01
9.67
8.75
20.73a
19.6b
18.21c
13.63d
B0
B30
B60
B90
Treatments
n- SOC of 1000-250 μm (g kg⁻¹)
25
20
15
10
5
0
Wheat-devived SOC
Maize-devived SOC
6.85
7.57
7.89
8.08
15.05a
14.31ab
13.20b
11.63c
B0
B30
B60
B90
Treatments
n-SOC of 250-53μm (g kg⁻¹)
8
7
6
5
4
3
2
1
0
Wheat-devived SOC
Maize-devived SOC
1.80
1.79
2.08
1.99
2.33c
2.62c
3.45b
3.97a
B0
B30
B60
B90
Treatments
SOC content (g kg⁻¹)
35
30
25
20
15
10
5
0
● B0
○ B30
▼ B60
△ B90
2000-1000 μm
1000-250 μm
250-53 μm
0
5
10
15
20
25
SOC content from wheat straw (g kg⁻¹)

Figure 4



Tables

Table 1. Soil aggregate size distribution of soil including biochar (BC) and excluding biochar at different biochar rates after 8-year application, i.e., B0, B30, B60, and B90 presenting the treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

Treatment	2000-1000 μm (g $100g^{-1}$)		1000-250 μm (g $100g^{-1}$)		250-53
	include BC	exclude BC	include BC	exclude BC	include BC
B0	2.23±0.09d	2.23±0.09d	12.83±0.35b	12.83±0.35b	41.43±1.80b
B30	4.03±0.18c	4.00±0.18c	11.47±0.18b	11.44±0.19b~~c~~	53.50±1.61a
B60	6.03±0.18b	5.98±0.17b	22.83±1.40a	22.68±1.40a~~b~~	33.23±1.30c
B90	6.61±0.15a	6.41±0.15a	10.43±0.41b	10.22±0.36b~~a~~	35.43±0.64c

†: no biochar detected in this aggregate size

Table 2. The loss rate using the ignition method, biochar content, and biochar C content of different aggregates size in the treatments, i.e., B0, B30, B60, and B90 presenting the treatments of 0, 30, 60 and 90 t ha^{-1} biochar application rates, respectively.

s	Treatments	Soil aggregates (μm)		
		2000-1000	1000-250	250-53
nition	B0	4.59±0.04c	4.04±0.04b	2.17±0.02a
	B30	4.99±0.10b	4.18±0.11b	2.19±0.06a
	B60	5.13±0.08b	4.44±0.06b	2.31±0.15a
	B90	6.32±0.12a	5.25±0.20a	2.35±0.08a
ntent	B0	0c	0b	0a
l)	B30	6.77±1.30b	3.74±0.92b	1.45±0.32a
	B60	9.06±1.65b	6.65±0.75b	2.31±2.23a
	B90	29.08±0.86a	20.22±3.71a	3.01±1.54a
content	B0	0c	0b	0a
l)	B30	3.47±0.68b	1.92±0.92b	0.75±0.17a
	B60	4.65±0.86b	3.41±0.38b	1.19±1.15a
	B90	14.91±0.63a	10.41±1.91a	1.55±0.79a