Long-term influence of maize stover and its derived biochar on soil structure and organo-mineral complexes in Northeast China

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Abstract: The effect of biochar on soil structure and aggregate stability is controversial in the literature. To explore the effect of biochar on soil aggregates, a long-term field experiment (5 years) was conducted in the Brown Earth soil of Northeastern China involving three treatments: control (annual application of 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}), biochar (control plus annual application of 2.625 t ha^{-1} maize stover biochar), and stover (control plus annual application of 7.5 t ha^{-1} maize stover). We determined the aggregate size distribution (>2000 μm, 250-2000 μm, 53-250 μm, <53 μm), and organic carbon (OC) and organo-mineral complex contents both in the bulk soil and within the soil aggregates in the plow layer (0-20 cm). The biochar and stover applications decreased soil bulk and particle densities significantly ($P<0.05$), and increased soil total porosity. Both the amendments significantly ($P<0.05$) increased the total OC, heavy fraction OC and organo-mineral complex quantities in the bulk soil as well as in all the studied aggregate fractions. Biochar and stover applications promoted the formation of small macroaggregates. A greater amount of organic matter was contained in macroaggregates, leading to the formation of more organo-mineral complexes, and the soil aggregate stability thus improved. Compared to stover application, biochar had lower carbon input, but it had a stronger effect on the organo-mineral complexes in different soil aggregate fractions by a unit carbon applied. Therefore, biochar application proved more useful than stover in improving the soil structure in this study.

Keywords: biochar, soil aggregates, organo-mineral complexes, soil structure, carbon sequestration

1 Introduction

Soil structure plays an important role in soil physical, chemical, and biological processes (Peng et al., 2015). Soil structure can influence plant growth and change the soil organic carbon (SOC) content; therefore, it is a key property affecting the soil fertility and quality (Peng et al., 2015). Soil aggregates are the basic units of soil structure, and the composition and distribution of soil aggregates are important indicators of the soil structure (Baiamonte et al., 2019, Six et al., 2000). Soil aggregates may provide physical protection for SOC, which plays a binding agent role, and is a vital substance in the formation of aggregates.

The organic carbon (OC) content in the soil is approximately 3.3 times than that in the air (Lal, 2004), and nearly 90% of the SOC is situated in soil aggregates in the topsoil (Jastrow, 1996). Thus, stable aggregates protect SOC, and SOC serves as a binding agent in the formation of soil aggregates (Luna et al., 2016). Organic colloids can improve soil aggregation, and soil organo-mineral complexes are formed by organic colloids and mineral particles. Organo-mineral complexes can significantly enhance soil aggregates and retain soil fertility. Organo-mineral complexes can promote the ability of OC to resist decomposition by microorganisms, which allows it to stay in the soil for a long time (Weng et al., 2017). Accumulated OC can bind to the mineral fraction to form organo-mineral complexes and then further polymerize to form soil aggregates (Jastrow, 1996). The soil organic matter stabilization occurs through several mechanisms, e.g., wrapping by mineral surfaces, embedding into layered mineral crystalline sheets, hydrophobic bonding, cation bridging, anion exchange, ligand exchange, coulombic attraction and van der Waals force (Bai et al., 2017, Sokol et al., 2019). But the binding capacity varies with different types of OM and minerals (Mikutta et al., 2007). Nevertheless, increasing soil organic matter and thus improving the formation of organo-mineral complexes and soil aggregates is a useful step for enhancing the soil quality (Zhang et al., 2015).

The most popular practice of crop residue management in Northeast China is to burn them in the field. This practice produces large amounts of ash and smoke, which pollute the environment. An alternative environmental-friendly management approach can be turning crop straw into biochar and return the product to soils to improve the SOC content. Biochar is the carbon-rich product of waste biomass pyrolysis performed in an oxygen-limited environment (Lehmann, 2011, Chen et al., 2019). Biochar can be used as a soil amendment to enhance carbon sequestration (Li et al., 2018) and reduce greenhouse gas emission (Lu et al., 2019). It has also been shown to reduce bioavailability of heavy metals (Yang et al., 2017; Xia et al., 2019) and organic contaminants (He et al., 2018; Qin et al., 2018), and improve soil nutrient supply (Li et al., 2019), resulting in increased crop yields and quality (Nie et al., 2018). Biochar also increases the cation exchange capacity (CEC) (Wu et al., 2012) and pH (Chen et al., 2019) of soils, and improve soil enzymatic and microbial activities (Palansooriya et al., 2019), in turn promoting crop growth. Biochar as an amendment is known to enhance the soil structure by improving the aggregate stability (Wang et al., 2017). However, this is inconclusive as some reports suggested that biochar had no positive effect on soil aggregates (Borchard et al., 2014, Rahman et al., 2017). These contradictory results were attributed to different crop residue feedstocks, soil types and environments. Specially, the effects of maize stover and stover-derived biochar on different SOC fractions in differently sized soil aggregates remain largely unknown.

The SOC content differs depending on aggregate size (Liu et al., 2014), and can be classified as light fraction organic carbon (LFOC) and heavy fraction organic carbon (HFOC) based on their density. The LFOC is free OC, an important component of labile OC, and is mainly derived from crop residues and decaying animal bodies (Christensen, 2010). The LFOC is not stored for long because it is easily degraded. The HFOC on the other hand exists in the form of organo-mineral complexes, which are not easily degraded, and are thus more stable than LFOC. The HFOC portion could account for up to 91% of total SOC (Kleber et al., 2015). It can therefore be assumed that different organic material inputs would have different effects on the formation of organo-mineral complexes in the field (Li and Wu, 2012).

Brown Earth (Gao et al., 2018) is the main soil type in the Liaoning province of China. This area is situated at one of the three gold maize (*Zea mays L*) belts of the world, and is the main grain-producing area in China (Yang et al., 2017). Historically, little organic amendments have been applied to the Brown Earth soil in this region. Although biochar as a soil amendment has many benefits, there was little information on the effect of biochar on soil structure in the Brown Earth region. How biochar affects the organo-mineral complexes in this soil was never studied before. Therefore, we designed a long-term field experiment (5 years) involving maize stover and stover-derived biochar incorporation to assess the soil aggregates and organo-mineral complexes. The purpose of this research was to investigate the long-term effects of maize stover and its biochar on (1) soil bulk density (BD), particle density (PD) and soil total porosity (TP); (2) soil water-stable aggregates and their stability; (3) SOC and soil organo-mineral complexes; and (4) the SOC and organo-mineral complexes within differently sized aggregates.

2 Materials and methods

2.1 Experimental site.

A 5-years long field experiment was conducted during May 2013 – October 2017 at Shenyang Agricultural University (41°49′N, 123°33′E). The site receives approximately 705 mm of annual precipitation. The average minimum and maximum temperatures were -25 and 35.3°C during the experimental period. This region is situated in Northeast China, Liaoning Province. The experimental site has a semi-humid warm-temperature climate. The soil type in this region is Brown Earth, and the soil is classified as a Hapli-Udic Cambisol according to the Food and Agriculture Organization (FAO) classification system (An et al., 2015, Yang et al., 2017). The frost-free period is about 150 days, while the whole growth period is 130~150 days. The annual precipitation during the whole growth period is 547 mm, and the average temperature is 20.7 °C (Lan et al., 2015). The type of agriculture in the Liaoning Province is dry land rain-fed agriculture. The basic properties of the topsoil (0-20 cm) at the start of the experiment are presented in Table 1. During the past five years, spring maize was continuously grown at this site with one harvest per year. The mineral NPK fertilizers applied annually contained urea (120 kg N ha^{-1}), calcium superphosphate (60 kg P_2O_5 ha^{-1}) and potassium sulfate (60 kg K_2O ha^{-1}). All fertilizers were applied once before sowing the seeds.

2.2 Maize stover and biochar

Maize stover was collected from the experimental field, and then chopped into sections with a length of 50~70 mm. The maize stover biochar used in this experiment was produced by Jinhefu Agriculture Development Company, Liaoning, China. The biochar was produced in a vertical kiln at 350-550°C temperature for 90 min. The properties of the maize stover and maize stover biochar are provided in Table 1.

Table 1. Physico-chemical properties of the experimental soil and amendment materials

	Topsoil (0-20 cm)	Maize stover	Biochar
pH	7.4	7.8	9.2
Bulk density (g cm^{-3})	1.31	/	/
Total C (g kg^{-1})	11.0	429.3	660.0
Total N (g kg^{-1})	1.2	5.4	12.7
Total P (g kg-^{1})	0.38	3.43	8.87
Total K (g kg^{-1})	20.1	17.6	32.2
Alkali-hydrolyzable N (mg kg^{-1})	84.5	/	/
Available P (mg kg^{-1})	15.9	/	/
Available K (mg kg^{-1})	158.7	/	/

Ash content (%)	/	3.78	15.57
Surface area (m^2 g^{-1})	/	3.43	8.87
Average pore size (nm)	/	10.75	16.23
Volatile matter (%)	/	80.14	21.94

2.3 Experimental design

Three treatments were selected: control (only the application of mineral NPK fertilizers: 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}), biochar (control plus annual application of 2.625 t.ha^{-1} maize stover biochar), and stover (control plus annual application of 7.5 t.ha^{-1} maize stover). On the carbon content basis, the applied amount of maize stover was almost equal to the stover biomass per year per hectare, and the biochar dosage was based on 35% output ratio in the kiln after pyrolysis. Maize stover pieces and biochar powder (passed through 2 mm sieve) were applied annually before conducting rotary tillage of the plots. Spring maize was sown in May, and harvested at the end of September each year. The seeding rate was 60,000 plants per hectare. A randomized block design with three replicates was used in the field experiment, and each plot had an area of 3.6 m × 10 m.

2.4 Soil sample preparation and analysis

After five growing seasons, in October 2017, topsoil (0-20 cm) samples were collected. Undisturbed soil samples (0-20 cm) were used to analyze soil aggregates collected in each plot, and the undisturbed soils were collected by a profile method (first dug a profile, cut the undisturbed soil with a vertical depth of 20 cm, and then held in aluminum boxes). Sub-samples were collected from five randomly selected spots in each plot, and then mixed to make one composite sample. The undisturbed soils were taken to the laboratory and air dried during which visible stones and plant residues were removed. The soils then were passed through an 8-mm sieve. The wet-sieving method was followed to assess the soil aggregate content (Elliott, 1986). Briefly, 50 g of air-dried soil was submerged in distilled water for 5 min on the top screen of the nested sieves. The sizes of the sieves were: 2000 μm, 250 μm, and 53 μm. Four aggregate size fractions were acquired: large macroaggregates (>2000 μm), small macroaggregates (250-2000 μm), microaggregates (53-250 μm), and the silt and clay fraction (<53 μm). The sieves were moved up and down by approximately 3 cm for 15 min, with approximately 20 strokes min^{-1}. The aggregate fractions remaining on each sieve were washed into aluminum boxes, oven dried at 60°C for 48 h, weighed, and stored in plastic bags. The soil BD was determined by the soil core and cutting ring method (Luo et al., 2016). The liquid pycnometer method was used to analyze PD (Walia and Dick, 2018).

The soil samples (0-20 cm) collected from five random spots in each plot were also subjected to physico-chemical analyses. The soil was air dried in the laboratory, and then passed through 2-mm and 1-mm sieves. Subsamples were also sieved through a 0.15-mm mesh to determine the SOC contents using an elemental analyzer (Elementar Macro Cube, Langenselbold, Germany). The soil organic fractions was determined using the relative density method (Fu et al., 1983). Briefly, 5 g of air-dried soil (<1 mm) was placed in a 100 ml centrifuge tube of known weight with 25 ml of sodium iodide aqueous solution (1.7 $g.cm^{-3}$). After shaking the mixture for 1 h and centrifuging at 3000 rpm for 10 min, the supernatant with floating material was filtered, and washed with deionized water. The sodium iodide solution was collected for reuse. The process was repeated twice. The soil remaining in the centrifuge tube, which consisted of heavy fractions, was washed twice with deionized water, oven dried at 40°C, weighed, and stored for further analysis. The SOC content in the heavy fraction was determined by an elemental analyzer as mentioned earlier.

2.5 Calculation and statistical analysis

The soil aggregate content was determined by using Eq. 1:

$$R_i=\frac{W_i}{50} \quad \text{(Eq. 1)}$$

where, R_i stands for the soil aggregate fraction (%); W_i stands for the weight of each soil aggregate fraction (g). The stability of soil aggregates was traditionally assessed by calculating the mean weight diameter (MWD), geometric mean diameter (GMD), macroaggregate content ($R_{>250}$), and fractal dimension (D). The formulae for these parameters are as follows (Eq. 2, 3, 4, 5):

$$MWD=\frac{\sum_{i=1}^{n}\bar{x}_i w_i}{\sum_{i=1}^{n} w_i} \quad \text{(Eq. 2)}$$

$$GMD=EXP\left[\frac{\sum_{i=1}^{n} w_i \ln\bar{x}_i}{\sum_{i=1}^{n} w_i}\right] \quad \text{(Eq. 3)}$$

$$R_{>250}=\frac{M_{>250}}{50} \quad \text{(Eq. 4)}$$

$$\frac{M_{(r<x_i)}}{20}=\left[\frac{x_i}{x_{max}}\right]^{3-D} \quad \text{(Eq. 5)}$$

where, $\bar{x}_i$ is the mean diameter of every soil aggregate size (mm), w_i is the weight percentage of every soil aggregate size (%), and $M_{>250}$ is the weight of the macroaggregates (g).

Total porosity (TP) was calculated using Eq. 6:

$$\text{Total porosity}=\left(1-\frac{BD}{PD}\right)\times100\% \quad \text{(Eq. 6)}$$

where, BD is the soil bulk density, and PD refers to soil particle density.

The soil organo-mineral complex index was calculated using Eq. 7–10 according to Fu et al. (1978):

$$QC=\frac{HC\times Hm}{m}\times100\% \quad \text{(Eq. 7)}$$

$$DC=\frac{HC\times Hm}{SOC\times m}\times100\% \quad \text{(Eq. 8)}$$

$$QAC=MQ-SQ \quad \text{(Eq. 9)}$$

$$DAC=\frac{MQ-SQ}{MC-SC} \quad \text{(Eq. 10)}$$

where, HC is the heavy SOC (%), Hm is the content of the heavy fraction (g), m is the weight of the sample (g), QC refers to the quantity of organo-mineral complexes in the soil (%), DC is the degree of organo-mineral complexes (%), QAC refers to the quantity of additional complexes (%), DAC refers to the degree of additional complexes (%), MQ represents the quantity of QCs under biochar and stover treatments (%), SQ represents the quantity of QCs under control (%), MC refers to the SOC under biochar and stover treatments (%), and SC refers to the SOC under control (%).

The relative contribution of SOC within different aggregate fractions was calculated as follows (Eq. 11):

$$\text{Relative contribution}=\frac{\text{SOC within aggregate}\times\text{Aggregate content (\%)}}{\text{SOC}} \qquad \text{(Eq. 11)}$$

All data gathered in this research are presented as the mean ± standard deviation. We used one-way analysis of variance (ANOVA) to test the differences in soil parameters among the treatments. The least significant difference (LSD) method was used to test for differences among the treatments ($p<0.05$).

3. Results

3.1 Soil bulk density, particle density and total porosity

Table 2 Effect of maize stover and its biochar on soil bulk density, particle density and total porosity

Treatment	BD ($g.cm^{-3}$)	PD ($g.cm^{-3}$)	TP (%)
Control	1.31±0.01a	2.55±0.01a	48.63±0.41b
Biochar	1.25±0.01b	2.48±0.01b	49.53±0.42b
Stover	1.23±0.01b	2.49±0.01b	50.60±0.42a

BD: bulk density; PD: particle density; TP: total porosity. Distinct lowercase letters indicate significant differences *($p<0.05$)* among the treatments in each column.

Maize stover biochar and maize stover returned to the soil both decreased the BD significantly ($p<0.05$) by 4.6 and 6.1% lower respectively than that under control after the five-year experiment (Table 2). The PD also changed in these two ways: biochar and stover decreased the PD significantly ($p<0.05$) as compared to the control, but their effects were not significantly different among themselves (Table 2). Although biochar decreased the BD and PD, the TP did not vary after the five-year experiment; conversely, stover returned increased the TP significantly after the experimental period (Table 2).

3.2 Soil aggregates and their stability

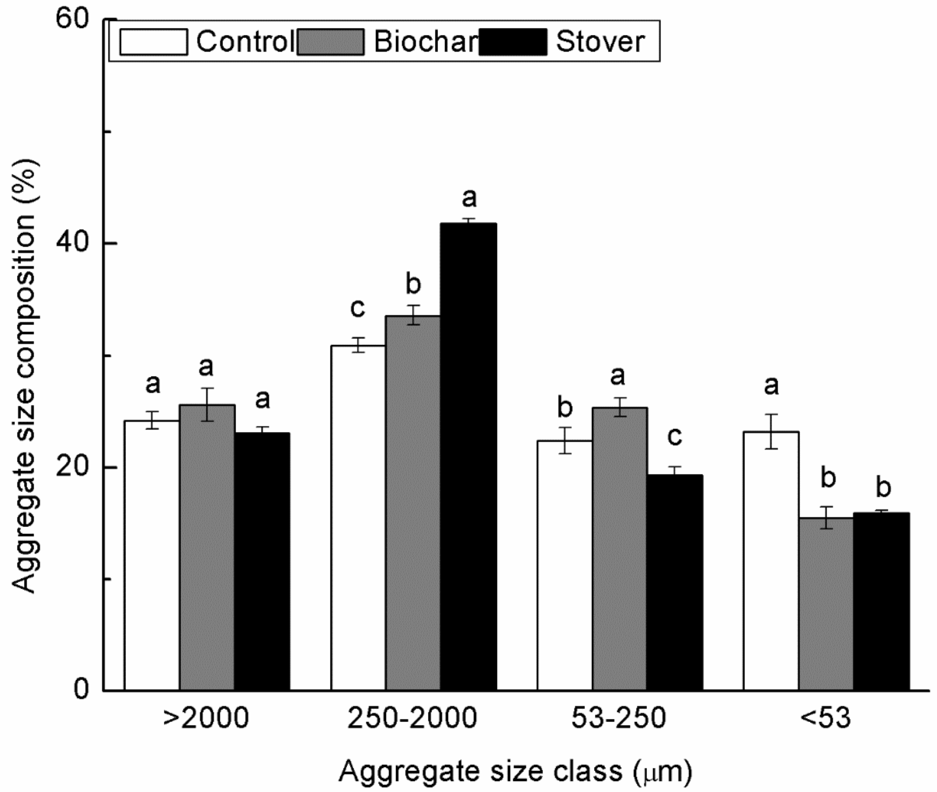


Figure 1. Effect of maize stover and its biochar on soil water-stable aggregates. Distinct lowercase letters indicate differences *(p<0.05)* among the treatments in one aggregate size class.

Biochar and maize stover changed the water-stable aggregate size and soil aggregate stability (Figure 1 and Table 3). The large macroaggregate (>2000 μm) fraction was not significantly different between biochar and stover returned treatments, but it was slightly lower under stover returned than under the control and biochar treatments. Compared to the control, biochar and stover enhanced the 250-2000 μm fraction by 14.91 and 55.69%, respectively.

However, the microaggregate fraction was the smallest under stover returned treatment, and not significantly different between control and biochar treatments. Finally, the silt and clay fractions (<53 μm) in the treatments followed the order: control > biochar = stover returned, indicating that biochar and stover returned to the soil both increased the large macroaggregates, and the effect of stover was stronger than that of biochar.

Table 3. Effect of maize stover and its biochar on water-stable aggregate stability

Treatment	MWD (mm)	GMD (mm)	$R_{>250\mu m}$ (%)	D
Control	1.60±0.04b	0.43±0.04b	55.10±1.27c	2.47±0.04a
Biochar	1.70±0.07a	0.56±0.04a	59.14±1.53b	2.40±0.03b
Stover	1.66±0.02ab	0.59±0.01a	64.85±0.69a	2.30±0.01c

MWD: mean weight diameter; GMD: geometric mean diameter; $R_{>250\mu m}$: macroaggregate content; D: fractal dimension. Distinct lowercase letters indicate differences *($p<0.05$)* among the treatments in one column.

The MWD, GMD and $R_{>250\mu m}$ of soil aggregates are important indices of soil aggregate stability. The MWD was not significantly different among the treatments after the five-year experiment, and the values changed from 1.60 to 1.70 mm (Table 3). The GMD in the treatments followed the order: stover returned = biochar > control treatments, and that under biochar and stover treatments was 23.91 and 37.72% higher than that under control, respectively, indicating that biochar and stover increased the soil aggregate stability. Biochar and stover returned treatments both increased the $R_{>250\mu m}$ values (macroaggregates), with the order of $R_{>250\mu m}$ values: stover returned > biochar > control, and the values under biochar and stover returned treatments were 7.98 and 22.52% higher than those under control (Table 3). The D values had a different trend from the other data, with an order of control > biochar > stover returned (Table 3). The order showed that organic input decreased the D value, and that stover had a stronger effect on soil structure than biochar.

3.3 Soil organic carbon, heavy fraction organic carbon and organo-mineral complexes

Table 4. Effect of maize stover and its biochar on soil organic carbon (SOC) content, heavy fraction organic carbon (HFOC) content, quantity of organo-mineral complexes (QC), degree of organo-mineral complexes (DC), quantity of additional complexes (QAC) and degree of additional complexes (DAC) in the bulk soil.

Treatment	SOC (%)	HFOC (%)	QC (%)	DC (%)	QAC (%)	DAC (%)
Control	1.08±0.11c	0.91±0.12c	0.91±0.12c	84.54±1.15b	--	--
Biochar	1.34±0.06b	1.09±0.16b	1.09±0.16b	81.30±1.21c	0.18±0.03b	67.91±6.24a
Stover	1.45±0.12a	1.28±0.29a	1.28±0.29a	88.26±1.99a	0.37±0.03a	99.06±7.75b

All data are shown as the mean ± standard error (n=3). Different lowercase letters in the same column indicate significant differences (p<0.05).

The SOC content was noticeably affected by biochar and maize stover applied as a soil amendment after five consecutive growing seasons. The SOC content increased by 24.21 and 34.49% under biochar and stover returned treatments, respectively (Table 4). The HFOC showed a trend similar to that of the SOC (stover returned > biochar > control), being 20.77 and 40.11% higher under biochar and stover returned than under control, respectively (Table 4). Biochar and stover improved the QC by 19.45 and 40.42%, respectively (Table 4). The DC in the biochar treatment was the lowest, and that in the stover returned treatment was the highest. The QAC increased with increasing SOC concentration. The DAC had the same tendency as the QAC in the biochar and stover returned treatments; the QAC under stover returned was approximately two times of that under biochar, and the DAC was significantly greater under stover than under biochar treatment (Table 4).

3.4 SOC content and organo-mineral complexes within aggregate fractions

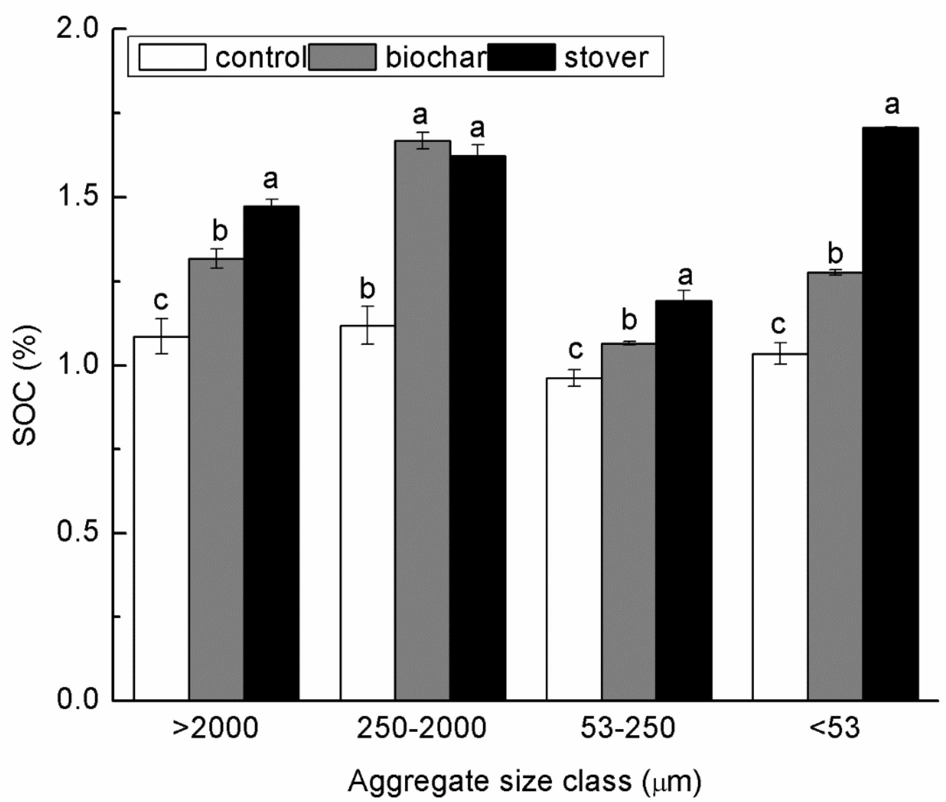


Figure 2. Effect of maize stover and its biochar on organic carbon within soil aggregates. Distinct lowercase letters indicate differences *(p<0.05)* among the treatments in an aggregate size class.

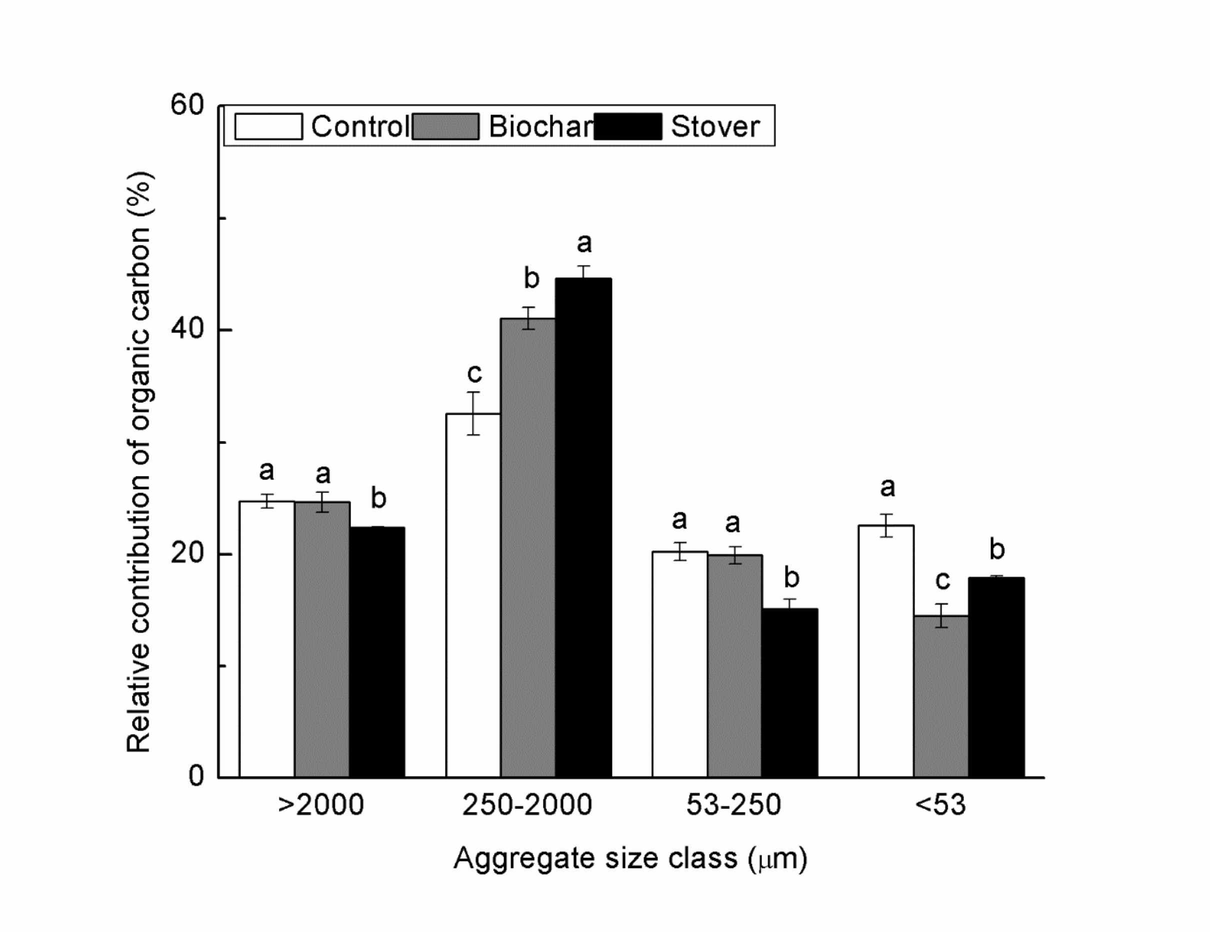


Figure 3. Relative contributions of organic carbon in different aggregate fractions. The lowercase letters above columns indicate differences *($p<0.05$)* among the treatments within each aggregate size class.

The distribution of SOC in water-stable aggregates was significantly impacted by the biochar and stover returned treatments (Figure 2). Biochar and stover both significantly enhanced the SOC content in differently sized aggregates ($p<0.05$). The SOC within large macroaggregates (>2000 μm) increased by 21.22 and 35.68% in the biochar and stover returned treatments, respectively, and that within small macroaggregates by 49.19 and 45.13%, respectively. The microaggregate fraction had the lowest SOC content of all aggregate sizes, with a 10.82 and 23.88% higher SOC under biochar and stover treatments than under control, respectively. The silt and clay fraction in the stover returned treatment had the highest SOC content, and the SOC concentration was improved by 64.99% compared to that under control. In contrast, the SOC concentration of this fraction under biochar improved by only

23.40% compared with that under control.

In general, the SOC contribution in the large macroaggregate fraction followed the order: biochar = control > stover returned (Figure 3). For the small macroaggregates, the SOC contribution rate was the highest under stover returned, followed by control and biochar. For the microaggregate fraction, the SOC contribution rate followed the order: biochar > control > stover returned. Moreover, in the silt and clay fraction, the order was: biochar > stover = control.

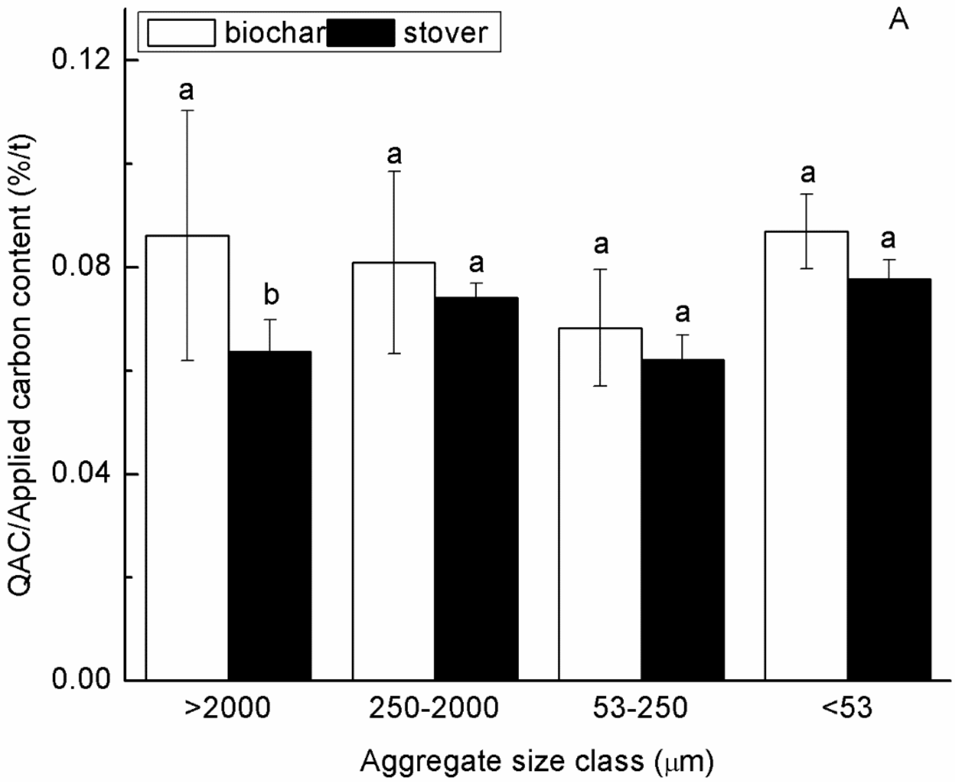
Table 5. Effect of maize stover and its biochar on heavy fraction organic carbon (HFOC) content, quantity of organo-mineral complexes (QC), degree of organo-mineral complexes (DC), quantity of additional complexes (QAC) and degree of additional complexes (DAC) in different aggregate size fractions.

Aggregate size (μm)	Treatment	HFOC (%)	QC (%)	DC (%)	QAC (%)	DAC (%)
>2000	control	0.82±0.47c	0.82±0.05c	75.61±4.29a	--	--
	biochar	0.94±0.42b	0.94±0.04b	71.15±3.18a	0.15±0.04b	64.63±9.02a
	stover	1.03±0.53a	1.03±0.05a	69.81±3.58a	0.21±0.01a	52.99±3.04b
250-2000	control	0.87±0.32c	0.87±0.03c	78.08±2.86a	--	--
	biochar	1.01±0.30b	1.01±0.03b	60.73±1.83c	0.14±0.06b	25.46±2.16b
	stover	1.11±0.09a	1.11±0.01a	68.50±0.56b	0.24±0.04a	47.27±7.83a
53-250	control	0.81±0.09c	0.81±0.01c	83.86±0.95b	--	--
	biochar	0.92±0.19b	0.92±0.02b	86.77±1.83a	0.12±0.02b	113.65±7.59a

	stover	1.01±0.15a	1.01±0.02a	84.49±1.29ab	0.20±0.02a	87.09±9.86b
	control	0.78±0.13c	0.78±0.01c	74.96±1.22a	--	--
<53	biochar	0.93±0.12b	0.93±0.01b	72.53±0.98a	0.15±0.01b	62.17±3.04b
	stover	1.03±0.12a	1.03±0.01a	60.10±0.72b	0.25±0.01a	37.25±0.39a

The HFOC concentration in each aggregate fraction had the same tendency as the HFOC in the bulk soil. These data might be explained by the increased organic material input in the aggregates. The QC in different aggregate fractions had the same tendency as the HFOC. These results showed that biochar and stover addition both increased the QC in different aggregate fractions. The DC in the large macroaggregate fraction was not significantly different from that in other fractions, but the DC in the microaggregate fraction was the highest among all fractions. In each fraction, the QAC in the stover treatment was higher than that under biochar treatment. The change in DAC differed among fractions; in the small macroaggregate fraction, stover returned treatment had a higher DAC than biochar treatment, and in the other fractions, the DAC had a stronger effect under biochar than the stover treatment (Table 5).

Biochar and stover application had different applied carbon contents. Maize stover treatment applied about 3.24 t ha^{-1} carbon per year, whereas biochar treatment applied about 1.74 t ha^{-1} carbon per year. By calculating the ratio of QAC and DAC to the applied carbon content (Figure 4), we found that in all aggregate fractions, biochar treatment had a higher ratio of QAC/applied carbon content, and it was significant in macroaggregate fractions ($P<0.05$). Meanwhile, the ratio of DAC/applied carbon content was significantly higher than stover treatment in biochar treatment except the small macroaggregate fraction ($P<0.05$).


biochar
stover
A
QAC/Applied carbon content (%/t)
0.00
0.04
0.08
0.12
a
b
a
a
a
a
a
a
>2000
250-2000
53-250
<53
Aggregate size class (μm)

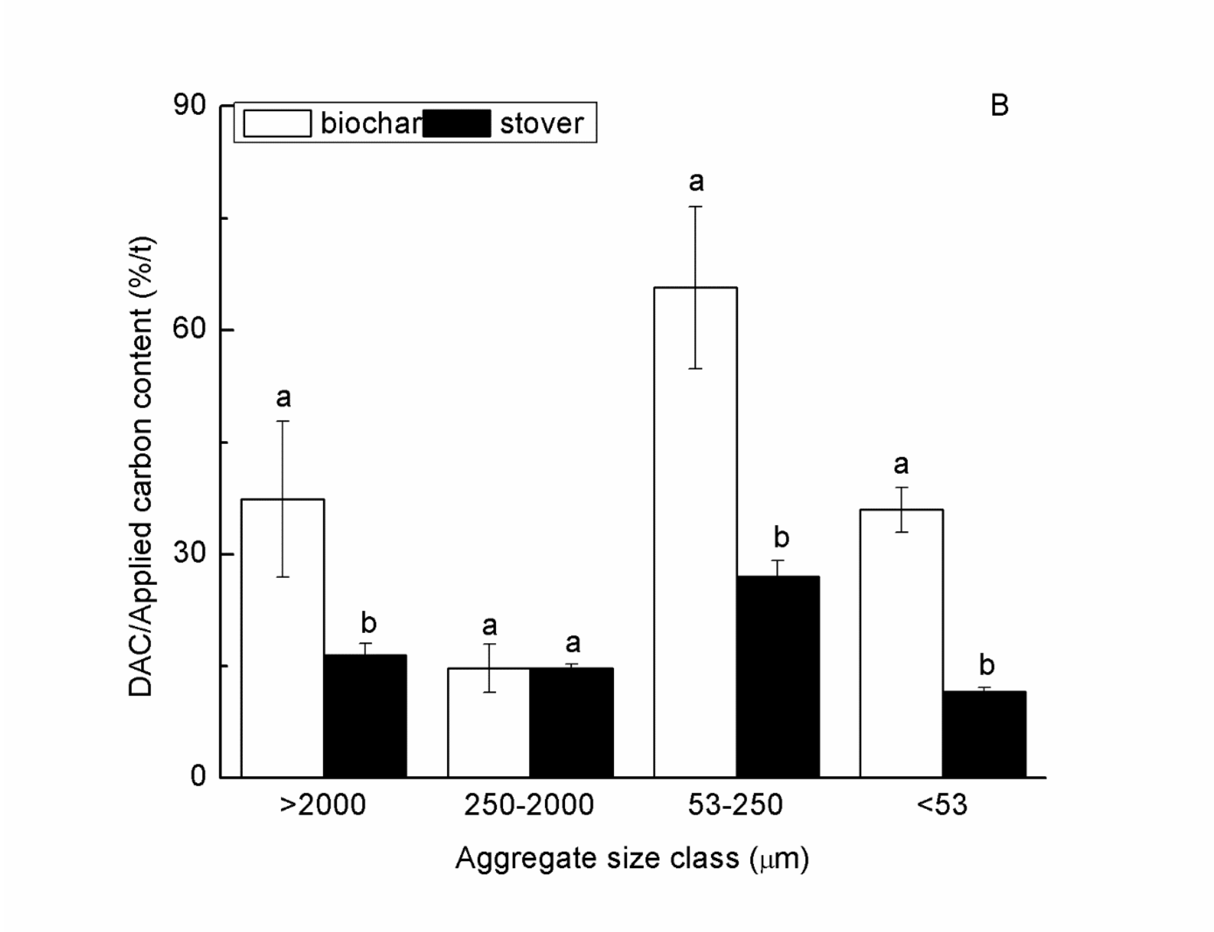


Figure 4. Ratio of QAC and DAC to carbon inputs within soil aggregates.

4. Discussion

4.1 Effect of maize stover and biochar on bulk density and total porosity

Both maize stover and its biochar significantly decreased soil BD after the five-year field experiment (Table 2). The difference between the effects of biochar and maize stover incorporation was not significant. In previous studies, biochar used as a soil amendment (Li et al., 2018) and stover (Getahun et al., 2018, Xu et al., 2018) both decreased soil BD, and the results of our study corroborated with those reports. In this study, PD significantly decreased with biochar and stover inputs. However, there was no significant difference between the biochar and stover treatments. Soil porosity is important for crops because of its direct effect on soil aeration and root growth (Walia and Dick, 2018). Biochar addition enhanced soil porosity in some previous studies (Obia et al., 2016), and biochar's effect on soil TP mainly depended on the addition rate of biochar (Głąb et al., 2018). In this study, TP decreased significantly as the BD increased. The TP increased by 2.00 and 4.06% under the biochar and maize stover amendments, respectively.

4.2 Effect of maize stover and biochar on soil aggregates and aggregate stability

In recent years, biochar has become a popular soil amendment used to improve soil quality, enhance carbon sequestration, mitigate greenhouse gas emission, and increase crop production (Purakayastha et al., 2019). The effects of biochar on soil aggregates were variable. Biochar applied at a rate of 16 t ha^{-1} increased the soil macroaggregate content (Zhang et al., 2017). Applications of 4.5 and 9.0 t ha^{-1} per year enhanced the macroaggregate content, but the effect was limited compared to that of returning straw to the field (Du et al., 2016). Biochar had a different effect on different soil textures, and biochar had little effect on soil aggregate in coarse textured soils (Wang et al., 2017). Additionally, some research showed that biochar had no or a negative effect on soil aggregates and aggregate stability. In a one-year short-term experiment, biochar had a significant effect neither on the soil aggregate content nor aggregate stability (Zhang et al., 2015). A three-year field experiment revealed that biochar had no effect on the MWD in sandy and silty soils (Borchard et al., 2014). In this study, both biochar and maize stover significantly enhanced the small macroaggregate (250-2000 μm) content and macroaggregate (>250 μm) content relative to the control treatment (Table 3 and Figure 1), and no significant differences in large macroaggregates (>2000 μm) were observed. Moreover, biochar and stover incorporation both decreased the silt and clay fraction in this study.

These results were similar to those of Du et al. (2016). Biochar serves as a binding agent in soil aggregate formation and enhances soil aggregate stability (Brodowski et al., 2005). Biochar has been proved to enhance soil organo-mineral interactions via adsorption and/or ligand exchange reactions, and thus stabilize SOC (Weng et al., 2017).

Our results showed that soil aggregates could be better bound under stover application than under biochar application probably because stover could promote the formation of fungal hyphae and production of root exudates (Jastrow, 1996). Most of the OC from maize stover is bioavailable OC, which can be easily used by microorganisms (Huang et al., 2018). The formation of organo-mienral complexes in areas of high microbial density (i.e., the rhizosphere and other microbial hotspots) might occur through the microbial turnover pathway, and carbon might be biosynthesized with high microbial carbon-use efficiency before binding together with mineral to form complexes. But in the areas of low microbial density, direct sorption might be the main mechanism for the formation of organo-mineral complexes, and these two mechanisms are not mutually exclusive, but rather spatially dictated (Sokol et al., 2019).

Biochar and maize stover also enhanced the soil aggregate stability, reflecting improved soil aggregates. Biochar amendment had a higher MWD, GMD, and $R_{>250\mu m}$ than control (Table 4), indicating that biochar could enhance soil structural stability. Stover also increased soil aggregate stability; however, the MWD and GMD did not differ significantly between the biochar and stover treatments. These results suggested that maize stover-derived biochar had an effect similar to that of stover on structural stability in the tested soil. Biochar significantly increased macroaggregates, but macroaggregates were more abundant in the stover treatment than in the biochar treatment in the present study, possibly because there was more inert carbon in biochar than in stover. Fractal dimension (D) is a proxy for soil particle size distribution (Tyler and Wheatcraft, 1992). Both biochar and stover decreased the D value in this study. The smaller the D value is, the more stable the soil aggregates are (Wu and Hong, 1999). The D value was significantly reduced by different biochar dosages, which might indicate that biochar could improve the resistance of soil aggregates to stress (Li et al., 2017). The D value is more appropriate than MWD and GMD for evaluating soil aggregate stability (Zhou et al., 2007). Therefore, both biochar and stover decreased the D value and enhanced the soil aggregate stability.

4.3 Effect of maize stover and its biochar on soil organic carbon, heavy fraction organic carbon and organo-mineral complexes

The SOC contents significantly increased with stover and biochar application in our study (Table 4). The highest SOC concentration was observed in the stover treatment during the current study because of the higher carbon input via the stover than its biochar. These results were similar to the results of previous studies (Huang et al., 2018, Yang et al., 2017). Our results indicated that stover and biochar application both significantly enhanced SOC contents. However, in a straw-mulch study, rice straw had no significant effect on SOC during crop growth (Li et al., 2016). The SOC content did not change significantly under straw incorporation in the first two years, but after 10 years, the SOC content increased significantly (Xu et al., 2011). This phenomenon was attributed to SOC being insensitive to short-term management, and the changes were slow, especially given the high background value of SOC (Xu et al., 2011). Therefore, the SOC content response to straw incorporation might differ depending on the type of straw, climate, soil type, geographical environment, tillage method and experimental duration.

The differences in the HFOC content were the same as those in SOC among the treatments. The highest HFOC concentration was observed in the maize stover treatment (Table 4). HFOC refers to the SOC fraction consisting of organo-mineral complexes, accounting for approximately 50~90% of SOC (Whalen et al., 2000). HFOC is extremely important for the maintenance of soil fertility and carbon sequestration. In recent years, many studies have considered the effect of biochar on LFOC, finding that biochar can enhance LFOC and SOC (Yang et al., 2017). However, little attention has been paid to HFOC. The HFOC content mainly consists of organo-mineral complexes, and the content is important for soil organo-mineral colloids (Weng et al., 2017). Due to the abundance of oxygen-containing functional groups, biochar could interact with mineral surfaces (Fe-, Al-, Mn-oxides, and phyllosilicates) or with dissolved metal ions (such as Ca^{2+}, Fe^{3+} and Al^{3+}) to form organo-mineral complexes (Lin et al., 2012, Qayyum et al., 2012). In our study, biochar and maize stover application increased HFOC concentrations by 20.77 and 40.11%, respectively, compared to the control.

Biochar application and stover treatment both increased the QC. The QC is an important quantitative index reflecting the organic matter and mineral particles in soils (Shi et al., 2002). Biochar increased the QC by 19.45%, and the stover treatment increased it by 40.42%. These results indicated that organic material inputs could enhance the QC, thereby promoting the formation of soil aggregates. However, the biochar treatment significantly decreased the DC, whereas the stover treatment increased the DC ($p<0.05$). These results were observed probably because biochar had refractory structure and poor accessibility to physically interact with the mineral matrix (Czimczik and Masiello, 2007).

Biochar application decreased the DC by 3.83%, and stover application increased it by 4.40% compared to CK. Long-term application of inorganic fertilizer, organic manure, or both increased the QC of fluvo-aquic soil and arid red soil, and combined inorganic and organic manure increased the QC in paddy soil (Shi et al., 2002). Chi et al. (2014) reported that the heavy fraction and QC were increased under different long-term fertilization treatments, and the DC decreased in the same way. In the present study, maize stover and biochar both increased the SOC content, HFOC content and QC, and biochar decreased the DC, while stover increased it. These responses mainly depended on the composition of organic material inputs and became stronger with an increase in stover input (Gao et al., 2017). This study confirmed the previous results that increasing soil organic matter promoted the formation of organo-mineral complexes and clustered soil aggregates.

4.4 SOC content and organo-mineral complexes within soil aggregate fractions

Soil macroaggregates and microaggregates affect the process of soil carbon sequestration (Six et al., 2004; Singh et al., 2019). SOC within soil aggregates has been regarded as a stable carbon sink in recent studies, and the formation and stability of aggregate are linked to soil C dynamics (Gao et al., 2017). On the one hand, soil aggregates provide physical protection to SOC against degradation, and then promotes soil C sequestration. The SOC could act as a binding agent in the formation of soil aggregates (Ghosh et al, 2018). In this study, almost all the size fractions in the stover retention treatment had higher OC concentrations than those in the biochar treatment because of more exogenous carbon input through the stover (Yang et al., 2017). Biochar and stover enhanced the relative carbon contributions in macroaggregates (>250 μm); in contrast, the relative contributions decreased in the <250 μm fractions, especially in the silt and clay fraction. These data further indicated that organic material input significantly increased carbon sequestration in macroaggregates, similar to a results reported by Du et al. (2016). Stable macroaggregates can protect SOC from degradation. This phenomenon indicated that biochar and stover amendments both increased the macroaggregate content, thus further promoted the stability of SOC. Macroaggregates could protect SOC from microbial degradation, and thus could contribute long-term storage of OC in soil (Grunwald et al, 2016). Therefore, biochar addition could stabilize SOC by macroaggregate formation, especially during the small macroaggregate formation process.

The order of HFOC in the aggregate fractions was as follows: stover > biochar > control. These data might be explained by the increased organic material inputs through the amendments than the control treatment. Fang et al. (2018) reported that 72.9~85.9% of biochar carbon was distributed in the LFOC, and the same results were shown in several other studies (Dharmakeerthi et al., 2015, Nimisha et al., 2014). In the present study, biochar and stover treatments increased the small macroaggregates significantly. With the increase in OC in aggregates, the QC increased gradually, but the DC decreased. These results indicated that organic matter addition enhanced OC in the soil, in turn improving the QC and increasing the macroaggregate content. In the present study, biochar and stover applications both increased the SOC and QC; ultimately, the macroaggregate content increased. The DC decreased. The QC increased in all aggregate fractions with organic material input, and stover retention had a more significant effect than biochar. However, biochar had a stronger effect on the DAC in the large macroaggregate, microaggregate, and silt and clay fractions than the stover treatment. A lower DC in macroaggregates could confirm that coarser OC was enclosed within the macroaggregates (Fu et al., 1983). In our study, the lower DC thus indicated that coarser OC was protected in the small macroaggregate fraction.

To better elucidate the organo-mineral complexes in different aggregate fractions, we normalized the ratio of the QAC and DAC to OC inputs in different fractions (Figure 4). Because the QAC reflects the quantity of colloidal complexes, the DAC refers to the degree of colloidal complexes (Fu et al., 1983). Both the QC and DC were important for soil aggregates, and the ratios indicated that the relative contribution of stover retention affected the soil organo-mineral complexes, and thus had a remarkable effect on soil aggregates. Biochar had a higher ratio of large macroaggregates in the QAC to OC input. In the other fractions, the ratio did not differ between the biochar and stover treatments. However, the ratio of DAC to OC input exhibited a different trend, and almost all the fractions had higher values in biochar than stover treatment. Although stover returned more OC back to the soil than biochar did, the later formed a greater quantity of and more stable large macroaggregates in the soil. These results were consistent with those obtained in earlier studies in which biochar was shown to protect soil aggregates (Brodowski et al., 2005). Biochar particles can chemically interact with mineral phases (Brodowski et al., 2005) where the cation bridging effect may prove to be a key mechanism for the formation of biochar-mineral complexes (Glaser et al., 2000; Lin et al., 2012). Thus, biochar played a stronger role than stover in binding minerals to form organo-mineral complexes in the present study.

5. Conclusions

The results of this long-term field study (five years) showed that maize stover and its biochar application had significant effects on the soil structure. Maize stover and its biochar increased the soil macroaggregate content, soil aggregate stability, and soil TP, but decreased soil BD and PD. The addition of biochar and maize stover resulted in an increased MWD, GMD, macroaggregate content, and a decreased D value. Both maize stover and its biochar increased the HFOC and SOC after the five-year experiment. Biochar and stover increased the QC, and enhanced the soil aggregate stability, but the DC was not affected by the SOC in the bulk soil. Biochar and stover both increased the OC in each aggregate fraction, and in the macroaggregate fraction, the relative contribution of SOC increased, indicating a positive role of biochar and stover in the process of soil aggregate formation. In this study, maize stover and equivalent stover-derived biochar both increased the SOC, soil aggregate stability, and organo-mineral complexes in different aggregate fractions, but due to higher OC input, maize stover had a stronger effect on soil aggregates and organo-mineral complexes. However, considering the amount of carbon input, biochar had a stronger effect on the QAC and DAC in different soil aggregate fractions. The present study suggests that maize stover and stover-derived biochar both improve soil aggregates and aggregate stability, and biochar application was a useful way to improve the soil structure.

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