POLISH JOURNAL OF SOIL SCIENCE
VOL. LIII/1 2020 PL ISSN 0079-2985

DOI: 10.17951/pjss/2020.53.1.41

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SOIL AGGREGATE SIZE DISTRIBUTION AND TOTAL ORGANIC CARBON IN INTRA-AGGREGATE FRACTIONS AS AFFECTED BY ADDITION OF BIOCHAR AND ORGANIC AMENDMENTS

Received: 19.02.2019
Accepted: 12.02.2020

Abstract. A two-year field trial on maize (*Zea mays* L.) production was established to determine the influence of biochar, maize straw, and poultry manure on soil aggregate stability, aggregate size distribution, total organic carbon (TOC), and soil microbial biomass carbon (MBC). Seven treatments with four replications, namely CK, control; S, 12.5 Mg ha^{-1} straw; B$_1$, 12.5 Mg ha^{-1} biochar; B$_2$, 25 Mg ha^{-1} biochar; SB$_1$, straw + 12.5 Mg ha^{-1} biochar; SB$_2$, straw + 25 Mg ha^{-1} biochar; and M, 25 Mg ha^{-1} manure were tested at four soil depths (0–10, 10–20, 20–30, and 30–40 cm). Aggregates were grouped into large macro-aggregates (5–2 mm), small macro-aggregates (2–0.25 mm), micro-aggregates (0.25–0.053 mm) and silt + clay (<0.053 mm). Biochar, straw, and manure applications all had significant effects ($p < 0.05$) on aggregate stability, with B$_2$ at 20 cm soil depth showing the greatest increase (62.1%). SB$_1$ of small macro-aggregate fraction showed the highest aggregate proportion (50.59% ± 10.48) at the 20–30 cm soil depth. The highest TOC was observed in SB$_2$ (40.9 g kg^{-1}) of large macro-aggregate at 10–20 cm soil depth. Treatment effects on soil MBC was high, with B$_1$ showing the greatest value (600.0 µg g^{-1}) at the 20–30 cm soil depth. Our results showed that application of biochar, straw, and manure to soil increased aggregate stability, TOC as well as MBC.

Keywords: biochar, aggregate stability, microbial biomass carbon, total organic carbon

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INTRODUCTION

Incorporation of biochars to improve soil quality and plant growth are of great importance, as biochar has been shown to have a significant influence on soil properties such as microbial activity and soil structural stability (Lehmann and Joseph 2009) as well as soil productivity (Biederman and Harpole 2013, Qian *et al*. 2015).

A soil aggregate is a group of primary soil particles that cohere to each other more strongly than the other surrounding particles (Nimmo 2004). Aggregate stability refers to the ability of soil aggregates to resist disintegration when exposed to forces such as water erosion and wind erosion, shrinking and swelling processes, and tillage (USDA 2008, Papadopoulos 2009). Wet aggregate stability suggests how well a soil can resist raindrop impact and water erosion, while size distribution of dry aggregates can be used to predict resistance to abrasion and wind erosion (USDA 2008). Soil structure affects a wide range of soil properties, including soil porosity, compactability and water retention (Cheng *et al*. 2015, Regelink *et al*. 2015). Incorporation of biochar into soil can lead to an improvement in soil aggregate stability (Liu *et al*. 2014, Zhang *et al*. 2015, Obia *et al*. 2016) by increasing exchangeable cation status of the soil, such as calcium (Enders *et al*. 2012, Jien and Wang 2013), thereby inhibiting clay dispersion and associated disruption of soil aggregates.

Soil organic matter and texture (clay content) are said to be the main abiotic binding agents in the formation and stabilization of aggregates (Duchicela *et al*. 2012, Portella *et al*. 2012), while soil microbes (bacteria and fungi) and plant roots have been reported as key biotic aggregating agents (Chaudhary *et al*. 2009, Duchicela *et al*. 2013). A desirable range of pore sizes for a tilled soil occurs when most of the clay fraction is flocculated into micro-aggregates, defined as <250 μm diameter, and secondly these micro-aggregates and other particles are bound together into macro-aggregates >250 μm diameter (Tisdall and Oades 1982). Micro-aggregates are supposed to be more stable against disruptive forces resulting from rain drops or tillage than macro-aggregates (Christensen 2001, Six *et al*. 2000). The addition of manure, slurry, or biochar to soil might exert different effects on the activity of microorganisms because of differences in their composition (e.g. C/N ratio, amount of low molecular compounds) (Helfrich *et al*. 2008, Le Guillou *et al*. 2012) and also provide substrate for microorganisms (An *et al*. 2015, Poirier *et al*. 2014). Soil organic carbon which is the metabolic product of microorganisms is stored in different fractions of soil aggregates or attached on clay particles during the processes of organic transformation and aggregate formation (Guggenberger *et al*. 1995, Six *et al*. 2004). Guan *et al*. (2015) and Hao *et al*. (2013) also reported that addition of crop residue to soil could alter the distribution of organic C in aggregates and increase the TOC content in aggregates, especially in macro-aggregate

(>250 µm). The influence of straw, manure and biochar on TOC, MBC and aggregate size and distribution will depend on soil properties, feedstock and environmental conditions.

Many research works, mostly pot experiments have been done to ascertain the influence of biochar on aggregate size distribution and TOC in soil aggregates but little attention has been given to comparing the effects of biochar and other organic amendments on the field at different soil depths. Therefore, the objective of this study was to determine how soil aggregate stability, size and distribution of soil aggregates, TOC contents in soil aggregates, and soil microbial biomass C are affected by addition of biochar, straw, and manure. We hypothesize that biochar, manure, and straw will increase TOC in intra-aggregate fractions, aggregate stability, and soil MBC.

MATERIALS AND METHODS

Experimental design and treatment

The experimental field was located at Harbin, Heilongjiang Province, China (45°41'N, 126°37'E). The experimental site has a monsoon-influenced, humid continental climate. The mean annual temperature is 3.4°C and the annual precipitation is 500–600 mm, with 90% of the precipitation falling as rain between April and September. The soil used is classified as Typic Hapludolls (USDA 1999). The experiment was laid out in a randomized complete block design (RCBD) with seven treatments, namely CK (control), S (12.5 Mg ha^{-1} maize straw), M (25 Mg ha^{-1} poultry manure), B$_1$ (12.5 Mg ha^{-1} biochar), B$_2$ (25 Mg ha^{-1} biochar), SB$_1$ (12.5 Mg ha^{-1} maize straw + 12.5 Mg ha^{-1} biochar), SB$_2$ (12.5 Mg ha^{-1} maize straw + 25 Mg ha^{-1} biochar), and four depths (0–10, 10–20, 20–30, and 30–40 cm). Biochar used for this study was sourced from Jin and Fu Agriculture Co., China. It was manufactured from maize at a pyrolysis temperature of 450°C and exhibited the following characteristics: C, 415.3 g kg^{-1}; Total N, 6.88 g kg^{-1}; Total P, 10.23 g kg^{-1}; Avail. P, 25.99 mg kg^{-1}; pH, 9.89. The amendments were applied once and were evenly spread on the soil surface, and then left over the winter. They were later incorporated into the soil via harrowing to a depth of 30 cm. The size of each plot was 20 m^2 (5 m × 4 m) and there were 28 experimental plots in total. The treatments were replicated four times. Biochar and straw were applied on October 27, 2014, while manure was applied on October 30, 2014. Maize (*Zea mays* L.) was sown by a mechanical planter on May 27, 2016, at one seed per hole at a spacing of 70 cm × 20 cm.

Soil sampling

Before the application of amendments, soil samples were taken randomly on each plot at the depth of 0–20 cm, bulked to form a composite sample, air dried and sieved through a 2 mm and 0.5 mm sieves, and analyzed to determine the basic properties. Soil samples at the depth of 0–20 cm were also collected on the plot with the aid of core sampler to determine soil bulk density. The soil basic properties are: pH (H_2O), 6.24; Total N, 0.42 g kg^{-1}; Org. C, 24.0 g kg^{-1}; Avail. P, 29.60 mg kg^{-1}; Exchangeable K, 0.2 C mol^{-1} kg^{-1}; and Na, 0.5 C mol^{-1} kg^{-1}. The soil textural class is clay loam (40% sand, 28% silt and 32% clay) with a bulk density of 1.32 Mg m^{-3}. Soil was sampled on October 13, 2016, after the harvest of maize, 24 months after application of amendments.

Determination of soil aggregate stability and microbial biomass C concentrations

Aggregate stability was determined for disturbed soil samples using the wet sieving method (Elliott 1986). Extraction of aggregate was performed with a Soil Aggregate Analyzer containing six sieves (5, 2, 1, 0.5, 0.25 and 0.106 mm). 80 g air-dried bulk soil sample from the field was placed on top of the 5 mm sieve and then gently plunged into de-ionized water for 10 min in order to soften the aggregates. The series of sieves were then automatically moved up and down, 30 times per minute over a distance of 5 cm under the water for 5 min in order to separate the aggregate fractions. At the end of the process, aggregates remaining on each sieve (2–0.106 mm) were collected in aluminium pans. The soil particles left in the water inside the container were <0.053 mm (silt + clay). The aggregates were oven-dried at 60°C to a constant weight. Total organic carbon (TOC) was determined on each of the aggregates using wet oxidation with $K_2Cr_2O_7$ method, and they were grouped into large macro-aggregates (5–2 mm), small macro-aggregates (2–0.25 mm), micro-aggregates (0.25–0.053 mm), and silt + clay (<0.053 mm). The soil was free of carbonates; hence soil organic carbon (SOC) was taken as TOC. The aggregate stability was calculated from the mean weight diameter (MWD) as:

$$\mathrm{MWD} = \sum_{i=1}^{n} \overline{Xi} \,.\, Wi$$

Where:

$\overline{xi}$ is the mean diameter of the openings of the two consecutive sieves

wi is the mass proportion of aggregate fraction remaining on each sieve to that of the bulk soil

n is the number of fractions

Microbial biomass carbon (MBC) was determined by Chloroform-Fumigation-Extraction as described by Vance *et al*. (1987) from fresh soil samples immediately after sampling from the field.

Statistical analysis

All data collected were subjected to two-way analysis of variance (ANOVA) using GenStat Discovery Edition 4 software in order to evaluate the significance of treatment and depth on aggregate MWD, aggregate size distribution, TOC and MBC. Means were compared using Least Significant Difference (LSD) test and Duncan's Multiple Range Test (DMRT) at the $p < 0.05$ level of significance. Simple linear regression was used to determine the relationship between MWD and aggregate-associated TOC at the 10–20 cm depth.

RESULTS

MWD of soil aggregates

Fig. 1 shows the values of MWD which ranged from 0.3298 mm to 0.7190 mm (mean ± SE = 0.5289 ± 0.0467). Significant differences ($p < 0.001$) were observed at all soil depths, with mean values ranging from 0.4623 mm to 0.588 mm. The highest MWD was shown by 20 cm soil depth followed by 10 cm soil depth, while 40 cm soil depth recorded the lowest MWD value. The two biochar levels, straw, manure and biochar-straw combinations all had significant effects on MWD. B_2 at 20 cm soil depth showed the greatest significant increase (0.2755 ± 0.04 mm; 62.1%) in MWD in comparison to the control. Also, at the

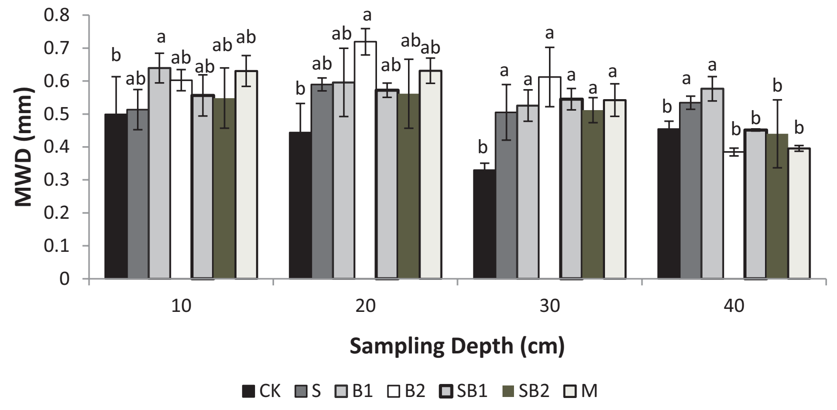

Fig. 1. Mean weight diameter (MWD) of soil aggregates as affected by biochar, straw, and manure additions at different soil depths (0–10, 10–20, 20–30, and 30–40 cm)

Note: CK, control; S, 12.5 Mg ha^{-1} straw; B_1, 12.5 Mg ha^{-1} biochar; B_2, 25 Mg ha^{-1} biochar; SB_1, straw + 12.5 Mg ha^{-1} biochar; SB_2, straw + 25 Mg ha^{-1}biochar; M, 25 Mg ha^{-1} manure. Error bars are standard error, n = 4, means with the same letter are not significantly different at $p < 0.05$.

30 cm soil depth, the control (CK) was significantly lower in MWD than all other treatments, with B_2 showing the greatest increase (0.2823 ± 0.09 mm). The combination of biochar and straw (SB_1 and SB_2) was not significantly different from straw (S) except at the 40 cm soil depth where straw was significantly higher ($p < 0.05$) than SB_1 and SB_2.

Size and distribution of soil aggregates

Small macro-aggregates (2–0.25 mm) were the most prominent of the aggregate fractions in all treatments across the four soil depths (Table 1), while silt + clay (S + C) and large macro-aggregates showed the lowest distribution of aggregates. For the large macro-aggregate (5–2 mm), significant difference was not observed among the treatments but there was significant difference ($p < 0.001$) among the soil depths. There was higher proportion of large macro-aggregates at the 10–20 cm soil depth than other soil depths. Straw-biochar combination (SB_1) was significantly ($p < 0.05$) higher at the 20–30 cm soil depth than straw (S) and control for small macro-aggregates (50.59 ± 10.48). Significant difference was observed in the size and distribution of micro-aggregate (0.25–0.053 mm) for both soil depth and treatment. The greatest increase in micro-aggregate proportion was shown by B_1 (29.91 ± 8.11) at the 20–30 cm soil depth, and it was significantly ($p < 0.05$) higher than other treatments. B_1 significantly ($p < 0.01$) increased the proportion of S + C fraction in comparison to other treatments at 0–10 cm and 30–40 cm soil depths by (8.63 ± 0.69, 139%) and (8.23 ± 0.69, ~133%), respectively.

Table 1. Distribution (%) of aggregate sizes (mm) following wet sieving of soils amended with biochar, straw, and manure (n = 4, ±S.E)

Depth (cm)	Treatment	Aggregate size (%)			
		Large Macro (5–2 mm)	Small Macro (2–0.25 mm)	Micro (0.25–0.053 mm)	Silt + Clay (<0.053 mm)
0–10	CK	4.87 ± 0.25	25.99 ± 10.64	15.21 ± 2.16	6.21 ± 0.63
	S	11.50 ± 2.72	45.76 ± 10.24	19.06 ± 3.97	8.59 ± 0.72
	B_1	6.28 ± 0.84	39.69 ± 5.84	28.14 ± 3.91	14.84 ± 1.32
	B_2	7.08 ± 0.37	46.11 ± 1.66	27.01 ± 0.94	13.34 ± 1.72
	SB_1	11.00 ± 2.82	44.58 ± 4.51	22.24 ± 3.81	12.34 ± 0.67
	SB_2	14.06 ± 1.38	44.21 ± 5.44	22.25 ± 7.97	9.50 ± 3.61
	M	14.66 ± 2.92	43.19 ± 6.20	25.49 ± 6.38	9.01 ± 1.06
10–20	CK	4.08 ± 0.77	28.96 ± 3.03	9.45 ± 1.21	7.53 ± 1.39
	S	10.63 ± 1.43	43.35 ± 1.84	16.41 ± 5.04	10.86 ± 1.67
	B_1	13.80 ± 6.08	50.03 ± 3.76	16.10 ± 4.39	8.78 ± 2.86
	B_2	8.18 ± 0.92	42.75 ± 3.66	14.76 ± 2.17	8.01 ± 0.90
	SB_1	14.63 ± 1.23	43.31 ± 4.99	13.28 ± 4.07	11.92 ± 1.64
	SB_2	9.19 ± 1.33	32.73 ± 1.69	14.91 ± 2.24	6.53 ± 1.45
	M	11.00 ± 2.03	41.94 ± 5.01	22.74 ± 6.33	7.55 ± 0.74

Depth (cm)	Treatment	Aggregate size (%)			
		Large Macro (5–2 mm)	Small Macro (2–0.25 mm)	Micro (0.25–0.053 mm)	Silt + Clay (<0.053 mm)
20–30	CK	8.40 ± 1.57	37.51 ± 2.93	15.54 ± 2.75	4.89 ± 0.75
	S	6.19 ± 1.28	35.55 ± 4.35	25.73 ± 11.34	12.15 ± 3.23
	B_1	6.41 ± 0.94	40.41 ± 6.47	29.91 ± 8.11	12.99 ± 1.25
	B_2	5.69 ± 0.87	41.08 ± 4.71	25.80 ± 7.63	11.41 ± 1.74
	SB_1	8.15 ± 1.50	50.59 ± 10.48	24.51 ± 6.09	9.25 ± 1.35
	SB_2	6.73 ± 0.97	48.36 ± 10.45	15.59 ± 3.31	8.56 ± 0.88
	M	8.46 ± 1.63	46.40 ± 1.51	27.80 ± 6.50	10.49 ± 1.98
30–40	CK	5.08 ± 0.75	46.96 ± 10.52	15.21 ± 2.16	6.21 ± 0.63
	S	2.25 ± 0.52	46.65 ± 0.47	23.79 ± 1.55	11.46 ± 0.40
	B_1	2.33 ± 0.21	43.48 ± 5.39	22.81 ± 1.03	14.44 ± 1.32
	B_2	2.61 ± 0.88	42.78 ± 0.97	18.23 ± 6.01	9.03 ± 0.67
	SB_1	5.48 ± 0.30	45.18 ± 5.62	21.56 ± 6.71	12.59 ± 0.87
	SB_2	2.58 ± 0.59	45.18 ± 10.57	20.19 ± 3.98	11.54 ± 0.50
	M	4.08 ± 0.46	49.58 ± 3.32	18.55 ± 3.41	8.55 ± 0.81
$LSD_{0.05}$	D	1.985***	4.119	3.651**	1.85
	T	2.626	5.449*	4.83*	2.447**
	D × T	5.252	10.898	9.66	4.894

Note: CK, control; S, 12.5 Mg ha^{-1} straw; B_1, 12.5 Mg ha^{-1} biochar; B_2, 25 Mg ha^{-1} biochar; SB_1, straw + 12.5 Mg ha^{-1} biochar; SB_2, straw + 25 Mg ha^{-1} biochar; M, 25 Mg ha^{-1} manure. D – depth, T – treatment, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

Proportion of TOC in soil aggregate fractions

The relative size of TOC found in aggregates is a function of depth and size of aggregate fraction (Fig. 2). Highest TOC was obtained at the 10–20 cm soil depth. The lowest proportion of TOC (14.8 g kg^{-1}) was located in silt + clay fraction (<0.053 mm). However, the highest TOC (40.9 g kg^{-1}) was located in 5–2 mm, and the same trend was observed at all soil depths. No significant difference was observed among the treatments in each of the aggregate fractions at the 30–40 cm soil depth. The TOC of straw and B_2 combination (SB_2) in the upper layer (0–10 cm) decreased from large macro-aggregate to small macro-aggregate to micro-aggregate and then to S + C by 14%, 27% and 40%, respectively. However, at the deepest layer (30–40 cm), SB_2 decrease in TOC within aggregate fractions was in the rate of ~5%, 20.6% and 26.3%, respectively. At the 20–30 cm soil depth, greatest TOC increase was observed in B_2 of large macro-aggregate, and the difference was significantly ($p < 0.05$) higher than S, M and CK while for the S + C fraction at the same depth, B_2 was also significantly ($p < 0.001$) higher than SB_1, S, M, and CK. The two levels of sole biochar additions (B_1 and B_2) showed the greatest increase in TOC of the micro-aggregate fraction at the 10–20 cm soil depth, and they were significantly ($p < 0.01$) different from CK.

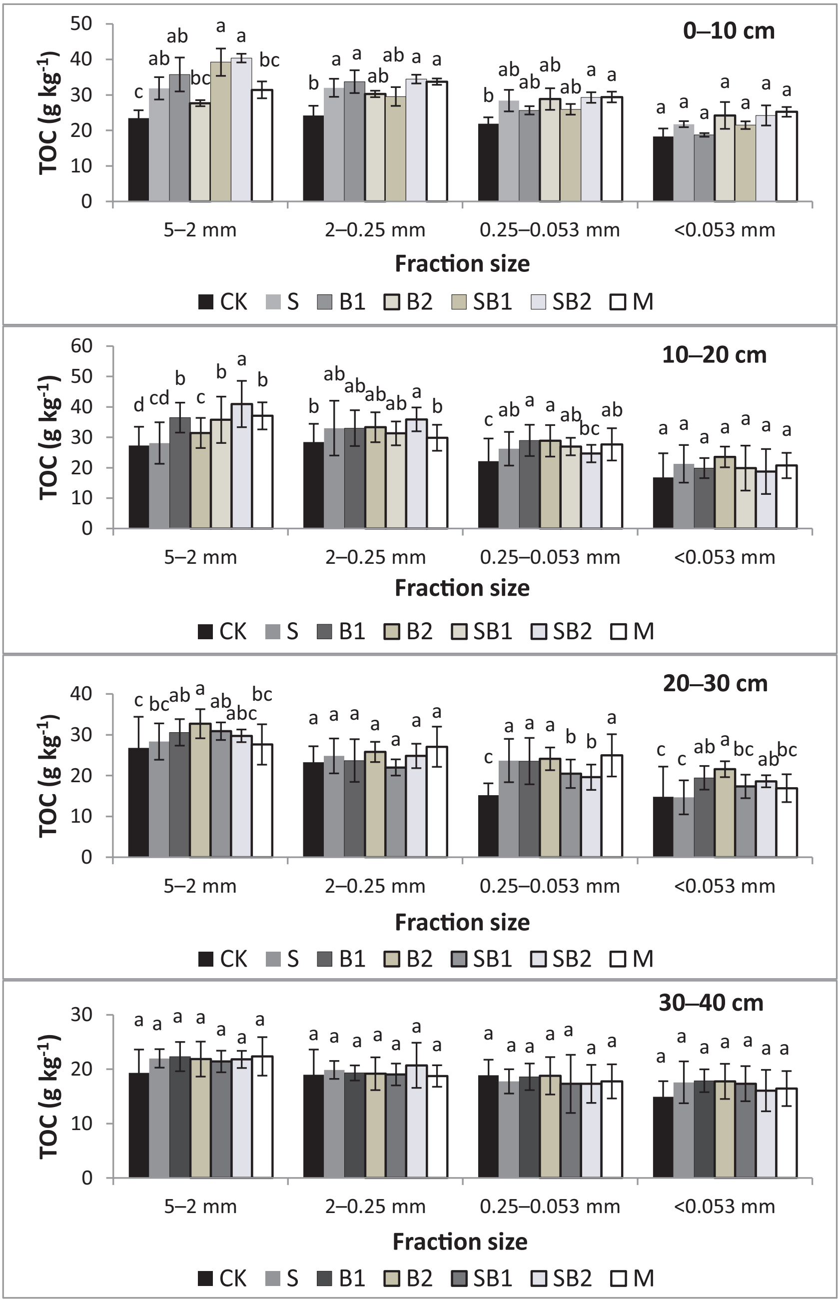

Fig. 2. TOC in soil aggregate fractions as affected by biochar, straw, and manure additions at four soil depths (0–10, 10–20, 20–30, and 30–40 cm)

Note: CK, control; S, 12.5 Mg ha^{-1} straw; B$_1$, 12.5 Mg ha^{-1} biochar; B$_2$, 25 Mg ha^{-1} biochar; SB$_1$, straw + 12.5 Mg ha^{-1} biochar; SB$_2$, straw + 25 Mg ha^{-1} biochar; M, 25 Mg ha^{-1} manure. Error bars are standard error, n = 4, means with the same letter are not significantly different at $p < 0.05$.

Microbial biomass carbon

There were great differences in MBC following the application of biochar, straw, and manure to soil at different soil depths (Fig. 3). The highest MBC value (600.0 µg g^{-1}) was shown by B_1 at the 20–30 cm depth. At depths of 0–10, 10–20, 20–30 and 30–40 cm, MBC levels vary between 331.6–552.6 µg g^{-1}, 457.9–568.4 µg g^{-1}, 457.9–600.0 µg g^{-1}, and 244.7–465.8 µg g^{-1}, respectively. B_1 also increased in MBC from 0–10 cm to 10–20 cm by 5.9% and to 20–30 cm by 11.8%. However, a decrease in MBC was observed for B_1 at the 30–40 cm depth but it was significantly (p = 0.001) higher than other treatments and control. Lowest accumulation of MBC was observed at the 30–40 cm depth, with CK showing the least value (244.7 µg g^{-1}).

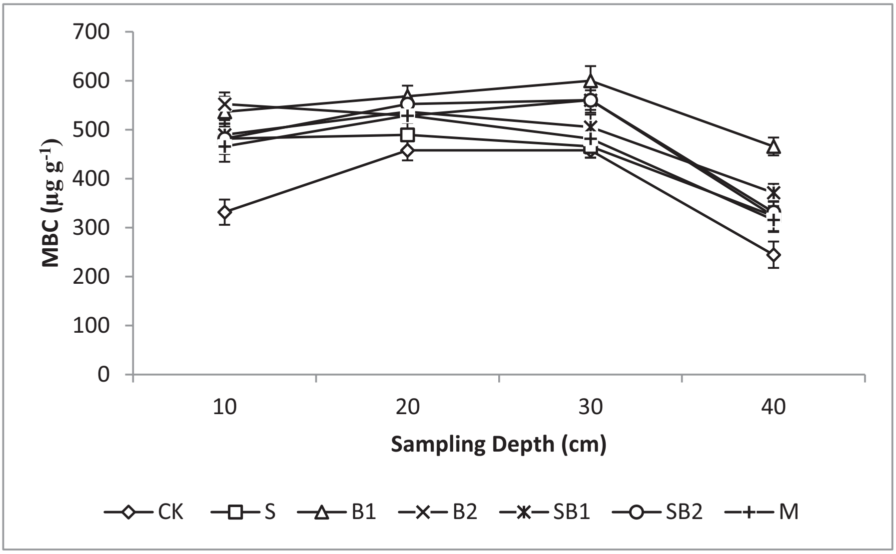

Fig. 3. Soil microbial biomass carbon (MBC) as affected by biochar, straw, and manure additions at different soil depths (0–10, 10–20, 20–30, and 30–40 cm)

Note: CK, control; S, 12.5 Mg ha^{-1} straw; B_1, 12.5 Mg ha^{-1} biochar; B_2, 25 Mg ha^{-1} biochar; SB_1, straw + 12.5 Mg ha^{-1} biochar; SB_2, straw + 25 Mg ha^{-1} biochar; M, 25 Mg ha^{-1} manure. Vertical bars represent standard error of means (n = 4).

Relationship between MWD and aggregate-associated TOC

At the 10–20 cm soil depth, a non-significant but positive correlation was observed between the MWD and large and small macro-aggregates (Fig. 4). However, at the same depth, a significant ($p < 0.05$) positive correlation was observed between MWD, micro-aggregates and silt + clay fractions (Fig. 4).

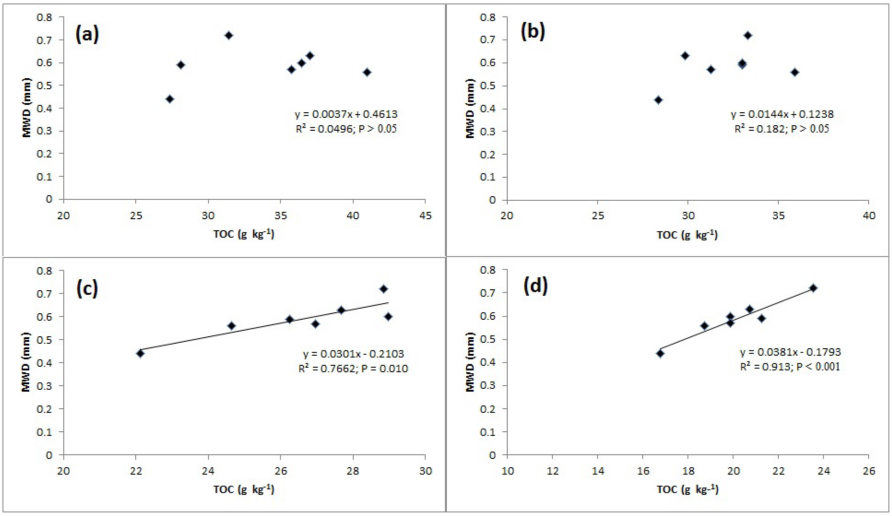

Fig. 4. Relationship between MWD and TOC contained in large macro-aggregates (a), small macro-aggregates (b), micro-aggregates (c), and silt + clay fractions (d) at the 20 cm soil depth

DISCUSSION

In this study, it was observed that the incorporation of biochar (either singly or when combined with straw) to soil led to an improvement in soil aggregates stability (MWD). This finding is in consonance with the works of (Sun and Lu 2013, Liu *et al*. 2014, Abdelhafez *et al*. 2014). Poultry manure addition also improved aggregate stability. This must have been possible because of the inter-layer cementing effects of manure that resulted in the consolidation of micro-aggregates into macro-aggregates. Similar results can be found in Nyamangara *et al*. (2001) where manure improved soil structural stability from 0.243 mm in control to 0.733 mm. Addition of organic amendment (biochar, straw, and manure) to soil must have led to the release of polysaccharides by soil microbes (predominantly bacteria and fungi) which helped in cementing/binding the soil particles. Kinsbursky *et al*. (1989) reported that the effectiveness of the binding agents in contributing to aggregate stability is dependent on soil textural characteristics and soil organic carbon. The textural class of the soil we used for this study is clay loam (medium-textured soil) and it contains higher clay content than light-textured soil, hence it is expected that aggregates of medium-textured soil would show more response to organic matter addition than coarse-textured soils. However, Gentile *et al*. (2010) reported an increase in aggregation following biochar addition to a light-textured soil.

There was higher concentration of small macro-aggregates in both amended soils and control. Organic materials are directly responsible for the formation of macro-aggregates through the actions of fungal hyphae and microbial extracellular polysaccharide gums (Six *et al.* 2004). Biochar treatment contributed to the formation of more small macro-aggregates. The combined application of biochar and straw also resulted in an increase in small macro-aggregate concentration at the 20–30 cm depth. This result suggests that application of biochar either singly or in combination with straw can improve small macro-aggregate formation in soils. Soil micro-aggregates also increased with additions of straw, biochar, and manure across all depths. This could be a result of the process of organic matter decomposition which involves the production of organic compounds such as hydrophilic polysaccharides that promote inter-particle cohesion through adsorption to mineral matter (Chenu 1989, Verchot *et al.* 2011, Demisie *et al.* 2014), thus increasing soil aggregation. Variability among treatments of the silt + clay fraction was relatively lower than the macro-aggregate and micro-aggregate fractions most likely due to its smaller particle size.

Biochar, manure, and straw amendments significantly increased the concentrations of TOC at the 0–30 cm soil depth. The highest soil TOC values were observed in the large macro-aggregate (5–2 mm) and small macro-aggregate (2–0.25 mm) fractions across the depths, which is an indication that there were higher microbial activities in the 5–0.25 mm fraction which resulted in an increase in organic carbon content. Similar results were also reported by Gioacchini *et al.* (2016). However in contrast, Hartley *et al.* (2016) reported that TOC was greatest in silt + clay fractions (<0.053 mm) within all soils irrespective of treatment. TOC increased with aggregate size, and the higher concentration of TOC in both large and small macro-aggregates than in micro-aggregate and silt + clay fractions can be useful for long-term C protection, long-term C storage and sequestration (Blanco-Canqui *et al.* 2017).

The carbon contained in bacteria and fungi of soil organic matter is known as soil MBC. The impact of biochar, straw, and manure incorporation into soil was evident on soil MBC at all soil depths considered. The increase that accompanied soil MBC, following the application of organic amendments, is an indication of increase in number and activities of soil microorganisms. Similar results were reported by Zhang *et al.* (2014) who found an increase in soil MBC after consecutive biochar application in North China. Odugbenro *et al.* (2019) also reported an increase in soil MBC following biochar and corn straw application to a clay loam soil. The greatest soil MBC was shown by sole-biochar treatments across all soil depths. Reason adduced to this is that sorption of relatively polar organic matter and nutrients could provide energy for microorganisms, while macro and micropores of biochar, which hold air and water, could likely support microorganisms' livable habitat (Lehmann *et al.* 2011).

The relationship between MWD and aggregates-associated TOC showed that there was a non-significant positive correlation between MWD and both large and small macro-aggregates (Figs. 4a and b). However, micro-aggregate and silt + clay fractions within the 0.25 to <0.053 mm range showed a significant positive correlation with MWD (Figs. 4c and d). This result suggests that the increase in TOC that follows application of organic amendments may contribute to aggregate stability, which has also been reported by several authors (Ma *et al*. 2016, Domingo-Olive *et al*. 2016).

CONCLUSIONS

Our study showed that application of biochar either singly or in combination with straw increased soil aggregate stability. Poultry manure and straw treatments also increased aggregate stability. Biochar, straw, and manure increased TOC in aggregates of all sizes in comparison to control. Biochar treatment showed the greatest soil MBC increase, which is an indication that biochar provided more favorable environment for microorganisms.

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