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RESEARCH ARTICLE

Pig manure biochar enhances soil organic carbon, aggregate stability and microbial biomass in the clay soil of Bangladesh

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Abstract

Biochar is a widely used soil amendment that can improve soil physical properties like aggregation. However, little information is available regarding how pig manure biochar improves soil structure formation and organic carbon content in clay soil. This short-term field experiment (120 days) investigated the impacts of biochar on soil organic carbon (SOC) and aggregate stability (MWD) coupling with microbial biomass carbon (MBC) in clay soil under Pumpkin (*Cucurbita maxima* L.). The experiment consisted of five treatments as follows: (i) Control as no biochar (T_0), (ii) inorganic fertilization (triple super phosphate) at 2 t ha^{-1} (T_1), (iii) biochar at 4 t ha^{-1} (T_2), (iv) biochar at 8 t ha^{-1} (T_3) and (v) biochar at 16 t ha^{-1} (T_4). Results showed that large macroaggregates were increased by 1.9, 2.2 and 2.7 times higher, while MWD was increased by 53, 75 and 103 % in the T_2, T_3 and T_4 treatments, respectively, upon biochar incorporation as compared to T_0 ($P < 0.01$). The SOC was increased significantly with all treatments compared to T_0 ($P < 0.001$). Moreover, MBC and GRSP were enhanced by 4.5 and 1.25-fold, respectively, with only T_4 biochar treatment ($P < 0.001$), while T_2 treatment had no impact on MBC and GRSP ($P > 0.05$). SOC, MBC and GRSP were significantly correlated with MWD ($P < 0.05$), while iron oxides had no impacts on MWD ($P > 0.05$). The study indicates that biochar, particularly at 16 t ha^{-1}, can potentially enhance MWD, boosting microbial activity and SOC in clay texture soils of southeast Bangladesh.

Keywords

aggregation; Fe oxides; GRSP; microbial activity; soil amendments

Introduction

Biochar, a carbon-rich material that originated from the thermal decomposition of biomass via pyrolysis, has been extensively studied for its beneficial impacts on the environment. Biochar is considered a suitable habitat and source of food for microbial communities and can enhance microbial function, thereby stabilizing soil structure (1). Soil fungi stabilize the larger soil particles, while bacteria interact to attach clay plus silt-size small soil mineral particles together. Soil microbial decomposition of organic amendments produces extracellular polysaccharide substances (EPS) coupling with low molecular weight metabolites, which can facilitate the stability of soil aggregates (2, 3). Biochar amendment considerably positively impacts soil structure formation (4). Biochar enhances soil aggregation through the interactions between the biochar surface C functional groups and soil minerals surface functional groups (5).

Applying biochar promotes the growth and activity of soil fungi by providing a higher carbon-to-nitrogen ratio, which is crucial for stabilizing soil aggregates. Fungal hyphae serve as a transitory binding agent, improving aggregation by interweaving and entangling soil particles (6). Fungal hyphae, particularly mycorrhiza fungi, can turn over rapidly for about 5-6 days and then the glomalin-related soil protein (GRSP) is released from dead mycelium (7). GRSP is closely associated with the walls of mycorrhizal fungal hyphae and spores and also plays a key function in enhancing aggregation by glueing soil particles together (8).

Soil aggregate formation after organic amendment incorporation also varies with soil clay mineralogy. The 2:1-type clay minerals, such as vermiculite and montmorillonite, promote soil aggregation through clay-organic matter bridge, while 1:1-type minerals, such as kaolinite, promote aggregation through mineral-mineral interaction (9). However, the impacts of biochar derived from different sources such as straw, wood and various waste materials on aggregation in the range of soil texture have been reported. In Bangladesh, pig farming produces a huge amount of manure, which leads to environmental pollution (10). Production of pig manure biochar might be an alternative option to minimize environmental pollution and improve sustainable soil management (10). However, the impact of biochar derived from pig manure remains largely illusive in clay-textured soil. Moreover, the published short-term reports of biochar addition on aggregation are largely inconsistent (10). For example, biochar influences positively or negatively, while no impact on aggregation is also reported in clay texture soil (11, 12, 13). These conflicting outcomes require investigating how biochar influences aggregate stability and SOC stock in clay soil. In the current investigation, it has been hypothesized that the biochar application would enhance the water stability of soil aggregates by enhancing SOC and boosting microbial activity under Pumpkin (*Cucurbita maxima*). The fruit type of pumpkin is a berry known as a pepo under the family Cucurbitaceae. The global production of pumpkins in 2022 was 23 mt, while in Bangladesh, it was 18-20 t/acre. In Bangladesh, it is widely cultivated over the country). The specific objectives of the current study were to determine the influence of biochar on the stability of soil aggregates, to measure the microbial activity under biochar application and to quantify the soil organic carbon after biochar application. The current study will explore the sustainable soil management approach to enhance crop productivity coupling with aggregation upscaling the soil carbon storage.

Materials and Methods

Soil and biochar

The study field was located under the experimental sites of Khulna University, Khulna, Bangladesh, specifically at coordinates 22°48' N latitude and 89°32' E longitude. The soil experimented with during the current investigation was classified as Typic Haplaquepts (14). The precipitation and temperature of the study area were 28 °C and 1280 mm, respectively. The biochar applied in this study was commercially obtained from Sanken Corporation, based in Hachimantai, Japan. It was manufactured through a specialized baking process using an oven at temperatures between 600°C and 700°C for ten minutes. This experiment used pig manure as raw material to manufacture the biochar. The final product exhibited a particle size of less than 10 mm. For further processing, it was mechanically crushed into smaller fragments and passed through a mesh containing 0.5 mm openings (15). The initial soil and biochar properties before implementing the experiment are presented in Table 1.

Table 1. The properties of initial soil and biochar before imposing the experiment.

	Properties	Results
Initial soil	Soil pH	8.00
	Electrical conductivity (EC)	3.83 dS/m
	Total N	1.60 g/kg
	SOC	8.59 g/kg
	Sand (2.00-0.05 mm)	7.12 %
	Silt (0.05-0.002 mm)	34.11 %
	Clay (< 0.002 mm)	58.76 %
	Available K, Na, Ca, Mg, S and P	0.14, 0.58, 0.56, 0.65, 0.09 and 0.03 g/kg, respectively
Biochar	pH	8.24
	EC	4.13 dS/m
	Carbon (C)	9.17 ± 0.02 %
	Nitrogen (N)	0.541 ± 0.03 %
	C/N Ratio	16.67 ± 0.06
	Source	Pig manure

Experimental design

The short-term field experiment started in February 2022 and continued for 120 days (4 months). The completely randomized design (CRD) was used to allocate the study plots because the soil properties did not differ spatially. The experiment consisted of five treatments: (i) control (T0; no biochar application), (ii) inorganic fertilizer (TSP, triple super phosphate) at 2 t ha^{-1} (T_1), (iii) biochar at 4 t ha^{-1} (T_2), (iv) biochar at 8 t ha^{-1} (T_3) and (v) biochar at 16 t ha^{-1} (T_4). Each treatment was replicated thrice and the plot size was 0.5 x 0.5 m^2. The biochar and inorganic fertilizer were incorporated on the surface of the respective plots and then mixed manually on the surface soil at a depth of 0-15 cm. The study plots were cultivated with pumpkin (*Cucurbita maxima* L.), popular locally during this season. It grows in all the districts of Bangladesh, but plenty of pumpkins are produced in Khulna, Jessore, Kustia, Chittagong and Dhaka. To maintain soil moisture content at field capacity during the growth period, the soil was irrigated every five days to replenish the water loss.

Soil sampling

The post-harvest samples were collected after the harvest of pumpkins (after 120 days). Five topsoil samples (0-15 cm depth) were collected from each study field. Then, these five samples were mixed to make a composite soil sample. This way, fifteen composite samples were prepared from seventy-five individual samples of the respective study field. Then, the samples were subjected to air-dry, crushed by a hammer and sieved by 4 for wet sieving and 2 mm mesh for biological and chemical analyses.

Soil sample analyses

Soil physicochemical characteristics

The samples' soil pH and electrical conductivity (EC) were determined using a soil-water ratio 1:2.5 (16). The total nitrogen concentration of the soil was determined using the Kjeldahl digestion procedure (17). Available soil magnesium (Mg), calcium (Ca) and potassium (K) were extracted with an ammonium acetate (1 M) solution at neutral pH, with a soil extractant ratio of 1:10 (17). Following the extraction, the concentration of K, Ca and Mg in the extract was determined using an Atomic Absorption Spectrophotometer (AAS, Shimadzu model AA-7000, Tokyo, Japan). The available soil S was extracted by a potassium dihydrogen phosphate (KH_2PO_4) solution with a soil: extractant ratio of 1:10. The available soil sulfur (S) was extracted using a potassium dihydrogen phosphate solution with a soil: extractant ratio of 1:10. The S content in the leachate was determined using the turbidity method (18). Soil available P was extracted at pH 8.5, using a 0.5 M sodium bicarbonate ($NaHCO_3$) solution and phosphorus was measured using the ascorbic acid blue colour method (18, 19).

Soil aggregation

The water stability of soil aggregate was determined by the wet sieving method as proposed by Elliott (20). The soil samples were placed on a 2 mm sieve and submerged in pure water for five minutes to pre-wet the aggregates. After pre-wetting, the aggregates on the 2 mm sieve were kept submerged under 1 cm of water and the sieve was stacked on top of 0.25 mm and 0.053 mm sieves. After that, the sieve stack was manually raised and lowered 50 times in a 2-min period within a 3 cm water column. The fractions left on each sieve were gathered and dried for 24 hours at 40°C in an oven. The following four water-stable aggregate size fractions were separated: (i) 2-5 mm (large macro-aggregates), (ii) 0.25-2 mm (small macro-aggregates), (iii) 0.053-0.25 mm (micro-aggregates) and (iv) < 0.053 mm fractions. The aggregate stability indicated by mean weight diameter (MWD) was calculated as per the Equation 1 formula:

$$MWD = \sum_{i=1}^{n} X_i W_i \qquad \text{.......(Eqn.1)}$$

X_i is the mean diameter of each aggregate fraction, W_i is the mass proportion of the aggregate fraction remaining on each sieve and n is the number of fractions.

Soil organic carbon (SOC) and microbial biomass carbon (MBC)

Soil organic carbon was quantified using the standard procedure proposed (21). Shortly, air-dried soil samples (about 2 g) were oxidized with potassium dichromate and sulfuric acid solution, with the leftover chromic acid solution titrated with ferrous sulfate to determine SOC content. The chloroform fumigation-extraction (CFE) method was used to estimate MBC (22). At first, a 10 g soil sample was split. One part was fumigated in dark conditions (24 hrs at 25 °C) with ethanol-free chloroform. The remaining non-fumigated soil portion was extracted using potassium sulfate solution (20 mL of 0.5 M) and then shaken for 30 min (180 rev. min^{-1}). The same extraction procedure was followed for fumigation. The SOC levels in fumigated and non-fumigated extracts were measured by following the oxidation procedure (21). MBC was calculated based on SOC differences between fumigated and non-fumigated extracts (22).

Iron (Fe) oxides

The determination of free iron oxide (Fe_{DCB}) was carried out using the Dithionite Citrate Bicarbonate procedure (DCB) (23). Amorphous or oxalate extractable iron (Fe_O) was extracted using an ammonium oxalate (0.2M) under acidic conditions at pH 3.0, maintaining a soil: solution ratio of 1:50. The samples were placed in a dark environment and shaken in a reciprocating shaker for 4 hrs (24, 25). The organically bound Fe (FeO) oxide extraction was conducted by sodium-pyrophosphate at pH 8.5 with 2 hrs of shaking (24). The extractants' Iron (Fe) concentration was analyzed using an AAS (AAS, Shimadzu model AA-7000, Tokyo, Japan).

Glomalin-related soil protein (GRSP)

The soils' GRSP content was obtained using a 50 mM citrate solution, following the method outlined by Wright and Upadhyaya (8). The Bradford dye-binding assay measured the extracted protein, with bovine serum albumin as the standard. Briefly, 0.25g of air-dried soil, measuring < 2 mm in size, was mixed with 2 mL of a 50 mM sodium citrate solution (at pH 8.0) and subjected to autoclaving at 121°C for 90 min. Following autoclaving, soil particles were removed by centrifugation at 10000 g for 10 min. This process was repeated five times until a clear, colourless supernatant was acquired. After extraction, the supernatants were combined and stored at 4°C for subsequent analyses.

Statistical analysis

The statistical analyses were conducted using SPSS 13. The effects of biochar addition on MWD, SOC, GRSP, various Fe oxides and soil nutrients were investigated using one-way ANOVA. The treatment mean (n = 3) was compared using the least significant difference (LSD at $P < 0.05$) analysis. The Origin 16.0 software (OriginLab, USA) performed linear regression to investigate the association between MWD and other parameters (SOC, MBC, GRSP and poorly crystalline Fe oxides).

Results

Soil aggregation

The impacts of biochar and inorganic fertilizer application on aggregate size distribution are displayed in Fig. 1. The significant fraction of aggregates was < 0.053 mm (~48 % of total mass). In contrast, the lowest fraction was 2–4 mm aggregate (~11 % of total mass) among the treatments (Fig. 1). Biochar application significantly increased the 2– 4 mm aggregates in the expanse of < 0.053 mm aggregates compared to the control treatments (P < 0.05). Large macroaggregates (2– 4 mm) were improved to 10, 11 and 14 % in the T_2, T_3 and T_4 biochar treatments, respectively, upon biochar incorporation compared to control (5 %) (P < 0.05). Biochar and inorganic fertilizer applications did not impact microaggregate formation, while biochar applications reduced the silt and clay size aggregates compared to the control (P > 0.05). The MWD was increased significantly with inorganic fertilization and biochar incorporation compared to the control (Fig. 2; P < 0.01). The biochar application in the T_2, T_3 and T_4 treatments significantly increased the MWD by 53, 75 and 103 % compared to control (P < 0.01). There was no significant impact of inorganic fertilization (TSP at 2 t ha^{-1}) and lower rates (4 and 8 t ha^{-1}) of biochar application were observed (P > 0.05).

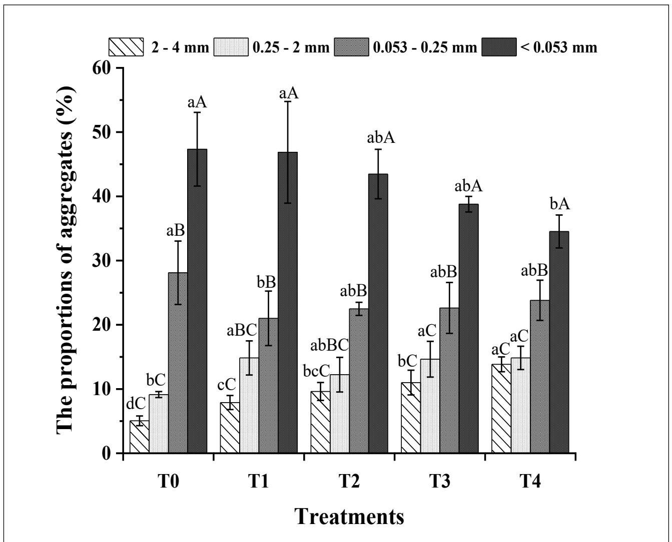

Fig. 1. The proportions of soil aggregates after biochar, inorganic fertilizer and control treatments. The vertical bars represent the standard deviation of three replicates (n = 3). Lowercase letters denote significant differences at P < 0.05 among the treatments for each aggregate size. Different capital letters indicate significant differences at P < 0.05 under the same treatments among the different aggregate size fractions. The **T0**, **T1**, **T2**, **T3** and **T4** denote the control, inorganic fertilizer (TSP) at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, and 16 t ha^{-1} treatments.

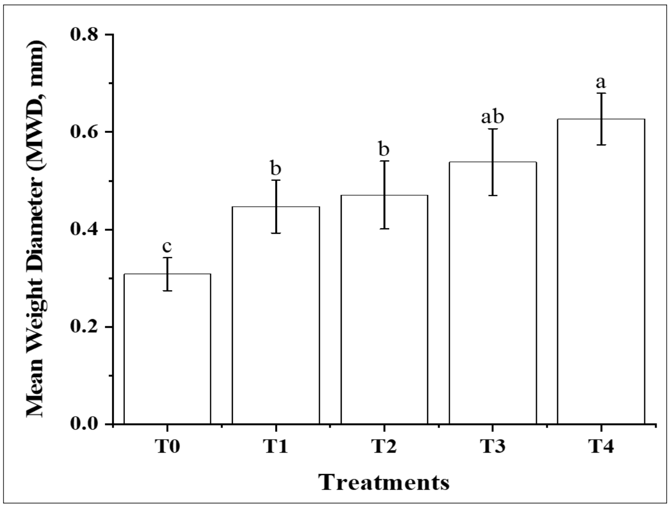

Fig. 2. The water stability of soil aggregates after biochar, inorganic fertilizer and control treatments. The vertical bars represent the standard deviation of three replicates (**n** = 3). Different lowercase letters denote significant differences at P < 0.01 among the treatments. The **T0**, **T1**, **T2**, **T3** and **T4** represent the control inorganic fertilizer (TSP) at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, and 16 t ha^{-1} treatments.

SOC concentration

The SOC content under different rates of biochar and inorganic fertilizer incorporation is listed in Table 2. The SOC contents were increased with increasing the biochar application rates in the order of $T_0 < T_2 < T_1 < T_3 < T_4$. After imposing the biochar (T_2, T_3 and T_4) and inorganic fertilizer (T_1) application, the SOC contents were increased by 25, 21, 29 and 38 % in the T_1, T_2, T_3 and T_4 treatments, respectively, compared to the control treatments (P < 0.001). The distribution of SOC in the soil aggregate size fraction is displayed in Fig. 3. In each treatment, SOC content was increased with increasing aggregate size (P < 0.05). Applying biochar significantly increased the SOC content in aggregates > 2 mm compared to the control. Specifically, the SOC levels in the > 2mm aggregates exhibited a marked increase of 47 % in the T_3 and T_4 biochar treatments. Conversely, applying biochar incorporation did not result in notable changes in SOC content within aggregate fractions < 0.25 mm. Finally, we found a strongly significant and linear relationship between the MWD and SOC (Fig. 4A; P < 0.001; R^2 = 0.62).

MBC and GRSP content

Soil MBC and GRSP were increased compared to the control after adding all the biochar treatments (Table 2). MBC was enhanced by 4.5 times, while GRSP was increased by 1.23 times higher after 16 t ha^{-1} (T_4) biochar addition (P < 0.001). On the other hand, there were no impacts of inorganic fertilizer, 4 and 8 t ha^{-1} biochar treatments on

Table 2. SOC, MBC and GRSP after four months of biochar and inorganic fertilizer application.

Soil Properties	Treatments				
	T_0	T_1	T_2	T_3	T_4
SOC (g/kg)	9.75±0.19c	12.23±0.19b	11.79±0.22b	12.55±1.27ab	13.44±0.29a
MBC (g/kg)	0.02±0.01b	0.05±0.03ab	0.05±0.03ab	0.07±0.02ab	0.09±0.04a
GRSP (g/kg)	1.46±0.15b	1.36±0.25b	1.33±0.27b	1.72±0.21ab	1.81±0.28a

Values are means ± SD (standard deviation), **n** = 3. Lowercase letters in the row indicate significant differences among treatments (P < 0.001). The T_0, T_1, T_2, T_3 and T_4 denote the control, inorganic fertilizer at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, 16 t ha^{-1} treatments, respectively.

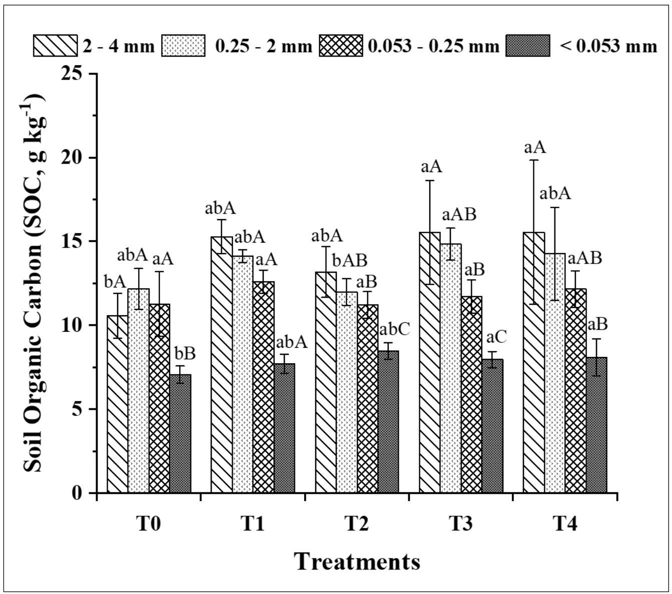

Fig. 3. The distribution of SOC in the four aggregate size fractions of control, inorganic fertilizer and different rates of biochar input. The vertical bar represents the standard deviation of three replicates (**n** = 3). Lowercase letters denote significant differences at P < 0.05 among the treatments for each aggregate size. Different capital letters indicate significant differences at P < 0.05 under the same treatments among the different aggregate size fractions. The $\mathbf{T_0}$, $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$ and $\mathbf{T_4}$ represent the control inorganic fertilizer (TSP) at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, and 16 t ha^{-1} treatments.

MBC and GRSP ($P > 0.05$). A significant positive relationship between the MWD and GRSP (Fig. 4B; $P < 0.05$; $R^2 = 0.29$) and the MWD and MBC (Fig. 4(D); $P < 0.05$; $R^2 = 0.41$) were observed.

Soil nutrient content

The study found that adding biochar and inorganic fertilizer enhanced the nutrient content compared to the control but was insignificant, except for available Ca and Mg (Table 3). Biochar increased available Mg content by 109 % in T_2 and T_4 treatments relative to the control (Table 3). The available Ca content was reduced by 1.4, 1.5, 1.4 and 1.5-fold upon T1, T_2, T_3 and T_4 treatments, respectively ($P < 0.05$). The contents of available S and total N showed an increase in the order of $T_0 < T_2 < T_4 < T_1 < T_3$, but no significant difference was observed among the treatments ($P > 0.05$).

Fe oxide content

Biochar treatments did not significantly impact different iron oxides and their ratio ($P > 0.05$; Table 4). The biochar application significantly increased the concentration of FeO except for T_4. Furthermore, the relationship between MWD and Fe_O oxides was non-significant (Fig. 4C); $P > 0.05$).

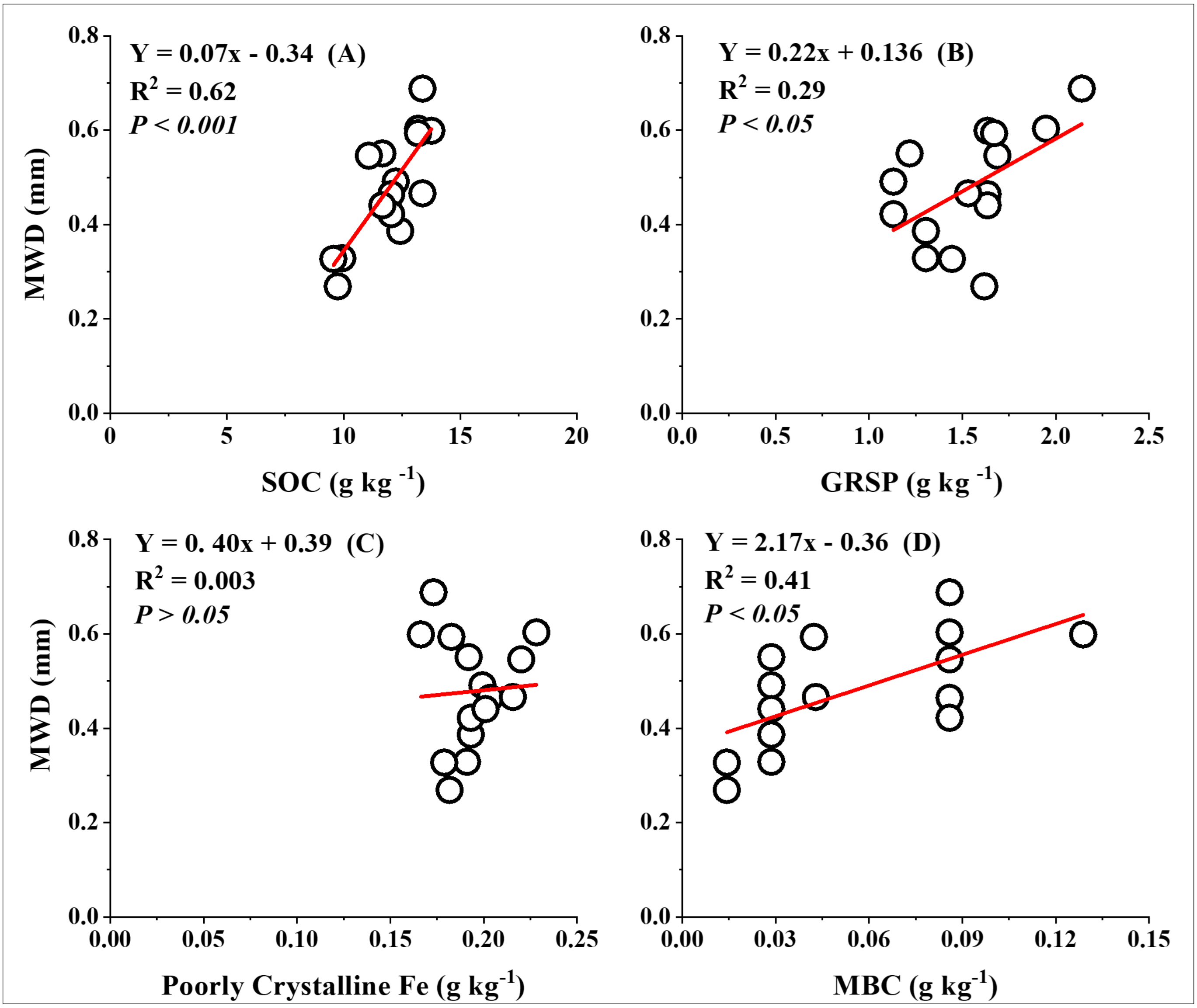

Fig. 4. The relationship between MWD and SOC, MWD and GRSP, MWD and poorly Crystalline Fe oxides and MWD and MBC.

Table 3. Changes in soil nutrient content upon different biochar and inorganic fertilizer application rates.

Nutrients (g/kg)	Treatments				
	T_0	T_1	T_2	T_3	T_4
Available Na	0.57±0.14a	0.90±0.14a	0.82±0.14a	0.90±0.14a	0.82±0.14a
Available K	0.14±0.01b	0.18±0.01a	0.16±0.01ab	0.18±0.01a	0.16±0.01ab
Available Ca	4.91±0.78a	3.45±0.10b	3.28±0.08b	3.45±0.10b	3.28±0.08b
Available Mg	0.98±0.02b	1.45±0.30ab	2.06±0.35a	1.45±0.30ab	2.06±0.35a
Available S	0.21±0.03a	0.32±0.05a	0.31±0.03a	0.32±0.05a	0.31±0.03a
Available P	0.25±0.03a	0.13±0.09a	0.20±0.01a	0.13±0.09a	0.20±0.01a
Total N	0.56±0.03a	0.70±0.10a	0.62±0.07a	0.70±0.10a	0.70±0.07a

. Values are means ± SD (standard deviation), n = 3. Lowercase letters in the row indicate significant differences among treatments (P < 0.05). The $\mathbf{T_0}$, $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$ and $\mathbf{T_4}$ denote the control, inorganic fertilizer at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, 16 t ha^{-1} treatments, respectively.

Table 4. Extractable Fe-oxides in the soil under biochar, inorganic fertilizer and control treatments.

Different Fe oxides	Treatments				
	T_0	T_1	T_2	T_3	T_4
Fe_{DCB} (g/kg)	2.98±0.05a	2.83±0.21a	2.86±0.17a	2.71±0.11a	2.89±0.15a
Fe_O (g/kg)	0.18±0.01b	0.20±0.01a	0.20±0.01a	0.22±0.01a	0.17±0.01b
Fe_P (g/kg)	0.05±0.00a	0.05±0.00a	0.05±0.00a	0.05±0.00a	0.05±0.00a
Fe_O / Fe_{DCB}	0.06±0.00bc	0.07±0.00b	0.06±0.00bc	0.08±0.00a	0.06±0.00c

. Values are means ± SD (standard deviation), **n** = 3. Lowercase letters in the row indicate significant differences among treatments (P < 0.05). The $\mathbf{T_0}$, $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_3}$ and $\mathbf{T_4}$ denote the control, inorganic fertilizer at 2 t ha^{-1}, biochar incorporation at 4 t ha^{-1}, 8 t ha^{-1}, 16 t ha^{-1} treatments, respectively.

Discussion

Effects of biochar on SOC and aggregation

In the current short-term field study, we observed that adding biochar led to a significant increase in macroaggregates, SOC and MWD compared to control. (P < 0.001). We also found a substantial and positive relationship between MWD and SOC, which indicates a strong influence of SOC on aggregate stability in the current study. The positive influence of biochar incorporation on SOC and MWD agreed with study reports that biochar application increased aggregation by 16.4 ± 2.5 % compared to control regardless of biochar properties, soil and experimental conditions (4, 26). Research indicates that biochar application at 16 t ha^{-1} significantly increased the SOC and MWD but not below 8 t ha^{-1} (26). The proportion of macroaggregate enhanced upon biochar input from 10 to 40 t ha^{-1} (27). Our current study found that the proportion of macro-aggregates and MWD was increased with biochar application from 4 to 16 t ha^{-1}. The impact of biochar addition on aggregation may depend on biochar properties and pyrolysis temperature (4). For exam-ple, wood biochar with higher pyrolysis temperature (> 600 °C) has higher favourable impacts on the aggregation than other plant-derived biochar (cereal straw, grain residue, manure). It has been widely suggested that aggregation is increased with the improvement of SOC, which might be due to the proliferation of microbial activity upon biochar incorporation (28). Biochar promotes microbial activity, which produces mucilage and hyphae, leading to the connection of micro-aggregates to macro-aggregates in the interface between soil particles and Biochar (28). Research indicates that extracellular polysaccharides produced upon microbial degradation of biochar are glue-like materials that have the potential to bind soil particles together (29). Moreover, research showed that the higher water stability of soil aggregates in polysaccharide treatments is due to the enmeshments of soil particles (30). In general, Biochar possesses a relatively higher C/N ratio, which is a favourable condition for the growth of fungi (31). Fungal hyphae and plant roots can intertwine with soil particles, potentially leading to increased MWD (32). The unique features of biochar include a highly porous structure, greater cation exchange capacity and specific surface area, which can facilitate aggregation by absorbing different minerals and OM with different molecular sizes and chemical properties (33). We also found that the aggregate fractions of 2-4 mm and 0.25-2 mm contained a higher proportion of organic carbon compared to the < 0.25 mm fractions (Fig. 3), which aligns with previous studies (34, 35). Applying stable ^{13}C isotope labelling techniques, macro-aggregates tend to accumulate newly added ^{13}C more than micro-aggregates (36). The more significant carbon accumulation in the macro-aggregates is due to the larger pores in macro-aggregates. In contrast, the lower carbon content in the micro-aggregates results from a greater surface area to volume ratio and more C loss during wet sieving due to shorter transfer pathways (35). The interaction of micro-aggregates through the cementing action of organic matter results in the formation of macro-aggregates, accumulating more carbon content in macro-aggregates (32).

GRSP and aggregation

In the current study, we found that applying biochar increased the soil GRSP (Table 2), in agreement with the findings of various studies (36,37, 38). The biochar significantly increased the GRSP synthesis, while Yuan *et al.* (38) found that biochar increase the GRSP coupling with soil carbon sequestration. In the current study, biochar incorporation increased the MBC (Table 2) by supplying the nutrients and carbon for the soil microbial community, which may stimulate fungal activity, growth and GRSP production (36, 37). Biochar application increased the abundance of a specialized group of GRSP-producing fungi (arbuscular mycorrhiza) in soil, thus increasing GRSP production (38). Arbuscular my-corrhiza fungi degrade the complex chemical structure of biochar, which is rich with aromatic C and lignin, resulting in higher GRSP synthesis (39). Arbuscular mycorrhiza hyphae contribute to increased GRSP storage in soil. The main processes for GRSP buildup in soil involve the turnover of arbuscular mycorrhiza hyphae, with GRSP being released from the decaying mycelium (7). Arbuscular mycorrhiza hyphae have a rapid turnover rate, with a calculated half-life of 5-6 days, resulting in higher GRSP accumulation in the soil (16). Furthermore, we found a positive relationship between GRSP content and aggregate formation (Fig. 4B); $P < 0.05$; $R^2 = 0.29$). Previous research findings have reported a positive relationship between GRSP and aggregation (40, 41). GRSP, produced from arbuscular mycorrhiza hyphae, is an essential binding material for soil particles together into larger aggregates, thus enhancing the MWD (8, 42). GRSP has been shown to increase water-stable aggregates, which are more erosion-resistant and contribute to improved soil quality (43). The hydrophobic properties of glomalin contribute to forming water-resistant soil aggregates (44). GRSP is a novel bioflocculant enriched with essential elements such as Fe (Fe^{2+} and Fe^{3+}), Ca^{2+}, Mg^{2+}, K^{+}, Zn^{2+}, Cu^{2+} and Mn^{2+} (16). These cations enhance biogeochemical processes by promoting soil particle flocculation (45). Furthermore, Fe and GRSP participate in flocculation with soil particles depending on the charge, absorption and bridging mechanism between GRSP and soil mineral particles (46). The mechanism through which GRSP bridges particles is influenced by its variety of functional groups and higher molecular weight, which create multiple sites for binding during flocculation and aggregation (47). GRSP includes the carboxyl (–COO–), amide (–CO –NH), hydroxyl (–OH), carbonyl (C– O–O-) and primary amine (–NH_2) groups (46). These specific functional groups play a crucial role in linking mineral particles, facilitating the formation of larger clusters during the aggregation process (47). GRSP binds the soil minerals to micro-aggregates (diameter < 0.25 mm) and then stable macro-aggregates by following the aggregates hierarchy (32).

Fe oxides and aggregation

In our current study, biochar incorporation did not significantly impact iron oxides ($P > 0.05$; Table 4). Consequently, we did not observe a significant relationship between amorphous iron oxide and MWD ($P > 0.05$;), which contradicts the findings of research (Fig. 4C) (48). A positive relationship between amorphous iron oxide and MWD ($r = 0.67$; $P < 0.05$) and the organic amendment incorporation promotes the conversion of crystalline Fe to amorphous Fe oxides and enhances soil aggregation (48). The soil mineralogy in the current investigation was dominated by illite and montmorillonite, which might be the reason for the absence of any correlation between amorphous iron oxides and aggregate stability (49).

Conclusion

Applying biochar in the clay soil significantly increased soil macroaggregate formation, thus promoting the stability of soil aggregates. Moreover, biochar at 16 t ha^{-1} significantly enhanced the current investigations' SOC, MBC and GRSP significantly. The biochar incorporation enhanced the SOC by boosting soil microbial activity, which plays a key role in soil aggregation, followed by fungal synthesis GRSP. On the other hand, Fe oxides had no impact on soil structure formation and SOC stock in the soil of southwestern Bangladesh. The findings demonstrate that biochar application at 16 ton/ha significantly promote soil aggregtaion and soil organic carbon stock boosting microbial activity in the clay soils of Bangladesh.

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Authors' contributions

MH and JC designed the work. TR and SJ performed the laboratory work. MU helped in data analysis. SN assisted in the laboratory work alongside TR and SJ, wrote the original draft, and MH reviewed and edited the original draft. All the authors reviewed and approved the submitted version of the article.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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