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Evaluating short-term effects of rice straw management on carbon fractions, composition and stability of soil aggregates in an acidic red soil with a vegetable planting history

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A R T I C L E I N F O

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A B S T R A C T

Red soils are characterised by acidic pH and limitations in carbon, nitrogen, water, and soil structure. To overcome such limitations, improved soil aggregation is the key to improving the physical and chemical properties of soil. Applying organic amendments such as straw can lead to corresponding soil aggregation and stability changes. Therefore, we explored the short-term effects of rice straw amendment, either alone or in combination with biochar, on improving the carbon fractions, stability, and composition of soil aggregates in red soil with a history of vegetable planting. The study consisted of four treatments: control (no organic material, CK), biochar alone (5% homemade straw biochar, B), straw alone (12% rice straw, S), and biochar with straw (5% homemade straw biochar + 12% rice straw, BS). Our results showed that equal amounts of straw and biochar substantially reduced the number of mechanically stable aggregates (*MSA),* mean weight diameter (*MWD*), and geometric mean diameter (*GMD*) of the soil. BS treatment reduced >0.25 mm aggregate content ($R_{0.25}$), *MWD* and *GMD* by 24.06%, 56.81%, and 62.19%, respectively, compared with that of the control. The addition of straw greatly enhanced the water-stable macromolecular content and stability coefficient of the soil, but treatment B had no obvious effect. The S treatment had the greatest effect on $R_{0.25}$, *MWD* and *GMD*, increasing them by 143.94%, 246.67%, and 181.82%, respectively, compared with that of the control. Soil organic carbon (*SOC*) was significantly increased by straw addition and carbonisation treatment, and the effect of the BS treatment was the best, with an increase of 325.63% compared with that of the control. The organic carbon content in the aggregates of different particle sizes treated with different organic materials also increased significantly. In the soil reactive organic carbon fraction, applying biochar alone did not affect microbial biomass carbon (*MBC*), dissolved organic carbon (*DOC*), or easily oxidized organic carbon (*EOC*) but could increase the particulate organic carbon (*POC*) content. All the treatments with straw application significantly increased the *MBC*, *DOC*, *EOC*, and *POC* content, and the highest effect was obtained by applying both straw and biochar in an integrated form, i.e., the BS treatment. In conclusion, the co-application of biochar

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and straw sequestered more carbon and revamped soil C pools than either biochar or straw alone and could be a promising option for the sustainable use of red soils to ameliorate the aforementioned limitations associated with this soil type.

1. Introduction

In the genetic soil classification of China, red soil belongs to the red soils great group, red soil subgroup, clayey red earth family, and red yellow earth with clayey bottom species, where the subgroups in soil taxonomy are plinthudults [1]. Red soil is one of the most important agricultural soils in China and is typically characterised by an acidic pH, low water-holding capacity, deficient nutrients, low organic matter, and poor soil aggregation. Such limitations adversely affect crop performance, owing to the limitations associated with soil composition regarding aggregation and carbon fractions. Aggregates, the basic structural components of soil, have large (>0.25 mm) and microaggregates (<0.25 mm), the two categories of soil aggregates. The role of aggregates of different particle sizes in retaining soil moisture and storing and supplying nutrients varies, and their distribution number and spatial arrangement determine the distribution and continuity of the soil pore space, which in turn becomes a key factor in determining soil quality [2,3]. Evaluation of soil structural stability usually relies on indicators such as >0.25 mm aggregate content ($R_{0.25}$), mean weight diameter (*MWD*), and geometric mean diameter (*GMD*) [4]. In contrast, the percentage of aggregate destruction (*PAD*) reflects the degree of soil structure degradation and erosion resistance [5].

Soil organic carbon (*SOC*) fractions are indicators of soil nutrient conservation and crop productivity and interact with aggregates to promote the stabilisation of the aggregate structure [6,7]. The composition of organic carbon (*OC*) in agricultural soils is spatially heterogeneous, complex, and dynamic [8]. The variation in *SOC* with fertilisation practices can be attributed to soil conditions [9]. Dissolved *OC* (*DOC*), microbial biomass C (*MBC*), easily oxidisable *OC* (*EOC*), and particulate *OC* (*POC*) are important indicators of *SOC* stocks under different soil management conditions [10]. The soil carbon fraction can regulate soil nutrient cycling by regulating soil microbial diversity, maintaining and enhancing soil carbon and nitrogen contents, and reducing fertiliser application and surface pollution [11].

Crop straw return is widely used as a viable soil management practice to improve *SOC* stocks in intensive agroecosystems, soil C sequestration, and soil stability. However, it has limitations because it can cause physiological damage to crops and stimulate more nitrous oxide (N_2O) emissions from the soil, thus negatively affecting the atmosphere [12,13]. The carbonisation of agricultural waste is the focus of biochar application in agriculture, and incorporating crop residues represents one of the vital aspects of sustainable development through recycling [14,15]. Biomass carbon is a kind of organic material with a large specific surface area, high porosity, high thermal stability, and rich C content formed by pyrolysis of biomass (such as plant residues, straw, manure, and wood chips) under high temperatures (i.e. 100–1000 °C) and anaerobic conditions [8]. The study showed that applying biochar induced soil aggregation, increased soil aggregate stability, and significantly increased *SOC* content by 11.47%–24.65% [16]. In addition, rice straw applied to the soil after carbonisation can improve soil quality and fertility by enhancing soil aeration, water-holding capacity, nutrient availability, and the activity of beneficial microorganisms and extracellular enzymes as soil conditioners [17].

Red soil is a common soil type in southern China, found mainly in the tropical and subtropical regions of China. It is one of the important agricultural production areas, covering 102 million hectares [18]. Although red soil has high production potential, it has weaknesses such as acidification, nutrient scarcity, low organic matter content, poor soil structural stability, credibility, and insufficient nutrient supply because of the spatial and temporal unevenness of precipitation in the distribution area and irrational human exploitation, especially the significant reduction in soil C content, resulting in a serious decline in productivity and crop quality in red soil areas [19,20]. Previous research has shown that biochar is alkaline and improves soil pH when applied to strongly acidic red soils [21]. Despite the well-documented benefits of biomass charcoal in improving acidic soils, few studies have analysed the interrelationships between aggregate stabilisation and *OC* composition of red soil with a vegetable planting history. To address these issues, we propose rice straw and biochar amendments: i) as important *OC* sources to supplement carbon fractions, ii) to maintain the accumulation of *OC* fractions in acidic red soils, and iii) to contribute to aggregate formation dynamics. In the context of looming red soil quality issues, information on aggregate stability and carbon stocks is important for developing strategies to improve soil structure and transform soil carbon pools by increasing aggregate stability and carbon stocks. Therefore, we cultivated red loam soil under constant temperature and humidity and used dry and wet sieves to determine the content of aggregates and the *OC* content, respectively, at different grain levels to reveal the stability of red loam aggregates and the distribution of *OC* at different grain levels in response to the addition of straw and its carbonisation. The aim of the study was to provide a theoretical basis for the sustainable development of acidic red loam soils and their resistance to erosion.

2. Materials and methods

2.1. Experimental materials

The tested red soil, which was used for vegetable cultivation for 12 months, was collected from the vegetable base (25°31′ N, 114°92′ E) of Wanxing Village (National Vegetable Quality Standard Center), Datangbu Town, Xinfeng County, Jiangxi Province, China. Soil was collected from the topsoil (0–20 cm). After air-drying, all impurities were removed, and the soil was sieved through a 2 mm sieve. The basic physical and chemical properties of the soil were as follows: pH 5.43, OC 12.60 g kg^{-1}, total N 3.24 g kg^{-1}, alkaline hydrolysable N 86.32 mg kg^{-1}, available K 51.20 mg kg^{-1}, and available P 10.93 mg kg^{-1}.

The rice straw used in this study was made from local late rice season straw, dried at 80 °C, and then crushed. Some of the straw used for crushing was taken as carbonised material and fired in a muffle furnace (SX-4-10 type box type resistance furnace; Tianjin Teste Instruments Co, Tianjin, China) at 500 °C under high-temperature anaerobic conditions for 2 h, then passed through a 60-mesh sieve and set aside. The carbonisation rate of the rice straw under these conditions was 42%. Carbonation rate data were obtained from indoor experiments, averaged after 30 repetitions, and analysed and calculated using Excel 2019.

The physicochemical characteristics of rice straw and its biochar were pH 6.68 and 9.92, organic carbon 340.05 g kg^{-1} and 325.82 g kg^{-1}, total nitrogen 4.61 g kg^{-1} and 3.13 g kg^{-1}, and effective phosphorus 2.33 g kg^{-1} and 2.13 g kg^{-1}. Nutrient data were obtained from indoor experiments with three replicates for both straw and biochar determinations, and the data were analysed using Excel 2019.

2.2. Design of experiment

On January 21, 2021, four treatments were set up in this experiment: CK (no material added), B (5% soil mass of biochar added), S (equal mass of straw added, according to a carbonisation rate of 42.0%, i.e. 12.0% soil mass of straw), and BS (cumulatively applied biochar and straw); each treatment was repeated four times. Each 1 L plastic bucket was filled with 500.00 g of air-dried soil, after which soil samples were mixed with different organic materials according to the experimental set ratio, mixed, and filled into buckets with distilled water at 70%–80% of the maximum water holding capacity in the field. The field water capacity of the soil was measured using the method described by Wu et al. [22]. A rubber tube with a clamp was connected to the lower end of the funnel, which was blocked with glass fibre. Subsequently, 50.00 g of soil was added into a funnel, the rubber tube was clamped, 50 mL of distilled water was added, and after 30 min, the clamp was opened to allow excess water to flow into the measuring cylinder. After 30 min, the amount of water flowing out and the soil moisture were measured. The plate was then closed using a plastic film with breathable filter paper in the middle and placed in an incubator. The soil was incubated at (25 ± 1) °C and 80% RH for 6 months, and the water content was kept constant during the incubation period by regularly replenishing the soil water using the weighing method every 7 days.

2.3. Soil sample collection

After 6 months of cultivation, we collected soil samples by destructive sampling, poured out each pot, and collected soil samples by quartering the content [23]. The soil was broken into 10 mm fragments along the natural fracture surface to eliminate the impact of mechanical stress. Then, part of the soil was dried and passed through a 2 mm sieve to determine the aggregate quantity, stability, and *SOC* content of each particle size. The *EOC* content was measured using a 0.25 mm sieve, and the *POC* content was measured using a 0.053 mm sieve. The other part of the soil sample was broken and separated by hand, passed through a 2 mm sieve, and maintained at 4 °C for the determination of *DOC* and *MBC* content.

2.4. Measurement indexes and laboratory methods

2.4.1. Determination of soil aggregates

The number of mechanically stable aggregates *(MSA)* and water-stable aggregates *(WSA)* in each grain level of air-dried soil were determined using dry and wet sieve methods [24].

Dry sieve method: 500 g of air-dried soil was weighed and placed on the top of the sieve with apertures of 2, 1, 0.5, and 0.25 mm. The sieve was covered and shaken to collect the aggregates on the sieve of each aperture, weighed, and set aside.

Wet sieve method: According to the percentage content of aggregates at each level obtained by the dry sieve method, the air-dried samples were mixed into 100 g of soil samples according to the proportion of each particle size, placed on a sieve with 2, 1, 0.5, 0.25 mm pore size, and analysed using the soil aggregate structure analyser (TPF-100, Zhejiang Topunnong Technology Co., Ltd, Zhejiang, China) after 15 min of wetting. After the end of the aggregates on the sieve at all levels were washed into the aluminium box, clarified, the supernatant removed, and dried to constant weight at the oven temperature of 50 °C and weighed.

2.4.2. Determination of soil organic carbon fraction and carbon content of each particle size aggregate

SOC in the soil and aggregates was determined using the oxidation-volumetric method using potassium dichromate heated to high temperatures [25]. *EOC* was measured using the 333 mmol L^{-1} $KMnO_4$ digestion method [26]. *MBC* was determined using chloroform fumigation - 0.5 mol L^{-1} K_2SO_4 leaching method [27]: chloroform-fumigated and unfumigated soil samples were leached with 0.5 mol L^{-1} K_2SO_4 solution, and then the carbon content in the leachate was determined using a *TOC* analyser, and the difference in organic carbon content of the two leachates could be calculated to obtain the content of soil *MBC*. Determination of *DOC* in soil according to the method of McGill et al. [28] was calculated using the following formula: $DOC(\%) = (B - S) \times 0.01 \times 0.003 \times 100/10$, where B and S are the amounts (mL) of 0.01 *N* ferrous ammonium sulphate for titration in blank and soil samples, respectively. *POC* content was determined using Cambardella's method [29]. Natural air-dried soil (or soil aggregates of each grain size separated by dry sieving) was ground and passed through a 100-mesh sieve. The *OC* content of aggregates of different grain sizes was determined using the potassium dichromate volumetric method [30].

2.4.3. Soil aggregates stability evaluation index

Aggregate stability was described using the mean weight diameter (*MWD*), geometric mean diameter (*GMD*), aggregate content greater than 0.25 mm ($R_{0.25}$), percentage of aggregate destruction (*PAD*), unstable aggregate index (E_{LT}), and fractal dimension (D) [31,32]. The calculation formulae are as follows:

$$MWD = \sum_{i=1}^{n} (\overline{x_i} w_i)$$

where $\overline{x_i}$ denotes the aggregate average diameter at the i th size (mm), and W_i is the quality of the level i aggregates,

$$GMD = Exp\left[\frac{\sum_{i=1}^{n} m_i \ln \overline{x}_i}{\sum_{i=1}^{n} m_i}\right]$$

where *GMD* is the weight of soil aggregates with different particle sizes,

$$R_{0.25} = M_{T>0.25} / M_T$$

where M_T is the total weight of the aggregates, and $M_{T>0.25}$ is the mass of aggregates with a particle size of >0.25 mm,

$$PAD(\%) = \left(W_{dry} - W_{wet}\right) / W_{dry} \times 100$$

where W_{dry} and W_{wet} are the mass percentages of aggregates in dry and wet sieves of >0.25 mm particle size, respectively,

$$E_{LT}(\%) = (W_T - W_{0.25}) / W_T \times 100$$

where W_T is the total weight of the test soil, and $W_{0.25}$ is the weight of *the WSA*, and

$$D = 3 - \frac{\lg\left[\frac{w(\delta < \overline{x_i})}{w_t}\right]}{\lg\left(\frac{\overline{x_i}}{x_{\max}}\right)}$$

where lg is a logarithmic function based on 10, W ($\delta < \overline{x_i}$) is the cumulative weight of soil particles with soil particle diameter $<\overline{x_i}$, w_t is the sum of the weight of soil particles, and $x_{\max}$ is the maximum average aggregate diameter.

2.4.4. Contribution of soil aggregates to soil organic carbon at each grain level

Contribution of aggregates to soil C (%) = [C content in aggregates at that grain level (g kg^{-1}) × aggregate content at that grain level (%)/soil C content in the cultivated layer] × 100.

2.5. Data analysis

The original values were recorded, and statistical analyses were performed using Excel 2019 and SPSS 24. All graphs were constructed using Origin 9.0. The least significant difference (LSD) method was used for multiple comparisons to determine significant differences between treatments.

Table 1
Effect of rice straw and its carbonisation on the content of aggregates at each grain level.

Method	Treatment	Aggregate size (mm)				
		>2	2.00–1.00	1.00–0.5	0.5–0.25	<0.25
Mechanical Stability Aggregates	CK	56.51 ± 3.23a	10.98 ± 3.62a	8.68 ± 4.33b	7.00 ± 3.27b	16.80 ± 6.40b
	B	18.66 ± 4.24b	14.71 ± 2.35a	18.36 ± 5.25a	15.53 ± 4.34a	32.74 ± 7.39a
	S	25.97 ± 5.84b	14.06 ± 3.63a	17.65 ± 2.86a	14.64 ± 2.16a	27.68 ± 3.40a
	BS	10.48 ± 2.03b	12.49 ± 1.83a	20.77 ± 1.44a	19.42 ± 0.53a	36.85 ± 1.54a
Water-Stable Aggregates	CK	0.05 ± 0.02c	3.86 ± 0.39b	10.69 ± 0.93b	13.94 ± 1.14a	71.46 ± 2.08a
	B	0.05 ± 0.02c	3.45 ± 0.25b	11.05 ± 1.30b	13.32 ± 3.16a	72.15 ± 2.77a
	S	25.08 ± 4.77a	12.48 ± 2.42a	18.04 ± 2.51a	15.02 ± 2.82a	29.39 ± 1.16c
	BS	9.69 ± 1.03b	10.86 ± 0.60a	16.50 ± 1.29a	12.75 ± 0.96a	50.25 ± 1.71b

Differences in *MSA* and *WSA* content of red soil between different treatments. Percentage of aggregate content at five-grain levels, different letters within a column indicates significant differences between different treatments at $P < 0.05$. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw.

3. Results

3.1. Soil aggregates and stability

3.1.1. Red soil aggregates

Among the soil *MSA*, adding straw and biochar reduced the soil macroaggregate content significantly. Different organic material treatments significantly reduced the content of *MSA* >2 mm particle size aggregates ($P < 0.05$) by 66.74%, 53.71%, and 81.32% in the B, S, and BS treatments, respectively, compared with CK (Table 1).

There was no significant difference in soil WSA between the B and CK treatments at any grain level ($P > 0.05$). The soil macroaggregate content significantly increased with the addition of straw, with the S treatment having the most prominent effect, followed by the BS treatment. The S and BS treatments increased the *WSA* > 2 mm agglomerate content by 500.60% and 192.80%, respectively, compared with CK. The S treatment improved soil macronutrients, the B treatment had no improvement effect on *WSA*, and the BS treatment had an intermediate effect.

3.1.2. Stability of red soil aggregates

The *MWD*, *GMD*, and $R_{0.25}$ of soil *MSA* decreased significantly after the addition of different organic materials (Table 2). Compared with CK, *MWD* of the B, S, and BS treatments decreased by 44.97%, 34.32%, and 56.81%; *GMD* decreased by 52.10%, 42.86%, and 62.19%; and $R_{0.25}$ decreased by 19.13%, 11.63%, and 24.06%, respectively. The D value of the soil *MSA* was significantly different after biochar application. Compared to CK, the *MSA* of the B and BS treatments increased by 5.70% and 6.58%, respectively. In addition, the differences in *MWD*, *GMD*, D, and $R_{0.25}$ of the B treatment in the soil *WSA* were not significant, while *MWD*, *GMD*, and $R_{0.25}$ of added straw treatments (S and BS) were significantly higher, and D values were significantly lower, with the S treatment having the best effect.

The addition of different organic materials significantly reduced soil *PAD*, with the B, S, and BS treatments decreasing *PAD* by 11.01%, 92.00%, and 67.85%, respectively, compared with CK (Table 3). The difference in E_{LT} was not significant for biochar application alone, whereas the addition of straw treatments produced significant differences in E_{LT}, with the S and BS treatments reducing E_{LT} by 57.49% and 29.75%, respectively. Overall, the best results were obtained with straw alone, whereas a combination of straw and biochar was superior to biochar alone.

3.2. Soil carbon fractions' concentrations

3.2.1. Total organic carbon

Significant differences in *SOC* content were observed between the treatments of added materials (Fig. 1A). The *SOC* content in the four treatments was ranked as follows: BS > S > B > CK. The maximum *SOC* content was 45.84 g kg^{-1} for BS treatment. Compared with the CK treatment, the *SOC* contents of the B, S, and BS treatments were significantly increased by 182.92%, 217.46%, and 325.63%, respectively. This indicates that the cumulative application of biochar and straw was more effective in increasing *SOC* content than straw and biochar alone.

Compared with CK, all treatments increased the soil carbon-to-nitrogen ratio (C/N) to different degrees. Treatment B had the highest soil C/N, with a significant increase of 152.35% compared with CK, followed by treatments BS and S (Fig. 1B). The soil C/N ratio did not differ significantly between treatments with straw addition. This indicates that the cumulative application of biochar and straw was more effective in improving *SOC* content than straw or biochar alone. However, the soil C/N ratio was the most significant for biochar alone.

3.2.2. Organic carbon content of aggregates

All treatments had the lowest *OC* content of *MSA* at the 1–2 mm aggregate grain level and the highest *OC* content at the <0.25 mm aggregate grain level when comparing different grain levels of the same treatment (Fig. 2A). The *OC* content in each treatment showed a decreasing trend and then gradually increased as the particle size of the soil aggregates decreased. Compared to the CK, the organic

Table 2
Effect of rice straw and its carbonation addition on the stability of soil aggregates.

Method	Treatment	MWD (mm)	GMD (mm)	D	$R_{0.25}$/%
Mechanical Stability Aggregates	CK	1.69 ± 0.27a	1.19 ± 0.31a	2.28 ± 0.11b	83.17 ± 6.43a
	B	0.93 ± 0.30c	0.57 ± 0.19b	2.41 ± 0.07a	67.26 ± 7.39bc
	S	1.11 ± 0.10b	0.68 ± 0.05b	2.35 ± 0.03 ab	73.50 ± 1.66b
	BS	0.73 ± 0.03c	0.45 ± 0.02b	2.43 ± 0.02a	63.16 ± 1.54c
Water-Stable Aggregates	CK	0.30 ± 0.01c	0.22 ± 0.01c	2.66 ± 0.01a	28.54 ± 2.08c
	B	0.29 ± 0.01c	0.22 ± 0.01c	2.66 ± 0.01a	27.85 ± 2.77c
	S	1.04 ± 0.08a	0.62 ± 0.05a	2.40 ± 0.01c	69.62 ± 1.10a
	BS	0.65 ± 0.03b	0.37 ± 0.02b	2.55 ± 0.01b	49.80 ± 1.44b

Differences in *MSA* and *WSA* content of red soil between treatments. *MWD*, *GMD*, D, and $R_{0.25}$ represent mean weight diameter, geometric mean diameter, fractal dimension, and >0.25 mm aggregate content, respectively. Different letters within a column indicate significant differences between different treatments at $P < 0.05$. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw.

Table 3
Effect of rice straw and its carbonation addition on aggregate destruction rate and unstable aggregate index.

Treatment	PAD (%)	E_{LT} (%)
CK	65.60 ± 2.57a	71.46 ± 2.08a
B	58.38 ± 4.70b	72.15 ± 2.77a
S	5.25 ± 2.35d	30.38 ± 1.10c
BS	21.09 ± 3.64c	50.20 ± 1.44b

Treatment S is more stable than all other treatments. Different letters within a column indicate significant differences between different treatments at $P < 0.05$. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw.

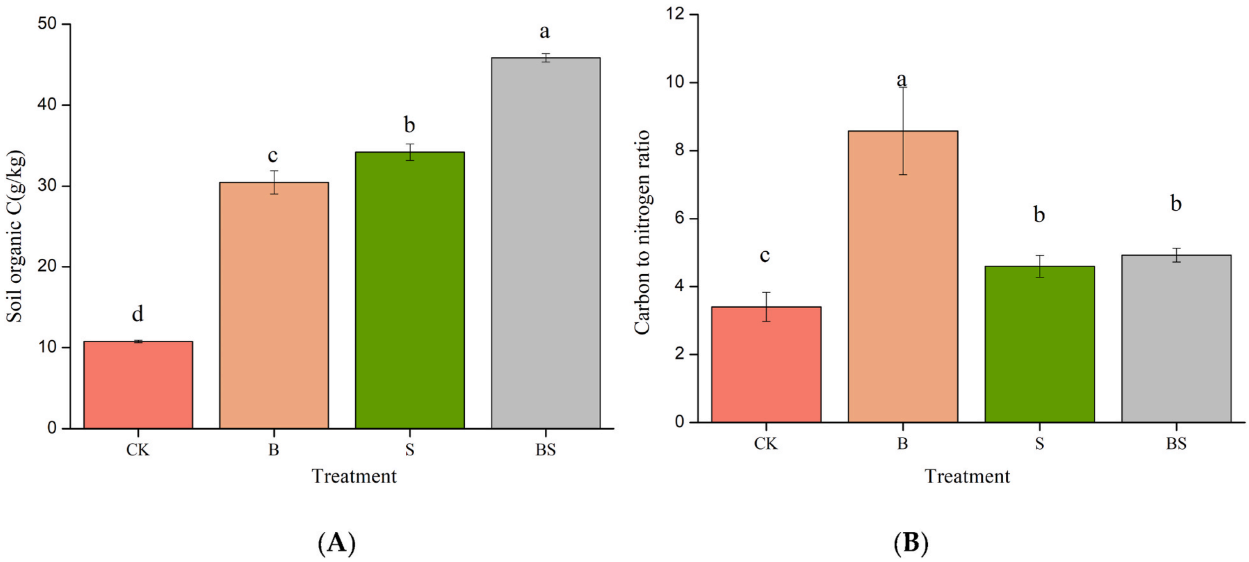


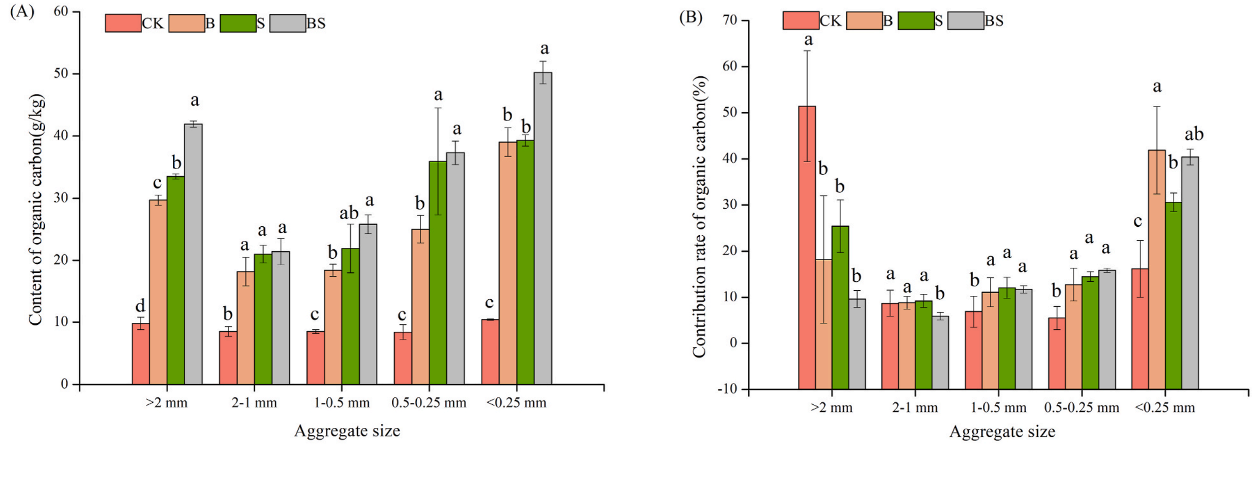
Fig. 1. Effect of rice straw and its carbonation addition on total organic carbon and carbon to nitrogen ratio in red loam soils. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw. Different lowercase letters indicate significant differences ($P < 0.05$) among the treatments.

material addition treatments differed significantly in *OC* content at all aggregate particle levels. The BS treatment was the most effective, followed by the S treatment. There was no significant difference in *OC* content at the particle level in 1–2 mm aggregates when comparing the three organic material addition treatments, whereas significant differences were found in all >2 mm aggregates, with the BS treatment increasing the level by 41.08% and 25.08% compared to the B and S treatments, respectively. The *OC* content of the B and S treatments did not differ significantly at the particle level of <0.25 mm aggregates, but both were significantly lower (by 22.31% and 21.71%, respectively) than the BS treatment.

This indicates that the addition of straw treatments (S and BS) increased the *OC* content in *MSA* of the soil at all grain levels, and the best performance was achieved by the co-application of biochar and straw. The *OC* content in the mechanically stable macroaggregates was significantly higher than that in the microaggregates for each treatment, and *OC* was mainly distributed in the macroaggregates.

3.2.3. Contribution of organic carbon from aggregates

The *OC* contribution of the different treatments decreased and then increased with decreasing soil grain size, with the lowest value reached in the 1–2 mm aggregates (Fig. 2B). The largest contribution of *OC* to *MSA* was made by the aggregates at the >2 mm and <0.25 mm particle size levels, while the aggregates at the 0.25–2 mm particle size level were less distributed. The *OC* contribution of <0.25 mm aggregates was the largest in the organic material addition treatment, ranging from 30.61% to 41.87%, whereas the *OC* contribution of >2 mm aggregates was the largest in the CK treatment (51.42 %). Adding organic material reduced the *OC* contribution in >2 mm aggregates significantly by 64.69%, 50.56%, and 81.34% in the B, S, and BS treatment aggregates, respectively, compared to CK. In <0.25 mm aggregates, the B, S, and BS treatments significantly increased the *OC* contribution by 159.09%, 89.42%, and 149.88%, respectively, compared to CK. Overall, biochar application and straw treatment affected the *OC* contribution of large aggregates only in >2 mm aggregates but significantly reduced the *OC* contribution of large aggregates and increased the *OC* contribution of macroaggregates. Most of the *OC* was deposited as >0.25 mm mechanically stable macroaggregates, which contributed 42.95%–72.38% of *SOC*.



(A) **(B)**

Fig. 2. Organic carbon content and contribution of mechanically stabilized agglomerates of red soil after 6 months of incubation. (A) and (B) are the organic carbon content and organic carbon contribution in the five aggregate grain classes of the four treatments, respectively. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw. Different lowercase letters indicate significant differences ($P < 0.05$) among the treatments.

3.2.4. Microbial biomass carbon

The *MBC* content under the organic material application treatments was ranked as follows: BS > S > CK > B (Fig. 3A). The maximum *MBC* content was 1903.96 mg kg^{-1}. The proportions of 349.74% and 575.11% were significantly higher in the S and BS treatments, respectively, than in the CK treatment. There was no significant difference in *MBC* content between the B and CK treatments, indicating that biochar application alone could not improve soil *MBC* content. The results from the BS, S, and CK treatments were significantly different ($P < 0.05$), indicating that the cumulative application of biochar and straw was the most effective.

3.2.5. Dissolved organic carbon

As shown in Fig. 3B, the additional application of straw significantly increased the soil *DOC* content, with significant levels of difference ($P < 0.05$) between all treatments and CK, with the highest *DOC* content of 1606.75 mg kg^{-1} in the BS treatment. Treatment B was not significantly different from CK. This showed that straw was more beneficial to the soil *DOC* content than additional biochar application, and the co-application of both could further increase the soil *DOC* fraction content.

3.2.6. Easily oxidized organic carbon

As shown in Fig. 3C, the *EOC* content of the straw treatments differed significantly ($P < 0.05$), whereas biochar application alone did not significantly change the *EOC* content. The maximum value of *EOC* content in BS treatment was 9.10 g kg^{-1}, indicating that the addition of straw treatment was beneficial to increase the soil *EOC* content, while the addition of biochar had little effect on the accumulation of *EOC* content.

3.2.7. Particulate organic carbon

The *POC* content of the acidic red loam vegetable soil was increased to different degrees by the organic material application treatments compared to CK (Fig. 3D). The B and BS treatments were more significant ($P < 0.05$) than the S treatment and were ranked as follows: BS > B > S > CK. The BS treatment had the highest *POC* content (4.44 g kg^{-1}. However, the difference between the B and BS treatments was not statistically significant. This indicates that the cumulative application of rice straw and biochar could increase *POC* content more effectively than straw alone.

3.2.8. Correlation analysis between soil stability indicators and organic carbon fractions

There is a certain correlation between the stability of soil aggregates and organic carbon components (Fig. 4), and the results showed that *SOC* was most affected by *DMWD*, *DGMD*, $DR_{0.25}$, and *PAD*, all of which show a highly significant negative correlation. The content of *MBC* was significantly positively correlated with *WMWD* and *WGMD*, a highly significant positive correlation with $WR_{0.25}$, and a highly significant negative correlation with *PAD* and E_{LT}; *DOC* content was significantly and positively correlated with *WMWD* and $WR_{0.25}$, significantly and negatively correlated with E_{LT}, and highly significantly and negatively correlated with *PAD*; *EOC* content was highly significantly and positively correlated with *WMWD*, *WGMD*, and $WR_{0.25}$, and highly significantly and negatively correlated with *PAD* and E_{LT}; *POC* was highly significant and negatively correlated with *DMWD*, *DGMD*, and $DR_{0.25}$.

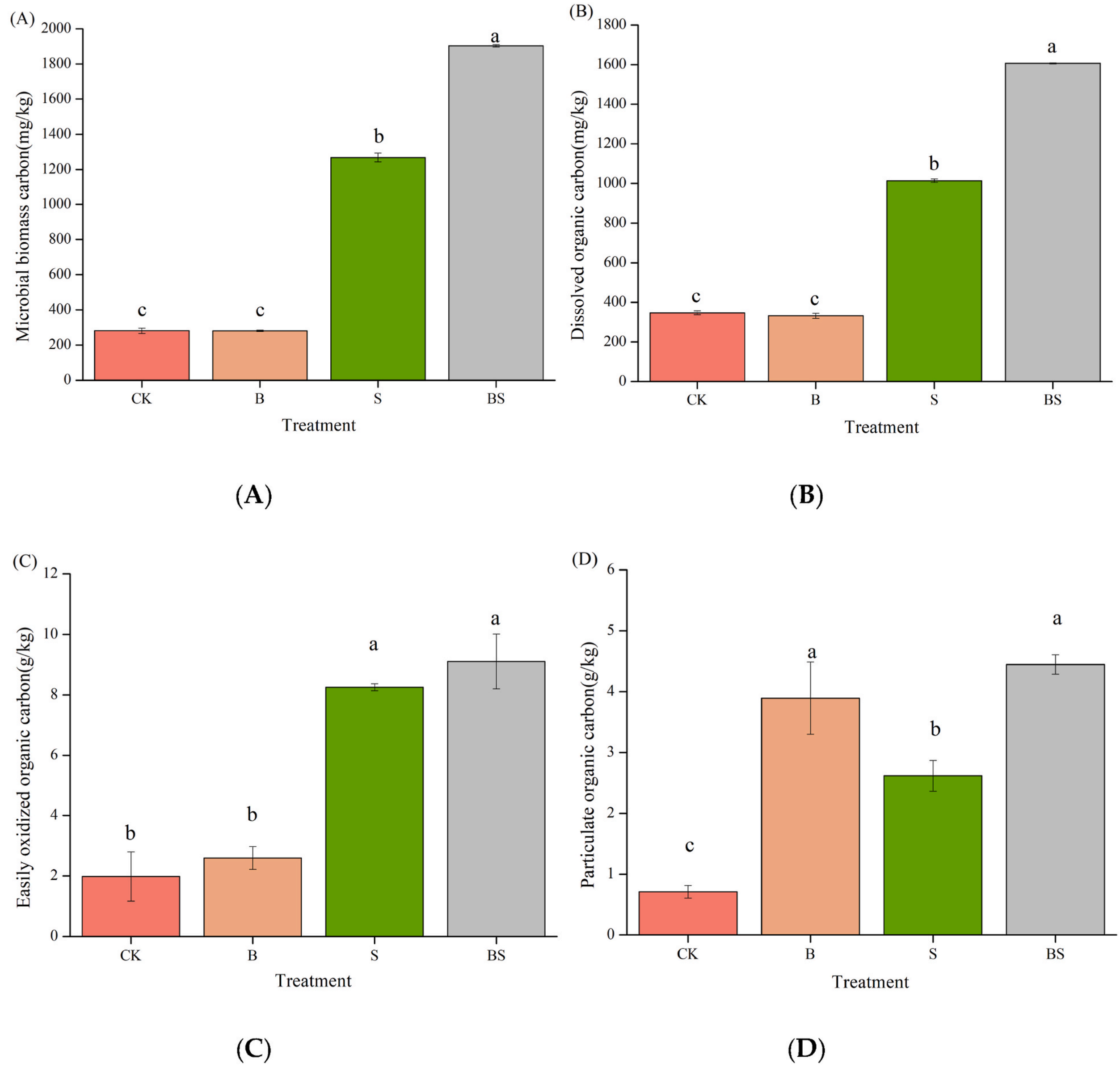


Fig. 3. Effect of rice straw and its carbonation addition on the content of soil organic carbon fraction. (A), (B), (C), and (D) are the microbial biomass carbon, dissolved organic carbon, easily oxidized organic carbon, and particulate organic carbon contents of the four different treatments, respectively. CK: no organic material; B: 5% homemade straw biochar; S: 12% rice straw; BS: 5% biochar + 12% rice straw. Different lowercase letters indicate significant differences ($P < 0.05$) among the treatments.

4. Discussion

4.1. Soil aggregates' composition and stability

In this study, we found that the addition of biochar and straw significantly reduced the content of *MSA* >2 mm particle size, *MWD*, *GMD*, *PAD*, and $R_{0.25}$ values and increased the aggregate content of the remaining particle sizes and D values compared to the control (Tables 1–3). This is similar to the results of Wang et al. [33], who concluded that the application of 1% straw biochar significantly reduced the content of *MSA* at particle sizes >2 mm, compared with that of no added material. However, Jiao et al. [34] concluded that the *MSA* content was significantly higher after applying $FeCl_3$-modified biochar and chitosan-modified biochar than without the added materials. This may be related to the raw materials used for the biochar preparation. Straw biochar has little decomposition ability and is more difficult for soil microorganisms to decompose; therefore, it has insufficient binding ability for large aggregates. In particular, there is an increasing number of small soil particles and more soil structural bodies through medium aggregate particle levels and

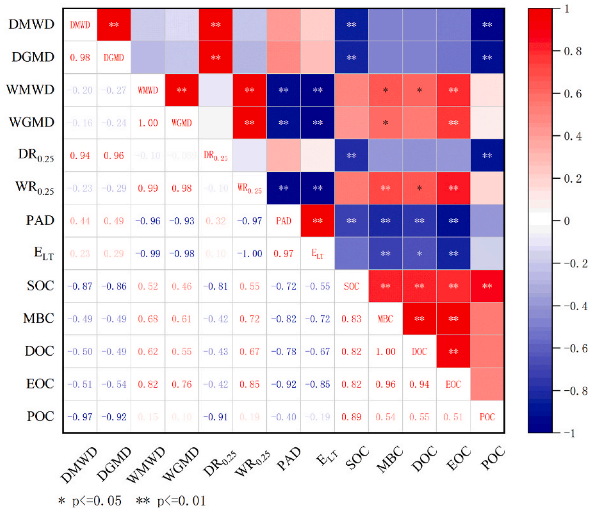


Fig. 4. Correlation analysis of different indexes of straw and its carbonisation addition. *DMWD*: mean weight diameter of dry sieve; *DGMD*: geometric mean diameter of dry sieve; *WMWD*: mean weight diameter of wet sieve; *WGMD*: geometric mean diameter of wet sieve; $DR_{0.25}$: dry sieve is larger than 0.25 mm particle size aggregate; $WR_{0.25}$: wet sieve is larger than 0.25 mm particle size aggregate; *PAD*: percentage of aggregate destruction; E_{LT}: unstable aggregate index; *SOC*: soil organic carbon; *MBC*: microbial biomass carbon; *DOC*: dissolved organic carbon; *EOC*: easily oxidizes organic carbon; *POC*: particulate organic carbon. Note: *, ** indicate significant correlation and extremely significant correlation respectively.

macroaggregate particle levels at high rates of addition, while modified biochar can promote soil microbial activity. With the increase in additions, microorganisms produce a variety of secretions, contributing to colloidal substances and forming large aggregates. The results of Sun et al. [35], who found that the full amount of straw returned to the field significantly reduced the *MSA* of >2 mm grain size and significantly increased the content of <2 mm grain size, were similar to those of this experiment, where the lack of topsoil moisture, weak soil pressure, and the addition of straw led to more fragmentation of the soil and increased the number of small grain size clumps. Macroaggregate content can affect soil structural stability; as organic material is applied to the soil, the mechanical

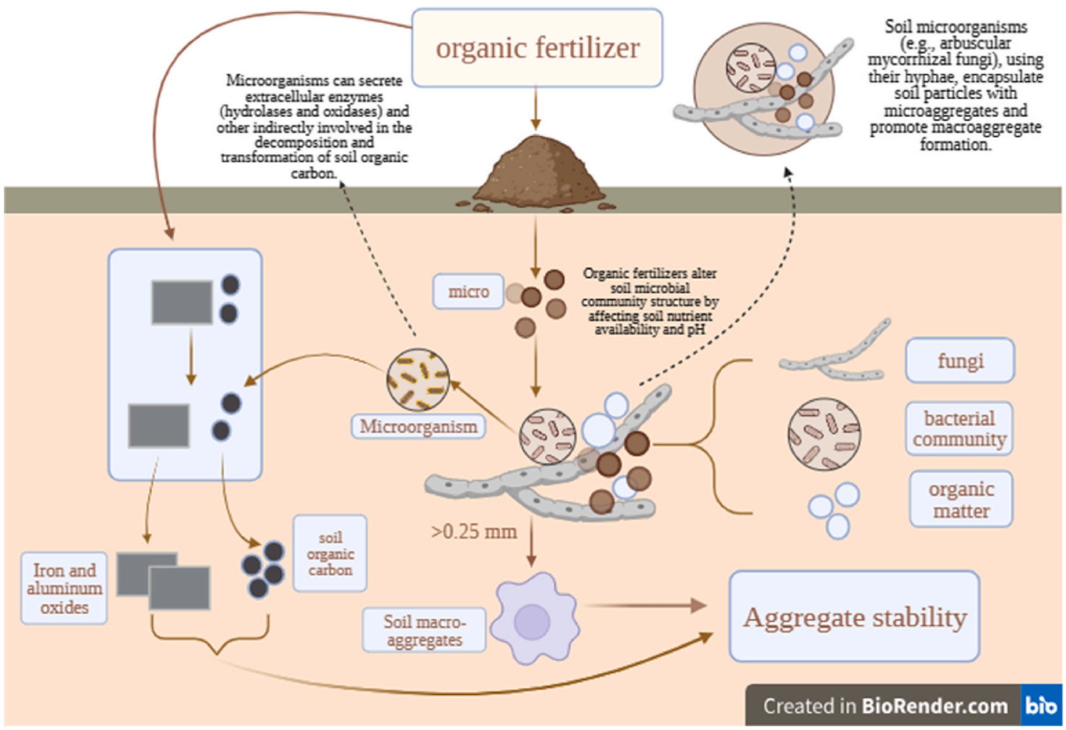


Fig. 5. Key processes of organic fertilizers regulating the stability of red soil aggregates and the distribution of organic carbon.

stability of the macroaggregate content decreases, resulting in a less mechanically stable soil structure.

A good soil structure requires many mechanically stable large aggregates and good water stability. The *WSA* at >0.25 mm particle size is generally used to judge the superiority of the soil structure, and the higher its content, the better the soil structure [36]. This study showed that the addition of straw treatments (S and BS) significantly increased the content of large aggregates at >0.5 mm particle size, significantly increased the values of $R_{0.25}$, *MWD*, and *GMD*, and significantly decreased the content of macroaggregates <0.25 mm and significantly decreased the D value compared to CK, which was similar to the results of Liu et al. and Rahman et al. [37, 38]. This is due to the decomposition process of straw, providing nutrients to the soil by affecting soil nutrient availability and pH to change the structure of the microbial community in the soil, improve microbial activity in the soil (e.g. arbuscular mycorrhizal fungi), and microbial decomposition to produce organic colloidal material with the fungal mycelia in the soil entangled to form granules and finally colloidal into large aggregates to promote soil structural stability (Fig. 5) [39]. The combination of biochar and straw applied to the soil is beneficial to the formation of humic substances after the decomposition of straw and biochar itself as a soil cementing substance, which has a positive effect on the formation and stability of large aggregates [40]. In the biochar addition treatment test, there was no significant difference between the aggregates of each particle size and CK, consistent with the results of Sun et al. [41].

Therefore, biochar was not as effective as straw in increasing soil aggregate content, whereas the cumulative application of biochar and straw significantly increased the water-stable macroaggregate content and aggregate stability and improved soil structure compared to biochar alone.

4.2. Organic carbon content and distribution of aggregates

The decomposition of straw can increase the input of soil organic matter, provide sufficient active C resources to the soil, and enhance *SOC* content [42]. Biochar is an aromatic compound containing 40%–75% C that can interact with soil to form a protective mechanism that inhibits the oxidation of soil organic matter and promotes its accumulation of soil organic matter [43–45]. Biochar prepared at low temperatures contains more *OC* components that can be easily decomposed, providing a C source available to soil microorganisms, while biochar prepared at high temperatures has strong chemical stability and behaves as mineral C when it enters the soil, reducing the *OC* content and increasing soil impoverishment [46]. In this study, the addition of biochar increased the *SOC* and *OC* content of the soil aggregates at each grain level. The application of biomass charcoal to the soil promotes the formation of soil humus and increases the *OC* content [47]. Organic fertilisers change the structure of microbial communities in the soil by affecting soil nutrient availability and pH, thus changing different enzyme activities [48]. Microorganisms can also secrete extracellular enzymes (hydrolases and oxidative enzymes) and others indirectly involved in the decomposition and transformation of *SOC*, which ultimately affect the content and quality of *SOC* (Fig. 5) [49]. Among the test materials, straw contained slightly more C than biochar; therefore, the S treatment contained more *OC* than the B treatment applied to the soil, whereas the BS treatment contained the highest amount of *SOC*, and the combination of the two was more effective than either application alone (Fig. 1A). The C/N ratio is an indicator of humification of organic matter humification [50]. The C/N ratio of treatment B was significantly higher than that of the other treatments (Fig. 1B), indicating that organic matter decomposition was low after biochar application to the soil. In contrast, organic matter decomposition was high in the straw treatment.

Iron-aluminium oxides and clay minerals in soils can tightly bind to *OC* through ligand substitution, high-valent ionic bond bridges, van der Waals forces, and complexation to spatially reduce the bioavailability of *OC*, thereby improving *SOC* stability (Fig. 5) [51]. The stabilisation mechanism of *OC* by aggregates is mainly manifested in the physical protection of *OC* by aggregates, that is, spatial segregation and the adsorption capacity of aggregates for *OC*. The variation in *SOC* content mainly depends on macroaggregates [52], and *OC* produced by organic matter is preferentially preserved in macroaggregates [53]. *OC* is the main cementing substance for the formation and stabilisation of soil aggregates. In this study, it was found that *OC* in aggregates was mainly distributed in large aggregates after the addition of organic materials, and the *OC* content of aggregates >2 mm in grain size was significantly higher than that of aggregates with other grain sizes (Fig. 2A). The formation of organic matter as the main cementing material in the aggregates may cement the soil particles and 0.25–2 mm particle size aggregates into >2 mm aggregates, and more mycelia in large aggregates can also increase the *OC* content of large aggregates [54]. Both the B and S treatments in this study increased the *OC* content of the aggregates, but the effect was less effective than that of the BS treatment. This is because biochar and straw, as exotic C sources, can enhance the soil C pool when returned to the field and are more effective when applied cumulatively. The contribution of *OC* was determined using the proportion of each particle level in *MSA*. The addition of biochar and straw significantly reduced the content of large aggregates and increased the content of macroaggregates. Therefore, the contribution of *OC* in large aggregates was significantly reduced, and the *OC* contribution of microaggregates was significantly increased (Fig. 2B).

4.3. Soil MBC, DOC, EOC, and POC

Exogenous carbon input is an important factor that influences the differences in the distribution characteristics of *OC* fractions. Soil *MBC* content is closely related to soil microbial activity and nutrient supply capacity [55]. In this study (Fig. 3A), the application of straw resulted in a higher soil *MBC* content because straw input both supplemented the soil carbon source and increased the number and activity of microorganisms, promoting the conversion of *SOC* to *MBC* in the straw [56]. Biochar application alone did not increase the *MBC* content in this study, probably because of the low carbon effectiveness of biochar and its reliance on reducing soil C metabolism to improve soil stability, which does not affect microbial abundance and activity [57]. The BS treatment was the most effective in increasing the *MBC* content and differed significantly from the other treatments. The high carbon effectiveness and microbial activity in straw, as well as the loose and porous structure of biochar, its large surface area, and high cation exchange provide a good habitat for soil microorganisms. The combination of the two can promote microbial growth, reproduction, and change the microbial community structure in the soil, and part of the soil active carbon is converted into a microbial amount of carbon through microbial action [58].

DOC is an important source of energy and carbon for the decomposition and transformation of organic materials by soil microorganisms [59]. Part of the applied organic material is used to renew the cellular components of the microorganisms themselves, whereas the other part is oxidized into the energy materials required for the activities of the microorganisms [60]. As shown in Fig. 3A and B, the change in *DOC* content was consistent with that of *MBC* content, and *MBC* and *DOC* content were significantly correlated, indicating that they may have a source-sink relationship, and microorganisms may use *DOC* dissolution to meet their growth and reproduction requirements [61]. In this study, treatment B had no effect on the *MBC* content, and the content of *DOC* utilised by microorganisms did not change significantly, probably because the C/N value of the biochar treatment was too high, which reduced the microbial activity, and the content of *DOC* utilised by microorganisms was low. Treatments S and BS increased the DOC content, and straw and biochar provided rich carbon and energy sources for microbial growth and reproduction, stimulated microbial growth, facilitated microbial decomposition of organic materials in the soil, and promoted the activation and decomposition of insoluble materials in the soil [62]. In addition, straw contains a large amount of material that can be directly decomposed and utilised by microorganisms, significantly promoting soil respiration when applied to the soil, thus increasing *DOC* consumption [63].

EOC is not only a sensitive indicator of the impact of agricultural management practices on soil quality but is also an important indicator for evaluating potential soil productivity [63]. Soil *EOC* content mainly depends on the input of active organic matter and the decomposition of *OC* [64]. Soil *EOC* content increased with straw application in Fig. 3C, but biochar application alone did not affect *EOC* content. Exogenous organic materials increase the source of organic matter and change the soil C/N value. In contrast, different organic materials' carbon availability varies, and the carbon source effectiveness of straw is higher than that of biochar. Moreover, homemade biochar was prepared at 500 °C with more ester and aromatic compounds, which showed high biological and chemical stability and limited components that microorganisms could use directly; therefore, the promotion of active organic carbon was not as good as that of straw [65,66].

POC is one of the newest and most biologically active *OCs*, consisting of partially decomposed fresh plant litter and microbial products [67] and depends mainly on the carbon replenishment of the soil [68]. In this study, all exogenous additions of organic materials significantly increased soil *POC* content (Fig. 3D). Therefore, a large C input can effectively increase the *POC* content. *POC* can be metabolised by microbial decomposition into biochemically stable carbon fractions, providing microbial respiration and carbon fluxes for stable soil organic matter fractions (aggregated *OC* and humic carbon), which also play important roles in the long-term sequestration of *SOC* [69]. Soil mineral-bound *OC* is the final decomposition product of *OC*, which has high stability under the protection of soil clay and powder particles and plays a strong role in the sequestration and protection of *SOC* [70]. In this study, we found that the mineral-bound *OC* of soil with biochar application was significantly higher than that with straw application alone, which might be attributed to the fact that biochar application had less of an effect on soil mucilage. Therefore, the mineral-bound *OC* was well protected and conducive to the long-term stable sequestration of *SOC*.

5. Conclusions

In the southern part of the country, inherent barriers in the red soil zone can lead to regional food shortages and instability, constraining regional agricultural development; therefore, a more detailed understanding of aggregates and carbon fractions in red vegetable soils is critical. As an indigenous option, straw application alone has been criticised for failing to integrate environmental sustainability into core considerations and address climate change impacts. The results of this study showed that the application of rice straw significantly increased the number of soil water-stable macroaggregates and erosion resistance, with the S treatment being the most effective, increasing *MWD*, *GMD*, and $R_{0.25}$ by 246.67%, 181.82%, and 143.94%, respectively, compared with the CK treatment. Second, BS treatment was generally effective, whereas B treatment was the least effective. The BS treatment was the most effective in improving the nutrients in the soil and aggregates, increasing the content of *OC* and its fractions in the red soil more than the application of biochar or straw alone and significantly increasing the utilisation rate of *OC*. Compared to straw or biochar alone, the combination of the two has greater potential for carbon sequestration and CO_2 mitigation because of the enhanced soil carbon sink function. In summary, applying biochar with straw can significantly improve the stability of soil aggregates in red soil vegetable plots, thus enhancing the soil carbon sequestration capacity, which is of great significance for the development of ecological low-carbon agriculture and the national goal of "carbon neutrality". However, the present study was only a short-term indoor incubation experiment, which only considered the effects of rice straw and its carbonisation additions on soil aggregates and organic carbon fractions in acidic red soil vegetable fields. In the future, it is necessary to study the effects of long-term cultivation of acidic red soil vegetable fields on microbial communities.

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Data availability statement

Data included in article/supp. Material/referenced the in article.

CRediT authorship contribution statement

Yawen Liao: Writing – original draft, Formal analysis, Data curation. **Masood Iqbal Awan:** Writing – review & editing. **Muhammad Aamer:** Writing – review & editing. **Jianxiu Liu:** Visualization, Investigation. **Jiahui Liu:** Investigation. **Bei Hu:** Investigation. **Zhiqiang Gao:** Resources. **Bo Zhu:** Resources. **Fengxian Yao:** Resources, Formal analysis. **Chen Cheng:** Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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