

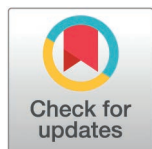
RESEARCH ARTICLE

Comparing the linkages between carbon components and soil aggregates in vegetable fields to continuous input of different carbon sources

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Abstract

Aims

To address the issue of declining soil organic carbon (SOC) in vegetable fields, this study investigated the effects of continuous input of different carbon sources on soil carbon pool dynamics.

Methods

A five-year field plot experiment was conducted to analyze the impact of organic fertilizer, biochar, and their combined application on soil organic carbon components, aggregate stability, and carbon pool characteristics.

Results

The continuous application of biochar and organic fertilizer significantly increased the content of soil organic carbon components. Organic fertilizer contributed more actively to labile organic carbon fractions, whereas biochar primarily supplied stable organic carbon. All treatments enhanced the proportion of macroaggregates (>0.25 mm) and improved aggregate stability, with organic fertilizer exhibiting superior effects compared to biochar. Organic fertilizer also promoted organic carbon sequestration in aggregates by enriching readily oxidizable organic carbon (ROC) and water-soluble organic carbon (DOC). In contrast, biochar application significantly increased inert organic carbon (IOC), leading to higher total SOC compared to organic fertilizer alone. Carbon pool characteristic indices improved significantly under continuous biochar and organic fertilizer application. Organic fertilizer treatment showed a higher carbon pool activity index, while biochar outperformed in other

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indices. The combined application yielded the most favorable results. Correlation analysis revealed that soil aggregate stability was significantly associated with DOC and microbial biomass carbon (MBC). Both biochar and organic fertilizer enhanced soil available nutrient content, which increased dynamically over time. Principal component analysis confirmed that the combined application of organic fertilizer and biochar was most effective for soil carbon sequestration and fertility improvement.

Conclusion

Biochar and organic fertilizer differentially influenced soil organic carbon composition. Organic fertilizer improved carbon pool characteristics by mediating macroaggregate formation through labile organic carbon (e.g., DOC and MBC), while biochar contributed directly to carbon sequestration due to its inert and stable nature. Their combined application synergistically enhanced the formation and stability of soil macroaggregates, improved soil carbon pool characteristics, and optimized nutrient availability in vegetable fields. This version improves clarity, conciseness, and flow while maintaining scientific accuracy. Let me know if you'd like any further refinements!.

1. Introduction

Soil organic carbon composition, aggregates and their stability are important indicators to measure soil fertility. Citation needed. Aggregate is the basic unit of soil structure and composition, and it is also an important place for the existence of organic carbon in soil. Its quantity and quality determine many physical and chemical properties and fertility status of soil [1]. At present, on a global scale, the total amount of soil organic carbon in different ecosystems varies significantly [2,3]. In forest ecosystems, the loss of soil organic carbon in the central and western regions may be as high as 6.0% per year [4]. Soil organic carbon and aggregates affect each other and are closely related. Organic carbon can not only enhance the agglomeration between soil particles, but also promote the formation of aggregates. Aggregates give physical protection to organic carbon, which can affect the characteristics of soil carbon pool [5].

Long-term single application of chemical fertilizers destroys the structure of soil aggregates and accelerates the decomposition of organic carbon in aggregates, which is not conducive to carbon sequestration [6]. Exogenous addition of biochar and organic fertilizers are effective measures to supplement soil carbon and improve the stability of soil aggregates [7]. The application of organic fertilizer significantly increased the proportion of macroaggregates in soil and the content of dissolved organic carbon (DOC) in this aggregate, as well as the content of carbon, nitrogen and phosphorus in each particle size aggregate, thereby promoting soil function and crop productivity [8–10]. found that single application of biochar could significantly increase soil total organic carbon (SOC) content. The combined application of AM fungi and biochar had no significant effect on medium soil aggregates, but it could

significantly increase DOC content and significantly reduce readily oxidizable organic carbon (ROC) content [11,12]. found that single application of biochar or combined application of biochar and organic fertilizer could significantly increase the content of POC and ROC in soil, and also contribute to the growth and yield of apple plants. Under the combined application of organic fertilizer and biochar, the degree of soil humification was higher when biochar was added alone, and microbial biomass organic carbon (MBC) and DOC had a significant effect on the cumulative emission of soil CO₂. [13,14] found that single application of biochar could significantly increase the content of carbon components in soil and the content of carbon components in aggregates of different particle sizes through the positioning test of paddy soil. Study by [15] showed a correlation between the stability of soil aggregates and SOC storage. These results revealed that the distribution of SOC and its fractions in soil aggregates were affected by many factors. In addition, the active organic carbon in the soil carbon pool has strong environmental sensitivity and plays a key role in the transformation of soil physical, chemical and biological characteristics Citations needed. It further highlights the necessity of in-depth study of the relationship between soil aggregates and SOC, in order to better understand the mechanism of soil SOC accumulation and transformation, and guide vegetable field management and soil protection measures. (After the application of organic fertilizer, several issues remain. First, the efficiency of soil carbon sequestration is low. Second, the accumulation pattern of organic carbon is ambiguous. Third, the mechanism by which microbial turnover influences the formation of soil aggregates is still unclear.) After the application of organic fertilizer, the efficiency of soil carbon sequestration is not high, the accumulation of organic carbon is not clear, and the mechanism of microbial turnover on the formation of aggregates is not clear. Application of biochar is mainly due to its stable physical properties and high stability after being applied to the soil, Under the combined application of biochar and organic fertilizer, organic fertilizer can provide active carbon source for soil microorganisms and promote the metabolism and breeding of microorganisms. Biochar adsorbs and stores substances of different types and components in micropores, which provides nutrients for microbial communities, accelerates the accumulation of microbial necrosis, and enriches carbon in aggregates [15]. Due to the differences in physical, chemical and biological properties of different soil types, the experimental results in different regions are also significantly different, which leads to different responses of carbon components in soil aggregates to organic fertilizer and biochar.

The vegetable industry in southwest China is developed. The intensive cultivation and the cultivation mode of large water and large fertilizer, especially the excessive application of nitrogen fertilizer, accelerate the decomposition of soil organic carbon and lead to the deterioration of soil structure [16,17]. In order to solve the problems of soil organic carbon reduction in vegetable fields, this paper explored the relationship between changes in soil organic carbon components and aggregates and their stability, and their effects on carbon pool characteristics through a five-year (2019–2023) long-term application of organic fertilizer and biochar and their combined use of positioning experiments. It is of great significance to optimize the management of vegetable soil carbon pool and the sustainable utilization of vegetable soil resources.

2. Materials and methods

2.1 Test site and materials

The experimental site (Fig 1) is located in Chengjiang City, Yuxi City, Yunnan Province (N24 ° 39 ' 6 " E102 ° 54 ' 48 "), Yunnan Agricultural University Agricultural Non-point Source Pollution Monitoring Station. The planting area belongs to the mid-subtropical plateau monsoon climate, with sufficient light, rain and heat in the same season, the annual average temperature is 11.9–17.5 °C, and the annual rainfall is 900–1200 mm. The typical vegetable field was selected, and the soil physical and chemical properties were as follows: pH 7.71, organic matter 19.88 g/kg, alkali-hydrolyzable nitrogen 79.03 mg/kg, available phosphorus 10.12 mg/kg, and available potassium 100.33 mg/kg. Biochar was prepared by pyrolysis of rice husk at 500 °C, N 0.35%, P₂O₅ 0.72%, K₂O 2.43%, pH 10.0, and the organic carbon content was 270.4 g/kg. Organic fertilizer is a commercial organic fertilizer (N 1.1%, P₂O₅ 1.61%, K₂O 3.92%) made from tobacco stem (leaf vein) as the main raw material. The pH is 7.6, and the organic carbon content is 218.5 g/kg, all of which are provided

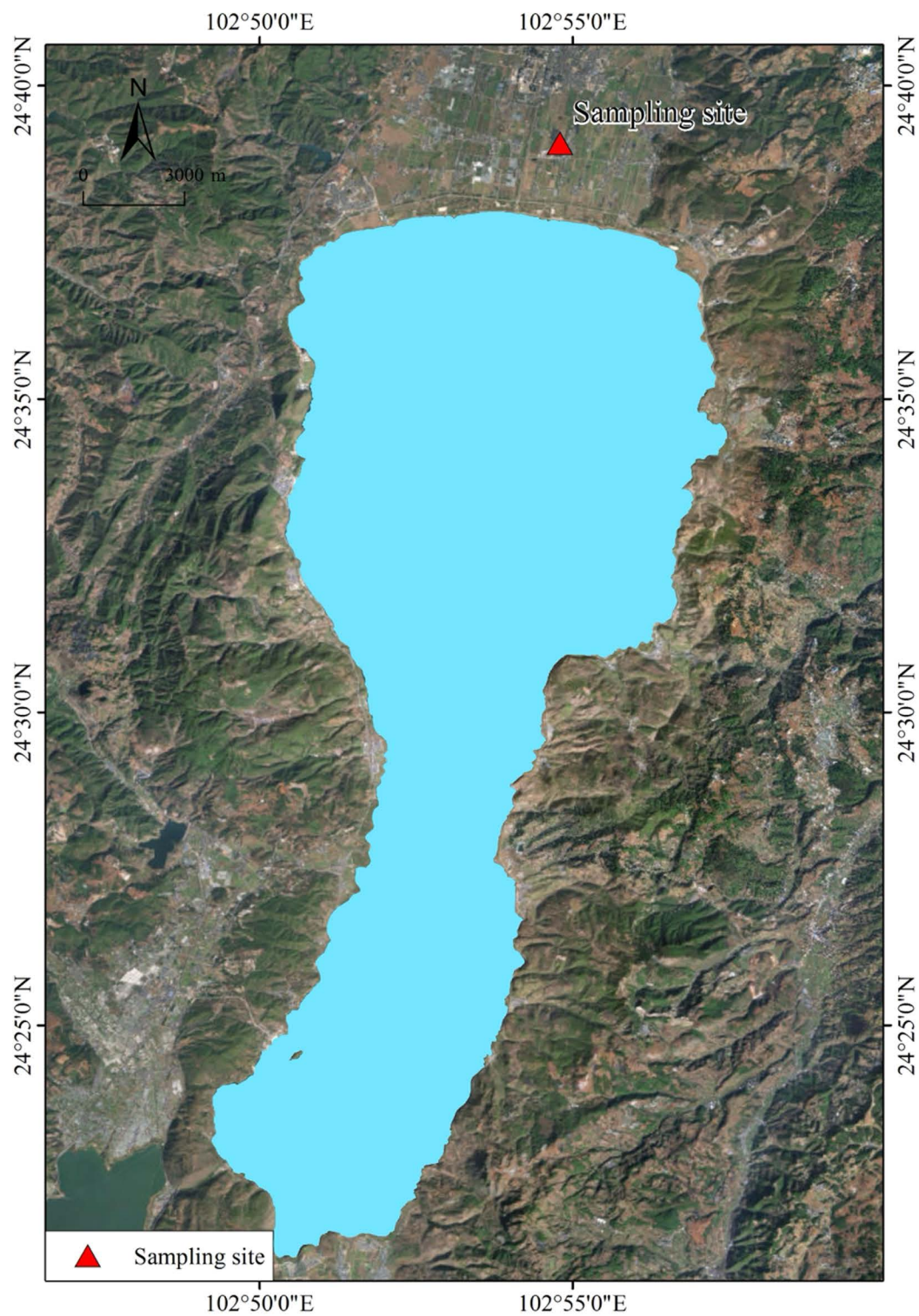


Fig 1. Positioning test topographic map.

<https://doi.org/10.1371/journal.pone.0323028.g001>

by Kunming Lishan Biotechnology Co., Ltd. Nutrient labeled amount of compound fertilizer was $N-P_2O_5-K_2O$: 18-17-17. During the five years of the experiment, the types of vegetable planting and the time of planting and harvesting were as follows: [Table 1](#).

2.2 experimental design

The field randomized block design was adopted in the positioning test. Two crops of vegetables were treated during soil preparation each year: Single application of chemical fertilizer as control (CK); organic fertilizer completely replaces chemical fertilizer (OF, alternative application of total nutrients such as CK); Organic carbon such as biochar and organic fertilizer was applied, and nutrient deficiency was supplemented by chemical fertilizer (B); organic fertilizer and biochar 1/1, with OF, B treatment and other carbon inputs, insufficient nutrients supplemented by chemical fertilizer (BF). Each treatment set three replicates, a total of 12 experimental plots, each plot area of 27 m², each plot interval of 20 cm. Biochar and organic fertilizer were fully mixed with 0–20 cm soil (0.2 m high, 2 m wide) before each vegetable planting. 30% of the compound fertilizer was mixed with the surface soil as the base fertilizer, and 70% was used as the top dressing and watering. A total of 10 crops of vegetables were planted in 5 years, and local seedlings were purchased for transplanting. The fertilization schemes of different kinds of vegetables are shown in [Table 2](#). Lettuce was applied twice after 10 days and 20 days of planting, 35% each. The first topdressing of broccoli was 20% at 20 days after transplanting, the second topdressing was 20% at budding, and the third topdressing was 30% at the stage of flower bulb expansion. The baby vegetables were

Table 1. Planting types and planting and harvesting time of vegetables.

crops	species of vegetable	implantation time	harvest time
The first crop	lettuce	August 2019	October 2019
The second crop	lettuce	November 2019	December 2019
The third stubble	baby cabbage	May 2020	July 2020
The fourth crop	lettuce	September 2020	October 2020
The fifth crop	lettuce	May 2021	June 2021
The sixth crop	baby cabbage	August 2021	October 2021
The seventh crop	broc-coli.	April 2022	July 2022
The eighth crop	lettuce	August 2022	September 2022
The ninth crop	broc-coli.	March 2023	June 2023
The tenth crop	baby cabbage	September 2023	November 2023

<https://doi.org/10.1371/journal.pone.0323028.t001>

Table 2. Fertilization amount of different vegetables per crop (kg/hm²).

		lettuce	broc-coli.	baby cabbage
CK	compound fertilizer	1067	1759	1680
OF	organic fertilizer	8369	13796	13176
B	biochar	6762	11148	10647
	compound fertilizer	612	1009	963
BF	organic fertilizer	4184	6898	6588
	biochar	3381	55784	5324
	compound fertilizer	306	504	482

Note: The amount of organic fertilizer applied in OF treatment was converted according to the total nutrient component of CK; the application amount of biochar in B treatment was converted to the organic carbon content such as OF, and the insufficient part of total nutrient content was supplemented by compound fertilizer. BF treatment of organic fertilizer and biochar 1/1, with OF and B treatment and other organic carbon application, the total nutrient content of the lack of part by the compound fertilizer supplement.

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applied four times at the seedling stage, rosette stage, early heading stage and late heading stage, and the fertilization method was watering. Pest prevention and weeding measures are carried out in accordance with local customs.

According to the calculation of [Tables 1](#) and [2](#), a total of 108965 kg/hm² organic fertilizer was applied to the OF treatment in the 5-year continuous test. A total of 88050 kg/hm² biochar was applied to the B treatment, and the three treatments applied a total of 23808 kg/hm² of organic carbon.

2.3 Sample collection and determination

From 2019 to 2023, in addition to measuring the yield of vegetables after each harvest, three mixed soil samples of 0–20 cm plough layer were collected. After air drying, the soil physical and chemical properties were determined by 20 mesh and 100 mesh sieves: alkali-hydrolyzable nitrogen, available phosphorus, available potassium, pH and other indicators. After the last harvest of vegetables in November 2023, five ring knife samples were taken from the plough layer of each treatment plot with stainless steel ring knife, and marked for the determination of soil bulk density. In each plot, the undisturbed soil samples of 0–20 cm soil layer were taken, and 5 points were fully mixed according to the ‘S’ route. Three mixed samples were taken from each experimental plot. After removing gravel and plant residues, they were divided into 2 soil samples. One fresh sample was sealed and stored at 4 °C for screening of aggregates, and the particle size of aggregates was determined. One was used for the determination of soil organic carbon components. They are Soil Organic Carbon (SOC), Particulate Organic Carbon (POC), Readily Oxidizable Organic Carbon (ROC), Microbial Biomass Carbon (MBC), Dissolved Organic Carbon (DOC). One for the determination of soil organic carbon components, respectively, soil organic carbon (Soil Organic SOC with potassium dichromate external heating method [\[18\]](#); ROC with potassium permanganate oxidation method [\[19\]](#); DOC was measured by TOC analyzer [\[19\]](#); MBC fumigation with chloroform [\[20\]](#); POC was determined by sodium hexametaphosphate dispersion method [\[21\]](#). In addition, the calculation formula of soil inert organic carbon (IOC) is: IOC = SOC - ROC.

The calculation formula of the determination index is as follows [\[22\]](#): (1)

$$\text{Yield (kg/m}^2\text{)} = \text{sample weight per plant (kg)} \times \text{number of plants/plot area (m}^2\text{)} \quad (1)$$

(2) Soil mechanical stability and water stability > 0.25 mm aggregate content calculation formula [\[23\]](#):

$$R_{0.25} = \frac{\text{> 0.25mm Total mass of aggregates}}{\text{Total soil mass before unscreening}} \times 100\% \quad (2)$$

(3) The calculation formula of the destruction rate of soil aggregate structure [\[23\]](#):

$$\text{PAD} = \left[\frac{(\text{R} > 0.25 \text{ dry screening} - \text{R} > 0.25 \text{ wet sieve})}{\text{R} > 0.25 \text{ dry screening}} \right] \times 100\% \quad (3)$$

(4) The calculation formula of average mass diameter and geometric mean diameter of aggregates [\[23\]](#):

$$\text{MWD} = \sum_i^n W_i X_i \quad (4)$$

$$\text{GMD} = \exp \left(\sum_{i=1}^n W_i \ln X_i \right)$$

In the formula, n , X_i and W_i are the number of particle size groups, the average diameter of the particle size aggregates (mm), and the percentage of the mass of the particle size aggregates to the soil mass (%).

(5) Fractal dimension D [23]:

$$D = 3 - \frac{\log\left(\frac{W_i}{W_0}\right)}{\log\left(\frac{X_i}{X_{\max}}\right)} \quad (5)$$

W_i , W_0 , X_i and $X_{\max}$ are the accumulated mass of soil particles with diameter less than 0.05mm, g; the sum of all grain-size soil mass, g; the average particle size of aggregates in the range of i particle size, mm; the average diameter of the largest grain size of soil particles, mm.

(6) Calculation of soil organic carbon storage [24]:

$$SOCs = SOC \times BD \times H \times 10 \quad (6)$$

In the formula: $SOCs$, SOC , BD , H , respectively, organic carbon storage (t/hm^2), soil organic carbon content (g/kg), bulk density (g/cm^3), soil thickness (0.2m).

(7) Soil carbon pool management index [25]:

Carbon pool index (CPI) = sample total carbon A content (g/kg) / original soil total carbon content (g/kg);

carbon pool activity (A) = active carbon content (g/kg) / inactive carbon content (g/kg);

carbon pool activity index (AI) = sample carbon pool activity / original soil carbon pool activity;

carbon pool management index (CPMI) = carbon pool index $\times$ carbon pool activity index $\times 100 = CPI \times AI \times 100$

2.4 Data analysis

Microsoft Excel 2021 was used to collate and summarize the original data, and SPSS.26 was used to analyze the variance of the data. The least significant difference method (Duncan) was used to test the significance of the difference ($P < 0.05$). Origin 2022 mapping and Canoco 5.0 were used for redundancy analysis.

3. Results and analysis

3.1 Changes of soil organic carbon fractions

As shown in Fig 2, compared with the control (CK), the contents of organic carbon (SOC), particulate organic carbon (POC), readily oxidizable organic carbon (ROC), soluble organic carbon (DOC), microbial biomass carbon (MBC) and inert organic carbon (IOC) in biochar (B), organic fertilizer (OF) and their combined application (BF) significantly increased. Among them, SOC, POC, IOC, B treatment were better than OF treatment, and showed significant ($P < 0.05$) differences, while ROC, DOC and MBC, OF treatment was higher than B treatment, and the difference was significant.

3.2 Effects of different carbon sources on the distribution and stability parameters of soil aggregates

Fig 3 is the distribution of soil aggregates under different fertilization treatments. Compared with CK, the soil water-stable aggregates with particle size < 0.25 mm in OF, B and BF treatments were significantly reduced by 10%, 3% and 10%, respectively. In Table 3, compared with CK, $R_{0.25}$ was significantly increased under OF and BF treatments, which increased by 10.51% and 10.7%, respectively, and the effect under BF treatment was the best. Compared with CK, the average mass diameter (MWD) of aggregates under OF and BF treatments was significantly increased by 17.0% and 15.0%, respectively. The geometric mean diameter (GMD) increased significantly under OF, B and BF treatments, increasing by 11.0%, 3.0% and 10.0%, respectively. The

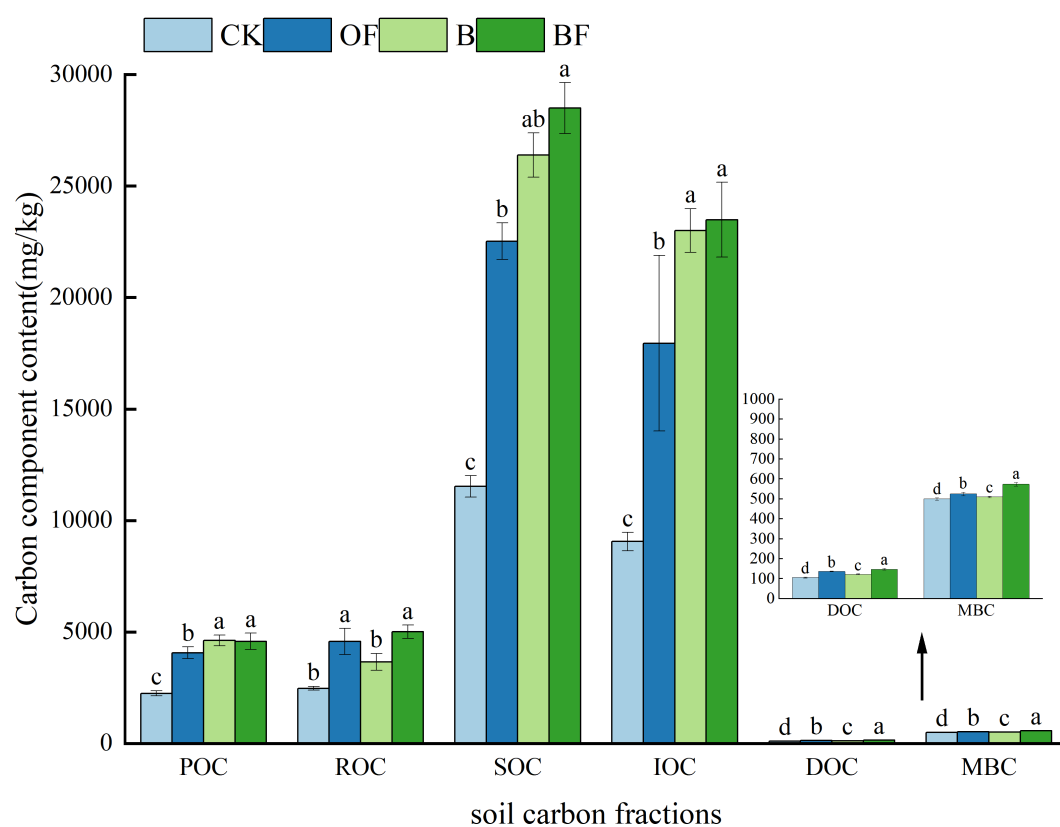


Fig 2. Organic carbon fractions in soil under different fertilization regimes.

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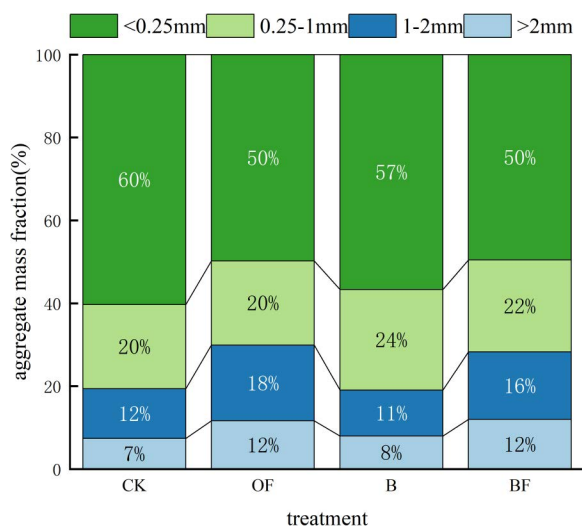


Fig 3. Stable soil water aggregates under different fertilization treatments.

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Table 3. Effects of different fertilization treatments on soil aggregate stability indexes.

Treatment	Water stability				
	MWD	GMD	R0.25%	PAD%	D
CK	0.53±0.09b	0.27±0.01c	39.72±3.7b	57±2.1a	2.25±0.22a
OF	0.70±0.01a	0.38±0.002a	50.23±0.6a	47.66±0.34b	1.77±0.05b
B	0.55±0.01b	0.30±0.002a	43.29±0.1b	54.22±0.36ab	2.22±0.02a
BF	0.68±0.02a	0.37±0.02a	50.42±1.9a	48.04±2.35b	1.94±0.14b

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data showed that the application of organic fertilizer (OF) had the best effect on increasing the MWD and GMD of soil aggregates. This is also true in reducing the soil aggregate structure destruction rate (PAD%) and fractal dimension (D).

3.3 Effects of different carbon sources on the distribution ratio of each carbon component in different size aggregates

After separating the aggregates of each particle size, the distribution of carbon components in the aggregates of each particle size was analyzed (Fig 4). The distribution ratio of R0.25 in different carbon fractions was increased under OF, B and BF treatments. The proportion of R0.25 in aggregates under the four treatments in DOC was BF > CK > OF > B; The distribution ratio of R0.25 in MBC was BF = B = OF = CK; the distribution ratio of R0.25 in POC was B > CK > OF > BF; the distribution ratio of R0.25 in EOC was CK = OF > BF > B.

3.4 Correlation analysis between soil organic carbon fractions and aggregates and their stability

The correlation analysis between soil aggregates and their stability and organic carbon components is shown in Fig 5. In Fig 5a, the content of soil aggregates with particle size > 0.25 mm was positively correlated with SOC, POC, ROC, IOC, MBC and DOC, among which the soil aggregates with particle size > 2 mm were significantly positively correlated with MBC and DOC. Among soil aggregates and aggregate stability, MWD, GMD and R0.25 were significantly positively correlated with soil aggregates with particle size > 1 mm, and significantly negatively correlated with soil aggregates with particle size > 0.25 mm. There was a significant negative correlation between D and soil aggregates with particle size > 1 mm, and a significant positive correlation with soil aggregates with particle size > 0.25 mm. Between soil carbon fractions and aggregate stability, each carbon fraction was negatively correlated with PAD and D, and positively correlated with MWD, GMD and R0.25. In Fig 5b, the content of soil aggregates with particle size > 2 mm was significantly positively correlated with MWD, GMD and R0.25, and significantly negatively correlated with PAD and D; the content of 1–2 mm soil aggregates was significantly positively correlated with MWD, GMD and R0.25, and significantly negatively correlated with PAD and D; the content of soil aggregates < 0.25 mm was significantly negatively correlated with MWD, GMD and R0.25, and significantly positively correlated with PAD and D.

3.5 Effects of continuous application of organic fertilizer and biochar on vegetable yield

Fig 6 shows the vegetable yield of continuous application of biochar and organic fertilizer for 5 years. The organic substitution (OF) significantly reduced the vegetable yield compared with the control, and the application of biochar (B) and the combined application (BF) increased the vegetable yield. In general, the order was BF treatment > B treatment > CK > OF treatment.

3.6 Effects of continuous application of organic fertilizer and biochar on the dynamic changes of soil conventional five items

As shown in a b c of Fig 7, the contents of alkali-hydrolyzable nitrogen, available phosphorus and available potassium in soil decreased first and then increased. By 2023, the contents of alkali-hydrolyzable nitrogen, available phosphorus, available potassium and organic matter in BF treatment were better than those in B and OF treatment, while the increase

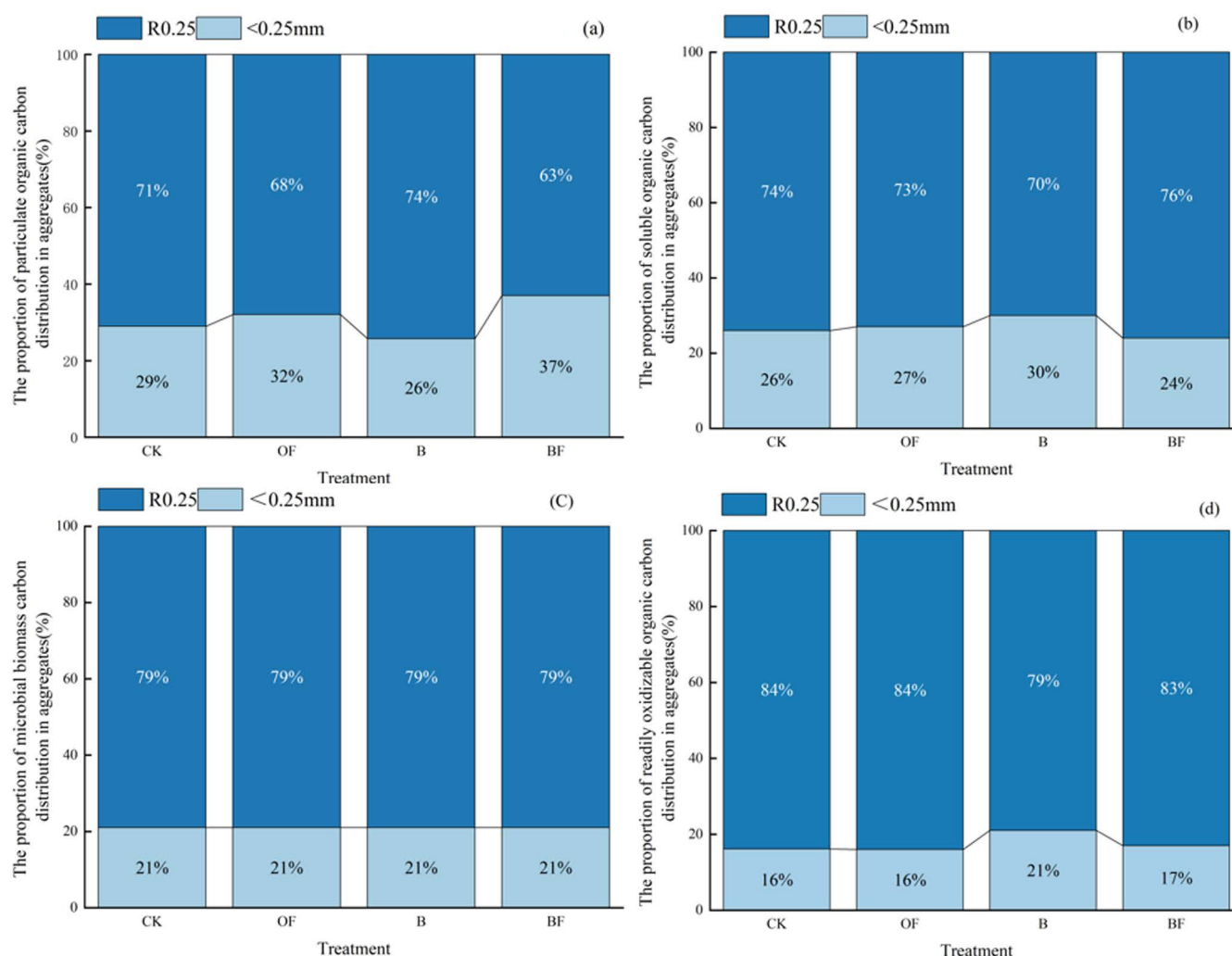


Fig 4. Distribution ratios of carbon components in aggregates of different grain sizes under different fertilization. (a) The proportion of particulate organic carbon distribution in aggregates (%); (b) The proportion of soluble organic carbon distribution in aggregates; (c) The proportion of microbial biomass carbon distribution in aggregates; (d) The proportion of readily oxidizable organic carbon distribution in aggregates.

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of available phosphorus content in OF treatment was better than that in B treatment, and the contents of alkali-hydrolyzable nitrogen, available potassium and organic matter in B treatment were better than those in OF treatment. Soil pH, compared with CK treatment, the other three treatments significantly increased pH. From 2021, the pH under OF, B, and BF treatments gradually increased. By 2023, each treatment showed B > BF > OF > CK.

3.7 Correlation between soil aggregates, organic carbon fractions and physical and chemical properties

As shown in Fig 8, the moisture content was significantly positively correlated with AK and AP, and negatively correlated with PAD and D; AP was significantly positively correlated with R0.25 and significantly negatively correlated with D; AK was positively correlated with R0.25 and negatively correlated with PAD. Soil moisture content was positively correlated with TOC, POC, DOC, ROC and IOC. pH, AN and AP were significantly positively correlated with POC and ROC, and significantly positively correlated with MBC and DOC. AK was significantly positively correlated with POC, ROC, IOC and DOC.

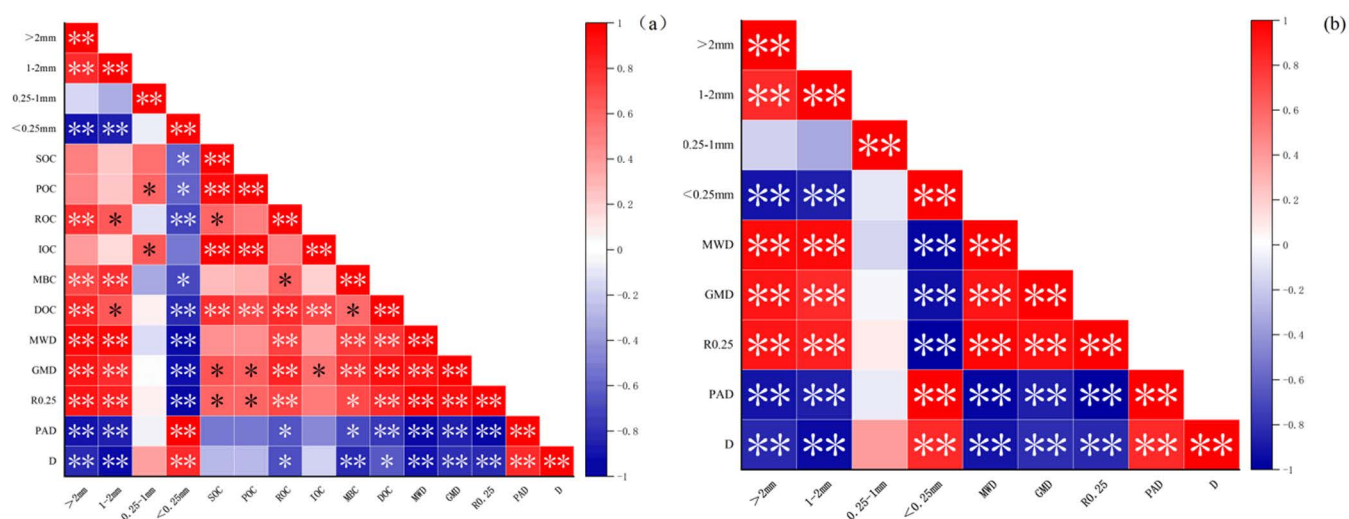


Fig 5. Correlation analysis between soil organic carbon fractions and aggregates and their stability. (a) correlation analysis of carbon composition and stability of aggregates; (b) correlation analysis of aggregate mass fraction and its stability.

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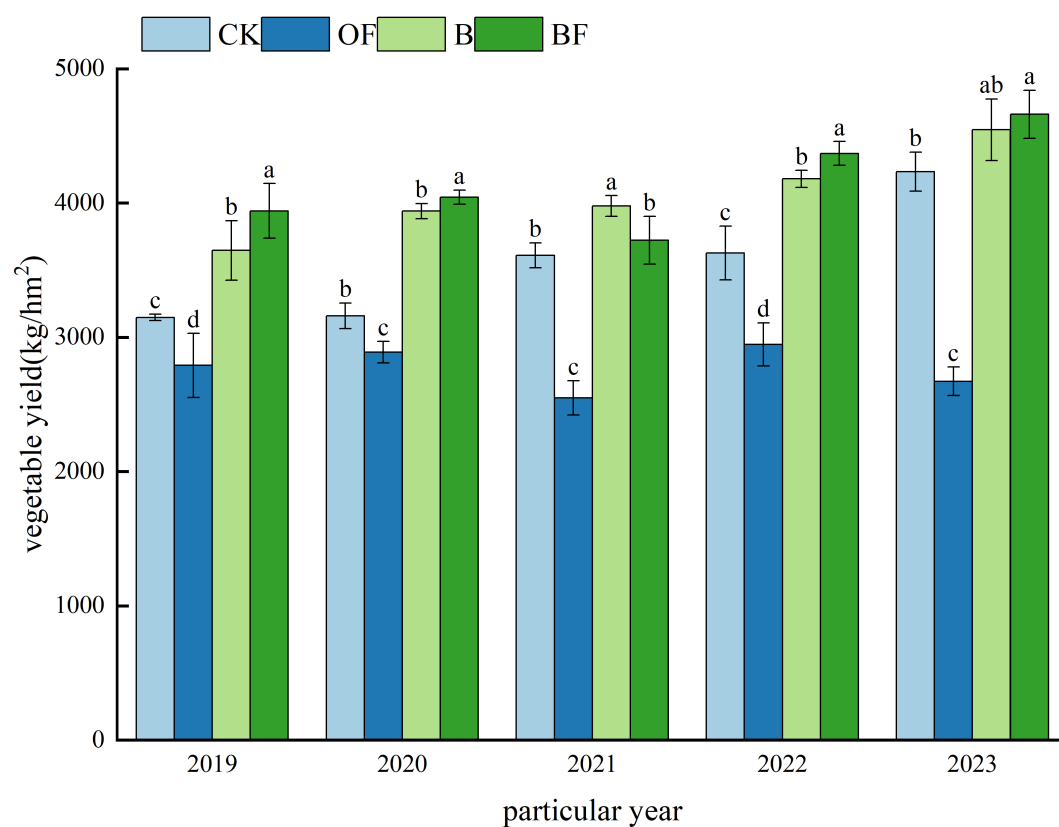


Fig 6. Vegetable yield under different treatments.

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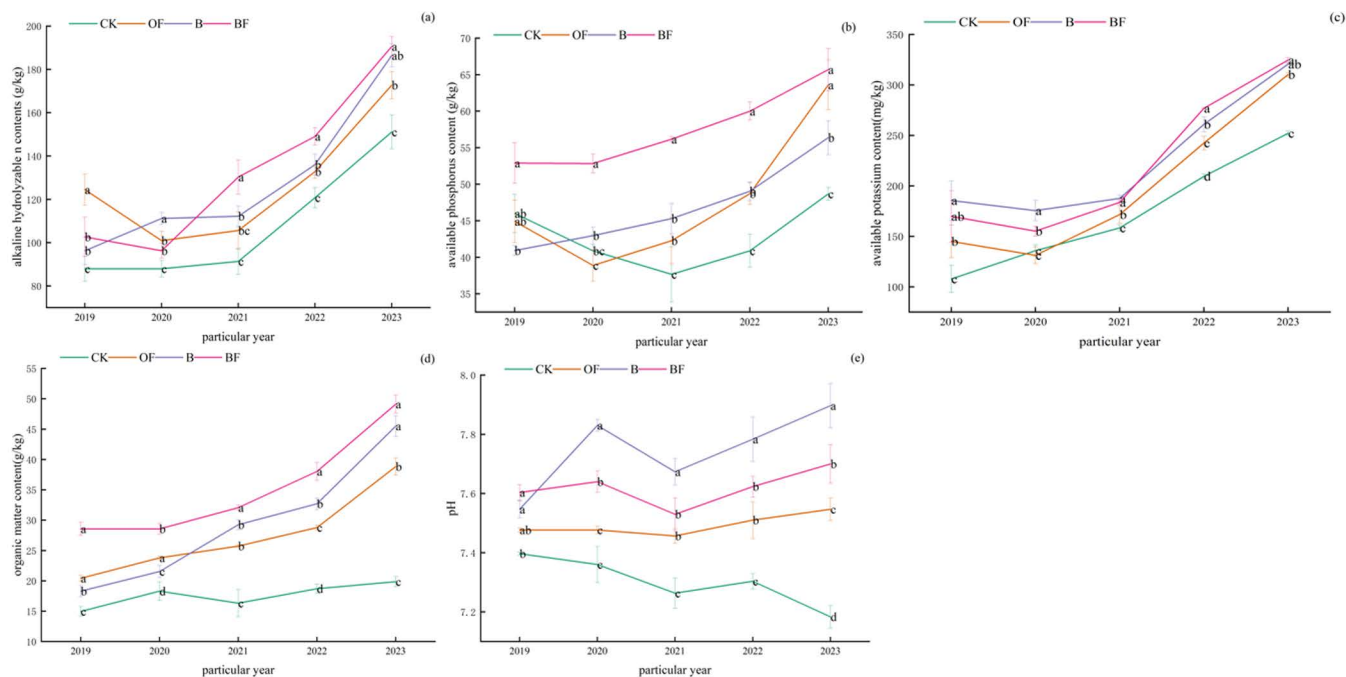


Fig 7. Dynamic changes of soil available nutrients. (a) alkaline hydrolysable n contents(g/kg);(b)available phosphorus content (g/kg); (c) available potassium content (mg/kg); (d) organic matter content (g/kg); (e) soil pH.

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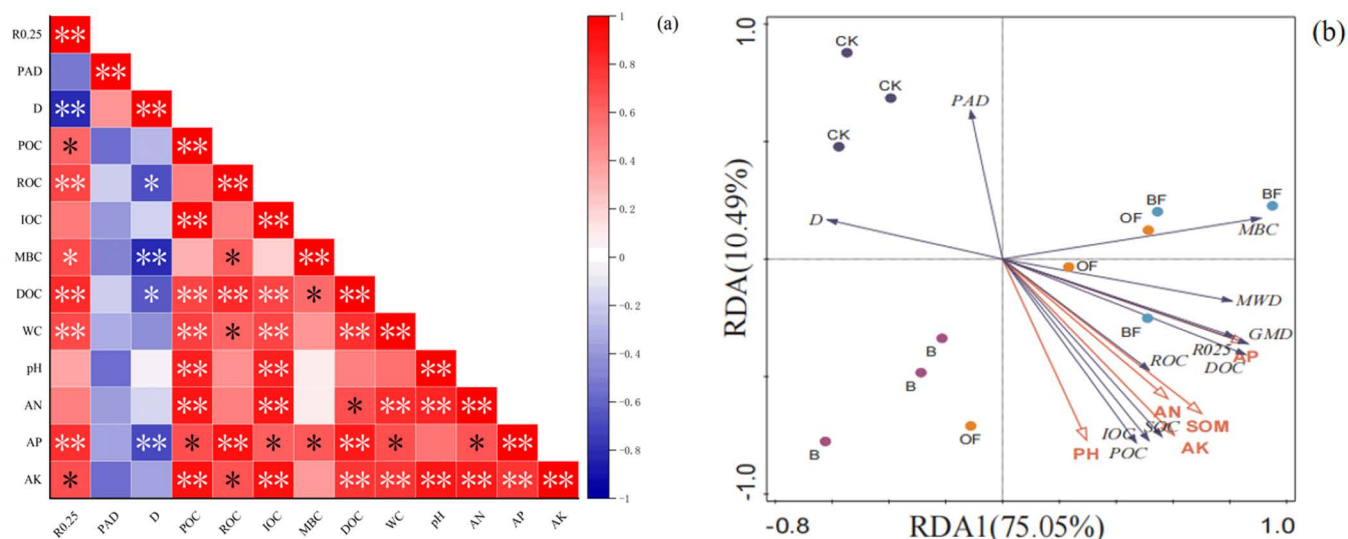


Fig 8. Correlation between aggregate stability and soil physical and chemical properties. (a) Correlation between aggregate stability and soil physical and chemical properties (b) Redundancy analysis of aggregate stability and soil physical and chemical properties.

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Correlation analysis showed that there was a strong correlation between soil physical and chemical properties and soil aggregates and organic carbon components. Therefore, redundancy analysis was carried out with soil physical and chemical properties as environmental variables and other indicators as response variables. The results could explain 85.54%. It can be seen from the figure that pH, AN, SOM, AK and AP were positively correlated with ROC, POC, DOC, MBC, IOC, TOC, R0.25, MWD and GMD, indicating that the increase of available nutrient content in soil contributed to the increase of soil organic carbon components. The pH, AN, SOM, AK and AP were negatively correlated with PAD and D, and AP and SOM were the main factors affecting the stability and carbon components of aggregates.

3.8 Effects of different fertilization treatments on the stability of soil organic carbon pool

[Table 4](#) shows the stability of soil organic carbon pool under different fertilization treatments. Compared with CK treatment, soil organic carbon storage increased significantly under OF, B and BF treatments, which increased by 29.73, 36.44 and 39.521000 kg/hm², respectively. B increased by 6.711000 kg/hm² compared with OF treatment, and the effect of combined application of the two was the best. BF increased by 9.791000 kg/hm² compared with OF treatment. The variation range of carbon pool activity index AI was 1.13~1.56. Compared with CK treatment, AI was significantly increased under BF treatment. The carbon pool index ranged from 1.26 to 1.96. Compared with CK treatment, the CPI under OF, B and BF treatments was significantly increased by 39.00%, 68.00% and 70.00%, respectively. It can be seen that when the CPI is increased, the effect of organic fertilizer and biochar is the best. The carbon pool management index ranged from 141.55 to 303.10. Compared with CK treatment, CPMI under OF, B and BF treatments was significantly increased by 140.49%, 66.29% and 161.55%, respectively. Among them, CPMI under B treatment was significantly lower than that under OF treatment, which was lower than that under OF treatment by 74.20%.

3.9 Comprehensive evaluation of different fertilization treatments on carbon pool characteristics and soil fertility in vegetable field

Principal component analysis was carried out on soil organic carbon components, soil aggregates, organic carbon storage, carbon pool activity index, carbon pool index, carbon pool management index and soil routine five items, and the principal components were extracted according to the standard of eigenvalue greater than 1 in [Table 5](#). The results showed that there were two principal components affecting the stability of soil carbon pool, as shown in [Table 6](#). The cumulative variance contribution rate was 90.24%, which had good representativeness and could be used to evaluate the effects of different fertilization treatments on the stability of soil carbon pool.

The first two principal components are selected, and the obtained feature vector is multiplied by the standardized data to obtain the principal component expression. According to the proportion of the eigenvalues corresponding to the principal component to the sum of the total eigenvalues of the extracted principal components, the principal component synthesis model is obtained. According to the principal component synthesis model, the comprehensive principal component value is calculated and sorted according to the comprehensive principal component value. As shown in [Table 6](#), the comprehensive scores of each treatment were ranked as BF treatment> OF treatment> B treatment> CK treatment, indicating

Table 4. Effects of different fertilization treatments on soil organic carbon pool characteristics.

Treatment	total soil organic carbon(1000 kg/hm ²)	AI	CPI	CPMI
CK	32.89±1.37c	1.13±0.11b	1.26±0.11c	141.55±2.02d
OF	62.62±2.84b	1.40±0.07ab	1.65±0.08b	282.04±2.16b
B	69.33±2.60ab	1.20±0.18b	1.94±0.11a	207.84±4.90c
BF	72.41±2.74a	1.56±0.23a	1.96±0.13a	303.10±8.32a

<https://doi.org/10.1371/journal.pone.0323028.t004>

Table 5. Coefficient and contribution rate of each index.

index	Principal component 1	Principal component 2
POC	0.89	0.39
DOC	0.93	-0.18
MBC	0.58	-0.63
ROC	0.81	-0.36
IOC	0.86	0.48
R _{0.25}	0.83	-0.41
<0.25mm	-0.83	0.41
total soil organic carbon	0.93	0.34
AI	0.59	-0.77
CPI	0.78	0.56
CPMI	0.94	-0.29
pH	0.75	0.55
AN	0.83	0.45
AP	0.92	-0.28
AK	0.95	0.26
SOM	0.97	-0.05
eigenvalue	11.41	3.03
variance contribution rate%	47.53	42.71
Cumulative contribution rate%	47.53	90.24

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Table 6. Common factor scores and comprehensive scores of different fertilization methods.

Treatment	Common factor score		comprehensive score	billing
	1st principal component	2nd principal component		
CK	-3.87	0.41	-267.86	4
OF	1.13	0.01	80.79	2
B	0.69	-0.71	36.05	3
BF	2.04	0.29	151.03	1

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that the combined application of organic fertilizer and biochar had the highest comprehensive evaluation in improving the stability of soil organic carbon pool and soil fertility.

4. Discussion

4.1 Effects of organic manure and biochar on soil carbon fractions, soil aggregates and stability

The proportion of water-stable soil aggregates, mean weight diameter, geometric mean diameter, aggregate destruction rate and fractal dimension of R0.25 are all important indicators for evaluating the degree of soil agglomeration [26]. This study found that organic substitution and application of biochar can significantly ($P < 0.05$) increase the content, geometric mean diameter and mean weight diameter of soil macroaggregates, and reduce aggregate destruction rate and fractal dimension [27]. The possible reason is that organic substitution accelerates the process of soil humification and promotes the cementation of aggregates. Biochar promotes the formation of aggregates by increasing organic matter, and because of the huge specific surface area and developed pore structure of biochar, it provides a rich living space for microorganisms and accelerates the accumulation of microbial necrosis to form stable aggregates [28]. Among them, the effect of biochar is better than that of organic fertilizer, which may be due to the low microbial activity of aggregates mediated

by biochar, which is not easy to be decomposed, while organic fertilizer is mainly formed by cementation. Aggregates have strong microbial activity, therefore, its ability to improve the stability of aggregates is lower than that of biochar [29]. Although both organic fertilizer and biochar can promote the formation of aggregates, the combination of biochar and organic fertilizer can best increase the content of soil macroaggregates in this study. The possible reason is that the two coordinate with each other to change the microbial community structure, accelerate the humification process, promote microbial metabolism and clay cementation of the bacteria themselves, and increase the content of macroaggregates and the stability of soil structure, which is consistent with the research conclusions of Song et al [30]. Correlation analysis showed that the particle size of aggregates was a significant factor affecting the stability of aggregates. The larger the aggregates, the stronger the stability. However, some studies show evidence with relevant citations have found that the higher the organic carbon content, the stronger the stability of aggregates.

4.2 Effects of organic fertilizer and biochar on vegetable yield and soil conventional five items

Vegetable yield is closely related to its genetic characteristics, soil type, nutrient status and climatic conditions. The supply of soil nutrients is one of the most important factors affecting yield [31]. Organic fertilizer instead of chemical fertilizer treatment and single application of biochar and organic fertilizer combined with biochar treatment can significantly increase vegetable yield [32]. This study found that the yield of vegetables was significantly reduced under the treatment of organic fertilizer completely replacing chemical fertilizer, while the yield was significantly increased under the treatment of single biochar and organic fertilizer combined with biochar. The slow release of organic fertilizer nutrients and the lack of coordination with the nutrients required for vegetable growth are the main reasons for the reduction of vegetable yield. The application of biochar increased vegetable yield, mainly due to the adsorption of nutrients by biochar, which increased the supply of nutrients required by plants [33]. The reason why the combined application of organic fertilizer and biochar can increase the yield of vegetables is that the interaction between biochar and organic fertilizer changes the soil physical and chemical conditions and microbial flora, thus improving the nutrient supply capacity [34]. There are also some reports that organic cultivation can reduce crop yield, depending on the fertilizer requirement characteristics of different crops and the length of the growth period [35]. Organic fertilizer completely replaces chemical fertilizer, that is, organic cultivation conditions can ensure crop yield. Under the condition of this experiment, the yield of organic fertilizer completely replacing chemical fertilizer was lower than that of the control chemical fertilizer treatment in 10 stubble experiments. The possible reason was that the growth period of leafy vegetables was shorter, and the organic fertilizer did not decompose and release enough nutrients during this period. In the organic substitution experiment of tomato and cucumber, our research group found that the biomass decreased in the early stage of growth, but it surpassed the single chemical fertilizer treatment in the later stage of yield formation. Therefore, the complete substitution of organic fertilizer for chemical fertilizer on crops with short growth period may reduce crop yield.

Soil available nutrients are important indicators to measure soil fertility. Organic fertilizer content, alkali-hydrolyzable nitrogen content, available phosphorus content, available potassium content and pH reflect soil nutrient status to a certain extent [36]. In this study, each treatment significantly increased the content of soil available nutrients, and changed significantly with time, showing BF treatment > B treatment > F treatment > CK. This is because with the input of organic carbon, the function of soil microorganisms was changed and the fixation of nutrients by microorganisms was accelerated [37]. In addition, since the input of organic carbon also accelerates the formation of soil aggregates, different nutrients are adsorbed inside the aggregates and further form large aggregates through cementation and other effects [38]. However, different carbon sources have different effects on the formation of aggregates, which may lead to different distribution of carbon sources and nutrients inside the aggregates, thereby affecting soil microecology. In this study, in general, the effect of biochar is better than that of organic fertilizer. This may be because biochar is a carbon-containing material formed by thermal cracking, which has a huge specific surface area and cation exchange capacity, and has an adsorption effect on nutrients.

4.3 Effects of organic fertilizer and biochar on carbon fractions in soil aggregates

DOC and EOC are important indicators of soil quality, and the increase in POC helps to improve soil quality [38]. In this study, the application of organic fertilizer and biochar changed the content of carbon components in aggregates with different particle sizes. Among them, BF significantly increased the content of POC and ROC in macroaggregates, while organic substitution and biochar application decreased the content of DOC in macroaggregates, which was basically consistent with the research conclusions of [28]. The reason is that the input of exogenous carbon source will increase the excitation effect of soil and accelerate the mineralization and decomposition of soil organic carbon. However, different carbon sources have different abilities to promote mineralization and decomposition. Stable carbon can promote the decomposition of organic carbon. In this study, the interaction between organic fertilizer and biochar improves soil function and enhances carbon sequestration. Organic substitution accelerates the consumption of organic carbon due to the high activity of microorganisms and reduces the sequestration of organic carbon. The application of biochar accelerates the decomposition of soil organic carbon and changes the content of POC and DOC to a certain extent [39] found that the application of organic fertilizer and biochar could significantly increase the content of easily oxidizable organic carbon, while Chen et al.'s research results showed that the application of organic fertilizer and biochar could reduce the content of easily oxidizable organic carbon. The contradictory conclusion may be due to the different cultivated crops, soil types and climatic conditions. In this study, the application of organic fertilizer and biochar reduced the content of easily oxidized organic carbon, because the application of organic fertilizer and biochar stimulated the activity of microorganisms, accelerated the reproduction of microorganisms and the consumption of easily oxidized organic carbon. The correlation analysis showed that the particle size of aggregates would affect the soil carbon components, which was similar to the conclusion of [40]. In summary, aggregates promoted the sequestration of organic carbon, but due to different carbon source inputs, the internal carbon components of different aggregates were also significantly different.

4.4 Effects of organic fertilizer and biochar on carbon storage and carbon pool characteristics

The application of organic fertilizer and biochar can change carbon components and increase soil carbon sequestration. In this study, the application of organic fertilizer and biochar significantly increased soil organic carbon content and increased carbon storage. The effect of biochar application was better than that of organic fertilizer, which was consistent with the research results of [41]. Exogenous carbon input is a measure to increase soil organic carbon, and the application of organic fertilizer and biochar can increase carbon storage. However, organic fertilizer is mainly rich in active organic carbon microorganisms, while biochar is mainly rich in stable organic carbon, which is not easy to be utilized by microorganisms. Therefore, the effect of biochar on improving organic carbon storage is better than that of organic fertilizer. Soil carbon pool management index and carbon pool activity index can sensitively reflect the dynamic changes of soil carbon [42]. Previous studies have shown that carbon pool management index and activity index are closely related to tillage measures and fertilization methods [43]. In this study, compared with single application of chemical fertilizer, the application of organic fertilizer and biochar increased the carbon pool management index and activity index, and the effect of organic fertilizer on carbon pool management index and activity index was better than that of biochar. This may be because organic fertilizer is mainly rich in active organic carbon and has high microbial utilization, while the combination of organic fertilizer and biochar has the best effect on improving carbon pool management index and activity index. This is because the application of organic fertilizer and biochar changed the soil carbon composition and increased the content of soil total organic carbon and active organic carbon [44], while the carbon pool management index was closely related to soil total organic carbon and easily oxidized organic carbon, and increased with the increase of soil organic carbon [45]. In addition, the interaction between biochar and organic fertilizer improved the soil microenvironment and increased microbial activity, so the carbon pool management index and activity index were significantly improved. Biochar is superior to organic fertilizer in improving carbon pool index, which is mainly related to the properties of biochar and organic fertilizer. Biochar contains many aromatic functional groups and is not easy to decompose, while organic fertilizer is mainly rich in active organic carbon such as glucose, and microbial utilization is large. The degree of mineralization

and decomposition of organic fertilizer is much higher than that of biochar. Therefore, the ability of biochar to improve carbon pool index is significantly better than that of organic fertilizer, but the combined application of biochar and organic fertilizer has the best effect. This is mainly because the combined application of biochar and organic fertilizer significantly changes microbial activity, increases microbial necrosis, agglomeration and cementation, and promotes the increase of soil carbon storage by increasing aggregate [46]. Therefore, we can see. Because biochar and organic fertilizer provide different carbon sources, soil carbon pool stability and carbon pool activity have different responses to different carbon sources. Active organic carbon enhances carbon pool activity, while inert organic carbon enhances carbon pool index, and the combination of the two has the best effect on increasing carbon storage. From the perspective of conducive to the conservation and fertilization of cultivated land, the combined application of biochar and organic fertilizer has the best effect on improving cultivated land fertility and soil carbon pool [47]. Principal component analysis was used to comprehensively evaluate each index, and the effects of different fertilization treatments on carbon pool characteristics and soil fertility were comprehensively analyzed. The results showed that compared with conventional fertilization, the application of biochar and organic fertilizer could improve soil carbon pool characteristics and soil fertility. The effect of organic fertilizer was better than that of biochar, and the combined application of the two had the best effect, which was consistent with the results of previous studies [20].

5. Conclusion

The application of biochar and organic fertilizer reorganized soil organic carbon from different components. Organic fertilizer improved the characteristics of carbon pool through the formation and development of large aggregates mediated by DOC and MBC, enhanced the aggregation ability of organic carbon components in aggregates, and promoted soil carbon sequestration in vegetable fields. Biochar itself is stable carbon and directly constitutes carbon sequestration. The combination of organic fertilizer and biochar has the optimum effect on soil fertilization and carbon sequestration.

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Supporting information

S1 Data.
(XLSX)

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