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Comparison of long-term effects of biochar application on soil organic carbon and its fractions in two ecological sites in karst regions

Minghua Jiang [a,b], Caibin Li [c], Weichang Gao [d], Kai Cai [d], Yuan Tang [e,*], Jianzhong Cheng [a,*]

[a] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, Guizhou Province, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Bijie Tobacco Company of Guizhou Province, Bijie 551700, Guizhou Province, China
[d] Upland Flue-Cured Tobacco Quality & Ecology Key Laboratory of China Tobacco, Guizhou Academy of Tobacco Science, Guiyang 550081, Guizhou Province, China
[e] School of Public Health, Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, Guizhou Province, China

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ABSTRACT

Biochar (BC) is widely used in soil to increase carbon sequestration. However, the long-term effects of BC application on the soil organic carbon (SOC) and labile organic carbon (LOC) fractions are unclear, especially in karst regions. In order to address this issue, two field experiments were designed to observe the changes in the SOC and its fractions after two years of BC application. In this study, we chose karst regions with two different ecological types, Linquan and Heishitou towns in Bijie City, China. Five BC treatment plots were established, with application rates of 0, 5, 15, 20, and 40 t·ha^{-1} (CK, B_5, B_{15}, B_{20}, and B_{40}, respectively). Compared with CK, the contents of SOC, readily oxidizable organic carbon (ROC), particulate organic carbon (POC) and recalcitrant carbon (RC) under the BC treatments in Linquan increased by 7.09–38.08%, 1.62–39.80%, 9.52–62.30% and 8.66–58.14%, respectively. In Heishitou, the increases in these contents were 3.60–38.58%, 4.40–115.23%, 19.57–50.98% and 10.94–53.87%, respectively. There are significant positive relationships between the SOC content and the ROC, POC and RC contents. However, the application of BC had no significant effect on the DOC content in the two ecological regions. In addition, the soil carbon pool index (CPI) and carbon pool management index (CPMI) increased with increasing BC application rate due to its unique physicochemical properties and nutrients. These results indicate that long-term BC amendment significantly improves the soil quality and carbon sequestration in karst regions by increasing SOC and its LOC fractions.

1. Introduction

As global warming progresses, soil organic carbon (SOC) has attracted more and more attention worldwide (Zhang et al., 2020). SOC represents the largest reservoir of organic carbon in the terrestrial biosphere, and it plays an important role in the global carbon cycle (Luo et al., 2019; Shedayi et al., 2016). In addition, the mineralization of SOC drives fundamental biogeochemical processes and influences soil nutrients and crop production (Cai et al., 2016). Thus, it has been suggested that SOC sequestration is of fundamental importance in agricultural soils, because it transfers and stores atmospheric carbon dioxide (CO_2) into the soil and enhances soil fertility (Chen et al., 2019). Therefore, increasing the SOC content is crucial for reducing the atmospheric CO_2 concentration and greenhouse effect.

Biochar (BC), a carbon-rich solid product, is produced by thermal decomposition of biomass under oxygen-limited conditions at relatively low temperatures (≤700 °C) (Bi et al., 2017). The pyrolysis of BC is characterized by a high carbon content, large surface area, high porosity, and high thermal stability. BC technology converts plant organic matter into stable carbon, which can be returned to the soil, reducing the release of CO_2 into the atmosphere during biomass decomposition and increasing the carbon sequestration in the soil (Lehmann, 2007; Lefebvre et al., 2020). Therefore, the effect of BC on SOC has received considerable critical attention. However, small or short-term changes in SOC are not easily monitored because of the large variable carbon background of the soil itself (Yang et al., 2018). Labile organic carbon (LOC) is the more active component of SOC, and it includes particulate organic carbon (POC), readily oxidizable organic

* Corresponding authors.
E-mail addresses: tangyuan@vip.gyig.ac.cn (Y. Tang), chengjianzhong@vip.gyig.ac.cn (J. Cheng).

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carbon (ROC), and dissolved organic carbon (DOC) (Sheng et al., 2015). Therefore, it is very important to evaluate the effect of BC on the soil LOC for carbon sequestration and emission reduction in farmland. Previous studies have shown that BC application can affect the SOC content and LOC fractions. In the history of continuous tomato planting field, the ROC content of soil after rice straw BC treatment was found to be significantly higher than that of the CK (Zhang et al., 2018a). However, the change of ROC content in the soil amended with Chinese chestnut wood BC was not apparent (Zhang et al., 2018b). Yang et al. (2018) found that in a 3-year field trial, the application of corn stover BC reduced the DOC content. However, Li et al. (2018) reported that low temperature BC significantly increased soil DOC content, while high temperature BC significantly decreased its concentration. Currently, there is no consensus regarding the effect of BC application on the SOC fractions. Recently, meta-analysis revealed that the decomposition rate of BC decreases exponentially with time, and the decomposition rate of BC within 0.5 years (average rate of 0.023%) is 4 times that over 1 year (average rate of 0.005%) (Wang et al., 2016). Therefore, the influence of BC on the SOC and LOC is related to the type of BC, application duration and environmental conditions.

At present, a large number of studies have focused on the short-term response of SOC to BC application, with typical study periods of less than 1 year. There is a lack of field studies on the long-term effects of BC on the SOC fractions, especially in karst regions. Karst regions are known to be sensitive to land degradation caused by human interference, such as agricultural activities (Pornaro et al., 2018). During the past few decades, most karst areas have been degraded due to SOC loss and soil erosion (Liu and Han, 2020). Theoretically, BC amendment of soil could mitigate climate change by enhancing soil carbon storage, improving the soil quality, and increasing plant growth. However, our knowledge of the impact of BC amendment on the SOC and its fractions in karst regions is limited.

Therefore, we selected two field sites with different ecological environmental conditions and evaluated the effects of BC application on the SOC and its fractions in karst regions in southwestern China after two years. Specifically, the effects of long-term BC application on the physicochemical properties of the two ecological sites were studied, as well as relationships between the SOC and its LOC fractions.

2. Materials and methods

2.1. BC preparation

The BC was provided by Guizhou Jinyefeng Agricultural Technology Co., Ltd., China, and was prepared using tobacco stalks as the substrate at 380 °C for 2 h with a limited supply of oxygen. The main properties of the BC are as follows: a total carbon content (TC) of 483.00 $g\cdot kg^{-1}$, a total nitrogen content (TN) of 15.00 $g\cdot kg^{-1}$, a total phosphorus content (TP) of 2.38 $g\cdot kg^{-1}$, a total potassium content (TK) of 24.37 $g\cdot kg^{-1}$, a pH of 9.18, surface area of 1. 47 $m^2\cdot g^{-1}$, and a mean pore diameter of 20.31 nm.

2.2. Field experiments

The two main tobacco-planting regions are situated in Bijie City, Guizhou Province, China. The first ecological region is located in Linquan Town (27°12′N, 105°24′E, 1319 m) in Qianxi County, Guizhou Province. This region is characterized by a subtropical monsoon climate, with a mean annual precipitation of approximately 1087 mm and a mean annual temperature of 14 °C. The soil type is yellow soil in the Genetic Soil Classification of China (GSCC), equivalent to Alisols in the World Reference Base for Soil Resources (WRB), with an SOC of 14.32 g kg^{-1}, a TN of 1.85 g kg^{-1}, and a pH of 6.47.

The second ecological region is located in Heishitou Town (26°45′N, 104°00′E, 2120 m) in Weining County, Guizhou Province, China. This region is also characterized by a subtropical monsoon climate, with a mean annual precipitation of approximately 926 mm and a mean annual temperature of 12 °C. The soil type is yellow-brown soil in the GSCC, equivalent to Alisols in the WRB, with an SOC of 14.18 g kg^{-1}, a TN of 1.24 g kg^{-1}, and a pH of 5.59.

2.3. Experimental design

The BC was mixed into the 0–20 cm soil layer at one time in May 2018. Five BC treatments were set up in two ecological sites, with application rates of 0, 5, 15, 20, and 40 t ha^{-1} (CK, B_5, B_{15}, B_{20}, and B_{40}, respectively). Each treatment was replicated three times in a randomized block design, i.e., a total of 15 plots. The area of each plot in Linquan was 67 m^2. Each plot was 8.7 m long × 7.7 m wide, and consisted of seven rows, with a 1.1 m inter row spacing. Fifteen tobacco plants were planted in each row, with a plant spacing of 0.55 m, and 105 tobacco plants were planted in each plot. The area of each plot in Heishitou was 74.8 m^2. Each plot was 11 m long × 6.8 m wide and consisted of ten rows, with a 1.1 m inter row spacing. Twelve tobacco plants were planted in each row, with a plant spacing of 0.55 m, and 120 tobacco plants were planted in each plot.

Flue-cured tobacco is transplanted in May and harvested in August every year. Fertilization and field management were implemented in accordance with local requirements for high-quality tobacco cultivation. The fertilization schedule of the Linquan site included 750 kg ha^{-1} of distiller's grain organic fertilizer as the base fertilizer and 37.5 and 300 kg ha^{-1} of compound fertilizer as seedling fertilizer and topdressing fertilizer, respectively. The fertilization schedule of the Heishitou site included 1800 kg ha^{-1} of distiller's grain organic fertilizer as the base fertilizer and 37.5 and 330 kg ha^{-1} of compound fertilizer as seedling fertilizer and topdressing fertilizer, respectively.

2.4. Soil sampling and analysis

Soil samples from the upper 0–20 cm of soil were collected in August 2020, and samples from five sites in each plot were mixed thoroughly to generate a soil sample. The soil samples were ground, air-dried, and sieved (100-mesh) to remove the gravel and roots. After removing the soil inorganic carbon using 2 M HCI, the SOC was analyzed using an elemental analyzer (Vario Elemental Analyzer, Germany). The DOC was determined using the extraction method with a soil-water ratio of 1:5 for the TOC instrument (Ghani et al., 2003). The ROC was determined using a spectrophotometer and the potassium permanganate oxidation method (Blair et al., 1995a). The POC was extracted using 5 g L^{-1} $(NaPO_3)_6$ solution and was then determined using an elemental analyzer (Wei et al., 2016). The RC was determined using an elemental analyzer after acid hydrolysis of 6 M HCL (Schwendenmann and Pendall, 2008). In addition, the detailed analytical procedures and methods used to analyze the soil organic matter (SOM), TN, hydrolyzed nitrogen (HN), TP, available phosphorus (AP), TK, available potassium (AK), and pH are described by Long et al. (2011).

2.5. Data analysis

The data analysis was performed in Origin 2017 and SPSS version 21.0. The data are presented as the average of three measurements and the standard error. The significances of the different BC treatments were tested using one-way analysis of variance (ANOVA) and the least significant difference (LSD) with a 95% confidence level. The Carbon pool management index (CPMI) was calculated as follows (Demisie et al., 2014):

Carbon pool index (CPI): CPI = SOCs/SOCc, (1)

C lability (L): L = (ROC)/(SOC − ROC), (2)

Lability index (LI): LI = Ls/Lc, (3)

Carbon pool management index (CPMI): CPMI = CPI x LI x 100. (4)

In Eqs. (1–4), SOCs and SOCc are the SOC concentrations of the BC treatments and CK, respectably; and Ls and Lc are the C labilities of the

BC treatments and CK, respectively.

3. Results

3.1. Physical and chemical properties of the soil

As is shown in Table 1, the SOM content increased as the amount of BC increased in the two ecological regions. In Linquan, compared with CK, the SOM contents of the B_5, B_{15}, B_{20}, and B_{40} treatments increased by 5.52%, 13.84%, 10.05%, and 22.95%, respectively. In Heishitou, BC addition significantly increased the soil pH from 6.03 in CK to 6.56 in the B_{40} treatment. The SOM contents also increased by 10.84%, 29.69%, 56.79%, and 67.80% in the B_5, B_{15}, B_{20}, and B_{40} treatments, respectively. Compared with CK, the TN, TP, AP, TK, and AK contents of the B_{40} treatment increased by 41.12%, 3.30%, 20.80%, 8.26%, and 143.98%, respectively. However, there were no significant differences in the TN, TP, AP, TK, and AK contents and the pH values among the different treatments in Linquan.

3.2. Effect of BC on SOC and its fractions

The BC application increased the SOC content in both ecological sites, especially for the higher application rates (Fig. 1). Compared with CK, the SOC contents in Linquan increased by 7.09%, 5.56%, and 38.08% in the B_{15}, B_{20}, and B_{40} treatments, respectively, whereas the SOC contents in Heishitou increased by 22.71%, 24.85%, and 38.58% in the B_{15}, B_{20}, and B_{40} treatments, respectively. However, there were no significant differences in the SOC contents of the B_5, B_{15}, B_{20}, and CK treatments in the two ecological regions.

Compared with CK, the ROC contents in Linquan increased by 6.80%, 1.62%, 4.35%, and 39.80% in the B_5, B_{15}, B_{20}, and B_{40} treatments, respectively, whereas the ROC contents in Heishitou increased by 4.40%, 35.42%, 79.81%, and 115.23%, respectively (Fig. 2a). Similarly, the application of BC generally increased the POC contents in the two ecological regions (Fig. 2c). Compared with CK, the POC contents of the B_{15}, B_{20}, and B_{40} treatments in Linquan increased by 9.52%, 25.79%, and 62.30%, respectively, whereas the POC contents in Heishitou increased by 25.80%, 30.38%, 19.57%, and 50.98%, respectively. The RC content increased with increasing BC application rate. There were significant differences between the RC contents of the B_{40} treatment and the other treatments in the two ecological sites (Fig. 2d). However, the application of BC did not have a significant effect on the DOC contents in the two ecological regions (Fig. 2b).

3.3. Relationships between SOC and its fractions in the two ecological sites

The ROC/SOC ratio increased with increasing BC application in Heishitou. However, BC addition did not have a significant effect on the ROC/SOC ratio in Linquan (Table 2). There is a significant correlation between the ROC content and the SOC content in the two ecological regions ($R^2 = 0.51^{**}$ in Linquan; $R^2 = 0.65^{**}$ in Heishitou, $p < 0.01$) (Fig. 3a). However, there were no significant correlations between the DOC and SOC contents (Fig. 3b). Compared with CK, the DOC/SOC ratios in Linquan decreased by 18.03%, 1.64%, 18.03% and 11.48% in B_5, B_{15}, B_{20}, and B_{40} treatments, respectively, but the DOC/SOC ratios in Heishitou increased by 60.42%, 4.17%, 68.75%, and 85.42%, respectively. In addition, there was a significant correlation between the POC and SOC contents ($R^2 = 0.58^{**}$ in Linquan; $R^2 = 0.52^{**}$ in Heishitou, $P < 0.01$) (Fig. 3c). However, none of the BC applications in any of the treatments had significant effects on the POC/SOC and RC/SOC ratios.

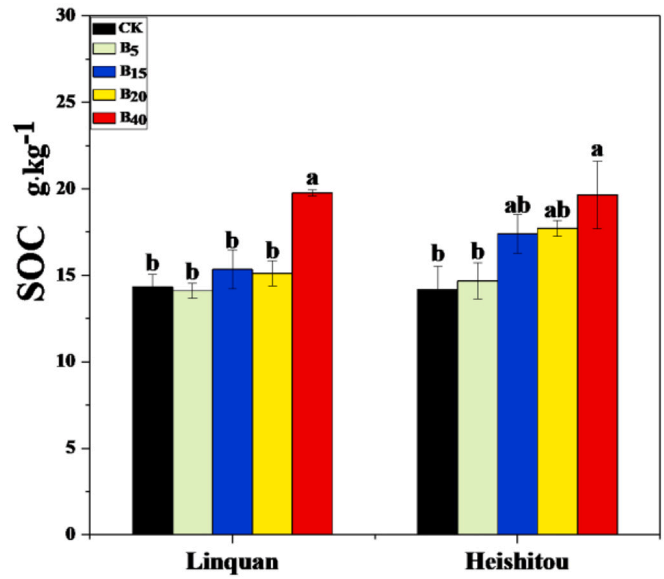

Fig. 1. Effects of BC on the SOC in the two ecological sites. Significant differences in the means are denoted by different letters ($p < 0.05$).

3.4. Carbon pool management index (CPMI)

Compared with CK, the application of BC in Linquan increased the CPI and CPMI by 7.00–38.00% and 2.60–39.23%, respectively (Table 3). Moreover, the B_{40} treatment significantly improved the CPI and CPMI, but the values of L and LI values were not significantly different among the treatments.

In Heishitou, the L, LI, CPI, and CPMI increased with increasing BC application rate. Compared with CK, the application of BC increased L, LI, CPI, and CPMI by 16.60–105.50%, 2.00–106.00%, 4.00–39.00%, and 6.06–176.95%, respectively. There were significant differences in the L, LI, CPI, and CPMI of the B_{40} treatment and CK.

Table 1
Chemical properties of the soil under the different BC treatments in the two ecological sites.

		TN ($g\ kg^{-1}$)	HN ($mg\ kg^{-1}$)	TP ($g\ kg^{-1}$)	AP ($mg\ kg^{-1}$)	TK ($g\ kg^{-1}$)	AK ($mg\ kg^{-1}$)	SOM ($g\ kg^{-1}$)	pH
Linquan	CK	1.85 ± 0.01^{a}	102.64 ± 1.86^{a}	1.33 ± 0.01^{ab}	75.15 ± 13.30^{ab}	27.93 ± 0.12^{b}	703.96 ± 48.45^{b}	24.27 ± 1.34^{a}	6.47 ± 0.61^{a}
	B_5	1.71 ± 0.01^{a}	101.24 ± 0.47^{a}	1.23 ± 0.01^{ab}	60.15 ± 6.89^{b}	31.03 ± 0.11^{a}	736.57 ± 30.13^{ab}	25.61 ± 1.78^{a}	5.97 ± 0.23^{a}
	B_{15}	1.81 ± 0.01^{a}	99.96 ± 2.94^{a}	1.10 ± 0.00^{b}	62.52 ± 11.38^{b}	23.90 ± 0.39^{b}	677.23 ± 62.41^{b}	27.63 ± 2.90^{a}	6.21 ± 0.42^{a}
	B_{20}	1.75 ± 0.01^{a}	105.44 ± 2.60^{a}	$1.22\pm0.00a^{b}$	51.63 ± 2.89^{b}	33.17 ± 0.13^{b}	703.13 ± 50.39^{b}	26.71 ± 1.04^{a}	6.18 ± 0.33^{a}
	B_{40}	1.96 ± 0.02^{a}	102.65 ± 3.07^{a}	1.40 ± 0.01^{a}	92.00 ± 4.61^{a}	28.43 ± 0.32^{b}	861.79 ± 14.02^{a}	29.84 ± 1.11^{a}	6.72 ± 0.43^{a}
Heishitou	CK	1.24 ± 0.01^{c}	94.24 ± 6.96^{a}	1.21 ± 0.01^{a}	36.44 ± 2.45^{b}	17.67 ± 0.15^{a}	335.47 ± 15.54^{d}	22.24 ± 1.38^{c}	5.59 ± 0.11^{c}
	B_5	1.33 ± 0.01^{b}	91.9 ± 1.23^{a}	1.24 ± 0.01^{a}	36.68 ± 2.65^{b}	16.77 ± 0.24^{a}	401.26 ± 42.12^{c}	24.65 ± 0.79^{c}	6.03 ± 0.14^{b}
	B_{15}	1.44 ± 0.01^{ab}	94.4 ± 10.20^{a}	1.23 ± 0.01^{a}	35.92 ± 1.95^{b}	19.03 ± 0.05^{a}	504.92 ± 31.52^{c}	28.62 ± 2.31^{bc}	6.15 ± 0.18^{b}
	B_{20}	1.74 ± 0.02^{ab}	88.41 ± 2.75^{a}	1.22 ± 0.01^{a}	39.35 ± 1.18^{ab}	19.50 ± 0.07^{a}	667.23 ± 11.58^{b}	34.87 ± 4.51^{ab}	6.27 ± 0.13^{ab}
	B_{40}	1.75 ± 0.01^{a}	87.95 ± 4.21^{a}	1.25 ± 0.01^{a}	44.02 ± 1.29^{a}	19.13 ± 0.12^{a}	818.51 ± 52.70^{a}	37.32 ± 2.01^{a}	6.56 ± 0.04^{a}

The values are expressed as mean ± standard error. *TN* - total N content; *HN* - hydrolysis N content; *TP* - total P content; *AP* - available P content; *TK* - total K content; *AK* - available K content; *SOM* - soil organic matter content. The different letters in the columns represent a significant difference ($p < 0.05$).

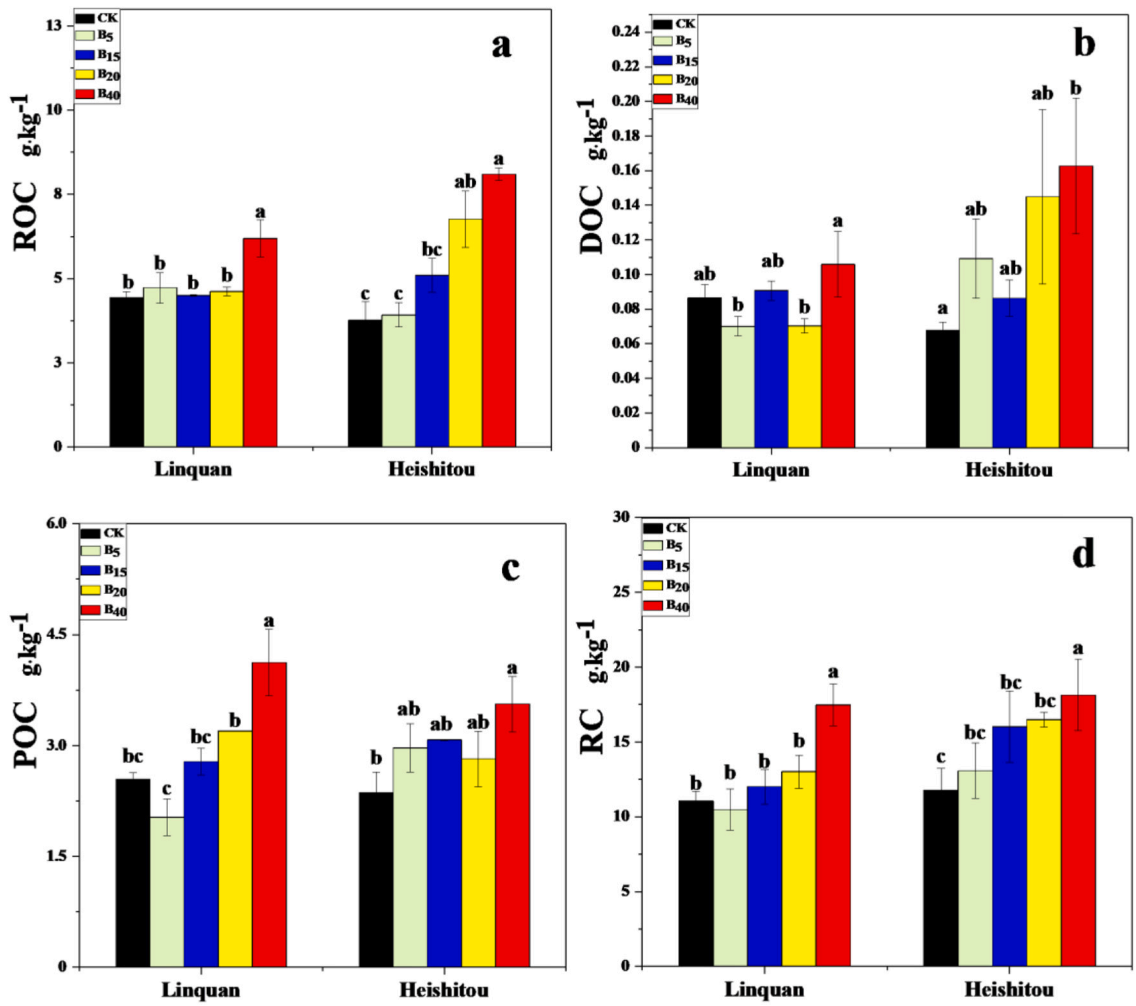

Fig. 2. Effects of BC on the (a) readily oxidized organic C (ROC), (b) dissolved organic C (DOC), (c) particulate organic C (POC), and (d) recalcitrant carbon (RC). Significant differences in the means are denoted by different letters ($p < 0.05$).

Table 2
Effect of BC on labile organic C fractions (%) in two ecological sites.

	Treatments	ROC/SOC	DOC/SOC	POC/SOC	RC/SOC
Linquan	CK	31.16 ± 2.50^{a}	0.61 ± 0.07^{a}	17.76 ± 0.28^{ab}	$77.16 \pm$ 0.02a
	B_5	33.42 ± 2.45^{a}	0.50 ± 0.04^{a}	14.34 ± 1.47^{b}	73.86 ± 0.08^{a}
	B_{15}	29.65 ± 2.11^{a}	0.60 ± 0.06^{a}	18.39 ± 1.96^{ab}	78.12 ± 0.03^{a}
	B_{20}	30.80 ± 2.31^{a}	0.50 ± 0.04^{a}	21.23 ± 1.05^{a}	85.75 ± 0.03^{a}
	B_{40}	31.28 ± 2.63^{a}	0.54 ± 0.10^{a}	20.83 ± 2.19^{a}	88.54 ± 0.08^{a}
Heishitou	CK	26.24 ± 1.58^{c}	0.48 ± 0.02^{a}	16.96 ± 1.71^{a}	82.65 ± 0.03^{a}
	B_5	26.70 ± 1.09^{c}	0.77 ± 0.21^{a}	20.52 ± 3.28^{a}	88.92 ± 0.01^{a}
	B_{15}	29.25 ± 1.96^{bc}	0.50 ± 0.04^{a}	17.84 ± 1.25^{a}	93.61 ± 0.16^{a}
	B_{20}	38.23 ± 4.61^{ab}	0.81 ± 0.26^{a}	16.06 ± 2.53^{a}	93.17 ± 0.01^{a}
	B_{40}	41.92 ± 3.60^{a}	$0.89 \pm 0.0.2^{a}$	18.20 ± 1.19^{a}	92.10 ± 0.06^{a}

The abbreviations are the same as in Table 1. The different letters indicate significant differences ($p < 0.05$).

4. Discussion

4.1. Effect of BC on SOC in two sites in a karst region

As the BC application rate increased, the SOC contents gradually increased in the two ecological regions, which is consistent with the results of previous studies (Yang et al., 2018; Sheng et al., 2015; Liu et al., 2018). The reason for this phenomenon is that BC is a carbon-rich material and contains a large amount of aromatic organic matter. Thus, BC has a strong ability to resist degradation under long-term environmental actions and to increase the SOC content (Spokas, 2010). In addition, BC may also increase the microbial quantity of crop roots. This promotes crop growth and atmospheric CO_2 fixation to recharge the SOC (Azeem et al., 2019). Except for the B_{40} treatment, there were no significant differences between the BC treatments and CK in the two ecological sites. This phenomenon is consistent with the results of previous studies (Gong et al., 2009; Shanthi et al., 2013) i.e., that BC amendment does not significantly increase the SOC contents of soils with high SOC backgrounds, but it significantly increases the SOC contents of soils with low SOC backgrounds. When the SOC background value is high, it is insensitive to changes caused by agricultural management measures at low BC application rates. In this study, the BC–C addition ratios of the B_5 B_{15}, B_{20}, and B_{40} treatments were calculated to be 0.71, 2.13, 2.84, and 5.68 g C·kg^{-1} in the upper 20 cm of topsoil. However, the initial SOC contents of the Linquan and Heishitou sites

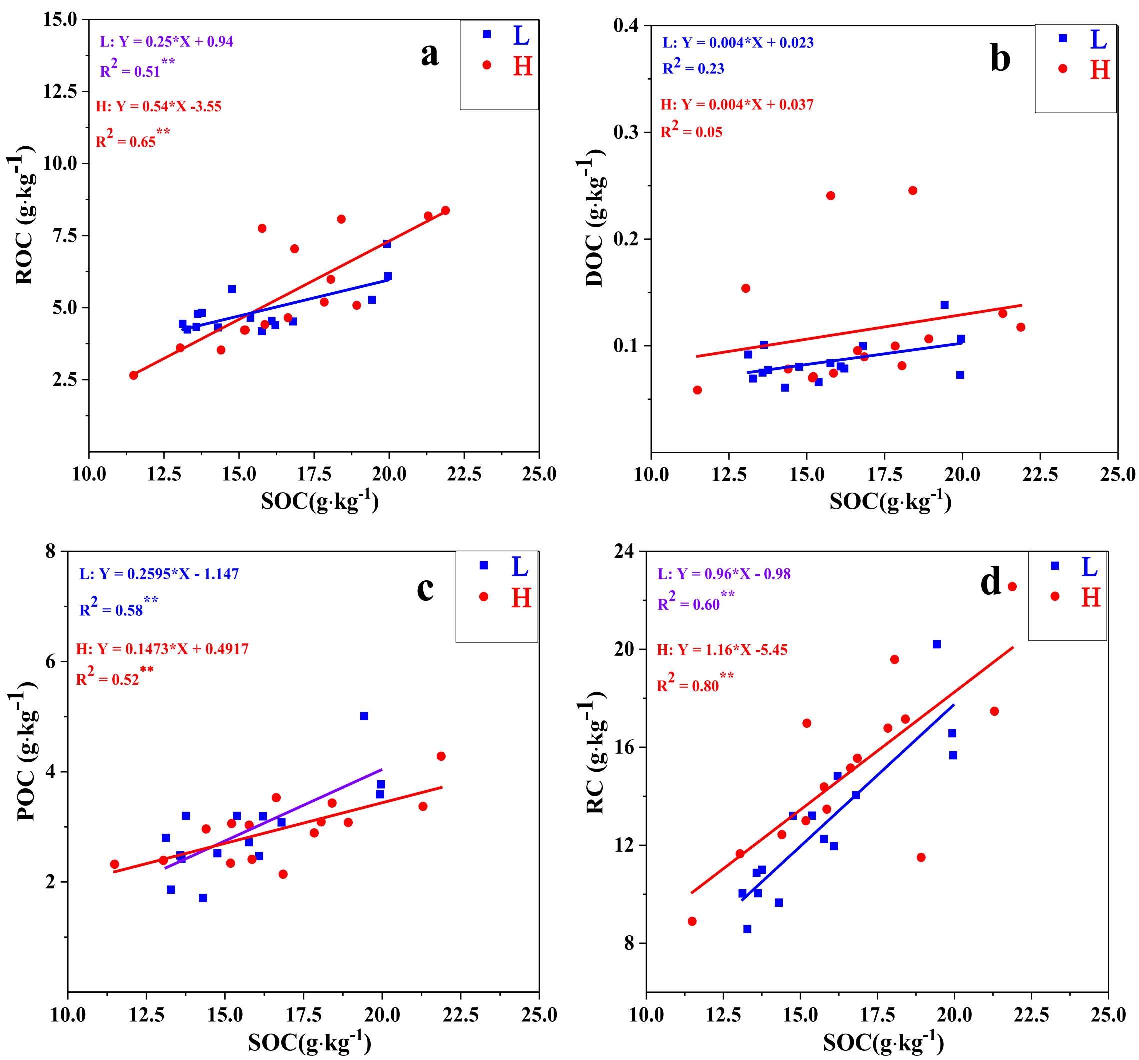

Fig. 3. Correlations between the SOC and its fractions. SOC - soil organic carbon; ROC - readily oxidizable organic carbon; DOC - dissolved organic carbon; POC - particulate organic carbon; and RC - recalcitrant carbon. **Significance at $p < 0.01$.

Table 3
Effect of BC on the soil carbon pool management index.

	Treatments	L	LI	CPI	CPMI
Linquan	CK	0.46 ± 0.05^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{b}	100.00 ± 0.00^{b}
	B_5	0.51 ± 0.06^{a}	1.11 ± 0.13^{a}	0.99 ± 0.03^{b}	109.77 ± 14.94^{b}
	B_{15}	0.42 ± 0.04^{a}	0.93 ± 0.10^{a}	1.07 ± 0.08^{b}	98.02 ± 2.34^{b}
	B_{20}	0.45 ± 0.05^{a}	0.98 ± 0.10^{a}	1.06 ± 0.05^{b}	102.60 ± 6.19^{b}
	B_{40}	0.46 ± 0.06^{a}	1.01 ± 0.12^{a}	1.38 ± 0.01^{a}	139.23 ± 18.09^{a}
Heishitou	CK	0.36 ± 0.03^{c}	1.00 ± 0.00^{c}	1.00 ± 0.00^{b}	100.00 ± 0.00^{b}
	B_5	0.36 ± 0.02^{c}	1.02 ± 0.06^{c}	1.04 ± 0.07^{b}	106.06 ± 10.86^{b}
	B_{15}	0.42 ± 0.04^{bc}	1.16 ± 0.11^{bc}	1.23 ± 0.08^{ab}	143.15 ± 17.96^{b}
	B_{20}	0.64 ± 0.11^{ab}	1.78 ± 0.32^{ab}	1.25 ± 0.03^{ab}	222.37 ± 41.03^{a}
	B_{40}	0.74 ± 0.11^{a}	2.06 ± 0.32^{a}	1.39 ± 0.14^{a}	276.95 ± 12.04^{a}

L, LI, CPI, and *CPMI* are the lability, lability index, carbon pool index, and carbon pool management index, respectively. The different lowercase superscript letters indicate significant differences in the same column ($p < 0.05$).

were 14.32 and 14.18 g C·kg^{-1} soil, respectively. Therefore, the lower application rates of BC had no significant effect on the SOC in such a high background region. However, the two ecological sites in the karst region experienced frequent and intense dry-wet alternations and annual average rainfalls of 1087 mm and 926 mm in Linquan and Heishitou towns, respectively. Moreover, the bedrock in karst regions is usually carbonate rock with strong cracks. Therefore, BC is easily decomposed and lost under such geological and climatic conditions.

4.2. Effect of BC on labile organic carbon

DOC is mainly derived from the decomposition of organic matter and plant residues by microorganisms, and it accounts for a small proportion of the SOC (Li et al., 2017). Previous studies have shown that the application of BC decreases the DOC content (Yang et al., 2018). For example, Meng et al. (2018) found that the soil DOC concentration with BC amendment decreased over time. However, some studies have found that the application of BC increased the DOC content (Li et al., 2018; Ouyang et al., 2014). In this study, the application of BC had no significant effect on the DOC content in the two ecological regions. The DOC content of BC-amended soil mainly depends on the DOC content of the BC, DOC loss, and BC and plant sorption. In recent years, several studies have reported the effect of BC on the DOC content, but universal or unidirectional patterns have not been observed, which is mainly related to the soil characteristics, BC and fertilizer types, application rates, and environmental conditions.

ROC is organic carbon that is easily oxidized by potassium permanganate, and its content is mainly affected by agriculture and other human factors (Li et al., 2021). POC is used as a soil quality indicator because it is sensitive to soil carbon management (Sruthi and Ramasamy, 2018). In this study, the application of BC generally increased the ROC and POC contents of the soil in the two ecological regions (Fig. 2a, c), which is consistent with previous the results of studies (Zhang et al., 2018a; Oladele and Adetunji, 2021). BC amendment promotes plant growth, soil aggregate formation and root biomass and activity (Abiven et al., 2015), which is the substrate for ROC formation. Moreover, the pyrolysis temperature of BC used in this study was about 380 °C, so the BC was not fully carbonized and contained a large amount of silicon and aluminum mineral ions, which prevent POC decomposition (Burrell et al., 2016).

4.3. *Effect of BC on the CPI and CPMI*

The CPMI is a comprehensive index that measures the quality and quantity of the soil carbon pools and reflects the C dynamics of the soil system (Blair et al., 1995b). Generally, a lower CPMI value indicates that the C is degrading, while a higher CPMI value indicates the restoration of C. Soils with a higher CPMI are considered to be better managed (Sainepo et al., 2018). The BC amendments increased the CPI and CPMI in the two ecological regions (Table 3). These results indicate that the BC amendment increased the activity and carbon sequestration of the soil carbon pool by increasing the SOC and ROC contents. In addition, there were no significant differences of in the L and LI among the BC treatments in Linquan. However, the L, LI, CPI, and CPMI in Heishitou significantly increased with increasing BC application rates. Therefore, the effect of the BC on soil carbon pool management index in Heishitou was better than that in Linquan. These results are similar to Yan et al. (2019), i.e., the effect of BC on the soil SOC and LOC fractions may be related to the soil type and environmental factors.

5. Conclusion

Compared with CK, long-term BC application increased the SOC, ROC, POC, and RC contents in two ecological sites in karst regions. However, only the high BC application rate (B_{40}) had a significant effect on the SOC and its fractions, which indicates that they are insensitive to changes caused by low BC application rates when the SOC background value is high. Significant positive correlations were found between the SOC content and the ROC, POC, and RC contents. However, the application of BC did not have a significant effect on the DOC content in the two ecological regions. Moreover, the CPI and CPMI increased with increasing BC application rate due to its unique physicochemical properties and nutrients. Therefore, long-term application of BC can not only improve soil fertility, but it can also increase carbon sequestration in karst regions, which is of great significance to mitigating climate change.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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