



The conversion of biomass to biochar decreases soil organic and inorganic carbon-derived CO₂ emissions under different water conditions in karst regions

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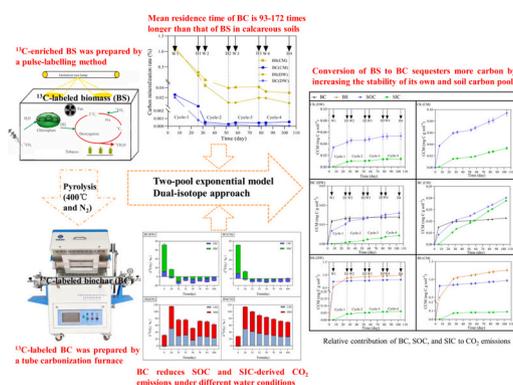
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HIGHLIGHTS

- Biochar significantly reduces SOC and SIC-derived CO₂ emissions under dry-wet alternating condition.
- The mean residence time of biochar is 93–172 times longer than that of biomass in the calcareous soil of karst region.
- Conversion of biomass to biochar sequesters more carbon by increasing the stability of its own and soil carbon pools.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Baoliang Chen

Keywords:

Biochar
Stability
Carbon sources
Soil inorganic carbon
Calcareous soil

ABSTRACT

Due to the unique geological background and climatic condition, karst soils in southwest China are mainly developed on carbonate rocks and accompanied by frequent dry-wet alternations. Therefore, the effects of biomass (BS) and biochar (BC) on the soil carbon pools (especially inorganic carbon) in karst regions are different from those in non-karst regions. In order to understand the responses of soil organic carbon (SOC) and soil inorganic carbon (SIC)-derived CO₂ emissions to BS and BC amendments under different water conditions in karst regions, a microscopic study under dry-wet alternate (DW) and constant moisture (CM) conditions was established to investigate the stability of BC, BS, and their effects on SOC and SIC pools by isotope double-labeling methods. Results showed that the contribution rates of SOC and SIC to soil CO₂ emissions were 51.55 %–99.52 %, 0.48 %–48.45 % and 60.55 %–97.72 %, 2.28 %–39.45 % under DW and CM conditions, respectively. Compared with the control, BS application under different water conditions increased SOC and SIC-derived CO₂ emissions by 929.78–1443.39 % and 169.69–335.71 %, respectively. However, BC amendment

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<https://doi.org/10.1016/j.scitotenv.2024.176865>

Received 4 June 2024; Received in revised form 29 September 2024; Accepted 9 October 2024

Available online 14 October 2024

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significantly decreased SOC and SIC-derived CO₂ emissions, especially in the DW condition. The mean residence time (the inverse of the decomposition rate) of BC in karst soils under different conditions ranged from 657 to 3105 years, which was much higher than those of BS (7–18 years). Therefore, the conversion of BS to BC in karst regions enhances carbon sequestration due to its stability of recalcitrant carbon, the decrease of SOC and SIC-derived CO₂ emissions. This study is helpful to understand the quantification of CO₂ sources from calcareous soils and elucidate the environmental behaviors and carbon sequestration potentials of BC or BS amendments in karst regions.

1. Introduction

In the global terrestrial ecosystem, farmland carbon sequestration is the only carbon pool that can be moderately regulated by reasonable measures on a relatively short time scale (Zhu et al., 2016). However, CO₂ emissions from farmland soils account for 21 % of total anthropogenic greenhouse gas emissions (Friedlingstein et al., 2020). Therefore, the potential of farmland carbon sequestration is huge (Freibauer et al., 2004; Fan et al., 2020), which plays an important role in mitigating global climate change. In order to deal with the increase of atmospheric CO₂, the direct return of straw and other biomass (BS) to agricultural fields can improve soil physicochemical properties, and promote plant growth and carbon sequestration (Liu et al., 2014). However, BS may stimulate soil respiration, and partially or completely offsetting its carbon sequestration (Lyu et al., 2018). Therefore, pyrolysis of BS to biochar (BC) under limited or absent oxygen conditions can increase its stability and carbon sequestration in the long-term (Muhammad et al., 2020; Tang et al., 2021). It is reported that BC can reduce greenhouse gas emissions by up to 1.8 Pg CO₂-equivalent per year, accounting for 12 % of total human greenhouse gas emissions (Woolf et al., 2010). However, the actual carbon sequestration potential of BC depends on its stability in the farmland. The stability of BC is not only related to raw materials and pyrolysis temperatures, but also depends on the environmental conditions in which it is applied (Kuzuyakov et al., 2014; Wang et al., 2016; Ventura et al., 2019).

Karst landforms cover about 10 % of land area in the world, particularly in southwest China, which contains one of the Earth's largest continuous karst regions (Li et al., 2024). Due to the unique geological background and climatic conditions, karst soils in southwest China are usually developed from carbonate parent rocks and accompanied with the phenomena of frequent dry-wet (DW) alternations (Wang et al., 2004; Cheng et al., 2018). Meanwhile, karst soils are usually thin and vulnerable to erosion and desertification (Wang et al., 2004). These unique properties make soil organic carbon (SOC) and soil inorganic carbon (SIC) losses and dynamic processes unusual in karst regions (Qin et al., 2022). Therefore, BC stability and its effect on the soil carbon pools (especially SIC) in karst regions are different from those in other regions, which may affect actual carbon sequestration potential. Previous studies showed that BC-induced a positive or negative priming effect (PE) of SOC (Watzinger et al., 2014; Ding et al., 2018). Besides, the pH of BC is relatively high, and it contains ash and Ca²⁺, which enhance SIC stock in the field (Dong et al., 2019; Shi et al., 2021). Therefore, the responses of SOC and SIC pools to BC application may be different, because the release of these two carbon sources depends on different driving mechanisms (Sun et al., 2023). However, the effects of BC amendments on SOC and SIC-derived CO₂ emissions in karst soils are still not fully understood. Hence, it is of great significance to investigate the stability of BC and its effect on soil carbon pools (SOC and SIC) to accurately assess the carbon sequestration potential.

The carbon isotopic compositions have obvious isotopic imprints and fractionations in various biogeochemical processes, which can be used to trace carbon sources and cycling (Pancost and Damsté, 2003). When there are three or more complex sources (SOC, SIC, and BC) of CO₂ emissions in BC-amended soils of the karst region, it is impossible to distinguish different end members by the traditional two-pool mixing model. In this case, the isotope double-labeling method was used to

distinguish the different sources by adding an additional isotope labeling treatment (Weng et al., 2020; Whitman and Lehmann, 2015). Using the double labeling method, the contributions of straw, glucose, and soil organic carbon to CO₂ emissions were 50.91 %, 43.79 %, and 5.30 %, respectively (Chen et al., 2022). Weng et al. (2020) also used this method to distinguish carbon mineralization between root respiration, BC, and SOC decompositions. However, a large number of studies have focused on the PE of BC on SOC mineralization, while the effect of BC on SIC pool is rarely reported (Lu et al., 2021; Shi et al., 2021). Compared to SOC, the turnover rate of SIC is assumed to be approximately 78,000 years and distributed in the deep soil layer, its contribution to soil CO₂ emissions induces the less attention (Wang et al., 2010; Raza et al., 2024). However, the SIC transformation in an agricultural system because of management practices (such as tillage, fertilization and irrigation) can occur on hourly to decadal timescales (Dong et al., 2019; Kim et al., 2020), which makes SIC an important contributor to atmospheric CO₂ (Raza et al., 2021; Sun et al., 2023). Many studies have also showed that the SIC is an important source of soil CO₂ emissions, especially in karst regions, which accounting for 13 %–40 % (Bertrand et al., 2007; Stevenson and Verburg, 2006; Tamir et al., 2011). These results suggest that SIC loss is more important than expected to stabilizing total carbon in karst soils, and the response of SIC-derived CO₂ emission to BS and BC amendments should be considered in the assessment of soil carbon cycles in karst regions. We hypothesized that: (1) when BS was applied to the soils, the BS decomposition and nitrification produces H⁺, which may lead to increase SIC dissolution and in turn induce SIC-derived CO₂ emissions; and (2) the conversion of BS to BC may decrease the SIC-derived CO₂ emission, due to the alkalinity and calcium ions of BC accelerating carbonate precipitation. To test these hypotheses, the carbon isotope double-labeling method was used to investigate the stabilities of BC and BS and their effects on SOC and SIC-derived CO₂ emissions in calcareous soils under different conditions, which will provide a theoretical basis for exploring the potential measures of carbon sequestration and mitigation in karst areas.

2. Materials and methods

2.1. Preparation of BS, BC, and soil

Higher ¹³C-enriched biomass (HBS in abbreviation), lower ¹³C-enriched biomass (LBS in abbreviation), higher ¹³C-enriched biochar (HBC in abbreviation), and lower ¹³C-enriched biochar (LBC in abbreviation) were labeled by a pulse-labeling method as previously described in detail (Lu et al., 2003; Whitman and Lehmann, 2015). In brief, a tobacco seedling is transplanted in Hoagland's nutrient solution, and then is placed in a transparent glass sealed box (50 × 30 × 40 cm) (Fig. S1). Plants were pulse-labeled with 500 mL of 99 % ¹³CO₂ at regular intervals (2–3 days) over the course of their growth in order to produce an even label. After labeling, the tobacco BS was harvested, oven-dried at 70 °C and grind finely for storage. Moreover, partially-labeled BS were heated to 400 °C at a rate of 5 °C·min⁻¹ under N₂ atmosphere and then carbonized for 2 h to obtain ¹³C-labeled BC.

Soil was collected from the plow layer (0–20 cm depth) of farmland in Qinglong County, Xingyi City, Guizhou Province. Soil sample was collected using a five-point sampling method in a tobacco field and placed into sealed bags by quartering after well-mixed. After air-dried,

roots and plant residues in the soils were removed. Then, soils were passed through a 100-mesh sieve and stored at room temperature. The soil type was classified as a calcareous soil (Chinese Soil Taxonomy), equivalent to a calcaric cambisol in the FAO/UNESCO Taxonomy. Detailed physicochemical properties and isotopic values of BS, BC, and soil are listed in Table 1.

2.2. Incubation experiment

Four alternate dry-wet (DW) cycles were designed in the incubation experiment, each consisting of a dry process and a wet process. The drought process takes about 20 days, as the soil slowly dries from the 40 % moisture content (field water holding capacity, FWHC) to 10 % moisture content. The wet cycle was performed under FWHC conditions (40 % moisture content) for 6 days. Meanwhile, the corresponding soil constant moisture (CM) condition was also designed, which requires the soil water content to keep constant at the FWHC.

Soil incubation experiments were conducted in 250 mL transparent flasks under DW and CM conditions. Each containing 50 g of soil mixed with 1 g of high- and low-labeled BC or BS and sealed with butyl rubber stoppers. Meanwhile, the blank control group (CK) without BC and BS was also established. The entire incubation experiment lasted for 104 days. Each treatment had three repetitions and set up eight destructive samples. The destructive soil sampling was performed at the end of each dry or wet period (i.e., days 6 (W1), 26 (D1), 32 (W2), 52 (D2), 58 (W3), 78 (D3), 84 (W4), and 104 (D4)) to determine soil dissolved organic carbon (DOC), SOC, and SIC contents.

2.3. Measurements of soil CO₂ flux and δ¹³C

Gas samples from the headspace in the flask were collected using a 20 mL gas-tight plastic syringe via a three-port valve during the incubation period. Two gas samples were collected daily for each treatment, and the interval between two samplings was 12 h. The CO₂ flux was calculated based on the change of CO₂ gas concentration during the two sampling times. CO₂ concentration was measured using a gas chromatograph (Agilent 7890A, Wilmington, USA).

The CO₂ for δ¹³C-SIC measurements was collected from a closed vial containing soil samples treated with H₃PO₄ solution for 12 h at 50 °C. The CO₂ for δ¹³C-SOC analysis was collected from sealed quartz tubes where HCl acidified soils were combusted with CuO at 850 °C for 2 h (Qin et al., 2022). The above collected CO₂ was purified on the vacuum line and analyzed for isotopic analysis using a mass spectrometer MAT-253 (Thermo Fisher Scientific, USA). Moreover, the δ¹³C-BC and δ¹³C-BS was also measured by an isotope ratio mass spectrometer (MAT-253, USA) (Wang et al., 2020). All isotopic measurements are presented in the delta (δ) notation:

$$\delta = \frac{R}{R_{\text{std}}} \times 1000$$

where R is the ¹³C/¹²C ratio in the sample and R_{std} is the ¹³C/¹²C ratio in the standard. All values are expressed as parts per mil (‰) and are

Table 1

Properties and isotopic values of BS, BC, and calcareous soil.

	pH	TC (mg·g ⁻¹)	TN (mg·g ⁻¹)	δ ¹³ C (‰)
HBS	5.66	390.00	16.20	144.26 ± 7.03
LBS	5.70	400.34	15.80	67.01 ± 2.010
HBC	10.74	480.10	22.10	133.78 ± 1.24
LBC	10.62	481.30	21.80	38.70 ± 0.57
SOC	–	10.80	–	–23.15 ± 0.08
SIC	–	7.40	–	–0.07 ± 0.06

HBS: High ¹³C-labeled biomass; LBS: Low ¹³C-labeled biomass; HBC: High ¹³C-labeled biochar; LBC: Low ¹³C-labeled biochar; SOC: Soil organic carbon; SIC: Soil inorganic carbon.

referenced to the Vienna Pee Dee Belemnite (V-PDB) standard. The precision of the δ¹³C measurements was ≤ 0.2 ‰.

2.4. Determination of soil properties

The pH and EC (1,5 mixture of solid and liquid) of soil, BC, and BS were measured by using a pH meter (PHS-3E, Leici, Shanghai, China) and conductivity meter (DDS-307, Leici, Shanghai, China) (Bai et al., 2018). For SOC, soil samples were acidified to remove inorganic C and were then combusted thoroughly determined with an automatic elemental analyzer (Analyzer vario MICRO cube, Elementar, Germany) (Tan et al., 2013). The soil total carbon (STC) and total nitrogen (TN) was measured without acidified by a dry combustion method using an elemental analyzer. The SIC content was calculated by subtracting the SOC content from the STC content (You et al., 2020). The dissolved organic carbon (DOC) in the different treatments was extracted with soil: water ratio of 1:6 (w/v) and determined by a TOC analyzer (Aurora 1030 W, USA) (Yang et al., 2018).

2.5. Calculations

Soil CO₂ efflux (F , mg CO₂·kg⁻¹·d⁻¹) was calculated as follows (Pan et al., 2016):

$$F = \frac{(C_t - C_0) \times V \times M \times 273.15}{22.4 \times W \times (273.15 + T)} \times t \times 1000$$

where C_t and C_0 is the concentration (μL·L⁻¹) of CO₂ in gases sampled t d and at zero-time after flask enclosure, respectively, M is the molecular weight of CO₂ (g·mol⁻¹), V is effective volume of flasks (mL), W is the soil mass (kg), t is enclosure time (d), and T is temperature of gas sampling (°C).

$$T = \sum_{i=1}^n F_i \times \frac{t}{1000}$$

where T is soil cumulative CO₂ emission (mg·CO₂·g⁻¹), F_i is soil CO₂ effluxes at the i -th day of incubation time (mg·CO₂·kg⁻¹·d⁻¹), t is incubation time (d).

The two-pool exponential model was fitted to the cumulative proportion of C mineralized from BC or BS over the 104 days incubation to estimate mean residence time (MRT) in different conditions (Wu et al., 2016).

$$M_t = M_1 \times (1 - e^{-k_1 t}) + M_2 \times (1 - e^{-k_2 t})$$

where M_t is the cumulative amount of C mineralized (%) and t is the incubation time (days). M_1 and M_2 represent the proportion (%) of the labile and recalcitrant C pools in BC or BS, respectively; k_1 and k_2 are the mineralization rate constants for the labile and recalcitrant pools, respectively; The MRT is the inverse of the mineralization rate constant ($1/k_1$ or $1/k_2$).

Dual-isotope approach to partitioning three sources: the sources of soil CO₂ were divided into SOC, SIC, and BC or BS in the present study. Taking BC as an example, the following equations were established (Whitman and Lehmann, 2015):

$$\delta^{13}\text{CO}_{2L} = f_{\text{SOC}}\delta^{13}\text{SOC} + f_{\text{SIC}}\delta^{13}\text{SIC} + f_{\text{BC}}\delta^{13}\text{LBC}$$

$$\delta^{13}\text{CO}_{2H} = f_{\text{SOC}}\delta^{13}\text{SOC} + f_{\text{SIC}}\delta^{13}\text{SIC} + f_{\text{BC}}\delta^{13}\text{HBC}$$

$$1 = f_{\text{SOC}} + f_{\text{SIC}} + f_{\text{BC}}$$

The δ¹³CO_{2L} and δ¹³CO_{2H} refer to the δ¹³C value of CO₂ emitted from soil after LBC and HBC amendments, respectively. δ¹³SOC, δ¹³SIC, δ¹³LBC, and δ¹³HBC represent the δ¹³C values of SOC, SIC, LBC, and HBC, respectively (Table 1). f_{SOC} , f_{SIC} , and f_{BC} refer to the proportion of soil CO₂ emissions originating from SOC, SIC, and BC, respectively.

2.6. Statistical analysis

Analysis of variance and the least significant difference (LSD) tests were performed to determine the significant differences between different conditions with BC or BS amendment at confidence level of $P < 0.05$ using SPSS 22.0 software. Graphic figures were constructed using GraphPad Prism 8, and data represent mean \pm standard error (SE).

3. Results

3.1. Effects of BC and BS amendments on DOC, SOC, and SIC contents

With the increase of incubation time, the content of DOC in soils under different treatments decreased gradually. Compared with CK, BC amendment significantly decreased on average soil DOC content by 17.40 % and 19.34 % under DW and CM conditions, respectively. Moreover, the soil DOC content in all treatments under DW conditions

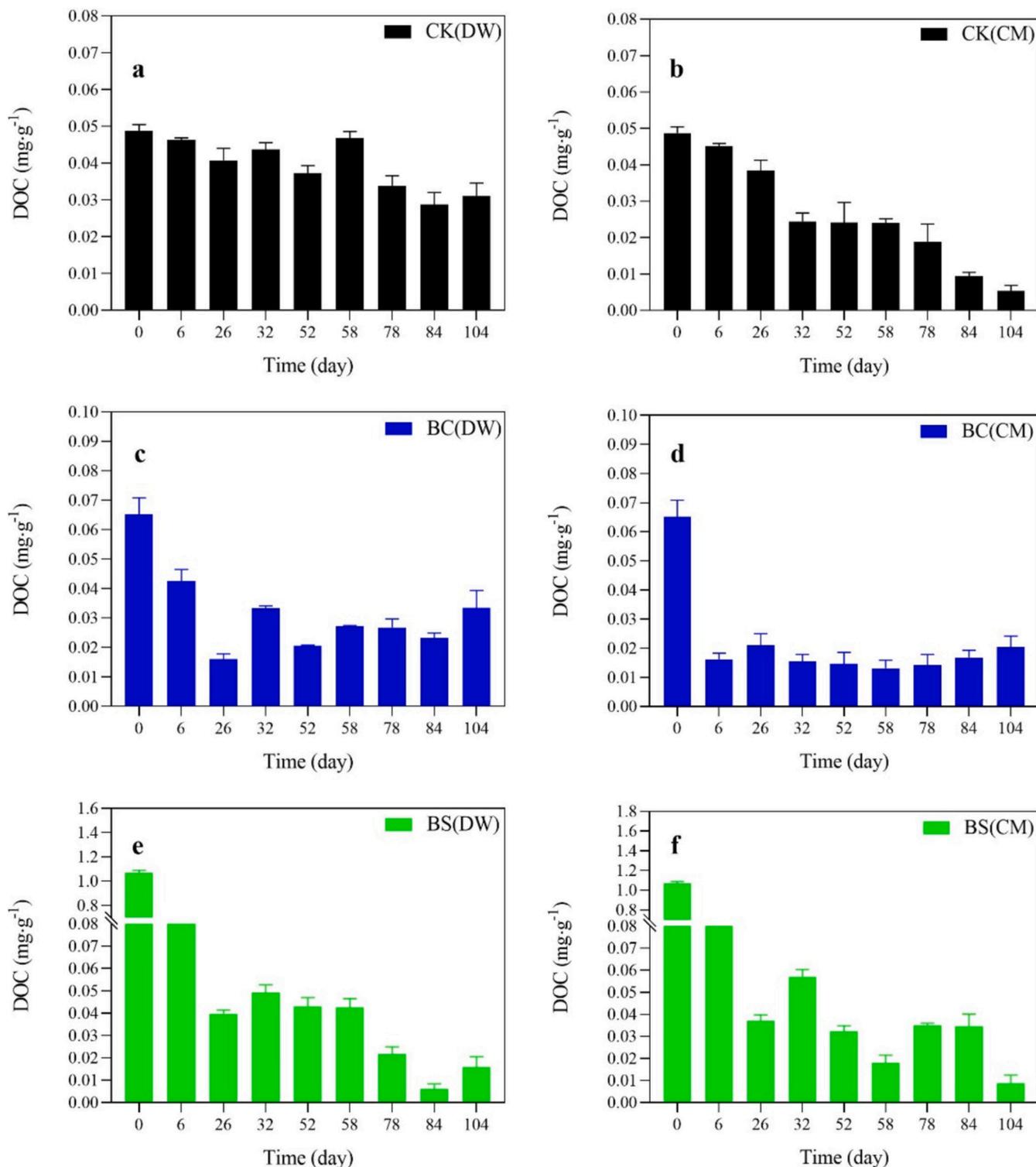


Fig. 1. Changes of DOC with the incubation time in different treatments under DW and CM conditions. DW: dry-wet alternations, CM: constant moisture.

was 1.03 %–49.77 % higher than that under CM conditions (Fig. 1). Compared with CK, BC and BS treatments during the whole incubation cycle significantly increased the SOC content by 61.54 %–80.81 % and 8.62 %–46.30 % under different water conditions, respectively ($p < 0.05$) (Fig. S2). In addition, BC amendment increased on average the SIC contents by 14.16 %–23.92 % under different water conditions. However, there was no significant difference in SIC content between BS and CK treatments, especially in CM conditions ($p = 0.488$, Fig. S2).

3.2. Effects of BC and BS amendments on soil CO₂ fluxes and cumulative emissions

Compared with CK, BS application significantly increased soil CO₂ emission rate and cumulative emission (Fig. 2). However, BC amendment reduced soil CO₂ cumulative emissions under both DW and CM conditions. Compared with DW conditions, soil CO₂ cumulative emissions in different treatments significantly increased by 26.89 %–308.72 % under CM conditions ($p < 0.05$, Fig. 2c, d). In addition, soil CO₂ fluxes in the CK and BC treatments under the DW condition showed a negative emission at the soil drought phase during cycles 3 and 4. The negative emission rate of soil CO₂ in BC treatment reached a maximum of $-6.39 \pm 0.43 \text{ mg kg}^{-1} \text{ d}^{-1}$, and the corresponding value in the CK treatment reached a maximum of $-2.10 \pm 0.62 \text{ mg kg}^{-1} \text{ d}^{-1}$ (Fig. 2).

3.3. Sources of soil CO₂ emissions in different treatments

After each DW cycle, the $\delta^{13}\text{C}$ of CO₂ emitted from all treatments will become more negative (Fig. S3). Except for the first wetting process (W1 stage), the contribution rates of SOC and SIC to soil CO₂ emissions under DW conditions were 51.55 %–73.81 % and 26.19 %–48.45 %, respectively. Under CM conditions, the contribution rates of SOC and SIC to soil CO₂ emissions were 60.55 %–68.94 % and 31.06 %–39.45 %, respectively (Fig. 3).

In the W1 stage, the contribution of BC to soil CO₂ emission under DW and CM conditions was 63.52 % and 64.14 %, respectively. In the remaining incubations, the contribution rate of BC to soil CO₂ emission was <10 % (Fig. 3). However, BS is the main source of soil CO₂ emission in the BS treatment during the whole incubation. The contributions of BS to soil CO₂ were 51.83 %–84.34 % and 56.09 %–83.30 % under DW and CM conditions, respectively. Compared with the dry period, the maximum contribution rates of SOC to CO₂ emissions after soil rewetting increased by 37.22 %, 63.61 %, and 528.00 % in CK, BC, and BS treatments, respectively (Fig. 3). The above results indicated that the sudden increase in water content stimulated soil microbial activity and promoted SOC mineralization, which was consistent with the changes of soil DOC content and CO₂ emission rate (Figs. 1 and 2).

Compared with CK, the amount of SOC mineralization in BS treatment significantly increased by 1443.39 % and 929.78 % under DW and CM conditions, respectively ($p < 0.05$) (Fig. 4). Moreover, BS application significantly increased the SIC-derived CO₂ emission by 335.71 %

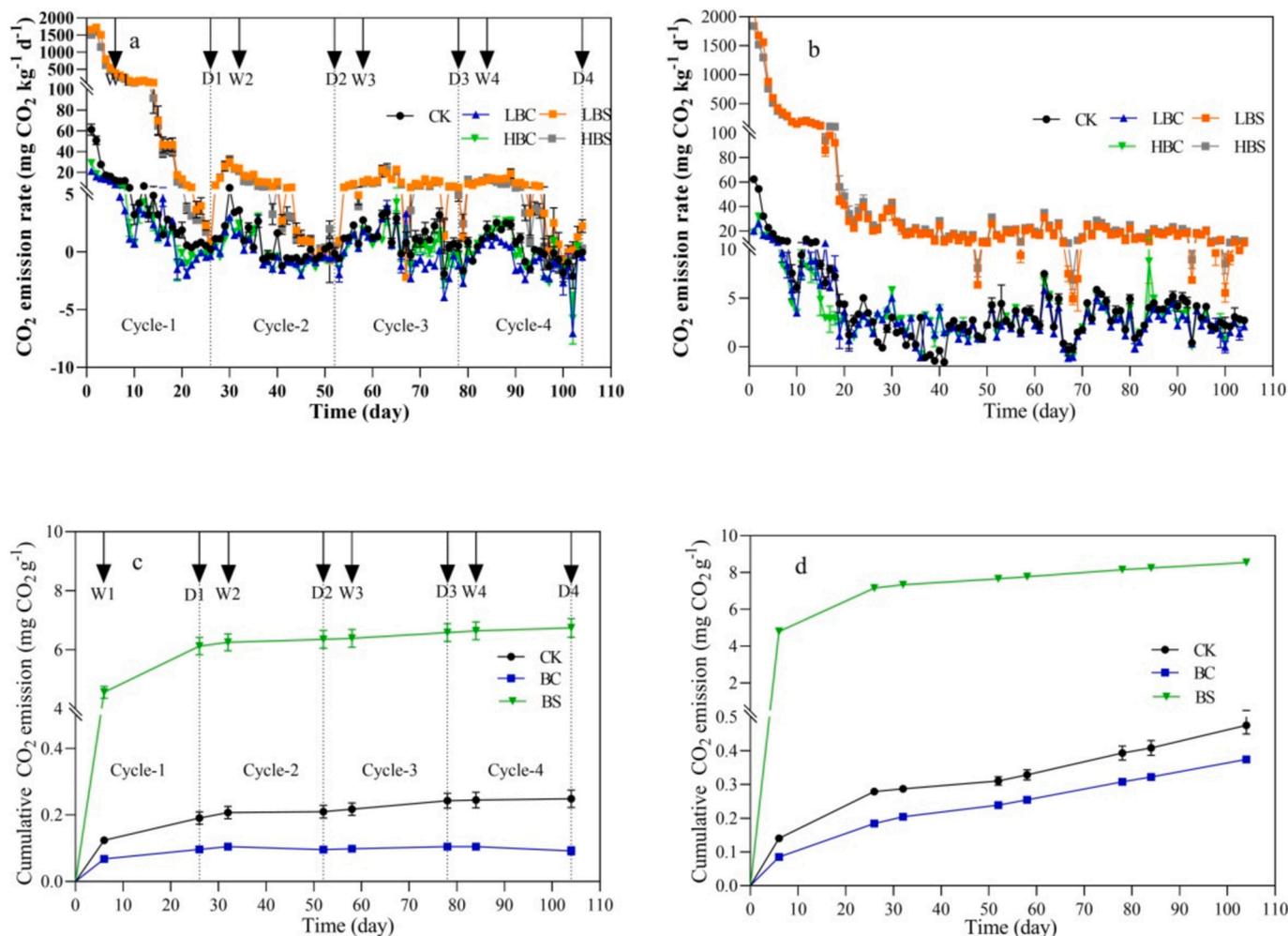


Fig. 2. Changes in soil CO₂ emission rates and cumulative emissions with the incubation time under DW (a, c) and CM (b, d) conditions, respectively. Arrows indicate the end of drying (d) and wetting (w) cycles.

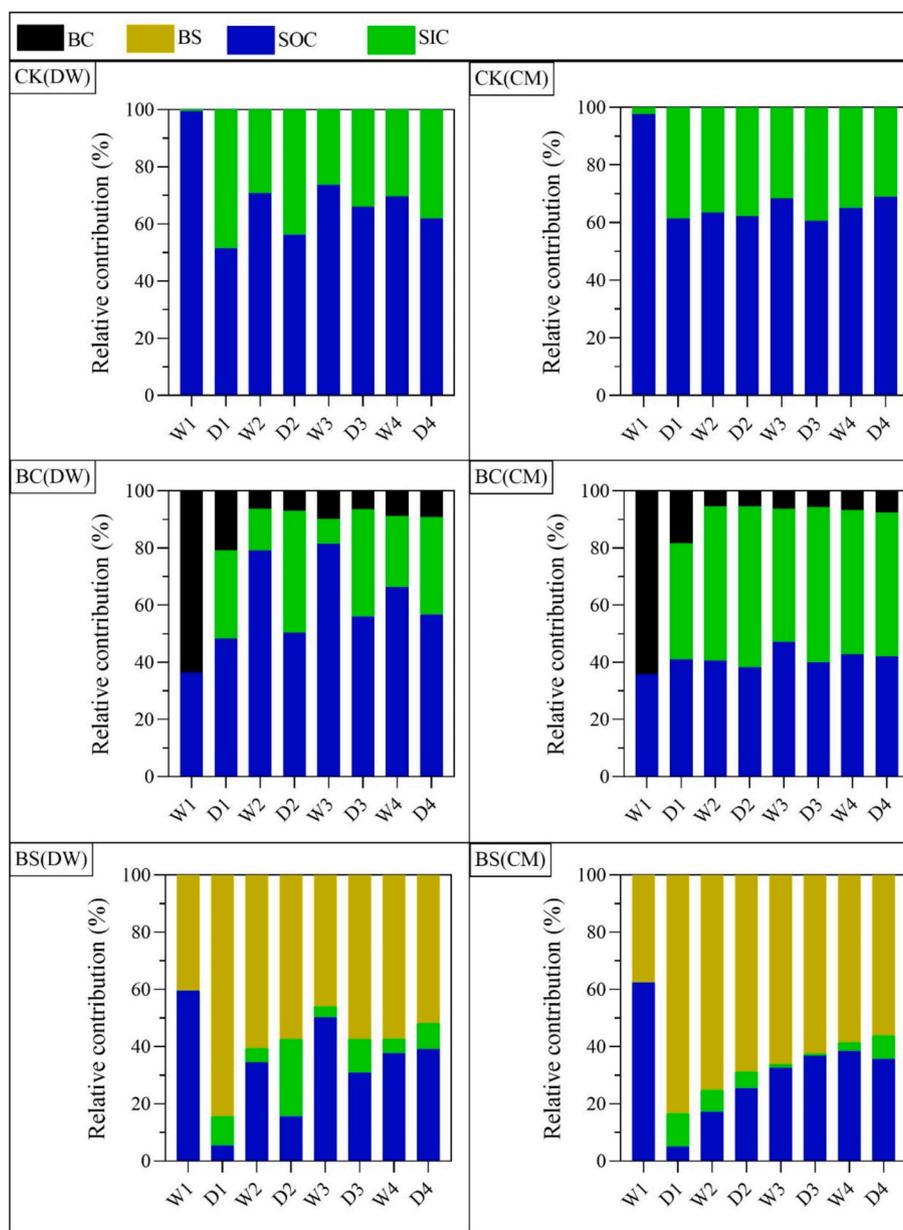


Fig. 3. Relative contribution of BC (BS), SOC, and SIC to soil CO₂ emissions (W1, W2, W3, and W4 represent the first, second, third, and fourth wetting processes, respectively; D1, D2, D3, and D4 represent the first, second, third, and fourth drought processes, respectively).

and 169.69 % under DW and CM conditions, respectively ($p < 0.05$). However, BC decreased SOC mineralization by 69.81 % and 57.45 % under DW and CM conditions, respectively (Fig. 4). Compared with CK, the addition of BC significantly also reduced the source of CO₂ from SIC sources by 71.42 % under DW conditions, but it had no significant effect under CM conditions (Fig. 4).

3.4. Mineralization amounts of BC and BS under different water conditions

The degradation rates of BC and BS exhibited a sharp decrease during cycles 1 and 2, and subsequently stabilized at a relatively low level during cycles 3 and 4 (Fig. 5). In the first cycle, BC was mineralized by 0.0132 mg C·g⁻¹ and 0.0196 mg C·g⁻¹ under DW and CM conditions, respectively. Only 0.0007 mg C·g⁻¹ and 0.0032 mg C·g⁻¹ were mineralized in the latter three cycles under DW and CM conditions, respectively. However, BS was mineralized by 0.98 mg C·g⁻¹ and 1.26 mg C·g⁻¹ under the whole DW and CM conditions, respectively (Fig. 5).

During the whole incubation experiment, the total mineralization amounts of BC were 0.15 % and 0.24 % under DW and CM conditions, respectively. However, the corresponding mineralization amounts of BS were 12.46 % and 15.97 %, respectively. Mean residence time (MRT) of BC in karst soils under DW and CM conditions were 3105 years and 657 years, respectively, which were much higher than those of BS (18 years and 7 years, respectively) (Table 2).

4. Discussion

4.1. Effects of BC and BS amendments on soil carbon components

The impact of BC amendment on soil DOC varies with BC feedstock and soil type. For example, Zhang et al. (2017) found that BC application significantly increased DOC content in the Loess Plateau. However, Yang et al. (2018) showed that DOC was decrease by 5.59 %–26.67 % in brown earth among different BC application rates. In the present study, a similar decrease of DOC contents (17.40 %–19.34 %) in BC-amended

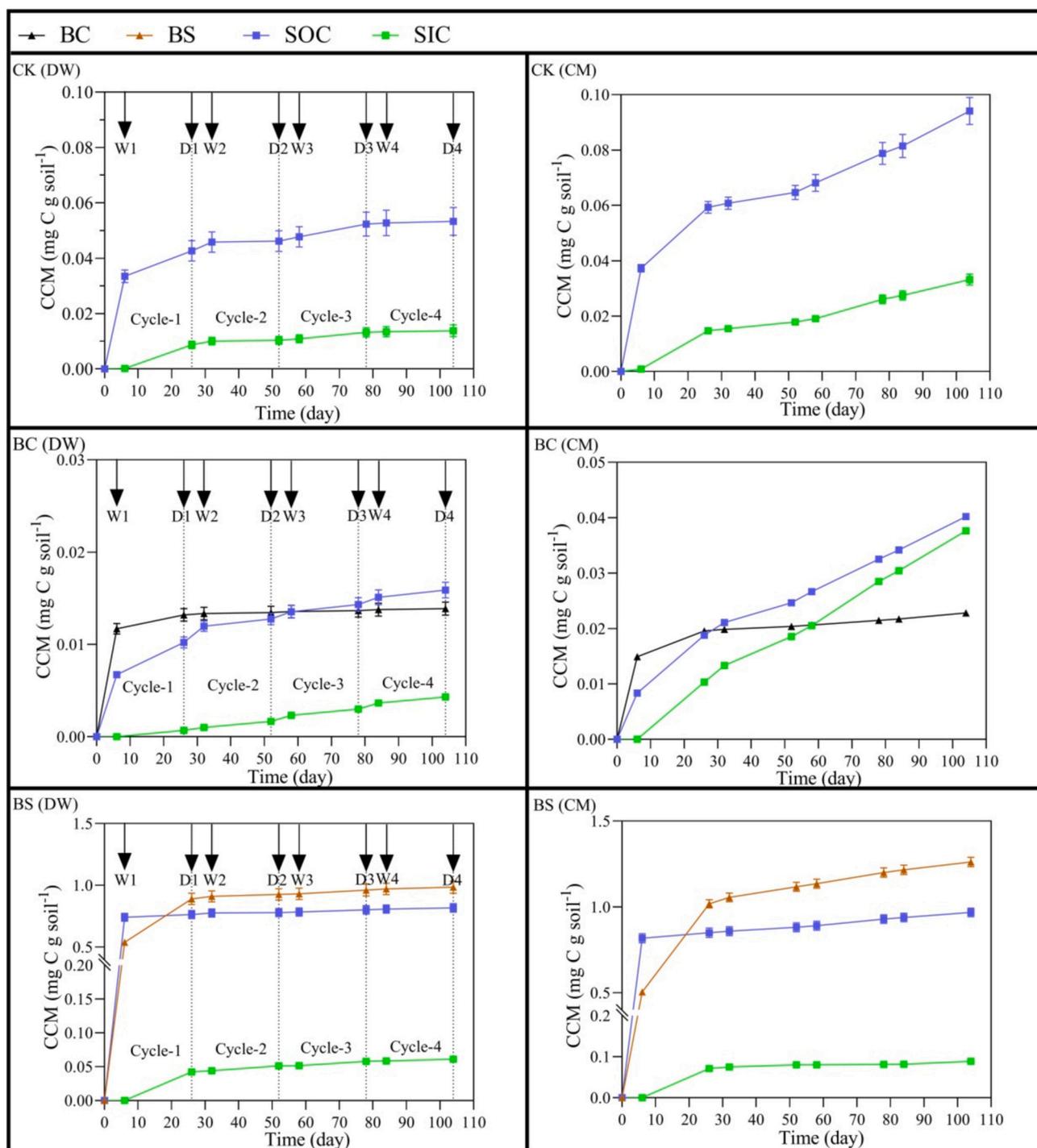


Fig. 4. Changes of cumulative carbon emissions (CCM) of BC (BS), SOC, and SIC with the incubation time. Arrows indicate the end of drying (d) and wetting (w) cycles.

soil was observed under different water conditions. On the one hand, BC can adsorb DOC on the surface or wrap it in pores (Pietikainen et al., 2000). On the other hand, the DOC adsorbed by the BC can quickly act as a carbon source for microorganisms (Jones et al., 2011). In our experiment, the reducing DOC also further explains for the observed reduction of CO₂ emissions and SOC mineralization in BC-amended soils by 21.22%–63.09% and 14.16%–23.92%, respectively (Figs. 2 and 4). In addition, the soil DOC content in all treatments under DW condition was 1.03%–49.77% higher than that under CM condition (Fig. 1). This phenomenon is mainly due to the fact that the DW cycle greatly reduces the microbial population, thereby decreasing the utilization of DOC by

microorganisms (Reverey et al., 2018). Moreover, DW cycle disrupts the soil structure, thus exposing insoluble organic matter and increasing the previously-sequestered carbon more readily available as DOC during the drying phase (Lundquist et al., 1999; Li et al., 2020).

Compared with CK, both BS and BC applications increased the content of SOC. BS contains a large amount of decomposable carbon, which is more easily decomposed and utilized by microorganisms (Rasul et al., 2022). However, BC is a carbon-rich substance with a highly aromatic structure and contains more stable carbon (Lehmann, 2007). Therefore, compared with the BC treatment, the SOC content in the BS treatment decreased significantly with the increase of the incubation time

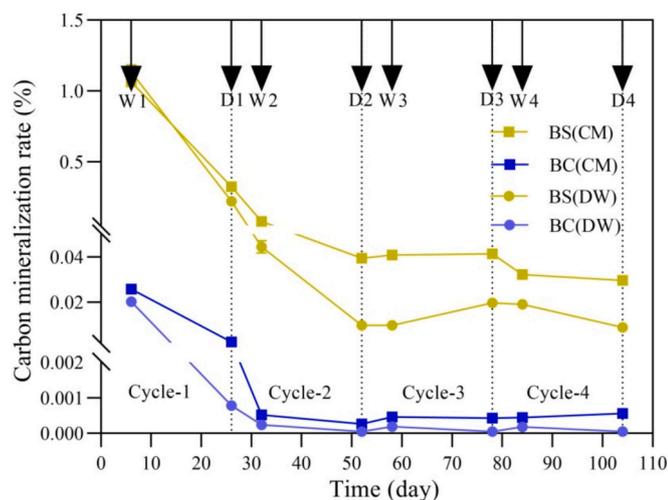


Fig. 5. Mineralization rates of BC and BS in the calcareous soil under DW and CM conditions. Arrows indicate the end of drying (d) and wetting (w) cycles.

Table 2

Mean residence time (MRT) and proportions of labile and recalcitrant components of BC and BS in DW and CM conditions during the 104-day period.

Treatments	MRT		Adjusted R ²	Labile C (%)	Recalcitrant C (%)
	Labile C (days)	Recalcitrant C (years)			
BC (DW)	3	3105	0.90	0.14	99.86
BC (CM)	4	657	0.98	0.19	99.81
BS (DW)	7	18	0.91	11.24	88.76
BS (CM)	9	7	0.98	12.72	87.28

(Fig. S2). In addition, BC amendment increased the SIC content, which is consistent with previous studies (Dong et al., 2019; Wang et al., 2023). This finding may be attributed to the high pH (10.68) and EC (430 $\mu\text{S}\cdot\text{cm}^{-1}$) in the tobacco, which may provide alkaline metals to soil and indirectly accelerate carbonate precipitation and SIC formation (Wang et al., 2014).

4.2. Contribution of different carbon components to CO₂ emissions

Compared to controls, BS application increased soil CO₂ emission rate during the whole incubation cycle. Meanwhile, the incorporation of BS caused significant increases in cumulative CO₂ emissions by 16.99–26.14 times under different water conditions, which is consistent with previous studies (Lou et al., 2007; Ibrahim et al., 2015). The main reason is that the addition of BS increases the more available carbon sources for microorganisms, which improves the activity of soil microorganisms and directly leads to the increase of SOC mineralization (Jones et al., 2011; Zhang et al., 2013). BS can also stimulate microorganisms to secrete extracellular enzymes to degrade BS and native SOC mineralization at the same time, leading to co-metabolism (Kuzuyakov et al., 2000). However, the addition of BC reduced soil CO₂ cumulative emissions by 21.22%–63.09% under different water conditions. Similar results obtained by Sun et al. (2014) who observed that BC amendment significantly decreased cumulative soil CO₂ emissions by 31.5%. BC amendment increased the co-localization of microorganism and nutrients, thus improving the efficiency of microbial carbon utilization (Lehmann et al., 2011). Moreover, the BC used in this study increased soil pH from 7.81 to 8.16 and EC from 291.38 to 1329.95 $\mu\text{S}\cdot\text{cm}^{-1}$, which may provide Ca²⁺ and alkaline metals to soil and led to the formation of carbonates (Case et al., 2014; Wang et al., 2014). The above biotic and abiotic mechanisms are also consistent with the results of the

reduction of SOC mineralization rate by 57.45%–69.81% and the increase of SIC content by 14.16%–23.92% in BC-amended soils, which can explain the inhibition effect of BC on soil CO₂ emission.

At present, numerous studies have shown that soil CO₂ production mainly comes from SOC mineralization and root respiration (Chen et al., 2022; Weng et al., 2020). However, the dissolution of soil carbonates can contribute up to 30% of total CO₂ emissions (Tamir et al., 2011), which is consistent with our results (26.19%–48.45% under different water conditions). Therefore, SIC is also an important source of CO₂ emission. Ignoring this process will result in overestimation of the CO₂ production from SOC source in karst regions. Moreover, BS application significantly increased the SIC-derived CO₂ emission under different water conditions. Firstly, the elevated CO₂ partial pressure in BS-amended soils is a result of enhanced CO₂ production induced by the decomposition of tobacco biomass, which would be beneficial for CaCO₃ dissolution by carbonic acid attack (Li and Keren, 2009). Secondly, BS amendment can produce acid microenvironment following its decomposition (Wang et al., 2018), which may promote the dissolution of carbonate and CO₂ emissions.

However, BC decreased SOC mineralization by 57.45%–69.81% under different water conditions (Fig. 4). This finding is mainly due to the fact that porous structure and large specific surface area of BC can directly adsorb and protect the unstable organic carbon to avoid microbial use (Kasozzi et al., 2010; Yang et al., 2019). Moreover, BC can increase the stability of organic and mineral components in soil, thus protecting and storing unstable carbon (Fang et al., 2014a; Keith et al., 2011). Moreover, BC reduces SIC-derived CO₂ emissions in karst soils. This is mainly due to the higher pH environment in BC-amended soils, which leads to the alkalization capacity of BC exceeding the acidification capacity of BC mineralization (Sun et al., 2023). Moreover, BC can be used as a precipitating core for carbonate formation when carbonate ions are present in soil waters (Joseph et al., 2010; Wang et al., 2023). In addition, BC addition can lead to the transformation of soil microbial communities into bacteria communities with slow carbon turnover (e.g., actinomycetes), which can help to increase the stability of soil aggregates (Zheng et al., 2018). However, the effect of BC on carbon cycling functional groups of microorganisms in karst soils needs further confirmation.

4.3. Stability of BC and BS in the calcareous soil of karst regions

Consistent with other studies (Fang et al., 2014a; Singh et al., 2012), the mineralization rates of BC and BS decreased rapidly in the first 32 and 52 days, respectively. During the whole incubation experiment, the total mineralization amounts of BC and BS under different conditions were 0.15%–0.24% and 12.46%–15.97%, respectively (Fig. 5). Previous studies showed that woody and rice straw BC were degraded by 0.10%–0.70% and 0.17%–0.28% during 120 and 390 days, respectively (Singh et al., 2012; Wu et al., 2016), which were similar to the results of tobacco BC in the present study. All of those results indicate the conversion of BS into BC will increase its stability in soils under both DW and CM conditions.

There are many factors that influence the mean residence time (MRT) of BC in soils. For example, the MRT of sludge and cow dung BC in the same soils were 201 years and 594 years, respectively (Singh et al., 2012). As the carbonization temperature increased from 450 °C to 550 °C, the MRT of oak BC increased from 463 years to 616 years (Fang et al., 2014b). Besides raw materials and pyrolysis temperature, environmental factors are also important in affecting the stability of BC. Wu et al. (2016) reported the average MRT of BC in soil with different SOC contents ranged from 617 years to 2829 years. Moreover, the average MRT of the same BC in different soil types is from 616 years to 1037 years (Fang et al., 2014b). In the present study, the MRT of BC ranged from 657 years to 3105 years, which is 93–172 times longer than that of BS in the calcareous soil of karst regions (Table 2). In addition, BC degradation rates measured in the field may be lower than those

measured in the laboratory due to relatively low field temperature and humidity conditions (Kuzyakov et al., 2014; Major et al., 2010). Therefore, the conversion of BS to BC in karst soils in southern China has a long MRT and stability, indicating a long-term carbon sequestration and mitigation at the field scale.

5. Conclusions

In the karst regions, BS is unstable and will decompose completely over about 20 years. Meanwhile, the application of BS to the farmland increased additional CO₂ emissions from soil carbon pools. Conversely, BC reduced CO₂ emissions from the sources of SOC and SIC, especially in DW conditions. Moreover, BC in karst soils can persist for 657–3105 years under different water conditions. Therefore, the conversion of BS to BC can achieve long-term carbon sequestration and mitigation in karst regions due to its high stability, the decrease of SOC and SIC-derived CO₂ emissions. However, it is important to note that laboratory conditions are different from field conditions, so caution is needed in extrapolating the results of this study to field conditions as well as to other soil types.

CRedit authorship contribution statement

Jianzhong Cheng: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Minghua Jiang:** Writing – original draft, Data curation. **Yuan Tang:** Writing – review & editing, Investigation, Formal analysis. **Weichang Gao:** Writing – review & editing. **Wenjie Pan:** Writing – review & editing. **Xinqing Lee:** Writing – review & editing.

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.

Acknowledgements

This study was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant no. XDB40020201), National Natural Science Foundation of China (42263013; 41773144), Guizhou Provincial Basic Research Program (Natural Science) (No. ZK[2022] 047), Key Research and Development Program of the China Tobacco Corporation (Grant No. 110202102038) and the Science and Technology Project of Bijie Tobacco Company of Guizhou Province (Grant No. 2022520500240192). Jianzhong Cheng was supported by the Outstanding Young Scientific and Technological Talents Project of Guizhou Province (YQK[2023]036) and the “Light of West China” Program of Chinese Academy of Sciences.

Appendix A. Supplementary data

The following are the supplementary data related to this article. The pulse-labelling method to generate ¹³C-enriched plant materials (¹³CO₂) (Fig. S1); Changes in SOC and SIC contents with the incubation time (Fig. S2); Variation of δ¹³C value of soil CO₂ emission with the incubation time (Fig. S3). Supplementary data to this article can be found online at [doi:<https://doi.org/10.1016/j.scitotenv.2024.176865>].

Data availability

Data will be made available on request.

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