

# Effects of nitrogen addition on carbonate-derived CO<sub>2</sub> emission after biochar addition

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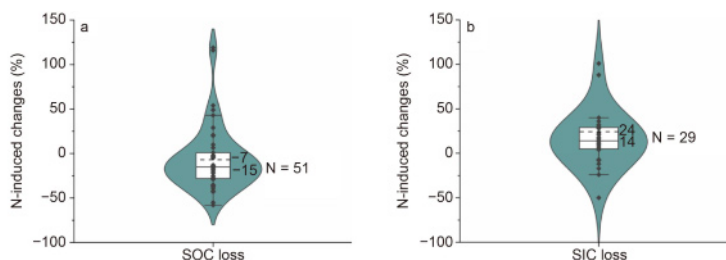
## ABSTRACT

- We studied the effect of nitrogen and biochar on CO<sub>2</sub> emission from SOC and SIC.
- Nitrogen increased SIC-derived CO<sub>2</sub> by 41% but decreased SOC-derived CO<sub>2</sub> by 20%.
- Biochar reduced total soil-derived CO<sub>2</sub> by neutralizing nitrogen-induced acidity.
- We proposed a method for 3- or 4-source partitioning CO<sub>2</sub> emission from calcareous soils.

Biochar addition generally increases the alkalinity regeneration to resist soil acidification driven by nitrogen (N)

fertilization. Calcareous soils contain soil organic carbon (SOC) and inorganic C (SIC). Owing to technical limitations in three-source partitioning CO<sub>2</sub>, how biochar addition affects SOC- and SIC-derived CO<sub>2</sub> emission has not been clarified yet. Therefore, we conducted a 70-day incubation experiment of ammonium-N and maize-straw-derived biochar additions to investigate the N plus biochar impacts on SOC- and SIC-derived CO<sub>2</sub> emission. Over the 70-day incubation, we found that the N-only addition increased the SIC-derived CO<sub>2</sub> emission by approximately 41% compared with the control, but decreased the SOC-derived CO<sub>2</sub> emission by approximately 20%. This suggests that the distinct responses of SIC- and SOC-derived CO<sub>2</sub> emission to N-only addition come from N-induced acidification and preferential substrate (N) utilization of soil microorganisms, respectively. Compared with N-only addition, N plus biochar addition decreased the SIC-derived CO<sub>2</sub> emission by 17%–20% during the first 20 days of incubation, but increased it by 54% during the next 50 days. This result suggested that biochar addition reduced the SIC-derived CO<sub>2</sub> emission likely due to the alkalization capacity of biochar exceeding the acidification capacity of ammonium-N in the short term, but it may increase the SIC-derived CO<sub>2</sub> emission induced by the weak acidity produced from biochar mineralization in the long term. This study is helpful to improve the quantification of CO<sub>2</sub> emission from calcareous soils.

**Keywords** biochar, soil organic carbon, soil inorganic carbon, three-source partitioning, soil CO<sub>2</sub> emission, <sup>13</sup>C isotope



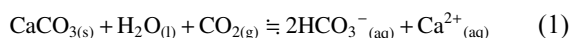
## 1 Introduction

Soil carbon (C) consists of soil organic C (SOC) and soil inorganic C (SIC). On a global scale, SOC and SIC storage is approximately 1460–1550 Pg C and 700–950 Pg C (Batjes, 1996; Lal, 2004; Monger et al., 2015) in the 1-m soil layer, and 1990–2460 Pg C (Batjes, 1996; Jobbagy and Jackson, 2000) and 2300 Pg C (Zamanian et al., 2021) in the 2-m soil layer, respectively. The SIC pool is distributed in

soil solids, soil solutions and soil gases in the form of calcium carbonate (CaCO<sub>3</sub>) and magnesium carbonate (MgCO<sub>3</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbon dioxide (CO<sub>2</sub>), of which CaCO<sub>3</sub> is absolutely dominant in terms of SIC storage (Pan, 1999; Ferdush and Paul, 2021). Generally, SIC pool was considered to be very stable, and even considered as dead C pool in terms of turnover rate (Sanderman, 2012; Li et al., 2016). Therefore, the contribution of SIC was rarely investigated in the previous studies on soil CO<sub>2</sub> emission (Ferdush and Paul, 2021; Sun et al., 2023). In the past decade, with the application of <sup>13</sup>C isotope in two-source distinguishing SOC- and SIC-derived CO<sub>2</sub>, it has been found

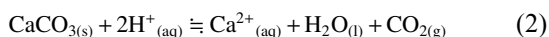
that the contribution of SIC to soil CO<sub>2</sub> emission is higher than expected, and even higher than that of SOC (Raza et al., 2021; Sun et al., 2023). We also found that the average contribution of SIC to soil CO<sub>2</sub> emission was 38% in calcareous soils, by synthesizing 27 studies on two-source distinguishing SOC- and SIC-derived CO<sub>2</sub> emission (Sun et al., 2023). Therefore, this result suggests that SIC loss is more important than expected to stabilizing total C in calcareous soils, and the SIC contribution to soil CO<sub>2</sub> emission should be considered in the assessment of soil C balance in calcareous soils (Zamanian and Kuzyakov, 2019; Sun et al., 2023).

The following chemical equilibrium applies to CaCO<sub>3</sub>-CO<sub>2</sub>-HCO<sub>3</sub><sup>-</sup> in calcareous soils (Raza et al., 2021; Sun et al., 2023):



This equilibrium of precipitation (Eq. (1) is shifted to the left) and dissolution (the opposite direction) is mainly controlled by the partial pressure of CO<sub>2</sub> in soil, soil pH value, soil moisture and the contents of calcium and magnesium ions (Ferdush and Paul, 2021; Raza et al., 2021; Sun et al., 2023). Changes in the above controlling factors may affect the dissolution and release of solid carbonate. For instance, straw incorporation usually increased the partial pressure of soil CO<sub>2</sub> by positive priming effect and straw decomposition, which may promote the dissolution and loss of CaCO<sub>3</sub> by driving the Eq. (1) to the right (Sun et al., 2021a).

Intensive agricultural measures often lead to soil acidification, resulting in a decrease in soil pH and SIC loss, among which N fertilization was the most crucial factor in cropland (Zamanian et al., 2018; Raza et al., 2021). However, in calcareous soils with typical pH between 7.5 and 8.5 (Chen and Barak, 1982), the increased strong acid (HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, organic acids) is neutralized by carbonate, and then CO<sub>2</sub> is produced as follows (Zamanian et al., 2018; Raza et al., 2021):



When high amount of ammonium-, amide-, and organic-N fertilizer is added to cropland, protons (H<sup>+</sup>) are generated through ammonium (directly from ammonium-N fertilizer or indirectly from amide- and organic-N fertilizer) nitrification of soil microorganisms, which directly react with solid carbonate, resulting in dissolution and release of carbonate (Eq. (2); Zamanian et al., 2018; Raza et al., 2021; Sun et al., 2023). Because straw-derived biochar (BC) is rich in alkalinity (including base cations, organic anions and carbonate), the addition of straw-derived biochar is an effective measure to decrease the acidifying effect induced by fertilizer-derived ammonium-N nitrification through alkalinity recycling (Raza et al., 2021; Azeem et al., 2022). After biochar addition in calcareous soils, the responses of SOC- and SIC-derived

CO<sub>2</sub> emission to biochar addition may be different, because the release of these two C sources depends on different driving mechanisms (Sun et al., 2023). In calcareous soils with biochar addition, soil CO<sub>2</sub> emission comes from at least three sources, i.e., biochar-C, SOC and SIC. Owing to technical limitations in three-source partitioning of soil CO<sub>2</sub> emission, the effects of biochar addition on SOC- and SIC-derived CO<sub>2</sub> emission is still not fully understood in calcareous soils (Sun et al., 2023). Therefore, this uncertainty needs urgent attention.

In the current study, in calcareous soils collected from a cropland of North China Plain, we conducted a 10-week laboratory incubation experiment fertilized with ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>], together with and without additions of maize-straw-derived biochar. To differentiate three sources (biochar-C, SOC and SIC) of soil CO<sub>2</sub> emission, the method was based on the <sup>13</sup>C three-source mixing model (SIMMR; Parnell et al., 2010, 2013). We hypothesized that: (1) After ammonium-N fertilizer was applied to the soils, the ammonium-N nitrification produces H<sup>+</sup>, which may result in the increase of SIC dissolution and subsequent SIC-derived CO<sub>2</sub> emission; and (2) the addition of N together with biochar may reduce the SIC-derived CO<sub>2</sub> emission, due to the alkalization capacity of biochar exceeding the acidification capacity of ammonium-N. We aimed: (1) to partition the soil CO<sub>2</sub> emission into SOC and SIC sources for the control, N-only addition or N together with biochar addition at different incubation stages, and (2) to investigate the effects of N-only or N together with biochar addition on SOC- and SIC-derived CO<sub>2</sub> emission at different incubation stages.

## 2 Materials and methods

### 2.1 Soils and maize-straw-derived biochar

The soil for the incubation experiment was taken from the topsoil (0–20 cm depth) from a cropland of Huantai County in Shandong Province, Northern China (36°57' N; 117°59' E). The soil had been used for winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) cropping per year. The soil of this region was derived from Yellow River alluvial sediments and was classified as a Fluvic Cambisol (a calcareous, fluvo-aquic sandy loam), with SOC of 8.4 g kg<sup>-1</sup> soil and SOC-δ<sup>13</sup>C value of -22.0‰, SIC of 5.4 g kg<sup>-1</sup> soil and SIC-δ<sup>13</sup>C value of -2.7‰, total N of 0.67 g kg<sup>-1</sup> soil and pH 8.1 (soil:water = 1.0:2.5). After sampling, the soil was air-dried, homogenized, and sieved with a 2-mm mesh sieve before incubation. The biochar was produced by pyrolysis of the maize straw at 350°C in a sealed high-temperature oven (DC-H8, Beijing DOTRUST Co., China). The concentration of biochar-C and the corresponding δ<sup>13</sup>C value were 17.6 g C kg<sup>-1</sup> and -13.7‰, respectively.

## 2.2 Incubation experiment

Three treatments with four replicates were set up: CK (control, no N and biochar addition), N [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> addition], N + B [both (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and maize-straw-derived biochar addition]. Biochar (< 2 mm) was mixed with soils, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added in the form of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution. The N and biochar addition rates were 300 mg N kg<sup>-1</sup> air-dried soil (corresponding to three times of local N fertilization rates; Meng et al., 2017) and 1.6 g C kg<sup>-1</sup> air-dried soil, respectively. We added 50 g air-dried soil to a 480-mL plastic incubation jar with the sealing cover. To minimize the potential effects of disturbance (e.g., sampling, sieving and rewetting) on soil microbial community, we adjusted soil moisture content in each jar to 55% water-holding capacity with deionized water and pre-incubated the soils (not sealed) for two weeks at room temperature. Then, soil moisture was re-adjusted to 75% water-holding capacity with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution for N and N + B treatments. Equivalent deionized water was added to the CK treatment. Each jar was sealed with the cover and incubated for 10 weeks at 25°C after treatment. To trap soil CO<sub>2</sub> emission, a plastic bottle (20 mL) containing 8 mL of 1 M sodium hydroxide (NaOH) solution was put into each incubation jar (Zhou et al., 2022).

## 2.3 Carbon and δ<sup>13</sup>C analysis of soil CO<sub>2</sub> emission

During the 70-d incubation, the bottle in each jar was replaced by a new bottle containing fresh NaOH solution at a 10-d interval. The NaOH trap solution of soil CO<sub>2</sub> was separated into three groups between the samplings for incubation periods of 0–10, 11–20 and 21–70 days after incubation (DAI). During 21–70 DAI, the NaOH trap solution of soil CO<sub>2</sub> over the samplings for five 10-d interval were mixed into a composite NaOH sample during the whole 50 d (Sun et al., 2019). The soil-respired CO<sub>2</sub> trapped in the NaOH solution was measured by the HCl (0.1 M)-SrCl<sub>2</sub> titration method. Blanks were included to correct for handling errors (Cheng et al., 2003; Zhu et al., 2014). To measure the δ<sup>13</sup>C of soil CO<sub>2</sub> emission, excess SrCl<sub>2</sub> (1 M) was added to the 5 mL NaOH trap solution of soil CO<sub>2</sub> to generate SrCO<sub>3</sub> precipitation (Harris et al., 1997). The SrCO<sub>3</sub> precipitation was dried at 60°C and analyzed for δ<sup>13</sup>C on the Isotope Ratio Mass Spectrometer (Vario EL III-Isoprime, Elementar, Hanau, Germany).

## 2.4 Calculations

In calcareous soils without biochar addition, a <sup>13</sup>C two-source mixing model was used to partition soil-respired CO<sub>2</sub> into two C sources (SOC and SIC; Tamir et al., 2011; Sun et al., 2023):

$$\delta_{\text{CO}_2} = \delta_{\text{SOC}} \times f_{\text{SOC}} + \delta_{\text{SIC}} \times f_{\text{SIC}} \quad (3)$$

$$1 = f_{\text{SOC}} + f_{\text{SIC}} \quad (4)$$

where  $f_{\text{SOC}}$  and  $f_{\text{SIC}}$  represent the contribution of SOC and SIC to soil total C (STC) emission, respectively;  $\delta_{\text{CO}_2}$ ,  $\delta_{\text{SOC}}$  and  $\delta_{\text{SIC}}$  are the δ<sup>13</sup>C values (‰) of soil-respired CO<sub>2</sub>, SOC and SIC, respectively.

In calcareous soils with biochar addition, soil CO<sub>2</sub> emission originates from three C sources (biochar-C, SOC and SIC). Therefore, Eqs. (3) and (4) were extended to a <sup>13</sup>C three-source mixing model for partitioning soil-respired CO<sub>2</sub> into biochar-C, SOC and SIC sources (Tamir et al., 2011; Sun et al., 2023):

$$\delta_{\text{CO}_2} = \delta_{\text{BC}} \times f_{\text{BC}} + \delta_{\text{SOC}} \times f_{\text{SOC}} + \delta_{\text{SIC}} \times f_{\text{SIC}} \quad (5)$$

$$1 = f_{\text{BC}} + f_{\text{SOC}} + f_{\text{SIC}} \quad (6)$$

where  $f_{\text{BC}}$  and  $\delta_{\text{BC}}$  represent the contribution of BC to STC emission and its δ<sup>13</sup>C value (‰), respectively. Two equations and three unknowns were solved using the isotopic mixing model, which allows us to calculate the potential contribution of multiple sources within a given mixture (CO<sub>2</sub> in this study) (Parnell et al., 2010, 2013). Here a novel Bayesian methodology was used to resolve multiple C sources by generating 10 000 potential solutions of source apportionment as the real probability distribution, implemented in the software package “SIMMR” (Stable Isotope Mixing Models in R).

N-induced changes in the emission SOC- or SIC-derived CO<sub>2</sub> (NC<sub>SOC or SIC</sub>) was calculated as the difference of SOC- or SIC-derived CO<sub>2</sub> between N-only addition and control treatments (Bramble et al., 2020; Sun et al., 2021b):

$$\text{NC}_{\text{SOC or SIC}} = \text{C}_{\text{SOC or SIC(N)}} - \text{C}_{\text{SOC or SIC(CK)}} \quad (7)$$

where  $\text{C}_{\text{SOC or SIC(N)}}$  is the SOC or SIC-derived CO<sub>2</sub> emission from the N-only addition soils, and  $\text{C}_{\text{SOC or SIC(CK)}}$  is the SOC or SIC-derived CO<sub>2</sub> emission from the control soils.

Biochar-induced changes in SOC- or SIC- derived CO<sub>2</sub> (BC<sub>SOC or SIC</sub>) emission was calculated as the difference of individual C source emission between B + N and N treatments (Luo et al., 2017; Bramble et al., 2020; Sun et al., 2021b):

$$\text{BC}_{\text{SOC or SIC}} = \text{C}_{\text{SOC or SIC(B+N)}} - \text{C}_{\text{SOC or SIC(N)}} \quad (8)$$

where  $\text{C}_{\text{SOC or SIC(B+N)}}$  is the SOC or SIC-derived CO<sub>2</sub> emission from the B + N addition soils.

Biochar- and N-induced changes in SOC- or SIC-derived CO<sub>2</sub> (BNC<sub>SOC or SIC</sub>) emission was calculated as the difference of individual C source emission between B + N and CK treatments (Luo et al., 2017; Bramble et al., 2020; Sun et al., 2021b):

$$\text{BNC}_{\text{SOC or SIC}} = \text{C}_{\text{SOC or SIC(B+N)}} - \text{C}_{\text{SOC or SIC(CK)}} \quad (9)$$

## 2.5 Statistical analysis

The data were analyzed by analysis of variance (ANOVA) to test the statistical significance of the impact of three treatments on soil CO<sub>2</sub> emission by SPSS (Version 18.0, 2009,

SPSS Inc., Chicago, IL, United States). Fisher's least significant difference (HSD;  $p < 0.05$ ) was used to determine statistical differences in the soil CO<sub>2</sub> emission among three treatments.

### 3 Results

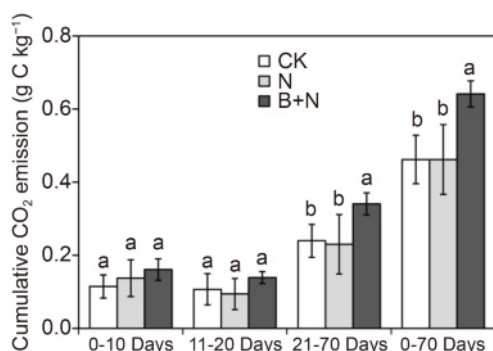
#### 3.1 Soil CO<sub>2</sub> emission and values of $\delta^{13}\text{C}\text{-CO}_2$

Over the whole 70-d incubation, the cumulative amount of soil CO<sub>2</sub> emission in the B + N treatment was significantly higher than that in the CK and N treatments, and there was no significant difference between CK and N treatments (Fig. 1). The significant difference between B + N and N (or CK) treatments in cumulative CO<sub>2</sub> emission occurred from the 21st d to the end of the 70-d incubation (Fig. 1).

Over the first 10 days of incubation, the  $\delta^{13}\text{C}$  values of CO<sub>2</sub> released from soils in the N and B + N treatments ( $-15.2\text{‰}$  and  $-13.9\text{‰}$ , respectively) were significantly more positive than that ( $-18.7\text{‰}$ ) in the CK treatment (Fig. 2), while there was no significant difference between N and B + N treatments. Over the second 10 days of incubation, soil  $\delta^{13}\text{C}\text{-CO}_2$  values of the three treatments increased, and there was no significant difference in soil  $\delta^{13}\text{C}\text{-CO}_2$  values among the three treatments (Fig. 2). Over the last 50 days of incubation, soil  $\delta^{13}\text{C}\text{-CO}_2$  values of CK and N treatments both decreased, while that of B + N treatment increased. Soil  $\delta^{13}\text{C}\text{-CO}_2$  values were in the order of B + N ( $-9.7\text{‰}$ ) > N ( $-12.6\text{‰}$ ) > CK ( $-15.7\text{‰}$ ) over the last 50 days of incubation (Fig. 2).

#### 3.2 Two/three-source partitioning of CO<sub>2</sub> emission

We used the two-pool mixing model (Fig. 3a) and the



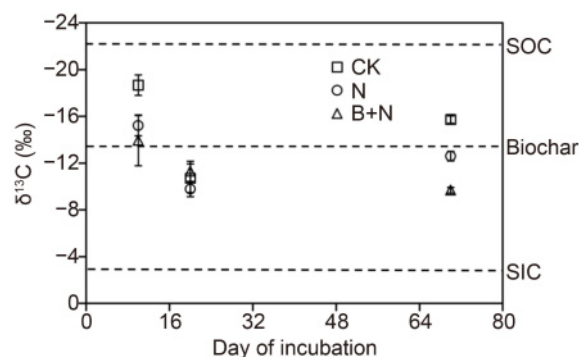
**Fig. 1** Cumulative amount of CO<sub>2</sub> emission from a calcareous soil in response to the addition of ammonium sulfate (N), ammonium sulfate plus maize-straw-derived biochar (B + N) and no addition (CK) during a 70-d incubation. Error bars indicate standard deviations ( $n = 4$ ). Different lowercase letters in the same period denote significant differences (HSD,  $p < 0.05$ ) in the total CO<sub>2</sub> emission among treatments.

SIMMR model (Fig. 3b) to partition the contribution of SOC and SIC to soil CO<sub>2</sub> emission in the CK and N treatments. The quantitative results of these two methods were approximate (Fig. 3). In the three periods of the 70-d incubation, the SIC contribution to soil CO<sub>2</sub> emission in the N treatment (34%–63%) was higher than that of the CK treatment (13%–59%; Fig. 3). We used the SIMMR model to partition the contribution of each source (biochar, SOC and SIC) to CO<sub>2</sub> emission from calcareous soils with biochar addition (Fig. 3b). As incubation period progressed, the contribution of biochar and SOC to CO<sub>2</sub> emission decreased from 36% and 43% during the first 10 days to 30% and 20% during the last 50 days of incubation respectively, while the contribution of SIC increased from 21% to 50% (Fig. 3b).

Over the whole 70-d incubation, the SIC contribution to soil CO<sub>2</sub> emission in the N treatment (47%; Fig. 4b) was 1.3 times that in the CK treatment (33%; Fig. 4a). For the B + N treatment, the contribution of SOC, SIC and biochar to soil CO<sub>2</sub> emission was 26%, 40%, and 34%, respectively (Fig. 4c).

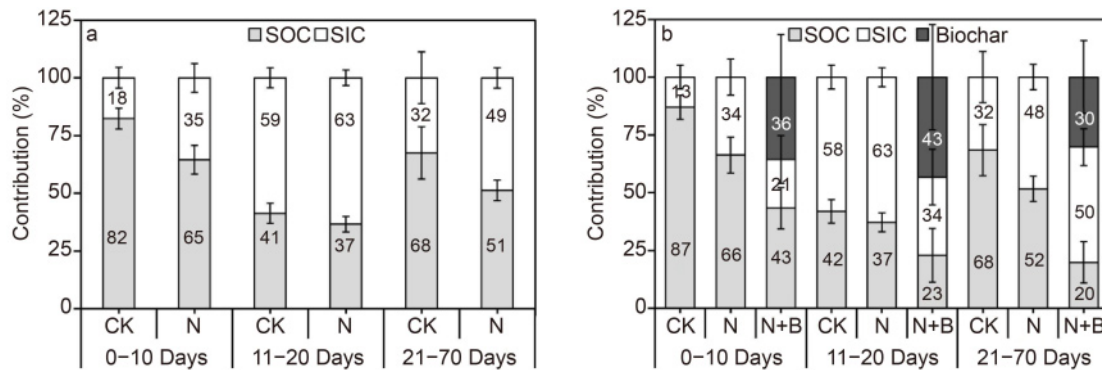
#### 3.3 Nitrogen- and biochar-induced SOC- and SIC-derived CO<sub>2</sub> emission

For each of the three incubation periods, the amount of SOC-derived CO<sub>2</sub> emission always followed the order of CK > N > B + N treatments (Fig. 5a). As incubation period progressed, the relative change of SOC-derived CO<sub>2</sub> emission induced by N-only addition and N plus biochar addition both continued to increase, while that induced by biochar addition showed a trend of decreasing first and then increasing (Fig. 5b). Over the whole 70-d incubation, the relative changes of N-, BC-, and N plus BC-induced SOC-derived CO<sub>2</sub> emission were  $-20.4\%$ ,  $-30.9\%$  and  $-45.0\%$ , respectively (Fig. 5b). Compared with the CK treatment, N and B + N treatments

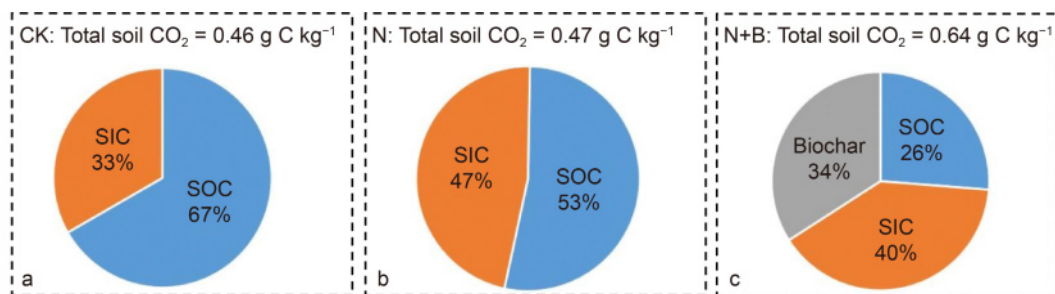


**Fig. 2**  $\delta^{13}\text{C}$  (‰) values of total CO<sub>2</sub> emission from a calcareous soil in response to the addition of ammonium sulfate (N), ammonium sulfate plus maize-straw-derived biochar (B + N) and no addition (CK) during a 70-d incubation. SOC and SIC represent soil organic carbon and soil inorganic carbon, respectively. Error bars represent standard deviations ( $n = 4$ ).





**Fig. 3** Two-source partitioning of total CO<sub>2</sub> emission from a calcareous soil with no biochar addition using the two-pool mixing model (a), and two/three-source partitioning of CO<sub>2</sub> emission from a calcareous soil with biochar addition using the SIMMR model (b). Error bars represent standard deviations ( $n = 4$ ).



**Fig. 4** Contribution of soil organic carbon (SOC), soil inorganic carbon (SIC), and biochar carbon to cumulative soil CO<sub>2</sub> emission over a 70-d incubation.

increased the SIC-derived CO<sub>2</sub> emission during the first 10 days and the last 50 days of incubation, while the two treatments decreased the SIC-derived CO<sub>2</sub> emission during the second 10 days of incubation (Fig. 5c). Over the whole 70-d incubation, the relative changes of N-, BC-, and N plus BC-induced SIC-derived CO<sub>2</sub> emission were 40.9%, 18.3% and 66.6%, respectively (Fig. 5d).

Over the whole 70-d incubation, the amount of SOC-derived CO<sub>2</sub> emission followed the order of B + N > N = CK treatments (Fig. 5e), and the relative changes of N-, BC-, and N plus BC-induced SOC-derived CO<sub>2</sub> emission were 0, -7.8%, and -7.8%, respectively (Fig. 5f).

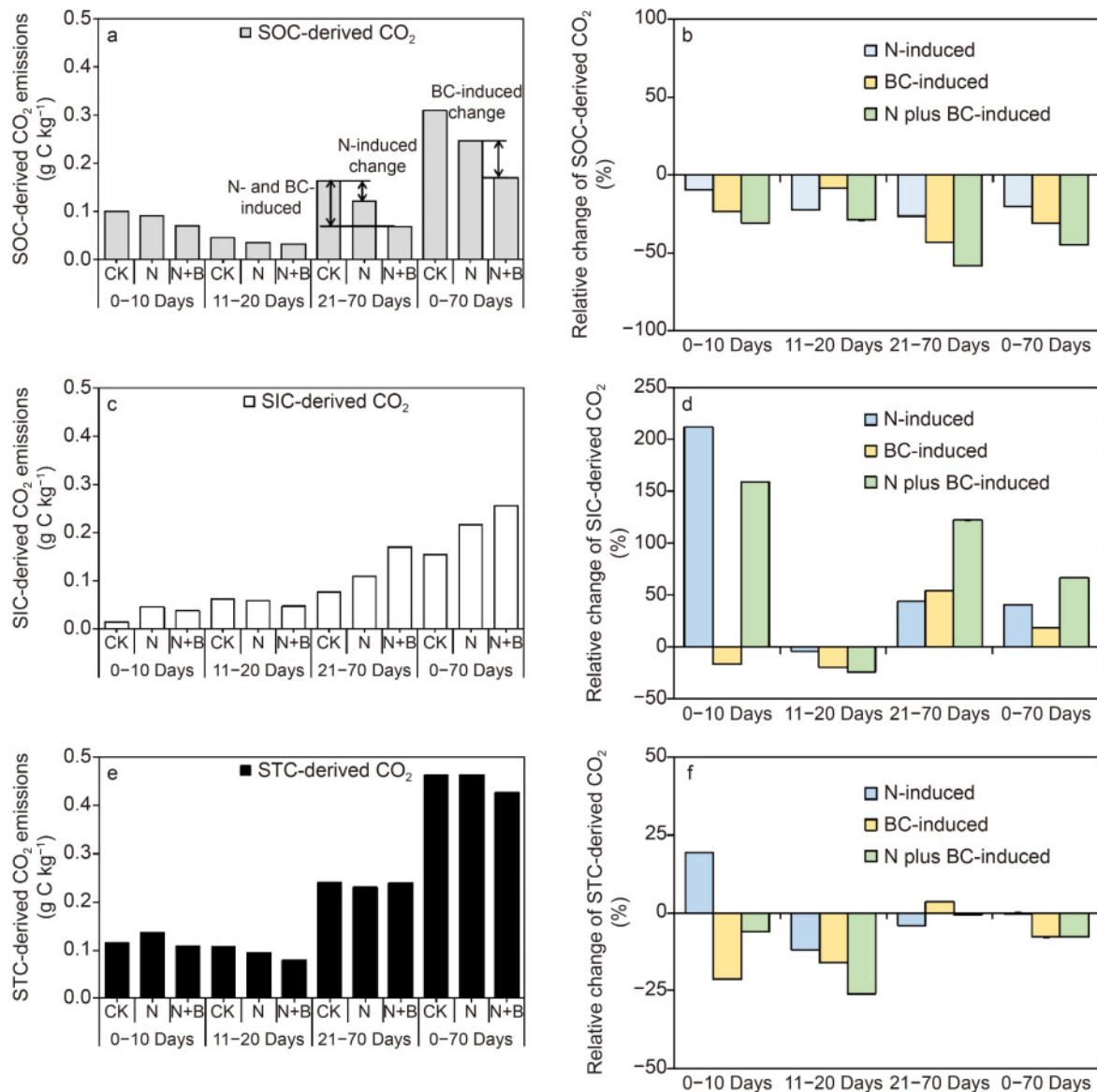
## 4 Discussion

### 4.1 Effects of N fertilization on SOC- and SIC-derived CO<sub>2</sub> emission

Nitrogen fertilization strongly reduced SOC mineralization and induced negative changes in SOC-derived CO<sub>2</sub> emission, which dropped by 20% compared with the control over the whole 70-d incubation (Fig. 5b). We also analyzed N fertilization effects on SOC mineralization from 16 studies with 51 observations in croplands (Table S1), and found that N fertilization decreased SOC-derived CO<sub>2</sub> emission by 7% on average, with a 95% confidence interval of [-17%, 3%]

(Fig. 6a). The decreased SOC mineralization under N fertilization may also be supported by the preferential substrate utilization theory (Kuzyakov, 2002; Wang et al., 2018). As such, N fertilization could decrease the magnitude of soil organic matter (SOM) mineralization, due to soil microbes preferentially using added fertilizer N and reducing mining for N from SOM (Kuzyakov, 2002; Wang et al., 2018; Feng and Zhu, 2021). In addition, in this study, the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> addition could lead to ammonium-N poisoning to soil microorganisms, leading the decrease in SOM mineralization (Treseder, 2008; Grandy et al., 2013; Wang et al., 2018). In most cases, N fertilization decreased the SOC-derived CO<sub>2</sub> emission (Fig. 6a) and increased the SIC-derived CO<sub>2</sub> emission (Fig. 6b), resulting in higher SIC contribution to soil total CO<sub>2</sub> emission compared with the control (Fig. 5a, 5c).

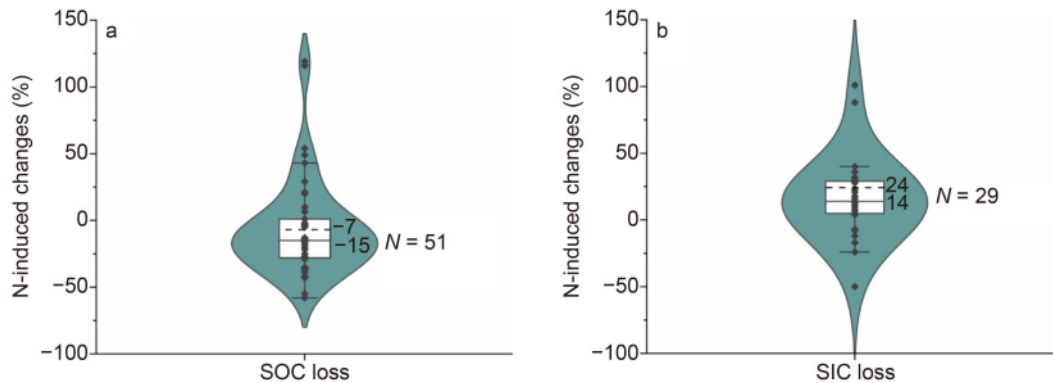
In support of our first hypothesis, this study showed that N fertilization promotes the SIC-derived CO<sub>2</sub> emission over the whole 70-d incubation (Fig. 5d). By integrating 10 studies about N-induced SIC loss from croplands (Table S2), we found that the average extent of N fertilization enhancing SIC loss was 24% ± 10% compared with no N addition, with a 95% confidence interval of [4%, 44%] (Fig. 6b). When high rates of ammonium-N, amide-N (Zamanian et al., 2018; Raza et al., 2021), and organic-N (Tamir et al., 2011) is applied to croplands, protons are produced through ammonium-N nitrification, which directly react with SIC, resulting in



**Fig. 5** Absolute and relative values (calculated as the difference in mean SOC-, SIC-, and STC-derived CO<sub>2</sub> between N and CK, N + B and N, N + B and CK) of SOC-derived CO<sub>2</sub> emission (a, b), SIC-derived CO<sub>2</sub> emission (c, d) and STC (SOC plus SIC)-derived CO<sub>2</sub> emission (e, f) from a calcareous soil in response to the addition of ammonium sulfate (N), and ammonium sulfate plus maize-straw-derived biochar (B + N) during a 70-d incubation. N-induced represents relative change of CO<sub>2</sub> emission from the N treatment compared with the CK treatment. BC-induced represents relative change of CO<sub>2</sub> emission from the B + N treatment compared with the CK treatment. BC-induced represents relative change of CO<sub>2</sub> emission from the B + N treatment compared with the CK treatment. SOC, soil organic carbon; SIC, soil inorganic carbon; STC, soil total carbon.

SIC dissolution and probably SIC-derived CO<sub>2</sub> emission (Zamanian et al., 2018; Raza et al., 2021; Sun et al., 2023). The degree of N-induced change was the highest during the first 10 days of incubation (212%), compared with the other two periods (-4% and 44%; Fig. 5d). When ammonium-N was added to calcareous soils, it is likely that the acidity generated from ammonium-N nitrification was immediate (usually within a week; Ju and Zhang, 2017), and thereby allowed the active carbonate in calcareous soils to readily solubilize (Tamir et al., 2012), leading to peak SIC-derived CO<sub>2</sub> emission in the first sampling time (Song et al., 2017;

Bramble et al., 2020). Since the retention time of nitrification in soil is generally within 10 days after the ammonium-N addition, the reverse was observed during the second 10 days of incubation, and thereafter N-induced SIC-derived CO<sub>2</sub> emission was negligible (-4%; Fig. 5d). In addition, compared with the CK treatment, N fertilization decreased soil-derived CO<sub>2</sub> production and the partial pressure of soil CO<sub>2</sub>, which may lead to the weak reduction of SIC dissolution (Fig. 5b). This is supported by Bramble et al. (2020) who found that there was a large initial increase in SIC-derived CO<sub>2</sub> emission following the ammonium-N added to



**Fig. 6** Fertilizer-N-induced changes of SOC (a) and SIC (b) loss in cropland. (a) and (b) are based on a literature survey including 51 observations from 16 articles of SOC loss (Table S1) and 29 observations from 11 articles of SIC loss (Table S2) under the N fertilization, respectively. The scattered closed symbols indicate data points in the violin figure; the  $N$  represents the number of data points; within the box, the solid line represents the median value ( $-15\%$  and  $14\%$ ), and the dashed line presents the mean value ( $-7\%$  and  $24\%$ ); the bottom and top edges of the box represent 25 and 75 percentiles of all data, respectively; and the bottom and top bars represent 5 and 95 percentiles of all data, respectively.

calcareous soils during the first 5 h of incubation, and the cumulative SIC-derived  $\text{CO}_2$  emission over the 23-d incubation was, however, statistically similar between N and control treatments. Hence, the effect of N fertilization on SIC-derived  $\text{CO}_2$  emission may mainly be attributed to the ammonium-N nitrification in the initial stage (Ju and Zhang, 2017; Song et al., 2017), and then may rely on the formation of  $\text{H}_2\text{CO}_3$  from the SOC decomposition after short-term nitrification (Bramble et al., 2020; Sun et al., 2023).

#### 4.2 Effects of N fertilization on SOC- and SIC-derived $\text{CO}_2$ emission after biochar addition

In this study, biochar retarded the native SOC mineralization (negative priming effect) by 31% compared with the soil without biochar addition under N fertilization (Fig. 5d). Preferential substrate utilization of soil microorganisms for the available biochar-C and fertilizer-N is likely responsible for the large negative priming effect (Kuzyakov, 2002; Wang et al., 2015). Our results are consistent with the meta-analysis of Wang et al. (2016) who also found negative priming effects of biochar addition on SOC mineralization. Considering the fact that the inhibition of SOC-derived  $\text{CO}_2$  emission by biochar addition exceeds the enhancement of SIC-derived  $\text{CO}_2$  emission (Fig. 5f), we conclude that biochar addition probably has a net positive effect on soil total C sequestration for calcareous soils of the North China Plain.

As we hypothesized, N combined with biochar addition decreased SIC-derived  $\text{CO}_2$  emission by 17%–20% in the initial 20 days of incubation (Fig. 5d). This result suggested that SIC loss could be mitigated through alkalinity regeneration using the alkalinity-rich biochar addition (Raza et al., 2021; Azeem et al., 2022). The biochar alkalinity includes not only the contribution of carbonate in the biochar, but also the contributions of acid-soluble inorganic (non-carbonate)

and organic compounds (Fidel et al., 2017a). For instance, Fidel et al. (2017a) found that the contributions of the carbonate, acid-soluble inorganic (non-carbonate) and organic alkalis to total alkalinity were 37%, 26%, and 37% in the corn-stover-derived biochar, respectively. However, after the 20-d incubation, N combined with biochar addition promoted SIC-derived  $\text{CO}_2$  emission compared with the sole N treatment (Fig. 5d). This might be attributed to the fact that N combined with biochar addition increased OC (BC plus SOC)-derived  $\text{CO}_2$  production by 50% compared with the sole N treatment, leading to increasing soil  $\text{CO}_2$  concentration and subsequent more SIC dissolution and loss (Fig. S3). Therefore, in the short-term, N combined with biochar addition may reduce the dissolution and loss of SIC by the biochar alkalinity neutralizing the strong acidity produced by the ammonium-N nitrification (Raza et al., 2021; Azeem et al., 2022); but in the long-term, it may increase SIC loss by the weak acidity induced by biochar mineralization (Sun et al., 2021a).

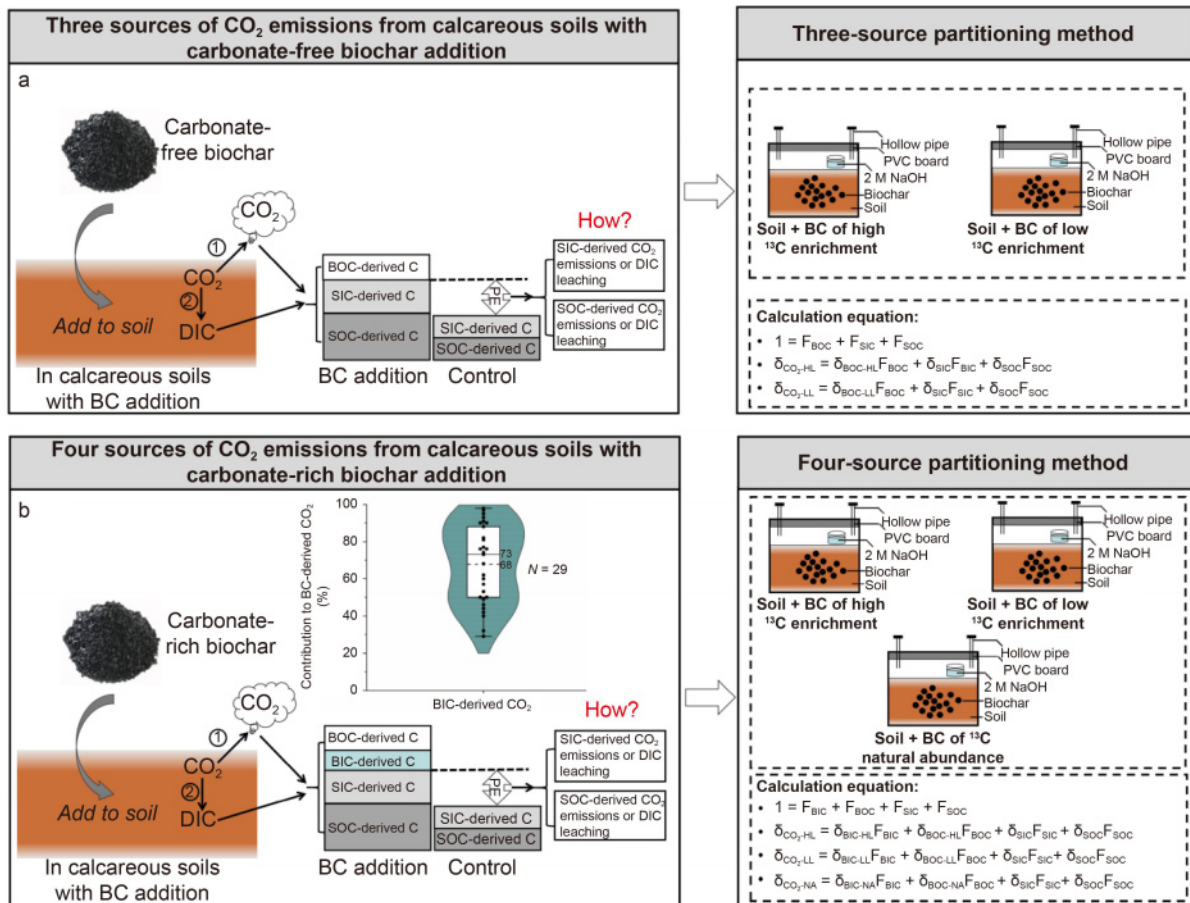
#### 4.3 Uncertainty in partitioning $\text{CO}_2$ emission from biochar-amended calcareous soils

Biochar total carbon (BTC) may include two components of biochar organic carbon (BOC) and biochar inorganic carbon (BIC; Fidel et al. 2017b). For example, Wang et al. (2014) found 58% of 117 biochar samples contained  $< 4 \text{ g BIC-C kg}^{-1}$ , while BIC-C was not detected in 38% of these samples. In biochar-amended soils, previous studies usually did not consider the contribution of BIC and SIC to soil  $\text{CO}_2$  emission, and the two-source partitioning of soil  $\text{CO}_2$  emission was mainly determined by  $\text{CO}_2$  difference method (Fiorentino et al., 2019),  $^{13}\text{C}$  natural abundance method (Luo et al., 2017; Chen et al., 2022a) and  $^{13}/^{14}\text{C}$ -labeled biochar addition (Bruun et al., 2014; Cui et al., 2017; Chalk

and Smith, 2022) or long-term <sup>13</sup>C/<sup>14</sup>C labeled SOC method (Jones et al., 2011). If the biochar does not contain detectable BIC-C, the contribution of BOC, SOC and SIC to total CO<sub>2</sub> emission from calcareous soils can be partitioned by the <sup>13</sup>C three-source model (Tamir et al., 2011; Sun et al., 2023). In this study, we neglected the contribution of BIC-derived CO<sub>2</sub> emission, due to the fact that biochar contains very low amount of BIC-C (0.8 g C kg<sup>-1</sup>). In this study, the average contribution of three C sources to total soil CO<sub>2</sub> emission was quantified using the SIMMR model (Parnell et al., 2010, 2013), which showed advantages compared with the two-source partitioning. By the SIMMR model, however, the contribution of three C sources may not be

accurately quantified, and only the range of contribution of different substrates to CO<sub>2</sub> emission is provided (Parnell et al., 2010, 2013). In the future, to improve the accuracy of three-source partitioning CO<sub>2</sub> emission from calcareous soils with carbonate-free biochar, we also propose a new method (Fig. 7a): (1) two types of biochars of high and low <sup>13</sup>C enrichment are added to the same soil system for incubation, respectively; and then (2) with the aid of the three-source <sup>13</sup>C mixing model, the contributions of BOC, SOC and SIC to total soil CO<sub>2</sub> emission may be successfully separated (Whitman and Lehmann, 2015; Chen et al., 2022b; Fang et al., 2022; Sun et al., 2023).

For biochar containing high amount of carbonate (e.g.,



**Fig. 7** Four-source partitioning of CO<sub>2</sub> emission from calcareous soils with biochar (BC) addition. Note: PE, priming effect; DIC, dissolved inorganic carbon; BOC, biochar organic C; BIC, biochar inorganic C; SOC, soil organic C; SIC, soil inorganic C; F<sub>BOC</sub>, BOC contribution to soil CO<sub>2</sub> emission; F<sub>BIC</sub>, BIC contribution to soil CO<sub>2</sub> emission; F<sub>SOC</sub>, SOC contribution to soil CO<sub>2</sub> emission; F<sub>SIC</sub>, SIC contribution to soil CO<sub>2</sub> emission; CO<sub>2</sub>, soil CO<sub>2</sub> emission; δ<sub>CO<sub>2</sub>-HL</sub>, δ<sup>13</sup>C (‰) values of CO<sub>2</sub> emission from soils with highly <sup>13</sup>C-enriched BC addition; δ<sub>CO<sub>2</sub>-LL</sub>, δ<sup>13</sup>C values of CO<sub>2</sub> emission from soils with lowly <sup>13</sup>C-enriched BC addition; δ<sub>CO<sub>2</sub>-NA</sub>, δ<sup>13</sup>C values of CO<sub>2</sub> emission from soils with non-labeled BC addition; δ<sub>SIC</sub>, δ<sup>13</sup>C values of SIC; δ<sub>SOC</sub>, δ<sup>13</sup>C values of SOC; δ<sub>BIC-HL</sub>, BIC-δ<sup>13</sup>C values of highly <sup>13</sup>C-enriched BC; δ<sub>BOC-HL</sub>, BOC-δ<sup>13</sup>C values of highly <sup>13</sup>C-enriched BC; δ<sub>BIC-LL</sub>, BIC-δ<sup>13</sup>C values of lowly <sup>13</sup>C-enriched BC; δ<sub>BOC-LL</sub>, BOC-δ<sup>13</sup>C values of lowly <sup>13</sup>C-enriched BC; δ<sub>BIC-NA</sub>, BIC-δ<sup>13</sup>C values of non-labeled BC; δ<sub>BOC-NA</sub>, BOC-δ<sup>13</sup>C values of non-labeled BC. This violin figure (inset in Fig. 7b) indicates literature survey of the contribution of BIC to BC-derived CO<sub>2</sub> emission, including 29 observations from four articles of BC-derived CO<sub>2</sub> partitioning (Table S3). In this violin figure: the scattered closed symbols indicate data points; within the box, the *N* represents the number of data points; the solid line represents the median value (73%), and the dashed line presents the mean value (68%); the bottom and top edges of the box represent 25 and 75 percentiles of all data, respectively; and the bottom and top bars represent 5 and 95 percentiles of all data, respectively.



> 3 g C kg<sup>-1</sup>; Wang et al., 2014), the BIC contents are sometimes in the same order of magnitude as the SIC contents (Dong et al., 2018, 2019). For example, although the BIC/BTC was only 0.31%–0.60% in barley-root-derived biochar (Bruun et al., 2014) and 2% in mainly rice-husk-derived biochar (Dong et al., 2019), the content of BIC derived from the barley root and rice-husk was 1.0–3.8 g C kg<sup>-1</sup> (Bruun et al., 2014) and 10.0 g C kg<sup>-1</sup> (Dong et al., 2019). Recent studies have shown that the BIC dissolution contributes greatly to the CO<sub>2</sub> emission from BTC when biochar is added to acidic soils, with an average contribution of 68% (Fig. 7b; Zimmerman, 2010; Jones et al., 2011; Yang, 2013; Bruun et al., 2014). However, most previous studies neglected the contribution of BIC to total soil CO<sub>2</sub> emission, and thus overestimated the microbial decomposition of biochar and further affected the calculation of the residence time and stability of biochar in soil (Sheng et al., 2016; Chalk and Smith, 2022). When there is a large amount of BIC, BIC can interfere with the <sup>13</sup>C signature measurement of BOC, due to the difference in the <sup>13</sup>C signal between BIC and BOC (Chalk and Smith, 2022). In this case, it requires measuring the <sup>13</sup>C signature of both BIC and BOC, and then quantifying the contribution of each C source to total soil CO<sub>2</sub> emission. After adding carbonate-rich biochar to calcareous soils, soil CO<sub>2</sub> emission can be released from up to four sources (BOC, BIC, SOC and SIC), which may be distinguished by sterilization combined with C labeling (Bruun et al., 2014). The process is as follows: (1) quantifying the IC-derived CO<sub>2</sub> emission by the sterilization method, and partitioning it into SIC- and BIC-derived CO<sub>2</sub> by the <sup>13</sup>C two-source mixing model; and (2) on the basis of the first step, the contribution of SOC- and BOC-derived CO<sub>2</sub> was further separated by the four-source <sup>13</sup>C mixing model (Sun et al., 2023). However, this method ignores the priming effect of biochar on SOC-derived CO<sub>2</sub> emission as well as the increase in soil CO<sub>2</sub> partial pressure from SOC- and BOC-derived CO<sub>2</sub>, which may lead to the enhancement of SIC and BIC dissolution (Sun et al., 2023). To accurately distinguish four sources of CO<sub>2</sub> emission from calcareous soils with carbonate-rich biochar addition, we also propose a new method (Fig. 7b): (1) three types of biochars of high/low <sup>13</sup>C enrichment and <sup>13</sup>C natural abundance are added to the same soil system for incubation, respectively; and finally (2) by the four-source <sup>13</sup>C mixing model, the contributions of BOC, BIC, SOC and SIC to total soil CO<sub>2</sub> emission may be successfully separated (Whitman and Lehmann, 2015; Sun et al., 2023).

## 5 Conclusion

Our results showed N-only addition decreased the SOC-derived CO<sub>2</sub> emission by 20%, but increased the SIC-

derived CO<sub>2</sub> emission by 41% over the 70-d incubation without changing total soil CO<sub>2</sub> emission. Compared with N-only addition, the addition of N together with biochar decreased and increased the SIC-derived CO<sub>2</sub> emission by 17%–20% and 54% in the initial two (0–10 and 11–20 d) and the third/last (21–70 d) periods of incubation respectively, while decreased the SOC-derived CO<sub>2</sub> emission by 8%–43% among the three periods of incubation. Our incubation experiment showed the evidence of distinct effects of N-only addition or combined N and biochar addition on SOC- and SIC-derived CO<sub>2</sub> emission without considering the possible BIC contribution to total CO<sub>2</sub> emission. However, to improve the quantification accuracy of soil native C emission in calcareous soils with carbonate-rich biochar addition, more work is needed to take into account the BIC-derived CO<sub>2</sub> to total soil CO<sub>2</sub> emission, and we propose a novel approach for distinguishing four sources of CO<sub>2</sub> emission from calcareous soils with carbonate-rich biochar addition (Fig. 7b).

## Abbreviations

SOC, soil organic carbon; SIC, soil inorganic carbon; STC, soil total carbon; BC, biochar; BTC, biochar total carbon; BOC, biochar organic carbon; BIC, biochar inorganic carbon.

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## Electronic supplementary material

Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s42832-022-0169-8> and is accessible for authorized users.

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