

REVIEW

Influencing factors and partitioning methods of carbonate contribution to CO₂ emissions from calcareous soils

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HIGHLIGHTS

- This study reviewed the contribution of carbonates to soil CO₂ emissions.
- The contribution was on average 27% in calcareous soils.
- The contribution was affected by both biotic and abiotic factors.
- We proposed a new method of distinguishing three CO₂ sources from calcareous soils.

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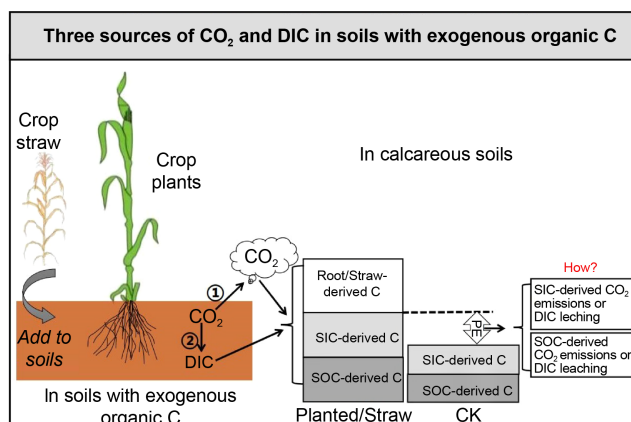
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GRAPHICAL ABSTRACT



ABSTRACT

In calcareous soils, recent studies have shown that soil-derived CO₂ originates from both soil organic carbon (SOC) decomposition and soil inorganic carbon (SIC) dissolution, a fact often ignored in earlier studies. This may lead to overestimation of the CO₂ emissions from SOC decomposition. In calcareous soils, there is a chemical balance between precipitation and dissolution of CaCO₃-CO₂-HCO₃⁻, which is affected by soil environmental factors (moisture, temperature, pH and depth), root growth (rhizosphere effect) and agricultural measures (organic materials input, nitrogen fertilization and straw removal). In this paper, we first introduced the contribution of SIC dissolution to CO₂ emissions from calcareous soils and their driving factors. Second, we reviewed the methods to distinguish two CO₂ sources released from calcareous soils and quantify the ¹³C fractionation coefficient between SIC and SIC-derived CO₂ and between SOC and SOC-derived CO₂, and to partition three CO₂ sources released from soils with plants and organic materials input. Finally, we proposed methods for accurately distinguishing three CO₂ sources released from calcareous soils. This review helps to improve the accuracy of soil C balance assessment in calcareous soils, and also proposes the direction of further investigations on SIC-derived CO₂ emissions responses to abiotic factors and agricultural measures.

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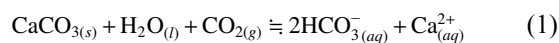
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1 Introduction

The soil carbon (C) pool includes soil organic C (SOC) and soil inorganic C (SIC). The global SOC and SIC storage at 0–1 m depth is approximately 1460–1550 Pg C (Batjes, 1996; Lal, 2004; Monger et al., 2015) and 700–950 Pg C (Batjes, 1996; Lal, 2004; Monger et al., 2015; Ferdush and Paul, 2021), respectively. In calcareous soils, the SIC pool includes not only calcium carbonate (CaCO_3) and magnesium carbonate (MgCO_3 ; solid phase) deposited in soil, but also HCO_3^- in soil solution (liquid phase) and CO_2 in soil air (gas phase; Pan, 1999; Monger et al., 2015; Ferdush and Paul, 2021), among which CaCO_3 and MgCO_3 are dominant (Pan, 1999; Mikhailova and Post, 2006; Sanderman, 2012). SIC is generally considered to be more stable than SOC, so the dynamics of SIC have seldom been considered in previous studies on soil C cycle (Bughio et al., 2017; Zamanian et al., 2018). However, under the development of C isotope technology for analysis of soil C cycle, SIC has recently been found to be a C source (carbonate dissolution and release; Ramnarine et al., 2012; Zamanian et al., 2018; Raza et al., 2021) or C sink (secondary carbonate formation; Wang et al., 2014; Zamanian et al., 2016; Bughio et al., 2017; Zhao et al., 2020). Therefore, the stability of SIC pool in calcareous soils directly affects atmospheric CO_2 concentration and soil C balance (Emmerich, 2003; Lal, 2009; Zamanian et al., 2021; Song et al., 2022).

The following chemical equilibrium ($\text{CaCO}_3\text{-CO}_2\text{-HCO}_3^-$) applies to calcareous soils (Chou et al., 1989; Serrano-Ortiz et al., 2010; Sanderman, 2012):



This equilibrium of precipitation and dissolution is mainly affected by the partial pressure of CO_2 in soil (Karberg et al., 2005; Nomeli and Riaz, 2017; Zhao et al., 2020), soil pH value (Blagodatskaya and Kuzyakov, 2008), soil moisture (Stevenson and Verburg, 2006; Inglima et al., 2009; Dong et al., 2014; Lardner et al., 2015) and the contents of calcium and magnesium ions (Dreybrodt et al., 1996; Bughio et al., 2017). Changes in these controlling factors may have an important effect on the dissolution and release of CaCO_3 . Recent studies have shown that factors such as soil depth (Mi et al., 2008; Cardinael et al., 2019), soil pH value (Shi et al., 2012; Lardner et al., 2015), root growth (Mubarak and Nortcliff, 2010; Ahmad et al., 2013, 2020; Sun et al., 2021a; Wen et al., 2021), organic materials input (Tamir et al., 2011, 2013) and soil acidification resulted mainly from high nitrogen (N) fertilizer inputs and straw removal (Berthrong et al., 2009; Guo et al., 2010; Gandois et al., 2011; Zamanian et al., 2018; Guo and Chen, 2021), affect the chemical equilibrium of CaCO_3 precipitation and dissolution. The aim of this paper is to review the SIC-derived CO_2 emissions responses to environmental factors or agricultural measures, and propose methods for accurately partitioning three CO_2 sources released from calcareous soils. The

results described in this paper can be applied to improving the accuracy of C balance assessment in calcareous soils.

2 Contribution and influencing factors of carbonate to soil CO_2 emissions

2.1 Importance of carbonate to soil CO_2 emissions

To date, the two-source apportionment of CO_2 released from calcareous soils has mainly been conducted via short-term incubation experiments without exogenous organic C (Table S1). On the basis of the synthesis of 28 studies on the contribution of SIC dissolution to CO_2 emissions (SOC-plus SIC-derived CO_2) from calcareous soils (Table S1), we found that the average contribution of SIC to soil CO_2 emissions was $27\% \pm 2\%$, with a 95% confidence interval of 22%–31% (Fig. 1). This suggests that SIC dissolution is more important than previously thought in stabilizing global soil C pool and regulating atmospheric CO_2 concentration (Bertrand et al., 2007; Zamanian et al., 2018; Raza et al., 2021; Zamanian et al., 2021). In long-term monitoring studies at a national or global scale, recent studies also found that the loss rates of carbonate from calcareous croplands were 7.5–8.2 Tg C year⁻¹ globally (Zamanian et al., 2018, 2021; Table 1) and 3.8–57.1 Tg C year⁻¹ at 30–98 cm soil depth in China (Wu et al., 2009; Raza et al., 2020; Song et al., 2022; Table 1). However, previous studies have mostly ignored the contribution of SIC dissolution to soil CO_2 emissions, which may overestimate SOC decomposition (Ferdush and Paul, 2021; Sun et al., 2021a). The contribution of SIC-derived CO_2 should be considered in the future assessment

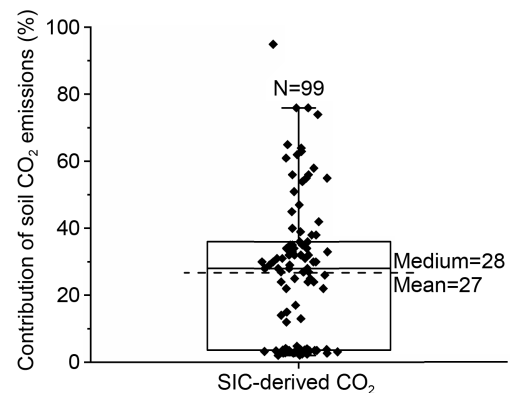


Fig. 1 Synthesis analysis of the contribution of SIC to CO_2 emissions from calcareous soils. Note: this figure is based on a literature synthesis that includes 99 observations from 28 studies of soil CO_2 partitioning in calcareous soils without exogenous carbon input (Table S1). The scattered black diamonds indicate data points; the N in the box represents the number of data points; the solid and dashed lines within the box represent the median and mean values of all data; 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.

Table 1 Soil carbonate losses from calcareous cropland.

Studied area	Duration (year)	Soil depth (cm)	Rate of carbonate loss(Tg C year ⁻¹)	References
Chinese cropland	28 (1980–2008)	0–98	57.1	Wu et al., 2009
Chinese cropland	40 (1980–2020)	0–40	3.8	Raza et al., 2020
Chinese cropland	30 (1980–2010)	0–30	16.0	Song et al., 2022
Global cropland	—	—	7.5	Zamanian et al., 2018
Global cropland	50 (1970–2020)	—	8.2	Zamanian et al., 2021

of soil C balance in calcareous soils (Ramnarine et al., 2012; Raza et al., 2021).

2.2 Soil moisture

In calcareous soils, increases in soil moisture usually stimulate CO₂ emissions from carbonate (Stevenson and Verburg, 2006; Dong et al., 2014; Lardner et al., 2015). This is caused by two main pathways: (1) the increase of soil moisture enhances SOC mineralization and reduces CO₂ diffusion (Nobel and Palta, 1989; Serrano-Ortiz et al., 2010), leading to the increase in soil CO₂ concentration, which may promote the dissolution of CaCO₃ (Inglima et al., 2009; Lardner et al., 2015); and (2) the soil moisture itself drives the CaCO₃-CO₂-HCO₃⁻ equilibrium equation (Eq. (1)) to the right (Inglima et al., 2009; Dong et al., 2014; Lardner et al., 2015). However, these two pathways co-exist temporally and spatially. To study the direct effect of soil moisture on the CaCO₃-CO₂-HCO₃⁻ equilibrium, soil sterilization is required to eliminate the indirect effect of the increase of soil CO₂ concentration caused by the extra SOC mineralization (Stevenson and Verburg, 2006). For instance, Stevenson and Verburg (2006) found that significant CO₂ emissions from SIC after wetting in calcareous soils by sterilization, suggesting that wetting directly causes Eq. (1) to shift to the right and fostering carbonate dissolution. High soil moisture usually stimulated SOC mineralization to a much larger extent than it stimulated SIC dissolution (Inglima et al., 2009; Schindlbacher et al., 2015). Therefore, the contribution of SIC to the total soil CO₂ emissions could be lower under wetting conditions (Inglima et al., 2009; Schindlbacher et al., 2015; Xu et al., 2022).

Although irrigation or rainfall can increase SOC mineralization by increasing soil moisture and then increasing soil CO₂ concentration, it does not necessarily increase SIC-derived CO₂ emissions under field conditions (Inglima et al., 2009). This is because soil CO₂ and H₂O react under field conditions to form H₂CO₃ in irrigation or rainfall transport to deep soil, thus leading to pH increase in the topsoil and no major increase in SIC-derived CO₂ emissions (Inglima et al., 2009). At present, most studies on the effect of soil moisture on SIC dissolution are carried out based on a closed-jar incubation (Stevenson and Verburg, 2006; Schindlbacher et al., 2015; Dong et al., 2014; Lardner et al., 2015; Xu et al., 2022), which only considers the SIC-derived CO₂ emissions and ignores the effect of soil dissolved inorganic C (DIC)

leaching (Inglima et al., 2009; Hodges et al., 2021). For example, Hodges et al. (2021) found that in the calcareous soils, 43% of CO₂ respired by roots and microbes dissolves and leaches from the soil rather than CO₂ emissions to the atmosphere. Accordingly, future studies taking SIC-derived DIC leaching into account are needed to better understand the response of SIC dissolution to soil moisture change. Additionally, because farmland is often irrigated with groundwater rich in DIC, it remains unclear whether this part of DIC is released in the form of CO₂ (Entry et al., 2004). To examine this problem, Hannam et al. (2016,2019) applied the ¹³C two-source model to reveal that the contribution of DIC in groundwater (approximately 17 mg C L⁻¹) to CO₂ emissions in irrigation soil represented 9%–45% of total CO₂ emissions. Therefore, if the contribution of DIC from irrigation water to soil CO₂ emissions is ignored, the SOC decomposition will be overestimated.

2.3 Soil temperature

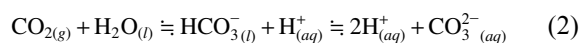
The equilibrium of carbonate dissolution and release is affected by temperature (Eq. (1)). Recent studies have shown that warming increases the CO₂ emissions from SIC dissolution in the laboratory-incubation (Stevenson and Verburg, 2006; Chevallier et al., 2016; Xu et al., 2022) and the field (Wang et al., 2013; Zhang et al., 2019; Sun et al., 2021b) experiments. For example, in the laboratory-incubation conditions, Chevallier et al. (2016) found that SIC-derived CO₂ emissions increased with the increase of incubation temperature between 20°C and 50°C. In the 5-year field warming experiment, Sun et al. (2021b) also found that the SIC-derived CO₂ emissions increased by 11%–14%, when the warming temperature was kept at 2.0°C above the ambient temperature. This may have occurred for the following reasons: (1) according to Henry's law, soil moisture and carbonate have a higher energy under the condition of higher temperature, which is favorable for the dissolution reaction (Wang et al., 2013; Xu et al., 2019); (2) the solubility of CO₂ in soil solution decreases with the increase of temperature (Zamanian et al., 2016), which may directly cause SIC-derived CO₂ emissions from soil; (3) elevated temperature usually promotes SOC decomposition (Davidson and Janssens, 2006; Chevallier et al., 2016; Zhang et al., 2019), and the increased SOC-derived CO₂ forms H₂CO₃ with water in soil solution, thus leading to the CaCO₃-CO₂-HCO₃⁻ equilibrium equation (Eq. (1)) to move to

the right and subsequent carbonate dissolution (Serrano-Ortiz et al., 2010); or (4) higher temperature usually increases microbial activity, which may enhance the nitrification of mineralized organic N and oxidation of reduced sulfur (Tamir et al., 2011, 2013; Nguyen et al., 2019; Raza et al., 2021), leading to more H⁺ ions production and the increase in carbonate dissolution. The responses of the increasing SOC- and SIC-derived CO₂ emissions to warming were substantially different (Stevenson and Verburg, 2006; Chevallier et al., 2016; Zhang et al., 2019; Sun et al., 2021b). In most cases, the temperature sensitivity of SOC-derived CO₂ emissions was greater than that of SIC-derived CO₂ emissions, resulting in lower contribution of CO₂ emissions from SIC dissolution in warming conditions (Stevenson and Verburg, 2006; Chevallier et al., 2016; Zhang et al., 2019; Sun et al., 2021b; Xu et al., 2022).

2.4 Soil pH

Recent studies found that the SIC-derived CO₂ emissions increased significantly with the decrease of pH value (Lardner et al., 2015; Nomeli and Riaz, 2017). For example, under the condition of field capacity above a third, soil pH decreased from 7.6 to 6.6, leading to the increase by 46 times in SIC-derived CO₂ emissions (Lardner et al., 2015). The pH decrease is neutralized by CaCO₃ and MgCO₃ in calcareous soils with pH ranging 6.5–8.5 (Bowman et al., 2008; Kuzyakov et al., 2021; Raza et al., 2021). When the pH is higher than 8.5, the pH decrease is largely buffered by Na₂CO₃ (Bowman et al., 2008; Raza et al., 2021). This implies that carbonates provide the strong neutralization capacity and maintain the soil pH > 6.5, unless carbonate is depleted by produced acid (Bowman et al., 2008; Guo et al., 2010; Kuzyakov et al., 2021; Raza et al., 2021).

The released CO₂ from soil is not directly discharged, but dissolved in soil solution, forming a CO₃²⁻-HCO₃⁻-H₂CO₃ balance, which is mainly affected by soil pH (Martens, 1987; Emmerich, 2003; Blagodatskaya and Kuzyakov, 2008):

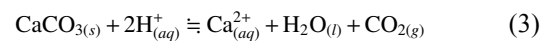


Previous studies on soil C release generally measured soil CO₂ emissions only and ignored the CO₂ dissolved in soil solution, which is correct for acidic and neutral soils (Blagodatskaya and Kuzyakov, 2008). However, because the soil pH value of calcareous soils in arid areas is generally between 7.5 and 8.5, a large amount of soil CO₂ is dissolved in soil solution and mainly forms HCO₃⁻ (Martens, 1987; Oren and Steinberger, 2008), which leads to the underestimation of soil CO₂ emissions by 10%–100% (Blagodatskaya and Kuzyakov, 2008). In such case, H₂CO₃ reacts with CaCO₃, which results in the formation of HCO₃⁻ until the CO₂-saturated capacity is reached; then, the excess HCO₃⁻ dissociates to release CO₂ from the soil (Blagodatskaya and Kuzyakov, 2008; Mehra et al., 2019). The CaCO₃ solubility is relatively high in CO₂-saturated

water (by the carbonation reaction), which is approximately 30 times that of pure water (by carbonate hydrolysis; Skidmore et al., 2004; Zamanian et al., 2018). Therefore, the proportion of SIC-derived CO₂ into DIC leaching and soil-respired CO₂ should be taken into account when quantifying SIC dissolution in calcareous cropland. Soil column experiments are a convenient way to quantify DIC leaching and soil-respired CO₂ resulting from SIC dissolution (Gandois et al., 2011). However, soil column studies of SIC dissolution are scarce.

In desert soil, the pH value of soil is very high (8.5–11), and the majority of DIC at this pH is as HCO₃⁻ and HCO₃²⁻ (Chou et al., 1989; Blagodatskaya and Kuzyakov, 2008). In this case, the HCO₃⁻/CO₃²⁻ and Ca²⁺/Mg²⁺ ions in soil solution form secondary carbonate, and even achieve inorganic C sequestration (Chou et al., 1989; Xie et al., 2009; Serrano-Ortiz et al., 2010; Wang et al., 2014). Additionally, in calcareous farmland in arid and semi-arid areas, fertilization, straw return and groundwater irrigation increased the concentration of Ca²⁺/Mg²⁺ ions, which intensified the absorption and fixation of CO₂ produced by roots and microbes, resulting in the accumulation rate of secondary carbonate exceeding that of SOC (Emmerich, 2003; Wang et al., 2014; Bughio et al., 2017). To solve the problem of CO₂ retention in calcareous soils, a CO₂-trapping system of closed circulation or continuous flushing driven by air pump is an effective method (Martens, 1987), which is better than the static alkali absorption method due to high CO₂-trapping efficiency (Cheng, 1996).

A variety of intensive agricultural practices affect soil acidification, resulting in a decrease in soil pH, among which application of N fertilizers and removal of crop residues are the most crucial in cropland (Guo et al., 2010; Zamanian et al., 2018; Raza et al., 2021). However, in calcareous soils within typically pH 7.5–8.5 (Chen and Barak, 1982), the increasing strong acid (HNO₃, H₂SO₄, organic acids) is neutralized by CaCO₃ and MgCO₃, and then CO₂ will be produced as follows (Ulrich, 1986; Oren and Steinberger, 2008; Tamir et al., 2011; Sanderman, 2012):



Nitrogen-application-induced soil acidification

When a large amount of ammonium N, amide N (Zamanian et al., 2018; Raza et al., 2021), and organic N fertilizer (Tamir et al., 2011, 2013) is applied to farmland, proton (H⁺) ions are generated through NH₄⁺ uptake of plants and nitrification of microorganisms (Barak et al., 1997), which directly reacts with carbonate, resulting in carbonate dissolution (Eq. (3); Gandois et al., 2011). By integrating the literature about fertilizer N acidification-induced carbonate losses from croplands, we found that the average degree of N fertilizer promoting carbonate losses was 40% ± 15%, with a 95% confidence interval of 11%–69% (Table 2). In the

Table 2 Nitrogen fertilization enhancement of soil carbonate loss from calcareous cropland.

Location	SIC/STC (%)	Degree of SIC loss increased by fertilizer N (%)	Soil type	Fertilizer N rate	Method	References
Finland	13	88	Peatland	59.5 kg N ha ⁻¹ (Slow-release fertilizer)	¹³ C natural abundance	Biasi et al., 2008
Israel	74	268	Typic Calciorthid	1755 kg N ha ⁻¹ (Chicken manure)	¹³ C natural abundance	Tamir et al., 2011
China	66	4	—	45 kg N ha ⁻¹ (Urea)	C difference method	Li et al., 2014
		7		90 kg N ha ⁻¹ (Urea)		
		11		135 kg N ha ⁻¹ (Urea)		
		14		180 kg N ha ⁻¹ (Urea)		
China	50	5	Eum-Orthic Anthrosol	0.2 g N kg ⁻¹ (Ammonium sulfate)	Soil sterilization with HgCl ₂	Meng et al., 2015
		9		0.3 g N kg ⁻¹ (Ammonium sulfate)		
		18		0.4 g N kg ⁻¹ (Ammonium sulfate)		
China	42	7	Eum-Orthic Anthrosol	0.3 g N kg ⁻¹ (Ammonium nitrate)	Soil sterilization at 550°C	Meng et al., 2017
		30		0.3 g N kg ⁻¹ (Potassium nitrate)		
		36		0.3 g N kg ⁻¹ (Ammonium sulfate)		
China	69	9	Eum-Orthic Anthrosol	120 kg N ha ⁻¹ (Urea)	C difference method	Jin et al., 2018
		16		240 kg N ha ⁻¹ (Urea)		
China	38	32	Eum-Orthic Anthrosol	160 kg N ha ⁻¹ (Urea)	¹³ C natural abundance	Yu, 2018
		29		160 kg N ha ⁻¹ (Urea) + Nitrification inhibitor		
		101		220 kg N ha ⁻¹ (Urea)		
		40		220 kg N ha ⁻¹ (Urea) + Nitrification inhibitor		
Mean ± SE		40 ± 15				
95% confidence interval		11–69				

North China cropland with relatively high CaCO₃ content (5%–10%), Guo et al. (2010) found that soil pH significantly declined from the 1980s to the 2000s by an average of 0.27 and 0.58 units under cereals crops (including wheat, maize, rice, and cotton) and cash crops (including fruit trees, vegetables, and tea), respectively. Therefore, excessive N fertilization is generally considered to be the first source-induced soil acidification in cropland (Guo et al., 2010; Raza et al., 2020; Zamanian et al., 2021). SIC reserves in cropland are continuously lost in the form of CO₂ because of the soil acidification caused by excessive N fertilization (Raza et al., 2020; Zamanian et al., 2021). For example, Raza et al. (2020) found that the SIC storage of Chinese croplands has decreased by approximately 7% in the past 40 years, due to the large amount of N application. Furthermore, in the past 50 years from the 1970s to the 2020s, Zamanian et al. (2021) estimated that at least 0.41 Gt CO₂-C was released into the atmosphere by global N fertilization-induced acidification.

Different types of N fertilizer lead to different soil acidification rates. For example, the acidification equivalent of ammonium sulfate, diammonium phosphate and urea

(acidification equivalent is the amount of CaCO₃ needed to neutralize the acid produced by 100 kg fertilizer) is 110, 79 and 74 kg CaCO₃, respectively, while calcium nitrate has a negative acidification effect (acidification equivalent is -50; Rengel, 2003). Zamanian et al. (2018) reported that 1 kg urea-N application resulted in 0.21 kg CaCO₃-derived CO₂-C emissions, while Goulding and Annis (1998) found that 1 kg ammonium-N application resulted in 1.2 kg CaCO₃-derived CO₂-C emissions. Therefore, it is necessary to optimize N management according to crop demand, avoid excessive N application, and select types of N fertilizer with a low or negative acidification equivalent. This is beneficial to slow soil acidification and ultimately reduce the carbonate loss in cropland.

Straw-removal-induced soil acidification

Crop plants usually absorb excess cations over anions, resulting in a net H⁺ release by roots to maintain the charge homeostasis in plants (Barak et al., 1997; Guo et al., 2010; Hao et al., 2022). The removal of crop aboveground organs containing the alkalinity as organic anions generated by N

assimilation (Slattery et al., 1991; Barak et al., 1997) results in an additional H^+ remaining in soil solution and is the second source of acidification in cropland (Berthrong et al., 2009; Guo et al., 2010; Hao et al., 2022). In intensively managed cropland, fertilizer N application increases H^+ release rates, mainly due to the N-promoted removal of base cations and nitrification. For example, the removal of crop aboveground organs in Chinese croplands resulted in a net residue of 20–33 $kmol H^+ ha^{-1} year^{-1}$ (Guo et al., 2010). Moreover, Hao et al. (2022) found that the removal of crop aboveground organs (dominated by straw removal) contributed over 50% of the total H^+ production in south-west China cropland. In calcareous cropland, the pH decline induced by aboveground removal is significantly lessened due to $CaCO_3$ and $MgCO_3$ dissolution (Chen and Barak, 1982; Guo et al., 2010), leading to an increase in base cations (mainly Ca^{2+} and Mg^{2+}) leaching. Because crop straw is rich in the base cations and alkalinity stored as organic anions, returning straw is an effective measure to compensate for the loss of soil base cations and decrease the acidifying effect on base cations leaching through alkalinity recycling (Barak et al., 1997; Guo et al., 2010; Hao

et al., 2022).

2.5 Soil depth

To explore the relationship between paired SOC and SIC concentration, and between SIC/soil total C (STC) ratio and soil depth, we integrated and analyzed the paired SOC and SIC concentration data of different soil depths in global cropland and natural ecosystems (including grassland, forest and desert; Table S2). A positive and significant correlation was found between SIC and SOC concentration in cropland ecosystems ($p < 0.001$; Fig. 2A). This may be attributed to two mechanisms: (1) carbonates are a large source of calcium, leading to the formation of Ca^{2+} -SOM complexes with organic matter (SOM) that can resist microbial decomposition; and (2) carbonates increase formation and stability of soil aggregates (Fernandez-Ugalde et al., 2014; Rowley et al., 2018; Raza et al., 2020). However, in natural ecosystems, there was no significant correlation between SOC and SIC concentration (Fig. 2B). In cropland and natural ecosystems, we found that the SIC/STC ratio increased significantly with the increase of soil depth ($p <$

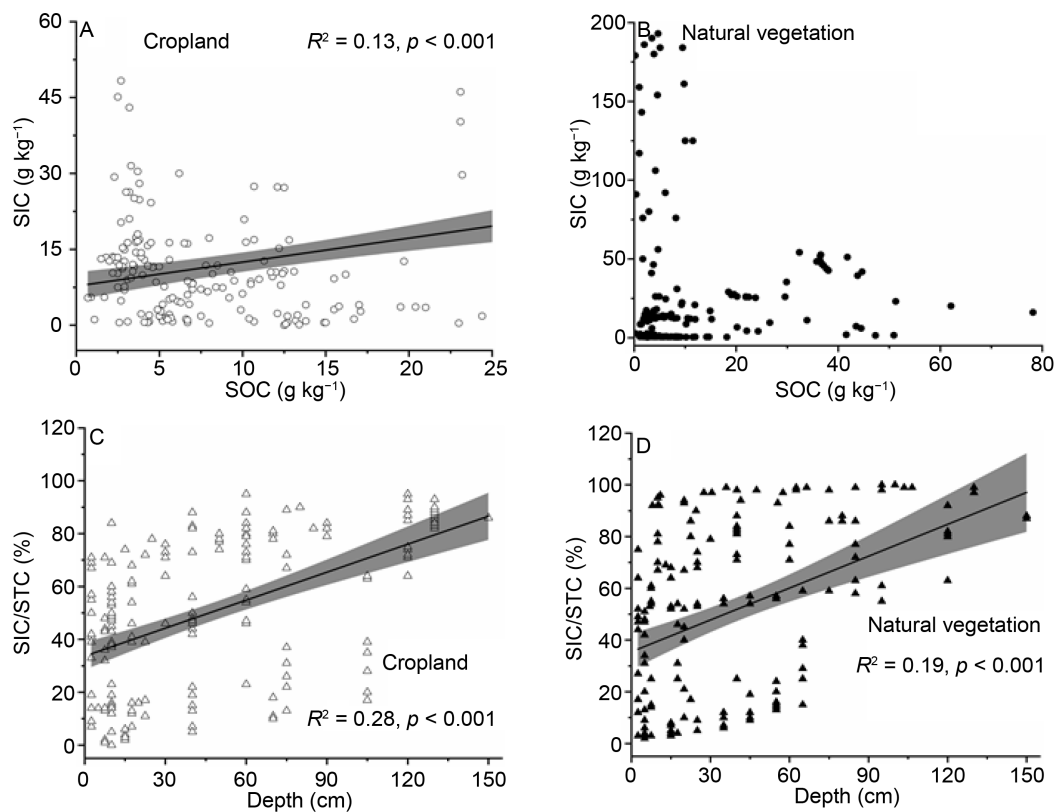


Fig. 2 Relationship between paired SIC and SOC concentration in cropland (A) and natural vegetation (B), and between paired SIC/STC ratio and soil depth in cropland (C) and natural vegetation (D). Note: (A) and (C) were based on literature synthesis including 189 observations from 17 studies of paired SIC and SOC concentrations, and paired SIC/STC ratio and soil depths in cropland (Table S2), respectively. (B) and (D) were based on literature synthesis including 168 observations from 11 studies of paired SIC and SOC concentrations, and paired SIC/STC ratio and soil depth in natural vegetation (Table S2), respectively. The scattered open symbols (cropland ecosystem) and closed symbols (natural ecosystems including grassland, forest and desert) indicate data points; black lines and gray shaded areas are the mean and 95% confidence intervals of the slope (all data combined), respectively.

0.001; Fig. 2C and Fig. 2D). This raises the question of whether the contribution of SIC to soil CO₂ emissions also increases with soil depth. Recent studies have clearly shown that this contribution increased significantly with soil depth (Dong et al., 2013; Cardinael et al., 2020; Xu et al., 2022). For example, in an *agroforestry ecosystem* with the increase of SIC/STC ratio with soil depth, Cardinael et al. (2020) found that the contribution of SIC to soil CO₂ emissions accounted for approximately 20% and 60% in topsoil and subsoil, respectively. Furthermore, the addition of CaCO₃ (Dong et al., 2014) was also shown to increase the amount and contribution of carbonate-derived CO₂ emissions. This suggests that the proportion of SIC to STC increases with soil depth, and then the contribution of SIC-derived CO₂ to total soil CO₂ emissions also increases with soil depth (Dong et al., 2013; Cardinael et al., 2020).

2.6 Rhizosphere effect

Crop growth promotes microbial decomposition of SOC by secreting labile C substrates from roots (Zhu et al., 2014; Huo et al., 2017) and enhances chemical dissolution of carbonate by secreting acidity (Mubarak and Nortcliff, 2010; Ahmad et al., 2020), leading to the subsequent increase in SIC-derived CO₂ emissions (Ahmad et al., 2013; Sun et al., 2021a). Recent studies have shown that the rhizosphere effect cannot only increase the SOC-derived CO₂ emissions, but also promote the CO₂ emissions from soil endogenous carbonate (Sun et al., 2021a) and exogenous carbonate (Ahmad et al., 2020). For example, in vegetated calcareous soils, Sun et al. (2021a) found that the rhizosphere effect increased SOC- and SIC-derived CO₂ emissions from maize-planted soils (by 54% for SOC and 159% for SIC) and wheat-planted soils (by 64% and 49%, respectively) compared with the unplanted soils. Moreover, Ahmad et al. (2020) also found that the root growth of soybean led to a 147% increase in CO₂ emissions from Ca¹³CO₃ addition compared with those of unplanted soils.

Most studies suggest that carbonate release is mainly caused by acidity secreted by roots and microbes (Mubarak and Nortcliff, 2010; Ahmad et al., 2020), ignoring the increase

of soil CO₂ concentration caused by root-derived CO₂ and extra SOC decomposition stimulated by the rhizosphere effect, which may lead to an increase in carbonate loss (Fig. 3A). For example, Deng et al. (2015) found that the soil CO₂ partial pressure increased from 12 to 77 bar, resulting in a doubling of the dissolution rate of CaCO₃. By integrating the ¹³C/¹⁴C tracer experiments of wheat and maize, we found that the root-derived CO₂ of maize and wheat accounted for more than 50% of the total soil CO₂ emissions (Sun et al., 2021a). Root-derived CO₂ and rhizosphere effect could increase the soil CO₂ concentration by two orders of magnitude relative to the atmospheric CO₂ concentration (Amundson and Davidson, 1990). Therefore, in calcareous soils with maize and wheat plants, root-derived CO₂ and positive rhizosphere effect increase soil CO₂ concentration (Sun et al., 2021a) or roots secrete acidity (Ahmad et al., 2020), both of which may increase the dissolution and release of CaCO₃ (Fig. 3A). Especially in intensively managed cropland, high ammonium- or urea-N input may increase the release of protons via the uptake of ammonium ions by roots (Tamir et al., 2021), and increase soil CO₂ partial pressure by enhancing rhizosphere respiration (Sun et al., 2021a; Wen et al., 2021), leading to a strong increase in CO₂ emissions by CaCO₃ acidification.

2.7 Straw or manure application

Under straw return to the soil, straw C usually has a positive priming effect on SOC decomposition. Compared with unamended soil, additional SOC decomposition and straw decomposition will further increase the partial pressure of CO₂ in soil (Fig. 3B; Meng et al., 2017; Wang et al., 2019). Concurrently high soil moisture will lead to the dissolution of carbonate, and the resulting HCO₃⁻ may react with H⁺ in soil solution to form H₂CO₃ (Bugchio et al., 2017; Zamanian et al., 2018). Therefore, CO₂ can be released from the soil when the soil solution is saturated with H₂CO₃. For example, we found that corn straw addition significantly increased the SOC- and SIC-derived CO₂ emissions from calcareous soils, using the stable isotope model IsoSource (Sun et al., 2021c). Furthermore, the decomposition of organic fertilizer

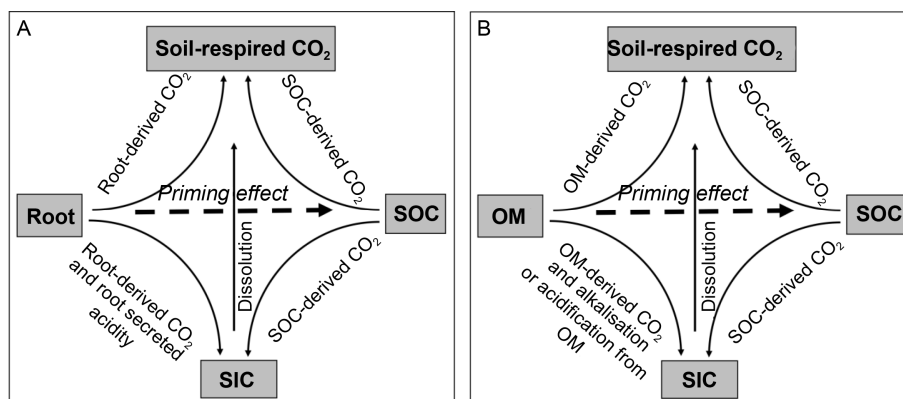


Fig. 3 Mechanism of CO₂ emissions from calcareous soils with plants or organic material input. Note: OM indicates organic material.

is accompanied by the production of organic acids, which will further enhance carbonate dissolution (Tamir et al., 2011, 2013). For example, Tamir et al. (2011) found that applying chicken manure to a calcareous soil resulted in a 2.7-fold increase in the SIC-derived CO₂ emissions. Therefore, the addition of organic materials not only increases the SOC-derived CO₂ emissions (Meng et al., 2017; Wang et al., 2019), but also enhances the SIC-derived CO₂ emissions (Tamir et al., 2011; Sun et al., 2021c).

In calcareous soils with cereal residues, the increase of CO₂ partial pressure may be the primary contributor of enhanced carbonate dissolution (Fig. 3B). This is because cereal residues have high alkalinity and low organic N, and the alkalinity of organic anions overwhelms the acidification induced by nitrification of mineralised residue N (Fig. 3B; Xu et al., 2006; Wang et al., 2011). Because of the buffering capacity of carbonate, pH values of calcareous soils have little response to the addition of exogenous cereal straw (Wang et al., 2018; Raza et al., 2021). On the contrary, in acidic soils, the biological decomposition of cereal residues can result in a significant increase in soil pH (Noble et al., 1996; Xu et al., 2006; Wang et al., 2011). In acid soils within pH 4.5–6.5, the acidification is largely buffered by exchange of H⁺ with base cations in the soil exchangeable complex (Ulrich, 1986; Gandois et al., 2011; Raza et al., 2021). Hence, when crop residues are added to an acid soil, neutralization of soil acidity is attributed not only to resupplying consumption of soil alkalinity, but also to compensating consumption of soil base cations from crop residues, which replenishes the soil exchangeable complex (Ulrich, 1986; Noble et al., 1996; Gandois et al., 2011; Wang et al., 2011). For example, in acidic soils receiving lime, Bramble et al. (2021) found that the addition of corn straw reduced the lime-derived CO₂ emissions compared with the control soil, which may be related to the increase of soil pH by straw addition.

3 Methods for partitioning soil CO₂ emissions

3.1 Two-source partitioning soil CO₂ emissions and quantification of ¹³C fractionation

The short-term change of SIC content is very small compared with the background value (Sun et al., 2021c). Therefore, it is difficult to be quantified directly by measuring the difference of SIC content, and it can be indirectly quantified by measuring the SIC-derived CO₂ emissions. Distinguishing between SOC- and SIC-derived CO₂ is the premise of assessing the individual responses to biotic and abiotic factors (Stevenson and Verburg, 2006; Chevallier et al., 2016; Sun et al., 2021a). To quantify the potential contribution of SIC to total CO₂ emissions, sterilization of soil is commonly used to minimize soil biological activity by mercuric chloride (HgCl₂; Wolf et al., 1989; Stevenson and Verburg, 2006; Meng et al., 2015) or high temperature (Stevenson and Verburg, 2006; Meng et al., 2017). Soil CO₂ emissions in

sterilised soils represent SIC-derived CO₂ emissions, and determination of SOC-derived CO₂ emissions is based on differences of CO₂ emissions between sterilised and unsterilised soils (Stevenson and Verburg, 2006; Meng et al., 2015; Wang et al., 2020). Sterilization changes the properties of soils, resulting in uncertainty in estimation of SIC-derived CO₂ emissions. For instance, previous studies showed that unbuffered HgCl₂ addition significantly increased CO₂ emissions compared with buffered HgCl₂ addition for Mojave Desert soils, due to the acidic nature of unbuffered HgCl₂ increasing SIC dissolution (Stevenson and Verburg, 2006; Dong, 2013; Meng et al., 2015). In addition, SIC dissolution is driven primarily by the soil CO₂ concentration (Eq. (1)), and sterilization inhibits SOC-derived CO₂ production by eliminating soil biological activity (Wolf et al., 1989; Stevenson and Verburg, 2006). In this case, the decrease of soil CO₂ concentration may lead to the underestimation of SIC-derived CO₂ emissions (Wang et al., 2020). Consequently, sterilization is not an accurate method for two-source partitioning soil CO₂ emissions into SIC and SOC sources.

$\delta^{13}\text{C}$ signatures measurement of CO₂ emissions is a useful way to trace the sources of soil CO₂ emissions originating from SOC and SIC (Tamir et al., 2011; Chevallier et al., 2016). Given that the $\delta^{13}\text{C}$ value of SIC is distinctly higher than that of SOC (Cerling, 1984; Tamir et al., 2011), the contribution of SOC and SIC to soil CO₂ emissions can be distinguished by the two-source mixing model based $\delta^{13}\text{C}$ signatures (Tamir et al., 2011; Chevallier et al., 2016; Cardinael et al., 2020). However, this method usually assumes that the $\delta^{13}\text{C}$ value is the same for SIC and SIC-derived CO₂, and for SOC and SOC-derived CO₂ (Tamir et al., 2011; Cardinael et al., 2020). Recent studies have shown that ¹³C fractionation occurs between them, but most studies ignore this (Chevallier et al., 2016; Cardinael et al., 2020). Even though this information for ¹³C fractionation between CaCO₃-derived CO₂ and CaCO₃ is available (Friedman and O'Neil, 1977; Chevallier et al., 2016), it would be important to have more experimental data about the variable. To improve the accuracy of separating CO₂ emissions from SIC and SOC, the ¹³C fractionation coefficient urgently needs to be quantified. Because SOC- and SIC-derived CO₂ emissions vary in both time and space, it is necessary to remove the interference of SOC decomposition to quantify the ¹³C fractionation between SIC and SIC-derived CO₂ emissions. Soil high temperature sterilization (Stevenson and Verburg, 2006) or chemical sterilization (Stevenson and Verburg, 2006; Meng et al., 2015) may be an effective method to remove SOC decomposition. Under soil sterilization, the difference in $\delta^{13}\text{C}$ between SIC and SIC-derived CO₂ emissions is equal to the ¹³C fractionation coefficient (Fig. 4). Similarly, to quantify the ¹³C fractionation between SOC and SOC-derived CO₂ emissions, SIC is removed by acid washing and then soil pH is adjusted to the original value (Fig. 4), which can eliminate the interference of SIC dissolution (Jones et al., 2011). In this case, the difference in the $\delta^{13}\text{C}$ value between SOC and SOC-derived

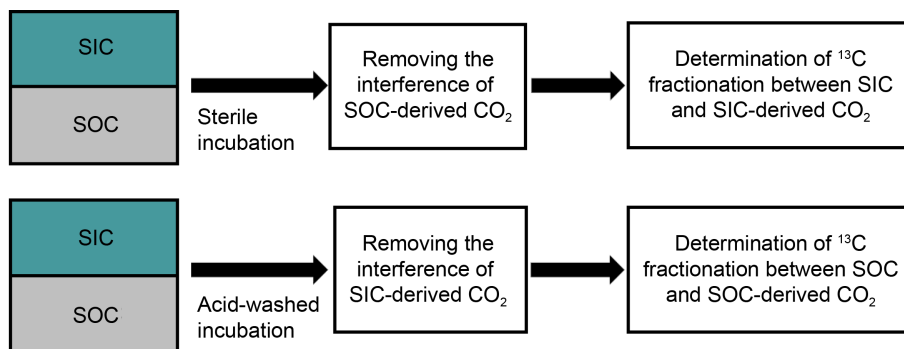


Fig. 4 Method of determining ¹³C isotope fractionation coefficient between SIC and SIC-derived CO₂ and between SOC and SOC-derived CO₂.

CO₂ emissions is the ¹³C fractionation coefficient (Fig. 4).

3.2 Methods for partitioning soil CO₂ emissions into three sources

To date, the two-source apportionment of CO₂ released from calcareous soils has mainly been conducted via incubation experiments without exogenous organic C, by ¹³C natural abundance or sterilization (Table S1). However, with exogenous organic C, soil CO₂ emissions may come from as many as three sources: decomposition of exogenous organic C, SOC decomposition and SIC dissolution (Sun et al., 2021a). Distinguishing the three sources of soil CO₂ emissions is required for quantifying the effects of root growth (Ahmad et al., 2020; Sun et al., 2021a) and organic material addition (Tamir et al., 2011; Fang et al., 2020; Sun et al., 2021c) on SOC- and SIC-derived CO₂ emissions. Although it is difficult to distinguish soil CO₂ emissions from the three sources, a series of methods for three-source CO₂ partitioning have been developed based on the ¹³C/¹⁴C isotope method, including the following main approaches (Table 3): (1) stable isotope analysis (Plestenjak et al., 2012; Sun et al., 2021c; Sun et al., 2021d); (2) ¹³C and ¹⁸O natural abundance (Lin et al., 1999); (3) ¹⁴C labeling combined with ¹³C natural abundance (Blagodatskaya et al., 2011; Tian et al., 2016; Cui et al., 2017; Luo et al., 2017); (4) double labeling with ¹⁴C and ¹³C (Shahbaz et al., 2018a, 2018b; Ji et al., 2019; Xiao et al., 2021); (5) ¹³C labeling and natural abundance (Kerre et al., 2016; Sun et al., 2021a; Chen et al., 2022); (6) high and low ¹³C-enriched labeling (Whitman and Lehmann, 2015; Weng et al., 2020); (7) the combination of C₃ and C₄ sources in different treatments (Kuzuyakov and Bol, 2004, 2005); and (8) the additive approach (Tamir et al., 2011; Fang et al., 2020). Among the above approaches to partitioning the three sources of CO₂ emissions, double labeling with ¹⁴C and ¹³C is currently considered to be the most accurate (Shahbaz et al., 2018a; Xiao et al., 2021). However, ¹⁴C material has radiation hazards and is highly regulated and limited to laboratory use. Compared with double labeling with ¹⁴C and ¹³C, high and low ¹³C-enriched labeling is an alternative (Whitman and Lehmann, 2015; Weng et al., 2020) that eliminates the

interference of fractionation in the decomposition process of ¹³C natural abundance material on the result of CO₂ discrimination and does not require specific C₃ plant and C₄ soil conditions (or C₄ plants and C₃ soil; Table 3). Therefore, the application of this approach with plants labeled with high and low enrichment of ¹³CO₂, or high and low enrichment of ¹³C labeled materials, can accurately differentiate three sources of soil CO₂ emissions with a three-source mixing model (Whitman and Lehmann, 2015; Weng et al., 2020).

Owing to method limitations in three-source CO₂ partitioning, it remains unclear how living roots and organic materials input affect SOC- and SIC-derived CO₂ emissions, and this uncertainty needs to be evaluated urgently in calcareous soils. To accurately distinguish CO₂ emissions and DIC from three C sources, we propose a new method: (1) in calcareous soils with plants, ¹³CO₂ at high and low enrichment is used to label the aboveground of plants in the two soil systems (Fig. 5A); (2) in calcareous soils with organic materials input, adding organic materials with high and low ¹³C enrichment to the two soil systems is used to distinguish the three sources (Fig. 5B); and then (3) the proportion of root- (or exogenous organic C), SOC- and SIC-derived CO₂ in soil CO₂ emissions and DIC is calculated using the three-pool ¹³C isotopic mixing model (Whitman and Lehmann, 2015; Sun et al., 2021a).

4 Conclusions

The global SIC stocks occupy approximately 25% of total soil C stocks, and are mainly located in calcareous soils of arid and semi-arid regions, covering approximately 50% of the Earth's land surface. In root- or residue-free calcareous soils, SIC contributed on average 27% of total CO₂ emissions. High pH of calcareous soils may lead to a substantial retention of CO₂ in the soil solution due to forming DIC. Neglecting this DIC component may lead to underestimation in soil CO₂ fluxes. To solve the problem, we recommend the use of soil columns connected to a CO₂-trapping system of closed circulation, to quantify both CO₂ emissions and DIC simultaneously.

To date, few studies have addressed the contribution of

Table 3 Comparison of the methods for partitioning soil CO₂ emissions into three sources.

Methods	Principle	Advantage	Disadvantage	References
Stable isotope analysis software	The contribution range of different substrate to soil CO ₂ emissions was quantified by isotope tracing software	Only the ¹³ C difference between CO ₂ and substrate can be used to quantify the contribution range of different substrate to CO ₂ emissions	The contribution of each substrate may not be accurately quantified	Plestenjak et al., 2012; Sun et al., 2021c; Sun et al., 2021d
¹³ C and ¹⁸ O natural abundance	The approach is based on using three-source mixing model of δ ¹³ C and δ ¹⁸ O to differentiate three sources of soil CO ₂ emissions	No ¹³ CO ₂ / ¹⁴ CO ₂ labeling is required; under the some assumptions, the potential contribution of three C sources to soil CO ₂ emissions can be distinguished	Due to the effect of ¹³ C fractionation, there is a certain uncertainty in the soil CO ₂ discrimination	Lin et al., 1999
¹⁴ C labeling combined with ¹³ C natural abundance	First, the amount of ¹⁴ C labeled C in soil CO ₂ emission was calculated based on the ¹⁴ C radioactivity of soil CO ₂ ; next, the δ ¹³ C values of C originating from non-labeled C sources were calculated based on the mass balance of stable isotopes; finally, the contribution of recent and old C in CO ₂ emissions from the C ₃ /C ₄ soil was calculated based on the ¹⁴ C labeled C-corrected δ ¹³ C values of soil CO ₂	The contribution of three sources to soil CO ₂ emissions can be accurately distinguished	¹⁴ C labeled materials have radiation hazards and their use is highly regulated; the ¹³ C fractionation of ¹³ C natural abundance method may result in the inaccuracy of soil CO ₂ discrimination	Blagodatskaya et al., 2011; Tian et al., 2016; Cui et al., 2017; Luo et al., 2017
Double labeling with ¹⁴ C and ¹³ C	First, the amount of ¹⁴ C labeled C in soil CO ₂ emissions was calculated based on the ¹⁴ C radioactivity of soil CO ₂ ; ¹³ C labeled C in soil CO ₂ was based on a ¹³ C mass balance equation	This approach accurately distinguishes three sources of soil CO ₂ emissions; ¹⁴ C and ¹³ C labeled materials can exclude the differential interference of ¹³ C fractionation effect	¹³ C and ¹⁴ C materials requiring uniform labeling; ¹⁴ C labeled materials have radiation hazards and their use is highly regulated	Shahbaz et al., 2018a, 2018b; Ji et al., 2019; Xiao et al., 2021
¹³ C labeling and natural abundance	Using ¹³ C labeled and natural abundance materials (only δ ¹³ C values of the two materials is different, other chemical components are the same) to distinguish soil CO ₂ emissions	Soil CO ₂ emissions can be distinguished from three sources by one ¹³ C tracer	¹³ C labeled material is required; because the δ ¹³ C difference between SOC and ¹³ C natural abundance materials is small, the ¹³ C fractionation may result in the inaccuracy of soil CO ₂ discrimination	Kerre et al., 2016; Sun et al., 2021a; Chen et al., 2022
Highly and lowly ¹³ C-enriched labeling	The approach is based on using three-source mixing model to differentiate three sources of CO ₂ with high and low enrichment of ¹³ C labeled materials	The contribution of soil CO ₂ emission can be distinguished accurately by three sources. This eliminates the effect of ¹³ C fractionation on the CO ₂ discrimination	¹³ C labeling materials with high and low enrichment and uniform labeling	Whitman and Lehmann, 2015; Weng et al., 2020
Combination of C ₃ and C ₄ sources in different treatments	The approach is based on the combination of C ₃ and C ₄ sources in different treatments to distinguish between three CO ₂ sources	The separation of three C sources in the CO ₂ emissions by one ¹³ C natural abundance tracer method	Paired carbon sources requiring similar δ ¹³ C values and decomposition rates; the ¹³ C discrimination by CO ₂ production from different C sources	Kuzyakov and Bol, 2004, 2005
Additive approach	It is assumed that the SOC- or SIC-derived CO ₂ emissions in the three source system (with exogenous C addition) is the same as that in the two C source system (without exogenous C addition control)	¹³ CO ₂ / ¹⁴ CO ₂ labeling is not required; under the assumption, the contribution of three C sources to soil CO ₂ emissions can be distinguished	It is assumed that the addition of exogenous C has no effect on the SOC- or SIC-derived CO ₂ emissions, resulting in the uncertainty of soil CO ₂ differentiation; ¹³ C fractionation may reduce the accuracy of soil CO ₂ discrimination	Tamir et al., 2011; Fang et al., 2020

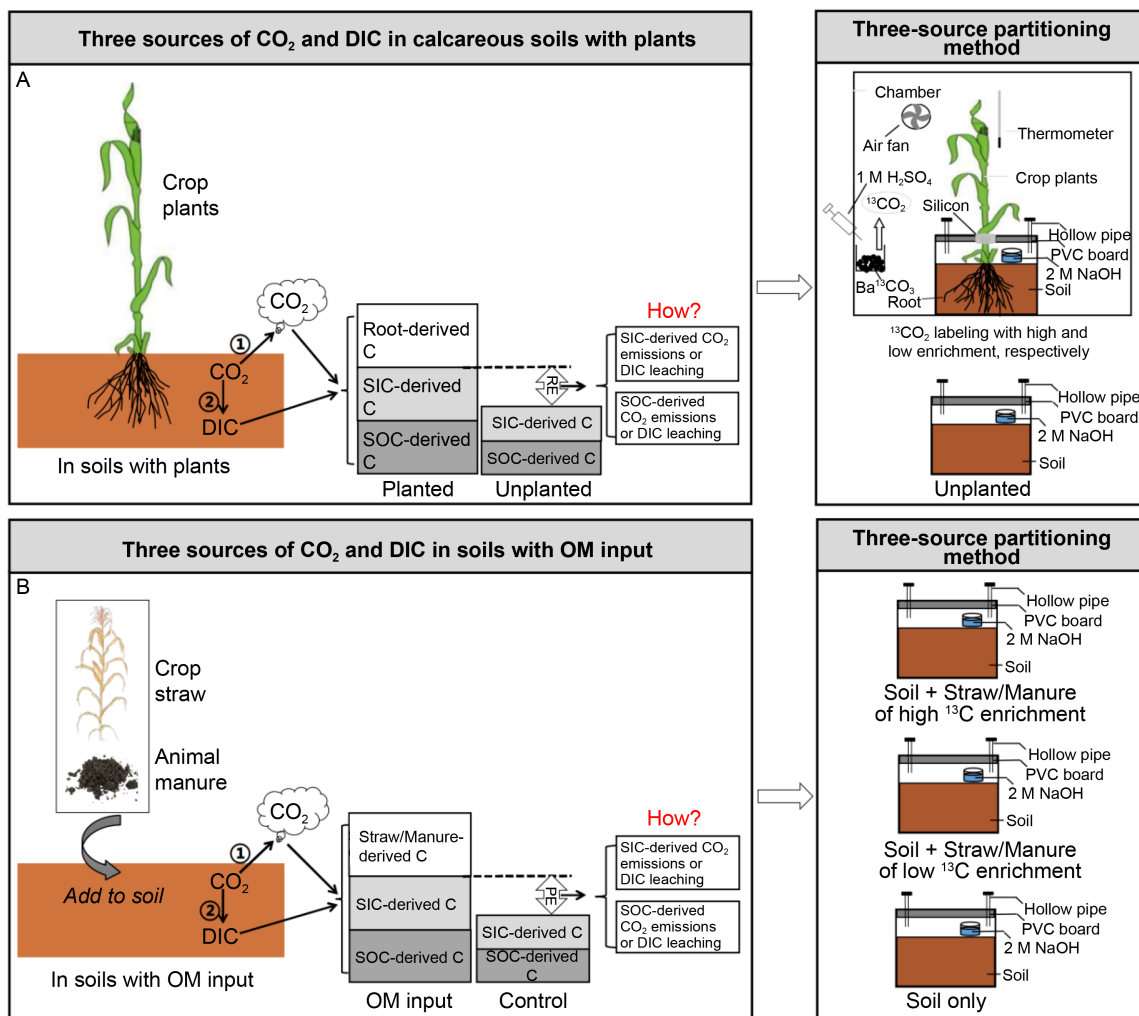


Fig. 5 Three-source partitioning of CO₂ released from calcareous soils with plants and organic material input. Note: RE and PE indicates rhizosphere effect and priming effect, respectively; DIC indicates dissolved inorganic carbon; OM represents organic material.

SIC to CO₂ emissions or DIC in planted or residue-amended calcareous soils. To increase the transferability from incubation results to field studies, further researches are essential to determine the contribution of CO₂ emissions from three C sources (root/residue, SOC, SIC) and the isotopic fractionation between SOC/SIC and CO₂ emissions. Especially in calcareous cropland, intensive agricultural practices can cause a large and rapid impact on the SIC dissolution. However, it is less known that how and why interactions between roots (or crop residues) and agricultural practices (irrigation, fertilization) affect CO₂ emissions from endogenous and exogenous C in calcareous cropland. Hence, to improve the estimates of calcareous cropland, these topics need further research.

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Electronic supplementary material

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