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CO_2 Emission and Source Partitioning from Carbonate and Non-carbonate Soils during Incubation

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ABSTRACT

The accurate quantification and source partitioning of CO_2 emitted from carbonate and non-carbonate soils are critically important for understanding terrestrial carbon (C) cycling. The two main methods to capture CO_2 released from soils are the alkali trap method and the direct gas sampling method. We conducted a 25-day laboratory incubation experiment to compare the efficacies of these two methods to analyze CO_2 emissions from Hapludult (non-carbonate) and Haplustalf (carbonate-rich) soils. An isotopic fraction was introduced into the calculations to determine the impacts on partitioning the sources of CO_2 into soil organic carbon (SOC) and soil inorganic carbon (SIC), and into C3 and/or C4 plant-derived SOC. The results indicated that CO_2 emissions from the non-carbonate soil measured by the alkali trap method and the gas sampling method were not significantly different. For the carbonate-rich soil, the CO_2 fluxes emission measured by the alkali trap method was significantly higher than those measured by the gas sampling method from the 14th day of incubation onwards. Although SOC and SIC each accounted for about 50% of total soil C in the carbonate-rich soil, SOC decomposition contributed 57%--72% of the total CO_2 emitted. For both non-carbonate and carbonate-rich soils, the SOC derived from C4 plants decomposed faster than that originating from C3 plants. We propose that for carbonate soil, CO_2 emissions may be overestimated using the alkali trap method because of decreasing CO_2 pressure within the incubation jar, but underestimated by the direct gas sampling method. The gas sampling interval and ambient air may be important sources of error, and steps should be taken to mitigate errors related to these factors in soil incubation and CO_2 quantification studies.

Key Words: alkali trap, carbonate soil, CO_2 emission, C3/C4, isotope fractionation, non-carbonate soil

INTRODUCTION

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Soil is the largest carbon (C) pool on the earth, with an organic carbon (OC) stock of 1550 Pg and an inorganic carbon (IC) stock of 950 Pg (Lal, 2007). In arid and semi-arid regions, which occupy a total land area of 4.9×10^7 km^2 (Lardner *et al*., 2015), the soil inorganic carbon (SIC) stock is approximately ten times greater than that of soil organic carbon (SOC) (Schlesinger, 1982). The SIC and SOC pools play a very important role in global C sequestration (Lal, 2009). Because SIC significantly contributes to CO_2 emissions, it should be paid more attention (Tamir *et al*., 2011; Ramnarine *et al*., 2012; Chevallier *et al*., 2016). Zamanian and Kuzyakov (2018) pointed out that, on a global scale, CO_2 emitted from carbonate soil by nitrogen (N) fertilization ($> 7.5 \times 10^{12}$ g C $year^{-1}$) and from liming of acidic soils ($> 273 \times 10^{12}$ g C $year^{-1}$) accounted for more than 30% of total global CO_2 emissions resulting from land-use changes (Zamanian *et al*., 2018).

The efflux of CO_2 from soils is recognized as one of the largest C fluxes within the global C cycle, and small changes can greatly affect atmospheric CO_2 (Schlesinger and Andrews, 2000) and consequently, the global climate (Lal, 2004; Luo *et al*., 2010). Since the 1970s, the importance of soil respiration as a source of CO_2 has been well recognized, and numerous studies have investigated CO_2 emissions from different ecosystems (Buyanovsky *et al*., 1986; Conant *et al*., 2000) and from soils underlying different types of vegetation (Raich and Schlesinger, 1992). Other studies have compared methods to quantify soil respiration (Jong and Schappert, 1972; Baldocchi *et al*., 1988). For non-carbonate soils, CO_2 emissions are mostly generated by soil respiration, i.e., SOC decomposition by microbes. However, for carbonate soils, SIC dissolution may also occur during the microbial decomposition of SOC (Stevenson and Verburg, 2006; Bertrand *et al*., 2007). The CO_2 produced by SOC decomposition and/or SIC dissolution dissolves in soil water and may react with Ca^{2+} and/or Mg^{2+} to re-precipitate as new carbonates (Nordt *et al*., 2000). In the field, such re-precipitation may occur on daily cycles if the hydrothermal conditions within the soil fluctuate intensively (Yates *et al*., 2012; Fa *et al*., 2016). However, under constant hydrothermal conditions in a laboratory setting, such re-precipitation may take several months (Ramnarine *et al*., 2012; Kuzyakov *et al*., 2006).

The two most commonly used methods to quantify soil CO_2 emissions are the alkali trap method and the direct gas sampling method (Rochette *et al*., 1997; Bruun *et al*., 2014; Lardner *et al*., 2015). Compared with field direct measurement, laboratory incubations have more stable hydrothermal conditions, which reduce interference from environmental factors. Measurements of soil CO_2 flux in laboratory incubations have been widely used to quantify soil microbial activity (Leita *et al.,* 1995), SOC decomposition (Kuzyakov and Bol, 2005), soil organic matter (SOM) pool dynamics (Haile-Mariam *et al*., 2008), and the turnover of different SOM fractions (Stevenson and Verburg, 2006; De Troyer *et al*., 2011). For example, using the alkali trap method, Ramnarine *et al.* (2012) found that 62%--74% of the CO_2 emitted during a 14-day incubation of typical Hapludalf soil was derived from SIC. Tamir *et al.* (2012) conducted a 168-day soil incubation experiment, and found that N application to calcareous soil enhanced the dissolution and re-crystallization of SIC. The alkali trap method may overestimate or underestimate CO_2 fluxes, because of variations in temperature and moisture and diffusion of external gases, which can result in excessive or insufficient absorption of the released CO_2 (Rochette *et al*., 1997; Ramnarine *et al*., 2012). Therefore, it is important to compare the efficacies and accuracies between the gas sampling method and the alkali trap method to quantify CO_2 released from incubated soil.

Isotopic measurement of ^{13}C content is an effective tool to identify CO_2 sources (Stevenson and Verburg, 2006; Tamir *et al*., 2011). Compared with SIC, SOC is less enriched with heavier ^{13}C. Therefore, $^{13}C_{CO2}$ from SOC ($\delta^{13}C_{CO2\text{-}SOC}$) is significantly different from that from SIC ($\delta^{13}C_{CO2\text{-}SIC}$) (Salomons and Mook, 1976; Magaritz and Amiel, 1980; Plestenjak *et al*., 2012). In addition, C3 and C4 plants have different $\delta^{13}C$ values because of physiological differences in the photosynthetic fixation of CO_2 (Balesdent *et al*., 1990; Bol *et al*., 2004; Krull *et al*., 2007). Based on the differences in $\delta^{13}C$ values between C3 and C4 plants, Kuzyakov and Bol (2005) distinguished three sources of CO_2 emitted from soils. Isotope fractionation may occur during the reactions that emit CO_2. The value of $\delta^{13}C_{CO2\text{-}SIC}$ is 7‰--9‰ lower than that of $\delta^{13}C_{SIC}$ in field conditions (Szaran, 1997; Chevallier *et al*., 2016). In laboratory

incubations, the differences between $\delta^{13}C_{CO2\text{-}SOC}$ and $\delta^{13}C_{SOC}$ range from 0 to 1‰ (Breecker *et al*., 2015; Boström *et al*., 2007). Some studies have also pointed out that, compared with field conditions, laboratory conditions lead to much smaller, or even negligible, isotope fractionation (Bertrand *et al.,* 2007; Tamir *et al.,* 2012). For instance, Chevallier *et al.* (2016) found that the estimated contribution of SIC to CO_2 emitted from soil exceeded total CO_2 emissions if isotopic fractionation between SIC and SIC-derived CO_2 was included in the calculation.

China has a large area of arid and semi-arid soils, which contain approximately 60 Pg SIC, accounting for 5%--6.7% of the global SIC pool (Pan, 1999). The Loess Plateau in northwestern China represents a typical carbonate soil area, in which the SIC content is approximately 50% of total carbon (TC) (Dong *et al*., 2014). In contrast, the northeastern plain in China is dominated by Hapludult, in which SOC accounts for > 99% of TC (Lan *et al*., 2016). Both of these regions are important agricultural production areas in China. Analyses of the amount and sources of CO_2 emissions from these two typical soils not only help us to understand the transformation of soil C, but also provide a basis for estimating CO_2 emissions in two major regions of China.

In this study, we collected Hapludult from the northeastern plain and Haplustalf from the Loess Plateau in northwestern China, and conducted a 25-day laboratory incubation experiment. The CO_2 emitted from the soil was collected and measured by both the alkali trap method and the gas sampling method. The differences between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{SOC}$ were compared to examine the effects of SOC fractionation during the Hapludult incubation process. An isotopic fraction was introduced into the calculation, to determine the impacts on partitioning CO_2 sources (Chevallier *et al*., 2016). We aimed to determine the differences in CO_2 capture between the alkali trap method and the gas sampling method, and to explore the contribution of plants with different types of photosynthesis (C3/C4) and soil carbon (SIC/SOC) to CO_2 emissions, as well as the errors associated with the calculations.

MATERIALS AND METHODS

Incubation experiment

Two types of soils were used for this experiment: the carbonate-rich soil, i.e., Udic Haplustalf soil, was collected in Shanxi Province in northwestern China (34°17′59.317″ N, 108°04′9.384″ E), at the southern edge of the Loess Plateau. This region is in a warm temperate zone with a semi-humid and semi-arid climate with an average annual temperature and precipitation of 13 °C and 600--650 mm, respectively. The soil is classified as Eum-Orthic Anthrosol in Chinese Soil Taxonomy, equivalent to Udic Haplustalf in USDA Soil Taxonomy. The soil texture is silt clay loam. The cropping system in the region is two crops per year, *i.e.*, winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). The non-carbonate soil (Hapludult) was collected in Liaoning Province in northeastern China (41°08′24.73″ N, 121°19′39.48″ E), where there is a warm temperate sub-humid climate, and the average annual temperature is 9 °C and the annual precipitation is 540--640 mm. The soil is Hapludult in USDA Soil Taxonomy. The soil texture is silt loam. The cropping system in the region is one crop per year, i.e., soybean (*Glycine max* L.) rotated with maize (*Zea mays* L.). Historically, maize is the main crop in the region. Before sampling, the last crop planted at both sites was maize. Soil samples (0--20 cm) were collected using a soil auger (diameter 3.0 cm) from the fields at the two sites. Three samples (each sample was a composite mix of five individual surface soil cores) were taken from each field, and then air-dried, passed through a 2-mm mesh sieve, and stored at room temperature.

To activate the microbial population and decrease fluctuations in SOC mineralization, we added deionized water to 20-g samples of the air-dried soil to achieve 70% of field water capacity, and then incubated each sample in a 100-mL beaker (not sealed) at 25 °C for 7 days before the start of the incubation experiment (Paul *et al*., 2006).

For the incubation experiment, each 20-g soil sample was placed in a 240-mL jar. To reduce the interference of carbonate re-precipitation, the incubation period was 25 days, because the dissolution of carbonates re-precipitation normally takes several months under laboratory conditions (Ramnarine *et al*.,

2012; Kuzyakov *et al*., 2006). Each jar was sealed with a layer of Parafilm and a plastic sealing cap and incubated for 25 days at 25 °C. At each sampling, after taking out the NaOH solution (alkali trap method) or collecting CO_2 with a needled syringe (gas sampling method), the jars were left open for 1 hour to allow the internal air pressure to adjust, and the soil water content was re-adjusted to 70% of field holding capacity by weight using deionized water (Tamir *et al*., 2011). Afterwards, new NaOH solution (alkali trap method) was added to the jars, and the jars resealed with new Parafilm and plastic sealing caps.

CO_2 emissions and $\delta^{13}C$ measurements

Alkali trap method. A 10-mL beaker (to hold NaOH solution) was fixed on the wall of the incubation jar with glue, about 2 cm above the soil in the jar. On day 0 of the incubation experiment, 10 mL 0.1 mol L^{-1} NaOH was added to the beaker. The NaOH solution was changed on days 2, 5, 7, 9, 14, 18 and 25 after the start of the incubation (corresponding to sampling times 1, 2, 3, 4, 5, 6, and 7, respectively), to collect the emitted CO_2. For the blank (no soil) treatment, sealed jars with only NaOH solution were used. Three replicate jars were prepared for each soil sample and the blank.

The CO_2 trapped in NaOH solution was measured using the HCl-$SrCl_2$ titration method (Ramnarine *et al*., 2012). To analyze $\delta^{13}C$ of the emitted CO_2, the suspension containing $SrCO_3$ was placed in a 250-mL centrifuge bottle and centrifuged at 8 000 × *g* for 20 min, and then washed three times with deionized water to remove excess $SrCl_2$ and NaCl (Ramnarine *et al*., 2012). The $SrCO_3$ samples were dried at 80 °C for 8 h, and then weighed into tin capsules. The $\delta^{13}C$ values of $SrCO_3$ were determined using an Elementar Vario EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California at Davis (CA, USA). All the $\delta^{13}C$ values reported are in VPDB.

Gas sampling method. The CO_2 analyses using the gas sampling method were conducted at the same time as those using the alkali trap method. Similarly, three replicates of each sample and the blank (no soil) were analyzed. The headspace of each incubation jar was gas-sampled using a 20-mL needled syringe. After removing the plastic sealing cap, the needle was inserted through the Parafilm. The gas sample in the syringe was compressed to 15 mL, after which the overpressure was released, and the volume was reduced to 12 mL. Then, the gas was transferred to a 12-mL vacuumed headspace bottle for analysis of the CO_2 concentration and $\delta^{13}C$ using a gas chromatography-isotope ratio mass spectrometer (DELTA plus, Thermo Fisher Scientific, Waltham, MA, USA) at the Stable Isotope Facility, University of California at Davis.

$\delta^{13}C$ calculation and correction. The amount of CO_2 (C_{CO2}) derived from the incubated soil was calculated by Eq. (1):

$$C_{CO2} = C_s - C_b \quad (1)$$

where for the alkali trap method, C_s (mg kg^{-1}) was the C in CO_2 emitted from the incubated soil quantified by back-titration with HCl, and C_b (mg kg^{-1}) was the C in CO_2 insides the blank jars. For the gas sampling method, C_s (mg kg^{-1}) was the C in CO_2 in the headspace within the jar containing soils quantified by gas chromatography, and C_b (mg kg^{-1}) was the C in CO_2 inside the blank jars.

The $\delta^{13}C$ of the CO_2 derived from the soil was calculated using Eq. (2) (Mary *et al*., 1992; Pataki *et al*., 2003):

$$\delta^{13}C_{CO2} = \left(C_s \times \delta^{13}C_s - C_b \times \delta^{13}C_b\right)/(C_s - C_b) \quad (2)$$

where for the alkali trap method, $\delta^{13}C_s$ was the $\delta^{13}C$ value of C in $SrCO_3$ from the incubated soils and $\delta^{13}C_b$ was the $\delta^{13}C$ value of C in $SrCO_3$ from the blank jar, which both were analyzed using a PDZ Europa 20-20 isotope ratio mass spectrometer. For the gas sampling method, $\delta^{13}C_s$ was the $\delta^{13}C$ value of CO_2 from the incubated soils and $\delta^{13}C_b$ was the $\delta^{13}C$ value of CO_2 from the blank jar, analyzed using a

DELTA plus isotope ratio mass spectrometer.

Total carbon, SIC, SOC, total nitrogen, $\delta^{13}C$, and pH analysis

Soil total carbon (TC) and total nitrogen (TN) contents were measured using a CN analyzer (Flash EA 2000, Thermo Electron Corporation, Italy) at the Chinese Academy of Agricultural Sciences. The SIC content was measured using the pressure-calcimeter method following Sherrod *et al.* (2002). The SOC content was calculated by subtracting the SIC content from the TC content. The $\delta^{13}C$ values were analyzed using a DELTA plus isotope ratio mass spectrometer at the Chinese Academy of Agricultural Sciences. Soil pH was measured with a pH meter (soil:water=1:5 w/v). The soil properties are presented in Table I.

Source partitioning of SOC and emitted CO_2

Source partitioning of SOC into C4 and C3 plant origins. The SOC derived from C4 and C3 plants in Hapludult (non-carbonate) and Haplustalf (carbonate-rich) soils were calculated by Eq. (3):

$$f_{C4} = (\delta^{13}C_{SOC} - \delta^{13}C_{C3})/(\delta^{13}C_{C4} - \delta^{13}C_{C3}) \quad (3)$$

where $\delta^{13}C_{SOC}$ was the $\delta^{13}C$ value of SOC in Hapludult or Haplustalf soils, $\delta^{13}C_{C4}$ was the $\delta^{13}C$ value of C4 plants (−12‰), and $\delta^{13}C_{C3}$ was the $\delta^{13}C$ value of C3 plants (−27‰). The proportion of SOC derived from C3 plants was $(1-f_{C4})$.

Estimation of CO_2 released from SOC and SIC. The proportion of CO_2 evolved from SOC (f_{SOC}) was estimated using the two-end-member mixing model of Eq. (4) (Balesdent *et al*., 1987) as follows:

$$f_{SOC} = (\delta^{13}C_{CO2} - \delta^{13}C_{CO2-SIC})/(\delta^{13}C_{CO2-SOC} - \delta^{13}C_{CO2-SIC}) \quad (4)$$

where for Hapludult, because SOC accounted for >99% of TC, we assumed all CO_2 emitted was from SOC and therefore f_{SOC}=1. For Haplustalf, $\delta^{13}C_{CO2}$ was the $\delta^{13}C$ value of the CO_2 emitted, $\delta^{13}C_{CO2\text{-}SOC}$ was the $\delta^{13}C$ value of SOC, and $\delta^{13}C_{CO2\text{-}SIC}$ was the $\delta^{13}C$ value of SIC. The proportion of CO_2 evolved from SIC (f_{SIC}) was $1-f_{SOC}$.

If isotopic fractionation was considered, for Hapludult, because SOC accounted for >99% of TC, we assumed all CO_2 emitted was from SOC, so that the difference between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{SOC}$ was due to isotopic fractionation during SOC decomposition into gaseous CO_2. For Haplustalf, we assumed isotopic fractionation during SOC decomposition into gaseous CO_2 was the same as that for Hapludult, i.e., $\delta^{13}C_{CO2\text{-}SOC}$ was the sum of $\delta^{13}C_{SOC}$ and the corresponding isotope fractionation. Isotopic fractionation in the process of SIC dissolution into gaseous CO_2 was set at 7‰, i.e., $\delta^{13}C_{CO2\text{-}SIC}$ was 7‰ lower than $\delta^{13}C_{SIC}$ (Chevallier *et al*., 2016).

Partitioning of CO_2 decomposed from SOC into C4 and C3 plant-derived SOC. The proportion of CO_2 decomposed from SOC derived from C4 plants (f_{C4}) was estimated by Eq. (5):

$$f_{C4} = (\delta^{13}C_{CO2-SOC} - \delta^{13}C_{CO2-C3}) * f_{SOC}/(\delta^{13}C_{CO2-C4} - \delta^{13}C_{CO2-C3}) \quad (5)$$

where for Hapludult, $\delta^{13}C_{CO2\text{-}SOC}$ was the $\delta^{13}C$ value of the CO_2 emitted, $\delta^{13}C_{CO2\text{-}C3}$ was the $\delta^{13}C$ value of SOC derived from C3 plants and $\delta^{13}C_{CO2\text{-}C4}$ was the $\delta^{13}C$ value of SOC derived from C4 plants. f_{SOC} was the proportion of CO_2 evolved from SOC. The proportion of CO_2 evolved from C3 plant-derived SOC (f_{C3}) was $(1-f_{C4})$.

If isotopic fractionation was considered, we assumed that $\delta^{13}C_{CO2\text{-}C4}$ and $\delta^{13}C_{CO2\text{-}C3}$ was 1‰ higher than the $\delta^{13}C$ value of SOC derived from C4 and C3 plants (Breecker *et al*., 2015; Boström *et al*., 2007).

For Haplustalf, $\delta^{13}C_{CO2\text{-}SOC}$ was the $\delta^{13}C$ value of SOC ($\delta^{13}C_{SOC}$), f_{SOC} was the proportion of CO_2

evolved from SOC, $\delta^{13}C_{CO2\text{-}C4}$ was the $\delta^{13}C$ value of C4 plants and $\delta^{13}C_{CO2\text{-}C3}$ was the $\delta^{13}C$ value of C3 plants.

If isotopic fractionation was considered, like in Eq. (4), $\delta^{13}C_{CO2\text{-}SOC}$ was the sum of $\delta^{13}C_{SOC}$ and the corresponding isotope fractionation. $\delta^{13}C_{CO2\text{-}C4}$ and $\delta^{13}C_{CO2\text{-}C3}$ were assumed to be 1‰ higher than the $\delta^{13}C$ values of C4 and C3 plants, respectively.

Statistical analysis

Data were analyzed using the statistical software SAS (SAS Institute Inc., 2000). *T*-tests were used to compare the physical and chemical properties of the two soils (Table I; $n = 3$). The CO_2 emission rates and $\delta^{13}C$ were tested for normality using the Shapiro-Wilk test. Then, one-way analysis of variance (ANOVA) was used to evaluate the differences between CO_2 collection methods in the same soil laboratory incubation experiment (Figs. 1--3; $n = 3$). If the effects of the sampling method on the measurement results were significant ($P < 0.05$), t-tests were used to compare the CO_2-^{13}C and CO_2 emissions between the two methods, and to compare the estimation results of the two methods for C sources (Tables II, III, and V; $n = 3$). The Bonferroni method was used to correct for multiple tests, and the significant difference level was set to $P < (0.05/N)$, where N was the number of CO_2 sampling times (seven times during the incubation study). Two-factor two-level ANOVA was used to detect soil type × sampling method interactions (Tables S3 and S5; $n = 3$), and the Bonferroni method was also used for post-hoc testing, with the significant difference level set to $P < (0.05/N)$.

RESULTS AND DISCUSSION

Soil C contents and $\delta^{13}C$ values

The C contents, $\delta^{13}C$ values, and pH differed significantly between Hapludult (non-carbonate soil) and Haplustalf (carbonate-rich soil) (Table I). The pH of Hapludult (7.2 ± 0.2) was significantly lower than that of Haplustalf (7.8 ± 0.2). The TC content of Hapludult was 10.3 ± 0.1 g kg^{-1}, of which 99% was SOC. In contrast, the TC content of Haplustalf was 17.1 ± 0.1 g kg^{-1}, comprising about 50% SOC and 50% SIC. The $\delta^{13}C$ value of TC ($\delta^{13}C_{TC}$, −20.6 ± 0.1‰) in Hapludult was significantly lower (more negative) than that of Haplustalf, reflecting the high SIC content in the TC of Haplustalf. However, the $\delta^{13}C$ value of SOC ($\delta^{13}C_{SOC}$) in Hapludult (−21.2 ± 0.1‰) was significantly higher than that of Haplustalf (−22.3 ± 0.1‰). The $\delta^{13}C$ values of SIC in Hapludult and Haplustalf were −7.6‰ and −5.8‰, respectively.

TABLE I Properties of Hapludult (non-carbonate soil) and Haplustalf (carbonate soil).

	Hapludult	Haplustalf
pH (KCL)	7.2±0.2b	7.8±0.2a
TC (g kg^{-1})	10.3±0.1b	17.1±0.1a
TC-^{13}C (‰VPDB)	-20.6±0.3b	-14.0±0.1a
SOC (g kg^{-1})	10.2±0.2a	8.6±0.3b
SOC-^{13}C (‰VPDB)	-21.2±0.1a	-22.3±0.1b
SIC (g kg^{-1})	0.1±0.1b	8.5±0.2a
SIC-^{13}C (‰VPDB)	-7.6±1.0 b	-5.8±0.0a
TN (g kg^{-1})	1.0±0.0a	1.0±0.0a

Values are mean ± standard deviation, $n = 3$. Different lower-case letters indicate significant differences between the two soils ($P < 0.05$).

Quantification of CO_2 fluxes during incubation

For Hapludult (non-carbonate soil), there were no significant differences in CO_2 emissions between the two gas collection methods during the whole incubation period (Fig. 1a). The cumulative CO_2 emissions quantified by the gas sampling and alkaline trap methods were similar, i.e., 147.7±5.3 mg kg^{-1} for the gas sampling method and 146.9±4.8 mg kg^{-1} for the alkaline trap method (Fig. 2a). For Haplustalf (carbonate-rich soil), the CO_2 emissions measured by gas sampling and alkali trap method were similar during the first 9 days of incubation; but from day 14 onwards, the CO_2 emissions at each sampling event measured by the gas sampling method were significantly lower than those measured using the alkali trap method (Fig. 1b). The cumulative CO_2 emissions during the whole 25 days of incubation measured using the gas sampling method and the alkali trap method were significantly different, i.e., 139.0±1.7 *vs.* 165.3±5.7 mg kg^{-1}, respectively (Fig. 2b).

For both Hapludult and Haplustalf, the cumulative CO_2 emissions in the first 9 days in the incubation experiment accounted for approximately 50% of the total CO_2 emitted during the whole 25-day incubation period (Fig. 2). This indicated that SOC decomposed faster in the early phase of soil incubation than in the later phase, consistent with the results of other studies (De Troyer *et al*., 2011; Tamir *et al*., 2011). For Haplustalf, the significant differences between the cumulative CO_2 quantified by the two methods during the later incubation period might be due to SIC dissolution, which is facilitated by the organic acids produced during SOC decomposition (Rovira and Vallejo, 2008; Tamir *et al*., 2011). In the alkali trap method, the CO_2 emitted was immediately absorbed by NaOH. Thus, the CO_2 concentration (pCO_2) in the sealed jars decreased. This would allow SIC dissolution and the CO_2 generation reaction to reach an equilibrium (Dong *et al*., 2013). In contrast, for the gas sampling method, the pCO_2 in the sealed jars continuously accumulated during incubation. The decrease in pO_2 and the increase in pCO_2 in the sealed jars would retard SOC decomposition by heterotrophic microorganisms (Ekschmitt *et al*., 2008) and reduce SIC dissolution driven by organic acids derived from SOC decomposition. Also, a high concentration of CO_2 may increase the assimilation of CO_2 by soil microorganisms (Ekschmitt *et al*., 2008). This may result in the amount of CO_2 collected being less than the amount of CO_2 actually emitted by the soil sample. In addition, in the gas sampling method, the increase in pCO_2 might reverse the equilibrium of SIC dissolution and reduce CO_2 generation. Therefore, for carbonate soil such as Haplustalf, CO_2 emissions may be overestimated using the alkali trap method and underestimated using the gas sampling method. The longer the sampling interval (duration between two gas collection events), the greater the differences in pCO_2 within the sealed jar between the two methods. Consistent with this, the difference in CO_2 emissions measured using the two methods was significant if the sampling interval was longer than 4 days (Fig. 1).

We also analyzed the effect of the interaction between gas collection method and soil type on CO_2 emissions, and found that in the later period of the incubation experiment (18--25 days), the interactive effect was significant (Table S3). This highlighted that the measured CO_2 emissions were also influenced by other factors such as the sampling interval, as well as the gas collection method and soil type.

Fig. 1 CO_2 emissions at each sampling event from a) Hapludult (non-carbonate soil) and b) Haplustalf (carbonate-rich soil) measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates significant difference between the two gas collection methods at a sampling day (P<(0.05/N), N = number of repeated CO_2 collections).

Fig. 2 Cumulative CO_2 emissions during incubation experiment from a) Hapludult (non-carbonate soil) and b) Haplustalf (carbonate-rich soil) measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates significant difference between the two gas collection methods at a sampling day (P<(0.05/N), N = number of repeated CO_2 collections).

$\delta^{13}C$ values of emitted CO_2

After correction by Eq. (2), the $\delta^{13}C$ values of the CO_2 emitted ($\delta^{13}C_{CO2}$) from Hapludult (non-carbonate soil) ranged from −19.4±0.6‰ to −19.1±0.6‰ (mean, −19.3±0.5‰) for the gas sampling method, and from −20.1±0.2‰ to −19.5±0.7‰ (mean, −19.8±0.7‰) for the alkali trap method (Fig. 3a). Overall, the values of $\delta^{13}C_{CO2}$ for Hapludult determined using the gas sampling and alkali trap methods were not significantly different, except at day 18 of incubation. For Hapludult, the $\delta^{13}C_{CO2}$ was similar to $\delta^{13}C$ values of SOC ($\delta^{13}C_{SOC}$, −21.2‰). The differences (1.4‰--1.9‰) may have come from three sources: (1) trace SIC in Hapludult that affected measured $\delta^{13}C_{CO2}$; (2) C isotope fractionation that occurred during the decomposition of SOC into gaseous CO_2, i.e., 0‰–1‰ during SOC decomposition and CO_2 diffusion from soil (Breecker *et al*., 2015; Boström *et al*., 2007; Cheng, 1996) (this was in line with the fractionation coefficient set in Eq. (5) (1‰)); and (3) operational errors during the experiment, such as measurement and sampling. Because soil contains active and inert OC pools (Amelung *et al*.,1999; Von Lützow *et al*., 2007), the preferential decomposition of active OC by microorganisms may be another reason for this difference (Amelung *et al*.,1999; Von Lützow *et al*., 2007). Therefore, for non-carbonate soil, quantifying genuine isotope fractionation during SOC decomposition into gaseous CO_2 is necessary for future studies.

For Hapludult (non-carbonate soil), we found that the $\delta^{13}C_{CO2}$ measured by the gas sampling method was slightly higher than that measured by the alkali trap method at each sampling event. There are several possible reasons for this: (1) contamination by trace ambient air in the gas collection needle: as we used a syringe for gas sampling, the trace air stored in the needle may have affected the result. We calculated these possible impacts, and found that in our experiment, the $\delta^{13}C_{CO2}$ was decreased (negative) by 0.05‰ if the ambient air stored in the needle (Table S4) was considered; (2) contamination by trace ambient air outside the incubation jar: in the current study, the incubation jars were sealed with a double-layer, i.e., Parafilm and a sealing cap. During the gas sampling process, ambient air may have entered the incubation jar when the outer sealing cap was opened and the gas sample was collected using the needle inserted through the Parafilm. The ambient air had $\delta^{13}C_{CO2}$ of −8‰, much higher than that of the CO_2 derived from SOC decomposition (−19.3±0.5‰). This implied that for the incubation experiment, contamination by ambient air during the gas collection process should be avoided, for example, by using real-time gas monitoring (Anan *et al*., 2014; Meijide *et al*., 2010).

For Haplustalf (carbonate-rich soil), the average $\delta^{13}C_{CO2}$ measured by the gas sampling method was −17.7±0.7‰ (range: −18.0±0.3‰ to −17.1±1.2‰) and that measured by the alkali trap method was −17.4±0.5‰ (range: −18.2±1.1‰ to −17.0±0.5‰) (Fig. 3b). There was no statistical difference in total $\delta^{13}C$ values between these two gas collection methods. However, compared with Hapludult (non-carbonate soil), Haplustalf showed higher fluctuations in $\delta^{13}C_{CO2}$ as measured using both methods during incubation. We concluded that this was due to SIC dissolution during incubation (Tamir *et al*., 2011).

For both Hapludult and Haplustalf, variations in $\delta^{13}C_{CO2}$ may have been due to re-precipitation of CO_2 into carbonate during incubation. However, because this re-precipitation usually takes several months under laboratory conditions (Ramnarine *et al*., 2012; Kuzyakov *et al*., 2006), we assumed its effects on $\delta^{13}C_{CO2}$ were negligible in our study. We also examined the effects of gas collection method and soil type on $\delta^{13}C_{CO2}$ (Table S5), and found that only soil type had a significant effect.

Fig. 3 $\delta^{13}C$ values of CO_2 emitted during the incubation period for a) Hapludult (non-carbonate soil) and b)

Haplustalf (carbonate-rich soil) measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates a significant difference between the two gas collection methods on a sampling day (P<(0.05/N), N = number of repeated CO_2 collections).

Partitioning sources of CO_2

In laboratory incubation experiments, the C isotope fractionation occurring during SOC decomposition and SIC dissolution is much smaller than that in the field, because (1) interference from ambient air is much higher in the field, because of wind; (2) the soil used in indoor incubation experiments is much more uniform than that in the field; (3) the constant temperature in laboratory conditions eliminates the influences of temperature and moisture variations on C isotope fractionation (Chevallier *et al*., 2016). In addition, CO_2 capture is more complete with timely replacement of NaOH (Bertrand *et al*., 2007), different from the previous study in 1985 by Fritz *et al*. Therefore, in most laboratory incubation studies, C isotope fractionation has not been considered (Bertrand *et al*., 2007; Tamir *et al*., 2012). However, to examine the effects of various influential factors, we compared scenarios where C isotope fractionation was considered or not considered in partitioning the sources of CO_2 emitted.

Using Eqs. (4) and (5), we first partitioned the emitted CO_2 into SOC and SIC, and then partitioned the CO_2 from SOC into C3 and C4 plant-derived SOC. In Hapludult (non-carbonate soil), as the SOC accounted for 99% of the TC (Table I), we assumed that all CO_2 came from C3 and C4 plant-derived SOC. The average proportion of C4 plant-derived CO_2 was 52±3% (range: 51±4%--53±6%) for the gas sampling method, and 48±4% (range: 46±1%--50±4%) for the alkali trap method (Table II). If we considered fractionation during SOC decomposition into gaseous CO_2 (for both C3 and C4 plant-derived SOC) to be 1‰, the average proportion of CO_2 derived from C4 plant-derived SOC was 45±3% (range: 44±4%--46±6%) for the gas sampling method, and 41±4% (range: 39±1%--43±4%) for the alkali trap method (Table II). For both scenarios (considering fractionation or not), we did not detect significant differences between the gas sampling and alkali trap methods in estimating the proportion of CO_2 released from C3 and C4 plant-derived SOC (Table II). Considering that the proportions of SOC derived from C3 and C4 plants in Hapludult were 61% (6.2 g kg^{-1}) and 39% (3.9 g kg^{-1}) (Eq. 3), respectively, we concluded that SOC derived from C4 plants decomposed faster than that derived from C3 plants, consistent with the results of other studies (Wynn and Bird, 2007; Ponphang-nga *et al.,* 2011).

TABLE II Proportion (%) of CO_2 released from C4 plant-derived SOC during incubation of Hapludult (non-carbonate soil). G, gas sampling method. A, alkali trap method.

		2 d	5 d	7 d	9 d	14 d	18 d	25 d
Without	G	51±3	53±4	51±2	51±4	53±6	52±1	52±3
Fractionation	A	48±9	50±4	50±4	48±4	47±5	46±1	48±4
With	G	44±3	46±4	44±2	44±4	46±6	45±1	45±3
Fractionation	A	41±9	43±4	43±4	41±4	40±5	39±1	41±4

Means ± standard deviation, n = 3. There were no significant differences between the two gas collection methods at the same sampling event (T test; P<(0.05/N), N = number of repeated CO_2 collections).

For Haplustalf (carbonate-rich soil), the contribution of SOC to emitted CO_2 was 72±4.3% (69±7%--74±5%) for the gas sampling method, and 71±3% (66±2%--76±3%) for the alkali trap method (Table III). If we considered fractionation in the processes of SOC decomposition (1.4‰--1.9‰, same as Hapludult) and SIC dissolution (7‰; Szaran, 1997; Chevallier *et al*., 2016) into gaseous CO_2, the contribution of SOC to emitted CO_2 was 65.4±10.7% (57±13%--71±11%) for the gas sampling method, and 57±7% (51±4%--65±7%) for the alkali trap method (Table III). The differences between the two methods were not statistically significant. As shown in Table I, the respective SOC and SIC contents were 8.6 and 8.5 g kg^{-1}. This provided further evidence that SOC decomposed faster than did SIC, consistent with the findings of many other studies (Table IV).

TABLE III Proportion (%) of CO_2 derived from SOC during incubation of Haplustalf (carbonate-rich soil). G, gas sampling method. A, alkali trap method.

		2 d	5 d	7 d	9 d	14 d	18 d	25 d
Without	G	69±7	74±5	71±4	74±2	72±1	73±6	71±4
Fractionation	A	68±3	70±2	66±2	68±4	75±7	76±3	72±2
With	G	57±13	71±11	62±8	69±8	66±10	68±15	64±8
Fractionation	A	52±3	59±9	51±4	53±11	57±8	65±7	60±8

Means ± standard deviation, n = 3. There were no significant differences between the two gas collection methods at the same sampling event (T test; $P<(0.05/N)$, N = number of repeated CO_2 collections).

TABLE IV Contribution of SOC to CO_2 emissions during incubation of Haplustalf (carbonate-rich soil). G, gas sampling method. A, alkali trap method.

Location	SOC/TC	Proportion of SOC derived CO_2 in total CO_2	Dominant source of CO_2	Soil	Incubation		Method
					Duration	Temperature	
	%	%			day	℃	
USA[a)]	4	87	SOC	Desert soil	14	25	G
France[b)]	26	73	SOC	Rendosol/Rendzina	91	15	A
Israel[c)]	14	70	SOC	Typic Haplocalcids	56	30	G
Canada[d)]	66--72	26--38	SIC	Typic Hapludalf	14	25	A
Tunisia[e)]	33	53--76	SOC	Calcari-Leptic Cambisol	28	20	A
Australia[f)]	15	5	SIC	Rudosols/Regosol	11	25	G
Current study	50	57--72	SOC	Udic Haplustalf	25	25	A and G

[a)] Stevenson and Verburg (2006); [b)] Bertrand *et al.* (2007); [c)] Tamir *et al.* (2011); [d)] Ramnarine *et al.* (2012); [e)] Chevallier *et al.* (2016); [f)] Lardner *et al.* (2015).

Similarly, we calculated the contribution of C4 and C3 plant-derived SOC to emitted CO_2 in Haplustalf (carbonate-rich soil) using Eq. (5). The contribution of C4 plant-derived SOC was 22.5±1.4% (21±2%--23±2%) for the gas sampling method, and 22.1±1% (21±1%--24±2%) for the alkali trap method (Table V). If the fractionation from C3 and C4 plant-derived SOC to gaseous CO_2 was set at 1‰, the contribution of C4 plant-derived SOC ranged from 21±4% to 28±6% (mean, 25.2±5.2%) for the gas sampling method, and from 18±6% to 23±10% (mean, 20.3±5.6%) for the alkali trap method (Table V). Considering that the ratio of C3/C4 plant-derived SOC was 117/55, this highlighted that the C4 plant-derived SOC decomposed faster than did C3 plant-derived SOC (Wynn and Bird, 2007; Ponphang-nga *et al*., 2011). Another reason may be that for both Haplustalf and Hapludult soils, the last crop before soil sampling was maize (a C4 plant), which tends to be stored in sand particles (Amelung *et al*., 1999; Von Lützow *et al*., 2007) and decomposes readily (Six *et al*., 2002; Drewitt *et al*., 2009).

TABLE V Proportion (%) of CO_2 derived from C4 during incubation of Haplustalf (carbonate-rich soil). G, gas sampling method. A, alkali method.

		2 d	5 d	7 d	9 d	14 d	18 d	25 d
Without	G	21±2	23±2	22±1	23±1	22±0	23±2	22±1
Fractionation	A	21±1	22±1	21±1	21±1	24±2	24±1	22±1
With	G	21±4	28±6	24±3	26±6	27±8	26±6	25±3
Fractionation	A	18±6	22±6	19±3	18±6	23±10	21±3	21±6

Means ± standard deviation, n = 3. There were no significant differences between the two gas collection methods at the same sampling event (T test; $P<(0.05/N)$, N = number of repeated CO_2 collections).

In this study, there were some uncertainties in our calculations and experimental procedure: (1) SOC fractionation in Haplustalf (carbonate-rich soil) may differ from that in Hapludult (non-carbonate soil); (2) the SIC fractionation coefficient of −7‰ derived from a field study (Szaran, 1997; Chevallier *et al*., 2016) might be higher than that of SIC fractionation in our laboratory incubation conditions; and (3) the fractionation of C3 and C4 plant-derived SOC into gaseous CO_2 may be higher or lower than 1‰. In most laboratory studies, the highest fractionation coefficient was <1‰ (Breecker *et al*., 2015; Boström *et al*., 2007). Using a lower fractionation coefficient in the partition calculation further supported the findings that C4 plant-derived SOC had a higher decomposition rate. (4) In this experiment, we analyzed three replicates for each sample (n = 3). Analysis of more replicates may improve the accuracy of the results. The coefficient of variation (CV) of CO_2 emissions (Fig. 1) was <10% (Fig. 1S), and that of ^{13}C values of emitted CO_2 (Fig. 3) was <8% (Fig. 2S). This suggested that three replicates provided a satisfactory level of accuracy, but a larger sample size is still recommended.

CONCLUSIONS

Both alkali trap and gas sampling methods can be used to quantify CO_2 emissions from soils in incubation studies. For carbonate soils, the alkali trap method may overestimate the CO_2 emissions due to decreasing CO_2 pressure within the incubation jar, while the direct gas sampling method may underestimate CO_2 emissions. We found that longer sampling intervals resulted in greater differences in measured CO_2 values between the two gas collection methods. Interference from ambient air is a major source of error in soil incubation and CO_2 emission analyses, and steps should be taken to avoid this. Carbon isotope fractionation during the processes of SOC decomposition and SIC dissolution can result in variations in partitioning sources of emitted CO_2. The general findings, i.e., that SOC and C4 plant-derived SOC decompose faster than do SIC and C3 plant-derived SOC, respectively, were not affected by whether the C fractionation coefficient was considered or not. This should be considered in future studies on CO_2 emissions, such as those related to land-use changes or from soils with different parent materials.

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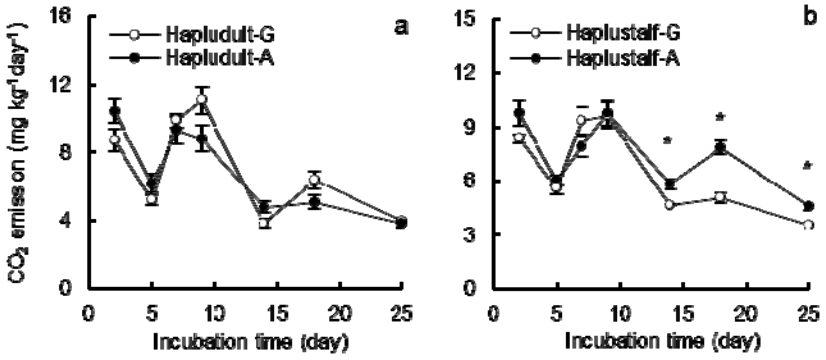

Fig. 1 CO_2 emissions at each sampling event from a) Hapludult (non-carbonate) and b) Haplustalf (carbonate-rich) soils measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates significant difference between the two gas collection methods at a sampling day (P<(0.05/N), N = number of repeated CO_2 collections).

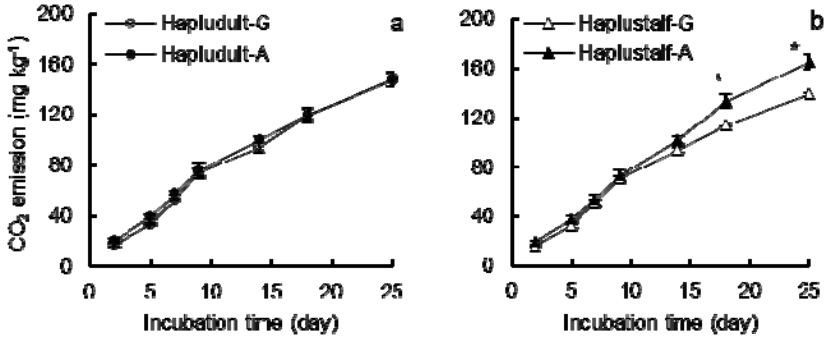

Fig. 2 Cumulative CO_2 emissions during incubation experiment from a) Hapludult (non-carbonate) and b) Haplustalf (carbonate-rich) soils measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates significant difference between the two gas collection methods at a sampling day (P<(0.05/N), N = number of repeated CO_2 collections).

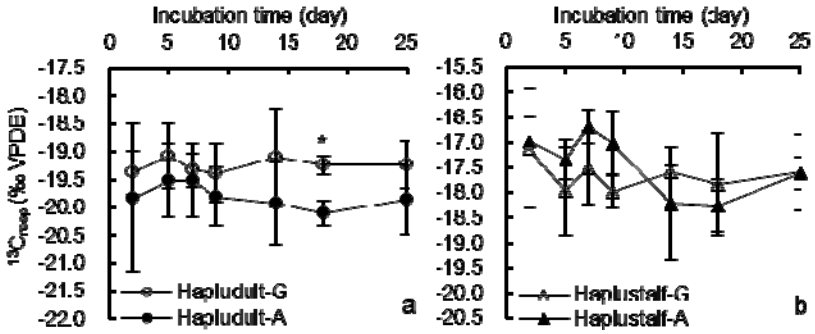

Fig. 3 $\delta^{13}C$ values of CO_2 emitted during incubation period for a) Hapludult (non-carbonate) and b) Haplustalf (carbonate-rich) soils measured by gas sampling method (G) and alkali trap method (A). Values are means and bars are standard deviation (n=3). * indicates significant difference between the two gas collection methods on a sampling day (P<(0.05/N), N = number of CO_2 collections).