CO2 Emission and Source Partitioning from Carbonate and Non-carbonate Soils during Laboratory Incubation

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ABSTRACT

Quantification and source partitioning of CO_2 from carbonate and non-carbonate soils are critical important to the terrestrial carbon (C) cycling study. Alkali trap (A) and direct gas sampling (G) methods are commonly used for capturing of CO_2 released from soils. We conducted a 25-day laboratory incubation experiment to compare the efficacies of these two methods and analyzed the influential factors on Hapludult (BS), a non-carbonate soil, and Haplustalf (LS), a carbonate-rich soil. Isotopic fraction was introduced into the calculation process, to determine its impacts on partitioning the sources of CO_2 emitted into soil organic carbon (SOC) and soil inorganic carbon (SIC), and into the C3/C4 plant-derived SOC. The results indicated that the CO_2 fluxes from BS determined by A and G methods were not significantly different. For the LS, the CO_2 fluxes and cumulative emission measured by A method were significantly higher than G method from the 14th day of the experiment. Although SOC and SIC both account for half of the soil total C in the LS, SOC decomposition contributed 57--72% of the total CO_2 emitted. For both BS and LS, the SOC derived from C4 plants was more easily decomposed than that originating from C3 plant. Our study highlighted that for carbonate soils, alkali trap method may overestimate the CO_2 emission due to the decreasing CO_2 pressure within the incubation jar, while direct gas sampling method exhibits a reverse effect. Gas sampling interval and ambient air are the important error sources in soil incubation and CO_2 quantification study.

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

INTRODUCTION

Soil is the largest carbon (C) pool on Earth, with the organic carbon (OC) stock of 1550 Pg and 950 Pg for inorganic carbon (IC) (Lal, 2007). Soil inorganic carbon (SIC) is located primarily in arid and semi-arid regions, which occupies a total land area of 4.9×10^7 km^2 (Lardner *et al*., 2015), and is mainly in the form of calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). In arid and semi-arid regions, the content of SIC is approximately ten times greater than that of soil organic carbon (SOC) (Schlesinger, 1982). SIC and SOC pools play a very important role in global C sequestration (Lal, 2009), and the contribution of SIC to CO_2 emissions accounts for a large proportion and should be given more attention (Tamir *et al*., 2011; Ramnarine *et al*., 2012; Chevallier *et al*., 2016).

CO_2 efflux from the soils is recognized as one of the largest C fluxes within the global C cycle; small changes may have a large effect on the concentration of atmospheric CO_2 (Schlesinger and Andrews, 2000), thereby effectively affecting the global climate (Lal, 2004; Luo *et al*., 2010). Since the 1970s, the importance of soil respiration as CO_2 has been recognized and numerous studies have been undertaken to investigate the soil respiration rates from different ecosystems (Buyanovsky *et al*., 1986; Conant *et al*., 2000), the relationship of soil respiration with vegetation (Raich and Schlesinger, 1992), as well as methods for quantifying soil respiration (Jong and Schappert, 1972; Baldocchi *et al*., 1988). For instance, some studies found that equating soil CO_2 emissions with soil respiration is not applicable for carbonate soils (Stevenson and Verburg, 2006; Bertrand *et al*., 2007).

Alkali trap (A) and direct gas sampling(G) methods are the most commonly used techniques for quantifying soil CO_2 emission in the field and laboratory studies (Rochette *et al*., 1997; Bruun *et al*., 2014; Lardner *et al*., 2015). Compared with field direct measurement, the laboratory incubation has more stable hydrothermal conditions which reduce the interference of these environmental factors. Determination of soil CO_2 flux in the laboratory incubation has been widely used to quantify soil microbial activity (Leita *et al.,* 1995), SOC decomposition (Kuzyakov and Bol, 2005), and soil organic matter (SOM) pool dynamics

(Haile-Mariam *et al*., 2008), and turnover of different SOM fractions in the short term (Stevenson and Verburg, 2006; De Troyer *et al*., 2011). Ramnarine *et al.* (2012) conducted a 14-day soil incubation (alkali trap method) and found that 62%--74% of the CO_2 emitted was derived from SIC. The 168-day incubation experiment by Tamir *et al.* (2012) indicated that N-rich amendment to calcareous soil enhanced the dissolution and re-crystallization of SIC. Alkali trap may overestimate or underestimate the CO_2 fluxes, because of the influences of temperature, moisture variations and external gases diffusion, resulting in excessive or insufficient absorption of CO_2 (Rochette *et al*., 1997; Ramnarine *et al*., 2012). Gas sampling has the uncertainties that rising CO_2 concentration in a sealed system affects SIC and SOC decomposition. Therefore, it is important to compare the efficacies and accuracies of the direct gas sampling and alkali trap methods on collecting CO_2 released from soil incubation.

Isotopic measurement of ^{13}C contents is an effective tool to estimate the CO_2 sources (Stevenson and Verburg, 2006; Tamir *et al*., 2011). Compared with SIC, SOC is less enriched with heavier ^{13}C and therefore $^{13}C_{CO2}$ from SOC ($\delta^{13}C_{CO2\text{-}SOC}$) is significantly different from SIC ($\delta^{13}C_{CO2\text{-}SIC}$) (Salomons and Mook, 1976; Magaritz and Amiel, 1980; Plestenjak *et al*., 2012). C3 and C4 plants have different $\delta^{13}C$ values owing to their physiological differences during the photosynthetic fixation of CO_2 (Balesdent *et al*., 1990; Bol *et al*., 2004; Krull *et al*., 2007). Based on the differences $\delta^{13}C$ values in C3 and C4 plants, Kuzyakov and Bol (2005) distinguished three sources of CO_2 efflux from soils. Isotope fractionation may occur during these reactions which emitted the CO_2. $\delta^{13}C_{CO2\text{-}SIC}$ is negative by 7--9‰ compared to $\delta^{13}C_{SIC}$ in field conditions (Szaran, 1997; Chevallier *et al*., 2016). $\delta^{13}C_{CO2\text{-}SOC}$ with amplitudes ranging from 0--1‰ compared to the $\delta^{13}C_{SOC}$ in the laboratory incubation (Breecker *et al*., 2015; Boström *et al*., 2007). Other studies pointed out that different from field condition, the isotope fractionation during the laboratory incubation is much smaller and may be negligible (Bertrand *et al.,* 2007; Tamir *et al.,* 2012), for instance, using alkali trapping, Chevallier *et al.* (2016) found that the estimated contribution of SIC to the soil CO_2 emitted exceeds total emission if isotopic fractionation between SIC and SIC-derived CO_2 was considered in the calculation.

China has a large area of arid and semi-arid soil, in which SIC is approximately 60 Pg, accounting for 1/20-1/15 of the global SIC pool (Pan, 1999). The Loess Plateau in northwestern China represents a typical carbonate soil area, of which the SIC content is approximately 50% of total carbon (TC) (Dong *et al*., 2014). China's northeastern plain is dominated by Hapludult and its SOC accounts for >99% of TC (Lan *et al*., 2016). Both of these regions are important agricultural production areas in China. Analysis of the CO_2 emissions and sources of these two typical soils under the same hydrothermal conditions not only helps to understand the transformation mechanisms of soil C, but also provides 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. The CO_2 emitted from the soil was collected and measured by both alkali trap and gas sampling methods. The differences between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{SOC}$ were compared to examine the SOC fractionation effects during the Hapludult incubation process. Isotopic fraction was introduced into the calculation process, to determine its impacts on partitioning CO_2 sources (Chevallier *et al*., 2016). We aimed to determine the differences of CO_2 capturing via the alkali traps and gas sampling methods, and to explore the contribution of different photosynthesis plants (C3/C4) and soil carbon (SIC/SOC) to CO_2 emission, as well as the errors accompanied with the calculation process.

MATERIALS AND METHODS

Incubation experiment

Two types of soils were used for the experiment: the Lou soil (LS), i.e., Udic Haplustalf soil, were collected in Shanxi Province in northwestern China (34º17'59.317"N, 108º04'9.384"E), at the southern edge of the Loess Plateau. The region is a warm temperate zone of semi-humid and semi-arid climate with an average annual temperature and precipitation of 13 ℃ and 600 to 650 mm, respectively. The soil is classified as Eum-Orthic Anthrosol in Chinese Soil Taxonomy, and equivalent to Udic Haplustalf in USDA Soil Taxonomy. The soil texture is silt clay loam. Cropping system is two crop season per year, i.e., winter

wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). The Brown soil (BS), i.e., 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 ℃ and the annual precipitation is 540--640 mm. The soil is equivalent to Hapludult in USDA Soil Taxonomy. The soil has the texture of silt loam. Cropping system is one crop season per year, and soybean (*Glycine max* L.) was rotated with maize (*Zea mays* L.). Historically, maize is the main crop in the region. Before sampling, the last crop planted on 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. Five replicates (each located at the 4 corners and center of a square with a side length of 30 m) were taken from each field, and then mixed uniformly, air dried, passed through a 2 mm sieve, and stored.

To activate microbial population and decrease fluctuations in SOC mineralization caused by drying and rewetting, we added deionized water to 20 g samples of the air-dried soil, and then incubated the samples in a 100 mL beaker at 25 ℃ for 7 days before the start of the incubation experiment (Paul *et al*., 2006).

For the incubation, 20 g soil was put into 240 mL jars. The jars were sealed with a layer of Parafilm and a plastic sealing cap and incubated for 25 days at 25 ℃, and the Parafilm was replaced by a new one after changing the NaOH solution (alkali trap method) or collecting the CO_2 by the needled syringe (gas sampling method). The deionized water was added on day 0, 2, 5, 7, 9, 14, and 18 to adjust soil moisture content to 70% of field holding capacity.

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

Alkali trap method (A). 10 mL of 0.1 M NaOH was added inside the incubation jar on day 0 of the incubation experiment. The NaOH solutions were changed on day 2, 5, 7, 9, 14, and 18. For blank (no soil) treatment, the sealed jars with only NaOH solution were prepared. Three jars were used as replicates for soil incubation and blank treatments.

The CO_2 trapped in NaOH solutions was measured using HCl-$SrCl_2$ titration method (Ramnarine *et al*., 2012). To analyze $\delta^{13}C$ of the emitted CO_2, suspension containing $SrCO_3$ was placed in 250 mL centrifuge bottle and centrifuged at 8000 r min^{-1} for 20 min, and washed three times with deionized water to remove excess $SrCl_2$ and NaCl (Ramnarine *et al*., 2012). The $SrCO_3$ samples was 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, Davis. All the $\delta^{13}C$ values reported were in VPDB.

Gas sampling method (G). The headspace of all incubation jars was gas sampled by a 20 mL needled syringe through the Parafilm covering the jars (after opening the plastic sealing cap). 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 which held the gas for analysis of CO_2 concentration and $\delta^{13}C$ using the gas chromatography-isotope ratio mass spectrometer DELTA plus (Thermo Fisher Scientific, USA) at the Stable Isotope Facility, University of California, Davis.

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

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

in which, for the A 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 G 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 within the blank jars.

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

2003):

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

in which, for the A method, $\delta^{13}C_s$ was the $\delta^{13}C$ value of $SrCO_3$ from the incubated soils and $\delta^{13}C_b$ was the $\delta^{13}C$ value of $SrCO_3$ from the blank jar, which both were analyzed by PDZ Europa 20-20 isotope ratio mass spectrometer. For the G 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 by isotope ratio mass spectrometer DELTA plus.

TC, SIC, SOC, TN, $\delta^{13}C$, and pH analysis

Total soil carbon (TC) and total soil nitrogen (TN) content were measured using CN analyzer (Flash EA 2000, Thermo Electron Corporation, Italy) at the Chinese Academy of Agricultural Sciences. SIC 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 with isotope ratio mass spectrometer DELTA plus (Thermo Fisher Scientific, USA) at the Chinese Academy of Agricultural Sciences. Soil pH was measured with a pH meter (soil:water=1:5). The soil properties were presented in Table I.

TABLE Ⅰ Properties of the Hapludult (BS) and Haplustalf soils (LS); means ± standard deviation, n = 5.

	BS	LS
pH (KCL)	7.2±0.2	7.8±0.2*
TC (g kg^{-1})	10.3±0.1	17.1±0.1*
TC-^{13}C (‰VPDB)	-20.6±0.1	-14.0±0.1*
SOC (g kg^{-1})	10.2±0.3*	8.6±0.3
SOC-^{13}C (‰VPDB)	-21.2±0.1	-22.3±0.1*
SIC (g kg^{-1})	0.1±0.2	8.5±0.2*
SIC-^{13}C (‰VPDB)	-7.6±1.1*	-5.8±0.0
TN (g kg^{-1})	1.0±0.0	1.0±0.0

* indicates significant differences between the two soils ($P < 0.05$).

Source partitioning of SOC and CO_2 emitted

Source partitioning of SOC into C4 and C3 plants origination. The SOC derived from C4 and C3 plants in BS and LS soils was calculated by Eq. (3):

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

where, $\delta^{13}C_{SOC}$ was the $\delta^{13}C$ value of SOC in BS or LS soils. $\delta^{13}C_{C4}$ was the $\delta^{13}C$ value of C4 plant (-12‰) and $\delta^{13}C_{C3}$ was the $\delta^{13}C$ value of C3 plant (-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 the SOC (f_{SOC}) was estimated using the two-end-member mixing model of Eq. (4) (Balesdent *et al.*, 1987):

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

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

If the isotopic fractionation was considered, for BS, as the SOC accounted for >99% of TC, we assumed all CO_2 emitted were from SOC, the differences between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{SOC}$ were due to isotopic fractionation in the process of SOC decomposition to gaseous CO_2. For LS, we assumed the isotopic fractionation in the process of SOC decomposition to gaseous CO_2 was same as BS, i.e., $\delta^{13}C_{CO2\text{-}SOC}$ was the sum of $\delta^{13}C_{SOC}$ and the corresponding isotope fractionation; the isotopic fractionation in the process of SIC dissolution to gaseous CO_2 was set at 7‰, i.e., $\delta^{13}C_{CO2\text{-}SIC}$ was negative by 7‰ 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 evolved from C4 plant-derived SOC (f_{C4}) was estimated by Eq. (5):

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

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

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

For LS, $\delta^{13}C_{CO2\text{-}SOC}$ was the $\delta^{13}C$ value of SOC ($\delta^{13}C_{SOC}$), f_{SOC} was the proportion of CO_2 evolved from the 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 the isotopic fractionation was considered, same to 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 positive by 1‰ 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). CO_2 emission rates and $\delta^{13}C$ were tested for normality using the Shapiro-Wilk test and then one-way analysis of variance (ANOVA) were used to evaluate the differences among CO_2 collection methods in the same soil laboratory incubation (in Fig.1--Fig.3; n = 3). If the effects of the sampling method on the measurement results were significant ($P < 0.05$), paired t-tests were used to compare the CO_2-^{13}C and CO_2 emissions between the two methods. T-tests were used to compare the physical and chemical properties of the two soils (Table Ⅰ; n=5) and compare the estimation results of the two methods for C sources (Table Ⅱ, Table Ⅲ and Table Ⅴ; n=3). Two-factor two-level ANOVA was used to analyze whether there are interactions between two soils and two gas collection methods (Table S1--S2; n=3).

RESULTS AND DISCUSSION

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

Hapludult soil (BS) and Haplustalf soil (LS) were significantly different on C contents, $\delta^{13}C$ values and pH (Table Ⅰ). The pH of BS (7.2±0.2) was significantly lower than LS (7.8±0.2). BS had the TC content of 10.3±0.1 g kg^{-1}, of which 99% was SOC. On the contrary, LS had the TC of 17.1±0.1 g kg^{-1}, of which SOC and SIC both accounted for about 50%. The $\delta^{13}C$ value of TC ($\delta^{13}C_{TC}$, -20.6±0.1‰) in BS was significantly lower (negative) than that of LS, reflecting the high content of SIC in TC of LS. However, the $\delta^{13}C$ value of SOC ($\delta^{13}C_{SOC}$) in BS (-21.2±0.1‰) were significantly higher (positive) than that of LS (-

22.3±0.1‰).

CO_2 fluxes quantified during the incubation

For BS, there were no significant differences in the CO_2 fluxes between the two gas collection methods during the whole incubation period (Fig. 1a). Therefore, the cumulative CO_2 emissions quantified by gas sampling (G) and alkaline trap methods (A) were similar, i.e., 147.7±5.3 mg kg^{-1} for method G and 146.9±4.8 mg kg^{-1} for method A (Fig. 2a). For LS, the CO_2 fluxes measured by G and A methods were similar during the first 9 days of the incubation experiment; however, since the 14th day after the incubation, the CO_2 flux at each sampling event measured by G method were significantly lower than that of A method (Fig. 1b). The cumulative CO_2 emissions during the whole 25 days of incubation for G and A methods were significantly different, i.e., 139.0±1.7 vs. 165.3±5.7 mg kg^{-1}, respectively (Fig. 2b).

For both BS and LS, 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 incubation period (Fig. 2), equivalent to that occurred in the later 16 days of the experiment. This is because SOC decomposition in the early phase of soil incubation was significantly higher than that in the later phase (De Troyer *et al*., 2011; Tamir *et al*., 2011). For LS, the significant differences between the cumulative CO_2 quantified by the two methods during the later period incubation might be due to the SIC dissolution which was facilitated by the organic acids produced during SOC decomposition (Kuzyakov, 2006; Rovira and Vallejo, 2008; Tamir *et al*., 2011). In the A method, the CO_2 emitted was immediately absorbed by NaOH used, thus the CO_2 concentration (pCO_2) in sealed jars decreased and afterwards, the equilibrium of SIC dissolution and the reaction of CO_2 generation were promoted (Dong *et al*., 2013). In contrast, for G method, the pCO_2 in the sealed jars continuously arose as the incubation proceeded. This inhibited microorganism activity for SOC decomposition (West *et al*., 2009) and the SIC dissolution which was driven by the organic acid from SOC decomposition. The increasing pCO_2 might also reverse the equilibrium of SIC dissolution and reduce the CO_2 generation in G method. This indicates that for carbonate soil such as LS, A method may overestimate the CO_2 emission while G method may underestimate the CO_2 emission. The longer the sampling interval (duration between two gas collection events), the greater differences in pCO_2 within the sealed jar between the two methods. Further examination found that the differences of CO_2 emission between the two methods were significant if the sampling interval was more than 4 days.

We also analyzed the interaction of gas collection methods and soil types on CO_2 emission and found that in the later period of the incubation experiment (18--25 days), the interactive effect was significant (Table S1). This highlighted that the CO_2 emission was also influenced by other factors such as sampling interval, other than the gas collection method and soil types.

Fig. 1 CO_2 fluxes from soils during the incubation experiment. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicate a significant difference between the two gas collection methods at a sampling day (P<0.05, n=3).

Fig. 2 Cumulative CO_2 emissions from soils during the incubation experiment. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicates a significant difference between the two gas collection methods at a sampling day (P<0.05, n=3).

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

After correction by Eq. (2), $\delta^{13}C$ values of the CO_2 emitted ($\delta^{13}C_{CO2}$) from BS ranged from -19.4±0.6‰

to -19.1±0.6‰ (mean -19.3±0.5‰) for the G method, and from -20.1±0.2‰ to -19.5±0.7‰ (mean -19.8±0.7‰) for the A method (Fig. 3a). Overall, the $\delta^{13}C_{CO2}$ for BS between G and A methods were not significantly different, except at the 18th day after the start of incubation. For BS, the $\delta^{13}C_{CO2}$ was similar to $\delta^{13}C$ values of the SOC ($\delta^{13}C_{SOC}$, -21.2‰) and the differences (1.4‰--1.9‰) may come from three sources: (1) the trace SIC in BS had impacts on the measured $\delta^{13}C_{CO2}$, (2) the C isotope fractionation occurred during the decomposition of SOC to gaseous CO_2, i.e., 0--1‰ during the SOC decomposition and CO_2 diffusion from soil to the incubation jar (Breecker *et al*., 2015; Boström *et al*., 2007; Cheng, 1996). This was in line with the fractionation coefficient set in the Eq. (5) (1‰); and (3) operation errors during the experiment, such as measurement and sampling.

For BS, we also observed that the $\delta^{13}C_{CO2}$ obtained by G method was slightly higher (positive) than that by A method at each sampling event. The reasons for this might be: (1) trace ambient air in the gas collection needle: as we used the syringe for gas sampling, the trace air stored in the needle may affect the result. We calculated the impact and found that in our experiment, the $\delta^{13}C_{CO2}$ was decreased (negative) by 0.05‰ if we considered the ambient air stored in the needle (Table S2); (2) trace ambient air outside the incubation jar: in current study, the incubation jars were sealed with a double-layer, i.e., sealing Parafilm and sealing cap. During the gas sampling process, the ambient air might come inside the incubation jar as the outer sealing cap was opened and gas sample was collected using a needle through the sealing Parafilm. The ambient air had the $\delta^{13}C_{CO2}$ of -8‰, much higher than that of the CO_2 derived from the SOC decomposition (-19.3±0.5‰). This implied that for the incubation experiment, the ambient air involvement during the gas collection process should be avoided, such as using real-time gas monitoring (Anan *et al*., 2014; Meijide *et al*., 2010).

For LS, the $\delta^{13}C_{CO2}$ measured by G method averaged at -17.7±0.7‰ (-18.0±0.3‰ to -17.1±1.2‰) and for method A, it averaged at -17.4±0.5‰ (-18.2±1.1‰ to -17.0±0.5‰) (Fig. 3b). We did not find the statistic differences of $\delta^{13}C$ values between these two gas collection methods. Compared with BS, the emitted $\delta^{13}C_{CO2}$ of LS had higher fluctuations for both gas collection methods during the incubation, and we guessed that this was due to the SIC dissolution during the incubation (Tamir *et al*., 2011).

For both the BS and LS, variations of $\delta^{13}C_{CO2}$ may also be from the reprecipitation of CO_2 as carbonate during the incubation. As this reprecipitation usually takes several months (Kuzyakov, 2006), which meant that it had negligible impacts on the $\delta^{13}C_{CO2}$ in our study. Similarly, we also examined the effects of gas collection method and soil type on $\delta^{13}C_{CO2}$ (Table S3), and found that only soil type exhibited a significant effect.

Fig. 3 $\delta^{13}C$ values of CO_2 emitted during the incubation period. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicates a significant difference between the two gas collection methods on a sampling day (P<0.05, n=3).

Partitioning the sources of CO_2

In laboratory incubation, the C isotope fractionation occurring during the SOC decomposition and SIC dissolution were much smaller than that in the field, because (1) interference from ambient air, such as wind speed, was much higher in the field; (2) the soil used in indoor incubation was much more uniform than that in the field; (3) constant temperature eliminated the influences of temperature and moisture variations on C isotope fractionation (Chevallier *et al*., 2016), and (4) a more complete CO_2 capture as NaOH was timely replaced (Bertrand *et al*., 2007), different from the previous study in 1985 by Fritz *et al*. Therefore, in most laboratory incubation studies, the C isotope fractionation was not considered (Bertrand *et al*., 2007; Tamir *et al*., 2012). However, in order to examine the effects of various influential factors, we did compare scenarios of considering and not considering the C isotope fractionation in partitioning the sources of CO_2 emitted.

Using the Eqs. (4) and (5), we firstly partitioned the CO_2 emitted into SOC and SIC, and then partitioned the CO_2 from SOC into C3 and C4 plant derived SOC. In the BS, 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 proportion of CO_2 derived from C4 plant averaged at 52±3% (51±4%--53±6%) for G method, and at 48±4% (46±1%--50±4%) for A method (Table Ⅱ). If we considered the fractionation during the SOC decomposition to gaseous CO_2 (for both C3 and C4 plant derived SOC at 1‰), the proportion CO_2 derived from C4 SOC was 45±3% (44±4%--46±6%) for G method, and 41±4% (39±1%--43±4%) for A method (Table Ⅱ). For both scenarios (considering and without consideration of fractionation), we didn't find significant differences between G and A methods in estimating the proportion of CO_2 released from C3 and C4 plant derived SOC (Table Ⅱ).Considering the of SOC derived from C3 and C4 plants in the BS was 61% (6.2 g kg^{-1}) and 39% (3.9 g kg^{-1}) (Eq. 3), respectively, which indicated that the SOC derived from C4 plant had a higher decomposition rate in our experiment (Wynn and Bird, 2007; Ponphang-nga *et al.,* 2011).

TABLE Ⅱ Proportion (%) of CO_2 released from C4 derived SOC during incubation of BS. 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±3a	53±4a	51±2a	51±4a	53±6a	52±1a	52±3a
Fractionation	A	48±9a	50±4a	50±4a	48±4a	47±5a	46±1a	48±4a
With	G	44±3a	46±4a	44±2a	44±4a	46±6a	45±1a	45±3a
Fractionation	A	41±9a	43±4a	43±4a	41±4a	40±5a	39±1a	41±4a

Different lower-case letters indicate significant differences ($P < 0.05$) between the two gas collection methods (n=3).

For the LS, the contribution of SOC to CO_2 emitted was 72±4.3% (69±7%--74±5%) for G method, and 71±3% (66±2%--76±3%) for A method (Table Ⅲ). If we considered the fractionation in the processes of SOC decomposition (1.4--1.9‰, same as BS) and SIC dissolution (7‰; Szaran, 1997; Chevallier *et al*., 2016) into gaseous CO_2, the contribution of SOC to CO_2 emitted was 65.4±10.7% (57±13%--71±11%) for G method, and 57±7% (51±4%--65±7%) for A method (Table Ⅲ). The differences between the two methods were not statistically significant. As stated in Table I, the respective SOC and SIC contents were 8.6 and 8.5 g kg^{-1}. This meant that SOC had a higher decomposition rate than SIC, consistent with most of other studies (Table IV).

TABLE Ⅲ Proportion (%) of CO_2 derived from SOC during incubation of LS. 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±7a	74±5a	71±4a	74±2a	72±1a	73±6a	71±4a
Fractionation	A	68±3a	70±2a	66±2a	68±4a	75±7a	76±3a	72±2a
With	G	57±13a	71±11a	62±8a	69±8a	66±10a	68±15a	64±8a
Fractionation	A	52±3a	59±9a	51±4a	53±11a	57±8a	65±7a	60±8a

Different lower-case letters indicate significant differences ($P < 0.05$) between the two gas collection methods (n=3).

TABLE Ⅳ Contribution of SOC to CO_2 emissions during incubation of LS. 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	Gray Brown Luvisol	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); G, gas sampling method. A, alkali trap method.

Similarly, we calculated the contribution of C4 and C3 plant-derived SOC to CO_2 emitted in LS using Eq. (5). The contribution of C4 plant-derived SOC was 22.5±1.4% (21±2%--23±2%) for G method, and 22.1±1% (21±1%--24±2%) for A method (Table Ⅴ). If the fractionation from C3 and C4 derived SOC to gaseous CO_2 was set at 1‰, the contribution of C4 plant derived SOC ranged from 21±4% to 28±6% with the mean of 25.2±5.2% for G method, and from 18±6% to 23±10% with the mean of 20.3±5.6% for A method (Table Ⅴ). Considering the fact that the ratio of C3/C4 plant-derived SOC was 117/55, again, this meant that the C4 plant-derived SOC had a higher decomposition rate than C3 plant (Wynn and Bird, 2007; Ponphang-nga *et al*., 2011). The other reason maybe that for LS and BS, the last crop before soil sampling was maize (C4), which tended to be stored in sand particles (Amelung *et al*., 1999; Von Lützow *et al*., 2007) and was more easily decomposed (Six *et al*., 2002; Drewitt *et al*., 2009).

TABLE Ⅴ Proportion (%) CO_2 derived from C4 during incubation of LS. G, gas sampling method. A, alkali method.

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

Different lower-case letters indicate significant differences ($P < 0.05$) between the two methods (n=3).

In this study, there were some uncertainties in our calculation: (1) SOC fractionation in LS may be different from that in BS; (2) SIC fractionation coefficient of -7‰ from field study (Szaran, 1997; Chevallier *et al*., 2016) might be higher than the real SIC fractionation in our incubation experiment; (3) the fractionation of C3 and C4 plant derived SOC to gaseous CO_2 might be different than 1‰. In most laboratory studies, the highest fractionation coefficient was lower than 1‰ (Breecker *et al*., 2015; Boström *et al*., 2007); using lower fractionation in the partition calculation further supported the findings that C4 plant derived SOC had a hither decomposition rate.

CONCLUSIONS

Both alkali trap and direct gas sampling methods are applicable for quantifying CO_2 emission from soils in incubation study. However, for carbonate soils, alkali trap method may overestimate the CO_2 emission due to the decreasing CO_2 pressure within incubation jar, while direct gas sampling method exhibits a reverse effect. Longer sampling interval causes increasing differences on CO_2 between the two gas collection methods. Ambient air is one major error source in soil incubation and CO_2 emission study and should be mitigated. Carbon isotope fractionation during the processes of SOC decomposition and SIC dissolution brings the variations in partitioning sources of CO_2 emitted, but the general findings, i.e., that SOC and C4 plant derived SOC exhibit a higher decomposition rate than SIC and C3 plant derived SOC, respectively, were not affected by whether the fractionation coefficient was considered or not.

411
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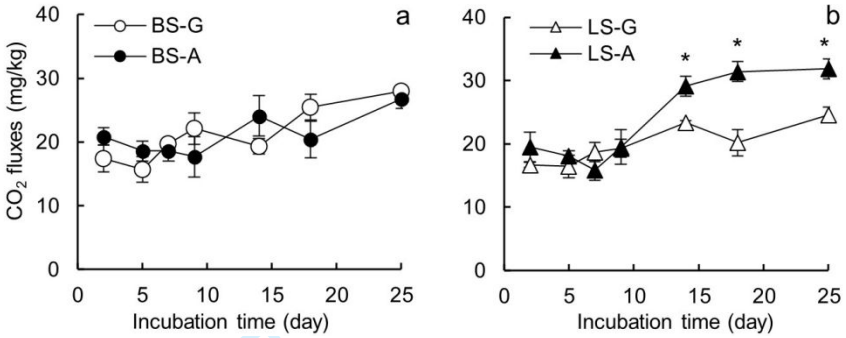

Fig. 1 CO_2 fluxes from soils during the incubation experiment. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicate a significant difference between the two gas collection methods at a sampling day (P<0.05, n=3).

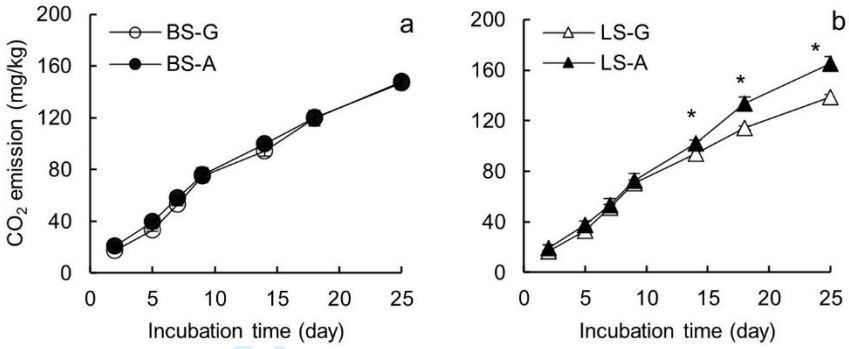

Fig. 2 Cumulative CO_2 emissions from soils during the incubation experiment. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicates a significant difference between the two gas collection methods at a sampling day (P<0.05, n=3).

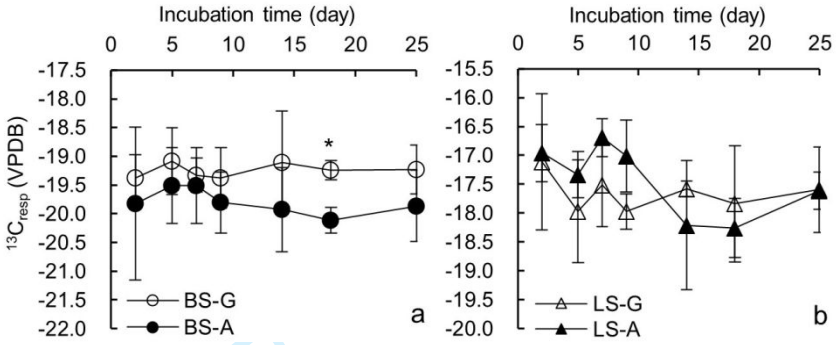

Fig. 3 $\delta^{13}C$ values of CO_2 emitted during the incubation period. a) Hapludult (BS) and b) Haplustalf (LS) measured using gas sampling method (G) and alkali trap method (A). * indicates a significant difference between the two gas collection methods on a sampling day (P<0.05, n=3).

SUPPORTING INFORMATION

CO2 Emission and Source Partitioning from Carbonate and Non-carbonate Soils during Laboratory Incubation

The Supporting Information contains:

Number of pages: 4

Number of tables: 3 (Table S1-S3)

Table S1 Two-way ANOVA analysis on CO_2 fluxes influenced by gas collection methods (M), soil types (S) and the interactive effects (M*S).

Day of gas collection after the incubation start (d)	Source	DF	SS	Mean Square	*F*	*P*
2	M	1	29.45	29.45	9.74	<0.05
	S	1	3.24	3.24	1.07	0.33
	M*S	1	0.34	0.34	0.11	0.75
5	M	1	13.24	13.24	5.06	0.054
	S	1	0.30	0.30	0.12	0.74
	M*S	1	1.81	1.81	0.69	0.43
7	M	1	12.06	12.06	6.00	<0.05
	S	1	10.95	10.95	5.45	<0.05
	M*S	1	2.12	2.12	1.05	0.33
9	M	1	13.66	13.66	2.11	0.18
	S	1	0.74	0.74	0.12	0.74
	M*S	1	16.30	16.30	2.52	0.15
14	M	1	83.40	83.40	24.69	<0.01
	S	1	62.58	62.58	18.53	<0.01
	M*S	1	0.68	0.68	0.2	0.67
18	M	1	28.46	28.46	7.09	<0.05
	S	1	24.53	24.53	6.11	<0.05
	M*S	1	198.80	198.80	49.49	<0.01
25	M	1	27.26	27.26	16.49	<0.01
	S	1	2.01	2.01	1.21	<0.05
	M*S	1	53.49	53.49	32.29	<0.01

Table S2 $\delta^{13}C$ values (‰) of the CO_2 fluxes with and without consideration of the air in the needle.

	2 d	5 d	7 d	9 d	14 d	18 d	25 d
Without consideration of the air	-19.37±0.4	-19.08±0.58	-19.33±0.3	-19.38±0.53	-19.1±0.89	-19.24±0.17	-19.23±0.43
With consideration of the air	-19.42±0.4	-19.13±0.58	-19.37±0.3	-19.42±0.53	-19.15±0.89	-19.29±0.17	-19.28±0.43

Note: The syringe used in gas sampling method (G) has 20 ml capacity and the needle attached to the syringe has 0.06 ml of capacity.

The $\delta^{13}C$ values of CO_2 fluxes with consideration of the air in the needle were calculated using the following equation:

$$\delta^{13}C = (\mathrm{V} * \delta^{13}C_{CO2} - 0.06 * \delta^{13}C_{air})/(\mathrm{V} - 0.06)$$

where, $\delta^{13}C_{CO2}$ was the $\delta^{13}C$ value of CO_2 fluxes in gas sampling method, $\delta^{13}C_{air}$ was the $\delta^{13}C$ value of ambient air (-8‰) and V was the volume of gas collected from the incubation jar, and 0.06 (ml) was the needle capacity.

Table S3 Two-way ANOVA analysis on the $\delta^{13}C$ values of CO_2 emitted influenced by gas collection methods (M), soil types (S) and the interactive effects (M*S).

Day of gas collection after the incubation start (d)	Source	DF	SS	Mean square	*F*	*P*
2	M	1	0.001	0.001	0.00	0.97
	S	1	20.01	20.01	21.17	< 0.01
	M*S	1	0.31	0.31	0.33	0.58
5	M	1	0.15	0.15	0.33	0.58
	S	1	8.12	8.12	18.46	< 0.01
	M*S	1	0.85	0.85	1.94	0.20
7	M	1	0.66	0.66	2.20	0.18
	S	1	16.6	16.6	55.47	< 0.01
	M*S	1	0.86	0.86	2.89	0.13
9	M	1	0.48	0.48	1.71	0.23
	S	1	13.41	13.41	47.66	< 0.01
	M*S	1	1.52	1.52	5.33	< 0.05
14	M	1	1.22	1.22	1.90	0.21
	S	1	7.90	7.90	12.23	< 0.05
	M*S	1	0.03	0.03	0.05	0.84
18	M	1	0.93	0.93	2.7	0.14
	S	1	7.86	7.86	22.91	< 0.01
	M*S	1	0.14	0.14	0.42	0.54
25	M	1	0.17	0.17	0.57	0.47
	S	1	11.37	11.37	37.36	< 0.01
	M*S	1	0.29	0.29	0.94	0.36