Application of Combustion Module Coupled with Cavity Ring-Down Spectroscopy for Simultaneous Measurement of SOC and $\delta^{13}$C-SOC

Dan Liu, 1 Zhiguo Yu, 2 and Junjie Lin 3

1Department of Agricultural and Forestry Science and Technology, Chongqing Three Gorges Vocational College, Chongqing 404000, China
2School of Hydrology and Water Resources, Nanjing University of Information Science and Technology, Nanjing 210044, China
3Key Laboratory of Water Environment Evolution and Pollution Control in Three Gorges Reservoir, Chongqing Three Gorges University, Chongqing 404100, China

Correspondence should be addressed to Junjie Lin; ybu_lin@126.com

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Quantifying the decomposition of soil organic carbon (SOC) fractions under climate change is essential to predict carbon-climate feedbacks and global C cycle [1, 2]. According to turnover times, SOC stocks can be divided into three fractions: annual cycling (aSOC), decadal cycling (dSOC), and millennial cycling, comprising of 0–5%, 60–85%, and 10–40% of total SOC stocks, respectively [3]. The approaches for investigating SOC fraction decomposition include improved measurement systems and labeling experiments using carbon isotope tracers ($^{13}$C or $^{14}$C). Soils enriched with $^{14}$C (“bomb carbon”) [4] or $^{13}$C (natural $^{13}$C tracer) [5] are used to investigate how climate change affects SOC decomposition in terrestrial ecosystems. Natural $^{13}$C tracer studies take advantage of the differences of $^{13}$C value between soil and plant, or soils had experienced a period of $C_3$-$C_4$ (or vice versa) vegetation switch (e.g., continuous paddy ($C_3 \approx -26\%$) cropping of fields previously dominated by $C_4$ input from $C_4$ vegetation $\approx -14\%$).

Previous studies have tested $^{13}$C-SOC by elemental analyzer coupled with isotope ratio mass spectrometry (IRMS) [6], gas chromatography (GC) coupled with IRMS [6], or nuclear magnetic resonance (NMR) spectrometry [7]. SOC is traditionally measured using either an elemental analyzer [8] or by the chromic acid titration method [9]. CM-CRDS system has been recently developed for isotope test in soil, plant, rock, and so forth. Onac et al. measured guano samples to explore guano-derived $^{13}$C-based paleohydroclimate record [10]. Li et al. analyzed leaf stable C isotope
composition by the CM-CRDS system with 0.2–0.3‰ precision [11]. Pettit et al. measured the total C content and the \( \delta^{13}C \) isotopic ratio of wood powder for decomposition of coarse woody debris about different elevation [12]. Qiu et al. used CM-CRDS to measure the \( \delta^{13}C \) isotope in C₃ and C₄ plant for calculating plant water use efficiency [13]. Rossier et al. used CM-CRDS to measure the \( \delta^{13}C \) signature for calculating biochar mass percentage [15]. Shi et al. discussed the effect of doubled CO₂ concentration on the accumulation of photosynthate in Lycium barbarum by CM-CRDS [16]. Hayes et al. tested total C and \( \delta^{13}C \) of mulch films to ensure the effect of weathering conditions on the physicochemical properties of biodegradable plastic mulches [17]. To date, few studies have reported the application of CM-CRDS system for simultaneous measurement of SOC and \( \delta^{13}C \)-SOC in soil samples.

In this study, combustion module (CM) coupled with CRDS system was explored as an alternative method for making bulk measurements of SOC and \( \delta^{13}C \)-SOC. CM-CRDS system provides a method that combines information about the quantity and isotopic signature of SOC for a sample in one measurement. With this method all organic carbon in samples is converted into CO₂ by 1600–1800°C combustion, avoiding \(^{13}C\) versus \(^{12}C\) fractionation effects due to derivatization processes. We tested the CO₂ conversion efficiency of the combustion using different organic standards with different molecular structure and stability. The recovery rates for standard substances were also determined. Finally, the method was applied to natural samples for SOC and \( \delta^{13}C \)-SOC, obtained with CM-CRDS system, and was compared with the results from other techniques.

### 2. Methods and Materials

#### 2.1. Soil Sampling and Preparation

The surface soil samples (0–20 cm) that had experienced a period of C₃–C₄ (or vice versa) vegetation switch were collected (Table 1). The samples were passed through a 2 mm sieve, thoroughly homogenized, and air-dried in the field. Visible roots and stones were carefully removed and milled for 5 min prior to the measure.

#### 2.2. CM-CRDS System

A combustion module (CM) coupled to CRDS system (G2131-i Analyzer, Picarro Inc., USA) was used for SOC and \( \delta^{13}C \)-SOC (Figure 1). The system was controlled by Picarro G2000 iCO₂ host software. Samples in tin capsules were loaded by an autosampler (Costech, USA) into the combustion module (980°C). The CO₂ collected by Picarro Liaison™ A0301 interface is inputted into CRDS for analysis.

Acetanilide (C₈H₇NO, C 71.09%, N 10.36%) and atropine (C₁₇H₂₃NO₃, C 70.56%, N 4.84%) were from Costech Analytical Technologies Inc., USA, and used as standards of carbon content. Glycine (C₂H₅NO₂, \( \delta^{13}C = -33.3\)‰) used as an isotopic reference material was purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Calcium carbonate (CaCO₃, \( \geq 99.9\) p.a.) and sodium bicarbonate (NaHCO₃, \( \geq 99.5\) p.a.) were purchased from Kermel Chemical Reagents, China.

#### 2.3. Comparison of Measurement Systems

Elemental analyzer (Vario EL III, Germany) was used to measure the SOC. Standard soil (carbon content = 2.01%) was procured from Starplex Scientific Inc. (Etobicoke, Canada). Acetanilide (C₈H₇NO, C 71.09%, N 10.36%), and atropine

### Table 1: Site information.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>MAT (°C)</th>
<th>MAP (mm)</th>
<th>Planted (years)</th>
<th>Vegetation</th>
<th>Soil type</th>
<th>Soil texture</th>
<th>C₃ to C₄ vegetation switch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shenyang</td>
<td>42.7°N 125.1°E</td>
<td>5.2</td>
<td>1518</td>
<td>1961</td>
<td>Zea mays L.</td>
<td>Aquic brown soil</td>
<td>Sandy loam</td>
<td>15 years</td>
</tr>
<tr>
<td>Haerbin</td>
<td>45.6°N 126.6°E</td>
<td>3.1</td>
<td>530</td>
<td>1988</td>
<td>Zea mays L.</td>
<td>Terra nera</td>
<td>Sandy loam</td>
<td>23 years</td>
</tr>
<tr>
<td>Guigang</td>
<td>22.9°N 109.5°E</td>
<td>22.0</td>
<td>653</td>
<td>1956</td>
<td>Saccharum officinarum L.</td>
<td>Laterite</td>
<td>Clay loam</td>
<td>55 years</td>
</tr>
</tbody>
</table>

MAP: mean annual precipitation; MAT: mean annual temperature.
(C_{17}H_{23}NO_{3}, C 70.56%, N 4.84%) were obtained from Costech Analytical Technologies Inc. (Valencia, California, USA) and used as standards of carbon content.

The elemental analyser (Flash EA 1112) coupled online via a ConFlo III interface with a Deltaplus XP isotope ratio mass spectrometer (EA-IRMS, Thermo Finnigan, USA) was used to determine δ^{13}C-SOC. Urea (δ^{13}C vs PDB = −45.380‰) and CO_{2} (δ^{13}C = −29.523 ± 0.181‰) were used as working reference standards.

2.4. Data Analysis. Recovery rates (RRs) were determined as follows:

\[ RR = \frac{C_i}{C_s} \times 100\% \]  

(1)

where \( C_i \) is the measured content of a sample by CRDS and \( C_s \) is the concentration obtained with the comparison system (EA).

For isotopic measurements, the stable carbon isotope ratios are reported in delta notation expressed in per mil [19]:

\[ \delta^{13}C_{\text{sample}} = \left( \frac{R_{\text{sample}}}{R_{\text{VPDB}}} - 1 \right) \times 1000, \]  

(2)

where \( R_{\text{sample}} \) is the \(^{13}C/^{12}C\) ratio of the sample and \( R_{\text{VPDB}} \) is the ratio of the international VPDB standard.

3. Results and Discussion

3.1. Standard Curve. We evaluated the linearity of the CM-CRDS method by plotting the measured \(^{12}CO_{2}\) concentration against the carbon content (0.15–1.07 mg C) of acetanilide standard (Figure 2). We found a linearity between C content and \(^{12}CO_{2}\) mean concentration with \( R^2 = 0.9972, P < 0.001 \). It is indicated that the CM-CRDS system has an acceptable \(^{12}CO_{2}\) conversion efficiency for detecting the C content.

3.2. Quantification of Soil Organic Carbon. Four standards can be divided into three sorts, including organic (atropine), inorganic (NaHCO_{3} and CaCO_{3}), and soil reference. Aliquots were used for analysis by CM-CRDS and EA. We investigated the linearity of the CM-CRDS system by comparing the measured values of different contents from comparison method. For different standards, we found the same \(^{12}CO_{2}\) peak areas at the equal C content with correlations constant of \( R^2 > 0.99 \) (\( R^2 = 0.9921 \) for atropine, \( R^2 = 0.9997 \) for NaHCO_{3}, and \( R^2 = 0.9999 \) for CaCO_{3}). The slopes ranged from 0.966 to 1.0434 (Figure 3). The average recovery rates (RRs) for atropine, NaHCO_{3}, and CaCO_{3} were 100.31%, 98.17%, and 95.29%, respectively (Table 2). We also applied CM-CRDS to reference soil. For the comparison of SOC content measured with CM-CRDS with those obtained with the EA system, we found a correlation with \( R^2 = 0.9989 \) and a slope of 1.0434 (Figure 3). According to the values of \( R^2 \) and RRs from the three sorts of standards, we found that CM-CRDS system can give us satisfactory results as comparing method.

With the increase of C content, the higher RRs for soil samples were found (Table 2). On the contrary, the RRs for inorganic materials were much lower than organic material and soil samples. It indicated that EA system is limited in comparison with the CM-CRDS system for organic and soil samples.

\[ y = 5133.6x + 130.65 \]  

\[ R^2 = 0.9972 \]  

\[ P < 0.0001 \]

\[ y = 0.966x - 0.0043 \]  

\[ R^2 = 0.9997 \]

\[ y = 1.0016x - 0.0092 \]  

\[ R^2 = 0.9999 \]

\[ y = 1.0434x - 0.01165 \]  

\[ R^2 = 0.9989 \]

Figure 2: Standard curve for \(^{12}CO_{2}\) concentration (ppm) versus C content (mg C).

Figure 3: Correlation between contents determined by CM-CRDS and comparison system for standard materials and reference soil.

Table 2: Measured C content and recovery rates (RRs) of three standard materials and three soils determined by CM-CRDS and comparison system (EA).

<table>
<thead>
<tr>
<th>Substance</th>
<th>EA (mg C)</th>
<th>CM-CRDS (mg C)</th>
<th>RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine (n = 4)</td>
<td>0.2929</td>
<td>0.2938</td>
<td>100.31</td>
</tr>
<tr>
<td>NaHCO_{3} (n = 4)</td>
<td>0.6226</td>
<td>0.6112</td>
<td>98.17</td>
</tr>
<tr>
<td>CaCO_{3} (n = 4)</td>
<td>0.2973</td>
<td>0.2833</td>
<td>95.29</td>
</tr>
<tr>
<td>Soil-1 (n = 5)</td>
<td>1.7000</td>
<td>1.7618</td>
<td>103.63</td>
</tr>
<tr>
<td>Soil-2 (n = 5)</td>
<td>2.0779</td>
<td>2.1690</td>
<td>104.38</td>
</tr>
<tr>
<td>Soil-3 (n = 5)</td>
<td>2.2637</td>
<td>2.3763</td>
<td>104.97</td>
</tr>
</tbody>
</table>
3.3. Isotope Values of Standard Materials and Soil Samples.

The different standard materials were used to express that the CM-CRDS system can determine a vast range of δ^{13}C values (Figure 4). We found a linear regression with a slope of 1.0603 and a \( R^2 = 0.9982 \). The heaviest δ^{13}C value was determined for NaHCO₃ (−7.639‰) and the lightest for acetanilide (−34.318‰). Balslev-Clausen et al. found the similar degree of correlation when they compared the δ^{13}C values measured with CM-CRDS with values determined by a continuous flow-(CF-) IRMS system for rock samples [18].

We also found C content-dependent variations in the δ^{13}C values for all standards materials within a C content range of 0.2–1.1 mg C (Figure 5). Below a content of 0.2 mg C, the δ^{13}C values appeared unsteady and seemed to be affected by background signal. However, the δ^{13}C values measured at contents > 0.2 mg C, which corresponded to 1000 ppmv of CO₂, were relatively stable. Moni and Rasse recently detected C content and δ^{13}C signature in vegetation samples by CM-CRDS [20]. However, δ^{13}C-labeled CO₂ was used to simulate a leak from geologically stored CO₂, which cannot represent the situation of samples in the natural δ^{13}C abundance. Ignoring the molecular structure and complexity of a sample, the combustion module (CM) facilitates the conversion of hydrocarbons to CO₂ and water. Thus, heterogeneous soils can also be analyzed with this system. This verifies the isotope analysis of rock [18] and volatile liquid [21], geologically stored CO₂ [20], amino acids [22], and insects [23].

To summarize, even small changes in soil CO₂ released from SOC decomposition can significantly affect atmospheric CO₂ concentration and global C cycle. Thus, the interest in estimating the contribution of different fractions of SOC to soil C respired under climate change in the terrestrial ecosystem has increased in recent years. The CM-CRDS system can be used to determine SOC and δ^{13}C-SOC simultaneously. In our investigation, the CM-CRDS system was characterized by a high signal to noise and more accurate measurements. To ensure the accuracy for a wide range of CO₂ concentration and reduce transient concentration response, the instrument needs to be recalibrated, and the processing software should be upgraded. Compared with EA and EA/IRMS systems, the CM-CRDS system has an equal or superior reproducibility, memory, and drift.

Conflicts of Interest

The authors declare they have no competing financial interests.

Authors’ Contributions

Dan Liu and Zhiguo Yu contributed equally to this work.

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