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Contributions of carbonates to soil CO2 emissions

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It is estimated that 700 to 900 Pg of inorganic carbon (C) is stored as carbonates in soils occupying >50% of the world’s land area (Batjes 1996; Schlesinger 1997; Adams and Post 1999), and may be a potential source of CO2 to the atmosphere. While previous studies have focused on the total CO2 efflux from the soil, our goal was to identify the various sources and their contribution to total CO2 emissions. Measurements of dissolved inorganic carbon (DIC) in soil solution have been used extensively to estimate CO2 production from calcareous soils (e.g., Adams and Post 1999). However, these studies have assumed that DIC produced by calcareous soils is derived from the dissolution of soil carbonates. We present results of a study to determine the proportion of DIC that is derived from soil carbonates.

Materials and methods

Soil samples were obtained from conventional tillage (CT) and no-tillage (NT) plots in southern Ontario, Canada. Soil samples (root- and residue-free) were laboratory-incubated for 14 d and the isotopic signature of the CO2 evolved from soil carbonates was determined using a two-end member mixing model. The isotopic signature of carbonates and soil organic matter decomposition was analyzed using isotope ratio mass spectrometry.

Key words: Soil CO2 emissions, δ13C, microbial respiration and carbonates

Abbreviations: δ13C, isotopic signature of carbon dioxide; CT, conventional tillage; LF, light fraction; HF, heavy fraction; MB, microbial biomass; NT, no-tillage; SIC, soil inorganic carbon; SOC, soil organic carbon; SOM, soil organic matter
availability of both Ca$^{2+}$ and HCO$_3^-$ ions in the soil solution. In calcareous soils the source of Ca$^{2+}$ is CaCO$_3$, but Ca$^{2+}$ can also originate from the weathering of primary silicate minerals (e.g., pyroxene, CaMgSi$_2$O$_6$) in inherently carbonate-free landscapes (Schlesinger 1985; Boettinger and Southard 1991).

Recent studies have indicated the importance of partitioning the potential sources of CO$_2$ emissions from soil, but the focus has mainly been on soil respiration (Haile-Mariam et al. 2008; Kayler et al. 2010). Soil respiration includes both heterotrophic (microbial) and autotrophic (root) respiration, and represents one of the largest terrestrial sources of CO$_2$ flux (Hanson et al. 2000; Kuzyakov 2006). CO$_2$ emissions from soil respiration and carbonates can significantly impact atmospheric CO$_2$ concentration, carbon sequestration, climate change and the global carbon balance (Schlesinger 1997; Lal and Kimble 2000; Intergovernmental Panel on Climate Change 2007). Thus, the separation of organic and inorganic CO$_2$ emissions from soil is essential for assessing the individual responses of both processes to environmental factors, and potential changes in climate or management.

The two major stable isotopes of carbon (C) are $^{12}$C and $^{13}$C with an average natural abundance of 98.9 and 1.1%, respectively. The term “isotopic composition (R, $^{13}$C/$^{12}$C)” refers to the molar ratio of the heavier to the lighter stable isotope. However, due to the large difference in the natural abundance between the isotopes, measurement of the absolute ratio is difficult and given the notation “delta”, δ. Therefore, δ$^{13}$C refers to the ratio of $^{13}$C/$^{12}$C and is calculated as:

$$\delta^{13}C(\%o) = [(R_{sample}/R_{standard} - 1) \times 1000]$$

where $R_{sample}$ and $R_{standard}$ is the $^{13}$C/$^{12}$C ratio of the sample and standard, respectively. δ$^{13}$C values are reported in parts per thousand (per mil, ‰) deviation from the standard reference material. An established reference standard for δ$^{13}$C measurement is PeeDee Belemnite (PDB), a marine limestone, with a δ$^{13}$C value of 0‰ by definition. Pedogenic and lithogenic carbonates have distinct isotopic values, with δ$^{13}$C values ranging from -10 to 0‰ and -2 to +2‰, respectively (Cerling 1984; Boutton 1991). Atmospheric CO$_2$ has a δ$^{13}$C value of -8‰ while that of C$_3$ and C$_4$ plants have mean values of -27‰ and -13‰, respectively (Smith and Epstein 1971).

Studies on soil respiration have noted the importance in differentiating the terms “soil CO$_2$” and “soil-respired CO$_2$” (Cerling et al. 1991; Amundson et al. 1998; Kayler et al. 2010). “Soil CO$_2$” is defined as the CO$_2$ that fills the soil pores at any given soil depth, while “soil-respired CO$_2$” refers to the CO$_2$ diffusing across the soil-atmosphere continuum (Cerling et al. 1991). It is also assumed that the isotopic signature of carbon dioxide (δ$^{13}$C$_{CO2}$) emitted from the soil surface reflects that of the originating component i.e., SOM (derived mainly from plant residues) or carbonates (Amundson et al. 1998; Emmerich and Martens 2006). Soil-respired CO$_2$ is $^{13}$C depleted due to molecular diffusion through the soil, which results in isotopic fractionation of $^{13}$C and $^{12}$C, since $^{13}$C is heavier and diffuses at a slower rate. It is estimated that the δ$^{13}$C value of soil-respired CO$_2$ is 4.4% lower than that of CO$_2$ in the soil profile, and this correction factor is essential for accurate interpretation of results (Cerling et al. 1991; Amundson et al. 1998). In our laboratory-incubation study, the δ$^{13}$C$_{CO2}$ values reported are that of “soil CO$_2$”, since isotopic fractionation due to diffusion is considered negligible.

Carbon substrates are organic materials used by soil microbes as a source of energy. The substrates vary in their availability (physical and chemical protection) and structural complexity (inherent nature of plant residues). Decomposition of fresh crop residues by the soil microbial biomass results in organic C being oxidized to CO$_2$ or stabilized as humified organic matter. SOM constituents can be characterized by density fractionation (physical separation of SOM using a liquid of known specific gravity), into a low- and high-density fraction, known as the light fraction (LF) and heavy fraction (HF), respectively (Strickland and Sollins 1987). The light fraction represents a transitory pool of organic material (mainly plant and microbial residues) with a high C concentration, and occurs in the early continuum of decomposition of fresh residues to humified SOM (Gregorich and Janzen 1996; Carter 2002). Light fraction also accounts for 1–18% of the soil organic carbon (SOC) in cultivated agricultural soils, and is considered a labile SOM pool providing a readily mineralizable source of C for soil microbes (Janzen et al. 1992). The LF is also more sensitive to changes in cropping and management practices than the total SOC pool, and is an important indicator of soil quality (Janzen et al. 1992; Carter 2002).

Soil microbial biomass (MB) refers to the dynamic, living microbial portion of SOM (mainly bacteria and fungi), but excludes living material larger than 5000 µm$^3$ (Jenkinson and Ladd 1981). Soil microbes are the agents of decomposition and represent the active pool of SOM (Sparling 1992). Soil microbial biomass carbon (MBC) accounts for 1–3% of the total SOC, and is small when compared with other soil C pools. However, its rapid turnover can contribute to major fluxes of nutrients and decomposition products such as CO$_2$ within the soil (Jenkinson and Ladd 1981). Studies have reported that C availability in the LF and MB is the driving factor for soil respiration (Alvarez et al. 1998).

This study uses the natural abundance of stable carbon isotopes together with a simple two-source mixing model (Balesdent et al. 1987) to quantify the proportions of CO$_2$ originating from SOM (microbial respiration) and carbonates (using root- and residue-free
soil) due to their unique isotopic signature. The soil samples were obtained from research plots managed under two tillage systems: conventional tillage (CT) and no-tillage (NT). Use of contrasting tillage systems allowed us to test the hypothesis that tillage has no effect on the isotopic signature of CO₂ emitted from the soil, and to determine the quantity and depth distribution of SOC, LF and MB in the soil profile. The objective of this study was to identify the sources (organic or inorganic) and determine their contribution to total CO₂ emissions, by measuring the isotopic signature of the CO₂ emitted from a calcareous soil during laboratory incubation.

**MATERIALS AND METHODS**

**Site Description**

The study site was located at the Elora Research Station, about 20 km northwest of Guelph, southern Ontario, Canada (lat. 43°38′N, long. 80°25′W, 376 m asl). At the site, the average monthly temperature ranges from −7.1°C in January to 19.8°C in July, and the mean annual precipitation is approximately 900 mm. The soils at the site belong to the London series (Hoffman et al. 1963) and are derived from a calcareous glacial till parent material. The London series is classified as a Gray Brown Luvisol in the Canadian System of Soil Classification (Soil Classification Working Group 1998) and as a Typic Hapludalf in US Soil Taxonomy (Soil Survey Staff 1999). Basic properties of the London silt loam soil are listed in Table 1.

The research site consisted of four large-scale plots (2 CT and 2 NT), each 100 m × 150 m (1.5 ha). Each plot was divided into six subplots (50 m × 50 m), and four of the six subplots were chosen for soil and plant sampling. All plots were initially managed under conventional tillage, but beginning in the year 2000, two of the plots were converted to no-tillage. From 2000, the site followed a crop rotation sequence of corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) and winter wheat (*Triticum aestivum* L.), but historical data show that corn was first grown in 1969 in rotation with alfalfa, winter wheat, barley, canola and soybean. With the exception of tillage management, agronomic practices were identical for all the plots. This study was carried out in 2005, at which time corn was grown in the crop rotation cycle.

**Soil Sampling and Preparation**

Undisturbed soil samples were taken in October 2005 (after corn harvest), with a hydraulic soil core sampler (38 mm i.d.) to a depth of 50 cm. The plant spacing for corn was 75 cm between rows and 20 cm within the rows. Within the subplots four cores were collected at four locations relative to the corn plant – on the plant, midway between plant within the rows, quarter-way (¼) and halfway (½) between plant rows. Each soil core was divided into four depth increments: 0–10, 10–20, 20–30, and 30–50 cm. The samples were divided by soil depth rather than soil horizons to account for the depth of tillage (20 cm) in CT plots and stratification of SOC, which may occur in NT plots (Kay and VandenBygaart 2002).

Soil samples were gently hand-crushed while still at field moisture content, and visible crop residues and roots were removed by handpicking. Soil samples were initially passed through a 4.75-mm sieve (due to the

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Clay, &lt;2 μm (g kg⁻¹ soil)</th>
<th>Silt, 2–50 μm (g kg⁻¹ soil)</th>
<th>Sand, 50–2000 μm (g kg⁻¹ soil)</th>
<th>Gravel, &gt;2000 μm (g kg⁻¹ soil)</th>
<th>Total organic carbon (g kg⁻¹ soil)</th>
<th>Total organic nitrogen (g kg⁻¹ soil)</th>
<th>Soil reaction (pH)</th>
<th>Bulk density (mg m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>NT</td>
<td>CT</td>
<td>NT</td>
<td>CT</td>
<td>NT</td>
<td>CT</td>
<td>NT</td>
</tr>
<tr>
<td>0–10</td>
<td>161 (5)</td>
<td>158 (4)</td>
<td>21.0 (0.8)</td>
<td>24.1 (0.5)</td>
<td>1.68 (0.07)</td>
<td>1.90 (0.06)</td>
<td>7.30 (0.09)</td>
<td>7.26 (0.05)</td>
</tr>
<tr>
<td>10–20</td>
<td>163 (6)</td>
<td>151 (1)</td>
<td>19.7 (0.7)</td>
<td>18.7 (0.2)</td>
<td>1.59 (0.05)</td>
<td>1.50 (0.04)</td>
<td>7.32 (0.07)</td>
<td>7.28 (0.04)</td>
</tr>
<tr>
<td>20–30</td>
<td>189 (5)</td>
<td>178 (3)</td>
<td>12.6 (0.9)</td>
<td>12.1 (0.1)</td>
<td>1.06 (0.07)</td>
<td>1.01 (0.03)</td>
<td>7.41 (0.06)</td>
<td>7.38 (0.03)</td>
</tr>
<tr>
<td>30–50</td>
<td>163 (2)</td>
<td>180 (2)</td>
<td>5.1 (0.2)</td>
<td>5.6 (0.2)</td>
<td>0.52 (0.02)</td>
<td>0.55 (0.02)</td>
<td>7.49 (0.03)</td>
<td>7.55 (0.02)</td>
</tr>
<tr>
<td>0–10</td>
<td>50 m</td>
<td>528 (7)</td>
<td>523 (3)</td>
<td>1.33 (0.01)</td>
<td>1.38 (0.01)</td>
<td>1.52 (0.02)</td>
<td>1.65 (0.02)</td>
<td>1.70 (0.02)</td>
</tr>
<tr>
<td>10–20</td>
<td>52 (8)</td>
<td>583 (4)</td>
<td>584 (4)</td>
<td>1.38 (0.01)</td>
<td>1.41 (0.01)</td>
<td>1.50 (0.02)</td>
<td>1.6 (0.02)</td>
<td>1.7 (0.02)</td>
</tr>
<tr>
<td>20–30</td>
<td>532 (4)</td>
<td>548 (4)</td>
<td>1.06 (0.07)</td>
<td>1.01 (0.03)</td>
<td>0.52 (0.02)</td>
<td>0.55 (0.02)</td>
<td>7.49 (0.03)</td>
<td>7.55 (0.02)</td>
</tr>
<tr>
<td>30–50</td>
<td>528 (7)</td>
<td>523 (3)</td>
<td>0.52 (0.02)</td>
<td>0.55 (0.02)</td>
<td>7.49 (0.03)</td>
<td>7.55 (0.02)</td>
<td>7.49 (0.03)</td>
<td>7.55 (0.02)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are the standard errors (SE) of the means (n = 16).*
difficulty in sieving moist soil samples) and then a subsample (250 g) was passed through a 2-mm sieve to obtain the fine-earth fraction used for analysis. Soil moisture content was obtained by drying a 10-g subsample of the soil at 105°C for 24 h. Root- and residue-free soil samples were then stored at 4°C at field moisture content until laboratory incubation. It should be noted that the isotopic signature of the LF and MB was determined only from soil samples taken at the 0- to 30-cm depth, since this represents the zone in the soil profile where the majority of the LF and MB occurs (Lorenz and Lal 2005).

Isolation of Light Fraction by Density Fractionation

Light fraction (LF) organic matter was isolated from the soil using density fractionation (Gregorich and Beare 2008). A 30-g subsample of soil was placed into a 250-mL centrifuge bottle and 100 mL of sodium iodide (NaI) solution (specific gravity 1.7) was added. The bottles were capped, placed upright and shaken on an end-to-end platform shaker for 1 h at 160 rpm. The particulate LF floating on the surface of the NaI solution was recovered using a water-jet vacuum filtration system. The LF was washed using 250-mL centrifuge bottles and 100 mL of sodium iodide solution (Murage and Voroney 2007). Extracts (Na2CO3) were dialyzed against deionized water in a 1.8-L dialysis reservoir on a magnetic stirrer at 500 to 600 rpm. The dialyzed extracts were freeze-dried and analyzed for δ 13C.

The δ 13C of the soil MBC was calculated from the δ 13C values of the C extracted from the fumigated sample and non-fumigated sample using the following equation:

$$\delta^{13}C_{\text{MBC}}(\%\text{oo}) = \frac{(\delta^{13}C_f \times C_f - \delta^{13}C_{nf} \times C_{nf})}{(C_f - C_{nf})}$$

where C_f and C_{nf} are the quantities of C extracted from the fumigated and non-fumigated samples, respectively. δ 13C_f and δ 13C_{nf} are the 13C natural abundances (‰) of the fumigated and non-fumigated samples, respectively.

Laboratory Incubation and δ 13C CO2

CO2 emission was measured by incubating the soil samples and collecting the released CO2 using sodium hydroxide (NaOH) solution traps (Anderson 1982). Soil samples stored at 4°C were allowed to equilibrate to room temperature in the laboratory and their moisture content adjusted to 55% water holding capacity (WHC). For determination of trapped CO2 a 50-g sample of soil was split into two subsamples (25 g each) and each subsample was placed into a 100-mL plastic vial. Samples were duplicated to ensure that an adequate quantity of C from CO2 emitted would be available for isotopic analysis. The vial with soil was placed into a gasket-sealed 1.5-L glass Mason jar (Bernadin®) with 8–10 mL distilled water placed at the base of the jar to maintain the humidity during incubation. NaOH solution (0.5 M, 20 mL) was dispensed into another 100-mL plastic vial and placed in the jar near the vial containing soil. The jar was closed ensuring an airtight seal and the samples were pre-incubated for 1 wk to account for the flush in soil microbial biomass which is expected due to the rewetting and increase in temperature of the soil.

After 7 d, the vial with the NaOH solution was removed (no titration or analysis) and replaced with a fresh quantity (0.5 M, 20 mL). The jars were then tightly sealed to ensure no exchange of atmospheric gases and incubated for 14 d at 25°C. Over the period of incubation, CO2 emitted from the soil was trapped by the NaOH solution and converted into sodium carbonate (Na2CO3).

$$\text{CO}_2(g) + 2\text{NaOH} \rightarrow \text{Na}_2\text{CO}_3(aq) + \text{H}_2\text{O} \ (1)$$

At the end of the incubation period the vials with NaOH solution were removed from the jars and titrated for quantification of released CO2. For titration, an excess amount of 0.5 M strontium chloride (SrCl2) was added to the vials to precipitate the Na2CO3 as strontium carbonate, SrCO3 (Harris et al. 1997).

$$\text{Na}_2\text{CO}_3(aq) + \text{SrCl}_2 \ (1) \rightarrow \text{SrCO}_3(s) + 2\text{NaCl(aq)} \ (2)$$

A few drops of phenolphthalein indicator were added, and the unneutralized NaOH solution was titrated using
standard 0.25 M HCl (back-titration). Triplicate blank samples (no soil, 20 mL of 0.5 M NaOH only) were also incubated with each batch of samples to correct for background CO₂ in the jar.

To analyze for δ¹³C, the suspension with SrCO₃ from duplicate samples was placed into a 250-mL centrifuge bottle, made up to ~200 mL volume (equal weight) with deionized water and left to settle overnight in the refrigerator. The samples were centrifuged at 8000 x g for 15 min and washed three times with deionized water to remove excess SrCl₂ and NaCl. Samples were dried at 60°C, stored in Wheaton® 4-mL vials and later placed into tin capsules and analyzed for δ¹³C. Note: The quantity of CO₂ in the incubation jars was determined during the 14-d incubation, and was therefore excluded from calculations on the proportion of CO₂ evolved from SIC.

Estimation of CO₂ Released from Soil Carbonates

The proportion of CO₂ evolved from inorganic carbon (fSIC) was estimated using a two-end member mixing model (Balesdent et al. 1987):

\[ \delta^{13}C_{CO2} = fSIC \times \delta^{13}C_{SIC} + (1 - fSIC) \times \delta^{13}C_{SOC} \]

The equation can be rewritten as:

\[ fSIC = \frac{(\delta^{13}C_{CO2} - \delta^{13}C_{SOC})}{(\delta^{13}C_{SIC} - \delta^{13}C_{SOC})} \]

where δ¹³C_CO₂ is the δ¹³C value of CO₂ emitted, δ¹³C_SOC is the δ¹³C of soil organic carbon and δ¹³C_SIC is the δ¹³C value of soil inorganic carbon from samples.

fSIC was also calculated by replacing “δ¹³C_SOC” in the above equation with the δ¹³C values from the light fraction carbon (δ¹³C_LFC) and microbial biomass carbon (δ¹³C_MBC), in order to determine if the proportions varied depending on the source of C substrates. fSIC was calculated for the 0–10 and 10–20 cm depth increments only, since this represent the zone most affected by tillage practices.

Statistical Analysis

Results were analyzed using the statistical software SAS 9.1 (SAS Institute, Inc., Cary, NC). Data were tested for normality using the Shapiro-Wilk test and then evaluated by analysis of variance (ANOVA) using the “proc GLM” procedure. Least significant differences (LSD) at \( P < 0.05 \) were used to determine significant differences between treatment means.

RESULTS AND DISCUSSION

A challenge in determining the source of CO₂ emitted from calcareous soil samples is differentiating CO₂ originating from carbonates from that of SOM decomposition. To elucidate the sources of CO₂ from the soil, it is essential to determine the isotopic signature of the potential contributing substrates. Methods used to estimate carbon respired from soil assume that CO₂ transport within the soil occurs only by diffusion and that soil CO₂ flux is at steady state (Cerling et al. 1991; Amundson et al. 1998). The assumption that CO₂ flux is at an isotopic steady-state means that the isotopic signature of the CO₂ leaving the soil surface is the same as the isotopic source or substrate (Amundson et al. 1998). This indicates that a correction for fractionation is unnecessary for samples incubated in a sealed jar. Even though the jars were not evacuated, atmospheric CO₂ present in the headspace was <5% of the total CO₂ recovered, and would have had minimal effect on δ¹³C_CO₂ due to length of time the samples remained incubated before CO₂ collection.

Isotopic Signature of Soil Inorganic Carbon

Preliminary work [part published in Ramnarine et al. (2011)] provided us with measurements of the concentration and isotopic signature (δ¹³C) of soil inorganic carbon (SIC) from soil samples obtained from the conventional tillage plots. Ideally, measurements would be taken from both tillage systems but since all the plots were under conventional tillage from 1969 to 1999, it was assumed that changes in soil carbonates would be very minor (if at all) after 6 yr of no-tillage. The SIC concentration at the experimental site was 0.82% for the 0–10 cm, 1.05% for the 10–20 cm, 1.58% for the 20–30 cm and 1.94% for the 30–50 cm depth increments. δ¹³C_SIC ranged from −6.79‰ for the 0–10 cm, −6.52‰ for the 10–20 cm, −4.26‰ for the 20–30 cm and −1.12‰ in the 30–50 cm depth. These results are similar to other locations in Canada where calcareous soils dominate the landscape. Wang and Anderson (2000) reported that the isotopic signature of pedogenic carbonate was −8‰ in the clay fraction of Cca horizons in soils of southeastern Saskatchewan. They also reported δ¹³C values of −1.4 to −1.1‰ for carbonates occurring in the Ck horizon.

Isotopic Signature of SOC, LFC and MBC

The δ¹³C of the SOC (δ¹³C_SOC) in the 0–10 cm depth of NT soils was significantly higher (¹³C-enriched) than...
that of the CT soils (Table 2). There were no significant differences in $\delta^{13}$C$_{SOC}$ for the lower soil depths between tillage treatments. The $\delta^{13}$C of the light fraction C ($\delta^{13}$C$_{LF}$) in the 0–10 and 10–20 cm depths of the NT soils was significantly higher than that of the CT soils. There was no significant difference in $\delta^{13}$C$_{LF}$ for the 20–30 cm depth between tillage treatments (Table 2). The $\delta^{13}$C of microbial biomass C ($\delta^{13}$C$_{MBC}$) measured on soil samples taken in fall 2005 was significantly higher in the 0–10 and 10–20 cm depths in the CT soils compared with the NT soils. However, there was no significant difference in the $\delta^{13}$C$_{MBC}$ for the 20–30 cm depth between tillage treatments (Table 2).

### Isotopic Signature of Carbon Dioxide ($\delta^{13}$C$_{CO2}$)

The isotopic signature of the CO$_2$ ($\delta^{13}$C$_{CO2}$) emitted from soil sampled in the CT and NT systems is presented in Table 2. The $\delta^{13}$C$_{CO2}$ from NT plots was significantly higher with depth, which can be attributed to the higher portion of lithogenic carbonates originating from the parent material. In addition, the $\delta^{13}$C$_{CO2}$ values increased with depth, which can be attributed to the higher portion of lithogenic carbonates originating from the parent material.

### Proportion of CO$_2$ Evolved from Soil Inorganic Carbon (fSIC)

Previous studies have reported that the isotopic composition of CO$_2$ emitted from soil should reflect the source of carbon substrates decomposed by the soil microbes (Cerling et al. 1991; Amundson et al. 1998). However, our results show that a significant contribution to the $\delta^{13}$C value of CO$_2$ emitted from soils is derived from soil carbonates enriched in $^{13}$C compared with that from microbial respiration. When the total SOC pool was considered as the substrate for microbial respiration, we estimated that 74% of the CO$_2$ emitted originated from carbonates, and was the same in both soil management systems (Table 3). Tamir et al. (2011) reported that the dissolution of carbonates can contribute up to 30% of the CO$_2$ emitted from calcareous soils obtained from Israel. The value of 30% obtained by Tamir et al. (2011) is possibly due to a lower rate of calcite dissolution, since air-dried soil samples were used in their study.

### Table 2. Isotopic signature of CO$_2$ ($\delta^{13}$C$_{CO2}$) emitted from soil after 14 d of laboratory incubation, total soil organic carbon ($\delta^{13}$C$_{SOC}$), light fraction carbon ($\delta^{13}$C$_{LF}$) and microbial biomass carbon ($\delta^{13}$C$_{MBC}$) in conventional tillage and no-tillage soils at fall 2005 sampling.

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>Conventional tillage</th>
<th>No-tillage</th>
<th>LSD*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm</td>
<td>$\delta^{13}$C$_{CO2}$ (%)</td>
<td>$\delta^{13}$C$_{SOC}$ (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>-11.05 (0.26)*</td>
<td>-10.32 (0.20)</td>
<td>0.64</td>
<td>0.028</td>
</tr>
<tr>
<td>10–20</td>
<td>-11.20 (0.28)</td>
<td>-9.85 (0.20)</td>
<td>0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20–30</td>
<td>-10.25 (0.34)</td>
<td>-10.24 (0.23)</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30–50</td>
<td>-9.34 (0.26)</td>
<td>-8.63 (0.30)</td>
<td>0.79</td>
<td>0.056</td>
</tr>
<tr>
<td>0–10</td>
<td>-23.41 (0.11)</td>
<td>-20.61 (0.07)</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>-23.50 (0.09)</td>
<td>-23.64 (0.05)</td>
<td>0.21</td>
<td>0.219</td>
</tr>
<tr>
<td>20–30</td>
<td>-25.41 (0.14)</td>
<td>-25.18 (0.07)</td>
<td>0.32</td>
<td>0.153</td>
</tr>
<tr>
<td>30–50</td>
<td>-27.57 (0.07)</td>
<td>-27.45 (0.07)</td>
<td>0.20</td>
<td>0.185</td>
</tr>
<tr>
<td>0–10</td>
<td>-18.29 (0.29)</td>
<td>-16.71 (0.26)</td>
<td>0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>-19.31 (0.24)</td>
<td>-18.33 (0.20)</td>
<td>0.62</td>
<td>0.003</td>
</tr>
<tr>
<td>20–30</td>
<td>-19.31 (0.28)</td>
<td>-19.35 (0.25)</td>
<td>0.75</td>
<td>0.931</td>
</tr>
<tr>
<td>30–50</td>
<td>ND*</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>-18.18 (0.10)</td>
<td>-19.27 (0.04)</td>
<td>0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>-18.69 (0.04)</td>
<td>-22.09 (0.06)</td>
<td>0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20–30</td>
<td>-23.82 (0.13)</td>
<td>-23.56 (0.06)</td>
<td>0.29</td>
<td>0.068</td>
</tr>
<tr>
<td>30–50</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Least significant difference calculated between two means at $P = 0.05$.

*Probability level for the $F$ value for tillage effect in ANOVA.

*Numbers in parentheses are the standard errors of the means ($n = 32$).

*Not determined.

### Table 3. The proportion of CO$_2$ evolved from soil inorganic carbon (fSIC) for the 0–10 and 10–20 cm depth of conventional and no-tillage soils after a 14-d laboratory incubation. The proportions are calculated using the light fraction carbon (LFC), microbial biomass carbon (MBC) or total soil organic carbon (SOC) as the C substrates for soil microbes.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conventional tillage</th>
<th>No-tillage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>LFC</td>
<td>0.63</td>
<td>0.64</td>
</tr>
<tr>
<td>MBC</td>
<td>0.63</td>
<td>0.72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate</th>
<th>10–20 cm depth</th>
<th>No-tillage</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>0.72</td>
<td>0.80</td>
</tr>
<tr>
<td>LFC</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td>MBC</td>
<td>0.62</td>
<td>0.76</td>
</tr>
</tbody>
</table>
Alternatively, since soil MBC and LFC represent the two most labile pools of SOM, and are the major C substrates of the soil microbes, we estimated the proportion of CO\textsubscript{2} emitted from carbonates. When the LFC pool was considered the sole source of C substrate for microbes, we determined that about 63% of the CO\textsubscript{2} originated from SIC. When the soil MBC pool was considered the source of C substrate, (fSIC) was 63% and 72% for the 0–10 cm depth of CT and NT soils, respectively (Table 3). Even though the majority of CO\textsubscript{2} emitted from the soil is expected to occur from the 0–10 cm depth, the proportions of CO\textsubscript{2} emitted from SIC in the 10–20 cm was similar to the topmost depth increment of each tillage system (Table 3). There were no significant differences in the proportions of CO\textsubscript{2} emitted from SIC among soil samples taken at different distances from the plant rows. This can be attributed to the small spatial variation in SIC and SOC concentrations within each depth increment.

In a system with only CaCO\textsubscript{3} present as soil carbonates, a possible mechanism for the dissolution of the CaCO\textsubscript{3} is shown below:

\[
\begin{align*}
\text{H}_2\text{O} (l) + \text{CO}_2 (g) & \rightarrow \text{H}_2\text{CO}_3 (aq) \\
\text{H}_2\text{CO}_3 (aq) + \text{CaCO}_3 & \rightarrow \text{Ca}^{2+} (aq) + 2\text{HCO}_3^- (aq) \\
\text{HCO}_3^- (aq) & \rightarrow \text{CO}_2 (g) + \text{OH}^- (aq)
\end{align*}
\]

The CO\textsubscript{2} produced from microbial respiration dissolves in the soil water to form carbonic acid (H\textsubscript{2}CO\textsubscript{3}). The carbonic acid reacts with CaCO\textsubscript{3} resulting in a release of bicarbonates (HCO\textsubscript{3}\textsuperscript{-}) which then dissociates releasing CO\textsubscript{2} from the soil. It is possible that there is a coupling mechanism between the CO\textsubscript{2} released from microbial respiration and the CO\textsubscript{2} originating from the dissolution of soil carbonates (Karberg et al. 2005).

The increase in CO\textsubscript{2} concentration (pCO\textsubscript{2}) due to microbial respiration is considered the main factor responsible for the dissolution of carbonates and isotopic exchange of C with soil carbonates (Cerling 1984; Kuzyakov et al. 2006; Rovira and Vallejo 2008). Given that the headspace concentration of CO\textsubscript{2} in the incubation jars is lower than ambient atmospheric concentration due to the NaOH traps, dissolution of soil carbonates is favoured. However, this effect would largely be restricted to those carbonates located nearby air-filled pores in surface soil, as CO\textsubscript{2} diffusion in water is about 10\textsuperscript{4} times slower than in air. Other scientists have shown that measurements of soil CO\textsubscript{2} emissions using alkali traps give results comparable with those obtained using gas chromatography analysis of incubation chamber, which suggests that the trapping method does not affect the contribution of carbonates to CO\textsubscript{2} emissions (Rochette et al. 1997). Thus, these emission measurements of carbonate-derived CO\textsubscript{2} conducted under laboratory conditions using the alkali trapping method should be similar to measurements conducted in the field under similar soil moisture and temperature conditions. Also, it is unlikely that dissolved carbonates would reprecipitate during the incubation since this usually occur when the soil moisture content decreases and normally takes many months or years (Kuzyakov et al. 2006). The dissolution of carbonates is related to soil water content; however, the incubated soils were kept at constant soil moisture content (thus not drying out) as indicated by measuring random samples after the 14-d incubation. It is also possible that organic acids released during SOM decomposition may have contributed to soil carbonate dissolution.

In our study, passing the soil through a 2-mm mesh sieve may have promoted the release of CO\textsubscript{2} from carbonates, since it would disrupt soil aggregates containing carbonates and increase their exposed surface area. CaCO\textsubscript{3} is considered one of the most surface-active constituent in calcareous soils and the specific surface area is inversely related to the CaCO\textsubscript{3} content of the soil (Holford and Mattingly 1975; Del Campillo et al. 1992). Pedogenic carbonates most commonly occur as microcrystalline aggregates and as coatings (cutans) on the surface of clay- and silt-sized particles, ped faces and pore spaces (Mermut and Arnaud 1981; Wang and Anderson 2000; Cailleau et al. 2005; Lynn et al. 2008). The gravel (material >2 mm) collected from the soil sample represented a small percentage of the total mass of soil. Also, addition of dilute HCl to a random set of gravel collected from the soil samples showed no or very weak effervescence, the latter more indicative of the less reactive dolomite [CaMg(CO\textsubscript{3})\textsubscript{2}]. Since the contribution of carbonate-derived C is similar in both tillage systems, one theory why the δ\textsuperscript{13}CCO\textsubscript{2} is still greater in the NT system compared with the CT system is that the labile LF pool was significantly higher in the NT system (−16.7\textsuperscript{‰}) than in the CT system (−18.3\textsuperscript{‰}). Many studies have reported that the LF pool has a faster turnover time than other recalcitrant C pools and is a source of C substrates for microbes (Gregorich and Janzen 1996).

Laboratory measurements of the δ\textsuperscript{13}CCO\textsubscript{2} emissions from sieved soils also differed from those taken in the field from which the soils were taken, during the fall period. A concurrent study on the same site by Drewitt et al. (2009) reported that the δ\textsuperscript{13}CCO\textsubscript{2} emissions for fall 2005 were −16.7\textsuperscript{‰} and −20.2\textsuperscript{‰} for the CT and NT plots, respectively. According to Cerling et al. (1991) and Amundson et al. (1998) the δ\textsuperscript{13}C of “soil-respired CO\textsubscript{2}” is reduced by 4.4\textsuperscript{‰} due to isotopic fractionation by diffusion. Considering that the δ\textsuperscript{13}CCO\textsubscript{2} values obtained from field measurements using micrometeorological techniques represents a 4.4\textsuperscript{‰} depletion, perhaps the higher values of δ\textsuperscript{13}CCO\textsubscript{2} compared with carbon substrates may be partly explained by the emissions of CO\textsubscript{2} from carbonates. However, further field studies are required to understand the partitioning of CO\textsubscript{2} emissions in calcareous soils.

Precautions should be taken with regard to the transferability of results from a laboratory incubation.
to a field study. This is because root (and associated rhizosphere) respiration can contribute about 50% of the total soil respiration (Kuziyakov and Larionova 2005). Therefore, the contributions of carbonates to CO₂ emissions can be much lower in a field study with plants. Further work is necessary to elucidate the δ¹³C signature of CO₂ from contributions of multiple carbon sources (root, microbes, carbonates) and the isotopic fractionation which occurs during soil gas transport (Kayler et al. 2010).

CONCLUSIONS

Isotopic measurement of emitted CO₂ is a useful method to quantify abiotic CO₂ production from carbonates, due to the unique δ¹³C signature of carbonates and soil organic matter. The δ¹³C of soil respired CO₂ can be used to identify and quantify labile C substrates, which contribute to CO₂ emissions to the atmosphere. In this 14-d laboratory incubation study, we estimated that the proportion of CO₂ originating from soil carbonates (inorganic carbon) was 62 to 74% for soil samples from CT plots, and 64 to 80% for NT soil samples. The use of the δ¹³C natural abundance technique as a means to partition CO₂ released from the decomposition of SOM and inorganic C from carbonates is advantageous since it identifies that organic carbon substrates are not the only contributors to CO₂ emissions from calcareous soils.

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