Contributions of carbonates to soil CO_2 emissions

Author(s): R. Ramnarine, C. Wagner-Riddle, K. E. Dunfield and R. P. Voroney
Source: Canadian Journal of Soil Science, 92(4):599-607.
Published By: Canadian Science Publishing
URL: http://www.bioone.org/doi/full/10.1139/CJSS2011-025

Contributions of carbonates to soil CO_2 emissions

R. Ramnarine[1,2], C. Wagner-Riddle[1], K. E. Dunfield[1], and R. P. Voroney[1]

[1]School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1 (e-mail: ravindra.ramnarine@agr.gc.ca); and [2]Agriculture and Agri-Food Canada, University of Manitoba, 13 Freedman Crescent, Winnipeg, Manitoba, Canada R3T 2N2. Received 15 February 2011, accepted 30 March 2012.

Ramnarine, R., Wagner-Riddle, C., Dunfield, K. E. and Voroney, R. P. 2012. **Contributions of carbonates to soil CO_2 emissions**. Can. J. Soil Sci. **92**: 599–607. Carbon dioxide (CO_2) is released in soil as a by-product of microbial and root respiration, but soil carbonates may also be a source of CO_2 emissions in calcareous soils. Global estimates of inorganic carbon range from 700 to 900 Pg as carbonates stored in soils, representing a significant potential source of CO_2 to the atmosphere. While previous studies have focused on the total CO_2 efflux from the soil, our goal was to identify the various sources and their contribution to total CO_2 emissions, by measuring the isotopic signature of the CO_2 emitted from the soil. Calcareous Luvisolic silt loam 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 CO_2 ($\delta^{13}C_{CO2}$) released was analyzed using isotope ratio mass spectrometry. Isotopic measurement was essential in quantifying the abiotic CO_2 production from carbonates, due to the unique $\delta^{13}C$ signature of carbonates and soil organic matter. A two-end member mixing model was used to estimate the proportion of CO_2 evolved from soil carbonates and soil organic matter decomposition. Analysis of emitted CO_2 collected after the 14-d incubation indicate that the proportion of CO_2 originating from soil inorganic carbon was 62 to 74% for CT soil samples, and 64 to 80% for NT soil samples. Further work is recommended in the quantification of CO_2 emissions from calcareous soils, and to determine the transferability of laboratory results to field studies.

Key words: Soil CO_2 emissions, $\delta^{13}C$, microbial respiration and carbonates

Ramnarine, R., Wagner-Riddle, C., Dunfield, K. E. et Voroney, R. P. 2012. **Apport des carbonates aux émissions de CO_2 du sol**. Can. J. Soil Sci. **92**: 599–607. Le sol libère du dioxyde de carbone (CO_2) en raison de la respiration des microorganismes et des racines, mais les carbonates pourraient également dégager du CO_2 dans les sols calcaires. On estime que les sols renferment entre 700 et 900 Pg de carbone minéral sous forme de carbonates dans le monde, ce qui représente une importante source potentielle de CO_2 pour l'atmosphère. Bien que des études antérieures se soient concentrées sur les émanations globales de CO_2 du sol, les auteurs voulaient identifier les origines diverses de ces dégagements et leur contribution aux émissions totales de CO_2 en précisant la signature isotopique du gaz libéré du sol. À cette fin, ils ont recueilli des échantillons de loam limoneux d'un luvisol calcaire dans des parcelles labourées de la manière classique (LC) ou non travaillées (NT) du sud de l'Ontario, au Canada. Les échantillons (débarrassés de leurs racines et de leurs résidus) ont été incubés en laboratoire pendant 14 jours, puis on a analysé la signature isotopique des dégagements de CO_2 ($\delta^{13}C_{CO2}$) par spectroscopie de masse à rapport isotopique. La quantification des isotopes s'est avérée essentielle au dosage du CO_2 abiotique produit par les carbonates, à cause de la signature $\delta^{13}C$ particulière de ces derniers et de la matière organique. Les auteurs ont recouru à un modèle de mélange à deux éléments pour estimer la proportion de CO_2 issue des carbonates et de la décomposition de la matière organique dans le sol. L'analyse des émissions de CO_2 recueillies après 14 jours d'incubation indique que la fraction du gaz émanant du carbone inorganique du sol se situe entre 62 et 74 % pour les échantillons LC et entre 64 et 80 % pour les échantillons NT. Il faudrait effectuer d'autres recherches pour quantifier les émissions de CO_2 des sols calcaires et déterminer si les résultats des analyses de laboratoire peuvent s'appliquer aux études sur le terrain.

Mots clés: Émissions de CO_2 du sol, $\delta^{13}C$, respiration microbienne, carbonates

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 CO_2 emitted to the atmosphere (Lal and Kimble 2000; Emmerich 2003; Emmerich and Martens 2006; Tamir et al. 2011). Soil carbonates (or soil inorganic carbon, SIC) can be divided into two types: lithogenic or primary and pedogenic or secondary (Salomons and Mook 1976). Pedogenic carbonates are formed when lithogenic carbonates (relict marine limestone) undergo dissolution, exchanging their C with that of CO_2 derived from biological sources (organic matter decomposition and root respiration), and reprecipitating as $CaCO_3$ in the soil (Cerling 1984; Nordt et al. 1996; Landi et al. 2003; Kuzyakov et al. 2006; Gocke et al. 2011). Pedogenic carbonate precipitation depends on the

Abbreviations: **$\delta^{13}C_{co2}$, 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, $CaMgSi_2O_6$) 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 (Ehleringer and Rundel 1989). For convenience, the R value is expressed relative to an international standard and given the notation "delta", δ. Therefore, $\delta^{13}C$ refers to the ratio of ^{13}C:^{12}C and is calculated as:

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

where R_{sample} and $R_{standard}$ is the ^{13}C:^{12}C ratio of the sample and standard, respectively. $\delta^{13}C$ values are reported in parts per thousand (per mil, ‰) deviation from the standard reference material. An established reference standard for $\delta^{13}C$ measurement is PeeDee Belemnite (PDB), a marine limestone, with a $\delta^{13}C$ value of 0‰ by definition. Pedogenic and lithogenic carbonates have distinct isotopic values, with $\delta^{13}C$ values ranging from -10 to 0‰ and -2 to $+2$‰, respectively (Cerling 1984; Boutton 1991). Atmospheric CO_2 has a $\delta^{13}C$ 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 ($\delta^{13}C_{CO2}$) 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 $\delta^{13}C$ 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 $\delta^{13}C_{CO2}$ 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 CO_2 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 CO_2 emissions, by measuring the isotopic signature of the CO_2 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 1. General physical and chemical properties of soils obtained from conventional tillage (CT) and no-tillage (NT) systems in fall 2005

Soil depth (cm)	CT	NT	CT	NT
	Clay, <2 μm (g kg^{-1} soil)		Total organic carbon (g kg^{-1} soil)	
0–10	161 (5)[z]	158 (4)	21.0 (0.8)	24.1 (0.5)
10–20	163 (6)	151 (1)	19.7 (0.7)	18.7 (0.2)
20–30	189 (5)	178 (3)	12.6 (0.9)	12.1 (0.1)
30–50	163 (2)	180 (2)	5.1 (0.2)	5.6 (0.2)
	Silt, 2–50 μm (g kg^{-1} soil)		Total organic nitrogen (g kg^{-1} soil)	
0–10	567 (4)	582 (4)	1.68 (0.07)	1.90 (0.06)
10–20	553 (8)	583 (4)	1.59 (0.05)	1.50 (0.04)
20–30	532 (4)	548 (4)	1.06 (0.07)	1.01 (0.03)
30–50	528 (7)	523 (3)	0.52 (0.02)	0.55 (0.02)
	Sand, 50–2000 μm (g kg^{-1} soil)		Soil reaction (pH)	
0–10	273 (3)	260 (2)	7.30 (0.09)	7.26 (0.05)
10–20	284 (2)	267 (5)	7.32 (0.07)	7.28 (0.04)
20–30	279 (2)	275 (3)	7.41 (0.06)	7.38 (0.03)
30–50	310 (7)	297 (2)	7.49 (0.03)	7.55 (0.02)
	Gravel, >2000 μm (g kg^{-1} soil)		Bulk density (mg m^{-3})	
0–10	14 (3)	8 (36)	1.33 (0.01)	1.33 (0.01)
10–20	13 (3)	8 (1)	1.38 (0.01)	1.41 (0.01)
20–30	20 (4)	11 (3)	1.52 (0.02)	1.50 (0.02)
30–50	67 (12)	38 (8)	1.65 (0.02)	1.70 (0.02)

[z]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 suspension was allowed to settle for 48 h at room temperature. 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 100 mL of 0.01 M $CaCl_2$ followed by 100 mL of distilled water. The LF was dried at 60°C for 16 h, weighed and then ground ($<$250 μm) using an agate mortar and pestle. The samples were analyzed for C, N and $\delta^{13}C$.

Soil Microbial Biomass Determination

Soil microbial biomass carbon (MBC) was measured using the chloroform fumigation extraction technique (Vance et al. 1987; Voroney et al. 2008). A 50-g sample of soil was split into two subsamples (25 g each) and placed into 240-mL glass bottles. One subsample was not fumigated and extracted immediately. Extraction was carried out by adding 100 mL of 2 M KCl (Murage and Voroney 2007) and shaking on an end-to-end platform shaker for 1 h at 160 rpm. The soil extracts were then vacuum-filtered using 1.0 μm glass fibre filter paper into 100-mL vials.

While the non-fumigated subsamples were shaking, subsamples for fumigation were placed in a 3.8-L Pyrex® vacuum desiccator with a beaker containing about 50 mL of ethanol-free chloroform. The desiccator was evacuated until the chloroform began boiling, then vacuum-sealed, and the samples incubated at 25°C for 24 h. After 24 h, residual chloroform vapors were removed by repeated evacuations and the fumigated samples extracted as previously described.

Dialysis was conducted to remove excess salts from the extract (Murage and Voroney 2007). Extracts ($\sim$50 mL) were transferred into Spectra/Por® Biotech Cellulose Ester dialysis membrane tubing with a Molecular Weight Cut Off (MWCO) of 100. The extracts 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 $\delta^{13}C$.

The $\delta^{13}C$ of the soil MBC was calculated from the $\delta^{13}C$ values of the C extracted from the fumigated sample and non-fumigated sample using the following equation:

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

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

Laboratory Incubation and $\delta^{13}C_{CO2}$

CO_2 emission was measured by incubating the soil samples and collecting the released CO_2 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 CO_2 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 CO_2 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, CO_2 emitted from the soil was trapped by the NaOH solution and converted into sodium carbonate (Na_2CO_3).

$$CO_2\ (g) + 2NaOH \rightarrow Na_2CO_3\ (aq) + H_2O\ (1)$$

At the end of the incubation period the vials with NaOH solution were removed from the jars and titrated for quantification of released CO_2. For titration, an excess amount of 0.5 M strontium chloride ($SrCl_2$) was added to the vials to precipitate the Na_2CO_3 as strontium carbonate, $SrCO_3$ (Harris et al. 1997).

$$Na_2CO_3\ (aq) + SrCl_2\ (1) \rightarrow SrCO_3\ (s) + 2NaCl\ (aq)$$

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 CO_2 in the jar.

To analyze for $\delta^{13}C$, the suspension with $SrCO_3$ 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 $8000 \times g$ for 15 min and washed three times with deionized water to remove excess $SrCl_2$ and NaCl. Samples were dried at 60°C, stored in Wheaton® 4-mL vials and later placed into tin capsules and analyzed for $\delta^{13}C$. Note: The quantity of CO_2 in the incubation jars was determined but represented a minor percentage of the total CO_2 collected during the 14-d incubation, and was therefore excluded from calculations on the proportion of CO_2 evolved from SIC.

Estimation of CO_2 Released from Soil Carbonates

The proportion of CO_2 evolved from inorganic carbon (fSIC) was estimated using a two-end member mixing model (Balesdent et al. 1987):

$$\delta^{13}C_{CO2} = f\text{SIC} \times \delta^{13}C_{SIC} + (1 - f\text{SIC}) \times \delta^{13}C_{SOC}$$

The equation can be rewritten as:

$$f\text{SIC} = (\delta^{13}C_{CO2} - \delta^{13}C_{SOC})/(\delta^{13}C_{SIC} - \delta^{13}C_{SOC})$$

where $\delta^{13}C_{CO2}$ is the $\delta^{13}C$ value of CO_2 emitted, $\delta^{13}C_{SOC}$ is the $\delta^{13}C$ of soil organic carbon and $\delta^{13}C_{SIC}$ is the $\delta^{13}C$ value of soil inorganic carbon from samples. fSIC was also calculated by replacing "$\delta^{13}C_{SOC}$" in the above equation with the $\delta^{13}C$ values from the light fraction carbon ($\delta^{13}C_{LFC}$) and microbial biomass carbon ($\delta^{13}C_{MBC}$), 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.

Isotopic Analysis

Samples were placed into 8×5 mm tin capsules with the sample sizes (weights) providing between 300 and 540 μg organic carbon required for mass spectrometer analysis. The samples were analyzed at the Stable Isotope Laboratory, Department of Soil Science, University of Saskatchewan. Carbon, nitrogen and $\delta^{13}C$ were simultaneously determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) using an ANCA-GSL elemental analyzer coupled to a Tracer/20 mass spectrometer (Europa Scientific, Crewe, UK). The stable carbon isotope ratio was expressed as $\delta^{13}C$ in per mil (‰) units and the precision of $\delta^{13}C$ measurements was <0.1‰.

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 CO_2 emitted from calcareous soil samples is differentiating CO_2 originating from carbonates from that of SOM decomposition. To elucidate the sources of CO_2 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 CO_2 transport within the soil occurs only by diffusion and that soil CO_2 flux is at steady state (Cerling et al. 1991; Amundson et al. 1998). The assumption that CO_2 flux is at an isotopic steady-state means that the isotopic signature of the CO_2 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 CO_2 present in the headspace was <5% of the total CO_2 recovered, and would have had minimal effect on $\delta^{13}C_{CO2}$ due to length of time the samples remained incubated before CO_2 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 ($\delta^{13}C$) 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. $\delta^{13}C_{SIC}$ 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 $\delta^{13}C$ values of −1.4 to −1.1‰ for carbonates occurring in the Ck horizon.

Isotopic Signature of SOC, LFC and MBC

The $\delta^{13}C$ of the SOC ($\delta^{13}C_{SOC}$) in the 0–10 cm depth of NT soils was significantly higher (^{13}C-enriched) than

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

Soil depth	Conventional tillage	No-tillage	LSD[z]	*P* value[y]
cm		$\delta^{13}C_{CO2}$ (‰)		
0–10	−11.05 (0.26)[x]	−10.32 (0.20)	0.64	0.028
10–20	−11.20 (0.28)	−9.85 (0.20)	0.69	<0.001
20–30	−10.25 (0.34)	−9.04 (0.23)	0.81	0.004
30–50	−9.34 (0.26)	−8.63 (0.30)	0.79	0.056
		$\delta^{13}C_{SOC}$ (‰)		
0–10	−23.41 (0.11)	−20.61 (0.07)	0.25	<0.001
10–20	−23.50 (0.09)	−23.64 (0.05)	0.21	0.219
20–30	−25.41 (0.14)	−25.18 (0.07)	0.32	0.153
30–50	−27.57 (0.07)	−27.45 (0.07)	0.20	0.185
		$\delta^{13}C_{LF}$ (‰)		
0–10	−18.29 (0.29)	−16.71 (0.26)	0.77	<0.001
10–20	−19.31 (0.24)	−18.33 (0.20)	0.62	0.003
20–30	−19.31 (0.28)	−19.35 (0.25)	0.75	0.931
30–50	ND[w]	ND		
		$\delta^{13}C_{MBC}$ (‰)		
0–10	−18.18 (0.10)	−19.27 (0.04)	0.21	<0.001
10–20	−18.69 (0.04)	−22.09 (0.06)	0.15	<0.001
20–30	−23.82 (0.13)	−23.56 (0.06)	0.29	0.068
30–50	ND	ND		

[z]Least significant difference calculated between two means at $P = 0.05$.
[y]Probability level for the *F* value for tillage effect in ANOVA.
[x]Numbers in parentheses are the standard errors of the means ($n = 32$).
[w]Not determined.

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 for the 0–10, 10–20 and 20–30 cm depth increments compared with the CT treatment. However, the $\delta^{13}C$ values for both tillage treatments are much higher than that of the total SOC or any of the individual fractions of SOM (Table 2). 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 (*f*SIC)

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 3. The proportion of CO_2 evolved from soil inorganic carbon (*f*SIC) 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

Substrate	Conventional tillage	No-tillage
0–10 cm depth	*f*SIC	
SOC	0.74	0.74
LFC	0.63	0.64
MBC	0.63	0.72
10–20 cm depth	*f*SIC	
SOC	0.72	0.80
LFC	0.63	0.70
MBC	0.62	0.76

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_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_2 originated from SIC. When the soil MBC pool was considered the source of C substrate, (*f*SIC) was 63% and 72% for the 0–10 cm depth of CT and NT soils, respectively (Table 3). Even though the majority of CO_2 emitted from the soil is expected to occur from the 0–10 cm depth, the proportions of CO_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_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 $CaCO_3$ present as soil carbonates, a possible mechanism for the dissolution of the $CaCO_3$ is shown below:

$$H_2O\ (1) + CO_2\ (g) \rightarrow H_2CO_3\ (aq)$$

$$H_2CO_3\ (aq) + CaCO_3 \rightarrow Ca^{2+}\ (aq) + 2HCO_3^-\ (aq)$$

$$HCO_3^-\ (aq) \rightarrow CO(g) + OH^-\ (aq)$$

The CO_2 produced from microbial respiration dissolves in the soil water to form carbonic acid (H_2CO_3). The carbonic acid reacts with $CaCO_3$ resulting in a release of bicarbonates (HCO_3^-) which then dissociates releasing CO_2 from the soil. It is possible that there is a coupling mechanism between the CO_2 released from microbial respiration and the CO_2 originating from the dissolution of soil carbonates (Karberg et al. 2005).

The increase in CO_2 concentration (pCO_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_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_2 diffusion in water is about 10^{-4} times slower than in air. Other scientists have shown that measurements of soil CO_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_2 emissions (Rochette et al. 1997). Thus, these emission measurements of carbonate-derived CO_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_2 from carbonates, since it would disrupt soil aggregates containing carbonates and increase their exposed surface area. $CaCO_3$ is considered one of the most surface-active constituent in calcareous soils and the specific surface area is inversely related to the $CaCO_3$ 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 [$CaMg(CO_3)_2$]. Since the contribution of carbonate-derived C is similar in both tillage systems, one theory why the $\delta^{13}C_{CO2}$ 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 ($-16.7‰$) than in the CT system ($-18.3‰$). 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 $\delta^{13}C_{CO2}$ 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 $\delta^{13}C_{CO2}$ emissions for fall 2005 were $-16.7‰$ and $-20.2‰$ for the CT and NT plots, respectively. According to Cerling et al. (1991) and Amundson et al. (1998) the $\delta^{13}C$ of "soil-respired CO_2" is reduced by 4.4‰ due to isotopic fractionation by diffusion. Considering that the $\delta^{13}C_{CO2}$ values obtained from field measurements using micrometeorological techniques represents a 4.4‰ depletion, perhaps the higher values of $\delta^{13}C_{CO2}$ compared with carbon substrates may be partly explained by the emissions of CO_2 from carbonates. However, further field studies are required to understand the partitioning of CO_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 (Kuzyakov and Larionova 2005). Therefore, the contributions of carbonates to CO_2 emissions can be much lower in a field study with plants. Further work is necessary to elucidate the $\delta^{13}C$ signature of CO_2 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 CO_2 is a useful method to quantify abiotic CO_2 production from carbonates, due to the unique $\delta^{13}C$ signature of carbonates and soil organic matter. The $\delta^{13}C$ of soil respired CO_2 can be used to identify and quantify labile C substrates, which contribute to CO_2 emissions to the atmosphere. In this 14-d laboratory incubation study, we estimated that the proportion of CO_2 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 $\delta^{13}C$ natural abundance technique as a means to partition CO_2 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 CO_2 emissions from calcareous soils.

ACKNOWLEDGEMENTS

Funding for this research was provided by the Natural Sciences and Engineering Research Council (NSERC) strategic grants program. We are grateful for the technical assistance of Myles Stocki from the Stable Isotope Laboratory, University of Saskatchewan.

Adams, J. M. and Post, W. M. 1999. A preliminary estimate of changing calcrete carbon storage on land since the Last Glacial Maximum. Global Planet. Change. **20**: 243–256.

Alvarez, C. R., Alvarez, R., Grigera, M. S. and Lavado, R. S. 1998. Associations between organic matter fractions and the active soil microbial biomass. Soil Biol. Biochem. **30**: 767–773.

Amundson, R. G., Stern, L., Baisden, T. and Wang, Y. 1998. The isotopic composition of soil and soil-respired CO_2. Geoderma **82**: 83–114.

Anderson, J. P. E. 1982. Soil respiration. Pages 831–872 *in* A. L. Page, R. H. Miller, and D. R. Keeney, eds. Methods of soil analysis: Microbiological and chemical properties. Part 2. 2nd ed. SSSA, Madison, WI.

Balesdent, J., Mariotti, A. and Guillet, B. 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biol. Biochem. **19**: 25–30.

Batjes, N. H. 1996. Total carbon and nitrogen in the soils of the world. Eur. J. Soil Sci. **47**: 151–163.

Boettinger, J. L. and Southard, R. J. 1991. Silicate and carbonate sources for aridisols on a granitic pediment, western Mojave Desert. Soil Sci. Soc. Am. J. **55**: 1057–1067.

Boutton, T. W. 1991. Stable carbon isotope ratios of natural materials. II. Atmospheric, terrestrial, marine, and freshwater environments. Pages 173–185 *in* D. C. Coleman and B. Fry, eds. Carbon isotope techniques. Academic Press, San Diego, CA.

Cailleau, G., Braissant, O., Dupraz, C., Aragno, M. and Verrecchia, E. P. 2005. Biologically induced accumulations of $CaCO_3$ in orthox soils of Biga, Ivory Coast. Catena **59**: 1–17.

Carter, M. R. 2002. Soil quality for sustainable land management: Organic matter and aggregation interactions that maintain soil functions. Agron. J. **94**: 38–47.

Cerling, T. E. 1984. The stable isotope composition of modern soil carbonate and its relationship to climate. Earth Plan. Sci. Lett. **71**: 229–240.

Cerling, T. E., Solomon, D. K., Quade, J. and Bowman, J. R. 1991. On the isotopic composition of carbon in soil carbon-dioxide. Geochim. Cosmochim. Acta. **55**: 3403–3405.

Del Campillo, M. C., Torrent, J. and Loeppert, R. H. 1992. The reactivity of carbonates in selected soils of southern Spain. Geoderma **52**: 149–160.

Drewitt, G., Wagner-Riddle, C. and Warland, J. 2009. Isotopic CO_2 measurements of soil respiration over conventional and no-till plots in fall and spring. Agric. For. Meteorol. **149**: 614–622.

Ehleringer, J. R. and Rundel, P. W. 1989. Stable isotopes: History, units, and instrumentation. Pages 1–15 *in* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, eds. Stable isotopes in ecological research. Springer-Verlag, New York, NY.

Emmerich, W. E. 2003. Carbon dioxide fluxes in a semiarid environment with high carbonate soils. Agric. For. Meteorol. **116**: 91–102.

Emmerich, W. E. and Martens, D. A. 2006. Separation of soil organic and inorganic CO_2 fluxes. ASA-CSSA-SSSA Annual Meeting November 12–16, 2006, Indianapolis, IN.

Gocke, M., Pustovoytov, K. and Kuzyakov, Y. 2011. Carbonate recrystallization in root-free soil and rhizosphere of *Triticum aestivum and Lolium perenne* estimated by ^{14}C labeling. Biogeochemistry **103**: 209–222.

Gregorich, E. G. and Janzen, H. H. 1996. Storage of soil carbon in the light fraction and macroorganic matter. Pages 167–190 *in* M. R. Carter and B. A. Stewart, eds. Structure and organic matter storage in agricultural soils. Advances in soil science. CRC Press, Inc., Lewis Publishers, Boca Raton, FL.

Gregorich, E. G. and Beare, M. H. 2008. Physically uncomplexed organic matter. Pages 607–616 *in* M. R. Carter and E. G. Gregorich, eds. Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL.

Haile-Mariam, S., Collins, H. P., Wright, S. and Paul, E. A. 2008. Fractionation and long-term laboratory incubation to measure soil organic matter dynamics. Soil Sci. Soc. Am. J. **72**: 370–378.

Hanson, P. J., Edwards, N. T., Garten, C. T. and Andrews J. A. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry **48**: 115–146.

Harris, D., Porter, L. K. and Paul, E. A. 1997. Continuous flow isotope ratio mass spectrometry of carbon dioxide trapped as strontium carbonate. Commun. Soil Sci. Plant Anal. **28**: 747–757.

Hoffman, D. W., Mathews, B. C. and Wicklund, R. E. 1963. Soil survey of Wellington County, Ontario. Ontario soil survey, report number 35. Canada Department of Agriculture, Research Branch, Ontario Agriculture. College Publ., Guelph, ON.

Holford, I. C. R. and Mattingly, G. E. G. 1975. Surface areas of calcium carbonate in soils. Geoderma **13**: 247–255.
Intergovernmental Panel on Climate Change. 2007. Climate change 2007: Impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the Intergovernmental Panel on Climate Change. M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, eds. Cambridge University Press, Cambridge, UK. 976 pp.
Janzen, H. H., Campbell, C. A., Brandt, S. A., Lafond, G. P. and Townley-Smith, L. 1992. Light fraction organic matter in soils from long-term crop rotations. Soil Sci. Soc. Am. J. **56**: 1799–1806.
Jenkinson, D. S. and Ladd, J. N. 1981. Microbial biomass in soil: Measurement and turnover. Pages 415–471 *in* E. A. Paul and J. N. Ladd, eds. Soil biochemistry. Vol. 5. Marcel Dekker, New York, NY.
Karberg, N. J., Pregitzer, K. S., King, J. S., Friend, A. L. and Wood, J. R. 2005. Soil carbon dioxide partial pressure and dissolved inorganic carbonate chemistry under elevated carbon dioxide and ozone. Oecologia **142**: 296–306.
Kay, B. D. and VandenBygaart, A. J. 2002. Conservation tillage and depth stratification of porosity and soil organic matter. Soil Tillage Res. **66**: 107–118.
Kayler, Z. E., Sulzman, E. W., Rugh, W. D., Mix, A. C. and Bond, B. J. 2010. Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration. Soil Biol. Biochem. **42**: 435–444.
Kuzyakov, Y. 2006. Sources of CO_2 efflux from soil and review of partitioning methods. Soil Biol. Biochem. **38**: 425–448.
Kuzyakov, Y. and Larionova, A. A. 2005. Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. J. Plant Nutr. Soil Sci. **168**: 503–520.
Kuzyakov, Y., Shevtzova, E. and Pustovoytov, K. 2006. Carbonate re-crystallization in soil revealed by ^{14}C labeling: Experiment, model and significance for paleo-environmental reconstructions. Geoderma **131**: 45–58.
Lal, R. and Kimble, J. M. 2000. Pedogenic carbonates and the global C cycle. Pages 1–14 *in* R. Lal, J. M. Kimble, and B. A. Stewart, eds. Global climate change and pedogenic carbonates. CRC Press, Inc., Lewis Publishers, Boca Raton, FL.
Landi, A., Mermut, A. R. and Anderson, D. W. 2003. Origin and rate of pedogenic carbonate accumulation in Saskatchewan soils, Canada. Geoderma **117**: 143–156.
Lorenz, K. and Lal, R. 2005. The depth distribution of soil carbon in relation to land use and management and the potential of carbon sequestration in subsoil horizons. Adv. Agron. **88**: 35–66.
Lynn, W., Thomas, J. E. and Moody, L. E. 2008. Petrographic microscope techniques for identifying soil minerals in grain mounts. Pages 161–190 *in* A. R. Ulery and L. R. Drees, eds. Methods of soil analysis. Part 5. Mineralogical methods. SSSA, Madison, WI.
Mermut, A. R. and Arnaud, R. J. 1981. A study of microcrystalline pedogenic carbonates using submicroscopic techniques. Can. J. Soil Sci. **61**: 261–272.
Murage, E. W. and Voroney, R. P. 2007. Modification of the original chloroform fumigation extraction technique to allow measurement of $\delta^{13}C$ of soil microbial biomass carbon. Soil Biol. Biochem. **39**: 1724–1729.
Nordt, L. C., Wilding, L. P., Hallmark, C. T. and Jacob, J. S. 1996. Stable carbon isotope composition of pedogenic carbonate and their use in studying pedogenesis. Pages 133–154 *in* T. W. Boutton and S. Yamasaki, eds. Mass spectrometry of soils. Marcel Dekker, New York, NY.
Ramnarine, R., Voroney, R. P., Wagner-Riddle, C. and Dunfield, K. E. 2011. Carbonate removal by acid fumigation for measuring the $\delta^{13}C$ of soil organic carbon. Can. J. Soil Sci. **91**: 247–250.
Rochette, P., Ellert, B., Gregorich, E. G., Desjardins, R. L., Pattey, E., Lessard, R. and Johnson, B. G. 1997. Description of a dynamic closed chamber for measuring soil respiration and its comparison with other techniques. Can. J. Soil Sci. **77**: 195–203.
Rovira, P. and Vallejo, V. R. 2008. Changes in 13C composition of soil carbonates driven by organic matter decomposition under Mediterranean climate: a field incubation experiment. Geoderma **144**: 517–534.
Salomons, W. and Mook, W. G. 1976. Isotope geochemistry of carbonate dissolution and reprecipitation in soils. Soil Sci. **122**: 15–24.
Schlesinger, W. H. 1985. The formation of caliche in soils of the Mojave Desert, California. Geochim. Cosmochim. Acta **49**: 57–66.
Schlesinger, W. H. 1997. Biogeochemistry: an analysis of global change. 2nd ed. Academic Press, San Diego, CA. 588 pp.
Smith, B. N. and Epstein, S. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol. **47**: 380–384.
Soil Classification Working Group. 1998. The Canadian system of soil classification. 3rd ed. Agriculture and Agri-Food Canada, NRC Research Press, Ottawa, ON. Publ. 1646. 187 pp.
Soil Survey Staff. 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. 2nd ed. Agricultural handbook number 436. USDA–NRCS, Washington, DC. 871 pp.
Sparling, G. P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soi1 organic matter. Aust. J. Soil Res. **30**: 195–207.
Strickland, T. C. and Sollins, P. 1987. Improved method for separating light- and heavy-fraction organic material from soil. Soil Sci. Soc. Am. J. **51**: 1390–1393.
Tamir, G., Shenker, M., Heller, H., Bloom, P. R. and Bar-Tal, P. 2011. Can soil carbonate dissolution lead to overestimation of soil respiration? Soil Sci. Soc. Am. J. **75**: 1414–1422.
Vance, E. D., Brookes, P. C. and Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. **19**: 703–707.
Voroney, R. P., Brookes, P. C. and Beyaert, R. P. 2008. Soil microbial biomass C, N, P, and S. Pages 637–651 *in* M. R. Carter and E. G. Gregorich, eds. Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL.
Wang, D. and Anderson, D. W. 2000. Pedogenic carbonate in Chernozemic soils and landscapes of southeastern Saskatchewan. Can. J. Soil Sci. **80**: 251–261.