

# Initial soil organic carbon concentration influences the short-term retention of crop-residue carbon in the fine fraction of a heavy clay soil

Vincent Poirier · Denis A. Angers · Philippe Rochette · Joann K. Whalen

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**Abstract** Among factors controlling decomposition and retention of residue C in soil, effect of initial soil organic C (SOC) concentration remains unclear. We evaluated, under controlled conditions, short-term retention of corn residue C and total soil CO<sub>2</sub> production in C-rich topsoil and C-poor subsoil samples of heavy clay. Topsoil (0–20 cm deep, 31.3 g SOC kg<sup>-1</sup> soil) and subsoil (30–70 cm deep, 4.5 g SOC kg<sup>-1</sup> soil) were mixed separately with <sup>13</sup>C–<sup>15</sup>N-labeled corn (*Zea mays* L.) residue at rates of 0 to 40 g residue C kg<sup>-1</sup> soil and incubated for 51 days. We measured soil CO<sub>2</sub>–C production and the retention of residue C in the whole soil and the fine particle-size fraction (<50 μm). Cumulative C mineralization was always greater in topsoil than subsoil. Whole-soil residue C retention was similar in topsoil and subsoil at rates up to 20 g residue C kg<sup>-1</sup>. There was more residue C retained in the fine fraction of topsoil than subsoil at low residue input levels (2.5 and 5 g residue C kg<sup>-1</sup>), but the trend was reversed with high residue inputs (20 and 40 g

residue C kg<sup>-1</sup>). Initial SOC concentration affected residue C retention in the fine fraction but not in the whole soil. At low residue input levels, greater microbial activity in topsoil resulted in greater residue fragmentation and more residue C retained in the fine fraction, compared to the subsoil. At high residue input levels, less residue C accumulated in the fine fraction of topsoil than subsoil likely due to greater C saturation in the topsoil. We conclude that SOC-poor soils receiving high C inputs have greater potential to accumulate C in stable forms than SOC-rich soils.

**Keywords** <sup>13</sup>C–<sup>15</sup>N-labeled residues · Soil organic carbon · Soil particle-size fractions · Residue input rate · Laboratory incubation

## Introduction

In agroecosystems, soil organic C (SOC) accrual is observed when crop residue C inputs exceed C losses (Paustian et al. 1997). Crop residue mineralization is controlled by their chemical composition (Johnson et al. 2007), soil texture (Oades 1988), soil environmental conditions (Fierer et al. 2003) and amount of residue added (Broadbent and Bartholomew 1948). These factors are generally considered in studies and models on SOC dynamics (Buisson and Aubinet 2010). However, current SOC models generally do not consider how initial SOC concentration affects soil CO<sub>2</sub> production and retention of C coming from newly incorporated crop residues.

The initial SOC concentration could influence short-term crop residue mineralization in two ways. First, soils with higher SOC content usually sustain greater microbial biomass and overall biological activity (Stevenson and Cole 1999), and this can result in greater and quicker decomposition of added

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V. Poirier · D. A. Angers · P. Rochette  
Soils and Crops Research and Development Centre,  
Agriculture and Agri-Food Canada, 2560 Hochelaga Blvd.,  
Québec, Québec, Canada G1V 2J3

V. Poirier · J. K. Whalen  
Department of Natural Resource Sciences, Macdonald Campus  
of McGill University, 21111 Lakeshore Road,  
Ste. Anne de Bellevue, Québec, Canada H9X 3V9

### Present Address:

V. Poirier (✉)  
Département des sciences du bois et de la forêt, Université Laval,  
2405 de la Terrasse,  
Québec, Québec, Canada G1V 0A6  
e-mail: vincent.poirier.1@ulaval.ca

residues (Broadbent and Bartholomew 1948; Stewart et al. 2008a). However, greater microbial activity in SOC-rich soil leads to more C being metabolized and consequently, higher stabilization of residue-derived C in the short-term compared to SOM-poor soils (Stewart et al. 2008a). Second, according to the theory of SOC saturation, mineral surfaces have a limited capacity to adsorb organic compounds and this can influence short-term residue decomposition (Hassink 1996, 1997). Silt and clay particles (<50  $\mu\text{m}$ ) can stabilize and protect decomposing residue C against microbial attack (Oades 1988), but this storage capacity appears to be limited and related to the amount of fine particles in the soil (Hassink 1997; Carter et al. 2003). Therefore, there is a balance between microbiological and adsorption processes influencing residue-derived C stabilization that may be affected by the initial SOC concentration.

The effect of initial SOC level and C saturation deficit on accumulation of residue C in the soil can be compared in topsoil and subsoil having similar physicochemical properties (Stewart et al. 2008b). Topsoil usually contains more SOC and has greater SOC saturation in the fine fraction than subsoil. Thus, subsoils may be more effective than topsoils in sorbing newly added residue C onto mineral surfaces (Kaiser and Guggenberger 2003; Rasse et al. 2005) and presumably have greater potential to retain SOC in the fine fraction (Lorenz and Lal 2005; Castellano et al. 2012).

Amounts and nature of organic residues applied to soil vary widely. From a practical perspective, understanding the impact of high organic residue input levels is particularly significant in situations such as high organic residue application rates sometimes used in soil reclamation to kick-start soil regeneration and restructuring processes (Grosbellet et al. 2011; Larney and Angers 2012). Different tillage practices also result in differential and high concentrations of residues at particular soil depths (Angers et al. 1995). In both these situations, organic residues will come in contact with SOC-poor soils or soil layers. In the soil C budget, understanding the short- and long-term fates of residue C throughout the soil profile requires consideration of how it is stabilized in response to the initial SOC concentration. Carbon saturation theory suggests that the proportion of new C accumulating in the stable fraction will be greater in a SOC-poor soil than a SOC-rich soil. Our objective was to test this hypothesis in soils with contrasting SOC levels receiving a wide range of residue C inputs in a short-term incubation study with  $^{13}\text{C}$ – $^{15}\text{N}$ -labeled residues.

## Materials and methods

### Soil sampling and properties

Heavy clay soil from the Kamouraska series classified as a Haplic Gleysol according to the World Reference Base for Soil Resource system (IUSS Working Group WRB 2006)

and as an Orthic Humic Gleysol according to the Canadian System of Soil Classification (Soil Classification Working Group 1998) was selected for this study. This soil is located in the St. Lawrence lowlands and was formed from marine and fluvial deposits. It is characterized by high content of clay particles (>650  $\text{g kg}^{-1}$ ), high specific surface area of the clay fraction (>200  $\text{m}^2 \text{g}^{-1}$ ), and uniform texture and mineralogy throughout the soil profile (De Kimpe et al. 1979).

The soil was sampled at the Harlaka Research Farm of Agriculture and Agri-Food Canada, Lévis, Québec, Canada (46°48' N, 71°23' W) in October 2007. Three pits were manually excavated (50 cm wide, 75 cm deep) at 3-m intervals along a 10-m transect in a plot that had a history of barley (*Hordeum vulgare* L.) production (2000 to 2003 and 2005 to 2007) and canola (*Brassica napus* L.) production (2004). The field was fertilized each spring by applying mineral fertilizers to the soil surface (not incorporated) according to local agronomic recommendations, i.e., 70 kg N  $\text{ha}^{-1}$ , 40 kg  $\text{P}_2\text{O}_5$   $\text{ha}^{-1}$ , and 40 kg  $\text{K}_2\text{O}$   $\text{ha}^{-1}$ . Visually, there was a clear distinction between the gray-brown-coloured topsoil and the light gray-coloured subsoil. Layers from the topsoil (0–20 cm) and subsoil (30–70 cm) horizons were removed from the pit by hand and with a shovel and placed in separate containers. Soils were initially kept at 4 °C, gently crumbled, and fresh roots and organic fragments were removed by hand. Then, soils were air-dried at room temperature for 7 days, sieved through a 6-mm mesh, and mixed to make homogeneous composite samples for each soil horizon. Several physical and chemical properties were determined on whole soil samples (<6 mm) (Table 1). The soil <6 mm was used for incubation. In such clayey soil, we believe that aggregation must be preserved and we used a 6-mm sieve to minimize aggregate breakdown and C losses during soil preparation procedures.

### Plant residue

The plant residue used in this study was from corn (*Zea mays* L. cv. Cargill 2610-L) grown in pots in a greenhouse. When the corn was at the V2–V3 vegetative stage, it was pulse-labeled with  $^{13}\text{CO}_2$  weekly and  $^{15}\text{N}$ - $\text{KNO}_3$  fertilizer was added after each pulse-labeling event. Corn was harvested at the V10–V12 vegetative stage, before tasseling. Corn residues (leaves + stems) were oven-dried at 50 °C for 24 h and ground using a Wiley laboratory mill. Since residue mineralization decreases with increasing particle size (Angers and Recous 1997), we used small residues (100  $\mu\text{m}$  to 1 mm) to avoid delayed mineralization. Corn residue used in this study contained 434.4 g C  $\text{kg}^{-1}$  and 69.7‰  $\delta^{13}\text{C}$ , with 16.5 gN  $\text{kg}^{-1}$  and 7.40 At%  $^{15}\text{N}$  (analyzed at Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada).

**Table 1** Selected physical and chemical properties of topsoil (0–20 cm) and subsoil (30–70 cm) from the heavy clay soil used for the incubation

	Particle-size distribution <sup>a</sup>			SOC <sup>b</sup> (gkg <sup>-1</sup> soil)	Total N <sup>b</sup> (gkg <sup>-1</sup> soil)	Mineral N <sup>c</sup> (mgkg <sup>-1</sup> soil)	C/N	pH		Mineralogy <sup>f</sup>
	<2 $\mu$ m (gkg <sup>-1</sup> soil)	<20 $\mu$ m (gkg <sup>-1</sup> soil)	<50 $\mu$ m (gkg <sup>-1</sup> soil)					H <sub>2</sub> O <sup>d</sup>	CaCl <sub>2</sub> <sup>e</sup>	
Topsoil	656	844	937	31.3	2.5	3.9	12.5	6.3	5.2	Q, F, A, Ch, S, V, I/M
Subsoil	675	847	946	4.5	0.5	1.0	9.0	7.2	6.3	Q, F, A, Ch, S, V, I/M

Q quartz, F feldspar, A amphibole, Ch chlorite, S smectite, V vermiculite, I/M illite/muscovite

<sup>a</sup> Determined by the pipette method (Kroetsch and Wang 2007)

<sup>b</sup> Measured by dry combustion (CNS-1000, LECO Corp., St. Joseph, MI, USA). No carbonates were detected by acidification analysis (SSM-5000A, Shimadzu Corp., Kyoto, Japan) and total C was considered equivalent to SOC in both topsoil and subsoil

<sup>c</sup> Mineral N =  $\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$ , determined by colorimetry after extraction with 2 M KCL (Maynard et al. 2007) using a 1:5 soil-to-KCL ratio

<sup>d</sup> Measured in 1:1 soil-to-H<sub>2</sub>O ratio

<sup>e</sup> Measured in 1:2 soil-to-CaCl<sub>2</sub> 0.01 M ratio

<sup>f</sup> Determined on random-oriented powders with an X-ray diffractometer (X'Pert Pro MPD, Panalytical) running at 40 kV and 40 mA using a Co K $\alpha$  radiation ( $\lambda=1.79$  Å) with a linear detector X'Celerator and a secondary flat monochromator. Whole soil samples were crushed in an agate mortar, put in cylindrical aluminium holders, and spun at 15 rpm. For each sample, a counting time of 3 s per 0.033° step was used for 2 $\theta$  in the 3.5–80° range

## Experimental design and incubation conditions

The incubation experiment was a completely randomized factorial design with initial SOC concentration (topsoil and subsoil) and residue C input as the two factors tested. The experimental units were 1-L glass jars containing 150 g of air-dried top- or subsoil mixed with 0 (control), 0.86, 1.73, 3.45, 6.91, and 13.81 g of <sup>13</sup>C–<sup>15</sup>N-labeled residues corresponding to 0, 2.5, 5, 10, 20, and 40 g residue C kg<sup>-1</sup> dry soil, respectively. All treatments were replicated four times for a total of 48 experimental units.

Soil N concentration at the time of residue incorporation influences C mineralization (Recous et al. 1995). Thus, mineral N in the form of a KNO<sub>3</sub> solution was added to facilitate microbial growth and avoid N limitation. Mineral N was added to the soil + residue mixture to achieve a C/N ratio of 10 considering soil initial mineral N ( $\text{NH}_4^+ - \text{N} + \text{NO}_3^- - \text{N}$ ) concentration and the amount of residue C added, similar to the method employed by Cosentino (2006). Since the initial mineral N concentration of the soil was low (<3.9 mgkg<sup>-1</sup>, Table 1), residue-amended topsoil and subsoil received the same amount of KNO<sub>3</sub> in each residue treatment. The moisture content in each experimental unit was adjusted at the start of the experiment to a water potential of –38 kPa (based on pressure plate measurements) by adding distilled water after accounting for water added with the KNO<sub>3</sub> solution. Soil and residue were mixed before and after the addition of water and KNO<sub>3</sub> solution to limit preferential segregation of soil and residue due to differences in particle size. Jars were open and placed in a controlled climate chamber and incubated at 25 °C for ~51 days (1,225.5 h) in the dark. Humidity of in-jar air was checked at each soil respiration monitoring event (see the following section), and

soil water content was adjusted every 3–4 days throughout the incubation period.

## Soil CO<sub>2</sub> production

Soil CO<sub>2</sub> production was measured 36 times during the 51-day incubation. At sampling time, the jars were sealed for 1, 2, and 4 h for sampling events that occurred between 0 and 1, 1 and 3, and 3 and 51 days of incubation, respectively. A gas sample was taken immediately after closing the jar ( $t_a$ ) and a second gas sample was taken at the end of the sampling event ( $t_b$ ). Gas samples (20 mL) were taken with a polypropylene syringe and injected into preevacuated vials (12 mL Exetainers, Labco, High Wycombe, UK). The CO<sub>2</sub> concentration in gas samples was analyzed within 1 week of sample collection using a gas chromatograph configured with a methanizer and a flame ionization detector according to the procedure of Rochette and Bertrand (2007). Carbon dioxide production (Fc, milligrams of CO<sub>2</sub>-C per kilogram of soil per hour) for a given sampling event was calculated using the equation of Rochette and Bertrand (2007):

$$Fc = \frac{dC/dt \cdot V}{M} \cdot \frac{Mm}{Mv} \cdot \frac{P}{e_a}$$

where  $dC/dt$  (moles of CO<sub>2</sub> per mole per hour) is the rate of change of headspace gas concentration between time  $t_a$  and  $t_b$  in dry air samples,  $V$  (cubic meters) is the volume of in-jar air,  $M$  (kilograms) is the mass of dry soil inside the jar,  $Mm$  (12,000 mg C mol<sup>-1</sup>) is the molecular weight of C in a CO<sub>2</sub> molecule,  $Mv$  (0.0244 m<sup>3</sup> mol<sup>-1</sup>) is the molecular volume at 25 °C,  $P$  (kilopascals) is the barometric pressure, and  $e_a$  (kilopascals) is the partial pressure of water vapor in the jar

headspace. The volume ( $V$ ) of in-jar air was calculated according to the following equation:

$$V = V_H + V_A + \alpha V_W$$

where  $V_H$  (cubic meters) is the headspace volume,  $V_A$  (cubic meters) is soil air porosity volume,  $\alpha$  is the dissolution constant of  $\text{CO}_2$  in water (in cubic meters of  $\text{CO}_2$  per cubic meter of water), and  $V_W$  (cubic meters) is the volume of water in soil at  $-38$  kPa. The term  $\alpha V_W$ , adapted from an equation given by Tiedje (1994) for  $\text{N}_2\text{O}$  measurements, accounts for the volume of  $\text{CO}_2$  dissolved in soil water at the moment of sampling. We used an  $\alpha$  value of 0.834 at  $25^\circ\text{C}$  and normal barometric pressure, calculated from the equation of Li and Tsui (1971). Cumulative amount of C lost upon mineralization (grams of  $\text{CO}_2\text{-C}$  per kilogram of dry soil) was calculated by linearly interpolating  $\text{CO}_2\text{-C}$  flux over time between sampling events. Residue-induced cumulative amount of  $\text{CO}_2\text{-C}$  lost was expressed as  $\text{CO}_2\text{-C}_{\text{treatment}}$  minus  $\text{CO}_2\text{-C}_{\text{control}}$ .

#### Soil organic C, total soil N, and $^{15}\text{N}$ and $^{13}\text{C}$ analyses

At the end of the incubation period, samples were air-dried for 7 days. The fine fraction was obtained as follows: 25 g of whole air-dried soil were shaken overnight with 100 mL of distilled water in a 250-mL plastic bottle with 10 glass beads (6 mm diameter), washed over a 50- $\mu\text{m}$  sieve, dried at  $50^\circ\text{C}$  for at least 24 h, and weighed (Balesdent et al. 1991). Samples from the whole soil and fine fraction were ground to  $\sim 10\ \mu\text{m}$  using a ball mill and then analyzed for SOC, total N,  $^{15}\text{N}$ , and  $^{13}\text{C}$ .

Total C and N concentrations in the whole soil and in the fine fraction were determined by dry combustion (CNS-1000, LECO Corp., St. Joseph, MI, USA). Residue C and N concentrations in the whole soil and in the fine fraction were calculated from the isotopic signature ( $\delta^{13}\text{C}$ ,  $\text{At}\%^{15}\text{N}$ ) given by isotope ratio mass spectrometry analysis (Stable Isotope Facility, UC Davis, CA, USA). The  $\delta^{13}\text{C}$  (in per mill) values were calculated according to the equation:

$$\delta^{13}\text{C} = \frac{^{13}\text{R}_{\text{sample}} - ^{13}\text{R}_{\text{standard}}}{^{13}\text{R}_{\text{standard}}} \times 1000$$

where  $^{13}\text{R} = ^{13}\text{C}/^{12}\text{C}$  and the standard is the international Pee Dee Belemnite. In the whole soil and the fine fraction, the fraction of SOC coming from residue C ( $f_C$ , in grams of residue C per gram of  $\text{SOC}_{\text{whole or fine}}$ ) was calculated as follows:

$$f_C = \frac{d_{\text{tr}} - d_c}{d_{\text{r}} - d_c}$$

where  $d_{\text{tr}} = \delta^{13}\text{C}$  of the whole soil or fine fraction in residue-amended soil,  $d_c = \delta^{13}\text{C}$  of the whole soil or fine fraction in unamended control soil, and  $d_r = \delta^{13}\text{C}$  of the corn residues. The amount of residue C (in grams of residue C per kilogram of

soil) retained in the whole soil or in the fine fraction was calculated as follows:

$$\text{Residue C} = f_C \times \text{SOC}_{\text{whole or fine}}$$

where  $[\text{SOC}]_{\text{whole or fine}}$  is the concentration of total C measured by dry combustion in the whole soil or in the fine fraction, expressed in grams of SOC per kilogram of soil.

The  $\text{At}\%^{15}\text{N}$  values were calculated according to the equation:

$$\text{At}\%^{15}\text{N} = \frac{\text{no. of } ^{15}\text{N atoms}}{\text{no. of } ^{15}\text{N} + ^{14}\text{N atoms}} \times 100.$$

In the whole soil and in the fine fraction, the proportion of total N coming from residue N ( $f_N$ , in grams of residue N per gram of total  $\text{N}_{\text{whole or fine}}$ ) was calculated as follows:

$$f_N = \frac{At_{\text{tr}} - At_c}{At_r - At_c}$$

where  $At_{\text{tr}} = \text{At}\%^{15}\text{N}$  of the whole soil or fine fraction in residue-amended soil,  $At_c = \text{At}\%^{15}\text{N}$  of the whole soil or fine fraction in unamended control soil, and  $At_r = \text{At}\%^{15}\text{N}$  of the corn residues. The amount of residue N (in grams of residue N per kilogram of soil) retained in the whole soil or in the fine fraction was calculated as follows:

$$\text{Residue N} = f_N \times \text{total N}_{\text{whole or fine}}$$

where  $[\text{total N}]_{\text{whole or fine}}$  is the concentration of total N measured by dry combustion in the whole soil or in the fine fraction, expressed in grams of total N per kilogram of soil.

#### Statistical analysis

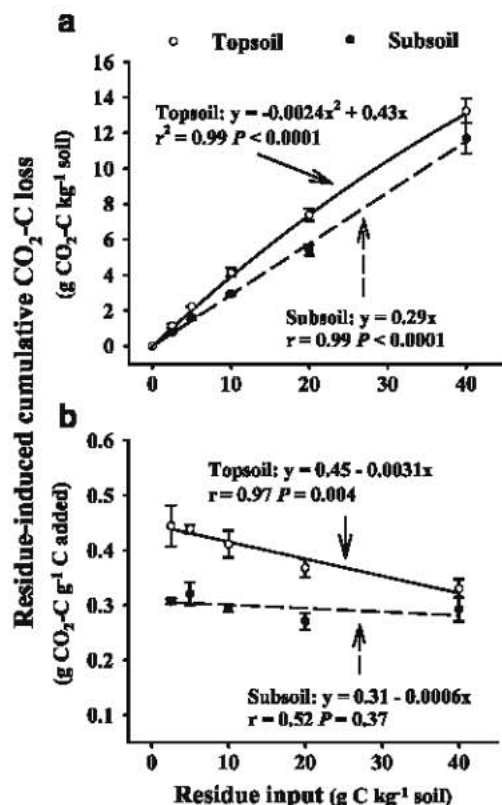
To test the effect of initial SOC concentration (i.e., topsoil vs. subsoil), residue C input, and their interactions on the dependent variables, we performed analysis of variance (ANOVA) with the general linear model (GLM) procedure of the SAS software (SAS Institute 2001). Normal distribution of the data was verified using the PLOT and UNIVARIATE procedures of SAS. The results for residue-induced cumulative  $\text{CO}_2\text{-C}$  losses and residue N retention in the fine fraction only were not normally distributed and were rank transformed prior to analysis. When ANOVA yielded significant ( $P < 0.05$ ) treatment effects at  $\alpha = 0.05$ , a post hoc least significant difference (LSD) test was used to detect differences among treatment means. Quantitative polynomial contrasts were also used to test linear and quadratic effects induced by the amount of residue C added with and without interaction with initial SOC concentration. Regression analyses were performed with SigmaPlot 9.0 (Systat Software Inc. 2004) and comparisons between regression parameters were achieved using Student's  $t$  test for bilateral pairwise comparisons.

# Results

Both topsoil and subsoil had very similar mineralogy and particle size distribution (Table 1). However, initial SOC concentration was seven times greater in the topsoil than the subsoil (Table 1).

## Soil CO<sub>2</sub> production

Cumulative CO<sub>2</sub>-C losses were about 14 times greater ( $P<0.0001$ ) in the unamended topsoil ( $0.55\pm0.05$  g CO<sub>2</sub>-C kg<sup>-1</sup> soil) than in the unamended subsoil ( $0.04\pm0.01$  g CO<sub>2</sub>-C kg<sup>-1</sup> soil). Residue-induced cumulative CO<sub>2</sub>-C losses were greater ( $P<0.03$ ) in topsoil than subsoil at each level of residue input (Fig. 1a). The relationships describing residue-induced cumulative CO<sub>2</sub> losses with increasing residue input were significantly ( $P<0.03$ ) different between topsoil and subsoil. When considered per kilogram of soil, the topsoil relationship was described by a quadratic increase, while the subsoil relationship was described by a linear increase (Fig. 1a). When considered per gram of residue C added, the topsoil showed a linear decline, while no relationship was observed in the subsoil (Fig. 1b).



**Fig. 1** Residue-induced cumulative CO<sub>2</sub>-C loss expressed **a** per kilograms of soil and **b** per grams of residue C added from topsoil (0–20 cm) and subsoil (30–70 cm) of a heavy clay soil after 51 days of incubation as a function of corn residue input (vertical bars on data points represent standard deviation from the mean)

## Soil organic C in the whole soil and the fine fraction

The SOC concentrations in the whole soil and the fine fraction are shown in Table 2 and were always greater ( $P<0.0001$ ) in topsoil than subsoil. Whole-soil SOC increased linearly ( $P<0.0001$ ) with residue input rate in topsoil and subsoil, and the effect of residue input was the same ( $P=0.14$ ) in both soils (data not shown).

## Residue-derived C in the whole soil and the fine fraction

The  $\delta^{13}\text{C}$  values in the unamended topsoil and subsoil were, on average,  $-26.1\pm3.1\%$  in the whole soil, similar to the  $-25$  to  $-27\%$   $\delta^{13}\text{C}$  reported by Angers et al. (1995) for autochthonous SOC in an agricultural soil of Eastern Canada. Whole-soil residue C retention was similar in topsoil and subsoil and approximately  $60\pm3\%$  of the residue C added was recovered in the whole soil, regardless of the residue input level, at the end of the incubation period (Fig. 2a and b). However, topsoil retained slightly more ( $P=0.054$ ) residue C than subsoil at the highest residue input level.

The relationship between residue C input and the amount of residue C retained in the fine fraction was described by a power function ( $y=ax^b$ ) in both soils. However, parameter  $a$  was greater ( $P=0.0009$ ) and parameter  $b$  was lower ( $P=0.0005$ ) in topsoil than subsoil (Fig. 2a and b). The topsoil retained  $2.3\pm0.8$  times more ( $P<0.03$ ) residue C in the fine fraction than the subsoil at the lowest residue inputs (i.e., 2.5 and 5 g residue C kg<sup>-1</sup>). The opposite trend was observed at the highest residue inputs (i.e., 20 and 40 g residue C kg<sup>-1</sup>) with  $1.3\pm0.3$  times more residue C retained in the fine fraction of subsoil than topsoil (significant at  $P<0.05$ ). The residue C-

**Table 2** Soil organic C (SOC) concentrations in the whole soil and fine (<50  $\mu\text{m}$ ) particle-size fraction in topsoil (0–20 cm) and subsoil (30–70 cm) of a heavy clay soil after 51 days of incubation with increasing amounts of corn residues

Residue input (g C kg <sup>-1</sup> soil)	Whole soil		Fine (<50 $\mu\text{m}$ )	
	Topsoil (g SOC kg <sup>-1</sup> soil)	Subsoil (g SOC kg <sup>-1</sup> soil)	Topsoil (g SOC kg <sup>-1</sup> soil)	Subsoil (g SOC kg <sup>-1</sup> soil)
0	29.3 <sup>Ae</sup>	3.9 <sup>Bf</sup>	2.5 <sup>Af</sup>	0.5 <sup>Bf</sup>
2.5	31.5 <sup>Ade</sup>	5.2 <sup>Be</sup>	2.7 <sup>Ae</sup>	0.8 <sup>Be</sup>
5	32.7 <sup>Ac</sup>	6.7 <sup>Bd</sup>	3.0 <sup>Ad</sup>	1.0 <sup>Bd</sup>
10	35.4 <sup>Ac</sup>	9.7 <sup>Bc</sup>	3.4 <sup>Ac</sup>	1.4 <sup>Bc</sup>
20	42.0 <sup>Ab</sup>	15.9 <sup>Bb</sup>	4.5 <sup>Ab</sup>	2.3 <sup>Bb</sup>
40	50.0 <sup>Aa</sup>	27.2 <sup>Ba</sup>	6.1 <sup>Aa</sup>	4.3 <sup>Ba</sup>

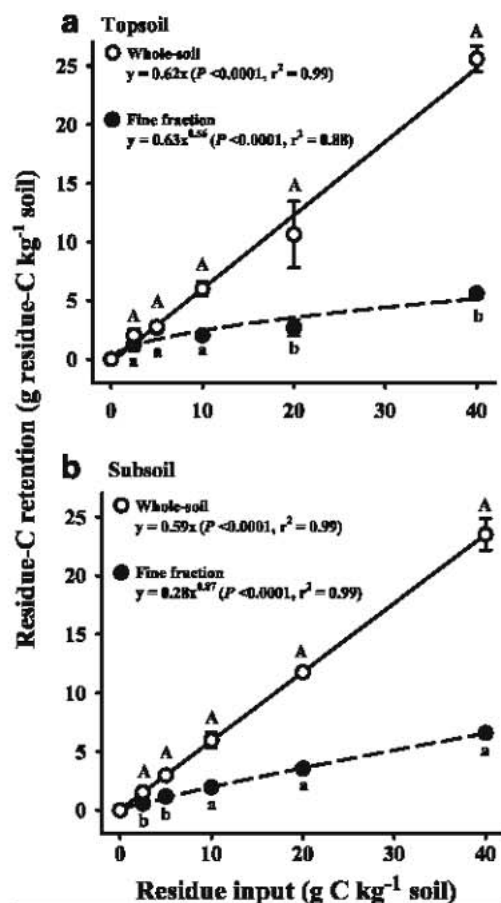
Means followed by uppercase letters are comparisons of soil types (in the same row) and means followed by lowercase letters are comparisons of residue input level (in the same column). Values followed by different letters are significantly different ( $P<0.05$ ) from each other according to the LSD test at  $\alpha=0.05$

to-residue N ratio of the fine fraction of topsoil was, on average,  $19.5 \pm 5.7$  at the lowest residue inputs and decreased to about  $5.6 \pm 1.0$  when 20 and 40 g residue C kg<sup>-1</sup> were added (Fig. 3). In contrast, the residue C-to-residue N ratio in the subsoil fine fraction was unaffected by residue inputs and averaged  $8.0 \pm 1.1$  across all residue input levels (Fig. 3).

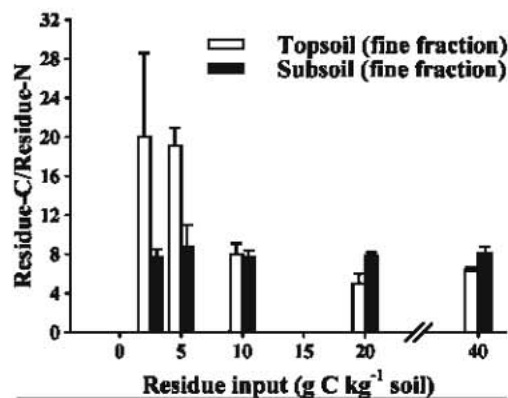
## Discussion

### Experimental conditions

Experimental conditions in this work were chosen to achieve rapid decomposition of crop residues through the addition of KNO<sub>3</sub> solution to reach a C/N ratio of 10 for every soil + residue



**Fig. 2** Residue-derived C retained in the whole soil and in the fine particle-size fraction in **a** topsoil (0–20 cm) and **b** subsoil (30–70 cm) of a heavy clay soil as a function of corn residue input after 51 days of incubation (vertical bars on data points represent standard deviation from the mean). At each level of residue input, the residue C retention was compared for topsoil vs. subsoil (uppercase letters for the whole soil; lowercase letters for the fine fraction). Data points with different letters were significantly different ( $P < 0.05$ ) according to the LSD test at  $\alpha = 0.05$ . At 40 g residue C kg<sup>-1</sup>, whole-soil residue C retention tended to be greater ( $P = 0.054$ ) in topsoil than subsoil



**Fig. 3** Residue C-to-residue N ratios of the fine (<50 μm) particle-size fraction of topsoil (0–20 cm) and subsoil (30–70 cm) from a heavy clay soil after 51 days of incubation with increasing amounts of corn residue (vertical bars represent standard deviation from the mean)

treatment and the use of finely ground (100–1,000 μm) residues that were thoroughly mixed with the soil. We expected these conditions to result in rapid stabilization of microbial products of decomposition in this heavy clay soil. Soil CO<sub>2</sub> production in unamended soils indicates that there were metabolically active microorganisms capable of degrading autochthonous SOC in the sieved, remoistened topsoil and subsoil. No lag phase in CO<sub>2</sub> production peaks was observed in either topsoil or subsoil within the same residue treatment (data not shown). This indicates that decomposition conditions and resource availability to decomposers were relatively similar between the two soils and allowed C mineralization to occur upon initiation of the experiment.

The greater apparent CO<sub>2</sub>-C losses observed in amended topsoil than subsoil, despite similar residue C retained in the whole soil, suggests enhanced mineralization of autochthonous SOC in topsoil, i.e., a positive priming effect occurred following residue addition. The presence of a priming effect is frequently observed when fresh organic material is added to soil (Blagodatskaya and Kuzyakov 2008), but this explanation cannot be experimentally confirmed in the present study since CO<sub>2</sub>-<sup>13</sup>C was not measured.

### Residue C in the whole soil and soil fractions

Residue C accumulated linearly in the whole soil but not in the fine fraction. The lower initial SOC concentration in the subsoil did not result in greater whole-soil residue C retention than in the topsoil at any residue input level. This differs from Hassink (1996), who found more labeled plant C remaining in the whole soil of less saturated than more saturated fine-textured soils after 53 days of incubation. However, our findings in this short-term incubation are consistent with current SOM models assuming linearity between C inputs and whole-soil SOC concentration (e.g., Century (Parton et al. 1987), RothC (Coleman et al. 1997)).

### *Residue input <10 g C kg<sup>-1</sup>*

Topsoil showed significantly greater residue C retention in the fine fraction than the subsoil when low amounts (<10 g C kg<sup>-1</sup> soil) of residues were added, contrary to our initial hypothesis. We suggest that this has been caused by greater biological activity in topsoil leading to more C being metabolized and consequently, more residue C being stabilized, compared to subsoil. The higher rate of CO<sub>2</sub>-C lost per unit of residue C added in the topsoil supports this conclusion. This is in agreement with results from laboratory incubations by Broadbent and Bartholomew (1948) and Sorensen (1981) with SOC-rich (≥20 g SOC kg<sup>-1</sup>) clayey soils that showed less CO<sub>2</sub>-C evolved per unit of substrate C added, with increasing substrate inputs. As well, Stewart et al. (2008a) indicated that after 0.5 year of incubation, greater amounts of residue-derived C were respired per unit of residue C added in topsoil than subsoil, and more residue-derived C was stabilized in topsoil than subsoil.

Greater microbial-driven decomposition in topsoil released compounds that were retained in the fine fraction. This likely resulted in a greater transfer of residue C from coarser soil fractions into the fine fraction when compared to the subsoil. This explanation agrees with Aita et al. (1997) who studied plant residue decomposition in surface soil in situ and found that the decrease in residue C concentration of coarse soil fractions led to an enrichment of residue C in finer ones. However, the residue-derived C-to-N ratios of the fine fraction (i.e., ~20) in topsoil indicate that this fraction is enriched in residue C and its composition resembles that of plant residues. The residues had a particle size between 100 and 1,000 μm and thus were broken down to enter the fine fraction, but the C-to-N ratio indicates they were likely only fragmented. Physical fragmentation of plant residues resulting from microbial-driven decomposition could be an important process by which residue-derived C is transferred from coarser to finer soil fractions (Aita et al. 1997).

### *Residue input >10 g C kg<sup>-1</sup>*

The subsoil retained significantly greater amounts of residue C in the fine fraction than the topsoil when high amounts of residues were added, which agrees with our hypothesis. The residue C retention in the fine fraction of the topsoil is expected to level off and approach SOC saturation (Hassink 1997; Stewart et al. 2008b) with increasing residue C inputs, and this is supported by the nonlinear residue C accumulation curve in the fine fraction of topsoil. The nonlinear residue C accumulation in topsoil could be due to a limitation of reactive mineral surfaces for SOC adsorption. In this soil, the SOC concentration of the fine fraction was at ~90 % of the SOC storage capacity as defined by Carter et al. (2003) when 40 g C kg<sup>-1</sup>

soil was added. In contrast, the SOC concentration of the subsoil fine fraction was at ~30 % of the SOC storage capacity (Carter et al. 2003) when 40 g C kg<sup>-1</sup> soil was added. Thus, more reactive mineral surfaces for SOC adsorption probably existed in the subsoil than in the topsoil.

In the SOC-rich topsoil, the nonlinear response of cumulative CO<sub>2</sub>-C losses with increasing residue input suggests that soil decomposition activity tended to be limited at high C addition rate. Such limitation might have been caused by the accumulation of coarse organic matter following saturation of the fine fraction. Indeed, in soils in which the clay fraction attained C saturation, Jagadamma and Lal (2010) argued that further SOC accrual is only possible in coarser soil fractions. Broadbent and Bartholomew (1948) and de Graaff et al. (2010) showed a levelling off of cumulative substrate-induced CO<sub>2</sub>-C loss in surface soils receiving up to 21.7 and 25.6 g C kg<sup>-1</sup> soil, respectively. Chantigny et al. (2000) observed a levelling off of the soil enzyme activity in field plots receiving the equivalent of 10 and 20 g C kg<sup>-1</sup> soil and suggested that this could be due to microbial community changes or nutrient limitation. In our study, N limitation was unlikely, since we added mineral N to facilitate C mineralization in order to reach a C/N of 10 (Recous et al. 1995) even with the highest C inputs. Nevertheless, a trend towards a “microbial saturation” and a levelling off of cumulative CO<sub>2</sub>-C losses in topsoil would favor residue C accumulation in this soil. The amount of residue C retained in the whole soil was slightly greater ( $P=0.054$ ) in topsoil than subsoil when 40 g C kg<sup>-1</sup> soil were added (Fig. 2a) and that was attributed to accumulation of residue C not associated with silt and clay particles.

## Conclusion

Short-term partitioning of residue C between the fine fraction and the whole soil was a function of initial SOC concentration, since this controlled biological and physical processes leading to short-term residue C stabilization. At low residue input levels, the greater biological activity in topsoil resulted in more physical fragmentation of the residue and greater residue C retention in the fine fraction, compared to the subsoil. At high residue input levels, the saturation of mineral surfaces in the fine fraction of the topsoil resulted in less residue C accumulating in this fraction than in the subsoil. One implication of this work is that SOC-poor soils receiving high C inputs (e.g., degraded and reclaimed soils, and subsoil horizons) would have greater potential to accumulate C in stable forms than SOC-rich soils.

Our short-term results are consistent with current SOC models assuming linearity between C inputs and whole-soil SOC stocks. However, in the long term, the saturation of the fine fraction might result in a faster turnover of newly added C present in coarser fractions, particularly in SOC-rich

topsoils. Future research and modeling efforts should focus on evaluating the role of initial SOC concentration on the partitioning of newly added C between coarse and fine soil fractions, and on the mean residence time of the C stored in each of these fractions.

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