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ORIGINAL PAPER

Carbon input from ^{13}C-labeled crops in four soil organic matter fractions

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Abstract This study quantified the fate of new carbon (C) in four crop sequences (lentil–wheat, canola–wheat, pea–wheat, and continuous wheat). Lentil–wheat and continuous wheat were grown in intact soil cores from a Brown Chernozem (BCz) and canola–wheat, pea–wheat, and continuous wheat in cores from a Dark Brown Chernozem (DBCz). In the first growing cycle, plants were pulse-labeled with ^{13}C-CO_2. Soil ^{13}C pools were measured once after the labeled growing cycle to quantify root biomass contribution to soil organic matter (SOM) in a single cycle and again after a second growing cycle to quantify the fate of labeled root and shoot residues. ^{13}C was quantified in four SOM fractions: very light (VLF), light (LF), heavy (HF), and water extractable organic matter (WEOM). For BCz lentil, BCz wheat, DBCz canola, DBCz pea, and DBCz wheat in the labeling year, root-derived C estimates were 838, 572, 512, 397, and 418 mg of C per kg soil, respectively. At the end of the second growing cycle, decreases in root-derived C were greater in the VLF, which lost 62 to 95 % of its labeled ^{13}C, than the LF (lost 21 to 56 %) or HF (lost 20 to 47 %). Root-derived C in WEOM increased 38 to 319 %. On DBCz, even though wheat and pea produced less aboveground biomass than canola, they generated similar amounts of SOC by fraction indicating that their residues were more efficiently stabilized into the soil than canola residues. Combining ^{13}C repeat-pulse labeling and SOM fractionation methods allowed new insights into C dynamics under different crop sequences and soil types. This combination of methods has great potential to improve our understanding of soil fertility and SOM stabilization.

Keywords Crop residues · Decomposition · Root biomass · Rhizodeposits · Canadian Prairie

Abbreviations

AAFC	Agriculture and Agri-Food Canada
C	Carbon
BCz	Brown Chernozem
DBCz	Dark Brown Chernozem
HF	Heavy fraction
LF	Light fraction
VLF	Very light fraction
SOC	Soil organic carbon
SOM	Soil organic matter
WEOM	Water extractable organic matter

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Introduction

Soil organic matter (SOM) is made of complex organo-mineral aggregates, and its genesis and structure are not yet fully understood (Schmidt et al. 2011). It is well known that C is the main constituent of all SOM, but there are still uncertainties about the rates of soil organic carbon (SOC) accumulation and mineralization in agricultural fields. Currently, total global SOC is estimated as 2,344 Gt (Stockmann et al. 2013), and several researchers believe that it is possible to substantially increase this amount (Lal 2005; Franzluebbers

2010; Macias and Arbestain 2010). Long-term studies have proven to be effective for determining which agricultural practices can enhance or decrease the net amount of SOC (Lemke et al. 2010). However, there is a gap in the scientific literature about annual gross production of SOC under different agricultural practices. This lack of knowledge is mainly due to the fact that the new C stabilized annually into the soil represents only a very small proportion of the total SOC and changes are hard to detect on an annual basis (Xu et al. 2011). As well, traditional methods to estimate root C production cannot account for microscopic root fragments and rhizodeposits that quickly mineralize in the soil. Without accurate estimates of belowground C production, accurate C flow models are difficult to produce.

Furthermore, to study SOC dynamics, bulk assessments of SOC production are not enough. The SOC contains different products of decomposition, which have different chemical recalcitrance levels and different stabilization mechanisms. Some of them will mineralize quickly, and others will bond with the mineral matrix and persist for long periods of time (Cotrufo et al. 2013; Smith et al. 2010; Bortolon et al. 2012). Soil density fractionation is a valuable technique to generate distinct SOM pools that may be correlated with theoretical SOM pools (Zimmermann et al. 2007). Density fractionation works on the premise that particles that float in dense liquid are composed mainly of freshly added, partially decomposed, and unstabilized organic matter (but can also contain charcoal material). In contrast, the particles that sink in heavy liquid are thought to be adsorbed to the clay and contain variable amounts of humified organic matter (Gregorich and Beare 2007). However, the process of crop residue decomposition and fresh SOM stabilization into operationally distinguishable pools has never been tracked directly.

Novel developments in stable isotope techniques have facilitated the tracking of crop C residues through different SOM pools and through time (Sangster et al. 2010). These isotopic techniques can be used to quantify root biomass production (Subedi et al. 2006). This study used pulse labeling with ^{13}C-CO_2 to address three scientific questions related to SOC input: Does the inclusion of legume crops in rotation with wheat in the Prairies increase the annual gross production of SOC? Into which soil pool is the new C going? What is the relative contribution of roots and shoots to the new SOC? To address these questions, the objectives of this study were (1) to determine, after a single growing cycle, the total belowground C production of four simple crop sequences (continuous wheat [Triticum aestivum], wheat–canola [T. aestivum–Brassica napus], wheat–lentil [*T. aestivum–Lens culinaris*], and wheat–pea [*T. aestivum–Pisum sativum*]) and (2) to assess the distribution of belowground residue C among soil fractions after a single growing cycle (with ^{13}C labeling) and, based on those residues, after a second growing cycle (without additional labeling).

Materials and methods

Due to the complexity of labeling crops with ^{13}C in the field, this study was designed to simulate field conditions in the greenhouse, focusing on typical crop sequences of the Canadian Prairies. One Gramineae (wheat, *T. aestivum cv. Lillian*), one oilseed (canola, *Brassica napus-T.*), and two Leguminosae (lentil, *Lens culinaris*; pea, *Pisum sativum*) were selected. In Canada, canola and pea are commonly cultivated in the Dark Brown Chernozomic (DBCz) soil zone and lentil in the Brown Chernozomic (BCz) soil zone. Wheat is widely cultivated in both soil zones. Agriculture and Agri-Food Canada (AAFC) research facilities at Swift Current (BCz) and Scott (DBCz), SK, Canada (Table 1) have long-term crop sequence studies with those respective crops. At both sites, the long-term plots are arranged in a randomized complete block design. Agriculture and Agri-Food Canada Swift Current has three field replications for each crop sequence and AAFC Scott has four. Large intact soil cores (12.5 L) were extracted from lentil–wheat and continuous wheat crop sequences at AAFC Swift Current and pea–wheat, canola–wheat, and continuous wheat sequences at AAFC Scott. For each crop sequence, six intact soil cores were extracted per field replicate. However, for practical reasons, the cores from field replicate #4 (AAFC Scott) of the DBCz canola–wheat and DBCz continuous wheat sequences were discarded. Ninety-six soil cores in total were used: 24 for DBCz canola–wheat and 18 for all other crop sequences. At the time of core extraction, all

Table 1 Soil and environmental features at AAFC-Scott and AAFC Swift-Current

Site	Location	Ecoregion	Soil type[a]	Soil texture	Clay (g kg^{-1})	pH	SOC (%)	N (%)	D_b[b] (g cm^{-3})
AAFC Swift	52°12'' N 107.24'' W	Mixed grassland	BCz	Sandy loam	220	6.4	2.0	0.2	1.16
AAFC Scott	52°23'' N 108.50'' W	Moist mixed grassland	DBCz	Loam	283	5.7	3.4	0.3	1.16

[a] *BCz* Orthic Brown Chernozem (Canadian System Soil Classification), Typic Haplustoll (U.S. Soil Taxonomy); *DBCz* Orthic Dark Brown Chernozem (Canadian System Soil Classification), Typic Hapludoll (U.S. Soil Taxonomy)

[b] D_b bulk density

sequences had completed a wheat phase the previous year. Aluminum cylinders (20 cm diameter and 39 cm depth) were inserted into the soil using a truck-mounted hydraulic punch (Stumborg et al. 2007) and carefully withdrawn to preserve soil structure. The cores were stored at 4 °C until seeding.

Greenhouse design and ^{13}C labeling

In July 2009, lentil and wheat from AAFC Swift Current (BCz) and canola, pea, and wheat from AAFC Scott (DBCz) were seeded into their respective soil cores according to crop sequence at a density of six seeds per soil core approximating seeding rates in the field (125 seeds m^{-2}). Prior to seeding, cores were fertilized with 15.2 kg P ha^{-1} as mono-ammonium phosphate (11–52–0) and 74.4 kg N ha^{-1} as mono-ammonium phosphate and urea (46–0–0). Lentil and pea were inoculated with a granular *Rhizobium leguminosarum* inoculant, "Nodulator" (Becker Underwood Inc., Saskatoon, SK, Canada). The inoculant was placed with the seed in the soil cores at the equivalent recommended rate of 8 kg ha^{-1}. Plants were grown in a photoperiod of 18 h at an average temperature of 22 ± 4 °C in a greenhouse. Four plants were grown to maturity in each soil core. Extra plants in cores were removed immediately after germination. The soil cores were arranged in six rows (Fig. 1). Each row had three cores each of BCz lentil, BCz wheat, DBCz pea, and DBCz wheat and four cores each of DBCz canola (BCz and DBCz in serial arrangement totaling 16 cores per row). Rows #1, 2, 3, and 4 were labeled with $^{13}CO_2$; rows #5 and 6 were not labeled and were kept in a separate room of the greenhouse to prevent ^{13}C contamination. All soil cores from both sites were watered every second day with approximately 1 L of tap water.

Following Sangster et al. (2010), labeling was carried out in sets of four cores (Fig. 1) in hermetic polymethyl methacrylate chambers. The soil cores were permanently fixed into the chamber base as a set of four, but chamber bases were randomly rotated on the greenhouse benches every week to ensure homogeneous plant illumination. Each set was pulse-labeled weekly for 2 h starting 20 days after germination and continuing to 75 days after planting, for a total of eight labeling sessions. Labeling was performed in two batches of four sets between 10 a.m. and 2 p.m. The order in which the sets were labeled was random. The soil surface was isolated from the enriched atmosphere during labeling by covering it and making a seal around plant stems with GLAD Press'n Seal Freezer® wrap (The Clorox Company, Oakland, CA, USA). During the labeling sessions, the total CO_2 concentration in the chambers was maintained at approximately 380 to 430 ppm, and the atmospheric enrichment was 33 atom % $^{13}CO_2$. The CO_2 was devolved into the chamber by injecting a saturated solution of ^{13}C-enriched $NaHCO_3$ (33 % atom ^{13}C) through a septum port into a beaker with 12 M HCl. Total CO_2 concentration was monitored with an infrared gas analyzer (IRGA) (S151 Infrared CO_2 Analyzer, Qubit Systems, Kingston, ON, Canada). Watering stopped 1 week after the last labeling session.

After watering stopped, plants were air-dried for 3 weeks in their soil cores and harvested to the soil surface. Dry weight of straw and grain was determined. The soil cores in rows #2, 4, and 6 were removed from the greenhouse (for soil and plant analyses), and cores in rows #1, 3, and 5 were kept intact for the second growth cycle. Shoot residues (leaves, stems, and pod/husks) from all harvested cores were ground (<5 mm) with a coffee grinder. Enriched ground plant residues from row #3 were mixed with the 0–10-cm soil of their analogical non-enriched core from row #5 (i.e., enriched shoot and non-enriched root). Non-enriched ground plant residues from row #5 were mixed with the 0–10-cm soil of their analogical enriched core in row #3 (i.e., enriched root and non-enriched shoot). Because canola produced notably more shoot residue than the other crops, more residues were added to the soil for canola than the other crops (15 g canola versus 10 g other crops, per soil core). For the second growing cycle, cores from rows #1, 3, and 5 were all seeded with wheat (four plants in each soil core); plants were grown and harvested as for the first growing cycle but without pulse labeling.

Soil and plant analysis

At the end of both growing cycles, samples (5 g) of roots, stems, leaves, pods/husks, and grains of lentil, canola, pea, and wheat were oven-dried at 50 °C, finely ground with a ball mill, and a subsample was analyzed for % C, % N, and $\delta^{13}C$ with a Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) coupled to a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.) in the Stable Isotope Facility at the University of Saskatchewan. Also, at the end of both growing cycles, soil cores were extruded, and the 0- to 10-cm soil layer was extracted for analysis. All soil from the 0–10-cm layer (including any roots present) was air dried, manually ground with a marble rolling pin, sieved to 2 mm, and homogenized prior to SOM characterization. Due to high clay content in the BCz and the DBCz, after air-drying, the soil shrank and became very hard. Hence, it was not possible to preserve the aggregates during grinding. Grinding coarse roots together with the bulk soil allowed estimation of total belowground C production for the labeled crops.

Water extractable organic matter (WEOM) consists of labile organic molecules that microorganisms can use as a source of energy; it was extracted from bulk soil samples with deionized water following Chantigny et al. (2007). The light fraction (LF) is believed to be a

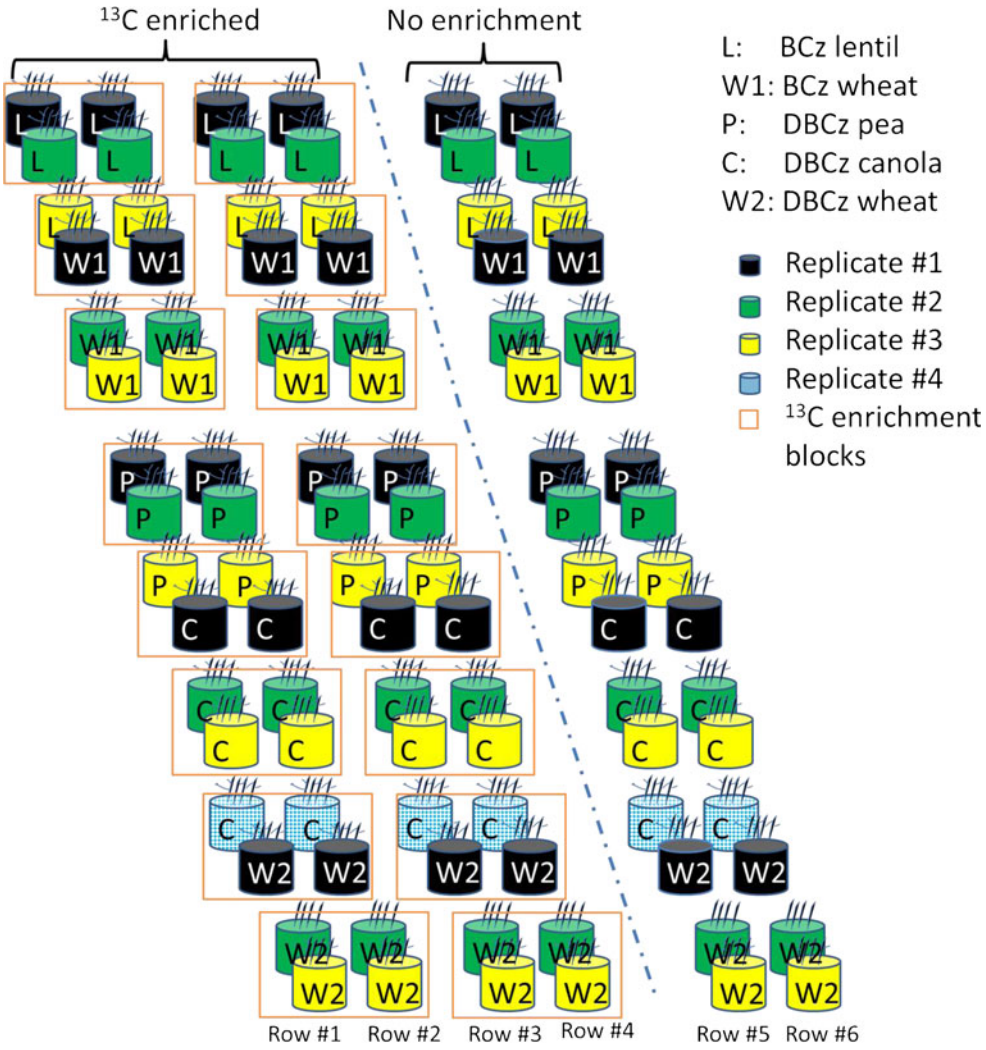

Fig. 1 Scheme of the experimental design applied in the greenhouse during the first growing season. Due to the design of the labeling chamber, labeling was performed in sets of four cores

transitional pool of organic matter between fresh materials and stabilized SOM, whereas the heavy fraction (HF) is bound with mineral particles and is considered to be more decomposed and stable (Gregorich and Beare 2007). The LF and HF were isolated with a dense liquid (NaI) at 1.7 g mL^{-1}, following Gregorich et al. (2006). The LF was further fractionated into very light fraction (VLF) (mainly fresh and identifiable plant residues) and LF with deionized water following the same Gregorich and Beare (2007) procedure. No energy source was used to disrupt the soil during the separation procedure. With only one exception (a canola grain), no plant fragments were observed in the LF and absolutely none in the HF. The fractionated materials were dried, finely ground with a ball mill, and subsequently, for each soil fraction, % C, % N, and $\delta^{13}C$ subsamples were analyzed as for the plant materials. No additional soil treatment or preparation was made prior to mass spectrometer analysis. Natural abundance of ^{13}C in soil and plant fractions was determined from the row #6 cores. The $\delta^{13}C$ values were calculated from the measured isotope ratios of the sample and standard as follow:

$$\delta^{13}C\ (‰) = (R\ \text{sample} / R\ \text{standard} - 1)\ \text{x}\ 1000$$

where $R = {}^{13}C/{}^{12}C$ (molar ratio).

From the $\delta^{13}C$ results, the milligrams of derived C per kg of soil for each soil fraction was calculated per adaptation of the Subedi et al. (2006) equation:

$$A = ((\text{mg of soil}(0-10\ \text{cm})) \times (\%\ \text{soil fraction}\ x/100) \times (\%\ \text{SOC}\ x/100))/\text{kg of soil}(0-10\ \text{cm})$$

The % ^{13}C of SOC for each soil fraction (B) was calculated as:

$$B = \frac{(\partial_{\text{enriched soil fraction}} - \partial_{\text{reference soil fraction}})}{(\partial_{\text{enriched roots or shoots}} - \partial_{\text{reference roots or shoots}})} \times 100$$

where the "enriched soil fraction" indicates the corresponding four soil fractions studied (VLF, LF, HF, and WEOM) and enriched with ^{13}C, "reference soil fraction" is the soil fractions

from the soil cores without ^{13}C labeling, "enriched roots or shoots" are the corresponding ^{13}C enriched root or shoot samples, and "reference roots or shoots" are the corresponding root and shoot samples from soil cores without labeling.

The derived *C* for each fraction was calculated by:

$$C = (A \times B)/100$$

The total belowground C production at the end of the first growing cycle was calculated as the sum of belowground derived C in each soil density fraction, which included all root materials. The root-derived C at the end of the first growing cycle in LF and HF was used as a proxy of unrecoverable roots and rhizodeposits (URaR) because, in these fractions, no plant parts were distinguishable. In effect, the LF and HF fractions would have been lost with traditional root estimation techniques, which would only capture the equivalent of our VLF. Accordingly, the URaR % was calculated as:

$$\text{URaR } \% = (\text{LF} + \text{HF})/(\text{VLF} + \text{LF} + \text{HF})$$

The root-derived C at the end of the second growing season was calculated using data from the cores with enriched root and non-enriched shoot. Accordingly, the shoot-derived C at the end of the second growing season was calculated using the data from the cores with enriched shoot and non-enriched root. Total root-derived SOC was calculated as the sum of the root-derived C in the VLF, LF, and HF. Percent change in root-derived ^{13}C in each SOM fraction was calculated as:

$$\%\ \text{change} = \left[1 - \frac{\text{derived C } 2^{\text{nd}}\ \text{growing cycle}}{\text{derived C } 1^{\text{st}}\ \text{growing cycle}}\right] \times 100$$

Statistical analysis

Data were checked for normality with the Shapiro–Wilk normality test. A factorial ANOVA was run separately for each soil type. In these ANOVAs, the derived C (mg of C per kg soil) was entered as response variable, and the crops (BCz lentil, BCz wheat, DBCz canola, DBCz pea, and DBCz wheat), the SOC fractions (WEOM, VLF, LF, and HF), and the enriched residue source/cycle (root at the end of the first growing sequence, root at the end of the second growing sequence, and shoot at the end of the second growing sequence) were entered as explanatory variables. In addition, a third factorial ANOVA was run with only the wheat crops. This ANOVA was run in the same fashion as the previous two but with the difference of having soil type (BCz or DBCz) instead of crops as first explanatory variable. Post hoc tests with the function pairwise *T*-test were made for the explanatory variables independently when the ANOVAs detected significant differences. No statistical test was made with the URaR and percent change in root-derived C because these results were calculated with values already processed in ANOVAs. Mathematical calculations and descriptive statistical analyses were done with Microsoft Excel XP®. Statistical testing was done using the statistical program R Foundation for Statistical Computing version 2.8.1 (R Development Core Team 2008); effects were deemed significant at $P < 0.05$.

Results

For BCz lentil, BCz wheat, DBCz canola, DBCz pea, and DBCz wheat, the total aboveground dry biomass values were 19.0, 16.7, 48.7, 16.6, and 18.5 g per soil

Table 2 Brown Chernozem (BCz) root and shoot-derived C in SOM fractions at the end of the first and second growing cycles

Crop	Organic matter fraction[a]					URaR %[b]
	WEOM	VLF	LF	HF	Total	
mg of derived C per kg of soil[c]						
Root 1st cycle						
Lentil	0.17 (0.02)	429 (76)	195 (88)	213 (41)	838 (148)	48.6 (6.4)
Wheat	0.12 (0.03)	218 (64)	156 (54)	197 (26)	572 (60)	61.7 (8.6)
Root 2nd cycle						
Lentil	0.49 (0.09)	26 (5)	149 (25)	171 (1)	347 (22)	
Wheat	0.51 (0.06)	17 (4)	69 (3)	139 (21)	225 (32)	
Shoot 2nd cycle						
Lentil	0.53 (0.05)	61 (25)	166 (27)	137 (16)	364 (71)	
Wheat	0.43 (0.18)	45 (8)	98 (18)	97 (21)	241 (56)	

[a] *WEOM* water extractable organic matter, *VLF* very light fraction, *LF* light fraction, *HF* heavy fraction

[b] *URaR* unrecoverable roots and rhizodeposits

[c] Mean values (standard error of the mean)

core, respectively. After the density fractionation, the VLF was composed mainly of recognizable root fragments; the LF looked like a coarse, gray powder; and the HF was brown or dark brown without recognizable vegetal material.

C input from the crops on BCz

Although BCz lentil produced notably more root-derived and shoot-derived total C than BCz wheat (Table 2), the factorial ANOVA showed no significant differences between these two crops (P=0.06). The ANOVA did show significant differences among soil fractions (P<0.01) and among residue source/cycles (P<0.01). The interactions between crops and soil fractions and between crops and residue source/cycle were not significant (P=0.63 and P=0.72), but the interaction between soil fractions and residue source/cycle was significant (P<0.01). This interaction was caused by the increase of root-derived C in WEOM between the first and second growing cycles while all the other soil fractions decreased (Table 4). The three-way interaction was not significant (P=0.54). The post hoc test for soil fractions showed that WEOM C was significantly lower than VLF, LF, and HF C (P<0.01 in all cases), but VLF, LF, and HF C were statistically equal (P>0.5 in all cases). The post hoc for residue source/cycle showed that the root-derived C at the end of the first growing cycle was significantly higher than the root-derived or shoot-derived C at the end of the second growing cycle (P<0.01 in both cases), but the root-derived and shoot-derived C at the end of the second growing cycle were statistically equal (P=0.91). The URaR % for BCz lentil and BCz wheat were 48.6 and 61.7, respectively (Table 4).

C input from the crops on DBCz

The factorial ANOVA for DBCz soil cores showed no significant differences among crops (P=0.08), but as for BCz, significant differences were found among soil fractions (P<0.01) and among residue source/cycles (P<0.01). The interaction between crop and root–shoot–first–second growing cycle was not significant (P=0.79), but the interactions between crop and soil fraction and between soil fraction and residue source/cycle were significant (P=0.02 and P<0.01). On DBCz, the HF had the highest values of derived C among the fractions, with the only exception being wheat root-derived VLF C at the end of the first growing cycle (Table 3). Accordingly, the post hoc test showed that HF C was significantly greater than VLF (P=0.02), LF (P=0.02), and WEOM (P<0.01). The WEOM was lower than all the other soil fractions (P<0.01 in all cases), and VLF and LF were statistically equal (P=0.89). Analogously to BCz, in DBCz, the root-derived C at the end of the first growing cycle was significantly greater than the root-derived or shoot-derived C at the end of the second growing cycle (P<0.01 in both cases), but there were no significant differences between the root-derived and shoot-derived C at the end of the second growing cycle (P=0.66). The URaR % for DBCz canola, DBCz pea, and DBCz wheat were 77.1, 70.0, and 44.7, respectively (Table 4).

C input from BCz wheat and DBCz wheat

The factorial ANOVA for BCz wheat versus DBCz wheat displayed significant differences between these two soils (P=0.02). As for previous ANOVAs, significant differences were found among soil fractions (P<0.01) and among residue source/cycles (P<0.01). The significantly higher amount of root- and shoot-derived C under BCz (P=0.02) was driven by greater amounts of derived C in the LF and HF at the end of the first and second growing cycles.

Change in remaining labeled ^{13}C between the first and second growing cycles

Both soil types (BCz and DBCz) had similar patterns of change in remaining derived SOC among soil fractions. For both, WEOM was the only SOM fraction that increased in derived C from the end of the first growing cycle to the end of the second growing cycle, and VLF lost notably more derived C than LF or HF for all studied crops (Table 4). In DBCz soil cores, pea had lower percent changes than canola or wheat in all soil fractions. In BCz soil cores, lentil had lower percent changes than wheat in all soil fractions except VLF.

Discussion

Belowground C inputs

Reliable estimates of belowground biomass production are essential for agronomic management, biogeochemical cycle research, and terrestrial C estimations (Russell and Ellis 1968; Yuan and Chen 2010). Chernozems/Mollisols are the most fertile soils and cover 7 % of ice-free lands (Soil Survey Staff 2010), yet only a few published studies quantify root biomass production in Chernozemic soils (Gan et al. 2009a; 2009b; 2011). In a prairie agro-ecosystem, Gan et al. (2011) estimated root biomass of legume, oilseed, and wheat crops in a Chernozemic soil using conventional techniques (i.e., root washing). The average estimates of C mass in the roots in the 0–20 cm depth in an irrigated field for lentil, wheat, canola, and pea were 182, 308, 466, and 167 kg ha^{-1}, respectively. Based on the soil bulk density of the BCz and DBCz (Table 1), it is

Table 3 Dark Brown Chernozem (DBCz) root and shoot-derived C in SOM fractions at the end of the first and second growing cycles

Crop	Organic matter fraction[a]					URaR %[b]
	WEOM	VLF	LF	HF	Total	
mg of derived C per kg of soil[c]						
Root 1st cycle						
Canola	0.09 (0.02)	117 (18)	180 (30)	215 (16)	512 (38)	77.1 (3.2)
Pea	0.12 (0.01)	119 (25)	126 (59)	152 (18)	397 (97)	70.0 (5.3)
Wheat	0.11 (0.03)	230 (53)	53 (10)	134 (11)	418 (71)	44.7 (5.3)
Root 2nd cycle						
Canola	0.36 (0.07)	15 (1)	88 (13)	137 (12)	242 (35)	
Pea	0.17 (0.03)	37 (24)	99 (54)	100 (17)	238 (58)	
Wheat	0.37 (0.11)	11 (1)	39 (7)	71 (17)	122 (17)	
Shoot 2nd cycle						
Canola	0.60 (0.15)	66 (10)	82 (9)	117 (26)	265 (48)	
Pea	0.40 (0.13)	84 (14)	46 (4)	91 (12)	222 (12)	
Wheat	0.31 (0.05)	49 (5)	50 (5)	78 (11)	178 (23)	

[a] *WEOM* water extractable organic matter, *VLF* very light fraction, *LF* light fraction, *HF* heavy fraction

[b] *URaR* unrecoverable roots and rhizodeposits

[c] Mean values (standard error of the mean)

possible to convert our results (total root first cycle in Tables 2 and 3) to kilograms per hectare. These converted estimates of root C mass in the 0–10-cm layer of BCz lentil, BCz wheat, DBCz canola, DBCz pea, and DBCz wheat were 972, 664, 595, 461, and 485 kg ha^{-1}, respectively. Thus, our estimates are noticeably higher than those reported by Gan et al. (2011), which is probably due to better accounting for roots that are no longer recoverable. As reported by Subedi et al. (2006), traditional root measurements do not account for microscopic roots, highly decomposed roots, root loss during washing, root exudates, and mucilage, and these authors found that traditional methods underestimated root biomass by 36 % in their pot experiment. However, higher amount of root C recovered compare to Gan et al. (2011) could also be due to greater stabilization. Even slight variations in clay percentage and mineralogy or variations in water regime could have produced notable effects on C stabilization (Dungait et al. 2012). For example, Wichern et al. (2007) used ^{13}C-glucose labeling to assess percentage of C from rhizodeposition with respect to total root C biomass in pea and oat in a Luvisol and obtained estimates of 29.6 and 30.8 %, respectively. In our study, the percentage of C from URaR for pea was 70.0 % (Table 2). This large difference between our results and these reported values is likely due to pedo-environmental differences that highlight the importance of taking into account the climate and soil characteristics when estimating rhizodeposition.

Table 4 Percentage change of the root-derived ^{13}C in each SOM fraction from the first to the second growing cycles

Crop	Organic matter fraction[a]			
	WEOM	VLF	LF	HF
% of increase (+) or decrease (-)[b]				
BCz				
Lentil	+177 (58)	-94 (4)	-23 (14)	-20 (15)
Wheat	+319 (89)	-92 (3)	-56 (39)	-30 (12)
DBCz				
Canola	+264 (104)	-87 (5)	-51 (25)	-36 (8)
Pea	+39 (34)	-62 (32)	-21 (140)	-34 (7)
Wheat	+230 (41)	-95 (1)	-27 (21)	-47 (8)

[a] *WEOM* water extractable organic matter, *VLF* very light fraction, *LF* light fraction, *HF* heavy fraction, *BCz* Brown Chernozem, *DBCz* Dark Brown Chernozem

[b] Mean values (standard error of the mean)

Because the DBCz in this study had more clay than the BCz (Table 1), it might be expected that more rhizodeposits would be stabilized under DBCz wheat compared to BCz wheat under comparable watering conditions, but this was not the case. The higher amount of rhizodeposits found under BCz wheat could reflect intrinsic soil fertility. Brown Chernozemic soils are less fertile than DBCz (lower SOC percentage); therefore, wheat plants would have to produce more root hairs, microscopic roots, and exudates on the BCz in order to obtain the same amount of nutrients (Campbell and Souster 1982; TaiWen et al. 2010).

To calculate total belowground C input of lentil, wheat, canola, and pea in the Canadian Prairies (Chernozemic soil), Gan et al. (2009a) estimated the C from rhizodeposits as 39 % of total C in roots. However, rhizodeposition can vary among

crops. For BCz lentil, BCz wheat, DBCz canola, DBCz pea, and DBCz wheat, our percentages of C from URaR were 48.6, 61.7, 77.1, 70.0, and 44.7, respectively (Tables 2 and 3). Consequently, the net primary production of these crops in Chernozemic soils could be higher than previously estimated.

A large variance in rhizodeposit production in the 0–10 cm depth among lentil, canola, and pea was also notable and might be the result of morphological differences. However, recent rooting system studies, made in the same agro-ecosystem, showed only slight differences in vertical distribution of the root surface area for lentil, canola, pea, and wheat (Gan et al. 2009a; Liu et al. 2011). Repeat pulse-labeling experiments should be reproduced directly in the field over several years to conclusively assess the horizontal and vertical distributions of rhizodeposits, the effects of weather on rhizodeposition, and the variation in rhizodeposition throughout the growing cycle.

Quantity and fate of derived SOC after a second growing cycle

At the end of the second growing cycle, in the cores with labeled root residues, all crops had similar patterns of residue decomposition reflected in the different SOM pools. The highest decay (reflected in losses of ^{13}C between the end of the first versus the end of the second cycle) occurred in VLF followed by LF and HF; in contrast, the WEOM increased substantially. In the VLF, pea had the least change in root-derived ^{13}C, lentil and wheat (BCz and DBCz) had the highest rates, and canola had an intermediate rate. The taproot of dicotyledonous plants, which include lentil, canola, and pea, is known to be more recalcitrant than the adventitious roots of monocotyledonous plants like wheat (Evert et al. 2006). The high decay in lentil was likely due to a high number of root hairs in the 0–10-cm soil layer and low C/N ratio (Campbell et al. 1992). Pea, the crop that produced the least amount of root-derived SOC, had the lowest decay rate, which suggests that although it produced lower amount of belowground residues, those residues could be more recalcitrant. It is also possible that on DBCz, root hairs from pea could penetrate better into the soil microaggregates than canola or wheat. Root hairs occluded in soil aggregates are assumed to be protected from quick decomposition (Rasse et al. 2005). A third possibility could be that pea roots produce molecular compounds that directly (or indirectly, after transformation by soil microbes) interact with and are stabilized by the organo-mineral surfaces (Sollins et al. 1996; von Lutzow et al. 2006).

Comparing the amount of shoot-derived SOC at the end of the second growing cycle, BCz lentil on average tended to have more remaining shoot-derived SOC than wheat, canola, or pea. From the perspective of residue recalcitrance, each of the taxa investigated in this study appears to have a strategy to resist microbial decomposition. The *Poaceae* (grass family) have a particular stele system that leads to a low lignin content, but it possesses silica phytoliths in the leaves that make the wheat residues hard to digest for soil biota (Judd et al. 1999). Oilseeds and legumes have a low C/N ratio that would promote micro-organism growth, but these plants produce more lignin and more alkaloids than wheat (Judd et al. 1999). Interestingly, canola did not produce more shoot-derived SOC even though it had a notably higher aboveground biomass than the other plants. As noted by Sangster et al. (2010), canola residues may be more recalcitrant than pea due to significantly greater proportions of lignin in the stems and leaves.

Two previous studies had investigated the effect of legume crops on SOC status in Chernozemic soils. Liang et al. (2003) found a significant positive impact of legume crops on the VLF–LF SOC among several sites in BCz and DBCz soils of Saskatchewan. On the other hand, Lemke et al. (2007) reported that even if legume crops were producing significantly less aboveground residues than canola and wheat, there was no significant difference in the amount of SOC per hectare among legume crops, oilseeds, and wheat in long-term rotations. The factorial ANOVAs made on BCz and DBCz were unable to detect significant differences among crops ($P=0.06$ and $P=0.08$). This is likely due to the fact that only the 0–10-cm increments of soil were analyzed; more indicative differences might have occurred deeper in the soil profile.

Evaluating the root- versus shoot-derived SOC at the end of the second growing cycle, the amounts of remaining SOC from the two sources were statistically equal. However, higher amounts were expected for shoot because at the end of the second growing cycle, the ^{13}C-labeled roots had been in contact with the soil for two cycles versus only one cycle for the ^{13}C-labeled shoots. The greater lability of the shoot may be attributable to grinding of the shoot residues and/or morpho-molecular differences between shoot and root, with the root having a stronger region of xylem in the center (Judd et al. 1999). Similar to our results, Rasse et al. (2005) estimated that the mean residence time in soils of root-derived C is higher than that of shoot-derived C. Those authors suggest that roots have specific SOM protection mechanisms, including physico-chemical protection, protection through root-hair activities, and chemical interactions with metal ions.

Dynamics of derived ^{13}C

The changes in derived C through the different SOM pools between the first and second growing cycles were comparable among studied crops. That is, all the crops had an increase in derived C in the WEOM and a decrease in the VLF greater than the decrease in LF or HF. The increase in the derived WEOM ^{13}C, when all the other soil fractions had lost labeled ^{13}C, suggests that several SOM pools might contribute to the organic C in the WEOM. The small amount of labeled ^{13}C in the VLF from the first growing cycle remaining at the end of the second growing cycle indicates that the VLF is a highly

labile fraction. The high loss of root-derived SOC in the HF indicates that a substantial proportion of newly fixed C in this fraction is still labile and can be accessed and metabolized by microorganisms. We suggest that fresh crop residues are initially part of the VLF; then, soil biota solubilizes a fraction of this VLF, and the partially degraded plant tissues move into the LF. With further decomposition, the fragmented tissues in the LF become free macromolecules and progress to the WEOM and/or are directly absorbed into the HF. In the WEOM, residues are consumed by soil microbes, and some of the water soluble compounds are fixed into the mineral matrix of the soil in the HF.

Conclusion

This study was the first to use the repeat ^{13}C pulse-labeling method to trace the fate of crop residue C in different SOM pools in Chernozemic soils. As well, this study was the first to generate estimates of annual gross production of SOC from roots versus shoots under different prairie crop sequences. The three primary findings were the following: (1) Even though wheat and legume crops produced less aboveground biomass than canola, they generated similar amounts of SOC in the different SOM pools. (2) In prairie agro-ecosystems, root and rhizodeposition SOC inputs might be significantly higher than previous estimates. (3) Despite roots producing less biomass than shoots, roots and shoots contributed equal amounts of derived SOC at the end of the second growing cycle, which corroborate that root C residues are more recalcitrant and/or stabilized more efficiently in Chernozomic soil than shoot C. Altogether, these results may help to generate soil management policies and strategies that optimize the utilization of crop residues without negatively affecting soil fertility and carbon balance. Nevertheless, to corroborate our findings and to determine exactly how long it takes for fresh plant residues to achieve a high level of stability in soils, long-term field studies using similar methodological approaches and considering the total rooting depth of crops are needed.

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