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Field ^{13}C Pulse Labeling of Pea, Wheat, and Vetch Plants for Subsequent Root and Shoot Decomposition Studies

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ABSTRACT: Isotopic labeling of plants is useful in tracking the fate of carbon (C) from different plant parts in a soil-plant system when these parts decompose simultaneously. Pulse labeling is a relatively simple technique and is amenable for use in the field. Therefore, we evaluated a $^{13}CO_2$ pulse-labeling method to label crop plants under subtropical field conditions for simultaneous root and shoot decomposition studies. Wheat (*Triticum aestivum L.*), pea (*Pisum sativum L.*), and vetch (*Vicia sativa L.*) plants were grown inside polyvinyl chloride (PVC) cylinders and pulse labeled once a week for a total of 11 times. After harvest, ''paired'' treatments were designed by combining ^{13}C-labeled shoots with unlabeled roots and unlabeled shoots with ^{13}C-labeled roots, resulting in six treatments (2 combinations × 3 species), plus an unamended control treatment. The ^{13}C enrichment of plant parts, chemical fractions, ^{13}C recovery, and distribution in roots, shoots, and soil were determined. Soil CO_2 emissions were measured continuously by the alkaline trap method for 180 days. Plant dry matter production and chemical composition were not modified by ^{13}C labeling. The maximum level of ^{13}C enrichment ($\delta^{13}C$) in plants was +495 ‰ in wheat, +426 ‰ in pea, and +378 ‰ in vetch plants. All three crops showed similar patterns of ^{13}C distribution in the following order: shoots > roots > soil. On average, 81 to 89 % of the recovered ^{13}C was in the shoots, 7 to 14 % was in the roots, and 2.7 to 4.3 % was in the soil. The rate of C mineralization and cumulative C mineralization were not different between ''paired'' treatments of the three crops, showing that the paired treatments were equally degradable. The pulse-labeling technique used under field conditions allowed for production of sufficiently labeled wheat, pea, and vetch plants. Therefore, it is a practical approach with respect to resource demand (tracer and labor costs), and it is suitable for *in situ* labeling.

Keywords: $^{13}CO_2$, C mineralization, chemical fractions, isotopic homogeneity, plant parts.

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INTRODUCTION

Crop residues are the primary source of all soil organic matter (SOM) and the type of crop residues influence carbon (C) sequestration in soils. Differences in the quality of above- and belowground plant residues and in their location during decomposition under a no-till (NT) system result in different C mineralization and stabilization pathways, which are likely to contribute to soil C in different ways and amounts. It is therefore important to distinguish between these two components of plants residues for a better understanding of their fate in soil.

Isotopic labeling techniques are reliable tools for tracking above- and belowground residue C dynamics in soil. In a literature survey, we found that most of the labeled residues that were used for C dynamic studies were labeled under controlled conditions (Aita et al., 1997; Sanaullah et al., 2011). This finding may call into question the actual estimates of C contributions to SOM, especially from root residues, because of possible bias introduced into root decomposition due to removing, washing, and drying these roots, which not only results in the loss of fine roots and soluble C, but also destroys root-rhizosphere interaction (Aulen et al., 2012; Tahir et al., 2016). Although it is a complicated task to label plants under field conditions (Comeau et al., 2013), it is necessary particularly where NT is practiced, to replicate actual field conditions of root (i.e., intact roots) and shoot decomposition.

Field labeling has shown potential in investigating C residue dynamics under actual field conditions (Puget and Drinkwater, 2001). However, until the present, few field experiments have been reported with the purpose of studying the simultaneous decomposition of above- and belowground residues by using *in situ* labeling (Puget and Drinkwater, 2001; Williams et al., 2006; Kong and Six, 2010). For field isotopic labeling, pulse labeling is better than continuous labeling because of its easy handling and simple instrumentation, but successful atmospheric labeling with $^{13}CO_2$ poses a number of challenges, e.g., homogeneous distribution of labels within the plants part and chemical fractions and the level of ^{13}C enrichment for subsequent detection. It has been suggested that if the label is applied at regular intervals that are frequent enough to represent C assimilation during the growing season, it may be possible to obtain adequate homogeneity for tracing plant residue decomposition through various C pools (Bromand et al., 2001). The field-labeling experiments that were developed in the past were mainly focused on the enrichment of residues with ^{13}C, and investigators did not check how the C isotope applied was distributed within the chemical fractions (Puget and Drinkwater, 2001; Williams et al., 2006). It is therefore important to know how the ^{13}C label is distributed among chemical fractions because different chemical fractions decompose at different rates (Trinsoutrot et al., 2000) and heterogeneous labeling may impact the interpretation of SOM analysis in subsequent decomposition studies (Sangster et al., 2010).

Although ^{13}C pulse labeling has been used to study carbon partitioning and dynamics under field conditions in various parts of the world, it has not been evaluated in a Brazilian subtropical climate under NT conditions. Therefore, we set up a ^{13}C pulse-labeling experiment for labeling crop residues of three winter crops (wheat, pea, and vetch) under subtropical NT field conditions. We hypothesized that uniform distribution of the label in plant parts and biochemical fractions can be achieved by applying $^{13}CO_2$ pulses at regular intervals over the growing season. The objectives were: (i) to assess the homogeneity and the degree of ^{13}C enrichment among plant parts and in chemical fractions; and (ii) to compare the decomposition dynamics of the labeled and unlabeled shoot and root residues by designing ''paired'' treatments, i.e. labeled root residues combined with unlabeled shoot residues and vice versa for the three crops.

MATERIALS AND METHODS

Site description and experimental setup

The field experiment was performed in the experimental area of the Soil Department of the *Universidade Federal de Santa Maria* (UFSM) in the state of Rio Grande de Sul (RS), Brazil (29° 41' S, 53° 48' W; at approximately 90 m a.s.l.). The soil is classified as a Typic Paleudalf (Soil Survey Staff, 2010) and as a *Argissolo Vermelho Distrófico arênico* (Santos et al., 2013) with 100 g kg^{-1} clay, 270 g kg^{-1} silt, 630 g kg^{-1} sand, 1.5 Mg m^{-3} bulk density, and 6.6 g kg^{-1} C and -19.17 $\delta^{13}C$ in the top 0.00-0.30 m soil layer. The experimental area has a humid subtropical climate by the Köppen classification system. Cumulative rainfall was 350 mm and mean air temperature ranged from 5.7 to 28.7 °C during the study period. Prior to the experiment, the site had been under NT crop rotation with oat (*Avena sativa*) or vetch (*Vicia sativa L.*) in the winter, and corn (*Zea mays*) or soybean (*Glycine max*) in the summer for 15 years.

On 15 May 2013, an area of 625 m^2 (25 × 25 m) within the experimental site was marked off, cleared, and fenced for the labeling of wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), and vetch (*Vicia sativa* L.). A split-plot randomized complete block design was used in the experiment, with three replications. The main plots consisting of wheat, pea, and vetch were divided into two equal microplots. One microplot in each main plot was used for pulse labeling with ^{13}C, while the other was kept unlabeled. The microplots had the same dimensions as the labeling chambers (0.80 × 0.80 m). Inside each microplot, nine open-ended polyvinyl chloride (PVC) cylinders (0.35 m height × 0.20 m diameter, area of 0.0314 m^2) were hydraulically forced to a soil depth of 0.30 m, with 0.05 m above the soil surface. In addition, twelve soil cylinders were similarly forced into soil in the area between each microplot as controls without plants. On 29 May 2013, wheat, pea, and vetch were sown in these soil cylinders. After emergence, the plants were thinned to four plants for wheat, two plants for pea, and four plants for vetch in each cylinder.

^{13}C labeling

Modified portable chambers (Figure 1) were used for *in situ* ^{13}C pulse labeling according to Sangster et al. (2010). The plants were pulse labeled with $^{13}CO_2$ from 22 June to 18 September, 2013. The ^{13}C-labeling events usually took place between 9:00 and 11:00 a.m. for a period of 1.5 h. The labeled microplots were enclosed inside labeling chambers, which rested on galvanized steel bases and were sealed with water. A 30 mL volume of 2 mol L^{-1} HCl was injected into the plastic container (100 mL) using a septum. The intended enrichment of CO_2 in the chamber was 33 atom% ^{13}C. Before every pulse-labeling event, the diluting effect of unlabeled CO_2 inside the labeling chamber was compensated by injecting $^{13}CO_2$ (99 atom % ^{13}C). For that reason, CO_2 concentration was allowed to fall to 266 mg L^{-1} by plant uptake. After the CO_2 concentration fell to 266 mg L^{-1}, 0.580 moles of $NaH^{13}CO_3$ (99 atom% ^{13}C) was injected into the plastic container through the septum, yielding an estimated ^{13}C abundance in the CO_2 of the air of the labeling chamber (192 L) close to the intended 33 atom%. When the height of the labeling chambers was raised to 384 L and 576 L due to growth of the plants, 1.16 and 1.74 moles of $NaH^{13}CO_3$ (99 atom% ^{13}C) were injected after CO_2 concentration had fallen to 266 mg L^{-1}. After the first injection, whenever the CO_2 concentration dropped to 266 mg L^{-1}, $NaH^{13}CO_3$ solution (33 % $NaH^{13}CO_3$ and 66 % $NaH^{12}CO_3$) was injected into the plastic container with an excess of HCl for 1.5 h to maintain a total CO_2 concentration between 266 mg L^{-1} and 400 mg L^{-1}. That way, the frequency of injections increased proportional to the rate of CO_2 removal and, by definition, $^{13}CO_2$ addition was therefore always proportional to photosynthesis, regardless of the plant growth stage (assuming all CO_2 removal was by photosynthesis). Ice packs were placed inside the chamber to minimize excess heating and to condense excess humidity.

Figure 1. Labeling chamber used for *in situ* ^{13}C pulse labeling. Components are: (1) labeling chamber, (2) galvanized steel base, (3) chamber extension, (4) fan, (5) rubber septum and plastic container (inside) for $^{13}CO_2$ production, and (6) IRGA.

The CO_2 levels were monitored by the infrared gas analyzer (IRGA) (SD800 CO_2 analyzer, Extech Instruments, Massachusetts, United States of America) throughout the labeling period, and average values were recorded every 20 seconds. Because of the difference in the wavelengths of $^{13}CO_2$ and $^{12}CO_2$, IRGA only provided an estimate of the total CO_2. After each labeling event, the chambers were placed over labeled microplots at sunset to capture overnight $^{13}CO_2$ respiration, thereby improving ^{13}C enrichment (Kong and Six, 2010). The chambers were removed the following morning after the CO_2 levels had fallen below 266 mg L^{-1}. The wheat, pea, and vetch plants were exposed to a total of 1.7, 2.0, and 1.8 g of $NaH^{13}CO_3$, respectively. The difference in amounts was related to differences in photosynthetic rates, as well as differences in the growth stages.

Plant biomass and cylinder sampling

The plants were harvested on 2 October 2013 by clipping them at the soil surface. They were separated into stems, leaves, pods/chaff, and grain and dried to constant weight at 40 °C. After harvesting the plants, three randomly selected soil cylinders from labeled and unlabeled microplots were excavated from the soil by digging down to 0.35 m. In addition, three randomly selected control soil cylinders (without plants) were also excavated. The cylinders were then carefully removed, placed in plastic bags, and transferred to the laboratory, where they were kept at 4 °C and processed within 2 days. Each undisturbed soil core (0.00-0.30 m) was taken out and separated into three soil layers (0.00-0.05, 0.05-0.15, and 0.15-0.30 m). The weight of each soil layer was recorded. All visible roots were immediately removed by hand from each soil layer. The soil of each layer was then thoroughly mixed, and 100 g of moist soil was subsampled and suspended in 200 mL of deionized water. Fine roots were removed by gentle shaking and rinsed further with tap water until clean. After root collection, a subsample of soil was used for determination of soil moisture. The remaining soil was passed through a 2-mm sieve, air-dried, and systematically mixed to ensure representative subsampling. The soil was finely ground in a steel ball mill for determination of total C and $\delta^{13}C$.

Paired treatments and CO_2-C measurements

''Paired'' treatments were designed by transferring wheat (W), pea (P), and vetch (V) shoot residues (mixture of stems and leaves at a rate of 5 Mg ha^{-1}) among labeled and unlabeled microplots within each block to create a root plot containing labeled roots and unlabeled shoots (LR) and a shoot plot containing unlabeled roots and labeled shoots (LS). This combination resulted in six treatments: WLS and WLR for wheat, PLS and PLR for pea, and VLS and VLR for vetch. For all treatments, the soil and roots remained undisturbed until the cylinders were sampled.

Carbon dioxide (CO_2) emissions began to be measured one day after the shoots were added back to the cylinders using closed chambers. Carbon dioxide was trapped in an alkaline solution (1 mol L^{-1} NaOH) according to the procedure described by Aita et al. (2006). The NaOH traps were changed at frequent intervals: daily for the first week, every 2 days for the next 3 weeks, every 3 to 5 days for the next 8 weeks, and then weekly for the remainder of the experiment. The traps were also changed immediately after rainfall throughout the experiment, and an amount of water equivalent to the rain was applied to the cylinders.

Chemical and isotopic analysis

Roots and shoots were dried to constant weight at 40 °C for 48 h in a forced-air oven. One subsample was oven dried at 65 °C for 48 h for dry matter correction. A second subsample dried at 40 °C was ground to 1 mm particles for soluble (SOL), cellulose (CEL), hemicellulose (HEM), and lignin (LIG) fractions for chemical analysis (Van Soest, 1963). A third subsample was first ground in a coffee grinder and then with a steel ball mill for C, N, and $\delta^{13}C$ analyses. The total N contents of root and shoot residues were analyzed using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Bremen, Germany). The total C and $\delta^{13}C$ of the soil, root, and shoot residues were analyzed using the elemental analyzer coupled to an isotope ratio mass spectrometer (Delta V Advantage, IRMS Thermo Fisher Scientific Inc., Germany) by an interface (ConFlowIV).

Calculations and statistical analysis

The amount of ^{13}C (mg) that was incorporated into the shoot, root, and soil was calculated using the equation 1 (An et al., 2015):

$$^{13}C_{amount} = C_{sample} \times (A_{labeled\ sample} - A_{unlabeled\ sample}) \quad \text{Eq. 1}$$

in which C_{sample} refers to the C content (mg) of each portion; $A_{labeled\ sample}$ and $A_{unlabeled\ sample}$ refer to the ^{13}C atom% of labeled samples and unlabeled samples, respectively. The percentage of the ^{13}C label that was partitioned into the shoots, roots, and bulk soil was expressed as a percentage of ^{13}C in the shoots, roots, and bulk soil as the total amount of recovered ^{13}C.

The results were subjected to analysis of variance and the means of the treatments were compared using the least significant difference (LSD) test at 5 % probability.

RESULTS

Plant dry matter, total C and N

Labeled plants tend to have a slightly higher DM (dry matter) than unlabeled plants for all plant parts except vetch roots (Table 1). The DM differences between shoot parts were only significant for vetch leaves. The root dry weights were not significantly different between labeled and unlabeled plants. Likewise, the total C and N content of the labeled and unlabeled shoots and roots of the three crops were not affected by labeling (Table 1).

Chemical composition

The chemical composition (SOL, CEL, HEM, and LIG) of the labeled plant parts (stems, leaves, and roots) are presented in figures 2a, 2b, and 2c. The labeled and unlabeled plant parts had similar compositions (data not shown). The three crops had higher SOL contents and lower LIG contents in the leaves than in the stems and roots. Overall, the distribution of C in the chemical fractions (SOL, CEL, HEM, and LIG) followed the distribution of DM and did not differ significantly between labeled and unlabeled plants (Table 2). Some differences were found for the HEM-C in vetch stems and pea leaves, for the LIG-C in wheat stems and pea roots, and for the N in the LIG in vetch roots. Nitrogen primarily

Table 1. Average shoot and root biomass production at plant harvest times and total C and N concentrations of labeled and unlabeled crop plants that were grown under field conditions

Crop plant	Shoots				Roots			
	Stem	Leaves	Chaff/Pod	Grain	0.00-0.05 m	0.05-0.15 m	0.15-0.30 m	0.00-0.30 m
				Dry matter (g m^{-2})				
Wheat-L	638.8a	145.4a	345.3a	267.0a	105.5a	16.3a	12.3a	134.1a
Wheat-U	550.4a	130.9a	274.6b	245.8b	95.8a	19.3a	14.7a	129.8a
Pea-L	618.4a	234.9a	216.5a	597.0a	43.7a	26.1a	24.3a	94.1a
Pea-U	566.4a	231.9a	210.8a	584.0a	40.3a	19.5a	19.0a	78.8a
Vetch-L	508.5a	796.7a	-	-	129.2a	35.5a	15.0a	179.7a
Vetch-U	481.8a	703.5b	-	-	145.5a	44.6a	18.7a	208.8a
				Total C (g kg^{-1})				
Wheat-L	437.9a	435.1a	436.9a	431.3a	398.2a	415.3b	396.9b	400.1a
Wheat-U	436.0a	442.0a	437.6a	431.4a	395.9a	419.4a	413.5a	402.2a
Pea-L	445.1a	425.9a	421.0a	424.5a	420.1a	417.7a	420.7a	420.1a
Pea-U	437.0a	426.5a	421.4a	424.9a	442.7a	425.5a	426.0a	434.3a
Vetch-L	428.9a	424.3a	-	-	397.2a	399.3a	415.0a	399.2a
Vetch-U	431.9a	425.3a	-	-	390.5a	393.6a	404.1a	392.0a
				Total N (g kg^{-1})				
Wheat-L	12.2a	24.7a	17.4a	26.6a	14.1a	17.0a	16.8a	14.7a
Wheat-U	11.7a	25.0a	17.7a	26.7a	14.0a	16.5a	16.3a	14.7a
Pea-L	14.8a	28.3a	20.1a	42.6a	32.1a	31.3a	29.3a	31.1a
Pea-U	14.6a	25.7a	19.9a	42.5a	33.3a	30.9a	32.3a	32.5a
Vetch-L	22.1a	40.5a	-	-	28.7a	35.8a	35.2a	30.6a
Vetch-U	18.7a	39.1a	-	-	30.3a	32.9a	31.0b	31.1a

Wheat-L: wheat labeled; Wheat-U: wheat unlabeled; Pea-L: pea labeled; Pea-U: pea unlabeled; Vetch-L: vetch labeled; Vetch-U: vetch unlabeled. -: not determined. The values are the means of three replicates. Means followed by different lowercase letters within a column represent significant differences at $p<0.05$ between labeled and unlabeled plants for a given component.

accumulated in the SOL fraction, ranging from 65 % in roots to 90 % in the stems and leaves of the total N present in the chemical fractions of the three crops (Table 2).

$\delta^{13}C$ enrichment of plant parts

The mean $\delta^{13}C$ enrichment of the plants increased significantly from -27 to 495 ‰ in wheat, -29 to 426 ‰ in pea, and -29 to 378 ‰ in vetch plants as a result of ^{13}C pulse labeling (Table 3). The $\delta^{13}C$ enrichment of the stems and leaves differ significantly for wheat and vetch plants, and homogeneous $\delta^{13}C$ enrichment was obtained for the leaves and stems of pea plants. The pod and grain fractions were the least enriched. Higher ^{13}C enrichment was observed in the roots of the three crops than in the aboveground parts (except for wheat leaves), and the differences in the ^{13}C enrichments between soil layers were small. Among the crops, the ^{13}C enrichment of stems was not different, and for the other plant parts, the enrichment was significantly different with the following ranking: wheat > pea > vetch. The ^{13}C was homogeneously distributed in the CEL, HEM, and LIG fractions of the wheat stems, leaves, and roots, given that the observed differences were not significantly different (Figures 2d, 2e, and 2f). The SOL fraction was least enriched, regardless of the plant part and the crop under consideration, except in the vetch root.

^{13}C recovery in plants and soil

The recovery of ^{13}C from plants (above- and belowground parts) and bulk soil was 44.8 % of the total ^{13}C applied to wheat, 34.7 % to pea, and 35.1 % to vetch from labeled microplots (data not shown). More than half of the recovered ^{13}C was found in the leaves

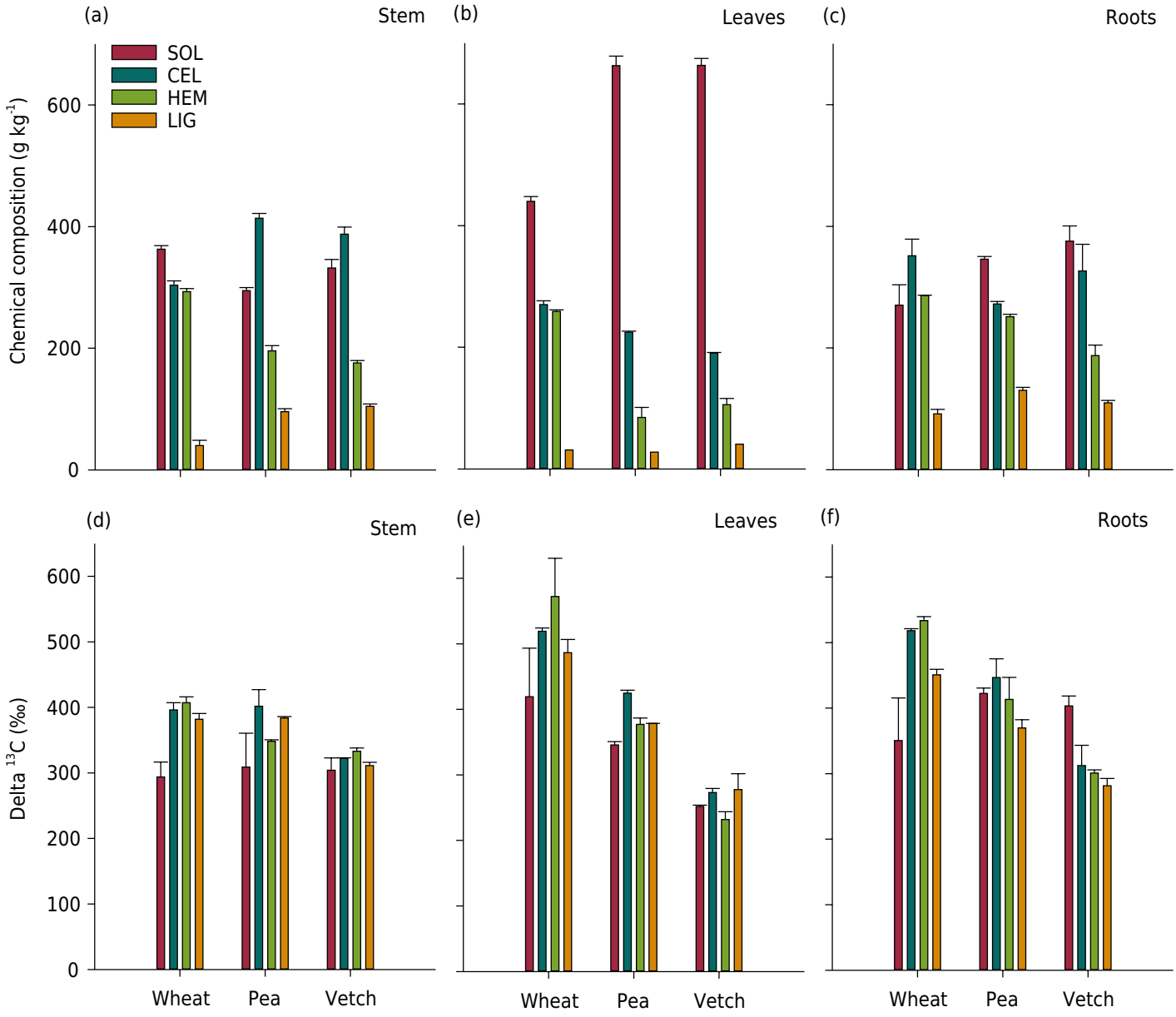

Figure 2. Chemical composition (a, b, and c) and δ^{13}C of chemical fractions (d, e, and f) in the stems, leaves, and roots of three crops grown under field conditions. Error bars indicate the standard error (n= 3). SOL: soluble fraction; CEL: cellulose; HEM: hemicellulose; LIG: lignin.

and stems, and the recovery of ^{13}C in the root fraction decreased along with decreasing root DM, i.e., with increasing soil depth. On average, 81 to 89 % of the net recovered ^{13}C remained in the shoots, 7 to 14 % was incorporated into the roots, and 2.7 to 4.3 % was retained in the soil (Figure 3), with no significant difference between crops. This ^{13}C recovered in soil was related to an isotopic shift in the soil δ^{13}C values of the labeled microplots observed after harvesting. The δ^{13}C value of the control soil was -19.17 ‰, which increased to -16.73 ‰ in wheat, -17.15 ‰ in pea, and -16.41 ‰ in vetch labeled microplots in the 0.00-0.30 m soil layer.

Total C mineralization of the paired treatments

The total CO_2-C evolved did not differ significantly between the "paired" treatments for wheat (WLS vs. WLR), pea (PLS vs. PLR), and vetch (VLS vs. VLR) at any time over 180 days of decomposition (Figures 4a, 4b, and 4c). The patterns of C mineralization rates were fairly similar among the species, marked by sharp fluctuations during the first 20 days, followed by a gradual decrease from day 45, and then an even more gradual decrease

Table 2. Total C and total N contents of chemical fractions from labeled and unlabeled crop plant parts (stems, leaves, and roots) grown under field conditions

Crop plant	Total C				Total N			
	SOL	CEL	HEM	LIG	SOL	CEL	HEM	LIG
	g kg^{-1} DM				g kg^{-1} DM			
				Stems				
Wheat-L	177.6a	128.3a	111.4a	20.5b	10.7a	0.4a	0.7a	0.2b
Wheat-U	162.3a	131.3a	117.4a	25.4a	10.1a	0.4a	0.8a	0.4a
Pea-L	130.3a	181.0a	79.2a	54.7b	12.8a	0.6a	0.3a	1.0a
Pea-U	125.9a	171.5a	76.3a	64.4a	12.4a	0.6a	0.4a	1.2a
Vetch-L	130.4a	174.9a	65.7b	57.8a	18.8a	0.8a	0.13a	1.1a
Vetch-U	131.6a	166.3a	74.1a	60.3a	15.4a	0.7a	0.11a	1.4a
				Leaves				
Wheat-L	197.6a	118.0a	107.9a	9.8a	22.3a	0.6a	1.8a	0.2a
Wheat-U	201.9a	118.1a	112.5a	10.1a	22.6a	0.6a	1.5a	0.3a
Pea-L	283.8ab	98.5a	34.5a	9.2a	24.5a	0.1a	2.2a	0.4a
Pea-U	300.9a	92.1a	25.3b	9.0a	22.2a	0.1a	1.6b	0.4a
Vetch-L	278.2a	81.1a	45.5a	19.7a	36.6a	0.6a	2.2a	0.7a
Vetch-U	288.7a	83.9a	30.9a	21.7a	35.4a	0.8a	1.7a	0.7a
				Roots				
Wheat-L	102.5a	156.3a	115.2a	26.1a	10.1a	2.8a	1.0a	0.8a
Wheat-U	104.7a	155.2a	117.8a	24.3a	10.1a	2.7a	1.1a	0.8a
Pea-L	136.5a	119.9a	104.1a	59.5b	20.6a	6.5b	1.1a	2.9a
Pea-U	136.0a	121.8a	107.1a	69.3a	20.2a	7.7a	0.6a	4.0a
Vetch-L	152.5a	140.3a	71.9a	34.3a	19.0a	8.4a	1.5b	1.7b
Vetch-U	155.2a	128.6a	73.1a	35.1a	17.3a	8.6a	2.2a	3.0a

DM: dry matter; Wheat-L: wheat labeled; Wheat-U: wheat unlabeled; Pea-L: pea labeled; Pea-U: pea unlabeled; Vetch-L: vetch labeled; Vetch-U: vetch unlabeled; SOL: soluble fraction; CEL: cellulose; HEM: hemicellulose; and LIG: lignin. The values are the means of three replicates. Means followed by different lowercase letters within a column represent significant differences at $p<0.05$ between labeled and unlabeled plants for a given component.

Table 3. $\delta^{13}C$ values of labeled and unlabeled parts of crop plants grown under field conditions

Crop plant	δ^{13}C-Shoot				δ^{13}C-Root		
	Stem	Leaves	Pod	Grain	0.00-0.05 m	0.05-0.15 m	0.15-0.30 m
	‰						
				Labeled			
Wheat	357.4 aC	493.4 aA	328.2 aD	192.8 aE	495.4 aA	490.4aA	453.9 aB
Pea	365.3 aB	365.7 bB	189.9 bC	124.3 bD	417.6 bA	426.6 bA	413.1 bA
Vetch	315.1 aB	254.8 cC	-	-	378.7 cA	324.5 cB	318.6 cB
				Unlabeled			
Wheat	-27.1 bB	-29.5 dC	-29.6 cC	-28.4 cB	-26.5 dA	-28.0 dB	-28.0 dB
Pea	-30.3 bB	-29.3 dA	-30.1 cB	-29.2 cA	-30.5 dB	-30.5 eB	-30.2 dB
Vetch	-30.9 bC	-29.4 dB	-	-	-28.8 dA	-30.2 eC	-29.8 dB

Means followed by different lowercase letters within each column and uppercase letters within each row represent significant differences at $p<0.05$ among labeled and unlabeled crops and between the plant parts of a given crop, respectively. -: not determined.

until the end of the experiment. The initial fluctuations in mineralization rates appeared to be affected by rain events. Total cumulative mineralization demonstrated non-significant differences between "paired" treatments for wheat, pea, and vetch (Figure 4d, 4e, and 4f). The cumulative CO_2 (total CO_2-C emitted minus CO_2-C emitted in the unamended control) was 1,565 kg ha^{-1} for WLS and 1,586 kg ha^{-1} for WLR, 1,481 kg ha^{-1} for PSL and

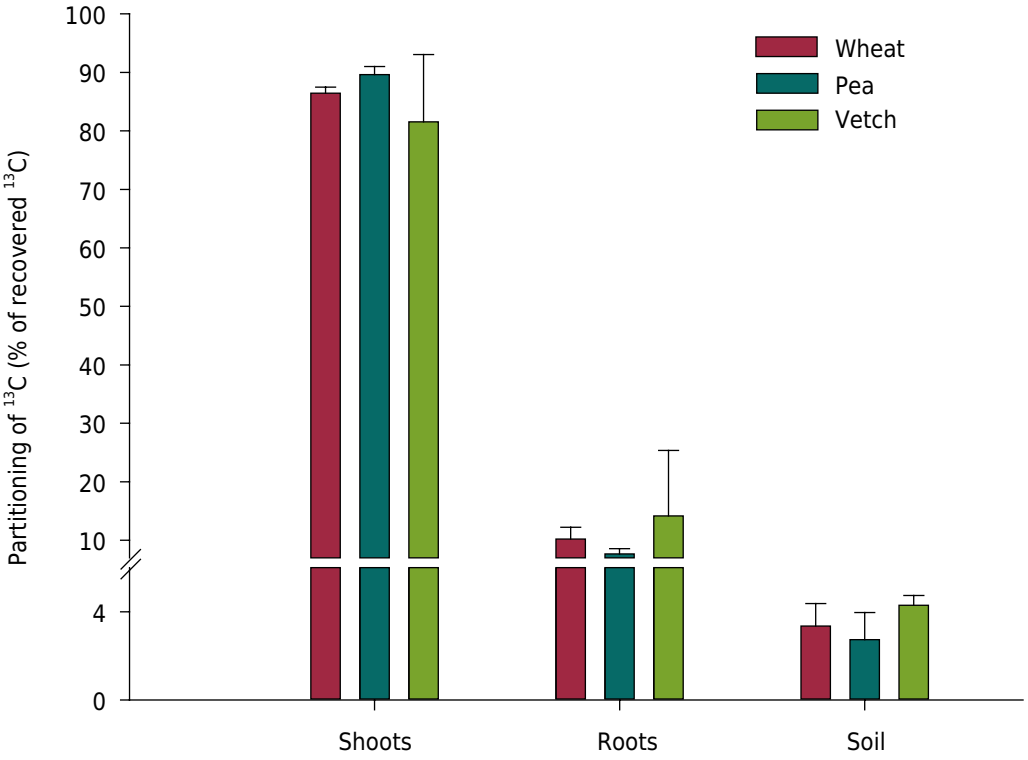

Figure 3. Partitioning of ^{13}C (% of recovered ^{13}C) in shoots, roots, and soil of three crop plants after repeat-pulse labeling under field conditions. The ^{13}C in shoots was the sum of the stems, leaves, pods, and grains for wheat and pea plants and the sum of stems and leaves for vetch plants. The error bar indicates the standard error (n= 3).

1,501 kg ha^{-1} for PRL, and 1,618 kg ha^{-1} for VSL and 1,632 kg ha^{-1} for VRL, assuming that the mineralization of SOM was equivalent in residue amended and unamended soils.

DISCUSSION

Plant production and chemical composition

This study was conducted in the field and involved repeat-pulse $^{13}CO_2$ labeling of three winter crops to obtain above- and belowground residues enriched with ^{13}C and identical to their unlabeled above- and belowground residue counterparts in terms of chemical composition and DM. This is fundamental to study the decomposition of aboveground and belowground residue C in soil simultaneously by combining labeled roots with unlabeled shoots and unlabeled roots with labeled shoots (''paired'' treatments). Differences in the chemical composition of labeled and unlabeled residues and in DM, particularly for roots, will not only jeopardize subsequent residue decomposition studies but also lead to misinterpretation of the results, if they are used. With the ^{13}C pulse method in this study, the above- and belowground DM and chemical composition of labeled and unlabeled plants did not vary significantly and were generally consistent with previously reported results for repeat-pulse-labeling experiments (Bromand et al., 2001; Puget and Drinkwater, 2001; Sangster et al., 2010; Meng et al., 2013). It is therefore not surprising that the similarity observed in kinetics and C mineralization rates between paired treatments was due to this similarity in DM and chemical composition. Similar C mineralization between ''paired'' treatments is an absolute prerequisite for applying the dilution equations to estimate the actual amount of C mineralizing from each source (i.e., SOM, shoots, roots) (Puget and Drinkwater, 2001).

The total C and N contents of the plant parts were within the range reported for similar crops grown under field conditions (Redin et al., 2014a,b) except for the wheat crop,

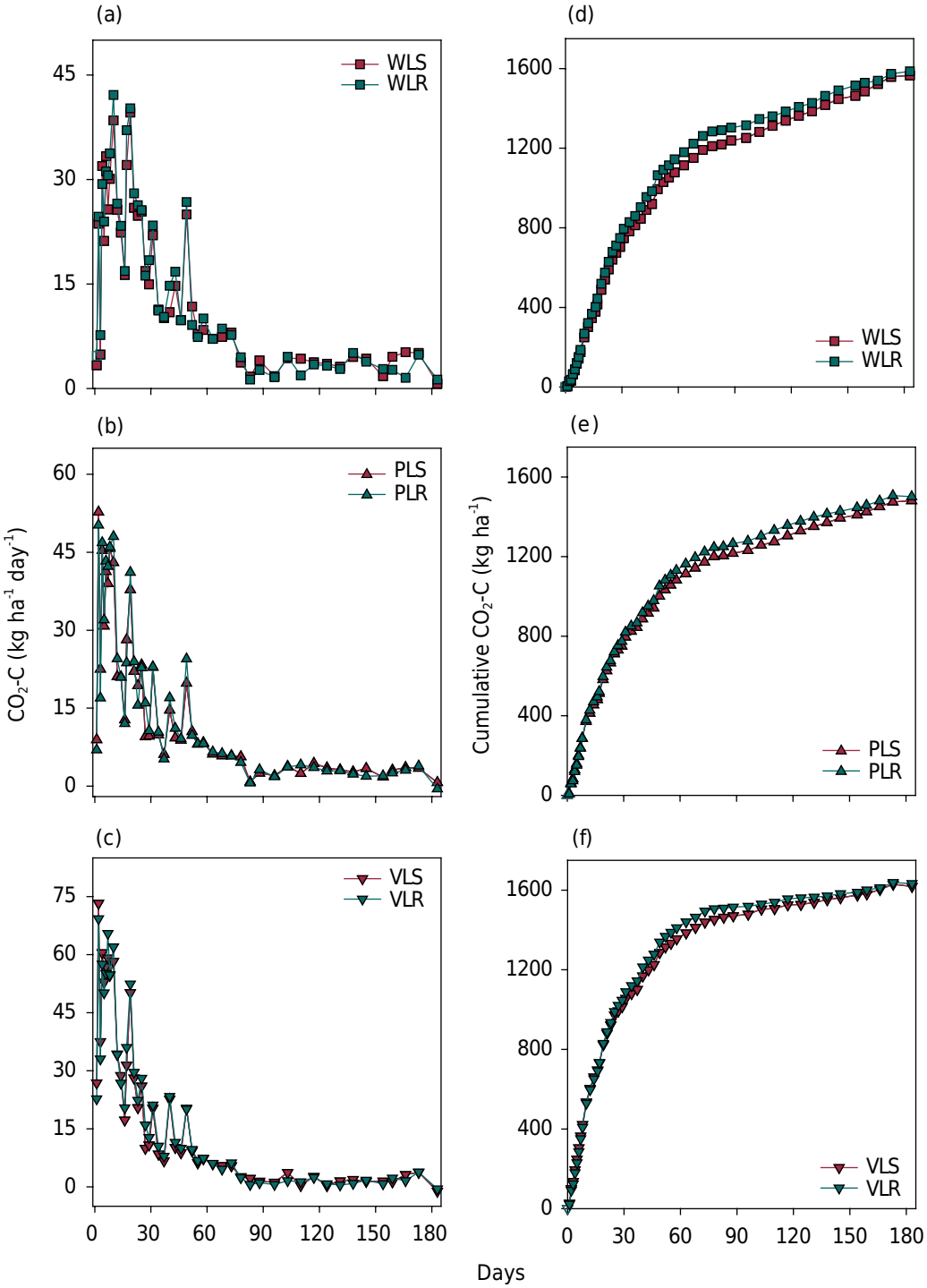

Figure 4. Carbon mineralization rates (a, b, c) and cumulative C mineralization (d, e, f) of wheat shoot labeled (WLS) and wheat root labeled (WLR) (a, d), pea shoot labeled (PLS) and pea root labeled (PLR) (b, e), and vetch shoot labeled (VLS) and vetch root labeled (VLR) (c, f) residues in soil during the 180-day experiment under field conditions.

which had a higher N content. This high N content for wheat may be related to the split application of N fertilizer and crop harvest in the early dough stage. The crop DM was likewise higher than the DM of similar crops grown in the field under similar climatic conditions [e.g., Boddey et al. (2010)]. The higher plant production in our experiment may be attributed to better management of crops (i.e., plant selection, staking of pea and vetch plants, split fertilization, etc.). Additionally, in this study, leaves that senesced during the growth period (from vetch and pea) were not allowed to decompose, i.e. they were collected and included in the total DM, which is not the case in the fields. We also acknowledge that the pre-mature harvesting of wheat plants also contributed

to the observed higher DM of wheat plants compared to field plants. We intended to use the above- and belowground residues of the three crop plants for subsequent decomposition studies at the same time under similar environmental conditions, which was the reason for harvesting wheat plants before maturity. Therefore, we recommend better consideration of the growth cycle of the different crops before the onset of labeling, even if this requires a more complex experimental design if several crops are labeled simultaneously, as was the case in this study. Taken together, these findings indicate that labeled residues obtained by $^{13}CO_2$ pulse labeling can be used for future reciprocal treatment experiments (i.e., the exchange of residues between labeled and unlabeled plots) for tracing root C compared with shoot C in SOM pools simultaneously [e.g., Gale and Cambardella (2000)].

The plants showed higher $\delta^{13}C$ enrichment in our experiment than in other experiments conducted under field conditions (Puget and Drinkwater, 2001; Williams et al., 2006; Kong and Six, 2010). The plants in these other experiments were pulse labeled with 99 atom% $^{13}CO_2$, whereas our intended atmospheric CO_2 enrichment was 33 atom% ^{13}C. Therefore, higher $\delta^{13}C$ enrichment of the plants in our experiment confirmed that the frequency of labeling events and labeling duration are critical issues for designing a pulse-labeling experiment in the field. Under subtropical climatic conditions, the 1.5 h duration of the labeling session appeared suitable for enrichment of plant residues; though ice packs were used to prevent excessive heating of the labeling chamber, we believe that duration longer than 1.5 h without a proper cooling system will not enhance ^{13}C enrichment. We also labeled plants between 9:00 and 11:00 a.m. to help keep labeling chamber temperature from 25 to 30 °C. This may raise the question of availability of sufficient photosynthetically active radiation (PAR) for photosynthesis compared to labeling around midday or later. However, the ^{13}C enrichment of residues in our experiment was greater than in other studies (Puget and Drinkwater, 2001; Williams et al., 2006; Kong and Six, 2010) where labeling was performed between 10:00 a.m. and 16:00 p.m., which suggests that labeling in early hours is possible. Another possible reason is the capture of $^{13}CO_2$ respired overnight and its contribution to building depleted leaf starch reserves during the following morning. An important finding in our experiment that is not consistent with other studies is the higher enrichment of roots compared to aboveground residues [e.g., Puget and Drinkwater (2001), Kong and Six (2010), Sangster et al. (2010)]. Similar results were also reported for wheat plants, and higher root enrichment was described as a result of succession of labeling pulses and effective translocation of ^{13}C to roots (Bromand et al., 2001). As we started the labeling at the early stages of plant growth, we assumed that the translocated assimilates were efficiently used in the buildup of roots, leading to the higher enrichment of root tissues.

As in plant parts, complete homogeneity in the distribution of ^{13}C labeling among chemical fractions (SOL, CEL, HEM, and LIG) of the plant parts was not achieved, possibly due to the relationship between the developmental stage of the plants and the labeling events (Sangster et al., 2010). However, the use of pulses at regular intervals minimized the heterogeneity of ^{13}C labeling in chemical fractions in this study compared to other studies [e.g., Moore-Kucera and Dick (2008) and Sangster et al. (2010)]. Another important issue regarding repeat-pulse labeling is enrichment of the residues for subsequent detection of labeled C in various soil organic matter pools. The ^{13}C enrichments of the shoot and root residues in our experiment was 2 to 12 times higher than reported by Puget and Drinkwater (2001), Williams et al. (2006), and Kong and Six (2010). We believe that the number of pulses [11 compared to around 5 in Puget and Drinkwater (2001), Williams et al. (2006), Kong and Six (2010)] were likely responsible for greater ^{13}C enrichment of shoot and root residues in our experiment. This supports our analysis that increasing the number of pulses, e.g., to twice a week, would not only enhance ^{13}C enrichment of the plant residues but would also result in increased homogeneity of distribution of labeling within plant parts and chemical fractions.

Recovery and distribution of ^{13}C in the plant parts and soil

The amount of fixed ^{13}C remaining in the plant-soil system was relatively low, but it was comparable with the values reported in the literature in which plants were labeled and harvested after maturity [i.e., Gregory and Atwell (1991), Wichern et al. (2007)]. The relatively low recovery of applied ^{13}C was possibly related to respiration by shoots and roots and microbial respiration (Ostle et al., 2000). Most of the recovered ^{13}C was incorporated into the shoots. Recovery of ^{14}C in the range of 91-95 % in wheat and barley shoots had previously been reported (Gregory and Atwell, 1991). In our experiment, the roots were generally more enriched than the shoots, and the total amount of ^{13}C was low. This finding suggests that there was an active belowground routing of ^{13}C during the active root growth period and that this decreased as the plant became mature. Higher recovery of photosynthates in the earlier plant growth stage than in the maturation stage was also reported (Lu et al., 2002). Additionally, a decrease in root biomass during plant growth also resulted in lower ^{13}C accumulation in the roots (Gregory and Atwell, 1991; Yevdokimov et al., 2006).

A minor proportion of the total ^{13}C was recovered in bulk soil (2.7 to 4.3 % of that applied) in our experiment, which was in line with earlier findings of Staddon et al. (2003) and Rangel-Castro et al. (2004). Most of the ^{13}C entering the soil during plant growth via rhizodeposition can stimulate microbial growth, which is important for soil organic matter formation (Austin et al., 2017). Additionally, rhizodeposits have been reported to be preferentially stabilized in aggregates on organo-mineral interactions due to their closer physical proximity than residue-derived C. Therefore, estimation of ^{13}C in the soil prior to a decomposition experiment is necessary for partitioning C derived from rhizodeposits from root-derived C, particularly under NT conditions (Tahir et al., 2016).

CONCLUSIONS

The *in situ* repeat-pulse-labeling method in this study revealed heterogeneous ^{13}C enrichment of different plant parts, which seems to be achieved by considering frequency and duration of labeling sessions in future studies.

Sufficient enrichment of plant material with ^{13}C was achieved without affecting the chemical composition compared to unlabeled plants, which is shown by the identical patterns of C mineralization between ''paired'' treatments of the crops.

Repeat-pulse labeling is therefore an appropriate approach for labeling crop residues considering ^{13}C tracer and labor cost and its suitability for C turnover studies under actual NT field conditions.

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