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Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops

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ABSTRACT

Quantifying the amount of carbon (C) incorporated from decomposing residues into soil organic carbon (C_S) requires knowing the rate of C stabilization (humification rate) into different soil organic matter pools. However, the differential humification rates of C derived from belowground and aboveground biomass into C_S pools has been poorly quantified. We estimated the contribution of aboveground and belowground biomass to the formation of C_S in four agricultural treatments by measuring changes in $\delta^{13}C$ natural abundance in particulate organic matter (C_{POM}) associated with manipulations of C_3 and C_4 biomass. The treatments were (1) continuous corn cropping (C_4 plant), (2) continuous soybean cropping (C_3), and two stubble exchange treatments (3 and 4) where the aboveground biomass left after the grain harvest was exchanged between corn and soybean plots, allowing the separation of aboveground and belowground C inputs to C_S based on the different $\delta^{13}C$ signatures. After two growing seasons, C_{POM} was primarily derived from belowground C inputs, even though they represented only ~10% of the total plant C inputs as residues. Belowground biomass contributed from 60% to almost 80% of the total new C present in the C_{POM} in the top 10 cm of soil. The humification rate of belowground C inputs into C_{POM} was 24% and 10%, while that of aboveground C inputs was only 0.5% and 1.0% for soybean and corn, respectively. Our results indicate that roots can play a disproportionately important role in the C_{POM} budget in soils. Keywords Particulate organic matter; root carbon inputs; carbon isotopes; humification rate; corn; soybean.

1. Introduction

In the soil–plant system, carbon (C) stabilized into soil organic matter has two distinct origins: aboveground inputs (leaves, stems, and floral structures) and belowground inputs (roots, and rhizodeposition). Studies examining the effect of management practices on soil organic carbon (C_S) in agricultural soils have often contrasted tilled and no-till systems (Lal, 1997; Peterson et al., 1998; Díaz-Zorita et al., 2002). Yet, far less attention has been given to the potential impact on the C_S balance of changes in C allocation to belowground and aboveground C inputs in different crops and cropping systems, in part because of the difficulties involved in quantifying and tracking C from belowground residues.

Decades ago, Broadbent and Nakashima (1974) proposed that belowground C inputs have a relatively greater influence on the C_S balance than aboveground C inputs. Subsequent reports from long-term residue management studies seemed to support this assertion (Campbell et al., 1991; Balesdent and Balabane, 1996; Clapp et al., 2000). The potential greater stabilization of belowground C inputs into C_S has been attributed principally to the inherent physical protection within soil aggregates of root-derived C (Gale et al., 2000; Wander and Yang, 2000; Puget and Drinkwater, 2001). However, studies quantifying the contributions of belowground

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and aboveground C inputs to C_S remain scarce (Rasse et al., 2005), particularly in crops of contrasting litter chemistry and in no-till agroecosystems. Consequently, a clear understanding of the dynamics of C derived from belowground inputs and its implications for agricultural management remains elusive (Kong and Six, 2010).

Our current understanding of the biological processes that explain the formation of soil organic matter has been summarized in several plant-soil simulation models. These models differ in the algorithms used to estimate aboveground and belowground biomass humification into C_S. The CENTURY model (Parton et al., 1988) separates biomass inputs into metabolic and structural pools. For the metabolic and structural pools the fraction of the decomposed C that is stabilized in the active C pool is slightly higher for belowground ($0.45\ g\ g^{-1}$) than for aboveground dead biomass ($0.40\ g\ g^{-1}$). Once in the active pool the origin of the C does not affect the stabilization in the slow or passive pools. In the ROTH-C model (Coleman and Jenkinson, 1996) the humification coefficient depends on the type of material but is the same for all C inputs above- and belowground. The more recent C-Farm model (Kemanian and Stöckle, 2010) also uses the same humification coefficient for shoot and root C inputs, and adds C from root exudates, albeit at a lower humification rate. Thus, these simulation models do not account for a potentially larger role of belowground inputs in C_S formation, probably because of the absence of quantitative data for model parameterization.

Estimating the humification of C from different biomass pools into C_S is especially challenging in the field. Several methods have been proposed for this purpose. One method is based on the use of long-term field experiments in which aboveground residues are either returned to the soil or removed after harvest, using a bare-soil treatment as a reference (Larson et al., 1972; Barber, 1979; Plénet et al., 1993; Kätterer et al., 2011). The issue with this method is that the residue removal creates an extreme condition, exposing the soil to rain and wind erosion. Another widely used approach is the use of litter-bag incubations (Parker et al., 1984; Robinson et al., 1997; Moretto et al., 2001). Here, the primary concern is soil disturbance that arises from placing litter bags into the soil, sometimes at different depths. A third method uses $\delta^{13}C$ natural abundance isotopic techniques in long-term experiments (Angers et al., 1995; Bolinder et al., 1999) or with pulse-labeled ^{14}C or ^{13}C (Kisselle et al., 2001; Kong and Six, 2010).

Isotopic tracing of natural ^{13}C is potentially useful for identifying the source of C in C_S and its turnover rate in many ecosystems. The method is useful when a system originally grown or managed with C_3 plants ($\delta^{13}C = -26‰$) is substituted with C_4 plants ($\delta^{13}C = -12‰$) or vice versa (Balesdent et al., 1988). A particularly useful case is when the soil exhibits an intermediate isotopic composition derived from mixed C_3 and C_4 vegetation ($\delta^{13}C = -18$ to $-21‰$), because it allows researchers to follow simultaneously the decline of $\delta^{13}C$ in the soil after the introduction of C_3 plants and its enrichment after the introduction of C_4 plants (Andriulo et al., 1999; Bayala et al., 2006). The tracing of natural ^{13}C abundance has been applied to both bulk soil and to different soil fractions (Martin et al., 1990; Gregorich et al., 1995; Desjardins et al., 2006).

Because C_S is heterogeneous in composition and turnover, it is often separated into different fractions via chemical or physical fractionation. One fractionation method separates organic matter (and therefore C_S) into particulate and mineral-associated organic matter (Cambardella and Elliott, 1992). Organic C in these fractions is defined as C_{POM} and C_{MAOM}, respectively. C_{POM} corresponds with relatively young, minimally transformed C compared to the fresh inputs, and is less associated with mineral constituents of the soil than C_{MAOM}. In contrast C_{MAOM} is more stable through time, likely due to association with the mineral fraction. Combining isotopic tracing with organic matter fractionation can provide a powerful method to unravel the fate of C in soils.

The objectives of this study were to estimate: (1) the proportion of C present in the C_{POM} fractions derived from aboveground or belowground biomass residues, and (2) the rate of humification of C from aboveground and belowground residues into C_{POM}. We tested the following hypotheses in this no-till system, where aboveground residues are not mechanically incorporated into the soil: (i) C_{POM} will be relatively rich in C derived from belowground biomass (i.e. a more than proportional representation of belowground inputs in C_{POM}) and, (ii) belowground C inputs will have a higher humification rate into C_{POM} than aboveground C inputs. To address these hypotheses, we studied C_{POM} decomposition and formation by measuring changes in soil $\delta^{13}C$ natural abundance in no-till plots of corn and soybean, two crops with contrasting C:N ratio in the residues left at harvest. These plots were established in a field that was previously a C_3/C_4 pasture, and in which we manipulated aboveground C inputs. Originally, our goal was to estimate the contribution of aboveground and belowground C inputs to the C_{MAOM} fraction as well. However, we reported previously that there is priming of C_{MAOM} under continuous corn (Mazzilli et al., 2014), and this makes it impossible to solve for the decomposition and humification rate into C_{MAOM} using the same methodology applied to C_{POM}. Therefore, this paper focuses exclusively on the dynamics of C_{POM} as affected by belowground and aboveground C inputs.

2. Materials and methods

2.1. Study site and experimental design

The experiment was located in northwest Uruguay, 10 km south of Paysandú (31° 21′ S and 58° 02′ W; 61 m above sea level), within the Northern Campos region of the Rio de la Plata grasslands (Soriano, 1992). The climate is meso-thermal sub-humid with a mean daily temperature of 25 and 13 °C in summer and winter, respectively, and a mean annual rainfall of 1200 mm distributed on average uniformly throughout the year, but with large inter-annual variations. The research site has a ~1% slope and soil that is a fertile Typic Argiudol. The soil particle-size distribution in the first 0.1 m is 212, 559 and 229 g kg^{-1} of sand, silt, and clay, respectively.

Between 1940 and 1970 the study site was under continuous annual cropping of wheat (one crop per year) in conventional tillage (inversion tillage plus several secondary operations). From 1970 to 1993 annual crops were rotated with pastures in a six-year rotation consisting of three years of white clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh.) and three years of crops at 1.7 crops per year (Ernst et al., 2009). From 1993 until the beginning of the experiment in 2007 the site was not cultivated and was gradually colonized by bermudagrass (*Cynodon dactylon* (L.) Pers. – a perennial C_4), maintaining a variable abundance of annual ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) and white clover (both C_3 species). This land use history provides an intermediate soil $\delta^{13}C$ signature ($-21.5‰ \pm 0.2$ in the first 10 cm depth for C_{POM}) and allows differentiating C inputs from both C_3 and C_4 plants.

The experiment was established in April of 2007. The experimental area was treated with glyphosate at a rate of 3.0 kg a.i. ha^{-1} in April of and subsequently at a rate of 1.5–2.0 kg a.i. ha^{-1} depending on weed infestation and weather conditions. Crops were no-till sown on December 6 in 2007 and November 28 in 2008. Pre- and post-emergent herbicides were applied in all treatments to control weeds as needed, and insects and diseases were controlled chemically based on regular insect and disease population monitoring. Prior to sowing, plots were fertilized with 150 kg ha^{-1} of ammonium phosphate (27 kg N ha^{-1} and 30 kg P ha^{-1}). At the six-leaf stage all corn plots were side dressed with 69 kg N ha^{-1} as urea.

The experiment has four treatments arranged in randomized blocks with three replicates. The treatments are: continuous corn (I_C^C), continuous soybean (I_S^S), and two stubble exchange treatments in which aboveground biomass was exchanged after harvest between corn and soybean plots, resulting in a treatment with C inputs from belowground biomass of corn and aboveground biomass of soybean (I_C^S), and a complementary treatment with C inputs from aboveground biomass of corn and belowground biomass of soybean (I_S^C) (Fig. 1). The belowground biomass includes roots and the crown of the plants. In these residue-exchange plots, all aboveground stubble (~100–150 kg per plot) from the corn and soybean plots was removed manually and exchanged from the corn (soybean) plots to the soybean (corn) plots within each replication.

2.2. Soil and crop sampling

Aboveground and belowground crop biomass at harvest was measured each year. Grain yield and total aboveground biomass of each plot was determined after physiological maturity by harvesting 2 m of two adjacent center rows (2.08 m^2). Belowground root biomass was measured at flowering using the soil core method. In each plot, two soil cores (diameter = 5 cm, depth = 100 cm) were taken in the row and in the inter-row space at the same depth intervals used for the soil samples (see below). The intact soil cores were frozen immediately and kept at −20 °C. To recover the roots, the samples were thawed and roots were separated from the soil using water and sieves of 2-, 0.5-, and 0.05-cm mesh. Roots were collected individually with tweezers and total plot root biomass was calculated by averaging the row and inter-row samples. Root biomass may be slightly underestimated because the freezing and subsequent thawing of roots may have caused cell membrane breakup and loss of soluble compounds from the root tissue in the washing process.

Soils were sampled before sowing crops in November 2007 and November 2009. Samples were taken at seven depths (0–5, 5–10, 10–20, 20-30. 30–50, 50–70 and 70–100 cm). Here, we report data for the top 10 cm of the soil profile, because deeper samples did not show changes over time across treatments for the first two years. In 2007 soil samples were taken, one in each plot, with a 7-cm diameter core, while in 2009 each sample was a composite of four 2-cm diameter cores per plot to minimize soil disturbance of the plots. Samples were weighed and approximately a third of the sample was dried to 105 °C to estimate soil moisture and bulk density. The reminder of each sample was gently crushed and sieved through a 2-mm mesh and dried to 60 °C. Thereafter, soil organic matter fractions were separated according to Cambardella and Elliott (1992). Carbon stocks for each plot were calculated on a constant soil mass basis (Davidson and Ackerman, 1993).

Sub-samples of corn and soybean aboveground and belowground biomass, and soil samples from each sampling time were milled and analyzed for C, N and $^{13}C/^{12}C$ ratio in an elemental C–N analyzer coupled to an isotope mass spectrometer (Finnigan MAT DeltaPlusXL mass spectrometer) at Duke University, USA. The organic C associated with the sand fraction corresponds to C_{POM} and that associated to the clay + silt fraction corresponds to C_{MAOM}. The C isotope ratios were expressed as $\delta^{13}C$ values:

$$\delta^{13}C\ (‰) = \left[\left(\frac{R_{sam}}{R_{std}}\right) - 1\right] \times 10^3 \qquad (1)$$

where $R_{sam} = {}^{13}C/{}^{12}C$ ratio for the sample, and $R_{std} = {}^{13}C/{}^{12}C$ ratio of the Pee Dee Belemnite standard.

Crop yield and biomass production in both years was greater than the average production of rainfed crops in this region, mostly due to a favorable amount and distribution of rain during the initial years of the experiment. Corn and soybean yields and biomass production did not differ between the intact plots where aboveground inputs were not manipulated (I_C^C and I_S^S) and the exchange residue plots (I_S^C and I_C^S). Thus, crop biomass production, biomass $\delta^{13}C$, and C and nitrogen (N) concentration data are presented as averages for corn and soybean, regardless of the stubble exchange treatment (Table 1).

2.3. Estimation of C_{POM} decomposition and humification in intact plots

C_{POM} was partitioned into old C (original or native C, i.e., present at the beginning of the experiment) and new C using the mixing equation proposed by Balesdent (1987):

$$C_{POMfnew} = \frac{(\delta e - \delta_i)}{(\delta_v - \delta_i)} \qquad (2)$$

$$C_{POMnew} = C_{POMfnew} \times C_{POMe} \qquad [3]$$

$$C_{POMold} = \left(1 - C_{POMfnew}\right) \times C_{POMe} \qquad [4]$$

where $C_{POMfnew}$ is the fraction of total C_{POM} derived from the new vegetation; C_{POMnew} (Mg ha^{-1}) is the C derived from the new vegetation (soybean or corn); δ_e is the $\delta^{13}C$ of the C_{POM} fraction at the end of the period analyzed; δ_i is the $\delta^{13}C$ of the C_{POM} fraction at the beginning of the experiment; δ_v is the $\delta^{13}C$ of the input biomass from the new vegetation (corn or soybean); C_{POMe} is the amount of C at the end of the time interval considered; and C_{POMold} is the amount of old or original C_{POM} present at the end of the time interval considered.

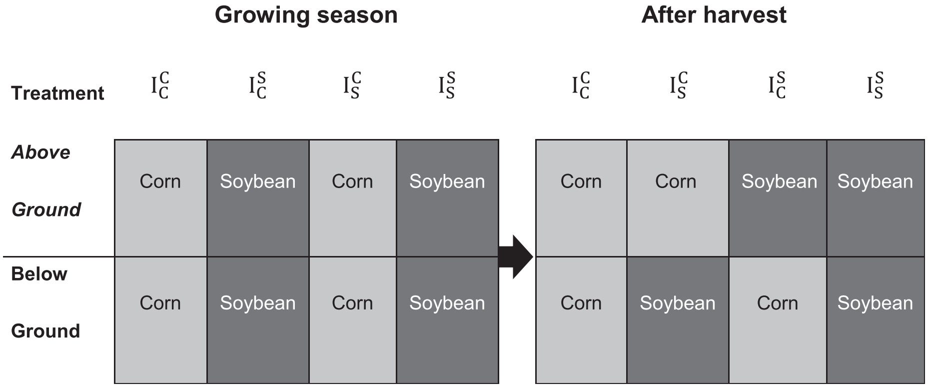

Fig. 1. Diagram of the intact corn and soybean treatments and the stubble exchange treatments.

Table 1
Cumulative two-year grain yields and crop biomass production, carbon and nitrogen concentration, C:N ratio and δ^{13}C in aboveground and belowground biomass of corn and soybean crops.

	Corn		Soybean	
	Aboveground	Belowground[b]	Aboveground	Belowground[b]
Grain yield (Mg of C ha^{-1})	8.5 ± 0.5	–	3.3 ± 0.2	–
Crop biomass production[a] (Mg of C ha^{-1})	8.9 ± 0.1	1.5 ± 0.1	6.5 ± 0.4	0.5 ± 0.1
Crop biomass carbon concentration (g Kg^{-1})	440 ± 12	372 ± 43	500 ± 12	385 ± 43
Crop biomass nitrogen (Mg of N ha^{-1})	0.12 ± 0.001	0.23 ± 0.06	0.37 ± 0.023	0.26 ± 0.06
Crop biomass nitrogen concentration (g Kg^{-1})	5.7 ± 0.7	6.2 ± 0.8	28.7 ± 0. 3	11.5 ± 0.5
Crop biomass C:N ratio	77.5 ± 5.0	57.3 ± 1.5	17.4 ± 0.3	33.3 ± 1.5
Crop biomass δ^{13}C (‰)	−13.00 ± 0.18	−13.66 ± 0.25	−27.10 ± 0.18	−25.07 ± 0.25

[a] Grain is not included.
[b] In the first 10 cm of soil. Values after the ± sign are standard errors.

Carbon decomposition was calculated for C_{POM} assuming an exponential decrease of the original amount through time as follows:

$$C_{POMold}(t) = C_{POMi} \times \exp^{-k_{POM}t} \qquad (5)$$

where k_{POM} is the C_{POM} decomposition rate, and t is the time in years after land conversion (two years in our experiment) and C_{POMi} is the amount of C (Mg ha^{-1}) at the beginning of the experiment. In the intact corn and soybean treatments (I^C_C, and I^S_S) the humification coefficient (h_{POM}) (fraction of decomposed C from residues that enters the C_{POM} pool) was computed by bulking aboveground and belowground C inputs per year (C_V) except for rhizodeposition. Thus, our estimates provide an upper boundary for humification rates, because some of the humified C may have been derived from unaccounted C inputs from rhizodeposition. The equation used was:

$$h_{POM} = \frac{C_{POMnew}}{\Delta t \times C_V} \qquad (6)$$

2.4. *Estimates of belowground and aboveground biomass humification in the C_{POM} fraction in the stubble exchange plots*

The humification of C from belowground and aboveground inputs can be separated using the stubble-exchange treatments. Specifically, the proportion of new C stabilized in the C_{POM} fraction derived from aboveground and belowground C inputs in I^C_S and I^S_C treatments can be estimated, provided that the following assumptions hold for the decomposition rate k_{POM}: (1) the k_{POM} of the original (or old) C in the C_{POM} fraction is the same as that of the newly stabilized C in C_{POM}; (2) the k_{POM} of the intact plots is the same as that of the exchange plots, and (3) k_{POM} is the same for both C_{POM} derived from aboveground or belowground C inputs.

We first calculated the k_{POM} in the pure corn (I^C_C) and soybean (I^S_S) treatments at each depth using Eq. (5); because no differences were detected between the k_{POM} of both crops (Table 3), we thereafter used one averaged value for all calculations involving k_{POM}. Based on k_{POM} and Eq. (5), we estimated the amount of C_{POMold} still present at the end of the experiment in the I^C_S and I^S_C treatments, allowing us to estimate $C_{POMfnew}$ in the treatments by solving Eq. (4):

$$C_{POMfnew} = (C_{POMe} - C_{POMold})/C_{POMe} \qquad [7]$$

With the $C_{POMfnew}$ estimated for the stubble exchange treatments, we calculated the δ^{13}C signal of the humified inputs (δ_V) that would explain the measured $\delta^{13}C_{POM}$ at the end of the experiment ($\delta^{13}C_{POMe}$) by rearranging Eq. (2):

$$\delta_V = \frac{(\delta e - \delta_i)}{C_{POMfnew}} + \delta_i \qquad (8)$$

Because we measured the δ^{13}C signal of the C inputs in the exchange stubble plots, where aboveground and belowground biomass had contrasting isotope signals from soybean or corn, it was possible to estimate the proportion of humified aboveground and belowground inputs that render the δ_V calculated with Eq. (9):

$$f_a = \frac{(\delta v - \delta_{vb})}{(\delta va - \delta_{vb})} \qquad (9)$$

where f_a is the proportion of aboveground biomass present in the C_{POMnew}, and δ_{va} and δ_{vb} are the δ^{13}C of the of aboveground and belowground biomass respectively (either soybean or corn). The proportion of belowground biomass present in the C_{POMnew} (f_b) is:

$$f_b = 1 - f_a \qquad (10)$$

Finally, aboveground and belowground humification rates in the C_{POM} fraction ($h_{A\text{-}POM}$ and $h_{B\text{-}POM}$, respectively) were calculated separately using Eq. (6).

Microbial fractionation was not accounted for in our calculations, as suggested by Clay et al. (2006, 2007), because these corrections apply mostly to relic soil organic matter (C_{MAOM} in our manuscript) and not to relatively fresh organic matter (Clay et al.,

Table 2
Average C_{POM} stocks and δ^{13}C of the different treatments analyzed at 0–5 and 0–10 cm depth.

Soil fraction	Variable	Unit	Sampling time	0–5					0–10				
				I^C_C	I^S_C	I^C_S	I^S_S	p-value[a]	I^C_C	I^S_C	I^C_S	I^S_S	p-value[a]
C_{POM}	C stocks	Mg ha^{-1}	2007	1.2	1.0	1.1	1.6	0.606	1.8	1.4	1.6	2.4	0.615
			2009	1.3	1.3	1.4	1.7		1.7	1.6	1.9	2.2	
C_{POM}	δ^{13}C	‰	2007	−22.5	−20.8	−21.7	−20.5	0.012	−21.1	−21.4	−21.9	−21.6	0.049
			2009	−20.5	−20.4	−21.4	−21.5		−20.5	−20.8	−21.7	−22.0	

[a] P-values denote the significance of the interaction of treatment by sampling time, indicating if treatments changed differentially through time.

Table 3
Mean and standard deviation of decomposition rates (k_{POM}) and humification rates of C inputs (h_{POM}) into the C_{POM} fraction, for soybean and corn plots.

Depth	Crop	k_{POM}	p-Value[a]	h_{POM}	p-Value
		%		%	
0–5	Corn	12.1% ± 3.1	0.44	2.6% ± 0.3	0.58
	Soybean	8.0% ± 4.0		3.8% ± 1.7	
0–10	Corn	5.4% ± 2.9	0.87	1.4% ± 0.9	0.32
	Soybean	6.3% ± 2.4		4.2% ± 1.5	

[a] P-values denote significant differences among crops at each depth.

2006, 2007) such as C_{POM}. Moreover, recent work by Menichetti et al. (2014) concluded that corrections for fractionation become relevant at decadal time scales, and not in shorter time frames such as were involved here.

2.5. Statistical analysis

The treatment effects on the response variables were analyzed using a split-plot design with treatment (I_C^C, I_S^S, I_C^S and I_S^C) as the main plot and time of sampling as the subplot. We focused our analysis in the differences in C_{POM} and $\delta^{13}C$ between sampling dates and the interactions between sampling time and treatments. Both k and h were obtained for each plot, and treatment effects were tested using analysis of variance and separation of means using Fisher's least significant difference. All statistical analyses used Infostat 2011/p.

3. Results

After two growing seasons, C_{POM} stocks did not change significantly through time across treatments (0–10 cm depth, $P > 0.6$), but the $\delta^{13}C$ of the C_{POM} fraction changed significantly in the topsoil (Table 2; $P = 0.012$ and 0.049 for the top 5 and 10 cm, respectively). Changes in C_{POM} allowed us to estimate decomposition and humification rates, as shown below. The initial $\delta^{13}C$ of C_{POM} (derived from the pre-treatment condition) was −21.5 ± 0.2‰, a value that resulted from a mixture of C_3 and C_4 vegetation (Fig. 2). As expected, $\delta^{13}C_{POM}$ became more negative in pure soybean treatments (I_S^S, towards −26.1 ± 0.1‰) and less negative in pure corn treatments (I_C^C, towards −13.3 ± 0.2‰) (Figs. 2 and 3), a change attributable to the observed $\delta^{13}C$ of the crop biomass (Table 1). The $\delta^{13}C_{POM}$ in the stubble exchange treatments shifted mostly towards the isotope signal of corn residues (above or belowground), probably because of its higher residue production (Figs. 2 and 3). When residues were exchanged, the magnitude of the $\delta^{13}C$ shift was more marked in the 0–5 cm than in the 0–10 cm layer due to the larger impact of the exchange in the top soil (Fig. 3).

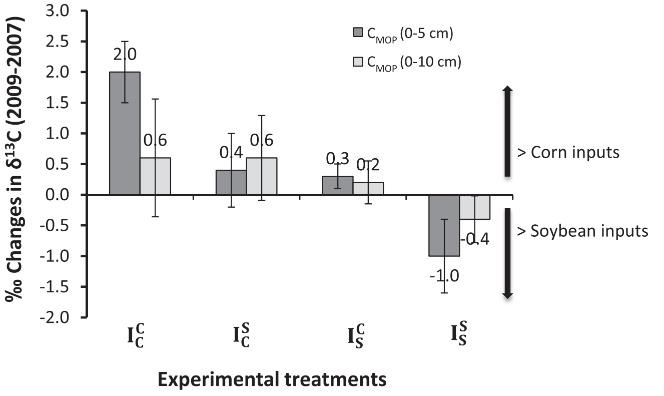

Fig. 3. Differences between initial (2007) and final (2009) $\delta^{13}C$ of the C_{POM} fraction for each treatment and for 0–5 and 0–10 cm depth. Bars indicate standard errors.

Decomposition rates (k_{POM}) of the C_{POM} fraction were not significantly different between intact soybean and corn plots, with higher k_{POM} in the top soil (0–5 cm) for both crops (Table 3). Since total C_{POM} stocks remained constant over the two-year period for both I_C^C and I_S^S, the proportion of new C derived from the crops was likely similar under both species (Table 3).

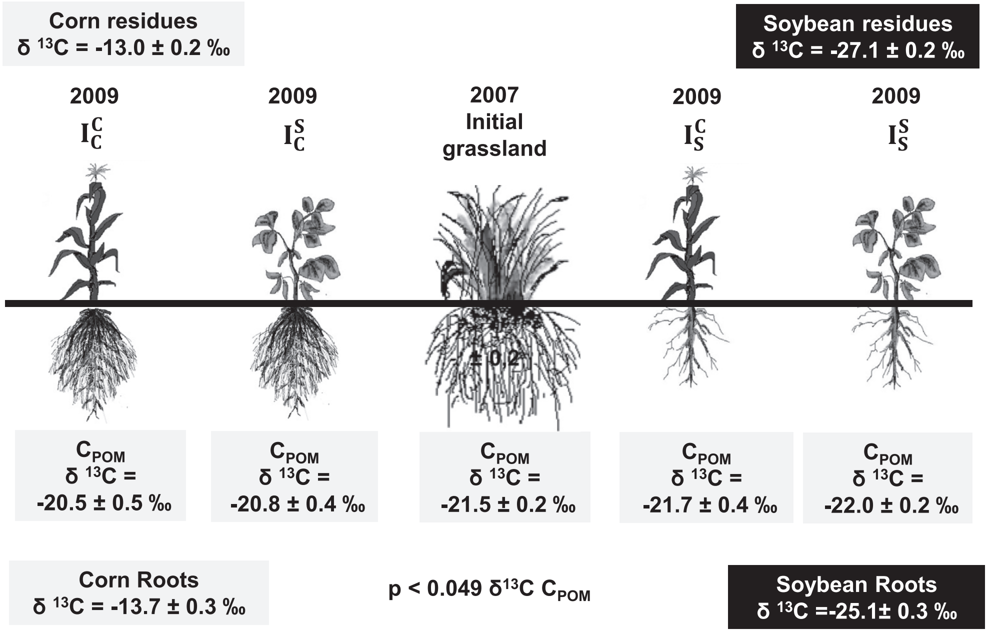

Fig. 2. Schematic representation of initial and final $\delta^{13}C_{POM}$ in different soil fractions (0–10 cm) for each treatment. To facilitate the visualization of $\delta^{13}C_{POM}$ shifts during the experiment, initial conditions are represented in the center of the figure, with pure treatments to the far left (I_C^C) and far right (I_S^S) and stubble exchange treatments in-between.

The proportion of new C in the C_{POM} fraction derived from belowground biomass was greater than the C derived from aboveground biomass. The estimated belowground biomass contribution of the total new C present in the C_{POM} fraction varied from ~56% to almost 80% depending on the depth and treatment analyzed (Table 4). The proportion of C_{POM} derived from belowground residues increased in the 0–10 cm depth compared with 0–5 cm depth in corn plots but remained stable in the soybean plots. Aboveground humification rates ($h_{A\text{-}POM}$) were similar for both crops (~1%) and an order of magnitude lower than belowground humification rates ($h_{B\text{-}POM}$) (Table 4). Humification rates for $h_{B\text{-}POM}$ in the top 10 cm of soil were 10 and 48 times higher than $h_{A\text{-}POM}$, for corn and soybean respectively. Belowground humification rates were higher for soybean roots than for corn roots (Table 4).

Our results indicate that only a small proportion of C from aboveground residues remain in the C_{POM} fraction after two years (≈2.4% of the total non-grain C production), and that a greater proportion of C_{POMnew} is derived from belowground biomass, despite the lower input of belowground compared to aboveground biomass (Fig. 4). Therefore, a major proportion of aboveground residues was respired during decomposition or incorporated into C_{MAOM} (Fig. 4).

4. Discussion

Our hypotheses that belowground C inputs would have a higher humification coefficient than aboveground C inputs and that most of the humified soil C would be derived from belowground biomass both appear to be correct for the C_{POM} fraction. This result is well illustrated by the I^S_C treatment (0–10 cm), where even though four times more C was contributed from soybean litter than from corn roots, the $\delta^{13}C_{POM}$ signal became closer to that of corn (Figs. 3 and 4). Therefore, the $\delta^{13}C$ signature of C_S may preferentially reflect the signal of belowground inputs, a conclusion with major implications for interpreting $\delta^{13}C$ data from soils. Nevertheless, it is possible that the humification coefficients of belowground C inputs were overestimated as rhizodeposition may add as much as 60% more C inputs than those measured in the root mass, at least for corn (Amos and Walters, 2006; Pausch et al., 2013).

The decomposition rates estimated here for the C_{POM} fraction in the top 10 cm of soil for the intact treatments (*ca.* 0.06 y^{-1}) are in the middle of the range reported in the literature (0.04–0.11 y^{-1}, Gregorich et al., 1995; Murage et al., 2007; Dorodnikov et al., 2011; Larionova et al., 2011). We did not detect statistically significant differences in the decomposition rates of C_{POM} under corn or soybean, despite the contrasting properties of the inputs from each crop (Table 1). Neither, we detected a change in the size of the C_{POM} pool in any treatment. The stability of C_{POM} stocks implies that the respired C was balanced by humified aboveground and belowground inputs, or that changes in C_{POM} stocks over the first two years of the experiment were too small to be detected.

Differences in $\delta^{13}C$ were mainly detectable in the C_{POM} fraction. This fraction is highly sensitive to changes produced by different land uses and management practices (Cambardella and Elliott, 1992; Bayer et al., 2001; Fabrizzi et al., 2003; Salvo et al., 2010). The changes in the C_{POM} fraction were mostly explained by the stabilization of belowground biomass, especially below the zone of influence of the aboveground residues, highlighting the important role of roots and belowground allocation in C_S formation, and supporting similar conclusions of recent studies (Kong and Six, 2010; Chirinda et al., 2014; DuPont et al., 2014).

The proportionally larger contribution of belowground inputs to soil C humification is rarely addressed in simulation models. Most models do not distinguish between aboveground and belowground C inputs, using similar humification rates regardless of the residue source (Parton et al., 1988; Coleman and Jenkinson, 1996; Kemanian and Stöckle, 2010). Therefore, it is likely that most simulation models underestimate the importance of roots and overestimate the importance of aboveground inputs in C_S formation. The distinction between these two sources of C in C_s formation is critical for many applications. Our results suggest that crops with larger C allocations to belowground biomass may store more C at least via C_{POM}.

That perennial pastures allocate proportionally more C to roots than annual crops do may be one of the reasons why crop-pasture rotations in the studied region and elsewhere tend to have higher C_S stocks than continuous annual cropping systems (Gentile et al., 2003; Bolinder et al., 2007; Ernst and Siri-Prieto, 2009). This fact might also explain seemingly paradoxical results where the removal of aboveground crop biomass produced either decreases or no changes in C_S stocks (Campbell et al., 1991; Rasmussen et al., 1998; Clapp et al., 2000), because the relative production of belowground biomass may be explaining the lack of response to aboveground residue manipulations.

A recent report by Schmer et al. (2014) can be interpreted as strongly supporting the importance of belowground C inputs in the C_S balance. After 10 years of continuous corn in Nebraska, the authors found a loss of C_3- derived C_S, as expected, and the accrual of C_4-derived C_S in the top 15 cm of soil under no-till (their Tables 5 and 6). This accrual, however, occurred with and without removal of the aboveground residue, albeit with more accumulation when residues were not removed (0.68 vs 0.33 Mg C ha^{-1} y^{-1} assuming 9.5 y between samples). Based on their data we calculated that under no-till, their C_3 stocks decreased during that period by ~0.33 Mg C ha^{-1} y^{-1}, or at about 3% y^{-1} (4.3% y^{-1} in tilled soils). Assuming a similar decomposition rate for the C_4 C_S in the top 15 cm of their system, the belowground biomass of corn prevented the loss of 0.83 Mg C ha^{-1} y^{-1} (the average size of the C_4 C_S times the decomposition rate) and in addition resulted in a net gain of 0.33 Mg C ha^{-1} yr^{-1}, for a net humification of 1.16 Mg C ha^{-1} y^{-1}.

Table 4

Fraction of soil C inputs derived for belowground biomass and aboveground and belowground humification rates into the C_{POM} fraction at 0–5 and 0–10 cm depth, in corn and soybean plots.

		0–5		0–10	
		I^C_S	I^S_C	I^C_S	I^S_C
Fraction of soil C inputs derived from belowground biomass	Soybean	0.57 ± 0.13a	na	0.56 ± 0.23a	na
	Corn	na	0.63 ± 0.21a	na	0.80 ± 0.42a
Aboveground	Soybean	na	1.2% ± 0.2a	na	0.5% ± 0.1a
$h_{A\text{-}POM}$ (%)	Corn	1.1% ± 0.1a	na	1.0% ± 0.03a	na
Belowground	Soybean	44.9% ± 3.5 a	na	24.0% ± 2.4a	na
$h_{B\text{-}POM}$ (%)	Corn	na	16.0 ± 0.8b	na	9.6% ± 0.92b

Values after the ± sign are standard errors. Different letters denote significant differences among crops ($p < 0.05$) per each depth. na = not applicable. $h_{A\text{-}POM}$ = aboveground humification rates into the C_{POM} fraction. $h_{B\text{-}POM}$ = belowground humification rates into the C_{POM} fraction.

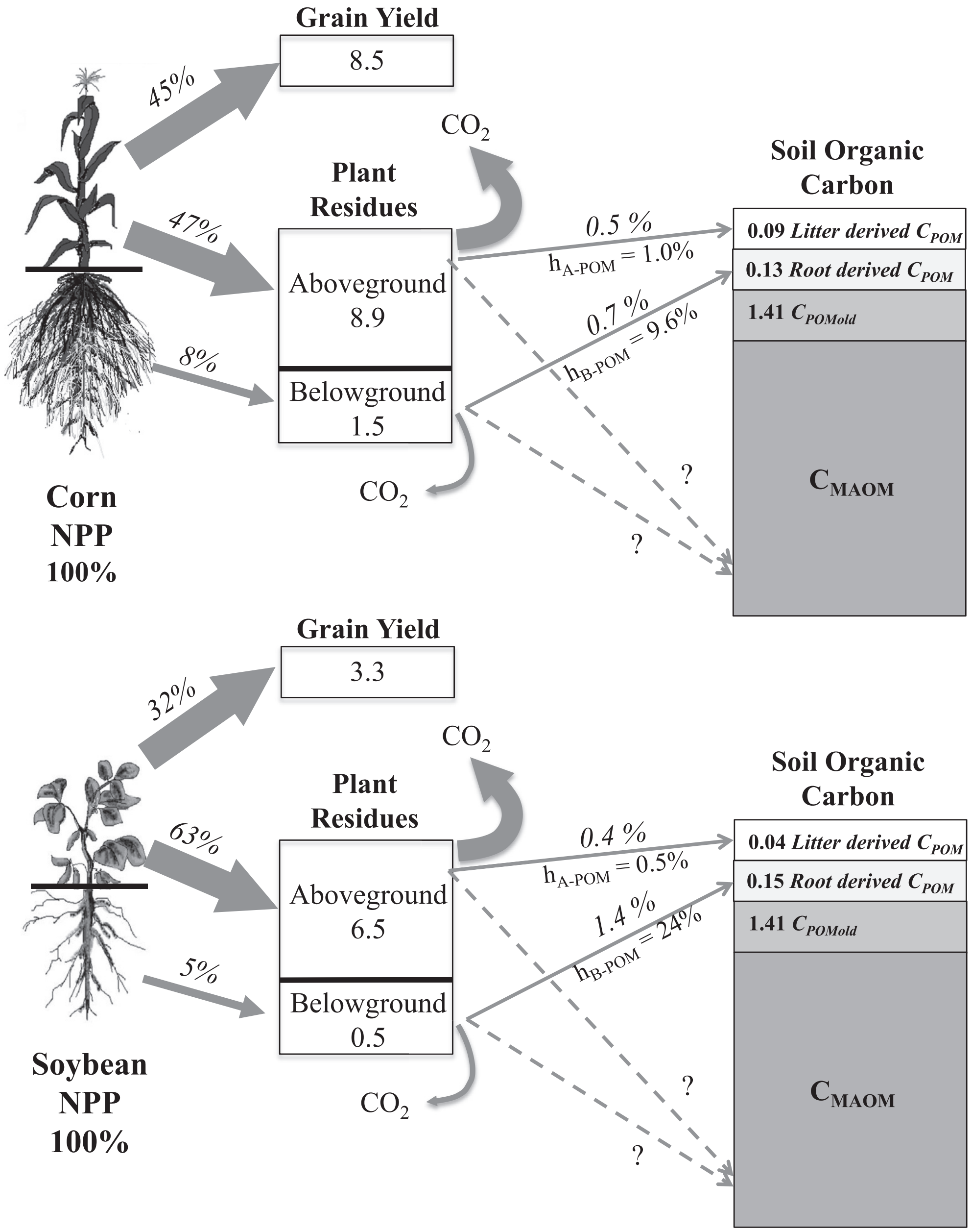

Fig. 4. Estimated C fluxes in soybean and corn crops in the top 10 cm of soil for the 2 years of the experiment. Numbers in italics next to the arrows represent the flux of C as a percentage of total net primary production (NPP) of the crop. Numbers in normal font inside the boxes represent the cumulative production of plant residues and grain, and the amount of C stabilized into the C_{POM} fraction from above and belowground residues after two years, in Mg of C ha^{-1} y^{-1}. The humification of aboveground residues ($h_{A\text{-}POM}$) was 1% and 0.5% y^{-1} while the humification of belowground residues (h_{B_POM}) was 9.6% and 24% y^{-1}, for corn and soybean, respectively. Estimates of C fluxes into the C_{MAOM} fraction were not possible due to the short duration (two year), except for the intact treatments (see Mazzilli et al., 2014).

Clearly, belowground biomass seems to have an outsized impact on the C_S balance of no-till soils, as suggested by the results obtained for C_{POM} in our experiment.

Overall, our results have important implications for soil C management and storage. Decreased C inputs to the soil arising from biomass removal for hay or bioenergy could potentially be compensated for by increasing inputs from belowground biomass. In addition, the selection of cover crops or green manures with a high C allocation to belowground organs could increase C humification and C_S storage. Perhaps, some of the diverse benefits of cover crops arise as much in their aboveground biomass as in their root allocation.

5. Conclusions

Starting from a pasture with a soil $\delta^{13}C$ signature intermediate between C_3 and C_4 biomass, our results show that most of the new C_{POM} was derived from belowground inputs after the first two years (range 56% to almost 80%). Our results also show that the humification to C_{POM} of belowground C inputs was much higher (~16%) than that of aboveground C inputs (~0.8%) for both crops. The influence of aboveground C inputs on the soil $\delta^{13}C$ signal was small and expressed only near the soil surface, even though aboveground inputs accounted for >80% of total C inputs. These results strongly indicate that belowground biomass has a disproportional impact on the balance of C_{POM}, and that a better understanding of belowground C dynamics is needed to describe, manage, and model C cycling in soils.

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