

Winter cover crops in soybean monoculture: Effects on soil organic carbon and its fractions



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ABSTRACT

The current agricultural production systems in the Pampas Region have been significantly simplified by cultivating large land areas under no tillage (NT), where soybean is the predominant crop. These systems with long periods of fall-winter fallow and poor annual input of carbon (C) into the soil lead to soil degradation, thereby affecting physical and chemical properties. A 6-year cover crop study was carried out on a Typic Argiudoll under NT in the south of Santa Fe, Argentina. Various winter species were used as cover crops: wheat (W), oat (O), vetch (V), an oat + vetch mixture (O+V) and a control (Ct) treatment without a cover crop. We examined the influence of cover crops on the following soil organic C-fractions: coarse particulate organic carbon (POC_c), fine particulate organic carbon (POC_f) and mineral-associated organic carbon (MOC) from 2008 to 2011. Aboveground carbon input by the cover crops was related to the June to October rainfalls. In general, the W and O treatments supplied a higher amount of C to the soil; these gramineous species produced 22 and 86% more biomass than O+V and V. The water cost of including cover crops ranged from 13 to 93 mm compared with Ct. However, this water-use did not affect soybean yields. On average, gramineous species (pure stand or mixture) supplied more than 3.0 Mg C ha⁻¹ year⁻¹ to the soil, whereas V supplied less than 2.0 Mg C ha⁻¹ year⁻¹. Increase in the mean annual C-input by residues into the soil (cover crop + soybean) explained most SOC variation (R² = 0.61; p < 0.05). This relationship was more evident with labile soil organic fractions, both for POC_c (R² = 0.91; p < 0.001) and POC_c + POC_f (R² = 0.81; p < 0.001). The stratification ratios of SOC (SI, 0–5: 10–20 cm) reflected differences among treatments, where >2.0 for W; 1.7 for O, O+V and V, and <1.5 for Ct. Soil physical fractionation by particle size showed that cover crops affected the most dynamic fraction directly associated with residue input (POC_c) at 0–5 and 5–10 cm. At 0–5 cm, the effects were observed in the most transformed fractions (MOC and POC_f) 4 years after the experiment started, whereas at 0–20 cm, differences in the labile fractions (POC_c and POC_f) were found at the end of the experiment (6 years). Although C-input by the cover crops fueled decomposition of labile soil organic fractions, concentration of surface SOC and its associated fractions (POC_c, POC_f and MOC) was modified after 6 years. This effect became noticeable during the third year when the plots under cover crops showed a higher SI than the traditional fallow.

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1. Introduction

Growing concerns on global warming has spurred interest on the need to enhance atmospheric sequestration of greenhouse gases such as carbon dioxide (CO₂) on terrestrial ecosystems

(Dolman et al., 2003). Some forms of CO₂ sequestration include the use of agricultural practices such as conservation tillage, cover crops, crop rotation and fertilization (Sainju et al., 2003).

Production systems involving large summer crop areas – mainly soybean (*Glycine max* L. Merr.) – under no tillage (NT) have experienced unfavorable changes in the Pampas Region over the last 15 years. These agricultural systems typically include long periods of fall-winter fallow with low annual carbon (C) input into the soil (2–3 Mg C ha⁻¹ year⁻¹) (Restovich et al., 2005), thereby promoting microbial activity and decomposition of soil organic

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matter (SOM) (Huggins et al., 2007). These effects deteriorate the physical and chemical fertility of soil (Andriulo and Cordone, 1998; Lavado, 2006). Considering the strong prevalence of soybean as the main crop in these production systems, absence of a soil cover is becoming a limiting factor. Sustainability of agricultural systems can be achieved by enhancing the C-balance through from higher plant biomass production. In this sense, cover crops are an agricultural tool that can supply significant amounts of C-rich residues to the soil, thus modifying the quantity and quality of SOM (Franzluebbbers, 2005; Restovich et al., 2011) and improving soil productivity (Sainju et al., 2003). In the Pampas Region, cover crops have been recommended for agricultural systems such as soybean monoculture, where crop residue production may be insufficient for proper soil cover and protection (Novelli et al., 2011). Furthermore, Muller et al. (2008) found that soybean in Vertisols favored soil loss by erosion, whereas inclusion of cover crops reduced it. Ding et al. (2006) demonstrated that cover crops increased SOM content, especially labile fractions. In soybean monoculture, inclusion of triticale helped to maintain SOM levels, which proved to be higher than the uncovered treatment (Álvarez et al., 2006). Several winter gramineous species such as, rye (*Secale cereale* L.), at (*Avena sativa* L.), barley (*Hordeum vulgare* L.), triticale *Triticosecale* Wittmack and ryegrass (*Lolium multiflorum* Lam.) are used as cover crops to maintain or improve soil organic carbon (SOC) levels (Álvarez et al., 2006). Even though changes in SOC rarely occur in the short term (3–4 years), the fractions most likely to be affected are the labile soil organic fractions associated to crop residues in early stages of decomposition and to coarser structural fractions of soil (coarse particulate organic carbon, POC_c) (Christensen, 2001). The POC is the most active SOC fraction and it is used as a soil quality indicator in the short term because it is sensitive to management-induced changes (Rovira et al., 2010; Duval et al., 2013). Also, these labile fractions have proven to be reliable indicators of changes in crop sequences (Salvo et al., 2010) and may show early soil changes resulting from the inclusion of cover crops.

The content of SOC is determined by the relationship between C-input into the soil ($k_1 A$) and C-losses ($k_2 SOC$); the temporal variation ($\delta C/\delta t$) is represented by the equation $\delta C/\delta t = -k_2 SOC + k_1 A$, where A is the annual C-input into the soil (residues + roots) ($Mg\ ha^{-1}\ year^{-1}$), SOC is the stock of total organic carbon in soil ($Mg\ ha^{-1}$), k_1 and k_2 represented the conversion rates of C added in SOC and the rate of decomposition of SOC, respectively. Therefore, long-term fallow cropping systems (soybean monoculture) reduce carbon input (A), whereas cover crops increase it significantly.

The role of cover crops in summer crop yields of the Pampas Region has been studied by many researchers (Restovich et al., 2012; Capurro et al., 2013; Vanzolini et al., 2013). However, little is known about their impact on soil properties, especially on SOC and

its labile organic fractions (Salvagiotti et al., 2013; Scianca et al., 2013).

Taking into account that soils under soybean monoculture supply N-rich organic materials, we hypothesize that C-input by cover crops under soybean monoculture will be rapidly degraded, thus affecting labile soil organic fractions in the long term.

The aim of this study was (i) to evaluate the effect of winter cover crop species on the balance and dynamics of soil organic carbon and its fractions, and (ii) to determine the impact of the quantity and quality of cover crop dry matter on soybean crop yields.

2. Materials and methods

2.1. Study site

The field research was carried out in a 30-ha area with 6 years of soybean monoculture and more than 40 years of continuous agriculture. The site is located in the province of Santa Fe, Argentina ($32^{\circ}57'21''\ S$, $61^{\circ}18'18''\ W$). The area is characterized by monsoon rainfall, 70% occurring mainly from October to March. The mean annual precipitation is 1019 mm and the average temperature is $17.5^{\circ}C$ (1957–2005).

At the beginning of the experiment, the soil had been under NT for the last 10 years. Crop rotation basically consisted of soybean monoculture (*Glycine max* L. Merr.), which remained under chemical fallow in fall and winter. Classified as a Typic Argiudoll (Soil Survey Staff, 2010), the soil is deep, well-drained, with a silt loam texture in the surface horizons (Horizons A). Table 1 shows the main characteristics of the study site.

2.2. Experimental design and treatments

A soybean monoculture with different winter cover crops was started in June 2006. The cover crops used for the experiment were wheat (W) (*Triticum aestivum* L.), oat (O) (*Avena sativa* L.), vetch (V) (*Vicia sativa* L.), an oat+vetch mixture (O+V) and a control treatment (Ct) (without a cover crop) that was kept weed-free with herbicide applications.

The experiment was established on $500\ m^2$ ($50\ m \times 10\ m$) plots in a randomized block design with five treatments and three replications. A soil with its natural vegetation was also evaluated (Ref). With a history of over 50 years without anthropogenic intervention, this soil was covered with gramineous plants, notably *Stipa* genus, bermudagrass (*Cynodon dactylon* L.) and johnsongrass (*Sorghum halepense* L.). The Ref is located about 250 m from the trial and provides a point of reference for the impact of crop production on soil properties. Therefore, this soil was used as an equilibrium reference among the organic fractions at different depths.

Table 1
Soil physical and chemical characteristics at the start of trial (2006).

Horizons	Thickness cm	Sand g kg ⁻¹	Silt	Clay	Texture	BD Mg m ⁻³	FC mm	PWP	PAW	SOC g kg ⁻¹
A	25	102	699	199	Silt loam	1.33	96	51	45	16.6
B22	25	–	–	–	–	1.38	106	67	39	–
B23	20	–	–	–	–	1.35	82	51	32	–
B3	25	–	–	–	–	1.33	89	50	39	–
C1	30	–	–	–	–	1.26	92	52	40	–
C2	35	–	–	–	–	1.26	107	60	47	–
Ck	40	–	–	–	–	1.25	122	69	53	–
	200					1.31	695	400	295	

BD: bulk density; FC: field capacity; PWP: permanent wilting point; PAW: plant-available water; SOC: total organic carbon.

2.3. Cover crops

From May to July, the cover crops were sown under NT on the same plot each year to evaluate their influence on soil organic carbon (SOC) and its fractions. The seeding density was 110, 60 and 45 kg seed ha⁻¹ for W, O and V, respectively. The O + V mixture was sown at a rate of 30 kg seed ha⁻¹ for both species. All cover crops were fertilized at seeding time with 7 kg ha⁻¹ of phosphorous (P) and 8.4 kg ha⁻¹ of sulphur (S) in the form of single superphosphate (0-20-0-12S). The gramineous species (W and O) were fertilized with 51 kg ha⁻¹ of N in the form of urea (46-0-0). In November, the cover crops were killed in their reproductive stage by glyphosate application (2.5/3 L ha⁻¹) (48% active ingredient) about 145 days after sowing. The criterion followed to decide on the proper termination time was that gramineous species should reach flowering to obtain a high production of total aerial dry matter (DM) without compromising the optimum date for soybean sowing, within 10–60 days after killing the cover crops (Table 2). Once the seed had been treated with inoculants and fungicides, the soybean was sown at 0.52-m row spacing and a density rate of 40 seeds m⁻². It was fertilized at seeding time with P, S and Ca at a rate of 6.8, 16.7 and 24 kg ha⁻¹, respectively, using mixtures of single superphosphate and calcium sulphate (0-0-0-18S).

2.4. Sampling cover crops and soybean

Cover crop DM was determined at cover crop termination (Table 2). Ten samples (0.5 m²) were collected from each experimental unit and the total fresh material obtained was weighed. Then, 200 g of each sample were placed in an oven (65 °C) to obtain a constant weight and determine the dry weight of the sample. On the basis of this value, DM production was determined. The samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen and the organic carbon was determined (g C kg⁻¹ DM) with an automatic analyzer (LECO, St. Joseph, MI). Total nitrogen (N) (g N kg⁻¹ DM) was determined for the 2009 and 2010 samples following the method of Kjeldahl (Bremner, 1996). The biochemical quality was assessed by neutral detergent fiber (Cellulose + Hemicellulose + Lignin + others), acid detergent fiber (Cellulose + Lignin + others) and acid detergent lignin (Lignin + others), using the sequential method, with α -amylase and sodium sulfite according to the procedure described by Van Soest et al. (1991). Contents of C and N (Mg ha⁻¹) in the aerial biomass of cover crops were determined by multiplying the DM weight by C and N concentrations, respectively.

Soybean DM was determined from five samples (1.0 m²) per experimental unit at harvest (Table 2). The harvested material was

dried at 65 °C, the grains were separated from the rest of the biomass, and DM and grain were determined. Both the grains and the rest of the plant were ground and sieved through a 1-mm screen to determine organic carbon (g C kg⁻¹) using an automatic analyzer (LECO, St. Joseph, MI). Soybean C-input into residues was determined by calculating the harvest index using the grain yield and the production of total aerial biomass. The harvest index was 0.47 for all treatments evaluated, similar to that reported by Johnson et al. (2006).

2.5. Soil water content

At the beginning of the experiment, soil physical properties were evaluated for each horizon (Table 1). Bulk density (BD) was determined from undisturbed samples using the core cylinder method (Blake and Hartge, 1986). Soil water content at field capacity and at permanent wilting point was obtained using pressure plate extractor (Soil Moisture Equipment Co., Santa Barbara, California) at 33 kPa (Klute, 1986) and at 1500 kPa (Richards, 1947), respectively.

After cover crop termination, soil moisture was estimated using neutron probes up to the 200-cm depth to determine the remaining available water in each treatment. Soil available water was determined as the sum of the differences between the volumetric water content and the permanent wilting point within each horizon.

2.6. Soil analysis

To evaluate the cumulative effect of 3-, 4- and 6-year cover crops, soil samples (three replicates) were randomly taken at 0–20 cm (2008, 2009 and 2011) and 0–5, 5–10 and 10–20 cm (2009 and 2011) depths from each plot every year.

2.6.1. Soil physical fractionation of SOM

Soil fractionation by particle size was conducted using the method described by Duval et al. (2013). The samples were passed through a pair of sieves of 53- and 105- μ m diameter mesh. Three fractions were obtained: the coarse fraction (105–2000 μ m) containing coarse particulate organic carbon (POC_c) and fine to coarse sands, the medium fraction (53–105 μ m) containing fine particulate organic carbon (POC_f) and very fine sands, and the fine fraction (<53 μ m) containing mineral-associated organic carbon (MOC), as well as silt and clay.

The C content of all soil samples and particulate labile fractions was determined by dry combustion (LECO, St. Joseph, MI). The value of MOC was obtained by calculating the difference between SOC and the C-content from the coarse and medium fractions.

Stratification ratios of SOC were calculated based on concentration of SOC at a depth of 0–5 cm, divided by the concentration of that property at a depth of 10–20 cm (Franzluibbers, 2002).

2.7. Carbon dynamic

The most common model used to describe the dynamic behavior of SOC, or its fractions, is the first-order model (Eq. (1)) (Six and Jastrow, 2002):

$$\frac{\delta \text{SOC}}{\delta t} = I - k \times \text{SOC} \quad (1)$$

where SOC: soil organic carbon; I: carbon input; k: decomposition rate.

The value of k is generally determined by isotopic techniques (Balesdent and Balabane, 1996). However, approximate k-values may also be obtained from long-term experiments. When a steady state is reached, no change in SOC or its fractions is expected over

Table 2
Cropping calendar and rainfall of the experimental plots.

Crop	Rainfall (mm) ^a	Seeding date	Harvest/Termination date ^b
Cover crop	168	June 16th, 2006	October 15th, 2006
Soybean	1042	November 8th, 2006	April 10th, 2007
Cover crop	329	May 31st, 2007	November 8th, 2007
Soybean	568	November 20th, 2007	April 5th, 2008
Cover crop	28	May 6th, 2008	October 8th, 2008
Soybean	584	December 5th, 2008	April 16th, 2009
Cover crop	353	May 19th, 2009	November 4th, 2009
Soybean	1568	November 17th, 2009	April 8th, 2010
Cover crop	120	July 5th, 2010	November 3rd, 2010
Soybean	473	December 2nd, 2010	April 25th, 2011
Cover crop	177	June 1st, 2011	November 3rd, 2011
Soybean	758	November 12th, 2011	April 3rd, 2012

^a Rainfall during the growing season.

^b For soybean and cover crops, respectively.

time ($\delta\text{SOC}/\delta t=0$). The k-value can be estimated as follows:

$$k = \frac{I}{\text{SOC}} \quad (2)$$

2.8. Data analysis

Analysis of variance (ANOVA) was applied for the statistical analysis of the results. When significant differences were detected, the comparison of means test was applied using the least significant difference (LSD) with a significance level of 0.05. The Pearson correlations analysis was conducted to evaluate the relationship between chemical variables and the quantity and quality of cover crop residues. All data were analyzed using Infostat statistical software (Di Rienzo et al., 2013).

3. Results and discussion

3.1. Soil available water

Cover crop inclusion demonstrated significant differences ($p < 0.05$) in soil available water (Fig. 1). At the time of cover crop termination, the soil water content in Ct was significantly higher than that in the cover crops during the years evaluated. In general, water content was 37% higher in Ct than in the cover crops. Other authors have reported lower water contents (30–50% less) depending on the cover crop species (Fernández et al., 2007). Soil water content at the end of the cover crop cycle was lower than that in the fallow (without cover crops) as a result of evapotranspiration by the cover crops; in fact the highest evapotranspiration values were observed in the gramineous species (Fig. 1). Legumes showed significant differences compared with gramineous species. The O+V treatment used the highest water amount and left the driest soil profile for soybean sowing in 2006, 2010 and 2011, whereas W and O showed the lowest soil water content in 2007 and 2008, respectively (Fig. 1).

3.2. Cover crop dry matter and carbon input

During 2006–2011, C-input into soil by cover crops differed among species ($p < 0.001$), among years ($p < 0.001$) and significant interaction ($p < 0.001$). In all years, W and O treatments supplied the highest C-input to the soil because of a larger biomass accumulation. On average, C-input by gramineous species was 22 and 86% higher than that by O+V and V, respectively (Table 3). Significant differences were also found between O+V and V, where the pure legume supplied the lowest C, except in 2011 (Table 3). Cover crops contributed with 1.15–4.92 Mg C ha⁻¹, of which gramineous species supplied more C (between 2.02 and 4.92 Mg C ha⁻¹) than the V treatment (between 1.15 and 2.61 Mg C ha⁻¹).

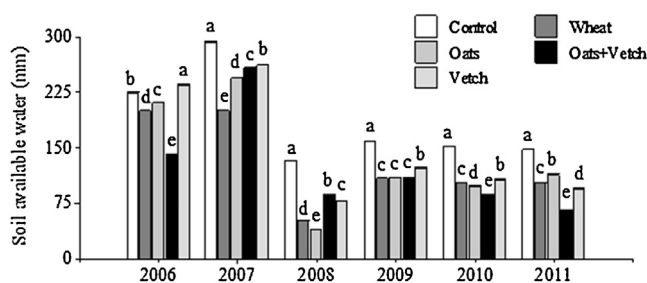


Fig. 1. Soil available water content (0–200 cm) at the time of cover crop termination. Different letters for each year indicate significant differences ($p < 0.05$) among treatments.

Table 3

Mean annual aboveground carbon input by cover crops.

Year	Wheat	Oat	Oat + Vetch	Vetch	Mean
2006	3.42 bA	3.20 bcAB	2.63 cB	1.42 cdC	2.67 (± 0.87)
2007	3.59 bAB	3.88 bA	3.77 aA	2.61 aB	3.46 (± 0.69)
2008	2.40 cA	2.32 cdA	2.16 dA	1.43 cdB	2.08 (± 0.43)
2009	4.65 aA	4.92 aA	3.37 bB	2.06 abC	3.75 (± 1.22)
2010	3.45 bA	3.19 bcA	2.57 cB	1.87 bcC	2.77 (± 0.67)
2011	2.02 cA	2.11 dA	1.56 eAB	1.15 dB	1.71 (± 0.50)
Mean	3.25 (± 0.91) ^a	3.27 (± 1.06)	2.68 (± 0.77)	1.75 (± 0.57)	

In each row, different upper-case letters indicate significant differences ($p < 0.05$) among cover crops. In each column, different lowercase letters indicate significant differences ($p < 0.05$) among years.

^a Values in parenthesis indicate the standard deviation of the mean.

Carbon input by O+V ranged from 1.56 to 3.77 Mg C ha⁻¹, with significant differences with pure gramineous species in 2006, 2009 and 2010 (Table 3). Inter-annual variations in C-input by the cover crops were strongly influenced by yearly rainfall (Table 3). These effects are reflected in the highly significant relationships between precipitation during the cover crop cycle and C-input: W ($R^2=0.46$, $p < 0.01$), O ($R^2=0.55$, $p < 0.001$), O+V ($R^2=0.54$, $p < 0.001$) and V ($R^2=0.36$, $p < 0.01$). In 2008 and 2011, cover crop growth was affected by scanty rainfall, which resulted in poor C-supply. According to Kuo and Jellum (2000), the aerial biomass of cover crops can fluctuate considerably from one year to another, mainly as a consequence of climatic conditions.

Variations in the amount of C supplied by cover crops were caused by differences in water-use efficiency. During low-rainfall years, W and O were capable of producing larger amounts of biomass than the V treatment. Unger and Vigil (1998) also observed that gramineous species adapted better than legumes because they produced higher yields under drought conditions. In this study we demonstrated that C-supply by gramineous species was clearly higher than by legumes, in accordance with results obtained by other authors (Neal et al., 2011; Restovich et al., 2012).

3.3. Cover crop quality

The amount of N accumulated in the aerial biomass ranged from 102 to 212 kg N ha⁻¹, with differences among years (Table 4). Differences among cover crop species were only observed in 2009, where N accumulation was higher in the V treatment (212 kg N ha⁻¹) than in the other cover crops. These results confirm the findings by Restovich et al. (2012), which revealed that the N accumulation at the time of cover crop termination differed between gramineous species and legumes. In our study, the differences in N concentration were observed in the plant tissues among cover crops ($W=O \leq O+V < V$) (Table 4). However, the largest amount of biomass production by gramineous species equaled N-supply by residues in 2010. This finding would indicate that gramineous species were more efficient in capturing the N fixed by soybean.

The C:N ratio related to the decomposition rate of crop residues (Heal et al., 1997) was used to differentiate among cover crop species, according to results observed by other authors (Restovich et al., 2012). In this study, the C:N ratio showed the sequence $O \geq W > O+V > V$. In the O+V and V treatments, the C:N ratio was below 30:1, indicating that net mineralization of the residues may have occurred (Clark et al., 1997).

Concentration of structural polymers, such as lignin and cellulose, also helped to clearly distinguish the different cover crop species, where concentration of cellulose and hemicelluloses was higher in W and O than in V. On the other hand, V showed higher concentration of non-structural carbohydrates and lignin as compared with gramineous species (Table 4).

Table 4

Cover crop quality: nitrogen, cellulose, hemicellulose, lignin, and non-structural carbohydrate concentration, C:N and lignin:nitrogen ratio.

Year	Wheat	Oat	Oat + Vetch	Vetch
	Nitrogen (g kg ⁻¹)			
2009	11.1 b*	9.2 b**	16.5 b	46.5 a*
2010	15.4 c	15.5 c	20.8 b	31.3 a
Mean	13.2	12.4	18.6	38.9
	Nitrogen (kg ha ⁻¹)			
2009	119 b	102 b	126 b	212 a*
2010	121 a	112 a	121 a	129 a
Mean	120	107	123	171
	Cellulose (g kg ⁻¹)			
2009	312 a**	297 a	300 a	220 b**
2010	329 a	290 b	286 b	279 b
Mean	320	294	293	249
	Hemicellulose (g kg ⁻¹)			
2009	317 a	319 a	235 b	201 b
2010	326 a	316 a	276 b	163 c
Mean	321	318	256	182
	Lignin (g kg ⁻¹)			
2009	42 b***	28 c***	43 b	60 a
2010	54 b	55 b	53 b	68 a
Mean	48	42	48	64
	Non-structural carbohydrates (g kg ⁻¹)			
2009	329	356	422	519
2010	292	338	384	490
Mean	311 d	347 c	403 b	504 a
	C:N			
2009	39 b*	49 a**	28 c	10 d*
2010	29 a	29 a	22 b	14 c
Mean	34	39	25	12
	Lignin:N			
2009	3.82	3.13	2.67	1.34
2010	3.56	3.59	2.62	2.17
Mean	3.69 a	3.36 a	2.65 b	1.76 c

For each year and property, different letters in each column indicate significant differences among treatments ($p < 0.05$). For each CC and property *, **, *** indicate significant differences between years at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Lower C:N and lignin:N ratios demonstrate the different qualities in vetch residues (Table 4). The lignin:N ratio is one of the most important factors in determining residue decomposition (Kachaka et al., 1993). Based on these parameters, we may infer that V had a lower potential to enhance soil cover and C-balance in soybean monoculture because these residues were quickly decomposed by soil microorganisms. Approximately 40–50% of V residues may be degraded 30 days after being incorporated (Sá Pereira et al., 2012). Scherer-Lorenzen (2008) assumed that residue mixtures of different species have intermediate chemical characteristics than each one individually. This effect was observed in the O+V mixture, where most of the chemical characteristics evaluated (C:N, Lignin:N, hemicellulose) showed intermediate values between both species (Table 4).

Table 5

Distribution and content of organic fractions in the reference soil.

Depth	SOC	POC _c	POC _f	MOC	POC _c :POC _f :MOC	SI
cm	Mg ha ⁻¹					(0–5/10–20)
0–5	49.0 (±5.2) ^a	13.61 (±1.93)	5.83 (±0.97)	29.5 (±3.8)	28:12:60	2.12 (±0.09)
5–10	28.3 (±2.3)	2.57 (±0.47)	3.63 (±0.80)	22.2 (±2.0)	9:13:78	
10–20	23.1 (±2.3)	1.75 (±0.98)	2.56 (±0.53)	18.8 (±1.4)	7:11:82	
0–20	30.9 (±2.9)	4.92 (±0.89)	3.64 (±0.57)	22.3 (±2.1)	16:12:72	
20–60	13.3 (±2.4)	0.74 (±0.61)	0.66 (±0.14)	11.9 (±1.9)	5:5:90	

SOC: soil organic carbon; POC_c: particulate organic carbon coarse; POC_f: particulate organic carbon fine; MOC: mineral-associated organic fraction; SI: stratification index.

^a Values in parenthesis indicate the standard deviation of the mean.

3.4. Soil organic carbon fractions

3.4.1. Natural environment

Distribution and quality of organic fractions in the natural soil (Ref) showed no differences among sampling dates. Table 5 shows the results for the different depths. Concentration of SOC and its fractions decreased with soil depth, and thus caused a natural stratification by residue accumulation on the surface. Stratification of SOC with soil depth is common in many natural ecosystems (Prescott et al., 1995). This effect is of major importance because SOC is essential for erosion control, water infiltration and nutrient conservation (Franzluebbers, 2002). Also, distribution of soil organic fractions fluctuated with depth, where POC_c was greatly reduced as depth increased (Table 5). The largest POC_c variation was probably due to the fact that this fraction is less transformed and more dependent of residue input than POC_f, whose proportion remained constant with respect to depth.

3.4.2. Agricultural soil

In 2008, after three years of C-input by the cover crops, no significant changes were observed in SOC levels compared with Ct (Fig. 2a). In Hapludolls from the northeastern area of Buenos Aires province, Álvarez and Scianca (2006) also failed to find differences in SOC levels after evaluating cover crops grown before summer crops for 3 years. In general, SOC content on the cultivated plots was 45% lower than the Ref treatment.

Four years after cover crop establishment (2009), significant differences were observed between the cover crops and Ct (Fig. 2a). The O and O+V treatments showed a significant SOC increase compared with Ct. In 2011, SOC levels for the W, O and O+V treatments rose by 14, 9 and 9%, respectively, with significant differences as compared with the Ct treatment. These cover crops hence improved the C-balance, which was the main goal of this management practice for the zone under study. Increase in SOC was significantly related to the amount of C supplied by the cover crops (Fig. 3, Table 7). At 0–5 cm, SOC levels rose by 7% for each Mg of C supplied by the cover crops. At 0–20 cm, cover crop effects on SOC were less marked; the response to C-input was slight because at the subsurface layers (5–10 and 10–20 cm) no significant differences occurred among treatments, as will be further explained. Unlike the Ct treatment, higher C-supply by the cover crops resulted in a significant SOC increase. This increase was significantly higher in 0–5 cm (higher slope) than in 0–20 cm (Fig. 3).

Concentration of labile soil organic carbon (POC_c and POC_f) did not reflect any differences between the cover crops and Ct in 2008 at 0–20 cm (Fig. 2b and c). This result suggests that 3 years of C-input by cover crops were insufficient to affect the most dynamic and labile fractions of SOM, despite C-input differences among treatments (Table 3). These results agree with those reported by

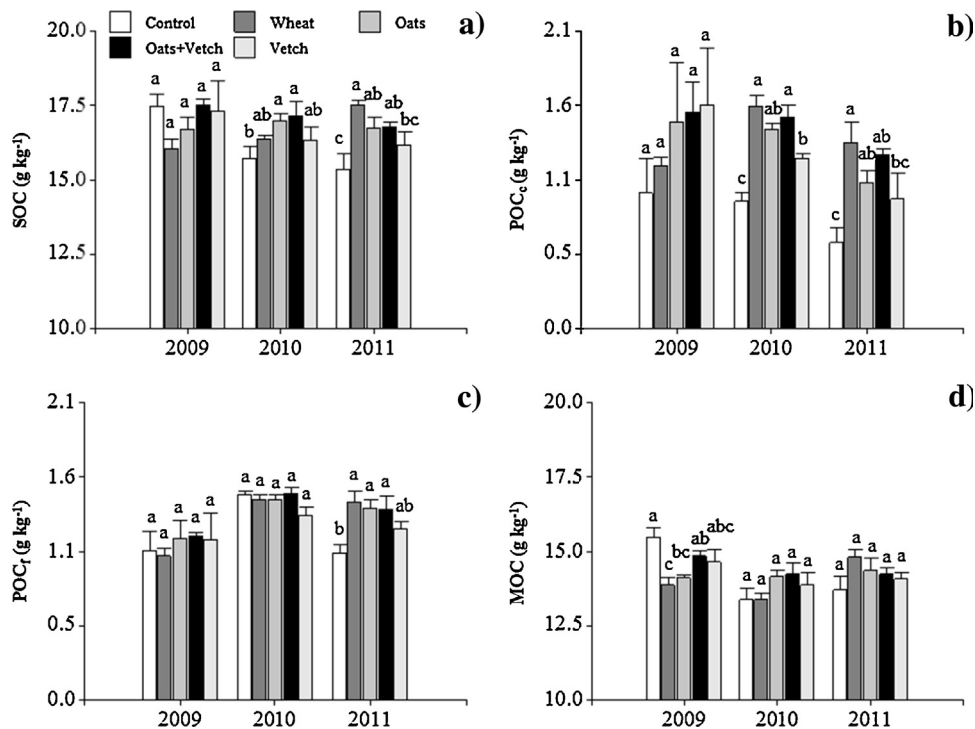


Fig. 2. Total organic carbon (SOC) (a), coarse particulate organic carbon (POC_c) (b), fine particulate organic carbon (POC_f) (c) and mineral-associated organic carbon (MOC) (d) as affected by cover crops at 0–20 cm depth.

Sainju et al. (2007), who found no cover crop effects on labile soil organic fractions after 2 years of cumulative effects. Only differences between the agricultural management and Ref were detected, where agricultural treatments (with and without cover crops) showed a 70% reduction in labile organic fractions (POC_c + POC_f). Although POC_c concentration was higher in cover crops than in Ct, the marked variability of the data evaluated (coefficient of variation = 40%) could have masked the differences among treatments; this variability in this labile soil organic fraction was found in other studies (Duval et al., 2013; Galantini et al., 2014).

As from 2009, SOC increase by the cover crops was mainly due to higher POC_c concentration (Fig. 2b). In 2009 and 2011, the cover crops significantly enhanced POC_c levels compared with Ct. Differences among cover crops were also found. In general, gramineous species showed higher POC_c concentration than V. This difference among species may have been caused by the higher quality of the legume contribution (lower C:N), which stimulated

residue decomposition and thus had a direct influence on POC_c. Regarding the Ct treatment, POC_c increased in the W, O and O + V treatments by 66 and 95% in 2009 y 2011, respectively, whereas in the V treatment, POC_c rose by 33 and 49% for the same periods.

Comparison of POC_f with POC_c revealed that POC_f degrades slower than POC_c and therefore it is relatively less labile (Benbi et al., 2012). This fraction showed significant differences among treatments as from 2011 (Fig. 2c). In that period, POC_f increased in W, O and O + V, whereas V did not differ from the Ct treatment. This fraction did not increase as much as POC_c, values being 32% for W, O and O + V over the last year.

Mineral-associated organic carbon (MOC) showed the lowest differences among treatments (Fig. 2d). Significant ones were only observed in 2009, when Ct showed the highest MOC levels compared with the other treatments. These effects may have been caused by the climatic conditions of that year, when scanty rainfall during the cover crop cycles in 2008 (28 mm) reduced water

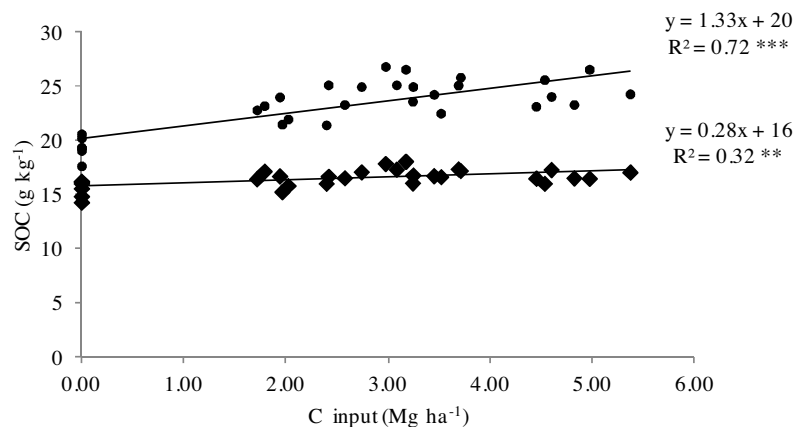


Fig. 3. Relationship between carbon input by cover crops and concentration of total organic carbon (SOC) at 0–5 cm (circles) and 0–20 cm (diamonds) (2009 and 2010). Significant level *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$) and ns: non-significant.

availability in the soil profile (22% of available water holding capacity) (Fig. 1). Crop residue decomposition was thus restrained, as reflected in the high POC_c levels of the cover crop treatments in 2008—not significant, though. In this year, moisture content was higher in Ct than in the cover crops (45% of available water holding capacity), thereby favoring humification processes (POC to MOC transformation), where POC_c tended to decline and MOC to increase (Fig. 2b and d).

3.4.3. Stratification of SOC and its fractions

In 2009 and 2011, the levels of SOC and its fractions showed significant effects among treatments ($p < 0.05$), among years ($p < 0.05$) and non-significant interaction ($p > 0.15$) (Fig. 2). Differences among years are not within the scope of this study; therefore, only the differences caused by the treatments were analyzed (Table 6). Increases in SOC by cover crop inclusion were mainly observed at 0–5 cm, where all the cover crops produced increases ranging from 16 to 31%. Varela et al. (2014) observed a 13% SOC increase at 0–5 cm after 5 years of cumulative effects of cover crop inclusion, as compared with the plots without cover crops. This surface effect was also reported by other authors in other parts of the world when evaluating different cover crop species (Balesdent et al., 2000; de Oliveira Ferreira et al., 2013).

Differences in the quantity (Table 3) and quality (Table 4) of cover crop DM also affected SOC, as reported in other studies (Bronick and Lal, 2005; Bayer et al., 2009). Higher SOC levels in W, O and O + V in comparison with V may have been attributed both to an increase in the C-input by gramineous species and to a lower quality of their residues (higher levels of cellulose + hemicellulose, and higher C:N and lignin:N rates). These results suggest that cover crops of gramineous species would enhance accumulation of more recalcitrant materials on the soil surface, thus promoting SOM increase. Also, larger residue amounts with a high concentration of soluble compounds and a low C:N ratio (vetch + soybean residues) would fuel microbial activity, stimulate decomposition and have a negative effect on organic fractions (Scherer-Lorenzen, 2008).

The stratification index (SI) is the ratio of a value determined for a soil property at the soil surface to that at deeper depths. This index has been used as an indicator of soil quality dynamics (Franzluebbers, 2002; Sá and Lal, 2009; Toledo et al., 2013). Different SI values of SOC were detected among treatments, where >2.0 in Ref and W; 1.7 in O, O + V and V; and <1.5 in Ct (Fig. 4). At many sites, the SI has been successfully used to distinguish between conservation practices and more disturbed managements systems (Papini et al., 2011; de Oliveira Ferreira et al., 2013; Toledo et al., 2013). According to Franzluebbers (2002), soils with

stratification levels higher than 2.0 could be considered as stabilized under conservation management conditions. In our study, the SI was a sensitive index which helped to detect management-induced changes. Differential SOC accumulation in the different treatments was attributed both to crop residue accumulation on the soil surface (higher in Ref and cover crops) and to higher decomposition rates caused by the fallow period length and the moisture content (higher in Ct). These greater stratification ratios of SOC enhance soil nutrient retention, soil structure, infiltration and aeration and capacity to transfer the nutrient excess (Battany and Grismer, 2000).

At 0–5 cm, POC_c concentration was lower in Ct (2.22 g kg⁻¹) than in the cover crops (4.82 in W; 3.98 in O; 4.56 in O + V; and 3.45 g kg⁻¹ in V) (Table 6). After 3 years, Salvagiotti et al. (2013) observed a POC_c increase of 21 and 32% at 0–3.5 and 0–7.5 cm with the inclusion of wheat cover crops compared with the chemical fallow under soybean monoculture; they reported no differences below the 15 cm depth. In our study, cover crop effects on POC_c were observed at 5–10 cm, where W and O + V showed an increase of 39 and 31%, respectively. At 10–20 cm, no differences were found in POC_c concentration among treatments. Increased POC_c levels were directly related to C-input and the quality of C-compounds throughout the 4 years after the start of the experiment (Table 7). Higher POC_c content in soil surface is consistent with that reported in other studies, which indicate a positive relationship between the aerial biomass produced by the cover crops and the POC_c (Salvagiotti et al., 2013). This relationship was higher in the top layer (0–5 cm), but lower and no significant in deeper layers.

Cover crop effects on the soybean monoculture differed when analyzing POC_f (Fig. 2, Table 6). This fraction showed no significant changes by cover crop inclusion compared with the Ct treatment for the first two sampling dates at all depths (Table 6). The POC_f had lower concentrations than Ref, with decreases of 56 and 72% in the cultivated plots. When working on the same fraction, Salvagiotti et al. (2013) found no differences between cover crops and the Ct treatment. In contrast, POC_f concentration steadily increased over time on the plots under cover crops, with significant differences compared with Ct in 2011. This soil organic fraction showed higher concentrations in W, O and O + V, but no differences between V and Ct at 0–5 cm. This finding confirms that decomposition dynamics increases and thus N-availability is enhanced with the inclusion of leguminous species (Galantini, 2008). For the other depths, no differences were found among treatments (Table 6).

In all the cultivated treatments, MOC accounted for more than 70% of SOC (Table 6). Differences in MOC levels were observed between Ct and the cover crops, the latter showing higher MOC

Table 6

Concentration of total organic carbon (SOC), coarse particulate organic carbon (POC_c), fine particulate organic carbon (POC_f), and mineral associated organic carbon (MOC) as affected by cropping systems at 0–5, 5–10 and 10–20 cm depths.

C-fraction	Depth (cm)	Control	Wheat	Oat	Oat + Vetch	Vetch
SOC (g kg ⁻¹)	0–5	19.4 c	25.5 a	24.3 a	24.5 a	22.5 b
	5–10	15.0 a	15.3 a	15.2 a	15.7 a	15.4 a
	10–20	13.9 a	13.4 a	14.0 a	13.9 a	13.6 a
POC _c (g kg ⁻¹)	0–5	2.22 d	4.82 a	3.98 bc	4.56 ab	3.45 c
	5–10	0.327 c	0.455 a	0.383 abc	0.428 ab	0.365 bc
	10–20	0.232 a	0.245 a	0.262 a	0.222 a	0.207 a
POC _f (g kg ⁻¹)	0–5	2.41 b	3.01 a	3.00 a	3.04 a	2.71 ab
	5–10	1.050 a	1.035 a	0.993 a	1.055 a	0.953 a
	10–20	0.758 a	0.795 a	0.777 a	0.767 a	0.687 a
MOC (g kg ⁻¹)	0–5	14.8 c	17.7 a	17.3 ab	16.9 ab	16.4 b
	5–10	13.6 a	13.8 a	13.8 a	14.2 a	14.1 a
	10–20	12.9 a	12.4 a	13.0 a	12.9 a	12.7 a
POC _c :POC _f :MOC	0–5	11:12:77	19:12:69	17:12:71	19:12:69	15:12:73
	5–10	2:7:91	3:7:90	3:7:90	3:7:90	2:6:92
	10–20	2:5:93	2:6:92	2:5:93	2:5:93	2:5:93

In each row, values followed by different letters indicate significant differences ($p < 0.05$) among treatments.

Table 7
Correlations (Pearson's correlation coefficients) of total organic carbon (SOC), coarse particulate organic carbon (POC_c), fine particulate organic carbon (POC_f) and mineral-associated organic carbon (MOC) with carbon inputs and components (Mg ha⁻¹) by cover crops.

C-fraction (g kg ⁻¹)	Depth (cm)	Carbon input	Cellulose + Hemicellulose	Lignin
SOC	0–5	0.86 ***	0.84 ***	0.91 ***
	0–20	0.58 **	0.56 **	0.58 **
POC _f	0–5	0.22 ns	0.19 ns	–0.03 ns
	0–20	0.45 *	0.46 *	0.38 *
POC _c	0–5	0.49 **	0.43 *	0.51 **
	0–20	0.05 ns	–0.04 ns	–0.12 ns
MOC	0–5	0.66 ***	0.68 ***	0.75 ***
	0–20	0.48 **	0.50 **	0.56 **

Significant level *** (p < 0.001), ** (p < 0.01), * (p < 0.05) and ns: non-significant.

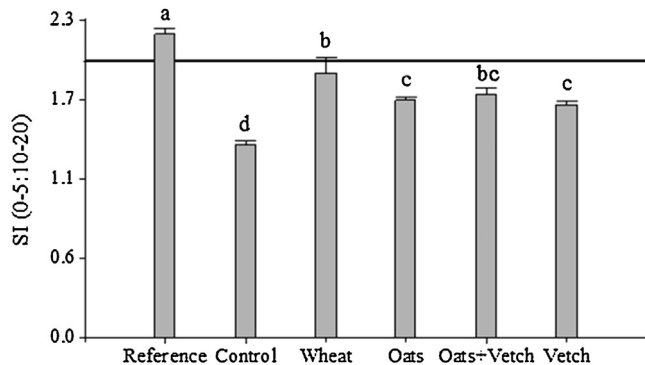


Fig. 4. Stratification ratios (SI) of soil organic carbon (SOC) concentrations. Different letters indicate significant differences between treatments (p < 0.05). The vertical bars indicate standard errors. The horizontal line is the stratification rate threshold.

concentration in the top layer (0–5 cm) in cover crops treatments. As in the labile soil organic fractions, this effect was mainly due to the annual C-input by the cover crops. Differences were also found among cover crops; MOC concentration was higher in W than in V, and no differences were detected with the other cover crops ($W \geq O \geq V \geq V$) (Table 6). These differences resulted both from a higher C-input, where W supplied twice as much C as V (3.25 Mg C ha⁻¹ vs 1.75 Mg C ha⁻¹) and from the different quality of residues in the two species. Furthermore, it is well known that the C:N ratio of residues is still considered as a major quality factor which modifies the efficiency in transforming residues to stable SOC forms. Residues with a high C:N ratio favor fungi, whereas those with a low C:N ratio favor bacteria (Bossuyt et al., 2001). Residues with a high C:N ratio (wheat = 34) are hence desirable for increasing more stable organic fractions (MOC), given that fungi prevail in the microbial community and enhance the physical environment and the recalcitrant C-forms in soil (Six et al., 2006).

Table 8
Soybean grain yield (kg ha⁻¹).

Years	Control	Wheat	Oat	Oat + Vetch	Vetch
2006	3885 (±150) ^a cA	3885 (±150) cA	3885 (±150) bcA	3885 (±150) bA	3885 (±150) bA
2007	4461 (±154) aA	4590 (±315) aA	4451 (±243) aA	4450 (±73) aA	4638 (±264) aA
2008	4214 (±120) bAB	4021 (±50) bcB	4036 (±144) abB	3932 (±344) bB	4529 (±113) aA
2009	3739 (±43) cA	3718 (±110) cA	3560 (±10) cA	3539 (±80) bA	3559 (±153) bcA
2010	4185 (±214) bA	4424 (±490) abA	4495 (±429) aA	4458 (±194) aA	4527 (±234) aA
2011	3341 (±52) dA	2717 (±219) dA	2808 (±344) dA	2959 (±334) cA	3228 (±275) cA
Mean	3971	3893	3873	3870	4061
SD	393	662	630	568	582
CV	10	17	16	15	14

SD: Standard deviation; CV: Coefficient of variation. In each row, different upper-case letters indicate significant differences (p < 0.05) among cover crops. In each column, different lower-case letters indicate significant differences (p < 0.05) among years.

^a Values in parenthesis indicate the standard deviation of the mean.

The average C-input of 3.1 Mg C ha⁻¹ year⁻¹ by the gramineous species had significant effects on the soybean monoculture, which tended to enhance SOC concentration in the upper layer after 3 years and to significantly increase after 4 years (Fig. 2, Table 6). Carbon input by soybean did not fluctuate among treatments because no yield differences were observed (Table 8). Therefore, the differences found in the concentration of SOC and its fractions should be interpreted as the cumulative effect of different residue contributions and their decomposition since cover crop implementation. Differences in SOC concentration among cover crops were caused by differences in C-input (4.9 to 1.5 Mg ha⁻¹), although C concentration in cover crops were similar (425 to 468 g kg⁻¹). Similar results were published by Sainju et al. (2003) after examining various gramineous and leguminous species. In general, C concentration in winter cover crop species is quite uniform, values ranging from 420 to 440 g kg⁻¹ (Wang et al., 2012). Therefore, DM production is one of the major characteristics when choosing a cover crop species.

Physical fractionation by particle size demonstrated that cover crops affected the most dynamic fraction, directly associated with residue input (POC_c), at 0–5 and 5–10 cm (Table 6). They also affected the most transformed fractions (POC_f and MOC) at the 0–5 cm depth (Table 6), 4 years after the beginning of the experiment, and the labile fractions (POC_c and POC_f) at the 0–20 cm depth, 6 years after the trial started (Fig. 2).

In this study, POC_c proved to be more sensitive to cover crops; in fact, POC_c concentration was higher for the 4-year cover crop treatments than for the control treatment (Fig. 2). Cover crop inclusion enhanced organic levels in soil by substituting the bare fallow period (source of C) for an additional period of C-assimilation (Lal, 2001). In turn, cover crops also increased SOC lability, as revealed by the increased proportion of POC_c in the top layer of the soil compared with MOC. In the cover crop treatments, POC_c accounted for 15–19% of SOC, whereas in the Ct treatment such value was 11% (Table 6). This increase in SOC lability was due to a steady surface accumulation of residues that

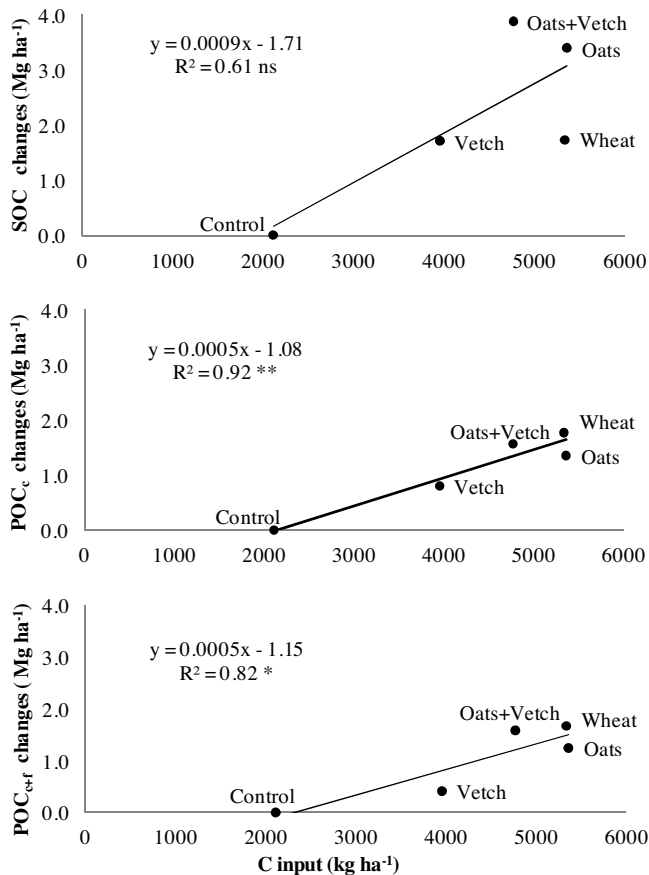


Fig. 5. Relationship between annual C-input and changes in the content of total organic carbon (SOC) and labile fractions (POC_c and POC_{c+f}). Significant level *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$) and ns: non-significant.

were incorporated to the particulate organic matter of soil more rapidly than to the mineral-associated fraction (MOC) (Table 6). The turnover of POC_c is faster compared with that of MOC and therefore POC_c is more sensitive to soil management practices. This increase in labile fractions has been frequently observed in soils under NT with a high residue-input (Bayer et al., 2001).

The quantity and quality of C supplied by the cover crops greatly affected soil C-levels (total content + fractions) (Table 7).

Levels of SOC, POC_c and POC_f at 0–5 and 0–20 cm in gramineous cover crops (mainly W) were similar to or higher than those in legume cover crops, then gramineous species were more efficient in supplying higher C-input to the soil. In addition, C-input by gramineous species had a higher C:N ratio (lower quality), which could have resulted in a slower residue decomposition in soil.

3.5. Soybean yields

Soybean grain yields showed significant differences among years ($p < 0.05$), but not among treatments ($p > 0.05$); non-significant interaction ($p > 0.15$) was detected (Table 8). Soybean yields ranged from 3011 to 4518 kg ha⁻¹ across the years. These differences were caused by rainfall during the crop cycle, with or without cover crops, with a highly significant relationship ($r = 0.83$, $p < 0.001$). Despite high water-use by the cover crops, the results obtained failed to show significant differences in grain production between the cover crops and the Ct treatment. Significant differences were only observed between V and the rest of the cover crops, with higher yields for legumes in 2007/2008. Yields of soybean with previous cover crops showed no significant

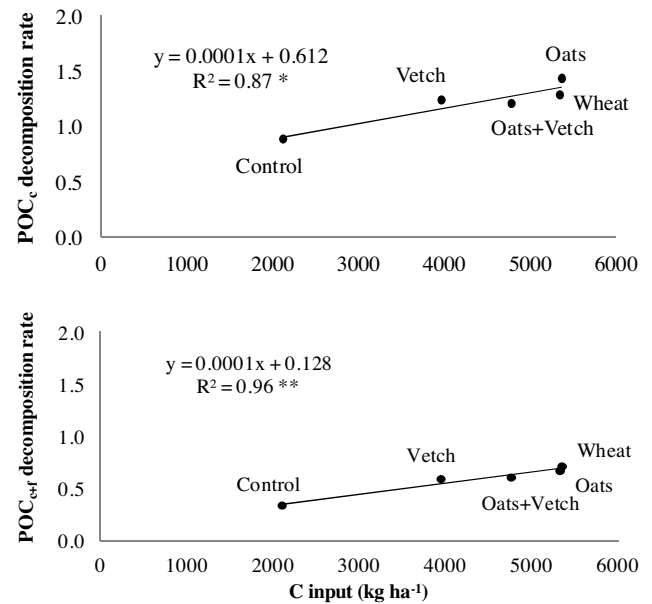


Fig. 6. Relationship between annual C-input and decomposition rate of labile fractions.

Significant level *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$) and ns: non-significant.

differences compared with Ct during the period evaluated (Table 8). These results agreed with those reported by Álvarez et al. (2008) who found no differences regarding grain production between the control and the cover crop treatments on Typic Argiudolls.

3.6. Carbon balance and dynamics

Labile soil organic carbon fractions reach an equilibrium or steady state in a shorter period of time (5–10 years) as compared with the recalcitrant fraction (decades or centuries). The transformation rate can thus be estimated from the average annual C-input and C content in the soil.

Higher mean annual C-input by residues (cover crops + soybean) into the soil accounted for much of the variation in SOC ($R^2 = 0.61$, $p < 0.05$). This association was more higher with the labile carbon fraction, both for POC_c ($R^2 = 0.91$, $p < 0.001$) and for POC_c + POC_f ($R^2 = 0.81$, $p < 0.001$) (Fig. 5).

The k-values estimated for POC_c and POC_c + POC_f ranged from 0.88 to 1.43 and from 0.34 to 0.71, respectively. This variation was strongly associated with C-input (Fig. 6), thus indicating that higher C-amounts, mainly supplied by the cover crops, enhanced the decomposition rate or the “priming effect”. These strong associations, both for POC_c ($R^2 = 0.87$, $p < 0.05$) and POC_c + POC_f ($R^2 = 0.96$, $p < 0.01$), reported the highest k-values, which corresponded to the gramineous species. This would clearly indicate a C-N unbalance caused by N-excess in the soybean monoculture. The slight differences among treatments in the first sampling period (3 years) could be associated with the immediate use of C by soil microorganisms. In this sense, the hypothesis proposed is confirmed for the first years after the start of the trial. Later, although a faster decomposition follows, labile fractions of the SOM started to accumulate.

4. Conclusions

After six growing seasons of cover crops on soybean monoculture, more than 3.0 Mg C ha⁻¹ year⁻¹ were supplied by gramineous species (pure stand or mixture), and less than 2.0 Mg C ha⁻¹ year⁻¹

by the vetch treatment. This additional C-input increased water-use by gramineous species, which was more efficient in producing C than vetch. This greater C-supply by gramineous species enhanced water storage after the cover crop cycle, thus promoting water-sequestration and reducing evaporation. Carbon input can be increased in continuous soybean under NT in Pampas Argiudolls by including gramineous cover crops.

The effect of cover crops on SOC concentration was proportional to DM production, but it is probably not enough to increase organic levels at deeper depths, at least in the short term. After four years, surface content of SOC and its associated fractions (POC_c, POC_f and MOC) were modified. This effect became noticeable during the third year, when plots under cover crops showed higher SI than the fallow. Therefore, from the point of view of the edaphic indicators, SOC stratification helped to differentiate among management practices, indicating that wheat was the most suitable species to be used as cover crops for the soybean monoculture under the conditions of this study.

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References

- Álvarez, C., Scianca, C., 2006. Cultivos de cobertura en Molisoles de la región pampeana. Aporte de carbono e influencia sobre propiedades edáficas. INTA EEA General Villegas. Día de campo. Jornada profesional agrícola.
- Álvarez, C., Barraco, M., Díaz-Zorita, M., Scianca, C., 2006. Uso de cultivos de cobertura en rotaciones con base soja: efecto sobre algunas propiedades edáficas y rendimiento de los cultivos en un Hapludol típico del noroeste bonaerense. XX Congreso Argentino de la Ciencia del Suelo. Salta, Argentina. 19 al 22 de Setiembre de.
- Álvarez, C., Scianca, C., Barraco, M., Díaz, Zorita, M., 2008. Impacto del manejo de cereales de cobertura invernal sobre propiedades edáficas y producción de soja. XXI Congreso Argentino de la Ciencia del Suelo. Potrero de los Funes (San Luis), Argentina. 13 al 16 de mayo del.
- Andriulo, A., Cordone, G., 1998. Impacto de labranzas y rotaciones sobre la materia orgánica de suelos de la Región Pampeana Húmeda. In: Panigatti, J.L., Marelli, H., Buschiazzi, D., Gil, R. (Eds.), Siembra Directa. Hemisferio Sur., pp. 65–95.
- Balesdent, J., Balabane, M., 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biol. Biochem.* 28, 1261–1263.
- Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil Tillage Res.* 53, 215–230.
- Battany, M.C., Grismer, M.E., 2000. Rainfall runoff and erosion in Napa Valley vineyards: effects of slope, cover and surface roughness. *Hydrol. Processes* 14 (7), 1289–1304.
- Bayer, C., Martin-Neto, L., Mielniczuk, J., Pillon, C.N., Sangoi, L., 2001. Changes in soil organic matter fractions under subtropical no-till cropping systems. *Soil Sci. Soc. Am. J.* 65, 1473–1478.
- Bayer, C., Dieckow, J., Amado, T.J.C., Eltz, F.L.F., Vieira, F.C.B., 2009. Cover crop effects increasing carbon storage in a subtropical no-till Sandy Acrisol. *Commun. Soil Sci. Plant Anal.* 40 (9–10), 1499–1511.
- Benbi, D.K., Toor, A.S., Kumar, S., 2012. Management of organic amendments in rice-wheat cropping system determines the pool where carbon is sequestered. *Plant Soil.* 360 (1–2), 145–162.
- Blake, G.R., Hartge, K.H., 1986. Bulk density, In: Klute, A. (Ed.), *Methods of Soil Analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI*, pp. 363–375.
- Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. *Appl. Soil Ecol.* 16, 195–208.
- Bremner, J.M., 1996. Nitrogen-total. In: Sparks, D.L. (Ed.), *Methods of Soil Analysis. Part 3—Chemical Methods. ASA, SSSA, CSSA, Madison, WI*, pp. 1085–1121 (Chapter 37).
- Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124 (1), 3–22.
- Capurro, J., Dickie, M.J., Surjack, J., Monti, J., Ninfi, D., Zazzarini, A., Tosi, E., Andriani, J., Gonzalez, M.C., 2013. Cultivos de cobertura en el sur de la provincia de Santa Fe. In: Álvarez, C., Quiroga, A., Santos, D., Bodrero, M., (Eds.). *Contribución de los cultivos de cobertura a la sustentabilidad de los sistemas de producción. INTA. EEA Anguil, Argentina*, pp. 92–104.
- Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur. J. Soil Sci.* 52 (3), 345–353.
- Clark, A.J., Decker, A.M., Meinsiger, J.J., McIntosh, M.S., 1997. Kill date of vetch, rye, and a vetch-rye mixture: I. Cover crops and corn nitrogen. *Agron. J.* 89, 427–434.
- de Oliveira Ferreira, A., Amado, T.J., Nicoloso, R., de Moraes Sá, J.C., Fiorin, J.E., Santos Hansel, D.A., Menefee, D., 2013. Soil carbon stratification affected by long-term tillage and cropping systems in southern Brazil. *Soil Tillage Res.* 133, 65–74.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2013. *InfoStat. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.*
- Ding, G., Liu, X., Herbert, S., Novak, J., Dula, A., Xing, B., 2006. Effect of cover crop management on soil organic matter. *Geoderma* 130, 229–239.
- Dolman, A.J., Verhagen, A., Rovers, C.A., 2003. *Global Environmental Change and Land Use. Kluwer Academic Publishers, Netherlands*, pp. 209.
- Duval, M.E., Galantini, J.A., Iglesias, J.O., Canelo, S., Martínez, J.M., Wall, L., 2013. Analysis of organic fractions as indicators of soil quality under natural and cultivated systems. *Soil Tillage Res.* 131, 11–19.
- Fernández, R., Quiroga, A., Arenas, F., Antonini, C., Saks, M., 2007. Contribución de los cultivos de cobertura y las napas freáticas a la conservación del agua, uso consuntivo y nutrición de los cultivos. In: Quiroga, A., Bono, A. (Eds.), *Manual de fertilidad y evaluación de suelos. INTA. EEA Anguil, Argentina*, pp. 51–59.
- Franzluebbers, A.J., 2002. Soil organic matter stratification ratio as an indicator of soil quality. *Soil Tillage Res.* 66, 95–106.
- Franzluebbers, A.J., 2005. Soil organic carbon sequestration and agricultural greenhouse gas emissions in the southeastern USA. *Soil Tillage Res.* 83, 120–147.
- Galantini, J.A., Duval, M., Iglesias, J.O., Krüger, H., 2014. Continuous wheat in semi-arid regions: long-term effects on stock and quality of soil organic carbon. *Soil Sci.* 179, 284–292.
- Galantini, J.A., 2008. Fraccionamiento de la materia orgánica del suelo. In: Galantini, J.A. (Ed.), *Estudio de las fracciones orgánicas en suelos de la Argentina. EdiUNS, Bahía Blanca, Argentina*, pp. 19–39.
- Heal, O.W., Anderson, J.M., Swift, M.J., 1997. Plant litter quality and decomposition: an historical overview. In: Cadish, G., Killer, K.E. (Eds.), *Driven by Nature: Plant Litter Quality and Decomposition.*, pp. 3–30.
- Huggins, D.R., Allmaras, R.R., Clapp, C.E., Lamb, J.A., Randall, G.W., 2007. Corn-soybean sequence and tillage effects on soil carbon dynamics and storage. *Soil Sci. Soc. Am. J.* 71, 145–154.
- Johnson, J.M.F., Allmaras, R.R., Reicosky, D.C., 2006. Estimating source carbon from crop residues, roots and rhizodeposits using the national grain-yield database. *Agron. J.* 98, 622–636.
- Kachaka, S., Vanlauwe, B., Merckx, R., 1993. Decomposition and nitrogen mineralization of prunings of different quality. In: Mulongoy, K., Merckx, R. (Eds.), *Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture. Wiley-Sayce, UK*, pp. 199–208.
- Klute, A., 1986. Water retention: laboratory methods, In: Klute, A. (Ed.), *Methods of Soil Analysis. Part 1. 2nd edition Agron. Monogr. 9. ASA and SSSA, Madison, WI*, pp. 635–661.
- Kuo, S., Jellum, E.J., 2000. Long-term winter cover cropping effects on corn (*Zea mays* L.) production and soil nitrogen availability. *Biol. Fertil. Soils* 31, 470–477.
- Lal, R., 2001. World cropland soils as a source or sink for atmospheric carbon. *Advances in Agronomy. Academic Press*, pp. 145–191.
- Lavado, R., 2006. La Región Pampeana: historia, característica y uso de sus suelos. In: Álvarez, R. (Ed.), *Materia orgánica: Valor agronómico y dinámica en suelos pampeanos. Editorial Facultad de Agronomía, Buenos Aires, Argentina*, pp. 1–11.
- Muller, D.G., Saluzzo, M.F., De Battista, J.J., 2008. Comparación de distintos cultivos de cobertura en suelo Vertisol. XXI Congreso Argentino de la Ciencia del Suelo. Potrero de los Funes (San Luis), Argentina. 13 al 16 de mayo.
- Neal, J., Fulkerson, W., Hacker, R., 2011. Differences in water use efficiency among annual forages used by the dairy industry under optimum and deficit irrigation. *Aust. Agric. Water Manage.* 98, 759–774.
- Novelli, L.E., Caviglia, O.P., Melchiori, R.J.M., 2011. Impact of soybean cropping frequency on soil carbon storage in Mollisols and Vertisols. *Geoderma* 167–168, 254–260.
- Papini, R., Valboa, G., Favilli, F., L'Abate, G., 2011. Influence of land use on organic carbon pool and chemical properties of Vertic Cambisols in central and southern Italy. *Agric. Ecosyst. Environ.* 140 (1), 68–79.
- Prescott, C.E., Weetman, G.F., DeMontigny, L.E., Preston, C.M., Keenan, R.J., 1995. Carbon chemistry and nutrient supply in cedar-hemlock and hemlock-amabilis fir forest floors. In: McFee, W.W., Kelley, J.M. (Eds.), *Carbon Forms and Functions in Forest Soils. Soil Sci. Soc. Am., Madison, WI*, pp. 377–396.
- Restovich, S.B., Sasal, M.C., Irizar, A.B., Rimatori, F., Darder, M.L., Andriulo, A.E., 2005. Rotación con maíz vs monocultivo de soja: efecto sobre los stocks de carbono y nitrógeno edáficos. VIII Congreso Nacional de Maíz. Rosario. Santa Fe. Argentina. pp. 208.

- Restovich, S.B., Andriulo, A.E., Amendola, C., 2011. Inclusion of cover crops in a soybean–corn rotation: effect on some soil properties. *Ci. Suelo (Argentina)* 29, 61–73.
- Restovich, S.B., Andriulo, A.E., Portela, S.I., 2012. Introduction of cover crops in a maize–soybean rotation of the Humid Pampas: effect on nitrogen and water dynamics. *Field Crops Res.* 128, 62–70.
- Richards, L.A., 1947. Pressure-membrane apparatus-construction and use. *Agric. Eng.* 28, 451–454.
- Rovira, P., Jorba, M., Romanyà, J., 2010. Active and passive organic matter fractions in Mediterranean forest soils. *Biol. Fertil. Soil* 46, 355–369.
- Sá Pereira, E.D., Galantini, J.A., Quiroga, A., 2012. Simulación de la dinámica de los residuos de cultivos de cobertura bajo siembra directa. XIX Congreso Latinoamericano de la Ciencia del Suelo y XXIII Congreso Argentino de la Ciencia del Suelo. Mar del Plata, Argentina. 16 al 20 de Abril.
- Sá, J.C.M., Lal, R., 2009. Stratification ratio of soil organic matter pools as an indicator of carbon sequestration in a tillage chronosequence on a Brazilian Oxisol. *Soil Tillage Res.* 103, 46–56.
- Sainju, U.M., Whitehead, W.F., Singh, B.P., 2003. Cover crops and nitrogen fertilization effects on soil aggregation and carbon and nitrogen pools. *Can. J. Soil Sci.* 83, 155–165.
- Sainju, U.M., Singh, B.P., Whitehead, W.F., Wang, S., 2007. Accumulation and crop uptake of soil mineral nitrogen as influenced by tillage, cover crops, and nitrogen fertilization. *Agron. J.* 99, 682–691.
- Salvagiotti, F., Vernizzi, A., Bodrero, M., Bacigaluppo, S., 2013. Cambios en el corto plazo en distintas fracciones de la materia orgánica en respuesta a la inclusión de cultivos de cobertura en secuencias basadas en soja. In: Álvarez, C., Quiroga, A., Santos, D., Bodrero, M., (Eds.). Contribución de los cultivos de cobertura a la sustentabilidad de los sistemas de producción. INTA. EEA Anguil, Argentina, pp. 88–91.
- Salvo, L., Hernandez, J., Ernst, O., 2010. Distribution of soil organic carbon in different size fractions, under pasture and crop rotations with conventional tillage and no-till systems. *Soil Tillage Res.* 109, 116–122.
- Scherer-Lorenzen, M., 2008. Functional diversity affects decomposition processes in experimental grasslands. *Funct. Ecol.* 22 (3), 547–555.
- Scianca, C., Varela, M.F., Barraco, M., Álvarez, C., Quiroga, A., 2013. Cultivos de cobertura en un Hapludol Thapto Árgico de La Pampa arenosa: análisis de cinco campañas. In: Álvarez, C., Quiroga, A., Santos, D., Bodrero, M., (Eds.). Contribución de los cultivos de cobertura a la sustentabilidad de los sistemas de producción. INTA. EEA Anguil, Argentina, pp. 105–116.
- Six, J., Jastrow, J.D., 2002. Organic matter turnover. In: Lal, R. (Ed.), *Encyclopedia of Soil Science*. Marcel 563 Dekker, New York, pp. 936–942.
- Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70, 555–569.
- Soil Survey Staff, 2010. *Keys to Soil Taxonomy*, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC.
- Toledo, D.M., Galantini, J.A., Ferreccio, E., Arzuaga, S., Gimenez, L., Vazquez, S., 2013. Indicadores e índices de calidad en suelos rojos bajo sistemas naturales y cultivados. *Ci Suelo (Argentina)* 31 (2), 201–212.
- Unger, P.W., Vigil, M.F., 1998. Cover crops effects on soil water relationships. *J. Soil Water Conserv.* 53, 200–207.
- Van Soest, P.V., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74 (10), 3583–3597.
- Vanzolini, J.I., Galantini, J., Agamennoni, R., 2013. Cultivos de cobertura de Vicia villosa Roth. en el valle bonaerense del Río Colorado. En: Álvarez, C., Quiroga, A., Santos, D., Bodrero, M., (Eds.). Contribución de los cultivos de cobertura a la sustentabilidad de los sistemas de producción. INTA. EEA Anguil, Argentina, pp. 21–28.
- Varela, M.F., Scianca, C.M., Taboada, M.A., Rubio, G., 2014. Cover crop effects on soybean residue decomposition and P release in no-tillage systems of Argentina. *Soil Tillage Res.* 143, 59–66.
- Wang, Q., Li, Y., Alva, A., 2012. Cover crops in mono-and biculture for accumulation of biomass and soil organic carbon. *J. Sustain. Agric.* 36 (4), 423–439.