Changes in soil organic carbon fractions in a sequence with cover crops

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Cambios en las fracciones de carbono orgánico del suelo en una secuencia con cultivos de cobertura
Alterações nas frações de carbono orgânico do solo numa sequência com culturas de cobertura

ABSTRACT

Advances in cover crops practice, in the context of potential benefics for annual crop production and sustained soil quality were studied. A soybean-maize sequence with five winter cover crops (CC) species were studied at the Marcos Juárez INTA Experimental Station, Córdoba, Argentina. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T) and hairy vetch (VV) + triticale (T) mixture were tested as well as a control treatment (Ct) without a CC. The CC effect on the dynamics and balance of the soil organic C (SOC) and its fractions were examined. Maize and soybean yields did not show significant differences between the control and the CC treatments. The SOC stratification (0-0.10 and 0.10-0.20 m) with accumulation of residue on surface was due to the concentration of SOC and fractions that decreased with depth. The gramineous crops were more efficient in biomass production with more C input into the soil. Triticale showed positive C balance in OC and in the easily degradable fraction (labile) and an increase in the residue decomposition rate. CC had a positive impact on the more stable C stock (recalcitrant OC) in the sub-superficial layer than in the superficial one. The gramineae input was evident in the superficial layer and the most stable OC fraction, was concentered in the sub-superficial layer. Organic soil fractioning by particle size have been shown to be useful indicators for detecting changes produced by management practices in most experiments. This study demonstrated that the effect of cover crops on SOC and the labile fraction in the upper soil layer was strongly related with the high residue production.

RESUMEN

Se estudió el manejo de los cultivos de cobertura y sus potenciales beneficios para la producción de cultivos anuales y la calidad sostenible de los suelos. Se estudió la secuencia soja–maíz con cinco especies de cultivos de cobertura (CC) invernales, en la Estación Experimental INTA Marcos Juárez, provincia de Córdoba, Argentina. Los CC utilizados fueron: vicia sativa (VS), vicia villosa (VV), centeno(C), triticale (T) y una consociación de vicia villosa y triticale (VV+T), así como un tratamiento control (Ct), sin CC. Se estudió el efecto de los CC sobre la dinámica y el balance del carbono orgánico del suelo (COS) y sus fracciones. Los rendimientos de maíz y de soja no mostraron diferencias significativas entre el tratamiento Ct y los CC. La concentración del carbono orgánico del suelo (COS) y sus fracciones disminuyeron a medida que aumentó la profundidad produciendo la estratificación del COS (0–0,10 y 0,10–0,20 m) con acumulación de residuos superficiales. Las gramíneas como CC fueron más eficientes en la producción de biomasa con la consecuente incorporación de C al suelo. Dentro de éstas, el triticale mostró un balance positivo de C tanto en el total del COS como en las fracciones fácilmente degradables (fracciones lábiles) con una mayor velocidad de descomposición de residuos. Los CC tuvieron un impacto positivo en el aumento del stock

del C más estable, carbono orgánico recalcitrante (COR), en el horizonte subsuperficial más que en superficial. El aporte de las gramíneas fue notorio en la capa superficial de suelo y la fracción de C recalcitrante se concentró en la capa subsuperficial. En la mayoría de los estudios se observó que el fraccionamiento del carbono orgánico del suelo es un indicador útil para detectar los cambios producidos por las prácticas de manejo. En este trabajo se demostró que en la capa superficial de suelo, el efecto significativo de los CC sobre el COS y la fracción lábil del mismo, estuvo fuertemente relacionado con la mayor producción de residuos.

RESUMO

Estudou-se a gestão de culturas de cobertura e os potenciais benefícios para a produção anual de culturas e a qualidade sustentável do solo. A sequência soja-milho foi estudada com cinco espécies de plantas de cobertura de inverno (CC), na Estação Experimental INTA Marcos Juárez, província de Córdoba, Argentina. As CC utilizadas foram: ervilhaca comum (VS), ervilhaca peluda (VV), centeio (C), triticale (T) e uma consociação de ervilhaca peluda (VV) + triticale (T), bem como um tratamento controlo (Ct) sem CC. Estudou-se o efeito dos CC sobre a dinâmica e o equilíbrio do carbono orgânico do solo (COS) e as suas frações. As produções de milho e soja não apresentaram diferenças significativas entre os tratamentos controle e CC. A estratificação do SOC (0-0,10 e 0,10-0,20 m) com a acumulação de resíduos na superfície foi devida à concentração de SOC e frações que diminuíram com a profundidade. As gramíneas foram mais eficientes na produção de biomassa com mais entrada de C no solo. O triticale apresentou um balanço positivo de C no CO e na fração facilmente degradável (lábil) e um aumento na taxa de decomposição do resíduo. O CC teve um maior impacto positivo na reserva do C mais estável (CO recalcitrante) na camada sub-superficial do que na camada superficial. O efeito das gramíneas foi evidente na camada superficial e a fração mais estável de OC concentrou-se na camada sub-superficial. O fracionamento do solo orgânico por tamanho das partículas demonstrou ser um indicador útil para detectar mudanças produzidas pelas práticas de gestão na maioria dos ensaios. Este estudo demonstrou que o efeito das culturas de cobertura no SOC e na fração lábil na camada superficial do solo estava fortemente relacionada com a elevada produção de resíduos.

1. Introduction

Cover crops (CC) are crops that are seeded between cash crops and are not harvested, not incorporated into the soil as green manure, and not intended for grazing, like annual forage. The species most commonly used as CC are gramineae, and to a lesser extent leguminosae, which have the benefit of fixing nitrogen (N) from the atmosphere (Rimsky-Korsakov et al. 2015). Cover crops grown during periods when the soil might otherwise be fallow are a valuable management option for reducing soil erosion and nitrogen losses from agroecosystems. They improve soil quality but the impacts on crop yield depend on the type of cover crop, the commercial crop considered and the climate (Álvarez et al. 2017).

Using cover crops (CC) would be an efficient alternative during winter periods for producing an increase in crop residues in production systems with a high frequency of soybean (*Glycine max* L.) rotation. Various authors agree that winter species as CC increase the dry matter production and improve the carbon (C) input into the soil (Sainju et al. 2007; Ghiotti and Basanta 2008). Significant amounts of carbon rich residues in the soil positively modify the composition and quality of the soil organic matter (SOM), improving soil productivity (Restovich et al. 2011; Sainju et al. 2003). In this context, CC has been recommended for soybean monoculture in Argentina, where the production of crop residues may be insufficient for proper soil cover and protection (Novelli et al. 2011). Soybean, characterized by a high N demand for grain production, also causes a negative soil N balance (Landriscini et al.

2019). On the other hand, maize produces more residues with high values in the C:N ratio, which would favor slow decomposition that is beneficial for stabilizing SOM evolution (Morón 2004).

The content of soil organic carbon (SOC) is determined by the relationship between the C input into the soil and C losses, therefore, long-term fallow cropping systems (soybean or maize monocultures) reduce the carbon input whereas cover crops increase it significantly (Duval et al. 2016). Several authors have recommended the annual C input necessary for maintaining the SOC levels in different soils in the Pampas region, e.g. in the southeast of Córdoba province, Argentina. It was estimated that 3 Mg ha^{-1} year^{-1} of C input from crop residues are needed for SOC levels of 36 Mg ha^{-1} (Cazorla 2013).

These agricultural systems typically include long periods of fall-winter fallow with a low annual C input into the soil (2-3 Mg C ha^{-1} year^{-1}) (Restovich et al. 2005).

In no-tillage systems, the C and N contents may differ between the surface and subsurface layers. The stratification ratio (SR) of SOC or total N (TN) is an index that relates these contents in two different soil layers and can be used as indicator of the dynamic soil quality (Franzluebbers 2002; Toledo et al. 2013). Furthermore, the SR of SOC is a good indicator of the SOC sequestration rate. Higher SR of SOC indicates that soil management enhances soil quality. This is because the topsoil layer is influenced by land management but the second and subsequent layers are less affected (Franzluebbers 2002). For SOC, stratification values higher than 2.0 are essential to maintain soil quality (Fernández-Romero et al. 2016). Stratification ratios allow a wide diversity of soils to be compared on the same assessment scale because of an internal normalization procedure that accounts for inherent soil differences (Franzluebbers 2002).

The SOC is unlikely to change in the short term (3-4 years), but not in the most labile fractions, associated with residues in the early stages of decomposition and linked to the coarser structural fractions of the soil (particulate organic carbon, POC) (Duval et al. 2016). The POC fraction is the most active and it is used as a soil quality indicator in the short term because it is sensitive to management-induced changes (Duval et al. 2013). In addition, these labile fractions were shown to be correct indicators of changes in crop sequences (Salvo et al. 2010) and they may also show early soil changes resulting from the inclusion of cover crops.

The SOC components can be compartmentalized into different fractions or pools. The labile fraction is composed of particulate organic matter, easily degradable by microorganisms with fast turnover rates, from months to a few years (Krull et al. 2003). The other stabilized or resistant fraction would be persistent in the soil over a scale of years (Bruun et al. 2007). This fraction can be defined as that which is slowly lost after cultivation and which increases proportionally as the SOC decreases.

There is little agreement about how to quantify soil organic matter (SOM) as a biochemical quality. Ideally, this "quality" must reflect biodegradability in the absence of physical protection and hence be based on chemical composition independent of physical position within the mineral matrix (Rovira and Vallejo 2007). A useful alternative is acid hydrolysis, proposed as a simple method to evaluate SOM quality. This procedure is easy to perform and can be applied to the large series of samples generally employed in ecological research. The non-hydrolyzable residue may include young SOM, but most radiocarbon studies have shown that the residue from acid hydrolysis is consistently older than the hydrolysable fraction, whether the hydrolysis is applied to the whole SOM (Pandey et al. 2014).

Even though there is no standard definition of "chemical recalcitrance" under natural conditions, operationally it refers to resistance to loss under selected chemical treatments (Pandey et al. 2014). Hydrolysis with acids like HCl (Plante et al. 2006) is a common procedure for obtaining the labile and recalcitrant fractions from the soil and for determining the SOM biochemical quality. Chemical recalcitrance removes proteins, nucleic acids and polysaccharides, whereas the non-hydrolysable residue contains resistant compounds such as lignin, aromatic, humified components and long-chain aliphatic compounds. The non-hydrolysable fraction

represents the recalcitrant pool and its relative size with respect to the total C is termed the "Recalcitrance Index" (RI) (Rovira and Vallejo 2002).

We hypothesized that cover crops mainly modify the distribution of the labile organic fractions and the quality of the recalcitrant compounds within the surface layer.

The objectives of this study were to: 1. Evaluate the effect of different CC inclusions sown after a soybean-maize (*Zea mays L.*) sequence, on the dynamics of the SOC and its fractions in a soil in the southeast of Córdoba province, Argentina, during a crop cycle; 2. Determine the SOC and its fractions balance after 7 years of a soybean-maize sequence; 3. Assess the changes in the organic fractions with different degrees of recalcitrance and the Recalcitrance Index variations.

2. Materials and Methods

2.1. Experimental Site

The study site was located at the Marcos Juárez INTA Experimental Station, Córdoba, Argentina (32°42'44.65"S; 62°05'46.07"W) (**Figure 1**). The area is characterized by a temperate climate with an average annual temperature of 16.9 ºC and a mean annual precipitation of about 894 mm (Andreucci et al. 2016). The soil is a Mollisol, i.e. Typic Argiudoll (Soil Survey Staff 2010); developed in aeolian sediments (loess), with a wide variability in depth, texture, soil OM content and fertility (Álvarez and Lavado 1998). It is a dark, deep and well-drained soil in a flat relief position with a silt loam texture in the surface horizons (Horizons A). It is a typical representative of the good soils of the area with a wide aptitude for crops, forage and pastures, although they present a slight climatic limitation, especially in the west and northwest sector

(Baigorria et al. 2019). The upper layer is slightly acidic (pH 6.4) with 32.6 g kg^{-1} OM, belonging to the Marcos Juárez series (INTA 1978). In semiarid and semihumid regions, the soils are characterized by a low OM content, and their dynamics are affected more strongly by water availability (Galantini et al. 2016).

Figure 1. Location of the experimental site at Cordoba province, Argentina.

2.2. Experimental design and treatments

A soybean-maize sequence with different winter cover crops used as predecessors was started in 2008, under no-tillage for the previous 9 years. The species used were common vetch (*Vicia sativa L.*) (VS), hairy vetch (*Vicia villosa* Roth) (VV), rye (*Secale cereale L.*) (R), triticale (*x Triticosecale Wittmack*) (T), hairy vetch (VV) + triticale (T) mixture, and a control treatment (Ct) without CC, that was kept weed-free with herbicide applications (**Figure 2** and **Table 1**). Rye and Triticale are gramineae (G) crop and the others leguminosae (L) species.

The long-term assay experiment was established on 150 m^2 (6 x 25 m) plots in a randomized split plot design with five treatments and three replications. The principal factor was the CC inclusion and the second factor was the CC fertilization (fertilized: f, and unfertilized: nf) applied to subplots. The fertilization factor was 100 kg N ha^{-1} for triticale and rye, 50 kg N ha^{-1} and 50 kg P ha^{-1} for the hairy vetch + triticale mixture and 50 kg P ha^{-1} for hairy and common vetch.

Table 1. Different cover crops species, scientific names and treatments code

Treatment	Scientific Name	Treatment Code
Cover Crop		CC
Common Vetch	*Vicia sativa L.*	VS
Hairy Vetch	*Vicia villosa Roth*	VV
Rye	*Secale cereal L.*	R
Triticale	*Triticosecale Wittmack*	T
Hairy Vetch + Triticale mixture	*Vicia villosa Roth x Triticosecale Wittmack*	VV + T
Control		Ct

CC   maize   CC   soybean

April-August   *October-March*   *April-September*   *November-April*
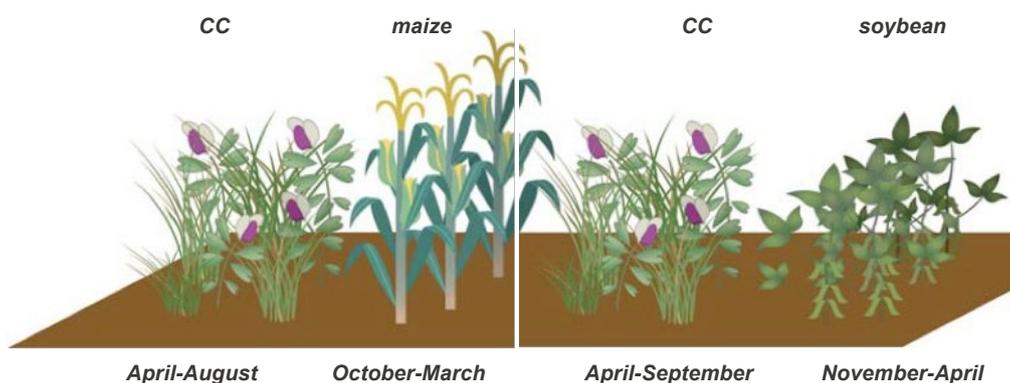
Figure 2. Scheme of the crop sequence in the long-term experiment with cover crops (CC): common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture. Harvest crop: maize and soybean. The months mentioned are the CC and crop sowing dates (González et al. 2017).

2.3. Cover crops and harvest crops

All CC were fertilized at seeding time and were killed in their reproductive stage by glyphosate application after harvesting the summer crop. The soybean and maize crops were sown between October and December each year. The seeding density was 270,000 and 65,000 plants ha^{-1} for soybean and maize, respectively.

At physiological maturity, the above-ground biomass of the cover crops was harvested manually from three 0.5 m^2 areas per sampling point at CC killing time. The total aerial dry matter yield was determined after drying in a forced-air oven at 65 ºC for at least 72 hours.

Soybean and maize yields were obtained by mechanical harvesting from three subsamples (1.0 m^2) per experimental unit. Grains were separated from all other vegetal material and dry matter weights were recorded separately.

2.4. Soil physical fractionation of SOM and soil analysis

After the soybean harvest, in 2017, soil samples (three replicates) were taken randomly at 0-0.10 and 0.10-0.20 m depths from each plot. The samples were air-dried, crushed and passed through a 2-mm aperture sieve.

The SOM was physically separated by wet sieving (Cambardella and Elliot 1992; Duval et al. 2013). Briefly, 50 g of previously air-dried and sieved soil was dispersed in 120-mL glass containers and mixed with 105 mL of distilled water. Ten glass beads (5 mm diameter) were added to increase aggregate destruction and reduce any potential problems created by sand. After dispersion, the soil suspension was sieved through 2 connected sieves of 53 and 105 µm diameter. The sieves were moved back and forth and the soil retained on the top of the sieve was sprinkled with distilled water until the water in the bottom sieve was clear to the naked eye. Three fractions were obtained: i) the coarse fraction (105-2000 mm) containing coarse particulate organic carbon (POCc) and fine to coarse sands, ii) the medium fraction (53-105 mm) containing fine particulate organic carbon (POCf) and very fine sands, and iii) the fine fraction (< 53 mm) containing mineral-associated organic carbon (MOC) as well as silt and clay. The material retained in each sieve was dried, homogenized and analyzed for OC. The C content was determined by dry combustion (LECO, St. Joseph, MI).

Each soil sample was tested with hydrochloric acid to verify the presence of carbonates (Schoenenberger et al. 1998). However, no carbonates were found in any of the samples at the 0-0.20 m soil depth.

Total N concentration was determined by the semi-micro method of Kjeldahl (Bremner 1996; IRAM-SAGyP 2018).

Stratification ratios (SR) of SOC and TN were calculated based on the concentration of SOC at a depth of 0-0.10 m, divided by the concentration of that property at a depth of 0.10-0.20 m (Franzluebbers 2002). The degree of stratification of soil organic C and TN pools with soil depth, expressed as a ratio, could indicate soil quality or soil ecosystem functioning, because surface organic matter is essential to erosion control, water infiltration, and conservation of nutrients.

The fine soil fraction was subjected to acid hydrolysis with a modified procedure of the method described by Paul et al. (1997). Briefly, 0.3-0.5 g of sample was refluxed at 118 °C for 16 hours in 20 ml of 6 M HCl. If insufficient material was recovered during the physical fractionation, individual replicates were combined. The hydrolysate was discarded. The unhydrolyzed residue was washed in deionized water with repeated centrifugation and decantation, and then transferred to pre-weighed vials and dried at 60 °C to constant weight. The resistant (residue) fraction, taken as the recalcitrance pool, was analyzed for ROC and RN. It was assumed that this residue only contained the recalcitrant pool. The degree of recalcitrance was expressed as the Recalcitrance Index (RIC) proposed by Rovira and Vallejo (2002):

RIc (%) = (non-hydrolyzed C (ROC)/SOC) * 100

2.5. Carbon balance and residues decomposition

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

$$\delta\ SOC/\ \delta\ t = I - k \times SOC$$

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 and no change in SOC or its fractions is expected over time ($\delta COS/\delta t = 0$), the k-value can be estimated as follows:

$$K = I/SOC$$

In this study, only the decomposition rate of POCc and POCc + POCf were calculated.

2.6. Data analysis

The statistical significance of the cover crop effect was determined using analysis of variance (ANOVA) for a split plot design. When significant differences were detected, the comparison of means test was applied using the least significant difference (LSD). When the interaction between CC and fertilization management was significant, each CC was analyzed independently.

The Pearson correlation analysis was conducted to evaluate the relationship between the chemical variables (% SOC and k) and the C input with CC residues. Contrast tests between the control and CC treatments were also applied. A Student t test was applied to analyze the differences between the 2010 and 2017 periods. All data were analyzed using Infostat statistical software (Di Rienzo et al. 2013). The significance level used for all the statistical analysis was 0.05.

3. Results and Discussion

3.1. Soil organic carbon and fractions

The effects of CC inclusion and N fertilization are shown in **Table 2**. The analysis of the results demonstrated significant differences in the SOC concentration. At the end of the CC cycle, the SOC at the 0-0.10 m depth was 16% higher than that in Ct (soil without cover crops). The SOC values were 22.3 vs. 19.2 g kg^{-1} (p < 0.001).

Cover crop fertilization did not show any differences between the CC, but nevertheless the SOC concentration was on average 21.0 g kg^{-1} (nf treatment) and 22.6 g kg^{-1} (f treatment), both 7.6% higher than Ct soil (p < 0.05). No significant interaction was found between CC inclusion and N fertilization. This result suggests that all treatments responded to N addition in the same way, therefore the average data from the treatments were used for the statistical analysis.

No significant differences were detected in SOC for the CC and fertilization effects at the 0.10-0.20 m depths. Because of this behavior, SOC and TN concentration decreased with soil depth and thus caused a natural stratification with residue accumulation on the surface. The SOC concentration was highest in the superficial layer (21.8 g kg^{-1}, average f and nf plots) followed by the 0.10-0.20 m layer (14.2 g kg^{-1}). Total N concentration showed a similar distribution at the different depths: 2.5 g kg^{-1} in the 0-0.10 m layer and 1.7 g kg^{-1} at 0.10-0.20 m depth. Other authors in other parts of the world have also reported this surface accumulation when evaluating different CC; the reduction in the intensity of soil tillage and the use of crops to maximize the amount of residue on the surface are commonly used management practices to maintain or increase SOC (Six et al. 1999).

Using contrast analysis for the 0.10-0.20 m depth, significant differences (p < 0.05) were observed in SOC levels between CC based on pure crops (PC) (14.5 g kg^{-1}, average of triticale, rye and vetch) compared with the vetch and VV + T mixture (13.5 g kg^{-1}). No significant differences were observed for the TN concentration with either CC inclusion or N fertilization, but the

Table 2. Distribution and concentration of soil organic carbon and total nitrogen in the control and with cover crops soils, in 0-0.10, 0.10-0.20 and 0-0.20 m depth

CC	SOC		X	TN		X	C:N
	nf	f		nf	f		
	g kg^{-1}			g kg^{-1}			
0-0.10 m							
Ct	18.4	20.0	19.2 a	2.3	2.3	2.3	8.4
VS	21.7	21.5	21.6 b	2.5	2.6	2.6	8.3
VV	21.5	23.6	22.6 b	2.6	2.8	2.7	8.4
VV+T	21.2	23.6	22.4 b	2.6	2.5	2.6	8.6
T	21.2	23.7	22.4 b	2.4	2.7	2.6	8.6
R	21.9	23.5	22.7 b	2.1	2.3	2.2	10.3
X	21.0 A	22.6 B		2.5	2.6		8.8
Ct vs CC			***				
0.10-0.20 m							
Ct	13.4	14.3	13.9	1.6	1.5	1.6	8.7
VS	14.8	14.3	14.6	2.0	1.9	1.9	7.7
VV	14.7	13.9	14.3	1.8	1.9	1.8	7.9
VV+T	13.1	13.8	13.5	1.7	1.8	1.7	7.9
T	14.0	15.1	14.6	1.7	1.6	1.7	8.6
R	14.3	15.1	14.7	1.6	1.4	1.5	9.8
X	14.1	14.4		1.7	1.7		8.4
VV+T vs PC			*				
L vs G						*	
0-0.20 m							
Ct	15.9	17.2	16.6 a	1.7	1.9	1.8 a	8.5
VS	18.3	17.9	18.1 b	2.3	2.3	2.3 b	8.0
VV	18.1	18.8	18.5 b	2.2	2.3	2.3 b	8.2
VV+T	17.2	18.7	18.0 b	2.1	2.2	2.2 ab	8.3
T	17.6	19.4	18.5 b	2.1	2.2	2.1 ab	8.6
R	18.1	19.3	18.7 b	1.9	1.9	1.9 ab	10.0
X	17.6 A	18.6 B		2.1	2.1		
Ct vs CC			***			*	

Different lowercase letters in the mean column (X) indicates significant differences ($p < 0.05$) between CC. Different capital letters in the mean row (X) indicates significant differences ($p < 0.05$) between nf and f treatment. *, ***, significant differences at 0.05, 0.001 probability levels, respectively. nf and f: unfertilized and fertilized treatments. SOC: soil organic carbon. TN: total nitrogen. C:N, carbon:nitrogen rate. X: mean values. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture, control (Ct), leguminosae (L), gramineae (G), pure crops (PC).

legume species increased significantly from 1.6 to 1.8 g kg^{-1}.

Summarizing the 0-0.20 m depths, the effects of SOC and TN concentrations were detected due to the inclusion of CC of different species.

Concentration of the labile fractions (POCc and POCf) showed similar behavior to the total SOC (**Table 3**).

The concentration of SOC and its fractions decreased with soil depth, and thus caused a natural stratification with residue accumulation on the surface. Higher labile soil organic levels

Table 3. Distribution and content of labile soil organic carbon fractions and mineral-associated organic carbon, in the control and with cover crops soils, in 0-0.10 and 0.10-0.20 m

CC	POCc		X	POCf		X	MOC		X
	nf	f		nf	f		nf	f	
				$g\ kg^{-1}$					
				0-0.10 m					
Ct	4.5	5.5	5.0	2.0	2.2	2.1 a	12.0	12.4	12.2
VS	5.1	6.1	5.6	2.8	3.0	2.9 b	13.8	12.4	13.1
VV	6.1	7.9	7.0	2.7	3.3	3.0 b	12.6	12.3	12.5
VV+T	5.3	7.4	6.4	3.2	3.3	3.3 b	12.7	12.8	12.8
T	4.9	6.5	5.7	2.8	3.7	3.3 b	13.4	13.4	13.4
R	5.7	6.2	6.0	2.8	3.3	3.0 b	13.4	14.1	13.7
X	5.3 A	6.6 B		2.7 A	3.1 B		13.0	12.9	
Ct vs CC						***			
				0.10-0.20 m					
Ct	2.3	2.8	2.6	0.7	0.8	0.8	10.4	10.7	10.6
VS	1.6	2.6	2.1	0.8	0.8	0.8	12.3	11.0	11.7
VV	2.4	3.3	2.8	0.8	0.8	0.8	11.4	9.9	10.7
VV+T	2.3	3.6	3.0	0.8	0.8	0.8	10.1	9.4	9.8
T	2.7	4.2	3.5	0.9	1.0	0.9	10.5	9.9	10.2
R	1.8	1.5	1.7	1.8	1.0	1.4	11.3	12.7	12.0
X	2.2	3.0		1.0	0.8		11.0	10.6	
VV+T vs PC									*
L vs G						*			

Different lowercase letters in the mean column (X) indicates significant differences ($p < 0.05$) between CC. Different capital letters in the mean row (X) indicates significant differences ($p < 0.05$) between nf and f treatment. *, ***, significant differences at 0.05, 0.001 probability levels, respectively. nf and f: unfertilized and fertilized treatments. POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon, MOC: mineral-associated organic carbon. X: mean values. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T), mixture, control (Ct), leguminosae (L), gramineae (G), pure crops (PC).

were observed in the 0-0.10 m layer than at the 0.10-0.20 m depth. The distribution and quality of the POCc labile fraction showed no differences between the control soil (Ct) and CC, therefore this fraction was analyzed as a whole. The results suggested that 9 years of C-input from cover crops were insufficient to affect the most dynamic and labile fractions of soil organic matter, despite the differences in C-input between the species. These effects agree with those reported by Sainju et al. (2003), who found no CC effects on the labile soil organic fractions after 2 years of cumulative effects.

Fertilization enhanced the POCc fraction significantly (24.5%), increasing from 5.3 to 6.6 g kg^{-1}, whereas POCf levels rose from 2.7 to 3.1 g kg^{-1} (14.0%) ($p < 0.05$). In this labile fraction the contrast analysis detected significant differences between CC inclusion and Ct ($p < 0.001$). Cover crops enhanced POCf concentration by 47% (3.3 vs 2.1 g kg^{-1}). This difference may have resulted from the quality of the gramineae and leguminosae species used as CC. It is to be noted that soil fractioning by particle size may reflect the changes in soil use and agricultural practices in more detail. The mineral-associated organic carbon fraction (MOC) showed minor variations due to soil depth.

Some authors found similar conclusions in labile CO fractions at all the depths analyzed, with the highest level in the 0-0.05 m surface

layer, corroborating that this fraction is a more sensitive indicator and would be able to detect management practice effects (Eiza 2005).

Cazorla et al. (2013) found increments in the same fractions by CC inclusion at all the depths studied in a Typic Argiudoll. The effect on the labile fraction of C could be in part due to greater addition of residues, higher input of crop roots and greater stability of aggregates in the system (Liu et al. 2005).

Cover crop fertilization did not show any changes in the CO fractions - neither the labile nor the stable ones - at 0.10-0.20 m depth. Concentration of fine labile soil organic carbon (POCf) increased significantly ($p < 0.05$) when G was used as a CC, compared with L (1.1 vs 0.8 g kg^{-1}), whereas pure crops increased the MOC concentration compared with that of a mixture (11.1 vs 9.8 g kg^{-1}).

Stratification of SOC showed similar values for the control (Ct) and CC soils. The stratification rate (SR) was 1.38 for the natural soil, lower than for CC, which had values varying from 1.53 to 1.66 (**Figure 3**). Regarding the SOC, stratification values higher than 2.0 are essential for soil quality maintenance (Franzluebbers 2002; Fernández-Romero et al. 2016). The degree of stratification with soil depth, expressed

as a ratio, might be related to soil quality or soil ecosystem functioning. A value of 2 for SR is considered the limit for good-quality agricultural soils. In this study, SR < 2 would indicate the negative effect of a soybean-maize sequence on the soil quality, although this management practice was slightly enhanced with respect to the control soil.

For TN pools, soils with CC inclusion showed a homogeneous N distribution for all treatments. The SR value was less than 2 (1.44), showing a fast decomposition of the superficial residue, in comparison with the SR of SOC.

3.2. C balance

During the 2008-2017 period, CC dry matter-input in the soil differed between years. In all years, VS supplied the lowest dry matter-input to the soil (4000 kg ha^{-1} year^{-1} on average) and T the highest (8700 kg ha^{-1} year^{-1}) due to a larger biomass accumulation. Cover crop fertilization did not reflect any significant differences except in 2012 with the VV+T mixture. The C-input into soil was estimated from this data. C-input by VS was 1600 kg ha^{-1} year^{-1} and by T was 3550 kg ha^{-1} year^{-1}, and the rest of CC supplied intermediate values. These results are consistent with other observations reported by Galantini et al. (2002),
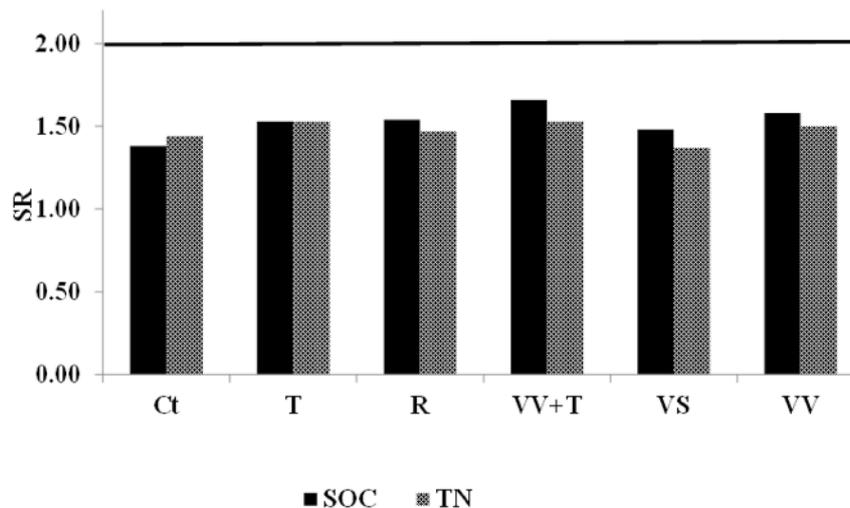

Figure 3. Stratification ratio (SR) of soil organic carbon (SOC) and total nitrogen (TN) concentrations. The horizontal line is the stratification rate threshold. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture.

which revealed that variations in the amount of C supplied by CC were caused by differences in water-use efficiency during the fallow period (in SOC decomposition) and during the crop cycle (in dry matter production and C-input into the soil).

The amount of residue C-input into soil from the crop harvest was estimated by taking account of a harvest index of 0.47 for soybean and 0.48 for maize (Duval et al. 2016). The biomass C-harvest crop (soybean and maize) input sequence was VS > VV > VV + T > R > T (**Figure 4**) and the C-CC input sequence was T > VV + T > R > VV > VS. The total C-input into the soil from both the harvest crop and CC showed the same sequence T > VV + T > R > VV > VS, corroborating that triticale improved the C balance whereas legume species supplied the smallest biomass residues. Differences in SOC and organic fraction concentrations could be due to the presence of different C-input quantities.
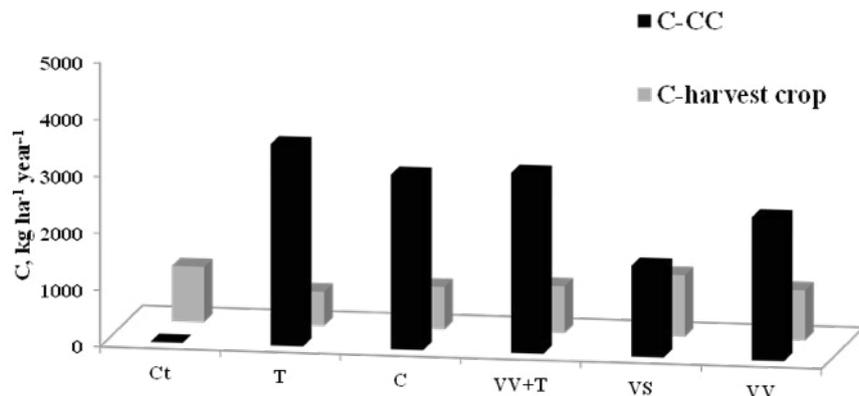

Figure 4. Relationship between C-input by cover crops and C-input by harvest crops and annually soil C content. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture.

Since the initial SOC in the control (Ct) plot in the experimental site was 16.4 g kg^{-1}, the CC treatments ranged from 18.1 g kg^{-1} with VS to 18.7 g kg^{-1} with R. The other CC showed medium values (**Figure 5**). The C-input into the soil showed an association with the TOC concentration ($R^2 = 0.59$, $p < 0.001$), according to previous information. The Ct plot needed at least 2.9 Mg C-input ha^{-1} year^{-1} to avoid a decrease in SOC at the 0-0.20 m depth.

This strong association was greater with the fine labile carbon fraction (POCf) ($R^2 = 0.91$, $p < 0.05$), than with the coarse labile carbon fraction (POCc) ($R^2 = 0.2948$, ns) and MOC ($R^2 = 0.0748$, ns) (**Figure 6**). The absence of association with the latter fractions could be due to fast residue decomposition that accumulated in POCf and is probably related to the low C:N rate and the low N stratification.
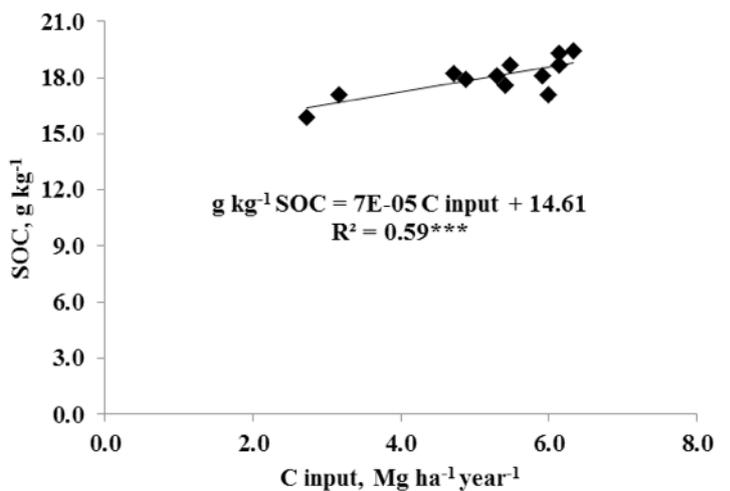

Figure 5. Relationship between C input by cover crops and concentration of soil organic carbon (SOC) at 0-0.20 m depth. Significant level, *** ($p < 0.001$).
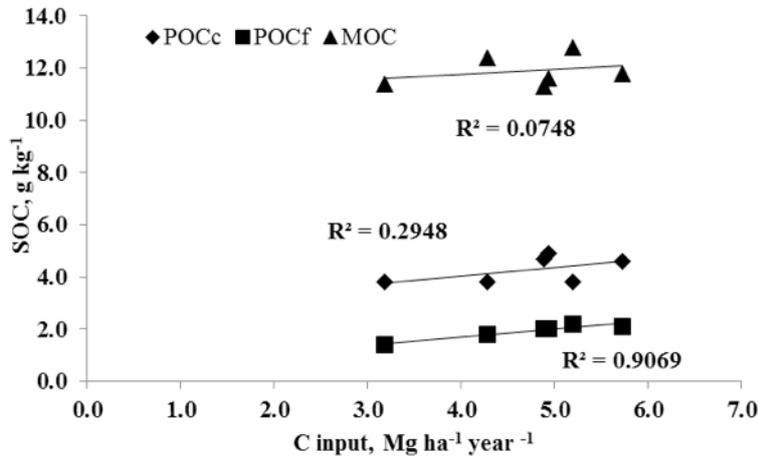
Figure 6. Relationship between C-input by cover crops, labile carbon fractions (coarse and fine) and the mineral-associated organic carbon (MOC) concentrations.

3.3. Residue decomposition

It is known that labile soil organic carbon fractions reach an equilibrium or steady state in a shorter period of time (5-10 years) than the recalcitrant fraction (decades or centuries) (Duval et al. 2015). The transformation rate (k) can thus be estimated from the average annual C-input and C content in the soil. Increase in TOC content (Δ) was significantly related to the amount of C supplied by the cover crops and harvested crops ($R^2 = 0.93$, $p < 0.0001$) (**Figure 7a**). In the 0-0.20 m layer the C-input into the soil (mostly by CC) improved the C-balance, which was the main goal of this management practice for the area

under study. The content of labile soil organic carbon (POCc+f) showed less association with the soil carbon changes and C-input into the soil ($R^2 = 0.77$, $p < 0.05$) (**Figure 8a**).

The k values estimated ranged from 0.02 to 0.09 for SOC and from 0.07 to 0.24 for POCc+f. This variation was associated with the C-input (**Figures 7b, 8b**). This was also found by Duval et al. (2016) and indicates that higher C-amounts, mainly supplied by the cover crops, enhanced the decomposition rate or the "priming effect". The more labile soil organic carbon fraction (POCc) with rye as the CC showed a faster decomposition rate with a value of 0.38 (**Table 4**).
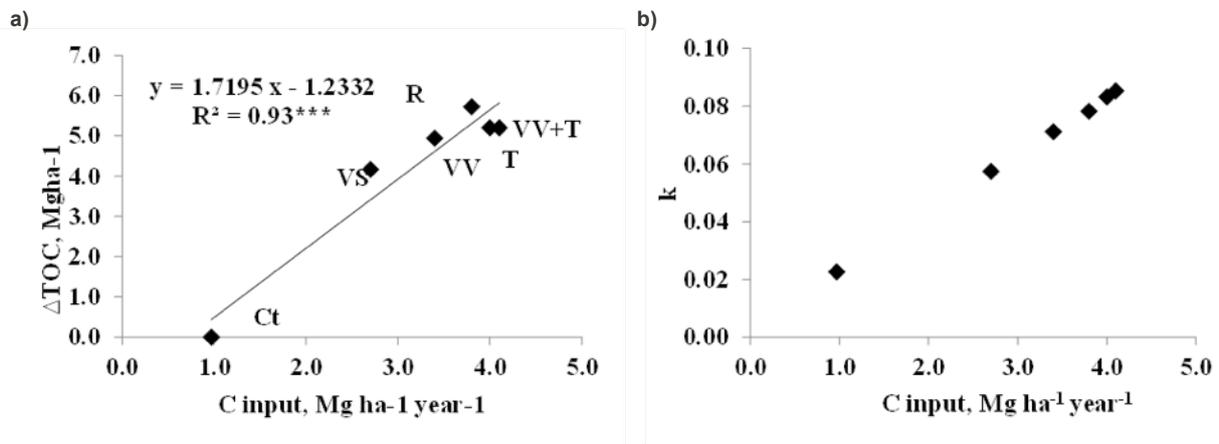
a)

b)

Figure 7. a) Relationship between annual C-input, changes in the content of total organic carbon (TOC). b) Decomposition rate (k). Significant level, *** ($p < 0.001$).
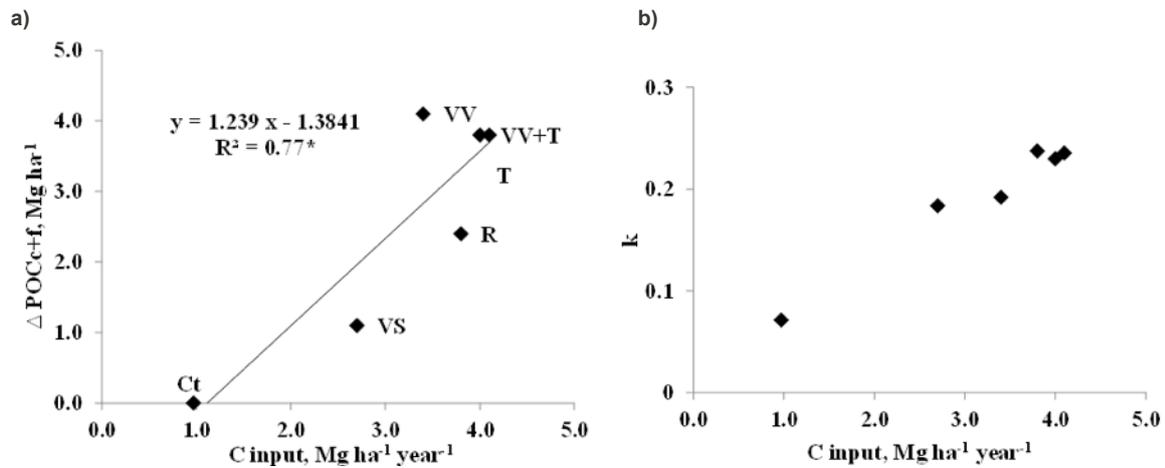
a)

b)

Figure 8. a) Relationship between annual C-input and changes in the content of labile organic carbon fractions (POCc+f). b) Decomposition rate (k). Significant level, * ($p < 0.05$).

In both cases, the greater k values corresponded to gramineous species, such as triticale and rye. The slight differences between treatments in the sampling period (9 years) could be associated with the immediate use of C by soil microorganisms and the fast residue decomposition accumulating in the labile organic carbon fraction.

Table 4. Decomposition rate of soil organic carbon and labile fractions under cover crops treatments

CC	k		
	SOC	POCc	POCc+f
Ct	0.02	0.10	0.07
VS	0.06	0.27	0.18
VV	0.07	0.27	0.19
VV+T	0.08	0.33	0.23
T	0.08	0.34	0.24
R	0.08	0.38	0.24

k: decomposition rate. SOC: soil organic carbon, POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T) hairy vetch (VV) + triticale (T) mixture, Control (Ct).

3.4. Changes in SOC after 7 years under gramineae species

After the 2010-2017 period of using gramineae (G) (triticale and rye) as the CC in a soybean-maize sequence, the levels of SOC and its fractions (labile organic carbon and mineral-associated organic carbon) at the 0-0.20 m depth generally increased, probably due to the surface accumulation of G residues (**Table 5**). Other authors in other parts of the world (Bella 2015) have also reported this surface effect when evaluating different cover crop species.

During this period, the maize yields were on average 8300 kg ha^{-1} less with G-CC compared with Ct, but nevertheless the soybean grain yields did not show any significant differences between the control and the CC treatments, with a production of 3500 kg ha^{-1} (data not showed) (Bella 2015).

The SOC concentration increased 20 and 22% ($p < 0.05$) with the inclusion of a triticale (f and nf) cover crop. This species had the highest production potential (annual average of 9000 kg ha^{-1}) with a fertilization response.

The labile carbon fraction (COPc and COPf) concentrations showed variable balances. The POCc fraction increased from 58 to 192% (p < 0.05), contrary to that observed with the Ct which decreased by 5%. Gramineae cover crop effects on the POCf concentration showed an increase of 64% when triticale f was used (p < 0.001). In the MOC fraction, these changes were observed less and they ranged from increases of 3.3% to losses of 7.1%.

In this study, POCc seemed to be more sensitive to a gramineae CC effect; the cover crops also increased SOC lability, as revealed by the increased proportion, especially of POCc in the 0-0.20 m layer soil compared with MOC.

Table 5. Soil organic carbon, coarse particulate organic carbon, fine particulate organic carbon, and mineral associated organic carbon contents under cover crops treatments at 0-0.20 m depth

CC	Year	SOC	POCc	POCf	MOC
		g kg^{-1}			
Ct	2010	16.3	4.0	1.4	10.9
	2017	16.5	3.8	1.4	11.4
	Δ	+ 1.2	-5.0	0	+ 4.5
R f	2010	17.5	2.4	1.8	13.3
	2017	19.3	3.8	2.1	13.4
	Δ	+ 10.3	+ 58.3	+ 16.7	+ 0.7
T nf	2010	14.4	1.3	1.8	11.2
	2017	17.6	3.8	1.8	12.0
	Δ	+ 22.2*	+ 192*	0	+ 7.1
T f	2010	16.1	2.7	1.4	12.0
	2017	19.4	5.4	2.3	11.6
		+ 20.5	+ 100	+ 64.3**	- 3.3

*: significant differences at 0.05; 0.001 probability levels. nf and f: unfertilized and fertilized treatment.
SOC: soil organic carbon, POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon, MOC: mineral-associated organic carbon. Rye (R), Triticale (T), Control (Ct).

3.5. Quality of the recalcitrant organic matter

No statistical differences for the mineral-associated organic carbon (MOC) fraction between treatments were observed. In order to verify if there were any changes in their quality, the recalcitrant fraction (ROC) and the Recalcitrance Index (RIc) of this fraction were determined.

The recalcitrant C behaved differently from SOC and did not show any significant increases with the use of CC (9.1 g kg^{-1} on average) (**Table 6**). This behavior could indicate that stabilization of carbon in a recalcitrant form may be limited by the organic matter input (Pandey et al. 2014). The gramineae species would produce slight increases in organic materials and resistant fractions, due to their high C: N ratio (9.8 g kg^{-1} on average). The soil recalcitrant pool obtained by acid hydrolysis is composed of organic molecules that are more difficult to be degraded naturally, hence they are stable over time and may be considered the lower limit of the carbon stock. It could be inferred from this chemical method that it would be possible to estimate the size of the labile C pool.

On average, the Recalcitrant Index of C (RIc) in the upper horizon was 46% compared with 71% in the sub-superficial layer, suggesting that the non-hydrolysable pool, such as the higher C stable fraction, would accumulate at depth. Generally, the greater biomass supplied by the residue input, such as CC, would contribute to OC accumulation, mostly in labile compounds.

Table 6. Soil organic carbon, recalcitrant carbon, total nitrogen, recalcitrant nitrogen contents and recalcitrance indices under cover crop treatments at the 0-0.10, 0.10-0.20 and 0-0.20 m depths

CC	CARBON				NITROGEN			
	SOC	MOC	ROC	RIc	TN	RN	RIn	ROC:RN
	g kg^{-1}							
	0-0.10 m							
Ct	18.4 a	11.4	9.0	48.9	2.5	0.4	17.2 a	22.5
T	21.2 b	13.4	10.2	48.0	2.4	0.6	24.1 b	17.0
R	21.9 b	13.4	9.6	43.9	2.2	0.5	25.1 b	19.2
	0.10-0.20 m							
Ct	13.5	10.5	9.2	68.2	1.7	0.6	37.6	15.3
T	14.0	10.5	9.4	75.4	1.7	0.5	33.1	18.8
R	14.3	11.3	10.2	70.8	1.6	0.6	36.8	17.0
	0-0.20 m							
Ct	15.9	10.9	9.1	58.5	2.1	0.5	27.4	18.9
T	17.6	11.9	9.8	61.7	2.0	0.5	28.6	17.9
R	18.1	12.3	9.9	57.3	1.9	0.5	30.9	18.1

Different lowercase letters in the column indicate significant differences ($p < 0.05$) between CC. SOC: soil organic carbon, MOC: mineral-associated organic carbon, ROC: recalcitrant organic carbon. RIc: recalcitrance index of carbon, TN: total nitrogen, RN: recalcitrant nitrogen, RIn: recalcitrance index of nitrogen.

This is especially important because the content of this fraction is associated with potential soil fertility. This index showed that the surface layer (0-0.10 m) had more labile material contents compared with the 0.10-0.20 m depth, where the most stable fractions of OC were accumulated. These results are consistent with other observations reported in the literature (Rovira and Vallejo 2007), which confirmed the extended concept that SOC stabilizes in deep horizons because it is more physically protected and it is in a more advanced state of biochemical stabilization relative to the SOC in surface horizons.

Unlike the ROC, the RN showed similar values for all the treatments and sampling depths (**Table 6**). RIn increased with soil depth; in the 0-0.10 m layer the control value was significantly lower than the gramineae ($p < 0.05$), but the trend was not observed in the 0.10-0.20 m depth.

There are conflicting results in the literature for changes in RIn values. In soils of the Argentinian pampas, N recalcitrance decreased with depth in most cases, in both burnt and unburnt soils (Sánchez and Lázzari 1999); N recalcitrance was more or less constant throughout the soil profile except for some N-poor horizons, in which the RIn values were much higher (Tan et al. 2004).

4. Conclusions

This study demonstrated that the effect of cover crops on SOC and the labile fraction (POCf) in the upper soil layer was strongly related with high residue production. However, it was insufficient to increase three physical fractions of soil organic carbon values in deep soil. No changes in soil organic carbon concentrations in the surface soil and in the stratification index were shown after 9-year long studied period with a summer soybean-maize sequence. Gramineae crops were more efficient in biomass production than the legume crops, with more C input into soils. Triticale was the most suitable cover crop showing a high positive C balance in both SOC and the labile fraction. An increase in the residue decomposition rate was also detected. CC had

a positive impact on recalcitrant C and the recalcitrance carbon index in the sub-superficial layer, showing more stable C stock and less labile C than in the superficial layer.

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