

Soil organic carbon. Stratification and spatial variation of different

fractions in an Argiudoll under no tillage

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Soil organic carbon (SOC) is an important factor for soil quality diagnosis. Physical and chemical fractionation of SOC are useful to characterize SOC, because some fractions are more sensitive indicators of the effects of different management practices. The aims of this study were (i) to determine values of SOC and different fractions of SOC at different depths and positions in an Argiudoll of the Argentinian Pampas under NT, and (ii) to determine the relation between physical and chemical fractions of SOC. In an experimental plot located in Chascomús, we determined SOC content, humic acids (HA), fulvic acids (FA), humins, coarse and fine particulate organic carbon (POCc and POCf) and mineral associated organic carbon (MOC), at different depths and in the row and inter-row. The content of SOC and different SOC fractions, as well as the contribution of each fraction to SOC was larger in the surface than in deeper layers, while humins' (older and more recalcitrant fraction) contribution to SOC increased with depth, and the contribution of FA, POCf and MOC to SOC remained relatively constant. There was no effect of row and inter-row in SOC content and composition. FA content was correlated to POCc, HA content to POCc and POCf and humins to MOC.

Key words: particulate organic carbon; humic acids; fulvic acids; humins; stratification

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El carbono orgánico del suelo (COS) es un factor importante para el diagnóstico de la calidad del suelo. El fraccionamiento del COS por métodos físicos y químicos resulta útil para su caracterización, ya que algunas fracciones son más sensibles al efecto de distintas prácticas de manejo. Los objetivos de este trabajo fueron (i) determinar el contenido de COS y de diferentes fracciones del COS a diferentes profundidades y posiciones en un Argiudol bajo siembra directa (SD), y (ii) determinar la relación entre fracciones físicas y químicas del COS. En un ensayo experimental localizado en Chascomús (región pampeana), determinamos COS, ácidos húmicos (AH), ácidos fúlvicos (AF), huminas, carbono orgánico particulado grueso y fino (COPg y COPf) y carbono orgánico asociado a minerales (COM), a distintas profundidades y en el surco y el entre surco. Los contenidos de COS y de las distintas fracciones mostraron distintas distribuciones verticales. La contribución de AH y COPg (fracciones más nuevas y más lábiles) al COS fue mayor en la superficie que en profundidad, mientras que la contribución de las huminas (fracción más vieja y recalcitrante) al COS aumentó en profundidad. La contribución de AF, POCf y COM al COS se mantuvo constante. No hubo efecto de la posición surco y entre surco en el contenido ni en la composición del COS. El contenido de AF se encontró principalmente correlacionado con el COPg, los AH con el COPf, y las huminas con el COM.

Palabras clave: carbono orgánico particulado; ácidos húmicos; ácidos fúlvicos; huminas; estratificación

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INTRODUCTION

Soil organic matter (SOM) is an important factor for soil fertility and functionality, and it is widely used for soil quality diagnosis, as it has an important role in plant nutrition (Cambardella & Elliott, 1992; Franzluebbers, 2002; Kay & VandenBygaart, 2002). Also, SOM is correlated with important chemical properties as pH, cation-exchange capacity and pollutants retention, and with many physical and hydraulic properties (Swift, 1996; Recio-Vázquez et al., 2014). It is well documented that SOM enhances aggregation, improving structure and structural stability (Novelli et al., 2013; 2017; Blanco-Canqui & Ruis, 2018), and stimulates soil fauna development (Domínguez & Bedano, 2016). Both structure and biota are responsible for creating a connected and functional pore system, and therefore generating proper physical and hydraulic properties for plant development and water entry (Kay & VandenBygaart, 2002; Bongiovanni & Lobartini, 2006; Sasal et al., 2017).

SOM changes in time and it is influenced by management practices, especially by tillage and by the amount and characteristics of organic residues entering the soil (Cambardella & Elliot, 1992; Ding et al., 2006). Thus, agricultural management changes SOM quantity, quality and distribution (Cambardella & Elliot, 1992; Franzluebbers, 2002). As most SOM inputs come from plant residues and fauna (Rasse et al., 2005), it is higher in the surface and diminishes with depth, presenting a natural stratification that can be more or less altered by soil use (Christensen, 2001; Franzluebbers, 2002; Álvarez et al., 2011). Tillage tends to redistribute and homogenize SOM in the upper layer (Hernanz et al., 2002; Kay & VandenBygaart, 2002), and tends to deplete it by enhancing SOM mineralization (Kay & VandenBygaart, 2002). No tillage (NT), on the other hand, minimizes soil disturbance and SOM mineralization, leading leaching of organic solutes and redistribution by faunal activities to be, as in a natural environment, the principal mechanisms for SOM transport (Kay & VandenBygaart, 2002; Álvarez et al., 2011). Therefore, stratification under NT is expected to be higher and closer to that found in natural environments (Franzluebbers, 2002). In this sense, stratification indexes (SI) have been proposed to be soil quality indicators by themselves, independent of soil type and climate. SI are defined as a soil property at the surface divided by the same soil property at a higher depth, such as the bottom of the tillage layer (Franzluebbers, 2002).

In addition, as SOM inputs are related to the rhizosphere and plant residues, a horizontal spatial variation between rows and inter-rows could be expected to be found. Binet et al. (1997), studying corn (*Zea mays* L.) crops, found higher abundance of earthworms in the rows, which was attributed to greater availability of trophic resources and microclimate induced by plant cover. On the other hand, Rasse et al. (2005) reviewed different studies and reported that living roots may have a priming effect, enhancing SOM mineralization. As organic matter takes a multitude of forms in soil and changes in space and time, so do other associated soil features. For this reason, gathering information on SOM spatial variation and

stratification may be useful to understand how management practices may affect productivity and hydrology of agricultural soils.

Soil organic carbon (SOC) is the most widely used estimative of SOM (Nelson & Sommers, 1996; Romaniuk et al., 2018). However, organic matter is a quite complicated cluster composed by many different pools of molecules, with highly variable chemical composition and properties, size, molecular weight, and thereby different resistance to degradation and function in the soil matrix (Swift, 1996; Olk et al., 2019). From this fact arises the need to describe more precisely SOM, in order to understand the effect of each pool on different soil properties (Recio-Vázquez et al., 2014) and be able to rapidly detect seasonal or management induced changes (Kay & VandenBygaart, 2002). Decomposition rates of organic molecules may vary from a few hours to several thousand years (Swift, 1996; Kay & VandenBygaart, 2002). As stable organic carbon represents an important fraction of SOC, SOC values are not always sensitive to identify changes over short periods of time (Plaza-Bonilla et al., 2014). Thus, separating and quantifying more dynamic carbon fractions (with an earlier response to changes in management) has been pointed out as an efficient tool to identify more sustainable agricultural practices (Bongiovanni & Lobartini, 2006; Ding et al., 2006; Duval et al., 2013; Plaza-Bonilla et al., 2014). In addition, many authors have found some fractions of SOC to have stronger correlations with specific physical, chemical and hydraulic properties (Recio-Vazquez et al., 2014).

Different physical and chemical fractions and fractionation methods have been studied. These fractions represent arbitrary groups and are method dependent, but have been proved to represent pools of SOM of different age and stability (Cambardella & Elliott, 1992) and with different degrees of resistance to turnover (Christensen, 2001). One way to classify and to fraction SOM molecules is according to their solubility at different pHs. This way, three major fractions are obtained: humic acids (HA), fulvic acids (FA) and humins (Olk et al., 2019). Each fraction represents a pool of molecules with different solubility that reflect similar molecular weight, chemical composition and degradation rate, related to their degree of humification (Benites et al., 2003; Yang et al., 2004).

However, it has been stated that these fractions of SOC may not be clearly related to the actual dynamic of organic matter in natural and cultivated systems (Cambardella & Elliott, 1992). This can be explained as SOM evolution and stabilization against degradation is not merely a process of chemical evolution but also of physicochemical protection occurring as SOM associates with minerals (Theng et al., 1992; Rasse et al., 2005: Bongiovanni & Lobartini, 2006: Duval et al., 2013). Therefore, other studies analyze SOC associated to different physical fractions, which emphasize different degrees of interaction with the mineral fraction (Christensen, 2001; Ding et al., 2006). By granulometrical separation, different fractions are obtained. For instance, following a widespread technique three fractions are obtained: coarse and fine particulate organic carbon (POCc, POCf), and mineral associated organic carbon (MOC), which is the finer fraction (Duval et al., 2018). The coarser fraction is considered to be the less transformed and more labile one, while the finest is in closer relation with the mineral fraction and therefore more resistant to biological degradation; intermediate fractions have intermediate age and lability (Cambardella & Elliott, 1992; Duval et al., 2013). The input of SOC attributed to NT is generally related to the particulate fraction that is directly linked to recent shoots and roots (Briedis et al., 2012).

The relation among fractions obtained by physical and chemical methods has not been clearly stated, and the meaning of most fractions is not yet fully understood (Plaza-Bonilla et al., 2014). Zalba & Quiroga (1999) studied the relationship between FA and POCc contents in agricultural soils under different management practices, finding a close relationship between both fractions. In addition, theoretical description of both pools of molecules include a quick, an intermediate and a slow fraction (Cambardella & Elliott, 1992; Yang et al., 2004).

Quantifying SOC fractions is especially important for understanding SOM dynamics in intensively managed systems as we move toward adoption of more sustainable practices (Cambardella & Elliott, 1992; Recio-Vazquez et al., 2014). For this reason, gathering knowledge about SOM composition and its spatial distribution and stratification may be useful to detect the effect of changing management practices and to predict their effect on other physical and hydraulic properties. For instance, studying the row and the inter-row of the previous crop may highlight the effect of the immediately previous crop on SOC. Furthermore, being able to determine whether a relation exists between different fractions of SOC might contribute to the understanding of what these fractions represent. We hypothesized that: (i) in an Argiudoll under NT, SOC composition among fractions shows vertical variation throughout the soil profile and spatial variation between row and inter-row, (ii) there is a relationship between physical and chemical fractions of SOC.

The aims of this study were (i) to determine the content of SOC and different fractions of SOC at different depths and positions in an Argiudoll of the Argentinian Pampas under NT, and (ii) to determine the relation between physical and chemical fractions of SOC.

MATERIALS AND METHODS

Soil sampling site

Samples were taken from an experiment set in Estación Experimental Manantiales (INTA), near Chascomús, Argentina (35 °44 '38" South and 58 °03 '10" West) (Figure 1). Soil was classified as a fine, illitic, thermic abruptic Argiudoll (Soil Survey Staff, 2014), Luvic Phaeozem (IUSS Working Group WRB, 2015). The A horizon was 0,30 m depth in average, with 23,3 % clay and 51,9 % silt (clay loam soil). Below, there is a Bt horizon with 40,5 % clay and 59 % silt. Soil pH is slightly acidic (6,0 ± 0.2 in the studied profile). The climate in the region is temperate (the temperature seldom goes below 0 °C) and the approximate annual rainfall reaches 1000 mm. The plot studied was 40 x 87 m and had been under NT for the last 7 years, with the following crop sequence: corn (2011) - natural grassland (NG) (2012) - NG (2013) - NG (2014) - NG/corn (2015) - corn (2016) corn (2017).

Sample collection and analytical procedures

Samples were taken in August 2018, during fallow and after corn harvest.



Figure 1. Location of the experimental trial.

Disturbed soil samples were collected from twelve different points, selected randomly and avoiding borders and visible wheel tracks. At each point, composite samples were taken at three different depths: 0-0,05 m, 0,05-0,10 m and 0,10-0,20 m, which corresponded to the A horizon. Six of the samples from the A horizon corresponded to the rows of the previous crop, and the other six to the immediate inter-rows. Additionally, at each sampling point samples were taken at 0,30-0,50 m depth (which corresponded to the Bt horizon) without distinction between row and inter-row. Samples were air-dried, pulverized to pass through a 2 mm sieve and frozen.

Chemical fractions' separation and quantification

Separation of different chemical fractions of SOC was achieved following the protocol described by Benites et al. (2003) that is a modification of the method described by Schnitzer (1982) and which's basic concepts are: dissolution of HA and FA with alkali and separation from humins by centrifugation; separation of FA from HA by acidification (HA precipitate) and filtration; and dissolution of the HA with alkali. Approximately 1 g of soil was weighted. HA and FA were extracted with sodium hydroxide (NaOH) (0,1 N) in 50 mL centrifuge tubes, for 24 h after manual agitation followed by a short extraction (1 h after manual agitation). The supernatant liquid (HA+FA) was separated from the solid phase (which included the humins fraction) by centrifugation for 20 min at 4000 rpm after the long and the short extraction, and then transferred to a unique tube. Liquid extracted was then acidified with H_2SO_4 to pH 1.0 ± 0.1 in order to precipitate HA and allowed to stand for 24 h. FA were separated from HA by vacuum filter employing a 0,45 µm pore size cellulose ester membrane filter. HA were then dissolved to pass through the filter with NaOH (0,1 N). Both FA and HA fractions were diluted to a known volume of 50 mL.

Physical fractions' separation and quantification

Different size fractions of SOC were obtained following the method described by Duval et al. (2018) which is based on Cambardella & Elliot (1992). Fifty g of soil sieved through a 2 mm sieve were physically dispersed by agitation with water and glass beads for 16 hours. The suspension was then sieved through 105 and 53 μ m until washing water was clear to the naked eye. Three fractions were obtained:

- coarse particulate organic carbon (POCc), which is the fraction between 105 and 2000 $\mu\text{m},$

- fine particulate organic carbon (POCf), which is the fraction between 53 and 105 $\mu m,$ and

- mineral associated organic carbon (MOC), the fraction smaller than 53 μ m, which is discarded and then estimated by difference.

The fraction retained in each sieve was dried at 40°C, weighted and then sieved through 500 μm before SOC quantification.

SOC quantification

SOC, POCc, POCf, HA and FA contents were

determined by the wet oxidation method described by Walkley and Black (1934). According to this method, SOC from a known amount of soil or soil's fraction was oxidized with potassium dichromate ($K_2Cr_2O_7$), at acidic pH obtained with sulfuric acid (H_2SO_4). The excessive $K_2Cr_2O_7$ was then determined by back titration by ferrous ammonium sulfate (Fe(NH4)₂(SO₄)_{2.6}H₂O). Organic carbon from the MOC and humins fractions was calculated by difference, substracting from SOC value the other two fractions (POCc and POCf for MOC, and HA and FA for humins). In all cases, SOC and SOC fractions' contents are expressed as g of oxidizable carbon per 100 g of soil dry mass (g/100g).

The relative contribution of each fraction to SOC was calculated dividing each fractions' content by SOC value and expressed as a ratio (organic carbon in the fraction/SOC). SI of SOC and SOC fractions (SOC_{SI}, FA_{SI}, HA_{SI}, humins_{SI}, POC_{CSI}, POCf_{SI} and MOC_{SI}) were calculated as the mean value measured at 0–0,05 m, divided by the mean value at 0,10–0,20 m (Duval et al., 2016) and are unitless.

Statistical analysis

Two way ANOVAs were performed using depth (three levels: 0-0,05 m, 0,05-0,10 m and 0,10-0,20 m) and position (two levels, row and inter-row) as factors, for SOC content, each fractions' content, SI and the contribution of each fraction to SOC. When a significant effect of the factors was found (p-value < 0.05), Fisher's LSD was used in order to compare mean values. Significant differences were expressed using letters (pvalue < 0.05), or uppercase letters when there was no interaction between factors and the effect of only one factor is shown. Relation between different fractions of SOC was tested by a correlation analysis (R expressed is from Pearson test) considering two levels of significance (p-value<0,05 and p-value < 0,005). All analyses were performed using INFOSTAT (Di Rienzo et al., 2008) and STATISTICA.

RESULTS

SOC composition and distribution with depth and between row and inter-row

For SOC, POCf, POCc, HA and FA, there was no interaction between position and depth. For MOC and humins there was interaction between position and depth. Both MOC and humins contents were significantly higher in the inter-row in the 0,10-0,20 m layer. SOC and all SOC fractions were affected by depth in both row and inter-row positions, being higher at surface and diminishing with depth (Figure 2). There was no significant effect of row and inter-row position, for any of the variables measured. As regards to the contribution of each fraction to SOC, there was no interaction between the studied factors (position and depth). There was no effect of depth nor position in FA/SOC, HA/SOC and humins/SOC. On the other hand, POCc/SOC, POCf/SOC and MOC/SOC, were significantly affected by depth but not by position (Table 1).



Figure 2. Soil organic carbon (SOC) contents and distribution among chemical fractions for different depths and positions (row and inter-row) expressed as g/100 g. Bars represent fulvic acids (FA), humic acids (HA), and humins. Letters on the right side of the bars indicate significant differences for SOC content at each depth (Fisher's LSD, p-value < 0,05). For each fraction, in the first 0,20 m of soil, uppercase letter indicates significant differences among depths for each fraction; lowercase case letter indicates significant differences among depth x position (Fisher's LSD, p-value < 0,05).

Table 1. Relative contribution of physical (coarse particulate organic carbon, POCc, fine particulate organic carbon, POCf and mineral associated organic carbon, MOC) and chemical fractions (fulvic acids, FA, humic acids, HA, and humins, h) to total organic carbon (SOC) at different depth and in the row and inter-row (i/row). * Uppercase letters indicates significant differences among depths for each fraction (Fisher's LSD, p-value < 0,05), as there was no interaction between factors nor effect of position.

		FA/SOC			HA/SOC		h/SOC		POCc/SOC		30C	MOC/SOC	;
	row	0,12		0,22		0,66		0,17		0,16		0,67	
0-0,05 m	i/row	0,11	A	0,23	A	0,66	A	0,17	A	0,17	В	0,69	В
0,05- 0,10 m	row	0,11		0,23		0,67		0,07		0,19		0,75	В
	i/row	0,11	A	0,24	A	0,65	A	0,06	В	0,28	A	0,65	
0,10- 0,20 m	row	0,10		0,25	A	0,65		0,06		0,18		0,75	A
	i/row	0,09	A	0,20		0,71	A	0,04	В	0,17	В	0,80	
0,30- 0,50 m	-	0,11		0,10		0,79		0,10		0,16		0,75	

SOC mean value at the surface was 2,45 g/100g and diminished with depth (Figure 2). There was no significant difference of SOC between the 0,05-0,10 and 0,10-0,20 m layers, showing a mean value of 1,98 g/100g (which represents 81% of SOC at surface). At 0,30-0,50 m, SOC content was 0,73 g/100g (30% of SOC at surface). FA, HA and humins contents also showed vertical variation in the soil profile (Figure 2). All three fractions had a similar vertical distribution to SOC, with higher values in the upper 0,05 m, and no differences between the 0,05-0,10 and the 0,10-0,20 m layers, with the exception of humins in the 0,10-0,20 m layer, where values where significantly higher in the inter-row. In relation to the contribution of each fraction to SOC content (Table 1), the largest fraction of SOC corresponded to humins (humins/SOC between 0,65 and 0.79 of SOC), followed by HA/SOC (between 0,11 and 0,25) and FA/SOC (0,09-0,12 of SOC). The contribution of each fraction remained relatively constant with depth, with no significant differences between layers. In the 0,30-0,50 m layer, values of FA were similar to those in the uper layers, while HA content was lower and humins content higher than in the upper layers. HA/FA ratio at surface was 1,8-2,0. Physical fractions of SOC were also stratified, with values that generally diminished with depth (Figure 3). For POCc, higher SOC contents were found in the 0-0,05 m layer (mean value 0,42 g/100g). In all the other layers, POCc values were between 3 and 5 times lower, with no significant differences between layers.

POCf remained relatively constant in the first 0,20 m (mean value of 0.40 g/100g). POCf was higher in the 0,05-0,10 m layer than in the 0,10-0,20 m layer. MOC showed a more gradual stratification. Higher values were found in the surface (around 1,66 g/100g). In the 0.05-0.10 m laver. MOC was around 88% of the value in the surface, being significantly lower only in the interrow. In the 0.30-0.50 m layer, POCc values were similar to those in the 0,10-0,20 m layer; POCf and MOC values had a big drop in the 0,30-0,50 m layer (values 3 to 6 times lower than in the upper layers). In relation to the contribution of each physical fraction to SOC content (Table 1) the largest fraction of SOC corresponded to MOC, with similar values to humins (mean value of MOC/SOC of 0,72). In the top layer, POCc/SOC and POCf/SOC were similar (around 0,17). In deeper layers POCf was the second most abundant fraction, while POCc/SOC only represented between 0,04 and 0,10. MOC/SOC had no significant differences between row and inter-row but was affected by depth. MOC/SOC was higher in the 0,10-0,20 m layer than in the upper layers. POCf/SOC was higher in the 0,05-0,10 m layer, and was lower in the 0.05-0,10 m and in the 0,10-0,20 m layers, with no significant difference between these layers. POCc/SOC was higher in the first 0,05 m, and then diminished with no significant differences between the 0,05-0,10 and 0,10-0,20 m layer. In the 0,30-0,50 m layer, POCc/SOC showed

higher values than in the 0,10-0,20 m layer.



Figure 3. Soil organic carbon (SOC) contents and distribution among physical fractions for different depths and positions (row and inter-row). Bars represent coarse particulate organic carbon (POCc), fine particulate organic carbon (POCf), and mineral associated organic carbon (MOC). Letters on the right side of the bars indicate significant differences for SOC content at each depth (Fisher's LSD, p-value < 0,05). For each fraction, uppercase letter indicates significant differences among depths for each fraction; lowercase case letters indicates significant differences among depth x position (Fisher's LSD, p-value < 0,05).

Soil organic carbon stratification

Stratification indexes are shown in Table 2. SOC_{SI} , HA_{SI} , humins_{SI}, COM_{SI} were affected by position, while FA_{SI} , $COPg_{SI}$ and $COPf_{SI}$ were not significantly affected by position. SOC_{SI} , humins_{SI} and COM_{SI} were higher in the row than in the inter-row. HA_{SI} , on the other hand, was higher in the inter-row than in the row.

Relationship between physical and chemical fractions of SOC

According to the correlation analyses (Figure 4), MOC was correlated to all chemical fractions (p-value < 0,005), showing the highest correlation coefficient with humins. POCf was also correlated with all chemical fractions, showing the highest correlation coefficient with HA (p-value < 0,005). POCc was only correlated with FA (p-value < 0,05).

Table 2. Stratification indexes of soil organic carbon (SOC_{SI}), coarse particulate organic carbon (POC_{CSI}), fine particulate organic carbon (POCf_{SI}), mineral associated organic carbon (MOC_{SI}), fulvic acids (FA_{SI}), humic acids (HA_{SI}) and humins (humins_{SI}). * For each fraction, different letters indicate significant differences between row and inter-row position (Fisher's LSD, p-value < 0,05).

							St	ratificatio	on inde	ex						
	SOC si		si FA si		HA si			humins _{SI}		POC	C SI	PO	Cf _{SI}	MO	C si	
row	1,36	а	1,57	а	1,2	6	b	1,76	а	5,69	a	1,43	а	1,18	а	
inter- row	1,21	b	1,54	a	1,4	2	a	1,10	b	5,82	a	1,27	a	1,03	b	
	2,00					2,00					2,00					
0g)	1,60		۰	••		1,60			• •		1,60					
/10	1,20		•			1,20					1,20			·		
C (g	0,80					0,80)				0,80					
W	0,40	•	R=	=0,91 *	•	0,40	•		R=0,	96 **	0,40	•		R=0,9	9 **	
	0,00	0,10	0,20	0,30	0,40	0,00	0	0.20	0.40	0.60	0,00	0,5	0 1,0	00 1,50	2,00	
POCf (g/100g)	0,60					0.60		0,200	0,110	.,	0,60					
	0,50		•			0,50				•	0,50			•		
	0,40			٠.		0,40				:	0,40			•		
	0,30		••			0,30			•	•	0,30			•••		
	0,20				0,20					0,20						
	0,10	•		R=0,7	79 *	0,10	• •	•	R=0,89 **		0,10	•	•	R=0	,81 *	
	0,00		0,20		0,4	0,00	0	0,20	0,40	0,60	0,00	0,40	0,80	1,20 1,	60 2,00	
	0,50					0,50					0,50					
POCc (g/100g)	0,40			•		0,40			:		0,40				•	
	0,30					0.30					0,30					
	0.20			R=0,81	•	0.20			R=0	0,64	0.20			R=0,6	8	
	0,10		÷			0.10				••	0.10			••		
1	0,10	:	•			0,10	•	•	•		0.00	•	-	•		
	0,00 0,00	0,10 FA	0,20 (g/100	0,30 Dg)	0,40	0,00	00	0,20 HA (g	0,40 (100g)	0,60	0,00	0,40 hu	0,80 Imins (1,20 1,0 (g/100g)	50 2,00	

Figure 4. Correlation between physical (coarse particulate organic carbon, POCc, fine particulate organic carbon, POCf and mineral associated organic carbon, MOC) and chemical fractions (fulvic acids, FA, humic acids, HA, and humins). R is from Pearson test, * indicates significant correlation (p-value < 0,05) and ** (p-value < 0,005).

DISCUSION

SOC composition and distribution with depth and between row and inter-row

SOC and all SOC fractions were higher at surface and diminished with depth (Figure 2), showing a clear stratification, which is in agreement with Franzluebbers (2002), Duval et al. (2018) and Guimarães et al. (2013). There was no interaction between factors nor effect of row and inter-row positions on SOC, POCf, POCc, HA and FA. As row and inter-row positions may change each cropping season, this treatment only reflects the effect of the immediately preceding crop. POCc and FA are thought to represent recently incorporated SOC and recently formed humic substances (Briedis et al., 2012). The fact that there were no significant differences at surface and in the row, where corn shoots from the previous crop were visible can be explained in different ways. This can indicate that one year of C inputs may not be enough to cause a change in SOC contents (even in the more sensitive fractions); that corn residues had not been transformed yet; or that corn residues were homogenously dispersed over both the row and inter-row. Regarding the first option, Reicosky et al. (2002), in a 30 years-old trial, found that corn stalks restitution vs. removal did not affect SOC content. Considering the second explanation, corn leaves residues of relatively high C/N ratio and with low surface (especially in the stems), and thus these residues are not easily degraded (Hadas et al., 2004). Samples were taken at the beginning of August, two months after corn harvest. Furthermore, corn residues were not incorporated to the soil, which would contribute to higher turnover rates (Yadvinder-Singh et al., 2005). In relation to the third hypothesis, it has been stated that most C in agricultural soils comes from roots in relation to that from shoots (Rasse et al., 2005). Mengel & Barber (1974) studied root distribution of corn finding that, in the top 0,15 m of soil, roots in the row doubled those in the inter-row. Thus, it is unlikely to think that C input from the previous crop was equal in both positions. At deeper layers, the fact that there was no difference between positions can be explained as corn has a well-developed root system that grows beyond the row (Mengel & Barber, 1974).

Humins and MOC content showed interaction between position and depth, with lower values in the row in the 0,10-0,20 m layer. In the row, MOC and humins contents in this layer were not different from the 0.05-0,10 m layer. In the inter-row, MOC and humins contents were significantly higher in the 0,10-0,20 m layer than in the 0,05-0,10 m layer. MOC and humins include older and more stabilized SOC (Ding et al., 2006; Yang et al., 2004), that is unlikely to form from the previous crop's residues. Therefore, lower MOC and humins content in the row may be explained because of higher degradation rate in the row. In the row, greater biological activity may be expected as more roots can enhance biological activity (Binet et al., 1997; Pausch et al., 2013). Although these fractions are characterized as stable SOC with very slow turnover rates, Jagadamma et al. (2014) found that MOC could be mineralized by microorganisms when fresh organic matter was added, reaching up to 11 % of MOC mineralized. In this study,

MOC was 12% lower in the row compared to the inter row, and humins 15 % lower.

Values of SOC were similar to those found for a Typic Argiudoll by Duval et al. (2014) and higher than those reported by Bongiovanni & Lobartini (2006) for a cultivated Haplustoll. SOC values were similar to those found by Soracco et al. (2018) in another trial in the same study site. Vertical distribution found in the present study agrees with Franzluebbers (2002), who reported that SOC content was relatively uniform in the first 0,20 m under long-term conventional tillage, and that in soils under NT, higher SOC levels in the surface may be explained because mineralization in the surface is slower than when residues are incorporated in the soil profile, primarily because of less optimal moisture conditions and lower interaction between the residue and soil in the upper layer (Yadvinder-Singh et al., 2005). This can also explain that less transformed fractions are more abundant in the surface.

Duval et al. (2018) studied the effect of management in SOC and POC in two Argiudolls in the Pampas Region. In both sites, they found higher SOC values in good agricultural practices (GAP) than in bad agricultural practices (BAP). Considering the first 0,10 m, SOC values reported in the present study are similar to those found in one of the sites under GAP, and higher than those found in the other site for both treatments. For the 0,10-0,20 m layer, values reported by Duval et al. (2018) are higher than those found in this study; thus, SI are lower. As regards the chemical fractions. Bongiovanni & Lobartini (2006) studied a cultivated and an undisturbed Haplustoll, finding lower FA and higher HA contents in the surface than the values reported in this study. Guimarães et al. (2013), studing natural and cultivated Ultisolls, found lower HA, FA and humins values than reported here.

The distribution of SOC among chemical fractions reported in this study (around 0,68 of SOC corresponding to humins, 0,21 to HA and 0,11 to FA) is in agreement with that reported by Recio-Vazquez et al. (2014) for many different types of soils, by Tan (1978) for Mollisols, and by Stevenson (1994) for mineral soils. Recio-Vazguez et al. (2014) reported that humins generally amount to more than half of SOC. In contrast, Zalba & Quiroga, (1999) reported that humins/SOC usually represent up to 0,20. Recio-Vazquez et al. (2014) found contents of the colloidal fraction (HA+FA)/SOC ranging from 0,33 in a forest soil, to 0,53 in an Histosoll. Orlov (1985) reported that (HA+FA)/SOC represented 0,57 ± 0,15 % in sub-podzolic soils (Olk et al., 2019). In our study (HA+FA)/SOC represented 0,30 ±0,06.

In this study, humins/TOC and HA/TOC remained contant in the first 0,20 m of the soil profile. In the 0,30-0,50 m layer, that corresponded to Bt horizon, humins/TOC value increased while HA/TOC diminished. FA remained constant throughout the soil profile including the Bt horizon. This is expectable, as FA is a soluble fraction, and thus can migrate through the soil profile (Zalba & Quiroga, 1999; Kay & VandenBygaart, 2002). Humins, on the other hand, represent a more stable and inert stock of C, that can persist for several thousand years in deeper layers where there is few biological activity (Paul, 2016). Furthermore, the 0,30-

0,50 m layer had higher clay contents that can enhance SOC stabilization and protection (Rasse et al., 2005). HA represent an intermediate pool of lower mobility than FA, and intermediate age between the other fractions (Benites et al., 2003). Thus, it is related to organic matter inputs, which are higher in the A horizon than in Bt. The values of HA/FA ratio at surface found in our study (mean value 1,9) are within the characteristic values for Mollisols (1,5-2,5) (Zalba & Quiroga, 1999). HA/FA ratios lower than one indicate poor humification process, while higher ratios may indicate good quality of SOM than can enhance soil physical properties or they may indicate FA loss (more common in sandy soils) (Guimarães et al., 2013).

In relation to the physical fractions of SOC, values of POCc, POCf and MOC for the 0-0,10 m layer were similar to those found for Argiudolls under GAP in the same region (Duval et al., 2013; Duval et al., 2018). POCc values in the 0,10-0,20 m layer were up to one order of magnitude higher than those found by Duval et al. (2018). Values of POC, MOC and SOC were higher to those measured by Bongiovanni & Lobartini (2006) for a cultivated Haplustoll. Considering the first 0,20 m, values of POCc, POCf and SOC were in the same range of those reported by Duval et al. (2018). POCc values were 3 times higher in the 0-0,05 m layer than in the 0,05-0,10 m layer, which represents an abrupt decrease in relation to that of POCf and MOC. POCc encompasses particulate, partly decomposed plant and animal residues, but also root fragments, spores, fungal hyphae and other biological materials (Christensen, 2001). Therefore, POCc is mainly related to top soil.

The largest fraction of SOC corresponded to MOC, with similar values to those of humins (around 0,65-0,80 of SOC). This is in agreement with Christensen (2001), who reported that, for temperate arable soils, most SOC is in the MOC fraction, with higher concentration in the clay-sized complexes (< 2 µm), followed by coarser particles. This can be explained as MOC represents SOC that is protected against degradation by the formation of organo-mineral complexes (Christensen, 2001). POCf/SOC represented between 0,12-0,28 and POCc/SOC was 0,04-0,17. This values are similar to those obtained for HA and FA respectively. Considering the first 0,20 m, POCc/SOC, was within the values found for Pergamino and Monte Buey (both Argiudolls of the Pampa region) by Duval et al. (2013). Christensen (2001) reported that in long cultivated arable soils POCc usually makes up less than 10 % of the OM in the tilled layer, and that it increases when under are soils native grassland. The (POCf+POCc)/SOC ratio in the first 0,20 m represented 0,13. This work showed a higher contribution of POCc to SOC than that found by Duval et al. (2014) for different rotations, pastures and soybean monoculture. Duval et al. (2014), for similar soils in the region, found that (POCf+POCc)/SOC represented around 0.17 in agricultural systems in Pergamino, and 0,40 in natural environments. Alvarez et al. (2011), for Argiudolls under under different rotations, found values of NT (POCf+POCc)/SOC of 0,20. Other studies showed that (POCf+POCc) generally represents between 0,15 and 0,24 (Duval et al., 2014). MOC/SOC was higher in the 0,10-0,20 m layer than in the upper layers, where the contribution of newer fractions was higher (Duval et al.,

2014). Considering the first 0,20 m, values reported in this study are similar to those found by Duval et al. (2014) for a natural environment in the same region.

Soil organic carbon stratification

SOC_{SI}, HA_{SI}, humins_{SI} and MOC_{SI} were significantly affected by position (row vs. inter-row), while the contents of SOC and SOC fractions were not affected by position. Therefore, SI were more sensitive to detect differences among positions than the fractions' content or SOC content alone. SOC was more stratified in the row, as did humins and MOC. This can be attributed to higher MOC and humins content in the inter-row in the 0,10-0,20 m layer. HA_{SI}, on the other hand, was higher in the inter-row than in the row. HA content showed no effect of position at any depth, though higher values of HA were observed in the inter-row in the 0,10-0,20 m layer, that can explain higher HA_{SI} in this position.

SOC_{SI} in this study were 1,36 for the row and 1,21 for the inter-row. These values agree with those reported or calculated using data from different authors, which were between 1,1-1,3 under NT (Kay & VandenBygaart, 2002; Duval et al., 2014; Soracco et al., 2018). Under conventional tillage, SI tended to be lower and rounded 1,1 (Kay & VandenBygaart, 2002; Soracco et al., 2018). Álvarez et al. (2011), for Argiudolls under NT calculated SI that ranged from 0,9 to 1,6. Duval et al. (2014) reported higher SI (around 1,4) for a natural environment and for a pasture. Franzluebbers (2002) reported SI that varied from 1,1 to 1,8 under conventional tillage and were greater than 2 under NT. According to this author, SOC_{SI} higher than 2 would be an indication that soil quality might be improving, independent of soil type and climatic regime. On the other hand, SI calculated for natural environments and natural grasslands in Pampas region were often lower than 2 (Duval et al., 2014; Duval et al., 2018), indicating this value of reference may not be valid for this region. Therefore, despite Franzluebbers (2002) proposed SI to be soil quality indicators independent of soil type and climate, values of reference for different soils may be needed.

The highest SI corresponded to POCc (5,69 for the row and 5,82 for the inter-row). These values are almost 4 times higher than values calculated for FA_{SI}, and are up to six times the values obtained for the other fractions. POCc, is closely related to dry matter input (Duval et al., 2013), and therefore higher SI can be expected. Values reported here are lower than those found by Duval et al. (2013), which were between 7,2 and 12 for POCc_{SI} and between 2,0 and 2,2 for POCf_{SI}. Álvarez et al. (2011) found that MOC_{SI} were lower than 2 while (POCc+POCf)_{SI} were lower than those reported in the present study, ranging between 4,7 and 5,3.

Relationship between physical and chemical fractions of SOC

MOC content was mainly correlated with humins, and also well correlated to HA and FA. POCf showed the highest correlation with HA and was also correlated to FA and humins. POCc was only correlated to FA. The fact that each chemical fraction correlated with more than one physical fraction may indicate that different physical and chemical methods seem to separate similar, though no perfectly matching fractions. For instance, taking into account the technique and the fact that SI of POCc are higher than those of FA, we can deduce that POCc fraction includes recently incorporated biological material (Haynes, 2005) that is not present in the FA fraction, while the FA fraction may contain more soluble and mobile molecules (Havnes, 2005), explaining higher levels in the deeper layers. Zalba & Quiroga (1999) also found a close relationship between FA and POCc. On the other hand, the humins fractions encompass all insoluble material, which can include coarse particulate solids that would be taken into account in the POCc fraction. However, other features were similar between fractions, for instance the relative contribution of each fraction to SOC and SI showed similar trends for FA and POCc, HA and POCf, and MOC and humins.

CONCLUSIONS

In an Argiudoll of the Argentinian Pampas under NT, the content of SOC and different SOC fractions show vertical variation throughout the soil profile, with higher contents in the surface that diminish with depth significantly within 0,50 m. This vertical distribution is different for each fraction of SOC, leading to different SI values. The contribution of POCc to SOC is larger in the surface than in deeper layers, MOC contribution to SOC increases with depth. On the other hand, the contribution of FA, HA and humins to SOC are not affected by depth. SOC content and composition are not different between row and inter-row positions, indicating that the last crop does not affect this distribution. Some physical and chemical fractions of SOC are correlated.

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