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# Winter cover crops effects on soil organic carbon and soil physical quality in a Typical Argiudoll under continuous soybean cropping

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**ABSTRACT:** The massive adoption of no-tillage (NT), along with the simplification of the cropping sequences has led to physical and chemical degradation of soils. To recover degraded soils, cover crops have been proposed as an alternative to increase soil organic carbon (SOC) and to improve soil physical quality (SPQ). This study aimed (i) to determine the content of SOC and its physical and chemical fractions at different layers and positions, in a soil with a soybean crop under NT with and without winter cover crops, and (ii) to determine SPQ indicators in a soybean crop under NT with and without winter cover crops. Measures and samples were made on a field experiment in a typical Argiudoll of the Argentinean Pampas. Soil organic carbon, coarse and fine particulate organic carbon (POCc and POCf), mineral associated organic carbon (MOC), fulvic acids (FA), humic acids (HA), and humins (H) were determined. Soil physical quality indicators determined were: soil bulk density and total porosity from field samples, and saturated hydraulic conductivity, water-conducting macro and mesoporosity, and total porosity connectivity from field water infiltration data. After eight years, cover crops did not cause any observable change in whole SOC content, but significant differences were observed for some SOC fractions. Humic acids and POCc had 40 and 25 % increases, respectively, in the cover crop treatment. Mineral associated organic carbon and H decreased by 9 and 7 % in cover crop treatment. Soil physical quality did not improve after eight years of cover crops. This can be related to degradation processes after 20 years of soybean monoculture under NT, and to the low ability of Argiudolls to recover from physical degradation.

**Keywords:** particulate organic carbon, humic acids, fulvic acids, saturated hydraulic conductivity, water-conducting porosity.

## INTRODUCTION

In Argentina, agricultural systems have gone through a big transformation since the adoption of no-tillage (NT), which allowed the expansion of the agricultural frontier and a constant increase in crop productivity (Domínguez and Bedano, 2016). Among other things, NT was implemented as a strategy to protect soils and increase soil organic carbon (SOC) contents (Lal et al., 2007). However, the adoption of NT has been accompanied by a simplification of cropping systems, characterized by the massive adoption of soybean monoculture and the disappearance of pastures and rotations (Carrasco et al., 2012; ISAAA, 2016). These simplified cropping systems under NT have led to physical and chemical degradation of soils (Irizar et al., 2015; Duval et al., 2016). Summer monocultures imply long fallow periods with low soil cover, leaving the soil unprotected to rain and temperature changes, and increasing degradation risk (Romaniuk et al., 2018). Álvarez et al. (2011) concluded that continuous NT management did not recovery SOC in the Argentinean Pampas. In particular, soybean monoculture causes a decrease in SOC level, because this crop leaves very little and easily degradable residues (Olson et al., 2010; Duval et al., 2016; Behrends Kraemer et al., 2017).

In addition, the conversion to simplified rotations under NT can lead to the formation of massive structure or platy structure, especially in soils with high silt contents like most soils in the Pampas region (Argiudolls) (Sasal et al., 2006, 2017). This way of physical degradation may be enhanced in soils under soybean monoculture (Lozano et al., 2014; Novelli et al., 2017), as soybean is harvested during the wettest period of the year and has a poorly developed root system, and thus is less able to generate porosity and structure than graminaceous crops (Imhoff et al., 2010). Processes of physical degradation may reduce crop yields and the ability of soils to conduct water (Lipiec et al., 1991; Sasal et al., 2006, 2017).

To recover degraded soils, cover crops have been proposed as an alternative to increase soil cover and C inputs (Poeplau and Don, 2015; Duval et al., 2016) and to improve soil physical quality (SPQ) (Recio-Vázquez et al., 2014). Many studies reported that cover crops increased SOC (Blanco-Canqui et al., 2011, 2015; Duval et al., 2016; Álvarez et al., 2017; Jian et al., 2020), being the effect greater in the top of the soil, and affecting deeper layers after longer periods (>6 years) (Duval et al., 2016). Many authors studied the effect of cover crop species on SOC, concluding that mixtures of legumes and gramineous can lead to a greater increase of SOC, due to greater biomass production (Blanco-Canqui et al., 2015). On the other hand, some authors did not find differences in SOC with the inclusion of cover crops (Yang et al., 2004; Fronning et al., 2008). In a meta-analysis, Jian et al. (2020) found that cover crops caused a mean increase in SOC of 15 %, which rose up to 30 % in studies under NT. However, some studies considered in this meta-analysis found no change on SOC, especially when the main crops were corn-soybean, corn-wheat/soybean or soybean monoculture (Jian et al., 2020). Blanco-Canqui et al. (2015) concluded that while cover crops generally increase SOC, this effect is not frequently detectable in the first few years after establishment. A possible explanation for this is that SOC content is not sensitive enough to detect short-term changes due to management practices (Purakayastha et al., 2008), while the content of different fractions of SOC may be more sensitive (Ding et al., 2006; Plaza-Bonilla et al., 2014). Romaniuk et al. (2018) and Duval et al. (2016) found that winter cover crops increased the content of coarse particulate organic carbon (POCc) before significant SOC changes could be observed. On the other hand, Duval et al. (2013) found that the POCf fraction was the most sensitive to management. Romaniuk et al. (2018) reported that the inclusion of wheat as a cover crop in a soybean monoculture did not increase mineral associated organic carbon (MOC) content, but MOC increased with the inclusion of corn and wheat in the crop rotation. Other studies determine the content of different fractions of SOC obtained by chemical fractionation, for instance, fulvic acids (FA), humic acids (HA), and humins (H), which are related to more labile, intermediate, and more resistant SOC pools, respectively.

Guimarães et al. (2013) and Yang et al. (2004) reported that cover crops increased the H fraction in the top 0.10 and 0.25 m of soil, respectively, and found no differences in the content of FA and HA. Arlauskienė et al. (2010) studied the effect of different cover crops on different kinds of HA on a clay loam soil and found that cover crops increased the more labile HA after the first year. These authors also reported that after the second year of treatment more stable HA were formed.

Cover crops can also improve SPQ, both by increasing SOC and because of the effect of roots and enhanced biological activity (Duval et al., 2016; Lozano et al., 2014; Behrends Kraemer et al., 2017). Many authors reported that cover crops improve SPQ, increasing porosity, macroporosity, and pore connectivity (Carof et al., 2007; Blanco-Canqui et al., 2015; Basche and DeLonge, 2017; Calonego et al., 2017). Increased water infiltration under cover crops as compared to bare fallow has been reported (Blanco-Canqui et al., 2011; Álvarez et al., 2017; Haruna et al., 2018; Chalise et al., 2019). In a review, Blanco-Canqui et al. (2015) reported that studies on the effect of cover crops on water infiltration were few and that an increase between 1.1 and 2.7 times was found. In another review, Basche and DeLonge (2019) found that cover crops increased infiltration rates, especially after the fourth year since cover crop inclusion (mean increment of 35 %). However, these authors found that this increment was greater in coarser soils and regions with annual precipitations below 650 mm. In contrast, some studies report cover crops may cause a decrease in saturated hydraulic conductivity, which may be temporal, and may be caused by pore-clogging by roots or by reduced fissures formation in a covered soil (Carof et al., 2007; Bodner et al., 2008). Other authors found no effect of cover crops on saturated hydraulic conductivity ($K_0$) (Wagger and Denton, 1989).

The effect of cover crops on SOC contents and SPQ frequently depends on cover crop species, soil texture, initial SOC contents, climatic conditions, and the amount and characteristics of the belowground development (Ding et al., 2002; Blanco-Canqui et al., 2015). Some authors found that soils from the Pampas region with high silt content have a low probability of recovering its topsoil porosity after several years of NT, even with higher organic carbon contents in surface layers (Taboada et al., 1998). The structure of these soils has low resilience (Taboada et al., 2008), affecting their ability to recover, even when more adequate management practices are applied. Thus, though cover crops are thought to increase SOC and positively affect SPQ, documentation is limited and needs to be broadened under different soil textural classes and crop species (Blanco-Canqui et al., 2015). Information about the effect of cover crops on SOC fractions obtained by chemical fractionation and in depth is scarce. Information on the potential of cover crops to accumulate SOC in depth is also scarce (Blanco-Canqui et al., 2015). Another aspect of SOC distribution is its spatial variation within a plot. There is literature on how SOC varies with topography, but there is little information on how it varies within short distances. In this sense, differences between the row and the inter-row of the previous crop can be expected to be found, as differences in root development and earthworms' abundance between these positions have been reported (Mengel and Barber, 1974; Binet et al., 1997). In addition, establishing relationships between SOC fractions and other soil properties that may define SPQ is still a challenge (Lal, 2009), that could provide valuable information to understand the functions of different SOC pools. The reviewed literature is summarized in tables 1 and 2.

We hypothesized that (i) winter cover crops induce a change in SOC distribution in the profile, especially by an increment in the top layer of soil and in the row; (ii) newer fractions of SOC (FA and POCc) are the most sensitive fractions to the inclusion of cover crops; and (iii) winter cover crops improve SPQ in a silty loam soil degraded by soybean monoculture. The aims of this study were (i) to determine the content of SOC and its physical and chemical fractions at different positions and layers, in a soil with a soybean crop under NT with and without winter cover crop; and (ii) to determine SPQ indicators in a soybean crop under NT with and without winter cover crop.

**Table 1.** Effect of cover crops on soil organic carbon (SOC) and SOC fractions in the reviewed literature

| Study | Type of study | Soil order | Soil texture | Main crop | Cover crop | Duration | Tillage | Effect on | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | SOC | POCc | POCf | MOC | FA | HA | H |
| | | | | | | year | | | | | | | | |
| Jian et al. (2020) | M | V | V | V | V | V | V | + | * | * | * | * | * | * |
| Beltrán et al. (2018) | | Typic Argiudoll | silt loam | S | oat | 8 | NT | + | + | + | ns | * | * | * |
| Romaniuk et al. (2018) | | Typic Argiudoll | silt loam | V | W | 8 | NT | ns | + | ns | ns | * | * | * |
| Álvarez et al. (2017) | M | V | V | V | V | V | V | + | * | * | * | * | * | * |
| Duval et al. (2016) | | Typic Argiudoll | silt loam | S | oat+ vetch | 3 4 6 | NT | ns + + | ns + + | ns ns+ | - | * | * | * |
| Blanco-Canqui et al. (2015) | R | V | V | V | V | V | V | + | * | * | * | * | * | * |
| Poeplau and Don (2015) | M | V | V | V | V | 1-54 | V | + | * | * | * | * | * | * |
| Varela et al. (2014) | | Thapto-Argic Hapludoll | silty clay | S | V | 5 | NT | ns or + | * | * | * | * | * | * |
| Guimarães et al. (2013) | | Ultisol (typic Hapludult) | sandy loam | coconut | legumes | * | T | + | * | * | * | ns | ns | + |
| Blanco-Canqui et al. (2011) | | Udic Argiustoll | silt loam | W- sorghum | V | 15 | NT | + | * | * | * | * | * | * |
| Arlauskienė et al. (2010) | | Cambisols | clay loam | W | V | 1 and 2 | * | + | * | * | * | * | + | * |
| Fronning et al. (2008) | | Haplaquolls | loam | C-S | rye | 1-3 | NT | ns | ns | ns | ns | * | * | * |
| Ding et al. (2006) | | Fluventic Dystrudept | sandy loam | C | V | 9 | T | ns or + | * | * | * | * | * | * |
| Yang et al. (2004) | | Fluvaqentic Humaquept | loam | cereals | V | 13 | NT | ns | * | * | * | ns | ns | + |

Effect of cover crops is expressed as increases (+), decreases (-), no change (ns), and not determined (*). Only studies that reported differences between cover crops and a control (without cover crop) treatment were included. Letter V stands for various crop species, soil classes, years of cover crops analyzed, or tillage systems. Study type is specified in the case of meta-analysis (M) and reviews (R). C: corn; S: soybean; W: wheat; T: tillage; NT: no-tillage; POCc: coarse particulate organic carbon; POCf: fine particulate organic carbon; MOC: mineral associated organic carbon; FA: fulvic acids; HA: humic acids; and H: humins.

## MATERIALS AND METHODS

### Sampling site

Samples were taken from an experiment set up at the Pergamino Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA), Pergamino (33° 51’ S, 60° 40’ W). The climate is temperate humid without a dry season, with a mean annual temperature of 16.5 °C and mean annual rainfall of 971 mm (Restovich et al., 2012). Soil at the study site is a Typic Argiudoll (Soil Survey Staff, 2014) of the Pergamino series. This soil is deep, well-developed, and well-drained. Soil texture, bulk density (BD), and pH($H_2O$) at a ratio of 1:2.5 are presented in table 3.

The trial consisted of a completely randomized design with 30 × 10 m plots, with two treatments and three repetitions. The sampled plots had the same relative position in the landscape. Treatments were: (i) soybean monoculture under NT (SBF) and (ii) soybean crop with a winter cover crop under NT (SCC). The plots under study (SCC and SBF treatments) have had soybean (*Glycine max* L.) under NT as the main crop since 1987 (for the last 30 years). The cover crop was included in the SCC plots in spring 2010 (eight years before sampling date), while SBF plots were left under soybean monoculture. Soybean was seeded with a distance between rows of 0.52 m. Cover crop was an oat (*Avena sativa* L.) and vetch (*Vicia sativa* L.) mixture with densities of 20 and 40 kg $ha^{-1}$, respectively. An experimental seeder with a cone plot planter was employed. Soybean

**Table 2.** Effect of cover crops on soil physical quality indicators in the reviewed literature

| Study | Type of study | Soil order | Soil texture | Main crop | Cover crop | Duration | Tillage | Effect on | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | BD | TP | K | $\varepsilon_{ma}$ | $\varepsilon_{me}$ | $Cw_{TP}$ |
| | | | | | | year | | | | | | | |
| Basche and DeLonge (2019) | M | V | V | V | V | V | NT/T | * | * | + | * | * | * |
| Chalise et al. (2019) | | Calcic/ Pachic Hapludolls | silt / silt loam | C - S | rye+ vetch | 9-11 | NT | - or ns | * | + | * | * | * |
| Haruna et al. (2018) | | Aeric Fluvaquents | silt loam | C | rye | 4 | NT/ T | ns | ns | ns | ns or + | ns or + | * |
| Álvarez et al. (2017) | M | V | V | V | V | 1-15 | * | - or ns | ns | + | * | * | * |
| Basche and DeLonge (2017) | M | V | V | V | V | V | V | * | + | * | * | * | * |
| Calonego et al. (2017) | | Typic Rhodudalf | clay | S/SF – S/W | V | 1, 3, and 9 | NT | ns | ns | * | * | * | * |
| Blanco-Canqui et al. (2015) | R | V | V | V | V | V | V | - or ns | + | + or ns | + | * | + |
| Blanco-Canqui et al. (2011) | | Udic Argiustoll | silt loam | W-sorghum | V | 15 | NT | - or ns | * | + or ns | * | * | * |
| Bodner et al. (2008) | | Calcareous chernozem | sandy loam/ loam | barley | V | 1 and 2 | T | * | * | - or ns | - or ns | - or ns | * |
| Carof et al. (2007) | | Orthic Luvisol | loamy | W | V | 3 and 4 | NT | * | + | - or … | + or ns | + or ns | * |
| Keisling et al. (1994) | | Typic Hapludalf + Aerie Ochraqualf | silty | cotton | V | 17 | T | - | + | + | + | * | * |
| Wagger and Denton (1989) | | Aquic Paleudults | sandy loam | C | V | 3 | NT | ns | ns | ns | * | * | * |

Effect of cover crops is expressed as increases (+), decreases (-), no change (ns), and not determined (*). Only studies that reported differences between cover crops and a control (without cover crop) treatment were included. Letter V stands for various crop species, soil classes, years of cover crops analyzed, or tillage systems. Study type is specified in the case of meta-analysis (M) and reviews (R). C: corn; S: soybean; W: wheat; SF: sun flower; T: tillage; NT: no-tillage; BD: bulk density; K: hydraulic conductivity; εma: effective macroporosity; εme: effective mesoporosity; CwTP: total porosity connectivity.

was fertilized at seeding with 12 kg $ha^{-1}$ of P; cover crop was not fertilized. The plot without cover crop was chemically maintained without weeds during fallow. Cover crop was dried with glyphosate (48 %, 3 L $ha^{-1}$) in spring (October 2018), at the reproductive stage, to seed soybean. Mean temperature and precipitations for that year were 16.7 °C and 970.6 mm.

### *Sample collection and analytical procedures*

Soil samples were collected at the end of November 2018 (after soybean seeding and in V2 development stage). For each treatment (SCC and SBF), position (row and inter-row of the previous soybean crop), and layer (0.00-0.05, 0.05-0.10, and 0.10-0.20 m), three homogenized samples were composed of over 15 randomly selected spots (final number of samples = 18). Additionally, for each treatment, three composite samples were taken at 0.20-0.40 m (Bt horizon), without differentiating between row and inter-row positions (final number of samples = 6). After measuring moisture, samples were air-dried and

**Table 3.** Particle size distribution, bulk density (BD), and pH (soil to water ratio 1:2.5) for soils under soybean monoculture (SBF) and soybean with cover crop (SCC)

| Layer | BD | | pH($H_2O$) | | Clay | Fine silt | Coarse silt | Sand |
|---|---|---|---|---|---|---|---|---|
| | SBF | SCC | SBF | SCC | | | | |
| m | Mg m$^{-3}$ | | | | g kg$^{-1}$ | | | |
| 0.00-0.05 | 1.24±0.08 | 1.20±0.03 | 6.20±0.39 | 6.04±0.06 | 220±20 | 320±30 | 260±10 | 190±30 |
| 0.05-0.10 | 1.29±0.04 | 1.25±0.06 | 5.95±0.02 | 5.98±0.06 | 230±20 | 340±20 | 230±20 | 200±40 |
| 0.10-0.20 | 1.36±0.05 | 1.37±0.03 | 5.98±0.08 | 6.07±0.06 | 250±10 | 360±20 | 220±30 | 170±50 |
| 0.20-0.40 | 1.37±0.06 | 1.33±0.04 | 6.37±0.02 | 6.56±0.03 | 240±20 | 340±40 | 220±10 | 200±50 |

Bulk density was determined according to Blake and Hartge (1986); pH (soil to water ratio 1:2.5); particle size distribution was determined according to Gee and Bauder (1986).

pulverized to pass through a 2 mm sieve and frozen. To determine BD and TP, four undisturbed soil samples were collected for each treatment and position, employing cylinders (5 cm diameter and 5 cm high, 98 cm$^3$ volume). A compacted layer was observed below 0.10 m depth.

### *Physical fractionation of SOC*

Different particle size fractions of SOC were obtained following the method described by Duval et al. (2018) which is based on Cambardella and Elliot (1992). Briefly, 50 g of dry soil were physically dispersed by 16 h of agitation in a rotary shaker with 500 mL of water and 10 glass beads. The suspension was then sieved through 105 and 53 µm sieves and washed until wash water was clean to the naked eye. Three fractions were obtained: (1) coarse particulate organic carbon (POCc), which is the fraction between 105 and 2000 µm; (2) fine particulate organic carbon (POCf), which is the fraction between 53 and 105 µm; and (3) mineral associated organic carbon (MOC), the fraction smaller than 53 µm, which is discarded and then estimated by difference. Each fraction was transferred to a Petri dish, dried at 40 °C until constant weight, and weighted. After this, the material was sieved through a 500 µm sieve for SOC determination.

### *Chemical fractionation of SOC*

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). Approximately 1 g of dry soil was weighed in 50 mL centrifuge tubes. Dissolution of HA and FA was achieved with a long extraction (24 h) with 20 mL of sodium hydroxide (NaOH) (0.1 mol L$^{-1}$). The supernatant (HA+FA) was separated from the solid by centrifugation (20 min at 4000 rpm). A second short extraction was performed (1 h with 20 mL of NaOH 0.1 mol L$^{-1}$) followed by centrifugation. Supernatants obtained from the two extractions were transferred to a single tube. Afterwards, HA were precipitated by acidification of the extracted solution to pH 1.0 ± 0.1 with sulfuric acid ($H_2SO_4$) 20 % v/v and allowed to stand for 24 h. Humic acids were separated from FA by filtration under vacuum, employing a 0.45 µm pore size cellulose ester membrane filter. After collecting the FA fraction (filtrate), HA were dissolved to pass through the filter with NaOH (0.1 mol L$^{-1}$). Both FA and HA fractions were diluted to a known volume of 50 mL.

### *Soil organic carbon quantification*

Soil organic carbon contents for whole soil, POCc, POCf, HA, and FA were determined by the wet oxidation method described by Walkley and Black (1934). The SOC from the MOC and H fractions was calculated by difference, subtracting from SOC value the other two fractions (POCc and POCf for MOC, and HA and FA for H). In all cases, SOC is expressed as g of oxidizable carbon per 100 g of soil dry mass (g 100 $g^{-1}$). The relative contribution of each fraction to SOC is expressed as a rate (content of the fraction/SOC).

### *SPQ indicators*

Soil BD was measured using the core method (Blake and Hartge, 1986). Total porosity (TP) was calculated from BD, using a particle density of 2.55 Mg $m^{-3}$ (Villarreal, 2018). Soil water infiltration was measured in the field employing disc infiltrometers (Perroux and White, 1988) (disc diameter = 12.5 cm), at four randomly selected sites for each treatment and position (row vs inter-row). After removing plant and mulch cover, infiltrometers were placed on a thin sand layer, to improve contact. Infiltration measurements were run at 6, 3, and 0 cm tension (h), in that order and the same place. Each infiltration run was performed for one hour, allowing to reach the steady state. Dynamic SPQ indicators were calculated from infiltrometer data. Hydraulic conductivity at different tensions was calculated using the multiple head method (Ankeny et al., 1991). Water-conducting macroporosity ($\varepsilon_{ma}$, equivalent r >0.5 mm) and water-conducting mesoporosity ($\varepsilon_{me}$, 0.5> r >0.25 mm) were calculated from K data according to Watson and Luxmoore (1986). Total porosity connectivity ($C_{wTP}$) was calculated as the quotient between $K_0$ and TP (Lozano et al., 2013). Particle size distribution was measured using the pipette method (Gee and Bauder, 1986).

### Statistical analysis

ANOVA was performed to determine the interaction and effect of the studied factors. Values were expressed as the mean value ± standard deviation. Means were compared using Fisher's $LSD_{50}$ test (p-value<0.05), and significant differences were expressed using different letters. The distribution of $K_0$, $\varepsilon_{ma}$, $\varepsilon_{me}$, and $CW_{TP}$ is log-normal, so values were log-transformed before statistical analysis. All analyses were performed using INFOSTAT software (Di Rienzo et al., 2008).

## RESULTS

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

ANOVA results are shown in table 4. There was no interaction between factors for any of the variables studied. Soil organic carbon and all SOC fractions were affected by depth. Humic acids, H, POCc, and MOC were also significantly affected by treatment (SCC vs SBF). Position (row vs inter-row) had no effect on the content of SOC or SOC fractions. Thus, mean values between the row and the inter-row are used in the discussion. The SOC and all SOC fractions contents were higher near the surface and diminished with depth (Table 5). Soil organic carbon, POCc, POCf, MOC, FA, and H showed similar vertical distribution, with higher values in the 0.00-0.05 m layer and with no significant differences between the 0.05-0.10 and 0.10-0.20 m layer. The HA content followed the order 0.00-0.05 m > 0.05-0.10 m > 0.10-0.20 m. At 0.20-0.40 m, values of SOC and SOC fractions diminished. Considering mean values for each fraction, SOC, H, and MOC values at the 0.20-0.40 m layer were around 50 % of those at the 0.10-0.20 m layer, HA content was around 25 % of that in the 0.10-0.20 m layer, while POCc, POCf, and FA contents were around 80 % of those in the 0.10-0.20 m layer.

Regarding the effect of treatment on SOC content, no difference was found between SBF and SCC. Among the physical fractions of SOC, POCc and MOC were affected by treatment. Considering the 0.00-0.20 m layer, POCc showed a 25 % increase in the SCC treatment in relation to SBF (Table 5). This difference was mainly due to higher POCc values in the 0.00-0.05 m layer in the SCC treatment (Table 5). In contrast, MOC content was 9 % higher in the SBF treatment. This difference was greater on the top 0.10 m of soil. Regarding the chemical fractions of SOC, HA content was higher in SCC than in SBF

**Table 4.** Results of the multifactorial ANOVA for the studied factors (treatment, T; position, P; and depth, D) and their interaction, on the variables studied. Variables were: soil organic carbon (SOC), coarse particulate organic carbon (POCc), fine particulate organic carbon (POCf), mineral associated organic carbon (MOC), fulvic acids (FA), humic acids (HA), and humins (H); the ratio of each of these fractions to SOC; saturated hydraulic conductivity ($K_0$), effective macro and meso-porosity ($\varepsilon_{ma}$ and $\varepsilon_{me}$), bulk density (BD), total porosity (TP), and pore connectivity ($CW_{TP}$)

| | SOC | POCc | POCf | MOC | FA | HA | H | POCc/SOC | POCf/SOC | MOC/SOC | FA/SOC | HA/SOC | H/SOC | $K_0$ | $\varepsilon_{ma}$ | $\varepsilon_{me}$ | BD | TP | $CW_{TP}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | ns | * | ns | * | ns | * | * | * | ns | ns | ns | * | ns | ns | ns | ns | ns | ns | ns |
| P | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| D | * | * | * | * | * | * | * | * | * | * | ns | ns | ns | - | - | - | - | - | - |
| T*P | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| T*D | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | - | - | - | - | - | - |
| P*D | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | - | - | - | - | - | - |
| T*P*D | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | - | - | - | - | - | - |

*: significant effect (p-value<0.05); ns: not significant.

**Table 5.** Soil organic carbon content of different physical and chemical fractions (coarse particulate organic carbon, POCc, fine particulate organic carbon, POCf, and mineral associated organic carbon, MOC; and fulvic acids, FA, humic acids, HA, and humins, H) at different layers and positions (row and inter-row) for the studied treatments: (i) soybean with cover crop (SCC) and (ii) soybean with winter bare fallow (SBF)

| Layer | | SOC | | FA | | HA | | H | | POCc | | POCf | | MOC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SBF | SCC | SBF | SCC | SBF | SCC | SBF | SCC | SBF | SCC | SBF | SCC | SBF | SCC |
| 0.00-0.5 m | Row | 1.69 ± 0.01a | 1.72 ± 0.06 a | 0.17 ± 0.06 ab | 0.18 ± 0.04 a | 0.20 ± 0.03 b | 0.26 ± 0.08 a | 1.31 ± 0.09 a | 1.28 ± 0.12 a | 0.26 ± 0.04 b | 0.32 ± 0.07 a | 0.3 ± 0.1 a | 0.41 ± 0.05 a | 1.1 ± 0.1 a | 1.0 ± 0.1 ab |
| | I-row | 1.66 ± 0.05 a | 1.73 ± 0.04 a | 0.14 ± 0.04 ab | 0.18 ± 0.05 a | 0.19 ± 0.06 b | 0.27 ± 0.06 a | 1.34 ± 0.09 a | 1.28 ± 0.11 a | 0.27 ± 0.01 b | 0.36 ± 0.06 a | 0.23 ± 0.02 a | 0.27 ± 0.04 a | 1.17 ± 0.03 a | 1.1 ± 0.1 ab |
| 0.05-0.10 m | Row | 1.10 ± 0.04 b | 1.03 ± 0.04 b | 0.10 ± 0.04 c | 0.10 ± 0.05 bc | 0.16 ± 0.04 b | 0.22 ± 0.09 ab | 0.84 ± 0.08 b | 0.71 ± 0.01 c | 0.04 ± 0.01 c | 0.05 ± 0.01 c | 0.11 ± 0.08 b | 0.09 ± 0.04 b | 0.95 ± 0.09 bc | 0.89 ± 0.05 d |
| | I-row | 1.12± 0.02 b | 0.99 ± 0.03 b | 0.10 ± 0.03 c | 0.11 ± 0.04 bc | 0.16 ± 0.03 b | 0.19 ± 0.05 ab | 0.85 ± 0.05 b | 0.70 ± 0.09 c | 0.05 ± 0.01 c | 0.04 ± 0.01 c | 0.12 ± 0.01 b | 0.14 ± 0.02 b | 0.95 ± 0.01 bc | 0.81 ± 0.01 d |
| 0.10-0.20 m | Row | 0.99 ± 0.05 c | 0.98 ± 0.01 c | 0.12 ± 0.01 bc | 0.11 ± 0.04 bc | 0.14 ± 0.05 b | 0.16 ± 0.04 b | 0.72 ± 0.06 bc | 0.71 ± 0.09 c | 0.03 ± 0.01 c | 0.03 ± 0.01 c | 0.08 ± 0.04 b | 0.09 ± 0.01 b | 0.88 ± 0.04 cd | 0.86 ± 0.01 cd |
| | I-row | 0.99 ± 0.05 c | 0.95 ± 0.05 c | 0.08 ± 0.05 bc | 0.08 ± 0.01 c | 0.17 ± 0.08 b | 0.16 ± 0.01 b | 0.7 ± 0.1 bc | 0.70 ± 0.01 c | 0.02 ± 0.01 c | 0.03 ± 0.01 c | 0.05 ± 0.05 b | 0.10 ± 0.01 b | 0.92 ± 0.06 cd | 0.82 ± 0.02 cd |
| 0.20-0.40 m | - | 0.57 ± 0.03 | 0.54 ± 0.03 | 0.07 ± 0.03 | 0.06 ± 0.01 | 0.06 ± 0.03 | 0.02 ± 0.01 | 0.44 ± 0.06 | 0.46 ± 0.01 | 0.05 ± 0.06 | 0.01 ± 0.01 | 0.04 ± 0.04 | 0.07 ± 0.04 | 0.5 ± 0.1 | 0.46 ± 0.04 |

The FA, HA, and H fractions were obtained according to Benites et al. (2003); POCc, POCf, and MOC fractions were obtained following Duval et al. (2018). Organic carbon content of each fraction and SOC was determined by Walkley and Black (1934). Values are expressed in g 100 $g^{-1}$. Values after ± indicate standard deviation. Different letters indicate significant differences between values of the same fraction of SOC (Fisher's LSD, p-value<0.05). I-row: inter-row.

(around 40 % increase in the 0.00-0.20 m layer), while H content was 7 % higher in the SBF treatment. Fulvic acids content was not affected by treatment. Higher HA values in SCC were mainly observed in the top 0.10 m of soil profile. The values of H content were higher in the SBF treatment through all the studied profiles, with the broader difference observed in the 0.05-0.10 m layer.

### Relative contribution of different fractions to SOC

There was no interaction between the studied factors (treatment, position, and depth) on fraction/SOC ratios (Table 4). There was no effect of row or inter-row positions on fraction/SOC ratios (Table 4). The POCc/SOC ratio was affected by both treatment and depth, while POCf/SOC and MOC/SOC were only affected by depth. The POCc/SOC was higher in the SCC than in the SBF treatment (0.09 vs 0.07, respectively). This difference was mainly due to higher values in the 0.00-0.05 m layer (0.20 for SCC and 0.16 for SBF). Regarding the vertical distribution, POCc/SOC and POCf/SOC had a similar behavior, with higher values at the surface (0.18 for both), and with no significant differences between the 0.05-0.10 and the 0.10-0.20 m layers (mean value 0.03 for POCc and 0.10 for POCf). The MOC/SOC, in contrast, was higher at 0.05-0.10 and 0.10-0.20 m layers with no differences between them (mean value 0.87), and lower at the 0.00-0.05 m layer (mean value 0.64). Regarding chemical fractions, FA/SOC and H/SOC ratios were not significantly affected by any of the factors studied, showing mean values of 0.10 and 0.75, respectively. The HA/SOC ratio was only affected by treatment, being higher

**Table 6.** Values of SPQ indicators: saturated hydraulic conductivity ($K_0$), effective macro and meso-porosity ($\varepsilon_{ma}$ and $\varepsilon_{me}$), bulk density (BD), total porosity (TP), and total porosity connectivity ($CW_{TP}$)

| Treatment | Position | $K_0$ | $\varepsilon_{ma}$ | $\varepsilon_{me}$ | BD | TP | $CW_{TP}$ |
|---|---|---|---|---|---|---|---|
| | | cm $h^{-1}$ | ppm | | Mg $m^{-3}$ | % | |
| SBF | Row | 1.0 ± 0.5 a | 3 ± 3 a | 7 ± 3 a | 1.24 ± 0.03 a | 51.0 ± 1.0 a | 1.9 ± 0.9 a |
| | Inter-row | 1.1 ± 0.4 a | 4 ± 3 a | 12 ± 6 a | 1.20 ± 0.10 a | 51.0 ± 5.0 a | 2.2 ± 0.8 a |
| SCC | Row | 0.9 ± 0.4 a | 5 ± 3 a | 6 ± 1 a | 1.19 ± 0.02 a | 53.4 ± 0.9 a | 1.8 ± 0.8 a |
| | Inter-row | 1.1 ± 0.5 a | 7 ± 4 a | 6 ± 3 a | 1.20 ± 0.05 a | 53.0 ± 2.0 a | 2.0 ± 1.0 a |

Soil BD was measured using the core method (Blake and Hartge, 1986); total porosity (TP) was calculated from BD, using a particle density of 2.55 Mg $m^{-3}$ (Villarreal, 2018); water infiltration was measured in the field employing disc infiltrometers (Perroux and White, 1988); $\varepsilon_{ma}$ (equivalent r >0.5 mm) and $\varepsilon_{me}$ (0.5> r >0.25 mm) were calculated according to Watson and Luxmoore (1986); $C_{wTP}$ was calculated according to Lozano et al. (2013). Values after ± indicate standard deviation. For each SPQ indicator, same letters indicate no significant differences (Fisher's LSD, p-value>0.05).

for SCC than for SBF (0.17 and 0.14, respectively). Most SOC was found in the MOC and H fractions, that represent older and more stabilized carbon (with MOC/SOC values ranging 0.58-0.93, and H/SOC 0.69-0.80). This distribution was relatively constant in the 0.20-0.40 m layer.

### Soil physical quality indicators

Soil physical quality indicators ($K_0$, $\varepsilon_{ma}$, $\varepsilon_{me}$, BD, TP, and $CW_{TP}$) showed no interaction between the studied factors and were not affected by treatment nor position (Table 4). Values obtained are shown in table 6.

## DISCUSSION

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

In the present study, eight years of an oat+vetch winter cover crop caused no significant differences on total SOC content compared to SBF treatment at any of the studied depths (Table 5). Other authors found no effect of cover crops on SOC. Fronning et al. (2008) found no changes on SOC in a loam Haplaquoll after three years of winter rye cover cropping in a corn-soybean rotation. Yang et al. (2004) found no changes on SOC after 13 years cover crops in a loam Fluvaqentic Humaquept (with cereals as the main crop and ryegrass and clover as cover crops), though they did find differences in the humin fraction. Plaza-Bonilla et al. (2014) reported that the time period needed to observe changes in SOC is normally more than 10 years. In contrast, many authors reported that cover crops increase SOC, even in shorter periods of time since the inclusion of cover crops (4 to 9 years) (Ding et al., 2006; Varela et al., 2014; Blanco-Canqui et al., 2015; Duval et al., 2016; Álvarez et al., 2017; Beltrán et al., 2018). In a meta-analysis, Jian et al. (2020) found that cover crops produced a mean SOC increase of 30 % on soils under NT. However, these authors also reported lower or no SOC increases in studies under soybean and in studies where cover crop was a grass and legume mixture. Other studies suggest that, when soybean is the main crop, gramineous cover crops can increase SOC content (as compared to leguminous or mixtures) (Varela et al., 2014; Duval et al., 2016; Beltrán et al., 2018). These results suggest that a proper balance between graminaceous and leguminous crops should be maintained to achieve SOC increases, and that graminaceous cover crops are more adequate for soybean monoculture.

It has been stated that the study of different fractions of SOC can be more sensitive to management induced changes than SOC content (Ding et al., 2006; Plaza-Bonilla et al., 2014; Blanco-Canqui et al., 2015). In this study, POCc was 25 % higher in the cover crop treatment, due to higher residue inputs in the soil surface (Franzluebbers, 2002; Eclesia et al., 2016). Other authors also found an increase in POCc caused by cover crops. Duval et al. (2016) reported 50 and 31 % increases of POCc at the 0.00-0.05 and 0.05-0.10 m layers, respectively, with an oat+vetch cover crop in relation to bare fallow. Beltrán et al. (2018) found that an oat cover crop increased POCc by 38.7 % in a soybean crop. Romaniuk et al. (2018) found that POCc was three times higher when wheat was included as a cover crop in a soybean crop.

The content of MOC was higher in the SBF treatment than in the SCC treatment, especially in the top 0.10 m of soil. This result was unexpected, as MOC is a stable fraction of SOC, physically protected against degradation because of its interaction with the mineral phase (Six et al., 2002). Higher values in a fraction of SOC can be explained by higher SOC formation or by lower SOC degradation in that fraction (Eclesia et al., 2016). Even though MOC is thought to be a stable SOC fraction with low turnover rates, some studies suggest that management changes can affect stabilized SOC (Six et al., 2002; Piñeiro et al., 2009; Hopkins et al., 2012). Some authors suggested that new residue inputs can have a priming effect on SOC mineralization (Galantini, 2008; Pausch et al., 2013; Varela et al., 2014; Poeplau and Don, 2015). Blanco-Canqui et al. (2015) reports that roots from cover crops have a disruptive effect that somehow resembles that of tillage, and thus could enhance SOC mineralization. Also, soil organic matter in these plots that had a long history of soybean monoculture can have a low C/N ratio (and thus can be easily degradable), as soil organic matter resembles in composition that of the residues that formed it (Six and Jastrow, 2002; Eclesia et al., 2016). Duval et al. (2016) also found lower MOC values under cover crops that were attributed to a dry year, in which the bare fallow treatment kept higher moisture content and had more favorable conditions for POC to MOC turnover than the cover crop treatment. In this study POCf was not affected by treatment nor position. On the contrary, other authors found that POCf was the most sensitive fraction to agricultural practices and to the inclusion of cover crops (Duval et al., 2013, 2014, 2018). No differences on POCf may indicate slow POCc to POCf turnover. In this study, POCc at 0.00-0.05 m was the physical fraction of SOC most affected by treatment.

Regarding the chemical fractions of SOC, HA and H were the fractions significantly affected by treatment. Humic acids content was around 40 % higher in SCC than in SBF, indicating cover crops residues were incorporated into the HA fraction (Arlauskienė et al., 2010). Other authors found no effect of cover crops on the HA fraction (Yang et al., 2004; Guimarães et al., 2013), though these studies were performed under different edaphoclimatic conditions. Humins content, in contrast, was 7 % higher for the SBF treatment, with higher values through all the studied profiles and the broader difference observed in the 0.05-0.10 m layer. These results suggest that cover crops may enhance H mineralization and H to HA turnover (Pausch et al., 2013), even though H represents a more stable and recalcitrant fraction of SOC (Stevenson, 1994). Furthermore, in this study the SBF treatment showed a root development strongly restricted to the top 0.05 to 0.08 m of soil, where the presence of platy structure was observed (at variable depth between 0.04 and 0.09 m). The SCC treatment, on the other hand, exhibited a weaker platy structure with abundant roots in deeper layers. In this sense the cover crop may have enhanced H mineralization in deeper layers, as well as SOC redistribution. On the contrary, other authors found an increment in H under cover crops (Yang et al., 2004; Guimarães et al., 2013), though these studies included more complex or heavily rotated managements, which would explain H buildup.

Fulvic acids content was not significantly affected by cover crop treatment. This result agrees with Yang et al. (2004) and Guimarães et al. (2013), who did not find differences in FA content in the cover crop treatments. Fulvic acids is a labile fraction that can be easily degraded. Also, FA is a mobile fraction that can percolate through the soil profile. Therefore, increments in FA might not be detectable, as it translocates to deeper layers, is mineralized or transformed to a more stable fraction (Guimarães et al., 2013). In this study, HA/FA was higher than 1 for both treatments and at all the studied depths, which indicates loss of the more labile FA fraction (Guimarães et al., 2013).

### Relative contribution of different fractions to SOC

The content of SOC and SOC fractions reported in this study, as well as the distribution of SOC between different fractions are within the values reported for similar soils (Duval et al., 2014, 2016; Recio-Vázquez et al., 2014). In relation to the effect of treatment on fractions contribution to SOC, POCc/SOC and HA/SOC were, as POCc and HA, affected by treatment, while the rest of the ratios were not affected by treatment. The relative contribution to SOC is, therefore, less sensitive to management induced changes than each fractions content. The POCc/SOC was higher in the SCC treatment due to higher POCc/SOC values for SCC in the 0.00-0.05 m layer. This indicates an increase in SOC lability due to the presence of cover crops (Duval et al., 2016).

### Soil physical quality indicators

Eight years of cover cropping in a soybean monoculture caused no significant effect on SPQ. Bulk density and TP measures performed in the top 0.05 m of soil were not affected by treatment nor position. This agrees with Álvarez et al. (2017), who made a revision of 67 studies in the Pampas region and found that cover crop impact on BD was minimal (with decreases or increases not greater than 0.1 Mg $m^{-3}$). Basche and DeLonge (2017), in a meta-analysis study, found an overall increase in TP of 8 % with cover crops. However, these authors found no significant effects on TP under the following situations: silt content >30 %, clay content >25 % and time since cover crop inclusion >7 years (conditions similar to this study). Values of TP and BD reported here are similar to those reported by Sasal et al. (2006) and by Villarreal (2018) for compacted soils with platy surface structure under NT in the same study site. In the SBF treatment, platy structure was observed within the first 0.10 m of soil (beginning at 0.04-0.06 m depth, and 0.02-0.03 m wide). This platy structure strongly limited soybean roots, causing the root system to develop only in the top 0.10 m of soil. In the SCC treatment, this structure was weaker and allowed root penetration. This difference between managements has also been described by Behrends Kraemer and Morrás (2018) for another Argiudoll in the Pampas region. However, no differences in SPQ values between treatments indicate that the effect of cover crops on the platy structure was not enough to improve SPQ indicators. It has been reported that platy structure causes a change in pore orientation and not necessarily in pore volume, which would explain the absence of significant differences in TP and BD (Lozano et al., 2013). Moreover, Soracco et al. (2015) found that static determinations such as BD or TP are not good SPQ indicators, since they cannot account for the connectivity of different pore size classes. Thus, relatively high BD and low TP measured in both treatments can be ascribed to compaction processes in soils that had been 30 years under NT (Sasal et al., 2017), and under soybean monoculture.

Saturated hydraulic conductivity was not affected by cover crops after eight years. This disagrees with most studies that report that K increases after winter cover cropping both in the long and in the short term (Keisling et al., 1994; Blanco-Canqui et al., 2011, 2015; Álvarez et al., 2017; Haruna et al., 2018). On a meta-analysis over a wide range of soils, Basche and DeLonge (2019) found cover crops generally increased infiltration rates, thought this effect was greater in coarser soils and in drier climatic conditions than those of this study. Other studies report that an intense cover crop growth can reduce K in the winter, as roots clog pores, while later root decay can increase K and porosity (Carof et al., 2007; Bodner et al., 2008). In the present study measures were made two months after cover crop was dried, when cover crop roots were still abundant. However, temporal pore clogging does not explain the absence of eight years cumulative effect on $K_0$. This reflects a poor pore system with low connectivity in a soil compacted by years of NT and soybean monoculture (both in the SBF and in the SCC treatments). Reduced $K_0$ can be a consequence of pore destruction by compaction or to the lack of pore generation (Sasal et al., 2006). Furthermore, Lozano (2014) suggested that infiltration was strongly limited because of platy structure's low permeability. Though soil in the SCC treatment showed more favorable visual structural features, these observed differences did not affect significantly SPQ indicators as $K_0$ and effective porosity. This can be attributed to the low ability of silty loam Argiudolls for natural pore regeneration under NT (Sasal et al., 2006). This is due to the abundance of illite clay with low expansive capacity (Sasal et al., 2006), the prevalence of fine silts within the silt fraction (Taboada et al., 1998) and the mild temperate climate, where freeze–thaw processes are negligible (Sasal et al., 2006). No effect on $K_0$ indicates 8 years of cover crops did not improve soil structural features (Sasal et al., 2006; Blanco-Canqui et al., 2015). In this study, cover crop had no significant effect on TP, $\varepsilon_{ma}$, $\varepsilon_{me}$ nor $CW_{TP}$.

Also, it has been stated that SPQ is closely related to soil organic matter (Sasal et al., 2006). This suggests that changes in SOC composition induced by the cover crop were not enough to cause a significant effect on SPQ. In this study, cover crops had no effect on SOC content, and did not affect FA and POCf fractions, which have been reported to be the fractions with higher correlation with SPQ indicators (Recio-Vázquez et al., 2014; Duval et al., 2018). However, rather than discourage the use of cover crops, these results encourage to try more intensified cropping sequences, with more gramineous crops in the sequence, to enhance SOC accumulation and structure improvement.

## CONCLUSIONS

Winter cover crops in soybean crops under no-tillage in a typical Argiudoll of the Argentinean Pampas, change the content and the relative contribution of different fractions of soil organic carbon. An oat+vetch cover crop did not increase soil organic carbon content in a soybean crop, which indicates that a more balanced rotation between leguminous and gramineous crops may be necessary. The cover crop did increase newer fractions of soil organic carbon. Soil organic carbon contents and soil organic carbon composition were not different when considering the row and the inter-row of the previous crop.

The determination of soil organic carbon fractions is more sensitive to evaluate changes induced by a cover crop in soil organic carbon. More sensitive fractions to treatment were humic acids and coarse particulate organic carbon, which represent intermediate and newer fractions of soil organic carbon, respectively.

Eight years of winter cover crop in a soybean crop under no-tillage did not improve soil physical quality. This was related to initial soil compaction after years of soybean monoculture under no-tillage and to the low ability of silty loam Argiudolls to recover soil physical quality. This also indicates that the possible enhancement of root activity and the increase in some of the fractions of soil organic carbon was not enough to cause changes in soil physical quality, at least for the studied time period.

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