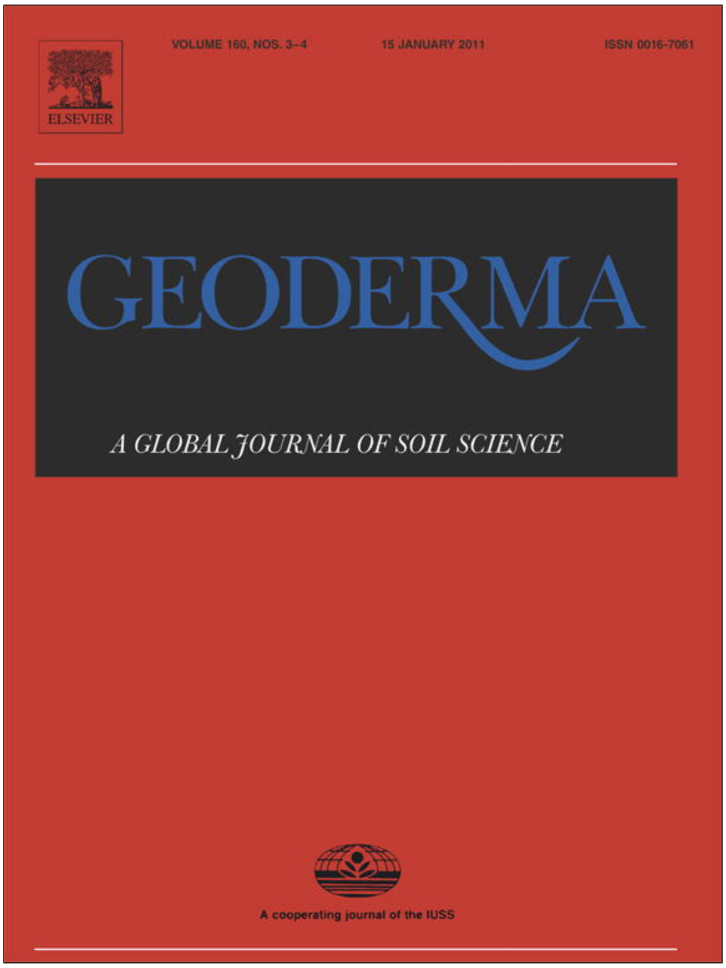

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Impact of soybean cropping frequency on soil carbon storage in Mollisols and Vertisols

L.E. Novelli [a,b,*], O.P. Caviglia [a,b,c], R.J.M. Melchiori [a]

[a] INTA EEA Paraná, Ruta 11, Km 12.5 (3100), Paraná, Argentina
[b] CONICET, Argentina
[c] Facultad de Ciencias Agropecuarias, Universidad Nacional de Entre Ríos, Ruta 11, Km 10 (3100), Paraná, Argentina

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A B S T R A C T

The high cropping frequency of soybean (*Glycine max* [L.] Merr.), mainly as a single annual crop, in the extensive agricultural systems of South America may adversely affect the soil organic carbon (SOC) storage, which may be different between soils depending on aggregation agents. The aim of this work was to evaluate the impact of the soybean cropping frequency on the SOC storage in different soil aggregate size classes in a Mollisol and in a Vertisol in the Northeastern Pampas of Argentina under no-tillage management. In each soil, the samples were collected at 0–5, 5–15 and 15–30 cm depths in eleven cropped and one uncropped fields. The number of months occupied with soybean in relation to the total number of months occupied with crops within crop sequences, over a 6-year period, was used to calculate the soybean cropping frequency. The SOC stocks in equivalent soil mass, the SOC concentration both in the whole sample and in different aggregate size classes, and the stratification ratio of the SOC stock and of the SOC concentration were determined. The increase in soybean cropping frequency reduced the SOC stock in both soils at 0–5 cm, and in the Vertisol at 5–15 and 0–30 cm but the change was evident only between the cropped and the uncropped situation. A decrease in soybean cropping frequency resulted in a higher amount of macroaggregates (>250 μm), a higher SOC concentration and a higher stratification ratio in the Mollisol at 0–5 cm, whereas in the Vertisol the soybean cropping frequency did not affect the stratification ratio or the aggregate distribution in any size class. The increase in soybean cropping frequency reduced SOC storage only in macroaggregates (>250 μm) in both soils at 0–5 cm, particularly in the largest macroaggregates (>2000 μm), and more in the Mollisol than in the Vertisol. Our results show that a high soybean cropping frequency may severely affect the SOC storage in the Mollisol, and suggest that in the Vertisol this effect may lead to a reduction in the SOC storage in the long term.

1. Introduction

The cropped area of South America, currently represents ca. 43% of the worldwide area sown with soybean (*Glycine max* [L.] Merr.) (FAOSTAT, 2011). However, the relation between the area sown with soybean and that sown with other summer crops such as corn (*Zea mays* L.) is ca. 6 in Argentina and Uruguay and ca.1.7 in Brazil (2005–2009) (FAOSTAT, 2011). Thus, in some countries, the extensive cropping systems are predominantly dominated by soybean, mainly as a single annual crop (Caviglia and Andrade, 2010). This scenario has been encouraged by the introduction of glyphosate-resistant genotypes and no-tillage, which have allowed the reduction of production costs, as well as by the favorable international price, in comparison to cereals (Satorre, 2005). Also, the high plasticity of soybean in different environments allows it to be cultivated at a wide range of latitudes, leading to a progressive cultivation toward more environmentally fragile areas that were traditionally occupied by livestock or native forests (Baldi and Paruelo, 2008; Paruelo et al., 2006).

However, the high soybean cropping frequency in the agricultural systems, mainly as a single annual crop, leads to an important waste of key resources (i.e. water and solar radiation) of the potential environmental productivity during the fallow period, thus dramatically reducing the efficiency and productivity of the system (Caviglia et al., 2004). Furthermore, soybean crops provide a limited amount of crop residues with a low carbon:nitrogen (C:N) ratio (Wrigth and Hons, 2004). This promotes rapid stubble degradation and exposes the soil to a greater erosion impact during the fallow period. There is evidence indicating that systems with a high proportion of soybean in crop sequences, which are associated with a low residue input and quality as compared with more balanced cropping systems, may affect soil organic carbon (SOC) storage and reduce macroaggregation

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* Corresponding author at: INTA EEA Paraná, Ruta 11, Km 12.5 (3100), Paraná, Argentina. Tel.: +54 343 4975200 237; fax: +54 343 4975200 275.
E-mail address: lnovelli@parana.inta.gov.ar (L.E. Novelli).

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(Franzluebbers et al., 1998; Studdert and Echeverría, 2000; Wrigth and Hons, 2004, 2005).

Several studies have reported that management practices, such as no-tillage, crop rotation and the intensification of crop sequences by the use of double crop or cover crop, increase SOC sequestration (Bronick and Lal, 2005b; Havlin et al., 1990; López-Fando and Pardo, 2011; Peterson et al., 1998; Villamil et al., 2006) and improve soil aggregation (Álvaro-Fuentes et al., 2009; Mikha and Rice, 2004; Wrigth and Hons, 2004). Soil aggregation is a mechanism that increases SOC storage (Six et al., 2004; Tisdall and Oades, 1982), due to the physical protection within aggregates (Balesdent et al., 2000; Beare et al., 1994). Nonetheless, the protection of SOC can change in soils with different agents that can stabilize the aggregates (Bronick and Lal, 2005a, 2005b; Denef and Six, 2005; Fabrizzi et al., 2009).

In Mollisols, SOC is considered one of the main agents that stabilizes soil aggregates (Fabrizzi et al., 2009), whereas in Oxisols, iron and aluminum oxides are the agents responsible for most of the stability of soil aggregates (Dalal and Bridge, 1996; Fabrizzi et al., 2009; Oades, 1993), and in Vertisols, aggregate stability may be attributed mostly to the high clay content, mainly smectite, which protects the SOC (Stephan et al., 1983). This suggests that SOC storage under a high soybean cropping frequency will be quite different depending on the soil type, since soils differ in textural, mineralogical and organic state.

While there are numerous studies that have evaluated the effect of tillage and fertilization practices on SOC storage and aggregate distribution (Fabrizzi et al., 2009; López-Fando and Pardo, 2011; Mikha and Rice, 2004; Wrigth and Hons, 2004, 2005), there is little evidence of the effect of an increase in the soybean cropping frequency on these variables in contrasting soils. As compared with Mollisols, Vertisols have received less attention and several questions have arisen about the impact of the soybean cropping frequency on SOC storage and aggregation. The knowledge of the processes involved in SOC storage has an importance that exceeds the farm level because it is a viable option that may help to mitigate the global warming potential by atmospheric CO_2 removal (Lal, 2004, 2010).

The enrichment of the SOC produced by no-tillage at surface level results in a stratification of the SOC (Franzluebbers, 2002). This has an important role because the surface is an interface that receives the greatest impact of the agricultural practices and rainfall, both of which drive the erosion process (Franzluebbers, 2010). Because the stratification of SOC can be used as an indicator of soil quality (Franzluebbers, 2002), the change in the stratification ratio by an increased soybean cropping frequency may indicate a trend to either the deterioration or the improvement of soils.

The study of the impact of the soybean cropping frequency on SOC storage and aggregation through a comparative study of contrasting soils can provide valuable information to achieve eco-efficient systems (Lal, 2010). The aim of this work was to evaluate the impact of the soybean cropping frequency on SOC storage in different soil aggregate size classes of a Mollisol and a Vertisol in the Northeastern Pampas of Argentina.

We hypothesized that: a) the soybean cropping frequency reduces SOC storage in the macroaggregates more markedly in the Mollisol than in the Vertisol and, b) the reduction in SOC storage in the Mollisol is more related to its lower structural stability than to its lower carbon concentration.

2. Materials and methods

2.1. Study site

The study was conducted in two sites with different soil types in Entre Ríos province in the Northeastern Pampas of Argentina. The region has a humid (annual rainfall ≈ 1000 mm) and temperate climate (annual temperature ≈ 18.3 °C). The Vertisol was located close to Las Tunas (31°51.5′ S, 59°45.05′ W). This soil was classified as a fine, smectitic, thermic Typic Hapluderts (Soil Survey Staff, 2010) (Table 1). The Mollisol was located close to the Experimental Station of INTA Paraná (31°50.9′ S; 60°32.3′ W). This soil was classified as a fine, mixed, thermic Aquic Argiudoll (Soil Survey Staff, 2010) (Table 1).

2.2. Field selection

Eleven fields under no-tillage with different soybean cropping frequencies within crop sequences and one uncropped situation were selected in each soil type (Table 2).

The production fields were set taking into account that they i) belong to the same series and erosion phase, ii) have been under a similar crop management and productivity, iii) have been under no-tillage for at least the last ten years, and iv) include a wide and similar range of soybean cropping frequencies. For the uncropped situation in each soil, we selected a site with native grassland (pristine situation) close to the production fields.

Information of the crop sequences was gathered from the farm record from a 6-year period, previous to the time of soil sampling.

2.3. Soil sampling and analysis

Soil samples were collected between March and October 2008 after the summer crop harvest and before planting the next summer crop. To minimize the spatial variability in the properties under evaluation, the sampling sites were located in a similar slope position, avoiding sampling areas with obvious erosion or deposition of the soil and every ca. 50 m through a linear transect. Soil samples (three replications) were taken from each field at 0–5, 5–15 and 15–30 cm depth using a shovel. In each replication, the soil sample consisted of at least ten sub-samples. Bulk density at each field was determined by the core method (Forsythe, 1975) at 0–5, 5–15 and 15–30 cm depth near the sampling place.

Soil samples were passed through a 10-mm sieve, roots removed, air-dried and stored at room temperature until analyzed. An aliquot of each sample was sieved through 0.5 mm and used to determine C and N by dry combustion using a LECO autoanalyzer model TRU SPEC (Leco Corp., St. Joseph, MI, USA).

Water-stable aggregates were separated using the wet-sieve method described by Wrigth and Hons (2004) with modifications. Briefly, 100 g soil samples were capillary-wetted to field capacity for 10 min to minimize slaking following immersion. The wetted soil was immersed in water on a nest of sieves (2000 μm, 250 μm and

Table 1
Description of textural characteristics of a Mollisol and a Vertisol (Plan Mapa de Suelos, 1998).

Order	Family	Horizon	Depth (cm)	% Sand	% Silt	% Clay	Texture Class
Mollisol	Aquic Argiudoll[a]	Ap	3–15	4.5	67.9	27.6	Silty clay loam
		B21t	21–33	3.9	54.6	41.5	Silty clay
Vertisol	Typic Hapludert[a]	A1	0–8	4.1	60.9	35	Silty clay loam
		B21t	24–38	4.5	53.5	42	Silty clay

[a] USDA classification (Soil Survey Staff, 2010).

Table 2
Crop sequence for 6 years preceding the soil sample for eleven cropped and one uncropped fields, in pristine situation, in a Mollisol and a Vertisol from the Northeastern Pampas of Argentina.

Year	Mollisol												Vertisol											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
2002–2003	Uncropped	W Sb	P C	W Sb	W Sb	C	Sb	C	Sb	W Sb	C	Sb	Uncropped	P	P	W Sb	W Sb	C	Sb	P Sb	Sb	W Sb	Sb	W Sb
2003–2004		W	Sb	C	C	Sb	C	Sb	C	Sb	Sb	Sb		P	P	C	Sb	Sb	W Sb	W Sb	W Sg	W Sb	W Sb	Sb
2004–2005		P	W Sb	W Sb	Sb	W Sb	W Sb	W Sb	W Sb	W Sb	Sb	W Sb		P	P	WSC	W Sb	W Sb	C	Sb	Sb	Sb	C	W Sb
2005–2006		P	C	C	W Sb	C	C	C	W Sb	C	W Sb	Sb		P	P	Sb	W Sb	W Sb	Sb	W Sb	Sb	W Sb	Sb	Sb
2006–2007		P	C	W Sb	C	W Sb	W Sb	Sb	Sb	Sb	C	W		P	P Sb	W Sb	C	C	W Sb	C	W Sb	C	W Sb	C
2007–2008		P Sb	W Sb	C	C	C	C	W Sb	C	W Sb	Sb	F Sb		W Sb	W Sb	C	W Sb	W Sb	C	W Sb	Sg	Sb	Sb	Sb

C: corn; F: flax; WSC: white sweet clover; P: pasture; Sb: soybean; Sg: sorghum; W: wheat.

53 μm) and shaken vertically 6 cm 60 times for a 2-min period. This time was selected to ensure a minimum amount of largest macroaggregates according to preliminary tests. We obtained four aggregate sizes: largest macroaggregates (>2000 μm), small macroaggregates (250–2000 μm), microaggregates (53–250 μm) and the fraction associated with minerals (<53 μm). This last fraction was obtained by the difference between whole soil and the sum of the three aggregate size fractions (>2000 μm + 250–2000 μm + 53–250 μm).

The soil aggregates retained on the sieves were backwashed with distilled water, transferred to containers, oven-dried at 60 °C for 3 days, weighed, ground and sieved through 0.5 mm and total C content determined by dry combustion using a LECO autoanalyzer. The SOC in the aggregates was not corrected for their contents of sand, because the sand contents were lower and quite similar between soils and depths (Table 1). Based on several previous works, we assumed that sand can be completely embedded into larger aggregates and clay can coat sand grains, and thus considered sand as part of the aggregates (Chung et al., 2009; Sainju et al., 2009; Wright and Inglett, 2009; Wrigth and Hons, 2005).

2.4. Calculations

2.4.1. Soybean cropping frequency

The soybean cropping frequency was calculated based on the number of months occupied with soybean in relation to the total number of months occupied with crops in the last 6 years. For the calculation of this index, we took into account an annual average occupancy of 6 months for corn and wheat (*Triticum aestivum* L.) and of 5 months for soybean. In some cases, other crops were present within crop sequences: for white sweet clover (*Melilotus albus* Medik) we took into account an annual average occupancy of 8 months, for sorghum (*Sorghum bicolor* L. Moench) one of 5 months, and for flax (*Linum usitatissimum* L.) one of 5.5 months. Table 3 shows an example for site 5.

2.4.2. Soil organic carbon (SOC) in equivalent soil mass

To quantify the SOC stocks at each depth and at 0–30 cm, the values were corrected to the equivalent soil mass (Lee et al., 2009), using the uncropped situation in each soil type as the baseline systems. For that, the following equations were used:

$$C_{\mathrm{equiv}(0-5\ \mathrm{cm})} = \left(M_{i(0-5\ \mathrm{cm})} - M_{i,\mathrm{add}(0-5\ \mathrm{cm})}\right) * \%\, C_{(0-5\ \mathrm{cm})} \quad (1)$$

$$C_{\mathrm{equiv}(5-15\ \mathrm{cm})} = \left(M_{i,\mathrm{add}(0-5\mathrm{cm})} * \%\, C_{(0-5\ \mathrm{cm})}\right) + \left(\left(M_{i(5-15\ \mathrm{cm})} - M_{i,\mathrm{add}(5-15\ \mathrm{cm})}\right) * \%\, C_{(5-15\ \mathrm{cm})}\right) \quad (2)$$

$$C_{\mathrm{equiv}(15-30\ \mathrm{cm})} = \left(M_{i,\mathrm{add}(5-15\ \mathrm{cm})} * \%\, C_{(5-15\ \mathrm{cm})}\right) + \left(\left(M_{i(15-30\ \mathrm{cm})} - M_{i,\mathrm{add}(0-30\ \mathrm{cm})}\right) * \%\, C_{(15-30\ \mathrm{cm})}\right) \quad (3)$$

$$C_{\mathrm{equiv}(0-30\ \mathrm{cm})} = (1) + (2) + (3) \quad (4)$$

where C_{equiv} is the equivalent C mass (Mg ha^{-1}), M_i is the dry soil mass (Mg ha^{-1}) for each layer obtained by the product between the thickness of the soil layer (m), the bulk density (Mg m^{-3}) and a factor conversion 10^4 (m^2 ha^{-1}), and $M_{i,\mathrm{add}}$ is the difference between M_i and mass in the baseline system.

The SOC stocks were recalculated in equivalent soil mass because the uncropped situation (baseline systems) had a lower bulk density than the cropped ones.

In fact, the soil bulk density for the uncropped situations was 1.05 Mg m^{-3} (0–5 cm) and 1.25 Mg m^{-3} (5–15 cm) for the Mollisol and 1.09 Mg m^{-3} (0–5 cm) and 1.06 Mg m^{-3} (5–15 cm) for the Vertisol, whereas that for the cropped situation was, on average, 1.14 Mg m^{-3} (0–5 cm) and 1.37 Mg m^{-3} (5–15 cm) for the Mollisol and 1.17 Mg m^{-3} (0–5 cm) and 1.28 Mg m^{-3} (5–15 cm) for the Vertisol.

Table 3
Example of calculation for the soybean cropping frequency for site 5 in a Mollisol from the Northeastern Pampas of Argentina.

Year	2002–2003	2003–2004	2004–2005	2005–2006	2006–2007	2007–2008
Crop sequence	W/Sb	C	Sb	W/Sb	C	C
Month of occupation	11	6	5	11	6	6

Soybean cropping frequency $= \frac{5_{(2002-2003)} + 5_{(2004-2005)} + 5_{(2005-2006)}}{11+6+5+11+6+6} = \frac{15}{45} = 0.33.$

2.4.3. Soil organic carbon (SOC) stratification ratio

The stratification ratio of the SOC concentration was calculated as the ratio between the C concentration at 0–5 cm and the C concentration at 5–15 cm (Franzluebbers, 2002). Likewise, the stratification ratio of the SOC stock was calculated as the ratio between $C_{equiv(0–5\ cm)}$ and $C_{equiv(5–15\ cm)}$ based on stock in equivalent soil mass from the uncropped situation in each soil type as the baseline systems.

2.4.4. Soil organic carbon (SOC) storage in aggregate size fractions

The storage of SOC in each aggregate fraction was obtained as the product of the C in the aggregate (%) and the mass of each aggregate size fraction (g).

2.5. Statistical analysis

Changes in SOC storage by effect of the soybean cropping frequency were analyzed using linear regression. We fitted the data using SAS PROC REG (SAS Institute, 2003) and SAS PROC NLIN (SAS Institute, 2003) for the linear function and the plateau-linear functions, respectively. We chose the models that had the smallest residuals, exhibited a random pattern and were normally distributed.

We performed a t-test to detect differences between uncropped and cropped situations in the Vertisol.

3. Results

3.1. Soil organic carbon (SOC) stock

The SOC stock ($C_{equiv\ 0–30\ cm}$) was not affected by the increase in soybean cropping frequency in the Mollisol, whereas in the Vertisol, changes in $C_{equiv\ 0–30\ cm}$ were evident only between the cropped and uncropped situations (Fig. 1). By pooling all the data, we found that the SOC stock ($C_{equiv\ 0–30\ cm}$) was 66 Mg C ha^{-1} in the Mollisol and 76 Mg C ha^{-1} in the Vertisol. The highest value of SOC stock ($C_{equiv\ 0–30\ cm}$) was found in the uncropped situation in the Vertisol (126 Mg C ha^{-1}) (Fig. 1), which differed significantly from the cropped situations ($P<0.0001$).

The soybean cropping frequency decreased the SOC stock ($C_{equiv\ 0–5\ cm}$) in both soils ($P<0.05$) (Fig. 2a), but a significant change ($P<0.0001$) in SOC stock ($C_{equiv\ 5–15\ cm}$) between the cropped and uncropped situations was found only in the Vertisol (Fig. 2b).

3.2. Soil organic carbon (SOC) concentration

The SOC concentration decreased as soybean cropping frequency increased at 0–5 cm in the Mollisol ($P<0.03$) (Fig. 3a) and showed

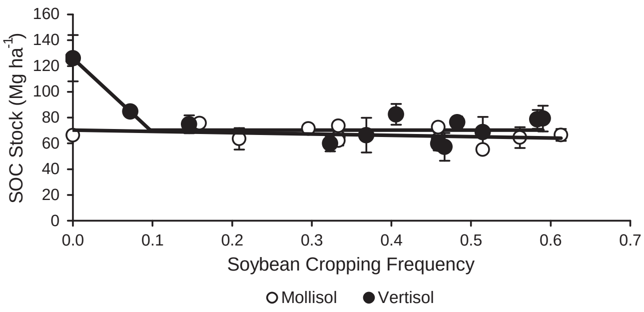

Fig. 1. Soil organic carbon (SOC) stocks ($C_{equiv\ 0–30\ cm}$) in equivalent soil mass as a function of soybean cropping frequency (SCF). C_{equiv} was calculated using uncropped situations in each soil type as the baseline systems. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: linear model ($y=-10.13\ SCF+70.23$), $R^2=0.10$, $P=NS$. Vertisol: plateau-linear model ($y=126.1–572.7\ SCF\ (SCF<0.1)+70.3\ (SCF>0.1)$), $R^2=0.95$, $P<0.001$.

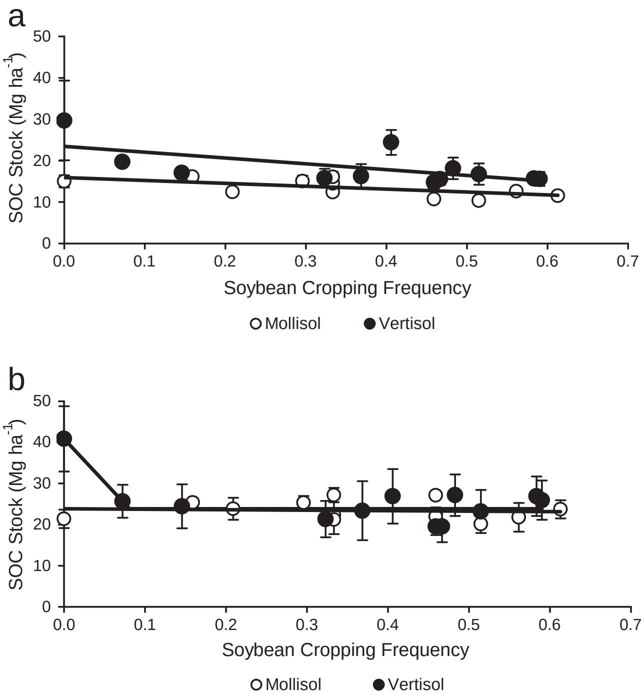

Fig. 2. Soil organic carbon (SOC) stock as affected by soybean cropping frequency (SCF) (a): $C_{equiv\ 0–5\ cm}$ and (b): $C_{equiv\ 5–15\ cm}$ using uncropped situations in each soil type as the baseline systems depending on the soybean cropping frequency. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: (a) linear model ($y=-7.04\ SCF+15.94$), $R^2=0.39$, $P<0.05$; (b) linear model ($y=-1.17\ SCF+23.87$), $R^2=0.01$, $P=NS$. Vertisol: (a) linear model ($y=-14.13\ SCF+23.48$), $R^2=0.39$, $P<0.05$; (b) plateau-linear model ($y=40.9–210.6\ SCF\ (SCF<0.08)+23.8\ (SCF>0.08)$), $R^2=0.94$, $P<0.001$.

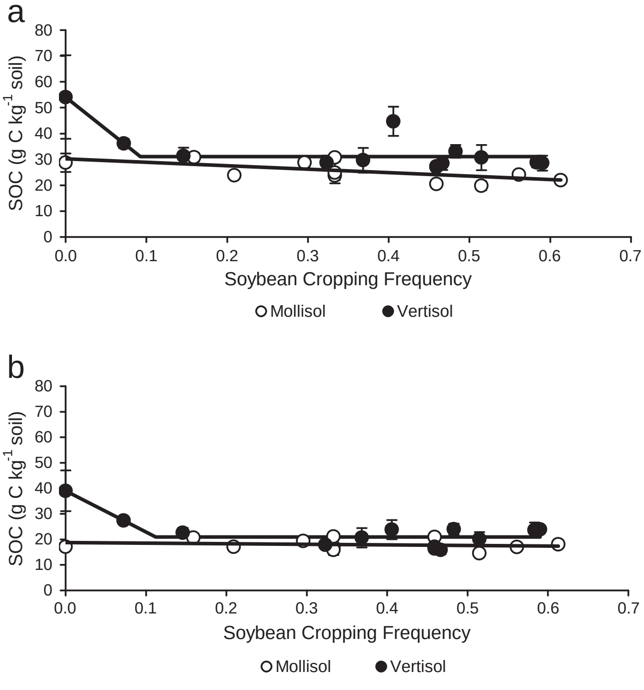

Fig. 3. Soil organic carbon (SOC) concentration as affected by soybean cropping frequency (SCF) (a): at 0–5 cm depth and (b): at 5–15 cm depth. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: (a) linear model ($y=-13.34\ SCF+30.19$), $R^2=0.39$, $P<0.03$ (b) linear model ($y=-2.23\ SCF+18.74$), $R^2=0.03$, $P=NS$. Vertisol: (a) plateau-linear model ($y=54.1–248.5\ SCF\ (SCF<0.09)+31.1\ (SCF>0.09)$), $R^2=0.92$, $P<0.001$; (b) plateau-linear model ($y=39–161.2\ SCF\ (SCF<0.11)+21\ (SCF>0.11)$), $R^2=0.95$, $P<0.001$.

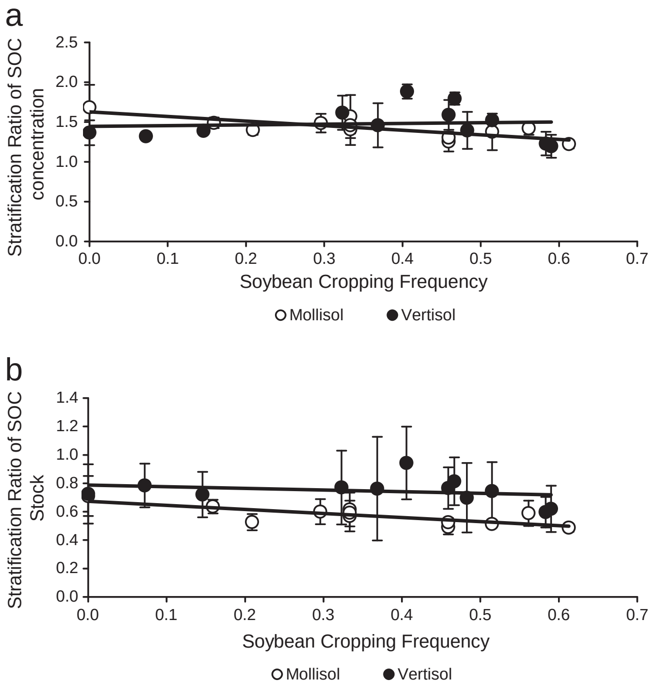

Fig. 4. Stratification ratio as affected by soybean cropping frequency (SCF) (a): SOC stock ($C_{equiv\ 0\text{–}5\ cm}/C_{equiv\ 5\text{–}15\ cm}$) and (b): SOC concentration (0–5 cm/5–15 cm). C_{equiv} was calculated using uncropped situations in each soil type as the baseline systems. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: (a) linear model ($y = -0.58\ SCF + 1.63$), $R^2 = 0.62$, $P < 0.01$, (b) linear model ($y = -0.28\ SCF + 0.67$), $R^2 = 0.59$, $P < 0.01$. Vertisol: (a) linear model ($y = 0.09\ SCF + 1.45$), $R^2 = 0.01$, $P = NS$, (b) linear model ($y = -0.12\ SCF + 0.79$), $R^2 = 0.06$, $P = NS$.

no evident changes at 5–15 cm (Fig. 3b). On the other hand, in the Vertisol there were significant differences ($P < 0.0001$) between cropped vs. uncropped situations at both depths (Fig. 3a and 3b).

The cropping frequency of cereals and pasture in the sequences did not significantly affect the SOC stock or concentration.

3.3. Soil organic carbon (SOC) stratification ratio

The stratification ratio of the SOC concentration in the Mollisol was affected ($P < 0.01$) by the increase in soybean cropping frequency, which ranged from 1.69 for the uncropped situation to 1.22 for the highest soybean cropping frequency (Fig. 4a). In contrast, in the Vertisol, the stratification ratio of the SOC concentration was unaffected by the increase in soybean cropping frequency.

The stratification ratio of the SOC stock was similar to that calculated using the SOC concentration (Fig. 4b). Although changes in the stratification ratio of the SOC stock were not evident in the Vertisol as soybean cropping frequency increased, the values of stratification were consistently higher than in the Mollisol.

3.4. Soil organic carbon (SOC) storage in aggregate-size fractions

The soybean cropping frequency decreased the amount of largest macroaggregates (>2000 μm) in the Mollisol at 0–5 cm ($P < 0.0005$), leading to a subsequent increase in the smaller aggregate size classes (250–2000 μm: $P < 0.01$; 53–250 μm: $P < 0.01$) (not shown). Similarly, the increase in soybean cropping frequency in the Mollisol decreased the amount of the largest plus small macroaggregates (>250 μm) and, as a result, increased the amount of aggregates <250 μm ($P < 0.005$) (Table 4). At 5–15 cm in the Mollisol, the soybean cropping frequency decreased only the amount of small macroaggregates ($P < 0.05$) (not shown). In the Vertisol, the soybean cropping frequency did not affect the amount of aggregates of any of the size classes at any of the depths analyzed (Table 4).

The SOC concentration decreased only in small macroaggregates ($P < 0.05$) and microaggregates ($P < 0.005$) at 0–5 cm in the Mollisol (not shown). In the Vertisol, a change in SOC concentration was recorded between the cropped and uncropped situations in all size classes and both depths (not shown).

Table 4
Soybean cropping frequency, aggregate size classes and soil organic carbon (SOC) storage in aggregates >250 μm and <250 μm for eleven cropped and one uncropped field, in pristine situation, in a Mollisol and a Vertisol from the Northeastern Pampas of Argentina.

Soil	Site	Soybean cropping frequency	Aggregate size classes (%)						SOC storage in aggregates (g C kg^{-1} soil)			
			0–5 cm			5–15 cm			0–5 cm		5–15 cm	
			>250 μm	<250 μm	SD[a]	>250 μm	<250 μm	SD	>250 μm	<250 μm	>250 μm	<250 μm
Mollisol	1	0	78.95	21.05	3.22	67.84	32.16	1.51	20.50	8.04	11.86	5.52
	2	0.16	79.20	20.80	2.10	87.01	12.99	3.13	20.14	10.69	16.44	4.26
	3	0.21	79.67	20.33	5.03	73.36	26.64	5.07	16.82	7.01	11.69	5.38
	4	0.30	72.17	27.83	5.74	65.04	34.96	1.69	19.10	9.70	12.75	6.89
	5	0.33	72.41	27.59	1.34	61.69	38.31	1.38	16.46	7.76	9.98	6.13
	6	0.33	58.45	41.55	4.47	64.44	35.56	2.90	12.49	10.24	9.71	5.64
	7	0.33	75.98	24.02	0.65	78.76	21.24	0.09	21.58	9.15	16.15	4.95
	8	0.46	62.26	37.74	5.70	60.25	39.75	4.50	12.13	8.37	9.25	6.67
	9	0.46	69.07	30.93	1.51	68.69	31.31	2.08	17.76	9.54	13.53	7.40
	10	0.51	66.47	33.53	8.31	66.63	33.37	0.82	11.66	8.18	9.50	5.03
	11	0.56	60.38	39.62	1.23	70.43	29.57	6.06	12.40	11.73	11.36	5.68
	12	0.61	62.78	37.22	2.37	60.17	39.83	2.51	11.80	10.20	11.20	8.07
Vertisol	13	0	72.67	27.33	12.14	78.90	21.10	1.23	41.19	12.91	29.02	10.01
	14	0.07	80.03	19.97	3.17	87.17	12.83	1.35	28.97	7.20	23.22	4.18
	15	0.15	80.86	19.14	1.87	68.39	31.61	1.96	26.84	4.52	14.26	8.31
	16	0.32	76.21	23.79	6.48	76.39	23.61	7.40	22.68	6.06	14.18	3.69
	17	0.37	74.96	25.04	5.18	85.47	14.53	3.45	21.68	8.02	18.32	2.31
	18	0.41	71.83	28.17	4.04	77.14	22.86	9.40	25.49	19.23	16.67	7.17
	19	0.46	78.23	21.77	7.64	71.08	28.92	5.47	18.74	8.26	12.64	4.46
	20	0.47	73.28	26.72	4.48	60.37	39.63	6.25	21.11	7.36	9.60	6.30
	21	0.48	78.74	21.26	8.29	81.48	18.52	10.35	23.88	9.22	18.93	5.04
	22	0.51	69.38	30.62	7.85	75.84	24.16	4.21	20.20	10.50	17.81	2.32
	23	0.58	82.68	17.32	5.56	77.85	22.15	6.45	24.59	4.27	18.22	5.45
	24	0.59	84.71	15.29	3.29	77.51	22.49	5.38	25.08	3.48	18.34	5.56

[a] SD: standard deviation of each mean.

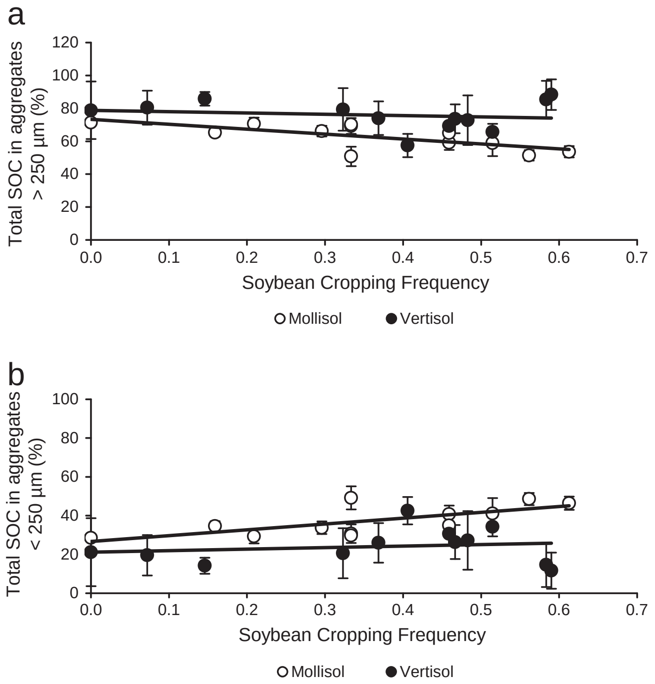

Fig. 5. Proportion of total SOC storage in aggregate size classes as a function of soybean cropping frequency (SCF) at 0–5 cm: (a): in aggregates >250 µm and (b): in aggregates <250 µm. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: (a) linear model ($y=-30.05$ SCF$+73.36$), $R^2=0.49$, $P<0.05$, (b) linear model ($y=30.05$ SCF$+73.36$), $R^2=0.49$, $P<0.05$. Vertisol: (a) linear model ($y=-7.91$ SCF$+78.83$), $R^2=0.03$, $P=$NS, (b) linear model ($y=7.91$ SCF$+21.17$), $R^2=0.03$, $P=$NS.

The proportion of total SOC storage in aggregates >250 µm at 0–5 cm was significantly ($P<0.05$) reduced as soybean cropping frequency increased in the Mollisol, without evident changes in the Vertisol (Fig. 5a). By pooling all the data, we found that the largest proportion of total SOC was stored in the largest plus small macroaggregates (>250 µm), reaching 50–71% and 57–88% in the Mollisol and the Vertisol, respectively. In the Mollisol, the soybean cropping frequency increased ($P<0.01$) the proportion of total SOC storage only in the <250 µm aggregates at 0–5 cm (Fig. 5b) and showed no changes in the Vertisol.

Soil carbon storage in the largest plus small macroaggregates (>250 µm) was related to the SOC storage in the largest macroaggregates (>2000 µm) in both soils at 0–5 cm including all cropped and uncropped situations (Mollisol: $R^2=0.80$, $P<0.0001$; Vertisol: $R^2=0.71$, $P<0.001$). A similar relationship was found at 5–15 cm (both soils, $P<0.0005$) (not shown).

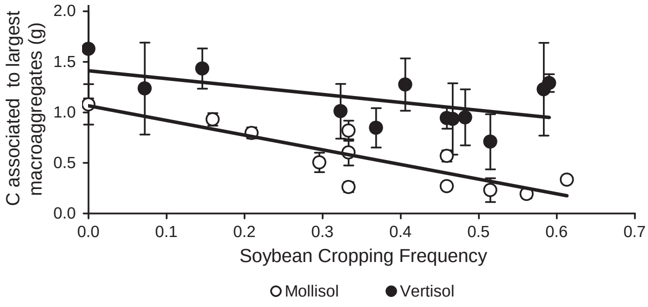

Fig. 6. Soil organic carbon (SOC) storage in largest macroaggregates as affected by soybean cropping frequency (SCF) at 0–5 cm. Open circles represent the Mollisol soil. Solid circles represent the Vertisol soil. Vertical bars represent the standard deviation of each mean. Mollisol: linear model ($y=-1.45$ SCF$+1.07$), $R^2=0.73$, $P<0.001$. Vertisol: linear model ($y=-0.78$ SCF$+1.41$), $R^2=0.33$, $P<0.05$.

The soybean cropping frequency reduced SOC storage only in macroaggregates (>250 µm) in both soils at 0–5 cm ($P<0.01$), more in the largest macroaggregates (>2000 µm) in the Mollisol ($P<0.001$) than in the Vertisol ($P<0.05$) (Fig. 6). At 5–15 cm, SOC storage in the largest plus small macroaggregates (>250 µm) and largest macroaggregates (>2000 µm) was unaffected by the soybean cropping frequency in either of the soils studied (not shown).

The SOC stock ($C_{\text{equiv 0–5 cm}}$) was related to SOC storage in macroaggregates in both soils (Mollisol: $R^2=0.81$, $P<0.001$; Vertisol: $R^2=0.73$, $P<0.001$) and to SOC storage in microaggregates only in the Vertisol ($R^2=0.51$, $P<0.05$) (not shown).

4. Discussion

A reduction in SOC stock in equivalent soil mass at 0–30 cm of the baseline systems (C_{equiv} 0–30 cm) was not evident between the cropped situations in either of the soils studied by the increase in the soybean cropping frequency, evidencing differences only between the cropped and uncropped situations of the Vertisol (Fig. 1). However, as the soybean cropping frequency increased, there was a decrease in SOC stock (C_{equiv} 0–5 cm) in both soils (Fig. 2a) and in SOC concentration on the surface (0–5 cm) for the cropped situations of the Mollisol (Fig. 3a). Accordingly, it has been widely shown that the changes in SOC stock occur mainly on the soil surface, where the soil receives the greatest impact both of agricultural practices and of rainfall (Franzluebbers, 2010), and that these changes become negligible when a higher depth of the soil profile is considered (Bowman et al., 1999; Franzluebbers, 2010).

Our findings support previous evidences on the negative impact of the frequent inclusion of soybean on SOC stock (Nicoleso et al., 2008) and SOC concentration (Dou et al., 2007; Studdert and Echeverría, 2000; Wrigth and Hons, 2004, 2005), and suggest that the use of no-tillage would be effective to maintain the SOC stocks in acceptable levels only if more balanced crop sequences are used.

The increase in soybean cropping frequency had an impact on the stratification ratio for the SOC concentration ($P<0.01$) and the SOC stock in the Mollisol, but showed no changes in the Vertisol (Fig. 4a, b). In addition, situations with low soybean cropping frequency increased the proportion of macroaggregates (>250 µm) in the Mollisol, but not in the Vertisol (Table 4).

In agreement with our results for the Mollisol, it has been previously reported that the largest macroaggregates from continuous soybean at 0–5 cm were importantly reduced as compared with crop sequences that included wheat/soybean double crop and wheat/soybean–sorghum in an experiment combining rotation and tillage systems (Wrigth and Hons, 2004).

The Vertisol not only had a higher content of clay from the surface (Table 1) than the Mollisol, but also differed importantly in clay mineralogy, which may confer a higher structural stability. The self-mixing of the shrink-swell smectitic clays, a property inherent in Vertisols, may minimize the expected stratification under no-tillage (Fabrizzi et al., 2009) and facilitate the restoration of the soil structure (Pillai and McGarry, 1999). This feature could explain the apparent higher resistance to the changes driven by the soybean cropping frequency in crop sequences (Fig. 4a, b and Table 4).

Aggregation is the key process to enhance SOC storage, because it protects the SOC within aggregates and reduces the access of degrading microorganisms (Beare et al., 1994). The composition of cropping sequences may affect the aggregation through the amount, quality and frequency of the crop residues returned to the soil (Wrigth and Hons, 2005). Our results highlight the role of the soybean cropping frequency in SOC storage only in macroaggregates (>250 µm) in both soils at 0–5 cm ($P<0.01$), more in the largest macroaggregates (>2000 µm) in the Mollisol ($P<0.001$) than in the Vertisol ($P<0.05$)

(Fig. 6). In the Mollisol, this reduction in SOC storage in the largest macroaggregates as soybean cropping frequency increased (Fig. 6) was driven mainly by the reduction in the amount of largest macroaggregates, since no significant changes were recorded in SOC concentration for that aggregate size class (not shown).

The significant reduction in SOC storage was observed in the largest plus small macroaggregates (>250 μm) in the Vertisol, despite the lower impact of the soybean cropping frequency on soil aggregation, suggesting that a high soybean cropping frequency in these soils may lead to a reduction in SOC storage in the long term.

5. Conclusions

Our results show that, in the Mollisol, the high soybean cropping frequency may have an important impact on soil aggregate distribution and, as a consequence, on SOC storage, reinforcing the concept that SOC plays an important role in the aggregation of these soils.

In the Vertisol, the increase in the soybean cropping frequency did not affect soil aggregation, although evident differences were observed between the cropped and uncropped situations. Despite the small impact of the soybean cropping frequency on soil aggregation and SOC stock in the Vertisol, a significant reduction in SOC storage in the largest plus small macroaggregates (>250 μm) was recorded, suggesting that the high soybean frequency in crop sequences in these soils may lead to a reduction in SOC storage in the long term.

Since the preservation of soil quality is fundamental to achieving eco-efficient systems that may satisfy the growing human needs, it is necessary to reduce the environmental impact and develop strategies that allow achieving the sustainability of systems. The composition of crop sequences is therefore a key issue to improve the SOC sequestration rates for the mitigation of high atmospheric CO_2 levels.

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