

Continuous Wheat in Semiarid Regions Long-term Effects on Stock and Quality of Soil Organic Carbon

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Abstract: Continuous wheat (*Triticum aestivum* L.) cropping in semiarid regions results in variable dry matter production. As a consequence, the balance of soil organic carbon (SOC) may vary across time. The aim of this research was to assess the dynamics and long-term changes of physically and chemically extracted SOC fractions. Soil samples (0- to 5-, 5- to 10-, and 10- to 20-cm depths) from continuous wheat with (f) and without (nf) fertilizer (N + P) under conventional tillage ((CT) for 25 years) and no-tillage ((NT) for 6 years) were taken during the experiment. Mineral-associated ((MOC) 0–0.053 mm), fine particulate ((POC_f) 0.053–0.100 mm), and coarse particulate ((POC_c) 0.1–2.0 mm) SOC and humic substances were obtained. The SOC variability depended on water availability during fallow periods (SOC decomposition) or crop cycles (dry matter production). The mean wheat yields were 1.33 (nf) and 2.09 (f) Mg grain ha⁻¹, with an estimated carbon input of 1.64 (nf) and 2.20 (f) Mg C ha⁻¹ year⁻¹. Losses from the initial level were higher in labile fractions, POC_c (–75%) and POC_f (–53%), than in MOC (–15%). Humic acids present slight differences in their structure and quantity as a result of long-term cropping. Conversion from CT to NT resulted in contrasting results. For an equivalent soil mass, fertilizer application increased SOC by 4.31 Mg ha⁻¹ (under CT) and 7.29 Mg ha⁻¹ (under NT). The SOC turned out to be higher under NT with fertilizer use and lower without application. No-tillage does not increase SOC content by itself; it must be combined with other agricultural practices such as fertilization and/or crop rotation.

Key Words: Fertilization, tillage system, physical organic carbon fractions, humic substances

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In semiarid regions, low and erratic rainfall coupled with high evapotranspiration rates leads to a low crop biomass production and thus to a limited residue input into the soil. These areas typically have a low soil organic matter (SOM) content, poor water and nutrient retention, and, consequently, low natural soil fertility (Lal, 2006). Soil organic matter content has an important effect

on physical, chemical, and biological soil properties, thereby influencing soil quality (Haynes, 2005).

Total SOM includes plant, animal, and microbial residues in all stages of decomposition. Many of these organic compounds in the soil are closely associated with inorganic soil particles. For this reason, the turnover rate of the different SOM compounds varies because of complex interactions between biological, chemical, and physical processes in the soil (Rasmussen and Collins, 1991; Balesdent et al., 2000).

Various land uses result in very rapid declines of SOM (Davidson and Ackerman, 1993). These losses have been largely attributed to reduced inputs of organic matter, increased decomposability of crop residues, and decreased physical protection against decomposition as a result of tillage practices (Jiang et al., 2011).

Cropping effects on soil organic carbon (SOC), the major SOM component, depend on the relationship between soil texture, weather conditions, cropping systems, and tillage characteristics (Franzuebbers, 2002; Galantini et al., 2004; Cookson et al., 2008). Control of SOC levels is therefore necessary to monitor management effects on soil quality under different agroecosystems. However, it is difficult to detect changes in total SOC in the short- and medium-terms because of the stability and spatial variability of humified materials (Blair et al., 1995).

In contrast, labile SOC fractions (i.e., microbial biomass C, dissolved organic C, particulate organic C, and easily oxidizable C) can respond more quickly to soil management than total organic C (Blair et al., 1995; Ghani et al., 2003; Duval et al., 2013). This is why they have been suggested as sensitive indicators of soil quality (Rudrappa et al., 2006; Yang et al., 2005). These fractions are typically more sensitive to changes in soil management practices than total SOC and, consequently, they are well established as early indicators of the change caused by different production systems (Galantini and Rosell, 2006; Huang et al., 2007; Fließbach et al., 2007; Cookson et al., 2008). However, labile fractions can also be changed by seasonal or weather conditions (Galantini and Rosell, 2006).

Although there may be a continuum of SOC compounds in terms of their decomposability and turnover time, physical fractionation techniques are often used to define and delineate various relatively discrete pools. Physically defined fractions, although containing a diverse array of organic compounds, integrate structural and functional properties of SOC (Christensen, 2001). Physical fractionation emphasizes the role of soil minerals and soil structure in SOC turnover and relates more directly to SOC dynamics *in situ* than classical wet chemical fractions (Oades, 1993; Christensen, 2001). Coarse particulate organic materials respond to management to a greater extent than fine ones and may thus be considered a more labile pool of SOC (Benbi et al., 2012; Duval et al., 2013).

On the other hand, humic substances (HS) are more stable organic matter compounds that make up a significant portion of total SOC (Stevenson, 1994; Galantini and Rosell, 2006).

The chemically reactive and refractory nature of humin (Hum), humic acid (HA), and fulvic acid (FA) contributes to their persistence in soils (Kiem and Kogel-Knabner, 2003). Quantity and quality changes can modify their important role in regulating

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nutrient flows through ecological systems and C emissions to the atmosphere (Lal, 2006).

The SOC stock is estimated as the product of organic carbon concentration, soil bulk density, and soil thickness. This calculation method, although widely used, is still insufficient to assess SOC storage (Van den Bygaart and Angers, 2006; Toledo et al., 2013).

Ellert and Bettany (1995) found that, for management-induced changes in SOC storage to be assessed reliably, the masses of soil being compared must be equivalent. The “equivalent soil mass” (ESM) is defined as the reference soil mass per unit area chosen in a layer, and the “equivalent C mass” is the C mass stored in an ESM (Ellert and Bettany, 1995).

The SOC values in semiarid regions might not be the most reliable indicators of soil quality because changes can be slow and have different turnover times. In such a case, the SOC status must be evaluated considering the short- and long-term changes in SOM fractions.

We hypothesized that, under semiarid conditions, highly variable rainfall patterns may sharply modify carbon input into the soil and, as a result, labile SOM fractions cannot be considered as sensitive indicators of soil quality; for this reason, the long-term effects can be found in the content of the SOC intermediate labile fraction and in the characteristics of the humified fraction. The objectives were (i) to evaluate the long-term SOC fraction dynamics in continuous wheat (*Triticum aestivum* L.) under conventional tillage (CT), (ii) to determine its long-term effects on HS quantity and quality, and (iii) to compare the effect of fertilization and tillage systems on SOC stock.

MATERIALS AND METHODS

Site Description

The experimental site is located in the Experimental Station of INTA Bordenave (33–41°S; 61–66°W). This site is representative of the central-southern semiarid Pampean region of Argentina, where the climate is temperate, continental, and semiarid, with a mean annual temperature of 15.2°C. The historical and mean annual precipitation during the experiment was 660 (1928–2010) and 778 (1983–2006) mm, respectively (Fig. 1). Rainfall is higher in the fall and the spring. However, during the grain-filling period of winter cereals, the rainfall rate was lower than the crop water requirements for this area (Fig. 2).

The predominant soils belong to a complex of coarse loamy, mixed, thermic Entic Haplustolls, with an A-AC-C1-C2 horizon sequence and a caliche (calcareous) layer at an average depth of 0.8 m (Table 1) (Gómez et al., 1981).

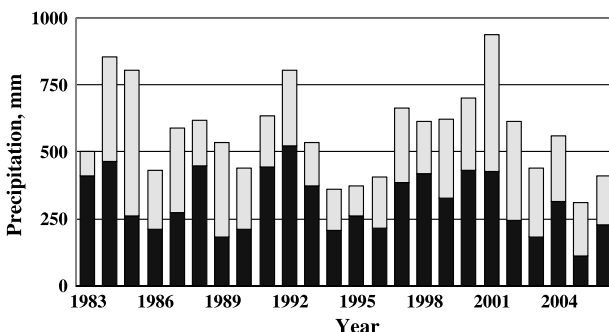


FIG. 1. Annual precipitation during the fallow period (dark) and wheat cycle (light) during the experiment (1983–2006).

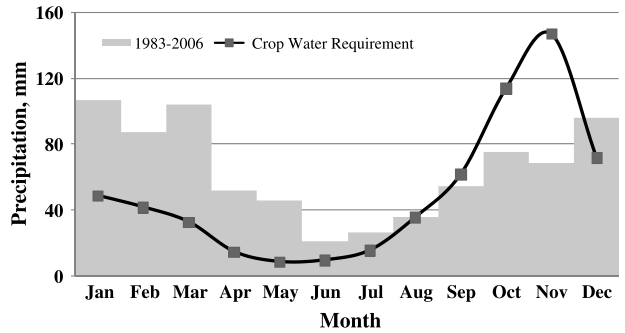


FIG. 2. Mean monthly precipitation (1983–2006) and estimated crop water requirement for wheat at Bordenave Experimental Station.

Experimental Design and Sampling

Continuous wheat (*T. aestivum* L.) (WW) was initiated in 1983 on a 20-year-old pasture (reference soil (REF)) under CT. The following management practices were implemented: harrowing (offset) after harvesting, chisel and disk plows for seed bed preparation, deep-furrow seed drilling, and chemical weed control. Crop residues were not removed.

The production system included nonfertilized (nf) and annually fertilized (f) treatments, with 16 kg P ha⁻¹ in the form of diammonium phosphate and 64 kg N ha⁻¹ as urea applied at seeding time (June).

After 1999, the main plots were split: half of them remained under CT (WW-CT) and the other half under no-tillage (NT) (WW-NT), in both cases with and without fertilizer application. No-tillage management was performed with chemical weed control and direct seeding without any soil disturbance.

A randomized block design was used, with three blocks (30 × 30 m). For each block, three composite samples were collected at a 0- to 20-cm depth during seeding time in 1986, 1991, 1992, 1993, 1994, 1995, 1996, 1997, and 2006. During 2006, soil samples were taken at 0- to 5-, 5- to 10-, and 10- to 20-cm depths.

TABLE 1. Description of Soil Profile

Soil Property	Horizon			
	A1	AC	C1	C2
Depth, cm	4–20	28–34	40–55	70–80
Soil organic carbon, %	0.86	0.37	0.29	0.15
Total nitrogen, %	0.069	0.046	—	—
Clay, %	6.1	11.2	10.6	6.9
<2 µm				
Silt, %				
2–20 µm	9.1	5.2	5.1	3.5
2–50 µm	16.6	10.6	8.7	9.0
Sand, %				
50–74 µm	3.0	3.4	3.1	3.1
74–100 µm	5.1	2.1	3.0	1.7
100–250 µm	57.1	58.0	59.1	67.8
>250 µm	12.1	14.7	15.5	11.5
Extractable phosphorus, mg kg ⁻¹	7.7	4.9	—	—
pH (soil:water, 1:2.5)	7.0	6.8	6.8	8.1
CaCO ₃ , g kg ⁻¹	0	0	0	360

The REF soil was sampled during 1991 in three plots under natural vegetation near each treatment block: at 0- to 5-, 5- to 10-, and 10- to 20-cm depths. During 1994 and 1996, same plots were sampled (0–20 cm). No differences in SOC (0–20 cm) were found across the years; for this reason, soil obtained in 1991 was selected as an older value and was used as the reference.

The soil samples were air-dried, ground, and passed through a 2-mm (10-mesh) sieve. As well as the samples taken every year, undisturbed soil samples were collected using stainless steel cylinders (height, 5 cm; diameter, 5 cm) to obtain bulk density values (4 cylinders \times 3 blocks \times 4 depths in any treatment and year).

Physical, Chemical, and Spectrometric Determinations

Total SOC was determined by dry combustion (LECO Carbon analyzer; LECO Corporation, St. Joseph, MI).

To avoid the effect of soil bulk density on the estimated SOC storage, all values were calculated as SOC content (Mg ha^{-1}) on an equivalent soil mass basis (2,400 Mg ha^{-1}) using the method outlined by Toledo et al. (2013).

Particle size fractionation of SOC was carried out by wet sieving (Cambardella and Elliott, 1992). Briefly, 50 g of air-dried sieved (2-mm) soil and 100 mL of distilled water (ratio of soil to water, 1:2) were put in 120-mL glass containers. Also, 10 glass beads were added to increase fragmentation and reduce potential problems created by different sand contents. The samples were subjected to mechanical dispersion on a rotary shaker for approximately 16 h (overnight) to disintegrate the aggregates. The sieving was done with a pair of 270- and 140-mesh sieves (53 and 105 μm , respectively), which moved back and forth until the water passing through them was reasonably clear. Three fractions were obtained: (i) a coarse fraction (105–2,000 μm) containing coarse particulate organic matter (POM) and fine + medium + coarse sands; (ii) a medium fraction (53–105 μm) with fine POM and very fine sand; (iii) a fine fraction (<53 μm) that included mineral-associated organic matter (MOM) and silt + clay minerals.

The material retained in each sieve was dried, homogenized, and analyzed for carbon so as to obtain the coarse particulate (POC_c), fine particulate (POC_f), and mineral-associated (MOC) organic carbon contents in the 105- to 2,000-, 53- to 105-, and 0- to 53- μm size fractions, respectively. The organic carbon detected in each particle size fraction was corrected by particle size content as follows:

$$\text{X-OC in soil (\%)} = \frac{\text{Determined X-OC (\%)} \times \text{X Fraction X-content (\%)}{100}$$

where X is mineral-associated (MOC), fine particulate (POC_f), or coarse particulate (POC_c) organic carbon fraction.

The isolation of HA was carried out following the standard International Humic Substances Society procedure (Swift, 1996). To avoid HA contamination with labile organic matter, only the soil fine fraction (0–53 μm) was used for HA isolation.

The HA and FA were extracted from the REF soil and from soils under WW-CT, both nf and f, sampled during 1991 and 1996.

Carbon, hydrogen, nitrogen, and sulfur were determined with a Fisons Instrument EA 1108 elemental analyzer. Oxygen was calculated by difference.

Total acidity was measured by titration of the HA sample with an excess of $\text{Ba}(\text{OH})_2$ under N_2 for 24 h, followed by titration with 0.1 M HCl. For carboxylic group determination, a sample of HA was shaken for 24 h with an excess of $\text{Ca}(\text{CH}_3\text{COO})_2$ solution and then back-titrated with 0.1 N NaOH. The phenolic hydroxyls were obtained by difference.

The $E_4:E_6$ ratios were determined by dissolving 1.0 mg of each HA in 5 mL of NaHCO_3 , and the pH was adjusted to 8.3

with NaOH. The absorbencies at 465 nm and 665 nm were measured on a Perkin Elmer Lambda 15 UV–vis spectrophotometer. The ratio of these absorbencies gives the $E_4:E_6$ ratio.

Fourier-transform infrared (FTIR) spectra of HA and FA were recorded on KBr pellets in 4,000- to 400- cm^{-1} wavenumber range using a Nicolet 5 PC FTIR spectrophotometer. The KBr pellets were obtained by pressing a mixture of 1 mg HA and 400 mg KBr, spectrometry grade, under reduced pressure.

Wheat Yields and Carbon Input

The wheat crops were sampled every soil sampling year. Total aboveground dry matter production was harvested to determine grain and straw yields (Mg ha^{-1}). During the other years, only wheat grain was harvested. Straw production was estimated using the harvest index (HI). The relation observed between HI and grain yield from 400 data sets of field experiments previously conducted in this region (data not shown) suggest a nonlinear behavior. The best estimation of HI resulted from the following equation:

$$\text{HI} = 0.143 y^{0.114} \quad (P < 0.001; R^2 = 0.40)$$

where y is grain yield (Mg ha^{-1})

This estimation showed that wheat HI varied with crop grain yield. This relationship suggests that, during dry years, grain yields were more depleted than total dry matter production. The application of a fixed HI may overestimate C input in wet years and underestimate it in dry ones.

The annual carbon input was estimated considering a shoot-to-root ratio of 4.4 (obtained for winter wheat by Bolinder et al. (2007) and a carbon concentration in the dry matter of 400 mg g^{-1} (mean value obtained from the harvested grain and straw).

Statistical Analysis

The statistical analysis of the results was carried out by applying the one- or two-way analysis of variance (Fertilization \times Tillage). The means were compared using the least significant difference (LSD) test ($P < 0.05$) with the InfoStat Software (Di Rienzo et al., 2011).

RESULTS AND DISCUSSION

Long-term Effects of WW-CT on Wheat Yields

The mean yields of wheat grain under CT were 1,333 and 2,093 kg ha^{-1} in the nf and f treatments, respectively (Table 2). Yield variability was lower under no fertilizer than under annual fertilizer application treatments presumably because of water availability differences during grain filling and of soil fertility decay. Two periods can thus be clearly distinguished: 1983 to 1997 and 1998 to 2006. The mean annual precipitation in both of them was similar (792 and 752 mm, respectively), but the crop yields were different. In the first period, the mean yields were 1,568 and 2,430 kg grain ha^{-1} for nf and f, respectively. In the second one, they were 942 and 1,530 kg grain ha^{-1} for nf and f, respectively.

The WW-CT f yields and the grain increase resulting from fertilizer application in the first period were strongly correlated to water availability from September to November ($P < 0.01$; $R^2 = 0.67$) (Fig. 3). No correlation was found in the second period, which was characterized by low yields, poor response to fertilizers, and no relationship with water availability. These results suggest that natural soil fertility at the beginning of the experiment sharply declined and that the fertilizer was not enough to maintain it.

The calculated carbon input, as well as the grain yields, showed low levels of carbon incorporation into the soil under

TABLE 2. Annual Wheat Grain Yields and Estimated Soil Carbon Input With and Without Fertilizer Application Under CT

	Grain Yield		Carbon Input	
	WW-CT nf	WW-CT f	WW-CT nf	WW-CT f
	kg ha ⁻¹			
1983	2,106	2,050	2,235	2,185
1984	2,482	3,805	2,567	3,676
1985	1,366	2,426	1,551	2,518
1986	2,222	3,366	2,339	3,317
1987	1,186	2,555	1,376	2,630
1988	1,490	2,426	1,669	2,518
1989	1,526	2,624	1,703	2,690
1990	1,586	2,386	1,759	2,483
1991	1,531	2,805	1,708	2,845
1992	1,275	2,228	1,463	2,344
1993	1,480	2,420	1,660	2,513
1994	1,362	1,271	1,547	1,459
1995	1,959	2,441	2,103	2,531
1996	781	1,046	2,278	1,237
1997	1,161	2,597	1,351	2,667
1998	92	867	1,192	1,055
1999	100	1,333	1,207	1,519
2000	875	2,890	1,063	2,918
2001	1,341	1,978	1,527	2,120
2002	1,100	1,388	1,291	1,572
2003	964	989	1,154	1,180
2004	946	1,079	1,136	1,270
2005	860	960	1,048	1,150
2006	2,200	2,300	2,319	2,408
Mean	1,333.0	2,092.9	1,635.3	2,200.2
S.D.	598.3	805.8	452.3	728.7
Time × fertilizer interaction	***			

the nf treatment in the first period and under both nf and f treatments in the second period (Table 2).

Long-term Effects of WW-CT on SOC Stock

The SOC concentration in the upper soil layer (0–20 cm) under CT showed a very rapid decline and remarkable variation across the years. Total SOC changed from 1.5% to 0.89% (WW-CT nf)

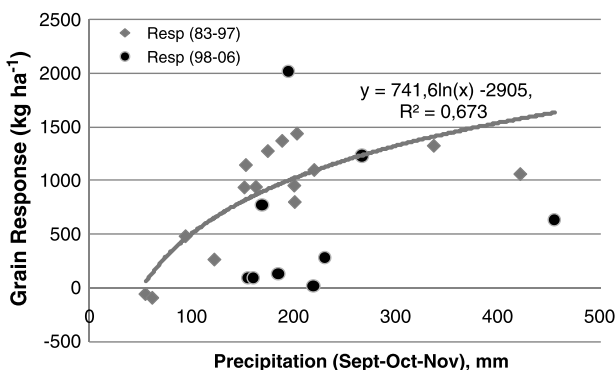


FIG. 3. Wheat grain response (GR = WWf - WWnf, kg ha⁻¹) during the initial (1983–1997) and final (1998–2006) periods of the experiment and precipitation rates in the critical season.

and from 1.5% to 1.03% (WW-CT f). This variation in SOC concentration may be related to changes in the quantity and distribution of annual precipitation (Galantini and Rosell, 2006) and/or to changes in soil bulk density along the study period (Ellert and Bettany, 1995; Toledo et al., 2013; Wang et al., 2009).

Considering that the variation in soil bulk density can modify the mass of soil sampled, SOC storage was calculated for an ESM of 2,400 Mg ha⁻¹ (Fig. 4). The SOC calculated for an ESM showed lower variations than when calculated as a percentage, but the trend was similar.

Previous results showed opposing effects of precipitation on SOC content, mainly the labile fractions (Galantini and Rosell, 2006). Higher rainfall during the fallow period can favor SOC decline, mainly the most labile fractions, by enhancing microbial activity; this may be caused by the combined effects of the previous crop residue incorporation and by water accumulation in the summer-fall period when temperatures are higher (Fig. 1).

On the other hand, higher precipitation during the crop cycle favors dry matter production. However, because of the negative balance between crop water requirements and water availability, mainly during the grain-filling period (Fig. 2), the grain yields did not follow the same tendency as dry matter production.

Fertilizer effect on SOC stock, the difference between the content in f and nf treatments, varied from 1.1 to 7.4 Mg ha⁻¹, with a mean value (1,986–2,006) of 4.66 Mg ha⁻¹.

Fertilization with N and P may be a useful practice for carbon sequestration, but the final outcome can be highly variable (Khan et al., 2007). Such variation may be accounted for by poor N use efficiency (Fageria and Baligar, 2005) and irregular water availability.

Long-term Effects of WW-CT on SOC Quality and Dynamics

Physical fractionation of SOC showed that the fractions exhibit varying degrees of transformation and sensitivity to crop management practices (Table 3). The POC content, both POC_f and POC_c, lost by cultivation was lower than MOC; however, the loss of labile fractions was proportionally higher. The decrease in SOC fractions was inversely linked to the particle size.

Fertilizer application reduced loss of all SOC fractions with a higher variability than the no fertilizer treatment. However, contrasting effects from fertilizer application were observed in the literature (Miglierina et al., 2000; Galantini et al., 2004; Andriulo et al., 2008; Zhang et al., 2010). When N from fertilizers is taken up by the crop, dry matter increase may contribute to a SOC

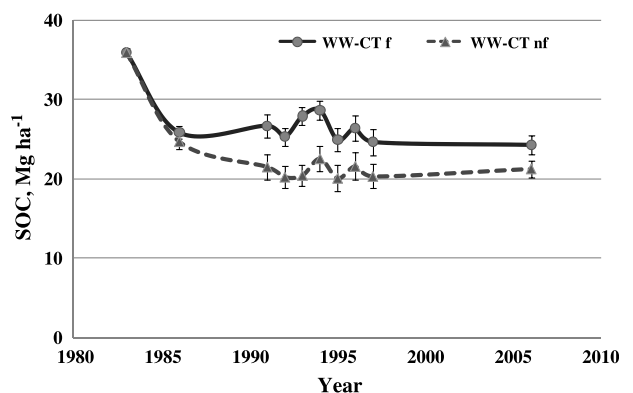


FIG. 4. Dynamics of SOC storage for an equivalent soil mass (2,400 Mg ha⁻¹) in continuous wheat with and without fertilizer application under CT.

TABLE 3. Dynamics of SOC Fractions (Mg ha⁻¹ for a 2,400-Mg soil mass) in Continuous Wheat With and Without Fertilizer Application Under CT

	WW nf			WW f		
	MOC	POC _f	POC _c	MOC	POC _f	POC _c
REF	22.45	3.72	9.39	22.45	3.72	9.39
1991	17.80	1.66	2.11	21.64**	2.35**	2.73*
1996	17.38	1.34	2.91	20.75**	2.02**	3.63**
2006	17.40	1.56	2.35	20.33**	1.64 ^{ns}	2.32 ^{ns}
Mean ¹	17.53	1.52	2.46	20.91	2.01	2.90
Change	23	58	75	9	56	75
S.D.	0.24	0.16	0.41	0.67	0.35	0.67
Time × fertilizer interaction				ns	**	*

¹Mean value of the cultivated soil samples.

S.D., standard deviation.

In each year and for each SOC fraction, ns, no significant differences; * and ** significant differences at the 0.05 and 0.01 levels, respectively.

increase. However, when this N is used by soil microorganisms, it may promote decomposition of labile organic materials. The final effect of fertilization is more likely related to N use efficiency rather than to the application rate.

For these reasons, WW-CT f may have shown a higher S.D. and greater variable differences in POC_f and POC_c fractions than WW-CT nf.

The dynamics of SOM fractions was better described by the following equations:

WW-CT nf

$$\text{MOC} = 22.2y^{-0.08} \quad R^2 = 0.944 \quad (1)$$

$$\text{POC}_f = 8.5y^{-0.45} \quad R^2 = 0.849 \quad (2)$$

$$\text{POC}_c = 3.5y^{-0.30} \quad R^2 = 0.898 \quad (3)$$

WW-CT f

$$\text{MOC} = 22.6y^{-0.03} \quad R^2 = 0.885 \quad (4)$$

$$\text{POC}_f = 9.0y^{-0.42} \quad R^2 = 0.901 \quad (5)$$

$$\text{POC}_c = 3.8y^{-0.34} \quad R^2 = 0.981 \quad (6)$$

where y means years with wheat crop.

A long-term effect of fertilizer application was found in the MOC decomposition rate, which dropped from $k = 0.08$ to $k = 0.03$.

The POC fractions were sensitive indicators of fertilization effects in the 1991 and 1996 sampling times. However, during 2006, an opposite conclusion can be drawn. Low yields from the previous dry year (199-mm rainfall during the crop cycle in 2005) were associated with poor use of N, which may enhance decomposition of labile SOM fractions during the following fallow.

The behavior of labile fractions, which depends on weather conditions, is in agreement with the hypothesis proposed.

Long-term Effects of WW-CT on Humic Substances

The main carbon concentration differences in Hum, HA, and FA occurred as a result of cultivation (REF vs. WW-CT) and fertilization (WW-CT nf vs. WW-CT f) but not between years (1991 vs. 1996) (Table 4).

TABLE 4. Carbon Content in Humic Acid, Fulvic Acid, and Humin Fractions in the REF and Wheat-Cultivated Soils

	C-Hum	C-HA	C-FA	C-HS	Hum:HA:FA
	%				
REF	0.570 ^a	0.323 ^a	0.154 ^a	1.05	4:2:1
1991					
WW-CT nf	0.420 ^b	0.235 ^c	0.083 ^b	0.74	5:3:1
WW-CT f	0.578 ^a	0.291 ^b	0.167 ^a	1.04	3:2:1
1996					
WW-CT nf	0.405 ^b	0.230 ^c	0.095 ^b	0.73	4:2:1
WW-CT f	0.538 ^a	0.289 ^b	0.178 ^a	1.01	3:2:1
Time × fertilizer interaction	ns	ns	ns		

Values within each column followed by different letters are significantly different according to the LSD test ($P < 0.05$).

Other studies have also found that changes in field management practices can modify HS concentration and alter the chemical properties of soil humic substances (Spaccini et al., 2006; Moraes et al., 2011).

The unfertilized wheat treatment decreased the initial concentration of C-Hum, C-HA, and C-FA. Annual fertilizer application tended to maintain C-Hum and C-FA.

The C-HA concentration decreased both in nf and f treatments; however, the decrease was less pronounced when the fertilizer was applied.

The Hum:HA:FA ratio was similar in the humic substances from the REF or cultivated soils; these similar values indicate a proportional loss of the three humic fractions.

The elemental composition of HA showed differences among treatments only for C and N contents (Table 5). Differences were found between nf and f treatments but not between years. The N concentration was increased and the C concentration was decreased by annual fertilizer application.

Total acidity of HA was lowered by cultivation mainly because of the decreased -OH phenolic groups, whereas carboxylic groups were similar in the REF and cultivated HA (Table 6). The HA from the REF soil had a higher -OH phenolic content than that from cultivated ones.

These results may be attributed to differences in the quantity and quality of residue input.

An increase of available N in semiarid environments improves water use efficiency and dry matter production. However, when water availability is low, the applied N could be taken up in excess by the crop, thus decreasing the C:N ratio of the residue input or it could remain available for microbial activity, thereby reducing labile carbon content. These two possibilities might account for the C:N ratio drop of HA in the fertilized soil, and wheat roots may be responsible for such -OH phenolic decrease (Senesi and D'Orazio, 2005).

The E₄:E₆ values of all HA were similar (Table 6), thereby indicating the same condensation of aromatic rings in natural and cultivated HA (Stevenson, 1994; Rivero et al., 1998). The E₄:E₆ ratio provides an index of aliphatic-aromatic balance, which, in turn, depends on the extent of coiling of the acid molecules (Sanyal, 2002). The E₄:E₆ ratio results from a greater absorbance by the aliphatic part (i.e., absorbance at 465 nm, E₄) than that by the aromatic part (i.e., absorbance at 665 nm, E₆).

The FTIR spectra from all HA were similar, with small variations among them (Fig. 5). No differences were found in spectra from cultivated soils between 1991 and 1996; because of this, the spectra of WW-CT nf and f shown in the figure correspond to the mean values between the 2 years.

TABLE 6. Acidic Group Content (cmol kg⁻¹) and E₄:E₆ Ratio of Humic Acids From the REF and Cultivated Soils

	Total Acidity	COOH	Phen-OH	E ₄ :E ₆
REF	10.8*	2.9 ^{ns}	7.9**	4.8 ^{ns}
1991				
WW-CT nf	7.0	3.1	3.8	4.8
WW-CT f	7.8	3.5	4.4	5.0
1996				
WW-CT nf	7.2	3.2	4.0	4.7
WW-CT f	8.7	3.2	4.5	5.0
Time × fertilizer interaction	ns	ns	ns	

Differences between REF and cultivated soil were, ns, no significant differences; * and ** significant differences at the 0.05 and 0.01 levels, respectively.

The main differences in HA spectra were found between REF and WW-CT nf treatments and the intermediate differences in WW-CT f.

The HA from WW-CT f showed a relative increase of the 3,400-cm⁻¹ peak (O-H and N-H stretching of the O- and N-containing groups) and a relative decrease of the 2,920- and 2,850-cm⁻¹ peaks (stretching of C-H bonds of aliphatic groups) compared with the nonfertilized treatment. These differences were in agreement with its highest N content by annual fertilization and a faster humification process. Fertilization can enhance biological activity, which produces a decrease of the labile groups and an increase of humic substances with higher N contents. These changes support the preferential oxidation of readily accessible moieties such as aliphatic side chains of lipidic and peptidic structures, leading to the production of highly functionalized aromatic macromolecules (Amir et al., 2004).

The FTIR spectra of the HA from the nonfertilized soil showed differences in the relative intensity between 1,715 (C=O stretching of COOH and ketones)- and 1,229-cm⁻¹ peaks (C-OH vibration of the phenolic OH and alcoholic groups). The peak in 1,618 cm⁻¹ (C=C aromatic region) was similar in all HA spectra.

The WW-CT f HA showed more intense peaks at 1,120, 1,080, and 1,030 cm⁻¹ (C-O of polysaccharide and polysaccharide-like substances) than the HA from the nonfertilized soil.

Consequently, the FTIR results indicated slight differences in the structural characteristics of the HA analyzed mainly attributed to a fertilization effect on N incorporation, a decrease in the

TABLE 5. Elemental Composition and Atomic Ratio of Humic Acids from the REF and Cultivated Soils

	C	N	H	S	O	C:N	C:H	O:C	C:S	N:S
REF	57.2 ^b	4.21 ^a	5.17 ^a	0.15 ^a	33.3 ^a	15.9	0.922	0.437	1,017	64.2
1991										
WW-CT nf	61.0 ^a	4.01 ^d	5.10 ^a	0.12 ^a	29.8 ^a	17.7	0.997	0.366	1,356	76.4
WW-CT f	56.4 ^b	4.11 ^{bc}	5.11 ^a	0.10 ^a	34.3 ^a	16.0	0.920	0.456	1,504	93.9
1996										
WW-CT nf	60.3 ^a	4.06 ^{cd}	5.05 ^a	0.10 ^a	29.9 ^a	17.3	0.995	0.372	1,608	92.8
WW-CT f	54.9 ^b	4.15 ^{ab}	5.09 ^a	0.14 ^a	36.9 ^a	15.4	0.899	0.504	1,046	67.8
Time × fertilizer interaction	ns	ns	ns	ns	ns					

Values within each column followed by different letters are significantly different according to the LSD test ($P < 0.05$).

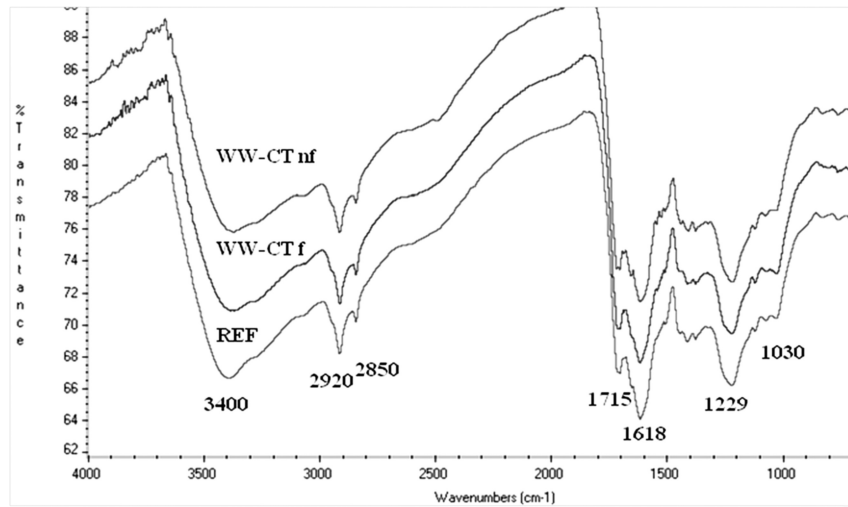


FIG. 5. The FTIR spectra of humic acids from the REF soil in continuous wheat under CT in fertilized (WW-CT f) and nonfertilized (WW-CT nf) soils.

relative content of aliphatic groups compared with aromatic groups, and an increase in polysaccharide compounds.

This behavior demonstrates HS stability, which presented slight differences in their quantity and structure as a result of long-term management practices.

Tillage and Fertilization Effects on Wheat Production

The WW-NT mean grain yields (2000–2006) were 967 and 1,660 kg ha⁻¹ in nf and f treatments (Table 7), similar to the 1,036 and 1,615 kg ha⁻¹ obtained in WW-CT nf and f, respectively.

A fertilization effect on grain yields was found in most of the years, with a significant interaction for Tillage × Fertilization.

In general, fertilization tended to increase grain yields. However, tillage effects tended to improve grain yields with fertilizer application or to decrease without it.

Under NT, wheat yields were higher when the fertilizer was applied, but they were lower when it was not; an opposite trend was observed during wet years.

The estimated carbon input was about 50% higher in the fertilized treatment; however, its variability was stronger than in WW-NT nf.

Tillage and Fertilization Effects on SOC Fractions

The accumulated effect on SOC fractions after 24 years of continuous wheat under CT and the conversion to NT for 8 years produced variable and significant effects from tillage and fertilizer application (Table 8).

Tillage-fertilization statistical analysis at each soil depth showed different behaviors among SOC fractions. Both POC_f and POC_c were able to show a tillage effect but not a fertilization effect. On the other hand, MOC showed a significant tillage-fertilization interaction, which was changing with depth. This interaction was a consequence of the negative effect observed in NT without fertilizer application.

A fertilizer effect was found in CT at a 10- to 20-cm depth, probably as a result of residue incorporation with tillage practices, and in all evaluated depths in NT soils. The POC_f was higher in NT than in CT, whereas the fertilizer effect was only observed at 5 to 10 cm of CT and 0 to 5 cm of NT. The POC_c was higher in NT than in CT plots for all depths, with greater carbon accumulation in the upper layer (0–5 cm).

Particulate organic materials, both fine and coarse POC, failed to demonstrate the differences between NT plots, either with or without fertilizer application. Dry weather conditions during the previous year (487 mm total rainfall in 2005) and the fallow period (60 mm accumulated rainfall from March to June) most likely affected dry matter input and decomposition.

These labile SOC fractions were suggested as early indicators of soil quality because of their sensitivity to management practices (Haynes, 2005). However, seasonal and weather variations may influence labile organic matter transformation; one sampling time may provide a different conclusion of the system dynamics.

Consequently, and as was proposed by the hypothesis, labile organic fractions can be considered as sensitive indicators of soil quality only if additional information on the most important factors affecting their dynamics is included (previous carbon input, nutrient status, water availability, and temperature).

When SOC fraction contents were analyzed on an ESM (2,400 Mg ha⁻¹) basis, fertilizer and tillage effects were clearly

TABLE 7. Annual Wheat Grain Yields and Estimated Soil Carbon Input With and Without Fertilizer Application Under NT

	Grain Yield		Carbon Input	
	WW-NT nf	WW-NT f	WW-NT nf	WW-NT f
1999	379	1,077**	455	1,400
2000	834	2,480**	959	3,100
2001	1,205	2,205**	1,355	2,550
2002	980	1,505 *	1,093	1,690
2003	945	1,150 ^{ns}	1,054	1,310
2004	790	1,055 ^{ns}	881	1,176
2005	805	1,225 *	925	1,380
2006	1,800	2,579**	2,007	2,876
Mean	967	1,660	1,091	1,935
S.D.	7,738	13,276	8,729	15,482
Time × fertilizer interaction	**			

Each year, the fertilization effect on grain yield was “ns,” no significant differences; * and ** significant differences at the 0.05 and 0.01 levels, respectively.

TABLE 8. SOC Fractions (Mg ha⁻¹) for Fixed Depths and an ESM (=2,400 Mg ha⁻¹) in Continuous Wheat Under Different Tillage Systems and Fertilizer Applications (Year 2006)

	WW-CT nf	WW-CT f	WW-NT nf	WW-NT f	Till	Fert.	Int.
MOC							
0–5	3.83	4.67	4.19	5.96*	—	—	*
5–10	4.49	5.07	4.11	5.86*	—	—	**
10–20	9.94	10.79*	7.62	11.02**	—	—	***
ESM	17.40	20.33**	14.58	21.19**	—	—	***
POC _f							
0–5	0.43	0.49*	0.67	0.75*	**	*	ns
5–10	0.40	0.45*	0.48	0.47	**	ns	ns
10–20	0.80	0.71	0.91	0.90	**	ns	ns
ESM	1.56	1.64	1.90	1.99	**	ns	ns
POC _c							
0–5	0.99	1.07	1.62	2.19*	**	ns	ns
5–10	0.72	0.68	0.86	0.84	**	ns	ns
10–20	0.70	0.59	1.11	1.14	**	ns	ns
ESM	2.35	2.32	3.40	3.99*	**	ns	ns

Mineral-associated (MOC, 0–0.053 μm), fine particulate (POC_f, 0.053–0.100 μm), coarse particulate (POC_c, 0.1–2.0 mm) organic carbon; CT, conventional tillage; NT, no-tillage; f and nf, with and without annual fertilizer application, respectively. Till, tillage (NT or CT), Fert., fertilization (nf or f); Int., tillage × fertilization interaction.

Values within each depth, SOC fraction, and tillage followed * and ** indicate differences at the 0.05 and 0.01 levels, respectively.

observed in SOC fractions. The MOC content was higher in fertilized than in nonfertilized treatments, both under CT and NT. On the other hand, tillage produced opposite effects in nonfertilized treatments, and MOC content was higher in the CT-nf than in the NT-nf treatment. Both POC_f and POC_c contents showed a significant tillage effect and a nonsignificant fertilizer effect.

Tillage can favor SOC decrease; on the other hand, no-tillage may reduce SOC oxidation produced by aeration and may enhance soil water conservation during the fallow period, thus boosting biological activity. Under these opposite trends, the remaining low soil fertility after 16 years of continuous wheat was probably not enough to produce sufficient crop dry matter to compensate for SOC decomposition. This is why no-tillage without fertilizer application turned out to be insufficient to maintain SOC levels in this study.

CONCLUSIONS

Continuous wheat on an Entic Haplustoll of the semiarid Pampas produced a sharp SOC decline, which becomes more pronounced when carbon input was low. The SOC dynamics strongly depended on water availability for SOM decomposition and crop dry matter production.

Sensitivity of labile SOC fractions to tillage and fertilization was variable across time, depending on residue input, water availability, and management practices.

Humified soil organic fractions showed slight differences in their structure and quantity as a result of long-term management practices.

No-tillage in low-fertility soils may increase SOC stock when fertilizers were applied or may produce SOC loss when they were not.

In this semiarid condition and shallow soils, the high dependence on both residue input (quantity and quality) and environmental controls of the organic fraction transformation (temperature and moisture) suggests that all factors should be considered as a whole.

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