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Influence of tillage practices on soil biologically active organic matter content over a growing season under semiarid Mediterranean climate

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Abstract

In semiarid areas, traditional, intensive tillage has led to the depletion of soil organic matter, which has resulted in reduced soil fertility. The aim of the present work was to evaluate the effects of different soil management systems, practised over 12 years, on soil organic carbon (SOC), nitrogen (SN) and biologically active organic matter [particulate organic matter (POM); potentially mineralisable nitrogen (PMN); microbial biomass (MB)]. A Mediterranean Alfisol, located in central Spain, was managed using combinations of conventional tillage (CT), minimum tillage (MT) or no-tillage (NT), plus a cropping background of either continuous wheat (WW) or a fallow/wheat/pea/barley rotation (FW). Soil was sampled at two depths on four occasions during 2006-2007. The results showed the sampling date and the cropping background to significantly affect the SOC ($p < 0.0057$ and $p < 0.0001$ respectively). Tillage practice, however, had no effect on SOC or SN. The C-and N-POM contents were significantly influenced by the date, tillage and rotation. These variables were significantly higher under NT than CT and under WW than FW. The PMN was influenced by date, tillage and rotation, while C-MB was significantly affected by tillage ($p < 0.0063$), but not by rotation. The NT plots accumulated 66% C-POM, 60% N-POM, 39% PMN and 84% C-MB more than the CT plots. After more than 12 years, the benefits of conservation practices were found in the considered soil properties, mainly under no tillage. In order to obtain a consistent data set to predict soil biological status, it is necessary further study over time.

Additional key words: conservation agriculture; crop rotation; fallow; labile soil organic matter; soil organic carbon.

Introduction

In Mediterranean dryland cropping systems, the main factor limiting crop production is water, the consequence of low and irregularly distributed rainfall and high evapotranspiration rates (Moret *et al.,* 2006; López-Bellido *et al.*, 2007). Leaving land fallow has traditionally been included in crop rotations as a way of conserving soil water and controlling weeds (Lampurlanés *et al*., 2002). However, many authors report that, in some areas, leaving land fallow provides no real advantage for the following crop (López & Arrúe, 1997; Halvorson *et al.*, 2002; Sainju *et al.*, 2006b; López-Bellido *et al.*, 2007); indeed, they indicate it to reduce soil water-use efficiency, accelerate soil organic matter (SOM) mineralisation, and to leave the soil vulnerable to erosion.

Under Mediterranean rainfed conditions, the yield potential of autumn-sown cereals is rather low (Calado *et al*., 2009). Biomass production is, therefore, also low, resulting in limited residue input to the soil. Increasing this input (*e.g.,* leaving straw to mulch, continuous cropping), together with management practices that reduce soil aeration, could, however, enhance SOM accumulation (Paustian *et al*., 2000; Halvorson *et al.,* 2002). However, in semiarid environments, experiments have shown that the tillage practice can affect SOM accumulation (and indeed other soil properties), the outcome is highly site-specific (Ordóñez Fernández *et al.,* 2007; Thomas *et al.,* 2007; Hernanz *et al*., 2009;

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Abbreviations used: BD (bulk density); CT (conventional tillage); FW (fallow-wheat); MB (microbial biomass); MT (minimum tillage); NT (no tillage); PMN (potentially mineralizable nitrogen); POM (particulate organic matter); SN (soil nitrogen); SOC (soil organic carbon); SOM (soil organic matter); WW (wheat-wheat).

Sainju *et al.,* 2009). While some authors report no-tillage to have a beneficial effect on SOM accumulation (López-Fando & Pardo, 2009; Sombrero & De Benito, 2010), others indicate it to have little or no impact (Thomas *et al.*, 2007; Sainju *et al.*, 2011).

The main soil characteristics that determine whether a tillage practice will have a significant influence on SOM accumulation are 1) the initial SOM level, and 2) the texture of the soil (Hassink & Whitmore, 1997; Campbell *et al.,* 2000; Denef *et al.*, 2004). However, the SOM content generally changes slowly with soil management practices since the pool size is usually large and heterogeneous in terms of its composition (Franzluebbers *et al.,* 1995). Thus, to study the effects of management practices, it can be more useful to measure the biologically active organic matter content of the soil, which includes its particulate organic matter (POM), potentially mineralisable nitrogen (PMN) and microbial biomass (MB) contents. These fractions usually change rapidly over time and might better reflect changes in soil quality (Gregorich *et al.,* 1994; Turco *et al.*, 1994).

The POM is a very heterogeneous SOM fraction since it consists of organic materials that have undergone varying degrees of decomposition (Cambardella & Elliot, 1992; Wander, 2004). The POM plays a role in aggregate formation in soils in which aggregate hierarchy applies (Six *et al.*, 1999). It provides a substrate for microorganisms, and has been associated with changes in the soil mineralisable organic nitrogen pool (Hassink, 1995a). Many studies have used the POM fraction as an index of labile SOM status, and it is recognised as a potential indicator of soil quality changes in the short term (Marriot & Wander, 2006; Yoo & Wander, 2008; Sequeira *et al.*, 2010). Some authors indicate POM to accumulate at the soil surface when tillage is reduced (Wander *et al.,* 1998; Álvaro-Fuentes *et al.*, 2008) and when cropping intensity is increased (Sainju *et al.,* 2006a). The influence of tillage practice on the soil POM content naturally depends on the soil type and quality of input residue (Sainju *et al.*, 2006b; Virto *et al.*, 2007; Álvaro-Fuentes *et al.*, 2008). Virto *et al.* (2007) and Álvaro-Fuentes *et al.* (2008) found a significant increase in POM content mainly in the surface soil when NT was practiced, while Sainju *et al.* (2006b) observed no significant effect of tillage practice on the POM at any depth.

The PMN content provides an estimate of the labile nitrogen in the soil and has been used as an indicator of SOM quality (Drinkwater *et al.,* 1996). The accumulation and decomposition of organic materials may influence the PMN over time (Willson *et al.*, 2001). The origin of the PMN is uncertain. Some authors indicate it to be a product of soil bacteria via mineralisation processes, and report a correlation between the PMN and the soil microbial biomass (MB) (Myrold, 1986). Others, however, suggest that the MB may be responsible for only 40-60% of the PMN (Bonde *et al.,* 1988), the POM fraction representing another possible source (Hassink, 1995b; Willson *et al.*, 2001). Since the MB is the living fraction of the SOM it has a rapid rate of turnover. Changes in the MB fraction due to soil management practices are therefore detectable long before any are seen in the SOM. This rapid response of the MB has been widely used as an indicator of change in soil quality (Dalal, 1998). Long term field experiments can provide valuable information on the impact of tillage practices on changes in soil quality over time (Benbi & Brar, 2009).

The aim of the present work was to assess the effects of the tillage practices and cropping backgrounds used for 12 years on the SOC, POM, PMN and MB contents of a semiarid Mediterranean soil over a single growing season (2006-2007).

Material and methods

Experimental design

The study was performed at the INIA experimental farm, 42 km northeast of Madrid (40° 32' N, 3° 20' W; altitude 600 m.a.s.l.). The area is given over to the typical dryland cereal farming practised in central Spain, which has a mild-continental Mediterranean climate with dry summers. Precipitations are irregularly distributed over the year and from one year to another; the average rainfall is 380.2 mm per year (calculated using data for 1957-2008) (Table 1). The mean air temperature ranges from 4.9°C in January to 23.7°C in July (mean annual temperature 13.3°C) (Mauri-Ablanque, 2000).

Experimental work was performed at a site involved in a long-term field experiment that began in 1994. The soil at this site is a sandy loam *Calcic Haploxeralf* (Soil Survey Staff, 2010). The initial SOC content (*i.e.,* that recorded in 1994) in the first 15 cm was low at around 6 Mg ha^{-1} (Martín-Rueda *et al.*, 2007). Table 2 shows the main soil properties.

The present experiment, which was carried out over one growing season (2006-2007), was designed on the

Table 1. Monthly total precipitation (mm) for the cropping seasons 2005-2006 and 2006-2007 (La Canaleja meteorological station), and the historic average (1957-2008, Mauri-Ablanque, 2000) at the experimental site

Month	2005-2006	2006-2007	Average (1957-208)
October	86.3	84.6	61.7
November	66.0	98.3	53.8
December	27.0	20.5	26.7
January	40.2	7.8	23.7
February	45.5	43.8	35.3
March	20.0	13.6	14.6
April	37.0	104.5	55.0
May	14.0	95.7	54.8
June	34.8	37.0	27.8
Total	370.8	505.8	353.3

basis of a split-plot randomised complete block with four replicates, in which the type of tillage since 1994 and the crop rotation were the primary and secondary variables respectively. The effects of three tillage systems were examined: conventional tillage (CT), minimum tillage (MT) and no-tillage (NT). CT consisted of mouldboard ploughing to a depth of 20 cm and a pass with a cultivator; MT consisted of chisel ploughing to 15 cm and a pass with a cultivator; NT consisted of direct seeding after the application of herbicides (glyphosate). The cropping backgrounds were either continuous wheat monoculture (WW) or a four-year rotation of fallow, winter wheat (*Triticum aestivum* L. var. Astral), pea (*Pisum sativum* L. var. Déclic) and barley (*Hordeum vulgare* L. var. Kika); the last condition before the present work began was "fallow" in all FW subplots. The dimensions of each subplot for each tillage practice/cropping background combination were 10×25 m. Wheat was sown in November and harvested in June. All WW and FW subplots received the standard husbandry practised in central Spain.

The unique C input in the present study comes from the incorporation of the crops residues into the soil after harvesting (straw and roots), thus the effect of tillage and cropping background on wheat grain yield and on straw biomass production in 2006 and 2007 harvests were examined. Wheat biomass and grain yield were determined by harvesting a surface of 0.7×0.7 m from each subplots. Wheat grain yield was adjusted to 120 g kg^{-1} moisture content for comparison purposes. After harvesting, all crop residues were chopped and left on the soil surface, independent of the tillage system.

Soil sampling and analysis

Soils were collected from 24 subplots, corresponding to combinations of the three tillage practices and two cropping backgrounds, with four replicates for each ($3 \times 2 \times 4 = 24$). Composite soil samples, three cores from each subplot, were randomly taken at depths of 0-7.5 and 7.5-15 cm on four sampling dates: 1) November 2006, after tilling and sowing, 2) May 2007, at wheat anthesis, 3) July 2007, after harvest, and 4) October 2007, after the next round of tilling. The study focused on the first 15 cm depth, which is where the influence of tillage on SOC fractions is most apparent (Wander *et al.,* 1998). Soil samples were air-dried and ground to pass a 2 mm mesh. A portion of the dried, ground soil was then milled for organic carbon and nitrogen analysis. The SOC concentration was determined using the Walkley-Black wet oxidation method (Nelson & Sommers, 1996). Total soil N (SN) was determined by the Kjeldahl method (Bremner, 1996). The POM fraction was obtained following the method of Cambardella & Elliot (1992). Briefly, 10 g of air-dried soil were dispersed in sodium polyphosphate (5 g L^{-1}) and shaken for 15 h before sieving through a 0.053 mm mesh. Both fractions, *i.e.,* that retained by the sieve (sand + POM) and the soil slurry that passed through [the mineral-associated organic matter fraction (C-MF) and the water-soluble C], were collected and dried at

Table 2. Main properties of the soil in the experimental plot

Soil depth (cm)	Sand[a] (50-2000 µm) (g kg^{-1})	Silt[a] (2-50 µm) (g kg^{-1})	Clay[a] (< 2 µm) (g kg^{-1})	$CaCO_3$ (g kg^{-1})	AWC[b] (g kg^{-1})	pH (1:2.5)	EC[c] (1:5) (dS m^{-1})
0-7.5	505	376	119	41.6	110	7.9	0.123
7.5-15	510	379	111	41.3	100	8.0	0.119
15-30	501	351	148	43.4	114	8.1	0.116

[a] Particle size distribution. [b] Available water content (between –30 and –1,500 kPa). [c] Electric conductivity.

50°C. The organic carbon and nitrogen contents of both fractions were analysed in the same way as for the determination of SOC and SN. The C- and N- POM contents (g kg^{-1} soil) were then calculated as the C and N concentrations (g C or g N g^{-1} POM) multiplied by the POM content of the soil (g POM g^{-1} soil).

The PMN was estimated using the anaerobic incubation method (Waring & Bremner, 1964). Air dried soil was saturated with distilled water and incubated for 7 days at 37°C. The ammonium produced during incubation was extracted with 2M KCl (1:10) and measured in a FIAstar 5000 injection flow system (Foss Tecator). Ammonium from non-incubated samples was also analysed. The PMN (mg kg^{-1}) was then calculated as the N-NH_4^+ from the incubated samples minus the initial N-NH_4^+ content of the soil.

The carbon associated with the microbial biomass (C-MB) was estimated using the fumigation-extraction method (Vance *et al.*, 1987). Prior to fumigation the air dried soil samples were moistened to 70% of field capacity and pre-incubated for 7 days at 30°C (Wander & Bidart, 2000). The soil samples were then fumigated with ethanol-free chloroform for 24 h, and extracted with 0.5 M K_2SO_4 (1:4). The extracted C was determined using the wet oxidation method. The same procedure was used for non-fumigated soil samples. The C-MB was calculated as the difference in soluble C between the fumigated and non-fumigated extracts (C_f-C_{nf}) divided by 0.41 [factor used to calculate total soil microbial biomass C (Dou *et al.,* 2008)]. All analyses were performed in triplicate. The C-MB was estimated only for the samples collected in May 2007.

In order to calculate the mass per hectare (Mg ha^{-1}) of the studied variables, soil bulk density (BD) was determined in all treatments from intact cores taken in May 2007 (Blake & Hartge, 1986). The SOC, SN, C and N-POM, PMN and C-MB stocks were corrected following the equivalent soil mass method (Ellert & Bettany, 1995) where an additional soil thickness (T_{ad}) was calculated as follows:

$$T_{ad}\,(\mathrm{m}) = \frac{(M_{soil,equiv} - M_{soil,surf}) \cdot 0.0001\ \mathrm{ha\ m^{-2}}}{BD_{subsurf}}$$

where $M_{soil,equiv}$ is the equivalent soil mass [mass of heaviest layer (Mg ha^{-1})], $M_{soil,surf}$ is the sum of soil mass in surface layer(s) (Mg ha^{-1}), and $BD_{subsurf}$ is the bulk density of the subsurface layer (Mg m^{-3}). The mass per area (Mg ha^{-1}) of the variables was then calculated by multiplying the concentration (kg Mg^{-1}) with BD (Mg m^{-3}) and depth: $T + T_{ad}$ (m).

To further characterise the soil, additional soil variables were also determined, including the pH (soil/water ratio 1:2.5); electric conductivity (soil/water ratio 1:5); texture, determined using the hydrometer method (Gee & Bauder, 1986); $CaCO_3$ concentration, determined by the titration method (Loeppert & Suárez, 1996); and the available water content, determined using a pressure cell apparatus and calculated as the difference between soil water content at field capacity, –33kPa, and the permanent wilting point, –1,500 kPa (Richards, 1965). These later variables were analysed once in soil samples collected in May 2007 (Table 2).

Statistical analysis

Data were analysed using the SAS statistical package (SAS Institute, 2003). Analysis of variance was performed using the PROC MIXED routine after testing for normality and the homogeneity of variance. Tillage practice and cropping background were considered fixed effects, the replicates were considered to represent the random effect (n = 4), and the sampling date represented a repeated measurement. Means were compared using the DIFF option of the LSMEANS routine. Significance was set at $p < 0.05$, unless otherwise stated. Pearson correlation coefficients were obtained using the PROC CORR routine.

Results

Wheat grain yield and straw biomass production

Table 3 shows the wheat grain yield and straw biomass (Mg ha^{-1}) harvested in June 2006 and June 2007. We found significant differences ($p < 0.0001$) in the wheat grain yield in the WW subplots between both years. The grain harvested in June 2006 under NT and MT practices, was more than double that recorded in June 2007, whereas under the CT no significant differences in grain yield between years were found ($p = 0.1093$). In June 2006, no significant differences in wheat grain yield and in straw biomass were seen with respect to tillage practice; in June 2007, however, significant differences were seen in grain yield and in straw biomass among tillage practices. Irrespective of rotations, under CT 2.18 Mg ha^{-1} of grain was obtained in June 2007 whereas for NT and MT wheat grain

Table 3. Analysis of variance (PROC MIXED) for 2006 and 2007 harvests, and mean values for wheat grain yield (at 12% water content) and dry straw biomass (mean ± standard deviation)

	Dependent variable	
	Wheat grain yield (Pr > F[a])	**Dry straw biomass(Pr > F[a])**
Date	< 0.0001	0.0212
Tillage	0.0434	0.1548
Cropping background	0.8538	0.0550

	Wheat grain yield ($Mg\ ha^{-1}$)		Dry straw biomass($Mg\ ha^{-1}$)	
	FW[b]	**WW**	**FW**	**WW**
Cropping background[c]				
June 2006				
NT	—	2.84 ± 0.83	—	3.81 ± 0.59
MT	—	2.76 ± 0.52	—	3.58 ± 0.48
CT	—	2.90 ± 0.50	—	3.74 ± 0.55
June 2007				
NT	1.64 ± 0.65 AB	1.39 ± 0.68 B	4.04 ± 0.78 aA	3.05 ± 0.88 b
MT	1.19 ± 0.67 B	1.19 ± 0.65 B	3.04 ± 0.62 B	2.71 ± 0.64
CT	2.13 ± 0.55 A	2.23 ± 0.87 A	3.75 ± 0.58 AB	3.52 ± 0.40

[a] Significance set at $p < 0.05$. [b] FW: fallow-wheat; WW: wheat-wheat. [c] NT: no tillage; MT: minimum tillage; CT: conventional tillage. Different lower case letters indicate significant differences between cropping backgrounds within a tillage practice. Different upper case letters indicate significant differences among tillage practices within a cropping background.

yields were significant lower: 1.51 and 1.19 Mg ha^{-1} respectively. Straw biomass obtained in June 2007 under NT was 1 Mg ha^{-1} higher than under MT in the FW rotation. Rotation effect was not significant for wheat yield at any year, while we found significant differences in straw production between rotations in June 2007: 3.61 and 3.09 Mg ha^{-1} under FW and WW respectively, mainly due to differences in the non-tilled plots.

Soil bulk density

Significant differences ($p = 0.0125$) in surface layer (0-7.5 cm) soil BD were found among the different tillage practices. The NT subplots returned the highest BD values, followed by the CT and MT subplots irrespective of cropping background (1.52, 1.38 and 1.24 Mg m^{-3} respectively). No such differences were observed at 7.5-15 cm (data not shown). Due to the significant differences found in the surface's BD values among tillage treatments, the equivalent mass method was applied in order to correct the differences in soil mass (Ellert & Bettany, 1995).

Soil organic carbon and total nitrogen

Table 4 shows that the SOC accumulated in the first 15 cm of the soil was significantly influenced by the sampling date and cropping rotation, whereas the SN content was influenced by cropping rotation and the interaction *Sampling date* × *Tillage practice*.

The sampling date had a significant influence on the SOC. Taking all treatments together, the SOC content in July 07 was significantly greater than in Nov 06 and May 07 and similar to that recorded in Oct 07.

Crop rotation had a significant effect on both the SOC and SN. Under WW, the mean values (*i.e.,* for the whole growing season) for the SOC and SN contents were respectively 16.1% and 11.0% higher than those recorded under FW over the entire soil depth (0-15 cm).

Fig. 1 shows the effect of tillage practice on the SOC and SN contents (Mg ha^{-1}). Tillage practice had no significant effect on SOC over the entire 15 cm of the soil when all sampling dates were considered together. However, significant differences were seen between the NT and CT conditions in Nov 06 and July 07 (31.6 and 29.7% higher respectively; $p = 0.029$ for Nov 06,

Table 4. Analysis of variance (PROC MIXED) for SOC (soil organic carbon), SN (soil nitrogen), POM (particulate organic matter), PMN (potentially mineralisable nitrogen) and MB (microbial biomass) accumulated in the first 15 cm of soil on the four sampling dates

	Dependent variable (Pr > F[a])					
	SOC	**SN**	**C-POM**	**N-POM**	**PMN**	**C-MB[b]**
Date	< 0.0001	0.3045	< 0.0001	< 0.0001	0.0001	—
Tillage (Till)	0.1350	0.1961	0.0036	0.0013	0.0204	0.0063
Date × Till	0.1818	0.0041	0.0082	0.0827	0.0288	—
Rotation (Rot)	0.0057	0.0170	0.0027	0.0041	< 0.0001	0.6097
Date × Rot	0.2328	0.9224	0.3113	0.6427	0.0890	—
Till × Rot	0.2688	0.2622	0.5617	0.6064	0.3525	0.7789
Date × Till × Rot	0.4678	0.9672	0.2082	0.5722	0.7302	—

[a] Significance set at $p < 0.05$. [b] The C-MB data are for May 2007 only.

and $p = 0.016$ for July 07). The SN values behaved in a manner similar to those recorded for SOC (Fig. 1b).

The C/N ratio was significantly higher in the surface than between 7.5-15 cm (data not shown). Taking all the sampling dates together, under CT the C/N ratio was 9.2, whereas under MT and NT the C/N ratio was slightly higher 9.5 and 9.7 respectively (0-7.5 cm depth).

Biologically active soil organic matter

The C-POM value for the first 15 cm of soil was significantly influenced by the sampling date, tillage practice, crop rotation and the interaction *sampling date × tillage practice* (Table 4). In general, the C-POM values for Nov 06 and May 07 were significantly lower than for July 07 and Oct 07 (before and after the wheat harvest respectively). The N-POM content (over the entire 15 cm of soil) was significantly influenced by sampling date, tillage practice and cropping background (Table 4); in general the behaviour recorded was similar to that recorded for C-POM.

Tillage practice had a significant influence on both the soil vertical distribution of C-POM and on its content (taking both crop rotations together). Under NT the mean C-POM values for the growing season were 3.1 Mg ha^{-1} and 1.5 Mg ha^{-1} at the upper and the lower soil depths respectively. Under CT, the C-POM content in the first 7.5 cm was 1.5 Mg ha^{-1}, and at 7.5-15 cm it was 1.3 Mg ha^{-1}, indicating greater homogeneity compared to NT. For the 15 cm of soil as a whole, the C-POM content was 59, 67, 106 and a 30% higher under NT than under CT in Nov 06, May 07, July 07 and Oct 07 respectively. Under MT, the C-POM content was intermediate between those of NT and CT. Over the entire 15 cm of the soil, and taking both crop rotations together, the C-POM content varied with sampling date, with two clear periods discernable under CT and MT: Nov 06-May 07, and July 07-Oct 07, the latter period returning higher values. The highest C-POM value recorded under NT was in July 07 (Fig. 2a).

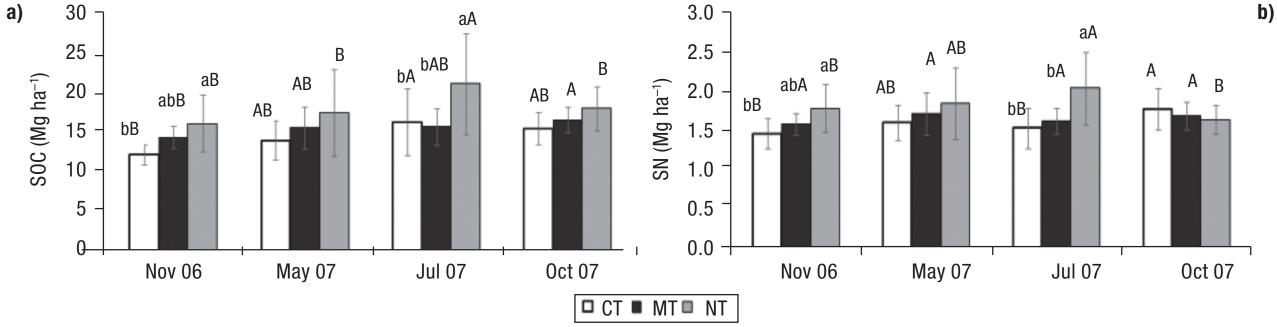

Figure 1. Effect of tillage practice on (a) soil organic carbon (SOC) and (b) soil nitrogen (SN) accumulated over the first 15 cm of soil (Mg ha^{-1}) on the four sampling dates (taking both cropping backgrounds together). CT: conventional tillage; MT: minimum tillage and NT: no tillage. Lower case letters indicate significant differences among tillage practices on the same date; upper case letters indicate significant differences among dates for the same tillage practice ($p < 0.05$). Bars indicate standard deviations.

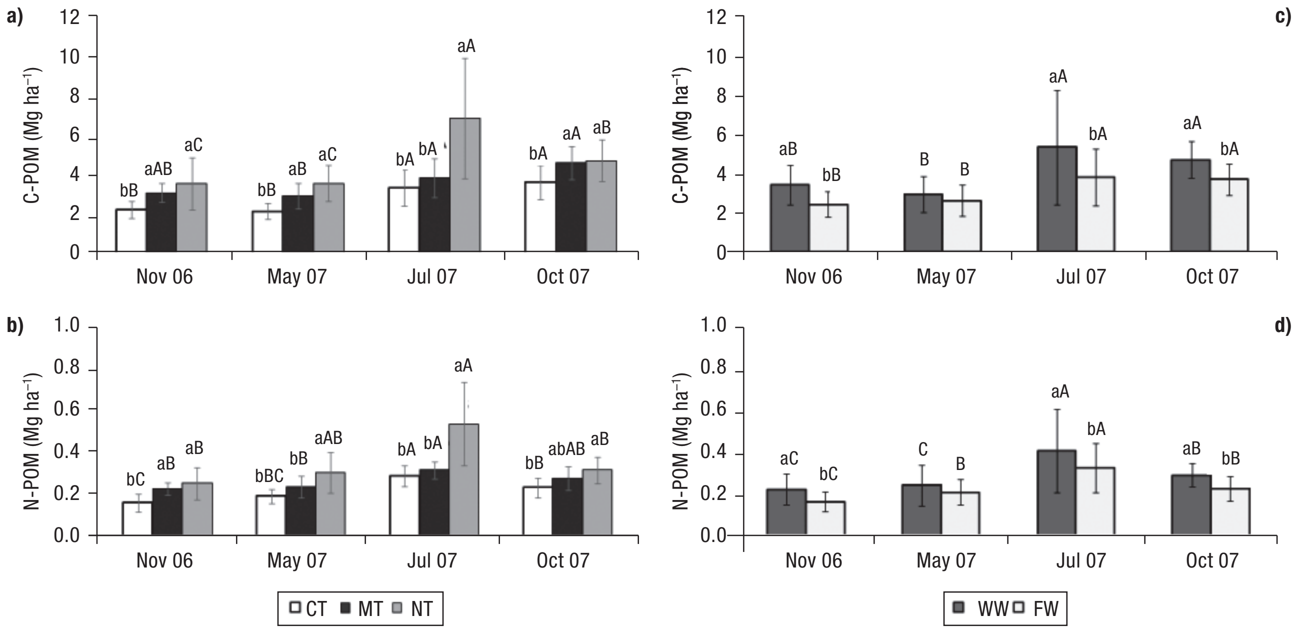

Figure 2. Effect of tillage practice on (a) C-POM and (b) N-POM and the effect of the cropping background on (c) C-POM and (d) N-POM accumulated over the first 15 cm soil of soil (Mg ha^{-1}) on the four sampling dates. CT: conventional tillage; MT: minimum tillage and NT: no tillage. WW: wheat-wheat; FW: fallow-wheat. Lower case letters indicate significant differences among tillage practices (or between crop rotations) on the same date; upper case letters indicate significant differences among dates for the same tillage practice (or the same crop rotation) ($p < 0.05$). Bars indicate standard deviations.

As for C-POM, significant differences were seen between the N-POM content under the NT and CT conditions throughout the growing season over the entire 15 cm of soil. Under MT, the content was intermediate between the values for NT and CT (Fig. 2b). The N-POM accumulated in the first 15 cm of the soil was 58, 59, 85 and a 36% greater under NT than under CT in Nov 06, May 07, July 07 and Oct 07 respectively. Both soil C-POM and N-POM values were higher under WW than under than FW throughout the growing season (Table 4, Fig. 2c and 2d3ii), with the greatest differences seen in July 07.

Under the CT tillage practice the C-POM/SOC ratio for the 15 cm of soil taken together ranged from 14.8 to 22.3% (corresponding to May 07 and Oct 07 respectively); under NT conditions it ranged from 18.9 to 30.3%. The N-POM/SN ratio for the 15 cm of soil taken together was strongly influenced by sampling date ($p < 0.0001$), and less strongly (but still significantly) by tillage practice ($p = 0.0135$) and cropping background ($p = 0.0111$). The proportion of N-POM in the total SN reached means of 13.1, 13.2, 20.8 and 15.6% for Nov 06, May 07, July 07 and Oct 07 respectively; under NT, this ratio was significantly higher than under CT; and, it was higher under the WW than FW conditions (data not shown).

The soil PMN values over the 0-15 cm soil profile were strongly influenced by sampling date and cropping background ($p < 0.001$), and to a lesser extent (although still significantly) by tillage practice and the interaction *Sampling date×Tillage practice* (Table 4). Regardless of tillage practice or cropping background, the PMN content varied over the year, with the highest values in July 07 (Fig. 3). The crop rotation significantly influenced the PMN content in the first 15 cm of the soil (Fig. 3a): higher values were found with the WW than with fallow background. Under NT, the soils contained 53, 22, 50 and 30% more PMN (over the 0-15 cm depth) than under CT in Nov 06, May 07, July 07 and Oct 07 respectively (Fig. 3b). The PMN/SN ratio varied significantly with sampling date and cropping background (data not shown). Mean values of 4.0, 4.1, 4.5 and 3.3% were recorded for Nov 06, May 07, July 07 and Oct 07 respectively (at the 0-15 cm depth), and higher ratios were recorded for the WW than the FW background in Nov 06 and Oct 07. Slightly higher PMN/SN ratios were found under NT and MT than under CT. The mean PMN/SN ratio values for the four sampling dates were 4.1%, 4.2%, and 3.7% under NT, MT and CT respectively (no significant differences).

The C-MB was only estimated for the May 07 samples. Table 5 shows the results obtained for the two soil

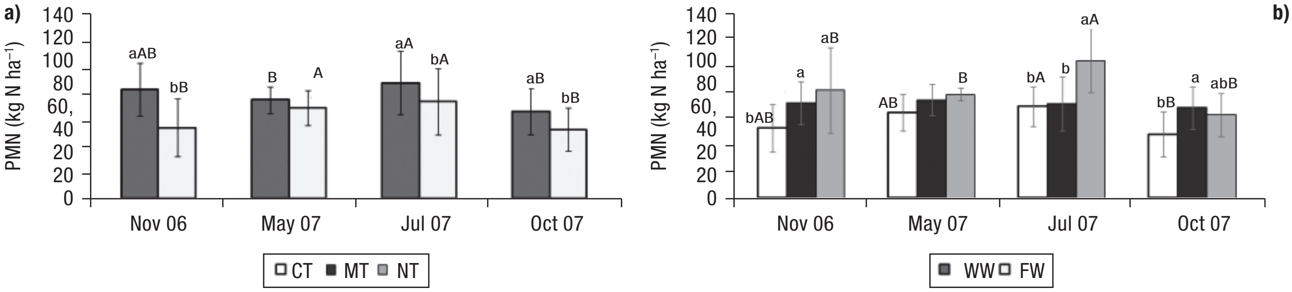

Figure 3. Effect of the cropping background (a) and tillage practice (b) on PMN accumulated (kg N ha^{-1}) over the first 15 cm of soil depth on the four sampling dates. WW: Wheat-wheat; FW: Fallow-wheat. CT: conventional tillage; MT: minimum tillage and NT: no tillage. Lower case letters indicate significant differences between crop rotations (or among tillage practices) on the same date; upper case letters indicate significant differences among dates for the same crop rotation (or the same tillage practice) ($p < 0.05$). Bars indicate standard deviations.

depths and under the different tillage practices and cropping background conditions. Taking both crop rotations together, tillage practice had a significant effect on C-MB at the 0-7.5 cm soil depth, but none was observed at 7.5-15 cm. Although the C-MB at the 7.5-15 cm depth was not significantly affected by tillage practice, this variable did have a significant influence on C-MB for the soil taken as a whole (0-15 cm) (Table 4). Under NT and MT the C-MB content (0-15 cm) was significantly higher than under CT. The mean C-MB value accounted for 1.5% of the total SOC; neither tillage practice nor cropping rotation presented significant differences.

Table 5. Analysis of variance (PROC MIXED) for C-MB accumulated at both soil depths: 0-7.5 and 7.5-15 cm (determined for samples taken in May 2007)

	Dependent variable (depth)	
	C-MB (0-7.5 cm)	**C-MB (7.5-15 cm)**
Pr > F[a]		
Tillage (Till)	0.0017	0.2185
Rotation (Rot)	0.5766	0.2839
Till × Rot	0.5695	0.9261
Mg ha⁻¹		
CT[b]	81.10 b	82.10
MT	149.62 a	108.46
NT	188.17 aA	112.30 B
WWc	145.49	109.10
FW	133.78	92.81

[a] Significance set at $p < 0.05$. [b] CT: conventional tillage; MT: minimum tillage; NT: no till. [c] WW: wheat-wheat; FW: fallow-wheat. Different lower case letters indicate significant differences among tillage practices. Different upper case letters indicate significant differences between depths within a tillage practice.

Table 6 shows the Pearson correlation coefficients and their significance levels among the studied variables. We found significant correlations among most of the studied variables like SOC, POM, PMN and C-MB.

Discussion

Crop yield and biomass

Differences in rainfall distribution and accumulation during the growing seasons 2005-2006 and 2006-2007 may have had a decisive effect on the grain yields and straw biomass productions recorded (Tables 1 and 3). In 2005-2006, rainfall was homogenously distributed, but in 2006-2007 there was very little rain from January to March (the latter being a critical month for wheat growth). The draught period in early spring and a greater weed development under NT and MT may have been the main reasons for the low wheat grain yields obtained under those practices in June 2007 (Santín-Montanyá *et al.*, 2008). On the other hand, under CT similar yields were obtained for both years. The high rainfall recorded during late spring in 2007 probably encouraged the high straw biomass production recorded for the 2007 harvest.

Soil organic carbon and nitrogen

Although there were found differences in straw biomass production between FW and WW in June 2007 the main reason of the differences in SOC and SN contents were likely the consequence of the absence of crops during the fallow period under the FW conditions. A year in fallow means a year without above- or

Table 6. Pearson correlation coefficients and significance levels for the studied variables in surface soil (data collected in May 2007)

	BD[a]	SOC	SN	C/N	C-POM	N-POM	C/N POM	PMN	C-MB
BD	1								
SOC	0.451	1							
SN	0.414	0.964***	1						
C/N	0.219	0.562**	0.336	1					
C-POM	0.547	0.857***	0.844***	0.457*	1				
N-POM	0.352	0.782***	0.773***	0.425*	0.918***	1			
C/N POM	0.457	0.221	0.227	0.085	0.250	–0.118	1		
PMN	0.491	0.486	0.518**	0.218	0.737***	0.792***	–0.075	1	
C-MB	0.273	0.569**	0.524**	0.418	0.714***	0.721***	–0.038	0.638**	1

[a] BD: bulk density; SOC: soil organic carbon; SN: soil nitrogen; POM: particulate organic matter; PMN: potentially mineralisable nitrogen; MB: microbial biomass. ***, **, *: significant at $p < 0.0001$, $p < 0.01$, $p < 0.05$, respectively.

below-ground biomass, resulting in reduced C inputs and consequently in lower SOC and SN values.

The higher SOC and SN values recorded under NT than either MT or CT at a soil depth of 0-15 cm on all sampling dates (Fig. 1) was mainly due to a greater accumulation of SOC in the first 7.5 cm of the soil (data not shown). Despite the stratification effect under NT, SOC accumulation remained significant when the total 15 cm depth layer was contemplated. In semiarid areas, the greater accumulation of SOM under conservative than under conventional practices has been reported by several authors (Álvaro-Fuentes *et al.*, 2008; Hernanz *et al.*, 2009; López-Fando & Pardo, 2011). Under intensive tillage (*e.g.,* CT) crop residues become mixed and incorporated with the soil, whereas under NT they are left on the soil surface. Thus, slower residue decomposition rates under no-tilled soil may lead to a greater SOM accumulation in the topsoil (Álvaro-Fuentes *et al.,* 2008).

Biologically active organic matter

The POM fraction is quite sensitive to crop residue inputs, fertiliser and soil disturbance, and may vary seasonally (Wander *et al*., 2007). In the present study, the POM fraction varied significantly over the growing season (Fig. 2). The highest C- and N-POM figures were recorded in July 07, and may have been due to the roots and crop residues left after the wheat harvest (Yoo & Wander, 2008). Values for C- and N-POM contents were significantly higher under NT than under CT over the entire growing season. In addition, higher values were recorded in the WW than the FW. These findings are in agreement with those of other studies performed under similar semiarid conditions (Virto *et al.,* 2007; Álvaro-Fuentes *et al*., 2008). A large proportion of the POM fraction belongs to the labile SOM pool and is physically protected within the soil aggregates (Jastrow & Miller, 1997; Wander, 2004). Thus, the POM may be mineralised more quickly if the soil is disturbed and mixed and these aggregates broken, as occurs with mouldboard ploughing. Reducing tillage intensity has been shown to increase aggregation (Martín-Lammerding *et al.,* 2011), which offers some protection to POM – this may be the first step in the C stabilisation process (Six *et al*., 2000).

The PMN values varied significantly among the studied sampling dates following the same trend as the POM fraction. Willson *et al.* (2001) also found a seasonal variability of the PMN in a corn-corn-soybean-wheat rotation. They found lower PMN values in early fall, when plants were near peak biomass (September-October), and higher PMN values in late spring, after plants senesce and incorporation of crop residues (April-June). In our study there was also found a response of PMN to residue incorporation which is probably associated to N immobilisation (July 07), however, during wheat growth (Nov-Jun) no differences in PMN were detected. The lower PMN values under FW were probably due to high mineralization rates from soil organic N pools and the lack of fertilization during the fallow period (Doran *et al.,* 1998; Needelman *et al.,* 1999). Doran *et al*. (1998) attributed the greater PMN with reduced tillage to a greater N immobilisation or to a less mineralisation (or to both). The mean value obtained for the proportion of PMN in the bulk SN was 4%, similar value was reported by other authors (Curtin & Wen, 1999).

The C-MB values recorded in this study were in the range reported for cultivated soils involved in long-term trials under semiarid conditions (Madejón *et al.*, 2009). The generally higher C-MB content found under NT than under CT may be related to the higher SOC and POM contents associated with NT. Other authors also report tillage practice to have a significant influence on the MB content (Madejón *et al.*, 2009; Melero *et al.*, 2009), and it is well known that the POM is usually the first fraction to be colonised by microorganisms (Wander *et al.*, 2007). In general, the NT treatment was associated with a gradient in C-MB over the soil profile; this was not seen with CT. Further, only under NT were significant differences seen in C-MB values between the two soil layers studied (Table 5). Under CT, the C-MB values were similar in both soil layers, a consequence of the soil being turned. These results are consistent with those reported in the literature (Hazarika *et al.,* 2009; Madejón *et al.,* 2009; López-Garrido *et al.*, 2011). The contribution of C-MB to the bulk SOC (on average 1.5%) was rather low; agricultural land values commonly range between 1 and 5%.

The C-POM, N-POM and PMN contents (under all treatment conditions) showed significant variation over the growing season, mainly due to differences in microbial activity, root exudation and C inputs after harvesting and climatic conditions. This finding is consistent with that reported in other studies (Willson *et al.,* 2001; Yoo & Wander, 2008). Therefore, when these variables are used as indicators of soil responses to specific management practices, one must take seasonal variations into account. In this study, trends in the POM fraction were most sensitive to differences in tillage practices probably due to its close relation to aggregation dynamics (Six *et al.,* 1999). On the other hand, the PMN appeared to be more sensitive to differences in crop rotation probably associated to the lack of fertilization and C inputs during the fallow period (Needelman *et al.,* 1999; Willson *et al.*, 2001).

Higher PMN values were strongly associated with higher SOC, SN, POM and C-MB values. In addition, the C-MB correlated strongly with N and C-POM and with both SOC and SN (Table 6). The POM fraction and the MB may be sources of PMN, first because POM contains a labile substrate for microorganisms and second because mineralisation is a microorganism-mediated process. The contribution of C-POM to the total SOC and N-POM/SN were relatively high (21.9 and 15.7% respectively) compared to finer-textured soils but similar to values reported for other semiarid soils of similar texture (Sainju *et al.,* 2006b). Consequently, the importance of the POM fraction to N retention and availability in sandy soils would appear to be high (Wander, 2004). The high correlation between POM and PMN found in this study ($R^2 = 0.77$) together with its high proportion in the soil, suggest that the POM may be an important source of available N to supply plant needs, consequently N fertilization could be efficiently adjusted.

Despite the inconsistent response of wheat grain production to tillage practices over the studied period, the benefits of conservation agriculture practices were found in the considered soil properties, mainly under no tillage. After more than 12 years, reducing tillage intensity enhanced surface SOC accumulation and significantly increased the soil POM, PMN and MB contents, with consequent benefits for soil fertility. The POM accumulation may account for a significant source of available N in sandy soils, which is an advantage in "low-input" agriculture.

In order to obtain a consistent data set to predict soil biological status, it is necessary to monitor those properties likely to be used as soil quality indices. Further study is needed to assess soil quality related to tillage practices in semiarid areas.

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