Soil biochemical properties in a semiarid Mediterranean agroecosystem as affected by long-term tillage and N fertilization

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Abstract

Tillage and N fertilization practices contribute to the balance between soil C inputs and outputs. Thus, the impacts of both practices and their interactions on soil organic C (SOC) dynamics must be studied. The main objective of this study was to determine long-term effects of tillage and N fertilization on soil biochemical properties in a long-term experiment established in 1996 on a dryland Typic Xerofluvent soil cropped with barley (*Hordeum vulgare* L.) in NE Spain. The response of SOC concentration, soil microbial biomass carbon (SMBC) and soil enzyme activities (DHA, dehydrogenase, and PRA, protease) to different tillage (no-tillage, NT; reduced tillage, RT; and conventional tillage, CT) and N treatments (zero, 0 kg N ha^{-1}; medium, 60 kg N ha^{-1}; and high, 120 kg N ha^{-1}) were measured in 2008 at four soil depths (i.e., 0-5, 5-10, 10-25 and 25-50 cm). All the soil biochemical properties studied showed significant differences for tillage, depth and the interaction between tillage and soil depth. However, N fertilization rates only affected the SMBC content, which was greater under 120 kg N ha^{-1} than under 0 kg N ha^{-1} in the 10-25 cm soil layer. In the soil surface layer (0-5 cm), SOC, SMBC and DHA levels in CT were about 50% of the levels in the NT plots. However, in the 10-25 cm soil layer, a greater SOC concentration in CT compared with NT and RT was also accompanied by SMBC and DHA values 30% higher in CT. Below 25 cm soil depth, similar values of soil biochemical properties were found among tillage systems. There was a significant correlation among almost all the parameters studied, with the greatest correlations between SOC and SMBC and between SOC and DHA. In semiarid Mediterranean conditions, after twelve years of experiment, tillage impacted soil biochemical properties in a greater extent compared with N fertilization even though this effect was only limited to the upper soil layers.

Keywords: Conservation tillage; Traditional tillage; Dryland farming; Soil microbial activity; Soil depth

1. Introduction

Several soil functions and properties are controlled by the content, characteristics and dynamics of soil organic matter (SOM). Thus, increases in SOM can be associated with the improvement of soil fertility and productivity (Johnston et al., 2009) and with the amelioration of major environmental issues such as climate change (Powlson et al., 2011).

Soil biochemical properties, such as soil microbial biomass and enzyme activities, are directly involved in SOM dynamics and thus highly correlated with soil organic C (SOC) levels (Acosta-Martínez et al., 2003; Melero et al., 2008). Soil microbial biomass is a pool of SOC as well as the main source of soil enzymes (Kladivko, 2001). These soil enzymes and their activities can offer indications regarding the response of microbial activity to agronomic practices. Dehydrogenases, for example, are intracellular enzymes involved in the respiration of cells and proteases are a group of hydrolytic extracellular enzymes involved in nutrient cycling, such as the cycling of soil N (Geisseler et al., 2010; Makoi and Ndakidemi, 2008).

In Mediterranean agroecosystems, tillage and N fertilization are key agronomic practices with positive or negative environmental impacts. Tillage results in aggregate breakdown stimulating SOM decomposition and diminishing soil quality (Álvaro-Fuentes et al., 2009; Fernández-Ugalde et al., 2009). No-tillage (NT) often results in greater surface soil microbial activity and enzymatic activities compared with conventionally tilled (CT) soils (Madejón et al., 2009; Melero, et al., 2009). However, despite the greater microbial activity in NT soils, NT adoption has been widely recognized as a viable practice to sequester SOC and thus to ameliorate global warming (Paustian et al., 2000). In a recent study it was estimated that in a hypothetical scenario with all the arable land in Mediterranean Spain under NT, agricultural soils could offset up to 8 Tg CO_2 yr^{-1} (Álvaro-Fuentes and Cantero-Martínez, 2010).

The addition of N fertilizers can also affect SOM dynamics, but this effect is not so clear. According to the review by Alvarez (2005), N addition generally results in an increase of SOC levels but only when crop residues are returned to the soil. However, this review, with data from more than 130 sites, showed a disparity in results with N both increasing or decreasing SOC levels. Similarly, a variable effect of mineral N addition on soil microbial activity has also been observed. Thus, while some authors, such as Li et al. (2010), reported a decrease of soil microbial biomass after the addition of mineral N, other authors found little or none effect of N on microbial biomass (Salinas-García et al., 1997; Treseder, 2008). Fauci and Dick (1994) observed that, in the short-term, the application of mineral N had limited effects on soil microbial biomass and enzyme activities whereas, in the long-term, N applications decreased microbial activity.

The N fertilization and tillage interaction effect on soil microbial biomass and enzyme activity has been scarcely studied (Melero et al., 2011; Salinas-García et al., 1997). In Mediterranean areas, Melero et al. (2011) presented data on the effects of tillage and N fertilization on some enzyme activities and SOC levels in a Vertisol located in SW Spain. However, the study was restricted to NT and CT and no information on microbial biomass was given. Therefore, the main objective of the present study was to determine long-term effects of different tillage systems and N fertilization rates on soil microbial biomass, soil enzyme activities and SOC levels in the 0-50 cm soil layer under dryland semiarid conditions. We hypothesized that (i) soil microbial biomass and enzymatic activity are higher in NT than in CT; (ii) the increase in N fertilization results in the stimulation of microbial activity due to N fertilization effects on crop growth; and (iii) microbial activity decreases with soil depth regardless of soil management.

2. Materials and methods

2.1. Location and site management

This study was conducted in a long-term experiment established in 1996 in Agramunt, Lleida (NE Spain). The mean annual rainfall in the area is 435 mm, and the soil is classified as a Typic Xerofluvent (Soil Survey Staff, 1994). At the beginning of the experiment, the soil in the Ap horizon (0-28 cm) contained 465 g kg^{-1} sand, 417 g kg^{-1} silt and 118 g kg-1 clay, with a pH (H_2O, 1:2.5) of 8.5. The SOC concentration was 11 and 8 g kg^{-1} in the 0-5 cm and 5-10 cm soil layers, respectively.

The experiment consisted of a factorial combination of three levels of N fertilization: zero (ZN) or 0 kg N ha^{-1}; medium (MN) or 60 kg N ha^{-1}; and high (HN) or 120 kg N ha^{-1} and three tillage systems: two conservation tillage systems (no-tillage, NT, and reduced tillage, RT) and one intensive tillage system (conventional tillage, CT). Tillage operations were conducted by the end of October or beginning of November. The CT treatment consisted of mouldboard ploughing to 25-30 cm depth with almost 100% of the residue incorporated in the soil. The RT treatment consisted of a cultivator pass to 10-15 cm depth with an incorporation of approximately 50% of the crop residue. In the NT treatment, no soil disturbances occurred, and sowing was done by direct drilling after spraying with herbicide (0.54 L a.i. *N*-(phosphonomethyl) glycine ha^{-1}). The cropping system consisted of barley (*Hordeum vulgare* L.) monoculture under rainfed conditions. By the end of June, at grain harvesting, the straw residue was spread over the plot in all the treatments.

The N fertilizer was broadcast, and its application was split with one-third before tillage as ammonium sulfate (21% N) and the other two-thirds at the beginning of tillering as ammonium nitrate (33.5% N). Further details of the agronomic practices can be found in previous publications (Cantero-Martínez et al., 2003; Morell et al., 2011b). The experimental design was a randomized complete block with three replicates and a plot size of 50 m x 6 m with 0.5 m between plots.

2.2. Soil sampling, measurements and statistical analysis

Sampling was carried out on the 25th of October 2008, after the summer-autumn fallow and before tillage operations. Soil samples were taken with a 4 cm diameter soil core sampler from four soil depths: 0-5, 5-10, 10-25 and 25-50 cm. In each plot, soil samples were collected at three different places 15 m apart on a longitudinal randomly selected transect. Soil samples were mixed to produce a composite sample for each soil layer, treatment, and block. In the laboratory, soil samples were sieved (<2 mm) and kept refrigerated at 4ºC. Soil water content was determined by drying a soil subsample in the oven at 105º C for 48 hours.

Total SOC concentration was determined by oxidation with potassium dichromate of dry soil (Walkley and Black, 1934). Soil microbial biomass carbon (SMBC) was determined by the chloroform fumigation-extraction method modified by Gregorich et al. (1990). Soil samples were fumigated with ethanol-free $CHCl_3$. Control samples (non-fumigated) were also established. Fumigated and non-fumigated soil samples were extracted with 0.5 M K_2SO_4. The extracts were bubbled with CO_2-free air for the removal of the $CHCl_3$, and the organic C in the extracts was quantified with a TOC-V-CSH/CSN Shimadzu analyser (Shimadzu Corporation, Tokyo, 101-8448, Japan). Soil dehydrogenase activity (DHA) was determined by the method of Trevors (1984) based on the determination of iodonitrotetrazolium formazan (INTF) produced from iodonitrophenyl tetrazolium after 20 hours incubation at room temperature. The INTF produced was measured spectrophotometrically at 490 nm. Soil protease activity (PRA) was determined after incubation of soil with casein at 50 ºC and measurement of the absorbance of the extracted tyrosine at 700 nm (Ladd and Butler, 1972).

Differences in SOC, SMBC, DHA, PRA, and the SMBC/SOC ratio among tillage systems, N fertilization rates and soil depths were determined with the R software version 2.15.0 (R Development Core Team, 2008). When significant differences were found at the 0.05 probability level, Tukey’s HSD tests were performed. Furthermore, a Pearson´s correlation analysis was performed among the measured variables.

3. Results

At the 0.01 probability level and with the exception of the SMBC/SOC ratio, all the soil chemical and biochemical properties studied showed significant differences for tillage, depth and the tillage x depth interaction (Table 1). Nitrogen fertilization affected the SMBC and the SMBC/SOC ratio and the N fertilization and tillage interaction only affected the SMBC/SOC ratio (Table 1).

The greatest SOC levels were found in the NT treatment followed by RT and CT. Differences in the total SOC concentration among tillage systems were higher in soil surface layers compared to the deepest soil layer (i.e., 25-50 cm) in which the SOC levels were similar among tillage treatments (Table 2). The greatest SOC concentration was observed in the top layer (0-5 cm) in the NT treatment followed by RT. However, in the soil surface (i.e, 0-5 cm), the SOC concentration in CT was about 50% the value in NT. On the contrary, in the 10-25 cm layer, the SOC concentration was about 20% lower in the NT and RT treatments compared with CT (Table 2). In the 25-50 cm soil layer, a similar SOC concentration was observed among tillage treatments. The SOC levels decreased with soil depth. Thus, SOC concentration in the 0-5 cm soil layer was about two-fold higher than the SOC levels in the 25-50 cm layer (Table 2).

The SMBC content was greater in NT compared with CT. The greatest SMBC values were found in the soil top layer (0-5 cm) in the NT treatment followed by RT (Table 3). As in the case of the SOC concentration, in the 0-5 cm soil layer the SMBC content in CT was about 50% the value in NT. Differences in SMBC among tillage treatments were only found in the surface soil layer (Table 3). Thus, below 5 cm soil depth the SMBC content was similar among tillage treatments. In general, SMBC decreased with soil depth. However, when analysed by tillage treatment, a similar SMBC content among soil depths was observed in the CT treatment (Table 3). Differences in SMBC among N fertilization rates were also found (Fig. 1). The SMBC content was greater under 120 kg N ha^{-1} than under 0 kg N ha^{-1}. With the addition of 120 kg N ha^{-1}, the SMBC content was about 25% higher compared with the unfertilized plots (Fig. 1).

The DHA values were higher in the NT and RT treatments compared with CT (Table 3). At the same time, differences among soil depths were also observed with a decrease in the DHA throughout the soil profile (Table 3). Similar to SMBC, the greatest DHA levels were found in the soil surface (i.e., 0-5 cm) and, in particular, in the NT and RT treatments. Below the 0-5 cm soil layer, no differences existed among tillage treatments within the same soil depth (Table 3).

The PRA values varied among tillage treatments in the next order: NT>RT>CT. Similar to the observed for the SMBC and DHA parameters, the PRA levels varied among soil layers with the highest values in the surface soil layers and the lowest values in the deep soil layers (Table 3). Thus, the greatest PRA levels were found in the 0-5 cm soil layer for the NT treatment and the lowest values in the 25-50 cm soil layer for all the tillage treatments. For the 0-5 cm soil layer, the PRA levels in the CT plots were about 30% the levels in NT. However, for the 25-50 cm soil layer, PRA values in the CT treatment were about 90% the levels in NT. As observed for the SMBC parameter, in the CT treatment no differences existed in PRA values among soil depths (Table 3).

As indicated before, the tillage x N fertilization interaction was significant for the SMBC/SOC ratio (Table 1). According to Table 4, the lowest ratios were observed in the CT and RT tillage treatments combined with 0 kg N ha^{-1} and in the NT and 60 kg N ha^{-1} treatment combination. Overall, the highest ratios were found for the 120 kg N ha^{-1} rate and the lowest for the 0 kg N ha^{-1} level (Table 4).

The correlation analysis showed significant correlation among all the parameters studied except for the SMBC/SOC ratio, which showed low correlation coefficients (Table 5). The greatest correlations were found between SOC and SMBC and between SOC and DHA (Table 5).

4. Discussion

After twelve years of experiment, soil tillage and N fertilization affected some biochemical properties. Compared to N fertilization, tillage influenced a higher number of soil properties. In particular, total SOC and the two soil enzyme activities measured (i.e., DHA and PRA) were affected by tillage treatments but not by N fertilization. In Mediterranean regions, the impact of tillage on SOC dynamics has been widely reviewed (Álvaro-Fuentes and Cantero-Martínez, 2010; Mrabet et al., 2001; Sommer et al., 2011). In most of these studies, NT adoption has resulted in an increase in the levels of SOC. However, this increase has been mostly restricted to the first centimetres of soil (Plaza-Bonilla et al., 2010). In the same experimental plots, there has been observed a 25% gain in the SOC levels of the NT plots (in the 0-30 cm soil layer) since the beginning of the experiment in 1996. However, SOC stocks have remained steady in the CT plots (Álvaro-Fuentes et al., 2012).

In the present study, significant differences in the DHA enzymatic activity were also found between tillage treatments. The dehydrogenase enzyme has been related with the oxidative capacity of soil microorganisms (Madejón et al., 2009). In the soil surface (0-5 cm), NT not only presented higher SOC and oxidative capacity, expressed as DHA, but also greater SMBC and PRA values compared with CT. Therefore, the first hypothesis of this experiment was supported by the data but only for the first soil layer. Proteases belong to the group of hydrolyses enzymes that help microbes assimilate proteins and other N containing soil organic products (Geisseler et al., 2010). Thus, despite the higher SMBC and enzymatic activity (expressed by DHA and PRA) in the soil surface, the SOC concentration was almost two-fold greater in NT compared with CT. In the same experimental plots, Morell et al. (2011a), measuring soil respiration with field chambers during two cropping seasons, observed a higher soil respiration in NT and RT compared with CT. Therefore, in our conditions, NT is stimulating higher soil microbial activity and SOC decomposition in the soil surface. During the 1997-2007 period, the NT system accumulated higher above-ground residue compared with CT specially in the 60 and 120 kg N ha^{-1} fertilization treatments (Table 6). This higher residue production under NT is constantly supplying fresh and labile organic substrates for microbial activity thus explaining the greater SMBC, DHA and PRA observed under NT compared with CT. Furthermore, it is important to mention the positive effect of soil microorganisms on SOC stabilization by soil aggregates in NT plots observed in several studies (Bossuyt et al., 2001; Six et al., 2004). In contrast, the annual intensive tillage in the CT plots prevented the accumulation of crop residue on the soil surface. Also, tillage led to the distribution of crop residues throughout the soil profile and their accumulation in deeper soil layers. This accumulation of crop residues in deep soil led to an increase in the SOC levels as observed in the 10-25 cm soil layer. In this 10-25 cm layer, SMBC and DHA were 30% higher in CT compared with NT. The relationship between SOC, DHA and SMBC was clearly reflected in the positive relationship found in the Pearson's correlation analysis performed in this study (Table 5).

In the NT plots the SMBC and DHA values decreased more than 70% from the 0-5 cm layer to the 10-25 cm layer, but in the CT plots these parameters were rather steady along the soil profile. Therefore, in CT the annual addition of fresh organic matter throughout the tilled soil layer could help to maintain microbial activity levels. Consequently, the data obtained in this study did not fully support the third hypothesis since the decrease in microbial activity with soil depth was only observed in the NT and RT treatments but not in the CT treatment.

The effects of N fertilization on soil biochemical properties were small. Actually, only two variables were significantly affected: the SMBC and the SMBC/SOC ratio. Interestingly, the higher the N fertilization rate the greater the value of SMBC. Despite significant differences were only found in the 10-25 cm layer, the 120 kg N ha^{-1} rate increased SMBC about 25% compared with the 0 kg N ha^{-1} rate. This increase in SMBC could be explained by the greater crop biomass observed in the fertilized plots compared with the unfertilized plots (Table 6), which partially supports the second hypothesis of this experiment. In different agroecosystems, other studies have not found differences in SMBC among N fertilization rates (Perucci et al., 1997; Salinas-Garcia et al., 1997). In particular, Salinas-Garcia et al. (1997) concluded that the effect of N fertilization on SMBC is indirect through the alteration of C inputs. In a global meta-analysis about the effects of N additions on microbial biomass, a 15% decline in microbial biomass was found under N fertilization (Treseder, 2008). In our study, soil samples were taken in October 2008 before crop planting and three months after crop harvest of the previous season. In this previous cropping season (i.e., 2007-2008), which was exceptionally dry (with a seasonal precipitation of only 266 mm), some of the treatments (in particular the CT plots) could not be harvested due to crop failure. However, despite the amount of crop residues being low, in the NT and RT plots the 120 kg N ha^{-1} rate compared with the 0 kg N ha^{-1} had 80% and 32% more grain yield, respectively (Morell et al., 2011b). Thus, in a cropping season with limited fresh C input, the greater C inputs observed in the 120 kg N ha^{-1} plots compared with the 0 kg N ha^{-1} plots could have stimulated microbial growth. Even though the tillage x N fertilization interaction was not significant, the higher C inputs in NT 120 kg N ha^{-1} during the 2007-2008 season could have mostly contributed to the highest SMBC in the 120 kg N ha^{-1}. This hypothesis is largely supported by the SMBC/SOC ratio in which the tillage x N fertilization interaction was significant. The highest ratio for this tillage x N fertilization interaction was obtained in the NT and 120 kg N ha^{-1} (Table 4). This difference in the ratio would confirm our hypothesis in which differences in crop residues from the previous cropping season resulted in differences in SMBC between N fertilization rates.

The lack of differences in total SOC concentration among N fertilization rates is in line with other similar studies reporting zero effects of N applications on SOC, even measuring increases in C inputs (Halvorson et al., 2002; Poirier et al., 2009). Morell et al. (2011a), measuring soil CO_2 fluxes in the same experimental plots, reported differences in the SOC concentration among N fertilization levels in the 0-5 cm soil layer. Despite the elapsed time between soil samplings in our study and sampling in the Morell et al. (2011a) study was only one year, we did not find differences in SOC values among N fertilization rates.

5. Conclusions

In semiarid Mediterranean conditions, long-term tillage affected SOC, soil microbial biomass carbon (SMBC) and the two soil-enzyme activities studied (i.e., dehydrogenase, DHA, and protease, PRA), but this influence varied with soil depth. In the soil surface, higher SOC and microbial activity was found in the NT treatment compared with the CT treatment. However, below 10 cm soil depth no differences were found among tillage systems neither in SMBC nor in DHA and PRA. Furthermore, whereas in the RT and NT systems soil biochemical properties decreased throughout the soil profile, in the CT plots similar values were observed among soil layers.

Compared to tillage, N fertilization only impacted soil microbial biomass, being greater in the fertilized plots compared with the unfertilized plots due to differences in crop growth among N fertilization rates.

Our results suggest that the two management practices evaluated impact differently on soil C cycling. Thus, while tillage has a significant impact on soil C cycling through its effects on soil microbial activity, N fertilization has little impact on soil C dynamics. Nevertheless, it is important to highlight that the effects of tillage on soil C cycling were restricted to the plough layer. These findings were obtained for particular soil, climate, and crop conditions typical of the Mediterranean areas. However, due to the lack of information available in the literature regarding the effects of the tillage and N fertilization interaction on soil microbial biomass and enzyme activity, the results of this experiment may provide useful information for other agro-climatic conditions.

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References

Acosta-Martínez, V., Zobeck, T.M., Gill, T.E., Kennedy, A.C. 2003. Enzyme activities and microbial community structure in semiarid agricultural soils. Biol. Fert. Soils 38, 216-227.

Alvarez, R. 2005. A review of nitrogen fertilizer and conservation tillage. Soil Use Manage. 21, 38-52.

Álvaro-Fuentes, J., Morell, F.J., Plaza-Bonilla, D., Arrúe, J.L., Cantero-Martínez, C. 2012. Modelling tillage and nitrogen fertilization effects on soil organic carbon dynamics. Soil Till. Res. 120, 32-39.

Álvaro-Fuentes, J., Cantero-Martínez, C. 2010. Potential to mitigate anthropogenic CO2 emissions by tillage reduction in dryland soils of Spain. Span. J. Agric. Res. 8, 1271-1276.

Álvaro-Fuentes, J., Cantero-Martínez, C., López, M.V., Paustian, K., Denef, K., Stewart, C.E., Arrúe, J.L. 2009. Soil Aggregation and Soil Organic Carbon Stabilization: Effects of Management in Semiarid Mediterranean Agroecosystems. Soil Sci. Soc. Am. J. 73, 1519-1529.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K. 2001. Influence of microbial populations and residue quality on aggregate stability. App. Soil Ecol. 16, 195-208.

Cantero-Martínez, C., Angas, P., Lampurlanes, J. 2003. Growth, yield and water productivity of barley (Hordeum vulgare L.) affected by tillage and N fertilization in Mediterranean semiarid, rainfed conditions of Spain. Field Crops Res. 84, 341-357.

Fauci, M.F., Dick, R.P. 1994. Soil microbial dynamics: short- and long-term effects of inorganic and organic nitrogen. Soil Sci. Soc. Am. J. 58, 801-806.

Fernández-Ugalde, O., Virto, I., Bescansa, P., Imaz, M.J., Enrique, A., Karlen, D.L. 2009. No-tillage improvement of soil physical quality in calcareous, degradation-prone, semiarid soils. Soil Till. Res. 106, 29-35.

Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B. 2010. Pathways of nitrogen utilization by soil microorganisms - A review. Soil Biol. Biochem. 42, 2058-2067.

Gregorich, E.G., Wen, G., Voroney, R.P., Kachanoski, R.G. 1990. Calibration of a rapid direct chloroform extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 22, 1009-1011.

Halvorson, A.D., Wienhold, B.J., Black, A.L., 2002. Tillage, nitrogen, and cropping system effects on soil carbon sequestration. Soil Sci. Soc. Am. J. 66, 906–912.

Johnston, A.E., Poulton, P.R., Coleman, K., 2009. Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. Adv. Agron. 101, 1–57.

Kladivko, E.J. 2001. Tillage systems and soil ecology. Soil Till. Res. 61, 61-76.

Ladd, J.N., Butler, J.H.A. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19-30.

Li, L.-J., Zeng, D.-H., Yu, Z.-Y., Fan, Z.-P., Mao, R. 2010. Soil microbial properties under N and P additions in a semi-arid, sandy grassland. Biol. Fertil. Soils 46, 653-658.

Madejón, E., Murillo, J.M., Moreno, F., López, M.V., Arrúe, J.L., Álvaro-Fuentes, J., Cantero, C. 2009. Effect of long-term conservation tillage on soil biochemical properties in Mediterranean Spanish areas. Soil Till. Res. 105, 55-62.

Makoi, J.H.J.R., Ndakidemi, P.A. 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. African J. Biotech. 7, 181-191.

Melero, S., Vanderlinden, K., Ruiz, J.C., Madejón, E. 2008. Long-term effect on soil biochemical status of a Vertisol under conservation tillage system in semi-arid Mediterranean conditions. Eur. J. Soil Biol. 44, 437-442.

Melero, S., Vanderlinden, K., Ruiz, J.C., Madejón, E. 2009. Soil biochemical response after 23 years of direct drilling under a dryland agriculture system in southwest Spain. J. Agric. Sci. 147, 9-15.

Melero, S., López-Bellido, R.J., López-Bellido, L., Muñoz-Romero, V., Moreno, F., Murillo, J.M. 2011. Long-term effect of tillage, rotation and nitrogen fertiliser on soil quality in a Mediterranean Vertisol. Soil Till. Res. 114, 97-107.

Morell, F.J., Cantero-Martínez, C., Lampurlanés, J., Plaza-Bonilla, D., Álvaro-Fuentes, J. 2011a. Soil carbon dioxide flux and organic carbon content: effects of tillage and nitrogen fertilization. Soil Sci. Soc. Am. J. 75, 1874-1884.

Morell, F.J., Lampurlanés, J., Álvaro-Fuentes, J., Cantero-Martínez, C. 2011b. Yield and water use efficiency of barley in a semiarid Mediterranean agroecosystem: Long-term effects of tillage and N fertilization. Soil Till. Res. 117, 76-84.

Mrabet, R., Saber, N., El-Brahli, A., Lahlou, S., Bessam, F. 2001. Total, particulate organic matter and structural stability of a Calcixeroll soil under different wheat rotations and tillage systems in a semiarid area of Morocco. Soil Till. Res. 57, 225-235.

Paustian, K., Six, J., Elliot, E.T., Hunt, H.W. 2000. Management options for reducing CO_2 emissions from agricultural soils. Biogeochemistry 48, 147-163.

Perucci, P., Bonciarelli, U., Santilocchi, R., Bianchi, A.A. 1997. Effect of rotation, nitrogen fertilization and management of crop residues on some chemical, microbiological and biochemical properties of soil. Biol. Fertil. Soils. 24, 311-316.

Plaza-Bonilla D, Cantero-Martínez C, Álvaro-Fuentes J. 2010. Tillage effects on soil aggregation and soil organic carbon profile distribution under Mediterranean semi-arid conditions. Soil Use Manage. 26, 465-474.

Poirier, V., Angers, D.A., Rochette, P., Chantigny, M.H., Ziadi, N., Tremblay, G., Fortin, J. 2009. Interactive effects of tillage and mineral fertilization on soil carbon profiles. Soil Sci. Soc. Am. J. 73, 255-261.

Powlson, D.S., Gregory, P.J., Whalley, W.R., Quinton, J.N., Hopkins, D.W., Whitmore, A.P., Hirsch, P.R., Goulding, K.W.T. 2011. Soil management in relation to sustainable agriculture and ecosystem services. Food Policy, 36, 72-87.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Salinas-Garcia, J.R., Hons, F.M., Matocha, J.E., Zuberer, D.A. 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. Biol. Fert. Soils 25, 182-188.

Six J., Bossuyt, H., Degryze, S., Denef, K. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res. 79, 7-31.

Soil Survey Staff. 1994. Keys to Soil Taxonomy. United States Department of Agriculture, Soil Conservation Service, Washigton, USA, 306 pp.

Sommer, R., Ryan, J., Masri, S., Singh, M., Diekmann, J. 2011. Effect of shallow tillage, moldboard plowing, straw management and compost addition on soil organic matter and nitrogen in a dryland barley/wheat-vetch rotation. Soil Till. Res. 115-116, 39-46.

Treseder, K.K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11, 1111-1120.

Trevors, J.T. 1984. Dehydrogenase activity in soil: A comparison between the INT and TTC assay. Soil Biol. Biochem. 16, 673-674.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

Figure caption

Fig. 1. Mean and standard error of soil microbial biomass carbon (SMBC) for different nitrogen (N) fertilization rates (0 kg N ha-1; 60 kg N ha-1, 120 kg N ha-1) after 12 years of experiment on a Typic Xerofluvent soil in NE Spain. Different lower case letters indicate significant differences among N rates ($P<0.05$).

Tables

Table 1

Analysis of variance (mean square) of the effects of tillage (Til), nitrogen fertilization (Nit), soil depth (Depth), and their interactions on total soil organic carbon concentration (SOC), soil microbial biomass carbon (SMBC), soil dehydrogenase activity (DHA), soil protease activity (PRA) and the SMBC/SOC ratio (n=108).

Source of variation	d.f.[a]	SOC (g kg^{-1} dry soil)	SMBC (mg C kg^{-1} dry soil)	DHA (mg INTF kg^{-1} dry soil h^{-1})	PRA (mg Tyr kg^{-1} dry soil h^{-1})	SMBC/SOC (%)
Til	2	32.62***	35995***	15.86**	21613***	0.24
Nit	2	0.38	28801**	1.31	509	4.00*
Til x Nit	4	0.41	9344	2.36	205	2.53**
Depth	3	228.08***	211859***	174.08***	8423***	0.15
Til x Depth	6	44.74***	39815***	19.07***	4200***	0.40
Nit x Depth	6	1.20	2766	2.58	417	0.31
Til x Nit x Depth	12	0.79	2479	1.21	360	0.29

[a] d.f., degrees of freedom; *, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2

Mean and standard error of total soil organic carbon (SOC) concentration for different tillage systems (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and soil depths after 12 years of experiment on a Typic Xerofluvent soil in NE Spain.

Soil depth (cm)	SOC (g kg^{-1} dry soil)			
	CT	RT	NT	**Depth**
0-5	7.86 ± 0.14ef[*]	12.35 ± 0.31b	15.81 ± 0.67a	12.00 ± 0.68a
5-10	8.05 ± 0.26de	10.73 ± 0.32c	9.39 ± 0.54cd	9.39 ± 0.30b
10-25	7.99 ± 0.24de	6.39 ± 0.18fg	6.58 ± 0.25efg	6.98 ± 0.18c
25-50	5.66 ± 0.28g	5.11 ± 0.21g	5.25 ± 0.25g	5.34 ± 0.14d
Til	7.39 ± 0.20c	8.65 ± 0.52b	9.25 ± 0.72a	

[*] Different lower case letters indicate significant differences at $P<0.05$. **Til** refers to the mean value of tillage systems. **Depth** refers to the mean value of each soil depth.

Table 3

Mean and standard error of soil microbial biomass carbon (SMBC), soil dehydrogenase activity (DHA) and soil protease activity (PRA) for different tillage systems (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) and soil depths after 12 years of experiment on a Typic Xerofluvent soil in NE Spain.

Soil depth (cm)	Tillage systems CT	 RT	 NT	 **Depth**
SMBC (mg C kg^{-1} dry soil)				
0-5	254 ± 18cd[*]	375 ± 34b	500 ± 32a	376 ± 25a
5-10	247 ± 18cd	304 ± 21bc	307 ± 22bc	286 ± 12b
10-25	254 ± 19cd	202 ± 16cd	195 ± 18d	218 ± 11c
25-50	170 ± 19d	170 ± 12d	178 ± 20d	173 ± 9d
Til	231 ± 10b	263 ± 17ab	295 ± 24a	
DHA (mg INTF kg^{-1} dry soil h^{-1})				
0-5	4.89 ± 0.50cd	7.90 ± 1.00ab	10.15 ± 0.57a	7.65 ± 0.58a
5-10	4.10 ± 0.22cde	5.93 ± 0.91bc	4.64 ± 0.65cd	4.89 ± 0.39b
10-25	3.48 ± 0.41cdef	3.07 ± 0.34def	2.60 ± 0.41def	3.05 ± 0.22c
25-50	1.85 ± 0.39ef	2.05 ± 0.28ef	1.51 ± 0.28ef	1.80 ± 0.18d
Til	3.58 ± 0.27b	4.74 ± 0.52a	4.73 ± 0.61a	
PRA (mg Tyr kg^{-1} dry soil h^{-1})				
0-5	44.4 ± 6.6e	101.4 ± 9.9bc	139.9 ± 9.1a	95.2 ± 9.1a
5-10	51.7 ± 5.6e	108.3 ± 6.3ab	119.1 ± 6.6ab	93.0 ± 7.8ab
10-25	66.4 ± 5.1cde	72.1 ± 3.2cde	91.2 ± 6.5bcd	76.5 ± 4.0b
25-50	54.8 ± 7.1e	55.3 ± 11.0e	61.3 ± 10.1de	57.1 ± 2.9c
Til	54.3 ± 3.2c	84.3 ± 5.4b	102.9 ± 6.4a	

[*] For each biochemical parameter, different lower case letters indicate significant differences at *P*<0.05. **Til** refers to the mean value of tillage systems. **Depth** refers to the mean value of each soil depth.

Table 4

Mean and standard error of the ratio between soil microbial biomass carbon and soil organic carbon concentration (SMBC/SOC) (%) for different nitrogen (N) fertilization rates (0 kg N ha^{-1}; 60 kg N ha^{-1}, 120 kg N ha^{-1}) and tillage systems (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) after 12 years of experiment on a Typic Xerofluvent soil in NE Spain.

Tillage	SMBC/SOC (%)		
system	0 kg N ha^{-1}	60 kg N ha^{-1}	120 kg N ha^{-1}
CT	2.63 ± 0.21b[*]	3.54 ± 0.17ab	3.18 ± 0.20ab
RT	2.87 ± 0.29b	3.21 ± 0.21ab	3.28 ± 0.18ab
NT	3.02 ± 0.25ab	2.71 ± 0.22b	4.06 ± 0.31a
Mean	2.84 ± 0.14B	3.15 ± 0.13AB	3.51 ± 0.15A

[*] Different lower case letters indicate significant differences among tillage treatments and N fertilization rates (*P*<0.05). Different upper case letters indicate significant differences among mean N rates (*P*<0.05).

Table 5

Pearson correlation coefficients among total soil organic carbon concentration (SOC), soil microbial biomass carbon (SMBC), dehydrogenase activity (DHA), protease activity (PRA), and the SMBC/SOC ratio.

	SOC	SMBC	DHA	PRA	SMBC/SOC
SOC	–	0.814^{***}	0.777^{***}	0.711^{***}	-0.137
SMBC		–	0.707^{***}	0.549^{***}	0.414^{***}
DHA			–	0.518^{***}	0.007
PRA				–	-0.141
SMBC/SOC					–

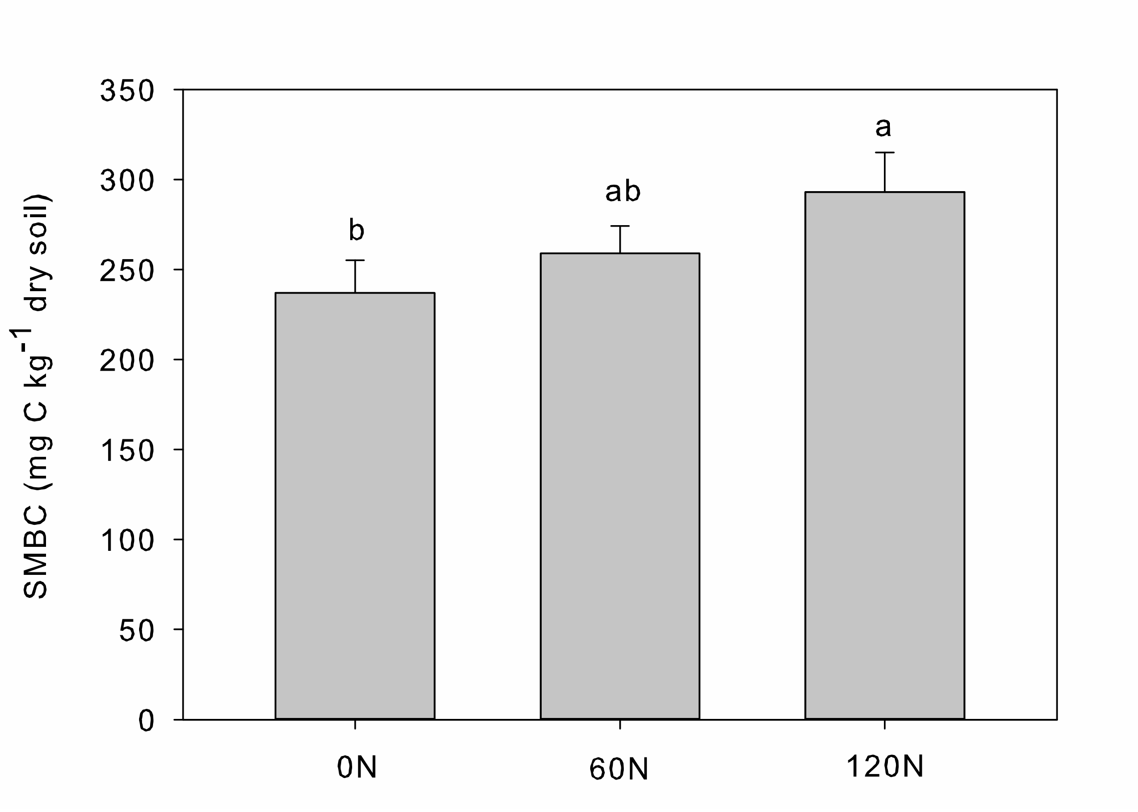
*** Significant at the 0.05 level.

Table 6

Mean above-ground residue input at harvest of a winter barley crop during the 1997-2007 period for different nitrogen (N) fertilization rates (0 kg N ha^{-1}; 60 kg N ha^{-1}, 120 kg N ha^{-1}) and tillage systems (CT, conventional tillage; RT, reduced tillage; NT, no-tillage) on a Typic Xerofluvent soil in NE Spain.

Tillage	Above-ground residue input (kg ha^{-1})		
system	0 kg N ha^{-1}	60 kg N ha^{-1}	120 kg N ha^{-1}
CT	1351 ± 12	1571 ± 12B*	1748 ± 15B
RT	1190 ± 9b	1679 ± 10aB	1612 ± 11aA
NT	1362 ± 11b	2083 ± 13aA	2265 ± 11aA

* Different lowercase letters indicate significant differences between N fertilization rates within tillage treatments (P<0.05). Different uppercase letters indicate significant differences between tillage treatments within N fertilization rates (P < 0.05).


SMBC (mg C kg^{-1} dry soil)
350
300
250
200
150
100
50
0
b
ab
a
0N
60N
120N