Carbon storage in a rainfed Mediterranean Vertisol: Effects of tillage and crop rotation in a long-term experiment

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Running title: Carbon storage in a rainfed Mediterranean Vertisol

Summary

The storage of carbon (C) in cultivated soils can be increased with the adoption of different practices. The objective of this study was to determine soil organic carbon (SOC) storage in the 0- to 90-cm depth profile, in four different soil layers (0–15, 15–30, 30–60 and 60–90 cm) in a long-term (29 years) experiment established in 1986 on a rainfed Mediterranean Vertisol in southern Spain. The treatments studied were: conventional tillage (CT) versus no-tillage (NT); five 2-year crop rotations (wheat–chickpea, wheat–sunflower, wheat–bare fallow, wheat–faba bean, and continuous wheat; and nitrogen (N) fertilizer applied to wheat at four rates (0, 50, 100 and 150 kg N ha–1). The SOC accumulation was higher in the 30–60-cm layer (9.2 Mg ha^{-1}) due to the size of the characteristic cracks of Vertisols under semiarid conditions. Over the 29-year study period, the SOC in the 0–90-cm layer increased by 23.6 Mg ha^{-1} due to the change in residue management. The NT treatment exhibited a higher mean annual rate of organic C accumulation compared with the CT treatment (1.0 and 0.66 Mg ha^{-1} year^{-1}, respectively) due to the retention of the mulched residue. Additionally, crop rotation influenced the rate of organic C accumulation, with wheat-faba bean, wheat-sunflower and continuous wheat exhibiting the highest levels of C storage in comparison to the other treatments. In rainfed Mediterranean agriculture, the selection of no-tillage along with a rotation with legumes is key to improving soil fertility and increasing C reserves and the rate of C accumulation by soil.

Keywords: Conventional tillage, crop residue, no tillage, soil organic carbon, wheat

1. Introduction

Carbon (C) sequestration by agricultural lands has generated great international interest due to its potential benefits and the corresponding effects on agriculture. Increased sequestration of C in soil occurs when a set of management practices promote the storage of C. The impacts of these practices, such as tillage system, crop rotation or fertilization, as well as improvements in water management, have been well-studied and differ according to soil type, cultivation system, crop residue management and climate (Baldock, Wheeler, McKenzie, & McBrateny, 2012; Lal, 2012; Lal, Negassa, & Lorenz, 2015; Sanderman, Farquharson, & Baldock, 2010; Snyder, Bruulsema, Jensen, & Fixen, 2009).

The main soil properties that affect C sequestration include the depth of the soil layer, the clay and silt content and the predominance of expansive 2:1 clay minerals (montmorillonite and vermiculite). Soils with characteristics that favour the formation of stable macro- and micro-aggregates have a greater capacity to act as sinks of organic C in comparison to soils that are “weakly structured” or “structurally inert” (Baldock et al., 2012). Soil texture is one of the properties with the greatest influence on soil organic C (SOC) levels. Numerous studies have found a correlation between the organic C content and the clay content of soil. Clay soils accumulate organic C relatively quickly, while sandy soils accumulate C to a much lesser extent (Lal, 2014).

Rainfed agrosystems in Mediterranean climates have unique characteristics that limit their ability to sequester C. These soils often experience high temperatures, scarce or irregular rainfall, minimal cloud cover and low quantities of crop residues, which act as poor surface covers for minimizing the impact of radiation and the effects of water and wind erosion. As a result, the soils of Mediterranean rainfed agrosystems inherently have low contents of organic matter and nutrients and rapidly lose large proportions of their C content in the form of carbon dioxide (CO_2) after tillage or similar conventional crop management practices. In general, the concentration of SOC should be maintained above the critical threshold of 1.2–1.5 % in the root zone (15–30 cm depth) (Lal, 2014). Decreasing the SOC has adverse effects on the soil structure, increases erosion and can favour the formation of crusting, compaction, runoff and erosion (Lal, 2004). The soils of many agrosystems in arid and semiarid zones have been continuously managed under extractive agricultural practices, which include uncontrolled grazing and the removal of crop residues for other uses (e.g., animal feed, construction materials, fuel and paper manufacturing) (Lal, 2014).

Conversion of native land for agriculture has typically resulted in decreases in SOC stock in the order of 40 to 60% from pre-clearing levels (Sanderman et al., 2010). The adoption of practices to increase the organic C content of Vertisols has not been well explored, although Lopez-Bellido, Fontan, Lopez-Bellido, and Lopez-Bellido (2010) suggest that C sequestration in rainfed Mediterranean Vertisols should not be considered negligible due to the aforementioned characteristics of this soil type. Tillage can affect soil heat capacity and thermal conductivity and, therefore, thermal diffusivity by changing soil organic matter, bulk density, inter-aggregate contact and moisture content (Shukla, Lal, & Ebinger, 2003; Van Wie, Adam, & Ullman, 2013). Olson, Al-Kaisi, Lal, and Lowery (2014) argue that there is ample evidence that SOC may be substantially increased by switching from conventional tillage (CT) to less intensive methods, known as conservation tillage. A metanalysis by Aguilera, Lassaletta, Gattinger, and Gimeno (2013) showed an average increase of 11.2% in SOC and a C sequestration rate of 0.44 Mg C ha^{-1} per year under no tillage (NT) management compared with conventional management in Mediterranean cropping systems.

Tillage increases SOC mineralization by bringing crop residue closer to microbes where soil moisture conditions favour mineralization (Gregorich, Greer, Anderson, & Liang, 1998), physically disrupts aggregates and exposes hitherto encapsulated C to decomposition. Both activities decrease soil moisture, increase maximum soil temperature and exacerbate the rate of SOC mineralization (Lal, 2004). In general, as tillage intensity decreases, soil C sequestration increases under continuous crop rotation, although this does not apply to wheat–bare fallow rotation (bare fallow is uncultivated). The stratification of C in surface soil layers and its varying bulk density in reduced tillage systems complicate comparisons and interpretations with respect to C capture among different tillage systems. Many studies have analysed the soil C content at a depth of 30 cm or less, which may lead to overestimation of the soil C capture in reduced tillage systems compared with conventional tillage systems (Snyder et al., 2009). When the effects of different management practices on organic C storage in soil are compared, the depth of the sample is a determinant factor. Hence, the entire soil profile should be considered to fully assess all the effects of tillage management practices on SOC gains or losses, rather than just considering the effect of tillage on topsoil layers only (Dimassi et al., 2014; Olson & Al-Kaisi, 2015; Powlson et al., 2014). Conventional tillage practices affect the whole profile of the soil and the stratification of crop residues depends on tillage depth and type of equipment.

In general, changes in the SOC content induced by management practices may occur over a period of several years or decades, until soil C reaches a new equilibrium. Considering such a time scale, it is evident that long-term experiments are the only means of studying the dynamics of soil organic C, especially with regard to the relationship between organic C content and management practices. The lack of relevant studies prevents a quantitative evaluation of the potential of agricultural soils to sequester C. Therefore, the objective of this study was to determine the effect of tillage system, crop rotation and N fertilization on SOC storage in the 0–90-cm depth profile in a long-term experiment conducted over 29 years on a Vertisol under rainfed Mediterranean conditions in southern Spain.

2. Materials and methods

2.1. Site

Field experiments were conducted in Cordoba, southern Spain (37.755 N, 4.536 W, 200 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region, where rainfed cropping is the standard practice (Table 1). The average annual rainfall during the study period was 549 ± 198 mm, varying from 233 mm (1998) to 1007 mm (1996) (Figure 1). The average maximum temperature for the period was 25 ± 0.96°C, and the average minimum temperature was 11 ± 0.76°C. The interannual temperature variability was notably lower than that of rainfall, which is characteristic of Mediterranean climates (Fig. 1).

2.2. Experimental design

The study took place over a 29-year period within the framework of a long-term experiment called `Malagon’ that began in 1986 and was designed as a randomized complete block with a split–split plot arrangement and three replications. The main plots tested the effects of the tillage system (no-tillage and conventional tillage); subplots tested 2-year crop rotations (wheat–wheat, wheat–fallow, wheat–

chickpea, wheat–faba bean and wheat–sunflower); and subsubplots tested N fertilizer rate (0, 50, 100 and 150 kg N ha^{-1}). Duplicate sets of plots were stablished within each block to allow all phases of the rotation to be present each year. The area of each sub-subplot was 50 m^2 (10 by 5 m).

The no-tillage (NT) plots were sown with a no-till seed drill. Weeds were controlled with glyphosate + MCPA [(4-chloro-2-methylphenoxy) acetic acid] at a rate of 0.5 + 0.5 L a.i. ha^{-1} prior to planting. The conventional tillage (CT) included moldboard plowing at 30-cm depth after crop harvest, disk harrowing in October and a vibrating tine cultivation to prepare the seedbed before planting. Information on cultivar, planting, and herbicides applied during the growing season is provided in Lopez-Bellido, Muñoz-Romero, Fernandez-Garcia, and Lopez-Bellido (2014). Nitrogen fertilizer was applied to wheat plots as broadcast ammonium nitrate. For all application rates, half of the N was applied before planting (incorporated by disk harrowing in CT plots and surface-broadcast in NT plots). The other half of the N was top dressed at the end of wheat tillering (Zadoks Growth Stage 29; Zadoks, Cheng, & Konzak., 1974). Each wheat year, a rate of 65 kg phosphorus (P) ha^{-1} as triple superphosphate was applied, incorporated into CT and banded in NT in the seed furrow at planting. Potassium fertilizer was not applied because the soil test K (ammonium acetate test) indicated that the level of K in the soil was adequate (≥530 mg kg^{-1}). Crop residues were returned to the soil in all plots. Before starting the experiment in 1986, the common practice was to harvest the wheat straw to make bales of straw and burn crop stubble.

2.3. Soil and aboveground residue analysis

Soil samples were collected every 3 years in November, starting in 1986. Prior to sowing, three soil cores (0–90 cm deep) were sampled from each wheat plot using a manual Eijkelkamp auger (Giesbeek, The Netherlands). Since 2012, the soil core samples have been collected up to 120 cm in order to investigate the presence of C in deeper layers. One sample consisting of two soil cores was used to determine SOC concentration and another, consisting of one core, to determine bulk density (Blake & Hartage, 1986). The samples were sectioned into four segments (0–15, 15–30, 30–60 and 60–90 cm) to analyse each soil depth. Because of the soil's high clay levels (Table 1), the samples were dried before milling and sieving. The samples were crumbled as finely as possible and spread on trays to facilitate air-drying for 48 hr. Subsequently, they were ground, sieved (2 mm) and maintained (frozen) in plastic bags at –30 °C until analysis to avoid decomposition of organic matter.

Organic C in the soil samples was determined by near infrared reflectance spectroscopy (NIRS) (Martin, Malley, Manning, & Fuller, 2002). Soil samples were scanned with a Foss NIRSystems 6,500 monochromator (Foss NIRSystems, Silver Spring, MD, USA). WinISI II 1.5 software (Infrasoft International, Port Matilda, PA, USA) was used for spectra data analysis and model development. The model was developed by Fontan, Lopez-Bellido, Garcia-Olmo, and Lopez-Bellido (2011) to predict the soil C (total, inorganic and organic). The reference method used to determine total C was dry combustion by an elemental analyzer (EA 3000 Eurovector SpA, Milan, Italy). Part of the sample was incinerated in a muffle furnace at 450_C for 16 hr, to eliminate all organic C (Nelson & Sommers, 1996). The non-incinerated and incinerated samples were analyzed, determining total and inorganic C, respectively. The organic C of the sample was calculated as the difference between both values. The calibration method was based on cross-validation. Calibrations were made using a modified partial least square regression for the chemical values of total, inorganic and organic C and their corresponding spectral data. The calibration set consisted of 492 samples and the validation set consisted of 161 samples, all of them from the same experiment. The predictive quality of the models obtained was confirmed by the results from the validation performed by Fontan et al. (2011). Soil organic C content (Mg ha^{-1}) (SOCS) for each soil depth was calculated using the equation:

SOCS =SOC × BD× SD,

where SOC is in g kg^{-1}, BD is bulk density (Mg m^{-3}) and SD is soil depth (m). The accumulation rate of SOC was determined as the difference between SOC stock in 2015 and 1986 divided by the number of years.

Each year, the harvested biomass returned was determined. For wheat, a 0.5 by 0.5-m quadrat was randomly placed in each of the plots at harvest. Crop residue dry matter was determined by drying the sampled plants at 80_C to constant weight. The organic C concentration of crop residues was analyzed by elemental analysis (EA 3000 Eurovector SpA, Milan, Italy). Aboveground residue C for wheat in each 2-year rotation was determined by multiplying the straw yield (Mg ha^{-1}) by its C concentration.

2.4. Statistical analysis

The data were analysed using a mixed-model analysis of variance (ANOVA) with three fixed factors: tillage system, crop rotation and N rate. Years and blocks (replications) were considered a random factor. Aboveground residue C, soil bulk density, SOC content as function of depth, total SOC and annual rate of C accumulation were subjected to ANOVA using a split–split plot design combined over the years, tillage system, preceding crop and N rate following the error term according to McIntosh (1983). For data obtained from soil samples, repeated measures analysis was used because depth was a repeated measurement. Analyses of variance were performed using the Analytical Software Statistix 8.1 program (Analytical Software, 2005) to determine treatment effects. When significant effects were found, treatment means were compared using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$.

3. Results

3.1. Soil Bulk Density

The soil bulk density varied significantly by year, tillage system and soil depth and by year × soil depth, tillage × soil depth and year × tillage × soil depth interactions (Table 2). In the experiment as a whole, the NT treatment exhibited higher soil bulk density than the CT treatment for all analysed soil layers (0–15, 15–30, 30–60, 60–90 cm) (Table 2). Soil bulk density increased with soil depth.

3.2. Aboveground residue carbon

The biennial and total aboveground residue C from wheat differed significantly for all of the sources of variation. Biennial aboveground wheat residues varied between 1.23 and 4.2 Mg ha^{-1} (Table 3). The NT treatment had significantly higher amounts of residue (5.91 Mg ha^{-1}) in comparison to CT (5.73 Mg ha^{-1}). The year × tillage system interaction differed between NT and CT, with no significance in some years and with residue carbon levels higher in CT than in NT in only one year (Figure 2). The continuous wheat rotation had the highest amount of residues, both biennially and overall, whereas the other rotations exhibited the following decreasing trend: wheat–faba bean > wheat–sunflower = wheat–chickpea > wheat–bare fallow (Table 3). Higher rates of N fertilizer applied to wheat significantly increased the amount of both biennial and total residues (Table 3). The tillage system × N rate interaction showed significant differences in the amounts of total residue C between tillage systems for the 100 and 150 kg N ha^{-1} rates, which were higher for NT (Figure 3).

3.3. Soil organic carbon storage

The storage of SOC over the 29-year study period varied significantly according to year, tillage system, crop rotation, N fertilizer rate and soil depth (Table 4). The SOC increased across all of the soil layers during the 1986–2015 period (Fig. 4), corresponding to an increase of 4.9, 4.4, 9.2 and 5.1 Mg ha^{-1} in the 0–15, 15–30, 30–60 and 60–90 cm layers, respectively. They were significant and linear, with the exception of the deepest soil layer (60–90 cm) (Fig. 4). The SOC accumulated int the total soil profile increased significantly and linearly by 23.6 Mg ha^{-1} in the 29-yr period relative to the baseline level of 30.6 Mg ha^{-1} in 1986 (Fig. 5). The SOC values in the 90-120 cm depth during the years in which the sampling depth was extended, were 7.9 and 7.7 Mg ha^{-1} in 2012 and 2015, respectively (data not shown). These values were significantly lower than in upper depths.

The third-order interaction year x tillage x soil depth (Fig. 5) differed throughout the study period (1986–2015) with respect to the accumulation of SOC between the two tillage systems and among the soil layers. Independent of the year-to-year variations derived from climate variability, especially rainfall, differences in SOC observed in the surface layer (0–15 cm) between tillage systems were always significant and consistently greater than those of the 15–30 cm layer, in which differences were not always significant (Fig.5). In the 30–60 and 60–90 cm layers, differences between the tillage systems were significant for all years (Fig. 5), and SOC accumulation was greater in the NT system; overall, these two layers accumulated more SOC than the more superficial soil layers. At the end of the study period (2015), SOC accumulation in the 0–90 cm profile was 28.4 Mg ha^{-1} in the NT system and 18.8 Mg ha^{-1} in the CT system, equivalent to 60 and 40 %, respectively, of the total storaged SOC. In the soil layers of the NT and CT systems, 5.9 and 4 Mg ha^{-1} were accumulated in the 0–15 cm layer, 5.6 and 3.2 Mg ha^{-1} in the 15–30 cm layer, 10 and 8.3 Mg ha^{-1} in the 30–60 cm layer and 6.9 and 3.3 Mg ha^{-1} in the 60–90 cm layer, respectively.

Accumulated SOC differed significantly according to the studied crop rotations (Table 4). The wheat-sunflower, continuous wheat and wheat-faba bean rotations exhibited the highest C stock, with no significant differences (28.6, 25.2 and 25.0 Mg ha^{-1}, respectively). The wheat-bare fallow and wheat-chickpea rotations accumulated lower quantities of SOC (i.e., 22.5 and 16.7 Mg ha^{-1}, respectively) (Fig. 6). The interaction of the rotations with the NT system enhanced SOC storage in comparison to CT for almost all rotations, especially for continuous wheat and wheat-bare fallow, which demonstrated greater differences in SOC between NT and CT. Except for the wheat-chickpea rotation, which had the lowest SOC content among the NT rotations (19.7 Mg ha^{-1}), the other NT rotations accumulated an average of approximately 30 Mg ha^{-1} during the study period, with few differences among them (Fig. 6).

The effect of N fertilizer on the accumulation of SOC was significant (Table 4). However, the results were contradictory, as there was no logical relationship between the rate of N fertilizer and the SOC content, except in the 0–15 cm layer, where the zero rate corresponded to the lowest SOC content; there were no differences in the other soil layers.

The mean annual rate of organic C accumulation in the 1986–2015 period was 0.82 ± 0.3 Mg ha^{-1} $year^{-1}$, and the differences between tillage systems were significant: 1.0 Mg ha^{-1} $year^{-1}$ in NT vs. 0.66 Mg ha^{-1} $year^{-1}$ in CT. This rate also differed according to rotation and was higher in the wheat-sunflower, continuous wheat and wheat-faba bean rotations (1.0, 0.9 and 0.85 Mg ha^{-1} $year^{-1}$, respectively). The rates were lowest for the wheat-bare fallow and wheat-chickpea rotations: 0.8 and 0.6 Mg ha^{-1} $year^{-1}$, respectively (Fig. 6). Figure 6 shows that climate conditions, especially the interannual variability in rainfall, resulted in strong variations in the storage of SOC and in some cases, negative rates of organic C accumulation.

4. Discussion

For the entire group of years studied, the soil bulk density was higher in NT than in CT across all of the soil layers. These results contrast with those found by Lopez-Bellido et al. (2010) for the same experiment, where differences were only found in the mean soil bulk density of the subsurface layer (15–30 cm). However, compaction from tractor traffic could have an effect on soil bulk density as a function of soil water content in some years. In contrast, Mazzoncini et al. (2016) found a higher soil bulk density in CT in comparison to NT in the 0–10, 10–20 and 20–30 cm soil layers as part of a 28-yr study on a silt-sandy soil in central Italy.

Overall, NT resulted in a greater quantity of aboveground wheat residues compared with CT, which is confirmed by the higher yield of the cereal in the NT treatment for the majority of studied years, especially in dry years due to the more favourable soil conditions (water content, organic matter, aggregation, temperature, etc.), as shown previously for the same experiment (Lopez-Bellido et al., 2012; Lopez-Bellido, Fuentes, Castillo, Lopez-Garrido & Fernandez, 1996; Lopez-Bellido, Lopez-Bellido, Benitez-Vega & Lopez-Bellido, 2007; Lopez-Bellido, Lopez-Bellido, Castillo & Lopez-Bellido, 2000; Muñoz-Romero, Lopez-Bellido & Lopez-Bellido, 2015). Turmel, Speratti, Baudron, Verhulst, and Govaerts (2015) reported that the greater input of residues to soil under NT and their maintenance on the soil surface improves soil aggregation, protects against erosion and soil loss, and increases soil compaction at the surface, favouring an increase in the soil organic C stock.

The amount of crop residue generated was higher for the continuous wheat in comparison to the other rotations. Similar results were obtained by Bonciarelli et al. (2016), where the highest total values were generally recorded with a higher presence of wheat in the rotation. However, the monoculture causes a loss of biodiversity in the soil, leading to a yield loss with respect to crop in rotation (Lopez-Bellido & Lopez-Bellido, 2013). Although the N fertilizer rates applied to wheat increased crop residue, there was no effect on the SOC content. The input of N fertilizer has a mixed effect on soil, as it stimulates both plant growth and microbial activity (Halvorson, Wienhold, & Black, 2002; Khan, Mulvaney, Ellsworth, & Boast, 2007), although differently, in all soil layers.

Over the course of the 29-yr study, the SOC content increased across all soil layers. The retention of residues in the soil, whether well-incorporated under CT or left on the surface in the NT system, is the main cause of this increase. The differences among layers, with greater C accumulation in the 30–60 cm layer, are explained by the formation of the characteristic cracks of Vertisols, especially under rainfed conditions in a Mediterranean climate. These cracks reach depths corresponding to the 30–60 cm layer, as shown in the same experiment (Lopez-Bellido, Muñoz-Romero, Lopez-Bellido, Guzman, & Lopez-Bellido, 2016), allowing C from the surface layers to naturally penetrate the deeper soil layers and become part of the stable SOC reserve. Recently, Alcantara, Don., Well, and Nieder (2016) proposed using deep ploughing to incorporate organic C on surface layers into deeper layers, thereby increasing the stable C reserves of the soil profile. However, this process occurs naturally in the Vertisols of semiarid climates. The majority of studies on C sequestration have focused on the upper soil layers, where the tillage practices only influence the portion of the soil profile where tillage is performed. Tillage causes stratification of residues and organic C reserves, although this depends on the depth of the tillage and the type of tool, and the majority of the residues are concentrated in the surface soil layer (Kahlon, Lal, & Ann-Varughese, 2013; Mazzoncini et al., 2016). The pattern of organic C storage in deeper soil layers has been less well studied (Blanco-Canqui & Lal, 2008). Although the SOC values at 90-120 cm depth during the 2 years studied were the lowest in the soil profile, they represented 12% of the total SOC in the complete profile, attributable to the special characteristics of the Mediterranean Vertisols because they reach great depth and have a great number of cracks. All this confirms the need to include deep soil sampling to study C and its accumulation in depth, particularly in deeply cracking soil types.

Overall, the accumulation of SOC increased more under NT than under CT, which may be attributed to the retention of the mulched residue on the soil surface, the greater stabilization of organic matter, and a reduction in the rate of organic matter decomposition, as well as improved macroaggregate formation. On the other hand, the rate of organic matter decomposition is affected by temperature and soil moisture. Muñoz-Romero et al. (2015) and Wang, Chen, Sun, and Zhang (2009) found that soil temperature was higher in the CT treatment than in the NT treatment. This is due to the tillage depth under CT, which makes the soil more porous and as a result the soil is likely to have lower thermal conductivity (Sarkar & Singh, 2007). This leads to greater heat retention under CT. In addition, the higher soil temperature under CT may be due to a surface difference because under NT the soil surface is partially covered by remnants of straw from the previous crop, causing the soil to absorb less solar radiation during the day (Wang et al., 2009). It is known that microbial metabolism and, therefore, C mineralization is slower at low than high temperatures, and warming is associated with larger releases. The temperature sensitivity (Q10) of soil respiration is crucial for carbon dynamics (Meyer, Welp, & Amelung, 2018). On the other hand, a reduced aggregate turnover due to less soil disturbance, as in NT relative to CT, enhances the formation of microaggregates within macroaggregates in which particulate OM is stabilized in the long term (Plaza, Courtier-Murias, Fernandez, Polo, & Simpson, 2013). Microbes and microbial by-products adsorbed on mineral surfaces and physically protected by entrapment within very small microaggregates appear to constitute an important pool of organic matter stabilization and C sequestration in soils under NT (Plaza et al., 2013). Several studies have reported greater C sequestration under NT (Conceição, Dieckow, & Bayer, 2013; Franzluebbers, 2005; Johnson et al., 2005; Kahlon et al., 2013; Kumar, Kadono, Lal, & Dick, 2012). However, according to Lopez-Bellido et al. (2010), the majority of these studies considered shallower soil depths (normally 30 cm or less), whereas our results are more comprehensive because a deeper soil profile was considered (0–90 cm). Cracks usually appear at the beginning of spring but they depend on the temperature and the precipitation that occurred in the growing season. Crack formation takes place in both tillage systems. In a study conducted with the same experiment by Lopez-Bellido et al. (2016), the volume and surface of cracks was higher in the CT than in the NT in the same experiment. However, there were no differences between depth, width and outline in the two tillage systems.

The wheat–sunflower, wheat–faba bean and continuous wheat rotations had the highest accumulated C during the 29 years of study. Although the continuous wheat residues were the largest, there were no differences between the SOC of these rotations. The different composition of faba bean, wheat and sunflower straw makes them more or less easy to decompose and, therefore, the different crop residues will provide more or less organic carbon to the soil. The storage of C in NT was greater compared with CT in almost all rotations, with distinct increases in SOC levels as a consequence of cropping diversification and the greater content of organic C in NT. West and Six (2007) estimated that the C stock increases more following conversion from CT to NT than with the diversification of rotations, whereas Conceição et al. (2013) reported the opposite in a sandy soil. The mean annual rate of organic C accumulation in the 29-year study period (0.82 Mg C ha^{-1} year^{-1}) was similar to the potential rate of mitigation found by Lorenz (2013). This rate was greater under NT than under CT, with an increase of 0.3 Mg C ha^{-1} year^{-1}. Zaher, Stöckle, Painter, and Higgins (2013) reported an average increase of 0.3 Mg C ha^{-1} year^{-1} in an area with average rainfall after switching from CT to NT. However, West and Post (2002) conducted a global analysis of long-term agricultural management experiments and found an average increase of 0.57 Mg C ha^{-1} year^{-1} after switching from CT to NT, over a period of 15–22 years. The rate of organic C accumulation was higher in the wheat–sunflower, continuous wheat and wheat–faba bean rotations, and lower in the other two rotations (wheat–chickpea and wheat–bare fallow). From the perspective of soil C sequestration under rainfed conditions in a Mediterranean climate, it is important to adopt an ideal tillage system and an adequate rotation method. Sanderman et al. (2010) conducted an experiment with different soil types in Australia and found an increase of 0.2–0.3 Mg C ha^{-1} $year^{-1}$ after improvements in crop management and rotations and the adoption of direct seeding or stubble retention in comparison to conventional tillage.

5. Conclusions

In rainfed Mediterranean Vertisols, the retention of crop residues, especially wheat (in contrast to the common local farming practices of removing residues), increases the stock of organic C in the soil in the long-term, with higher increases for no-tillage in comparison to conventional tillage.

The formation of cracks, characteristic of Vertisols, enhanced by the warm and semiarid Mediterranean climate, constitutes a natural means of organic C penetration directly from the incorporation of intact crop residues into the deeper soil layers, which contributes to increasing the proportion of stable organic C in the soil.

Selection of the no-tillage system, along with rotation diversification could be adequate for increasing organic C reserves, the rate of C sequestration and the fertility of soils under rainfed Mediterranean conditions.

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Table 1. The properties of the Vertisol used in field experiments. Córdoba (Spain).

Properties	Soil depth (cm)		
	0-30	30-60	60-90
Fine sand (g kg^{-1})	127	143	187
Silt (g kg^{-1})	179	152	26
Clay (g kg^{-1})	694	705	787
pH (water)	7.7	7.6	7.6
Organic matter (g kg^{-1})	10.2	7.4	5.3
Calcium carbonate equivalent (g kg^{-1})	75	93	71
CEC (cmol kg^{-1})	46.5	36.6	30
Available P (mg kg^{-1})			
Beginning of the experiment	4.9	1.5	1.5
End of the experiment	9.1	7.2	5.2
Total N (%)			
Beginning of the experiment	0.07	0.06	0.04
End of the experiment	0.1	0.07	0.06

Table 2. Effect of tillage system (CT: conventional tillage, NT: no-tillage) and soil depth on soil bulk density.

Treatment	**Source**	**Bulk density /Mg m^{-3}**			
Depth /cm		0-15	15-30	30-60	60-90
Tillage System	CT	0.95	0.97	1.03	1.06
	NT	0.99	1.02	1.06	1.09
	p	0.0026	0.0000	0.0000	0.0001
	SE	0.0113	4.829x 10^{-3}	5.49 10^{-3}	6.759 10^{-3}
	LSD	0.0238	0.0101	0.0115	0.0142

Table 3. Effects of tillage system, crop rotation and N fertilizer on C in anual and total aboveground wheat residue from 1989 to 2015

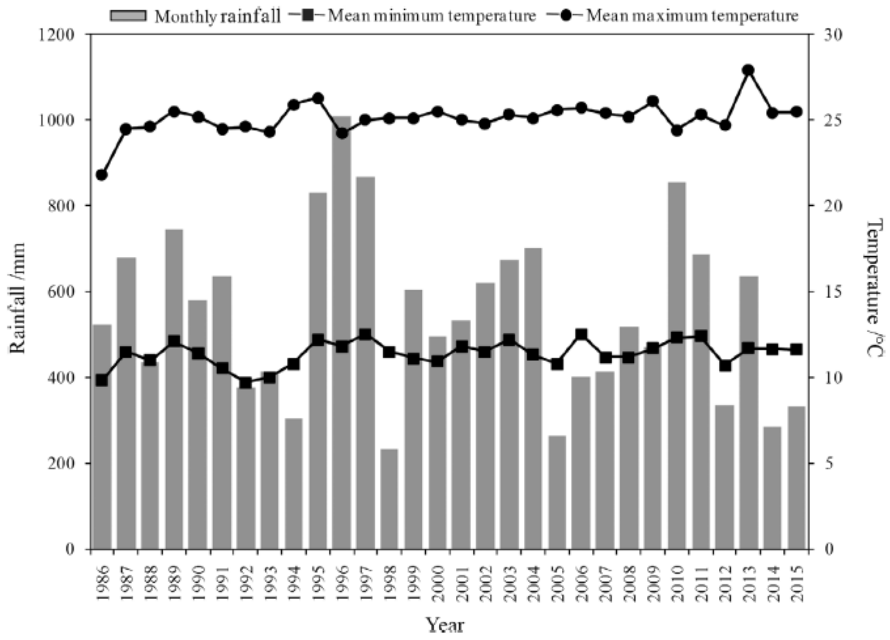
Treatments	**Source**	**Residue C /Mg ha^{-1}**	
		Annual	Total
Tillage system	No tillage	1.74	22.6
	Tillage	1.65	21.5
	p	0.0040	0.0016
	SE	0.0271	0.0984
	LSD	0.0547	0.3131
Rotation	Faba bean	2.11	27.4
	Fallow	1.93	25.1
	Chickpea	1.81	23.6
	Sunflower	1.34	17.4
	Wheat	1.29	16.7
	p	0.0000	0.0000
	SE	0.0411	0.5819
	LSD	0.0808	1.2009
Nitrogen fertilizer /kg ha^{-1}	0	1.38	17.9
	50	1.71	22.2
	100	1.83	23.8
	150	1.87	24.3
	p	0.0000	0.0000
	SE	0.0198	0.2474
	LSD	0.0388	0.4915

Table 3. Effects of tillage system, crop rotation and N fertilizer on C in anual and total aboveground wheat residue from 1989 to 2015

Table 4. Effects of year, tillage system, crop rotation, N fertilizer and soil depth on soil organic carbon content for layers /Mg ha^{-1}

Source	**Depth /cm**	**df**	**SS**	**MS**	**F**	***p***
	0-15					
Year		8	3557.65	444.706	62.51	0.0000
Tillage		1	865.11	865.107	367.59	0.0000
Rotacion		4	403.09	100.772	60.84	0.0000
Nitrogen		3	54.76	18.253	14.24	0.0000
	15-30					
Year		8	3056.84	382.106	49.66	0.0000
Tillage		1	276.07	276.074	140.44	0.0000
Rotacion		4	153.49	38.371	19.48	0.0000
Nitrogen		3	19.16	6.386	5.11	0.0017
	30-60					
Year		8	7229.9	903.73	18.78	0.0000
Tillage		1	1095	1094.95	69.97	0.0000
Rotacion		4	280.8	70.2	5.01	0.0008
Nitrogen		3	66.1	22.04	3.1	0.0262
	60-90					
Year		8	5836.6	729.58	17.57	0.0000
Tillage		1	1536.4	1536.37	67.04	0.0000
Rotacion		4	267.3	66.83	3.36	0.0116
Nitrogen		3	35.6	11.87	1.06	0.3674

FIGURE 1 Annual rainfall and soil temperature over the 29-year study at Cordoba (Spain)



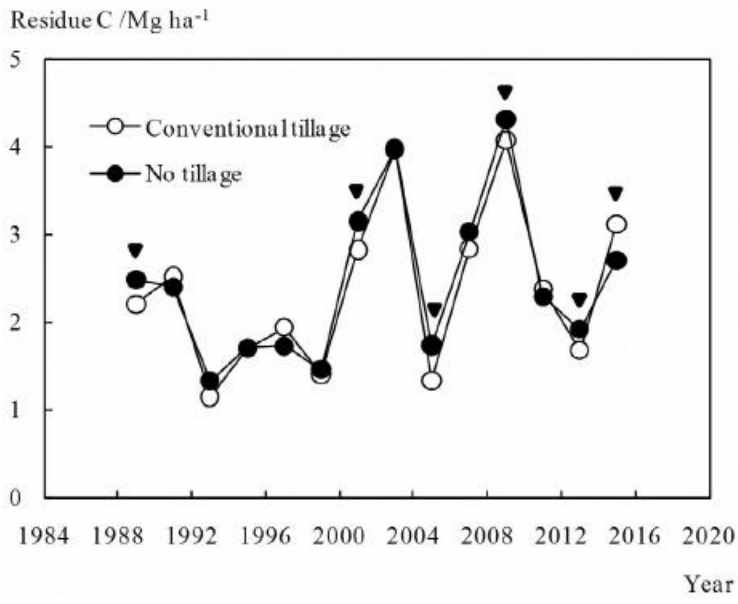


FIGURE 2 Effects of year and tillage system on C in aboveground residues. The triangle represents significant differences between tillage systems

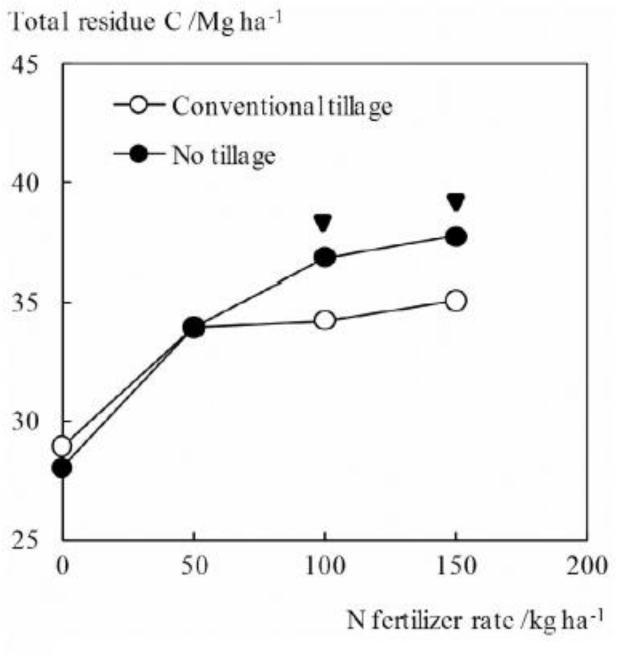


FIGURE 3 Effects of tillage system and N fertilizer rate on C in aboveground residues. The triangle represents significant differences between tillage systems

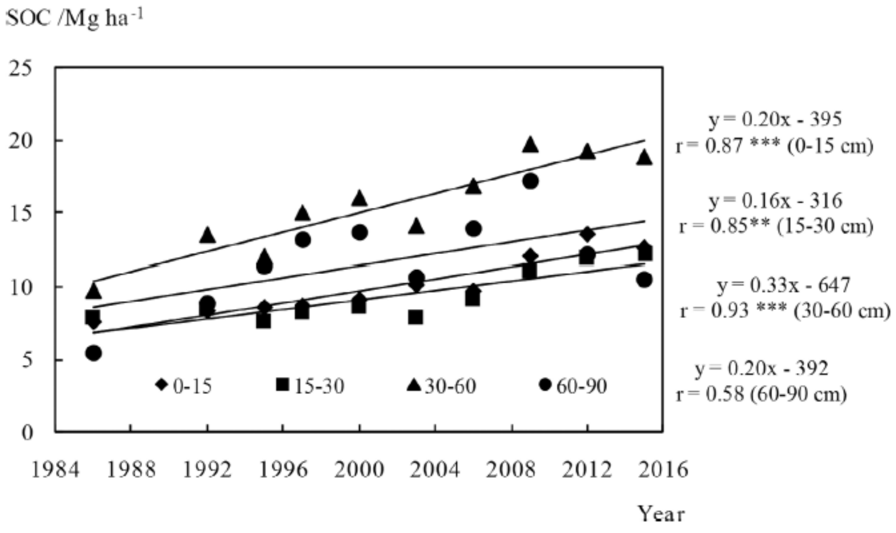
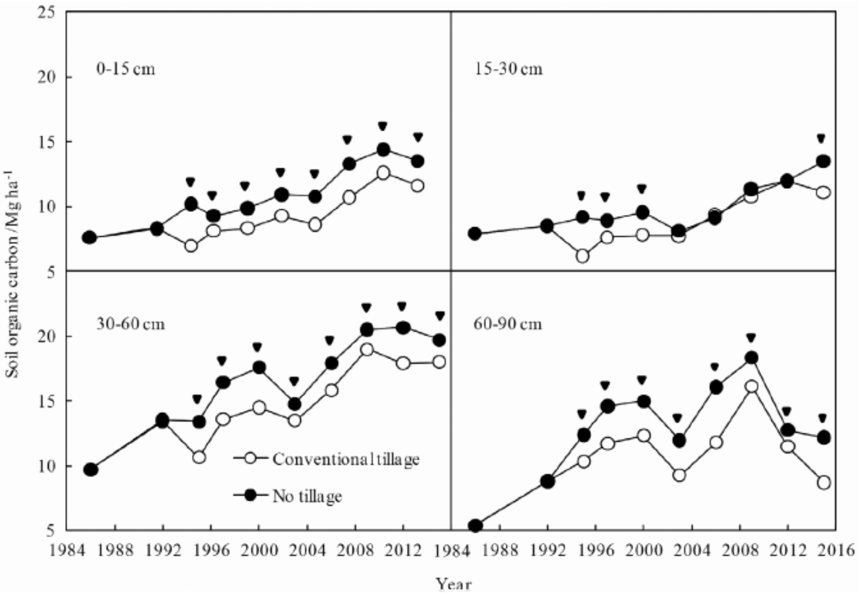
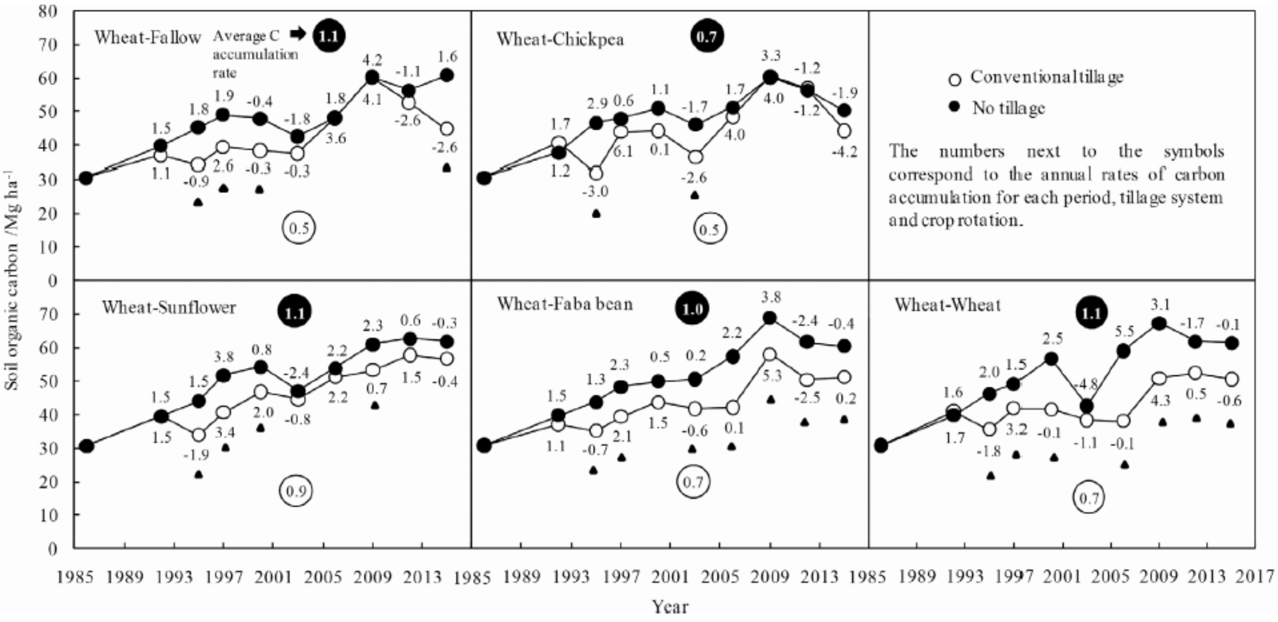


FIGURE 4 Relationship between year and soil organic carbon content in soil layers (0–15, 15–30, 30–60 and 60–90 cm). ** Significant at the 0.01 probability level; *** significant at the 0.001 probability level

FIGURE 5 Effects of year, tillage system and depth on soil organic carbon stock. The triangle represents significant differences between tillage systems





The numbers next to the symbols correspond to the annual rates of carbon accumulation for each period, tillage system and crop rotation.

FIGURE 6 Effects of year, tillage system and crop rotation on total soil organic carbon content (0–90 cm) and carbon accumulation rate, respectively. The triangle represents significant differences between tillage systems for each year and rotation