No-till: A key tool for sequestering C and N in microaggregates on a Mediterranean Vertisol

Rafael J. Lopez-Bellido [a], Veronica Muñoz-Romero [a], Rafael Fuentes-Guerra [a], Purificacion Fernandez-Garcia [a], Luis Lopez-Bellido [a *]

[a] *Eco-efficient Cropping Systems, University of Cordoba, Edificio C4 "Celestino Mutis", Ctra. Madrid-Cadiz km 396, 14071 Cordoba, Spain*

A B S T R A C T

Soil aggregate stability is crucial for agroecosystem sustainability and for mitigating the effects of greenhouse gases. Within soil aggregates, increases in the microaggregate-associated C and N contents are of paramount importance. The aim of this study was to determine the effect of agricultural practices on macro- and microaggregate-associated C and N through a 27-year continuous field experiment in a dryland Mediterranean Vertisol. The treatments included a tillage system (no-till and conventional), 2-year crop rotations [wheat (*Triticum aestivum* L.)-faba bean (*Vicia faba* L.) and wheat-sunflower (*Helianthus annuus* L.)], and the application of N fertilizer (0 and 100 kg ha^{-1}) to wheat. Soil samples were collected from depths of 0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm. Higher proportions of microaggregates and macroaggregates were observed under no-till than under conventional tillage. Crop rotations did not affect the microaggregate concentration, and the microaggregate-associated C and N stocks (0–90 cm) were higher in the no-till treatment compared with conventional tillage (6.9 *vs.* 4.4 Mg ha^{-1} and 957 *vs.* 672 kg ha^{-1}, respectively). The microaggregate-associated C stocks obtained from the no-till treatment were significantly higher at depths of 0 to 15, 15 to 30, and 60 to 90 cm. At certain depths, the no-till treatment resulted in greater microaggregate N stocks than conventional tillage, regardless of crop rotation. Conventional tillage yielded significantly lower macroaggregate-associated C and N stocks (0–90 cm) than no-till. This finding was also observed at certain depths in both crop rotations. The wheat-faba bean treatments yielded greater macroaggregate C stocks than the wheat-sunflower treatments under both tillage systems at certain depths. We determined that no-till is a key practice for sequestering C and N in microaggregates and that crop rotation and N fertilization do not contribute to the C and N sequestration. As a result, no-till appears to be the only truly effective practice for the long-term encapsulation of C and N in microaggregates.

Keywords: tillage system; crop rotation; macroaggregates

* Corresponding author.

E-mail addresses: rjlopezbellido@uco.es (R.J. Lopez-Bellido), g82murov@uco.es (V. Muñoz-Romero), rafafuentesguerra@gmail.com (R. Fuentes-Guerra), , g72fegap@uco.es (P. Fernandez-Garcia), cr1lobel@uco.es (L. Lopez-Bellido).

1. Introduction

Macroaggregates are known to provide physical protection to soil organic matter, shielding it from rapid decomposition (Beare et al., 1994), and are thus regarded as key elements enabling soil C sequestration in microaggregates through strong encapsulation (McCarthy et al., 2008). Prior studies indicate that macroaggregate C (macroaggregate-associated organic C) is the C source for C sequestration in microaggregates through isolation. Six and Paustian (2014) recommended further clarification of the dynamics of microaggregates within macroaggregates in different soils and under different environmental conditions.

First, C sequestration in microaggregates is influenced by the soil type and climate (Denef et al., 2004; Lenka and Lal, 2013; Six and Paustian, 2014). The microaggregate C content can be increased through agricultural practices (Paustian et al., 1998; Lenka and Lal, 2013). However, we have a limited capacity to increase C sequestration in microaggregates because we can only modify the soil, not the climate, and would therefore affect the physical, chemical, biological, and microclimate conditions of soils (Bronick and Lal, 2005; Plante et al., 2006; Nie et al., 2014). These four factors combined with the spatial location and temporal dynamics of C, which are responsible for C encapsulation in microaggregates, are strongly linked and cannot be separately studied. As a result, any study of these variables is not easy, and many aspects of this subject have therefore not been thoroughly elucidated (Gupta and Germida, 2015).

According to Follett (2000) and Blanco-Canqui and Lal (2004), soil and crop management practices, such as the tillage system, crop residue, crop sequence, nutrient management, and irrigation, impact C sequestration in aggregates. The literature over the last decade indicates an incremental increase in the number of studies examining the effects of agricultural practices on soil C within aggregates in different environments, i.e., the relationship between climate and soil. Nevertheless, few studies have investigated the effects of agricultural management on C and N sequestration in aggregates in Mediterranean Vertisols. In fact, we only found one publication describing a study conducted under similar conditions by Ortas and Lal (2012), who demonstrated that soils treated with high levels of P fertilizer contained higher C and N concentrations in macroaggregates compared with microaggregates. Prior research has been conducted under Mediterranean conditions or in Vertisols but not both, e.g., Alvaro-Fuentes et al. (2008) studied a Mediterranean Aridisol and found that no-till and the suppression of long-term fallow increased soil aggregation; Blair and Crocker (2000) studied a Marine-Mild Winter Vertisol and determined that eliminating long fallow periods and adopting no-till combined with residue retention increased the soil C content and its structure; and in a sub-humid sub-tropical Vertisol, Hati et al. (2008) found that the application of N fertilizer had no effect on the macroaggregate concentration and soil organic C.

In general, the literature states that agricultural practices affect C in aggregates as follows (Blanco-Canqui and Lal, 2004; Bronick and Lal, 2005): (i) no-till promotes C-enriched macro- and microaggregates more than conventional tillage; (ii) crop rotations can influence C sequestration in microaggregates but the effect depends on the tillage system, the quantity and quality of organic residues, the crop types, and site-specific soil characteristics; (iii) inorganic fertilization may or may not improve the microaggregate C levels; and (iv) irrigation has the potential to enhance C sequestration in microaggregates. However, few studies have investigated the effect of agricultural practices on N in aggregates. Wright and Hons (2005) stated that no-till increases C and N sequestration in microaggregates.

Therefore, the objectives of this study were to determine the effect of the tillage system, crop rotation, and N fertilizer application rate on soil aggregate size distribution and the associated C and N concentrations in a dryland Mediterranean Vertisol through a 27-year continuous experiment. In particular, the study emphasized the effect of treatments on C and N sequestration in microaggregates.

2. Materials and Methods

2.1. Site characteristics and experimental design

The study was carried out in a field experiment that was initiated in 1986, called Malagon, which is located in Cordoba in southern Spain (37° 46´ N, 4° 31´ W, 280 m above sea level) on a dryland Vertisol (Typic Haploxererts) under Mediterranean conditions. The results of a soil analysis performed at the beginning of the experiment in 1986 were described by Lopez-Bellido et al. (1997). The average annual rainfall in this area is 584 mm (39% from October to December, 37% from January to March, 19% in June, and 5% from July to September); the average of annual potential evapotranspiration is 1000 mm; the average annual temperature is 17.5°C; the average temperature in the coldest month is 9.5°C; and the average temperature during the warmest month is 27.5°C.

The field experiment was designed taken up in a split-split plot randomized block, and each block was replicated four times. The main plots were tillage systems (no-till and conventional tillage), whereas the subplots were divided based on crop rotations, with four 2-year rotations [wheat-sunflower, wheat-chickpea (*Cicer arietinum* L.), wheat-faba bean (*Vicia faba* L.), and wheat-bare fallow] and continuous wheat, and the sub-subplots were divided based on the N fertilizer rates (0, 50, 100, and 150 kg N ha^{-1}) applied to wheat. Duplicate sets of plots were established within each block to allow the presence of all phases of the rotation each year. For this study, the following treatments were selected: tillage system (no-till and conventional tillage), crop rotation (wheat-sunflower and wheat-faba bean), and N fertilizer rate (0 and 100 kg ha^{-1}). The area of each sub-subplot was 50 m^2 (10 by 5 m).

2.2. Crop management

Specific seed drills (Great Plains) were used for each tillage system. Weeds were controlled by the application of glyphosate and MCPA [(4-chloro-2-methylphenoxy) acetic acid] at rates of 0.87 and 10.60 kg active ingredient ha^{-1} before planting, respectively. The conventional tillage treatment included mouldboard ploughing and disk harrowing at the beginning of autumn or vibrating tine cultivation to prepare seedbeds for planting. Information about the cultivars, planting, harvesting and herbicides applied during the growing season were provided by Lopez-Bellido et al. (2007a, 2007b). Nitrogen fertilizer was applied to the wheat plots as ammonium nitrate. Under all application rates, half of the N fertilizer was applied at tillering, and the rest was applied at the time of stem elongation. Every year, the wheat plots were also supplied P fertilizer at a rate of 65 kg ha^{-1}. The soil-available K was adequate (530 mg kg^{-1}).

2.3. Soil sampling and aggregate C and N analysis

In November 2013, three soil samples from each of four depths (0–15, 15–30, 30–60, and 60–90 cm) were collected at each plot using a manual Eijkelkamp auger (53 mm Ø). One sample was used to determine the bulk density (Grossman and Reinsch, 2002), and the other (two soil cores) was used to analyse the C and N within the aggregate-size class. Once in the laboratory, the field-moist soil was passed through an 8-mm sieve by gently breaking apart the soil, air drying, and storing at room temperature.

Soil aggregates were separated with wet sieving using a methodology adapted by Elliott (1986) proposed by Six et al. (2000a) and modified by Novelli et al. (2011) for heavy clay soils. The aggregates were separated by wet sieving the air-dried soil through a series of three sieves (2000, 250, and 53 μm). A 100-g air-drying at room temperature subsample was capillary-wetted to field capacity for 10 min to minimize slaking following immersion (Novelli et al., 2011). The wetted soil was immersed in water on a nest of sieves (2000 μm, 250 μm, and 53 μm) and shaken vertically a distance of 6 cm during a 2-min period (60 times). Four aggregate sizes were obtained: large macroaggregates (>2000 μm), macroaggregates (250–2000 μm), microaggregates (53–250 μm), and a fraction associated with minerals (<53 μm). This last fraction was obtained based on the difference between whole soil and the sum of the three aggregate size fractions (>2000 μm + 250–2000 μm + 53–250 μm). The aggregates were oven-dried (50°C) and weighed.

The sand content of the aggregates was determined based on the dispersion of subsample of aggregates with sodium hexametaphosphate (5 g L^{-1}). The weights of the macro- and microaggregates were corrected for the content of sand (Six et al., 2000b).

The aggregate C and N contents were quantified through a dry combustion method (Nelson and Sommers, 1996) with a Vario Max CN analyser (Elementar Instrument, Hanau, Germany). The soil was analysed to quantify the inorganic C using a modified pressure calcimeter method (Sherrod et al., 2002). The organic C concentration was calculated by subtracting the inorganic C from the total C. Within each aggregate size class, the C and N aggregate stocks (C_t and N_t Mg ha^{-1}) were calculated as follows:

$C_t = BD \times C_c\% \times D$

$N_t = BD \times N_c\% \times D$

where BD is soil bulk density (g cm^{-3}), $C_c\%$ is soil carbon concentration, $N_c\%$ is soil nitrogen concentration, and D is soil sampling depth increment (cm).

2.4. Statistical analysis

All data were analyzed using a mixed model ANOVA approach to a randomized split-split plot design. The blocks were considered a random effect, whereas the tillage system, crop rotation, N fertilizer, soil depth, and aggregate-size class were considered fixed effects. Analysis of variance for each measurement was performed as function of depth and whole profile (0−90 cm). Another analysis of variance was performed to compare the aggregate-size classes. However, in order to simplify some of the analysis of variance were not shown in table 1. Least significant differences (LSD) for different main effects and interactions were calculated using the appropriate standard error term according to Gomez and Gomez (1984). Different simple linear regressions were also calculated. The statistical analyses were performed using Version 9.1 of SAS (Statistical Analyses System, SAS Institute, 2003).

3. Results

3.1. Bulk density and total C and N stocks

The bulk density was significantly greater in the no-till treatment than in the plots subjected to conventional tillage at depths of 0 to 15 and 60 to 90 cm, indicating that this variable was only affected by the tillage and depth (Table 2). The total C was significantly greater under no-till than under conventional tillage at all depths. A higher C content was concentrated in the 0-to-30-cm depth under both tillage systems. At a depth of 0 to 90 cm, the total C stocks in the plots subjected to no-till and conventional tillage were 48 and 37 Mg ha^{-1}, respectively (i.e., an increment of 30%; Table 2). The total C content was also significantly affected by the crop rotation × depth, tillage system × crop rotation × depth, and tillage system × crop rotation × N fertilizer × depth. However, these results are not presented because the present study focused more on microaggregates and these interactions were not significant for microaggregate-associated C and N. In contrast, the total N stock was only significantly affected by the depth and tillage system × depth. At all depths, significant differences were observed in the total N content between tillage systems, except at the 15-to-30-cm depth. At depths of 0 to 90 cm, the total N was 17% lower under conventional tillage compared with no-till (Table 2).

3.2. Aggregate-size distribution

The aggregate-size distribution at a depth of 0 to 90 cm was dominated by macroaggregates (250–2000 μm), which on average accounted for 48% of the total aggregates (Fig. 1). The microaggregates comprised 13% of the aggregates, which was the lowest proportion obtained. All of the aggregate-size concentrations were significantly different between them. The crop rotations did not significantly affect the microaggregate content (Table 1). However, the tillage system significantly affected all aggregate sizes with the exception of the silt and clay fraction (<53 μm) (Table 1). Significantly higher proportion of microaggregates and macroaggregates were observed under no-till than under conventional tillage in total average profile (Fig. 1). In contrast, the large macroaggregate (>2000 μm) concentration was significantly greater under conventional tillage (211 g kg^{-1} *vs.* 65 g kg^{-1}; Fig. 1).

Significantly higher values of microaggregate concentrations were obtained under no-till compared with conventional tillage at depths of 0 to 15, 15 to 30, and 60 to 90 cm (Fig. 1). Similar results were found for macroaggregates and for the silt and clay fraction at a depth of 60 to 90 cm. At depths of 15 to 30 and 60 to 90 cm, the concentrations of large macroaggregates were significantly greater under conventional tillage (Fig. 1). There was practically no significant difference in the aggregate concentration at depth for the silt and clay fraction under both tillage systems, for microaggregates under conventional tillage, and for macroaggregates and large macroaggregates under no-till. It is remarkable that the greatest difference between no-till and conventional tillage was localized in the 15-to-30-cm depth (in terms of microaggregates) and the 60-to-90-cm depth (in terms of macroaggregates and large macroaggregates; Fig. 1).

3.3. Microaggregate and macroaggregate C stocks

The microaggregate C stock was only significantly affected by tillage at the 0-to-90-cm depth and by depth and the tillage × depth interaction in all soil depths (Table 1). The microaggregate C stock at a depth of 0 to 90 cm was on average 57% higher under no-till compared with conventional tillage (6.9 *vs.* 4.4 Mg ha^{-1}; Fig. 2). The microaggregate C stock under no-till represented 14% of the total C stock and under conventional tillage represented 12% (Fig. 2 and Table 2). The major difference in the microaggregate C content between no-till and conventional tillage was observed in the 15-to-30-cm depth (Fig. 2). The sum of the microaggregate C at depths of 0 to 15 cm and 15 to 30 cm in the plots subjected to no-till and conventional tillage equalled 3.18 and 1.33 Mg ha^{-1}, respectively (Fig. 2). These values indicated that the microaggregate C under no-till decreased with depth ($y = -34.599x + 124.57$, $r = 0.999$***), whereas the maximum value obtained under conventional tillage was obtained at a depth of 30 to 60 cm (2.17 Mg ha^{-1}) (Fig. 2).

The macroaggregate C stock at a depth of 0 to 90 cm was only significantly affected by the tillage system, and was significantly affected by the depth, crop rotation × depth, crop rotation × N fertilizer rate × depth, and tillage system × crop rotation × N fertilizer rate × depth in all soil depths (Table 1). A significant relationship was found among the microaggregate C and macroaggregate C at a depth of 0 to 90 cm ($y = 0.295x - 2.549$; n = 8, $r = 0.88$**). The macroaggregate C stock (0–90 cm) was significantly lower under conventional tillage than under no-till (24.7 *vs.* 30.9 Mg ha^{-1}; Fig. 3). The analysis of the tillage system × crop rotation × N fertilizer rate × depth interaction indicated that the macroaggregate C stock in the plots subjected to no-till was greater than that obtained with conventional tillage under a wheat-sunflower rotation at all studied depths (Fig. 3). No-till macroaggregate C stocks that were greater than those obtained with conventional tillage were detected under the following treatments and at the following depths: wheat-faba beans applied fertilizer at a rate of 0 kg N ha^{-1} (at a depth of 0 to 15 cm); wheat-sunflower treatment applied fertilizer at a rate of 0 kg N ha^{-1} (at depths of 30 to 60 cm and 60 to 90 cm); and wheat-sunflower treatment applied fertilizer at a rate of 100 kg N ha^{-1} (at a depth of 15 to 30 cm; Fig. 3). Because the two first depths are lower than the other two, we observed a decline in the macroaggregate C stock with increasing depth in all of the treatments. Therefore, the 0-to-30- and 30-to-60-cm depths exhibited a minimal difference compared with the 60-to-90-cm depth (Fig. 3). In the plots subjected to no-till, the C stock was significantly greater under 100 kg N ha^{-1} than under 0 kg N ha^{-1} at a depth of 15 to 30 cm in the plots treated with wheat-sunflower; however, the opposite finding was obtained with the wheat-sunflower treatment at a depth of 60 to 90 cm and under conventional tillage with the wheat-faba bean treatment at a depth of 15 to 30 cm (Fig. 3). Greater macroaggregate C stock values were obtained with the wheat-faba bean treatment compared with the wheat-sunflower treatment for both tillage systems with an N fertilizer application rate of 100 kg N ha^{-1} at the 15-to-30-cm depth and for conventional tillage with an N fertilizer application rate of 0 kg N ha^{-1} at the 15-to-30-cm depth. However, the wheat-sunflower macroaggregate C stock was greater than that obtained with the wheat-faba bean treatment under conventional tillage with an N fertilizer application rate of 0 kg N ha^{-1} at the 30-to-60-cm depth (Fig. 3).

3.4. Microaggregate and macroaggregate N stocks

The microaggregate N stock was only significantly affected by tillage at the 0-to-90-cm depth and by the tillage × rotation × depth and N fertilizer rate × depth interactions in all soil depths (Table 1). The microaggregate N stocks at a depth of 0 to 90 cm under no-till and conventional tillage were 957 and 672 kg ha^{-1}, respectively (Fig. 4). The no-till microaggregate N stock represented 14% of the total N stock, and whereas that of conventional tillage represented 12% (Fig. 4 and Table 2). By adding the two first depths, we found that a significant difference only between the 0-to-30, 30-to-60 cm, and 60-to-90 cm depths. No-till yielded greater microaggregate N stocks than conventional tillage in the wheat-sunflower treatment at depth of 15 to 30, 30 to 60, and 60 to 90 cm and in the wheat-faba bean treatment at a depth of 60 to 90 cm (Fig. 4a). Only at 15 to 30 cm there was a significant difference between crop rotations: the sunflower treatment exhibited higher values than the wheat-faba bean treatment under no-till, whereas the opposite result was observed under conventional tillage (Fig. 4a). The microaggregate N stock obtained with an N fertilizer rate of 100 kg ha^{-1} was only greater than that obtained with an N fertilizer rate of 0 kg ha^{-1} at the 30-to-60-cm depth (Fig. 4b).

The microaggregate and macroaggregate N stocks at the 0-to-90-cm depth were related each other (microaggregate N stock = 0.293 macroaggregate N stock – 0.118; n = 8, r = 0.76*). The macroaggregate N stock at a depth of 0 to 90 cm under no-till was significantly greater than that obtained under conventional tillage (3.42 *vs.* 2.95 Mg ha^{-1}; Fig. 5). The no-till macroaggregate N stock represented 50% of the total N stock, whereas that of conventional tillage represented 51% (Fig. 5 and Table 2). In general, the macroaggregate N stock values were similar at depth of 0 to 60 cm, indicating a significant decline at the 60-to-90-cm depth (Fig. 5). The N stock under no-till was only significantly higher than that obtained under conventional tillage for the wheat-sunflower rotation with an N fertilizer rate of 100 kg ha^{-1} at the 60-to-90-cm depth (Fig. 5a). Under conventional tillage, the macroaggregate N stock was greater in the wheat-faba bean than the wheat-sunflower rotation and was also higher with a fertilizer application rate of 0 kg N ha^{-1} compared with 100 kg ha^{-1} (Fig. 5b).

4. Discussion

After the 27-year continuous experiment, the bulk density was only affected by the tillage system, as indicated in a previous study in the same experiment (Lopez-Bellido et al., 2010). However, no significant differences were found in bulk density between the tillage systems from the beginning of the experiment. The first year when we identified a difference was in 1997, 10 years after the start of the experiment. Even afterwards, no significant difference was detected in some years, e.g., 2003 (Lopez-Bellido et al., 2010). This finding is due to the particular characteristics of Vertisols in the Mediterranean climate.

A significantly higher proportion of microaggregates and macroaggregates were observed under no-till than under conventional tillage. It seems reasonable to hypothesize that cracking Vertisol soils could have an important effect on microaggregation due to the breakdown of large aggregates by root penetration through the cracks.. However, there is no information available regarding this phenomenon. Only Millan et al. (2007) have recommended that further studies search and interpret scaling in Vertisols and investigate the role of stabilizing materials on aggregation processes. Moreover, the changes in C stocks in a Vertisol under Mediterranean conditions can be pronounced due to periods with severe drying, which occur regularly; as a consequence, more C and N from organic matter is released after drying and rewetting (Gestel et al., 1991).

In comparison to the linear trend for the total C stock observed from the beginning of the same experiment (Lopez-Bellido et al., 2010), the results decreased under both tillage systems. According to Lopez-Bellido et al. (2010), the variations between different 3-year periods are common due to the erratic weather conditions. Gestel et al. (1991) argued that mechanical disruption and rearrangement of soil components caused by soil desiccation and rewetting make organic compounds available for subsequent mineralization in amounts that are determined by the aggregation state. These researchers concluded that in a well-aggregated Vertisol with high cationic interchange capacity, relatively more C and N from non-living organic matter is released after drying and rewetting than would be observed in an Alfisol. However, despite the existence of these small tri-annual fluctuations in our experiment, the trend line since 1986 is always increasing in both tillage systems, which indicates the degree of soil degradation due to previous agricultural practices, e.g., removing straw, burning crop residue, and increasing tillage intensity. It appears that the C content of the agroecosystem has not yet reached a steady state, but more time is necessary to draw this conclusion. The total soil N stock was greater under no-till compared with conventional tillage. Melero et al. (2011) found the same results for the same experiment in 2008.

In a Vertisol in a temperate climate, Dalal et al. (1991) also found higher C and N contents under no-till compared with conventional tillage. Ryan et al. (2011) clearly highlighted that the soil organic C and N values were inversely related to the cultivation intensity in a clay soil and suggested that minimum tillage or no-till could promote resilience and mitigate the adverse effects of conventional tillage that have already occurred.

The effect of conventional tillage on the microaggregate C stock is devastating, decreasing it by 36% compared with that observed under no-till. McCarthy et al. (2008) described the relevant mechanisms for C protection in soil microaggregates and suggested that their findings contribute to the development of strategies for enhancing C sequestration in soils through changes in agricultural management practices and land use. However, as our results suggest, it appears that we only have one effective agricultural practice, i.e., no-till. Soil type is an important factor in microaggregate formation. In soils with a weak structure under Mediterranean conditions, Alvaro-Fuentes et al. (2009) found a greater proportion of microaggregates under no-till compared with conventional tillage in one of three long-term experiments, but the microaggregate C was greater under no-till conditions at the three locations but only at depths of 0 to 5 cm.

According to Mikha et al. (2005), the drying and rewetting of soil is an important process in soil aggregation, soil organic matter decomposition, and nutrient cycling. These drying-rewetting cycles are common under Mediterranean conditions, both at a small scale during the growing season and at a large scale in summer. The results presented by Mikha et al. (2005) suggest that drying-rewetting cycles cause a significant C flush, but these researchers also recommended further studies using soils with different C contents and textures to further determine the effect of drying-rewetting cycles on soil aggregation and nutrient release. Our results are similar to those obtained by Ortas and Lal (2012) under the same climate and conditions; these researchers found that the concentrations of C and N are greater in macroaggregates compared with microaggregates. We did not identify C and N concentrations based on aggregate size, but our results indicated a greater amount in macroaggregates than microaggregates (Fig. 1) and a greater concentration of C and N in macroaggregates. The C and N concentrations in macroaggregates were 7.9 and 1.19 g kg^{-1}, respectively, and those in microaggregates were 6.2 and 0.89 g kg^{-1}, respectively (data not shown). According to Blanco-Canqui and Lal (2004), the inclusion of legumes in crop rotations changes the dynamics of macro- and microaggregation for soil organic C storage. The residues of legumes are often rich in the labile organic fraction, which increases soil aggregation and the soil organic C concentration; however, this effect is often transient because the labile fraction is easily degraded. This fact could explain why we did not identify a significant difference in the microaggregate C stock between crop rotations but does not explain why the macroaggregate C stock was greater in the wheat-faba bean treatment compared with the wheat-sunflower treatment at the 30-to-60-cm depth (Fig. 3).

According to Six et al. (2000b), the rate of macroaggregate formation and degradation (i.e., aggregate turnover) declines under no-till compared with conventional tillage and leads to the formation of stable microaggregates in which C is stabilized and sequestered over the long term. Therefore, the link between macroaggregate turnover, microaggregate formation, and C stabilization within microaggregates partly determines the observed soil organic matter increases under no-till (Six et al., 2000b). This finding can be supported by determining the microaggregate-to-macroaggregate ratio under both tillage systems in terms of the aggregate concentration (Fig. 1) and C stock (Figs. 2 and 3) throughout the whole profile (0−90 cm). The ratios of aggregate concentration and C stock under no-till and conventional tillage conditions are 0.30 *vs.* 0.22 and 0.22 *vs.* 0.17, respectively. According to Six et al. (2000a), in soils dominated by a 2:1 clay mineral profile, the C content of the macroaggregates is 1.65-fold greater than that of the microaggregates, but a markedly higher value of 4.9 was found in this study (Figs. 2 and 3). Vertisol soils are dominant in this type of clay, but Six et al. (2000a) conducted their study under completely different climate conditions. However, in general terms, their conclusions were similar to those reached in this study: conventional tillage induced a loss of C-rich macroaggregates and a gain of C-depleted microaggregates, resulting in an overall loss of soil organic C.

5. Conclusions

After a 27-year field experimentation, no-till was identified as the key practice for the sequestration of C and N in microaggregates in a dryland Mediterranean Vertisol. We wanted to highlight this fact in a general manner because many previous studies in different environments have reached the same conclusion and because no-till appears to be the only truly effective practice for the long-term encapsulation of C and N in microaggregates. Under the study conditions, crop rotation and N fertilization did not contribute to the pool of C and N sequestration.

Acknowledgements

This work was funded by Spain’s National R&D Plan (Project AGL2015-65548-R). The authors would like to thank ABECERA for providing the land and allowing us to use their field facilities. Special thanks are also expressed to Joaquin Muñoz, Jose Muñoz, and Auxiliadora Lopez-Bellido for their excellent assistance in the laboratory and field work.

References

Alvaro-Fuentes, J., Arrue, J.L., Gracia, R., Lopez, M.V., 2008. Tillage and cropping intensification effects on soil aggregation: Temporal dynamics and controlling factors under semiarid conditions. Soil Till. Res. 145, 390–396.

Alvaro-Fuentes, J., Cantero-Martinez, C., Lopez, M.V., Paustian, K., Denef, K., Stewart, C.E., Arrue, J.L., 2009. Soil aggregation and soil organic carbon stabilization: Effects of management in semiarid Mediterranean agroecosystems. Soil Sci. Soc. Am. J. 7, 1519–1529.

Beare, M.H., Cabrera, M.L., Hendrix, P.F., Coleman, D.C., 1994. Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils. Soil Sci. Soc. Am. J. 58, 787–795.

Blair, N., Crocker, G.J., 2000. Crop rotation effects on soil carbon and physical fertility of two Australian soils. Aust J. Soil Res. 38, 71–84.

Blanco-Canqui, H., Lal, R., 2004. Mechanisms of carbon sequestration in soil aggregates. Crit. Rev. Plant Sci. 23, 481–504.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.

Dalal, R.C., Henderson, P.A., Glasby, J.M., 1991. Organic matter and microbial biomass in a Vertisol after 20 yr of zero-tillage. Soil Biol. Biochem. 23, 435–441.

Denef, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. Soil Sci. Soc. Am. J. 68, 1935–1944.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.

Follett, R.F., 2000. Soil management concepts and carbon sequestration in cropland soils. Soil Till. Res. 61, 77–92.

Gestel van, M., Ladd, J.N., Amato, M., 1991. Carbon and nitrogen mineralization from two soils of contrasting texture and microaggregate stability: Influence of sequential fumigation, drying and storage. Soil Biol. Biochem. 4, 313–322.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. Wiley, New York.

Grossman, R.B., Reinsch, T.G., 2002. Bulk density and linear extensibility, in: Dane, J.H., Topp, G.C. (Eds.), Methods of soil analysis, Part 4, SSSA Book Ser. 5. SSSA, Madison, pp. 201–254.

Gupta, V.V.S.R., Germida, J.J., 2015. Soil aggregation: Influence on microbial biomass and implications for biological processes. Soil Biol. Biochem. 80, A3–A9.

Hati, K.M., Swarup, A., Mishra, B., Manna, M.C., Waniari, R.H., Mandal, K.G., Misra, A.K., 2008. Impact of long-term application of fertilizer, manure and lime under intensive cropping on physical properties and organic carbon content of an Alfisol. Geoderma 148, 173–179.

Lenka, N.K., Lal, R., 2013. Soil aggregation and greenhouse gas flux after 15 years of wheat straw and fertilizer management in a no-till system. Soil Till. Res. 126, 78–89.

Lopez-Bellido, R.J., Fontan, J.M., Lopez-Bellido, F.J., Lopez-Bellido, L., 2010. Carbon sequestration by tillage, rotation, and nitrogen fertilization in a Mediterranean Vertisol. Agron. J. 102, 310–318.

Lopez-Bellido, L., Lopez-Garrido, F.J., Fuentes, M., Castillo, J.E., Fernandez, E.J., 1997. Influence of tillage, crop rotation and nitrogen fertilization on soil organic matter and nitrogen under rainfed Mediterranean conditions. Soil Till. Res. 43, 277–293.

Lopez-Bellido, R.J., Lopez-Bellido, L., Benitez-Vega, J., Lopez-Bellido, F.J., 2007a. Tillage system, preceding crop, and nitrogen fertilizer in wheat crop: I. Soil water content. Agron. J. 99, 59–65.

Lopez-Bellido, R.J., Lopez-Bellido, L., Benitez-Vega, J., Lopez-Bellido, F.J., 2007b. Tillage system, preceding crop, and nitrogen fertilizer in wheat crop: II. Water utilization. Agron. J. 99, 66–72.

McCarthy, J.F., Ilavsky, J., Jastrow, J.D., Mayer, L.M., Perfect, E., Zhuang, J., 2008. Protection of organic carbon in soil microaggregates via restructuring of aggregate porosity and filling of pores with accumulating organic matter. Geochim. Cosmochim. Acta 72, 4725–4744.

Melero, S., Lopez-Bellido, R.J., Lopez-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.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol. Biochem. 37, 339–347.

Millan, H., Gonzalez-Posada, M., Morilla, A.A., Perez, E., 2007. Self-similar organization of Vertisol microstructure: A pore–solid fractal interpretation. Geoderma 138, 185–190.

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter, in: Sparks, D.L. et al (Eds.), Methods of soil analysis. Part 3 SSSA Book Ser. 5. SSSA, Madison, pp. 961–1010.

Nie, M., Pendall, E., Bell, C., Wallenstein, M.D., 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil Biol. Biochem. 68, 357–365.

Novelli, L.E., Caviglia, O.P., Melchiori, R.J.M., 2011. Impact of soybean cropping frequency on soil carbon storage in Mollisols and Vertisols. Geoderma 167–168, 254–260.

Ortas, I., Lal, R., 2012. Long-Term phosphorus application impacts on aggregate-associated carbon and nitrogen sequestration in a Vertisol in the Mediterranean Turkey. Soil Sci. 177, 241–250.

Paustian, K., Cole, C.V., Sauerbeck, D., Sampson, N., 1998. CO2 mitigation by agriculture: An overview. Clim. Change 40, 135–162.

Plante, A.F., Conant, R.T., Stewart, C.E., Paustian, K., Six, J., 2006. Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. Soil Sci. Soc. Am. J. 70, 287–296.

Ryan, J., Kapur, S., Ibrikci, H., Singh, M., 2011. Cultivation intensity in relation to organic matter and related properties in a Vertisol in southern Turkey. J. Sustain. Agric. 35, 613–623.

SAS Institute, 2003. SAS/STAT user's guide. Release 9.1 ed. SAS Inst., Cary, NC.

Sherrod, L.A., Dunn, G., Peterson, G.A., Kolberg, R.L., 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Sci. Soc. Am. J. 66, 299–305.

Six, J., Elliott, E.T., Paustian, K., 2000b. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103.

Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biol. Biochem. 68, A4–A9.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000a. Soil structure and soil organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Sci. Soc. Am. J. 64, 681–689.

Wright, A.L., Hons, F.M., 2005. Soil carbon and nitrogen storage in aggregates from different tillage and crop regimes. Soil Sci. Soc. Am. J. 69, 141–147.

Table 1. Significant effects of tillage system, crop rotation, N fertilizer, and soil depth on aggregate-size class and micro- and macroaggregate C and N stock in 4-depths and total soil profile in a dryland Mediterranean Vertisol

Source of variation	Aggregate-size class [μm]				Microaggregate C stock		Macroaggregate C stock		Microaggregate N stock		Macroaggregate N stock	
	< 53	53–250	250–2000	> 2000	Depth	Profile	Depth	Profile	Depth	Profile	Depth	Profile
Tillage system [T]	ns[a]	***	**	**	**	**	*	*	**	**	*	*
Crop rotation [R]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T × R	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N Fertilizer [N]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T × R × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Depth [D]	ns	***	***	***	***	–	***	–	***	–	***	–
T × D	*	***	***	***	***	–	ns	–	**	–	***	–
R × D	ns	ns	**	***	ns	–	**	–	ns	–	***	–
N × D	ns	ns	ns	ns	ns	–	ns	–	*	–	*	–
T × R × D	**	ns	ns	ns	ns	–	ns	–	**	–	*	–
T × N × D	ns	ns	ns	*	ns	–	ns	–	ns	–	**	–
R × N × D	ns	ns	ns	ns	ns	–	*	–	ns	–	ns	–
T × R × N × D	*	ns	ns	ns	ns	–	*	–	ns	–	ns	–

[a] *, **, *** Significant at 0.05, 0.01, and 0.001 probability level, respectively; ns, not significant.

Table 2, Bulk density and total C and N stocks affected by tillage system and soil depth in a dryland Mediterranean Vertisol

Depth [cm]	Bulk density [Mg m^{-3}]		Total C stock [Mg ha^{-1}]		Total N stock [Mg ha^{-1}]	
	No-till	Conventional tillage	No-till	Conventional tillage	No-till	Conventional tillage
0–15	0.92a[a]	0.84b	12.9a	10.7b	1.61a	1.23b
15–30	0.98a	0.95a	10.8a	11.3b	1.32a	1.34a
30–60	1.06a	1.05a	15.3a	9.6b	2.40a	1.91b
60-90	1.10a	1.04b	9.0a	5.8b	1.57a	1.28b
0–90	1.04A	1.00A	48.0A	37.4B	6.90A	5.76B

[a] Within depth, means followed by the same letter are not significantly different at P < 0.05 according to LSD.

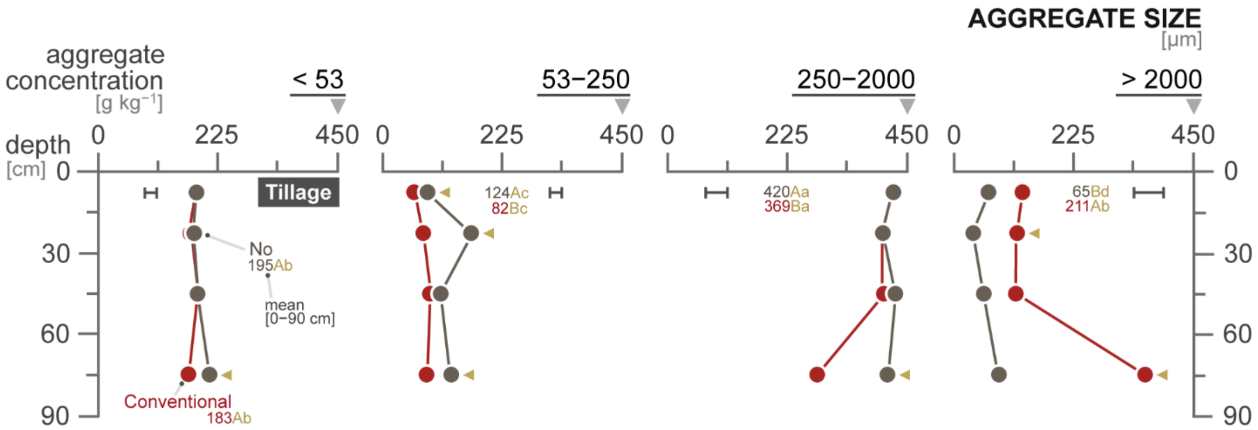

Fig. 1. Aggregate size concentration of four classes as affected by tillage system and soil depth. Triangle represents significant difference between tillage systems within aggregate-size and depth. Horizontal bars represent LSD for comparison between depths within aggregate-size and tillage system. Means followed by the same letter are not significantly different: (i) capital letter for comparison between tillage systems within aggregate-size; (ii) lowercase for comparison between aggregate-sizes within tillage system.

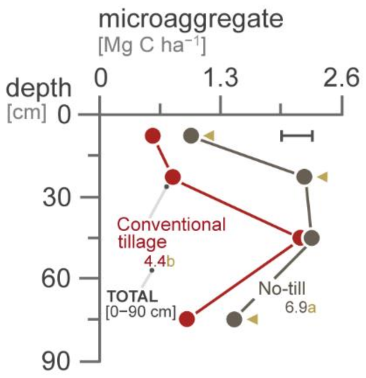

Fig. 2. Microaggregate C stock as affected by tillage system and soil depth. Triangles represent significant difference between tillage systems within depth. Horizontal bar represent LSD for comparison between depths within tillage system. Means followed by a different letter are significantly different.

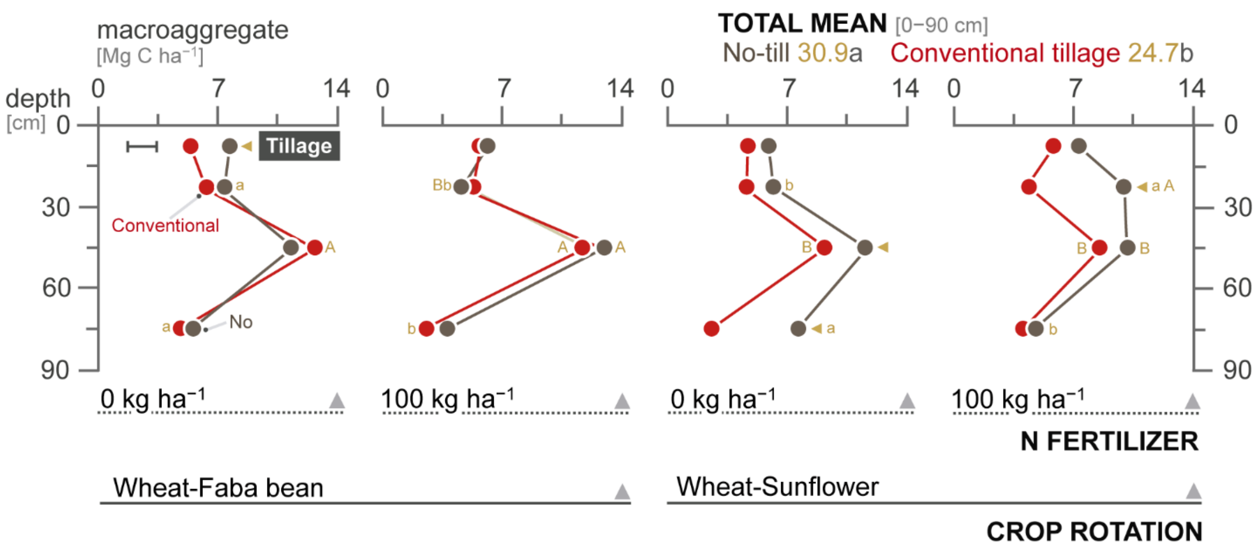

Fig. 3. Macroaggregate C stock as affected by tillage system, crop rotation, N fertilizer rate and soil depth. Triangle represents significant difference between tillage systems within crop rotation, N fertilizer, and depth. Horizontal bar represent LSD for comparison between depths within tillage system, crop rotation, and N fertilizer. Means followed by the same letter are not significantly different: (i) lower case for comparison between N fertilizer rates within tillage system, crop rotation, and depth; (ii) capital letter for comparison between crop rotations within tillage system, N fertilizer rate, and depth.

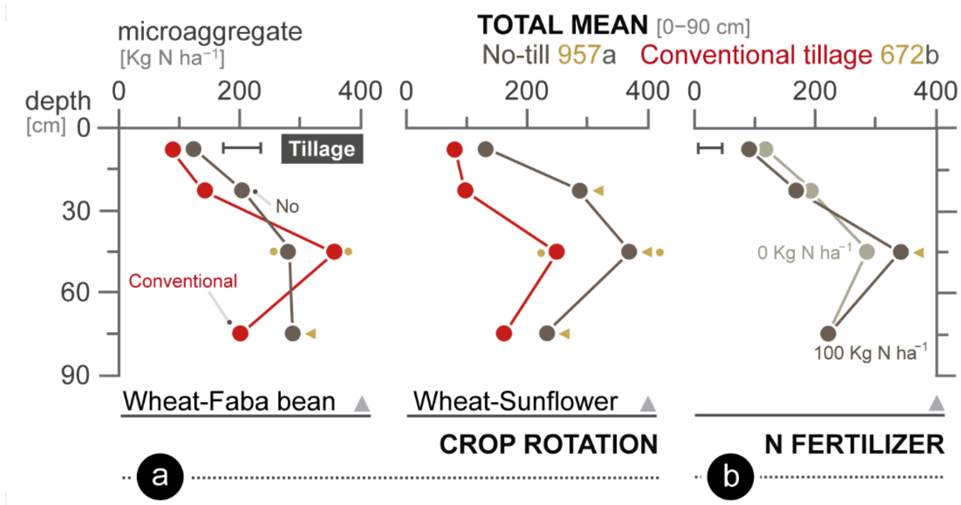

Fig. 4. Microaggregate N stock as affected by a) tillage system, crop rotation and soil depth and b) N fertilizer rate and soil depth. Triangle represents significant difference between tillage systems or N fertilizer rates within crop rotation and depth in the first interaction and within depth in the second one. Horizontal bar represent LSD for comparison between depths within tillage system, and crop rotation or within N fertilizer rate. Yellow circle represents significant difference between crop rotations within tillage system and depth. Means followed by the same letter are not significantly different.

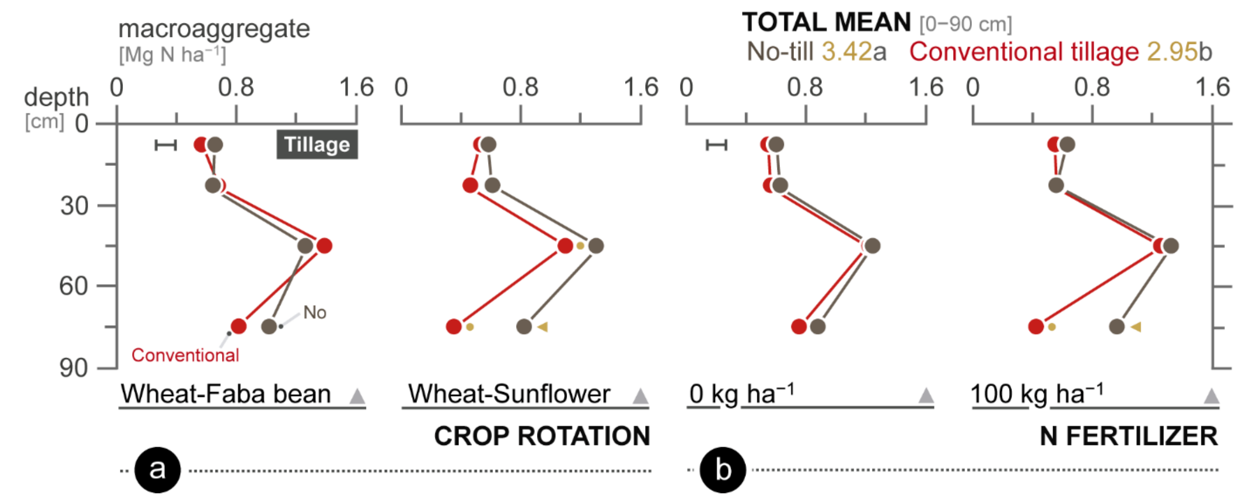

Fig. 5. Macroaggregate N stock as affected by a) tillage system, crop rotation and soil depth and b) tillage system, N fertilizer rate and soil depth. Triangle represents significant difference between tillage systems within crop rotation and depth in the first interaction, and within N fertilizer rate and depth in the second one. Horizontal bar represent LSD for comparison between depths within tillage system, and crop rotation or within N fertilizer rate. Yellow circle represents significant difference between crop rotations within tillage system and depth for the first interaction, and between N fertilizer rates within tillage system and depth in the second one. Means followed by the same letter are not significantly different.