**SOIL ORGANIC MATTER FRACTIONS AS AFFECTED BY TILLAGE
AND SOIL TEXTURE UNDER SEMIARID MEDITERRANEAN CONDITIONS**

Nuria Blanco-Moure, Ricardo Gracia, Ana C. Bielsa, M. Victoria López*

Departamento de Suelo y Agua, Estación Experimental de Aula Dei, Consejo Superior
de Investigaciones Científicas (CSIC), POB 13034, 50080 Zaragoza, Spain

* Corresponding author. Tel.: 34-976-71 61 44; fax: 34-976-71 61 45

E-mail address: vlopez@eead.csic.es

Abstract

The inherent complexity of soil organic matter (SOM) and its stabilization processes make suitable the identification of SOM fractions that reflect the management-induced changes in soil organic carbon (SOC) dynamics. This is of special interest in semiarid regions where the capacity of soil for agricultural production is limited. This study aims to evaluate the effect of different tillage and soil management practices on the distribution of C among SOM fractions and determine the influence of soil texture on the protection of SOC in a semiarid Mediterranean region (Aragon, NE Spain). Under on-farm conditions, pairs of adjacent fields under long-term no tillage (NT) and conventional tillage (CT) were compared in five different cereal production areas. In all cases, a nearby undisturbed soil under native vegetation (NAT) was included. Results indicate that the two isolated mineral-associated OM (Min) fractions, d-Min and μ agg-Min (outside and within stable microaggregates, respectively), constituted the main part of total SOC (mean contributions of 54 and 26% respectively) and were not consistently affected by soil management. Soil clay was a determinant factor for d-Min-C and total SOC ($r^2=0.60-0.70$; $P<0.001$), indicating that chemical stabilization, through clay-organic complexes, seems to be a main preservation mechanism in the studied soils. Physical protection seems to be another SOC stabilization process in these soils due to strong correlation found between μ agg-Min-C and the mass of water-stable microaggregates ($r=0.900$; $P<0.0001$). With smaller contributions to total SOC, the two labile fractions, coarse and fine particulate OM (cPOM and fPOM) were sensitive to soil management and their concentrations decreased as soil disturbance increased (NAT>NT>CT). The highest differences between NT and CT corresponded to fPOM at the soil surface where this fraction was 1.2-3 times higher under NT. Higher soil stratification ratios in NT, always >2 for the POM fractions, indicate an improvement in soil quality with long-term NT adoption in this semiarid Mediterranean region.

Keywords: Soil organic carbon, particulate organic matter, mineral-associated organic matter, conservation tillage, rainfed agriculture

Abbreviations: cPOM, coarse particulate organic matter fraction ($>250\ \mu\text{m}$ in size); fPOM, fine particulate organic matter fraction ($250\text{--}53\ \mu\text{m}$); d-Min, easily dispersed mineral-associated organic matter fraction ($<53\ \mu\text{m}$, outside water-stable microaggregates); $\mu\text{agg-Min}$, mineral-associated organic matter fraction in water-stable microaggregates ($<53\ \mu\text{m}$); μagg , microaggregate fraction ($250\text{--}53\ \mu\text{m}$ in size).

1. Introduction

Soil organic matter (SOM) has been described as the most complex and least understood component of soils (Magdoff and Weil, 2004). The complexity is due to the fact that SOM is a heterogeneous mixture of organic substances with different chemical composition and turnover rates. This is one reason why total soil organic carbon (SOC) is not always the best indicator of changes in soil management, especially in semiarid regions where low precipitation and high temperature are limiting factors for SOC storage (Chan et al., 2003; Melero et al., 2012). Under these conditions, significant increments in SOC are only to be expected several years after adoption of sustainable management practices (Laudicina et al., 2015).

With the aim of elucidating the complex composition of SOM and understanding its dynamics and mechanisms of stabilization, the scientific community has made considerable efforts to develop techniques to separate SOM into fractions of different composition and stability (see reviews by Wander, 2004; von Lützow et al., 2007). These advances have allowed differentiate labile and recalcitrant SOC pools and identify those that can serve as early indicators of changes in soil quality. This seems to be the case of the particulate organic matter (POM, $>53\text{ }\mu\text{m}$ in size) since it has been reported to be a sensitive labile fraction to management-induced changes in soil (Cambardella and Elliot, 1992; Wander, 2004). Increments in POM with the adoption of conservation tillage and, specially, with no tillage (NT) have been reported in several studies (Six et al., 1999, 2000; Dou and Hons, 2006; Virto et al., 2007; Álvaro-Fuentes et al., 2008; Martín-Lammerding, 2013). However, the magnitude of these increments is variable and the POM effectiveness as soil indicator seems to depend on different factors such as quantity and quality of crop residues, management historical and soil texture (Domínguez et al., 2009; Martín-Lammerding, 2013; Blanco-Moure et al., 2013).

1 As POM decomposes and fragments into finer organic matter, it becomes physically
2 and/or chemically stabilized within the soil mineral component as mineral-associated organic
3 matter (Min, <53 μm in size). The long persistence of Min-C in the soil and its high
4 contribution to total SOC, make this fraction of particular interest in the context of the global
5 C cycle (Schmidt et al., 2011; Feng et al., 2013). Considerable progress has been made in the
6 characterization of this fraction and, however, our knowledge is still limited due to its
7 heterogeneity and to the different stabilization mechanisms involved and their interactions
8 (von Lützow et al., 2007; Moni et al., 2010; O'Brien and Jastrow, 2013). Although there is
9 evidence that SOC in the Min fraction is controlled by soil texture and particularly by clay
10 content, the literature reveals that there is not always a close and direct relationship between
11 clay and SOC concentration (McLauchlan, 2006; Sleutel et al., 2006; Zhao et al., 2006). The
12 reason seems to be complex and can involve not only many influential factors, such as the
13 clay mineralogy (and specific surface area) or the composition of organic C inputs, but also
14 mechanisms other than simple monolayer sorption of SOM onto mineral surfaces as recent
15 studies have shown (O'Brien and Jastrow, 2013; Vogel et al., 2014).

16 All the above information suggests that more research is necessary to advance in the
17 characterization of SOC fractions and their dynamics, and thus, to develop soil management
18 strategies to increase the quantity and stability of SOM. No tillage can be a sustainable
19 strategy due to its potential to increase SOC (Govaerts et al., 2009; Cerdà et al., 2009). This is
20 of special relevance to the Mediterranean region where a 74% of land has a surface soil
21 horizon with less than 20 g kg^{-1} of SOC (Van-Camp et al., 2004). In Aragon (NE Spain), as in
22 the rest of Spain, the interest of farmers in NT has been increasing (López et al., 2012;
23 Gonzalez-Sanchez et al., 2015). However, there is still little available information on SOM
24 and its fractions in agricultural soils of the region and it comes from small research plots and
25 single soil types. The objectives of this study were to (1) determine the effect of soil tillage on

the distribution of C among SOM fractions by comparing traditional tillage with long-term NT and with undisturbed soils under native vegetation, and (2) evaluate the influence of soil texture, and other basic soil properties, on SOC protection, giving special attention to the Min-C pool. The study was conducted under on-farm conditions in different cereal production areas of Aragon across different soils, microclimates and agronomic practices.

2. Materials and methods

2.1. Description of the study sites

The study was conducted at six long-term NT fields (9-21 years) representative of the different scenarios of NT in Aragon (NE Spain) and located in areas receiving a mean annual precipitation ranging from 350 to 740 mm (Table 1). These fields were selected from a previous study where 22 soils under NT were characterized across different rainfed cereal areas of the region (López et al., 2012). With the exception of the Peñaflor site, the study was carried out under on-farm conditions (fields of collaborating farmers) where pairs of adjacent fields under NT and conventional tillage (CT) were compared. In Peñaflor, the study was conducted in the research plots from a long-term tillage experiment at the dryland research farm of the Estación Experimental de Aula Dei (Consejo Superior de Investigaciones Científicas). In this case, tillage treatments (NT, CT and reduced tillage, RT) were arranged in a randomized complete block design with 3 replicates. More details about the Peñaflor site can be found in López et al. (1996). In all sites, an undisturbed soil under native vegetation (NAT) and close to the NT and CT fields was included in the study for comparison purposes.

Information on location and soil management characteristics for each site are shown in Table 1 and detailed in a previous study (Blanco-Moure et al., 2012). It should be noted, briefly, the case of Artieda, the study site located in the area with the highest rainfall and hence highest production. As a common practice in this area, farmer removes the straw from

the NT and CT fields to prevent later problems with seeding. The information in Table 1 reflects the diversity of cropping systems and the reality of the conservation agriculture in the region (López et al., 2012). Therefore, following the remark made by Blanco-Canqui and Lal (2008), the present study shows data on the effect of NT- and CT-based cropping systems on SOC rather than those of tillage alone.

All soils were medium-textured soils (from sandy loam to silty clay loam), alkaline ($\text{pH} > 8$; CaCO_3 contents of 60-560 g kg^{-1}) and generally low in OC content ($< 20 \text{ g kg}^{-1}$) (Table 2). In each site, both NT and CT fields were contiguous and the NAT soil close to them, thus ensuring that soil type and topography were as similar as possible. All fields were nearly level with the exception of those of Torres de Alcanadre (hereafter, Torres) where a slight slope (3-4%) was present.

2.2. Soil sampling and analyses

Soil samples were collected at three depths: 0-5, 5-20 and 20-40 cm. In the farmer fields, soil sampling was made in three different zones within each field (NT, CT and NAT) where two samples per depth were collected and mixed to make a composite sample. In the Peñaflor site, each of the composite samples came from each of the three tillage plots per treatment (NT, RT and CT). Thus, a total of 27 composite samples were obtained from each site (36 in Peñaflor) (3 or 4 treatments x 3 depths x 3 replicates). The study was conducted in two growing seasons (2009-2010 and 2010-2011), with soil samplings restricted to a similar period of the season. Thus, at the sites where continuous cropping is practiced, samplings were carried out after planting during the fall period and, at the fields under cereal-fallow rotation, during the early fallow phase after cereal harvest in June. At each site, all soil samples were collected on the same day.

1 Once in the laboratory, the field moist soil samples were passed through an 8 mm sieve
2 by gently breaking up the soil clods along their natural planes of failure. Before air drying at
3 room temperature, large rocks, visible roots and organic debris were removed by hand. Air-
4 dry soil from each depth, field and site was physically fractionated to isolate different organic
5 matter fractions in a simple, two-step process, modified from the Six et al. (2002a) and Plante
6 et al. (2006a) procedures.

7 In a first step, whole-soil samples were partially dispersed using the microaggregate
8 isolation method of Six et al. (2000, 2002a) to obtain three size fractions: coarse particulate
9 organic matter fraction (cPOM, $>250\ \mu\text{m}$), microaggregate fraction (μagg , $250\text{--}53\ \mu\text{m}$), and
10 easily dispersed mineral-associated organic matter fraction (d-Min, $<53\ \mu\text{m}$). Briefly, 50 g of
11 air-dried soil was placed on a $250\ \mu\text{m}$ sieve and submerged in deionized water for 30 min to
12 allow slaking. After this time, soil was gently shaken with 50 glass beads (6 mm in diameter)
13 in running water over the $250\ \mu\text{m}$ sieve until complete macroaggregate disruption. The
14 continuous and steady water flow prevented the disruption of microaggregates which, together
15 with finer particles, were immediately flushed on a $53\ \mu\text{m}$ sieve. The material retained on the
16 $53\ \mu\text{m}$ sieve was wet sieved by hand for 50 strokes in 2 min to separate the water-stable
17 microaggregates from the d-Min fraction. The three fractions were oven dried at $60\ ^\circ\text{C}$ and
18 weighed.

19 In the second step, the isolated microaggregates were subject to further fractionation to
20 obtain two fractions: the fine particulate organic matter fraction (fPOM, $250\text{--}53\ \mu\text{m}$) and the
21 mineral-associated fraction occluded within microaggregates ($\mu\text{agg-Min}$, $<53\ \mu\text{m}$). For this,
22 10 g of the microaggregate samples were dispersed by shaking for 18 h with 15 glass beads (4
23 mm in diameter) in 50 ml deionized water. After shaking, the suspension was poured over the
24 $53\ \mu\text{m}$ sieve and washed to separate fPOM and $\mu\text{agg-Min}$ fractions. Fractions were oven dried
25 ($60\ ^\circ\text{C}$) and weighed.

Organic carbon content was measured in the four isolated fractions (cPOM, fPOM, d-Min and μ agg-Min) by using a LECO analyser (RC-612 model). Total SOC was calculated as the sum of the OC fractions and, in some soil samples, total OC was also measured to compare and determine the efficiency of the fractionation procedure. Soil particle size distribution was obtained by laser diffraction analysis (Coulter LS230), CaCO_3 content by dry combustion with the LECO analyzer, and electrical conductivity (EC), pH and gypsum content by standard methods (Page et al., 1982).

2.3. Statistical analyses

As the study was conducted under on-farm conditions, the NT, CT, and NAT treatments were not field replicated. In each of the sites, however, the three fields were contiguous and sited on similar landscape position and same soil. Therefore, the three sampling locations within each field were used as pseudoreplicates and statistical comparisons among treatments were made using one-way ANOVA, assuming a randomized experiment (Christopher et al., 2009). In the case of the Peñaflor site, the randomized complete block design with three replicates per tillage treatment (CT, RT and NT) was also applied and statistical results were compared with those obtained from the pseudoreplicate analysis. Duncan's multiple range test was used to compare treatment means ($P < 0.05$). When data showed non-normality, transformations were made and ANOVA conducted with the transformed data. Computations were performed using SPSS 19.0 statistical software.

3. Results and discussion

Mass recovery after the two-step fractionation process was $97.6 \pm 1.7\%$ (mean $\pm$ standard deviation) and OC recovery was $99.3 \pm 2.9\%$. These values, close to 100% and similar to recovery rates registered in the literature (Plante et al., 2006a,b; Moni et al., 2010;

O'Brien and Jastrow, 2013), indicate that the loss of material was low, thus confirming the efficiency of the fractionation procedure.

Organic C concentrations of the four isolated fractions for each study site, soil management and soil depth are shown in Table 3. Average total SOC concentration in the 0-40 cm layer varied from 10 g kg⁻¹ at Torres and Lanaja to 18 g kg⁻¹ at Undués de Lerda (hereafter, Undués), being ≈11 g kg⁻¹ at Peñaflor and 12 g kg⁻¹ at Artieda (Table 2). These contents, lower than 20 g kg⁻¹, are in agreement with the levels of SOC estimated for this region in the Map of Topsoil Organic Carbon in Europe (Jones et al., 2005) and, in general, for Southern Europe where a 74% of the land has a surface soil horizon with less than 20 g kg⁻¹ of OC (Van-Camp et al., 2004). The further characterization of SOC in the present study showed that the highest contribution to total SOC corresponded to the d-Min-C fraction, accounting for ca. 40-70% of total OC (Table 3). It was followed by the μagg-Min-C fraction (15-40%) and, finally, by the fPOM-C (6-30%) and cPOM-C (<10%) fractions.

3.1. Mineral-associated organic matter fractions

The d-Min-C plus the μagg-Min-C form the total Min-C fraction (<53 μm) which constituted 70-90% of total OC in these soils.

3.1.1. Easily dispersed mineral-associated organic matter

The d-Min-C fraction (<53 μm outside water-stable microaggregates) was not greatly affected by soil management with the exception of the Undués and Artieda sites where NAT soils had the highest carbon contents (Table 3). Although this effect was significant ($P<0.05$) in all soil depths, the greatest differences occurred at the surface where the d-Min-C concentration was 1.5-2.7 times higher in NAT than in the agricultural soils. A different pattern was observed at the most arid sites where the d-Min-C was equal or even less in NAT than in agricultural soils, as can be observed in the deeper layers at Peñaflor and Lanaja (Table

3). This is due to the low and variable precipitation received at these sites which limits primary production and OC storage also in the NAT soils. At Lanaja, the lowest d-Min-C is explained, in addition, by the semi-natural conditions of this field (Table 1). In the cultivated soils, the d-Min-C content was not significantly affected by tillage with the exception to the Peñaflor site where the soil surface (0-5 cm) under NT had a significant higher d-Min-C than the soils under CT and RT (Table 3). However, in depth (20-40 cm), this behavior was reversed, showing CT the highest concentration.

There are few studies in the literature that evaluate the effect of tillage on the d-Min-C fraction. These include the work of Dou and Hons (2006) who found that, after 20 years of NT, the content of this fraction at the soil surface was higher under NT than CT. However, they also observed that this difference between treatments was declining with decreasing the crop rotation intensity and it was always lower than the quantified for the coarser size fractions ($>53\ \mu\text{m}$). In other study, Plante et al. (2006b), comparing forest and agricultural soils under CT and NT (NT for ≈ 24 years), observed that the OC content of the easily dispersed silt- and clay-sized fractions were not greatly affected by either land use or tillage system.

The relatively low responsiveness of the d-Min fraction to soil management can be explained by the nature of this fraction. The d-Min fraction consists of OM associated with soil minerals (clay and silt particles) through many different organomineral interactions (hydrogen bonds, polyvalent cation bridging, van der Waals forces, etc.) (Feng et al., 2013; Lopez-Sangil and Rovira, 2013). Although the chemical stabilization is, therefore, the main preservation mechanism of d-Min-C, the biochemical stabilization is also involved due to the presence of recalcitrant OM in this pool (Dou and Hons, 2006; Plante et al. 2006b; Stewart et al., 2009). The high degree of OC protection from decomposition exerted by the inherent biochemical recalcitrance of the OM and by the strong associations with the active mineral

surfaces explains the persistence of the Min-C during decades (biochemical stabilization) or centuries-millennia (chemical stabilization) (Schmidt et al., 2011).

Of particular interest is the role of clay particles in the OM stabilization due to their high specific surface area and charge. In fact, clay minerals are considered the most active constituents in the formation of organomineral complexes (Chenu et al., 2006) and responsible for the long-term preservation of SOM, even over millennia (Zhou et al., 2014). This predominant role of clay particles seems to be confirmed in our study through the relationships between soil texture and OC content. Although soil texture was dominated by the silt-sized particles in all of the study sites except Torres with a more sandy soil (Table 2), stronger relations with SOC were obtained by using clay instead of silt+clay as predictor variable. Significant positive relationships ($P<0.001$) between clay content and d-Min-C, Min-C or total SOC concentrations were found for both agricultural and NAT soils at either 0-5 or 0-40 cm depths (Table 4 and Fig. 1). Considering the total soil layer characterized (0-40 cm), the percentage of variation explained by linear regressions was, on average, 68% for d-Min-C and Min-C fractions, and 65% for total SOC. At the soil surface, these percentages reached 80% in the NAT soils and decreased to 30-50% in the agricultural soils (Table 4), reflecting the mixing effect of tillage in the plough layer. The greater regression slopes in NAT (Table 4) indicate that the differences in OC between NAT and agricultural soils were higher in the soils with higher clay contents. This observation was more pronounced at the soil surface and, especially, for total SOC. Similar results were found by Hevia et al. (2003) comparing cultivated and virgin soils in the Semiarid Pampas of Argentina, and indicate that cultivation of natural lands can lead to higher losses of OM in fine textured soils than in coarser soils.

Different previous studies have reported positive relationships between clay or clay+silt content and Min-C or total SOC using a wide variety of regression models (linear, exponential, quadratic, two-segment linear) and also showing very different coefficients of

determination ($r^2 = 0.20-0.70$; $P < 0.05$) (Six et al., 2002b; Hevia et al., 2003; Hao and Kravchenko, 2007; Shrestha et al., 2007; Gami et al., 2009). However, there are also works reporting no or little effect of soil texture on SOC (McLauchlan, 2006; Sleutel et al., 2006) probably due to differences in clay mineralogy, limited textural range, high variations in SOC pools or in environmental conditions, and, finally, to the complexity of the mechanisms of SOM stabilization (Plante et al., 2006a). In this sense, the significant relationships obtained in the present study are considered satisfactory and provide further information on the key role of the d-Min-C fraction in the stabilization of SOC by fine mineral particles since no other isolated OM fractions were correlated with soil texture.

3.1.2. Mineral-associated organic matter within microaggregates

The μ agg-Min-C fraction ($< 53 \mu\text{m}$ occluded within water-stable microaggregates) in all cases contributed to total Min-C in a lesser degree than the d-Min-C fraction (20-50% vs. 50-80%). No consistent results were found concerning the effect of land use and tillage on μ agg-Min-C (Table 3). At the soil surface (0-5 cm depth), this fraction was higher in the NAT than in the CT soils at Undués, Lanaja and Torres (40-50% more) whereas the opposite occurred at Peñaflor where it was, on average, 30% lower in the NAT than in the agricultural soils. Considering only the cultivated soils, with the exception of the Peñaflor site, the μ agg-Min-C content at the soil surface was in all cases higher under NT than under CT (30-50% more). Dou and Hons (2006) also reported increments between 30 to 50% of this OC fraction in the first 5 cm of a NT soil with respect to CT for different cropping systems. Likewise, in three soils with different texture and clay mineralogy, Denef et al. (2004) found that the Min-C associated with microaggregates within macroaggregates was significantly greater under NT than under CT even to a soil depth of 20 cm. On the contrary, in the current study, at Peñaflor CC and for total soil depth (0-40 cm), the μ agg-Min-C was ca. 30 and 40% higher in CT than in NT and NAT, respectively.

1 The unexpected effect of CT at the Peñaflor site, and especially under the CC system,
2 could be explained because, in contrast to the other sites, crop residues are left in the field
3 after harvest also in this tillage treatment (see Table 1). During 19 years, the annual
4 incorporation of fresh organic material into the soil by mouldboard ploughing has probably
5 favored the occlusion of OC within soil aggregates, increasing in this way the $\mu\text{agg-Min-C}$ in
6 the plough layer of CT compared to no tilled soils. This interpretation is supported by the
7 results of Gregorich et al. (2009) and Andruschkewitsch et al. (2014) who found greater OC
8 contents within stable soil aggregates in CT than in NT soils at or near the bottom of the
9 plough layer and, sometimes, also in the surface layers.

10 In contrast to d-Min-C, the $\mu\text{agg-Min-C}$ fraction was not significantly correlated with
11 soil texture variables. This implies that, at the study sites, the stabilization of Min-C within
12 stable microaggregates was not dependent on the soil clay and/or silt content. This difference
13 between the two Min fractions seems to be in line with previous observations on the distinct
14 nature and behavior of both pools (Plante et al., 2006c; Stewart et al., 2009). Recent studies
15 have demonstrated that mechanisms other than simple monolayer adsorption of SOM onto
16 clay surfaces are implied in the SOM stabilization by fine soil particles (McCarthy et al.,
17 2008; Moni et al., 2010; O'Brien and Jastrow, 2013; Vogel et al., 2014). More complex
18 multilayer arrangements involving multiple mineral surfaces, i.e., clay- and silt-sized
19 aggregates, zonal structures or OM-filled pores, would mean a Min-C content higher than that
20 expected on the basis of the specific surface area of clays (O'Brien and Jastrow, 2013).
21 Recently, Vogel et al. (2014), with incubation experiments, observed that OM was
22 preferentially attached to organo-mineral clusters with rough surfaces containing pre-existing
23 OM and that less than 19% of the total soil mineral area was covered by OM. These findings
24 could explain why clay and/or silt content is sometimes weakly or not related to SOC, as it
25 can be the case of $\mu\text{agg-Min-C}$ in the present study.

The physical protection of SOM provided by soil microaggregates was confirmed by the positive correlation found between the $\mu\text{agg-Min-C}$ concentration and the mass of water stable microaggregates (sand-free basis) at either 0-5 or 0-40 cm soil depth ($r \approx 0.900$; $P < 0.0001$). The importance of SOM as cementing and stabilizing agent in soil aggregation can also be inferred from this significant relationship. Considering together all sites and treatments, multiple regression analysis showed that 80-90% of the total variation in the microaggregate mass was explained by $\mu\text{agg-Min-C}$ and, to a much lesser extent, by soil clay and CaCO_3 contents (Table 5 and Fig. 2). The very weak influence of clay and CaCO_3 (explaining only 10% of the variation) would indicate that, in the studied soils, these properties were not highly involved in the formation and persistence of water-stable microaggregates. These results are supported by a previous study on the dynamics of aggregate destabilization by water in these same soils (Blanco-Moure et al., 2012). That study showed that slaking was the dominant process of aggregate destabilization by water in these soils, being strongly and negatively affected by the aggregate-associated OC content and not by other soil variables such as clay or CaCO_3 . In this same sense, Wuddivira and Camps-Roach (2007) showed that the positive influence of SOM on structural stability was more pronounced in soils with low clay content. Furthermore, these authors reported that both clay and calcium may either favour or prevent slaking and dispersion depending on their interactions with SOM and on the clay mineralogy. In Mediterranean soils, Boix-Fayos et al. (2001) found that, at low SOC concentration, the water stability of macroaggregates was enhanced by carbonates but not in the case of microaggregates. Anyway, further research on the interaction between organic and inorganic soil components is required to improve our understanding of protection mechanisms of SOM in microaggregates.

3.2. Particulate organic matter fractions

1 The cPOM-C plus the fPOM-C form the total POM-C fraction which constituted 10-
2 30% of total OC in these soils. The fPOM-C was always present at a higher concentration than
3 cPOM-C (Table 3). The fPOM/cPOM ratio averaged 2 at the soil surface and increased to
4 over 6 in the deeper soil layer. Moreover, in some cases, as Lanaja or Undués, fPOM was the
5 only POM fraction present in depth since the amount of cPOM was negligible. It is important
6 to remind that, in the present study, the total POM was divided by size and that we did not
7 differentiate between free and occluded POM. However, the cPOM-C (>250 μ m) constitutes
8 an unprotected OC pool (as free or within macroaggregates) and represents the easily
9 decomposable SOM (Six et al., 2002b). In contrast, part of the fPOM-C isolated in our study
10 is a physically protected OC pool since it was protected within stable microaggregates. The
11 rest of fPOM-C, resulting from the breaking up of macroaggregates (inter-microaggregate
12 fPOM) and unstable microaggregates, can be considered unprotected OC (Six et al., 2002b).
13 The fPOM represents an intermediate or transitional status between the cPOM and the Min
14 fractions and, in general, it is not as dynamic and variable as cPOM (Duval et al., 2013).

15 In contrast to the Min-C fractions, the POM-C fractions were clearly influenced not only
16 by land use but also by tillage system (Table 3). The fPOM-C concentration in the agricultural
17 soils was from 24 (Undués) to 84% (Lanaja) of that in the NAT soils for 0-40 cm with
18 differences being statistically significant throughout the whole soil profile. In the case of the
19 cPOM-C, these percentages were lower (10-63%) and the differences between agricultural
20 and NAT soils decreased with soil depth, disappearing in the 20-40 cm layer. For different
21 regions of the Argentinean pampas, Duval et al. (2013) also found a depletion of fPOM and,
22 especially, of cPOM by cultivation with reductions of 20-70% as compared to natural soils.

23 Within the cultivated soils, the largest differences among tillage systems were found at
24 the soil surface (Table 3). In the upper 5 cm depth, the fPOM-C content was between 1.2
25 (Artieda) and 3 times higher (Peñaflor CC) under NT than under CT. With the exception of

Artieda, the cPOM fraction followed a similar pattern as fPOM although the tillage effect was less pronounced. At Artieda, the lower cPOM-C content under NT than under CT in the three soil layers (2-2.6 times less) can be explained by the farmer's practice of removing crop residues from the NT field (see Table 1). At the Peñaflor site, under both CF and CC cropping systems, the RT response was intermediate between CT and NT with cPOM-C and fPOM-C concentrations significantly higher than those under CT (1.6-1.9 times higher).

In our study conditions, both fPOM and cPOM fractions contributed to the differences among sites based on the relative gain or loss of OC under NT with respect to CT at each site (i.e. (NT-CT)/CT). With the exception of cPOM at Artieda and Undués, POM-C was always higher under NT than under CT in the surface layer (Table 3). These values represent between 24 and 191% more fPOM-C with NT, averaging about 105% more. For cPOM, this range varied from 48% less to 187% more, with an average value of 74% more. Figure 3 shows negative and strong relationships ($P < 0.01$) between the relative difference in the concentration of both fPOM-C and cPOM-C at 0-5 cm depth and the mean annual precipitation at each site thus indicating that the relative gain of OC with the adoption of NT decreases as precipitation increases. Total SOC was also influenced by precipitation but was much less responsive than POM fractions ($r^2 = 0.588$). As discussed in our previous study (Blanco-Moure et al., 2013), although climatic factors, such as precipitation, have been shown to exert a control on the potential of NT to store SOC, the direction and magnitude of this potential with respect to CT systems can vary widely. However, our finding on the increase in the relative benefit of NT with reduction in precipitation is supported by Gregorich et al. (2009) who compare SOC storage in different Canadian regions. Similar conclusions are made by Luo et al. (2010) for agricultural soils in Australia. In these studies the authors suggest that compared to low rainfall areas, the faster decomposition of SOM in the surface layer under a more humid

environment minimizes the overall change of SOC balance between input and output under NT and, therefore, the relative OC gain with respect to CT.

3.3. Soil organic matter stratification ratio

For both cPOM and fPOM fractions, the decreasing pattern in OC concentration with depth was more prominent under conservation tillage, especially NT, in such a way that the average concentrations in the 0-40 cm profile were not significantly different from those under CT. This depth gradient was reflected in the values of the stratification ratio, SR (Franzluebbers, 2002), calculated as the OC concentration at 0-5 cm depth divided by that at 20-40 cm (Table 6). In addition to NAT soils, in NT and RT soils the SR for both POM fractions was greater than the threshold value of 2. Even under CT, $SR > 2$ were obtained for these fractions at the most humid sites. This contrasts with the relatively low SR for μ agg-Min-C, d-Min-C and SOC. The marked stratification of POM-C is generally observed under continuous NT management (Álvaro-Fuentes et al., 2008; Salvo et al., 2010) and is produced by the maintenance of crop residues at the soil surface and the absence of soil disturbance.

The POM-C, disproportionately to its small contribution to total SOC, has a large effect on nutrient-supplying capacity and structural stability of soils, and for these reasons it is considered a key attribute of soil quality (Haynes, 2005). Of the two POM fractions isolated in the present study, fPOM was, in general, more sensitive to soil tillage and land use than cPOM. On the other hand, cPOM is more dependent on plant derived C inputs and, therefore, more variable in time and space (also in depth) than fPOM (Lee et al., 2009; Duval et al., 2013). For those reasons, fPOM can be considered more reliable and useful indicator of soil changes associated with tillage and crop residue management.

4. Conclusions

This on-farm study reinforces the importance of analyzing SOC fractions to improve our understanding of the management-induced changes in SOC dynamics. Among the four isolated fractions, the d-Min-C (<53 μm , outside water-stable microaggregates) provided the highest contribution to total SOC (40-70%) and was not greatly affected by soil management. Soil clay content was a determinant factor for d-Min-C, indicating that chemical stabilization, through clay-organic complexes, seems to be a main preservation mechanism of OC in the studied soils. In contrast, soil texture did not influence the Min-C content occluded within water-stable microaggregates ($\mu\text{agg-Min-C}$, 15-40% of total SOC). However, the strong relationship found between this fraction and the mass of stable microaggregates indicated that physical protection is also a stabilization process of OC involved in these soils. The two labile fractions isolated, cPOM- and fPOM-C, despite their small contributions to total SOC (<10% and 6-30%, respectively), were sensitive to soil management and their concentrations decreased as soil disturbance increased (NAT>NT>RT>CT). These fractions were also sensitive to management of crop residues after harvest both in NT and CT. The highest differences between NT and CT corresponded to fPOM-C and were generally restricted to the soil surface where this fraction was 1.2-3 times higher under NT. This relative gain with respect to CT increased in the most arid locations of the agroclimatic gradient. Likewise, higher soil stratification ratios under NT, always >2 for the POM-C fractions, suggest an improvement in soil quality with the long-term NT adoption in this semiarid Mediterranean region.

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References

- Álvaro-Fuentes, J., López, M.V., Cantero-Martínez, C., Arrúe, J.L., 2008. Tillage effects on soil organic carbon fractions in Mediterranean dryland agroecosystems. *Soil Sci. Soc. Am. J.* 72, 541-547.
- Andruschkewitsch, R., Koch, H., Ludwig, B., 2014. Effect of long-term tillage treatments on the temporal dynamics of water-stable aggregates and on macro-aggregate turnover at three German sites. *Geoderma* 217-218, 57-64.
- Blanco-Canqui, H., Lal, R., 2008. No-tillage and soil profile carbon sequestration: An on-farm assessment. *Soil Sci. Soc. Am. J.* 72, 693-701.
- Blanco-Moure, N., Moret-Fernández, D., López, M.V., 2012. Dynamics of aggregate destabilization by water in soils under long-term conservation tillage in semiarid Spain. *Catena* 99, 34-41.
- Blanco-Moure, N., Gracia, R., Bielsa, A., López, M.V., 2013. Long-term no tillage effects on particulate and mineral associated soil organic matter under rainfed Mediterranean conditions. *Soil Use Manage.* 29, 250-259.
- Boix-Fayos, C., Calvo-Cases, A., Imeson, A.C., Soriano-Soto, M.D., 2001. Influence of soil properties on the aggregation of some Mediterranean soils and the use of aggregate size and stability as land degradation indicators. *Catena* 44, 47-67.
- Cambardella, C.A., Elliot, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56, 777-783.
- Cerdà, A., Flanagan, D.C., le Bissonnais, Y., Boardman, J. 2009. Soil erosion and agriculture. *Soil Till. Res.* 106, 107-108.
- Chan, K.Y., Heenan, D.P., So, H.B., 2003. Sequestration of carbon and changes in soil quality under conservation tillage on light-textured soils in Australia: a review. *Aus. J. Exp. Agric.* 43, 325-334.

1 Chenu, C., Plante, A.F., Puget, P., 2006. Organo-mineral relationships. In: Lal, R. (Ed.)
2 Encyclopedia of Soil Science. CRC Press, Boca Raton, pp. 1227-1230.

3 Christopher, S.F., Lal, R., Mishra, U., 2009. Regional study of no-till effects on carbon
4 sequestration in the Midwestern United States. *Soil Sci. Soc. Am. J.* 73, 207-216.

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

7 Domínguez, G.F., Diovisalvi, N.V., Studdert, G.A., Monterubbianesi, M.G., 2009. Soil
8 organic C and N fractions under continuous cropping with contrasting tillage systems on
9 mollisols of the southeastern Pampas. *Soil Till. Res.* 102, 93-100.

10 Dou, F., Hons, F.M., 2006. Tillage and nitrogen effects on soil organic matter fractions in
11 wheat-based systems. *Soil Sci. Soc. Am. J.* 70, 1896-1905.

12 Duval, M.E., Galantini, J.A., Iglesias, J.O., Canelo, S., Martinez, J.M., Wall, L., 2013.
13 Analysis of organic fractions as indicators of soil quality under natural and cultivated
14 systems. *Soil Till. Res.* 131, 11-19.

15 Feng, W., Plante, A.F., Six, J., 2013. Improving estimates of maximal organic carbon
16 stabilization by fine soil particles. *Biogeochemistry* 112, 81-93.

17 Franzluebbers, A.J., 2002. Soil organic matter stratification ratio as an indicator of soil
18 quality. *Soil Till. Res.* 66, 95-106.

19 Gami, S.K., Laure, J.G., Duxbury, J.M., 2009. Influence of soil texture and cultivation on
20 carbon and nitrogen levels in soils of the eastern Indo-Gangetic Plains. *Geoderma* 153,
21 304-311.

22 Gonzalez-Sanchez, E.J., Veroz-Gonzalez, O., Blanco-Roldan, G.L., Marquez-Garcia, F. and
23 Carbonell-Bojolo, R., 2015. A renewed view of conservation agriculture and its evolution
24 over the last decade in Spain. *Soil Till. Res.* 146, 204-212.

- 1 Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K. D., Dixon, J., Dendooven, L.,
2 2009. Conservation agriculture and soil carbon sequestration: Between myth and farmer
3 reality. *Crit. Rev. Plant Sci.* 28, 97-122.
- 4 Gregorich, E.G., Carter, M.R., Angers, D.A., Drury, C.F., 2009. Using a sequential density
5 and particle-size fractionation to evaluate carbon and nitrogen storage in the profile of
6 tilled and no-till soils in eastern Canada. *Can. J. Soil Sci.* 89, 255-267.
- 7 Hao, X., Kravchenko, A.N., 2007. Management practice effects on surface total carbon:
8 differences along a textural gradient. *Agron. J.* 99, 18-26.
- 9 Haynes, R.J., 2005. Labile organic matter fractions as central components of the quality of
10 agricultural soils: an overview. *Adv. Agron.* 85, 221-268.
- 11 Hevia, G.G., Buschiazzo, D.E., Hepper, E.N., Urioste, A.M., Antón, E.L., 2003. Organic
12 matter in size fractions of soils of the semiarid Argentina. Effects of climate, soil texture
13 and management. *Geoderma* 116, 265-277.
- 14 Jones, A., Montanarella, L., Jones, R., 2005. Soil Atlas of Europe. European Soil Bureau
15 Network. European Commission, 128 pp. Office for Official Publications of the European
16 Communities, Luxembourg.
- 17 Laudicina, V.A., Novara, A., Barbera, V., Egli, M., Badalucco, L., 2012. Long-term tillage
18 and cropping system effects on chemical and biochemical characteristics of soil organic
19 matter in a Mediterranean environment. *Land Degrad. Develop.* 26, 45-63.
- 20 Lee, J., Laca, E.A., van Kessel, C., Rolston, D.E., Hopmans, J.W., Six, J., 2009. Tillage
21 effects on spatiotemporal variability of particulate organic matter. *Appl. Environ. Soil Sci.*
22 76, 1-14.
- 23 López, M.V., Arrúe, J.L., Sánchez-Girón, V., 1996. A comparison between seasonal changes
24 in soil water storage and penetration resistance under conventional and conservation
25 tillage systems in Aragón. *Soil Till. Res.* 37, 251-271.

- 1 López, M.V., Blanco-Moure, N., Limón, M.A., Gracia, R., 2012. No tillage in rainfed Aragon
2 (NE Spain): Effect on organic carbon in the soil surface horizon. *Soil Till. Res.* 118, 61-
3 65.
- 4 Lopez-Sangil, L., Rovira, P., 2013. Sequential chemical extractions of the mineral-associated
5 soil organic matter: An integrated approach for the fractionation of organo-mineral
6 complexes. *Soil Biol. Biochem.* 62, 57-67.
- 7 Luo, Z., Wang, E., Sun, O.J., 2010. Soil carbon change and its responses to agricultural
8 practices in Australian agro-ecosystems: A review and synthesis. *Geoderma* 155, 211-223.
- 9 Magdoff, F., Weil, R.R., 2004. *Soil Organic Matter in Sustainable Agriculture*. CRC Press,
10 Boca Raton, FL, USA, 398 p.
- 11 Martín-Lammerding, D., Tenorio, J.L., Albarrán, M.M., Zambrana, E., Walter, I., 2013.
12 Influence of tillage practices on soil biologically active organic matter content over a
13 growing season under semiarid Mediterranean climate. *Spanish J. Agric. Res.* 11, 232-243.
- 14 McCarthy, J.F., Ilavsky, J., Jastrow, J.D., Mayer, L.M., Perfect, E., Zhuang, J., 2008.
15 Protection of organic carbon in soil microaggregates occur via restructuring of aggregate
16 porosity and filling pores with accumulating organic matter. *Geochim. Cosmochim. Ac.*
17 72, 4725-4744.
- 18 McLauchlan, K.K., 2006. Effects of soil texture on soil carbon and nitrogen dynamics after
19 cessation of agriculture. *Geoderma* 136, 289-299.
- 20 Melero, S., López-Bellido, R.J., López-Bellido, L., Muñoz-Romero, V., Moreno, F., Murillo,
21 J.M., Franzluebbers, A.J., 2012. Stratification ratio in a rainfed Mediterranean Vertisol in
22 wheat under different tillage, rotation and N fertilization rates. *Soil Till. Res.* 119, 7-12.
- 23 Moni, C., Rumpel, C., Virto, I., Chabbi, A., Chenu, C., 2010. Relative importance of sorption
24 versus aggregation for organic matter storage in subsoil horizons of two contrasting soils.
25 *Eur. J. Soil Sci.* 61, 958-969.

- 1 O'Brien, S.L., Jastrow, J.D., 2013. Physical and chemical protection in hierarchical soil
2 aggregates regulates soil carbon and nitrogen recovery in restored perennial grasslands.
3 *Soil Biol. Biochem.* 61, 1-13.
- 4 Page, A.L., Miller, R.H., Keeney, D.R., (Eds.), 1982. *Methods of Soil Analysis: Part 2.*
5 *Chemical and Microbiological Properties*, 2nd edn. Agronomy No. 9. ASA, Madison, WI.
- 6 Plante, A.F., Conant, R.T., Stewart, C.E., Paustian, K., Six, J., 2006a. Impact of soil texture on
7 the distribution of soil organic matter in physical and chemical fractions. *Soil Sci. Soc.*
8 *Am. J.* 70, 287-296.
- 9 Plante, A.F., Stewart, C.E., Conant, R.T., Paustian, K., Six, J., 2006b. Soil management
10 effects on organic carbon in isolated fractions of a Grey Luvisol. *Can. J. Soil Sci.* 86, 141-
11 151.
- 12 Plante, A.F., Conant, R.T., Paul, E.A., Paustian, K., Six, J., 2006c. Acid hydrolysis of easily
13 dispersed and microaggregate-derived silt- and clay-sized fractions to isolate resistant soil
14 organic matter. *Eur. J. Soil Sci.* 57, 456-467.
- 15 Salvo, L., Hernández, J., Ernst, O., 2010. Distribution of soil organic carbon in different size
16 fractions, under pasture and crop rotations with conventional tillage and no-till systems.
17 *Soil Till. Res.* 109, 116-122.
- 18 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A.,
19 Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse,
20 D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem
21 property. *Nature* 478, 49-56.
- 22 Shrestha, B.M., Singh, B.R., Sitaula, B.K., Lal, R., Bajracharya, R.M., 2007. Soil aggregate-
23 and particle-associated organic carbon under different land uses in Nepal. *Soil Sci. Soc.*
24 *Am. J.* 71, 1194-1203.

- 1 Six, J., Elliott, E. T., and Paustian, K., 1999. Aggregate and soil organic matter dynamics
2 under conventional and no-tillage systems. *Soil Sci. Soc. Am. J.* 63, 1350-1358.
- 3 Six, J., Elliot, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate
4 formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol.*
5 *Biochem.* 32, 2099-2103.
- 6 Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul, E.A.,
7 Paustian, K., 2002a. Measuring and understanding carbon storage in afforested soils by
8 physical fractionation. *Soil Sci. Soc. Am. J.* 66, 1981-1987.
- 9 Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002b. Stabilization mechanisms of soil organic
10 matter: Implications for C-saturation of soils. *Plant Soil* 241, 155-176.
- 11 Sleutel, S., De Neve, S., Singier, B., Hofman, G., 2006. Organic C levels in intensively
12 managed arable soils—long-term regional trends and characterization of fractions. *Soil Use*
13 *Manage.* 22, 188-196.
- 14 Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2009. Soil carbon saturation:
15 Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biol.*
16 *Biochem.* 41, 357-366.
- 17 Van-Camp, L., Bujarrabal, B., Gentile, A-R., Jones, R.J.A., Montanarella, L., Olazabal, C.,
18 Selvaradjou, S-K., 2004. Reports of the Technical Working Groups Established under the
19 Thematic Strategy for Soil Protection. EUR 21319 EN/3, 872 pp. Office for Official
20 Publications of the European Communities, Luxembourg.
- 21 Virto, I., Imaz, M.J., Enrique, A., Hoogmoed, W., Bescansa, P., 2007. Burning crop residues
22 under no-till in semi-arid land, Northern Spain-effects on soil organic matter, aggregation,
23 and earthworm populations. *Aust. J. Soil Res.* 45, 414-421.

- 1 Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schlöter, M.,
2 Kögel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and
3 nitrogen sequestration in soils. *Nature Comm.* 5(2947), DOI: [10.1038/ncomms3947](https://doi.org/10.1038/ncomms3947)
- 4 von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner,
5 E., and Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools
6 and to stabilization mechanisms. *Soil Biol. Biochem.* 39, 2183-2207.
- 7 Wander, M., 2004. Soil organic matter fractions and their relevance to soil function. In:
8 Magdoff, F. and Weil, R.R. (Eds.), *Soil Organic Matter in Sustainable Agriculture*. CRC
9 Press, Boca Raton, FL, USA, pp. 67-102.
- 10 Wuddivira, M.M., Camps-Roach, G., 2007. Effects of organic matter and calcium on soil
11 structural stability. *Eur. J. Soil Sci.* 58, 722-727.
- 12 Zhao, L., Sun, Y., Zhang, X., Yang, X., and Drury, C.F., 2006. Soil organic carbon in clay and
13 silt sized particles in Chinese mollisols: relationship to the predicted capacity. *Geoderma*,
14 132, 315-323.
- 15 Zhou, Z.G., Chen, N., Cao, X.Y., Chua, T., Mao, J.D., Mandel, R.D., Bettis, E.A., Thompson,
16 M.L., 2014. Composition of clay-fraction organic matter in Holocene paleosols revealed
17 by advanced solid-state NMR spectroscopy. *Geoderma* 223, 54-61.

Figure legends

Figure 1. Relationships between the concentrations of total soil organic carbon (SOC, ---○---) and easily dispersed mineral-associated organic C (d-Min-C, —●—), and the clay content in agricultural soils at 0-40 cm depth (a) and in natural soils at 0-5 cm depth (b).

Figure 2. Relationship between measured and predicted mass of water-stable soil microaggregates (sand-free basis) using equation in Table 5 for the 0-40 cm soil depth. Data come from soils under different tillage and management systems (CT, conventional tillage; RT, reduced tillage; NT, no tillage; NAT, natural soil).

Figure 3. Relative difference between no tillage (NT) and conventional tillage (CT) in fine (fPOM-C, —●—; g C kg⁻¹ soil) and coarse particulate organic C (cPOM-C, ---○---) at 0-5 cm soil depth as a function of mean annual precipitation (MAP).

Table 1. Location and management characteristics of the studied sites (NT, no tillage; RT, reduced tillage; CT, conventional tillage; NAT, natural soil; CC, continuous cropping; CF, cereal-fallow rotation; CL, cereal-legume rotation; MP, mouldboard ploughing; Ch, chisel ploughing).

Site	Location	MAP ^a		Land use and management
		mm	Soil type ^b	
Peñaflor CC	41° 44' 30" N 0° 46' 18" O (259 m elev.)	355	Hypercalcic Calcisol	19-yr NT-CC barley. 19-yr CT-CC (MP) barley. 19-yr RT-CC (Ch) barley. Maintenance of crop residues in the field. Straw chopped and spread in NT/RT (>30% of soil cover by crop residues) and incorporated into the soil in CT. NAT: Typical semiarid grassland.
Peñaflor CF	41° 44' 22" N 0° 46' 30" O (259 m elev.)	355	Hypercalcic Calcisol	20-yr NT-CF. 20-yr CT-CF (MP). 20-yr RT-CF (Ch). Maintenance of crop residues. Straw chopped and spread in NT/RT (>30% residue cover) and incorporated into the soil in CT. NAT: Typical semiarid grassland.
Lanaja	41° 43' 22" N 0° 21' 19" O (422 m elev.)	433	Hypocalcic Calcisol	10-yr NT-CL followed by 4-yr NT-CC barley with maintenance of crop residues (>30% residue cover). >14-yr CT-CF (MP) and straw removed. NAT: Frequently grazed area developed over an abandoned terrace (>40-yr) with sparse vegetation and patches of low shrubs.
Torres de Alcanadre	41° 57' 52" N 0° 05' 00" O (431 m elev.)	468	Calcaric Cambisol	9-yr NT-CC cereal with maintenance of crop residues (>30% residue cover). >9-yr CT-CC cereal (MP/Ch) and straw removed. NAT: Typical Mediterranean shrubland and <i>Pinus halepensis</i> . Soil surface covered with mosses and algae.
Undués de Lerda	42° 33' 43" N 1° 07' 26" O (860 m elev.)	676	Haplic Calcisol	13-yr NT-CF. Maintenance of crop residues (>30% residue cover). >13-yr CT-CF (MP) and straw removed. NAT: Typical Mediterranean shrubland and <i>Pinus halepensis</i> .
Artieda	42° 35' 46" N 0° 59' 39" O (526 m elev.)	741	Hypocalcic Calcisol	19-yr NT-CC cereal followed by 2-yr NT-CL and straw removed (≈10-15% residue cover). >21-yr CT-CC cereal (MP/Ch) and straw removed. NAT: Typical Mediterranean shrubland.

^a Mean annual precipitation.

^b World Reference Base for Soil Resources, 2007.

Table 2. Selected properties of the studied soils in the 0-40 cm depth (CT, conventional tillage; RT, reduced tillage; NT, no tillage; NAT, natural soil). In Peñaflores, CC refers to the continuous cropping system and CF to the cereal-fallow rotation.

Site	Treatment	pH	EC (1:5) ^a	CO ₃ Ca	Gypsum	Organic carbon	Sand	Silt	Clay
		(H ₂ O, 1:2.5)	dS m ⁻¹	g kg ⁻¹					
Peñaflores CC	CT	8.2	0.46	453	48	11.5	320	436	244
	RT	8.5	0.16	460	46	11.0	340	440	220
	NT	8.5	0.17	467	43	10.6	367	422	211
	NAT	8.3	0.79	560	44	11.2	492	351	157
Peñaflores CF	CT	8.4	0.18	471	46	10.6	333	433	234
	RT	8.4	0.16	482	46	10.2	316	445	239
	NT	8.4	0.18	485	47	10.5	318	445	237
	NAT	8.3	0.79	560	44	11.2	492	351	157
Lanaja	CT	8.3	0.31	439	34	10.0	117	600	283
	NT	8.4	0.23	405	34	10.9	125	608	266
	NAT	8.3	0.64	324	40	8.7	272	537	191
Torres de Alcanadre	CT	8.5	0.12	237	29	8.8	576	284	140
	NT	8.6	0.13	229	28	9.4	571	294	135
	NAT	8.5	0.14	245	29	11.5	619	264	118
Undués de Lerda	CT	8.3	0.13	56	66	14.7	106	531	363
	NT	8.3	0.13	89	65	14.5	115	533	352
	NAT	8.2	0.20	119	64	25.2	209	487	303
Artieda	CT	8.1	0.19	177	46	10.6	370	394	236
	NT	8.2	0.18	239	44	10.2	314	451	235
	NAT	8.2	0.15	84	65	16.4	308	416	276

^a EC, electrical conductivity

Table 3. Concentrations of different soil organic C fractions (cPOM-C and fPOM-C, coarse and fine particulate organic C; μ agg-Min-C, mineral-associated organic C occluded within stable microaggregates; d-Min-C, easily dispersed mineral-associated organic C) at different soil depths as affected by soil management and tillage (CT, conventional tillage; RT, reduced tillage; NT, no tillage; NAT natural soil). In Peñaflor, CC refers to the continuous cropping-system and CF to the cereal-fallow rotation.

Site	Treatment	0-5 cm				5-20 cm				20-40 cm			
		cPOM-C	fPOM-C	μ agg-Min-C	d-Min-C	cPOM-C	fPOM-C	μ agg-Min-C	d-Min-C	cPOM-C	fPOM-C	μ agg-Min-C	d-Min-C
		g C kg ⁻¹ soil				g C kg ⁻¹ soil				g C kg ⁻¹ soil			
Peñaflor CC	CT	0.46	1.12	5.64	4.75	0.41	1.32	4.58	5.21	0.32	1.05	4.51	5.56
	RT	0.74	2.12	4.72	5.37	0.50	1.52	3.86	5.50	0.25	0.74	4.17	5.01
	NT	1.33	3.27	3.51	6.95	0.38	1.21	3.99	4.73	0.25	0.81	3.51	5.17
	NAT	1.98	4.71	2.96	6.42	0.62	2.36	3.17	5.23	0.33	1.93	3.37	4.16
	LSD ^a	0.50	0.44	0.85	0.80	0.18	0.47	0.78	ns	ns	0.53	ns	0.34
Peñaflor CF	CT	0.30	0.64	4.30	5.50	0.22	0.85	3.36	6.18	0.21	0.70	4.11	5.44
	RT	0.53	1.04	3.75	5.83	0.38	0.94	2.66	6.50	0.18	0.60	4.28	4.76
	NT	0.82	1.42	4.04	7.02	0.24	0.64	3.65	5.82	0.16	0.49	4.04	5.19
	NAT	1.98	4.71	2.96	6.42	0.62	2.36	3.17	5.23	0.33	1.93	3.37	4.16
	LSD	0.14	0.39	0.67	1.29	0.14	0.28	ns	0.98	0.08	0.55	ns	1.14
Lanaja	CT	0.78	1.44	2.08	6.38	0.02	1.40	2.41	6.30	0.00	1.27	2.05	6.51
	NT	1.65	3.46	2.81	7.39	0.15	1.93	2.68	6.22	0.35	1.34	1.67	6.56
	NAT	2.92	5.30	3.17	6.26	0.42	1.85	2.15	4.75	0.01	1.05	1.90	3.27
	LSD	1.25	2.02	ns	ns	ns	0.28	ns	1.34	0.14	ns	ns	1.37
Torres de Alcanadre	CT	1.11	1.68	1.87	5.80	0.76	1.20	1.44	5.84	0.35	0.83	2.02	4.79
	NT	1.47	3.35	2.89	5.64	0.62	1.37	1.90	5.57	0.30	0.87	2.57	4.58
	NAT	3.95	4.24	2.69	5.95	1.83	2.13	2.04	5.28	0.92	1.64	2.97	4.80
	LSD	0.51	1.26	0.99	ns	0.22	0.77	0.22	ns	0.21	0.41	0.73	ns
Undués de Lerda	CT	0.56	2.66	3.07	10.62	0.20	1.83	2.89	10.14	0.00	1.36	2.79	9.84
	NT	0.45	3.96	3.89	11.59	0.12	2.11	3.12	9.51	0.00	1.16	3.37	8.89
	NAT	5.61	13.05	4.27	16.46	1.30	7.94	3.37	13.78	0.00	5.53	4.56	11.29
	LSD	3.00	1.55	0.99	3.07	0.67	1.95	ns	3.92	ns	1.92	1.69	ns
Artieda	CT	2.12	2.26	3.26	5.42	1.05	1.41	2.51	5.64	0.68	1.38	2.87	5.14
	NT	1.10	2.80	4.38	4.97	0.41	1.60	2.61	5.42	0.30	1.33	2.90	5.00
	NAT	3.58	9.08	3.41	13.85	1.33	3.78	2.67	9.23	0.72	2.34	2.05	7.37
	LSD	1.62	1.49	0.95	1.16	0.61	0.25	ns	0.81	0.24	0.48	ns	0.83

^a LSD, least significant difference ($P < 0.05$).

Table 4. Regression equations relating concentrations (g kg^{-1} soil) of the easily dispersed mineral-associated organic C (d-Min-C), total mineral-associated organic C (Min-C) and total soil organic C (SOC) to soil clay content (g kg^{-1}).

Soil depth		Equation	r^2	P	n
cm	Soil type				
0-5	Agricultural	d-Min-C = $1.73 + 0.021 \text{ clay}$	0.429	<0.0001	42
		Min-C = $4.57 + 0.024 \text{ clay}$	0.534	<0.0001	42
		SOC = $8.36 + 0.021 \text{ clay}$	0.257	<0.001	42
	Natural	d-Min-C = $0.53 + 0.050 \text{ clay}$	0.803	<0.0001	15
		Min-C = $2.78 + 0.056 \text{ clay}$	0.808	<0.0001	15
		SOC = $4.79 + 0.104 \text{ clay}$	0.768	<0.0001	15
0-40	Agricultural	d-Min-C = $1.31 + 0.020 \text{ clay}$	0.680	<0.0001	42
		Min-C = $3.83 + 0.023 \text{ clay}$	0.734	<0.0001	42
		SOC = $5.43 + 0.023 \text{ clay}$	0.723	<0.0001	42
	Natural	d-Min-C = $-1.19 + 0.040 \text{ clay}$	0.688	<0.0001	15
		Min-C = $0.87 + 0.044 \text{ clay}$	0.624	<0.001	15
		SOC = $0.63 + 0.067 \text{ clay}$	0.586	<0.001	15

Table 5. The optimum regression equations to estimate the mass of water-stable soil microaggregates (sand-free basis; $\text{Mass}_{\mu\text{agg}}$, $\text{g kg}^{-1}\text{soil}$) as a function of the mineral-associated organic C occluded within stable microaggregates ($\mu\text{agg-Min-C}$, $\text{g C kg}^{-1}\text{soil}$) and clay and CaCO_3 contents (g kg^{-1}).

Soil depth cm	Equation	r^2	P	n
0-5	$\text{Mass}_{\mu\text{agg}} = 13 + 56 \mu\text{agg-Min-C} - 9076/\text{clay} + 0.121 \text{ CaCO}_3$	0.841	<0.0001	55
0-40	$\text{Mass}_{\mu\text{agg}} = 43 + 53 \mu\text{agg-Min-C} - 8709/\text{clay} + 0.120 \text{ CaCO}_3$	0.899	<0.0001	55

Table 6. Stratification ratios (0-5 cm/20-40 cm) for total soil organic carbon (SOC) and different fractions of SOC (cPOM-C and fPOM-C, coarse and fine particulate organic C; μ agg-Min-C, mineral-associated organic C occluded within stable microaggregates; d-Min-C, easily dispersed mineral-associated organic C) as affected by soil management and tillage (CT, conventional tillage; RT, reduced tillage; NT, no tillage; NAT natural soil). In Peñaflo, CC refers to the continuous cropping-system and CF to the cereal-fallow rotation.

Site		cPOM-C	fPOM-C	μ agg-Min-C	d-Min-C	SOC
Peñaflo CC	CT	1.44	1.07	1.25	0.85	1.05
	RT	2.92	2.85	1.13	1.07	1.28
	NT	5.33	4.06	1.00	1.34	1.55
	NAT	5.91	2.44	0.88	1.54	1.65
	LSDa	1.54	0.75	0.25	0.18	0.21
Peñaflo CF	CT	1.39	0.91	1.05	1.01	1.03
	RT	2.94	1.74	0.88	1.22	1.14
	NT	5.11	2.91	1.00	1.35	1.34
	NAT	5.91	2.44	0.88	1.54	1.65
	LSD	1.57	0.84	ns	0.28	0.20
Lanaja	CT	-	1.14	1.02	0.98	1.09
	NT	4.75	2.58	1.68	1.13	1.54
	NAT	-	5.07	1.67	1.91	2.89
	LSD	-	2.61	ns	0.15	0.44
Torres de Alcanadre	CT	3.15	2.01	0.93	1.21	1.31
	NT	4.86	3.85	1.13	1.23	1.61
	NAT	4.28	2.59	0.91	1.24	1.63
	LSD	1.49	1.62	ns	ns	0.28
Undués de Lerda	CT	-	1.96	1.10	1.08	1.21
	NT	-	3.40	1.16	1.30	1.48
	NAT	-	2.36	0.94	1.46	1.89
	LSD	-	1.00	ns	ns	0.26
Artieda	CT	3.13	1.63	1.14	1.06	1.31
	NT	3.70	2.10	1.51	0.99	1.39
	NAT	4.98	3.88	1.66	1.88	2.40
	LSD	ns	0.76	0.37	0.18	0.34

^aLSD, least significant difference ($P < 0.05$).

Fig. 1

Fig. 2

Fig. 3