**Long-term no tillage effects on particulate and mineral-associated soil
organic matter under rainfed Mediterranean conditions**

N. BLANCO-MOURE, R. GRACIA, A. C. BIELSA & M. V. LÓPEZ

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

Correspondence: M.V. López. E-mail: vlopez@eead.csic.es

Running title: Long-term no tillage effects on soil organic matter fractions

Summary

Soil organic carbon (SOC) plays an essential role in the sustainability of natural and agricultural systems. The identification of sensitive SOC fractions can be crucial for an understanding of SOC dynamics and stabilization. The objective of this study was to assess the effect of long-term no tillage (NT) on SOC content and its distribution between particulate organic matter (POM) and mineral-associated organic matter (Min) fractions in five different cereal production areas of Aragon (north east Spain). The study was conducted under on-farm conditions where pairs of adjacent fields under NT and conventional tillage (CT) were compared. An undisturbed soil nearby under native vegetation was included. The results indicate that SOC was significantly affected by tillage in the first 5 cm with the greatest concentrations found in NT (1.5 - 43% more than in CT). Below 40 cm, SOC under NT decreased (20 - 40%) to values similar or less than those under CT. However, the stratification ratio (SR) never reached the threshold value of 2. The POM-C fraction, disproportionate to its small contribution to total SOC (10-30%), was greatly affected by soil management. The pronounced stratification in this fraction ($SR > 2$ in NT) and its usefulness for differentiating the study sites in terms of response to NT make POM-C a good indicator of changes in soil management under the study conditions. Results from this on-farm study indicate that NT can be recommended as an alternative strategy to increase organic carbon at the soil surface in the cereal production areas of Aragon and in other analogous areas.

Keywords: Soil organic carbon (SOC), agriculture, tillage, carbon storage, land use, soil management

Introduction

Soil organic matter (SOM) plays an essential role in maintaining the necessary chemical, physical and biological conditions for sustainability and environmental integrity of ecosystems (Lal, 2004). Unfortunately, 20th century tillage practices have caused a worldwide decline in SOM with serious environmental and agricultural implications. According to the Thematic Strategy for Soil Protection (European Commission, 2006), the decrease of SOM is one of the main processes of soil degradation in Europe. Besides climatic reasons, human activities are the most relevant driving forces. The need to adopt sustainable management practices that can contribute to improving SOM, such as conservation tillage, is of particular relevance to the Mediterranean region where 74% of the land has a surface soil horizon (0-30 cm) with $<20 \text{ g kg}^{-1}$ of organic carbon (Van-Camp *et al.*, 2004).

Many studies have shown the potential of conservation tillage, and especially of no tillage (NT), to increase SOM and improve soil quality (Govaerts *et al.*, 2009) though the rate of adoption of these alternative systems has been relatively slow in Europe. No tillage is practised on about 117 million ha throughout the world but only 1.15 million ha are in Europe (Derpsch & Friedrich, 2010). For Spain, 650,000 ha of agricultural land are under NT. This indicates that NT adoption is still low in Spain. A recent on-farm study in Aragon (north east Spain) has shown the increasing interest of farmers in NT systems but, at the same time, there is lack of knowledge about the soils on which these techniques are being applied (López *et al.*, 2012). A greater adoption rate of NT could help to reduce the high risk of land degradation by wind and water erosion in this semiarid region of Aragon (López *et al.*, 2001; García-Ruiz, 2010).

Total soil organic C (SOC) is not always the best indicator of changes in soil management, especially under semiarid climatic conditions where low soil moisture and high temperature are limiting factors for SOC accumulation (Chan *et al.*, 2003; Moreno *et al.*,

2006; Melero *et al.*, 2012). Under these conditions significant changes in SOC are only to be expected after several years of NT adoption. The advance in SOM fractionation techniques has made possible the separation of the heterogeneous organic material into labile and recalcitrant pools defined not only on the basis of their composition and turnover rates but also in their response to soil management (Cambardella & Elliot, 1992; Six *et al.*, 2002a; Haynes, 2005). Thus, while labile SOC pools seem to be influenced by management practices, the recalcitrant SOC fractions may or may not be affected by the tillage system (Álvaro-Fuentes *et al.*, 2008; Jagadamma & Lal, 2010). The reasons for this variable response seem to be complex and can involve not only many influential factors (such as soil texture and mineralogy), but also the inherent nature of the fraction and the different mechanisms of C stabilization (biochemical recalcitrance and organo-mineral associations) (Six *et al.*, 2002b; Moni *et al.*, 2010). Thus research into the characterization of the different SOC fractions is needed to increase our understanding of the specific contribution of each fraction to soil function and quality.

The few available data on SOC fractions in agricultural soils of Aragon have been derived from small research plots and from single soil types (Álvaro-Fuentes *et al.*, 2008). However, direct measurements under on-farm conditions across different soils, microclimates and agronomic practices are necessary since farming practices are very diverse and can differ from those in experimental plots (López *et al.*, 2012). The objective of this study was to assess the effect of long-term NT on SOC content and its distribution between particulate organic matter-C (POM-C) and mineral-associated organic C (Min-C) fractions under rainfed Mediterranean conditions. With this aim, six fields of NT were compared with adjacent conventional tillage fields and with undisturbed soils under native vegetation in different cereal production areas of Aragon.

Materials and methods

Description of the study sites

The study was based on six long-term NT fields (9 - 21 yr) representative of the different scenarios of NT in Aragon and located in areas receiving between 350 and 740 mm mean annual rainfall (Table 1 and Figure 1). These fields were selected from a previous study where 22 soils under NT were characterized across different rainfed cereal areas in the region (Lopez *et al.*, 2012). With the exception of the Peñaflor site, the study was carried out under on-farm conditions using fields of collaborating farmers where pairs of adjacent fields under NT and conventional tillage (CT) were compared. In Peñaflor, the study used 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. In all sites, an undisturbed soil under native vegetation (NAT) and close to the NT and CT fields was included in the study for comparative 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). The site at Artieda has the highest rainfall and hence highest agricultural production. A common practice in the area is for farmers to remove the straw from NT and CT fields to prevent later problems with seeding. The information in Table 1 reflects the diversity of cropping systems and conservation agriculture in the region (López *et al.*, 2012). Therefore, the results from the present study provide data on the effect of NT- and CT-based cropping systems on SOC rather than on tillage alone.

The soils were medium-textured (sandy loam to silty clay loam), alkaline ($\text{pH} > 8$; CaCO_3 contents of 60-560 g kg^{-1}) and generally low in OC ($< 20 \text{ g kg}^{-1}$) (Table 2). At 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 there was a slight slope (3-4%).

Soil sampling and analyses

Soil samples for OC analysis were collected at three depths (0-5, 5-20 and 20-40 cm) and from three different zones within each field (NT, CT and NAT) where two samples per depth were collected and mixed to form a composite sample. At the Peñaflor site, each of the composite samples came from each of the 3 tillage plots per treatment. 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). Six undisturbed soil samples were also taken per depth and field (2 in each of the 3 sampling zones per field) to determine the dry bulk density by the core method. The study was over two growing seasons (2009-2010 and 2010-2011), with sampling done in similar periods. Thus, at the sites where there was continuous cropping, sampling was after planting during the fallow period and at the fields under the cereal-fallow rotation during the early fallow phase after the cereal harvest in June. At each site all soil samples were collected on the same day.

In the lab visible roots and organic debris were removed by hand and then air-dry soil (<2 mm) from each depth, field and site, was physically fractionated following the Cambardella & Elliot (1992) procedure. With this method using soil dispersion and sieving, particulate organic matter (POM, >53 μm) and mineral-associated organic matter (Min, <53 μm) were isolated.

The OC concentration in both SOM fractions was determined by dry combustion with a LECO analyser (RC-612 model). Total SOC content was calculated as the sum of the POM-C and Min-C contents and expressed on a mass per unit area basis by multiplying the OC

concentration value by the corresponding soil bulk density. Soil particle size was obtained by laser diffraction analysis (Coulter LS230), CaCO_3 content by dry combustion with the LECO analyser, and electrical conductivity (EC), pH and gypsum content by standard methods.

Statistical analyses

Since the study was conducted under farm conditions, the NT, CT, and NAT treatments were not field replicated. However, in each of the sites, the fields were contiguous and sited on similar landscape positions and 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 was also applied and statistical results were compared with those obtained from the pseudoreplicate analysis. Treatments means were compared by the Duncan's multiple range test ($P < 0.05$). When data showed non-normality, transformations were made and ANOVA conducted with the transformed data. Computations were performed using SPSS 19.0 statistical software.

Results and discussion

Tillage effects on total soil organic carbon

With the exception of the NAT soil of Undués and Artieda, total SOC contents in the 0-40 cm layer were $< 20 \text{ g kg}^{-1}$ (Figure 2), in agreement with SOC as estimated for this region in the Map of Topsoil Organic Carbon in Europe (Jones *et al.*, 2005). Although this range is less than for other European regions, it corresponds well to estimated data for Southern Europe where 74% of the land has a surface soil horizon (0-30 cm) with $< 20 \text{ g kg}^{-1}$ of organic C (Van-Camp *et al.*, 2004).

At the most humid sites (Artieda and Undués) the greatest SOC contents were in the NAT soils, and in the driest ones they were similar (1 and 7% more in NAT at Peñaflor CF and Peñaflor CC, respectively) or even less than those in the agricultural soils (nearly 20% less at Lanaja). These relatively small SOC contents of the NAT soils in the driest areas are due to these fields often being in the marginal lands and are not suitable for crop production due to limited depth or excessive stoniness. In the case of the Lanaja site, the lowest SOC concentration of the NAT field is due to their semi-natural conditions since it is in an abandoned agricultural terrace (>40 yr) that is frequently grazed by livestock (Table 1). From a comprehensive database on Spanish grassland and agricultural soils, Romanyà & Rovira (2011) also found greater differences in SOC concentration between grassland and arable areas as the climate became wetter.

For cultivated soils, SOC was significantly affected by tillage only in the first 5 cm of soil with the highest concentrations found generally in NT (Figure 2). The magnitude of this increase with respect to CT varied considerably between sites, ranging from only 1.5% at Artieda to 43% at Lanaja. At the other sites, this increase varied between 18 and 28%. Below 5 cm depth, SOC concentrations under NT decreased considerably (22-38%) up to values similar to those under CT or slightly lower (Figure 2). Thus, the greater SOC at the soil surface in NT was offset by the lower concentration at depth, so that the average SOC concentration in the 0-40 cm profile was not significantly different from that of CT. At Artieda, the lack of difference between tillage systems even in the top layer (Figure 2) can be explained by the farmer's practice of removing crop residues from the NT field (Table 1); this negated the expected increase in SOC associated with conservation tillage. SOC contents under NT were nearly or greater than 12 g kg⁻¹ in the 0-20 cm depth. Although there is no clear evidence of a unique threshold/critical level in SOC (Loveland & Webb, 2003), the

value of ca. 12 g kg⁻¹ (20 g kg⁻¹ SOM) is considered the minimum for optimal agricultural use in Western Europe (Bullock, 1997).

Greater stratification of SOC with depth under NT than under CT has been widely reported under different agroclimatic regions, including semiarid areas of Spain (Álvaro-Fuentes *et al.*, 2008; Hernanz *et al.*, 2009; López-Fando & Pardo, 2011; Martin-Lammerding *et al.*, 2011; Melero *et al.*, 2012). As stated in these studies, the lack of tillage in NT involves the retention of crop residues at the soil surface, thus slowing decomposition rates and increasing SOC in the topsoil. In contrast, mouldboard ploughing in CT incorporates and distributes throughout the plough layer almost 90-100% of surface crop residues. The stratification ratio, SR (Franzluebbers, 2002), calculated as the SOC concentration at 0-5 cm depth divided by that at 20-40 cm, increased with the reduction in intensity of soil disturbance (CT<RT<NT<NAT) (Table 3). Although SR values greater than the threshold value of 2 were only found in the NAT soils, the improvement of soil quality with the adoption of NT can be deduced from SR values in all cases higher than those in the tilled soils.

Tillage effects on soil organic carbon fractions

The effect of land use and tillage on SOC differed between the two analysed organic matter fractions, POM and Min (Table 4). In all cases, the highest contribution to total SOC corresponded to the Min-C fraction, accounting for ca. 70-90% of total C (Figure 3). The Min fraction is a relatively inert SOM pool, selectively protected by its high stabilization by silt and clay particles and/or its inherent recalcitrance (Cambardella & Elliot, 1992; Six *et al.*, 2002b). In our study the concentration of this fraction was not greatly affected by soil management with the exception of the Undués and Artieda sites, where NAT soils had the highest Min-C contents. This effect was significant ($P<0.05$) for all soil depths but especially so for the surface where the Min-C concentration was 1.4-1.9 times higher than in the

agricultural soils. In contrast, at the most arid sites Min-C was similar or even less in NAT than in agricultural soils, as can be observed at Peñaflor and Lanaja in the deeper layers (Table 4). At these sites, low and variable precipitation limit primary production and soil C storage. Also, the lowest Min-C at Lanaja is explained by the semi-natural conditions of this field (Table 1). Despite the differences in Min-C concentration among NAT soils, the contribution to total SOC was similar, accounting for ca. 50-60% in the surface layer and 70-80% in the deep one (Figure 3). In all cases, these percentages were significantly lower than those from the agricultural soils which included 80-90% of total SOC. Similarly, Llorente *et al.* (2010) in another semiarid region of Spain reported for the first 10 cm of soil contribution percentages of Min-C of 83 and 53% under cropped land and tree cover respectively. For different climatic conditions, Christensen (2001) and John *et al.* (2005) also found lower percentages in soils under permanent vegetation cover than in cultivated soils (50-85% vs. $\geq 90\%$). This difference between land uses is explained by the reduced predominance of the POM fraction in the agricultural soils compared to the NAT soils. In cultivated fields, lower C inputs, and disruption of soil aggregates by tillage and other agronomic practices led to a preferential loss of SOC from the POM fraction (Haynes, 2005; Kögel-Knabner *et al.*, 2008), to thus increase the contribution of the Min fraction with respect to undisturbed soils.

The POM fraction is a labile SOM pool with relatively fast turnover times and consists mainly of partially decomposed plant residues (Cambardella & Elliot, 1992; Haynes, 2005). In our study, in contrast to Min-C, the C associated with this fraction was influenced not only by land use but also by tillage system (Table 4). The POM-C concentration in the agricultural soils was from 22% (Undués) to 77% (Lanaja) of that in the NAT soils for 0-40 cm with differences being statistically significant throughout the whole soil profile. Within the cultivated soils, the POM-C fraction followed the same pattern as the total SOC although the effect of tillage was more pronounced. With the exception of Artieda, where crop residues

were removed from the field, in the upper 5 cm soil depth the POM-C content was between 1.2 (Undués) and 3 times higher (Peñaflor CC and Lanaja) under NT than under CT (Table 4). At the Peñaflor site, the RT response under both CF and CC cropping systems was intermediate between CT and NT with POM-C concentrations significantly higher than those under CT (1.7-1.8 times higher). Again, the decreasing pattern in C 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 SR for POM-C which were greater than 2 in the NT and RT soils (Table 3). Even under CT, $SR > 2$ were obtained for this fraction at the most humid sites. This contrasts with the relatively low SR for Min-C and SOC, suggesting that the POM-C fraction can be a good indicator of soil quality under rainfed conditions of semiarid Aragon. The marked stratification of POM-C is generally observed under continuous NT management (Franzluebbers, 2004; 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 fraction represented a relatively small percentage of the total SOC in terms of C storage (9-30%; Table 5), in agreement with typical values of 10-30% as reported in the literature (Wander, 2004; Álvaro-Fuentes *et al.*, 2008; Martin-Lammerding *et al.*, 2011). However, despite its small contribution, it has a large effect on nutrient-supplying capacity and structural stability of soils and for these reasons is considered a key attribute of soil quality (Haynes, 2005).

In our study the POM-C fraction contributed to the differences between our sites based on the relative gain or loss of POM-C stock (Mg C ha^{-1}) under NT with respect to CT at each site (i.e. $(\text{NT}-\text{CT})/\text{CT}$). With the exception only of Artieda, POM-C was always higher under NT than under CT in the surface layer (ratios > 0) (Figure 4). Such values represent between 1% less and 225% more POM-C with NT, averaging about 111% more. Figure 4 shows a

negative and strong relationship ($P < 0.01$) between the relative difference in POM-C stock at 0-5 cm depth and the mean annual precipitation at each site and indicates that the relative gain of C with the adoption of NT decreases as precipitation increases. A significant relationship was also obtained for total SOC but this property was not as responsive to precipitation as POM-C (Figure 4). For the Min-C fraction, no significant influence of precipitation was observed at any of the soil depths. 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 (VandenBygaart *et al.*, 2003; Franzluebbers, 2004; Govaerts *et al.*, 2009; Blanco-Canqui *et al.*, 2011). The increase in the relative benefit of NT with reduction in precipitation is also reported 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 C gain with respect to CT.

In a similar way to previous studies, the differences between treatments in C storage at the soil surface are reduced when the whole soil profile (0-40 cm) is considered (Table 5). The depth of soil sampling can greatly affect conclusions on SOC sequestration rates (Blanco-Canqui & Lal, 2008; Christopher *et al.*, 2009). At the most arid sites, Peñaflor and Lanaja, C stocks in the NAT soils were similar or even lower than those in cultivated soils, supporting the idea that native SOC levels do not necessarily represent the upper limit for SOC storage and can be exceeded by those under agricultural management (Six *et al.*, 2002b; VandenBygaart *et al.*, 2003). In contrast, in the more humid areas, C stocks were enhanced in the NAT soils (Table 5). Within the cultivated soils, although a trend of higher SOC stored

under NT than under CT was generally found when the whole 0-40 cm layer was considered, the additional C was only statistically significant at Lanaja (15% more; Table 5) and can be attributed to the intensification of cropping system in NT (continuous cereal cropping vs. cereal-fallow rotation in CT; Table 1). Regardless of the potential of NT to sequester SOC, the numerous benefits to soil quality derived from the greater accumulation of SOC at the surface cannot be questioned. A study carried out under semiarid rainfed conditions in south Spain (López-Garrido *et al.*, 2012) shows that although the increase in SOC after 16 yr of reduced tillage was only significant down to 5 cm, biological soil properties were significantly improved. Enhanced structural stability of soil is of special importance in semiarid Aragon to reduce soil susceptibility to the two main degradation processes of wind and water erosion that affect agricultural land (López *et al.*, 2001; Blanco-Moure *et al.*, 2012).

Conclusion

The results from this on-farm study show that the increase in SOC due to long-term NT was generally restricted to the soil surface (0-5 cm depth) where, on average, 23% more SOC was found under NT than under CT. The Min-C fraction provided the greatest contribution to total SOC in all cases (70-90%). However, the POM-C fraction was more sensitive to soil tillage and land use than Min-C and total SOC. In addition, and in contrast to total SOC, stratification ratios of POM-C were >2 under NT at all studied sites. For these reasons, POM-C can be considered a useful indicator of soil changes associated with NT. A negative and strong relationship was found between the relative difference in the surface POM-C stock (i.e. $(NT-CT)/CT$) and mean annual precipitation, suggesting that the relative gain in C with NT increases as precipitation decreases. Regardless of the similar potential of NT and CT to sequester SOC in whole soil profile (0-40 cm), the environmental benefits derived from the

higher accumulation of SOC at the surface under NT cannot be questioned. Thus NT can be recommended as a sustainable management alternative to CT in rainfed cereal areas of Aragon in north east Spain and in other analogous areas.

Acknowledgements

The authors thank M.J. Salvador for field and laboratory support and Dr. J. Machín for his assistance with the characterization and classification of soils. We are also grateful to all farmers for allowing access to their fields and to M. Pérez-Berges (CTA-DGA) and AGRACON for helping us to contact no-tillage farmers in Aragon. This research was supported by the Comisión Interministerial de Ciencia y Tecnología of Spain (Grants AGL2010-22050-CO3-02/AGR and AGL2007-66320-C02-02/AGR) and the European Union (FEDER funds). N. Blanco-Moure was awarded a FPI Fellowship by the Spanish Ministry of Science and Innovation.

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 Science Society of America Journal*, **72**, 541-547.
- Blanco-Canqui, H. & Lal, R. 2008. No-tillage and soil profile carbon sequestration: an on-farm assessment. *Soil Science Society of America Journal*, **72**, 693-701.
- Blanco-Canqui, H., Schlegel, A.J. & Heer, W.F. 2011. Soil-profile distribution of carbon and associated properties in no-till along a precipitation gradient in the central Great Plains. *Agriculture, Ecosystems & Environment*, **144**, 107-116.
- 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.
- Bullock, P. 1997. Sustainable development of soils in Western Europe—An overview. *Edafología. Conferences in the 50th Commemorative Meeting of the Spanish Soil Science Society*, p. 109-123. Madrid, Spain.
- Cambardella, C.A. & Elliot, E.T. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Science Society of America Journal*, **56**, 777-783.
- 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. *Australian Journal of Experimental Agriculture*, **43**, 325-334.
- Christensen, B.T. 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *European Journal of Soil Science*, **52**, 345-353.
- Christopher, S.F., Lal, R. & Mishra, U. 2009. Regional study of no-till effects on carbon sequestration in the Midwestern United States. *Soil Science Society of America Journal*, **73**, 207-216.

- 354 Derpsch, R. & Friedrich, T. 2010. Sustainable crop production intensification -The adoption
355 of conservation agriculture worldwide-. In: *Proceedings of the 16th ISCO Conference*, pp.
356 265-293. Santiago, Chile. <http://www.rolf-derpsch.com/sustainablecropproduction.pdf>
357 (consulted in November 2012).
- 358 European Commission. 2006. Thematic Strategy for Soil Protection. Communication
359 (COM(2006) 231), EU Brussels. http://ec.europa.eu/environment/soil/three_en.htm
360 (consulted in November 2012).
- 361 Franzluebbers, A.J. 2002. Soil organic matter stratification ratio as an indicator of soil
362 quality. *Soil & Tillage Research*, **66**, 95-106.
- 363 Franzluebbers, A.J. 2004. Tillage and residue management effects on soil organic matter. In:
364 *Soil Organic Matter in Sustainable Agriculture* (eds F. Magdoff & R.R. Weil), pp. 227-
365 268. CRC Press, Boca Raton, FL.
- 366 García-Ruiz, J.M. 2010. The effects of land uses on soil erosion in Spain: a review. *Catena*,
367 **81**, 1-11.
- 368 Gregorich, E.G., Carter, M.R., Angers, D.A. & Drury, C.F. 2009. Using a sequential density
369 and particle-size fractionation to evaluate carbon and nitrogen storage in the profile of
370 tilled and no-till soils in eastern Canada. *Canadian Journal of Soil Science*, **89**, 255-267.
- 371 Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K.D., Dixon, J. & Dendooven,
372 L. 2009. Conservation agriculture and soil carbon sequestration: between myth and farmer
373 reality. *Critical Reviews in Plant Sciences*, **28**, 97-122.
- 374 Haynes, R.J. 2005. Labile organic matter fractions as central components of the quality of
375 agricultural soils: an overview. *Advances in Agronomy*, **85**, 221-268.
- 376 Hernanz, J.L., Sánchez-Girón, V. & Navarrete, L. 2009. Soil carbon sequestration and
377 stratification in a cereal/leguminous crop rotation with three tillage systems in semiarid
378 conditions. *Agriculture, Ecosystems & Environment*, **133**, 114-122.

- 379 Jagadamma, S. & Lal, R. 2010. Distribution of organic carbon in physical fractions of soils as
380 affected by agricultural management. *Biology and Fertility of Soils*, **46**, 543-554.
- 381 John, B., Yamashita, T., Ludwig, B. & Flessa, H. 2005. Storage of organic carbon in
382 aggregate and density fractions of silty soils under different types of land use. *Geoderma*,
383 **128**, 63-79.
- 384 Jones, A., Montanarella, L. & Jones, R. 2005. *Soil Atlas of Europe*. European Soil Bureau
385 Network. European Commission, 128 pp. Office for Official Publications of the European
386 Communities, Luxembourg.
- 387 Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S.,
388 Eusterhues, K. & Leinweber, P. 2008. Organo-mineral associations in temperate soils:
389 Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition*
390 *and Soil Science*, **171**, 61-82.
- 391 Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security.
392 *Science*, **304**, 1623-1627.
- 393 Llorente, M., Glaser, B. & Turrión, M.B. 2010. Storage of organic carbon and Black carbon
394 in density fractions of calcareous soils under different land uses. *Geoderma*, **159**, 31-38.
- 395 López-Fando, C. & Pardo, M.T. 2011. Soil carbon storage and stratification under different
396 tillage systems in a semi-arid region. *Soil & Tillage Research*, **111**, 224-230.
- 397 López-Garrido, R., Deurer, M., Madejón, E., Murillo, J.M. & Moreno, F. 2012. Tillage
398 influence on biophysical soil properties: The example of a long-term tillage experiment
399 under Mediterranean rainfed conditions in South Spain. *Soil & Tillage Research*, **118**, 52-
400 60.
- 401 López, M.V., Gracia, R. & Arrúe, J.L. 2001. An evaluation of wind erosion hazard in fallow
402 lands of semiarid Aragon (NE Spain). *Journal of Soil and Water Conservation*, **56**, 212-
403 219.

- 404 López, M.V., Blanco-Moure, N., Limón, M.A. & Gracia, R. 2012. No tillage in rainfed
405 Aragon (NE Spain): Effect on organic carbon in the soil surface horizon. *Soil & Tillage*
406 *Research*, **118**, 61-65.
- 407 Loveland, P. & Webb, J. 2003. Is there a critical level of organic matter in the agricultural
408 soils of temperate regions? A review. *Soil & Tillage Research*, **70**, 1-18.
- 409 Luo, Z., Wang, E. & Sun, O.J. 2010. Soil carbon change and its responses to agricultural
410 practices in Australian agro-ecosystems: A review and synthesis. *Geoderma*, **155**, 211-
411 223.
- 412 Martin-Lammerding, D., Hontoria, C., Tenorio, J.L. & Walter, I. 2011. Mediterranean
413 dryland farming: effect of tillage practices on selected soil properties. *Agronomy Journal*,
414 **103**, 382-389.
- 415 Melero, S., López-Bellido, R.J., López-Bellido, L., Muñoz-Romero, V., Moreno, F., Murillo,
416 J.M. & Franzluebbers, A.J. 2012. Stratification ratio in a rainfed Mediterranean Vertisol
417 in wheat under different tillage, rotation and N fertilization rates. *Soil & Tillage Research*,
418 **119**, 7-12.
- 419 Moni, C., Rumpel, C., Virto, I., Chabbi, A. & Chenu, C. 2010. Relative importance of
420 sorption versus aggregation for organic matter storage in subsoil horizons of two
421 contrasting soils. *European Journal of Soil Science*, **61**, 958-969.
- 422 Moreno, F., Murillo, J.M., Pelegrín, F. & Girón, I.F. 2006. Long-term impact of conservation
423 tillage on stratification ratio of soil organic carbon and loss of total and active CaCO₃.
424 *Soil & Tillage Research*, **85**, 86-93.
- 425 Romanyà, J. & Rovira, P. 2011. An appraisal of soil organic C content in Mediterranean
426 agricultural soils. *Soil Use and Management*, **27**, 321-332.

- 427 Salvo, L., Hernández, J. & Ernst, O. 2010. Distribution of soil organic carbon in different size
428 fractions, under pasture and crop rotations with conventional tillage and no-till systems.
429 *Soil & Tillage Research*, **109**, 116-122.
- 430 Six, J., Feller, C. Denef, K., Ogle, S.M., de Moraes Sa, J.C. & Albrecht, A. 2002a. Soil
431 organic matter, biota and aggregation in temperate and tropical soils – Effects of no-
432 tillage. *Agronomie*, **22**, 755-775.
- 433 Six, J., Conant, R.T., Paul, E.A. & Paustian, K. 2002b. Stabilization mechanism of soil
434 organic matter: implication for C-saturation of soils. *Plant and Soil*, **241**, 155-176.
- 435 VandenBygaart, A.J., Gregorich, E.G. & Angers, D.A. 2003. Influence of agricultural
436 management on soil organic carbon: A compendium and assessment of Canadian studies.
437 *Canadian Journal of Soil Science*, **83**, 363-380.
- 438 Van-Camp. L., Bujarrabal, B., Gentile, A-R., Jones, R.J.A., Montanarella, L., Olazabal, C. &
439 Selvaradjou, S-K. 2004. *Reports of the Technical Working Groups Established under the*
440 *Thematic Strategy for Soil Protection*. EUR 21319 EN/3, 872 pp. Office for Official
441 Publications of the European Communities, Luxembourg.
- 442 Wander, M. 2004. Soil organic matter fractions and their relevance to soil function. In: *Soil*
443 *Organic Matter in Sustainable Agriculture* (eds F. Magdoff & R.R. Weil), pp. 227-268.
444 CRC Press, Boca Raton, FL.

Figure legends

Figure 1. Location of the study sites and average annual rainfall isohyets (mm).

Figure 2. Depth distribution of soil organic carbon concentration under different tillage and management systems (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. Horizontal bars indicate LSD ($P<0.05$) for comparisons at the same soil depth, where significant differences were found.

Figure 3. Relative proportion of particulate organic carbon (POM-C, □) and mineral-associated organic carbon (Min-C, ■) to total soil organic carbon (SOC) 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. For the same study site and soil depth, different letters indicate significant differences at $P<0.05$.

Figure 4. Relative difference between no tillage (NT) and conventional tillage (CT) in total soil organic carbon content (SOC, —○—; Mg ha^{-1}) and particulate organic carbon content (POM-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	Hypocalcic 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	Hypocalcic 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 Peñaflo, 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					
Peñaflo 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
Peñaflo 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. Stratification ratios (0-5 cm/20-40 cm) for total soil organic carbon (SOC), particulate organic C (POM-C) and mineral-associated organic C (Min-C) 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.

Fraction	Treatment	Peñaflor	Peñaflor	Lanaja	Torres	Undués	Artieda
		CC	CF		de Alcanadre	de Lerda	
SOC	CT	1.05	1.03	1.09	1.31	1.21	1.31
	RT	1.28	1.14	—	—	—	—
	NT	1.55	1.34	1.54	1.61	1.48	1.39
	NAT	1.65	1.65	2.89	1.63	1.89	2.40
	LSD ^a	0.21	0.20	0.44	0.28	0.26	0.34
POM-C	CT	1.16	1.05	1.75	2.38	2.48	2.11
	RT	2.85	2.08	—	—	—	—
	NT	4.37	3.47	3.06	4.27	3.79	2.38
	NAT	2.96	2.96	7.84	3.22	3.48	4.19
	LSD	0.63	0.87	1.29	1.55	ns	1.13
Min-C	CT	1.03	1.03	0.99	1.13	1.08	1.09
	RT	1.10	1.06	—	—	—	—
	NT	1.21	1.20	1.24	1.19	1.26	1.18
	NAT	1.26	1.26	1.85	1.11	1.36	1.83
	LSD	0.18	0.20	0.24	ns	ns	0.15

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

Table 4. Particulate organic C (POM-C) and mineral-associated organic C (Min-C) concentrations at different soil depths 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	Treatment	POM-C			Min-C		
		0-5 cm	5-20 cm	20-40 cm	0-5 cm	5-20 cm	20-40 cm
		(g C kg ⁻¹ soil)					
Peñaflor CC	CT	1.59	1.73	1.37	10.38	9.80	10.07
	RT	2.86	2.02	1.00	10.09	9.36	9.19
	NT	4.60	1.59	1.06	10.46	8.72	8.69
	NAT	6.68	2.98	2.26	9.37	8.40	7.54
	LSD ^a	0.74	0.60	0.31	ns	ns	1.24
Peñaflor CF	CT	0.93	1.07	0.91	9.80	9.55	9.55
	RT	1.56	1.32	0.77	9.58	9.17	9.04
	NT	2.25	0.88	0.65	11.06	9.47	9.24
	NAT	6.68	2.98	2.26	9.37	8.40	7.54
	LSD	0.54	0.39	0.61	ns	ns	1.52
Lanaja	CT	2.21	1.41	1.27	8.47	8.71	8.56
	NT	5.11	2.08	1.69	10.20	8.89	8.23
	NAT	8.22	2.28	1.05	9.43	6.90	5.17
	LSD	1.39	0.43	0.47	ns	0.89	1.39
Torres de Alcanadre	CT	2.79	1.95	1.19	7.68	7.29	6.81
	NT	4.82	2.00	1.17	8.53	7.46	7.15
	NAT	8.19	3.96	2.56	8.64	7.32	7.77
	LSD	1.63	0.93	0.56	ns	ns	ns
Undués de Lerda	CT	3.22	2.03	1.36	13.69	13.03	12.63
	NT	4.41	2.22	1.16	15.49	12.63	12.26
	NAT	18.66	9.24	5.53	20.73	17.15	15.85
	LSD	4.35	2.59	1.92	3.06	3.33	ns
Artieda	CT	4.38	2.46	2.06	8.68	8.15	8.01
	NT	3.90	2.02	1.63	9.35	8.03	7.90
	NAT	12.66	5.11	3.06	17.26	11.90	9.42
	LSD	2.96	0.77	0.56	1.48	1.23	1.28

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

Table 5. Soil organic carbon (SOC), particulate organic C (POM-C) and mineral-associated organic C (Min-C) stocks in the 0-40 cm soil layer 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	Treatment	SOC	POM-C	Min-C
		Mg C ha ⁻¹		
Peñaflo CC	CT	57.36	7.60	49.76
	RT	54.89	7.95	46.94
	NT	60.23	9.57	50.66
	NAT	57.31	15.66	41.65
	LSD ^a	ns	3.30	ns
Peñaflo CF	CT	54.91	5.07	49.84
	RT	51.38	5.34	46.04
	NT	59.17	5.26	53.59
	NAT	57.31	15.66	41.65
	LSD	ns	3.21	7.77
Lanaja	CT	54.57	7.76	46.81
	NT	62.75	12.89	49.86
	NAT	48.18	12.87	35.31
	LSD	5.38	2.03	5.62
Torres de Alcanadre	CT	55.12	10.31	44.81
	NT	58.78	12.05	46.73
	NAT	63.94	20.67	43.27
	LSD	ns	4.50	ns
Undués de Lerda	CT	89.42	10.88	78.54
	NT	88.54	11.36	77.18
	NAT	129.91	42.15	87.77
	LSD	23.73	7.63	ns
Artieda	CT	59.07	13.65	45.41
	NT	65.18	12.95	52.23
	NAT	97.07	29.28	67.79
	LSD	7.09	3.00	7.50

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