EFFECT OF LONG-TERM CONSERVATION TILLAGE ON SOIL BIOCHEMICAL PROPERTIES IN MEDITERRANEAN SPANISH AREAS

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Abstract

In semi-arid Mediterranean areas, studies of the performance of conservation tillage systems have largely demonstrated advantages in crop yield, soil water storage and soil protection against wind and water erosion. However, little attention has been given to interactions between soil biochemical properties under different tillage practices. Biochemical properties are useful tools to assess changes caused by different soil tillage systems in long-term field experiments. This study deals with the effect of long–term tillage practices (reduced tillage and no tillage vs. traditional tillage) on soil chemical properties and microbial functions in three different sites of Spain two of them located in the Northeast and one in the Southwest under semi-arid Mediterranean conditions. Soil biological status, as index of soil quality, was evaluated by measuring microbial biomass carbon (MBC) and dehydrogenase (an oxidoreductase) and protease (a hydrolase) activities at three soil depths (0-5, 5-10 and 10-25 cm). In the three experimental areas, increases in soil organic matter content, MBC and enzymatic activities were found at the superficial layers of soil under conservation tillage (reduced tillage and no tillage) in comparison with traditional tillage. Values of the stratification ratio of some biochemical properties were significantly correlated with yield production in Northeast sites.

Conservation tillage has proven to be an effective strategy to improve soil quality and fertility in Mediterranean areas of Spain.

Keywords: Soil tillage, soil organic carbon, soil enzymatic activities, soil microbial biomass carbon; semi-arid areas.

1. Introduction

Benefits from conservation tillage, including improvement of soil properties, savings of time, energy and water, and wind erosion control, have been reported in many studies carried out under different environment conditions (Griffith et al., 1986; Lal, 1989). Thus, traditional, intensive inversion tillage (TT) is being replaced by conservation tillage systems. Conservation tillage systems reduce labour, fuel, and machinery expenses and also have some agronomic and environmental implications. Conservation tillage protects the soil against water and wind erosion and reduces soil evaporation by leaving crop residues on the soil surface, thus promoting greater soil moisture content (Lafond, 1994).

To be considered conservation tillage (CT), any tillage and planting system must maintain at least 30 percent of the soil surface covered by residue after planting to reduce soil erosion by water. Where soil erosion by wind is a primary concern, the system must maintain a 1.1 Mg ha^{-1} flat small grain residue equivalent on the surface during the critical wind erosion period (Gajri et al., 2002). Some times, there is no distinction between CT, MT (minimum tillage) or reduced tillage (RT) (Bradford and Peterson, 2000). Types of CT include no-tillage (NT), ridge tillage, mulch tillage and zone tillage (Hill, 1996).

Under semiarid climate, CT is one of the best options to store and conserve soil water (Rawitz and Hadas, 1994). Many short-term studies and a few long-term studies have evaluated the effect of tillage systems on plant productivity (Cantero-Martínez et al., 2003; Moreno et al., 1997). In general, the lack of negative effect on yield, make conservation tillage attractive attending the reduction in operating costs and soil quality increase (Franzluebbers, 2004). Soil quality can be defined as its capacity to work properly within ecosystem boundaries maintaining biological productivity, environment quality and also to promote plant and animal health (Doran and Safley, 1997). The definition of soil quality has focused on some properties that affect soil health and quality (Doran and Safley, 1997). Soil microbial biomass and enzymes have been suggested as potential indicators of soil quality because of their relationship to soil biology, ease of measurement (i.e., potential to be adopted by commercial laboratories for routine soil testing), rapid response to changes in soil management and high sensitivity to temporary soil changes originated by management and environment factors (Nannipieri 1994). Conservation tillage increases organic matter levels in superficial layers of the soil (Franzluebbers, 2004). Thus, biological activity has been found to be higher in soils under CT than under TT, (Bolinder et al., 1999). Also, under CT, an increase of the activity of some enzymes (acid phosphomonoesterase, arylsulphatase, dehydrogenase, urease and β-glucosidase) has been found (Angers et al., 1993, Eivazi et al., 2003).

During the past 20 years, conservation tillage practices, as RT or NT, have been introduced in the Mediterranean areas with different success (Cantero-Martínez et al., 2003; López et al., 1996, 2005; Moreno et al., 1997). In these areas, studies of the performance of conservation tillage systems have demonstrated advantages in yield, water profitability (water storage, water use by crops) and protection of the soil against erosion by water and wind (Álvaro-Fuentes et al., 2008a; Cantero-Martínez et al., 2003; Mrabet, 2002, Muñoz et al., 2007). Reduction of CO_2 fluxes to the atmosphere derived from conservation tillage adoption has also been reported (Sánchez et al., 2002; Álvaro-Fuentes et al. 2008b). Despite some disadvantages such as the increase of the use of herbicides, CT appears to be the most important sustainable alternative system to traditional agriculture to cope with negative agro-environmental problems derived from TT, including the diminution in soil biodiversity. However, comparatively little attention has been given to soil biochemical properties under different tillage systems (Madejón et al., 2007).

The aims of the study were to determine the effects of long-term conservation tillage on soil chemical properties and microbial function in three sites of Spain under semi-arid

Mediterranean conditions. We hypothesized that conservation tillage would have a positive effect by increasing soil organic matter and fertility, and, enhancing soil microbial functionality.

2. Materials and methods

The study was carried out in three different experimental sites of the semi-arid Spain located specifically in the provinces of Lleida (LLE), Zaragoza (ZAR) and Sevilla (SEV) (Fig. 1). All the sites have a long history of experimentation of conservation tillage (12-18 years).

2.1. Experiment at Lleida

The experiment was established in the fall of 1996 in a farm of Agramunt in the Lleida province (NE Spain). Soil was classified as Typic Xerofluvent (USDA, 1996). The area has a temperate continental Mediterranean climate with rainfall variable ranging between 350–550 mm. The rainfall distribution has two peaks, in autumn and late spring, respectively, with little rain in the winter and summer months. More details of the site and soil characteristics are shown in Table 1. Three tillage systems (TT, RT and NT) were established. The TT consisted of one moldboard ploughing (25–30 cm depth) plus one or two cultivator passes (15 cm depth) before sowing during August or September each year, depending upon the soil moisture. The RT was conducted with one or two cultivator passes (10–15 cm depth) in each September in the same soil moisture conditions as the TT. The NT consisted of sowing by direct drilling after spraying with herbicide (1.5 L 36% glyphosate [N-(phosphonomethyl)-glycine] plus 1 L of 40% MCPA (2-(4chloro 2-metilfenoxi) acetic acid) per ha). Barley (*Hordeum vulgare* L. cv. “Hispanic”) was sown in late October to early November each year. Sowing was performed with a no-till disc and harvesting was done with a standard, medium-size combine. Nine replicate plots (50 m x 6 m) for each tillage system were randomly established. More details of the crop management practices are given in Cantero-Martínez et al. (2003).

2.2. Experiment at Zaragoza

The experiment was established in 1990 in the dryland research farm of the “Estación Experimental de Aula Dei (CSIC)”, in the Zaragoza province (NE Spain). The soil was classified as Xerollic Calciorthid (USDA, 1996). The area is characterized by a semiarid climate. More details of the site and soil characteristics are shown in Table 1. Three tillage treatments (TT, RT and NT) were established The TT treatment consisted of mouldboard ploughing to a depth of 30–40 cm in followed by secondary tillage to a depth of 10–15 cm with a sweep cultivator in late spring. In the RT treatment, primary tillage was chisel ploughing to a depth of 25–30 cm (non-inverting action), followed, as in TT, by a pass with the sweep cultivator in the same dates. After this cultivation, the plots were not ploughed again until November–December when seedbed preparation with a point cultivator was carried out prior to sowing. Weeds on NT plots were controlled with herbicide application (2 L ha^{-1} of 36% glyphosate {N-(phosphonomethyl)-glycine}). A conventional planter was used in the TT and RT treatments. In NT, sowing was performed directly into the crop residues from the previous harvest using a hoe drill. Barley (*Hordeum vulgare* L. cv.“Albacete”) was cropped

Tillage treatments were arranged in a randomized complete block design with six replications per treatment. The subplot size was 33.5 m × 10 m. Details about crop management practices and experimental design have been previously given (López et al., 1996).

2.3 Experiment at Sevilla

The experiment was established in 1992 at the experimental farm of the “Instituto de Recursos Naturales y Agrobiología (CSIC)” in the Sevilla province (SW Spain). Soil was classified as Xerofluvent, (USDA, 1996). Climate of the zone is typically Mediterranean, with mild rainy winters (500 mm mean rainfall, average of 1971-2004) and very hot, dry summers. More details of the site and soil characteristics are shown in Table 1. An area of about 2,500 m^2 was selected to establish the experimental plots in 1991, which was cropped with wheat under rainfed conditions. Two tillage treatments were established: TT used in the area for rainfed agriculture and a RT. The TT consisted of mouldboard ploughing (30 cm depth), after burning the straw of the preceding crop. Straw burning was suppressed since 2003. The RT was characterized by not using mouldboard ploughing, by reduction of the number of tillage operations and leaving the crop residues on the surface (Moreno et al., 1997). A wheat (*Triticum aestivum*, L.)-sunflower (*Helianthus annuus*, L.) crop rotation was established for both treatments. In 2005 a fodder pea crop (*Pisum arvense*, L. cv. "Ideal") was included in the rotation. Before sowing wheat in first December 2005, a pre-emergence herbicide (18% glyphosate + 18% MCPA) was applied in RT at a high rate of 7L ha^{-1} Three replicate plots (22 x 14 m) for each tillage system were randomly established. More details of the experimental management and agronomic practices are given in Moreno et al. (1997).

2.4 Soil sampling and analysis

Soil sampling in the three sites of study was carried out in March 2006 at depths of 0-5, 5-10 and 10-25 cm. At each plot five soil cores were taken to make a composite sample representative of each plot and depth. (ca. 200 g per soil core sample at each depth). Surface residue was left in the corresponding depth (mainly in the 0-5 cm). Field moist soil was sieved (2 mm) and divided into two subsamples. One was immediately stored at 4 ºC in plastic bags loosely tied to ensure sufficient aeration and to prevent moisture loss until assaying of microbiological and enzymatic activities. The other was air-dried for chemical analysis.

Soil total organic carbon (TOC) content was determined according to Walkley and Black (1934), water soluble carbon (WSC) was determined in an (1/10) aqueous extract using a TOC-V-CSH/CSN analyser. The MBC content was determined by the chloroform fumigation-extraction method modified by Gregorich et al. (1990). Dehydrogenase activity (DHA) was determined in a 1 M TRIS–HCl buffer (pH 7.5) by the method of Trevors (1984), using INT

(2(p-iodophenyl)-3-(p-nytrophenyl) 5-phenyl tetrazolium chloride) as the electron acceptor. The iodonitrotetrazolium formazan (INTF) produced was measured spectrophotometrically at 490 nm. Protease activity was measured after incubation of soil with casein and measurement of the absorbance of the extracted tyrosine at 700 nm following the procedure described by Ladd and Butler, (1972).

Stratification ratio for each variable was calculated dividing the values of the variable determined at 0-5 cm by the value determined at 10-25 cm.

2.5 Statistical analysis

In ZAR and LLE experiments, the data were analysed by ANOVA, considering the tillage system as the independent variable. The means were separated by the Tukey's test, using a significance level of $p<0.05$.

In SEV, response variable between both tillage systems were assessed by the Student t-test. All statistical analyses were carried out with the program SPSS 15.0 for Windows**.**

3. Results and discussion

3.1 Soil total organic carbon and water soluble carbon

As a rule, TOC and WSC values were higher in the soils under RT and NT that under TT at the superficial layer (0-5 cm) (Figures 2a and 2b). For both parameters, differences among treatments were only significant at LLE, where the increase of TOC and WSC with conservation tillage was noticeable even at intermediate layers (5-10 cm) (Figures 2c and 2d). In the three experimental sites, soil organic matter content decreased with depth and at the deepest layer (10-25 cm) the values of TOC and WSC similar for the different tillage treatments (Figures 2e and 2f).

Conservation tillage systems have been shown to maintain soil organic matter at higher levels than traditional tillage especially at surface (Díaz-Zorita and Grove, 2002). This increase is particularly important in the Mediterranean area, where the levels of organic matter in semi-arid agricultural soils are low (around 10 g kg^{-1}) (Acosta-Martínez et al., 2003).

Water soluble carbon accounts for only a small proportion of the TOC in soil (Mc Gill et al., 1986). However, it is widely recognised that this fraction influences soil biological activity (Flessa et al., 2000). Soil tillage has been found to affect WSC. Thus, Linn and Doran (1984) reported higher WSC values in NT system than in conventionally tilled soil in the top 7.5 cm. These authors reported also that no differences were recorded at deeper layers. In accordance, Leinweber et al. (2001) found that intensive tillage enhanced oxidative chemical and microbial activity and consequently decreased the WSC content.

3.2 Soil microbial biomass carbon and enzymatic activities

Soil MBC values at the LLE site were significantly higher under RT and NT that under TT up to 10 cm depth (Figures 3a and 3b). In ZAR, only soil under NT significantly increased MBC values with respect to TT at the superficial layer (Figure 3a). In SEV, no differences between treatments were found for MBC (Figure 3). As occurred with TOC and WSC, MBC values decreased with depth in the three experimental sites.

The microbial biomass contained in the crop residues and the addition of substrate-C could account for the increase of MBC in the RT and NT soils in the most superficial layer. Soil subjected to conservation tillage accumulates crop residues and, consequently, increases organic carbon in soil, speciality at the most superficial layer (Kanderler et al., 1999). This carbon is the substrate for soil microorganisms and, consequently, the microbial biomass tended to increase in the soil surface. This stimulating effect of high levels of soil organic matter on MBC is supported by the high correlation found between TOC (r^2= 0.590 $p<0.01$) and WSC (r^2= 0.394 $p<0.01$) and MBC. Numerous authors have found strong relationships between organic matter and microbial population under different conditions (Melero et al., 2007).

Values of DHA were higher in NT than in TT in LLE and ZAR only at the superficial layer (0-5 cm) whereas in SEV, no differences between treatments were found (Figure 4). DHA, which depends on the metabolic state of the soil biota, has been proposed as a sensitive indicator of changes in microbial activity in soils of semi-arid Mediterranean areas (García et al., 1997). As DHA uses oxygen and different compounds as terminal electron acceptors, this enzyme should reflect the oxidative capacity of the total soil microflora. DHA responded to tillage treatments in a similar manner as soil organic C, increasing under conservation tillage and reflecting a better oxidative capacity of the soil.

The highest values of protease were found in the RT and NT soils of LLE for all soil profile considered (Figure 5). In ZAR, effect of tillage was only observed in the most superficial layer and for soils under NT (Figure 5). In SEV, no differences between treatments were found (Figure 5).

Both enzymes (dehydrogenase and protease) were positively correlated with each other (r^2= 0.647 $p<0.01$) and with WSC, TOC and MBC. This behaviour has been also reported by other authors (Alvear et al., 2005; Melero et al., 2007). Kunito et al. (2001) attributed this positive correlation between enzyme activities and MBC to an indirect effect of the increase of the soil organic carbon.

Values of all the biochemical parameters measured were, in general, higher in LLE than in the other two experimental sites, indicating a better soil quality in this site. Moreover, differences among treatments clearly showed the positive effect of conservation tillage on biochemical status of the LLE soil. In contrast, in ZAR, the soil showed the smallest values of biochemical properties, indicating the low activity of the microorganisms, probably due to the adverse climatic conditions of the area, related mostly with the low precipitation recorded in the period previous to the sampling. Nevertheless, in this soil slight increases in soil biochemical properties with conservation tillage were also observed.

The lack of tillage effect in organic carbon and biochemical properties in SEV can be due to the presence of the leguminous (pea) in the previous season. This crop improves the microbiological status of the soil in any tillage system. Leguminous add organic matter and N to the soil (Ashraf et al., 2004), increasing soil fertility and favouring soil microorganisms development. Dinesh et al. (2004) also observed that the leguminous cover crop enhanced soil microbial activity and enzyme synthesis and accumulation due to increased C turnover and nutrient availability. In fact, in previous years before leguminous cropping, increases of biochemical properties were clearly observed in conservation tillage soils (Madejón et al., 2007). Moreover the rotation of crop in this area (in the other two areas no rotation of crop was carried out) could also contribute to maintain a higher biochemical activity.

Some authors have shown that herbicides could reduce the microbial functional diversity in soil but did not affect other microbiological parameters (Moreno et al 2009). These authors also revealed that the positive effect of the cover crop and the organic matter predominated in microbiological status in presence or not of herbicides. In the present experiments a reduction in the microbial activity due to the application of herbicides was not observed. Nevertheless further investigations are required to asses the potential influence of the herbicides applied under conservation tillage on microbial activity.

3.3 Stratification Ratio

Climatic conditions of the Mediterranean areas are the limiting factor for the accumulation of organic carbon in the top soil layers. Thus, the simple determination of TOC can not be the best indicator of the improvement caused by conservation tillage. Under these conditions it may be more interesting to study the stratification ratios (STR) of TOC. In general, under any condition, high STR for TOC indicates a good quality of the soil. In contrast, ratios lower than 2 are frequent in degraded soils (Franzluebbers, 2002). This approach could also be applied to other variables (Figure 6). Thus, in LLE, STR was clearly higher in soil under RT and NT than under

TT and the differences among treatments were even higher than those found when comparing values of each parameter. In ZAR, a general increase in the values of the STR was also observed, although differences were only significant in the case of MBC and PRA. In SEV, high values of STR were observed in all treatments, pointing out the benefits of the leguminous crop on the improvement of the superficial layer with conservation tillage.

These results could also show the importance of the values of STR of the biochemical properties to indicate the advantages derived from the adoption of conservation tillage in semi-arid areas. Soils with low inherent levels of organic matter can be the most functionally improved with conservation tillage, despite modest or no change in total standing stock of organic matter within the rooting zone (Franzluebbers, 2004). In general, the enrichment of the soil surface with crop residues usually led to significantly greater and more stable soil aggregates, especially in soils with coarse texture (Franzsluebber, 2004). Other authors have also confirmed the positive relation between soil biochemical parameters such as dehydrogenase and microbial biomass carbon and soil macroaggregation (Roldan et al., 2005). Moreover, data of STR were significantly correlated in some cases with yield production in LLE and ZAR (Tables 2 and 3). In SEV, although correlations were also positive and sometimes significant, the results were not consistent due to the low number of cases (6 plots in total). These positive relationships could point out the importance of STR data not only on determining soil quality but also yield production in the studied areas. These results pointed out the importance of these indexes to assess soil quality and fertility. Nevertheless, further studies in the same areas should be necessary to asses the positive correlation between STR and productivity before to define these ratios as indexes of productivity for Mediterranean conditions.

4. Conclusion

Long-term use of conservation tillage has become a proven strategy to increase soil organic matter, especially surface soil biochemical quality in Mediterranean areas of Spain. Changes in soil biochemical properties with tillage and in their stratification ratios should provide practical tools to complement physical and chemical test and, thus, evaluate the effect of tillage in Mediterranean semi-arid conditions. As the climatic conditions of the semi-arid Mediterranean areas are an important limiting factor for the accumulation of organic carbon in the top soil layers, the simple determination of TOC not always is the best indicator of the improvement caused by conservation tillage. Under these conditions it may be more interesting to study the stratification ratios (STR). Results in this study have shown the importance of the values of stratification ratio of TOC and other variables related to soil biology for these purposes by their correlations with yield production.

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Table 1. Site and soil characteristics in the Ap soil layer

Site and soil characteristics	Experimental site		
	Lleida	Zaragoza	Seville
Latitude	41º 48’ N	41º 44’N	37º 17’ N
Longitude	1º 07’E	0º 46’W	6º 3’ W
Elevation (m)	330	270	30
Mean annual air temperature (ºC)	14.2	14.5	17.5
Mean annual precipitation (mm)	430	390	494
Soil classification*	Xerofluvent typic	Xerollic Calciorthid	Xerofluvent
Ap horizon depth (cm)	28	30	30
pH (H2O, 1:2.5)	8.5	8.2	7.9
EC1:5 (dS m^{-1})	0.15	0.29	-
Water retention (g g^{-1})			
-33 kPa	0.16	0.20	0.23
-1500 kPa	0.05	0.11	0.12
TOC (g kg^{-1})	5.58	11.4	9.20
Particle size distribution (%)			
Sand (2000-50 µm)	30.1	32.4	49.8
Silt (50-2 µm)	51.9	45.5	29.1
Clay (<2 µm)	17.9	22.2	21.1

*USDA classification (USDA, 1996).

Table 2.

Correlation coefficients between stratification ratio of biochemical properties and organic matter in soil samples and yield production in Lleida experiment. (n = 27)

	STR TOC	STR WSC	STR CMB	STR DHA	STR PRA	Yield
STR TOC	-	0.626**	0.447*	0.627**	0.404**	**0.554****
STR WSC		-	0.409*	0.901**	0.601**	**0.851****
STR CMB			-	0.403*	0.565**	**0.266**
STR DHA				-	0.565**	**0.811****
STR PRA					-	**0.481***

** correlation is significant at the 0.01 level.

*   correlation is significant at the 0.05 level.

TOC: total organic carbon, WSC: water soluble carbon, CMB: carbon biomass; DHA: dehydrogenase activity;
PRA: protease activity;

Table 3.

Correlation coefficients between stratification ratio of biochemical properties and organic matter in soil samples and yield production in Zaragoza experiment n = 18

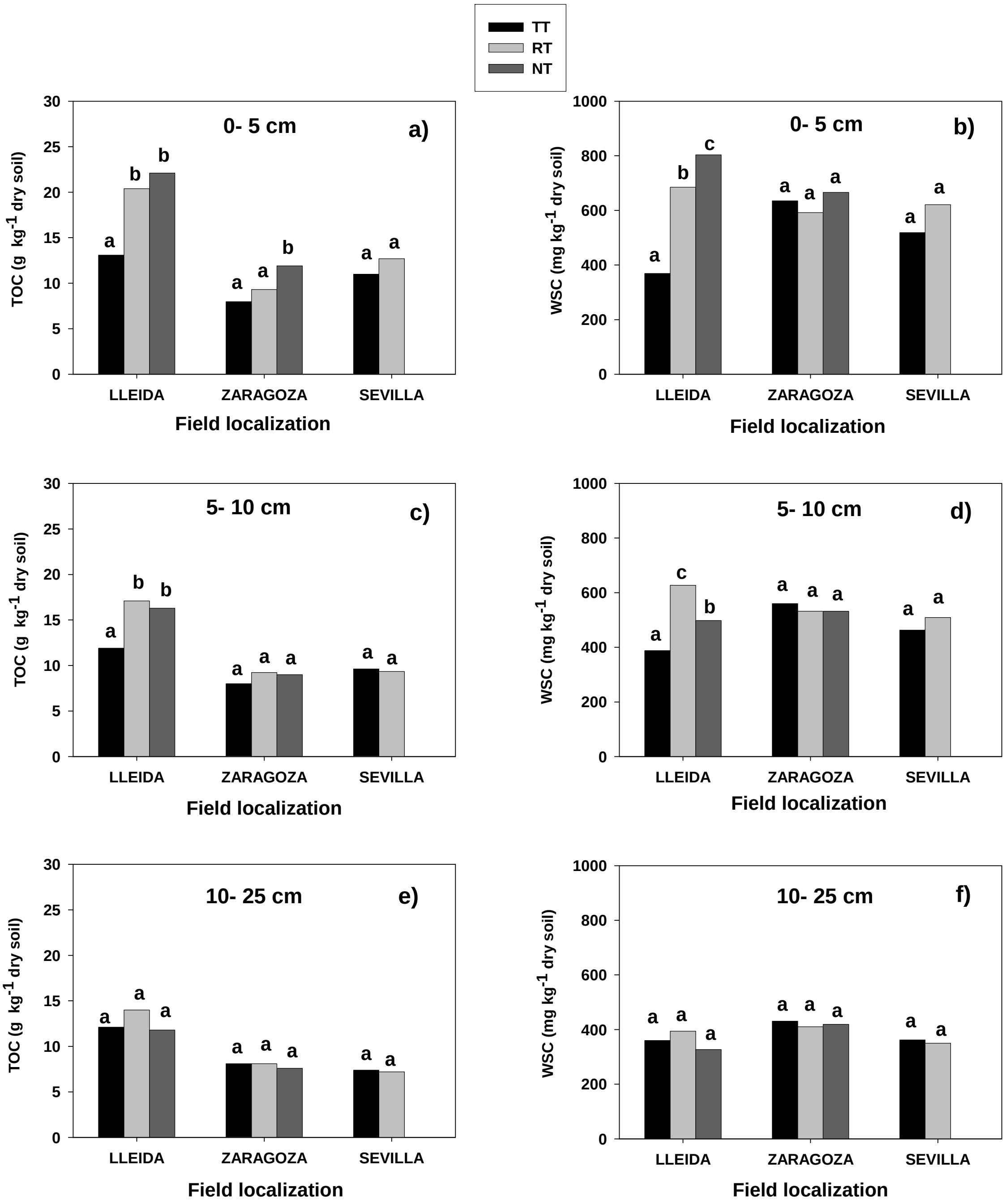
	STR TOC	**STR WSC**	**STR CMB**	**STR DHA**	**STR PRA**	**Yield**
STR TOC	-	0.355	0.616**	0.694**	0.319	**0.237**
STR WSC		-	0.419	0.730**	0.529*	**0.614****
STR CMB			-	0.462	0.462	**0.224**
STR DHA				-	0.660**	**0.498***
STR PRA					-	**0.581***

** correlation is significant at the 0.01 level.
* correlation is significant at the 0.05 level.
TOC: total organic carbon, WSC: water soluble carbon, CMB: carbon biomass; DHA: dehydrogenase activity;
PRA: protease activity;


France
Zaragoza
Lleida
Madrid
SPAIN
Portugal
Sevilla
★ Sampling Areas

Figure 1

Figure 2


TT
RT
NT
0- 5 cm
a)
TOC (g kg^{-1} dry soil)
0
5
10
15
20
25
30
a
b
b
a
a
b
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
0- 5 cm
b)
WSC (mg kg^{-1} dry soil)
0
200
400
600
800
1000
a
b
c
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
5- 10 cm
c)
TOC (g kg^{-1} dry soil)
0
5
10
15
20
25
30
a
b
b
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
5- 10 cm
d)
WSC (mg kg^{-1} dry soil)
0
200
400
600
800
1000
a
c
b
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
10- 25 cm
e)
TOC (g kg^{-1} dry soil)
0
5
10
15
20
25
30
a
a
a
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
10- 25 cm
f)
WSC (mg kg^{-1} dry soil)
0
200
400
600
800
1000
a
a
a
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization

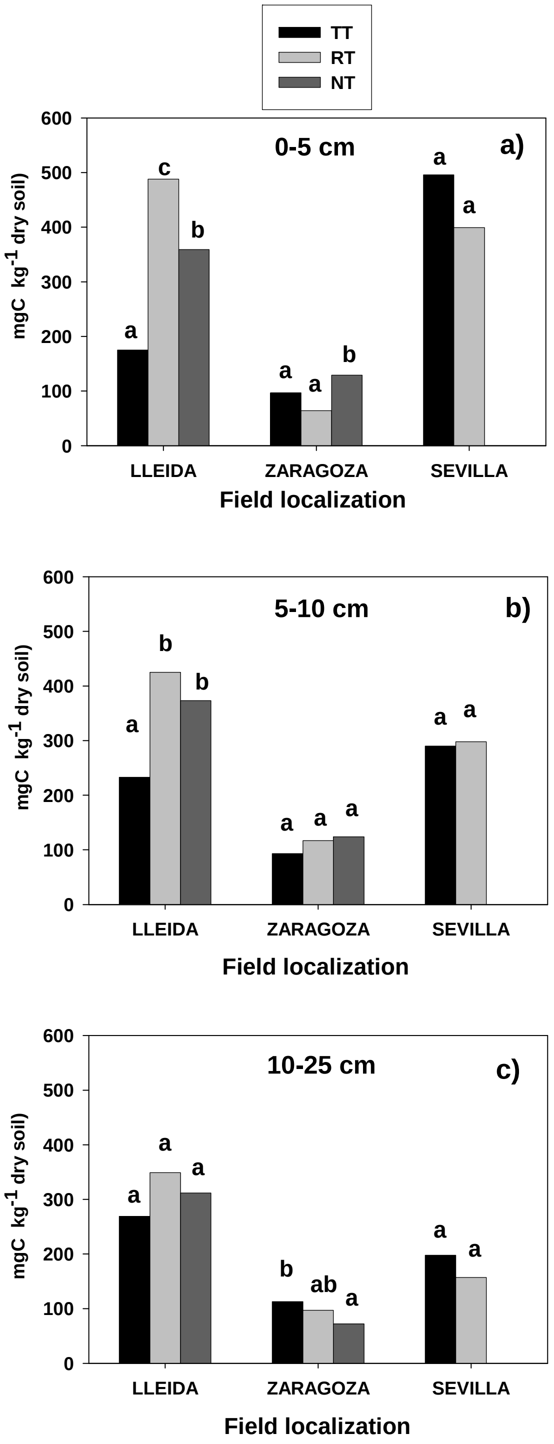

TT
RT
NT
mgC kg^{-1} dry soil)
0
100
200
300
400
500
600
0-5 cm
a)
a
c
b
a
a
b
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
5-10 cm
b)
a
b
b
a
a
a
a
a
10-25 cm
c)
a
a
a
b
ab
a
a
a

Figure 3

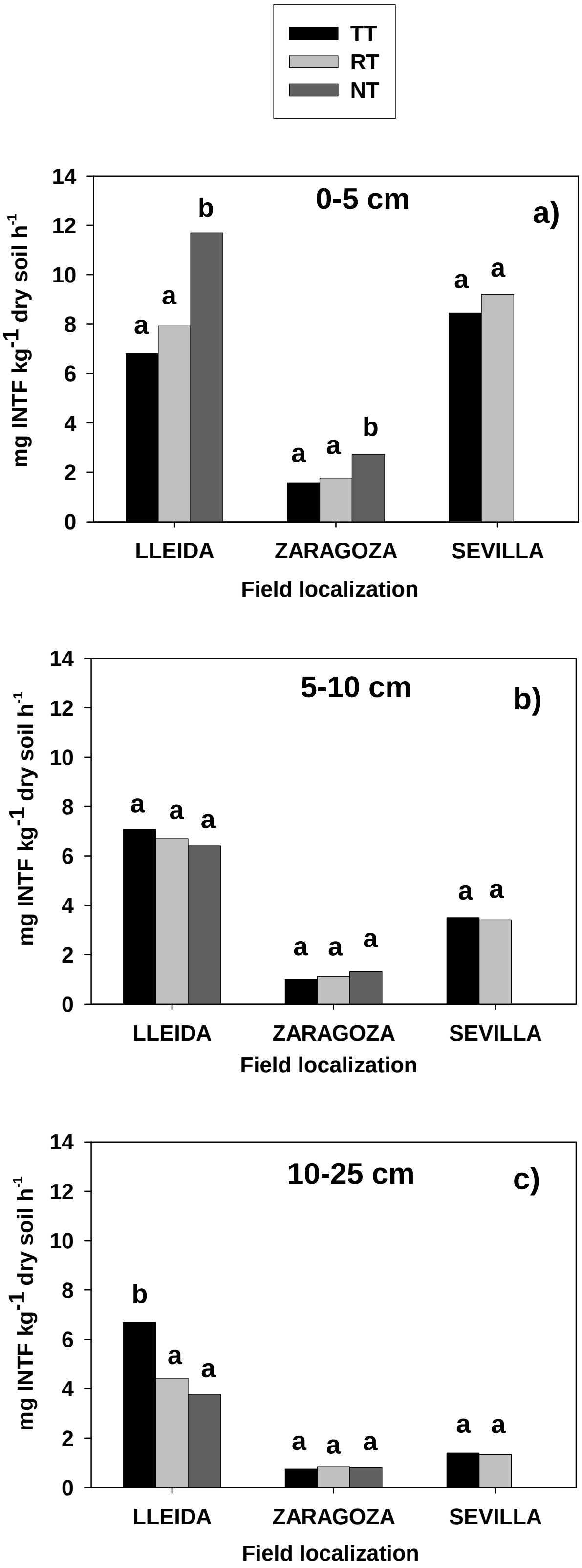

TT
RT
NT
0-5 cm
a)
b
a
a
a
a
a
a
b
0
2
4
6
8
10
12
14
mg INTF kg^{-1} dry soil h^{-1}
LLEIDA
ZARAGOZA
SEVILLA
Field localization
5-10 cm
b)
a
a
a
a
a
a
a
a
0
2
4
6
8
10
12
14
mg INTF kg^{-1} dry soil h^{-1}
LLEIDA
ZARAGOZA
SEVILLA
Field localization
10-25 cm
c)
b
a
a
a
a
a
a
a
0
2
4
6
8
10
12
14
mg INTF kg^{-1} dry soil h^{-1}
LLEIDA
ZARAGOZA
SEVILLA
Field localization

Figure 4

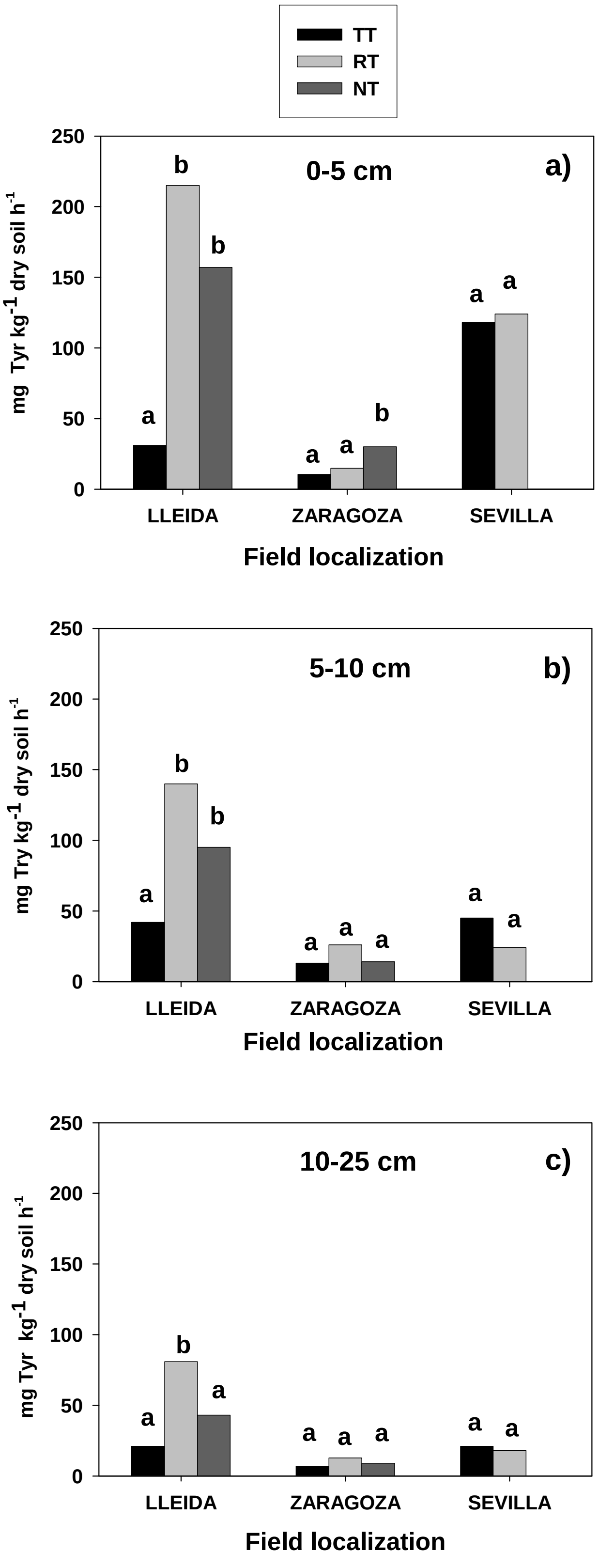

TT
RT
NT
0-5 cm
a)
mg Tyr kg-1 dry soil h-1
250
200
150
100
50
0
b
b
a
a
a
a
b
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
5-10 cm
b)
mg Try kg-1 dry soil h-1
250
200
150
100
50
0
b
b
a
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
10-25 cm
c)
mg Tyr kg-1 dry soil h-1
250
200
150
100
50
0
b
a
a
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization

Figure 5

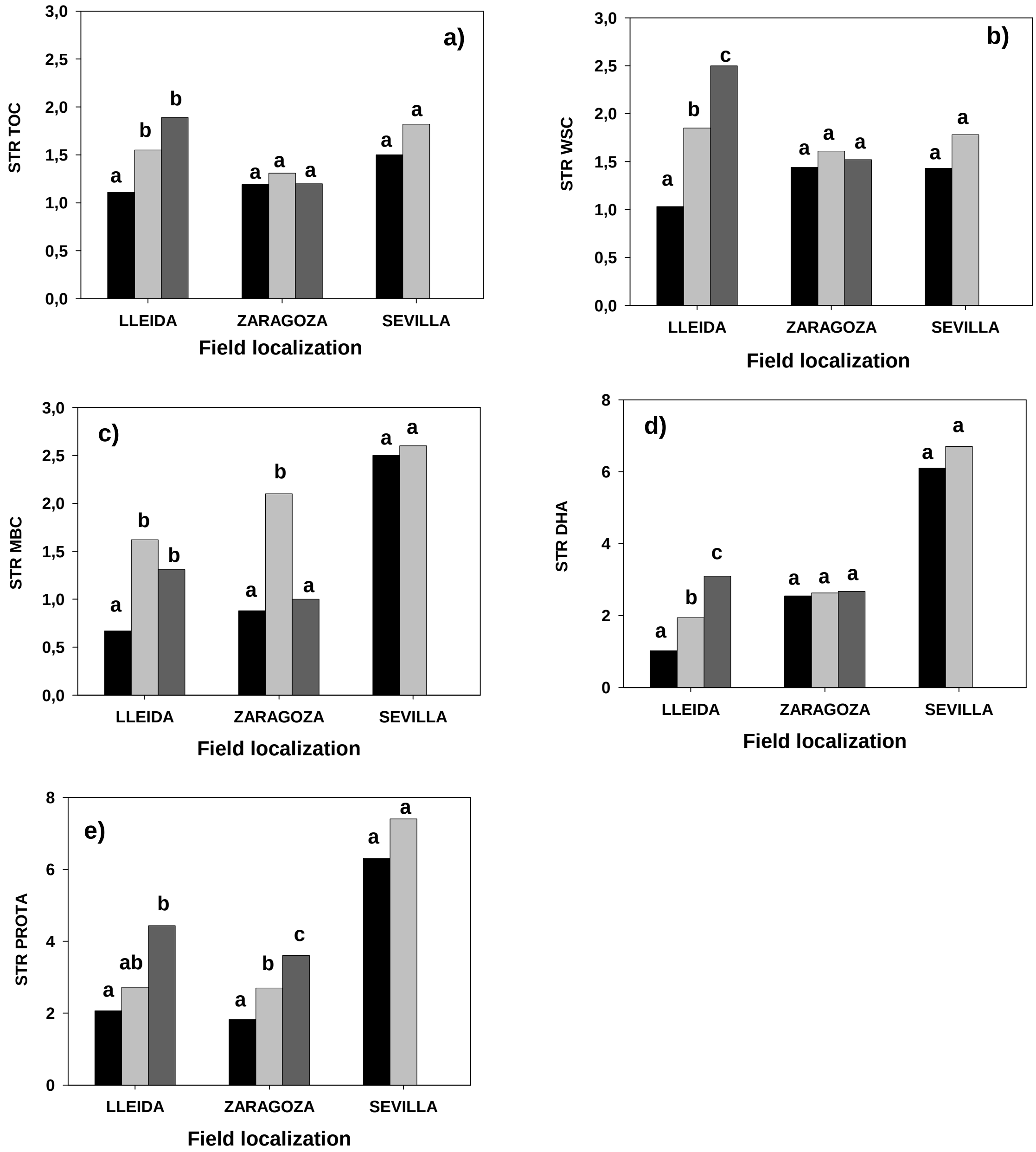

a)
STR TOC
3,0
2,5
2,0
1,5
1,0
0,5
0,0
a
b
b
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
b)
STR WSC
3,0
2,5
2,0
1,5
1,0
0,5
0,0
a
b
c
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
c)
STR MBC
3,0
2,5
2,0
1,5
1,0
0,5
0,0
a
b
b
a
b
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
d)
STR DHA
8
6
4
2
0
a
b
c
a
a
a
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization
e)
STR PROTA
8
6
4
2
0
a
ab
b
a
b
c
a
a
LLEIDA
ZARAGOZA
SEVILLA
Field localization

Figure 6

LEGENDS OF FIGURES

Figure 1. Location of the sampling areas

Figure 2. Total (TOC) and water soluble (WSC) organic carbon at different soil depths of each treatment: traditional tillage (TT) in black; reduced (RT) and no-tillage (NT) following an increasing grey scale. Columns with the same letter, for each sampling area, do not differ significantly ($p < 0.05$).

Figure 3. Microbial biomass carbon (MBC) values at different soil depths of each treatment: traditional tillage (TT) in black; reduced (RT) and no-tillage (NT) following an increasing grey scale. Columns with the same letter, for each sampling area, do not differ significantly ($p < 0.05$).

Figure 4. Dehydrogenase (DHA) values at different soil depths of each treatment: traditional tillage (TT) in black; reduced (RT) and no-tillage (NT) following an increasing grey scale. Columns with the same letter, for each sampling area, do not differ significantly ($p < 0.05$).

Figure 5. Protease (PROT) values at different soil depths of each treatment: traditional tillage (TT) in black; reduced (RT) and no-tillage (NT) following an increasing grey scale. Columns with the same letter, for each sampling area, do not differ significantly ($p < 0.05$).

Figure 6. Values of the stratification ratios (STR) of different soil properties at different soil depths of each treatment: traditional tillage (TT) in black; reduced (RT) and no-tillage (NT) following an increasing grey scale. Columns with the same letter, for each sampling area, do not differ significantly ($p < 0.05$).