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CONSERVATION TILLAGE: SHORT AND LONG-TERM EFFECTS ON SOIL CARBON FRACTIONS AND ENZYMATIC ACTIVITIES UNDER DRYLAND MEDITERRANEAN CONDITIONS

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Abstract

Short- and long-term field experiments are necessary to provide important information about how soil carbon sequestration is affected by soil tillage systems; such systems can also be useful for developing sustainable crop production systems. In this study, we evaluated the short- and long-term effects of conservation tillage (CT) on soil organic carbon fractions and biological properties in a sandy clay loam soil. Both trials consisted of rainfed crop rotation systems (cereal-sunflower-legumes) located in semi-arid SW Spain. In both trials, results were compared to those obtained using traditional tillage (TT). Soil samples were taken during flowering and after harvesting of the pea crop and collected at three depths (0-5, 5-10 and 10-20 cm). The soil organic carbon fractions were measured by the determination of total organic carbon (TOC), active carbon (AC) and water-soluble carbon (WSC). Biological status was evaluated by the measurement of soil microbial biomass carbon (MBC) and enzymatic activities [dehydrogenase activity (DHA), *o*-diphenol oxidase activity (DphOx), and β-glucosidase activity (β-glu)].

The contents of AC and MBC in the long-term trial and contents of AC in the short-term trial were higher for CT than TT in the upper layer. Furthermore, DHA and β-glucosidase values in the July sampling were higher in the topsoil under conservation management in both trials (short- and long-term). The studied parameters decreased as depth increased for both tillage system (TT and CT) and in both trials with the exception of the DphOx values, which tended to be higher at deeper layers.

Values of DHA and β-glu presented high correlation coefficients (r from 0.338 to 0.751, $p \leq 0.01$) with AC, WSC and TOC values in the long-term trial. However, there was no correlation between either TOC or MBC and the other parameters in the short-

term trial. In general, only the stratification ratios of AC were higher in CT than in TT in both trials. The results of this study showed that AC content was the most sensible and reliable indicator for assessing the impact of different soil management on soil quality in the two experiments (short- and long-term).

Conservation management in dryland farming systems improved the quality of soil under our conditions, especially at the surface layers, by enhancing its storage of organic matter and its biological properties, mainly in the long-term.

Key-words: sustainable agriculture; tillage; soil active carbon; enzymatic activities; microbial biomass carbon.

1. Introduction

Long-term traditional tillage (TT) practices may result in significant losses of soil organic matter, thus inducing an increase in soil erosion and a loss of soil structure (Álvarez and Álvarez, 2000; Nachtergaele, et al., 2002). Consequently, agricultural practices that reduce soil degradation are needed to improve soil quality and agricultural sustainability. Conservation tillage (CT) planting with minimal soil disturbance combined with crop rotation protects the soil against degradation toward sustainability (Balota et al., 2004). CT and, in particular, no-tillage (NT) induce changes in the distribution of organic pools in the soil profile (Álvarez and Álvarez, 2000). In general, the long-term effects of soil management practices on the size and activity of the microbial biomass have been found to be closely related to changes in total soil organic matter content (Haynes and Beare 1996). In long-term field experiments, marked stratification of the total soil microbial biomass and its activity has been observed as a

consequence of the application of NT to previously tilled soils. In short-term field experiments, it is often difficult to detect changes in soil organic matter following the implementation of new management practices (Álvarez and Álvarez, 2000). The short-term (≤ 10 years) effects of management on soil organic carbon (SOC) are complex and vary with soil conditions such as soil texture, climate, cropping system and kind of crop residue, as well as with the management itself (Paustian et al., 1997; Al-Kaisi et al., 2005; Muñoz et al., 2007). NT practices generally increase the sequestration of soil carbon (C), but this increase might not be apparent for approximately five to ten years (West and Post, 2002; Franzluebbers and Arshad, 1996). However, Franzluebbers and Arshad (1996) noted that there was little or no detectable increase in SOC content in the first two to five years after implementing conservation tillage. Weil et al. (2003) found active carbon (AC) to be a more sensitive indicator of soil management than TOC.

Soil microbial properties such as microbial biomass and soil enzymes, have been used to predict soil biological status and the effects of farm management as it relates to soil quality (Eivazi et al., 2003). Soil enzyme activities have also been used as discriminatory indicators for a wide range of soil management practices (Eivazi et al., 2003; De la Horra et al., 2003; Roldán et al., 2005; Melero et al., 2008 a,b). Researchers have observed a marked stratification in total soil microbial biomass and its activity as a consequence of the application of no-tillage to previously tilled soils in long-term experiments (fourteen years) (Álvarez et al. 1995).

Although several studies have been published comparing the effects of different tillage systems on soil biological properties (De la Horra et al., 2003; Balota et al., 2004; Roldán et al., 2005), there is comparatively less information (short- and long-term) on the soil biological status found in rainfed-agriculture under semi-arid Mediterranean conservation agriculture systems. In Spain, dryland crops constitute a

much larger agricultural area than irrigated crops and are thus particularly economically important. Our objective was to study the evolution of the soil organic C fractions (total organic carbon, active carbon, and water-soluble carbon), microbial biomass carbon, and enzymatic activities (β-glucosidase, *o*-diphenol oxidase activity and dehydrogenase activity) in short- and long-term field experiments in which CT and TT were compared. We hypothesised that CT would have a positive effect on soil quality by increasing soil organic matter and enhancing soil microbial functionality, especially over the long-term. We also discussed these parameters as reliable indicators of change in soils with both long and short histories of conservation management.

2. Materials and methods

2.1 Localization of the experimental area and tillage systems

Short- and long-term field trials using soil conservation management have been conducted on a sandy clay loam soil, Entisol (Xerofluvent, Soil Survey Staff, 1999), at the experimental farm at the 'Institute of Natural Resources and Agrobiology at Seville (IRNAS-CSIC) (37º 17' N, 6º 3' W), located 13 km southwest of the city of Seville (Spain). The soil has a pH of around 7.8 (calcareous), and a clay content of about 22% (15% montmorillonite, 6% illite, and 4% caolinite). The climate of the zone is typically Mediterranean, with mild rainy winters (484 mm mean rainfall) and very hot and dry summers. The mean annual daily temperature is around 17º C, with maximum and minimum temperatures of 33.5 ºC and 5.2 ºC in July and January, respectively.

An area of about 2500 m^2 was selected for establishing the experimental plots in 1991. In autumn of that year, wheat was grown. After harvesting the wheat in June

1992, the area was divided into six plots of approximately 300 m^2 (22 m x 14 m) each in a completely randomised experimental design (three replicates per treatment). In 2005, a short-term experiment was established in the same area following the same experimental design, but with 200 m^2 plots.

Two tillage treatments were compared: TT and CT. In both short- and long-term trials, TT consisted of mouldboard ploughing (to a depth of 30 cm) after the straw of the preceding crop had been burned. We should note here that straw burning has not occurred since 2003, when it was banned by the local government. In the long-term trial, CT was characterized by lack of mouldboard ploughing and a reduction in the number of tillage operations (retaining only chiselling at a depth of 25-30 cm) as well as by leaving the crop residues on the soil surface. CT in the short-term trial was characterized by the absence of tillage (direct drilling) in which the residue is left on the soil surface until it decays, except sunflower stalks, which were broken into smaller pieces before the next crop was sown.

At the beginning of the long-term trial, a wheat (*Triticum aestivum*, L.)–sunflower (*Helianthus annus*, L.) crop rotation was established for both TT and CT. However, in 2005, a fodder pea crop (*Pisum arvense*, L.) was included in the rotation for both tillage methods. Thus, from 2005 on, the annual crop rotation consisted of a basic cereal-sunflower-legumes rotation for both trials and treatments.

The sunflower and fodder pea crops were not fertilized (as is traditional in this zone), while wheat received deep fertilization with 400 kg ha^{-1} of a complex fertilizer (15N–$15P_2O_5$– $15K_2O$) before sowing and a top dressing with 200 kg ha^{-1} urea (46% N). Since 2002, fertilization has been reduced to 100 kg ha^{-1} (fertilizer complex) with no top dressing fertilizer. Weeds are controlled by tillage in TT and by the application of pre-

emergence herbicides in CT, at a rate of 2 l ha^{-1} trifluraline (18%) (sunflower) and 4 l ha^{-1} glyphosate (18%) (wheat, fodder pea).

2.2 Sampling and soil chemical and biochemical analysis

In both short- and long-term field trials, soil sampling was carried out in March 2008 during the pea crop-growing period and in July 2008 after harvesting at three sites of each individual plot (a total of nine samples per treatment); soil was collected at three depths: 0-5 cm, 5-10 cm and 10-20 cm. The moist field soil was sieved (2 mm) and divided into two sub-samples. One was immediately stored at 4 ºC in loosely tied plastic bags to ensure sufficient aeration and prevent moisture loss prior to assaying for microbiological and enzymatic activities. The other was air-dried for chemical analysis. Biochemical analyses were carried out within two weeks.

TOC was analysed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). WSC was determined in a 1/10 aqueous extract using a TOC-V-CSH/CSN analyser. AC was determined by oxidation with 0.2 M $KMnO_4$ in 1M $CaCl_2$ (pH 7.2) and non-reduced Mn^{7+} was colorimetrically determined at 550nm (Weil et al., 2003).

MBC content was determined by the chloroform fumigation-extraction method modified by Gregorich et al. (1990). *o*-Diphenol oxidase activity was measured following the procedure described by Perucci et al. (2000). Dehydrogenase activity was determined according to Trevors (1984), and β-glucosidase activity was measured as indicated by Eivazi and Tabatabai (1988).

Stratification ratios were calculated from soil properties (TOC, MBC and enzymatic activities) at 0-5 cm divided by those at a deeper layer (10-20 cm) (Franzluebbers, 2002).

For each microbiological analysis, three replicates per collected sample were done. Results were based on the oven-dried weight of the soil.

2.3 Statistical analysis

Statistical analyses were carried out using SPSS 11.0 for Windows, and the results were expressed as mean values. Significant differences between management systems (TT, CT) were shown by a Student's t-test at $p<0.05$. One-way analysis of variance (ANOVA) was carried out to assess the spatial variability of all parameters for each individual treatment. A correlation matrix of different properties was based on Pearson correlation coefficients ($p<0.01$ and $p<0.05$).

Data normality was tested prior to analysis; when necessary, variables were transformed logarithmically. If, after transformation, the data still did not have a normal distribution, we used non-parametric tests: the Mann-Whitney U test for comparison of mean values and the Kruskal-Wallis ANOVA by ranks test for variance analysis.

3. Results

3.1 Soil TOC fractions to long- and short -term.

In the long-term trial, only AC and MBC mean values were statistically different between treatments at 0-5 cm depth for both sampling periods and at 5-10 cm depth in March (Table 1). AC and MBC mean values in March and TOC, AC, WSC and MBC values in July showed differences between different soil depths under CT, whereas only

WSC mean values showed significant differences between the different soil depths in soils under TT in both sampling periods (Table 1).

In the short-term trial, only AC mean values presented statistical differences between treatments (CT and TT) at a depth of 0-5 cm in both sampling periods (Table 2). Under conservation tillage, significant differences between different depths were found for AC in the March samples and for AC, WSC and MBC in the July samples (Table 2).

3.2 Enzymatic activities in long- and short-term trials

In the March samples during the long-term trial, no significant differences in enzymatic activity values were found between treatments, although the highest enzymatic activity was observed in soils under CT. In the July samples, significant differences were observed in DHA and β-glucosidase values between treatments at 0-5 cm depth (Table 3), with the highest values in soils under CT. Significant differences among the different depths were observed only for β-glucosidase activity in both soil management systems in March, while DHA and β-glucosidase activity showed significant differences between the different soil depths in soils under CT in the July samples (Table 3).

In the short-term, DphOx values were higher in soils under TT than in soils under CT in March and July samples (Table 4), but not at long-term. In July, DHA and β-glucosidase values showed significant differences between treatments (CT and TT) at 5-10 and 10-20 cm depth, showing the highest values in soils under TT (Table 4). In both sampling periods, significant differences among different depths were observed only for DHA values in both treatments and in DphOx values under TT (Table 4).

On the whole, we observed a decrease in the studied properties as depth increased (Tables 1 to 4) in both trials (long- and short-term) and in both treatments (TT and CT)

with the exception of DphOx. DphOx values tended to be higher in lower layers than in upper layers, with significant differences in the short-term trial and with the TT treatment (Tables 3 and 4).

3.3 Correlation coefficients among soil properties and stratification ratio values in the long- and short-term.

In the long-term trial, β-glucosidase and DHA were highly correlated with AC, WSC and TOC contents (r from 0.338 to 0.751, $p \leq 0.01$), as well as with each other (r =0.751, $p \leq 0.01$). DphOx was found to be positively correlated with only AC (r = 0.348, $p < 0.05$) and MBC (r = 0.624, $p < 0.01$). In the short-term trial, lower correlations were found between both TOC and MBC and other properties, while AC and WSC contents were positive correlated with β-glucosidase and DHA activities. DphOx showed a negative correlation with AC, WSC, β-glucosidase and DHA (Table 5).

In general, stratification ratio values of the studied variables were greater in CT than in TT in both trials, although the differences were not always significant (Fig. 1). Only AC content showed significant differences in all trials (long- and short-term) and in both sampling periods (Fig. 1).

4. Discussion

The climatic conditions in southern Spain (mild winters, warm springs and high temperatures during summer) are the limiting factor for the accumulation of organic carbon in the top layer of soil. However, CT may limit mitigation of TOC losses due to an increase in C inputs through crop residues left on the soil surface.

The increase in TOC under CT in the long-term has been observed by other researchers (De la Horra et al., 2003; Madejón et al., 2007; Melero et al., 2008 b). In short-term studies, several authors have found an increase in TOC in the top layer when using NT in the first three years of transition from TT to NT (McCarty et al. 1998; Muñoz et al., 2007). However, Liang et al. (2007) reported that in the short-term (3-year), NT tended to stratify TOC, but did not lead to a significant increase in TOC in topsoil (0-5 cm) as compared to TT. Franzluebbers and Arshad (1996) also noted little or no detectable increase in TOC content during the first two to five years, but a significant increase often occurred five to ten years after converting from TT to CT. In our experiments, we recorded a noticeable increase in TOC in the soil upper layer (0-5 cm depth) only under CT (compared to TT) in the long-term trial (1.1 fold in March and 1.4 fold in July). These increases were not found in the short-term trial. The highest accumulation of crop residues in the soil occurred under NT could be because poor residue-soil contact reduces the decomposition of structural plant constituents through delayed colonisation by microorganisms degrading cellulose and hemicellulose (Roldán et al., 2005). In general, these results suggest that CT is an effective soil management technique for increasing sequestration of soil C, especially in the long-term.

Monitoring soil properties is a key point for the technical changes implied by conservation tillage, since farmers have to adapt their practices to the new states of the system. This requires the development of indicators characterizing this system; these indicators cannot be the same ones used in conventional agriculture. Suitable indicators of conservation tillage are required (Murillo et al., 2006). In our case, AC was the only soil property that showed a significant increase in the topsoil in both trials, with a significantly greater stratification ratio under conservation tillage than under traditional

tillage. The AC variable also showed a better correlation with the other studied variables (Table 5).

Thus, this study shows that under our experimental conditions, AC content is the most sensible and reliable indicator for assessing the impact of different soil management techniques on soil quality for both the short and long-term. Oyonarte et al. (2007) also proposed AC as a good indicator of the organic fraction in environmental monitoring programmes for arid environments. AC determination (Weil et al. 2003) is a relatively simple and promising method that could be adopted by soil test laboratories for use in routine soil analyses.

Microbial biomass may also represent a useful indicator of tillage-induced changes (Álvarez and Álvarez, 2000). The distribution of MBC may be related to the placement of crop residues. Álvarez et al. (1995) observed marked stratification in total soil microbial biomass and activity as a consequence of the application of NT to previously tilled soils in long-term experiments. Álvarez and Álvarez (2000) reported that total microbial biomass did not reflect the changes in the management of residues at 0-5 cm depth in the first crop cycle after implementing NT; therefore, total microbial biomass does not seem to be an early indicator of changes across soil management techniques. In contrast, Gupta et al. (1994) found higher values of microbial biomass in the first 5 cm of the soil profile under NT than under CT after one year of conservation management. Our results showed more MBC in the upper layers for soils under CT in the long-term trial, whereas the reported results in the short-term trial reflect those obtained by Álvarez and Álvarez (2000).

In general, in both trials (short and long-term), enzymatic activity (DHA and β-glucosidase) was found to be higher under CT than under TT. The same results have been observed by several other authors (Eivazi et al., 2003; De la Horra et al., 2003;

Roldán et al., 2005). Eivazi et al. (2003) reported that changes in enzyme activities in the profiles of tilled and no-tilled plots may be a consequence of large relative changes in the populations of aerobic and facultative anaerobic microorganisms. These changes may be due to the fact that the biochemical environments of no-tilled soils are less oxidative than those of soils under TT.

Moreover, the long-term effects of crop rotation could also have a positive influence on the accumulation of organic matter in the upper layers in both tillage systems, especially CT due to crop residues left on the surface (Magdoff and Weil, 2004). The crop rotation effects (different exudates, organic components from root systems and crop residues) also influence microbial activity (Balota et al., 2004). The high concentration of residue and roots of previous crops in the surface soil under CT can affect microbial activity. One of the benefits derived from conservation tillage may occur due to the "rhizosphere effect", which probably contributes significantly to higher enzyme activity than TT (Balota et al., 2004).

In both short- and long-term trials, β-glucosidase was the soil enzymatic activity with more pronounced statistical differences between depths under conservation tillage in both sampling periods. The same finding has been observed by other authors as well (De la Horra et al., 2003). This can be associated with a decrease in the easily decomposable organic C contents (grass roots and top material) with depth under CT. The accumulation of organic carbon in surface soils, as well as greater accumulation of inorganic nutrients under no tillage, tends to increase enzyme activities, especially β-glucosidase (De la Horra et al., 2003). This may be due to the fact that β-glucosidase is closely involved in the C cycle and is related to the composition, transformation and recycling of soil organic matter. In contrast, greater DphOx values were found at deeper layers, which may be related to a greater proportion of less available, humified soil

organic matter present in deeper soil layers (Haynes, 1999**)**. The *o*-diphenol oxidase is an oxidoreductase that catalyses the oxidation of phenolic compounds to quinines, participates in the formation of humic acids, and is an important measure of the soil microflora capacity to degrade recalcitrant organics (Perucci et al., 2000). In general, few differences were found in DphOx activity between tillage systems, which seems to indicate that DphOx is a poor indicator for soil quality in our experimental conditions. Some studies have found that seasonal changes affect soil microbial communities in agroecosystems (Schloter et al., 2003). In general, we observed greater MBC contents in the March sampling than in the July sampling in both long- and short-term trials, while DHA values showed the opposite trend. Schloter et al. (2003) found that the amount of microbial biomass in summer was reduced, which was closely related to the low water content and the high temperature in the topsoil. In addition, the DHA content was higher in July than March, which could be related to situational stress that strengthened the defence mechanisms of the microorganism population by increasing their activity (Gianfreda and Bollag, 1996). This shows the importance of taking into account the seasonal variation of biochemical parameters when these are used as indicators of soil responses to specific treatments.

5. Conclusion

In our study, conservation tillage promoted an accumulation of crop residues at soil upper layers, increasing the storage of organic matter and improving biological properties, especially in the long-term. Thus, conservation tillage may contribute to the long-term sustainability of agricultural ecosystems under dryland semi-arid Mediterranean conditions.

In both trials, active carbon content was an appropriate soil indicator of changes by different soil tillage systems, showing a significant increase under conservation tillage. Therefore, AC could be utilised as a sensible and early warning indicator for assessing the impact of soil quality under conservation tillage, a key point to overcoming farmer resistance to the establishment of these new conservation management techniques.

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Tables
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Table 1.

Mean values ± standard errors of total organic carbon (TOC), active carbon (AC), water soluble carbon (WSC) and microbial biomass carbon (MBC) in soil under traditional tillage (TT) and conservation tillage (CT) in the long-term experiment. Results of a one way analysis of variance for each soil property ([a] $p < 0.05$) at the different depths are also included.

	Treatment	Depth (cm)			F (2,24)	[a]P value
		0-5	5-10	10-20		
March 2008						
TOC(g kg^{-1})	**TT**	9.84±0.97	9.03±1.00	8.23±1.60	0.43	0.66
	CT	10.8±0.78	8.87±0.30	8.65±0.70	3.70	0.06
AC(mgkg^{-1})	**TT**	780±49.5	694±0.90	695±1.47	3.02	0.09
	CT	1680*±205	1039*±19.0	693±3.10	9.54	0.006[a]
WSC(mgkg^{-1})	**TT**	60.4±5.20	50.7±1.60	42.4±0.01	8.24	0.01[a]
	CT	82.9±24.0	65.6±8.15	46.6±3.20	1.51	0.27
MBC (mgkg^{-1})	**TT**	814±41.7	806±40.0	780±53.40	0.15	0.86
	CT	1058*±32.2	978*±52.5	879±25.1	5.44	0.03[a]
July 2008						
TOC (g kg^{-1})	**TT**	9.30±0.56	9.26±0.35	8.08±0.91	1.12	0.38
	CT	12.7*±0.49	9.35±0.26	7.62±0.30	50.48	0.00[a]
AC (mgkg^{-1})	**TT**	700±3.17	702±2.07	698±3.60	0.37	0.70
	CT	1380*±2.40	704±1.20	694±2.07	40192	0.00[a]
WSC (mgkg^{-1})	**TT**	72.4±3.70	61±3.60	52.7±5.30	5.34	0.04[a]
	CT	147±31.6	70.1±7.80	53.7±4.00	6.88	0.03[a]
MBC (mgkg^{-1})	**TT**	406±27.0	387±41.4	325±36.8	1.42	0.31
	CT	654*±40.0	405±16.8	283±19.6	47.6	0.00[a]

Differences between treatments are indicated by (*) ($p < 0.05$).

Table 2.

Mean values ± standard errors of total organic carbon (TOC), active carbon (AC), water soluble carbon (WSC) and microbial biomass carbon (MBC) in soil under traditional tillage (TT) and conservation tillage (CT) in the short-term experiment. Results of a one way analysis of variance for each soil property ([a] $p < 0.05$) at the different depths are also included.

	Treatment	Depth (cm)				
		0-5	5-10	10-20	F (2,24)	[a]P value
March 2008						
TOC(g kg^{-1})	**TT**	9.4±0.78	9.6±0.33	9.22±0.45	1.93	0.20
	CT	9.98±0.50	9.54±1.50	9.53±0.20	0.07	0.92
AC(mgkg^{-1})	**TT**	705±2.70	692±7.80	644±37.0	2.12	0.17
	CT	1368*±2.90	700±1.04	696±0.90	42638	0.00[a]
WSC(mgkg^{-1})	**TT**	56.9±2.11	56.3±3.31	51.06±0.95	1.90	0.20
	CT	81.1±11.2	62.6±7.55	56.8±1.38	2.61	0.13
MBC (mgkg^{-1})	**TT**	791±215	790±252	550±241	0.34	0.72
	CT	354±148	156±52.2	127±29.5	1.78	0.22
July 2008						
TOC (g kg^{-1})	**TT**	9.49±0.60	9.22±0.18	9.32*±0.26	0.13	0.88
	CT	9.46±0.58	8.10±0.38	7.72±0.39	3.91	0.08
AC (mgkg^{-1})	**TT**	704±1.20	704±1.20	702±2.10	0.80	0.49
	CT	1360*±4.10	696±2.40	692±3.20	13417	0.00[a]
WSC (mgkg^{-1})	**TT**	77.2±5.60	58.0±2.40	58.5±0.90	9.43	0.01[a]
	CT	83.1±2.20	62.8±6.60	50.8±3.50	13.15	0.006[a]
MBC (mgkg^{-1})	**TT**	472±53.0	363±13.6	362±13.0	1.56	0.28
	CT	509±23.8	360±52.0	291±18.6	10.3	0.01[a]

Differences between treatments are indicated by (*) ($p < 0.05$).

Table 3.

Mean values ± standard errors of enzymatic activities (dehydrogenase, diphenol oxidase, β-glucosidase), in soil under traditional tillage (TT) and conservation tillage (CT) in the long-term experiment. Results of a one way analysis of variance for each soil property ([a] $p < 0.05$) at the different depths are also included.

	Treatment	Depth (cm)				
		0-5	5-10	10-20	F(2,24)	[a]P value
March 2008						
DHA	**TT**	1.16±0.31	0.66±0.20	0.49±0.18	2.12	0.17
	CT	1.15±0.39	0.72±0.20	0.26±0.20	2.50	0.14
DphOx	**TT**	1.64±0.03	1.82±0.03	1.87±0.15	1.79	0.22
	CT	2.53±0.37	2.31±0.34	1.75±0.02	1.85	0.21
β-Glu	**TT**	140±18.8	84.2±11.0	55.6±11.0	4.94	0.04[a]
	CT	169±19.5	108±13.0	98.8[*]±4.70	13.5	0.002[a]
July 2008						
DHA	**TT**	2.41±0.43	1.79±0.41	1.24±0.46	1.78	0.24
	CT	4.32[*]±0.19	1.35±0.15	0.43±0.29	82.2	0.00[a]
DphOx	**TT**	1.33±0.04	1.43±0.48	1.41±0.04	1.45	0.30
	CT	1.55±0.09	1.39±0.03	1.61±0.13	1.44	0.31
β-Glu	**TT**	122±24.6	115±19.0	84±18.60	0.92	0.44
	CT	236[*]±20.5	136±27.0	66±16.4	15.2	0.004[a]

DHA: Dehydrogenase activity (mg TPF dwt kg^{-1} h^{-1});DphOx: Diphenol oxidase (mg cathecol 10 $min^{-1}g^{-1}$ dwt); β-Glu: β-glucosidase activity (mg p-nitrophenol kg^{-1} dwt h^{-1}).
Differences between treatments are indicated by (*) ($p < 0.05$).

Table 4.

Mean values ± standard errors of enzymatic activities (dehydrogenase, diphenol oxidase, β-glucosidase), in soil under traditional tillage (TT) and conservation tillage (CT) in the short-term experiment. Results of a one way analysis of variance for each soil property ([a] $p < 0.05$) at the different depths are also included.

	Treatment	Depth (cm)				
		0-5	5-10	10-20	F(2,24)	[a]P value
March 2008						
DHA	**TT**	1.16±0.30	0.46±0.20	0.22±0.10	5.07	0.03[a]
	CT	1.43±0.55	0.40±0.14	0.09±0.04	4.55	0.04[a]
DphOx	**TT**	1.97*±0.07	2.04±0.04	2.26*±0.07	5.64	0.02[a]
	CT	1.73±0.05	2.00±0.10	1.98±0.06	4.05	0.05
β-Glu	**TT**	97.2±5.60	71.6±9.70	48.2±8.40	9.15	0.007[a]
	CT	103±12.4	50.1±7.36	36.5±3.90	16.7	0.001[a]
July 2008						
DHA	**TT**	2.17±0.26	1.42*±0.10	1.29*±0.13	6.88	0.03[a]
	CT	2.36±0.28	0.86±0.10	0.36±0.10	32.6	0.001[a]
DphOx	**TT**	1.74*±0.05	1.79±0.02	1.94±0.04	6.52	0.03[a]
	CT	1.50±0.01	1.62±0.22	1.76±0.23	0.53	0.61
β-Glu	**TT**	81.6±4.60	83.7*±3.50	85.3*±6.20	0.143	0.87
	CT	106±11.0	55.2±4.80	40.2±2.80	23.5	0.001[a]

DHA: Dehydrogenase activity (mg TPF dwt kg^{-1} h^{-1});DphOx: Diphenol oxidase (mg cathecol 10 $min^{-1}g^{-1}$ dwt); β-Glu: β-glucosidase activity (mg p-nitrophenol kg^{-1} dwt h^{-1}).
Differences between treatments are indicated by (*) ($p < 0.05$).

Table 5.

Correlation coefficients between the different variables in the long-term and short-term experiments.

Long-term experiment							
	TOC	**AC**	**WSC**	**MBC**	**DHA**	**DphOx**	**β-Glu**
TOC	-	**0.540****	**0.539****	0.233	0.568**	0.056	**0.648****
AC		-	**0.625****	**0.475****	**0.338***	**0.348***	**0.648****
WSC			-	0.024	**0.677****	-0.147	**0.751****
MBC				-	-0.182	**0.624****	0.152
DHA					-	-0.241	**0.751****
DphOx						-	-0.039
β-Glu							-

n= 108
** correlation is significant at the 0.01 level.
* correlation is significant at the 0.05 level.
TOC: total organic carbon; AC: active carbon; WSC: water soluble carbon; MBC: microbial biomass carbon; DHA: dehydrogenase activity;Dph Ox: Diphenol oxidase activity, β-Glu: glucosidase activity.

Short-term experiment							
	TOC	**AC**	**WSC**	**MBC**	**DHA**	**DphOx**	**β-Glu**
TOC	-	0.149	0.065	0.148	-0.023	0.271	0.169
AC		-	**0.649****	-0.068	**0.495****	**-0.469****	**0.565****
WSC			-	-0.190	**0.481****	**-0.343***	**0.414****
MBC				-	-0.180	0.301	0.168
DHA					-	**-0.499***	**0.744****
DphOx						-	**-0.349***
β-Glu							-

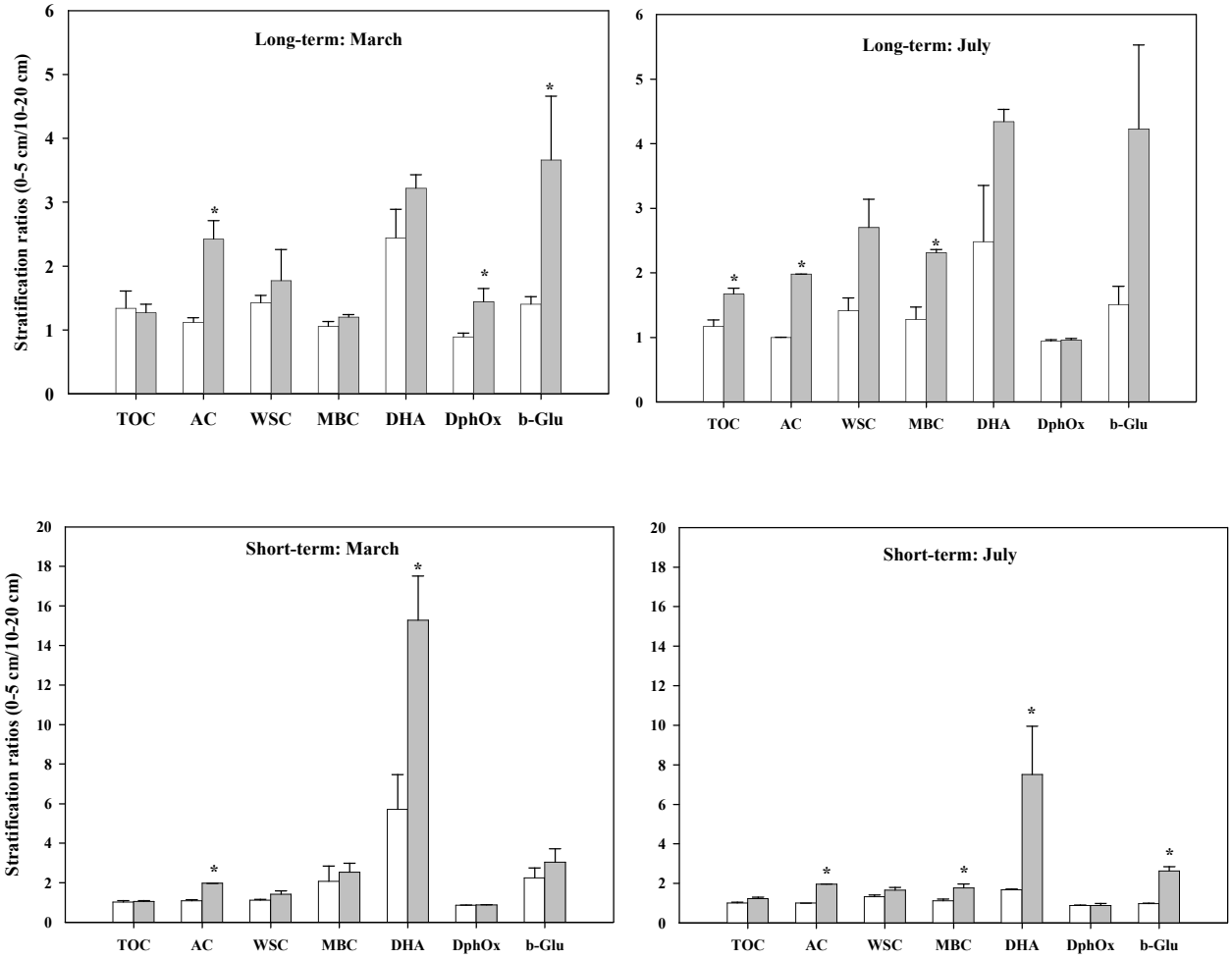
n= 108
** correlation is significant at the 0.01 level.
* correlation is significant at the 0.05 level.
TOC: total organic carbon; AC: active carbon; WSC: water soluble carbon; MBC: microbial biomass carbon; DHA: dehydrogenase activity;Dph Ox: Diphenol oxidase activity, β-Glu: glucosidase activity.

Figure Legends.

Figure 1.

Stratification ratio values (0-5cm/10-20cm) for: total organic carbon (TOC), active carbon (AC), water soluble carbon (WSC), microbial biomass carbon (MBC), and soil enzymatic activities (DHA: dehydrogenase activity; DphOx: Diphenol oxidase activity, Glu: β-glucosidase activity) under traditional tillage (TT) (white bars) and conservation tillage (CT) (grey bars). Mean values ± standard errors. Significant difference between treatments is indicated with asterisk (*) ($p < 0.05$).

Figure


Long-term: March
Long-term: July
Short-term: March
Short-term: July
Stratification ratios (0-5 cm/10-20 cm)
TOC
AC
WSC
MBC
DHA
DphOx
b-Glu