

Effects of tillage, crop rotation and N application rate on labile and recalcitrant soil carbon in a Mediterranean Vertisol

Verónica Muñoz-Romero^a, Rafael J. López-Bellido^a, Purificación Fernández-García^a, Ramon Redondo^b, Sergio Murillo^a, Luis López-Bellido^{a,*}

^a Departamento de Ciencias y Recursos Agrícolas y Forestales, University of Córdoba, Campus de Rabanales, Edificio C-4 “Celestino Mutis”, Ctra. Madrid km 396, 14071 Córdoba, Spain

^b Laboratorio de Isótopos Estables, Autonomous University of Madrid, Spain

* Corresponding author. Tel.: +34 957 218 495; fax: +34 957 218 440

E-mail address: cr1lobel@uco.es

ABSTRACT

The quantification of labile and recalcitrant fractions of organic C could provide valuable information in the study of soil organic carbon (SOC) changes in agrosystems. Few studies have determined the effects of farming practices on the recalcitrant and labile fractions of SOC at depth in a Mediterranean Vertisol. Our objective was to determine the effects of tillage, crop rotation and N fertilization on labile and recalcitrant SOC fractions and characterize $\delta^{13}\text{C}$ in a soil profile (0–120 cm) from a long-term experiment established in 1986 on rainfed Mediterranean Vertisols in southern Spain. The following treatments were studied: conventional tillage (CT) vs. no-tillage (NT); three crop rotations (wheat [*Triticum aestivum* L.]–chickpea [*Cicer arietinum* L.], wheat–sunflower [*Helianthus annuus* L.] and wheat–faba-bean [*Vicia faba* L.]); and two N fertilizer application rates (0 and 100 kg N ha⁻¹). The SOC contents of the soil samples from five soil layers (0–15, 15–30, 30–60, 60–90 and 90–120 cm) were determined. Throughout the experiment, the SOC content was greater in surface than in deep layers. The NT resulted in a greater SOC content than CT (10.7 Mg ha⁻¹ and 8 Mg ha⁻¹, respectively) in the most superficial soil layer. The SOC content was greater in the recalcitrant fraction than in the labile fraction (62% and 38% of total SOC, respectively). The recalcitrant SOC fraction was greater under CT than under NT. In the uppermost 15 cm, the labile organic C fraction was greater in the NT treatments than in the CT treatments because tillage resulted in a greater mineralization of the least stable forms of SOC. The influence of N rate and crop rotation on SOC was very low. The stable C isotopic composition ($\delta^{13}\text{C}$) was greater at depth than at the surface for both total SOC and the recalcitrant fraction. The labile fraction had more ¹³C than the recalcitrant fraction. The $\delta^{13}\text{C}$ value was greater under CT than under NT for both soil fractions. Tillage system exerted a

notable influence in both soil fractions and therefore in the organic matter quality since the labile fraction is an indicator of this quality.

Keywords: no-tillage, conventional tillage, $\delta^{13}\text{C}$

INTRODUCTION

Maintaining soil organic carbon (SOC) is particularly important for sustaining agroecosystem productivity because SOC plays a central role in soil quality and function by influencing soil physical, chemical, and biological properties (Lal et al., 1999; Carter,

2002). The SOC can change over time with different cultivation practices, such as tillage systems, crop rotations and nitrogen fertilization (Lopez-Bellido et al., 2010). Also, SOC is closely related to soil crack patterns (Zhang et al., 2016).

The capture and storage atmospheric C in a stable form is known as C sequestration. Agricultural soils provide an alternative for mitigating the greenhouse effect through C biosequestration by acting as C sinks or storage.

For C sequestration to occur, C should be converted from active C to less reactive intermediate or passive C and consequently stored in the soil for decades or longer (Wang and Hsieh, 2002). The results obtained by Lopez-Bellido et al. (2010), who studied Mediterranean rainfed Vertisols after 20 years of cultivation, demonstrate that no-till (NT) sequesters more C than conventional tillage (CT). In CT with legumes, rotation also affects C sequestration. In addition, the N fertilizer application rate did not affect C sequestration in these soils, despite the increase in biomass production (Lopez-Bellido et al., 2010). The components of SOC can be compartmentalized into different fractions or pools. The labile fraction is composed of particulate organic matter (OM), microbial biomass C, soluble C which is mineralizable and extractable with various reagents (Haynes, 2005); and the recalcitrant fraction is composed of organic materials that are highly resistant to microbial decomposition because of their chemical structure and association with soil minerals. Due to its resistance to decomposition, the recalcitrant C pool is likely more important for C storage and sequestration than the labile C pool (Laganière et al., 2010). In contrast, the labile fractions of SOC exhibit relatively fast turnover rates and therefore do not appear to significantly contribute to C sequestration in soils. The separation and quantification of labile and recalcitrant SOC into pools can be useful to understand SOC changes and their underlying mechanisms (Belay-Tedla et al., 2009). The stabilities and turnover rates of different SOC fractions are important for determining the influences of agricultural management on soil quality (Silveira et al., 2008). Altering the sizes of the labile and recalcitrant pools would influence the longevity of soil C sequestration (Paul et al., 2003).

The study of $\delta^{13}\text{C}$ natural abundance is useful as a tool to confirm the crop history of the study areas, as well as to show how the crop residues of sowed plants in those areas are contributing to the formation of C in certain environments (Siqueira Neto et al., 2009; Guareschi et al., 2012).

Few studies have determined the effects of farming practices on the recalcitrant and labile fractions of SOC at depth, particularly for rainfed Vertisols, which are typical for the Mediterranean region.

The objectives of this study were to i) determine the effects of tillage, crop rotation and N fertilization on the SOC contents and $\delta^{13}\text{C}$ values in the labile and recalcitrant fractions and ii) characterize the $\delta^{13}\text{C}$ values throughout the soil profile (0–120 cm) in the labile and recalcitrant fractions in a Vertisol under rainfed Mediterranean conditions.

MATERIALS AND METHODS

2.1. Site and experimental design

Field experiments were conducted in Córdoba, southern Spain (37° 46' N and 4° 31' W, 280 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region, where rainfed cropping is the standard practice. The study took place within the framework of a long-term experiment called “Malagón” that began in 1986 and was designed as a randomised complete block with a split-split plot arrangement and 3 replications. The main plots tested the effects of the tillage system (no-tillage and conventional tillage); subplots tested 2-year crop rotations (wheat-chickpea, wheat-faba bean and wheat-sunflower); and sub-subplots tested N fertiliser rate (0, 50, 100 and 150 kg N ha⁻¹). Each rotation was duplicated in a reverse crop sequence to obtain data for all crops on a yearly basis. The area of each sub-subplot was 50 m² (10 by 5 m).

2.2. Crop management

The NT plots were seeded with a no-till seed drill. Weeds were controlled by applying glyphosate (N-[phosphonomethyl] glycine) + MCPA ([4-chloro-2-methylphenoxy] acetic acid) at a rate of 0.5 + 0.5 L a.i. ha⁻¹ before planting. The CT treatment included mouldboard ploughing, disk harrowing and/or vibrating tine cultivation to prepare the seedbed. During the chickpea and faba bean growing seasons, weeds were controlled by applying cyanazine (2-[4-chloro-6-ethylamino-1,3,5-triazin-2-yl-amino]-2-methyl

propionitrile) at 2 L a.i. ha⁻¹. Ascochyta blight (*Didymella rabiei*) was controlled in chickpea by applying chlorothalonil (2, 4, 5, 6-tetra-chloroisophthalonitrile) at 0.75 a.i. ha⁻¹, and glyphosate was applied to faba bean plots at a rate of 0.065 L a.i. ha⁻¹ as a post-emergence spray when broomrape (*Orobanche crenata* Forsk) was approximately 0.5–1 cm tall (García-Torres et al., 1987).

Corresponding with the common practices in the area, kabuli chickpea (cv Sinaloa) was planted in 0.38-m-wide rows in February at a seeding rate of 80 kg ha⁻¹; faba bean (cv Alameda) was planted in 50-cm-wide rows in early December at 170 kg ha⁻¹; sunflower (cv Pioneer) was planted in 50-cm-wide rows in February at 5 kg ha⁻¹; and hard red spring wheat (cv Gazul) was planted in 18-cm-wide rows in late November at 150 kg ha⁻¹. Each year, the wheat plots were supplied with P fertilizer at a rate of 65 kg P ha⁻¹. The fertilizer was incorporated according to standard conventional tillage practices and was applied in bands when drilling in the no-tillage plots.

2.3. Soil sampling and pre-treatment

Three soil core samples were collected from each wheat plot after harvest at a depth of 0–120 cm using a manual Eijkelkamp auger. Samples were obtained at the following soil depths: 0–30, 30–60, 60–90 and 90–120 cm. The first layer (0–30 cm) was divided in two sub-layers (0–15 and 15–30 cm) to study the superficial layer in more detail. It was named “superficial layer” for 0–30 cm depth and “most superficial layer” for 0–15 cm depth. Bulk density was determined following the method of Grossman and Reinsch (2002). Due to the high soil clay content, the samples were dried before grinding and sieving. Samples were crumbled, as finely as possible, and spread out on trays to facilitate air drying for 48 h. Following the methods of Leavitt et al. (1996), samples were ground and sieved (1 mm) to remove large plant fragments and pebbles, and a subsample of 10 g was acidified with 1 N HCl to remove the carbonate fraction found in the samples. Next, the samples were immersed in a 1.2 g cm⁻³ NaCl solution to remove the floating fine plant residue fragments. Sample immersion and the removal of the floating fragments was repeated until no more plant residues floated to the surface. Then, the soil was filtered, rinsed with deionized (DI) water free of salts, and dried on a hot plate at 60 °C. After drying, the soil sample was ground using a mortar and pestle and examined again under a 20 × microscope to remove any remaining identifiable plant fragments.

2.4. Soil pool separation

After the removal of carbonates and plant fragments from all samples collected from each soil layer, acid hydrolysis was performed using 6N HCl. The acid hydrolysis procedure was modified from Leavitt et al. (1996). The samples were placed into a 500 ml round-bottom flask with 150 ml of 6 N HCl. With a water-cooled condenser installed above the flask, the mixture was heated to boiling at 116 °C using an electric heating mantle for ca. 18 h. After hydrolysis, the mixture was allowed to cool and was centrifuged at 1500 g for 20 min. The supernatant liquid was decanted into a pre-weighed beaker, and non-hydrolysed residue was rinsed with 20 ml of DI water. The rinsing solutions were decanted into a beaker containing the supernatant. The process of centrifuging and decanting was repeated twice with DI water to remove all soluble materials from the residue. Next, the residue was recovered by transferring it to pre-weighed vials. The hydrolysate (supernatant) fraction, classified as the labile pool, and the resistant (residue) fraction, taken as the recalcitrant pool, were both dried on a hot plate at 60 °C until a constant weight was achieved before grinding, passing through a 1-mm sieve and analysing for C and $\delta^{13}\text{C}$.

2.5. Analysis and calculations

The whole soil and soil fractions (labile and recalcitrant pools) were analysed for total C content and $\delta^{13}\text{C}$. The total C and $\delta^{13}\text{C}$ were equivalent to those of organic C because the soil was pre-treated to remove carbonates. The ^{13}C and total C concentration of the soil samples were determined using a Thermo FlashEA 1112 elemental analyser coupled to a Thermo isotope-ratio mass spectrometer in continuous flow and using the Dumas combustion method (EA 3000 Eurovector SpA, Milan, Italy).

The stable C isotopic composition was expressed relative to the international standard PDB (the international Vienna Pee Dee Belemnite) as $\delta^{13}\text{C}$ in units of per mil (‰).

2.6. Statistical analysis

The annual data for each variable were subjected to analysis of variance (ANOVA) using a randomized complete block design combined with the error term according to McIntosh (1983). Tillage system and blocks (replications) were considered random effects, while

crop rotation, N fertilizer and soil depth were considered fixed effects. Means were compared using Fisher's protected least significant difference (LSD) test at $P < 0.05$. LSDs for the different main effects and interaction comparisons were calculated using appropriate standard error terms. The Statistix v. 9.0 (Analytical Software, 2008) package was used for this purpose.

RESULTS

Soil organic carbon (SOC)

The main effects and significant interactions of the different treatments are shown in Table 1. The SOC in the full soil profile (0–120 cm) was greater at the surface than at depth. The SOC concentrations were highest (16.1 Mg ha^{-1}) in the most superficial soil layer (0–30 cm), followed by the 30–60 and 60–90 cm horizons (11.6 and 10.6 Mg ha^{-1} , respectively), with the 90–120 cm layer having the lowest concentration (9.5 Mg ha^{-1}) (Fig. 1). The SOC concentrations in the 30–60 and 60–90 cm layers represented 46% of the SOC in the full soil profile, and the SOC concentration in the 0–30 cm layer represented 34% of the SOC in the full soil profile.

Throughout the experiment, tillage system was not significant in the total SOC of the full soil profile (48 Mg ha^{-1} and 47 Mg ha^{-1} under CT and NT, respectively). When considering the soil depth, the SOC was greater under NT than under CT only in the most superficial soil layer (0–15 cm) (10.7 Mg ha^{-1} compared to 8 Mg ha^{-1} , respectively).

Labile and recalcitrant organic C

Throughout the experiment, the SOC content was greater in the recalcitrant fraction than in the labile fraction (29.7 Mg ha^{-1} compared to 18.1 Mg ha^{-1} , respectively), which represented 62% recalcitrant C and 38% labile C in the total SOC (Fig. 2). When considering the soil depth, the recalcitrant C contents were greater than the labile C contents at all depths (Fig. 2).

The influence of N rate and crop rotation on SOC was low. The only significant interaction was rotation \times N \times depth (Table 1). A higher recalcitrant C content was only observed in the wheat-faba bean rotation in the most superficial soil layer (0–15 cm) when

the rate of N applied to the wheat was 0 kg ha⁻¹ than when 100 kg N ha⁻¹ was applied (data not shown).

Regarding the tillage systems, the recalcitrant SOC fraction was greater under CT than under NT (32.3 Mg ha⁻¹ compared with 27.0 Mg ha⁻¹, respectively), and no differences were observed for the labile fraction. The C content was greater in the recalcitrant fraction under CT than under NT at soil depths of 30-60 and 60-90 cm (Fig. 3a). Although no significant difference in the labile fraction was observed between the tillage systems at a depth of 0-30 cm (Fig. 3b), if we consider only the most superficial soil layer (0-15 cm) the labile fraction was greater under NT than under CT (data not shown).

Relationships between total SOC and its fractions

Significant positive correlations were observed between the total SOC and C in the labile and recalcitrant fractions (Fig. 4).

Natural abundance of $\delta^{13}\text{C}$

Throughout the experiment, the $\delta^{13}\text{C}$ in the C labile fraction (-22.4‰) was greater than the $\delta^{13}\text{C}$ in the recalcitrant fraction (-26.2‰). In the tillage system, the $\delta^{13}\text{C}$ in the labile and recalcitrant fractions was greater in the CT systems than in the NT systems (Table 2). Additionally, in this same fraction, the $\delta^{13}\text{C}$ was greater when the N application rate was 100 kg ha⁻¹ than when the N application rate was 0 kg ha⁻¹ of N (-22.2‰ and -22.6‰, respectively). The $\delta^{13}\text{C}$ values of the recalcitrant SOC fractions at the surface (0-15 cm) were less than the $\delta^{13}\text{C}$ values at the other depths analysed (Fig 5 and Table 2). Crop rotation did not influence the $\delta^{13}\text{C}$ values in either fraction and total SOC.

DISCUSSION

Similar to other studies (Wang et al., 2013), and particularly in rainfed Mediterranean Vertisols (Lopez-Bellido et al., 2010), the SOC decreased with increasing soil depth throughout the experiment. However, as indicated by Lopez Bellido et al. (2010), a significant amount of C exists in the deeper soil horizons of these soils from the formation of extensive cracks due to frequent drought conditions. Cracks of up to 30 cm deep and 3

cm wide have been observed to result from the contraction and expansion of expandable clays (unpublished data).

Of the fractions recovered after hydrolysis, the recalcitrant fraction was the largest. These data are consistent with those of other studies (Paul et al., 2006).

It is known that any factor, such as tillage system, that disturbs the physical protection of C in the soil may lead to rapid C loss (Pandey et al., 2014). In our study, the lower SOC content in the labile fraction occurred in the CT treatment at soil depths of 0-15 cm was attributed to the mineralization of these labile soil fractions caused by tillage (Ding et al., 2012). Compared with NT, the increased aeration and soil temperatures that occurred under CT (Muñoz-Romero et al., 2015) facilitated greater SOC degradation, which occurred at a greater extent in the materials that were easier to break down, such as the materials in the labile fraction. The labile fractions of SOC exhibit relatively fast turnover rates and, therefore, may not contribute significantly to C sequestration in soils (Silveira et al., 2008). Shrestha et al. (2008) indicated that tillage has little influence on the recalcitrant pool due to its relatively high stability. Our study confirmed it in the first 15 cm of soil. Most studies have indicated that the presence of higher C contents in deeper layers is equivalent under NT and CT or higher under CT (Baker et al., 2007). In this experiment, the SOC in the recalcitrant fraction was greater under CT in the soil layers between 30 and 90 cm. This was likely because the greater displacement of SOC from the first centimetres of soil to deeper soil layers due to the cracks under CT where the crack volume was greater than under NT (Lopez-Bellido et al., 2016). The lower $\delta^{13}\text{C}$ values observed at the soil surface relative to the other soil depths are consistent with previous findings (Fernandes et al., 2014; Ehleringer et al., 2000). The enrichment of ^{13}C with depth can be due to the fact that SOM components are differentially digested by microbes (Ehleringer et al., 2000). Also, the $\delta^{13}\text{C}$ ratios of atmospheric CO_2 have been decreasing since the beginning of the industrial revolution because of the combustion of ^{13}C -depleted fossil fuels (Friedli et al., 1987; Troler et al., 1996). Since SOM at deeper soil depths is older (Trumbore, 2000), the C in these soil layers should have originated at a time when the $\delta^{13}\text{C}$ values of atmospheric CO_2 were more positive. Few studies have considered the natural abundance of $\delta^{13}\text{C}$ in the labile and recalcitrant C fractions; however, a study conducted by Cheng et al. (2008) based on 30 years of C_3 vegetation, obtained similar results to those presented in this research, in which the labile fraction had greater ^{13}C than the recalcitrant fraction. The positive relationship between the total SOC and the labile and recalcitrant C indicates that changes in soil organic matter caused

by agricultural management can be better understood by considering the alterations in the different C fractions separated by acid hydrolysis (Ding et al., 2012). For C to be sequestered within the soil for decades or longer, the soil active pools of C must be converted to less reactive intermediate or passive pools (Wang and Hsieh, 2002). The stabilities and turnover rates of SOC fractions are important variables for determining the influences of agricultural management on organic matter quality (Silveira et al., 2008). According to Haynes (2005), the recalcitrant fraction is largely unaffected by management practices imposed on the soil, however, the labile one can be considered as useful indicators of soil quality that influence soil function in specific ways and that are much more sensitive than recalcitrant fraction to changes in soil management practice.

CONCLUSIONS

The surface layer of soil (0-15 cm) has the greatest SOC content within the entire soil profile.

The SOC content was greater in the recalcitrant fraction than in the labile fraction. The greater total SOC content and labile fraction C in the NT systems than in the CT systems in the surface layer of soil was attributed to the mineralization of less stable forms of SOC due to the aeration and temperature changes that resulted from tillage. N application rates and crop rotations did not significantly affect the amounts of organic C found in the different soil fractions. The labile fraction had more ^{13}C than the recalcitrant fraction. The stable C isotopic composition was greater at depth than at the surface for both total SOC and the recalcitrant fraction probably due to the progressive impoverishment of d^{13}C at the surface of long-cultivated soils.

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Table 1. Analysis of variance (mean squares) of the soil organic carbon content as affected by tillage system, crop rotation, N rate, soil depth and fraction.

Source	df	Mean squares
Tillage (T)	1	19093ns
Rotation (R)	2	75332ns
Nitrogen (N)	1	63ns
Soil depth (D)	4	583171***
Fraction (F)	1	4785465***
T × D	4	173174***
T × F	1	742712*
D × F	4	98585***
R × N × D	8	48578***
R × D × F	8	25794***

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

Ns: not significant

Table 2. $\delta^{13}\text{C}$ (‰) mean values for total organic C, labile carbon and recalcitrant carbon as influenced by tillage (NT, no tillage; CT, conventional tillage), rotation, N rate and soil depth .

		$\delta^{13}\text{C}$ total	$\delta^{13}\text{C}$ recalcitrant	$\delta^{13}\text{C}$ labile
Tillage	NT	-25.1 a	-26.5 b	-22.7 b
	CT	-24.9 a	-25.8 a	-22.0 a
Rotation	Faba bean	-25.0 a	-26.3 a	-22.5 a
	Chickpea	-25.0 a	-26.1 a	-22.1 a
	Sunflower	-25.0 a	-26.2 a	-22.5 a
N rate (kg ha ⁻¹)	0	-25.1 a	-26.1 a	-22.6 b
	100	-24.9 a	-26.2 a	-22.2 a
Soil depth (cm)	0-15	-25.4 c	-26.9 c	-22.7 a
	15-30	-25.0 b	-26.2 b	-22.3 a
	30-60	-24.8 ab	-25.8 a	-22.0 a
	60-90	-24.7 a	-26.0 ab	-22.4 a
	90-120	-24.9 ab	-25.8 a	-22.4 a

Within each treatment, means followed by the same letters are not significantly different at $p < 0.05$ according to LSD.

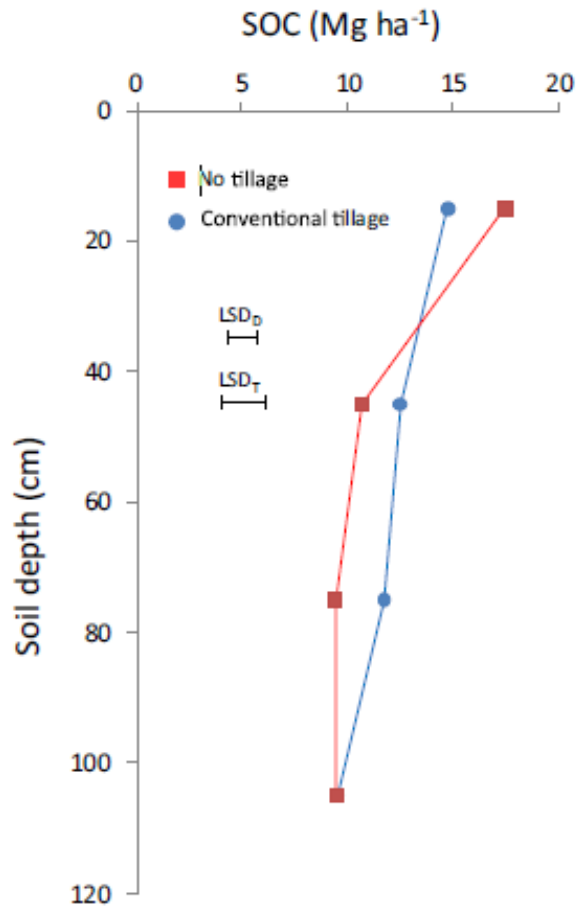


Fig 1. Soil organic carbon (SOC) as influenced of soil depth. Within soil depth, same letters indicate no significant differences at $p < 0.05$ according to LSD.

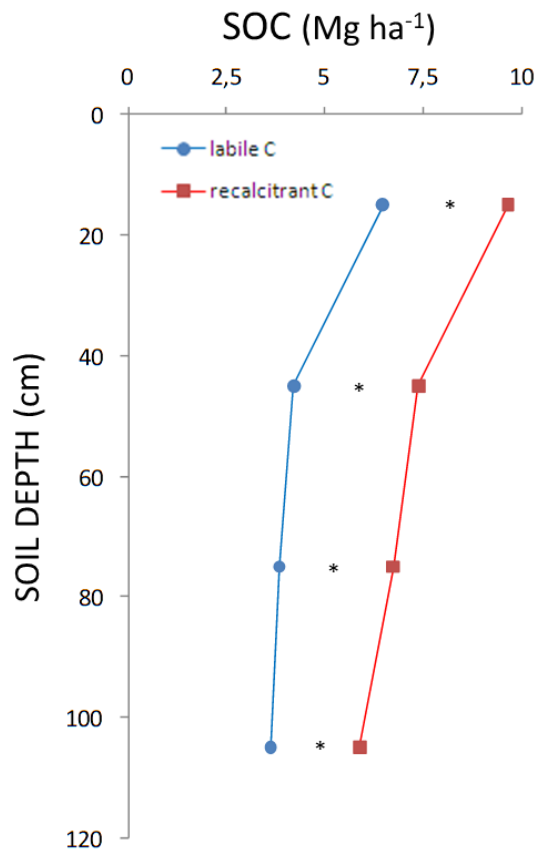


Fig 2. Soil organic carbon (SOC) as influenced of soil depth in labile and recalcitrant fractions. The asterisk (*) represents significant difference between fractions at the same soil depth.

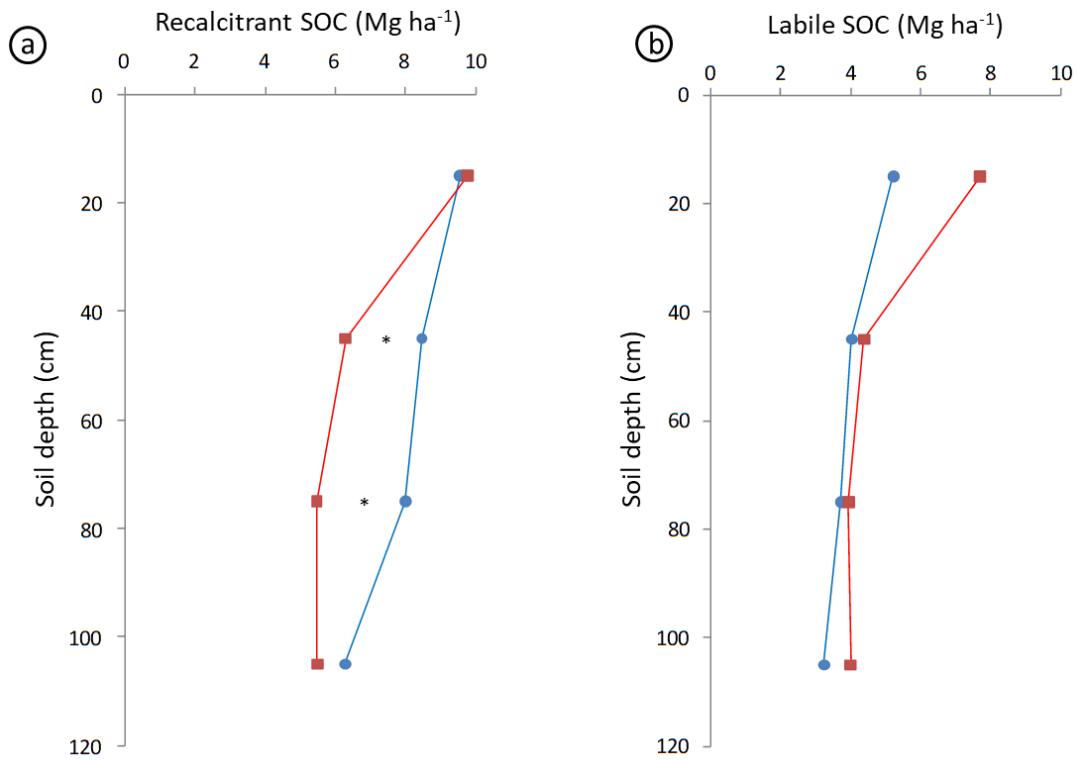


Fig 3. Soil organic carbon (SOC) as influenced of tillage system and soil depth on a) recalcitrant fraction, b) labile fraction. The asterisk (*) represents significant difference between tillage systems at the same soil depth.

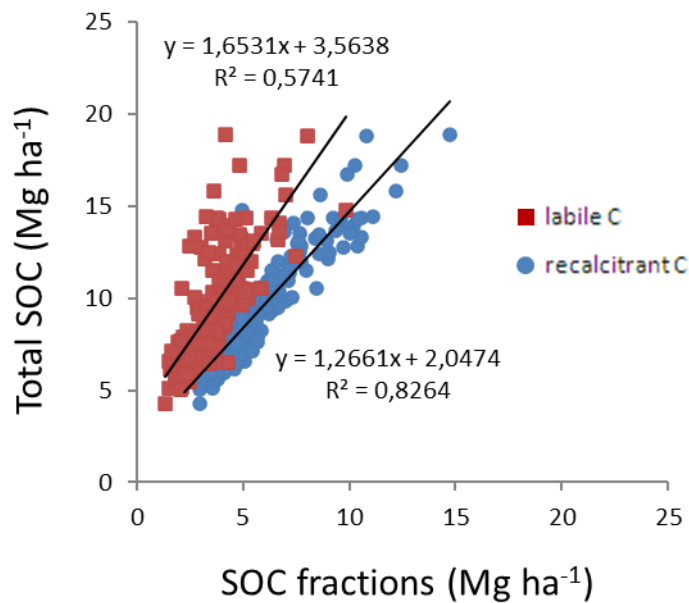


Fig 4. Relationship between soil organic C (SOC) on labile and recalcitrant fraction and total soil organic C.

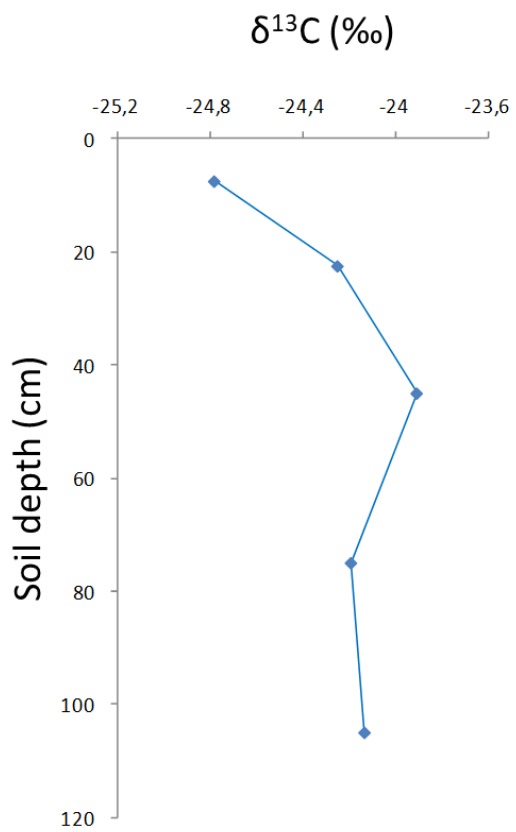


Fig 5. $\delta^{13}\text{C}$ as influenced of soil depth. Within soil depth, same letters indicate no significant differences at $p < 0.05$ according to LSD.