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LONG-TERM CROPPING SYSTEMS AND TILLAGE MANAGEMENT EFFECTS ON SOIL ORGANIC CARBON STOCK AND STEADY STATE LEVEL OF C SEQUESTRATION RATES IN A SEMIARID ENVIRONMENT

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ABSTRACT

A calcareous and clayey xeric Chromic Haploxerept of a long-term experimental site in Sicily (Italy) was sampled (0–15 cm depth) under different land use management and cropping systems (CSs) to study their effect on soil aggregate stability and organic carbon (SOC). The experimental site had three tillage managements (no till [NT], dual-layer [DL] and conventional tillage [CT]) and two CSs (durum wheat monocropping [W] and durum wheat/faba bean rotation [WB]). The annually sequestered SOC with W was 2·75-times higher than with WB. SOC concentrations were also higher. Both NT and CT management systems were the most effective in SOC sequestration whereas with DL system no C was sequestered. The differences in SOC concentrations between NT and CT were surprisingly small. Cumulative C input of all cropping and tillage systems and the annually sequestered SOC indicated that a steady state occurred at a sequestration rate of 7·4 Mg C ha^{-1} y^{-1}. Independent of the CSs, most of the SOC was stored in the silt and clay fraction. This fraction had a high N content which is typical for organic matter interacting with minerals. Macroaggregates (>250 µm) and large microaggregates (75–250 µm) were influenced by the treatments whereas the finest fractions were not. DL reduced the SOC in macroaggregates while NT and CT gave rise to higher SOC contents. In Mediterranean areas with Vertisols, agricultural strategies aimed at increasing the SOC contents should probably consider enhancing the proportion of coarser soil fractions so that, in the short-term, organic C can be accumulated.

KEY WORDS: carbon sequestration; particle-size fraction; soil aggregates; soil organic matter pools; Sicily

INTRODUCTION

Agricultural management practices influence organic matter in soil (Reicosky and Lindstrom, 1995), micro- and macroaggregates distribution, rate of soil organic matter (SOM) turnover, saturation limit and SOM stability and their steady state level (Stewart *et al*., 2007). Agriculture and intensive tillage have caused a decrease in soil C of between approximately 30 and 50 per cent due to the fact that many soils were brought into cultivation more than a 100 years ago (Schlesinger, 1986). Soil aggregation can provide physical protection of organic matter against rapid decomposition (Pulleman and Marinissen, 2004). Soil aggregation itself and consequently the C stock, soil organic carbon (SOC) dynamics and organic matter quality is strongly influenced by agricultural management intensity (e.g. Bono *et al*., 2008; Alvaro-Fuentes *et al*., 2009; Blano-Canqui *et al*., 2009; Simon *et al*., 2009). Soil structure and organic matter storage are also affected by the quality and quantity of organic inputs and the use of pesticides, fertilisers and manure (Droogers and Bouma, 1997). In addition, SOM and soil structure are mutually related: SOM binds with mineral particles to form soil aggregates and, in turn, stable aggregation can provide physical protection of otherwise mineralisable SOM (Tisdall and Oades, 1982; Elliott, 1986; Gupta and Germida, 1988; Beare *et al*., 1994b). Since the start of large-scale farming in the 20th century, agricultural practices have caused the loss of SOM from cultivated soils (Lal and Kimble, 1997; Smith *et al*., 2000). Apart from the detrimental effects on soil structure and soil quality (Reeves, 1997; Six *et al*., 1998), the released organic C contributes to global warming (IPCC, 2001, 2007). An important objective of sustainable use of soil resources is, therefore, to increase the pool of soil organic C (Paustian *et al*., 1997). However, the understanding of the mechanisms of SOM protection in aggregates and the management conditions that favour this process needs to be improved. The hierarchical model of aggregate formation by Tisdall and Oades (1982) illustrates the role of various organic binding agents in the organisation of stable soil aggregates. This model distinguishes between

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micro- and macroaggregates at a size of 250 μm. Generally, microaggregates are relatively stable and bound by persistent polysaccharide-based glues produced by roots and microbes and by calcium bridges. On the other hand, microaggregates are bound into macroaggregates by a network of roots and hyphae. Therefore, macroaggregate stability is thought to respond more rapidly to changes in soil management such as tillage and organic inputs (Tisdall and Oades, 1982; Elliott, 1986). Several authors found that aggregate stability and SOM contents of stable macroaggregates were higher in native grassland or reduced tillage compared to conventionally tilled and cultivated systems (Elliott, 1986; Cambardella and Elliott, 1994; Beare *et al.*, 1994a,b; Jastrow *et al.*, 1996; Six *et al.*, 1998). Others reported higher mean residence times of SOM for less-tilled soils (Balesdent *et al.*, 1990; Six *et al.*, 1998; Collins *et al.*, 2000; Dalal *et al.*, 2007; Metay *et al.*, 2007) that were attributed to a better physical protection of soil aggregates in the absence of conventional tillage (CT) practices.

Generally, most soils of dry-land ecosystems are characterised by low SOC contents and in regions having winter rainfall, rain-fed cropping is dominated by cereals (e.g. wheat, barley) and legumes (e.g. chickpea, faba bean, peas, forage legumes) combined with livestock production. Specifically in the semiarid environment, conservative soil management techniques allow the increase of C only in the soil surface, while CT shows a higher C content that is probably due to the higher root biomass production (Alvaro-Fuentes *et al.*, 2008; Bell *et al.*, 2003). Furthermore, the contribution to SOM concentration is determined by the incorporation of new organic matter in the coarse fraction and the reduction of mineralisation processes in the finest ones (Ouédraogo *et al.*, 2005). But in any case, it is not clear if a kind of SOC saturation limit can be reached as a function of agricultural management, since in the semiarid environment and specifically on Vertisols no studies were carried out about the relationship between soil management and SOC dynamics. Interaction between climate and high clay content could be a factor affecting SOM mineralisation rates and accumulation in soil. However, SOC concentrations in clayey soils in Sicily are usually not lower than 15 g kg^{-1}, despite the high environmental (temperature and rain) pressure on the mineralisation rate.

The aim of this study was to evaluate the long-term impact of different tillage management practices and crop rotation on: (i) changes in soil C stocks due to different cropping systems (CSs) and soil tillage managements; (ii) the contribution of soil aggregate size on C stocks; (iii) SOM stability; (iv) the role of the finest soil fractions in protection against SOM mineralisation. Using an experimental approach, we assumed that after 19 years of different land management techniques differences in the SOC can be detected. We hypothesised that no tillage (NT) should lead to a better SOC stabilisation and consequently to a higher content of organic C in the soil. The high clay content of the soils of interest is expected to protect SOM from degradation. Consequently, differences in SOC due to land use should be detected preferentially in the coarse fraction of the soil.

MATERIALS AND METHODS

Study Area

The research was carried out at the Pietranera farm, located in the southern part of central Sicily (Italy) (37°32′74'' N, 13°31′53'' E; elevation 236 m; mean annual precipitation 481 mm; mean annual air temperature 19 °C) on a soil of a 19-year long-term experiment covering an area of 4440 m^2.

The geology of the area is characterised by clays, sandy-clays and yellow sandstones of Tortonian age.

The soil of the experimental site is classified as a fine-clayey, calcareous, mixed, xeric Chromic Haploxerert having a slope of 4 per cent. The soil sampled before the start of the experiment (0–15 cm depth) had 471 g kg^{-1} clay, 225 g kg^{-1} silt, 304 g kg^{-1} sand and an average of 18·1 g SOC kg^{-1} (standard deviation ±0·5; $n = 12$) and 1·29 g N kg^{-1} and a pH of 8·1 in 1990 (start of experiment). The previous history since 1952 of the experimental area was a durum wheat/vetch crop rotation under a deep ploughing regime.

Experimental Design

Twelve plots of a 19-year long-term experiment were used with two-crop rotations, wheat (*Triticum durum*) [W] and wheat/faba bean (*Vicia fava*) [WB]) and three different soil managements: (i) CT consisting of ploughing to a depth of 30–35 cm and a surface tillage (5 cm depth); (ii) dual-layer tillage (DL) with a chisel to a depth of 40 cm and a subsequent 15 cm depth light tillage; (iii) NT. The individual plot size was 370 m^2, so that the use of agricultural machinery was possible. To minimise the annual climatic variability effects, all crops had a yearly-based rotation. Wheat seed density was 350 seeds m^{-2} in rows 16 cm apart. Following normal farm practice, every year before seeding 92 kg ha^{-1} of P_2O_5 and 82 kg ha^{-1} of N (urea 46 per cent) was applied in WB (120 kg ha^{-1} under W). In NT, weeds were controlled by a pre-planting *glyphosate* treatment (using different concentrations depending on the height of the weeds). Weeds in continuous wheat were controlled by herbicides at the 3rd to 4th leaf stage using different categories of herbicides depending on the amount of weeds present. A density of 40 seeds m^{-2} in rows 70 cm apart was used for the faba beans. Fertilisation was performed before seeding, using 18 kg ha^{-1} of N and 46 kg ha^{-1} of P_2O_5. Weeds were controlled by a combination of herbicides

(*Imazethapyr* and *Pendimethalin* using a concentration of 66 and 966 g ha^{-1}, respectively) and an inter-row tillage in March. Annual biomass production was estimated by weighing one square metres of biomass for each plot (4·28 Mg ha^{-1} y^{-1} for wheat/conventional tillage [W/CT], 3·84 Mg ha^{-1} y^{-1} for wheat/dual-layer [W/DL], 4·32 Mg ha^{-1} y^{-1} for wheat/no tillage [W/NT], 3·76 Mg ha^{-1} y^{-1} for wheat/bean/conventional tillage [WB/CT], 3·71 Mg ha^{-1} y^{-1} for wheat/bean/dual-layer [WB/DL] and 3·93 Mg ha^{-1} y^{-1} for wheat/bean/no tillage [WB/NT]). Wheat and faba bean straws were not manured, but removed according to the farm practices of the area. Thus, only the biomass of the roots was considered as C input to the soil and was calculated according to Kong *et al*. (2005) and Chung *et al*. (2008) with:

$$\begin{aligned}&\text{Carbon root biomass }(\text{Mg ha}^{-1})\\&= 0\cdot 22 \times \text{aboveground biomass }(\text{Mg ha}^{-1})\\&\times \text{root carbon content }(\text{per cent})\end{aligned}$$

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For both species a 40 per cent carbon content was assumed (data not shown). The value 0·22 for root to shoot ratio is an average that has been used in several papers both for legumes and cereals. This value has been published in the IPCC Guidelines for National Greenhouse Gas Inventories Chapter 11 (2006) and is the mean between 0·24 for wheat and 0·19 for legumes.

Annual SOC sequestered was calculated as the difference between the first (18·1 g kg^{-1}) and the last experimental year SOC values (Kong *et al*., 2005). We used the mean C value of the field at the beginning of the experiments since the variability of SOM was low (standard deviation = 0·5; $n = 12$) which is typical for arable land under CT where soil mixing by tillage reduced organic C variability (see also Röver and Kaiser, 1999; Kravchenko *et al*., 2006; de Oliveira Machado *et al*., 2007). Positive and negative values were considered as SOC gains or losses for the CSs, respectively.

Soil Sampling and Fractionation

Soil samples (two replicates *per* each plot; 12 plots with 2 subsamples gives a total of 24 samples) were collected after wheat harvesting at 0–15 cm depth (twice in each plot), air-dried and sieved at 2 mm. In order to reduce the error tolerance to less than ±5 per cent, around 2–4 kg of soil material (Hitz *et al*., 2002) were collected *per* sample. Wet aggregate-size fractions, with no prior chemical dispersion, were isolated by mechanical shaking of 50 g air-dried fine earth on a column with sieves of 2000, 1000, 500, 250, 75 and 25 μm using a Shaker AS 200 Sieve (RETSCH analytical-203 mm ϕ sieves) (amplitude of 2 cm, frequency of 1·6 Hz and a water flux of 2 L min^{-1}). After the physical fractionation, we distinguished four main fractions: 250–2000 μm (macroaggregates), 75–250 μm (large microaggregates), 25–75 μm (small microaggregates) and <25 μm (silt and clay fraction). We measured the relative weight distribution of these fractions and the C content. Total N and ^{13}C natural abundance were measured solely in the bulk soil (2 mm sieved) and the finer fractions (75–250, 25–75 and <25 μm) because of the limited amount of coarser fractions.

Soil bulk density was measured using the tube core method (Baruah and Barthakur, 1997). Soil bulk density was measured for each treatment.

SOC Analysis

Soil organic content was analysed by the Walkley–Black method (Walkley and Black, 1934) and N by the Kjeldahl procedure. For the ^{13}C analysis, an EA-IRMS (elemental analyser isotope ratio mass spectrometry) was used. Prior to analysis, the samples were treated to remove the total carbonates according to Harris *et al*. (2001). The reference material used for analysis was IA-R001 (Iso-Analytical Limited wheat flour standard, $\delta^{13}C_{\text{V-PDB}} = -26{\cdot}43$ ‰). IA-R001 is traceable to IAEA-CH-6 (cane sugar, $\delta^{13}C_{\text{V-PDB}} = -10{\cdot}43$ ‰). IA-R001, IA-R005 (Iso-Analytical Limited beet sugar standard, $\delta^{13}C_{\text{V-PDB}} = -26{\cdot}03$ ‰) and IA-R006 (Iso-Analytical Limited cane sugar standard, $\delta^{13}C_{\text{V-PDB}} = -11{\cdot}64$ ‰) were used as quality control check samples for the analysis. The International Atomic Energy Agency (IAEA), Vienna, distribute IAEA-CH-6 as a reference standard material.

The results of the isotope analysis are expressed as a δ value (‰), relative to the international Pee Dee Belemnite standard as follows:

$$\delta(‰) = \frac{(R_s - R_{st})}{R_{st}} \times 1000$$

where: $\delta = \delta^{13}C$; $R = {}^{13}C/{}^{12}C$; s = sample; st = standard.

Total carbonate was measured using the gas volumetric method after HCl treatment (Dietrich-Frühling Calcimeter).

Statistical Analysis

All data were analysed according to a split-plot design with two replicates and two cores *per* plot to test both the tillage (T) and the CS effects using the Anova procedure of SAS (SAS Institute, 2001) with T and CS as fixed effects (main plot) and replicates as random effect. Significance was checked using the least square difference (LSD) test. No significant interaction between tillage and CS was found. Consequently, main effects of tillage and copping systems on soil parameters could be analysed.

RESULTS AND DISCUSSION

SOM in Bulk Soil Samples

After 19 years of crop rotation and different tillage techniques, the SOC and N contents were significantly influenced by the treatments (Table I). The C content in the different experimental plots ranged from 18·3 up to 21·2 g kg^{-1} (Table I).

Surprisingly, the highest C content was found with the W/NT (21·2 g kg^{-1}) and W/CT (20·8 g kg^{-1}) treatments, respectively. The SOC content with WB was significantly lower than with W. The values ranged between 18·3 and 19·6 g kg^{-1} (average 18·8 g kg^{-1}). The comparison of the three different soil management techniques showed that NT had a greater influence on organic C accumulation (20·4 g kg^{-1}, $n = 8$), although the SOC values did not significantly differ from CT (19·7 g kg^{-1}, $n = 8$). A lower C content was found with the DL system (18·3 g kg^{-1}, $n = 8$). A lack of a significant difference in SOC stock between LNT (long-term NT) and long-term mouldboard ploughing (LMP) was also detected in the Midwest USA (Dick *et al.*, 1991; Wander *et al.*, 1998; Yang and Wander, 1999), in Eastern Canada (Carter and Rennie, 1982; Angers *et al.*, 1995, 1997; Deen and Kataki, 2003; VandenBygaart and Kay, 2004; Dolan *et al.*, 2006), and in the rolling Pampas area of Argentina (Alvarez *et al.*, 1998). Although many studies suggest that NT increases SOC within the soil profile compared to mouldboard ploughing, some other studies indicated that no net change occurred. The latter studies suggested that NT only stratified the SOC, where a near-surface increase in SOC was offset by a concomitant decrease in the subsurface (Yang *et al.*, 2008). However, lands with a history of intensive cultivation, which is not the case for Sicily, respond to changes in agricultural management on lands having the highest SOC accumulation rates, because soils that have lost the most C stand to gain the greatest (Johnson *et al.*, 1995). West and Post (2002) found, on average, that a change from CT to NT can give rise to a sequestration rate of 57 ± 14 g C m^{-2} y^{-1}, excluding wheat-fallow systems which may not result in a SOC accumulation with a change from CT to NT. The small differences between NT and CT could be also due to the high clay content of the soils (Ouédraogo *et al.*, 2005) that finally protects SOM from a quick degradation under a more intensive use.

Similarly to the SOC results, the N concentration (Table I) is significantly higher with W (1·25 g kg^{-1}, $n = 12$) than with WB (1·09 g kg^{-1}, $n = 12$) although the values varied within a relatively small range of 1·04–1·37 g kg^{-1}. The lower value of N under WB rotation can be explained either by a higher SOM stability under W (lower C/N ratio) or by a higher N fertilisation under W than WB.

The DL management led to the lowest mean values of C and N and the highest C/N ratio (Table I). This demonstrates

Table I. Cumulative C input (over a period of 19 years), annual SOC sequestration, SOC and N concentrations of the bulk soil and particle-size fractions as a function of the tillage and CS

Tillage and CS	Cumulative C input(Mg C ha^{-1})	Annual SOC sequestered (Mg C ha^{-1} y^{-1})	SOC bulk (g kg^{-1})	SOC (g C$_{org}$ kg^{-1}bulk soil)				SOC (g C$_{org}$ kg^{-1})			N (g kg^{-1})		N (g kg^{-1} bulk soil)	C/N (g kg^{-1} bulk soil)	
				75–250 μm	25–75 μm	<25 μm	>250 μm	75–250 μm	25–75 μm	<25 μm	bulk	<25 μm	<25 μm	bulk	<25 μm
WCT	7·2a[a]	0·15ab	20·8ab	2·3ab	2·5	14·0	2·0a	24·1ab	18·3b	19·9	1·37a	1·54a	1·09a	15·1c	12·9c
WBCT	6·4b	0·03c	18·6c	3·2a	2·6	12·2	0·6b	25·1a	22·0a	18·2	1·04b	1·32bc	0·88ab	18·0a	13·8c
WDL	6·4b	0·01c	18·3c	2·1b	2·3	13·5	0·4b	23·5b	17·7b	19·4	1·04b	1·37ab	0·95ab	17·9a	14·1c
WBDL	6·2c	0·01c	18·3c	2·4ab	3·3	13·7	0·0c	23·3b	19·1b	20·2	1·12b	1·18bc	0·80b	16·3b	17·2ab
WNT	7·2a	0·17a	21·2a	2·3ab	3·5	13·8	1·6a	24·0ab	19·1b	20·8	1·34a	1·29bc	0·86b	15·8bc	16·2b
WBNT	6·6b	0·08bc	19·6bc	1·8b	2·9	14·6	0·3b	23·3b	19·6ab	20·1	1·12b	1·12c	0·81b	17·6a	18·1a
W	6·9a	0·11a	20·1a	2·2a	2·8	13·8	1·3a	23·9a	18·3b	20·0	1·25a	1·40a	0·97a	16·3b	14·4b
WB	6·4b	0·04b	18·8b	2·4a	2·9	13·5	0·0b	23·9a	20·2a	19·5	1·09b	1·20b	0·83b	17·3a	16·4a
CT	6·8ab	0·09a	19·7a	2·8a	2·5	13·1	1·3a	24·6a	20·1a	19·1	1·20a	1·43a	0·98a	16·6a	13·4c
DL	6·3b	0·01b	18·3b	2·2ab	2·8	13·6	0·0b	23·4b	18·4a	19·8	1·08b	1·27b	0·88a	17·1a	15·7b
NT	6·9a	0·13a	20·4a	2·0a	3·2	14·2	1·0a	23·7ab	19·3a	20·4	1·23a	1·20b	0·84a	16·7a	17·1a

W, wheat; WB, wheat/faba bean rotation; CT, conventional tillage; DL, dual-layer tillage; NT, no tillage; WCT, wheat conventional tillage; WBCT, wheat/bean rotation conventional tillage; WDL, wheat dual-layer tillage; WBDL, wheat/faba bean rotation dual-layer tillage; WNT, wheat no tillage; WBNT, wheat/faba bean rotation no tillage.

[a] Values followed by a different lowercase letter within one column are significantly different ($p \leq 0{\cdot}05$) between cropping and tillage systems. Values followed by the same letter within a column are not significantly different at $p \leq 0{\cdot}05$ (LSD test). Values with no letters do not differ from each other. The statistics rare related to three different groups of tillage and CSs: WCT–WBNT; W–WB; CT–NT.

that this treatment has an unfavourable effect on organic matter accumulation in soils under such climatic and pedologic conditions. The DL management was generally less effective in weed control, since the seed bank of weeds is not transported in a deeper layer, as may occur under CT. In addition, non-inversion tillage is known to be associated with an increasing speed of development of resistance to weeds herbicides and cereal disease fungicides and in wet conditions having increased nitrous oxide emissions, thus reducing crop yield and residue production of the main crop. A lower wheat production, both as biomass and grain, with DL compared to CT and NT is common in rain-fed cropland having fine textured soils (Al-Issa and Samarah, 2006; Omonode *et al*., 2006). As a consequence of lower wheat yield and higher weed biomass, a different amount and type of organic matter is returned to the soil as crop residue. Soil carbon sequestration, through conversion to a restorative land use and adoption of recommended management practices, is more intense in cooler than it is warmer, and higher in wetter than it is in drier climates, and larger in clayey than sandy soils (Lal, 2009). Differences in the SOM content between W and WB can be explained by different annual root biomass inputs into the soil. The estimated cumulative C input (Table I) ranged from 6·2 Mg C ha^{-1} in WB/DL to 7·2 Mg C ha^{-1} in W/NT and W/CT. The WB CS had a lower input of C (6·4 Mg C ha^{-1} against 6·9 Mg C ha^{-1}). In this study we found an increasing annual SOC sequestration with increasing cumulative C input up to values of 7·38 Mg C ha^{-1} (Figure 1). This value can be considered as a steady-state level with crop cultivation at the present-day climatic and pedologic conditions at the investigated site. The annual SOC sequestered was 2·75 times higher with W than with WB (0·11 Mg C ha^{-1} y^{-1} vs. 0·04 Mg C ha^{-1} y^{-1}, $p < 0{\cdot}05$). Similar results were found by Sainju *et al*. (2007) who demonstrated that the inclusion of legumes in rotation with spring wheat did not influence the residue amount and SOC content. Carbon sequestration in agricultural soils can contribute to offsetting CO_2 anthropogenic emissions and also enhance soil fertility, soil water retention and crop production (Alvaro-Fuentes *et al*., 2009). The sequestration rates were comparable to those measured by Alvaro-Fuentes *et al*. (2009) in Mediterranean semiarid agroecosystems. Compared to results of other long-term agricultural experiments sites, Sicilian rain-fed CSs seem, however, to have relatively low C sequestration efficiency in relation to the C inputs (Miglierina *et al*., 2000; Bono *et al*., 2008; Alvaro-Fuentes *et al*., 2009; Simon *et al*., 2009). The low annual C sequestration was probably due to (and almost attained) low biomass production, high SOM mineralisation processes and the limited storage capacity of the soil. An asymptotic end-value (steady-state level) seems to be reached at about 7·4 Mg C ha^{-1} y^{-1}. According to Bell *et al*. (2003) and Alvaro-Fuentes *et al*. (2008), agricultural management that increases biomass input into soils and reduces tillage intensity leads to a higher accumulation of total C in the soil.

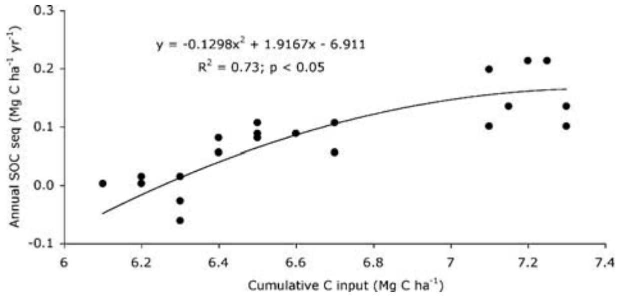

Figure 1. Relationship between annually sequestered SOC and cumulative C input (over the 19 years duration of the experiment) across the two different CSs and 3 different soil tillage management practices ($n = 24$).

Organic Matter in Particle-Size Fractions

Regarding the different soil aggregates, the <25 μm fraction was the most abundant one (mean 688 g kg^{-1}), followed by the 25–75 μm fraction (mean 147 g kg^{-1}) and then by the 75–250 μm fraction (98 g kg^{-1}) (Table II). Small differences were found for the 25–75 and 75–250 μm fractions between the treatments. Regarding the C contribution of the particle-size fractions (aggregate) to the bulk SOC, the following order was obtained: 0–25 > 25–75 μm > 75–250 > 250–2000 μm. The higher accumulation of SOC in the finest fraction was due to the higher mass of the silt-clay fraction in the soils while the sandy fractions in general account less for the total soil mass (Figure 2). However, taking the average SOC concentration of each fraction into account, then the 75–250 μm aggregate size showed an SOC enrichment compared to the bulk SOC concentration (23·9 g kg^{-1}), while the corresponding values of the 25–75 and <25 μm fractions did not statistically differ (19·3 and 19·8 g kg^{-1}, respectively) (Figure 3). The average SOC concentration of the fraction >250 μm was the lowest. The standard deviation was, however, by far the highest. This means very low up to very high concentrations were found in this fraction. Similarly to Gerzabek *et al*. (2001), we compared the SOC mass distributions among the size fractions and found that large microaggregates (75–250 μm) were enriched in SOC (C mass distribution/aggregate size distribution ratio = 1·20), whereas small microaggregates (25–75 μm) and the silt and clay fraction (<25 μm) showed no enrichment or rather a depletion (0·98 and 0·99). This observation partially disagrees with other authors who found that the silt fractions (2–63 μm) act as a medium-term sink for the introduced organic C (Gerzabek *et al*., 2001). Kong *et al*. (2005), however, reported a preferential stabilisation of SOM in the microaggregate fraction. The treatments only affected the SOC content of large microaggregates (Table I)

Table II. Particle-size fractions SIC and ^{13}C natural abundance in the soil fractions as a function of the tillage and CS

Tillage and CS	Aggregate size ($g\,kg^{-1}$)			SIC ($g\,kg^{-1}$)		$\delta^{13}C$			
	250–2000 µm	75–250 µm	25–75 µm	<25 µm	bulk	bulk	75–250 µm	25–75 µm	<25 µm
WCT	45[a]	97ab	137ab	707	23a	−25·59a	−26·17	−26·52	−25·45
WBCT	54	129a	119b	669	22a	−25·65ab	−26·36	−26·45	−25·47
WDL	47	89ab	128ab	694	38b	−25·66ab	−26·29	−26·09	−25·50
WBDL	49	100ab	169ab	674	30ab	−25·83b	−26·29	−26·05	−25·55
WNT	52	95ab	182a	664	38b	−25·85b	−26·25	−26·15	−25·45
WBNT	54	76b	148ab	723	29ab	−25·66ab	−26·27	−26·31	−25·58
W	48	94a	149a	688	35a	−25·70a	−26·24	−26·25	−25·47
WB	52	102a	145a	689	28b	−25·71a	−26·31	−26·27	−25·53
CT	49	113a	128a	688	23a	−25·62a	−26·26	−26·48	−25·46
DL	48	95a	148a	684	34b	−25·74a	−26·29	−26·07	−25·52
NT	53	85a	165a	694	33b	−25·75a	−26·26	−26·23	−25·52

W, wheat; WB, wheat/faba bean rotation; CT, conventional tillage; DL, dual-layer tillage; NT, no tillage; WCT, wheat conventional tillage; WBCT, wheat/bean rotation conventional tillage; WDL, wheat dual-layer tillage; WBDL wheat/faba bean rotation dual-layer tillage; WNT, wheat no tillage; WBNT, wheat/faba bean rotation no tillage.

[a] Values followed by a different lowercase letter within one column are significantly different ($p \leq 0{\cdot}05$) between cropping and tillage systems. Values followed by the same letter within a column are not significantly different at $p \leq 0{\cdot}05$ (LSD test). Values with no letters do not differ from each other. The statistics rare related to three different groups of tillage and CSs: WCT–WBNT; W–WB; CT–NT.

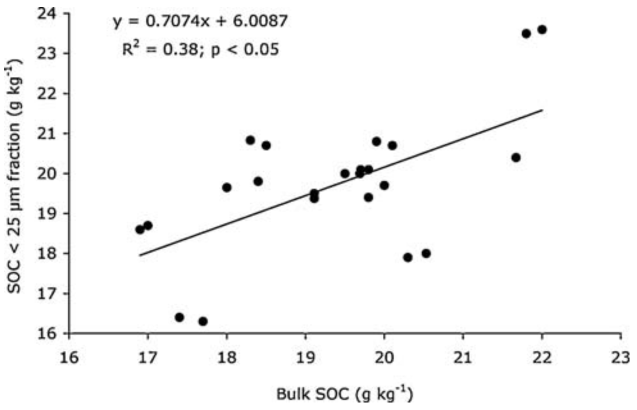

Figure 2. Relationship between the organic C concentration in the bulk soil and in <25 µm fraction of all samples.

and coarser fractions. In fact, crop residues incorporated into the soil were first stored in >75 µm fractions and were then transferred to the silt and clay fractions. The differences among the CSs W and WB were not significant. Tillage seemed, however, to influence the SOC concentration in the large macroaggregate (75–250 µm) fraction. Differences could be detected between CT and DL whereas NT did not differ from these. The contribution of large macroaggregates (>250 µm) to the total SOC is definitely influenced by the tillage and CSs. This is also reflected by the high standard deviation of the SOC concentration. With WB, the contribution of the macroaggregates to the total SOC was almost zero. Distinct differences can be seen between DL and CT or NT. In addition and similarly to the WB crop rotation, the DL tillage reduced the contribution of the macroaggregates to SOC to almost zero. CT and NT showed similar values of SOC in the macroaggregates.

^{13}C is a useful tracer for studying the decomposition and incorporation of organic material into more stable SOC (Andreux *et al.*, 1989; Zaccheo *et al.*, 1993; Desjardins *et al.*, 1994; Garcia-Olivia *et al.*, 1994; Piccolo *et al.*, 1994; Golchin *et al.*, 1995). Microbial and chemical transform-

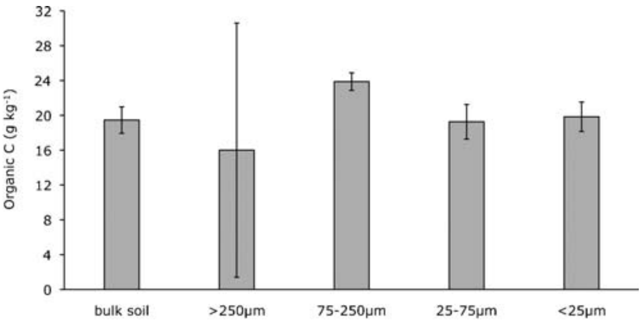

Figure 3. Average SOC concentrations (with standard deviation) in the different soil fractions.

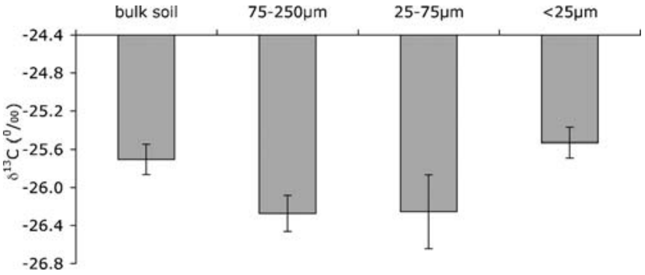

Figure 4. Average δ^{13}C values (with standard deviation) across the different soil fractions.

ation during humification enriches ^{13}C in silt and clay (Gregorich *et al*., 1994). δ^{13}C values significantly differed among the fractions. There was a trend of increasing δ^{13}C values with decreasing particle size. Since the majority of the organic C was present in the silt-clay fraction ($<25\ \mu$m), the δ^{13}C values of this fraction were close to the bulk soil and significantly differed ($p < 0{\cdot}05$) from the 25–75 and 75–250 μm fractions (Figure 4). Similar findings were also made in previous studies by Gerzabek *et al*. (2001), Baldesdent and Mariotti (1996) and Stemmer *et al*. (1999).

The mechanisms involved are not yet fully clear. An explanation might be that (i) ^{12}C is preferentially oxidised during microbial digestion of SOC (small organic particles that have been subjected to repeated microbial attack would be correspondingly more depleted in ^{12}C) or (ii) organic compounds such as lignin that are enriched in ^{13}C and are less available for microbial digestion (Roscoe *et al*., 2000). However, it is not possible to find unequivocal results in literature, since several authors also reported a depletion of ^{13}C in the more-stable fractions (Benner *et al*., 1987; Roscoe *et al*., 2000). These discrepancies were probably due to several factors such as soil type, mineralogy, the history of agricultural land use and agricultural management. We expected, furthermore, that the cropping and tillage systems would affect the δ^{13}C value due to either increased decomposition or SOC accumulation processes. This, however, was not the case. The δ^{13}C value is obviously not a parameter that is sensitive enough to trace back these small changes.

The higher resistance (stability) to mineralisation processes of silt-clay associated SOM was also due to the higher concentration of N in this fraction (Kleber *et al*., 2007) than in the bulk soil (average $N = 1{\cdot}30$ and $1{\cdot}17\ \mathrm{g\,kg^{-1}}$, respectively; $p < 0{\cdot}05$). Kleber *et al*. (2007), Kögel-Knabner *et al*. (2008) and others showed the importance of the mineral fraction in SOM protection. According to the model proposed by Kleber *et al*. (2007), the formation of particularly strong organo-mineral associations appears to be favoured in the so-called ‘contact zone’ by situations where proteinaceous materials unfold upon adsorption, thus increasing adhesive strength by adding hydrophobic interactions to electrostatic binding.

Significant differences were also found for the C/N ratio between the bulk soil and the $<25\ \mu$m fraction (16·8 vs. 15·5, $p = 0{\cdot}05$). Organic N compounds contribute to the stability of SOM as they can be strongly bound to mineral surfaces. Barbera *et al*. (2008) found that the resilient organic matter fraction that resisted H_2O_2 oxidation was enriched in aliphatic components and in N when compared to untreated samples. This is also in agreement with Leifeld and Kögel-Knabner (2001), Cuypers *et al*. (2002) and Eusterhues *et al*. (2005). Oxidation-resistant SOM is highly aliphatic and often enriched in N-containing compounds (Cheshire *et al*., 2000). Baldock *et al*. (2004) found that carbohydrates distinctly decreased during decomposition of organic matter. Proteins, lignins and a lesser extent lipids increased relatively and were more protected from decay in forest and agricultural soils. This protection from decay was explained by an immobilisation of proteins and by the greater biochemical recalcitrance of lipids and lignins (Marschner *et al*., 2008).

Soil Inorganic Carbon

Emission of C from soil is caused by mineralisation/oxidation of SOC pools as well as acidification and leaching of carbonates from the soil inorganic C pool (SIC) (Lal, 2002, 2004). The amount of inorganic C in the soils was also influenced by the treatments (Figure 5). The SIC content was higher with W than with the WB crop rotation. Conservation tillage led to a higher SIC content than the conventionally tilled variants (DL, CT). A reduction in tillage frequency and an increase in the cropping intensity increased the $CaCO_3$ content. The mechanism involved in this process is not fully clear. A possible explanation could be that the higher biomass input (root biomass) led to increased Ca and Mg inputs and consequently enhanced $CaCO_3$ precipitation or

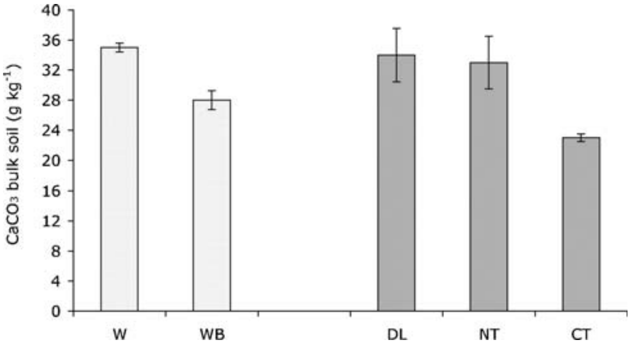

Figure 5. Average $CACO_3$ contents (with standard deviation) of the soil samples from the plots having different tillage management and CSs. W, durum wheat monocropping; WB, wheat–faba bean crop rotation; DL, dual-layers tillage; NT, no till; CT, conventional tillage.

hindered the leaching of $CaCO_3$ (see Sainju *et al*., 2007). Increased biological activities or an increased cropping intensity can influence the formation of $CaCO_3$ (Cerling, 1984; Monger, 2002). Furthermore, increased SIC can also be the result of the addition of soil treatments, such as fertilisers containing Ca, which could lead to an increased formation of $CaCO_3$ (Amundson and Lund, 1987; Mikhailova and Post, 2006). An increased SIC content, when alternative management practices are used, may also be an indication of increased C sequestration in the soil, since $CaCO_3$ at a depth of 0–20 cm may represent an important CO_2 sink in the environment (see Verrecchia *et al*., 2006).

CONCLUSIONS

The physical soil fractionation method used allowed the investigation of the long-term effects of different soil management techniques and crop rotation on soil structure and organic C storage.

Here are the major findings:

- After an experiment lasting 19 years, the differences with respect to soil C accumulation between the tillage and crop rotation systems were generally low. Nonetheless, these differences were in part significant.
- Sicilian rain-fed CSs seem to have a relatively low C sequestration efficiency in relation to the C inputs
- Wheat monocropping produced a higher cumulative C input over this period and a greater annual SOC sequestration: this resulted in a higher concentration of SOC in the bulk soil.
- Among the different tillage techniques, NT and CT seemed to improve the SOC content compared to the DL tillage. Conservative tillage coupled with wheat monocropping increased the C sequestration in the soil, also in its inorganic form.
- Surprisingly, the differences between NT and CT were small to none. NT does not improve the SOC content in soils in every case. The reasons for the lack of difference are not as yet fully clear. We hypothesise that the crop rotation *Triticum durum*/wheat is less susceptible to SOM degradation. Compared to NT, accumulation of SOC is similar to the NT system. Furthermore, the high clay content in the soils protects SOM from decay, also when CT is used. A change to NT has consequently no or only a small effect on SOM.
- Most of the SOC was stored in the silt-clay fraction. Furthermore, this fraction seemed to be more resistant against biodegradation, which was reflected in the $\delta^{13}C$ value and N content.
- Wheat residues (root biomass) seemed to be less mineralisable (decomposition to CO_2) and more stable against decomposition than faba/bean residues.
- Large microaggregates were enriched in C (C mass distribution/aggregate size distribution ratio = 1:20) and characterised by a lower $\delta^{13}C$. Organic residues seemed, consequently, to be transferred firstly into macroaggregates and then, with proceeding biodegradation, into smaller aggregates.
- The coarser fractions and consequently macroaggregates were more influenced by the treatments than the finest fraction. The DL tillage strongly reduced the SOC in macroaggregates while the differences between NT and CT were small. Thus, in Mediterranean areas with Vertisols, agricultural strategies aimed at reducing the impact on soils and increasing the SOC contents should probably consider increasing the proportion of coarser soil fractions so that, in the short-term, organic C can be accumulated. This suggestion, however, would need to be substantiated with additional field-experiments.

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