Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions

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INTRODUCTION

Soil aggregates are the arrangement of soil particles of different sizes joined by organic and inorganic materials (Amezketa, 1999) and their stability can be used as an index of soil structure (Bronick and Lal, 2005). Soil aggregates physically protect SOC from its degradation by soil microorganisms (Beare et al., 1994a; Tisdall and Oades, 1982) and it is evidenced by the flush of carbon dioxide observed upon soil aggregates disruption (Beare et al., 1994a).

Soil structure and SOC are extremely sensitive to crop and soil management (Blanco-Canqui and Lal, 2004). It is well established that NT adoption in a previously conventionally-tilled soil results in the physical stabilization of SOC within soil aggregates (e.g., Six et al., 1999; Álvaro-Fuentes et al., 2009). During the process of SOC stabilization within soil aggregates, plant roots and fungal-derived hyphae play an important role as initial binding agents (Jastrow, 1996). According to the conceptual scheme proposed by Six et al. (2000), macroaggregate turnover is greatly reduced under NT promoting the formation of C-enriched microaggregates within macroaggregates. Moreover, the SOC sequestered within these microaggregates remains protected from microbial attack resulting in longer residence time (Blanco-Canqui and Lal, 2004). Compared to macroaggregates, the biochemical structure of the SOC that stabilizes microaggregates tends to be highly processed and recalcitrant (Elliott, 1986). Also, soil biological activity under NT is increased (Madejon et al., 2009) promoting the production of organic binding by-products that stabilize soil aggregates.

When NT is maintained over time, soil aggregate stability is enhanced (Beare et al., 1994b) leading to the increase of total SOC (West and Post, 2002). In Florida, Ochoa et al., (2009) studied a NT chronosequence of 0, 6, 10 and 15 years under NT in commercial plots. They observed a relationship between the increase in surface soil water-stable macroaggregates and the hydrolysable organic carbon with longer years under NT. Thus, they concluded that continuous NT is beneficial for SOC buildup in soil macroaggregates.

In the Mediterranean semiarid agroecosystems, intensive tillage practices have led to the loss of soil structure and soil degradation (Álvaro-Fuentes et al., 2007). Recently, conservation tillage systems (e.g., reduced tillage or NT) have been increasingly adopted in these areas due to its agricultural and environmental benefits (Kassam et al., 2009). In these semiarid systems, several studies have investigated the impacts of adoption of continuous NT on soil aggregation and physical C stabilization (e.g., Álvaro-Fuentes et al., 2009; Plaza-Bonilla et al., 2010). Nevertheless, the vast majority of these studies have been based on time-point comparisons (Staley et al., 1988). As a result, there is a lack of information about the continuous maintenance of NT on soil aggregation and SOC protection. Consequently, the objective of this experiment was to study the temporal dynamics of soil aggregation and SOC protection after the conversion of CT to NT in a rainfed Mediterranean agroecosystem. In order to achieve this objective we established a NT chronosequence 20 years ago in a representative Mediterranean dryland agroecosystems located in northeast Spain. We hypothesized that the maintenance of NT results in greater SOC protection within C-enriched water-stable macroaggregates.

MATERIALS AND METHODS

Experimental site

A NT chronosequence experiment located in the semiarid Ebro river valley, NE Spain (41º48’ N, 1º07` E, 330 m), was established 20 years ago in a previously intensive-tilled field of 7500 m^2. Mean annual precipitation, mean air temperature and mean annual evapotranspiration in the area are 430 mm, 13.8 ºC and 855 mm, respectively. The soil was classified as Typic Xerofluvent (Soil Survey Staff, 1994), with the following properties in the Ap horizon (0-28 cm) at the start of the experiment: pH (H_2O, 1:2.5): 8.5; electrical conductivity (1:5): 0.15 dS m^{-1}; $CaCO_3$ eq. (%): 40; Water retention (kg kg^{-1}): 0.16 and 0.05 at -33 and -1500 kPa, respectively; sand (2000-50 µm), silt (50-2 µm) and clay (<2 µm) content: 475, 417 and 118 g kg^{-1}, respectively. The edaphoclimatic conditions of the experiment could be considered as representative of the most part of cropping systems located in the dryland Mediterranean areas. In 1990, 1999, 2006 and 2009 successive portions of 1500 m^2 of the intensive-tilled field (i.e., 7500 m^2) were transformed to NT. Thus, in 2010, a surface of 1500 m^2 remained under CT and 6000 m^2 under NT with different years: 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years. In all five chronosequence phases the cropping system consisted in winter cereals rotation. Fertilization was based on pig slurry homogeneously applied for the whole experimental area in a dose of 50 kg N ha^{-1} yr^{-1} depending of the slurry composition. The CT treatment consisted of one pass of a moldboard plough to 25 cm depth immediately followed by one or two passes with a cultivator to 15 cm, both in September. The NT treatments consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. Planting was performed with a direct drilling disk machine set to 2-4 cm in November. Prior to the set up of the experiment, the historical management of the field was based on conventional intensive tillage with moldboard ploughing and pig slurry additions, similar to the management applied to the CT phase of the chronosequence. Neither slope nor differences in soil characteristics in the whole experimental area were found; as a result the treatments were arranged in a randomized design. Three sampling locations within each chronosequence phase were used as pseudo-replicates.

Soil sampling and analyses

Soil sampling was performed in July 2010, right after crop harvest. Each phase of the chronosequence was divided in three areas. In each area, a composite sample was collected from three samples randomly selected. Soil samples were obtained using a flat spade in four soil layers from 0 to 30 cm depth (0-5, 5-10, 10-20 and 20-30 cm) and were stored in crush-resistant airtight containers. Once in the laboratory, soil was sieved with a 8 mm-sieve and air-dried at room temperature. For each sample, dry soil aggregate and water-stable aggregate distributions were obtained. Water-stable aggregate size separation was performed according to a modified wet sieving method adapted from (Elliott, 1986). The method is extensively described in a previous work (Plaza-Bonilla et al., 2010). Four water-stable aggregate fractions were obtained: (i) large macroaggregates (2 – 8 mm), (ii) small macroaggregates (0.250 – 2 mm), (iii) microaggregates (0.053 – 0.250 mm) and (iv) silt-plus clay-sized particles (< 0.053 mm). All water-stable aggregate fractions were oven-dried at 50 ºC (48 h) in aluminum trays and weighed. Sand content of the aggregate classes (> 0.053 mm) was determined dispersing 5 g of a subsample in a sodium hexametaphosphate solution (5 g L^{-1}) using a reciprocal shaker. Sand correction was performed in each aggregate-size class because sand was not considered part of those aggregates (Elliott et al., 1991). The dry aggregate size distribution was conducted placing 100 g of air-dried sub-sample (8 mm sieved) on an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain) with the same sieves used for the water-stable aggregate size distribution. A sieving time of 1 min and the lowest power program of the machine were used.

SOC concentrations from the bulk soil and from each water-stable aggregate size-class were determined using the wet oxidation of the Walkley-Black method described by Nelson and Sommers (1996). In some treatments the amount of large macroaggregates (2-8 mm) was not enough to determine SOC concentration. Consequently, large (2-8 mm) and small (0.250-2 mm) macroaggregates were mixed and SOC determined as macroaggregate-C. The method was modified to increase the digestion of SOC. The modification consisted in extensive heating of the sample during the digestion, boiling the sample and the extraction solution at 150 ºC for 30 minutes (Mebius, 1960).

In each chronosequence phase, the stratification ratio (SR) was calculated dividing the SOC concentration in the 0-5 cm soil depth by those in the 5-10 cm, 10-20 cm and 20-30 cm soil layers (Franzluebbers, 2002). A regression analysis was performed between the SR of SOC and the number of years under NT to assess the changes of this ratio over time.

The data were analyzed using the SAS statistical software (SAS institute, 1990). To compare the effects of tillage treatments and soil depths, analysis of variance (ANOVA) for a randomized design was performed using the procedure general linear model. When significant, differences among treatments and depths were identified at the 0.05 probability level of significance using Duncan’s test.

RESULTS

No-tillage maintenance effects on soil organic carbon concentration

In the 0-5 cm soil depth, total SOC concentration was significantly greater in the NT-11 and NT-20 phases compared with the NT-4, NT-1 and CT phases. Furthermore, SOC concentration in the NT-4 phase was significantly greater than in the NT-1 and CT phases. However, below 5 cm soil depth no significant differences were found among treatments (Table 1).

The stratification of SOC on soil surface increased with the time under NT (Table 1 and Fig. 1). In the 0-5 and 20-30 cm soil layers, SOC concentration values ranged between 8.6 and 10.5, 8.5 and 20.0, 7.1 and 24.0 and 6.5 and 24.0 g C kg^{-1} dry soil in the NT-1, NT-4, NT-11 and NT-20 phases, respectively (Table 1). In the NT-11 and NT-20 phases, SOC concentration among soil depths was significantly different in the next order: 0-5 > 5-10 > 10-20 and 20-30 cm depth. However, in the NT-4 phase differences were only found between 0-5 cm and the rest of the analyzed depths. Moreover, in the NT-1 phase, total SOC concentration did not show any stratification trend with depth (Table 1). The regressions between SOC stratification ratios and the number of years under NT showed significant logarithmic relationships (Fig. 1). The SR for 0-5:20-30 cm varied between 1.2 and 4.1 for the NT-1 and NT-20 treatments, respectively. When the number of years under NT increased, changes in the SR for the 0-5:5-10 and the 0-5:10-20 depths were minimal. However, for the 0-5:20-30 depth, the regression showed an increase in the SR over the 20-yr period (Fig. 1).

No-tillage maintenance effects on dry and water-stable aggregate-size classes

Differences between treatments on dry macroaggregates were only found in the 10-20 and 20-30 cm soil depths (Fig. 2). In the 10-20 cm depth, the NT-4, NT-11 and NT-20 chronosequence phases showed greater proportion of large dry-sieved macroaggregates when compared with the NT-1 and CT phases, but this fact was compensated with a lower proportion of small dry-sieved macroaggregates (Fig. 2). In the 20-30 cm depth, greater large dry-sieved macroaggregates were found when NT was maintained over time (Fig. 2). Interestingly, between depths, greater dry-sieved small macroaggregates content was found in the 10-20 and 20-30 cm than in the 0-5 and 5-10 cm soil depths in the CT and NT-1 phases (Fig. 2).

In the 0-5 cm soil depth, water-stable macroaggregates ranged between 0.01 and 0.32 g aggregate g^{-1} dry soil (Fig. 3). Differences in water-stable aggregates between treatments were only found in the 0-5 and 5-10 cm depths (Fig. 3). In the 0-5 cm soil depth, greater amount of large water-stable macroaggregates was found in the NT-11 and NT-20 phases compared with the other three phases (i.e., CT, NT-1 and NT-4). A similar trend was observed in the small macroaggregates, with greater amount in the NT-4, NT-11 and NT-20 phases compared with the NT-1 and CT phases. In the 0-5 cm soil depth, a significant decrease in the proportion of water-stable microaggregates was observed when increasing the number of years under NT with the greatest amount of water-stable microaggregates in the CT treatment (Fig. 3). In the 5-10 cm soil depth, significant differences in large water-stable macroaggregates were found between the NT-20 phase and the NT-1 and CT phases. Furthermore, for this soil depth, the proportion of water-stable microaggregates also significantly differed between the CT phase and the NT-11 and NT-20 phases.

No-tillage maintenance effects on C concentration in the water-stable aggregate fractions

In the 0-5 cm soil layer, no sand-corrected C concentration of the water-stable macroaggregates was similar among chronosequence phases with values ranging from 18.6 to 30.7 g kg^{-1} (Table 2). However, in the 10-20 and the 20-30 cm soil layers, significant differences were found between phases with the greatest C concentration in the CT and NT-1 phases. Likewise, no-sand corrected C concentration in microaggregates (0.053-0.250 mm) and silt-plus clay-sized particles (< 0.053 mm) was significantly greater in the NT-4, NT-11 and NT-20 phases compared with the CT and NT-1 phases in the 0-5 cm soil layer (Table 2). Differences in no-sand corrected macroaggregate-C between soil depths were found in all NT chronosequence phases. However, for the no-sand corrected microaggregate-C and the C associated to the silt-plus clay-sized particles, differences between soil depths were only found in some phases (i.e., the NT-4, NT-11 and NT-20 phases for microaggregates and the NT-11 and NT-20 phases for the silt-plus clay-sized particles). In both cases, the no-sand corrected C concentration decreased with increasing soil depths (Table 2).

In the 0-5, 10-20 and 20-30 cm soil layers, the sand-corrected C concentration of soil macroaggregates was significantly different between chronosequence phases with the greatest macroaggregate-C concentration in the CT phase (Table 3). On the contrary, in the 0-5 and 5-10 cm soil layers, greater sand-free C concentration of microaggregates, was observed in the NT-11 and NT-20 phases compared to the NT-4, NT-1 and CT phases (Table 3). Differences in sand-free C concentration between depths were found in the three aggregate-size classes and in some chronosequence phases (Table 3). For instance, for the macroaggregates differences between soil layers were found in the NT-1, NT-4, NT-11 and NT-20 phases. In general, the sand-free C concentration decreased with increasing soil depth (Table 3).

DISCUSSION

The maintenance of no-tillage (NT) over time increased total SOC concentration. However, differences between chronosequence phases were only observed in soil surface. Those differences were related to the proportion of water-stable macroaggregates in the 0-5 and 5-10 cm depths. Thus, for example, in the 0-5 cm depth the maintenance of NT during 4 and 11 years (i.e., NT-4 and NT-11 phases) promoted 6-fold and 17-fold increase of water-stable large macroaggregates, respectively, compared to the NT-1 phase.

After NT adoption, several authors have reported increases in the proportion of soil water-stable macroaggregates together with gains in SOC concentration (Álvaro-Fuentes et al., 2008; Beare et al., 1994a). In an experiment with contrasting tillage systems and different number of years since the implementation of NT, Plaza-Bonilla et al., (2010) found greater differences in SOC levels and water-stable macroaggregates between CT and NT treatments when NT was maintained longer time. Adoption of NT promotes soil microbial activity in soil surface (Madejon et al., 2009; Staley et al., 1988) leading to greater production of organic binding by-products when decomposing fresh organic inputs (Abiven et al., 2009; Golchin et al., 1995; Golchin et al., 1994). These organic by-products play an important role in macroaggregate formation and stability, according to the hierarchy concept proposed by Tisdall and Oades, (1982). However, in our experiment, despite water-stable macroaggregates in soil surface (0-10 cm) increased significantly with the number of years under NT, differences in aggregate-C were only found in the microaggregate fraction. Under NT, the reduction in soil disturbance leads to the protection of SOC within macroaggregates. In particular, C within macroaggregates is stabilized in the form of microaggregate-sized particulate organic matter, enhancing the formation of C-enriched microaggregates (Denef et al., 2001). In the same area of this study, Álvaro-Fuentes et al., (2009) found greater microaggregate-C within macroaggregates in NT when compared to CT in three tillage experiments. Six et al., (2000) stated that slower macroaggregate turnover and subsequent formation and liberation of C-enriched microaggregates occluded within macroaggregates could explain the greater SOC stocks usually found under NT. Our results corroborate this theory, with increasing proportions of stable macroaggregates and greater C concentration within microaggregates when the number of years under NT increased.

SOC stratification with depth increased with the number of years under NT. Franzluebbers, (2002) suggested that the stratification ratio (i.e. the proportion of SOC at the soil surface in relation to the SOC in deeper soil layers) could be a better indicator of soil quality than total SOC alone. In Mediterranean conditions, higher SR's under NT than under CT have been reported (López-Fando et al. 2007; Lopez-Garrido et al., 2011). In similar Central Spanish conditions, Hernanz et al. (2009) reported an increase in the SR over time in a NT system throughout a 20-year experiment. In our study, the SR in the 0-5:20-30 depths increased according to the years under NT with starting values of 1.2 in NT-1 up to 4.1 in the NT-20 phase. In a subtropical climate, Sa and Lal, (2009) also observed an increase in the stratification ratio of SOC when increasing the number of years under NT. Under NT crop residues are placed on the top of soil surface where their decomposition is reduced (Paustian et al., 1997). The stratification of SOC was closely related with the decrease with depth in the proportion of water-stable macroaggregates in the phases with more years under NT (i.e. NT-11, NT-20). Significant differences were found between depths in the sand-free C concentration in the macroaggregates of the NT-4, NT-11 and NT-20 cases. In the microaggregate fraction, differences in C concentration between soil depths were only found in the NT-

4, NT-11 and NT-20 phases. Similarly, in the silt-plus clay-sized fraction, these differences were only found in the NT-11 and NT-20 phases. According to the data obtained, it could be hypothesized that the stratification of C concentration under NT is dependant on the size of soil aggregates. Consequently, C concentration in the greatest fractions (i.e. macroaggregates) showed faster stratification compared with the finest fractions (i.e. silt-plus clay-sized particles). In soil surface in the CT and NT-1 phases, the differences between the proportions of the water-stable and the dry-sieved macroaggregates were greater than in the NT-11 and NT-20 phases. On the contrary, in deeper soil, differences in the proportions of dry-sieved and water-stable macroaggregates were significant for all the chronosequence phases. Thus, it could be assumed that in soil surface macroaggregates were more stable when the number of years under NT increased. The small amount of water-stable macroaggregates located at deeper soil layers (i.e. 10-20 and 20-30 cm) in the CT and NT-1 phases, could be an explanation to the absence of differences in SOC concentration between chronosequence phases.

Different results were obtained in C concentration of the different aggregate fractions when this C concentration within aggregates was not corrected for sand content. Similar trend was observed by Plaza-Bonilla et al., (2010) who hypothesized that the redistribution of sand particles and/or the erosion of the silt and clay particles under inversion CT could explain the greater C concentration in soil macroaggregates under CT or the NT phase with only one year since the implementation (i.e. NT-1) when corrected for sand content.

CONCLUSIONS

Our results show that the maintenance of NT over time enhanced SOC concentration in soil surface reaching its maximum value after 11 years. Both the proportion of water-stable macroaggregates and the C concentration of microaggregates in soil surface increased according to the increase of the years under NT. Thus, the greater proportion of water-stable macroaggregates and the greater C-concentration within microaggregates were the main mechanisms of SOC protection in the NT chronosequence. A significant logarithmic stratification with depth over the NT chronosequence was observed in SOC concentration, which was related with the stratification of water-stable macroaggregates in soil depth. In these Mediterranean semiarid agroecosystems, the increase in the proportion of stable macroaggregates and the enrichment of C concentration of microaggregates are the main mechanisms of SOC protection when NT is maintained over time.

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FIGURE CAPTIONS

Figure 1 Regression analysis between soil organic carbon (SOC) stratification ratio and the number of years under no-tillage (NT) for three ratio depths (0-5:5-10 cm; 0-5: 10-20 cm; 0-5:20-30 cm)

* Regression significant at $P<0.05$; **Regression significant at $P<0.01$.

Figure 2 Dry aggregate size distribution at the 0-5, 5-10, 10-20 and 20-30 cm soil depths in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years under NT.

Error bars represent standard errors. Within the same soil depth and aggregate fraction, different lowercase letters indicate significant differences between years under no-tillage at $P<0.05$. For the same year treatment and aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$

Figure 3 Water-stable aggregate size distribution at the 0-5, 5-10, 10-20 and 20-30 cm soil depths in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years under NT.

Error bars represent standard errors. Within the same soil depth and aggregate fraction, different lowercase letters indicate significant differences between years under no-tillage at $P<0.05$. For the same year treatment and aggregate fraction, different uppercase letters indicate significant differences between depths at $P<0.05$

Table 1 Total soil organic carbon (SOC) concentration in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth	SOC (g kg^{-1})				
(cm)	CT	NT-1	NT-4	NT-11	NT-20
0 – 5	11.9 (0.3)‡ cA*¶	10.5 (0.5) cAB	17.3 (1.7) bA	24.0 (1.2) aA	24.0 (0.6) aA
5 – 10	11.7 (0.4) AB	12.1 (2.1) A	13.0 (1.2) B	15.0 (3.5) B	14.1 (1.6) B
10 – 20	10.3 (1.9) AB	11.0 (1.1) AB	9.3 (0.9) B	8.8 (0.8) C	9.0 (1.3) C
20 – 30	9.9 (0.4) B	8.6 (1.7) B	8.5 (2.1) B	7.1 (0.9) C	6.5 (2.3) C

* Within each depth values are significantly different between chronosequence phases at P<0.05

¶ Within each chronosequence phase, different letters indicate significant differences between depths at P<0.05.

‡ Values in parenthesis are the standard errors of the mean.

Table 2 Linear relationship between soil organic carbon (SOC) concentration and the proportion of water-stable large macroaggregates (2 – 8 mm), small macroaggregates (0.250 – 2 mm), microaggregates (0.053 – 0.250 mm) and silt-plus clay-sized particles (< 0.053 mm) for the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Water-stable aggregate fraction (mm)	CT	NT-1	NT-4	NT-11	NT-20
2-8	0.33*	n.s.	0.67**	0.92***	0.83***
0.250-2	n.s.	n.s.	0.79***	0.91***	0.90***
0.050-0.250	0.46*	0.49*	n.s.	n.s.	-0.35*
<0.050	-0.55**	-0.49*	-0.75***	-0.90***	-0.96***

n.s.: no significant; *P<0.05; **P<0.01; *** P<0.001

Table 3 Soil organic carbon (SOC) concentration in different water-stable aggregate classes in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth (cm)	Water-stable Aggregate classes (mm)	SOC (g kg^{-1})				
		CT	NT-1	NT-4	NT-11	NT-20
0-5	0.250 – > 2	24.9 (1.4)‡ A¶	18.6 (5.3)	25.8 (9.3) A	29.5 (2.0) A	30.7 (1.7) A
	0.053 – 0.250	10.2 (1.4) b*	10.1 (2.3) b	16.4 (5.6) aA	18.1 (2.0) aA	19.5 (0.8) aA
	< 0.053	10.3 (2.3) b	8.8b (4.5)	19.5 (7.5) a	19.9 (2.7) aA	13.4 (1.7) abA
5-10	0.250 – > 2	23.5 (1.3) AB	22.1 (4.6)	19.5 (6.7) AB	22.7 (7.4) B	27.0 (9.5) A
	0.053 – 0.250	10.2 (0.9)	10.9 (2.7)	11.4 (2.8) AB	12.3 (2.2) B	13.1 (0.7) B
	< 0.053	10.0 (1.1)	9.3 (4.1)	11.9 (3.9)	11.8 (1.5) B	9.0 (3.3) B
10-20	0.250 – > 2	24.7 (2.2) aA	21.2 (6.6) ab	13.1 (6.7) bcB	10.4 (2.1) cC	14.7 (2.2) bcB
	0.053 – 0.250	10.7 (1.3)	10.1 (2.3)	8.2 (1.6) B	8.1 (1.7) C	8.8 (0.9) C
	< 0.053	6.0 (2.8)	8.3 (1.1)	9.7 (3.6)	8.9 (2.7) BC	7.4 (1.2) B
20-30	0.250 – > 2	18.6 (5.5) aB	13.8 (5.7) ab	8.8 (4.5) bB	6.8 (1.3) bC	10.1 (0.2) bBC
	0.053 – 0.250	9.9 (0.5)	9.2 (1.9)	7.5 (2.5) B	6.7 (0.6) C	6.9 (1.3) D
	< 0.053	7.9 (1.3)	6.5 (2.2)	9.9 (2.6)	7.7 (2.3) BC	7.5 (0.9) B

* For a given depth and water-stable aggregate fraction, different lowercase letters indicate significant differences between chronosequence phases at P<0.05

¶ For a given chronosequence phase and water-stable aggregate fraction, different uppercase letters indicate significant differences between depths at P<0.05.

‡ Values in parenthesis are the standard errors of the mean.

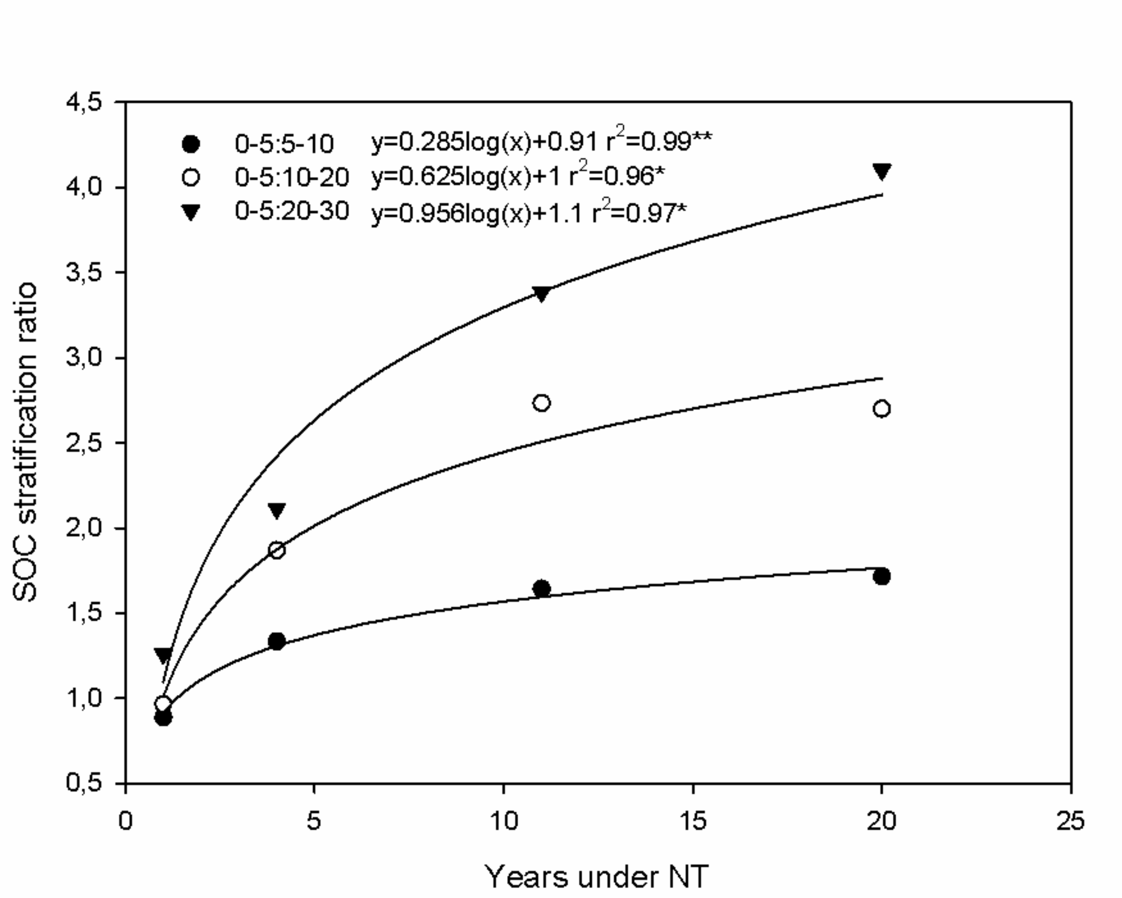
Table 4 Sand-free soil organic carbon (SOC sand-free) concentration in different water-stable aggregate classes in the 0-30 cm soil depth in a no-tillage (NT) chronosequence with the following phases: conventional tillage (CT) and NT under 1 (NT-1), 4 (NT-4), 11 (NT-11) and 20 (NT-20) years.

Soil depth (cm)	Water-stable Aggregate classes (mm)	SOC (g kg^{-1}) sand-free				
		CT	NT-1	NT-4	NT-11	NT-20
0-5	0.250 – > 2	64.3 (7.3)‡ a*	54.8 (6.8) abA¶	46.0 (8.5) bA	49.5 (4.0) bA	50.9 (3.9) bA
	0.053 – 0.250	16.2 (2.2) c	18.3 (0.4) c	26.2 (4.8) bA	33.9 (4.5) aA	36.7 (2.6) aA
	< 0.053	10.3 (2.3) b	8.8 (4.5) b	19.5 (7.5) a	19.9 (2.7) aA	13.4 (1.7) abA
5-10	0.250 – > 2	62.9 (10.2)	52.7 (9.4) A	37.9 (5.6) AB	42.9 (11.0) A	49.2 (14.7) A
	0.053 – 0.250	17.7 (2.1) b	19.3 (1.8) b	19.2 (2.2) bB	24.7 (4.9) abB	28.0 (5.5) aB
	< 0.053	10.0 (1.1)	9.3 (4.1)	11.9 (3.9)	11.8 (1.5) B	9.0 (3.3) B
10-20	0.250 – > 2	63.5 (10.5) a	49.6 (5.7) bAB	33.7 (9.5) cAB	29.6 (0.7) cB	36.7 (6.1) bcAB
	0.053 – 0.250	19.0 (1.7)	18.1 (2.5)	14.0 (1.8) C	17.6 (3.4) C	16.0 (1.0) C
	< 0.053	6.0 (2.8)	8.3 (1.1)	9.7 (3.6)	8.9 (2.7) BC	7.4 (1.2) B
20-30	0.250 – > 2	51.2 (16.9) a	37 (9.1) abB	26.3 (6.3) bB	23.5 (4.7) bB	26.5 (3.8) bB
	0.053 – 0.250	17.6 (0.5) a	17.7 (1.9) a	12.3 (2.4) bC	12.3 (0.6) bC	13.9 (3.9) abC
	< 0.053	7.9 (1.3)	6.5 (2.2)	9.9 (2.6)	7.7 (2.3) BC	7.5 (0.9) B

* For a given depth and water-stable aggregate fraction, different lowercase letters indicate significant differences between chronosequence phases at P<0.05

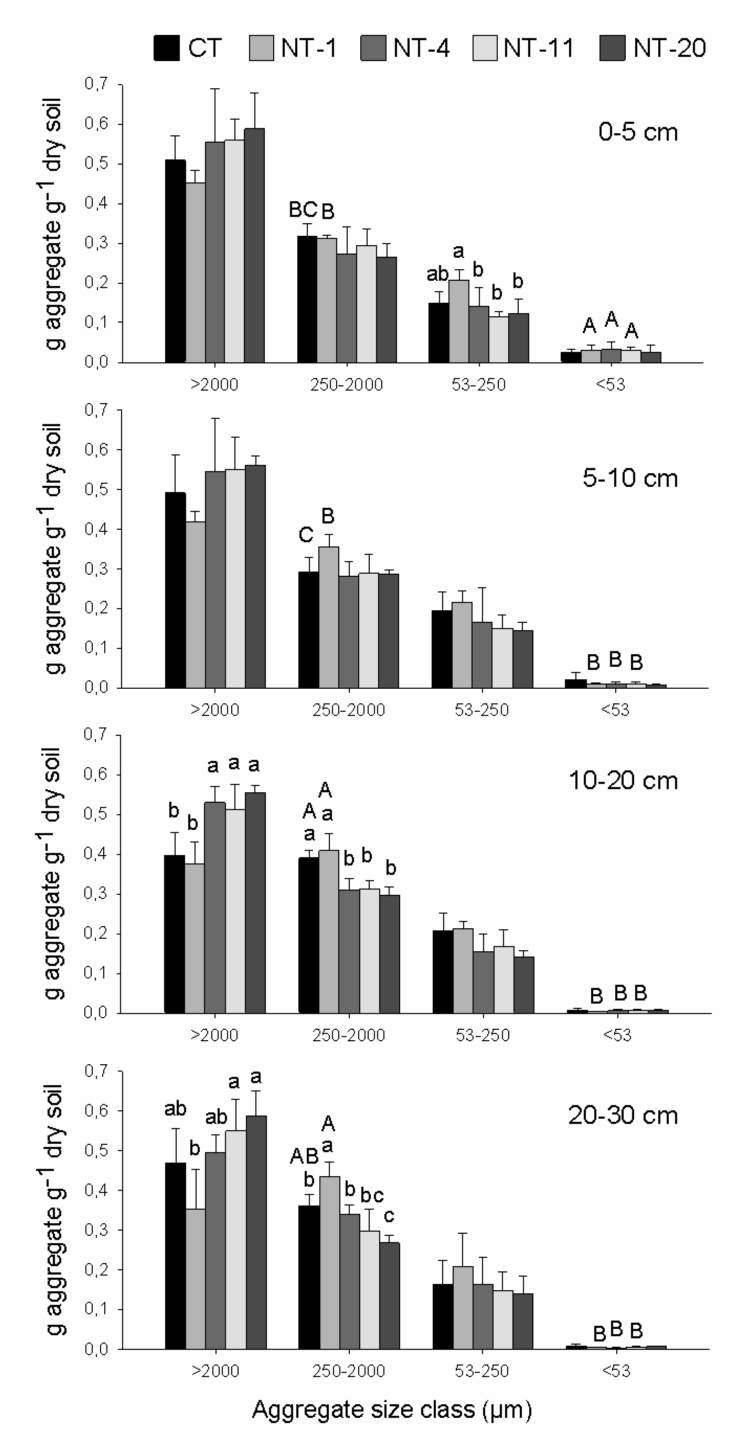
¶ For a given chronosequence phase and water-stable aggregate fraction, different uppercase letters indicate significant differences between depths at P<0.05.

‡ Values in parenthesis are the standard errors of the mean



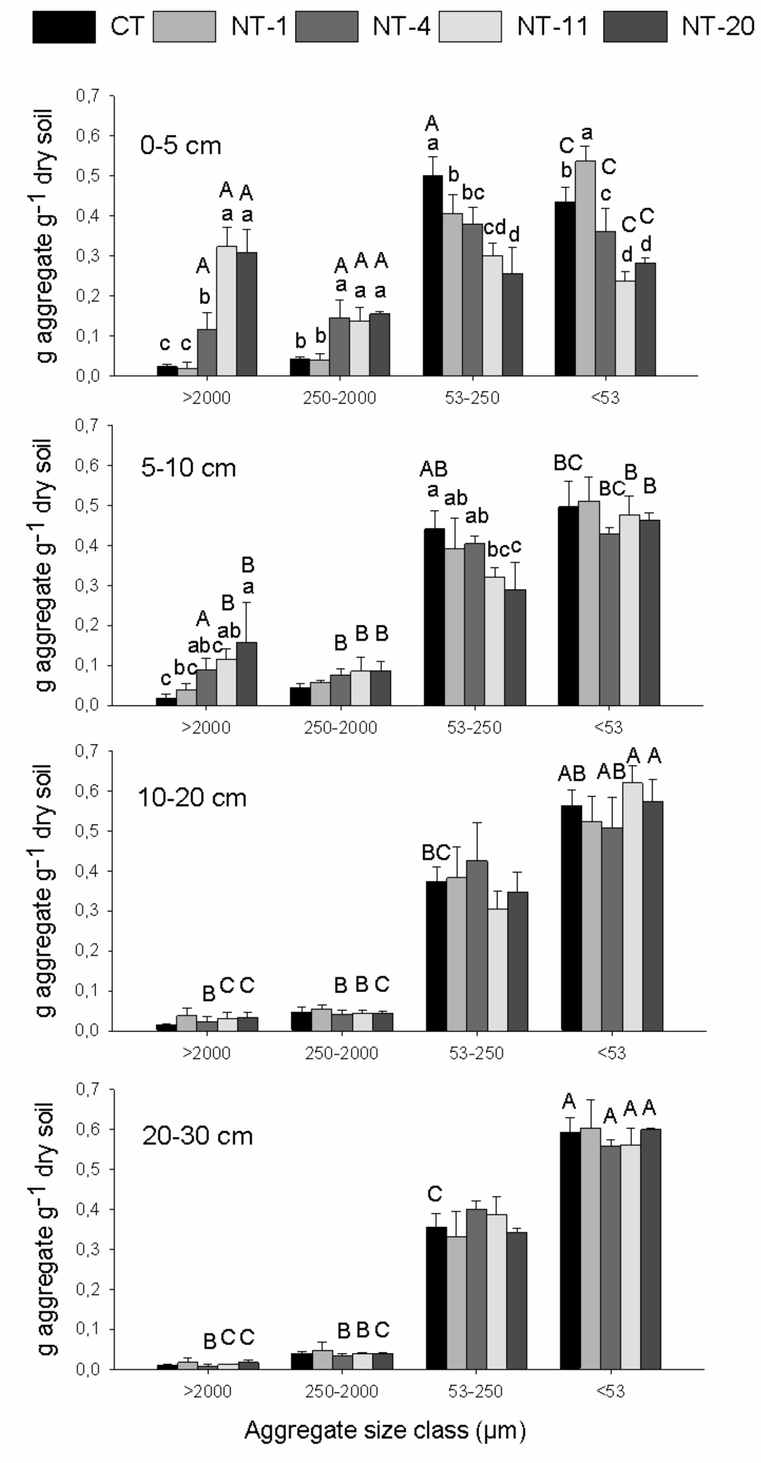
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0
5
10
15
20
25
SOC stratification ratio
Years under NT
0-5:5-10 y=0.285log(x)+0.91 r2=0.99**
0-5:10-20 y=0.625log(x)+1 r2=0.96*
0-5:20-30 y=0.956log(x)+1.1 r2=0.97*

Figure 1



CT
NT-1
NT-4
NT-11
NT-20
g aggregate g-1 dry soil
0-5 cm
5-10 cm
10-20 cm
20-30 cm
>2000
250-2000
53-250
<53
Aggregate size class (µm)

Figure 2



CT
NT-1
NT-4
NT-11
NT-20
0-5 cm
5-10 cm
10-20 cm
20-30 cm
g aggregate g-1 dry soil
>2000
250-2000
53-250
<53
Aggregate size class (µm)

Figure 3