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Soil Aggregation and Soil Organic Carbon Stabilization: Effects of Management in Semiarid Mediterranean Agroecosystems

Álvaro-Fuentes, J.[1*], Cantero-Martínez, C.[2], López, M.V.[1], Paustian, K.[3,4], Denef, K.[5], Stewart, C.E.[6], Arrúe, J.L.[1]

[1]Departamento de Suelo y Agua. Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (CSIC), POB 202, 50080-Zaragoza, Spain. [2]Departament de Producció Vegetal i Ciencia Forestal, Universitat de Lleida–IRTA, Rovira Roure 177, 25198 Lleida, Spain. [3]Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA. [4]Department of Soil and Crop Science, Colorado State University, Fort Collins, CO 80523, USA [5]Laboratory of Applied Physical Chemistry, Ghent University, Ghent, Belgium. [6]Department of Geological Sciences, University of Colorado, Boulder, CO 80309 USA

* Corresponding author. Present address: Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA.

E-mail address: jalvaro.fuentes@gmail.com

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ABSTRACT

In semiarid agroecosystems of the Ebro valley (NE Spain) soils are characterized by low soil organic matter (SOM) and a weak structure. In this study we investigated the individual and combined effect of tillage system (no-tillage, NT; reduced tillage, RT; conventional tillage, CT) and cropping system (barley-fallow rotation, PN-BF and continuous barley, PN-BB) on soil organic carbon (SOC) storage as well as the physical protection of SOM fractions by soil aggregates in three long-term experimental sites. In both cropping systems, total SOC content was more than 30% higher in NT compared with CT in the 0- to 5-cm depth. The suppression of fallowing in the PN-BB cropping system led to a greater SOC stabilization only in NT. In all the three sites, greater proportion of water-stable macroaggregates (> 250 µm) was found under NT than under CT in the 0- to 5-cm depth. Macroaggregate organic C concentration (250-2000 µm) was greater in NT compared with CT in the BB cropping system, but did not differ with tillage treatment in the PN-BF rotation. Greater proportion of microaggregates within macroaggregates in NT compared with CT was only found in AG. However, greater C stabilized inside these microaggregates was observed in AG, SV and PN-BB in the 0-5 cm depth. The results of this study demonstrate that in the semiarid Mediterranean agroecosystems of the Ebro valley, the adoption of NT together with the suppression of long-fallowing period can significantly increase the amount of SOC stabilized in the soil surface and improve soil structure and aggregation.

Abbreviations: AG, Agramunt site; CT, conventional tillage; IC, inorganic carbon; iPOM, particulate organic matter occurring within aggregates; LF, light fraction; mSOC, mineral associated soil organic carbon; NT, no-tillage; PN, Peñaflor site; PN-BB continuous barley at Peñaflor site; PN-BF, barley-fallow rotation at Peñaflor site; POM, particulate organic matter; RT, reduced tillage; SOC, soil organic carbon; SOM, soil organic matter; SV, Selvanera site; Total mM-C, total microaggregate-associated carbon.

The SOM plays an important role in a wide range of soil properties and processes such as soil structure (Oades 1993), water relations (Lado et al. 2004) and cation exchange capacity (Smettem et al. 1992). Improving SOM levels helps to maintain nutrient availability and agricultural sustainability as well as to improve carbon sequestration in order to mitigate CO_2 emissions to the atmosphere. Recently, Freibauer et al. (2004) reviewed numerous strategies for increasing soil carbon stocks, identifying the environmental side effects and impacts on farm income for each strategy. Two of these practices were the intensification of cropping systems and the reduction of tillage intensity.

Intensification of cropping systems increases the amount of carbon returned to the soil through a reduction of the bare fallow duration. In the semiarid Great Plains of North America, Halvorson et al. (2002) reported more than 27% greater annual carbon return to the soil from a continuous wheat system as compared with a fallow-wheat rotation. Similar studies have concluded that a reduction of the fallow period is associated with greater residue production and, therefore, with an increase in SOC content (Campbell et al. 1995; Potter et al. 1997; McConkey et al. 2003).

Tillage, in particular mouldboard ploughing, contributes to the mixing of fresh crop residues with soil thus modifying soil profile characteristics (e.g., aeration, moisture and temperature regimes) and promoting soil microbial activity (Reicosky et al. 1995; Paustian et al. 1998). Also, tillage continually exposes soil to wetting/drying and freeze/thaw cycles at the surface, making aggregates more susceptible to break down (Six et al. 1998). Upon aggregate disruption, aggregate-occluded SOM is released and becomes more available for decomposition (Paustian et al. 1997).

Agricultural soils in the Mediterranean region of Spain are characterized by low SOM levels due to the limited rainfall, resulting in low crop production (Cantero-Martínez et al. 2003). Traditional soil management practices in these agricultural areas are long-fallowing (to build up stored water) and conventional tillage with mouldboard ploughing and deep subsoiling as the main tillage practices. Few studies have been carried out to determine the effects of different management practices on SOC content in semiarid Spain (Hernanz et al., 2002; Moreno et al., 2006; Bescansa et al., 2007). In semiarid Ebro valley, Álvaro-Fuentes et al. (2008) found greater total SOC in NT compared with CT but only in the soil surface. However, in this previous study, no attempt was made to study the physical mechanisms that control SOC stabilization in surface soil as affected by management practices.

Consequently, the overall objective of the present work was to determine the effects of different tillage and cropping systems on soil C stabilization by soil aggregates in Mediterranean semiarid conditions. We hypothesized that: (i) macroaggregation levels and microaggregate formation within macroaggregates are higher under NT than under CT and under the continuous barley system than under the barley-fallow rotation and (ii) the SOC occluded within microaggregates is responsible for the greater SOC sequestration under NT compared with CT. To test these hypotheses two experiments were performed during two consecutive years. In the first experiment (Experiment 1), soil C fractions were isolated from size-class aggregates in different tillage and cropping systems. The second experiment (Experiment 2) was set up to investigate the role of microaggregates occluded within macroaggregates in the long-term SOC sequestration in Mediterranean semiarid conditions.

MATERIALS AND METHODS

Site description

Soils were collected in July 2003 and July 2004 from three long-term tillage experiments located in northeast Spain (Ebro valley). These sites span a range from higher to lower annual precipitation: Selvanera (Lleida Province, latitude 41º 50’N; longitude 1º 17’E; altitude 475 m), Agramunt (Lleida province, latitude 41º 48’N; longitude 1º 07’E; altitude 330 m) and Peñaflor (Zaragoza province, latitude 41º 44’N; longitude 0º 46’W; altitude 270 m). Site and soil characteristics are presented in Table 1.

The experiment at Selvanera (SV) was established at 1987. This experiment consisted of a wheat (*Triticum aestivum* L.)-barley (*Hordeum vulgare* L.)-wheat-rapeseed (*Brassica napus* L.) rotation with two tillage treatments: conventional tillage (CT) consisting of subsoiler tilling at 50 cm in August followed by a pass with a field cultivator to a depth of 15 cm in October before sowing, and no-tillage (NT).

The experiment in Agramunt (AG) was established at 1990. This experiment consisted of a barley-wheat rotation with two tillage treatments: a CT treatment consisting of moldboard plowing to a depth of 25-30 cm depth in October followed by a pass with a field cultivator to a depth of 15 cm, and NT.

The experiment in Peñaflor (PN) was established in 1989. This experiment consisted of two cropping system: a continuous barley system (PN-BB) and a barley-fallow rotation (PN-BF), with three tillage systems compared in each cropping system: CT, reduced tillage (RT) and NT. The CT treatment consisted of one pass with a moldboard plow to a depth of 30 to 35 cm plus a pass with a tractor-mounted scrubber consisting of a metal beam passed over the soil surface in order to break down large clods. The RT plots were chisel plowed to a depth of 25 to 30 cm. In the CT and RT plots of the PN-BB system, primary tillage was implemented every year in October followed by a pass of a sweep cultivator to a depth of 10-15 cm as secondary tillage. However, in the PN-BF rotation, primary tillage was implemented in March every two seasons, during the fallow phase of the rotation. At the three experimental sites, in the NT treatment no tillage operations were done and for sowing a direct drill planter was used. In this treatment, the soil was kept free of weeds with herbicide (glyphosate). Prior to the establishment of these three long-term experiments, fields had been under CT for several decades.

At SV and AG, tillage treatments were arranged in a randomized complete block design with three replicates in SV and with four replicates in AG. The size of each plot was 7x50 m at SV and 9x50 m at AG. At PN, tillage and cropping systems were arranged in a split block design with three replications and a subplot size of 10x33.

Soil sampling and aggregate separation

Experiment 1

In July 2003, immediately after harvest, soil samples were collected at three depths (0-5, 5-10 and 10-20 cm) in the CT, RT and NT treatments of both PN-BF and PN-BB cropping systems. From each subplot and depth, a composite soil sample for aggregation analyses was prepared from two samples taken at two points 15 m apart with a flat spade and placed in crush-resistant, air-tight containers in order to avoid aggregate break down during sample transportation. Once in the laboratory, field-moist soil was passed through a 8-mm sieve. The sieved soil was air dried and stored at room temperature. A soil subsample was taken from 0-5, 5-10 and 10-20 cm soil depths and analyzed for total SOC concentration. At the same time, four undisturbed soil cores (height 51 mm, diameter 50 mm, volume 100 cm^3) were taken per plot and soil depth for soil dry bulk density determination.

Aggregate size separation was performed by a wet sieving method adapted from Elliot (1986). Briefly, 100-g air-dried (8 mm sieved) soil sample was placed on the top of a 2000-µm sieve and submerged for 5 min in deionized water at room temperature. Sieving was manually done by moving the sieve up and down 3 cm, 50 times in 2 min in order to achieve aggregate separation. A series of three sieves (2000, 250 and 53 µm) was used to obtain four aggregate fractions: i) >2000-µm (large macroaggregates), ii) 250 to 2000-µm (small macroaggregates), iii) 53 to 250-µm (microaggregates), and iv) <53-µm (silt- plus clay-size particles). Aggregate fractions were oven dried (50 ºC), weighed and stored in glass jars at room temperature (21 ºC). Sand correction was performed in each aggregate size class because sand was not considered part of those aggregate (Elliot et al. 1991). Sand-corrected aggregate size classes were expressed as:

Sand-corrected aggregate size class (% w/w) =

$$\left(\frac{\text{size}-\text{class weight} \;-\; \text{sand weight of same size}-\text{class}}{\sum \text{sand}-\text{corrected weights}}\right)100 \qquad [1]$$

Separation of free light fraction, LF, (POM, occurring between aggregates) and iPOM (POM occurring within aggregates) was performed according to Six et al. (1998). Briefly, free LF was isolated by density flotation by placing aggregate fractions in 35 mL of 1.85 g cm^{-3} sodium polytungstate. The liquid was gently stirred to avoid breaking up the aggregates and the floating material (free LF) was aspirated and filtered using a 20-µm nylon filter and the heavy fraction (iPOM + sand) was dispersed in 5 g L^{-1} sodium hexametaphosphate. After shaking for 18 h, the dispersed heavy fraction was passed through 2000-, 250-, and 53-µm sieves, depending on the aggregate size being analysed.

Experiment 2

In July 2004, immediately after harvest, soil samples were collected at three depths (0-5, 5-10 and 10-20 cm) from the CT and NT treatments at the SV, AG and PN-BB sites. Similar soil sampling procedures, soil bulk density determination and aggregate separations were followed as in the Experiment 1.

Microaggregates contained within stable macroaggregates (>2000 and 250- to 2000-µm) were mechanically isolated according to the methodology described by Six et al. (2000) and Denef et al. (2004). Briefly, a 10-g macroaggregate subsample was immersed in deionized water on top of a 250-µm mesh screen inside a cylinder. Macroaggregates were shaken together with 50 glass beads (4-mm diameter) until complete macroaggregate disruption was observed. Once the macroaggregates were broken up, microaggregates and other <250-µm material passed through the mesh screen with the help of a continuous water flow until a 53-µm sieve. The material retained on the 53-µm sieve was wet sieved to ensure that the isolated microaggregates were water-stable (Six et al. 2000).

Carbon analyses

Total SOC was measured according to the wet oxidation method of Walkley and Black (Nelson and Sommers 1982). Possible differences in soil C determinations between the different methods used in this experiment were tested. Data obtained by López (personal communication, 2008) in the same experimental plots showed similar SOC concentrations between the wet oxidation and the dry combustion methods. In the Experiment 1, total C content of each isolated SOM fractions was measured by dry combustion, on a LECO CHN-1000 analyzer (Leco Corp., St. Joseph, MI). During the dry combustion procedure organic C is oxidized to CO_2 and carbonates are decomposed. Because of the presence of carbonates in the soil samples used in this experiment, inorganic carbon (IC) content of each fraction was determined in order to calculate organic carbon as:

Organic carbon = Total carbon (from dry combustion) - Inorganic carbon [2]

The IC content was measured by the modified pressure-calcimeter method (Sherrod et al. 2002). The C concentrations for free LF from the Experiment 1 and the total microaggregate-associated C (total mM-C) from the Experiment 2 were determined on a Carlo Erba NA 1500 CN analyzer (Carlo Erba, Milan, Italy) due to the smaller sample sizes.

The mineral associated soil organic C (mSOC) concentration from the Experiment 1 was determined by difference between total aggregate C and particulate organic matter C (free LF and iPOM-C):

mSOC = total aggregate C – (free LF-C + iPOM-C) [3]

The C concentration of each SOM fraction was expressed on a sand-free basis due to the fact that sand particles do not specially contribute to the dynamics of soil organic matter (Elliot et al. 1991). Sand-free C concentrations (g kg^{-1} sand-free macroaggregates) were calculated as follows:

$$\text{Sand} - \text{free}\ C_{\text{fraction}} = \frac{C_{\text{fraction}}}{\left[1 - (\text{sand proportion})_{\text{fraction}}\right]} \quad [4]$$

Data were analyzed using the SAS statistical package (SAS Institute 1990). In Experiment 1, the effects of tillage, cropping systems and each interaction were tested with variance analyses (ANOVA). Interactions were tested with the PDIFF option of the LSMEANS statement. In Experiment 2, ANOVA analyses for each site were also made and differences between tillage treatments were tested with Duncan's multiple range test.

RESULTS

Total soil organic carbon content

In three sites and in both cropping systems at PN (PN-BB and PN-BF), total SOC content was significantly greater under NT than CT in the 0- to 5-cm depth. In the PN-BB system greater SOC in NT compared with CT was also observed in the 5- to 10-cm layer. However, in the PN-BF rotation and at SV and AG no significant differences between tillage systems were observed in the 5- to 10-cm layer (Table 2). Similar SOC contents were observed between tillage systems in the 10- to 20-cm depth. In the 0- to 20-cm interval, differences between tillage systems were only found in the PN-BB system with greatest SOC under NT (Table 2). Differences between cropping systems were only observed in the NT treatment for the 0- to 5-cm layer where greater SOC was found in the PN-BB system compared with the PN-BF rotation (Table 2).

In the 0-20 cm depth, in an equivalent mass basis although differences between tillage systems decreased, greater SOC was measured under NT than under CT in the PN-BB, PN-BF and AG. At SV, SOC kept similar between NT and CT (Table 2).

Aggregate size distribution

Water-stable aggregate size distributions in the PN-BB and PN-BF systems presented a similar trend among cropping systems and tillage systems. Microaggregates (53-250 µm) accounted for more than 50% of the total soil and were the predominant water-stable size class in both cropping systems and tillage treatments (Fig. 1). The silt and clay fraction (<53 µm) were similar among tillage treatments and soil depth (Fig. 1). The proportion of microaggregates was lower in NT compared with CT and RT in the 0-5 cm depth in both cropping systems and in the 5-10 cm in the PN-BB system (Fig. 1).

Both large and small macroaggregates (>2000 µm and 250-2000 µm, respectively) accounted for the lowest proportion of aggregates with less than 40% of the total dry soil mass (Fig. 1 and Table 3). However, in all the four sites studied greater proportion of large and small macroaggregates were found in NT compared with CT in the 0-5 cm depth except for the small macroaggregates fraction at SV (Fig. 1 and Table 3). In the 5-10 cm depth, small macroaggregates were greater in NT compared with CT at PN-BB and AG (Fig.1 and Table 3). At SV, greater large macroaggregates were found in NT compared with CT in all the soil depths (Table 3). The amount of large macroaggregates at the CT treatment of AG and PN-BB was small and negligible.

Differences in aggregate size-distribution between cropping systems were only found in soil surface (0-5 cm) and in NT. Greater proportion of large macroaggregates and silt and clay fraction and lower proportion of microaggregates was observed in the PN-BB system compared with the PN-BF rotation (Fig. 1).

Aggregate-associated carbon concentrations

In the PN-BB system, total aggregate C concentration of the small macroaggregates differed in the order NT > RT > CT for the 0-5 cm depth (Fig. 2). In the same cropping system, microaggregate C concentration under NT was greater than under CT and RT in the 0-5 cm depth. However, below 5 cm depth no differences in total aggregate C concentrations were observed in this cropping system (Fig. 2). Not enough stable large macroaggregates (>2000 µm) were obtained from the wet sieving procedure at PN-BB and PN-BF for total aggregate C determination and subsequent SOM fractionation (Fig. 2).

In the PN-BF rotation, similar total aggregate C concentration among tillage treatments was observed in all the soil layers except in the 0-5 depth where greater microaggregate C concentration was observed in NT and CT compared with RT (Fig. 2).

Differences between cropping system were found in the 0-5 and 5-10 cm depths in the NT treatment where greater total macroaggregate and microaggregate C concentrations were observed in the PN-BB system compared with the PN-BF rotation (Fig. 2).

In the 0-5 cm depth, neither coarse iPOM C (250-2000 µm) from small macroaggregates (250c) nor microaggregate iPOM C (53f) were affected by tillage in either cropping systems (Fig. 3). However, in both cropping systems, the fine iPOM C (53-250 µm) concentration of the small macroaggregates (250f) decreased among tillage treatments in the following order: NT > RT > CT (Fig. 3).

In the 5-10 cm and 10-20 cm depths, no differences were observed among tillage treatments in any iPOM C size fraction and in any cropping systems (Fig. 3).

Differences between cropping systems were found in the soil surface (0-5 cm) in the NT treatment where greater coarse and small iPOM C concentrations of the small macroaggregates were observed in the PN-BB system compared with the PN-BF rotation (Fig. 3).

In the PN-BB system, greater mineral-associated carbon (mSOC) was observed in NT compared with RT in the microaggregates in the 0-5 cm depth (Fig. 4). Below 5 cm depth, no differences in mSOC were observed in the PN-BB system. The exception was observed in the 10-20 cm depth where greater mSOC was measured under NT than under RT (Fig. 4). In the PN-BF rotation, no differences in mSOC among tillage treatments were observed in any soil depth. No differences were observed in mSOC between cropping systems in any soil depth (Fig. 4).

Results of the free light fraction (LF) were not shown due to the low and similar values among tillage treatments and cropping systems with values ranging from 0.05 to 0.24 g C kg^{-1} sand-free aggregate.

Tillage x cropping system

Interactions between tillage treatments and cropping systems (PN-BB vs. PN-BF) are shown in Tables 4 and 5. Tillage and cropping system interaction was only observed in the soil surface (0-5 cm depth) and in total SOC, aggregate stability and total aggregate C concentration. The aggregate stability of the >2000, 53-250 and <53 μm fractions showed interaction between tillage and cropping system and also the total C concentration of the small macroaggregates (Tables 4 and 5).

Proportion of microaggregates within macroaggregates and microaggregate-C

Not enough large macroaggregates were obtained from the CT treatment at AG and PN-BB for the microaggregate isolation procedure. At AG greater proportion of microaggregates within macroaggregates was observed in NT compared with CT for the 0-5 cm layer (Fig. 5). However, for the same soil depth, at SV and PN-BB similar proportion of microaggregates was observed between tillage treatments. In deeper soil layers similar proportion of microaggregates within macroaggregates between tillage systems was observed in all the sites.

In all the three sites, greater total microaggregate C isolated from small macroaggregates (total mM-C) was observed in NT compared with CT for the 0-5 cm depth. However, similar total mM-C was observed between NT and CT for both 5-10 cm and 10-20 cm depths (Table 6).

DISCUSSION

At all the sites and cropping systems, greater SOC content was observed in NT compared with CT in the soil surface. In NT systems, crop residues are left on the soil surface implying a much slower crop residue incorporation and decomposition and a lower soil susceptibility to physical disruptive forces (e.g. drying/wetting, freeze/thaw) due to the presence of a mulch layer (Paustian et al. 1997). This slower decomposition of crop residues under NT than under CT led to the accumulation of SOC in the upper soil layers as observed in similar studies carried out in other semiarid Mediterranean areas (Mrabet et al. 2001; Hernanz et al. 2002; Moreno et al. 2006).

As mentioned in the Methods section, previous to the establishment of the experiment these fields had been ploughed for several decades. A shift from a CT system to a more conservative system as NT or RT increased the proportion of stable macroaggregate proportion as observed in other experiments (Franzluebbers and Arshad 1996; Mikha and Rice 2004). The NT system promotes the formation of stable macroaggregates in the soil due to the lower turnover rates compared with CT (Six et al. 1999). Tillage increases the susceptibility of aggregates to wet-dry and freeze-thaw cycles and to raindrop impact leading to a greater aggregate disruption (Six et al. 2000). The lower proportion of macroaggregates under CT resulted in an increase in the proportion of microaggregates especially in the soil surface. Elliot (1986) and Cambardella and Elliot (1993) suggested that the breakdown of unstable soil macroaggregates into smaller aggregates is related to the hierarchical formation model of soil aggregates (Tisdall and Oades 1982). In the PN-BB system in soil surface (0-5 cm), total aggregate organic C concentration from small macroaggregates (250-2000 µm) increased as tillage intensity decreased. The decrease in the disruption of soil macroaggregates under NT permitted a greater accumulation of SOC within these macroaggregates. The influence of aggregation on SOM protection and accumulation has been widely studied and reviewed (Six et al. 2002, 2004). The accumulation of SOC in macroaggregates of NT compared with CT was only observed in the PN-BB system. In the PN-BF rotation, total aggregate organic C was similar among tillage treatments even though the proportion of both large and small macroaggregates was increased under NT compared to CT and RT. Tisdall and Oades (1982) suggested that the disposition rather than the amount of organic carbon plays a major role in the stabilization of aggregates. Also, Chaney and Swift (1984) suggested that total SOC may not be sufficient to explain differences in aggregate stability and that certain SOM fractions may play a more important role. Beare et al. (1994) in a similar experiment found differences in mineral-associated carbon (mSOC) in the surface layer (0-5 cm) in both macro- and microaggregates and Six et al. (1998) observed greater fine iPOM in the soil macroaggregates of NT compared with CT. In our study, in both cropping systems, greater fine iPOM C concentrations were observed in NT compared with CT in the 0-5 cm depth. Six et al. (1998, 1999, 2000) developed a conceptual model of macroaggregate formation and C stabilization in relation with tillage. In this model, slower macroaggregate turnover in NT compared with CT leads to a greater microaggregate formation within macroaggregates formed around fine iPOM and to a long-term stabilization of SOC occluded in these microaggregates. However, in our study, significantly greater proportion of microaggregates within macroaggregates in NT compared with CT was only observed in one (AG) of the three sites studied and only in soil surface (0-5 cm depth). Although no significant differences were found at SV and PN-BB slightly greater proportion of microaggregates was also found in the NT treatment especially at SV. Consequently, in these Mediterranean agroecosystems the concept of slower macroaggregate turnover in NT compared with CT, leading to greater formation of microaggregates within macroaggregates, may also be applicable.

Denef et al. (2004), studying differences in C from microaggregates isolated within macroaggregates between tillage treatments in soils with different mineralogy, observed greater total C of these microaggregates occluded within macroaggrates (total mM-C) in NT compared with CT. In our study, total mM-C was only significantly greater in NT compared with CT in the 0-5 cm depth.

The suppression of the long fallow phase from the rotation led to a slightly increased of total SOC content of all depths and tillage treatments. This increment was significantly in the 0-5 cm depth of the NT treatment. Several studies have concluded that the greater production of crop residues in more intensive cropping systems results in an increase of SOC (Collins et al. 1992; Potter et al. 1997; Halvorson et al. 2002). In our study, the annual crop residue production average from 1999 to 2005 was 2063 and 1351 kg of dry matter ha^{-1} in the PN-BB system and in the PN-BF rotation, respectively. Shaver et al. (2002) concluded that under semiarid conditions, the intensification of cropping systems leads to an increase in crop residue production and an increase in stable macroaggregates. Gillabel et al. (2007), comparing dryland and irrigated farming systems, observed similar macroaggregate levels between the two farming systems despite the greater C inputs under irrigation. They suggested that because tillage was used in both farming systems, it had an overriding effect on soil aggregation. In our study, more stable large macroaggregates in PN-BB compared with PN-BF were only found under NT plots in the 0- to 5-cm depth. At the same time, macroaggregate C concentration and iPOM C was greater under NT in the PN-BB system than under the PN-BF rotation.

Interaction effects between tillage and cropping system were only found for total SOC and macroaggregate C concentration in the first 5 cm depth. We hypothesized that the large differences found in SOC in soil surface between tillage treatments and cropping systems led to an interaction effect between the two factors. However, these differences disappeared with soil depth and consequently the interaction effects as well. Consequently, in these agroecosystems, the response of total SOC and total C concentration of aggregates to tillage in soil surface generally depends on the cropping system considered.

CONCLUSIONS

In semiarid Mediterranean agroecosystems, tillage and cropping system affected total SOC, aggregation and aggregate-SOM fractions. A reduction in tillage intensity led to a greater SOC accumulation in the upper soil layers (0-5 and 5-10 cm). Increased cropping intensity resulted also in greater SOC accumulation in the soil surface (0-5 cm) but only under NT. The response of total SOC and total C concentration of aggregates to tillage generally depended on cropping system and vice versa. While NT resulted in a greater proportion of macroaggregates in both types of cropping systems, macroaggregate-C concentration was only increased under NT in the continuous barley system. Under these semiarid conditions, the fine iPOM of the small macroaggregates was the only SOM fraction that explained differences in total SOC among tillage systems under both cropping systems. At the same time, greater proportion of microaggregates located within macroaggregates and greater C associated to these microaggregates were found in NT compared with CT in soil surface. Therefore, in the Mediterranean semiarid agroecosystems studied, slower aggregate turnover under NT than under CT leads to greater microaggregate formation within macroaggregates and to the stabilization of SOC within these microaggregates occluded within macroaggregates.

In semiarid Mediterranean agroecosystems of the Ebro valley, the adoption of NT and the intensification of cropping systems are two management strategies that can enhance SOC sequestration by improving macroaggregate stability and the consequent stabilization of SOC as particulate organic matter occluded inside microaggregates formed within stable macroaggregates. However, this effect was only observed in the soil surface.

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TABLES

Table 1. Site and soil properties at the experimental sites.

Site and soil characteristics	Experimental sites		
	Selvanera (SV)	Agramunt (AG)	Peñaflor (PN)
Mean annual air temperature (ºC)	13.9	14.2	14.5
Mean annual precipitation (mm)	475	430	390
Soil classification †	Xerocrept fluventic	Xerofluvent typic	Xerollic Calciorthid
Soil characteristics (Ap horizon)			
Depth (cm)	37	28	30
pH (H_2O, 1:2.5)	8.3	8.5	8.2
$EC_{1:5}$ (dS m^{-1})	0.16	0.15	0.29
Particle size distribution (%)			
Sand (2000-50 µm)	36.5	30.1	32.4
Silt (50-2 µm)	46.4	51.9	45.5
Clay (< 2 µm)	17.1	17.9	22.2

†USDA classification (Soil Survey Staff 1975).

Table 2. Soil organic carbon (SOC) content at Peñaflor in a continuous barley system (PN-BB) and in a barley-fallow rotation (PN-BF), Selvanera (SV) and Agramunt (AG) as affected by tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage).

Site	Depth (cm)	NT		RT		CT	
		Bulk density (kg m^{-3})	SOC (g m^{-2})	Bulk density (kg m^{-3})	SOC (g m^{-2})	Bulk density (kg m^{-3})	SOC (g m^{-2})
PN-BB	0-5	1343a	853aA†	1148b	576bA	1194b	547bA
	5-10	1482a	723aA	1248b	590bA	1269b	582bA
	10-20	1407a	1167aA	1311b	1119aA	1283b	1148aA
	0-20	-	2743aA	-	2285bA	-	2278bA
	0-20 (eq. mass.) ‡		2743a		2576b		2556b
PN-BF	0-5	1292a	671aB	1189a	515bA	1220a	490bA
	5-10	1403a	573aA	1263b	566aA	1304ab	515aA
	10-20	1375a	1062aA	1311a	1073aA	1339a	1016aA
	0-20	-	2306aB	-	2154aA	-	2021aA
	0-20 (eq. mass.)		2306a		2398a		2193b
SV	0-5	1289a	1207a	-	-	1351a	851b
	5-10	1655a	673a	-	-	1550a	774a
	10-20	1666a	1061b	-	-	1548a	1255a
	0-20	-	2941a	-	-	-	2880a
	0-20 (eq. mass.)		3002a				3016a
AG	0-5	1317a	932a	-	-	1454a	666b
	5-10	1526a	782a	-	-	1436a	629b
	10-20	1522a	1322a	-	-	1530a	1305a
	0-20	-	3037a	-	-	-	2600a
	0-20 (eq. mass.)		3117a				2699b

†Different lower case letters indicate significant differences among tillage treatments within the same site and soil depth ($P<0.05$). Different upper case letters indicate significant differences between PN-BB and PN-BF within the same tillage treatment and soil depth ($P<0.05$). PN-BB and PN-BF data from 2003 sampling and SV and AG data from 2004 sampling.

‡SOC on an equivalent soil mass basis (equivalent soil mass: 2820 Mg ha^{-1}, 3012 Mg ha^{-1} and 3170 Mg ha^{-1} in PN-BB and PN-BF, SV and AG, respectively).

Table 3. Proportion of large and small macroaggregates in the 0-5, 5-10 and 10-20 cm soil depths under no-tillage (NT) and conventional tillage (CT) at the continuous barley system at Peñaflor (PN-BB), Selvanera (SV) and Agramunt (AG).

Site	Depth (cm)	Proportion of macroaggregates (g macroaggregate g^{-1} soil)			
		Large macroaggregates (>2000 µm)		Small macroaggregates (250-2000 µm)	
		NT	CT	NT	CT
PN-BB	0-5	0.16	-	0.16a†	0.11b
	5-10	0.08	-	0.09a	0.07b
	10-20	0.06	-	0.09a	0.08a
SV	0-5	0.37a	0.15b	0.21a	0.20a
	5-10	0.21a	0.10b	0.12a	0.12a
	10-20	0.17a	0.06b	0.07a	0.09a
AG	0-5	0.29	-	0.13a	0.06b
	5-10	0.25	-	0.10a	0.04b
	10-20	0.15	-	0.06a	0.04a

†Different lower case letters indicate significant differences among tillage treatments within the same site, soil depth and macroaggregate size class (P<0.05).

Table 4. Analysis of variance for total soil organic carbon (SOC) and water-stable aggregates at Peñaflor in a continuous barley system (PN-BB) and in a barley-fallow rotation (PN-BF) in the 0-5, 5-10 and 10-20 cm soil depths.

Variables	Aggregate size class	Source of variation	DF†	*Pr > F*		
				0-5 cm	5-10 cm	10-20 cm
Total SOC		Tillage	2	0.008	0.11	0.94
		Crop. Syst.	1	0.14	0.12	0.08
		Till x Crop. Syst.	2	0.004	0.19	0.74
Water-stable aggregates	>2000	Tillage	2	0.008	0.01	0.07
		Crop. Syst.	1	0.14	0.75	0.64
		Till x Crop. Syst.	2	0.004	0.65	0.82
	250-2000	Tillage	2	0.0006	0.10	0.25
		Crop. Syst.	1	0.27	0.60	0.78
		Till x Crop. Syst.	2	0.84	0.57	0.20
	53-250	Tillage	2	0.001	0.001	0.16
		Crop. Syst.	1	0.10	0.71	0.21
		Till x Crop. Syst.	2	0.01	0.37	0.63
	<53	Tillage	2	0.89	0.85	0.25
		Crop. Syst.	1	0.69	0.51	0.88
		Till x Crop. Syst.	1	0.005	0.38	0.22

†Degrees of freedom.

Table 5. Analysis of variance for total aggregate C concentration, intra-particulate organic matter C (iPOM) and mineral-associated C (mSOC) at Peñaflor in a continuous barley system (PN-BB) and in a barley-fallow rotation (PN-BF), in the 0-5, 5-10 and 10-20 cm soil depths.

Variables	Aggregate size class	Source of variation	DF†	*Pr > F*		
				0-5 cm	5-10 cm	10-20 cm
Total aggregate C	250-2000	Tillage	2	0.04	0.30	0.70
		Crop. Syst.	1	0.03	0.03	0.60
		Till x Crop. Syst.	2	0.03	0.57	0.98
	53-250	Tillage	2	0.03	0.36	0.10
		Crop. Syst.	1	0.04	0.61	0.48
		Till x Crop. Syst.	2	0.17	0.10	0.43
	<53	Tillage	2	0.14	0.19	0.008
		Crop. Syst.	1	0.99	0.34	0.12
		Till x Crop. Syst.	2	0.87	0.61	0.78
iPOM C	250c	Tillage	2	0.78	0.72	0.17
		Crop. Syst.	1	0.71	0.92	0.87
		Till x Crop. Syst.	2	0.61	0.52	0.49
	250f	Tillage	2	0.003	0.09	0.10
		Crop. Syst.	1	0.03	0.18	0.62
		Till x Crop. Syst.	2	0.27	0.33	0.73
	53f	Tillage	2	0.07	0.15	0.21
		Crop. Syst.	1	0.55	0.64	0.14
		Till x Crop. Syst.	2	0.41	0.25	0.44
mSOC	250-2000	Tillage	2	0.78	0.27	0.90
		Crop. Syst.	1	0.63	0.08	0.51
		Till x Crop. Syst.	2	0.18	0.22	0.62
	53-250	Tillage	2	0.04	0.53	0.06
		Crop. Syst.	1	0.08	0.41	0.45
		Till x Crop. Syst.	2	0.56	0.09	0.55

†Degrees of freedom.

Table 6. Total microaggregate-C (total mM-C) isolated from small macroaggregates (250-2000 µm) in the 0-5, 5-10 and 10-20 cm soil depths under no-tillage (NT) and conventional tillage (CT) at the continuous barley system at Peñaflor (PN-BB), Selvanera (SV) and Agramunt (AG).

Site	Depth (cm)	Total mM-C (g C kg^{-1} sand-free macroaggregate)	
		NT	CT
PN-BB	0-5	9.89a†	5.81b
	5-10	8.08a	7.56a
	10-20	7.11a	6.59a
SV	0-5	17.55a	15.42b
	5-10	11.15a	11.39a
	10-20	8.62a	8.93a
AG	0-5	15.9a	10.2b
	5-10	16.2a	12.9a
	10-20	10.7a	10.7a

†Different lower case letters indicate significant differences among tillage treatments within the same site, soil depth and macroaggregate size class (*P*<0.05).

FIGURE CAPTIONS

Fig. 1. Water-stable aggregate size distribution in 0-5, 5-10 and 10-20 cm soil layers as affected by cropping system at Peñaflor (PN-BB, continuous barley system; PN-BF, barley-fallow rotation) and tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). For the same cropping system and depth, different letters indicate significant differences among tillage treatments at $P<0.05$. For the same tillage treatment and depth * indicate significant differences among cropping systems at $P<0.05$.

Fig. 2. Sand-free aggregate C concentration distribution in 0-5, 5-10 and 10-20 cm soil layers as affected by cropping system at Peñaflor (PN-BB, continuous barley system; PN-BF, barley-fallow rotation) and tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). For the same cropping system and depth, different letters indicate significant differences among tillage treatments at $P<0.05$. For the same tillage treatment and depth * indicate significant differences among cropping systems at $P<0.05$.

Fig. 3. Distribution of sand-free intra-aggregate particulate organic matter C (iPOM C) in 0-5, 5-10 and 10-20 cm soil layers as affected by cropping system at Peñaflor (PN-BB, continuous barley system; PN-BF, barley-fallow rotation) and tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). 250c: coarse (250-2000 µm) iPOM C in small macroaggregates (250-2000 µm); 250f: fine (53-250 µm) iPOM C in small macroaggregates (53-250 µm); 53f: iPOM C (53-250 µm) in microaggregates (53-250 µm). For the same cropping system and depth, different letters indicate significant

differences among tillage treatments at *P*<0.05. For the same tillage treatment and depth * indicate significant differences among cropping systems at *P*<0.05.

Fig. 4. Mineral associated soil organic C (mSOC) in 0-5, 5-10 and 10-20 cm soil layers as affected by cropping system at Peñaflor (PN-BB, continuous barley system; PN-BF, barley-fallow rotation) and tillage (CT, conventional tillage; RT, reduced tillage; NT, no-tillage). For the same cropping system and depth, different letters indicate significant differences among tillage treatments at *P*<0.05. For the same tillage treatment and depth * indicate significant differences among cropping systems at *P*<0.05.

Fig. 5. Proportion of soil microaggregates (250-2000 µm) contained within large (>2000 µm) and small (250-2000 µm) macroaggregates in 0-5, 5-10 and 10-20 cm soil layers under conventional tillage (CT) and no-tillage (NT) at Selvanera (SV), Agramunt (AG) and the continuous barley system at Peñaflor (PN-BB). Values followed by a * within a site and soil depth are significantly different between tillage treatments (P<0.05). ND: Not determined.

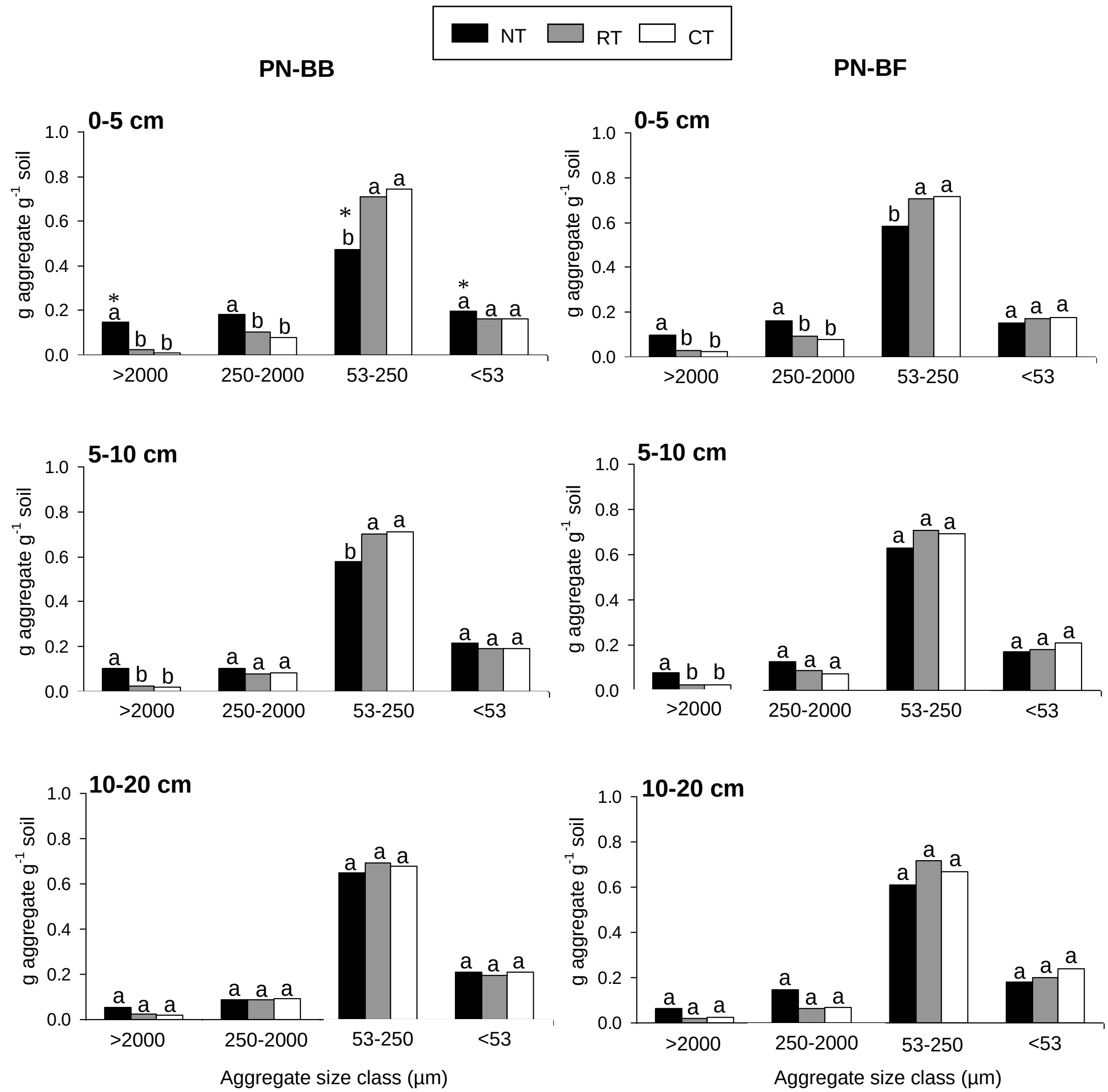
NT
RT
CT
PN-BB
PN-BF
0-5 cm
5-10 cm
10-20 cm
g aggregate g-1 soil
>2000
250-2000
53-250
<53
Aggregate size class (µm)

Fig. 1.

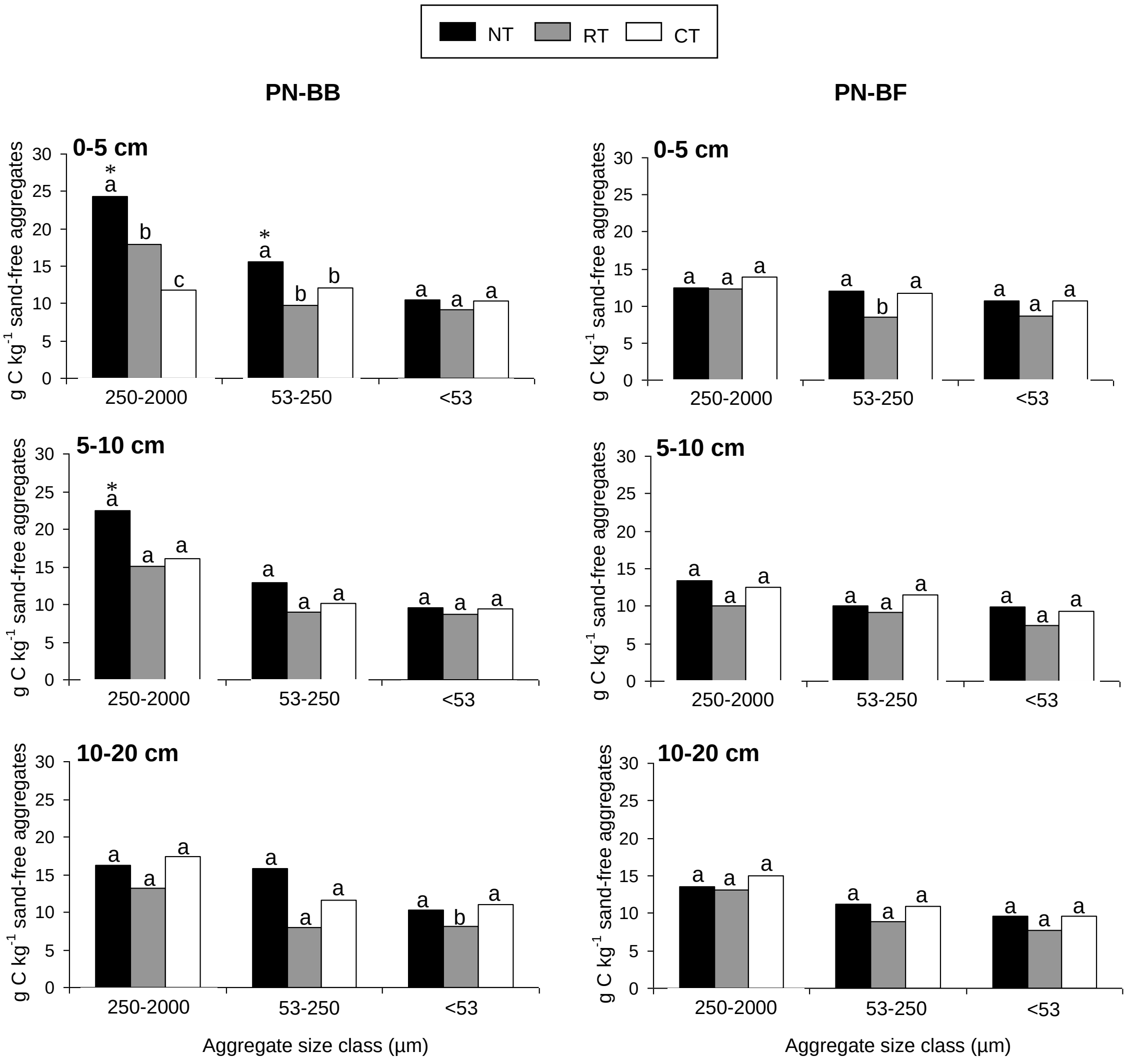
NT
RT
CT
PN-BB
PN-BF
0-5 cm
5-10 cm
10-20 cm
g C kg-1 sand-free aggregates
250-2000
53-250
<53
Aggregate size class (µm)

Fig. 2.

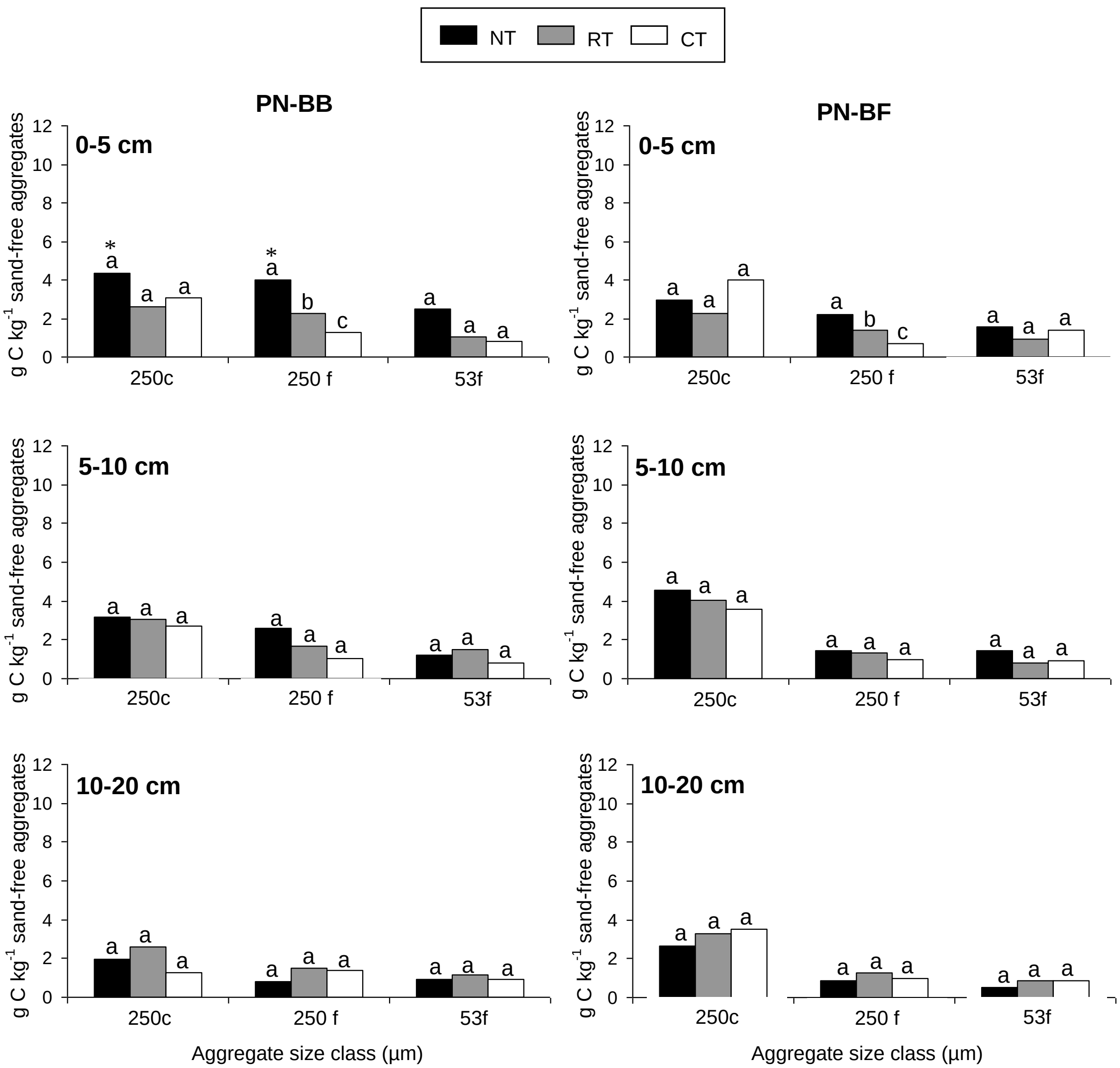
NT
RT
CT
PN-BB
PN-BF
0-5 cm
5-10 cm
10-20 cm
g C kg-1 sand-free aggregates
250c
250 f
53f
Aggregate size class (µm)

Fig. 3.

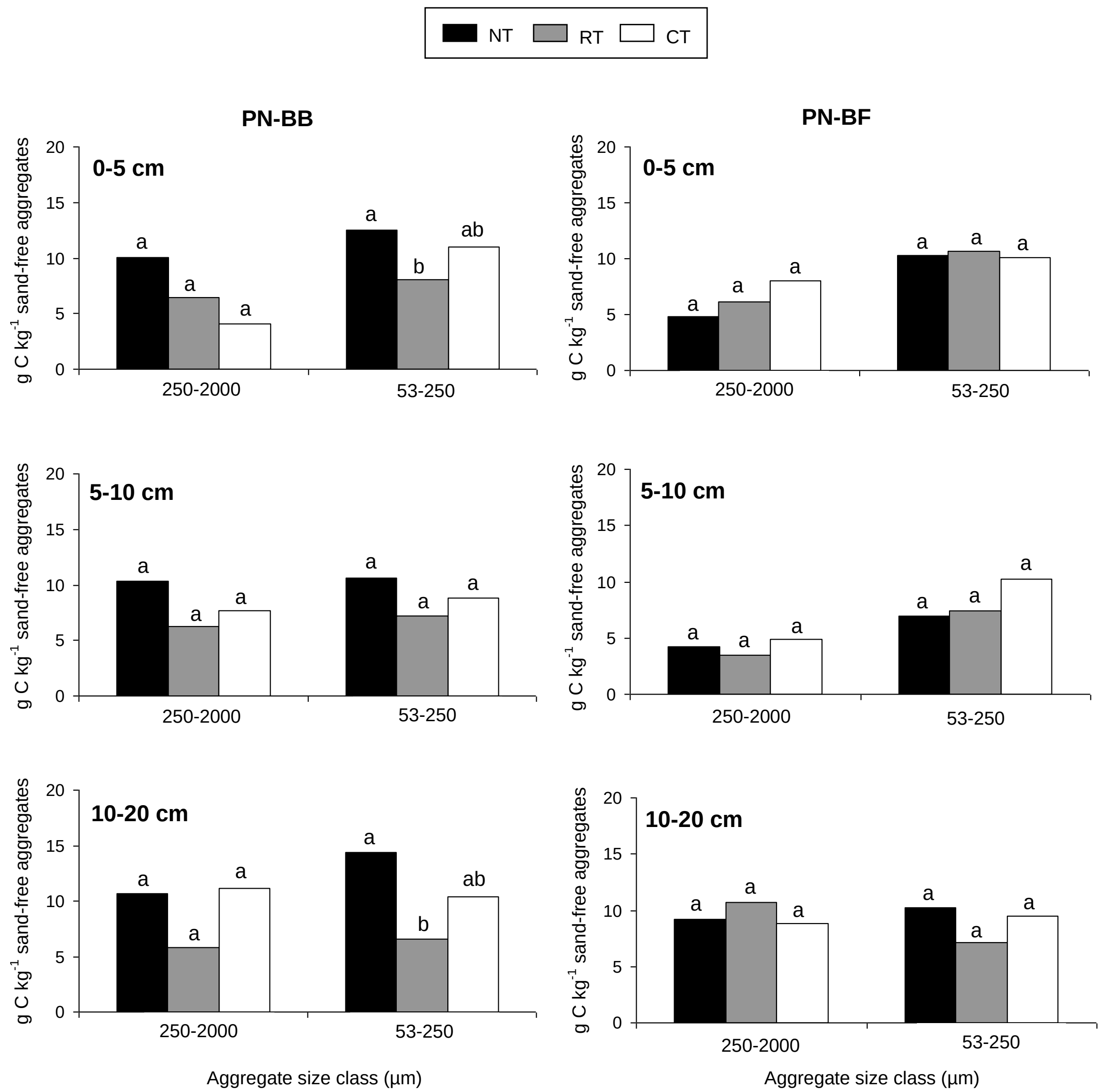
NT
RT
CT
PN-BB
PN-BF
0-5 cm
5-10 cm
10-20 cm
g C kg^{-1} sand-free aggregates
250-2000
53-250
Aggregate size class (µm)

Fig. 4.

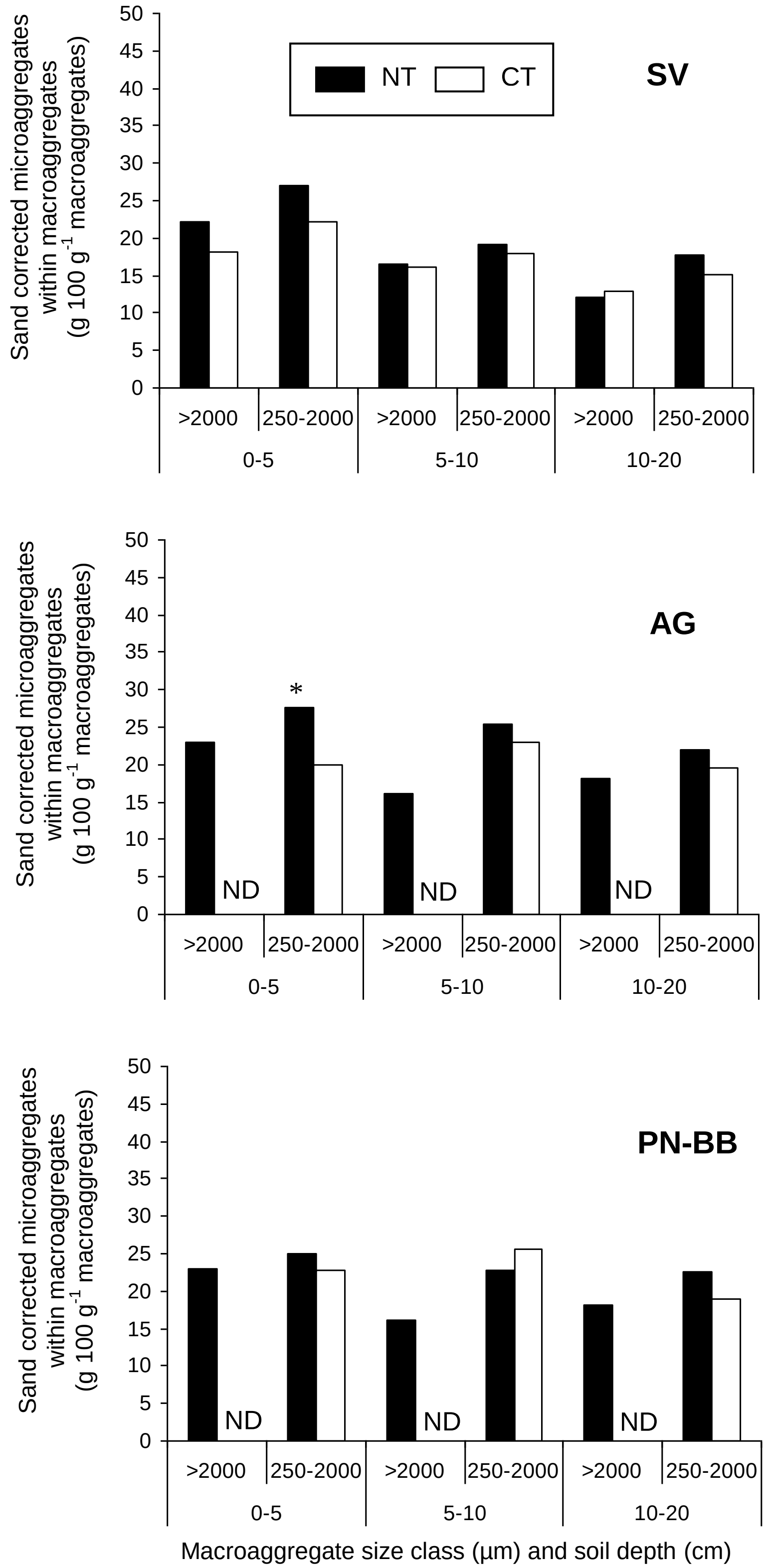
NT
CT
SV
AG
PN-BB
Sand corrected microaggregates within macroaggregates (g 100 g-1 macroaggregates)
0
5
10
15
20
25
30
35
40
45
50
*
ND
>2000
250-2000
0-5
5-10
10-20
Macroaggregate size class (µm) and soil depth (cm)

Fig. 5.