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No-Tillage Does Not Always Stop the Soil Degradation in Relation to Aggregation and Soil Carbon Storage in Mediterranean Olive Orchards

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Abstract: Intensive tillage (CT) application in Mediterranean olive orchards has threatened soil quality and led to soil degradation. No tillage with bare soil (NT+H) has been considered as an alternative practice to this management system; however, there are discrepancies in the literature on the results of the application of this practice. Our main objective was to assess the impact of continuous tillage and zero tillage on soil aggregate stability, SOC and SON stocks. The study was conducted in a Mediterranean rainfed olive grove under semi-arid conditions in a Calcaric Cambisol, for 16 years evaluating complete soil profiles (0–120 cm depth). In the long-term, the management practices CT1 and NT+H significantly affected aggregate particle size by reducing the percentage of macro-aggregates (>250 μm) and promoting a higher number of micro-aggregates (<250 μm). Nevertheless, NT+H affected the Bw and BC horizons with the increase in the large macroaggregates (>2000 μm) percentage. In relation to these results, the soil structural stability indices showed a significant decrease in both Mean Weight Diameter (MWD) and Geometric Mean Diameter (GMD) values with losses of more than 50% with respect to the initial period (CT0) in the first two horizons. In the long term, both in CT1 and in NT+H, higher SOC concentrations were found in deep horizons showing a C redistribution in depth and important losses in TN values—while, in CT0, macroaggregates contained the highest CPC values, after the long-term both management practices (CT1 and NT+H) affected the C dynamics and were characterised by higher C pool in the microaggregates than in the macroaggregate fractions. Therefore, long-term NT+H and CT1 showed an SOC storage deterioration and increased susceptibility to decomposition, CO_2 emissions and fertility losses. This trend i.e., decreases in SOC stocks following NT, confirms previous studies on the subject and points to nutrient balance impacts.

Keywords: long-term no till; aggregate stability; aggregate-associated C; soil organic carbon preservation capacity

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1. Introduction

The intensification process of the agricultural farming system is a response to the world population growth and the new food consumption pattern in recent decades [1,2]. In order to increase the crop productivity, a series of changes in mechanisation, the use of fertilisers and pesticides, and the establishment of mono-cropping have been implemented with the aim of maximised production [3,4]. Looking back through history, it is evident that soil degradation and desertification are not new issues [5,6]. This pattern reflects both a long history of tillage-induced erosion and loss of biodiversity that are threatening global food security [7–11].

In the Mediterranean basin olive orchard is one of the main woody crops, with a long history of farming in the area and being a fundamental element of the traditional crop species in Mediterranean landscapes [12,13]. In Spain, olive groves are particularly relevant as they are the second largest crop in terms of extension, after cereals [14]. In addition, with 2.6 million hectares of olive groves, it is the country with the largest surface area of this woody crop in the world and the world's highest producer of olive oil, with 1.35 million tonnes in the 2020/21 campaign [15]. The Andalusia region accounts for 63% (1.6 Mha) of the olive-growing land in Spain and is the most representative agroecosystem in this territory, with great socio-economic influence for the region [16,17].

Soil structure is the assemblage of mineral and organic particles that associate to form aggregates of different sizes [18]. According to the size of the aggregation, aggregates generally can be classified as macroaggregates (>0.25 mm) or microaggregates (<0.25 mm) [19]. In this sense, structural stability is defined as the resistance of the soil to the aggressive action of external agents [20]. Soil aggregation processes have a fundamental role in multiple soil functions such as water [21] and nutrient retention [22] or Carbon (C) sequestration and stabilisation [23].

In the soil aggregate formation and stabilisation, soil organic matter (SOM) has been detected as one of the main particle binding agents [24,25]. The decrease in the soil organic carbon (SOC) content in agricultural soils is related to unsustainable management and the macroaggregates destruction mainly due to intensive tillage [26,27]. As a result, agricultural soils become structurally less stable and more exposed to erosive processes [28]. Soil aggregates and associated C can be important indicators of soil properties' modifications caused by the changes both in soil management [29,30] and land use [31–33]. In this sense, there is a wide amount of scientific literature related to this issue and in Mediterranean soils is also well documented [34–36]. However, the vast majority of them are focused on the first centimetres of soils (0–30 cm) and only a few studies analyse changes in the deep horizons (100 or 120 cm depth). The deep horizons have been shown as important C pools and the analysis of aggregation dynamics, and SOC sequestration and storage along the soil profile seem to be fundamental [37–39]. This is especially important nowadays where climate change mitigation strategies are being designed and where agricultural soils play a central role in these strategies [7,8,40,41].

This study focused on the particle size distribution of soil aggregates and the physical fractions of SOC, and Total Nitrogen (TN) associated with the aggregates to understand the effect of long-term management practices in a calcaric Cambisol soil under a rainfed olive orchard in semiarid conditions. The main purposes of the study were to assess the effects of long-term conventional tillage (CT) and no tillage with bare soil by using herbicides (NT+H) on: i) stability of soil aggregates and their distribution along the profile, and ii) changes in SOC and TN associated with aggregates and subfractions in depth in a semiarid environment.

2. Materials and Methods

2.1. Study Area

The present study was conducted in an experimental olive farm in Torredelcampo, a municipality located in the west of the province of Jaén (Andalusia, Spain, 37°46′26.0″N, 3°54′41.5″W) (Figure 1). The farm where the study was carried out covers an area of 10 ha. The history of this farm is characterised by a long agricultural activity with small hills and unirrigated conventional olive trees (*Olea europea* var. picual) with 2–3 trunks under a monocropping system (12 m × 12 m pattern).

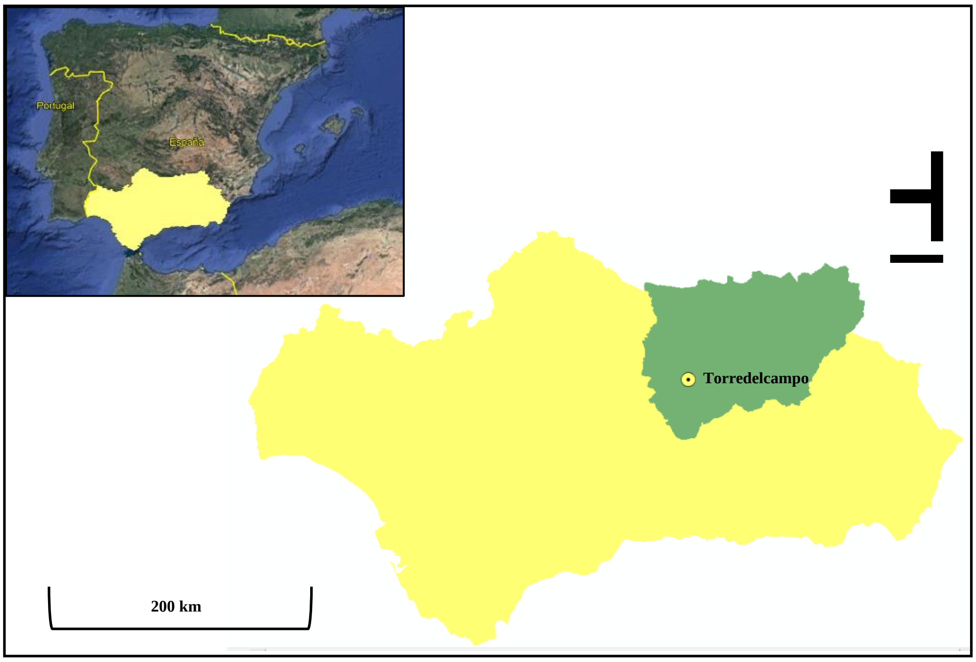

Figure 1. Location of the study area. Map of Andalusian region (yellow colour) Jaen province (green colour) and Torredelcampo municipality (point yellow and black). https://www.google.com/maps/@37.848303,-3.8662558,320m/data=!3m1!1e3 (accessed on 1 December 2021).

The area has a typical Mediterranean climate characterised by dry and cold winters with average minimum temperatures of 8.3 °C in January and very warm and dry summers which reach an average of over 27 °C in July. The historical mean annual precipitation in the area is around 493.2 mm, with a maximum in the autumn and early spring months, while the summer months are characterised by a drought period. During the period studied (2004–2020), the average annual air temperature of the experimental area was 16.7 °C, and the mean annual precipitation was 451.9 mm.

2.2. Experimental Design and Soil Sampling

The study was conducted from 2004 to 2020 in the mentioned research area. Until 2004, the farm was managed only under conventional tillage (CT0), but, from 2004, the farm was subdivided in two plots, one of which was maintained under conventional tillage (CT1) and in the other one no tillage with bare soil by using herbicides (NT+H) management was implemented. In both plots, the same fertilization rate was applied (100 kg ha^{-1} urea, N richness 46%) in alternate years after the olives had been harvested. In addition, in both plots, the pruning remains were incorporated every two years after the pruning of the olive trees and fungicides (copper oxychloride 34.5% w.p.) were applied. In both treatments, vegetation was eliminated by applying preemergence herbicides (1.0 L 36% glyphosate ha^{-1}) in autumn to control weeds. The NT+H plot was managed without mechanical practices and the plot managed under CT was tilled (25–30 cm) with a cultivator in spring followed by tine and disc harrowing in summer.

Soil samples were collected in 2004 (CT0) and 2020 (CT1 and NT+H). In the experimental plots, under each management, five random sampling points were selected (5 soil profiles × 3 plots = 15 complete soil profiles), pits were dug with a mini-excavator and soil samples were collected horizon by horizon. Soil profiles showed a depth of 120 cm on average and four horizons were identified in each open profile (Ap, Bw, BC and C). Therefore, five soil samples were taken from the analysed soil horizons.

In the experimental area, soil was classified as Calcaric Cambisol according to the World Reference Base for Soil Resources [42] and general physico-chemical properties of the soil before the beginning of the experiment were: (i) clayey soils (sand: 5%, silt: 22% and clay: 72%); (ii) soil pH values of 7.9; (iii) bulk density up to 1.40 Mg m^{-3}, and (iv) organic matter content ranged between 1.2 and 0.7% [38].

2.3. Soil Particle-Size Separation, Water Stable Aggregates and Aggregate Associated Carbon and Nitrogen

Soil samples collected in the experimental plots were air-dried in a laboratory and passed through an 8 mm sieve to remove roots and rock fragments prior to the wet sieving process. The aggregate size distribution of soil was conducted by a wet sieving method [43,44]. For the aggregate analysis, three sieves were used (2000, 250, 53 μm). In the soil fractionation process, a 100 g air-dried soil sample was placed in a 2-mm sieve and submerged in distilled water for five minutes. After slaking, the 2-mm sieve was manually moved up and down 50 times during a 2-min period. The soil samples retained on the 2-mm sieve were collected. The suspension and soil that passed through the 2-mm sieve were washed through a 250 μm sieve. These processes were repeated to obtain soils fractions that were classified as >2000, 2000–250, 250–53, and <53 μm, and all classified fractions were oven-dried at 50 °C and then weighed.

Soil mean weight diameter (MWD) and geometric mean diameter (GMD) were evaluated to determine soil aggregate stability index [43]. The MWD (mm) was calculated as follows:

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i w_i$$

where x_i indicates the mean diameter of aggregates remaining on the respective sieves, w_i indicates the ratio between the weight of the aggregate remaining on the sieve to the total weight of the sample and n denotes the number of sieves used for aggregate separation.

The GMD (mm) was calculated as follows:

$$\mathrm{GMD} = \exp\left[\frac{\sum_{i=1}^{n} w_i \log x_i}{\sum_{i=1}^{n} w_i}\right]$$

where x_i denotes the mean diameter of each aggregate oversize of each sieve, w_i indicates the total dry weight of obtained aggregates and n denotes for the total number of sieves.

Soil aggregate fractions were ground with mortar after the removal of any stone or coarse organic particles for the determination of aggregate associated TN by the dry combustion method using a CN analyser. Aggregate associated SOC in each size fraction was determined by the Walkley and Black method [45].

The formula for calculating the soil organic carbon preservation capacity (CPC) of soil aggregates was determined as follows [26,46]:

$$\mathrm{CPC} = \frac{\mathrm{WCi} \times \mathrm{WAi}}{100}$$

where *WCi* is the SOC content of the given aggregate particle size level in g kg^{-1} (>2000 μm, 2000–250 μm, 250–53 μm, <53 μm) and *WAi* is the content of aggregates of a given particle size level in grams (>2000 μm, 2000–250 μm, 250–53 μm, <53 μm).

2.4. Statistical Analysis

Data for aggregate size distribution, water stable aggregates and aggregate-associated SOC and TN were tested for normality to verify the model assumptions using a Kolmogorv–Smirnov test. As the data failed the normality test, non-parametric tests were used (Kruskal–Wallis ANOVA). Significant differences among treatments were determined with one-way analysis of variance in the different treatments with the same aggregate sizes followed by the significant difference (Tukey test) at $p < 0.05$. All computations were made using SigmaPlot v14.0.

3. Results and Discussion

3.1. Soil Aggregate Size Distribution

Distribution of soil aggregate size under the wet sieving method in the study period is shown in Figure 2. Results showed clear differences in the four size aggregate fractions (>2000 μm, 2000–250 μm, 250–53 μm, and <53 μm) from the start of the study (CT0-2004) to the end of the study period (CT1 and NT+H-2020). CT0 was the management with the highest macroaggregate (large macroaggregate + small macroaggregate) percentage along the whole profile. Over time, macroaggregates decrease in all horizons under the two management systems implemented, although there are some differences.

Macroaggregates (>2000 μm + 250–2000 μm) significantly changed the percentages obtained in the Ap horizon (Figure 2a) with a substantial decrease from CT0 (66.3%) to CT1 (29.3%) and NT+H (20.8%) with applied treatments. In the Bw and BC horizon (Figure 2b and 2c), the macroaggregates' distribution maintained the same trend with significant decreases compared to the initial situation (CT0; 73 and 65.8% in Bw and BC, respectively) as shown in the surface horizon. However, in Bw and BC horizons, soil management affected macroaggregates, and, after 16 years of study, a significantly higher percentage of macroaggregates were found under NT+H management (40.5 and 54.1%) regarding CT1 management (28.2 and 29.1%). In the C horizon (Figure 2d), macroaggregate values decreased significantly in CT0 (57.8%) and NT+H (34.2%) with respect to the BC horizon, while, in CT1 (30.3%), the value of macroaggregates remains similar.

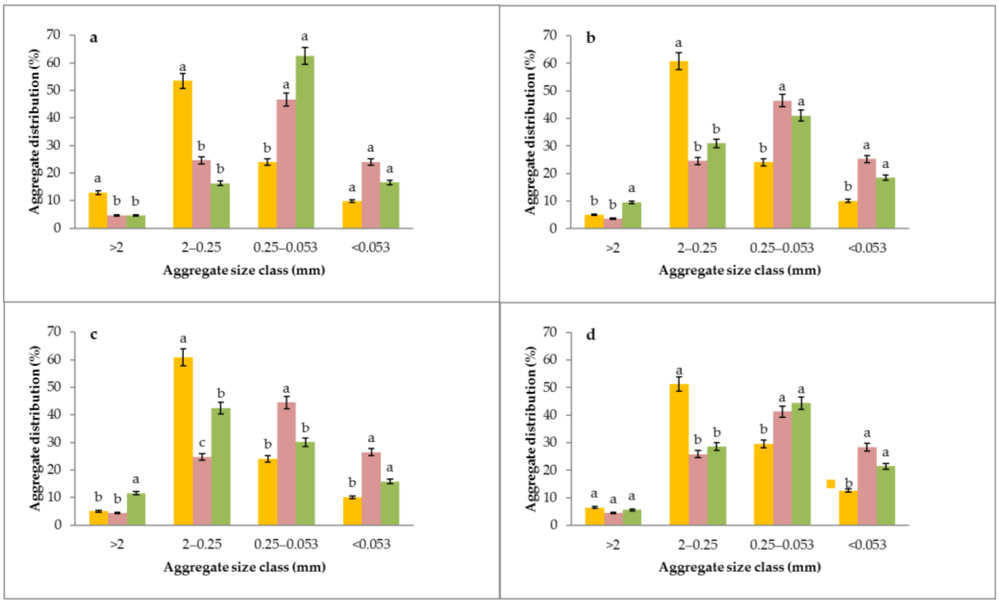

Figure 2. Soil water-stable aggregate size distribution (%) for initial situation (CT0; yellow bars) conventional-tillage (CT1; red bars) and no-till (NT+H; green bars) treatments at different horizons for (**a**) Ap horizon, (**b**) Bw horizon, (**c**) BC horizon and (**d**) C horizon. Values are given as mean ± standard error ($n = 5$). The different lowercase letters above the error bars for the same aggregate fraction indicate significant differences based on post-hoc tests and Tukey's honestly significant difference (HSD) at $p < 0.05$ between management.

When we focus our attention on the two sizes of macroaggregates separately, related to the large macroaggregates (>2000 μm), we obtained in the Ap horizon the highest percentages under CT0 with 12.9% with regard to those found in NT+H (4.5%) and CT1 (4.7%). However, NT+H management produced the highest proportion of large macroaggregates in the two following horizons—in the Bw (9.6%) and BC (11.6%) horizons, while CT0 reached 6.5 and 5%, and CT1 3.6 and 4.4% in each horizon, respectively. In the C horizon, large macroaggregates showed similar values between the different management practices ranged between 6.5% (CT0), 5.6% (NT+H) and 4.4% (CT1). On the other hand, in small macroaggregates' size fraction (250–2000 μm), CT0 widely dominated the higher values with percentages above 50% in all analysed horizons. Both CT1 and NT+H management remained below the levels found in this fraction in the initial period of the study with percentages varying between 24.5 and 25.8% under CT1 and 16.3 and 42.5% under NT+H.

Regarding the microaggregates (<250 μm), this fraction accounted for the majority of the aggregates (in the range between 69.7 and 71.8%) for CT1 management in each soil horizon. In NT+H, microaggregates were the fraction with the highest percentage ranging between 45.9 and 79.2% except in the BC horizon where macroaggregates (54.1%) obtained a higher percentage than microaggregates (45.9%).

In the study area within the microaggregates fraction, the subfraction of large microaggregates (53–250 μm) accounted for the highest number of aggregates under NT+H management in the Ap horizon (62.5%) above the percentages registered in CT1 46.7% and CT0 23.9% in the same horizon. However, in the Bw horizon, CT1 (46.5%) reached higher percentages in this fraction than NT+H (41%) and CT0 (19.4%).

In the two deepest horizons, CT1 reached the highest values (44.4% in BC and 41.3% in C) while NT+H remained at 30.1 and 44.3% and CT0 with the lowest values 24.1 and 29.6%, respectively. On the other hand, the silt+clay fraction (<53 μm) remained without significant differences in the surface horizon, but there was an increasing percentage in depth between the baseline situation and both management after the long-term, with CT0 accounting between 7.6 and 12.6%; CT1 between 25.3 and 28.4% and NT+H between 15.8 and 21.5%.

In the study period, soil management clearly affected soil structure. In this way, the soil structure in the study area moved from a predominance of macroaggregates (>250 μm) to a soil characterised by the prevalence of microaggregates (<250 μm). Results of our study showed that the content of aggregates >250 μm was significantly decreased from de initial period (CT0). Sixteen years of olive orchards managed under continuous tillage (CT1) led to macroaggregate breakdown; however, when the soil suffered a management change to no till (NT+H), the macroaggregates percentage also reduced considerably. Our results were in line with previous studies that reported reduced soil macroaggregate percentages and increased microaggregate amounts in cropland under continuous tillage [47] and showed a general shift of the size distribution towards smaller aggregate classes [48,49].

Soil macroaggregation is essential for several processes such as root development, water retention, gas exchange and resistance to erosion forces [50–54]; in this sense, the massive loss of macroaggregates under CT1 and NT+H over a period of 16 years leads to a significant soil quality decline.

These results showed that tillage removal and the maintenance of bare soil had a worse effect on the macroaggregates development in the shallow horizon, while the application of these management practices could promote the formation of larger aggregates in the subsurface horizons in olive orchards under Mediterranean conditions. This could be related to the soil exposure to environmental factors that cause erosion processes due to the absence of vegetation cover and root systems in the olive orchard alleys, so that macroaggregate formation and stabilisation were not promoted under these management practices. The lack of increase in the macroaggregate fraction can be related to low levels of organic matter (OM) and pesticides application since, according to [55], biotic aggregation in subsoils is promoted by a higher supply of readily available OM in the rhizosphere. This is a major issue because these management practices are predominant in olive orchard management, and even NT+H management has been promoted as a sustainable practice by many farmers because it reduces the amount of labour.

3.2. Stability of Water Stable Aggregates

The stability of the different aggregate size fractions in the study area was measured by MWD and GMD under different management. The MWD and GMD were significantly influenced by the applied treatments with significant variations over the long term of study (Figure 3). The highest MWD and GMD values were found at the beginning of the analysed period (CT0), where, according to the classification of [56], in all horizons, the MDW values showed a medium stability with values decreasing in depth from 1.09 and 1.01 mm in the first two horizons to 0.90 and 0.85 mm in the deepest ones, and the GMD values ranged between 0.61 mm and 0.41 mm.

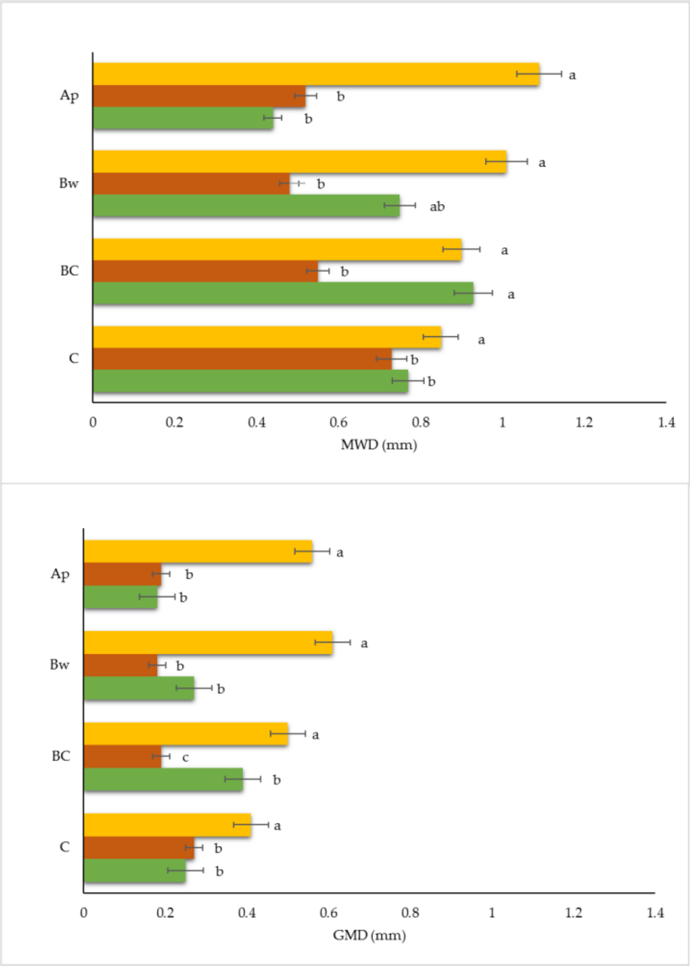

Figure 3. Mean weight diameter (MWD) and geometric mean diameter (GMD) of soil aggregates for initial situation (CT0; yellow bars), conventional-tillage (CT1; red bars) and no-till (NT+H; green bars) treatments at different horizons. Values are given as mean ± standard error ($n = 5$). The different lowercase letters above the error bars for the same soil horizon indicate significant differences based on post-hoc tests, Tukey's honestly significant difference (HSD) between management at $p <$ 0.05.

After 16 years under CT and NT+H management, a significant decrease was observed in MWD and GMD values resulting in unstable soil [56]. The MWD and GMD of soil aggregates suffered a decrease of 52.6 and 66.5% (0.52 and 0.19 mm) in the Ap horizon under CT1 management, while, under NT+H management, the decrease was 59.4 and 67.7% (0.44 and 0.18 mm), respectively. A similar percentage of loss was observed in the Bw horizon in MWD and GMD under CT1 (0.48 and 0.18 mm); however, in the Bw horizon under NT+H, the decrease in MWD and GMD values was lower (0.75 and 0.27 mm). In the BC horizon, the decrease in MDW and GMD in CT1 is maintained, reaching values of

0.55 mm and 0.19 mm, respectively, while, in NT+H, the values obtained (0.93 and 0.39 mm) were similar to that found at the initial situation. In the C horizon, the MWD values under CT1 and NT+H suffered a slight decrease and remain at 0.73 mm and 0.77 mm, respectively, while GMD reaches 0.27 and 0.25 mm.

In the present study, CT1 and NT+H treatments significantly decreased MWD and GMD values. Soil aggregates stability determined in relation to MWD and GMD in the study area reflects how their ability to resist fractions' breakdown and disaggregation due to common disruptive forces in the area such as wet and dry cycles, freeze-thaw events and torrential precipitation events has been drastically decreased over the long-term period analysed. Therefore, these soils are more exposed to erosive processes that lead to soil loss, CO_2 emissions and nutrient mobilisation and deposition.

It is widely established that intensive tillage destroys stable aggregates and greatly affects the larger soil aggregates, which are considered less stable [57–59]. In line with [60], our results showed that, in the long-term, the stability reduction occurred not only in the surface layers but also in deeper ones, although in smaller proportions. On the contrary, the NT soil management system has been defined as one of the agricultural conservation methods, as it has been shown in numerous studies as an agronomic practice that reduces soil disturbance and leads to increased aggregate size compared to intensive tillage [61,62]. However, our results after 16 years did not show this relationship between no tillage implementation and the increase in structural stability with respect to the initial situation because soil was bare through herbicide application. Even though similar stability values were found under CT1 and NT+H in the superficial horizon, the reduction in soil stability under NT+H on Bw and BC horizons was much smaller than the reduction under CT1 referred to the initial situation (CT0), suggesting that soil structure in deep horizons can be better maintained when tillage is removed. This lower stability in the Ap horizon could be due to the fact that NT management was maintained with bare soil; however, NT is usually associated with soil cover either by the establishment of a vegetative cover or the maintenance of crop residues, as mulching and root development that have been shown to be fundamental in macroaggregates formation and structural stability increase [63–65]. In this sense, several studies that analyse the soil management effects on structural stability do not define in detail the residue management practices in association with NT management. In accordance with [66], organic crop residues management under an NT system can be essential in order to define this management practice as a positive strategy in agricultural soils conservation and structural stability improvement.

3.3. Aggregate Associated SOC and TN Concentration

The SOC and TN concentrations within different soil aggregate fractions were significantly influenced by management (Figures 4 and 5). In general, the highest aggregate SOC and TN concentrations were found under a macroaggregates size class, this finding was consistent with the results reported by other authors [67,68], although other authors demonstrated an opposite pattern [69].

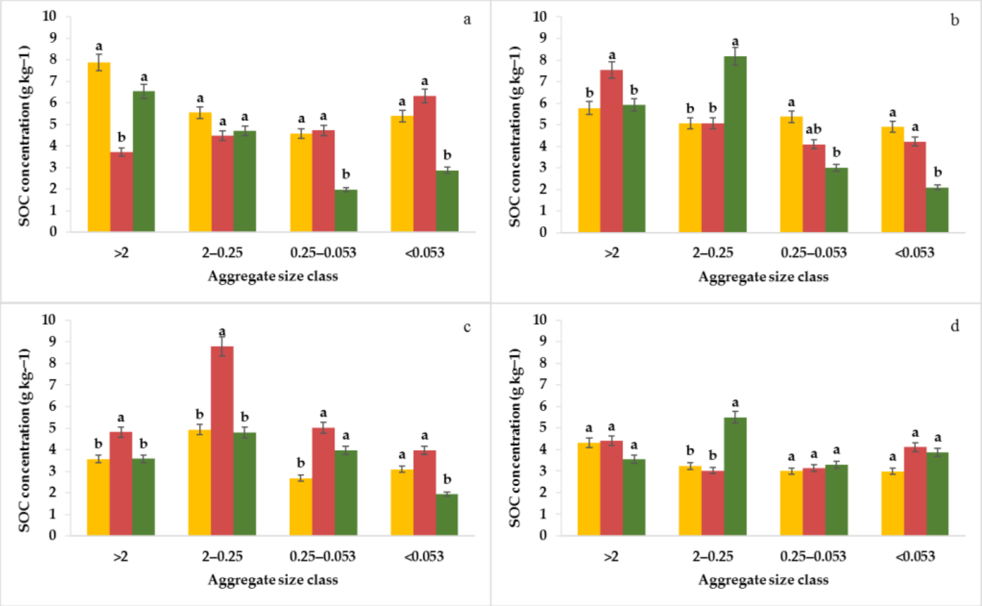

Figure 4. Soil organic carbon (SOC) concentration (g kg^{-1}) in different size of aggregates for initial situation (CT0; yellow bars), conventional-tillage (CT1; red bars) and no-till (NT+H; green bars) treatments at different horizons for (**a**) Ap horizon, (**b**) Bw horizon, (**c**) BC horizon and (**d**) C horizon. Values are given as mean ± standard error ($n = 5$). The different lowercase letters above the error bars for the same aggregate fraction indicate significant differences based on post-hoc tests, Tukey's honestly significant difference (HSD) at $p < 0.05$ between management.

Figure 4 shows that the major SOC concentrations in the aggregate fractions differed between the studied management in the Ap horizon. CT0 and NT+H recorded the highest SOC concentrations in the large macroaggregates while CT1 obtained the highest concentrations in silt+clay fraction. In addition, a reduction in SOC concentrations was observed with regard to the initial situation, especially in the microaggregate fractions of the NT+H due to a reduction in the input of organic components into the soil and in the large macroaggregates of the CT1 management showing an increase in the SOC mineralization rate related to intensive tillage that disrupts soil macroaggregates into microaggregates and thus improves OM decomposition [70]. In this sense, greater C additions from increased organic residues could increase the formation of soil aggregates [71,72] and consequently a greater stabilisation of SOC because macroaggregates formation has been proposed as one of the main mechanisms for SOC protection into the soil [73,74].

In the Bw horizon, SOC concentration values both under tillage (CT1) and no tillage (NT+H) were higher than those found in the initial situation (CT0) in the macroaggregates while in the microaggregates concentrations decreased. At this soil depth, NT+H management obtained the highest aggregate SOC concentration under 250–2000 μm fraction (8.2 g kg^{-1}), while CT1 reached the highest values of large macroaggregates (7.5 g kg^{-1}). In the BC horizon, the SOC concentration values were higher in all fractions under CT1, especially in the small macroaggregates fraction where a SOC concentration of 8.8 g kg^{-1} was reached. The increase in SOC concentration values under CT1 in the BC horizon may be caused by tillage that included small amounts of organic residues in the first soil horizons, favouring C redistribution disproportionately across soil depths and fractions [75].

The TN concentrations of different particle sizes of aggregates in the Ap horizon were higher in CT0 in all the aggregate fractions than in CT1 and NT+H treatments (Figure 5a). The TN concentration of macroaggregate fractions was on average 23.2 and 15.9% higher in CT0 than CT1 and NT+H, respectively. In the microaggregate fractions, the differences were increased with a loss of 51.8% in CT1 and 48.8% in NT+H compared to the initial situation. However, in the Bw and BC horizon (Figure 5b,c), CT1 obtained the highest values in macroaggregate fractions (1.3 g kg⁻¹), while the greatest TN values in microaggregate fractions were found in CT0 (0.75 and 0.56 g kg⁻¹). In the BC horizon, a similar trend to SOC concentration was observed and the content of TN in macroaggregate fractions was increased under CT1, while, in the C horizon, significantly higher values were found in the initial situation in all fractions. In our study, generally the lowest values were observed in NT+H in all aggregate size fractions. Therefore, the implementation of NT+H for a longer period reduced TN concentration in aggregate fractions by removing tillage operations and keeping low residue coverage on the surface, which were easily transported off the farm by water erosion and deteriorated by climatic conditions specific to the region.

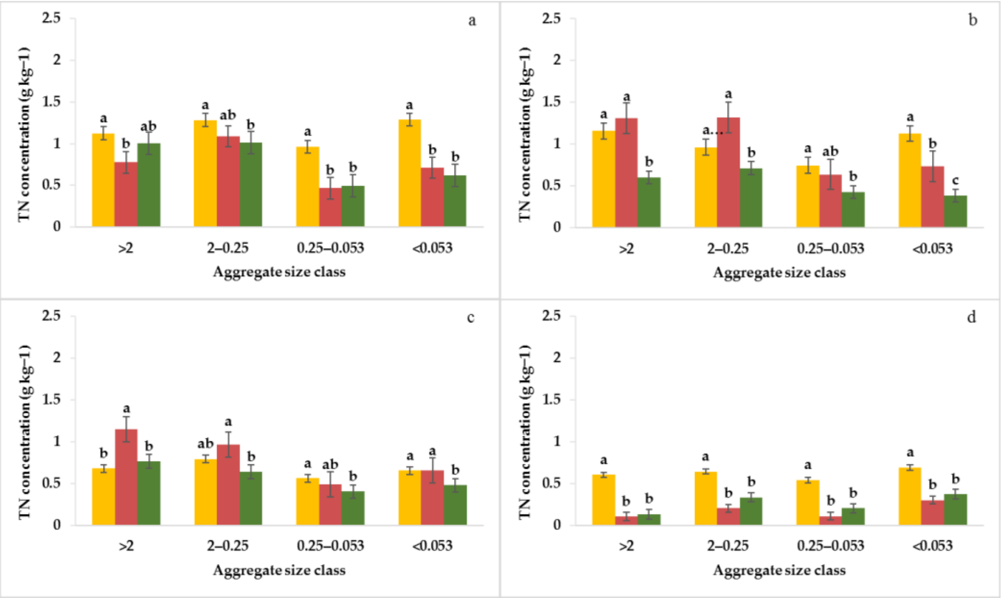

Figure 5. Total nitrogen (TN) concentration (g kg⁻¹) in different size of aggregates for initial situation (CT0; yellow bars), conventional-tillage (CT1; red bars) and no-till (NT+H; green bars) treatments at different horizons for (**a**) Ap horizon, (**b**) Bw horizon, (**c**) BC horizon and (**d**) C horizon. Values are given as mean ± standard error (n = 5). The different lowercase letters above the error bars for the same aggregate fraction indicate significant differences based on post-hoc tests, Tukey's honestly significant difference (HSD) at $p < 0.05$ between management.

3.4. Soil Organic Carbon Preservation Capacity

Across all aggregate sizes to analyse the soil organic carbon preservation capacity of soil aggregates, the organic CPC was calculated (Figure 6). CPC exhibited diverse effects of management on the cumulative C and different sizes of soil aggregates in olive orchard soils. The association between soil aggregates and SOC showed that the SOC pool was greater in macroaggregates (>250 μm) in CT0 in all horizons, while, in CT1, the SOC pool was larger in microaggregates (<250 μm) and in NT+H varied depending on the horizon, with the microaggregates SOC pool predominating in Ap and C and macroaggregates in Bw and BC. As shown in Figure 6, the C storing capacity within the aggregate sizes class was higher in the Ap horizon, although the Bw horizon obtaining similar values showed the important C storing capacity of subsoil horizons. In CT0 among the aggregate fractions, small macroaggregate (2000–250 μm) fractions stored more SOC (5.8 g kg⁻¹) and represented the highest C storing capacity, followed by 250–53 μm aggregate fraction (2.1 g kg⁻¹). The order was as follows: small macroaggregates > microaggregates > large macroaggregates > silt+clay. This CPC aggregate sizes order differs under CT1 and NT+H where microagreggates fraction stored the most SOC (4.5 and 4.4 g kg⁻¹), followed by small macroaggregates (3.5 and 2.0 g kg⁻¹), silt+clay fraction (2.2 and 1.5 g kg⁻¹) and large macroaggregates (0.31 and 0.62 g kg⁻¹), respectively. Therefore, in this management, the

C sequestration effect of microaggregates was stronger than that of macroaggregates in line with other studies [76,77]. The predominance of SOC in the microaggregates in CT1 and NT+H showed that, under these management strategies, the aggregates-associated SOC had less physical protection, lost macroaggregate integrity and reduced soil binding agents. All of these factors promoted a significantly decreased SOC storage capacity of the soil aggregates. Related to this finding, modification in the soil C pool showed how microaggregate fractions are primarily responsible for SOC storage because they are formed by permanent binding agents, can withstand strong mechanical soil disturbance and persist in soils for the long term, while macroaggregates are formed mainly due to transient organic-binding agents, and they are highly susceptible to degradation by soil management practices [78–80].

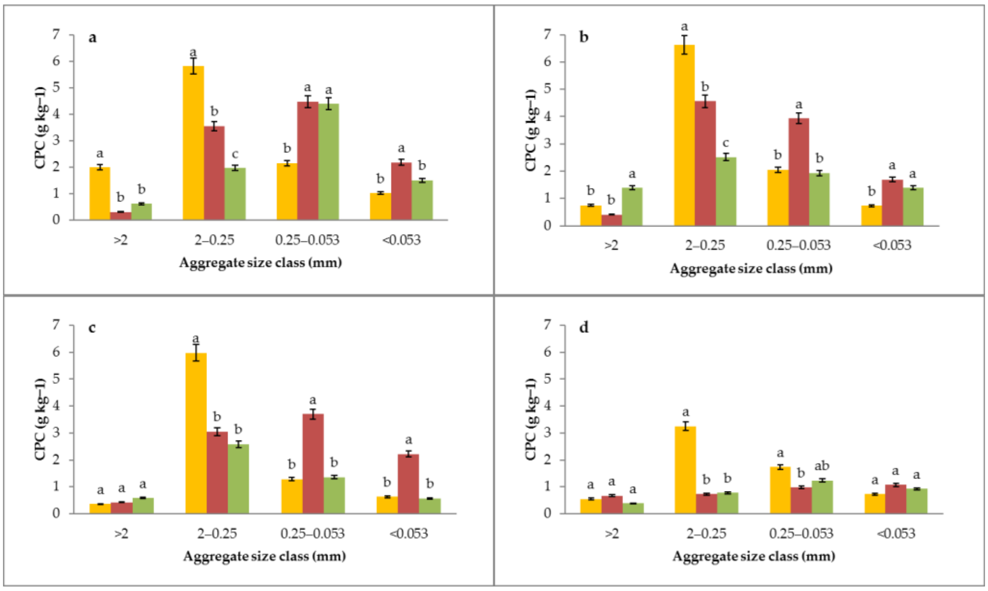

Figure 6. Soil organic carbon preservation capacity (CPC) in g kg^{-1} in different sizes of aggregates for the initial situation (CT0; yellow bars), conventional-till (CT1; red bars) and no-till (NT+H; green bars) treatments at different horizons for (**a**) Ap horizon, (**b**) Bw horizon, (**c**) BC horizon and (**d**) C horizons. Values are given as mean ± standard error (n = 5). The different lowercase letters above the error bars for the same aggregate fraction indicate significant differences based on post-hoc tests, Tukey's honestly significant difference (HSD) at $p < 0.05$ between management.

In more detail, the data obtained showed the relevance of deep horizons in carbon storage and how these horizons are also sensitive to changes produced by different management. In this sense, in horizons Bw and BC (Figure 6b,c), NT+H increased the SOC pool in the large macroaggregates (1.39 g kg^{-1}; 0.58 g kg^{-1}), while CT0 (6.6 g kg^{-1}; 5.9 g kg^{-1}) and CT1 (4.6 g kg^{-1}; 3.0 g kg^{-1}) showed higher values in small macroaggregates. Therefore, in these horizons, it was shown that no tillage limited soil disturbance and enhanced the physical protection of C by aggregates in line with a wide amount of scientific literature [26,72], while tillage promotes the deterioration of large macroaggregates, which are very susceptible to oxidation [81].

In this sense, long-term NT+H resulted in values similar to those followed by CT1, which shows that these management strategies in olive groves deteriorate the C sequestration capacity of soils, showing a greater susceptibility to CO_2 emissions and fertility losses. This SOC storage deterioration was observed not only in the surface layers but also in the deepest ones, especially under CT1. There are large discrepancies and the reasons for this continuous decrease in SOC reserves under NT. Some authors interpret the lack of C storage in croplands due to the suppression of tillage or cover crops as a lack of key plant nutrients [82]. This author indicates that, when faced with nutrient deficiency, plants decompose soil organic matter to extract nutrients, and carbon is released into the atmosphere, leading to land degradation. Therefore, in order to restore at least the values of the initial stage of the study, it seems necessary to implement strategies that increase the input of organic components in the soil, such as the installation of vegetation covering the olive grove alleys or the incorporation of organic amendments [83–85]. These strategies seem necessary to meet the objectives set in the different European strategies concerning climate change adaptation and the improvement of soil properties such as the European Green Deal [86] and Horizon Europe Mission on Soil Health and Food [41].

4. Conclusions

The long-term effects of management practices on the aggregate stability and SOC and TN associated with soil aggregates of different particle sizes were investigated in olive orchards in southern Spain. No till with bare soil did not outperform conventional tillage systems in terms of aggregation and soil aggregate-associated C and N. Both the implementation of NT+H and the maintenance of tillage led to a significant reduction in macroaggregate fractions (>250 µm) and a displacement of aggregate size towards smaller particles. In line with these results, the stability indices analysed in this study, MDW and GMD, showed a significant reduction in stability levels after 16 years of application of these management practices resulting in soil with increased instability.

Moreover, aggregate-associated SOC pools differed over the study period and across aggregate particle sizes. In this sense, the application of the management practices CT1 and NT+H caused a reduction in CPC and the displacement of the main SOC pool in the study area from macroaggregates to microaggregates along the soil profile. Therefore, under these management techniques, soil C protection, C storage was decreased, and CO_2 emissions were enhanced. In conclusion, in light of these results, it seems urgent to change management practices to increase C inputs and promote the formation of macroaggregates for greater C protection and sequestration. This would contribute to strategies that favour climate change mitigation and adaptation.

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