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Crop Diversification Effects on Soil Aggregation and Aggregate-Associated Carbon and Nitrogen in Short-Term Rainfed Olive Groves under Semiarid Mediterranean Conditions

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Abstract: Soil particle aggregation and their associated carbon (C) and nitrogen (N) content can provide valuable diagnostic indicators of changes in soil properties in response to the implementation of different agricultural management practices. In this sense, there is limited knowledge regarding the impact of intercropping on soil organic carbon (SOC) and total nitrogen (TN) pools in aggregates. This study aimed to evaluate the short-term effect (4 years) of three crop diversifications in rainfed olive orchards on soil aggregation, SOC and TN concentration and SOC stocks (SOC-S) under semi-arid Mediterranean conditions. Olive orchards were diversified with *Crocus sativus* (D-S), *Vicia sativa* and *Avena sativa* in rotation (D-O) and *Lavandula x intermedia* (D-L) and compared with monocropping system (CT). Soil samples were collected at two depths (0–10 and 10–30 cm) and analysed for soil aggregate mass, SOC and TN content in aggregate-size fractions obtained by the wet-sieving method. Changes caused by crop diversifications on SOC-S were also determined. Overall, after 4 years, a reduction in aggregation values was observed. However, D-S increased the macroaggregates (>250 µm) percentage, Mean Weigh Diameter values, and Geometric Mean Value in the 0–10 cm. Across treatments, aggregate-associated C in 0–10 cm was higher in the D-S treatment, while in the 10–30 cm soil layer, the greatest values were found in CT. Regarding the SOC-S, after 4 years, significant losses were recorded under CT management in 0–10 cm (-1.21 Mg ha^{-1}) and 10–30 cm (-0.84 Mg ha^{-1}), while D-O and D-L showed similar values to those obtained at the beginning of the study. The highest increases in SOC-S were found in D-S, with an increase of 5.88% in the 0–10 cm and 14.47% in the 10–30 cm. Our results showed the high potential of the diversified cropping system to increase soil stability and SOC sequestration.

Keywords: olive orchards; intercropping; aggregate-associated organic carbon

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1. Introduction

The severe impacts expected from climate change in the Mediterranean area require that adaptation to these environmental conditions is essential for the agricultural sector [1–3]. Particularly vulnerable to these effects are semiarid zones, which have been identified as areas highly exposed to adverse environmental changes [4] such as increased temperature [5] and modifications of the rainfall regimes with increased drought risk [6] and rainfall erosivity [7] and, consequently, soil losses due to water erosion [8].

Considering these environmental risks associated with monocropping and high-input systems, increasing attention is being focused on sustainable management systems and optimized land resource use, contributing to the long-term sustainability of agroecosystems and climate change mitigation and adaptation [9,10]. The transition from intensive soil management practices to a sustainable agricultural system has become one of the significant challenges of contemporary agriculture. In this sense, management practices such as intensive tillage degrade the soil quality and compromise their productive capacity and aptitude to provide ecosystem services [11–14]. On the other hand, the implementation of sustainable management practices, such as reduced tillage with minimal or no-tillage, crop rotation, or establishment of soil coverage, are essential to mitigate CO_2 emissions and increase the C sequestration rates in agricultural soils [5,15]. In addition, sustainable management practices can lead to soil organic matter (SOM) increase and thus improve soil quality by increasing soil fertility [16,17], water retention capacity [18], greater resistance to prolonged periods of drought [19] and erosive processes [20]. In this regard, sustainable management practices have been identified as a crucial element in the achievement of relevant climate change mitigation strategies and policies such as the 4‰ [21], European Green Deal [22], or Horizon Europe Mission on Soil Health and Food [23].

In the soil C balance, soil organic carbon (SOC) inputs or outputs can predominate, generating a flux in which C stored in the soil can be returned to the atmosphere. These SOC losses are caused by soil disruption processes such as erosion, land-use change, and conventional tillage [24–26]. Management practices applied to the soil can modify soil structure, aggregate stability, and organic C distribution, and, consequently, is a critical factor in C sequestration and storage processes [27–29]. SOM is the primary binding agent in the constitution of aggregates, which can follow a process of formation or degradation according to the disturbances suffered by the soil [30]. Through the aggregation process, SOC is protected [31,32], and thus different C pools with variable degrees of stabilization are established [33,34]. Indeed, aggregate formation and breakdown and the SOC distribution in the different soil aggregate fractions play a key role in understanding C sequestration and stabilization processes [35,36]. Moreover, the diverse aggregates composition of different sizes class is influenced by human impacts, among which land use modification and management plays a vital role in altering the soil aggregates composition [37].

The intercropping system, i.e., cultivating two or more crop species simultaneously in the same field area [38], has been proposed as a sustainable management practice that enhances soil productivity as well as encouraging the more efficient use of resources such as water or nutrients [39–41] and promoting bacterial community structure changes [42]. In addition, several studies have demonstrated that intercropping significantly promotes agroecosystem services [43–46]. In this sense, the implementation of an intercropping system could increase the parameter values such as SOC, total nitrogen (TN) content, or soil aggregate stability [47–49], accordingly enhancing soil quality [50,51]. However, the combination of species selected to constitute the intercropping system is critical to achieving a sustainable cropping system that provides the benefits mentioned above [52] because some crop diversifications are more effective than others [53]. Furthermore, aspects such as competition for light, water, or nutrients must be assessed before developing these management systems. In woody crops, and specifically in rainfed olive groves, little attention has been paid to the impacts of crop diversification on C stock associated with aggregate size fractions and the influence of the management system on their distribution with a low input rainfed management regime in semi-arid Mediterranean conditions.

Therefore, this study assessed the implementation of three crop diversifications (*Crocus sativus*, *Vicia sativa*, and *Avena sativa* in rotation and *Lavandula x intermedia*) in the alley between olive orchard rows and their impact on the particle size distribution of soil aggregates, the concentrations of SOC and TN associated with the aggregates size and SOC-S in aggregates to understand the effect of the intercropping system in a Calcaric Cambisol soil under semiarid conditions. We hypothesized that olive orchards diversification would improve soil aggregation and C storage compared to the monocropping olive system. The specific purposes of this study were to evaluate the impact of the crop diversification system on: (i) soil aggregate size distribution and structural stability; (ii) aggregate-associated SOC and TN; and (iii) SOC-S in aggregate fractions balance under low-input rainfed olive cropping system.

2. Materials and Methods

2.1. Study Site

This research was performed on an experimental olive farm (slope gradient 4–8%; elevation 450 m) in Torredelcampo (Jaén, Andalusia, Spain, 37°46′26.0″ N, 3°54′41.5″ W) (Figure 1). Due to historical reasons, the degradation of the olive grove in this area has been severe since the middle of the last century due to agricultural management intensification. The site is characterised by low hills with centenary rainfed olive trees (*Olea europea* var. picual) with 2–3 trunks under monocropping conditions and large growing frames (12 m × 12 m pattern). This is the most common olive farm typology in the region.

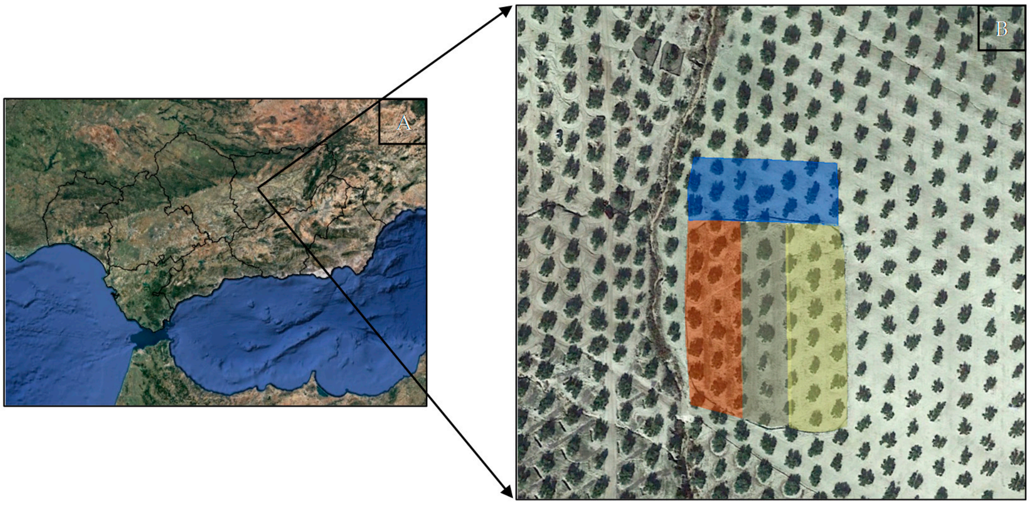

Figure 1. Location map Andalusia region (**A**) and aerial view of the experimental treatments (**B**); conventional tillage (blue); saffron (orange); oats (grey), and lavender (yellow bars).

The Mediterranean climate dominates the area, with average precipitation and temperature of 493 mm and 16.6 °C, respectively. There are important thermal oscillations throughout the study area throughout the year, winters with average minimum temperatures of 8.3 °C in January and hot and dry summers that reach an average of over 27 °C in July.

2.2. Experimental Detail

The research methodology was included in the H2020—Diverfarming project (Crop diversification and low-input farming cross Europe: from practitioners' engagement and ecosystems services to increased revenues and value chain organisation). The experimental design included four scenarios varying in the cropping system, tillage, intercropping crop, and residue management practices (Figure 2). The study was conducted for four years, from 2018 to 2021, in the mentioned study area. The traditional management of the farm was conventional tillage (CT), but in 2018, a portion of the area dedicated to CT changed to agricultural management with three different crop diversifications, *Crocus sativus* (saffron) (D-S), *Vicia sativa* (vetch), and *Avena sativa* (oat) in rotation (D-O) and *Lavandula x intermedia* (lavender) (D-L), diversifications crops were implemented in the olive alleys and each diversification covered about 2000 m^2. In CT, fertilizer was applied (100 kg ha^{-1} urea, N richness 46%) in alternate years after the olives had been harvested. In addition, the pruning remains were incorporated every two years after the pruning of the olive trees, and fungicides (copper oxychloride 34.5% w.p.) were spread. In CT treatment, vegetation was eliminated by applying preemergence herbicides (1.0 L 36% glyphosate ha^{-1}) in autumn to control weeds. The plot managed under CT was tilled (25 cm) with a cultivator in spring, followed by tine and disc harrowing in summer. Diversification plots were established without irrigation, no herbicides or fertilizer were applied, and cultivation work consisted of incorporating olive pruning residues, mechanical weeding carried out using a manually operated star cono weeder, and minimum tillage (10 cm) for the seedbed (in D-O, tillage was carried out annually). Diversification was selected according to soil and environmental conditions, looking for crops with the capacity to grow in poor soils, with low water requirements, and resistant to drought periods. In addition, the Diverfarming project aimed at economic profitability for the farmer. *Crocus sativus* was sown only once with seed rates of 2000 kg ha^{-1}, and *Lavandula x intermedia* needed replanting in the second year (12,000 plants ha^{-1}). On the other hand, *Vicia sativa* and *Avena sativa* were grown annually with a seed rate of 120 kg ha^{-1} and 140 kg ha^{-1}, respectively.

Figure 2. Management systems and crop diversifications implemented in the study area (**A**) Olive intercropping with *Crocus sativus*, (**B**) Olive intercropping with *Lavandula x Intermedia*, (**C**) Conventional Tillage, and (**D**) Olive intercropping with *Vicia Sativa* and *Avena sativa* in rotation.

2.3. Soil Sampling

Soil samples were collected in September 2018 and 2021 in all research plots simultaneously. A randomized block design with three replicate plots per treatment was established to compare the diversification managements (D-S, D-O, and D-L) with the monocrop system (CT). Under each management, nine random sampling points were selected in the experimental plots, and soil samples were collected in the olive alleys at 0–10 cm and 10–30 cm depth. The soils were characterised by low organic matter levels [11] and high erosion rates [12] that have deteriorated soil properties and quality. According to World Reference Base for Soil Resources, the soil of the study site was classified as Calcaric Cambisol [54]. The general physicochemical 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 1.30 mg m^{-3}, and (iv) organic matter content ranging between 1.2% and 0.7%.

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

According to the wet-sieving process, soil samples collected were passed through an 8 mm sieve to remove roots and rock fragments before the continuous oscillating soil aggregates in water. The aggregate stability analysis of soil was performed by the wet sieving method [55]. Three sieves were used for the aggregate size distribution (2000, 250, 53 µm). In the wet-sieving procedure, 100 g air-dried soil samples were placed in a 2000 µm sieve and submerged in distilled water for five minutes. After the slaking process, manual wet sieving was performed. The soil aggregates were oscillated in water at 50 cycles for 2 min and passed through progressively smaller sieves (i.e., 250 and 53 µm mesh sizes). After the oscillating process, the remaining soil aggregates on each mesh screen were washed from the sieves into aluminium pans, oven-dried at 50 °C for 24 h, and weighed separately by aggregate-size class (i.e., >2000, 2000–250, 250–53, and <53 µm).

Soil Mean Weight Diameter (MWD) Equation (1), Geometric Mean Diameter (GMD) Equation (2) and GMD sensitivity index (SI_{GMD}) Equation (3) were calculated to analyse soil aggregate stability index [56,57]. The MWD, GMD and SI_{GMD} were determined as follows:

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

where, n indicates the number of aggregate size fractions, x_i represents the mean diameter in millimetres of aggregates remaining on the respective sieves, w_i represents the proportion of the total soil sample weight associated with each size fraction.

$$\mathrm{GMD} = \exp\left[\frac{\sum_{i=1}^{n} w_i \log x_i}{\sum_{i=1}^{n} w_i}\right] \tag{2}$$

where, n represents the number of aggregate size fractions x_i indicates the mean diameter of each aggregate oversize of each sieve, w_i denotes the dry weight of the total sample in the corresponding size fraction.

$$SI_{GMD} = \mathrm{GMDt}/\mathrm{GMDc} \tag{3}$$

where GMDt is the GMD of each management, and GMDc is the GMD of the control treatment in the corresponding soil layer.

Soil aggregate fractions were ground with mortar for the aggregate-associated SOC and TN measurement by the dry combustion method with a CN elemental analyser [58]. The aggregates SOC stock (SOC-S) Equation (4) was calculated as follows [59]:

$$\text{SOC-S} = \mathrm{Bd} \times \mathrm{H}\,(1-\delta)\sum_{i=1}^{n} m_i \mathrm{SOC}_i \tag{4}$$

where Bd denotes the bulk density (mg m^{-3}) of the soil sample, H represents the soil horizon thickness (cm), δ is the gravel mass (%), n indicates a number of aggregate size fractions, m_i is the aggregate mass ratio (%), and SOC_i is the soil organic carbon content in each size aggregate fraction (g kg^{-1}).

2.5. Statistical Analyses

The interactive effects of intercropping and monocropping systems related to aggregate size distribution, soil aggregate stability, aggregate-associated SOC and TN, and aggregates SOC-S were previously tested for normality to verify the model assumptions using the Kolmogorov–Smirnov test. When significant differences among treatments were identified, mean comparisons were performed with one-way analysis of variance (ANOVA) followed by post hoc tests, Tukey's honestly significant difference (HSD), to compare soil properties data among the treatments (significance level of $p < 0.05$). Two-way ANOVA was applied to compare changes in soil properties in factors depth and time, Dunnett's was used for comparisons between diversifications and the control system. Statistical analyses were carried out using SigmaPlot v14.0 (San Jose, CA, USA).

3. Results

3.1. Aggregate Size Distribution

In the study period, the soil aggregate size distribution under the wet sieving method is shown in Figure 3 (surface layer) and Figure 4 (subsurface layer). 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 (2018) to the end of the study period (2021). In the surface layer (0–10) (Figure 3), at the beginning of the research, all managements presented similar macroaggregate (>250 µm) values D-L and D-O registered 36.9 and 36.4%, respectively, followed by D-S (33.1%) and CT (31.9%) (Figure 3a,b). Over time, the amount of macroaggregates decreased in managements under D-O (−8%), D-L (−5.5%), CT (−4.3%) and increased in D-S (1.2%). In the 10–30 cm depth, the highest macroaggregate percentage was recorded in 2018 under D-L (47.1%), while the rest of the management obtained similar values, ranging between 38 and 38.5% (Figure 4a,b). After four years, the higher macroaggregate losses were detected under D-L (−10.5%) and CT (−9.4%), while D-S (−4.1%) and D-O (−3.7%) obtained lower decreases in macroaggregates amount.

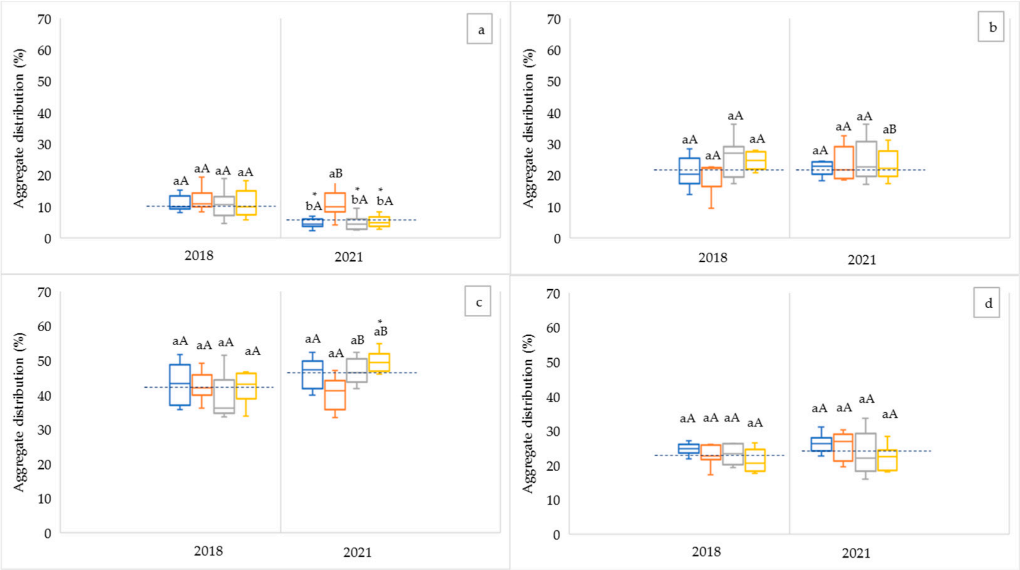

Figure 3. Soil water-stable aggregate size distribution (%) for conventional-tillage (CT; blue bars); saffron (D-S; orange bars); oats (D-O; grey bars), and lavender (D-L; yellow bars) treatments at 0–10 cm depth for (**a**) >2000 µm, (**b**) 250–2000 µm, (**c**) 53–250 µm and (**d**) <53 µm. The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Different capital letters are indicating differences in depth between 0–10 cm layer and 10–30 cm layer which is represented in Figure 4. Each cultivation system (*) represents significant differences between managements compared to olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test ($p < 0.01$); missing asterisks denote non-significant differences.

The dominant aggregate size class in the 0–10 cm soil layer was the 250–53 µm fraction, both at the beginning and the end of the research period, with percentages between 42% and 58% of the total soil sample, followed by the 250–2000 µm and <53 µm fractions (Figure 3b–d). While in the 10–30 cm soil layer, the aggregate size class predominant was also 250–53 µm (Figure 4c). This showed that the intercrop establishment in olive groves did not affect the dominance of the predominant fraction in the short term under the soil management implemented. In addition, the hierarchical distribution of soil aggregates in the analysed area was not modified.

In depth significant differences were found in the D-S (>2000 µm fraction) and D-L plots (250–2000 and 250–53 µm fractions) in both years (2018 and 2021). These significant differences showed higher large macroaggregates (>2000 µm) in D-S in 0–10 cm and higher small macroaggregates (250–2000 µm) and large microaggregates (250–53 µm) fractions in D-L in 10–30 cm and 0–10 cm soil layer, respectively.

The main changes in the aggregate fractions of the analysed managements were observed in the >2000 µm fraction of the 0–10 cm layer, where all the analysed managements reduced the percentage of this fraction significantly about the conventional tillage system (Figure 3a) except D-S management, which maintained the rate of this aggregate size fraction above 10%. In addition, significant differences were found in some aggregate size fractions at 10–30 soil layers. As in the topsoil layer, a reduction in a large macroaggregate (>2000 µm) was found, which was especially significant in CT and D-O management, while in the rest of the management, similar values to the initial ones were obtained. The other analysed fractions remained identical to the values recorded at the beginning of the experiment, both in 0–10 and 10–30 cm layers, except for the 53–250 µm fraction under D-L diversification, which recorded an increase reaching 49.6% in the surface layer.

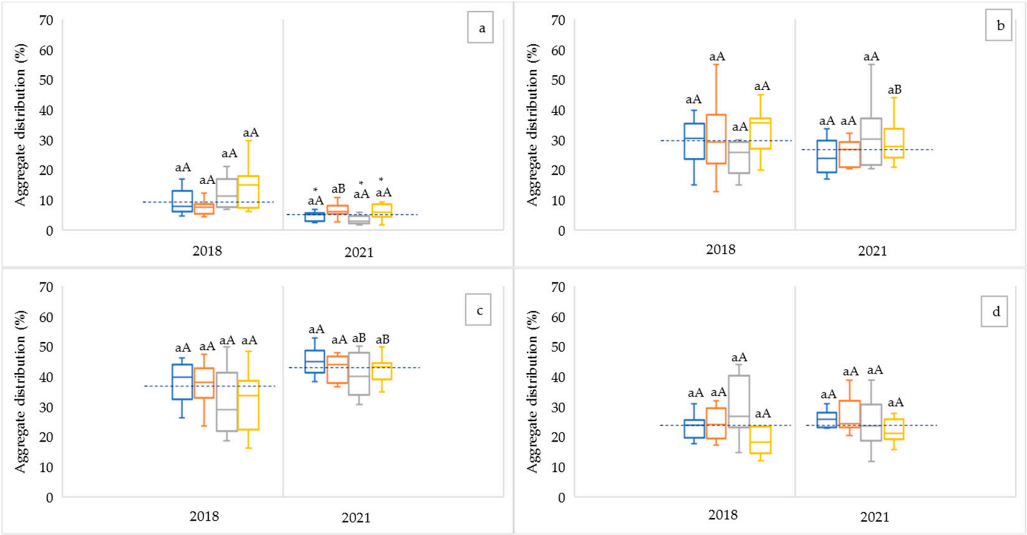

Figure 4. Soil water-stable aggregate size distribution (%) for conventional-tillage (CT; blue bars); saffron (D-S; orange bars); oats (D-O; grey bars), and lavender (D-L; yellow bars) treatments at 10–30 cm depth for (**a**) >2000 µm, (**b**) 250–2000 µm, (**c**) 53–250 µm and (**d**) <53 µm. The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Different capital letters are indicating differences in depth between 10–30 cm layer and 0–10 cm layer which is represented in Figure 3. Each cultivation system (*) represents significant differences with respect to the olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test ($p < 0.01$); missing asterisks denote non-significant differences.

3.2. Soil Aggregate Stability

In the study area, soil aggregate stability was determined by calculating three different soil aggregation indices: MWD, GMD, and SI_{GMD} (Figure 5 and Table 1). In the 0–10 cm soil layer, the MWD ranged between 0.50 and 0.83 mm, and similar values were found in the 10–30 cm (0.50–0.92 mm). The highest MWD values were observed in the surface layer at the end of the analysed period (2021) in D-S management (0.83 mm). In the short-term, significant variations were detected in this layer (Figure 5a), while in D-S management, an increase was registered. In the rest of the management (CT, D-O, and D-L), MWD values decreased by 0.48, 0.52 and 0.50 mm, respectively. In the 10–30 cm soil layer (Figure 5b), the trend found was a significant decline concerning the control (0.72 mm; 2018) under the annually tilled management CT (0.50 mm) and D-O (0.53 mm), while in the non-tilled managements, the decreases were smaller: D-S (0.62 mm) and D-L (0.65 mm).

Regarding GMD, similar values for the two analysed soil layers were found. However, the GMD of the aggregates presented significant differences between treatments in the surface layer, with higher GMD values in the D-S diversification plot (0.26 mm) (Figure 5c). In comparison, D-L (0.24 mm) obtained the higher values in the 10–30 cm soil layer (Figure 5d). However, significant differences with the introduction of diversifications crops regarding the control management in 2018 were not detected in this aggregation index.

In depth significant MWD differences were only detected in the D-S plot in 2021 due to the increase in MWD values in the 0–10 cm layer (Figure 5a,b). In GMD, differences were observed in the D-L plot in 2018 but not in 2021 (Figure 5c,d).

On the other hand, SI_{GMD} values (Table 1) showed both in the surface layer (0–10 cm) and in the 10–30 cm layer significant differences with continued CT management (0.81 and 0.79 mm, respectively) with regard to the same management in 2018. However, although the other studied treatments showed no differences between the surface layer, it is evident that the D-S treatment presented 24% higher than the reference treatment (CT; 2018). Significant differences in depth were found in the D-S plot with higher SI_{GMD} values in 0–10 cm (1.24 mm) than in the 10–30 cm (0.90 mm).

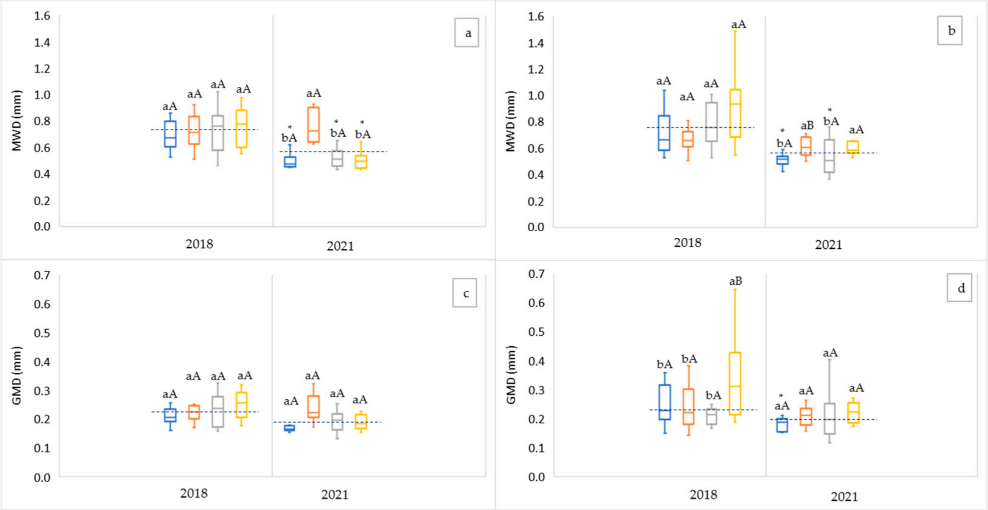

Figure 5. Mean weight diameter (MWD) and geometric mean diameter (GMD), for conventional-tillage (CT; blue bars); saffron (D-S; orange bars); oats (D-O; grey bars) and lavender (D-L; yellow bars) treatments at (**a**) 0–10 cm depth, (**b**) 10–30 cm depth, (**c**) 0–10 cm depth and (**d**) 10–30 cm depth. The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Different capital letters are indicating differences in depth. Each cultivation system (*) represents significant differences with respect to the olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test ($p < 0.001$). Missing asterisks denote non-significant differences.

Table 1. GMD sensitivity index (SI_{GMD}) in the 0–10, and 10–30 cm layers.

Treatments	SI_{GMD}	
	0–10	**10–30**
D-S	1.24 aA	0.90 aB
D-O	0.94 aA	0.91 aA
D-L	0.90 aA	1.05 aA
CT (2021)	0.81 bA	0.79 bA
CT (2018)	1.00 aA	1.00 aA

Treatments; saffron (D-S), oats (D-O), lavender (D-L), conventional tillage (CT 2021), and conventional tillage (CT 2018). Means followed by the different lowercase letters in the columns are significantly different from each other ($p < 0.05$). Different capital letters are indicating differences in depth.

3.3. Aggregate Associated SOC and TN

Higher SOC and TN contents were detected in macroaggregate fractions (>250 µm) in all treatments and soil layers. The aggregate-associated SOC results (Figure 6) showed improvements in the surface layer for intercropping with D-S regarding CT. However, this increase was only significant in the <53 µm aggregate size fraction. D-S reached the highest SOC levels in all analysed fractions in this layer. At the same time, D-O and D-L registered SOC losses in the different aggregate sizes, which were especially significant in the 250–2000 µm fraction under D-L, showing a similar trend to that found under CT management for the studied period. In the 10–30 cm layer, no significant differences were recorded with respect to CT in the initial situation after four years. However, in D-S diversification, SOC increases were observed in all fractions while D-O and D-L showed the opposite trend.

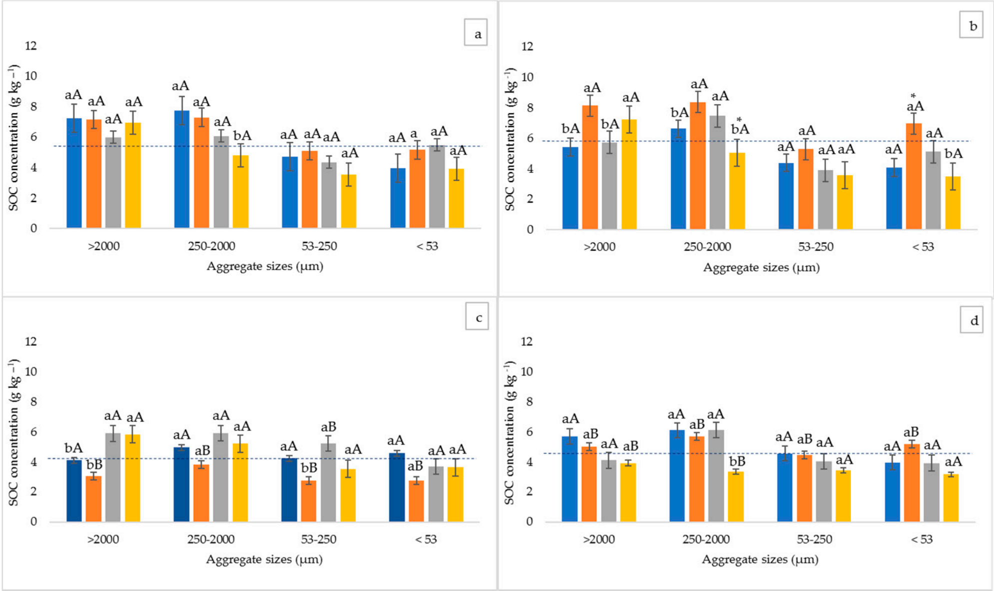

Figure 6. Aggregate-associated soil organic carbon (SOC) concentration (g kg^{-1}) in different sizes of soil water-stable aggregates for conventional-tillage (CT; blue bars); saffron (D-S; orange bars); oats (D-O; grey bars) and lavender (D-L; yellow bars) treatments at (**a**) 0–10 cm depth (2018), (**b**) 0–10 cm depth (2021), (**c**) 10–30 cm depth (2018) and (**d**) 10–30 cm depth (2021). The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Different capital letters are indicating differences in depth. Each cultivation system (*) represents significant differences with respect to the olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test (* $p < 0.05$). Missing asterisks denote non-significant differences.

Considering the variations in the aggregate-associated TN between different sizes of soil water-stable aggregates and treatments, significant differences were found in the surface soil layer in >2000 µm, where an increase was detected under D-O and D-S, reaching 1.72 and 1.11 g kg^{-1}, respectively, while in the initial situation under CT values they were 0.72 g kg^{-1}. Meanwhile, D-L recorded significant TN losses in the 250–2000 µm and <53 µm fractions, which amounted to −0.40 and −0.37 g kg^{-1} with regard to the initial situation, respectively. In the 10–30 cm layer, CT significatively increased the TN concentration in >2000 µm (+0.52 g kg^{-1}), while D-S increased TN values in 53–250 µm (+0.64 g kg^{-1}).

Regarding depth, the main variations were detected in D-S diversification where all fractions showed significant differences between the two analysed layers with higher SOC values in 0–10 cm in 2018 and these differences were maintained in 2021 (Figure 6a–d). In D-L, differences were only found in the macroaggregate fractions (>2000 and 250–2000 µm) in 2021 due to a reduction in SOC content in the 10–30 cm layer in these aggregate size fractions. In TN, differences in depth were detected in the macroaggregates (>250 µm), thus CT registered variations in >2000 µm in 2021 due to the increase in TN in 10–30 cm (Figure 7d), while in the same aggregate size fraction D-O increased TN content in 0–10 cm and showed significant differences with the 10–30 cm layer (Figure 7b). In the 250–2000 fraction, differences were found in D-S and D-L in 2021 due to a reduction in TN content in the 10–30 cm layer (Figure 7d).

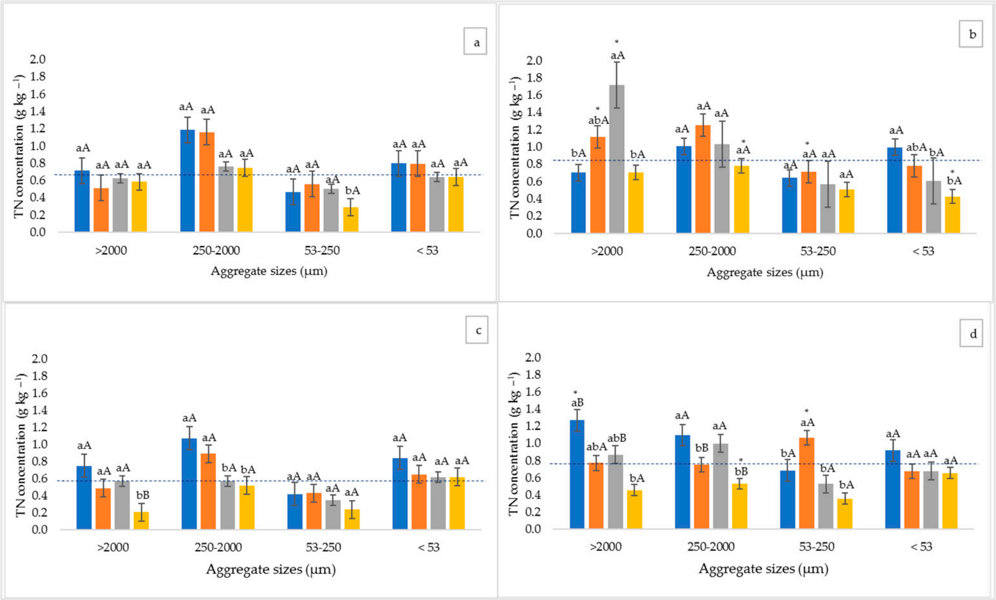

Figure 7. Aggregate-associated total nitrogen (TN) concentration (g kg^{-1}) in different sizes of soil water-stable aggregates for conventional-tillage (CT; blue bars); saffron (D-S; orange bars); oats (D-O; grey bars) and lavender (D-L; yellow bars) treatments at (**a**) 0–10 cm depth (2018), (**b**) 0–10 cm depth (2021), (**c**) 10–30 cm depth (2018) and (**d**) 10–30 cm depth (2021). The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Different capital letters are indicating differences in depth. Each cultivation system (*) represents significant differences with respect to the olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test (* $p < 0.05$). Missing asterisks denote non-significant differences.

3.4. SOC Stock in Soil Aggregates Size Class

In the short-term period, the reserves of SOC-S related to soil aggregates size class and soil depths were determined (Figure 8). In all analysed managements, periods, and layers, microaggregates (<250 µm) reached a higher SOC-S content than macroaggregates (>250 µm). In more detail, the aggregate fractions analysed showed the following order 53–250 µm > 250–2000 µm > 53 µm > 2000 µm, except for the D-L management in 2018 at 10–30 cm depth, which showed a higher content in 250–2000 µm than the 53–250 µm size fraction.

In the surface layer, significant differences were found between the respective treatments in 2021 (Figure 8a,b). In this sense, the highest SOC-S values were found under D-S management in 2018 (4.9 Mg ha^{-1}) and 2021 (5.2 Mg ha^{-1}), followed by D-O (4.1 Mg $ha^{-1)}$ and D-L (3.7 Mg ha^{-1}), which maintained the same SOC-S level both in 2018 and 2021. Furthermore, the lowest values were detected under CT in 2021 (2.6 Mg ha^{-1}) These results implied significant losses under this management in the studied period (−1.2 Mg ha^{-1}).

In contrast, CT treatment reached the highest SOC-S values in the 10–30 cm soil layer in 2018 (9.3 Mg ha^{-1}), while in 2021, the highest levels were found in D-S (9.9 Mg ha^{-1}); therefore, in the short-term under this management, the SOC-S content increased in 1.2 Mg ha^{-1} (Figure 8c,d). In this soil layer under the diversifications, D-O and D-L, the lowest SOC-S values, were registered with equivalent results in the initial and final periods of the research (7.3 and 6.4 Mg ha^{-1}, respectively), showing significant differences compared to the olive monocrop in the initial stage.

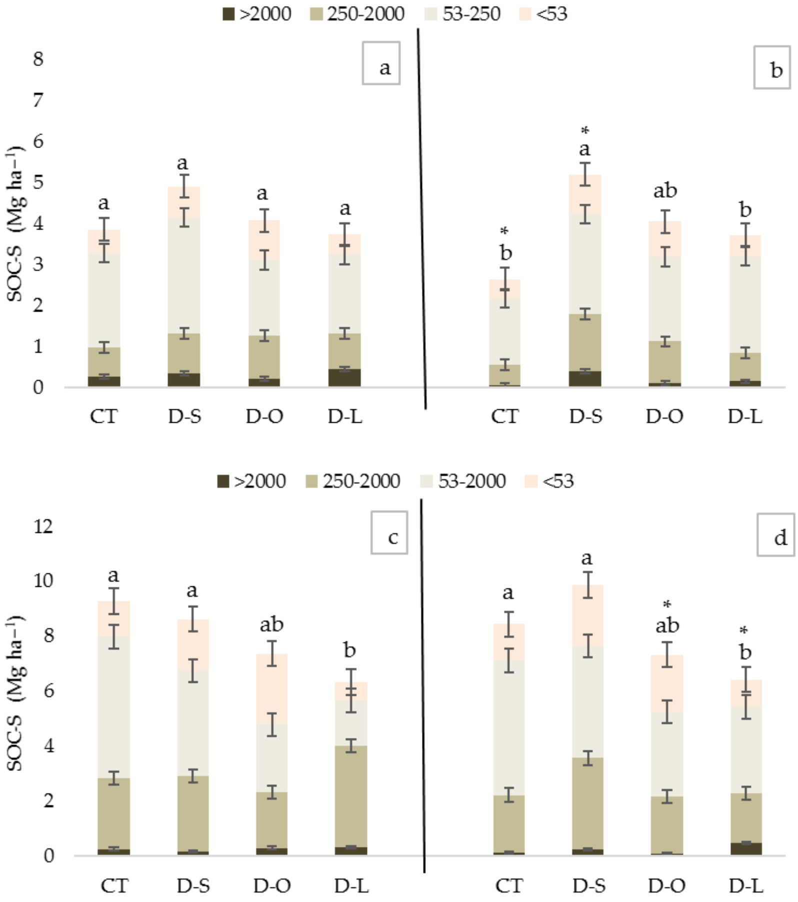

Figure 8. Aggregate-associated soil organic carbon stocks (SOC-S) distribution (Mg ha^{-1}) within soil water-stable aggregates for conventional-tillage (CT); saffron (D-S); oats (D-O) and lavender (D-L) treatments at (**a**) 0–10 cm depth (2018), (**b**) 0–10 cm depth (2021), (**c**) 10–30 cm depth (2018) and (**d**) 10–30 cm depth (2021). The different lowercase letters above the error bars for the same soil layer indicate significant differences between managements at $p < 0.05$. Each cultivation system (*) represents significant differences with respect to the olive monocrop conventional tillage system in 2018 (control treatment) by Dunnett's test (* $p < 0.05$). Missing asterisks denote non-significant differences.

4. Discussion

4.1. Soil Aggregate Distribution and Stability

Crop diversification is recognized as an environmentally sustainable agricultural practice that improves the efficiency of agroecosystems and increases the sustainable use of resources [44,60,61]. Although several studies have been conducted on cover crops in olive groves (i.e., herbaceous plants established in the inter-rows of olive groves) [62–65], little is known about integrating a second crop in the olive grove alleys.

Regardless of the established system (monocrop or diversification), the microaggregates played predominant roles in the 0–10 and 10–30 cm soil layers, indicating a lack of transformation in soil aggregate composition in the hierarchy of the dominant soil aggregate fractions in the short term. In relation to this, the microaggregates predominance in the soil structure has been detected in several studies as a crucial factor in the dynamics of different soil functions such as infiltration, water retention, and carbon sequestration [66]. The dominance of microaggregates in the surface layer is especially relevant due to the soil degradation process. Therefore, it can aggravate erosive processes [67] because the predominance of fine particles reduces the porosity and promotes surface soil sealing, thus enhancing runoff rates. In the study area, a long period of intensive tillage has caused significant erosion rates detected in previous studies [13]. In this sense, soil erosion, transport, and sedimentation promote the modification of aggregate size distributions [68,69]. This soil structural configuration in the context of torrential precipitations characteristic of the Mediterranean region hinders the implementation of diversifications and soil properties restoration that increase soil quality, since erosive processes encourage soil loss and thus reduce fertile soil layers and fertility [70,71].

During the study period, all managements reduced the macroaggregate percentage (>250 µm) in the short term except D-S, which increased slightly. The lowest macroaggregate levels were detected in 2021 in CT management in the surface and 10–30 cm soil layers. In relation to these results, it has been widely reported that tillage destroys the macroaggregates contributing to the microaggregates predominance [72,73]. Although important soil structure and aggregate size distribution modifications under different managements were not detected, and intercropping did not significantly increase the levels of macroaggregates, in D-S diversification, a different trend was observed because no significant losses in macroaggregates were recorded. The level of large macroaggregates (>2000) increased with regard to the reference treatment (CT) and the other diversifications (D-O and D-L). This was reflected in the aggregation indices MWD, GMD, and SI_{GMD}, which showed a higher soil aggregation under D-S, especially in the 0–10 cm layer (0.83, 0.26, and 1.24 mm, respectively). At the same time, D-O (0.52, 0.19 and 0.94 mm), D-L (0.50, 0.19 and 0.90 mm) and CT (0.48, 016, 0.81 mm) remained at lower values. The higher aggregation index values found in D-S diversification in the soil surface layer could be due to the greater development of the vegetation cover associated with saffron cultivation. In this sense, the root system affects the macroaggregation process and soil stability through the natural release of exudates of spontaneous vegetation into the soil, promoting a more stable soil structure around the roots [74]. On the other hand, a greater amount of plant residues associated with the spontaneous biomass could also affect larger particle size aggregation [67]. In this sense, the D-S management was the only management that obtained significantly higher MWD values in the 0–10 cm than in the 10–30 cm layer, showing the greater effect of the management change on the aggregation rates in the surface layer. Similar findings were observed for Kumar et al. [75] under the zero tillage and crop diversification system. The lack of spontaneous cover crop development in combination with lavender cultivation due to the high levels of soil degradation and the tillage applied to the D-O diversification for the annual planting of the cover crop could be the reasons for which the results obtained in the D-S diversification were not found in the other diversifications implemented in the study area. Jat et al. [76] evaluated the effect of zero tillage and crop diversification on soil aggregation and found an improvement in total water-stable aggregates after 4 years of the experiment. Similarly, Nunes et al. [77] observed the beneficial effect of the cropping system diversification on the formation and preservation of water-stable aggregates during the same temporal period on plots under no-till. In these studies, crop residue retention and tillage removal were highlighted as key factors in increasing soil aggregation levels. In line with these results, Singh et al. [78], in a 3-year study of the semi-arid soil of India under rainfed conditions, stated that diverse crop groups did not affect the soil aggregation; however, the crop residue retention resulted in about 20% increase in aggregation indices.

4.2. SOC Stock and Aggregate-Associated C and N

Numerous studies have demonstrated that sustainable soil management, such as soil coverage and spontaneous vegetation in the inter-row areas, significantly affects the dynamics of SOC and nutrients in olive orchards [79–81]. However, reports of the impacts of intercropping and diversification on the aggregate-associated SOC, total nitrogen, and SOC aggregate storage are few.

In our study area, aggregates SOC concentration decreased with soil depth from 0 to 10 to 10−30 cm depth. In D-S diversification, significant differences in SOC concentration were found in all fractions in the two analysed periods and in D-L in 2021 linked to a SOC decrease in the macroaggregates fractions of 10–30 cm layer. TN showed significant differences between the soil analysed layers, mainly in 2021, due to a higher increase in TN concentration in the macroaggregates in diversification plots in 0–10 cm and in CT in

10–30 cm. Differences in depth were caused by an increase in SOC and TN in the 0–10 layer or a reduction in the 10–30 layer under the diversifications plots and the contrary trend in the monocropping system. Therefore, in line with other research [82,83], in diversifications plots surface layer, (0–10 cm) was shown to be more sensitive to increased SOC and TN than the subsurface layer (10–30 cm). The macroaggregates fraction (>250 µm) obtained the highest mean SOC and TN concentration in soil for all the treatments and depths (Figures 6 and 7); these aggregate size fractions contained between 15–48% and 14–40% higher SOC and TN contents, respectively than microaggregates (<250 µm) in soil. This finding is related to the fact that organic matter acts as an essential binding agent in soil aggregation processes, and microaggregates are occluded into macroaggregates establishing the soil aggregate hierarchy model [84]. However, considering the SOC-S in soil aggregates size classes (Figure 8), it was observed that microaggregate fractions concentrated the largest proportion of SOC-S in the soil, since microaggregates obtained the highest aggregate soil mass. These results showed that although macroaggregates have a greater capacity to contain SOC and TN, these soils, highly degraded due to a long history of unsustainable management, have lost the majority of the SOC-S available in macroaggregates, which are extremely sensitive to management alterations and are easily disrupted. In contrast, microaggregates were the largest reservoir of SOC in these soils because they provide physical protection to the SOC, thus promoting the long-term fixation of SOC into the soil [85–87]. In this regard, the sustainable management practices aimed at increasing C stocks and C sequestration in agricultural soils should encourage the development and preservation of macroaggregates in line with Zheng et al. [88], whose evaluation and monitoring can serve as a key parameter in the impact assessment of the implemented crop management and diversifications.

Under D-S, saffron associated with spontaneous cover for a large part of the crop cycle enhanced SOC and TN content in all aggregate fractions in the surface and subsurface soil layer (Figures 6 and 7). These results are connected to better soil aggregation (macroaggregate percentage and MWD). Under D-O and D-L diversifications, the results obtained in SOC concentration were not as positive as those shown in D-S and were similar to those found under CT. However, in the superficial layer, TN content showed a significant increase, especially in the large macroaggregate fraction (>2000 µm) under D-O diversification. However, in the subsurface layer, the values were similar to or lower than those observed in CT. Our data suggest that the presence of crop residues on the soil surface enhances soil responses under D-S, not only the lack of soil disturbance. However, in the diversifications under D-O, where soil disturbance by tillage was maintained, and D-L, where no spontaneous vegetation associated with lavender was generated, the results were close to those obtained in the control treatment. Hence, the quantity, quality, and residues covering soil appear to influence the aggregate fractions' C and N attributes [49]. Related to these aspects, intercropping under long-term sustainable practices is expected to significantly increase the macroaggregate amount, the structural stability, and the SOC and N associated with the aggregates.

In the study area, aggregate-associated SOC-S in microaggregates (53–250 µm) presented a higher contribution to the soil C storage in the surface layer (0–10 cm) and subsurface layer (10–30 cm), indicating that microaggregates were the main SOC reservoir in the soil. Based on the above findings, the associated SOC-S in soil aggregates was determined by the mass proportion of aggregate size class in the short-term analysed period. The aggregate-associated SOC-S distribution in the intercropping olive orchards showed different trends according to the management and intercropping system implemented. In D-S treatment, SOC-S were increased by, respectively, 5.88% and 14.47% compared with those in the 0–10 and 10–30 cm soil layer in 2018, reaching 5.19 and 9.87 Mg ha^{-1}. This implied that SOC accumulation was three times higher in the subsurface layer than in the topsoil during the analysed period. This pattern has been reported in other studies assessing tillage removal and cover crop installation [89,90], even in the short-term under semiarid climatic conditions [48,91]. Under D-S in the two soil layers analysed, major increases in SOC-S were found in the 250–2000 μm fraction. This is a particularly interesting finding since it showed a change in the trend of C distribution in the different soil fractions where microaggregates were predominant, and macroaggregates increased their storage capacity due to less soil disturbance and surface organic residues. Consequently, these results could indicate the beginning of a process of soil re-carbonisation and a return to a period where macroaggregates predominated in C storage in the study area and which were deteriorated by long periods of unsustainable management [92].

In our study site, D-O and D-L crop diversification practices did not enhance SOC-S in the rainfed olive grove and showed similar values to those obtained at the initial stage. These diversification practices did not record the SOC-S losses found under CT management in the topsoil (-1.21 Mg ha^{-1}) and subsurface layer (-0.84 Mg ha^{-1}). In addition, D-O and D-L diversification systems showed a better SOC-S balance with respect to the monocrop system; however, the absence of crop residues in considerable quantities and annual tillage for sowing were driving factors for the reduced accumulation of soil C in the different aggregate size classes. Similar findings were observed in Oliveira et al. [93] and Martinez Mena et al. [94], where SOC-S was not affected in the short-term. Moreover, the short-term period of this field study (i.e., 4 years) to assess the diversification effect combined with severe environmental conditions, especially in the dry season months (temperatures above 40 °C, no rainfall events, extreme evapotranspiration rates), SOM-poor soils, low structural stability and parent material consisting of the sedimentary substrate (mainly, limestones and marls), which are easily erodible, hindered the diversification development and contributed to decelerate the SOC dynamics and C sequestration processes. In this sense, under the mentioned conditions, a longer period is required to evaluate changes in soil structure and SOC related to these intercropping systems.

The results showed that diversified cropping systems could improve stability conditions and C sequestration in the aggregates with respect to the monocropping olive system, as demonstrated under D-S diversification. In accordance with these results, on a larger scale, the inclusion of diversifications in olive orchards could provide considerable environmental benefits and lead to healthy soils, as proposed by the EU soil strategy 2030 [23]. However, under Mediterranean conditions the establishment of diversified systems could require long periods before their impacts are observed. These results were in line with Yan et al. [95], who found an increase in C and N stocks in diversified systems versus traditional systems in a recent study. However, in semiarid Mediterranean under rainfed conditions, these processes may not be observed in the first years of intercropping and low input agricultural strategies, as found in D-O and D-L diversification. Consequently, a longer period may be necessary to improve soil quality and agroecosystems sustainability, as highlighted by Martinez Mena et al. [94] in similar conditions in woody cropping systems. In this regard, the first years after the implementation of an intercrop in a monocrop system can be difficult in terms of improving soil properties, especially when soils, such as those analysed in this study, have suffered long processes of degradation that have left adverse conditions, not only for the development of a crop with specific productive yields but also for the development of vegetation covers with a high percentage of coverage and plant development. These aspects are of vital importance because the exposure of the soil to Mediterranean climatic conditions, especially torrential rainfall episodes, causes alterations in the soil C balance and the rapid deterioration of the improvements obtained with sustainable management practices such as intercropping.

5. Conclusions

The intercropping system showed differences among the different diversification strategies implemented in the study area for 4 years. D-S significantly improved the SOC and TN content in all aggregate fractions and SOC-S and showed higher aggregation rates. Our results indicated that in this management system, D-S intercropping with ground cover and without soil disturbance improves soil properties in terms of fertility, SOC sequestration, and soil aggregation. However, our results showed that D-O and D-L diversification did not improve soil aggregation processes or structural stability in our study and did not affect SOC stocks, although the severe losses found under the monocropping system were not recorded.

The SOC-S were primarily dominated by small microaggregates (53–250 µm) in the topsoil and subsurface soil layers for all cropping systems. However, in the short-term study under D-S, the largest increases were recorded in the 2000–250 µm fraction. This showed a process of C redistribution towards a larger aggregates size class that increased sequestration and storage capacity in these soils with the implemented system.

Our findings reveal the high potential of intercropping associated with vegetation coverage and without soil disturbance in rainfed olive groves under semiarid conditions to increase aggregation levels and SOC sequestration. Consequently, it was demonstrated that this management strategy can be positive in re-carbonising highly degraded soils, thus mitigating climate change impacts. Further long-term research on the effects caused by intercropping systems related to aggregation and SOC-S dynamics under Mediterranean conditions is needed.

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