Crop diversification effects on soil organic carbon and nitrogen storage and stabilization is mediated by soil management practices in semiarid woody crops

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Abstract

Crop diversification is a promising strategy to mitigate climate change through soil carbon sequestration while ensuring soil fertility maintenance and food security. Here, we assess the impact of different inter-cropping practices on soil organic carbon (SOC) and nitrogen (N) storage and stabilization under rainfed and irrigated semiarid conditions. Under rainfed conditions, an almond (*Prunus dulcis* Mill.) monocrop was compared with an almond inter-cropped with caper (*Capparis spinosa*) or with winter thyme (*Thymus hyemalis*). Under irrigated conditions, a mandarin (*Citrus reticulata* Blanco) monocrop was compared with a mandarin inter-cropped with an annual crop rotation (including *Hordeum vulgare, Vicia sativa* and *Vicia faba*) or with a triennial crop rotation (including *Vicia faba, Portulaca oleracea* and *Vigna unguiculata* L.). The SOC and N stocks, SOC mineralization rates, water-stable aggregates and associated OC and N contents were estimated at 0-10 and 10-30 cm depth in each monocrop and inter-cropping system after three years. Contrasting effects of inter-cropping on SOC and N stabilization were found in the rainfed and irrigated systems. The combination of inter-cropping and no tillage did not affect the SOC stocks or mineralization rates at any soil depth in the rainfed system, while it significantly increased the OC and N contents within the large macro-aggregates in the subsoil. Compared to the irrigated mandarin monocrop, the mandarins inter-cropped with the annual crop rotation showed a significant increase of 10% and 18% in the topsoil and subsoil N stocks, respectively, while the SOC mineralization rates were reduced by 30% at both soil depths. On the contrary, the topsoil SOC stock in the mandarins inter-cropped with the triennial crop rotation were reduced by 38% compared to the monocrop. Significant reductions ranging between 24% and 66% were also observed in the OC and N contents associated to the macro- and micro-aggregates in the topsoil and subsoil of both crop diversifications compared to the monocrop system. Our results highlight the potential of inter-cropping rainfed woody crops with perennials for boosting SOC and N storage and stabilization. In irrigated woody monocrops, inter-cropping with annual crop rotation schemes that include two or more leguminous and reducing the frequency of and planting operations seems to be more appropriate in terms of SOC and N stabilization.

Keywords: Inter-cropping; crop rotations; soil aggregates; soil organic carbon and nitrogen pools; organic carbon mineralization; climate change mitigation

1. Introduction

Soils play a very important role in the global carbon cycle and are crucial to combat climate change (Rumpel et al., 2018). Agricultural use of soils, which is expected to keep expanding to support a growing demand scenario, usually depletes soil organic carbon (SOC) contents (Guo and Gifford, 2002; Wei et al., 2014). In light of the ongoing discussion on the post-2020 European Common Agricultural Policy, and the current sustainability agenda (e.g., Paris Climate Agreement, European Strategy of Biodiversity and the European Mission of Soil, Sustainable Development Goals), there is a need to promote sustainable agricultural management practices that contribute to SOC sequestration while reducing external inputs such as fertilizers and pesticides (Pe'Er et al., 2019). In this regard, crop diversification (either adding more crops into an existing crop rotation scheme or inter-cropping, *i.e.*, growing annual or perennial crops in the inter-tree-rows of woody crops) is a promising strategy to mitigate climate change and land degradation through soil carbon sequestration while ensuring food security and maintaining natural resources (soil, water and biodiversity), particularly in dry regions (Francaviglia et al., 2017; Sanz et al., 2017; Yang et al., 2021; Ebbisa, 2022).

Although the agro-environmental benefits of crop diversification, including promising soil carbon sequestration rates and fertility maintenance, have been worldwide reported (Paustian et al., 1997; Abdalla et al., 2014; Tamburini et al., 2020; Carranca et al., 2022) (Yan et al., 2022), there is a need for improving our understanding of the different SOC and nitrogen (N) stabilization mechanisms fostered by inter-cropping and how local environmental and management conditions influence the efficiency of the SOC and N sequestration process (Six et al., 2002; DeGryze et al., 2004), particularly in semiarid regions (Blanco-Canqui and Lal, 2004; Hobley et al., 2018; Garcia-Franco et al., 2021;

González-Rosado et al., 2022; Sánchez-Navarro et al., 2023). Therefore, the analysis of the different mechanisms favouring SOC and N storage and stabilization in semiarid soils is a stepping stone to understand how C and N fluxes may be affected by different agricultural management scenarios, and will contribute to identify early warning indicators as well as to design sustainability metrics tailored for these dry regions.

The storage and stabilization of organic carbon and nitrogen inputs in agricultural soils depends on multiple environmental and management factors that modulate the interplay between stability and mineralization (Kan et al., 2021). Previous research has highlighted the relevance of fresh carbon inputs that provide labile organic sources to boost microbial activity and the incorporation of C inputs into soils (Cotrufo et al., 2015; Chenu et al., 2019; Lavergne et al., 2021; Thapa et al., 2022). However, higher SOC mineralization rates have been also reported worldwide after fresh labile carbon inputs has been added to soils due to priming effects (Kuzyakov et al., 2000; Blagodatsky et al., 2010; Bailey et al., 2019; Siles et al., 2022). On the other hand, the role of recalcitrant organic sources (e.g., roots) in soil aggregation and OC occlusion has been proved in no tillage systems (Gale et al., 2000; Liu et al., 2021). Therefore, SOC and N stabilization is a complex mechanism that depends not only on the type of fresh plant residues returned to the soil and tillage management, but also on the local pedoclimatic conditions and microbial community, all together modulating the different physical, chemical and bio-chemical protection mechanisms that occur during the organic matter decomposition and mineralization processes (Cotrufo et al., 2013; Kan et al., 2022; Thapa et al., 2022).

The aim of this study was to assess the effectiveness of different inter-cropping practices for enhancing SOC and N storage and stabilization in two woody monocrop systems under contrasting management conditions (a low-input rainfed almond and a ferti-irrigated mandarin) in order to promote changes in existing conventional agronomic practices from a climate change mitigation and soil conservation perspective in semiarid regions. The specific objectives of this study were to assess the effects of inter-cropping after three years from establishment on: (1) changes in SOC mineralization rates and SOC and N stocks; 2) changes in soil aggregation distribution; and 3) changes in SOC and N pools associated with different aggregate-size classes. Here we ask ourselves whether the new C and N inputs from the secondary crops contribute to the formation of soil aggregates and the incorporation and stabilization of OC and N within them (Yan et al., 2022) or, by contrast, SOC mineralization is enhanced by the so-called priming effect (Kan et al., 2021). The following hypotheses were tested: (i) the new C and N inputs from the secondary crops will activate soil microorganisms, promoting biogenic aggregation and the physical protection of OC and N in the relatively short-term (Ledo et al., 2020; Liu et al., 2021), (ii) the cessation of tillage reduces soil aggregate disruption and increases the physical protection of SOC in macro-aggregates (Six et al., 2004; (Zhang et al., 2014); Nunes et al., 2018), and (iii) the degree of soil aggregation and OC and N stabilization in these semiarid agroecosystems will be mediated by the type of fresh plant residues returned to the soil in combination with tillage management practices (Bailey et al., 2019; Lavergne et al., 2021; Thapa et al., 2022).

2. Materials and Methods

2.1. Study site

The study was conducted in two nearby woody monocrop systems located in the Region of Murcia, South-Eastern Spain: (i) a rainfed organic almond (*Prunus dulcis* Mill.) orchard with an extension of 2.63 ha, cultivated on terraces with a 7 m x 7 m spacing in 1950, and tilled between two- and three-times a year to control weeds (37° 57’ 31’’ N, 0° 56’ 17’’ W; 167 m a.s.l.); and (ii) an irrigated mandarin (*Citrus reticulata* Blanco) orchard cultivated in 2000 with a 6 m x 4 m spacing, where tillage operations are performed between four- and five-times a year and herbicides are applied to control weeds (37° 57’ 33’’ N, 0° 56’ 13.8’’ W; 155 m a.s.l). Both low- and high-input woody monocrop systems are representative of the South-Eastern Spain landscape.

The climate is semiarid Mediterranean, with warm, dry summers and cold, relatively wet winters. The annual precipitation averages 280 mm (1996-2021; SIAM) and it is concentrated in the fall and spring months, but with great inter- and intra-annual variability. The annual air temperature averages 17.5 ºC and the mean potential evapotranspiration reaches 1300 mm yr^{-1} (calculated by the Thornthwaite method), so the mean annual water deficit is around 1000 mm.

The soils in both woody monocrop systems are developed on marl, classified as Calcaric Eutric Regosols (IUSS Working Group WRB, 2015), have a silt-loam texture and a high content of $CaCO_3$ (~ 55% on average). Slight differences in soil properties between the two woody monocrop systems at the beginning of the experiment are probably explained by the historical management. The soil organic carbon (SOC) and nitrogen (N) contents (relatively low in both woody monocrop systems) were twice lower in the rainfed (4.5 and 0.7 g kg^{-1} for SOC and N, respectively) than in the irrigated (8.2 and 1.3 g kg^{-1} for SOC and N, respectively) system. The pH (H_2O, 1:5) showed values of 8.5 and 7.5 in the rainfed and irrigated monocrop, respectively, while the electrical conductivity oscillated between 0.20 and 1.37 dS m^{-1} under rainfed and irrigated conditions, respectively. For more details on the initial soil characteristics of all crop management treatments see Sánchez-Navarro et al. (2022) and Almagro et al. (2023).

Soils in the study area have suffered severe degradation and erosion processes due to a combination of geomorphological factors (lithology, topography, and climatology), land use history and plant cover changes typical of Mediterranean regions (García-Ruiz, 2010). Indeed, about 27% of the Region of Murcia agricultural surface is classified as having a moderate erosion risk, with mean annual soil loss rates ranging between 3 and 10 Mg ha^{-1} (Ortiz et al., 1999), which represents soil carbon losses by erosion at rates ranging from 0.25 to 0.3 Mg C ha^{-1} yr^{-1} in cultivated areas (Martínez-Mena et al., 2008; Almagro et al., 2010). On the other hand, the limiting climatic conditions (e.g., high temperatures and evapotranspiration rates together with low precipitation) leading to low crop growth rates together with inappropriate crop and soil management practices (e.g., long fallow periods, lack of plant cover in the inter-tree rows of permanent crops, crop residue removal, intensive-tillage) make it difficult to expedite land recovery after degradation (Martínez-Mena et al., 2002; Asner et al., 2003; Vallejo, 2005).

Table 1. Description of the different farming activities performed in each crop management practice.

Crop management practice	Overview	Spacing	Crop management	Soil and crop residue management	Water and fertilization management
Almond monocrop (M1)		7 m x 7 m	Annual almond harvesting in August	Chisel ploughing to 15 cm depth twice yr^{-1}	Rainfed No fertilizer application
Almond inter-cropped with caper (D1)		3.5 m x 3.5 m	Annual almond harvesting in August Caper plantation in November 2018	No tillage	Rainfed No fertilizer application
Almond inter-cropped with thyme (D2)		1 m x 0.5 m	Annual almond harvesting in August Winter thyme plantation in November 2018 Annual winter thyme harvesting in April	No tillage	Rainfed No fertilizer application
Mandarin monocrop (M2)		6 m x 6 m	Annual mandarin harvesting in January	Chisel ploughing to 30 cm depth 4-5 times yr^{-1} Pruning residue chopped & mulched once yr^{-1}	Drip irrigation N, P and K application at 250, 85 and 200 kg ha^{-1} yr^{-1}
Mandarin inter-cropped with a 1-yr cycle crop rotation: barley/vetch and fava bean (D3)		1 m x 0.4 m	Annual mandarin harvesting in January Annual green manure seeding (Feb.) & harvesting (June) Annual fava bean seeding (Sep.) & harvesting (Jan.)	Chisel ploughing to 30 cm depth 6-7 times yr^{-1} Incorporation of crop residues after harvesting	Regulated deficit irrigation N, P and K application at 250, 85 and 200 kg ha^{-1} yr^{-1}
Mandarin inter-cropped with a 3-yr cycle crop rotation: barley/vetch, fava bean, purslane and cowpea (D4)		1 m x 0.4 m (1^{st} yr) 1 m x 1 m (2^{nd} yr) 1 m x 0.2 m (3^{rd} yr)	Annual mandarin harvesting in January Green manure seeding (Feb.2018)/harvesting (June 2018) Fava bean seeding (Sep. 2018)/ harvesting (Jan. 2019) Purslane seeding (May 2019)/ harvesting (July 2019) Cowpea seeding (June 2020)/ harvesting (Sep. 2020)	Chisel ploughing to 30 cm depth 6-7 times yr^{-1} Incorporation of crop residues after harvesting	Regulated deficit irrigation N, P and K application at 250, 85 and 200 kg ha^{-1} yr^{-1}

2.2. Crop diversification practices description and experimental design

To increase the soil cover, sustainability and profitability of the rainfed and irrigated woody monocrop, different inter-cropping practices were implemented in autumn 2018 (Table 1). To do so, a proportion of the rainfed almond monocrop was intercropped with *Capparis spinosa* (hereafter caper, for food production; Diversification 1; D1) or with *Thymus hyemalis* (hereafter winter thyme, for essential oil production; Diversification 2; D2), leaving the remaining part of the almond crop as a monocrop (our control treatment under rainfed conditions; M1). In the rainfed almond monocrop, tillage was performed by chisel ploughing to 15-20 cm depth twice or three times a year after important rainfall events to control weeds, so the soil in the inter-tree rows is uncovered almost all year round and biomass inputs from spontaneous vegetation are negligible. In the rainfed diversified cropping systems, no tillage operations were performed due to the presence of the secondary crops in the inter-tree rows (see pictures in Table 1).

Likewise, a proportion of the irrigated mandarin monocrop was inter-cropped annually with a mixture of *Hordeum vulgare* and *Vicia sativa* (for livestock feeding) followed by *Vicia faba* (for human consumption; Diversification 3; D3) or with a 3-year crop rotation cycle for human consumption, including *Vicia faba*, *Portulaca oleracea* and *Vigna unguiculata* L. (Diversification 4; D4), leaving the remaining part of the mandarin crop as a monocrop (our control treatment under irrigated conditions; M2). Barley and vetch seeds (1:3 ratio) were manually sown at 150 kg ha^{-1}, covering the entire alley surface, and crop residues were incorporated in the soil as green manure after harvest. Fava bean, purslane and cowpea seeds were manually sown under drip irrigation in three rows in each alley at different plant densities (2.5, 1 and 5 plants m^{-2}, respectively). After harvest, crop residues were left on the soil surface as mulching. Tillage was performed by chisel ploughing to 30-40 cm depth between four and five times per year to control weeds in all crop management treatments. Additional passes were performed in both crop diversification systems immediately before sowing each secondary crop to prepare the soil and after harvest to incorporate the crop residues into the soil (in total, between three and four additional passes per year compared to the monocrop). On the other hand, pruning residues were annually chopped and incorporated as mulching on the soil surface only in the mandarin monocrop system, because the inter-tree rows of the diversified systems were covered by the secondary crops.

A drip irrigation system was installed in all mandarin tree rows, with one line per tree row and 3 pressure-compensated emitters (4 L h^{-1}) per tree. In the crop diversification treatments, three additional drip irrigation lines were established for irrigation with pressure-compensated emitters (4 L h^{-1}) every 40 cm. Regulated deficit irrigation (RDI) was applied to the main crop in the inter-cropping treatments to save water in order to be used for the secondary crops. Fertilizers were applied as ferti-irrigation by use of the commercial products Neptuno PK 28, Neptuno Triton and Neptuno Pandora (Medifer, Constantino Gutiérrez, SA), as a mixture of soluble N, P, K, Ca, Mg and chelated oligoelements. Application rates for N, P and K were 250, 85 and 200 kg ha^{-1} yr^{-1}, respectively. For more details on the irrigation and fertilization management, see Sánchez-Navarro et al. (2023).

To compare the crop diversification systems with their respective monocrop ones a split-plot design with three replicate plots per each crop management treatment was established in both the rainfed (almond monocrop, almond inter-cropped with caper, and almond inter-cropped with winter thyme) and the irrigated (mandarin monocrop, mandarin inter-cropped with an annual crop rotation, and mandarin inter-cropped with a triennial crop rotation) orchard. Thus, nine plots (7 m x 30 m, each enclosing five almond trees) and 6 plots (12 m x 280 m, each enclosing six mandarin trees) were established in the rainfed almond and the irrigated mandarin orchards, respectively. For more details on the experimental design layout see Martínez-Mena et al. (2021).

2.3. Soil sampling and analysis

Soil samples were collected at 0-10 cm and 10-30 cm depth in April 2021, three years after the diversification practices were implemented. The soils were sampled in the alleys between the trees, 2 m from the tree trunks. Three disturbed composite soil samples (each one from five randomly collected subsamples) were taken per crop management practice and block in each cropping system for physical, chemical and microbial activity (measured as basal respiration) analyses. Undisturbed samples were also collected at the same spots using steel cylinders (100 cm^3 core volume) for bulk density (BD, in g cm^{-3}) determinations according to (Burke W, Gabriels D, Bouma J, 1986). The disturbed soil samples were air-dried and sieved to < 2 mm for physical (texture) and chemical (C and N) analyses. The remaining non-sieved soil samples were stored for aggregate stability analysis.

2.3.1. Soil organic carbon mineralization and CO_2 analysis

Soil subsamples (40 g) were moistened to 60% WHC prior to incubation at 28 °C in air-tight, 125-cm^3 containers under aerobic, controlled conditions. The CO_2 (%) evolved from the containers was regularly measured with an infrared gas analyzer (CheckMate II, PBI Dansensor, Denmark) and the containers were opened for 1 h after each measurement to balance the atmosphere inside and outside them. Measurements were performed more frequently during the first phase of the incubation (on days 2, 4, 8, 11, 18, 25, 32, 39, 46, 54, 61, 68, 75, 90 and 105). The moisture content of samples was also checked periodically by weighting the mesocosms but replacement of evaporated water was not necessary during the experiment. We used linear interpolations between sampling dates and then summed them across all dates to estimate the cumulative amount of CO_2 released (mineralized) after 105 days of incubation, to estimate the potential SOC mineralization rates (mg CO_2 kg^{-1} soil; Nannipieri et al., 1990). The mineralized OC was calculated as the averaged C content respired daily per gram of SOC, and was expressed as mg CO_2-C g^{-1} OC per day.

2.3.2. Determination of chemical and physical soil properties

Soil texture was determined using a Coulter LS200 'Laser particle sizer' (Coulter corporation, Miami, Florida). Previously, soil samples were treated with hydrogen peroxide to remove organic matter before being dispersed using sodium hexametaphosphate for 12 h. Bulk soil organic carbon (SOC, in g kg^{-1}) and nitrogen (Nt, in g kg^{-1}) were analyzed using an N/C Analyzer (Flash 1112 EA, Thermo-153 Finnigan, Bremen, Germany). Before N/C Analyzer, soil carbonates were eliminated using 1 M HCl. The SOC and N stocks (g m^{-2}) of bulk soils were calculated on an "equivalent mass", except for those whose BD was comparable (Wendt and Hauser, 2013).

2.3.3. Determination of soil organic carbon and nitrogen stocks

The soil organic carbon and nitrogen stocks at 0–10 cm and 10-30 cm depth (in g m^{-2}) were calculated as follows:

$SOC_{stock} = SOC \times BD \times D \times (1 - S) \times 10$

$Soil\ N_{stock} = N \times BD \times D \times (1 - S) \times 10$

Where SOC_{stock} and Soil N_{stock} are the stored carbon and nitrogen in the soil in g m^{-2}, respectively; SOC and N are the concentration of soil organic carbon and nitrogen in g kg^{-1}, respectively; BD is the soil bulk density in g cm^{-3}; D is the soil depth in cm; and S is the proportion of the volumetric coarse fragment fraction (particles > 2 mm) in g 100 g^{-1}. Noteworthy, the SOC and N stocks in the rainfed system were calculated on an "equivalent mass" following Wendt and Hauser (2013).

2.3.4. Fractionation of water-stable soil aggregates and OC and N determination

Water-stable aggregates were separated from soil composite samples of each crop management practice and soil depth following the wet-sieving method proposed by Elliott (1986). Briefly, a 100-g subsample of air dried soil was placed on top of a 2000-μm sieve and gently moistened by sprinkling to minimise aggregate slaking before immersed in water at room temperature. The sieving was performed manually by moving the sieve up and down 3 cm, 50 times in 2 min, to achieve aggregate separation. A series of three sieves (2000, 250, and 53 μm) was used to obtain four aggregate-size classes: i) large macro-aggregates (LM; > 2000 μm); ii) small macro-aggregates (SM; 250–2000 μm); iii) micro-aggregates (m; 53–250 μm); and iv) silt plus clay-sized particles (s + c; < 53 μm). The aggregate size classes were oven-dried at 50 ºC, weighed, and stored in glass jars at room temperature (21 ºC).

Organic carbon (OC, in g kg^{-1}) and nitrogen (N, in g kg^{-1}) were analyzed separately for each water-stable aggregate-size class using the same elemental C/N analyzer mentioned above, after soil carbonates had been eliminated by acid digestion with 2N HCl. Briefly, between 0.05 and 0.07 g of sample was weighted in a tin capsule and placed on a stainless-steel heating plate at 120 ◦C. Drops (~100 μL) of HCl 2N were added to each soil sample until bubbling stopped. The digested soil sample was them combusted and total carbon as CO_2 was determined by infrared analysis. A paired soil sample was analyzed in the same way but without previous digestion. The samples were analyzed in triplicate. The OC concentration within each water-stable aggregate-size class was expressed on a sand-free aggregate basis (Elliott et al., 1991).

2.4. Statistical analyses

Statistical analyses were performed for each woody crop management system (rainfed and irrigated) and soil depth (0-10 and 10-30 cm) separately. Prior to statistical analyses, the data were tested for ANOVA assumptions. Since most of the variables did not meet the equality of variances assumption (e.g., SOC stock, N and OC and N pools associated with the different aggregate-size classes), the Kruskal-Wallis non parametric test was used to test for significant differences in SOC mineralization rates (i.e., CO_2 released), as well as in SOC and N stocks and pools, between the monocrop and their respective inter-cropping treatments within each crop management system (rainfed and irrigated) and soil depth. When significant crop management treatment and/or soil depth effects were found, pairwise comparison tests with Bonferroni adjustment were used to detect differences between crop management treatments (monocrop *vs* crop diversification) in each soil depth. The Spearman correlation test was used to examine the relationships between soil organic carbon and nitrogen pools and SOC mineralization rates for each crop management treatment under rainfed and irrigated conditions. All the statistical analyses were performed using SPSS 28.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil organic carbon mineralization rates

The effect of crop diversification on SOC mineralization rates differed between the rainfed and the irrigated system (Fig. 1; Table 2). After three years, SOC mineralization rates did not significantly differ between the monocrop (M1) and the crop diversification treatments (D1 and D2) at any soil depth in the rainfed system (Fig.1; Table 2). In the irrigated system, however, SOC mineralization rates were 30% lower in the mandarins inter-cropped with the annual crop rotation (D3) than in the monocrop (M2) at both soil depths. On the other hand, SOC mineralization rates did not significantly change in the mandarins inter-cropped with the triennial crop rotation (D4) with respect to those observed in the monocrop at any soil depth, although somewhat higher values were observed in the topsoil of the former than in the later.

3.2. Soil organic carbon and nitrogen stocks

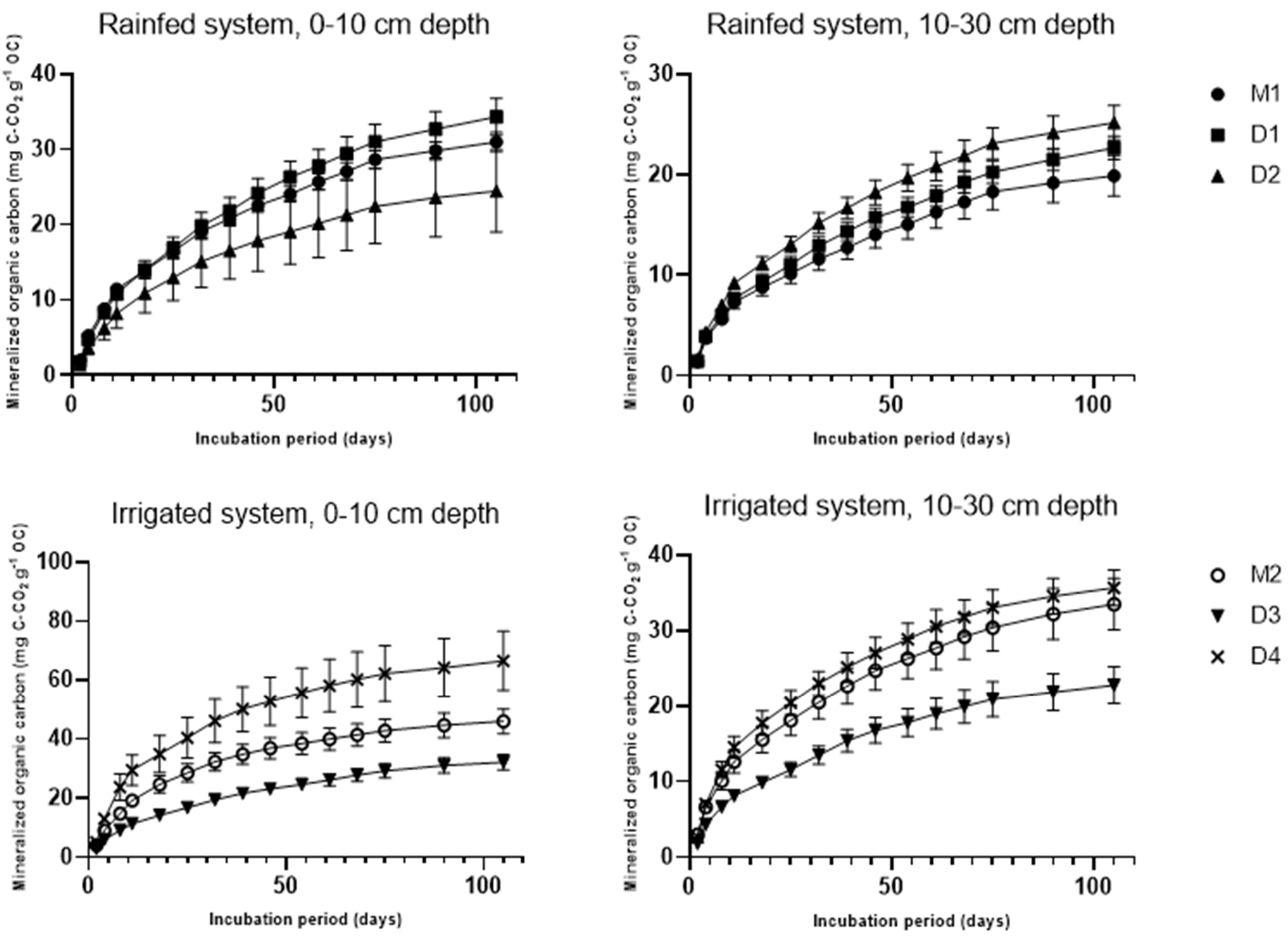
After three years, no differences were observed in the topsoil or subsoil organic carbon stocks between the monocrop (M1) and the crop diversification treatments (D1 and D2) in the rainfed system. The topsoil nitrogen stock was 37% lower in the almond trees inter-cropped with winter thyme than in the almond monocrop, but no differences in the subsoil nitrogen stocks were observed between the monocrop and the crop diversification treatments (Table 2). In the irrigated system, a significant reduction of 38% in the topsoil organic carbon stock was found in the mandarins inter-cropped with the triennial crop rotation (D4 treatment) compared to the

Table 2. Soil organic carbon mineralization rates, and organic carbon and nitrogen stocks in the topsoil and subsoil of the different rainfed systems (almond monocrop, almond inter-cropped with caper, and almond inter-cropped with winter thyme) as well as in the irrigated systems (mandarin monocrop, mandarin inter-cropped with an annual crop rotation, and mandarin inter-cropped with a triennial crop rotation) after three years from establishment (values on dry weight basis). Standard errors are given in parentheses.

		Rainfed cropping system					**Irrigated cropping system**				
Soil variables	**Soil depth**	**Almond monocrop (M1)**	**Almond-caper (D1)**	**Almond-thyme (D2)**	***F***	***P***	**Mandarin monocrop (D2)**	**Mandarin-annual rotation (D3)**	**Mandarin-triennial rotation (D4)**	***F***	***P***
SOC mineralization rate (mg C-CO_2 g^{-1} OC d^{-1})	Topsoil (0-10 cm)	0.30 (0.01) **a**	0.33 (0.02) **a**	0.29 (0.03) **a**	2.7	0.25	0.44 (0.04) **b**	0.31 (0.02) **a**	0.63 (0.1) **b**	14.2	<0.01
	Subsoil (10-30 cm)	0.19 (0.02) **a**	0.22 (0.01) **a**	0.24 (0.02) **a**	4.7	0.09	0.32 (0.03) **b**	0.22 (0.02) **a**	0.34 (0.02) **b**	8.5	0.01
SOC stock (g m^{-2})	Topsoil (0-10 cm)	555 (53) **a**	601 (50) **a**	539 (40) **a**	3.9	0.13	1236 (49) **b**	1288 (72) **b**	760 (98) **a**	10.9	0.04
	Subsoil (10-30 cm)	1060 (113) **a**	1010(73) **a**	896 (88) **a**	2.3	0.3	1437 (155) **a**	1604(115) **a**	1170 (103) **a**	4.8	0.1
N stock (g m^{-2})	Topsoil (0-10 cm)	111.8 (8) **b**	107 (8) **b**	70 (7) **a**	11	0.04	150 (5) **ab**	166 (4) **b**	118 (9) **a**	14.3	<0.01
	Subsoil (10-30 cm)	216.8 (39) **a**	180 (10) **a**	165 (8) **a**	1.2	0.5	211 (7) **a**	250 (9) **b**	239 (10) **a**	10.0	<0.01

*Within each rainfed and irrigated cropping system and soil depth, different lowercase letters indicate significant differences between crop management treatments (monocrop vs crop diversification), accordingly to the Kruskall-Wallis test ($P < 0.05$) ("a" denotes lower values than "b") mandarin monocrop system, while the subsoil organic carbon stock did not differ between the monocrop and the crop diversification treatments (Table 2). Significant increments were observed in the topsoil and subsoil nitrogen stocks (by 10% and 18%, respectively) of the mandarins inter-cropped with the annual crop rotation (D3 treatment) compared to the mandarin monocrop system.

Figure 1. Mineralized soil organic carbon (mg C-CO_2 g^{-1} OC) during the incubation period for each crop management treatment and soil depth in the rainfed and irrigated system after three years from implementation (M1: Almond monocrop; D1: Almond inter-cropped with caper; D2: Almond inter-cropped with winter thyme; M2: Mandarin monocrop; D3: Mandarin inter-cropped with a 1-yr crop rotation cycle; D4: Mandarin inter-cropped with a 3-yr crop rotation cycle.



3.3. Soil aggregate size-classes distribution

The effect of inter-cropping under rainfed conditions on the proportion of the different aggregate-size classes differed from that of inter-cropping under irrigated conditions. In the rainfed system, the proportion of large macro-aggregates (LM) significantly increased in the almond inter-cropped with winter thyme compared to the almond monocrop in the subsoil (Fig. 2). In the irrigated system, the proportion of the total macro-aggregates (large and small) was significantly reduced (by 41% and 51% in the D3 and D4 treatment, respectively) in the topsoil of both crop diversification treatments compared to that in the mandarin monocrop. Moreover, the proportion of small macro-aggregates was significantly reduced by 38% in the mandarins inter-cropped with the triennial crop rotation (D4) compared to the mandarin monocrop system (M2) in the subsoil. These reductions were paralleled by increments in the silt plus clay-size fraction.

3.4. OC and N pools associated with different aggregates-size classes

The OC and N concentrations of the different aggregate-size classes were low, particularly in the rainfed cropping system. Differences in the OC and N concentrations of the different aggregate-size classes between monocrop and inter-cropping were observed under rainfed and irrigated conditions, but showed no consistent patterns (Table 3). In the rainfed system, the OC concentration of the large macro-aggregates (LM) in the topsoil of the almond inter-cropped with caper was 61% higher than that of the almond monocrop. On the other hand, the N concentration of the silt plus clay fraction was 30% lower in the topsoil of the almond inter-cropped with winter thyme than in the almond monocrop. In the irrigated system, the OC and N concentrations of the large macro-aggregates (LM) in the subsoil, as well as the OC and N concentrations of the small macro-aggregates (LM) in the topsoil, were reduced between 26% and 46% when the mandarin monocrop was inter-cropped with the triennial crop rotation (D4 treatment; Table 3). The C:N ratios of the different aggregate-size classes did not differ between the monocrop and inter-cropping in the rainfed nor in the irrigated system.

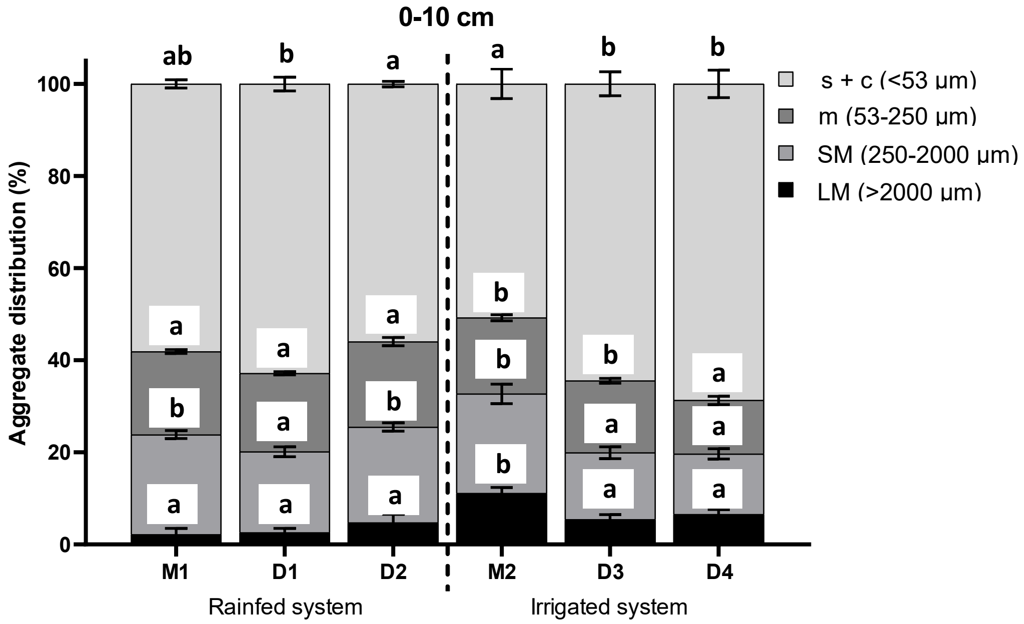
Contrasting crop diversification effects on the OC and N contents associated to the different aggregate-size classes were observed in the rainfed and the irrigated system (Fig. 3A-D). In the rainfed system, the OC and N contents associated to the large macro-aggregates were larger in both crop diversification treatments compared to the almond monocrop treatment, particularly in the subsoil, although statistical significant differences were only detected in the case of the almond trees inter-cropped with winter thyme (Fig. 3B; 3D). These increments in the OC and N contents associated to the largest macro-aggregates were paralleled by decreases in the OC and N contents associated to the micro-aggregates. In the irrigated system, however, the OC and N contents associated to the macro- and micro-aggregate size classes were between 24% and 66% lower in both crop diversification treatments compared to the mandarin monocrop system, particularly in the topsoil (Figs. 3A-D)

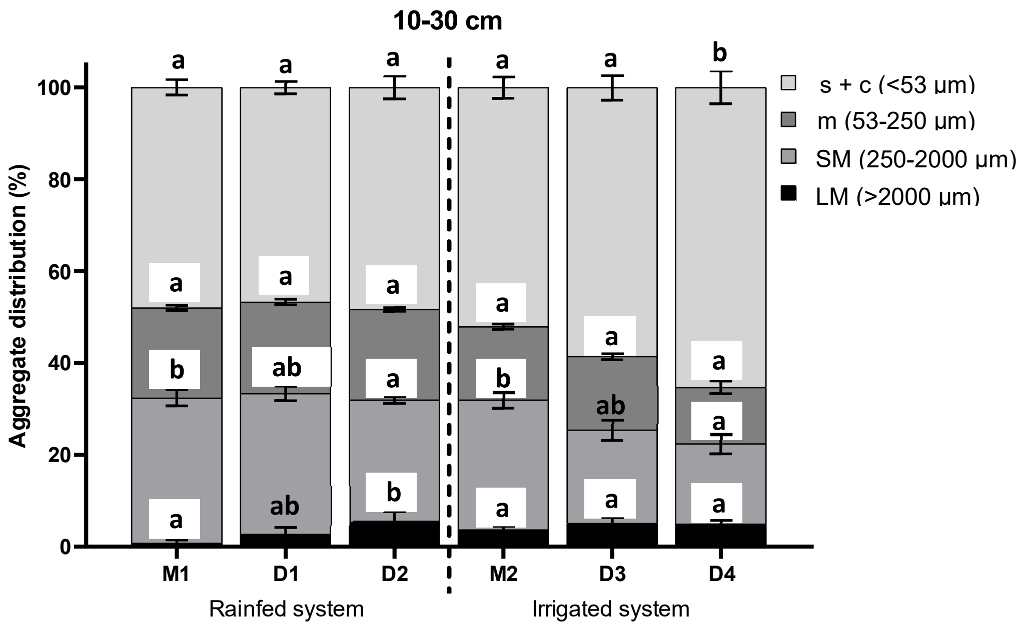
	Soil depth	Rainfed cropping system: Almond monocrop (M1)	Almond-caper (D1)	Almond-thyme (D2)	*F*	*P*	Irrigated cropping system: Mandarin monocrop (D2)	Mandarin-annual rotation (D3)	Mandarin-triennial rotation (D4)	*F*	*P*
OC (mg g^{-1} aggregate)											
LM (>2000 µm)	0-10 cm	4.9 (0.5) a	7.9 (0.4) b	5.0 (0.2) a	11.0	**0.04**	11.2 (1.3) a	11.5 (1.9) a	8.0 (1.3) a	3.28	0.19
	10-30 cm	3.7 (0.2) a	3.7 (0.6) a	4.4 (0.4) a	2.76	0.25	10.5 (0.9) b	9.0 (1.0) b	5.6 (0.7) a	9.47	**<0.01**
SM (250–2000 µm)	0-10 cm	5.2 (0.7) a	6.9 (0.8) a	5.0 (0.7) a	3.6	0.16	13.4 (1.0) b	9.9 (0.9) b	7.7 (0.9) a	6.48	**0.04**
	10-30 cm	3.9 (0.5) a	3.8 (0.4) a	4.2 (0.3) a	0.1	0.96	6.4 (0.1) a	7.0 (0.6) a	6.1 (0.5) a	1.95	0.37
m (53–250 µm)	0-10 cm	4.2 (0.6) a	4.7 (0.7) a	3.8 (0.3) a	0.9	0.62	11.3 (0.2) a	9.0 (0.6) a	7.8 (1.5) a	5.60	**0.06**
	10-30 cm	3.0 (0.7) ab	4.1 (0.3) b	2.5 (0.2) a	6.9	**0.03**	6.7 (0.6) a	5.8 (0.4) a	5.8 (0.3) a	1.10	0.58
s + c (<53 µm)	0-10 cm	3.1 (0.3) a	4.0 (0.3) a	3.5 (0.3) a	3.2	0.19	6.4 (0.1) a	6.0 (0.7) a	5.0 (0.5) a	3.82	0.14
	10-30 cm	3.1 (0.3) a	3.9 (0.4) a	3.3 (0.2) a	3.1	0.21	5.0 (0.4) ab	5.1 (0.4) b	3.7 (0.3) a	9.15	**0.01**
N (mg g^{-1} aggregate)											
LM (>2000 µm)	0-10 cm	0.9 (0.1) ab	1.1 (0.1) b	0.8 (0.1) a	6.7	**0.03**	1.5 (0.1) a	1.3 (0.1) a	1.1 (0.1) a	5.00	**0.08**
	10-30 cm	0.6 (0.0) a	0.7 (0.0) a	0.6 (0.0) a	1.8	0.4	1.2 (0.1) b	1.2 (0.1) b	0.8 (0.1) a	14.37	**<0.01**
SM (250–2000 µm)	0-10 cm	1.1 (0.1) ab	1.1 (0.1) b	0.8 (0.0) a	5.8	**0.05**	1.5 (0.0) b	1.3 (0.1) ab	1.1 (0.1) a	5.90	**0.05**
	10-30 cm	0.8 (0.0) ab	0.9 (0.1) b	0.6 (0.0) a	6.5	**0.04**	0.8 (0.0) a	0.9 (0.0) a	0.8 (0.0) a	2.81	0.24
m (53–250 µm)	0-10 cm	0.8 (0.1) ab	1.0 (0.1) a	0.6 (0.1) b	7.2	**0.02**	1.3 (0.0) a	1.0 (0.1) a	1.1 (0.1) a	3.82	0.15
	10-30 cm	0.7 (0.0) a	0.7 (0.1) a	0.6 (0.1) a	5.4	0.06	0.8 (0.1) a	0.9 (0.1) a	0.8 (0.1) a	0.27	0.87
s + c (<53 µm)	0-10 cm	1.0 (0.0) a	0.7 (0.0) a	0.6 (0.1) b	10.3	**<0.01**	1.0 (0.0) a	0.9 (0.1) a	0.8 (0.1) a	4.62	0.1
	10-30 cm	0.6 (0.0) a	0.7 (0.1) a	0.6 (0.0) a	1.7	0.42	0.8 (0.0) ab	0.9 (0.1) b	0.7 (0.0) a	7.17	**0.03**
OC:N											
LM (>2000 µm)	0-10 cm	5.6 (0.7) a	7.3 (0.4) a	6.9 (0.9) a	2.5	0.28	6.5 (0.0) a	8.1 (0.4) a	5.6 (0.9) a	5.60	0.06
	10-30 cm	5.7 (0.2) ab	5.2 (0.5) a	7.2 (0.4) b	7.52	0.02	6.3 (1.0) a	7.7 (0.7) a	7.1 (0.5) a	1.08	0.58
SM (250–2000 µm)	0-10 cm	4.9 (0.5) a	6.4 (0.6) a	6.0 (0.6) a	3.4	0.18	6.0 (1.1) a	6.8 (0.9) a	3.6 (0.8) a	3.82	0.14
	10-30 cm	4.9 (0.6) a	4.6(0.6) a	6.4 (0.2) a	4.9	0.08	6.8 (0.8) a	7.7 (0.5) a	7.3 (0.4) a	0.65	0.72
m (53–250 µm)	0-10 cm	4.9 (0.3) a	4.9 (0.2) a	5.9 (0.3) a	7.2	>0.05	4.8 (0.5) a	5.0 (0.3) a	5.4 (0.5) a	0.62	0.73
	10-30 cm	4.0 (1.0) ab	5.7 (0.4) b	4.6 (0.2) a	3.4	0.18	6.7 (0.6) a	6.8 (0.5) a	7.3 (0.4) a	1.39	0.49
s + c (<53 µm)	0-10 cm	4.5 (0.3) a	5.7 (0.3) a	5.9 (0.4) a	6.9	>0.05	5.8 (0.5) a	6.3 (0.1) a	5.6 (0.8) a	2.75	0.25
	10-30 cm	5.2 (0.4) ab	5.6 (0.7) b	5.3 (0.3) a	0.2	0.89	5.8 (0.7) a	6.0 (0.6) a	5.4 (0.4) a	0.35	0.83

Table 3. OC and N concentration (mg g^{-1} aggregate) of aggregate-size classes LM (>2000 µm), SM (250–2000 µm), m (53–250 µm), and s + c (<53 µm) in the topsoil and subsoil of the different rainfed systems (almond monocrop, almond inter-cropped with caper, and almond inter-cropped with winter thyme) as well as in the irrigated

systems (mandarin monocrop, mandarin inter-cropped with an annual crop rotation, and mandarin inter-cropped with a triennial crop rotation) after three years from establishment (values on dry weight basis). Numerical values are means ± standard errors. Within each rainfed and irrigated woody cropping system and soil depth, different lower-case letters in rows indicate significant differences between crop management treatments (monocrop vs inter-cropping) for each aggregate-size classes (Kruskal-Wallis test, $P < 0.05$) ("a" denotes lower values than "b")

Figure 2. Aggregate size class distribution (g aggregate 100 g^{-1} soil): LM (> 2000 μm), SM (250 -2000 μm), m (53 - 250 μm), and s + c (< 53 μm) in the 0-10 and 10-30 cm soil layers for each crop management treatment in the rainfed and irrigated system after three years from implementation: M1 (Almond monocrop), D1 (Almond inter-cropped with caper), D2 (Almond inter-cropped with winter thyme), M2 (Mandarin monocrop), D3 (Mandarin inter-cropped with a 1-yr crop rotation cycle) and D4 (Mandarin inter-cropped with a 3-yr crop rotation cycle). Means ± standard errors are shown. For each rainfed and irrigated management system, different lowercase letters in bars indicate significant differences between crop management treatments for each aggregate size according to Bonferroni test (P <0.05).





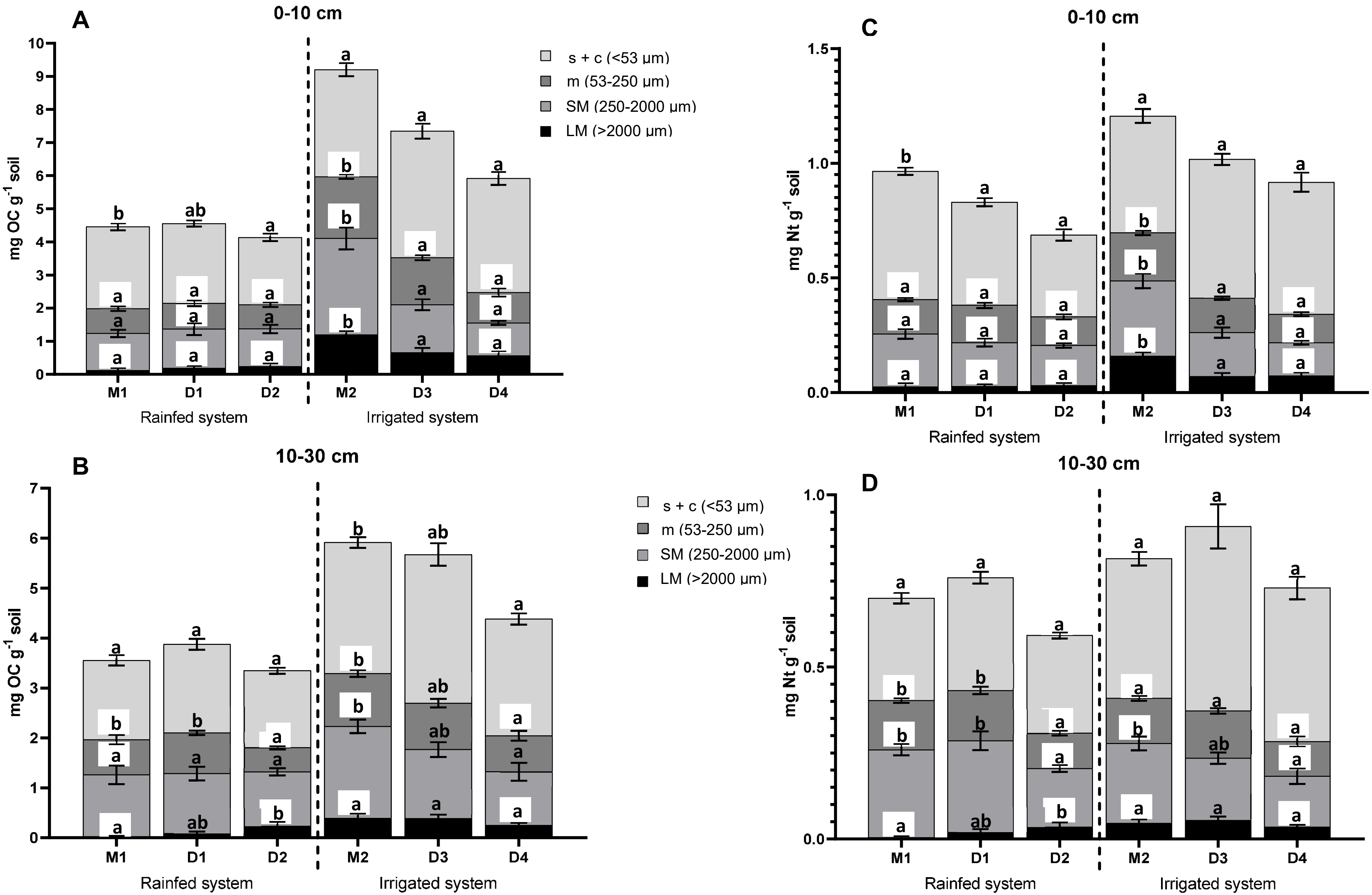

A
0-10 cm
mg OC g^{-1} soil
s + c (<53 µm)
m (53-250 µm)
SM (250-2000 µm)
LM (>2000 µm)
M1
D1
D2
M2
D3
D4
Rainfed system
Irrigated system
B
10-30 cm
mg OC g^{-1} soil
C
0-10 cm
mg Nt g^{-1} soil
D
10-30 cm
mg Nt g^{-1} soil

Figure 3. Contribution of OC (left) and N (right) contents (mg g^{-1} soil) of aggregate size classes to total SOC and N of bulk soils (LM (> 2000 µm), SM (250-2000 µm), m (53-250 µm) and s + c (< 53 µm) in the 0-10 and 10-30 cm soil layers for each crop management treatment in the rainfed and irrigated system after three years from implementation: M1 (Almond monocrop), D1 (Almond inter-cropped with caper), D2 (Almond inter-cropped with winter thyme), M2 (Mandarin monocrop), D3 (Mandarin inter-cropped with a 1-yr crop rotation cycle) and D4 (Mandarin inter-cropped with a 3-yr crop rotation cycle). Means ± standard errors are shown. For each rainfed and irrigated management system separately, different lowercase letters in bars indicate significant differences between crop management treatments for each aggregate size according to Kruskall-Wallis test after Bonferroni adjustment ($P < 0.05$).

Table S1. Spearman correlations between soil and particulate organic carbon and nitrogen contents, water-stable aggregate sizes and associated OC and N contents, and SOC mineralization rates considering the whole soil profile (0-30 cm depth) for each crop management treatment under rainfed and irrigated conditions at the end of the experimental period (three years).

	Monocrop and crop diversification management treatments					
	Rainfed cropping system			**Irrigated cropping system**		
variable	**M1**	**D1**	**D2**	**M2**	**D3**	**D4**
SOC mineralization rate (mg CO_2 kg^{-1} d^{-1})						
Particulate Organic Carbon (g kg^{-1})	.921**	.836**	.633**	.773**	.864**	.251
SOC (g kg^{-1})	.364	.655**	.639**	.631**	.728**	.674**
N (g kg^{-1})	.421	.725**	.203	.682**	.752**	.426
LM:m	.483*	.150	.254	.713**	.197	.315
Total macro-aggregates (LM+SM) (%)	-.638**	-.568*	.113	.228	-.162	.280
OC in total macro-aggregates (g kg^{-1})	.256	0.363	.528*	.713**	.391	.703**
N in total macro-aggregates (g kg^{-1})	-.075	-0.011	.548*	.713**	.245	.657**
Micro-aggregates (%)	-.526*	-.671**	-.293	.259	.154	.379
OC in micro-aggregates (g kg^{-1})	.000	.218	.494*	.792**	.657**	.505*
N in micro-aggregates (g kg^{-1})	.228	.549*	.464	.767**	.410	.670**
silt +clay (%)	.633**	.582*	.216	-.261	.096	-.249
OC in the silt +clay fraction (g kg^{-1})	.770**	.830**	.363	.273	.325	.554*
N in the silt +clay fraction (g kg^{-1})	.714**	.791**	.330	.422	.001	.146
Particulate Organic Carbon (g kg^{-1})						
SOC (g kg^{-1})	.331	.806**	.753**	.707**	.624**	-0.009
N (g kg^{-1})	.476*	.705**	.515*	.802**	.668**	.057
LM:m	.396	.004	-.051	.672**	-.051	-.024
OC in total macro-aggregates (g kg^{-1})	-.619**	-.537*	-.022	.069	-.172	.108
N in total macro-aggregates (g kg^{-1})	.262	.386	.629**	.678**	.319	.313
Micro-aggregates (%)	.011	.035	.565*	.670**	.156	.321
OC in micro-aggregates (g kg^{-1})	-.347	-.427	.096	.146	.302	.119
N in micro-aggregates (g kg^{-1})	.231	.345	.724**	.711**	.717**	.049
silt +clay (%)	.436	.630**	.857**	.744**	.395	.193
OC in the silt +clay fraction (g kg^{-1})	.542*	.558*	.194	-.092	.049	-.030
N in the silt +clay fraction (g kg^{-1})	.733**	.842**	.391	.441	.327	.108
OC in total macro-aggregates (g kg^{-1})	.710**	.758**	.389	.387	-.073	.092

Discussion

4.1. Impact of crop diversification and tillage management on soil organic carbon and nitrogen stocks, and SOC mineralization

Overall, inter-cropping rainfed almond trees with perennials such as caper or winter thyme under no-tillage did not enhance topsoil nor subsoil OC and N stocks after three years from establishment. Our results corroborate those by Martínez-Mena et al. (2021) in the same study site, who reported no changes in the topsoil OC stock 18 months after these crop diversifications were established. This highlights that soil carbon and nitrogen dynamics are slow in these low-input semiarid agroecosystems, particularly if intercropped with perennials returning recalcitrant plant residues to the soil (Chenu et al., 2019; Almagro et al., 2021). This statement is supported by the fact that SOC mineralization rates in the almond trees inter-cropped with winter thyme or caper were similar to those in the almond monocrop system despite larger C inputs in the former than in the latter.

In the irrigated high-input system, however, inter-cropping significantly affected the SOC and N stocks after three years from establishment, although contrasting effects were observed depending on the crop diversification practice. Compared to the mandarin monocrop, inter-cropping with the annual crop rotation (barley/vetch followed by fava bean) significantly increased the soil N stocks and reduced the SOC mineralization rates at 0-30 cm depth. On the contrary, inter-cropping with the triennial crop rotation (fava bean, purslane, and cowpea) led to a significant reduction in the topsoil OC stock paralleled by a slight (although no statistically significant) increase in the SOC mineralization rates. The differences in SOC mineralization rates between inter-cropping treatments in the irrigated system can be explained by the different amount and quality (i.e., chemical composition) of the fresh plant residues returned to the soil after harvest in each crop rotation that modulates the soil microbial response by the so-called priming effect (Kuzyakov et al., 2000; Blagodatsky et al., 2010). It is well known that soil microorganisms respond differently depending on the availability (amount and quality) of organic matter inputs, and particularly when new carbon and nitrogen sources are added to the system, as it is the case of inter-cropping in formerly bare soils. The N-rich crop residues from the legumes in the D3 treatment contributed to increase the soil N stock while decreasing the SOC mineralization rates, probably because the new OC and N sources satisfied microorganisms demand and no soil mineral N-mining was necessary. In the triennial crop rotation, however, there was only one leguminous in the crop rotation scheme and the soil was uncovered around nine months each year (see section 2.2.), which together with increasing tillage frequency, explain the abrupt decrease in the topsoil OC stock. Together, these results highlight the importance of combining inter-cropping with other sustainable agricultural management practices under semiarid Mediterranean conditions, such as organic or green manure addition in low-input systems, reducing tillage frequency operations, and including two or more leguminous species in the crop rotation schemes, supporting what was previously claimed by several authors for similar environmental conditions (Vicente-Vicente et al., 2016; Morugán-Coronado et al., 2020; Ruiz et al., 2020; Soto et al., 2021).

4.2. Impact of crop diversification and tillage management on soil aggregation, and OC and N pools

The distribution of water-stable aggregates and their associated OC and N contents were differently affected by inter-cropping in the rainfed and the irrigated system. In the rainfed system, an increment in the proportion of large-macroaggregates as well as in their associated OC and N contents was found in the subsoil of the almond trees inter-cropped with winter thyme compared to the almond monocrop system. Noteworthy, the increment in the OC and N contained in the large macro-aggregates was paralleled by a reduction of the OC and N contained in the micro-aggregates together with reductions in the N contained in the small macro-aggregates and the micro-aggregates. This pattern is explained by the combined effect of tillage cessation, avoiding soil macro-aggregates disruption, and winter thyme root growth and exudates acting as binding agents, altogether leading to a positive feedback loop that promotes the formation and preservation of large macro-aggregates and the occlusion of the micro-aggregates within them (a physical protection and stabilization mechanism; García-Franco et al., 2015, 2021; Gale et al., 2000; Denef and Six, 2006; Virto et al., 2007; Cotrufo et al., 2013; Ye et al., 2021). Our statement is supported by the observed significant positive correlations between the particulate organic carbon fraction (a precursor of macro-aggregate formation) and the OC and N contents associated to the macro- and micro-aggregates in the almond inter-cropped with winter thyme (D2 treatment; see Table S1). Other authors have reported significant positive correlations between labile carbon fractions and macro-aggregates (Bhattacharyya et al., 2012; Shen et al., 2021). Over time, the initial effect of tillage cessation will be gradually maintained by fresh plant material entering the soil, derived from the growth of the secondary crop, and likely further stabilization mechanisms will be activated in the long run, such as the formation of stable microaggregates (Edwards and Bremner, 1967; Golchin et al., 1994). Although previous research has reported micro-aggregate formation in similar period time as that from our experiment (Denef and Six, 2006; Shahbaz et al., 2017), the harsh pedoclimatic conditions in our study site (*i.e*, very low soil organic matter content together with scarce water availability that constrain the production of plant biomass inputs and the microbial activity) and the recalcitrant nature of the plant residues derived from perennials (winter thyme and caper) were unfavourable for short-term microaggregate formation and the process will unfold very slowly.

Our results also highlight the importance of the amount and quality of fresh plant residues to foster soil aggregation and OC and N stabilization in semiarid environments. It is worth mentioning that inter-cropping almond trees with caper did not have the same effect on aggregation than did inter-cropping almond trees with winter thyme. This is explained not only by the lower plantation density of caper compared to that of winter thyme but also by phenological differences between both secondary crops leading to distinct amounts of annual fresh plant residues between them. Namely, while winter thyme provides a permanent plant cover and continuous fresh plant inputs to the soil from its establishment, caper shoots are lost annually from November to April, and therefore additional carbon inputs from this crop can be only accounted for half of the year. Moreover, it is well known that above- and below-ground plant residues have different effects on soil aggregation (Puget and Drinkwater, 2001; Denef and Six, 2006; Almagro et al., 2021), and that the ability to foster new macro-aggregate formation depends on their chemical composition due to its double action as a binding agent and as a stimulating microbial resource (Homulle et al., 2021).

In the irrigated system, on the contrary, reductions in the proportion of the large and small macroaggregates paralleled by an increase in the silt plus clay fraction were found in both crop diversification systems compared to the monocrop system, being this effect more pronounced in the topsoil. Noteworthy, the negative effect of inter-cropping on soil aggregation and OC and N stabilization was more abrupt when mandarin trees were inter-cropped with the triennial crop rotation (D4 treatment) than with the annual crop rotation (D3 treatment) since significant reductions in the proportion of stable micro-aggregates were also found in the former. Despite larger plant biomass inputs into the soil from the secondary crops, the OC and N contents associated to the macro-aggregates (LM and SM) and microaggregates (m) of the topsoil significantly decreased in both crop diversification systems. In the subsoil, although only in the case of the mandarins inter-cropped with the triennial crop rotation, the OC content was also reduced in all aggregate sizes except for the large macro-aggregates (LM). These results can be explained by the fact that in both crop diversification systems the frequency of tillage operations was increased while no significant increments in the amount of plant C and N inputs into the soil occurred (*i.e.*, all secondary crops are harvested) compared to the mandarin monocrop system. This has probably promoted the disruption of the new macro-aggregates potentially formed by the roots of the secondary crops that otherwise would have contributed to the incorporation and physical protection of the plant OC inputs.

Conclusions

Our results highlight that decreasing tillage frequency and improving the amount and quality of crop residues is pivotal to foster SOC and N sequestration and stabilization in semiarid degraded agricultural soils. Despite the short-term nature of our study (3 years), inter-cropping rainfed almond monocrops with perennials such as winter thyme in combination with tillage cessation contributed to climate change mitigation and soil restoration by fostering aggregation and organic carbon and nitrogen stabilization without increasing SOC mineralisation rates. However, inter-cropping irrigated mandarin monocrops with annual crops caused reductions in the OC content associated to the macro- and micro-aggregates despite increased plant C inputs, reinforcing the idea that improving the quality of fresh plant residues by incorporating two or more leguminous species in the crop rotation schemes is pivotal to offset the potential reductions in the SOC and N resistant pools (at least in the subsoil). Moreover, using cover crops as green manure instead of harvesting them for livestock feeding (as it is the case in our study) during the first years is highly recommended to boost soil restoration processes in these fragile and degraded agricultural soils. Further research to understand the soil response to new carbon and nitrogen sources will improve our capacity to predict how the adoption of inter-cropping will influence SOC and N stocks in agricultural areas. Likewise, long-term experiments are needed to monitor whether the observed patterns is this study are consistent over time before drawing definitive conclusions on the impacts of inter-cropping on SOC and N storage and preservation.

Acknowledgements

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