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Soil organic matter fractions in the topsoil and subsoil of woody crop systems: Impact of reduced tillage plus cover crops under rainfed semi-arid Mediterranean conditions

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ABSTRACT

Carbon dynamics in deep layers have potential for soil C sequestration and contribute to the fight against global climate change, however, in Mediterranean regions, most studies focus on the top 20–30 cm soil layer. To advance this knowledge, this study proposes to investigate the behavior of different SOC compartments in the topsoil (0–15 cm) and subsoil (15–60 cm) in two rainfed almond orchards under conditions typical of Mediterranean regions. The treatments evaluated included seeded cover crops (SCC), and spontaneous cover crops (NCC) combined with reduced tillage and traditional tillage (TT). Moreover, an undisturbed natural reference area (forest) representing the pre-cultivated conditions was included. Samples from two experimental sites (Burete and Cagitán) were collected at intervals of 0–15, 15–30, 30–50 and 50–60 cm, and quantified bulk density, soil organic carbon (SOC), particulate organic carbon (POC) and Nitrogen (PON), hot water extractable carbon (HWOC) and short-term mineralizable carbon (SMC). The results showed that the use of cover crops and reduced tillage improve microbial activity, increase soil organic matter fractions, and improve carbon storage, both in the topsoil and subsoil, being an effective and useful strategy for the improvement of soil C sequestration under rainfed almond crop systems in semi-arid areas. In addition, the positive effect of cover crops on SOC storage would have been underestimated by about 35–40 % without accounting for the stock at the 30–60 cm layer. The use of different organic carbon and nitrogen fractions has furthered the understanding of soil carbon dynamics and proved effective in detecting differences among soil management strategies.

1. Introduction

Soil organic carbon (SOC) is a vital element of terrestrial ecosystems, contributing significantly to their overall resilience and productivity (Georgiou et al., 2022). In vulnerable regions such as the Mediterranean, high climate variability, including changes in temperature and precipitation, directly affects the potential of soils to store carbon (Funes et al., 2022). Estimating SOC content in these environments is essential to support sustainable land-use practices that enhance SOC sequestration and contribute to climate change mitigation (Shen et al., 2024).

SOC accumulation largely depends on carbon inputs through plant biomass, organic residues and decomposition processes. However, typical Mediterranean conditions, such as water scarcity, nutrient limitations and soil disturbance, reduce net primary productivity and, consequently, SOC inputs from litter, roots and crop residues (Funes et al., 2019; Rabbi et al., 2015). Land management strategies such as cover cropping, reduced tillage, crop diversification and agroforestry have shown potential to improve SOC and nitrogen (N) in these areas (Fernández-Soler et al., 2024; Funes et al., 2022; Vicente-Vicente et al., 2017).

Changes in carbon stocks are more evident in the surface layers (0–30 cm), due to the greater residue deposition and climatic sensitivity, which directly affects the decomposition of organic matter and carbon mineralization (Albaladejo et al., 2013). The potential of cropland subsoils to store additional C remains an open question, as most studies focus on the top 20–30 cm layer of soil (Guillaume et al., 2022). Several

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authors highlight that deep soils may have a greater potential to store persistent SOC than surface soils, due to their lower susceptibility to biotic and abiotic factors that contribute to climate change mitigation (Hu et al., 2023; Pries et al., 2023; Zheng et al., 2025). However, most studies have focused on cereal crops in humid and sub-humid regions, while studies on woody crop systems under Mediterranean conditions have typically limited soil sampling to 30 cm (e.g., Addesso et al., 2025), leaving subsoil SOC dynamics largely unexplored.

In addition, most of the studies evaluating SOC in deep soils focus on total SOC content, which can obscure critical differences in carbon stabilization mechanisms (Ghimire et al., 2019; Okolo et al., 2023). However, SOC comprises functionally distinct fractions such as particulate organic carbon (POC), hot water-soluble carbon (HWOC), microbial biomass carbon (MBC, SMC), and mineral-associated organic carbon (MAOC) each characterized by specific origins, turnover rates, and roles in long-term sequestration (Cotrufo and Lavallee, 2022; Hu et al., 2023; Liang et al., 2017). These fractions are more responsive to management practices and offer more accurate insights into SOC dynamics than total SOC alone. However, the influence of reduced tillage and cover cropping on the distribution of SOC fractions in subsoil layers remains poorly understood, representing a critical research gap (Fohrafellner et al., 2023; Sapkota et al., 2024).

To advance this knowledge, this study proposes to investigate the behavior of different SOC compartments in the topsoil (0–15 cm) and subsoil (15–60 cm) in two rainfed almond orchards, under conditions typical of Mediterranean regions, where different combinations of reduced tillage and cover crops (natural and seeded cover crops) have been implemented for 14 and 9 years. The specific objectives are: i) To quantify the total SOC and N sequestration at different depths and management; ii) To explore the relative contribution of each OC and N pool to the total SOC and N at different depths and management; and iii) To investigate the relationships between SOC, N and various OC and N pools across depths.

2. Materials and methods

2.1. Study site

The study was carried out in the high steppe plateau of the province of Murcia on relatively shallow soils (average soil depth of about 60 cm) and low slope (< 10 %) in south-east Spain. The two experimental sites were located in Cehegín (38°3′15’’N 1°46’12’’W; 633 m a.s.l.) and Cieza (38°11’23’’N 1°29’52’’W; 431 m a.s.l.) hereafter referred to as “Burete” and “Cagitán”, respectively. The soils were classified as Petric Calcisol (Burete) and Regosol (Cagitán) according to the World Reference Base (IUSS Working Group, WRB 2015) with loam (Burete) and silty loam (Cagitán) texture, and developed on colluvium. Both soils had a basic pH (8.5), attributed to their high carbonate content (~60 %), together with low electrical conductivity (<250 μS cm^{-1}), low SOC (<1.5 g kg^{-1}) and low tN (<1.2 g kg^{-1}) at 0–60 cm depth. An undisturbed natural area on the steep upper slopes and adjacent to both sites served as a reference to represent the pre-cultivated conditions. These reference soils were shallow (< 30 cm depth) and had a moderate slope (about 20 %), in contrast to the cultivated soils. Physical and chemical properties of the soil at both sites under the different land uses, management and depths are showed in Table S1.

The climate is semi-arid Mediterranean. The average annual precipitation is 370 mm (Burete) and 250 mm (Cagitán), concentrated in autumn and spring, but with great inter and intra-annual variability. The mean annual temperatures were 16.6 ºC in Burete and 17.2 ºC in Cagitán. The mean potential evapotranspiration at both sites was 1200 mm year^{-1} (calculated by Thornthwaite’s method) and the average annual deficit was 500 mm (period of 30 years, Fernández-Soler et al., 2024). In the last 14 years (since the beginning of the Burete experiment), mean annual precipitation has slightly decreased, being 304.4 mm and 228.2 mm for Burete and Cagitán, respectively (Fig. 1).

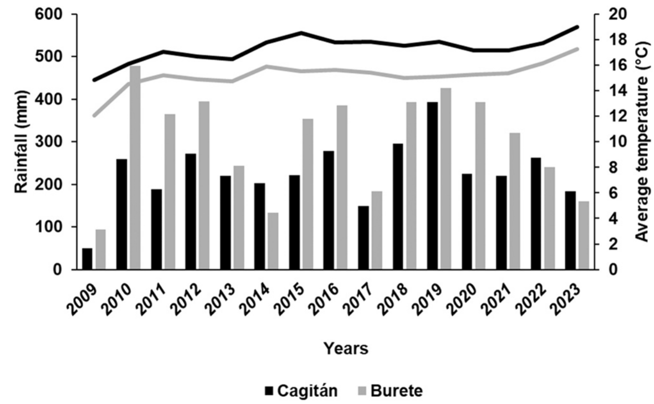

Fig. 1. Average annual rainfall and temperature in Burete and Cagitán from 2009 to 2023. Source: Sistema de Información Agrario de Murcia (SIAM). https://www.siam.carm.es.

2.2. Experimental design

The experiment consisted of implementing different soil management practices in two organic rainfed almond orchards (*Prunus dulcis* Mill.) with a planting framework of 7 m x 7 m. The usual soil management practice in the experimental farms, referred to as traditional tillage (TT), consisted of frequent tillage, (three to five times a year) to control weeds after heavy rainfall events, leaving the soil bare for most of the year.

In Burete, two additional soil management practices were applied in 2009: i) reduce tillage plus natural cover crops (NCC) and ii) reduce tillage plus seeded cover crops (SCC). The reduced tillage was carried out using the same cultivator as in the TT, twice a year (autumn and spring). The natural cover crops consisted of annual and perennial vegetation growing between the rows (*Hordeum murinum* L., *Lolium perenne* L., *Eruca vesicaria* (L.) Cav). The seeded cover crops were composed of a seed mixture of common vetch (*Vicia sativa* L.) and common oats (*Avena sativa* L.) in a 3:1 proportion. The seed mixtures were sown annually during early autumn at a density of 150 kg ha^{-1}, manually mowed and then incorporated into the soil by tillage during spring (see more detail in Almagro et al., 2017). In the Cagitán experimental site, reduced tillage was implemented for six years (2014–2020) and no tillage for three years (2021–2023) plus natural cover crops (NCC). At this site, annual and perennial vegetation growing between rows and residues were incorporated into the soil (2014–2020) and left on the soil surface for two years (2021–2023). The most representative species were *Sonchus* sp. *Anacyclus clavatus* (Desf.) Pers., *Moricandia arvensis* L. (DC.), *Diplotaxis* sp. and *Scorpiurus muricatus* L. All tillage (TT, NCC and SCC) described above consisted of chisel ploughing to a depth of 15 cm, using a cultivator and affecting the entire plot area, including the soil surrounding each base tree.

At both sites, the undisturbed natural area was covered by a typical Mediterranean shrubland with scattered Aleppo pines (*Pinus halepensis* Mill.), hereafter, forest. The dominant species composition differed between sites: Burete was characterized by *Rosmarinus officinalis* L., *Quercus coccifera* L., *Rhamnus lycioides* L., and *Juniperus oxycedrus* L, - whereas Cagitán’s natural area hosted *Stipa tenacissima L.*, *Rosmarinus officinalis L.*, *Salsola genistoides* Juss. ex Poir., *Asparagus acutifolius* L., *Globularia alypum* L. and *Anthyllis cytisoide* L.

2.3. Soil sampling and sample preparation

Soil samples were collected in November 2023 in each undisturbed natural and in each cultivated area by making a soil pit. Due to the shallow soil profiles (<30 cm) of the undisturbed natural areas, two soil layers of 0–15 and 15–30 cm were established for soil sample collection.

In the cultivated areas, soil samples were collected from a depth interval of 0–60 cm, and further subdivided according to sampling depth: 0–15 cm, 15–30 cm, 30–50 cm and 50–60 cm. Three samples of disturbed composite soil were collected per management and depth. Three undisturbed samples were also collected using steel cylinders (100 cm core volume) to determine bulk density. The disturbed soil samples were air-dried and sieved to < 2 mm for determining texture, pH, carbonates content, and organic carbon and nitrogen pools.

3. Methods

3.1. Soil physicochemical properties

Soil bulk density (BD, g cm^{-3}) was calculated from the oven-dried mass (105 ºC, 24 h) according to Burke et al. (1986). Soil texture (particle size distribution) was determined using a Coulter LS200 'Laser particle sizer' (Coulter Corporation, Miami, Florida). Soil samples were previously treated with hydrogen peroxide (H_2O_2) to remove organic matter before dispersing them with sodium hexametaphosphate for 12 h. Soil pH was determined using an electrode method (H_2O, 1:5). Soil calcium carbonate ($CaCO_3$) was calculated with the Bernard Calcimeter. Total soil organic carbon (SOC, g kg^{-1}) and nitrogen (tN, g kg^{-1}) were analyzed using an N/C Analyzer (Flash 1112 EA, Thermo-153 Finnigan, Bremen, Germany) after eliminating soil carbonates with 2 M HCl according to the International Organization for Standardization (1998).

3.2. Soil OC and N stocks

Soil OC and N stocks were calculated in the 0–15 cm, 0–30 cm and 0–60 cm layers. Using bulk density measurements, the soil mass in each sampled layer was determined. Any excess soil mass of a specific profile was discounted from its deepest layer (15–30 cm and 50–60 cm for forest and cultivated, respectively) using the total soil mass associated with the lowest bulk density value (indicating the least amount of compaction). This procedure is mathematically described by Sisti et al. (2004).

3.3. Soil organic matter fractionation

Particulate organic carbon (POC, g kg^{-1}), nitrogen (PON, g kg^{-1}) and mineral-associated organic carbon (MAOC) were determined following the method described by Cambardella and Elliott (1992). Briefly, 20 g of the ground air-dried simples were dispersed by shaking in a 100 ml solution of sodium hexametaphosphate (5 g l^{-1}) for at least 12 h. The mixture was then sieved through a 53 µm sieve and gently rinsed with deionized water to remove reagent remnants. The material retained on the sieve was filtered, dried in an oven at 60 ºC for 48 h, weighted and finely ground with a ball mixer mill. The organic carbon and nitrogen content of these pools was analyzed with the same N/C Analyzer described above. The material that passed through the 53 µm sieve, consisting of MAOC from the silt and clay fractions, was quantified by the difference between total organic C and POC. POC, PON and MAOC were then calculated by multiplying the percentages of organic carbon and nitrogen by the weight percentage of dried retained material.

The POC, PON and MAOC stocks were calculated as follows:

$$(\text{POC, PON or MAOC})_{\text{Stock}} = (\text{POC, PON or MAOC}) \cdot \text{BD} \cdot \text{D} \cdot (1\text{-P}) \quad (1)$$

Where POC, PON and MAOC represent particulate organic carbon and nitrogen and mineral-associated organic carbon, respectively in the bulk soil, BD is the bulk density, D is depth, and P represents the volumetric stone fraction (> 2 mm).

3.4. Easily decomposable SOC and N

Hot water extractable organic carbon (HWOC) and nitrogen (HWN) were determined following the method described by Ghani et al. (2003). Briefly, 10 g of the ground air-dried samples were shaken (30 rpm for 30 min) with 40 ml of distilled water in Falcon tubes. The tubes were then placed in a water bath at a temperature of 80 ºC for 16 h. Afterwards, centrifugation was performed at 3000 rpm for 20 min and the supernatant was filtered through 0.45 µm cellulose membrane filters. The HWOC and HWN fraction corresponds to the dissolved organic carbon and nitrogen in the extracted water passed through the filter described above.

Short-term mineralizable carbon (SMC) was obtained by the lab incubation method (Franzluebbers et al., 2000). Soil subsamples (30 g) were moistened to 60 % WHC before incubation in 125 ml air-tight containers for an incubation period under controlled conditions for 72 h at 28 °C. The CO_2 (%) released from the containers was measured with an infrared gas analyzer (CheckMate 232 II, PBI Dansensor, Denmark). SMC was calculated as the C average content respired daily per gram of OC in the soil and was expressed as mg C-CO_2 kg^{-1} soil.

3.5. Data Analysis

For the statistical analysis, homogeneity of error variances and normality of the data were tested using the Shapiro–Wilk test. ANOVA was then performed, followed by a Tukey's post-hoc test, using RStudio software Version 4.3.0 (R code Team, 2018) to analyze the interaction between depth and land use management systems ($n = 3$). The graphs were generated using the mean of each treatment and the standard error of the mean ($n = 3$), using SigmaPlot (Version 14.0). Correlation analysis between the different variables studied was performed using the Spearman's non-parametric rank test, implemented through the *Hmisc* R package (v 5.2–3) (Harrell Jr., 2025). These correlation results were visualized as correlation plots using ggcorrplot (Kassambara, 2023) (v 0.1.4.1), with additional graphical refinements of ggplot2 (Wickham, 2016) and ggtext (Wilke and Wiernik, 2022) (v 0.1.2) R packages. All of the above inferential analyses were performed and considered significant at a P-value < 0.05.

4. Results and discussion

4.1. Effects of land use and managements on carbon and nitrogen sequestration in the deeper layers

The results illustrate the patterns of OC and N in soils managed under different land use systems in two experimental areas under semi-arid Mediterranean conditions (Table 1). In Burete, the mean of SOC and tN concentrations ranged from 7.66 to 29.96 g kg^{-1} and from 0.61 to 2.09 g kg^{-1} respectively, depending on depth and management. In Cagitán, SOC and tN concentrations ranged from 5.22 to 12.36 g kg^{-1} and from 0.61 to 1.30 g kg^{-1} respectively, depending on depth and management. In general, the highest mean concentrations of SOC, tN and C/N ratio were observed in the surface layer (0–15 cm), with forest soil having the highest values (almost double, Table 1), and it decreased with depth. These patterns are commonly observed under Mediterranean soil conditions in experiments involving natural vegetation, pasture, woody crops, and no-tillage systems (Aguilera-Huertas et al., 2022; Albaladejo et al., 2013; Funes et al., 2019, 2022; Torres et al., 2021). It can be explained by the greater accumulation of plant biomass (and the influence of root systems) in the topsoil, and the rapid decrease with depth of above- and below-ground biomass in forest and cultivated areas (Cerli et al., 2012; Six et al., 2002).

Table 1
Soil organic carbon, total nitrogen and C/N ratio (mean ± standard error) under different land management practices and soil depths.

Site	Properties	Depth (cm)	Management			
			TT	NCC	SCC	Forest
Burete						
	SOC (g kg^{-1})	0–15	12.03 ± 1.54 b	10.06 ± 0.86 b	12.19 ± 0.96 b	29.96 ± 1.06 a
		15–30	10.10 ± 0.94 b	8.62 ± 0.78 b	10.16 ± 0.50 b	24.33 ± 2.92 a
		30–50	11.11 ± 2.04	8.91 ± 0.86	10.01 ± 0.33	-
		50–60	10.59 ± 0.82 a	7.66 ± 0.19 b	11.47 ± 0.00 a	-
	tN (g kg^{-1})	0–15	1.21 ± 0.03 b A	0.93 ± 0.08 b A	1.01 ± 0.10 b	2.09 ± 0.10 a A
		15–30	1.04 ± 0.01 b AB	0.87 ± 0.02c A	0.82 ± 0.04c	1.84 ± 0.04 a B
		30–50	1.00 ± 0.11 AB	0.89 ± 0.07 A	0.81 ± 0.06	-
		50–60	0.86 ± 0.05 a B	0.61 ± 0.06 b B	0.98 ± 0.00 a	-
	C/N ratio	0–15	10.01 ± 1.53 b	10.87 ± 1.17 b	12.17 ± 1.10 ab	14.32 ± 0.24 a
		15–30	9.63 ± 0.88 b	9.93 ± 0.79 ab	12.47 ± 0.37 ab	13.17 ± 1.26 a
		30–50	10.97 ± 1.03	9.92 ± 0.24	12.31 ± 0.38	-
		50–60	12.46 ± 1.35	12.82 ± 1.70	11.67 ± 0.00	-
Cagitán						
			TT	NCC		Forest
	SOC (g kg^{-1})	0–15	6.41 ± 0.16 b AB	11.12 ± 0.90 a A		12.36 ± 0.48 a
		15–30	7.34 ± 0.67 A	9.47 ± 0.92 A		10.68 ± 1.10
		30–50	5.88 ± 0.26 AB	7.50 ± 0.89 B		-
		50–60	5.22 ± 0.39 b B	7.62 ± 0.39 a B		-
	tN (g kg^{-1})	0–15	0.67 ± 0.04 b	1.30 ± 0.01 a A		1.17 ± 0.07 a A
		15–30	0.72 ± 0.03 b	1.02 ± 0.06 a B		0.80 ± 0.03 b B
		30–50	0.61 ± 0.02 b	0.87 ± 0.003 a BC		-
		50–60	0.64 ± 0.02 b	0.76 ± 0.06 a C		-
	C/N ratio	0–15	9.55 ± 0.29	8.54 ± 0.73		10.63 ± 0.75
		15–30	10.13 ± 0.71	9.28 ± 0.64		13.43 ± 1.85
		30–50	9.66 ± 0.63	8.58 ± 0.98		-
		50–60	8.17 ± 0.64 b	10.08 ± 0.42 a		-

TT: traditional tillage; NCC: natural cover crops; SCC: seeded cover crops; Forest: undisturbed natural soil. Different lowercase letters in rows means significant differences between managements at each soil depth. Different uppercase letters in columns indicate significant differences in depth within each management under the Tukey test ($P < 0.05$).

No significant differences in BD were observed with depth, and therefore, the results of OC and N stocks are mainly attributed to their concentration in soil (Table S2). Both SOC and tN stocks in Burete are significantly higher in Forest compared to cultivated areas, independent of management. In the 0–60 cm layer, higher mean SOC and tN stocks were found under SCC and TT management compared to NCC (Fig. 2A, B). When TT was converted to SCC, sequestration rates were almost zero at 0–15 (-0.01 Mg OC ha^{-1} year^{-1}) but positive at 0–30 (0.29 Mg OC ha^{-1} year^{-1}) and 0–60 cm depths (0.49 Mg OC ha^{-1} year^{-1}). Conversion of undisturbed natural soils (Forest) to cultivated areas in Burete resulted in a loss of OC and tN of between 49 % and 56 %, with no significant differences between sustainable practices and traditional tillage, neither at 0–15 nor at 0–30 cm depth. Therefore, excluding the 30–60 cm layer from C accounting would have resulted in a 40 % underestimation of the potential of croplands to store additional SOC by introducing seeded cover crops. This underestimation value is in a similar range than that reported in a Swedish study where the effect of temporary grasslands on SOC storage was underestimated by 58 % without accounting for subsoil stocks (Guillaume et al., 2022). In order to evaluate the potential of reduced tillage and the contribution of legumes to the accumulation of OC in the surface and subsurface layers of an Acrisol in Southern Brazil, Veloso et al. (2018) demonstrated that approximately half of the OC storage is due to increased OC in the subsurface layer (30–100 cm). The direct effect of deep roots or the transport of organic residues by soil organisms to deeper layers favors a higher accumulation of OC and N at depth. This result reinforces the importance of understanding the dynamics of OC and N in deeper soil layers.

In Cagitán, converting forest to cultivated land under traditional tillage led to a 36 % loss of OC in the 0–30 cm soil layer. In contrast, NCC maintained similar SOC stocks to forest, suggesting that reducing and halting tillage enhanced carbon storage. NCC also showed higher tN stocks in the 0–60 cm layer (7.2 vs. 5.3 Mg ha^{-1}) and higher, though not statistically significant, OC stocks compared to TT (Figs. 2C, 2D). SOC and tN sequestration rates increased with depth under NCC, reaching up to 1.58 Mg OC ha^{-1} year^{-1} and 0.22 Mg N ha^{-1} year^{-1} at 0–60 cm. Thomaz and Kurasz (2023) found that the forest area showed 20 % more carbon stock than no-till at 0–20 cm soil depth, while the entire no-till soil profile (0–60 cm) showed similar soil carbon to that of forest soil (probably due to the effect of bulk density values). In this site, the change from traditional tillage to NCC led to a higher SOC stock, not only in the top surface (0–15 cm) but also in the subsoil. However, as in the case of the Burete site, the potential sequestration rate under NCC (considering 9 years of reduced tillage prior to no tillage) was 35 % higher when the 30–60 cm layer was considered, rather than just the top surface layer.

Cover crops can increase SOC stocks through plant input, its decomposition and subsequent stabilization (Cotrufo and Lavallee, 2022, Hu et al., 2023). However, the climate scenario in the Mediterranean region, characterized by rising temperatures and reduced soil water availability, could negatively impact crop yields and soil C inputs (Funes et al., 2019). At the Burete experimental site, an increase in temperature (~ 1.5ºC) and a reduction in precipitation (11 %) have been observed in the three years prior to sampling compared to a longer period of 14 years (Fig. 1), which may have reduced crop productivity and carbon inputs, leading to similar SOC and tN stocks between TT and cover crop systems. The reduction in the number of tillage passes in TT due to this drier condition further minimized differences between traditional and cover crops managements. In fact, the SOC and tN content obtained in TT, NCC and SCC in the top layer was lower than that reported in a study conducted three years earlier (Fernández-Soler et al., 2024). This reduction was more pronounced under cover crops than under TT, probably due to greater exposure to solar radiation under the former (Fig. S1A). These environmental conditions could accelerate residue decomposition, promoting the mineralization of existing OC and leading to subsequent loss and decrease of SOC. The introduction of

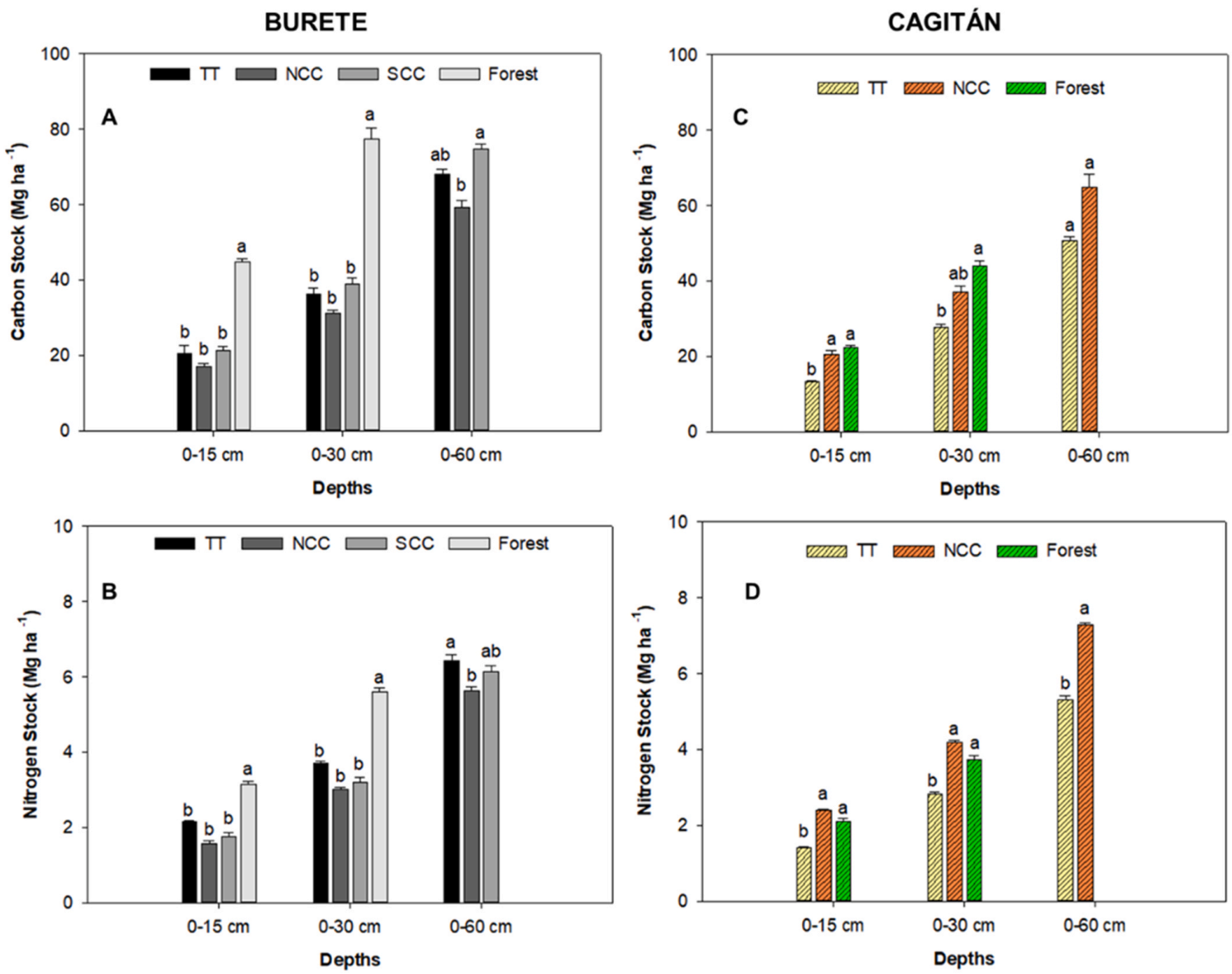

Fig. 2. Stocks of soil organic carbon (A, B) and total nitrogen (C, D), at 0–15, 0–30 and 0–60 cm soil layer under TT: traditional tillage; NCC: natural cover crops; SCC: seeded cover crops and undisturbed natural area: Forest. Means ± standard error. Bars followed by the same letter do not differ from each other using the Tukey test ($P < 0.05$).

cover crops (with more labile carbon) likely promoted the mineralization of plant material, resulting in the formation of more stable organic matter through a phenomenon known as priming effect (Liu et al., 2017).

The pattern of change in temperature and precipitation observed in Burete was negligible in Cagitán (0.6°C increase in temperature and 3 % reduction in precipitation). Moreover, in this site, there is not differences in the exposure to solar radiation among cultivated fields (Fig. S1B), which could explain the greater differences observed between traditional and cover crops management practices regarding SOC and tN sequestration compared to Burete site. In addition, SOC and tN contents in the top layer in NCC obtained in the present study were higher than values obtained in a previous sampling conducted three years earlier (data not displayed), while SOC and tN contents in TT were similar, suggesting that environmental conditions did not hide the positive impact of cover crops on OC and N sequestration. According to Hartley et al. (2021), the effects of temperature increase on carbon stocks are more noticeable in sandy than in finer soils, which is consistent with the loam and clay loam texture in Burete and Cagitán, respectively (Table S1).

4.2. *Effects of land use and managements on soil organic carbon fractions stocks at different depths*

Table 2 illustrates the fractions of soil organic matter at the two experimental sites. POC, PON, and MAOC exhibited distinct distribution patterns across soil layers depending on land use. At the Burete site, the Forest area showed higher mean concentrations of POC, PON, and MAOC compared to cultivated areas within the top 30 cm of soil, regardless of management practice (NCC, SCC, or TT). This resulted in significantly higher stocks of these fractions in the Forest at 0–15 and 0–30 cm depths (Fig. 3A, B, C). A decrease in mean POC and PON was observed from the surface (0–15 cm) to deeper layers under the different managements, being significant for TT and SCC (Table 2). Under SCC, a higher PON concentration was observed at 50–60 cm depth, which may be attributed to root exudates or nitrogen transport by roots from legume and grass cover crops to deeper soil layers. At 0–15 and 0–30 cm depth, the contribution of POC to total soil carbon stock ranged from 25 % to 42 %, with the lowest value observed in the NCC system and the highest in the Forest system. In contrast, the trend was reverted for MAOC, with a higher contribution in NCC (75 %) compared to Forest (58 %). At 0–60 cm depth, the cultivated areas showed a similar contribution of POC and MAOC to the total SOC, with an average of approximately 24 % and 76 % of POC and MAOC, respectively.

Table 2
Soil organic carbon fractions concentration (mean ± standard error) under different land management practices and soil depths.

Site	Properties	Depth (cm)	Management			
			TT	NCC	SCC	Forest
Burete						
	POC (g kg^{-1})	0–15	4.06 ± 0.15 b A	2.93 ± 0.32 b	3.90 ± 0.17 b A	12.63 ± 0.18 a
		15–30	2.44 ± 0.04 b B	1.86 ± 0.01 b	1.96 ± 0.15 b B	10.24 ± 1.03 a
		30–50	1.83 ± 0.14 B	1.80 ± 0.03	1.85 ± 0.12 B	-
		50–60	2.00 ± 0.09 B	1.91 ± 0.02	2.42 ± 0.003 B	-
	PON (g kg^{-1})	0–15	0.37 ± 0.01 b A	0.50 ± 0.08 b	0.36 ± 0.02 b A	0.96 ± 0.03 a
		15–30	0.17 ± 0.01 b B	0.20 ± 0.02 b	0.18 ± 0.02 b B	0.63 ± 0.05 a
		30–50	0.11 ± 0.01 B	0.23 ± 0.05	0.15 ± 0.01 B	-
		50–60	0.10 ± 0.01 B	0.20 ± 0.03	0.25 ± 0.002 AB	-
	MAOC (g kg^{-1})	0–15	7.97 ± 0.87 b	7.13 ± 0.43 b	8.28 ± 0.36 b	17.33 ± 0.44 a
		15–30	7.67 ± 0.45 b	6.76 ± 0.36 b	8.20 ± 0.23 b	14.09 ± 0.70 a
		30–50	9.28 ± 0.85	7.11 ± 0.43	8.16 ± 0.12	-
		50–60	8.59 ± 0.36 a	5.75 ± 0.08 b	9.05 ± 0.01 a	-
Cagitán			TT	NCC		Forest
	POC (g kg^{-1})	0–15	1.20 ± 0.07c	3.12 ± 0.16 b A		6.98 ± 0.51 a A
		15–30	1.10 ± 0.03 b	2.28 ± 0.08 a A		2.23 ± 0.31 a B
		30–50	1.02 ± 0.02	1.30 ± 0.05 B		
		50–60	1.00 ± 0.01	1.16 ± 0.07 B		
	PON (g kg^{-1})	0–15	0.09 ± 0.002 b A	0.31 ± 0.01 a A		0.37 ± 0.05 a A
		15–30	0.05 ± 0.004 b AB	0.14 ± 0.005 a B		0.08 ± 0.002 b B
		30–50	0.04 ± 0.001 B	0.04 ± 0.006 C		
		50–60	0.03 ± 0.001 B	0.04 ± 0.006 C		
	MAOC (g kg^{-1})	0–15	5.21 ± 0.09 b AB	8.00 ± 0.33 a		5.38 ± 0.44 b
		15–30	6.24 ± 0.34 A	7.19 ± 0.49		6.96 ± 0.63
		30–50	4.85 ± 0.10 AB	6.20 ± 0.37		
		50–60	4.22 ± 0.19 B	6.46 ± 0.25		

TT: traditional tillage; NCC: natural cover crops; SCC: reduced tillage plus seeded cover crops; Undisturbed natural area: Forest; POC: particulate organic carbon; PON: particulate organic nitrogen; MAOC: mineral-associated organic carbon. Different lowercase letters in rows means significant differences between managements at each soil depth. Different uppercase letters in columns indicate significant differences in depth within each management. Tukey test ($P < 0.05$).

In Cagitán, higher mean concentrations of POC were observed in Forest, followed by NCC and TT at 0–15 cm while no differences were observed between Forest and NCC at 15–30 cm depth (Table 2). In addition, a decrease in these fractions was observed with increasing soil depth in all managements. Higher mean values of PON were observed for Forest and NCC than for TT in the surface layer (0–15 cm) while NCC had the highest PON values at 15–30 cm. Depth directly influenced the dynamics of accumulation and distribution of MAOC in the soil. Thus, in the top layer (0–15 cm), NCC showed the highest mean MAOC concentration, even surpassing Forest, while below this depth, similar MAOC concentrations were observed between managements. The contribution of POC to total SOC was about 19 %, 28 % and 56 % for TT, NCC and Forest, respectively, in the surface layer (0–15 cm) while MAOC accounted for about 80 % and 70 % of total SOC in TT and NCC, respectively, but about 45 % in Forest. At 0–60 cm depth, similar proportions of POC (~ 18 %) and MAOC (~ 80 %) in TT and NCC were observed.

Cover crops were found to have a positive impact on SOC fractions (Cotrufo and Lavallee, 2022; Hu et al., 2023), as verified in SCC and NCC in Burete and Cagitán, respectively. Vicente-Vicente et al. (2017) observed that the implementation of plant cover in olive orchards in Granada (Mediterranean) doubled SOC compared to bare soil management, with most of the SOC being physically, chemically or biochemically protected from microbial activity. However, spontaneous soil cover (NCC) in Burete generally showed lower stocks and fractions of SOC compared to traditional tillage (Figs. 2 and 3). The increase in SOC stock in the deepest layer (50–60 cm) observed in both SCC and NCC in Burete and Cagitán, respectively, together with the highest contribution of MAOC to total SOC compared to TT in both sites (Table 2), indicates a higher protection of SOC from environmental and anthropic factors in deeper layers. However, spontaneous cover crops in Burete did not show the same protection role as SCC, as NCC generally had lower stocks (Table 1) and fractions of SOC (Table 2) throughout the soil profile compared to traditional tillage. Overall, these results highlight that the balance between POC and MAOC fractions leads to greater C stocks, the extent of which depends on the intrinsic characteristics of each region and land use. Fulton-Smith et al. (2024) reported that efforts to increase soil carbon sequestration should focus on both fractions, with the balance between them improving both carbon sequestration and soil health in agroecosystems.

4.3. Effects of land use and managements on easily decomposable OC fractions at different depths and managements

Soil organisms play a crucial role in ecosystems, including the decomposition of organic matter, nutrient cycling, increasing agricultural productivity and ultimately environmental sustainability (Baretta et al., 2007; Mendes et al., 2017). The dynamics of C and N interact closely during the decomposition of plant materials due to the simultaneous assimilation of these nutrients by the soil microorganisms involved in this process (Mohanty et al., 2013). Due to their rapid response to changes in land use and the influence of biotic and abiotic factors on soil properties, the results of these activities are considered sensitive indicators for climate change mitigation and help to guide sustainable land management practices (Bankó et al., 2021; Lado et al., 2023).

In Burete, mean concentrations of HWOC and HWN ranged between 0.32 and 1.29 g OC kg^{-1} and between 0.04 and 0.15 g N kg^{-1}, respectively depending on depth and management, with the lowest values for the cultivated soils and the highest for the forest soil at all depths. Among the cultivated soils, significantly higher mean HWOC and HWN concentrations were obtained under SCC compared to NCC and TT in the deepest layer (50–60 cm) (Table 3). However, no differences between them were detected in the remaining layers, which is consistent with the trend for total SOC and POC fractions explained above and with the significant positive correlations obtained between those pools (Fig. 4A and B). Mean SMC concentrations ranged from 7.58 to 63.54 mg C-CO_2 kg^{-1} soil depending on depth and management, with the lowest values

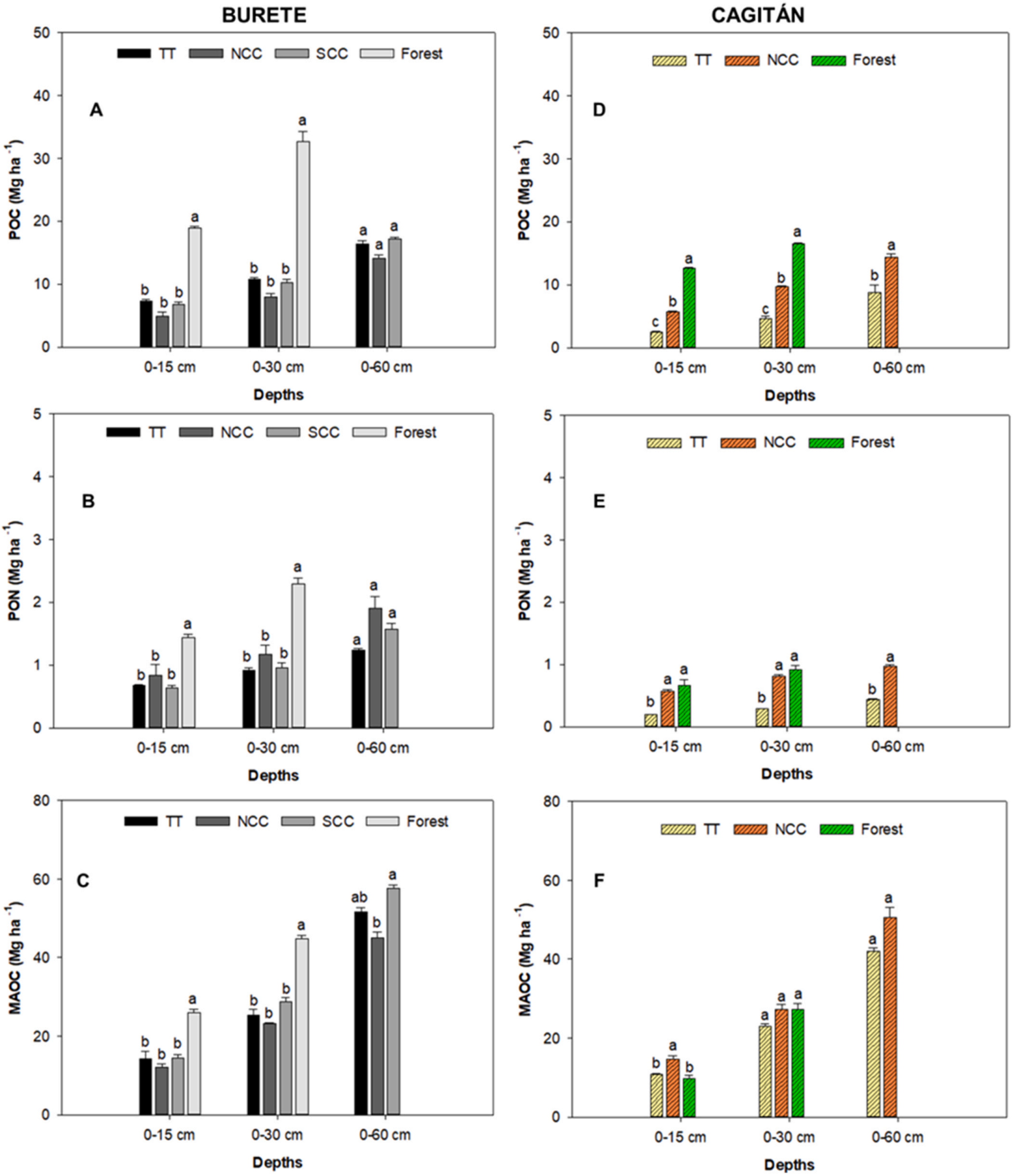

Fig. 3. Stocks of particulate organic carbon; POC (A, D), particulate organic nitrogen; PON (B, E), and mineral-associated organic carbon; MAOC (C, E) at 0–15, 0–30 and 0–60 cm soil layer under TT: traditional tillage; NCC: natural cover crops; SCC: seeded cover crops and undisturbed natural area: Forest. Means ± standard error. Bars followed by the same letter do not differ from each other using the Tukey test ($P < 0.05$).

for cultivated soils and the highest for forest soil at 0–30 cm depth (Table 3). Among the cultivated soils, significantly higher mean SMC concentrations were observed in SCC and TT compared to NCC at a depth of 50–60 cm.

In Cagitán, mean HWOC and HWN concentrations ranged between 0.23 and 1.09 g OC kg^{-1} and 0.028 and 0.11 g N kg^{-1}, respectively and across all management practices and soil depths. The highest mean concentrations of HWOC were obtained under Forest followed by NCC and TT in the top layer, while no significant differences were observed between Forest and NCC at 15–30 cm depth, both being higher than TT

Table 3
Total organic carbon and nitrogen easily decomposable (mean ± standard error) under different land management practices and soil depths.

Site	Properties	Depth (cm)	Management			
			TT	NCC	SCC	Forest
Burete	HWOC (g kg^{-1})	0–15	0.73 ± 0.02 b A	0.63 ± 0.04 b A	0.66 ± 0.08 b A	1.29 ± 0.23 a
		15–30	0.66 ± 0.22 ab AB	0.41 ± 0.06 b B	0.38 ± 0.05 b B	1.12 ± 0.29 a
		30–50	0.38 ± 0.04 B	0.42 ± 0.03 B	0.46 ± 0.07 AB	-
		50–60	0.34 ± 0.01 b B	0.32 ± 0.02 b B	0.50 ± 0.00 a AB	-
	HWN (g kg^{-1})	0–15	0.091 ± 0.006 ab A	0.07 ± 0.004 b A	0.08 ± 0.010 b A	0.146 ± 0.027 a
		15–30	0.068 ± 0.014 ab AB	0.05 ± 0.007 b B	0.05 ± 0.002 b B	0.123 ± 0.024 a
		30–50	0.046 ± 0.005 B	0.05 ± 0.001 B	0.06 ± 0.009 B	-
		50–60	0.040 ± 0.002 b B	0.04 ± 0.002 b B	0.06 ± 0.000 a B	-
	SMC (mg C-CO_2 kg^{-1} soil)	0–15	28.02 ± 3.14 b A	26.91 ± 2.38 b A	22.76 ± 2.55 b A	63.54 ± 20.36 a A
		15–30	10.23 ± 3.33 b B	8.54 ± 0.83 b B	8.44 ± 2.73 b C	29.09 ± 5.20 a B
		30–50	9.19 ± 0.66 B	11.31 ± 1.95 B	8.98 ± 2.56 BC	-
		50–60	12.81 ± 3.67 a B	7.58 ± 0.13 b B	14.18 ± 0.00 a B	-
Cagitán			TT	NCC		Forest
	HWOC (g kg^{-1})	0–15	0.40 ± 0.02c A	0.84 ± 0.06 b A		1.09 ± 0.04 a A
		15–30	0.32 ± 0.02 b B	0.58 ± 0.02 a B		0.53 ± 0.06 a B
		30–50	0.24 ± 0.01 b C	0.37 ± 0.02 a C		-
		50–60	0.23 ± 0.01 b C	0.34 ± 0.01 a C		-
	HWN (g kg^{-1})	0–15	0.05 ± 0.002 b A	0.11 ± 0.007 a A		0.11 ± 0.004 a A
		15–30	0.04 ± 0.003 b AB	0.08 ± 0.002 a B		0.05 ± 0.006 b B
		30–50	0.03 ± 0.003 b B	0.05 ± 0.002 a C		-
		50–60	0.03 ± 0.002 b B	0.04 ± 0.002 a C		-
	SMC (mg C-CO_2 kg^{-1} soil)	0–15	11.02 ± 0.74c A	41.01 ± 1.68 b A		81.80 ± 0.13 a A
		15–30	7.98 ± 0.40c AB	23.75 ± 2.80 b B		44.79 ± 0.86 a B
		30–50	6.00 ± 0.01 b B	15.57 ± 1.16 a C		-
		50–60	6.25 ± 0.41 b B	10.05 ± 0.00 a C		-

TT: traditional tillage; NCC: natural cover crops; SCC: seeded cover crops; Undisturbed natural soil: Forest; HWOC: Total hot water extractable organic carbon; HWN: total hot water extractable nitrogen; SMC: Short-term mineralizable carbon. Different lowercase letters in rows means significant differences between managements at each soil depth. Different uppercase letters in columns indicate significant differences in depth within each management. Tukey test ($P < 0.05$).

(Table 3). Below 30 cm depth, NCC showed higher HWOC concentrations than TT. In addition, NCC did not differ from the Forest in HWN concentrations in the top layer (both being significantly higher than TT), but was significantly higher at 15–30 cm depth. A significant decrease in HWOC and HWN concentrations was observed with depth under both forest and cultivated soils, with these reductions being less pronounced under TT (Table 3). The SMC pool performed similarly to HWOC, with the highest mean values under Forest, followed by NCC and TT at 0–15 and 15–30 cm depth. Below 30 cm depth, the mean SMC values of NCC remained higher than those of TT. Those results suggest that cover crops significantly increase the activity of soil microbiota and the degradation of plant residues, leading to higher extractable SOM, POC, MAOC and, consequently, to an increase in SOC stocks. Hu et al. (2023) also observed that the incorporation of cover crops significantly increased the effect sizes of DOC, MBC and POC. Understanding carbon dynamics at depth is considered crucial to combat climate change, however, the presence of shallow soils (<30 cm) in some regions, such as Murcia (Spain), is a significant obstacle for this purpose.

The undisturbed natural areas at both sites had the highest concentrations of HWOC, HWN and SMC in the top layer (0–15 cm). The preservation and continuous deposition of plant residues in these areas promotes microbial activity that increases decomposition and, consequently, carbon accumulation in the soil. The increase in SMC and HWOC reflects an increase in the labile fractions of SOC and heralds a long-term increase in soil C storage (Alvarez and Alvarez, 2000). In fact, high correlations of POC, HWOC and SMC with SOC were observed only in Cagitán (POC: $r = 0.82$, $p < 0.01$; HWOC: $r = 0.77$, $p < 0.05$ at 0–15 cm and SMC: $r = 0.89$, $p < 0.01$ at 15–60 cm; Fig. 4C and D, respectively). In addition, a decrease of those pools with depth was observed in all managements and sites (Tables 2 and 3). In this sense, a decrease in soluble organic matter concentrations and SMC with soil depth is a common phenomenon observed in several studies (Min et al., 2021; Pries et al., 2018). This decrease in easily decomposable OC pools with depth was more pronounced in forest soils than in cultivated soils and under cover crops compared to traditional tillage, due to the higher input of OM and microbial activity in the topsoil. Agricultural practices mix soil OM throughout the profile, resulting in a more gradual decline in easily decomposable OC with depth.

In general, the use of the easily decomposable organic carbon fractions such as HWOC, HWN and SMC helped to understand the impact of land use and management, even in deeper layers. According to these results, the changes between land use managements (forest and cultivated areas) were higher for the intermediate labile pools such as POC (OC associated to plants). However, the most easily decomposable organic carbon fractions represented by SMC and HWOC, seem to be more sensitive than POC to establish differences between agricultural management practices in the soil profile, despite representing a low percentage of the total SOC (< 5 %). Other authors have also found low variability and a relatively fast response from SMC compared to POC, suggesting that it is a particularly useful variable for assessing the effects of management on deep C (Ladoni et al., 2015). According to the latter authors, the statistical power for POC never reached an acceptable level due to the very high variability of POC data. However, this could not be verified in the present study due to the limited number of samples.

5. Conclusions

Carbon sequestration in Mediterranean agricultural soils is essential to mitigate climate change, but depends on climate conditions, management practices and soil depth. While conversion from natural to agricultural areas with traditional tillage reduces soil OC and N stocks by about 36–56 %, sustainable management practices such as cover crops

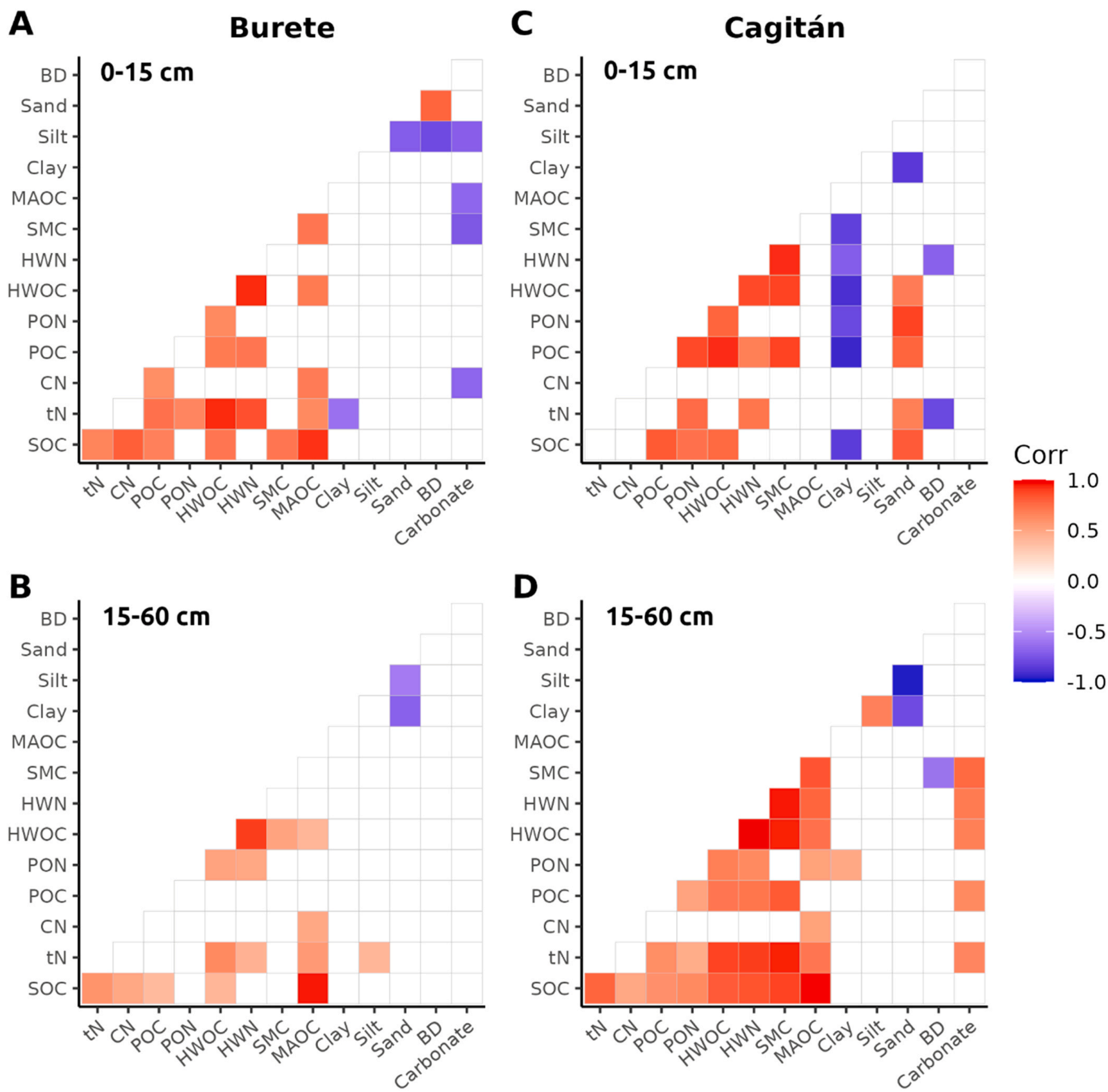

Fig. 4. Spearman correlation matrix of all variables assessed in this study across land uses and managements at different depths (topsoil and subsoil). Only significant correlations are displayed.

and reduced tillage improve microbial activity and SOM fractions, increasing carbon storage throughout the soil profile. This study demonstrates that the 30–60 cm layer plays a critical role in SOC sequestration, with cover crops improving SOC protection and stocks at depth, effects that would be underestimated without considering subsoil layers. Notably, reduced tillage combined with cover crops in Cagitán restored OC and N stocks to levels comparable to undisturbed soils. The analysis of different SOM fractions proved effective in detecting management impacts and highlights the need for future research to assess OC and N pools at greater depths and over time under various land covers in Mediterranean systems.

CRediT authorship contribution statement

María Martínez-Mena: Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Stallone Da Costa Soares:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristina Fernández-Soler:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Efraín Carrillo-López:** Writing – review & editing, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: María Martínez-Mena reports financial support was provided by Spanish National. Maria Martinez Mena reports financial support was provided by European Union (Next Generation EU- PRTR- C17.I1. Maria Martinez Mena reports financial support was provided by Fundación Séneca AGROALNEXT. Stallone da Costa Soares reports a relationship with Global Research Alliance on Agricultural Greenhouse Gases (CLIFF-GRADS program) that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109821.

Data availability

Data will be made available on request.

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