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Does spontaneous cover crop increase the stocks of soil organic carbon and nitrogen in commercial olive orchard?

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ABSTRACT

Management practices in the inter-row area of olive orchards are undergoing significant transformations. Current regulations and recommendations are increasingly advocating the implementation of temporary spontaneous cover crops (TSCV) mainly to reduce erosion. Existing research has predominantly focused on evaluating the effectiveness of TSCV in mitigating soil erosion in olive orchards, with limited attention given to carbon (C) cycling, despite the potential of TSCV for contributing to the removal of atmospheric CO_2 and in the reduction of eroded carbon. Moreover, the limited number of studies on the effects of TSCV on C cycling have been confined to a few experimental sites and at the short term. This study aimed to assess the potential of TSCV to enhance carbon sequestration and nitrogen retention in rainfed commercial olive orchards under semi-arid conditions. To achieve this, we evaluated the annual aboveground organic carbon input due to TSCV, as well as the stocks of soil organic (SOC) and inorganic (SIC) carbon and total N (STN) in 24 commercial olive groves with calcareous Regosols and calcium Cambisols as the predominant soil types that have implemented TSCV for at least the last 8 years. These were compared with 24 comparable groves with bare soil (BS). Net aboveground annual carbon and CO_2 fixation of the TSCV averaged 125.7 kg C ha^{-1} y^{-1} and 460 kg CO_2 ha^{-1} y^{-1}, respectively, which are figures relatively low mainly due to the low area covered by the TSCV. After eight years of implementing TSCV, the SOC stocks increased by an average of 2.03 Mg C ha^{-1} (in the top 30 cm of soil) compared to BS olive orchards. Moreover, SOC content of unprotected (>250 μm) and physically protected (53–250 μm) fractions were 82 and 38 % higher in the TSCV olive farms. Although there was a tendency of lower SIC content in TSCV olive orchards, differences were not significant. The STN content and the potentially mineralizable nitrogen in TSCV farms were on average 26 % and 77 % higher than in BS olive orchards. These findings underscore the potential of TSCV for organic carbon accumulation and nitrogen retention in the soil, contributing to climate change mitigation and soil fertility enhancement. Increasing vegetation coverage and productivity can enhance their effectiveness.

1. Introduction

In recent years, the dynamics of the stocks of soil organic carbon (SOC) in cultivated land and the practices which contribute to increase the reservoir of SOC have been paid attention because of their potential contribution to climate change mitigation by moving atmospheric CO_2 into SOC (Pareja-Sánchez et al., 2020; Sanz-Cobena et al., 2017; Plaza-Bonilla et al., 2015; Álvaro-Fuentes et al., 2014). Currently, authorities and policy makers face the challenge of developing and implementing effective SOC accretion strategies for agriculture, which requires the identification of the best management practices for each crop and pedoclimatic conditions. Several agricultural management techniques are recognized for their ability to capture SOC by augmenting carbon inputs into the soil and improving various soil processes that safeguard carbon from microbial decomposition (Singh et al., 2018).

The utilization of cover crops in combination with the subsequent incorporation of their residues into the soil represents a promising and sustainable approach for mitigating the risk of soil erosion in woody crops (Alliaume et al., 2014; Gómez et al., 2009). This practice not only offers potential benefits in terms of soil conservation but also serves as a means to offset carbon losses resulting from changes in land use and tillage practices within agricultural landscapes (Gómez-Muñoz et al., 2014; Ramos et al., 2010). Indeed, it has been demonstrated that the incorporation of cover crops in woody crops, such as olive and almond

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orchards, in Southern Spain is crucial for mitigating soil erosion (López-Vicente et al., 2021; Vicente-Vicente et al., 2016; Gómez et al., 2011). These crops have traditionally been cultivated in marginal Mediterranean regions characterized by relatively shallow soils, reduced tree density, and a high proportion of bare ground between tree canopies, all of which contribute to soil erosion, particularly on sloping terrain. The adoption of cover crops in olive groves and other woody crops has been boosted by the regional authorities of Andalusia. Approximately 1.6 million hectares in this region are dedicated to olive cultivation. These authorities have implemented a policy known as Good Agricultural and Environmental Practice for olive farming. This policy entails linking subsidies for olive cultivation to the condition of incorporating additional cover plants under specific circumstances, such as when the mean slope exceeds 7 %. Furthermore, the new eco-regimes of the Common Agricultural Policy (CAP) stipulate that the cover must have a width equivalent to 40 % of the free canopy projection width (European Commission, 2023).

Previous research has predominantly concentrated on evaluating the effectiveness of cover crops in olive orchards in terms of soil erosion mitigation (Gómez et al., 2004). However, there has been relatively less emphasis on examining the intricacies of carbon cycling associated with this practice (Castro et al., 2008). In a meta-analysis, Vicente-Vicente et al. (2016) arrived at the conclusion that the utilization of cover crops within woody crop systems, such as olive and almond orchards and vineyards, made a substantial and noteworthy contribution to the accrual of SOC. Their findings revealed an average annual SOC accumulation rate of approximately 1.1 Mg C ha^{-1}. In a more recent study conducted by Torrús-Castillo et al. (2022), it was similarly observed that the implementation of cover crops contributed significantly to the positive SOC balances of a wide selection of olive farms. However, most of the studies investigating the specific role of cover crops on SOC accreation have been focused in a limited number of experimental olive farms and after very few years of cover crops. Taking into account the great spatial variability in the cover crops annual net primary production, and thus in the atmospheric CO_2 fixation, studies which include many commercial olive farms are needed to provide robust data sets on the effects of cover crops on the increase in SOC stocks. Moreover, SOC can persist within the soil as a storage repository divided into various compartments subject to varying degrees of safeguarding. Stabilizing SOC through soil particle aggregation, which includes the physical protection of SOC, constitutes a fundamental aspect for understanding the intricacies of soil carbon dynamics. This process begins with the unprotected fraction of organic carbon, primarily derived from plant residues. The capacity of aggregates to protect and stabilise SOC is widely documented (Lee et al., 2021; Novelli et al., 2017). This process is primarily associated with the aggregates' ability, through particle aggregation, to spatially segregate organic matter and microorganisms, along with diminishing microbial activity attributed to reduced oxygen diffusion within the soil aggregates (Xue et al., 2019). Consequently, the dynamics and effective management of these reservoirs play pivotal roles in determining whether soil functions as a carbon source or serves as a carbon storage medium (Gonzalez-Rosado et al., 2020).

On the other hand, the dynamic of soil inorganic carbon (SIC) has received limited attention in terms of estimation, despite its presence in more stable forms like calcium carbonate (Yang et al., 2012). On a worldwide scale, arid and semi-arid areas encompass over 30 % of the Earth's land expanse (Lal and Kimble, 2000; Romanyà and Rovira, 2011), and soils in which carbonates ($CaCO_3$) are predominant, as Calcisols or calcaric Regosols (IUSS Working Group WRB, 2014), might account up a half of the global C pool (Scharififar et al., 2023). Most area where olive farming are implemented within the Andalusian region is considered as semiarid regions (mean annual rainfall between 200 and 700 mm) and soils in these regions are usually calcareous and contain large amounts of inorganic carbon in the form of carbonates (Junta de Andalucía, 1999). Many studies, especially C-cycle models, typically treat carbonates and SIC as a stable carbon pool within soils. However, it is noteworthy that the carbon flow originating from the inorganic carbon cycle may exert significant impact, even when considering relatively brief timescales (Sanchez-Cañete et al., 2011; Serrano-Ortiz et al., 2009, 2010). The dynamics of SIC are related to the existence of carbonate dissolution and precipitation cycles in soil. These cycles are interrelated with biological processes that generate CO_2. In fact, a portion of the CO_2 generated during the breakdown of residues from cover crops in soils rich in carbonate content has the potential to precipitate as carbonate compounds (Gocke et al., 2011). Additionally, biological reactions that impact pH, such as nitrification resulting from the decomposition of organic matter within cover crop residues, can produce hydrogen ions or organic acids. These byproducts may, in turn, facilitate the dissolution of carbonates and enhance the release of CO_2 from the soil (de Soto et al., 2017; Tamir et al., 2013). Therefore, the implementation of the cover crops could result in a SOC accumulation but also in a decrease in SIC throughout the latter process.

We hypothesized the following: (1) cover crop increases the total SOC and soil inorganic nitrogen contents compared to the non-cover management in commercial olive groves; (2) this increase is mainly due to a rise in the unprotected, as well as in the physically protected SOC, (3) the contribution of SIC to the total soil carbon (SOC+SIC) is much higher than that of the SOC, and (4) SIC in olive groves with cover crop is significantly lower than that of bare olive groves.

The main purpose of this study was to assess the effectiveness of plant cover for enhancing soil C sequestration in semiarid rain-fed olive oil orchards, to promote changes in existing conventional agronomic practices from a climate change mitigation perspective. Specifically, the objectives were: (1) to determine the variability of annual aboveground organic carbon input due to the presence of a plant cover; (2) to assess the effects of plant cover residue addition to the soil on SIC and SOC accretion and SOC fractions of different protective levels.

2. Materials and methods

2.1. Experimental site and treatments

This study was conducted during April and May 2018 in commercial olive groves at Estepa (Seville province), SW Spain (37°17′30″N 4°52′45″W, 535 m asl). The climate is Mediterranean with marked signs of continentality. Mean annual precipitation and potential evapotranspiration, and temperature in the area was 404 mm, 1379 mm and 17.5 °C, respectively (data from 1993 to 2023; Junta de Andalucía, 2023). The monthly precipitation was highly variable during the study period, ranging between 0 and 227 mm in July and March, respectively, being the accumulated annual precipitation and potential evapotranspiration of 653 mm and 1335 mm, respectively. The yearly air temperature followed the typical Mediterranean pattern with hot summers, with air temperatures above 35°C, and mild to cold winters.

The study was carried out on 24 sites with commercial olive groves from the Protected Origin designation of Estepa (Fig. 1). Within each site, a pair consisting of two nearby (< 50 mts) comparable (similar orientation, soil type and same weather conditions) commercial olive groves was selected (Table S1, Supplementary material). In one olive grove of each pair, temporary spontaneous cover crops (TSCV) comprised wild herbaceous annual plants that had grown spontaneously in the plot. These cover crops had been implemented for at least 8–12 years before the start of this study. Cover crops in these olive groves typically grow from early autumn and are controlled the following April to avoid competition for water and nutrients with the trees. Despite our goal was not the description of the spontaneous cover crop communities, a preliminary sampling in the area, consisting of 65 experimental units of 0.5 m x 0.5 m, revealed that the most predominant species were *Diplotaxis virgata*, *Erodium malacoides*, *Urtica urens*, *Centaurea melitensis*, *Anthemis cotula*, and *Bromus madritensis*. Only two legume species were identified: *Medicago polymorpha* and *Medicago doliata*. In contrast, in the other olive grove of each pair, the soil was bare during that time by pre

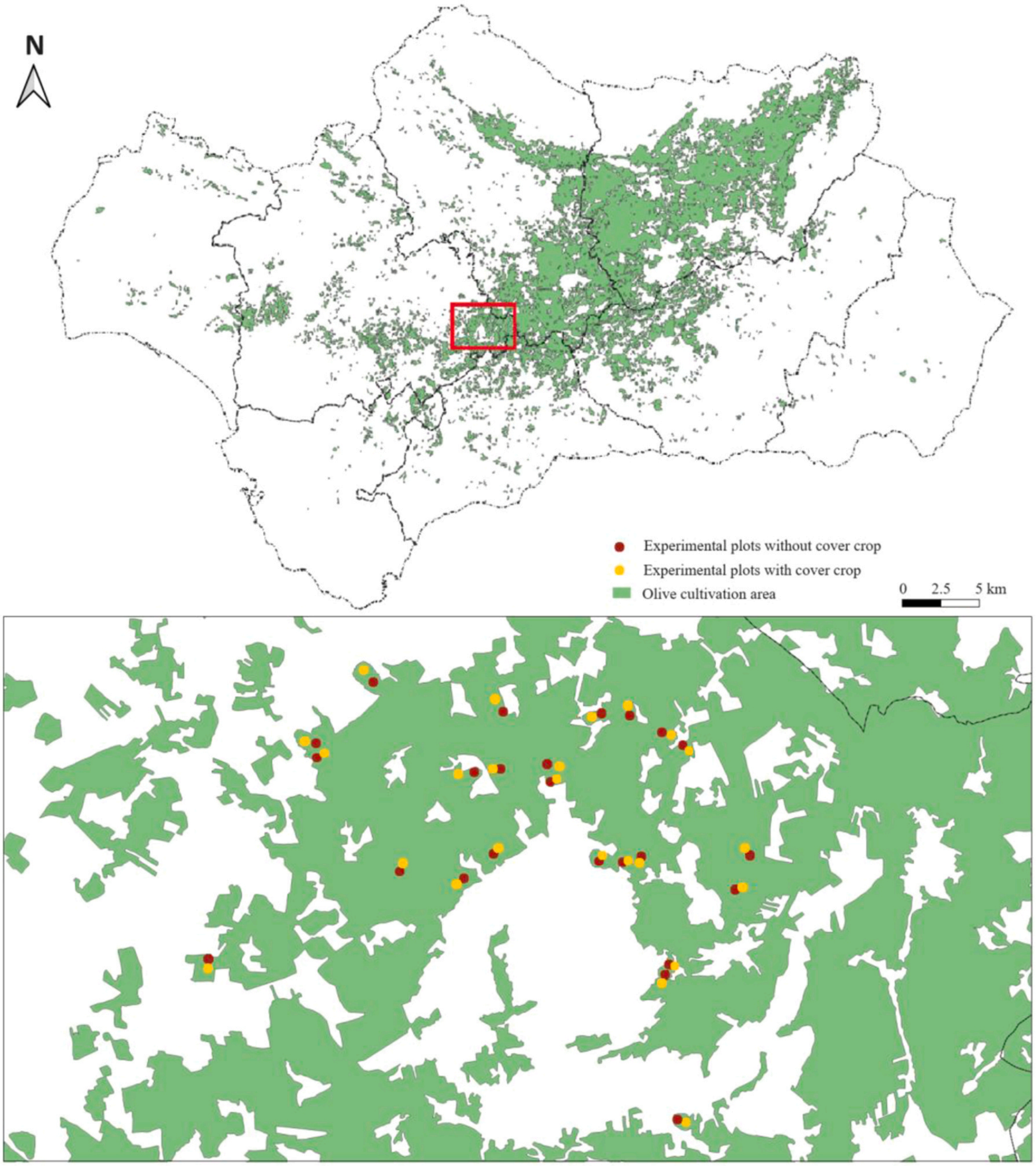

Fig. 1. Location of the selected olive groves in Andalucia (Spain).

and post-emergency herbicides applications and/or by tillage (BS) according to the traditional practices of olive grove cultivation in the area. Therefore, a total of 48 olive groves were selected; 24 of each were TSCV and 24 BS. Total area of TSCV and BS were 363 and 238 ha, respectively. The average slope for the whole TSCV and BS were 6.7 and 9.7 %, respectively and differences were not significant.

Soils in the TSCV and BS were rather homogeneous. These are calcareous Regosols and calcium Cambisols (classified as Calcisols in FAO, 1998, and later WRB editions) over Miocene and Paleocene calcaereous marls and Plio-quaternary conglomerates and clays, with fine textures. The soil clay and sand contents varied little in the area, averaging 32.8 % and 24 % for BS olive groves, and 32.7 % and 25.1 % for TSCV olive groves, respectively (Table S2, Supplementary material). Many of the soils in the area have suffered severe erosion (Rodríguez-Sousa et al., 2019), so the natural A horizon has been partially lost, emerging subsurface horizons rich in carbonates ($CaCO_3$ equi. was > 40 % in most of sites). The presence of such a high carbonate content conditioned a relatively high pH values. The soil pH averaged 8.0 (Table S2, Supplementary material), while cation exchange capacity (CEC) averaged 19.9 cmol(+) kg^{-1}and 20.0 cmol (+) kg^{-1}, for BS and TSCV olive groves, respectively. Soil Olsen P was measured according to Olsen et al. (1954). Available P was highly variable, ranging between very low levels (3 mg kg^{-1}) up to quite high (44 mg kg^{-1}), although the average contents were that expected for fine-textured soils (15 – 40 mg kg^{-1}). Exchangeable K was measured using the 1 N pH 7 ammonium acetate saturation method (Rhoades, 1982). Exchangeable K also showed wide variations, with levels ranging from very low (75 mg kg^{-1}) to as high as 600 mg kg^{-1}.

2.2. *Soil and aboveground cover crops biomass sampling*

Soil and aboveground cover crops biomass measurements were carried out during 15th April to 15th May 2018. In TSCV and BS olive groves, 4 top-30 cm soil samples were taken at random in the between trees area, within the 1–5 meter band were temporary spontaneous cover crops were growing. The samples were stored in plastic bags previously labeled and transported to the lab the same day. In the TSCV olive groves, and after soil sampling, the aboveground biomass of cover crops was also sampled. Five replicates squared frame (0.5 m x 0.5 m) was thrown at randon in the area covered by temporarily spontaneous cover crops in each of the TSCV farms and all the biomass at ground level was collected, stored in plastic bags and transported to the laboratory the same day. The width (m) of the band covered by temporarily spontaneous cover crops was recorded in five inter-row lines per olive grove and varied between 1 and 5 m.

2.3. *Organic and inorganic carbon contents of the soil and aboveground net primary estimation*

Once in the laboratory, soil samples were air dried for a week and were gently passed through an < 2 mm sieve. The percentage of soil particles which passed through the sieve and the soil particles retained in the 2 mm mesh were recorded. Soil samples < 2 mm were oven dried for 48 hours at 80° C in aluminum trays and organic and inorganic carbon were analysed. Organic carbon was determined through an acid digestion with potassium dichromate, following the methodology of Anderson and Ingram (1993). The inorganic carbon content was estimated by the volumetric method based on Bernard's calcimeter by measuring the amount of CO_2 released estimated by the pressure it exerts on a saturated solution of CO_2 in a gas burette (Page et al., 1982).

Aboveground biomass samples were dried in an oven at 60° C for 48 hours in aluminum trays and weighed. Taking into account the aboveground biomass (g m^2) and the area covered by the temporarily spontaneous cover crops (number of inter-row lines in a 100 m line draw perpendicular to the tree lines times the mean width of the band covered times 100 m) in a hectare of the TSCV olive groves, the aboveground biomass was estimated. Temporarily spontaneous cover crops was not controlled since the previous season (April-May 2017), and therefore the aboveground biomass was assumed to be the aboveground net primary production. The organic carbon content of the aboveground biomass was determined following the method proposed by Anderson and Ingram (1993), once they were ground (< 1 mm) by a knife mill.

2.4. *Stocks of soil organic and inorganic carbon and organic carbon of the aboveground plant biomass determinations*

Bulk density of the < 2 mm soil samples were estimated according to Howard et al. (1995) and Aguilera et al. (2013), whereas the bulk density of the soil particles > 2 mm was assumed to be 2.4 g cm^{-3} (Santos-Francés, 1979). The amounts of organic and inorganic carbon in the top 30 cm of soil and in a hectare were calculated according to equations 1 and 2, respectively.

$$SOC_{pool}\ (Mg\ C\ ha^{-1}\ top\ 30\ cm) = SOC \times 0.3 \times 10000\ m^2 \times [(BD_{<2\ mm} \times f) + (BD_{>2\ mm} \times g)] \times (1-g)$$

$$SIC_{pool}\ (Mg\ C\ ha^{-1}\ top\ 30\ cm) = SIC \times 0.3 \times 10000\ m^2 \times [(BD_{<2\ mm} \times f) + (BD_{>2\ mm} \times g)] \times (1-g)$$

where, SOC and SIC are the soil organic and inorganic carbon content (g g^{-1} soil), respectively, BD < 2 mm is the bulk density of the < 2 mm soil samples, BD > 2 mm is the bulk density of the soil particles > 2 mm (2.4 g cm^{-3}), and f and g are the fractions (over 1) of soil particles lower and higher than 2 mm, respectively.

The atmospheric CO_2 captured annually (kg CO_2 ha^{-1} y^{-1}) by the TSCV was calculated from the C accumulated in the aerial biomass (kg C ha^{-1} y^{-1}), using a molecular weight ratio (1.0 g carbon = 3.66 g CO_2) and by assuming that the C taken up by plants comes exclusively from the atmospheric CO_2.

2.5. *SOC fractionation*

Soil C fractionation was performed in three replicates of soil samples of 12 pairs of TSCV and BS olive groves (24 in total). Separation of the various soil C pools was accomplished by a physical wet fractionation. 50 g of air-dried soil were dispersed with distilled water and the resulting slurries were passed through two consecutive meshes with a pore size of 250 and 53 μm. The soil particles retained in the 250 μm and 53 μm meshes and those which passed throughout the 53 μm mesh where dried at 105 °C during two days and weighted. Organic carbon contents in the $>$ 250 μm, 53–250 μm and $<$ 53 μm soil fractions were analysed as above and were considered the coarse non-protected organic carbon (cPOM), microaggregate (μagg) C or physically protected carbon, and easily dispersed silt and clay (dSilt+dClay) or chemically protected organic carbon.

2.6. *Total soil nitrogen and potentially mineralizable nitrogen*

Total nitrogen in the soil (STN) was determined using the Kjeldahl method (1993). The amount of STN in the top 30 cm of soil and in a hectare was calculated according to equation 3.

$$STN_{pool}\ (Mg\ C\ ha^{-1}\ top\ 30\ cm) = STN \times 0.3 \times 10000\ m^2 \times [(BD_{<2\ mm} \times f) + (BD_{>2\ mm} \times g)] \times (1-g),$$

where, STN is the soil total nitrogen content (g g^{-1}), BD < 2 mm is the bulk density of the < 2 mm soil samples, BD > 2 mm is the bulk density of the soil particles > 2 mm (2.4 g cm^{-3}), and f and g are the fractions (over 1) of soil particles lower and higher than 2 mm, respectively.

The potentially mineralizable nitrogen content (PMN) in the soil samples was determined following the method of Kandeler (Schinner et al., 1995). A 5 g aliquot of soil sample was incubated with 15 ml of distilled water in Falcon tubes, previously shaking, during 7 days at 40 °C. After incubation, 15 ml of KCl 2 M was added, shaker for one hour and filtered. The amount of potential N mineralization was calculated as the differences between the ammonium content in the post incubation samples respect to soil samples not incubated. Ammonium concentration in the filtrated was analysed according to the indophenols blue method described by Keeney and Nelson (1982).

2.7. *Statistic analysis*

Statistical analyzes were performed using the statistical software STATISTICA v.10 (Stat Soft Inc.). Data were checked for normality by plotting a normal quantile plot. A logarithmic transformation was carried out to normalize soil inorganic carbon stock. An analysis of variance (ANOVA) was performed with management, site and their interaction as effects. When significant, differences among treatments were identified at 0.05 probability level of significance with a Fisher's post-test.

3. Results

3.1. *Management and annual aboveground net primary production and carbon and CO_2 fixation of the cover crops*

The width of the band with temporarily spontaneous cover crops on the commercial TSCV olive groves of Estepa ranged from one to five meters, with an average of 2.45 ms (Fig. 2a). This band is located within the width of between 3 and 8 mts between the limits of the tree canopy of two adjacent trees. Only in four out of the twenty-four commercial olive groves with cover crops the band width was over three meters (Fig. 2a). This resulted in between 1000 m^2 ha^{-1} to 5460 m^2 ha^{-1} and an average of 2710.2 m^2 ha^{-1}(Fig. 2b) of area covered, accounting for between 14 % and 100 % (mean of 47 %) of the between-tree canopy area (Fig. 2c). Productivity in the band covered averaged 140.9 g m^{-2}, although was very variable ranging with a coefficient o variation of

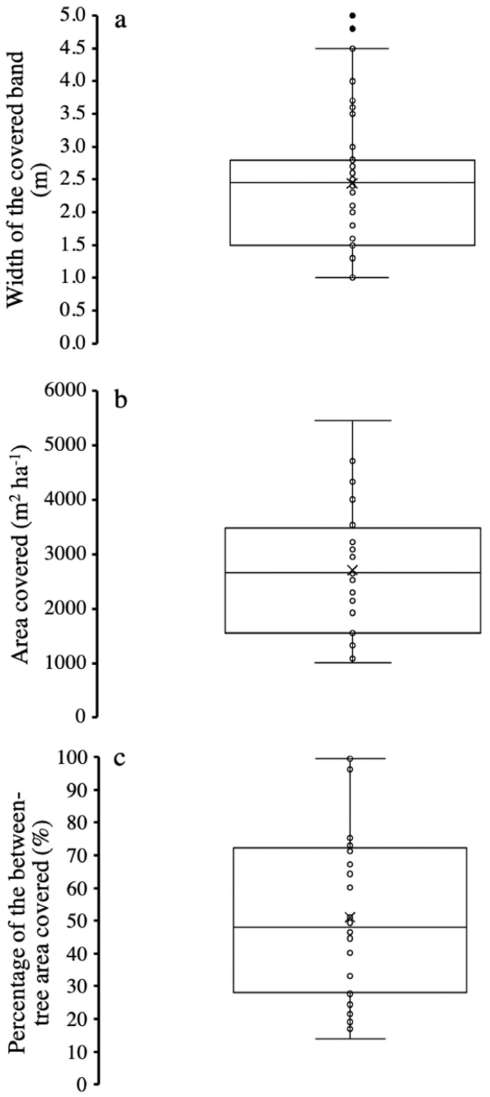

Fig. 2. Box-plot representation of width of the covered band (m) (a) area covered (m^2 ha^{-1}) (b) and percentage of the between-tree area covered (%) (c). Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin lines within the box mark the median and the X mark average. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots.

67.2 %. The aboveground net primary production averaged 355 kg DW ha^{-1} y^{-1} (Fig. 3a).

Organic carbon content of the cover crop averaged 35.9 % with a coefficient of variation of 13.9 %. Aboveground annual net C fixation averaged 125.7 kg C ha^{-1} y^{-1} in the TSCV olive groves (Fig. 3b). The aboveground net CO_2 fixation averaged 460 kg CO_2 ha^{-1} y^{-1} with minimum and maximum values of 85.6 and 1366 kg CO_2 ha^{-1} y^{-1} (Fig. 3c), respectively.

3.2. Organic and inorganic C in the temporarily spontaneous cover crops and bare soils

Organic carbon content (g kg^{-1}) of the top 30 cm of soil of TSCV olive groves averaged 13.7 g kg^{-1}, and overall was 25 % higher than that of BS olive groves (10.9 g kg^{-1} on average), being the differences significant ($P < 0.05$) (Table 1). In ten out of 24 TSCV-BS pairs olive groves, SOC was significantly higher in the former olive groves with respect to the later (Fig. 4a). It is important to highlight that the observed differences in SOC are not influenced by soil texture. This is evidenced by the similar clay content in both the TSCV and BS plots, with no significant differences between them ($P = 0.86$).

Soil inorganic carbon (g kg^{-1}) was on averaged 4.3 and 5.5 times higher than that of SOC in the TSCV and BS olive groves, respectively, with lowest and highest SIC/SOC ratio of between 1.9 and 11.6. SIC averaged 59.2 and 60.0 g kg^{-1} in the soils of the TSCV and BS olive groves, respectively and there were not significant differences between both type of soil management (Table 1), although in 7 out of the 24 pairs differences between TSCV and BS were significant (Fig. 4b).

Pooling up data of the 24 TSCV olive farms, the pool of soil organic carbon averaged 32.5 Mg C ha^{-1} 30 cm of soils, whereas that of the 24 BS olive farms were 30.6, being the differences significant (Fig. 5). There were not significant difference between the pool of soil inorganic carbon content of the TSCV and BS soils (Table 1).

In the selected 12 TSCV-BS pairs of olive groves, organic carbon concentration (g C kg^{-1} fraction) in the 250–2000 μm and 53–250 μm particles fractions of soils under TSCV were significantly higher than that of the BS in 8 out of the 12 sites. This was true for the organic carbon concentration of the silt plus clay soil fraction in 5 out of 12 sites (Table 2). Per gram of soil in 8, 4 and 5 sites out of the 12, soil organic carbon in the 250–2000 μm, 53–250 μm and silt+clay (< 53 μm) were significantly higher in the soils under TSCV, respectively. Grouping data from the 12 TSCV and 12 BS, the organic carbon concentration (g C kg^{-1} fraction) in the particle fractions of 250–2000 μm and 53–250 μm in soils under TSCV was significantly higher than that of the BS olive groves, whereas the silt+clay (< 53 μm) fraction showed no significant differences. However, per gram of soil, soil organic carbon only showed significant differences in the 250–2000 μm particle fraction, being higher in TSCV (Table 2).

3.3. Organic nitrogen and potential N mineralization in the temporarily spontaneous cover crops and bare soils

Soil total organic nitrogen content (STN) (g kg^{-1}) of the top 30 cm of soil was significantly higher in TSCV than in BS in six out of 24 TSCV-BS pairs olive groves (Fig. 6a). Organic nitrogen content in TSCV averaged 1.1 g kg^{-1}, and overall was 26 % higher than that of BS olive groves (0.9 g kg^{-1} on average), being the differences significant ($P < 0.05$) (Fig. 6b).

Soil potentially mineralizable nitrogen (PMN) was significantly affected by both the interaction between management and site and by the individual effects of these factors (Table 1). In this regard, PMN in soils of olive groves with temporarily spontaneous cover crops was 17.6 mg N-NH_4^+ kg^{-1}, although it was highly very variable, ranging with a coefficient o variation of 67.9 %. (Fig. 7). In BS olive groves, potencially mineralizable nitrogen averaged 9.9 mg N-NH_4^+ kg^{-1} (Fig. 7) and with a coefficient o variation of 49.2 %.

4. Discussion

4.1. Spontaneous cover crops contributed significantly to SOC and STN accreation

Temporary spontaneous cover crops can contribute to C accumulation and N retention in the soil, but the extent of their impact depends on several factors, including the productivity of the cover crops and the area they cover. In the selection of commercial olive groves under the umbrella of DOP Estepa, TSCV showed differences in the amount of biomass produced and in the percentage of total area covered, affecting C accumulation and N retention in the soil. With only 28 % of one hectare covered by spontaneous cover crops on average, the potential for C accumulation and N retention in the soil was limited compared to areas with higher vegetation coverage. Consequently, in some of the TSCV olive groves, the organic matter input into the soil, which is critical for long-term carbon storage, may not be significant.

The aboveground net primary production found in the 24 TSCV olive

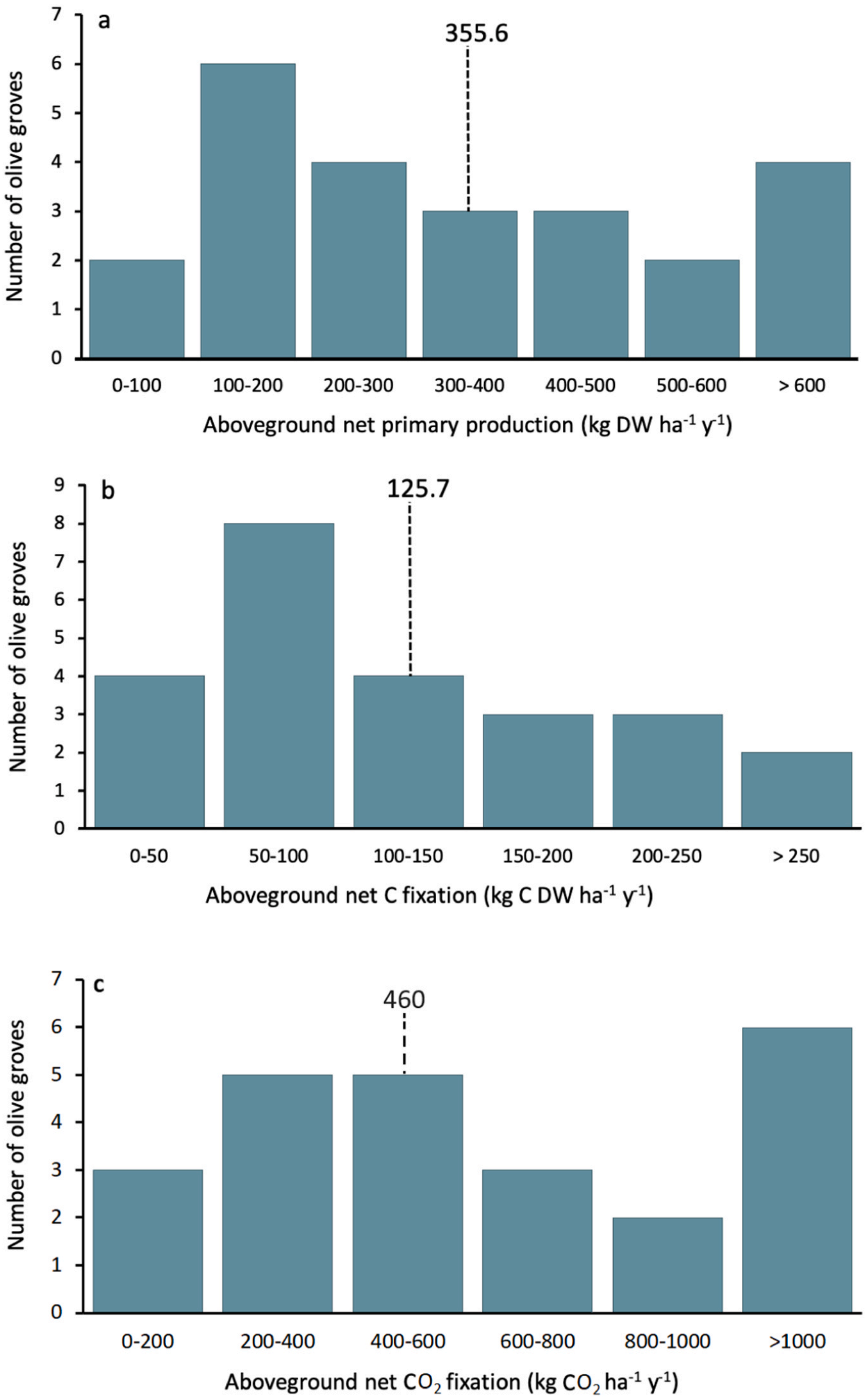

Fig. 3. Frequency distribution of the annual aboveground net primary production (kg DW ha^{-1} y^{-1}) (a), aboveground net C fixation (kg C DW ha^{-1} y^{-1}) (b) and aboveground net CO_2 fixation (kg CO_2 DW ha^{-1} y^{-1}) (c) in the olive groves.

Table 1

Analysis of variance (*P*-values) of soil organic carbon (SOC), soil inorganic carbon (SIC) and soil organic nitrogen (STN) contents (g kg^{-1} soil) and pools (Mg C or N ha^{-1}), and content of potentially mineralizable nitrogen (PMN), of the top 30 cm of soil as affected by the presence of TSCV, site and their interactions.

Source of variation	**SOC (g kg^{-1})**	**SIC (g kg^{-1})**	**STN (g kg^{-1})**	**SOC pool (Mg C ha^{-1})**	**SIC pool (Mg C ha^{-1})**	**STN pool (Mg N ha^{-1})**	**PMN (mg N-NH$_4^+$ kg^{-1})**
Management	<0.001	ns	<0.001	0.001	ns	ns	<0.001
Site	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Management*Site	<0.001	<0.001	0.005	ns	ns	ns	<0.001
ns, non-significant							

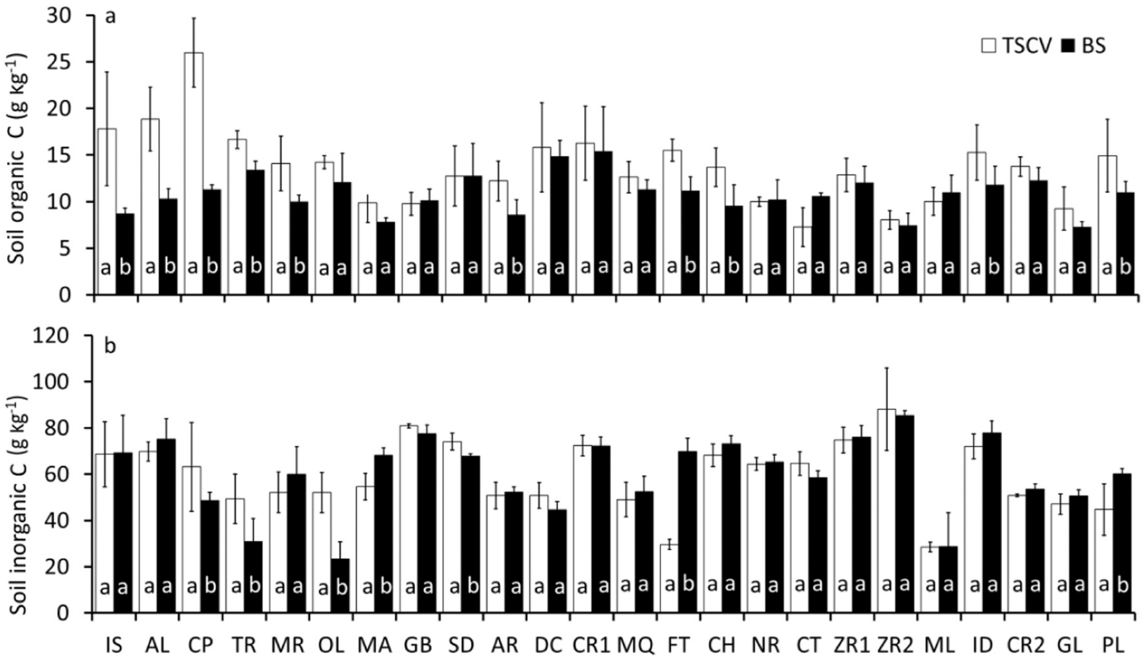

Fig. 4. Soil organic (g kg^{-1}) (a) and inorganic carbon (g kg^{-1}) (b) as affected by management (TSCV; spontaneous cover crops and BS; bare soil). For a given site, different lowercase letters indicate significant differences between management treatments at $P< 0.05$. Vertical bars indicate standard deviation.

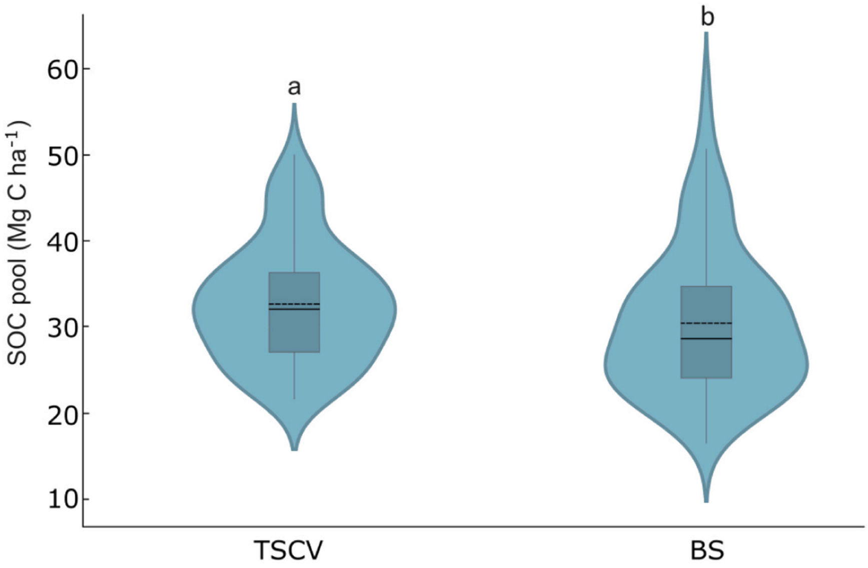

Fig. 5. Violin plot of the stock of soil organic carbon (Mg C ha^{-1}, top 30 cm soil) in soils of the TSCV and BS olive groves. Boundaries of the boxes closest to, and furthest from zero indicate the 25^{t}h and 75th percentiles, respectively. The thin lines within the box mark the median and the dotted line average. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Different lowercase letters indicate significant differences between soil management (TSCV; spontaneous cover crops and BS; bare soil) at $P< 0.05$.

groves of the DOP Estepa were lower than these found by Vicente-Vicente et al. (2017), Castro et al. (2008) and Márquez-García et al. (2013) in olive groves of Andalucía. These studies observed values of annual aboveground biomass production within the range of 650–2550 kg DW ha^{-1} y^{-1}, 2617–4060 kg DW ha^{-1} y^{-1} and 3500–4500 kg DW ha^{-1} y^{-1}, respectively. The presence of spontaneous cover crops occupying the whole the inter-row area in the referenced studies contributed to the higher biomass production. However, Torrús-Castillo et al. (2022) observed similar results to our study in olive groves where cover crops covered bands with an average width of 3.2 m in the inter-row areas. Therefore, the relatively low percentage of area covered by spontaneous vegetation in our study's olive groves would have contributed to the relatively low annual net primary production observed (range of 61–913 kg DW ha^{-1}y^{-1}). Therefore, the promotion of higher area covered by TSCV in orchard crops, such as olive groves, by the regional, national and EU authorities could contribute significantly to increase the transference of atmospheric CO_2 into the soil via TSCV. The new CAP 2023–2027 (European Commission, 2023), which partially links economic subsidies to the implementation of cover crops covering 40 % of orchard areas, is expected to expand the coverage of TSCV and thereby enhance the role of TSCV in orchards in mitigating climate change.

In our study, the amount of carbon accumulated in the aboveground biomass of the TSCV was lower compared to values reported in other

Table 2

Soil organic carbon concentration as density (g C kg^{-1} fraction) and soil organic carbon content (g C kg^{-1} soil) of different fraction (200–2000, 53–200 and <53 µm) as affected by management (TSCV; spontaneous cover crops and BS; bare soil) and site of field experiments. For a given site, different lowercase letters indicate significant differences between management treatments at $P < 0.05$.

		Density (g C kg^{-1} fraction)			Soil organic carbon content (g C kg^{-1} soil)		
Sites	**Management**	**200–2000 µm**	**53–200 µm**	**< 53 µm**	**200–2000 µm**	**53–200 µm**	**< 53 µm**
IS	**TSCV**	$19.7^{a}\pm6.6$	$12.0^{a}\pm2.5$	$11.6^{a}\pm2.2$	$6.2^{a}\pm2.7$	$5.1^{a}\pm1.1$	$3.1^{a}\pm0.9$
	BS	$8.8^{b}\pm1.9$	$8.7^{b}\pm0.3$	$9.9^{a}\pm1.0$	$2.3^{b}\pm0.6$	$4.2^{a}\pm0.4$	$2.5^{a}\pm0.2$
AL	**TSCV**	$22.1^{a}\pm3.2$	$13.9^{a}\pm1.1$	$13.3^{a}\pm1.6$	$4.4^{a}\pm1.4$	$6.1^{a}\pm1.0$	$4.7^{a}\pm0.6$
	BS	$11.0^{b}\pm1.1$	$9.7^{b}\pm0.8$	$10.9^{a}\pm0.5$	$2.0^{b}\pm0.3$	$4.3^{b}\pm0.4$	$3.9^{a}\pm0.3$
CP	**TSCV**	$48.1^{a}\pm6.8$	$20.4^{a}\pm3.3$	$16.5^{a}\pm1.7$	$7.7^{a}\pm1.7$	$8.3^{a}\pm1.6$	$7.1^{a}\pm0.6$
	BS	$15.5^{b}\pm2.2$	$10.9^{b}\pm0.9$	$12.9^{b}\pm0.3$	$1.7^{b}\pm0.2$	$5.5^{b}\pm0.4$	$4.9^{b}\pm0.6$
MR	**TSCV**	$24.4^{a}\pm12.4$	$14.3^{a}\pm3.6$	$12.3^{a}\pm1.1$	$2.5^{a}\pm1.5$	$6.5^{a}\pm1.2$	$5.4^{a}\pm0.7$
	BS	$11.5^{b}\pm2.4$	$10.7^{a}\pm0.3$	$11.9^{a}\pm1.0$	$0.9^{b}\pm0.1$	$4.6^{a}\pm0.3$	$5.7^{a}\pm0.4$
MA	**TSCV**	$8.7^{a}\pm2.9$	$9.1^{a}\pm2.1$	$11.9^{a}\pm3.2$	$2.1^{a}\pm0.9$	$4.2^{a}\pm0.9$	$3.4^{a}\pm0.4$
	BS	$9.9^{a}\pm1.0$	$8.6^{a}\pm0.7$	$9.5^{a}\pm1.1$	$1.3^{a}\pm0.1$	$3.9^{a}\pm0.3$	$3.8^{a}\pm0.5$
AR	**TSCV**	$13.3^{a}\pm3.5$	$13.4^{a}\pm2.2$	$13.4^{a}\pm1.1$	$2.5^{a}\pm0.7$	$5.6^{a}\pm0.9$	$5.2^{a}\pm0.4$
	BS	$8.0^{b}\pm0.9$	$8.9^{b}\pm0.5$	$9.7^{b}\pm0.4$	$1.1^{b}\pm0.1$	$4.3^{a}\pm0.6$	$3.6^{b}\pm0.4$
FT	**TSCV**	$12.2^{a}\pm3.7$	$15.5^{a}\pm1.5$	$15.6^{a}\pm1.3$	$1.0^{a}\pm0.3$	$7.2^{a}\pm0.8$	$7.0^{a}\pm0.6$
	BS	$8.4^{a}\pm1.1$	$10.6^{b}\pm0.9$	$14.0^{a}\pm0.7$	$1.68^{a}\pm0.2$	$4.8^{b}\pm0.7$	$4.8^{b}\pm0.2$
CH	**TSCV**	$28.6^{a}\pm4.0$	$14.5^{a}\pm1.1$	$12.9^{a}\pm1.6$	$1.9^{a}\pm0.6$	$4.9^{a}\pm1.1$	$7.5^{a}\pm0.1$
	BS	$18.7^{b}\pm7.3$	$9.7^{b}\pm1.3$	$9.3^{b}\pm1.6$	$0.9^{b}\pm0.1$	$3.8^{a}\pm1.1$	$5.0^{b}\pm0.4$
CT	**TSCV**	$14.4^{a}\pm1.0$	$7.8^{a}\pm0.4$	$7.3^{a}\pm1.5$	$1.4^{a}\pm0.1$	$2.7^{a}\pm0.5$	$3.9^{a}\pm0.4$
	BS	$7.23^{b}\pm0.8$	$9.6^{a}\pm0.7$	$12.2^{b}\pm0.7$	$0.7^{b}\pm0.1$	$4.5^{b}\pm0.3$	$5.1^{b}\pm0.4$
ZR2	**TSCV**	$17.3^{a}\pm10.0$	$6.6^{a}\pm1.1$	$7.1^{a}\pm0.7$	$1.1^{a}\pm0.3$	$3.7^{a}\pm0.6$	$2.5^{a}\pm0.4$
	BS	$15.9^{a}\pm2.9$	$6.0^{a}\pm1.1$	$8.6^{a}\pm1.6$	$0.7^{a}\pm0.1$	$3.4^{a}\pm0.8$	$3.3^{a}\pm0.4$
GL	**TSCV**	$9.3^{a}\pm3.6$	$9.6^{a}\pm1.2$	$10.4^{a}\pm2.0$	$1.4^{a}\pm0.5$	$4.0^{a}\pm1.0$	$4.3^{a}\pm0.4$
	BS	$3.5^{b}\pm0.4$	$6.8^{b}\pm0.4$	$8.3^{a}\pm1.2$	$0.4^{b}\pm0.1$	$3.0^{a}\pm0.2$	$3.6^{a}\pm0.3$
PL	**TSCV**	$14.4^{a}\pm6.7$	$14.3^{a}\pm1.8$	$14.7^{a}\pm0.8$	$1.1^{a}\pm0.6$	$5.9^{a}\pm1.5$	$7.4^{a}\pm1.1$
	BS	$10.5^{a}\pm0.4$	$11.2^{b}\pm0.7$	$11.9^{b}\pm0.6$	$1.0^{a}\pm0.2$	$4.8^{a}\pm0.3$	$5.4^{b}\pm0.1$
Whole olive groves	**TSCV**	$19.6^{a}\pm12.4$	$12.8^{a}\pm4.2$	$12.5^{a}\pm3.2$	$2.8^{a}\pm2.4$	$5.5^{a}\pm1.8$	$5.2^{a}\pm1.8$
	BS	$10.7^{b}\pm4.8$	$9.3^{b}\pm1.7$	$10.8^{a}\pm2.0$	$1.2^{b}\pm0.6$	$4.3^{a}\pm0.8$	$4.3^{a}\pm1.0$

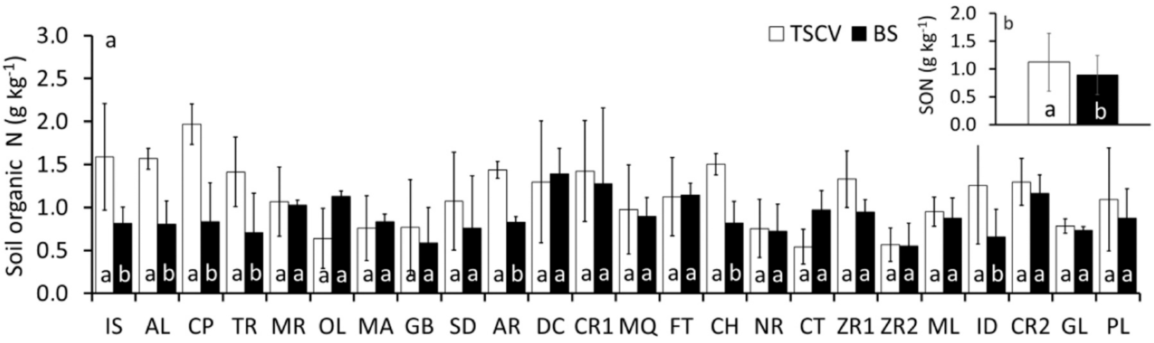

Fig. 6. Soil organic nitrogen (g kg^{-1}) as affected by management (TSCV; spontaneous cover crops and BS; bare soil) (a and b). For a given site, different lowercase letters indicate significant differences between management treatments at P< 0.05. Vertical bars indicate standard deviation.

studies, such as Rodríguez-Lizana et al. (2020) and Hutchinson et al. (2007), attributable to the relatively low percentage of area covered by spontaneous vegetation.

Although the annual aboveground primary production and organic carbon accumulation associated with the implementation of TSCV are relatively low, the SOC and total N concentrations in the top 30 cm of TSCV olive groves were, on average, 25 % and 26 % higher than those in the BS olive groves. For SOC, there was a tendency of higher values in 19 out of the 24 pairs, although the differences were only significant for 10 pairs for SOC and 9 for total N. This underscores the fact that due to the relatively low biomass of the temporary spontaneous cover crops, more time is required to detect statistically significant differences in changes in soil carbon concentration in the top 30 cm, which must be greater as the organic carbon stock rises. The higher total N of the top 30 cm of soils of the TSCV indicates higher N retention due to the presence of the TSCV. The rapid growth rate typical of the herbaceous vegetation of the TSCV, permits the interception and storage of highly mobile nutrients, such as N-nitrate, as organic N of the plant biomass. This might reduce significantly the N that are prone to be lost by leaching, soil erosion and runoff. A fraction of the plant derived N will be progressively released during decomposition at rates which are more in accordance to those required for acquisition by olive trees and/or the following TSCV development, contributing to more efficient nutrient cycling by boosting the soil-plant subcycle. Therefore, the TSCV farms of our study had higher capacity to retain N while providing annually more available N throughout N mineralization as indicated by the 77 % higher potential N mineralization of the TSCV soils.

After eight years of implementing TSCV, the SOC pool was found to be significantly higher (by an average of 2.03 Mg C ha^{-1} top 30 cm soil) compared to BS, with an approximate annual rate of 0.25 Mg C ha^{-1} y^{-1}. This figure, which is an average of the difference in the stock of C for the 24 pairs, were lower than those reported by Vicente-Vicente et al. (2016) in a meta-analysis, where an average annual SOC accumulation rate of approximately 1.1 Mg C ha^{-1} was observed. It is worth mentioning that all the farms included in this meta-analysis had the entire area covered with a cover crop, resulting in higher values compared to ours, where only 28 % of the soil was covered with cover crops. However, our figure is similar to that of Hernanz et al. (2002),

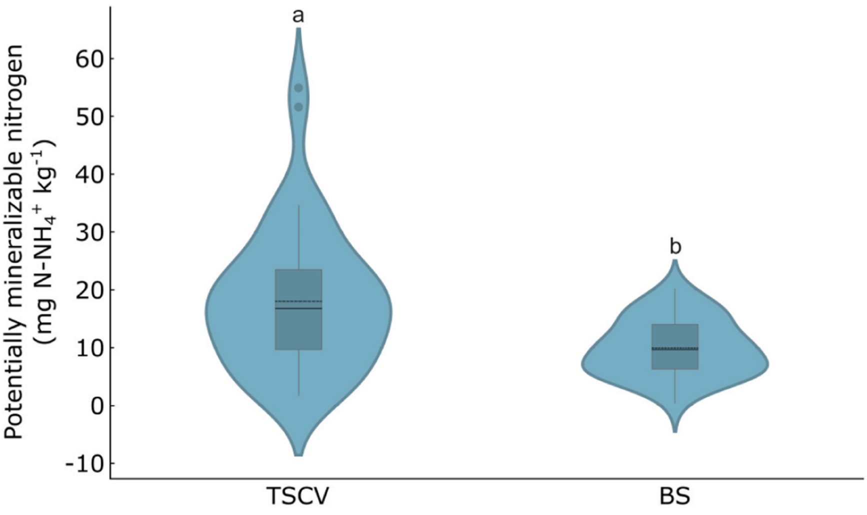

Fig. 7. Violin plot of soil potencially mineralizable nitrogen (mg N-NH$_4^+$ kg^{-1}) in soils of the TSCV and BS olive groves. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin lines within the box mark the median and the dotted line average. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as dark dots. Different lowercase letters indicate significant differences between soil management (TSCV; spontaneous cover crops and BS; bare soil) at $P< 0.05$.

who found a mean value (0.38 Mg C ha^{-1} y^{-1}) in herbaceous cultivations to a depth of 0– 30 cm in semi-arid central Spain. Nevertheless, the findings from our study were slightly higher than the 0.10 Mg C ha^{-1} y^{-1} reported by Castro et al. (2008) for an olive grove with 28-year of cover crops that primarily consisted of spontaneous cover crops.

In any case, our research reveals that, following about 8 years of cover crop, the organic carbon derived from plant residues is substantial enough to augment SOC stocks in most of the investigated olive groves. However, the expected soil erosion reduction in the TSCV olive groves could explain a portion of the differences in the SOC stocks between TSCV and BS olive groves. Márquez-García et al. (2013) conducted a study in different rain-fed olive regions in Andalusia, demonstrating that TSCV practices led to 80.5 % reduction in erosion and a substantial 67.7 % reduction in organic carbon transport, being carbon losses of 13.18 and 46.38 kg ha^{-1} yr^{-1} for TSCV and BS, respectively. In another study, López-Vicente et al. (2021) showed in a olive groves that soil organic carbon loss in TSCV plots averaged 148 kg C ha^{-1} yr^{-1} whereas under BS the lost was 222 kg C ha^{-1} yr^{-1}. The difference in the quantity of carbon lost due to erosion between the TSCV and BS systems could, to some extent, account for the observed increase in SOC stock within TSCV-managed olive groves.

Despite the relatively low amount of carbon transferred from the atmosphere to the soil via TSCV of our study, still contributed significantly to climate change mitigation.

4.2. Unprotected and physically protected SOC pools were the fractions increased with cover crops

Once the aboveground biomass of the TSCV olive groves has been cleared and residues applied on top of the soil, decomposition of these and their roots commences. The organic carbon that is not released as CO_2 during this process contributes to the enrichment of unprotected carbon and various forms of protected carbon. Both unprotected (250–2000 μm) and protected (53–250 and < 53 μm) SOC fractions were significantly higher under olive farms with TSVC compared to BS. The increase in the physically protected SOC fraction (53–250 μm) indicates that cover crops residues and roots contribute to the promotion of the formation of macro- and/or microaggregates in the soil. Indeed, soil aggregate stability has been shown to increase with spontaneous cover crops (López-Vicente et al., 2021; García-Franco et al., 2015). While plant residues undergo decomposition, microorganisms release organic compounds that act as binding agents, helping to hold soil particles together and form aggregates (Jastrow et al., 1998). These aggregates act as protective structures, enclosing and shielding the SOC from microbial decomposition and other forms of degradation (Killham et al., 1993) and promoting SOC preservation over a longer period resulting in a net C sequestration into the soil rather than loss to the atmosphere as CO_2 (Xiao et al., 2021).

Organic carbon density (g C kg^{-1} soil particles < 53 mm) and content (g C kg^{-1} of soil) of the chemically protected C tended to be higher (15 and 20 % respectively) in soils of the TSCV olive groves, but differences were not significant. This lack of significant differences indicates that the annual rate of organic carbon compounds produced by the microbial activity while decomposing the unprotected C of the soils of the TSCV, is not enough to increase by adsorption the organic carbon density of the silt+clay soil fractions.

The chemically protected carbon fraction of the silt + clay (< 53 μm) did not show significant differences between treatments when considering the mean of all study plots. However, it was observed that under TSVC treatment, this protected SOC fraction tended to be 16.9 % higher, compared to BS treatment.

4.3. Soil inorganic C pool is much higher than SOC and the presence of cover crops did not affect significantly to SIC pool

Pools of soil inorganic carbon (SIC) were on average 167 and 171 Mg C ha^{-1} (top 30 cm of soil), which is 4.3 and 5.5 times higher than organic carbon (SOC) in the TSCV and BS olive groves, respectively. These large amounts of SIC are due to the pedoclimatic features of the studied area, where the process of calcification is favored over decalcification because of the relatively low annual precipitation and very high potential evapotranspiration (Sánchez et al., 2004).

Despite the SIC content and stock in the top 30 cm of the BS soils tending to be higher than those in the TSCV olive groves, our results do not support the hypothesis that the higher biological activity associated with the decomposition of temporary cover crop residues in olive groves may have influenced the dissolution and precipitation of carbonates in the soil, thereby affecting the dynamics of SIC. This contrasts with other studies that have identified negative correlations between SOC and SIC stocks within the 0–50 cm soil layer across various vegetation cover types (Zhao et al., 2016), suggesting that SOC gain due to vegetation residues is compensated by SIC loss, resulting in a lack of significant change in total soil carbon due to vegetation.

The lack of significant differences in our study could also be partially explained by the very high concentration and stock of SIC, which require very large changes in stock to achieve significant differences. Xiaoyang et al. (2018) found that an average accumulation of 1 kg SOC was associated with a 0.73 kg loss of SIC in the 0–40 cm layer following revegetation, demonstrating that a significant measurable change in SOC can be associated with a non-measurable change in SIC due to the large amount of SIC compared to SOC.

5. Conclusion

This study demonstrates that temporary spontaneous cover crops (TSCV) can increase both SOC and STN compared to bare managed olive orchards. Despite the low annual biomass production of cover crops, prolonged TSCV presence led to significant SOC accumulation and improved total nitrogen retention. TSCV contributed to the increase in physically protected organic carbon fractions, suggesting enhanced soil aggregate stability, favoring long-term carbon preservation. Although no significant differences were observed, a trend towards an increase in the chemically protected organic carbon fraction in TSCV soils suggests potential for further stable carbon accumulation. While TSCV significantly contributed to SOC accumulation, no significant differences were observed in SIC contents between olive groves with and without TSCV, indicating a limited impact on SIC dynamics. In summary, implementing temporary spontaneous cover crops appears to be an effective strategy for enhancing soil quality by increasing organic carbon and improving nitrogen retention. However, further research is needed to fully understand the underlying mechanisms and long-term effects of TSCV on soil carbon and nitrogen dynamics.

CRediT authorship contribution statement

Evangelina Pareja Sánchez: Data curation, Writing – original draft, Writing – review & editing. **Roberto Garcia-Ruiz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Writing – review & editing. **Julio Calero:** Writing – review & editing, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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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.still.2024.106237.

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