



The effects of olive orchard diversifications on greenhouse gas emissions and its influencing factors

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Abstract

Purpose In recent years, intercropping in olive groves has become a sustainable practice for mitigating climate change. Therefore, the objective of this study was to determine whether crop diversification in rainfed olive groves modifies carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄) emissions and key chemical and enzymatic properties of the soil in the short term.

Materials and methods A field experiment was conducted under semi-arid Mediterranean conditions using four treatments: a conventional olive monocrop (CP) and three crop diversification systems: saffron (*Crocus sativus*) (D-S), oats and vetch (*Vicia sativa* and *Avena sativa*) (D-O) and lavandin (*Lavandula* × *intermedia*) (D-L). CO₂, N₂O, and CH₄ emissions were measured periodically using closed static chamber techniques. Simultaneously, soil samples were analyzed to determine physicochemical parameters (pH, total nitrogen (TN), total organic carbon (TOC), P available, K, and Fe) and enzymatic activities (β-1,4-glucosidase (BG), leucine aminopeptidase (LA), N-acetylglucosaminidase (NAG), Arylsulfatase (AS) and phosphatase (AP) and dehydrogenase (DH)) in order to evaluate the interactions between soil properties and gas fluxes.

Results and discussion The results showed that CO₂ emissions were significantly higher in D-S (385.2 mg CO₂ m⁻² h⁻¹) and D-O (335.2 mg CO₂ m⁻² h⁻¹) compared to CP, which was attributed to increased microbial and root activity promoted by the vegetation cover. In contrast, N₂O fluxes (0.002 mg N₂O m⁻² h⁻¹ in CP, 0.007 in D-S, 0.001 in D-O, and -0.002 in D-L) and CH₄ (-0.2 mg CH₄ m⁻² h⁻¹ in CP, 0.1 in D-S, 0.2 in D-O, and 0.2 in D-L) did not show significant differences between all treatments ($p > 0.05$), probably due to the prevailing semi-arid conditions. D-O had higher values of P available, Fe, and TN, while D-S and D-L stood out for higher values of K and TOC, both compared to CP. Enzymatic activities were particularly higher in D-S and D-O, specifically BG, LA, and AS. In addition, positive and significant correlations were observed between CO₂ accumulation and several soil parameters: in D-S, with POC, total Zn, exchangeable Na, total Cu, and BG, LA, and AS activities; and in D-L, with POC, exchangeable Na, total Mn, P available, TN, and LA and AS activities, highlighting that these indicators contribute to the regulation of greenhouse gas (GHG) emissions.

Conclusion It was concluded that crop diversification in rainfed olive groves increases CO₂ emissions in the short term due to intensified biological activity, but at the same time improves soil fertility and biochemical functionality. However, long-term monitoring is required to determine whether these systems contribute to carbon stabilization and net sequestration, ensuring their potential as sustainable strategies for climate change mitigation in Mediterranean agroecosystems.

Keywords Intercropping · GHG emissions · Enzymatic activities · Conventional tillage · Olive orchards

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1 Introduction

It is well known that soil functions and soil ecosystem services are threatened by poor management (e.g., monocrops and heavy mechanization) (Morugán-Coronado et al. 2020), which accelerates soil degradation with dramatic consequences for productivity (Ferreira et al. 2022). In the European Union, 60–70% of soils are degraded because of unsustainable agricultural management practices (EC 2020b), leading to adverse environmental effects, such as increased soil erosion, loss of biodiversity, and higher greenhouse gas (GHG) emissions.

In the Mediterranean area, olive crops are one of the most important land uses, accounting for 67% of the world's olive oil production (Eurostat 2019). However, this region is also highly vulnerable to soil degradation (Lahmar and Ruellan 2007), mainly due to the reduced soil organic matter (SOM) and highest erosion rates in Europe (Panagos et al. 2020; Aguilera-Huertas et al. 2022) resulting from agriculture intensification (Bourlion and Ferrer 2018; IPCC 2019).

Traditional olive orchards managed under conventional tillage (CT) often present bare soils for long periods, which accelerates erosion, reduces soil carbon inputs and may increase GHG emissions through enhanced mineralisation and lower structural stability (FAO 2017; Zornoza et al. 2018). Therefore, agricultural soils in Mediterranean environment can become in a source of GHG emissions, although they have a considerable potential to be carbon sinks (Cerdà et al. 2010; Aguilera-Huertas et al. 2022). Therefore, implementing sustainable cropping systems is essential to foster the potential of agricultural soils to become large carbon sinks (Chabbi et al. 2017; González-Rosado et al. 2022). According to Morugán-Coronado et al. (2020), crop diversification together with reduced tillage are the most widely accepted sustainable agricultural management practices. In olive orchards, this strategy is favorable for decreasing GHG emissions (Zikeli and Gruber 2017), increasing soil carbon storage (Janzen et al. 2022), improving soil quality and fertility (Panagos et al. 2020; Almagro et al. 2023), reducing soil and water losses (Raza et al. 2019) and improving productivity through perennial biomass (García Azcárate 2017; Kay et al. 2019). These combined effects not only improve soil fertility but also contribute to its long-term sustainability, creating a healthier environment for crop growth and potentially influencing GHG emissions (Paramesh et al. 2023).

Variations in soil nutrients can have a direct effect on GHG emissions. For instance, greater nitrogen availability in the soil can increase the production of nitrous oxide

(N₂O), a potent GHG, due to microbial processes such as nitrification and denitrification (Ramzan et al. 2020). Conversely, adequate levels of exchangeable cations and micronutrients can improve soil structure and plant health, reducing the need for synthetic fertilizers and, therefore, the emissions associated with their use (Dahmani et al. 2023). Improvements in SOM and particulate organic carbon (POC) can also contribute to higher C sequestration in the soil and modify carbon dioxide (CO₂) emissions (Rocci et al. 2021; González-Rosado et al. 2021). Collectively, these improvements in soil quality through crop diversification not only promote agricultural sustainability but also have the potential to mitigate GHG emissions (Chabbi et al. 2017).

Soil enzymatic activity also plays a crucial role in regulating GHG emissions (Marcos-Pérez et al. 2023; Jiang et al. 2021). Enzymes such as β -1,4-glucosidase (BG), N-acetylglucosaminidase (NAG), arylsulfatase (AS), phosphatase (AP), leucine aminopeptidase (LA), and dehydrogenase (DH) play a key role in the decomposition of SOM and the nutrient cycle (Liu et al. 2024). These enzymes regulate carbon and nitrogen transformations and are therefore closely related to microbial respiration and CO₂ generation, as well as to processes that influence N₂O and CH₄ emissions by modulating the availability of mineral nitrogen and labile carbon compounds, which regulate nitrification-denitrification pathways and support microbial groups involved in CH₄ oxidation. (Ma et al. 2022; Guan et al. 2022; Peng et al. 2024). However, it should be noted that higher enzymatic activity indicates a high rate of SOM decomposition, which can lead to an increase in GHG emissions if not properly managed (Raza et al. 2023). Crop diversification can stimulate favorable enzymatic activity, improving soil health and contributing to the mitigation of GHG emissions (Cuartero et al. 2022; Zou et al. 2024).

This study is notable for its innovative approach as it comprehensively addresses the relationship between sustainable agricultural management, specifically crop diversification, and its impact on GHG emissions, as well as its potential to mitigate climate change in Mediterranean olive farming systems. A link is established between soil chemical properties and enzymatic activity in relation to GHG emissions, with a particular focus on olive groves. This strategy could serve as a reference to reverse the current unfavorable situation, contributing to the development of new practices for sustainable soil management at a global level, improving soil health and quality, and strengthening its role in the fight against climate change.

We hypothesize that crop diversification in Mediterranean rainfed olive groves, with the introduction of herbaceous and aromatic crops, would modify CO₂, N₂O, and

CH₄ emission fluxes and be associated with changes in soil chemical properties and enzymatic activities compared to conventional tillage.

The objective of this study was to evaluate the short-term effect of alley cropping with crop diversification such as *Crocus sativus*, *Vicia sativa* and *Avena sativa* in rotation, and *Lavandula x intermedia* on CO₂, N₂O and CH₄ emissions, total cumulative emissions and their relationship with variations in soil chemical and enzymatic properties in a Mediterranean rainfed olive grove.

2 Materials and methods

2.1 Study site and experimental detail, soil sampling and laboratory analysis

This study was developed in an experimental rainfed olive farm with an area of 10 hectares and a planting frame of 12 m × 12 m in the Giennese municipality of Torredelcampo (Jaén-Spain) (37°51'0.81" N–3°51'55.26" W) (Fig. 1) during the period 2018–2021. The study area is characterized

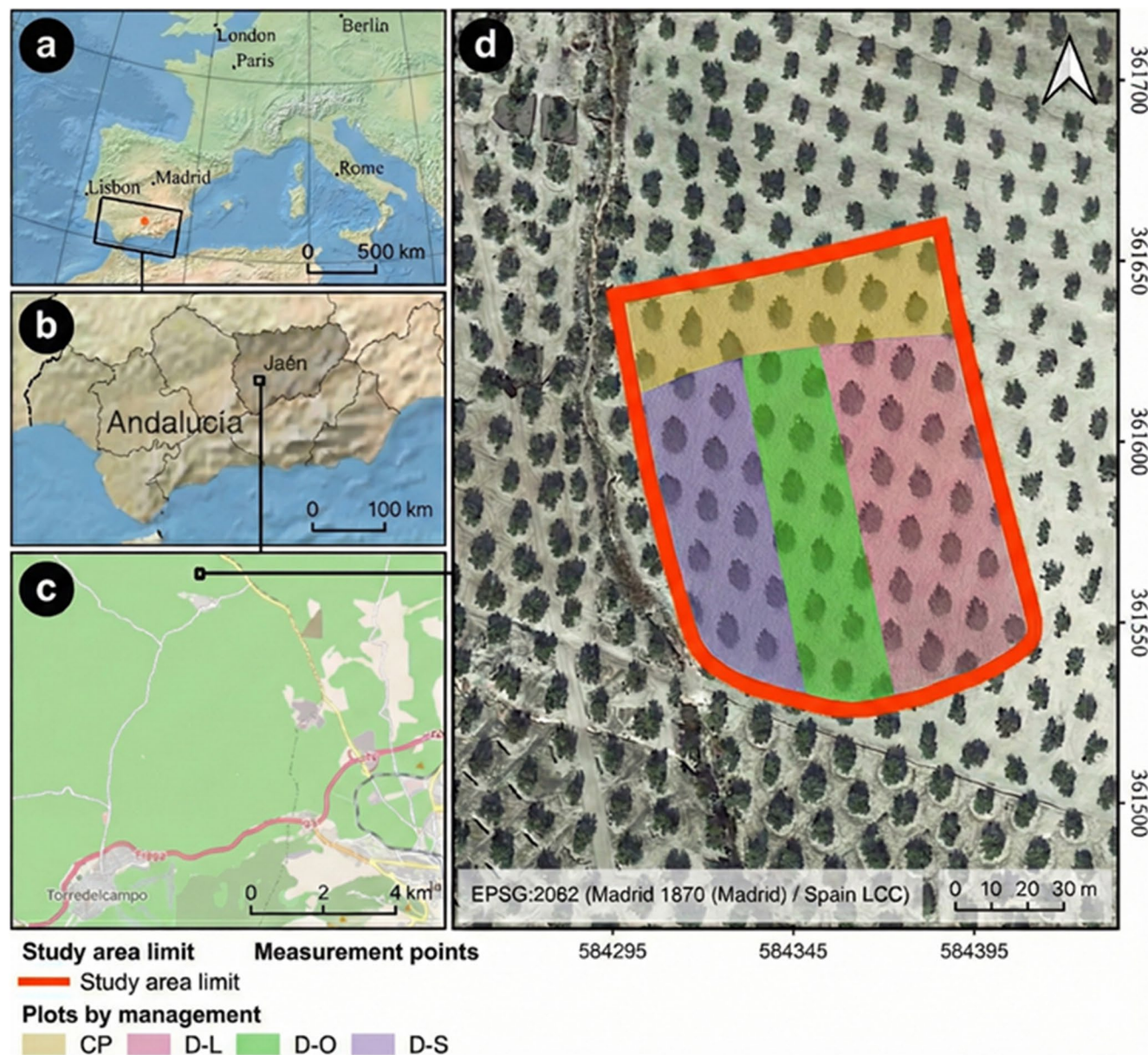


Fig. 1 Location of the study area. Map of Spain (a). Region of Andalusia where the province of Jaén and the municipality of Torredelcampo are highlighted in dark gray (black box) (b). Municipality of Torredelcampo where the study area is marked (black box) (c). Aerial view of its delimitation (red outline) and of the experimental treatments

(d). CP: olive monocrop under conventional tillage; D-S: olive intercropped with *Crocus sativus*; D-O: olive intercropped with *Vicia sativa* and *Avena sativa* in rotation; D-L: olive intercropped with *Lavandula x intermedia*. Each treatment plot covers 2000 m² and consists of three olive tree rows within a 12 m × 12 m planting frame

by an average altitude of 541 m above sea level, with a S-SW orientation, with slopes that are not very steep, varying between 1% and 6%. The climate is Mediterranean, with a great thermal contrast during the different seasons. The average rainfall is 493 mm. The average annual temperature (T) is 17.1 °C, relative humidity is 59%, and insolation is 237 h month⁻¹ (according to (AEMET 2020)). The soils are classified as Calcareous Cambisols (FAO-ISRIC-ISSS 2015) With a high percentage of clays (72% clay), with high erosion and low amounts of SOM, i.e., we found poor quality soils that were degraded.

The experimental design included land management change and crop diversification. Four experimental plots with an area of 2000 m² formed by three rows of olive trees were established. The original management on the farm (CP) was CT, characterized by the application of mineral fertilization in intercalary years, after the olive harvest, applying an amount of 100 kg ha⁻¹ of urea (46% N). In addition, in two-year periods, the olive grove was pruned, and the shredded remains of this pruning were applied to the soil and added to the soil; this was followed by tillage using a disc harrow to a depth of 25 cm. Finally, in autumn, herbicide (Glyphosate 36%, soluble concentrate; Roundup®, Bayer CropScience, Germany) was applied to control weeds and vegetation. At the beginning of the experiment, new plots were set up by planting three crop diversifications. The treatments tested were: (i) *Vicia sativa* (vetch) and *Avena sativa* (oats) in rotation (D-O) with an annual crop, (ii) *Crocus sativus* (saffron) (D-S), sown only once (iii) *Lavandula x intermedia* (lavandin) (D-L). These diversifications were established in the center of the olive grove alleys with a seed rate of 120 kg ha⁻¹ and 140 kg ha⁻¹ for vetch and oats respectively, 2,000 kg ha⁻¹ for saffron and 12,000 plants per hectare for lavandin. Saffron, oats and vetch were planted 4.5 m from the trunk of the olive tree. However, lavandin was planted at 5.5 m from the trunk of the olive tree because this diversification is perennial and had to allow the passage of machinery during olive harvesting. No fertilizers or herbicides were applied to the three diversified plots, and the management also included pruning the olive trees and incorporating the residues on the soil and mechanical weeding and with minimum tillage (MT) to a depth of 10 cm for the seedbed, which was conducted manually. None of the four treatments studied involved the application of water through irrigation, as it was an experimental area of rainfed olive groves. The diversifications may have been affected by local fauna (e.g., rabbits) that used the seeds and small shoots as food. The management change towards MT and the lack of herbicide application allowed spontaneous vegetation growth. Therefore, a mixed vegetation cover was obtained, which developed to a greater extent in the *Crocus sativus* plot and the *Vicia sativa* and *Avena sativa* plot in rotation, compared to

the *Lavandula x intermedia* plot. However, its development in this plot (D-L) was also significant. During this period, a higher percentage of cover developed in the D-S and D-O plots. The reason for a lower rate of alley cover in D-L is on the one hand, the lower surface cover because of the necessity to allow the passage of machinery during olive harvesting, and on the other hand, because lavandin was a perennial cover without vegetative debris.

A randomized block design with three replicates and five random sampling points per experimental plot (D-S, D-O, D-L, and CP) was employed, resulting in a total of 60 soil samples (3 replicates × 5 sampling points × 4 experimental plots = 60). Soil samples were collected from the olive tree alleys at a depth of 0–10 cm. In the field, samples were split into two aliquots: one was kept at ambient T for chemical analyses, while the other was stored in a cooler with ice for biological studies. The samples were transported immediately to the laboratory, where the soil was dried at room T and sieved to less than 2 mm. The soil designated for biological analysis was stored at –20 °C.

The laboratory analyses carried out to determine the different physicochemical properties, nutrients and enzymatic activities of the soil were performed according to the methods described by Aguilera-Huertas et al. (2023).

2.2 Soil greenhouse gas measurements

Measurements of CO₂, N₂O and CH₄ were made every 7–40 days, depending on weather conditions, in triplicate for all replicated treatments in the period 2020–2021, specifically from 09/01/2020 to 22/07/2021 between 9:00 am and 11:00 am. Three measurements were made per plot (Fig. 1), obtaining a total of nine measurements per treatment and 36 measurements per day, during a period of 49 days within the period indicated above, obtaining a total of 1764 measurements in all plots. (3 measurements per plot per day × 3 sub-plots in each treatment × 4 treatments = 36 measurements per day × 49 days = 1764 measurements of CO₂, N₂O, and CH₄ during the study period). The method used was the dynamic gas chamber technique (Álvaro-Fuentes et al. 2019). The chambers were made of non-oxidizable steel, with a diameter of 7.5 cm and a height of 20 cm. They were mounted on a non-oxidizable steel base that was randomly inserted into the soil at a depth of 15 cm between two olive trees in CP and between diversifications in D-S, D-O and D-L at a minimum distance of 2.5 m from the tree. The emission rates of N₂O, CO₂ and CH₄ were expressed as the difference between the quantification at the end and at the beginning of the measurement period divided by time. Emission rates of N₂O, CO₂ and CH₄ (F_{gas}, mg m⁻² h⁻¹) were calculated as:

$$F_{\text{gas}} = (Q_{\text{end}} - Q_{\text{start}}) / \Delta t$$

where Q_{start} and Q_{end} are the gas quantifications at the beginning and at the end of the measurement period (mg m^{-2}), and Δt is the duration of the measurement (h).

Cumulative CO_2 , N_2O and CH_4 emissions for each treatment were estimated by numerical integration and converted to CO_2 equivalent (CO_2e) following the methodology described by Abdeldaym et al. (2019). Cumulative CO_2 , N_2O and CH_4 emissions (E_{gas}) were obtained by numerical integration of the discrete fluxes using the trapezoidal rule:

$$E_{\text{gas}} = \sum_{i=1}^{n-1} \frac{F_i + F_{i+1}}{2} (t_{i+1} - t_i)$$

where F_i and F_{i+1} are consecutive emission rates ($\text{mg m}^{-2} \text{h}^{-1}$) and $(t_{i+1} - t_i)$ is the time interval between measurements (h). Emissions were then converted from mg m^{-2} to g m^{-2} by dividing by 1000. Cumulative emissions were expressed as CO_2e (CO_2e , $\text{g CO}_2\text{e m}^{-2}$) as:

$$E_{\text{CO}_2\text{e}} = E_{\text{CO}_2} + \text{GWP}_{\text{N}_2\text{O}} E_{\text{N}_2\text{O}} + \text{GWP}_{\text{CH}_4} E_{\text{CH}_4}$$

Where E_{CO_2} , $E_{\text{N}_2\text{O}}$ and E_{CH_4} are the cumulative emissions of each gas (g m^{-2}), and $\text{GWP}_{\{\text{N}_2\text{O}\}}$ and $\text{GWP}_{\{\text{CH}_4\}}$ are the 100-year global warming potential factors used according to IPCC.

Soil T and moisture (M) were measured using a Pro-Check, and 5TM sensors (Decagon Devices, USA) were inserted at a depth of 10 cm close to the location of GHG measurements.

2.3 Chemical soil parameters and enzymatic activity

Chemical soil parameters were determined for all experimental areas at the beginning of the experiment (2018) and at the end (2021). total nitrogen (TN) and total organic carbon (TOC) were measured by dry combustion method using a CN 802 elemental carbon and nitrogen analyzer (VELP Scientifica, Usmate, Italy) (Diaz-Pereira et al. 2019; Alvaro-Fuentes et al. 2019). In this method, high-T combustion occurs in an oxygen-rich atmosphere, converting all compounds present in the soil, and quantified using a specific detector. The contents of P available was determined using the Olsen method (Velazco-Sánchez et al. 2024) with the aim of determining the amount of P available in a soil sample, i.e., the fraction of phosphorus that plants can absorb and utilize. Using colorimetry and spectrophotometry, this method allows the amount of phosphorus present to be determined, as it is directly proportional to the amount of reduced phosphomolybdate and, therefore, to the color developed. exchangeable Mg, Na, Ca, K, and total Fe, Cu, Zn, Mn, were determined using the Mehlich-3

multi-element extractant, and element concentrations in the extracts were quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES), following the procedures described in the Handbook of Plant and Soil Analysis for Agricultural Systems (Mehlich 1984; Alvaro-Fuentes et al. 2019). Particulate organic carbon (POC) was quantified after dispersing the soil with sodium hexameta-phosphate, separating the $\geq 53 \mu\text{m}$ particulate fraction by wet sieving, drying the material retained on the sieve and determining its C concentration by dry combustion, following Cambardella and Elliott (1992) as reported in Alvaro-Fuentes et al. (2019).

Enzyme activity data (Aguilera-Huertas et al. 2023) were used to relate to greenhouse gas emissions. The extracellular enzyme activities BG, NAG, AS, AP and LA were measured using a fluorogenic microplate assay according to Marx et al. (2001). Briefly, fresh soil (equivalent to 1–2 g dry soil) was homogenised in buffered solution, and aliquots were dispensed into 96-well microplates together with the corresponding fluorogenic substrates (4-methylumbelliferyl- β -D-glucoside for BG, 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide for NAG, 4-methylumbelliferyl-sulfate for AS, 4-methylumbelliferyl-phosphate for AP and L-leucine-7-amino-4-methylcoumarin for LA). Plates were incubated at controlled T and fluorescence was read at the appropriate excitation and emission wavelengths; activities were expressed as nmol product g^{-1} dry soil h^{-1} .

DH was determined following von Mersi and Schinner (1991) by incubating moist soil with iodonitrotetrazolium chloride (INT) and quantifying the formation of INT-formazan colorimetrically. After incubation, INT-formazan was extracted with organic solvent and absorbance was measured spectrophotometrically, and DH activity was expressed as $\mu\text{g INT-formazan g}^{-1}$ dry soil h^{-1} . Detailed protocols for all assays are described in Alvaro-Fuentes et al. (2019)

2.4 Statistical analysis

Before analysis, the normality of GHG flux data (CO_2 , N_2O , and CH_4) was verified using the Kolmogorov-Smirnov test. When the assumptions of normality and homoscedasticity ($p > 0.05$) were met, the effects of management (CP, D-S, D-O, D-L), sampling date, and their interaction on instantaneous emission rates were analyzed using a two-way ANOVA. When significant effects were detected ($p < 0.05$), the means of the treatments were separated using Tukey's post hoc test. The cumulative emissions of each gas and the total CO_2e during the monitoring period were compared between treatments using a one-way ANOVA followed by Tukey's test ($p < 0.05$). These analyses were performed using SigmaPlot v.14.0 (San Jose, California, USA), and

GHG emission figures were obtained using Plotly Chart Studio (v.5.13.1).

To evaluate changes in soil chemical properties over time, data from 2018 to 2021 were used to calculate, for each plot and parameter, a relative increase as $(\text{value}_{2021} - \text{value}_{2018})/\text{value}_{2021}$. Statistical analysis of soil parameters were performed in R v.4.2.1 (R Core Team 2021). Normality and homoscedasticity were checked, and since most soil chemical variables did not meet these assumptions, differences between CP and each diversification treatment (D-S, D-O, D-L) were evaluated using the nonparametric Wilcoxon test ($p < 0.05$).

Multivariate analyses were used to explore the joint response of soil variables to different management practices. Principal component analysis (PCA) was performed to visualize the separation of treatments based on soil chemical properties using the factoextra package (Kassambara and Mundt 2016), and heat maps were generated with the ggplots package (Warnes et al. 2005) to illustrate differences in the distribution of variables between treatments, and statistical significance was assessed using Wilcoxon test using FSA package (Ogle et al. 2025). Finally, to investigate the relationships between CO₂ accumulation and changes in soil chemical parameters and enzyme activities, simple linear regressions were fitted for each treatment. Significant relationships ($p < 0.05$) were identified, and the corresponding regression coefficients were visualized using the ggplot2 package (Wickham 2016) to highlight the most relevant soil and enzymatic indicators associated with CO₂ accumulation (Figs. 2, 3, 4, and 5).

3 Results and discussion

3.1 Effects of crop diversification on soil chemical and enzymatic properties

Crop diversification and intercropping are widely recognised as key sustainable management strategies that enhance soil fertility, nutrient cycling and biological activity in Mediterranean agroecosystems (Chabbi et al. 2017; Janzen et al. 2022). Our results showed that the different diversification treatments significantly altered soil chemical properties compared to CP (Table S1 and S2). These findings are consistent with previous research indicating that crop diversification and intercropping influence soil fertility and nutrient dynamics through plant-microbe interactions and SOM incorporation (Volsi et al. 2022). An exploratory analysis of all data was performed using PCA (Fig. 6), including both soil chemical data and enzyme activity. A clear differentiation between CP and the other management systems (D-L, D-O, and D-S) was observed along the first

principal component (PC1, 22.7%), while along the second component (PC2) a separation between D-O and D-L had been seen, with D-S located between them.

When the PCA was performed using only soil chemical variables (Fig. S1 (a)), the D-O treatment was separated along PC1 (26.6%), whereas CP, D-L, and D-S clustered together on the opposite side of this axis. Along PC2 (18.6%), CP was separated from D-L and D-S. The biplot representation further illustrates how individual soil chemical variables contributed to the observed sample separation. Variable loadings are displayed as vectors, with direction and length indicating their influence on the ordination. total Fe showed a strong positive loading on PC1, pointing towards the D-O treatment, while P available was oriented in the opposite direction, indicating a contrasting contribution along this axis. TN loaded positively on PC2, pointing towards CP, whereas exchangeable K and TOC showed a negative loading on PC2. Those differentiations suggest the presence of different nutrient and carbon recycling pathways, as documented in studies analyzing nutrient and microbial community variability in diversified systems (Bai et al. 2019; Yu et al. 2022).

In contrast, when the PCA was performed using only enzyme activity data (Fig. S1 (b)), CP and D-L were separated from D-O and D-S along PC1 (41.5%), with AS, AG, and LA contributing most to this separation. PC2 (29.3%) did not differentiate between D-O and D-L, but allowed the separation of CP from D-L, mainly driven by DH and BG activities.

Focusing on soil chemical properties, the heatmap (Fig. 7) shows the distribution of relative changes among the four management systems (CP, D-S, D-O, and D-L) across the different soil variables. Data in the heatmap were standardized by variable to allow direct comparison across soil parameters. To complement the heatmap, Wilcoxon tests were performed and identified five soil chemical variables that significantly differentiated CP from the diversification treatments ($p < 0.05$; Fig. 7, Table S2). Specifically, D-O differed from CP in P available, total Fe, and TN, whereas D-S and D-L differed from CP in exchangeable K and TOC, being this results consistent with the PCA, in which D-O is clearly separated along PC1, primarily associated with total Fe and P available, while CP is distinguished from D-S and D-L along PC2, related to TN, exchangeable K, and TOC.

The significant increase in P available levels in D-O suggests a more active P cycle mediated by microbial and plant activity, consistent with studies reporting that crop diversity and plant cover promote organic P mineralization and inorganic P solubilization (Richardson and Simpson 2011). Similarly, total Fe, which is crucial for enzymatic processes and redox reactions, showed significant differences between D-O and CP, which may be directly related to the presence

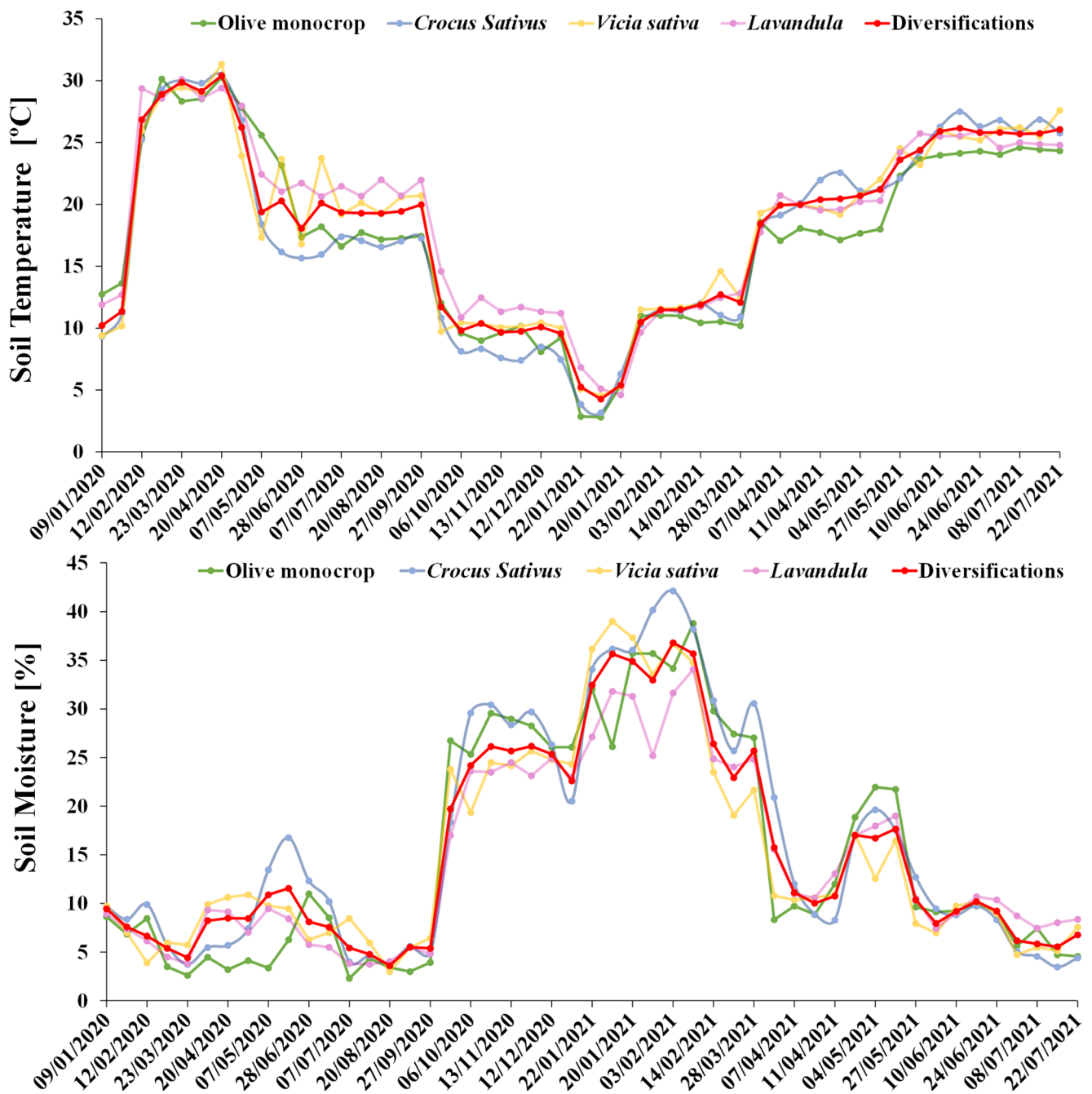


Fig. 2 Evolution of soil temperature and moisture under Olive monocrop (Green color); Olive intercropped with *Crocus sativus* (Blue color); Olive intercropped with *Vicia sativa* and *Avena sativa* in rota-

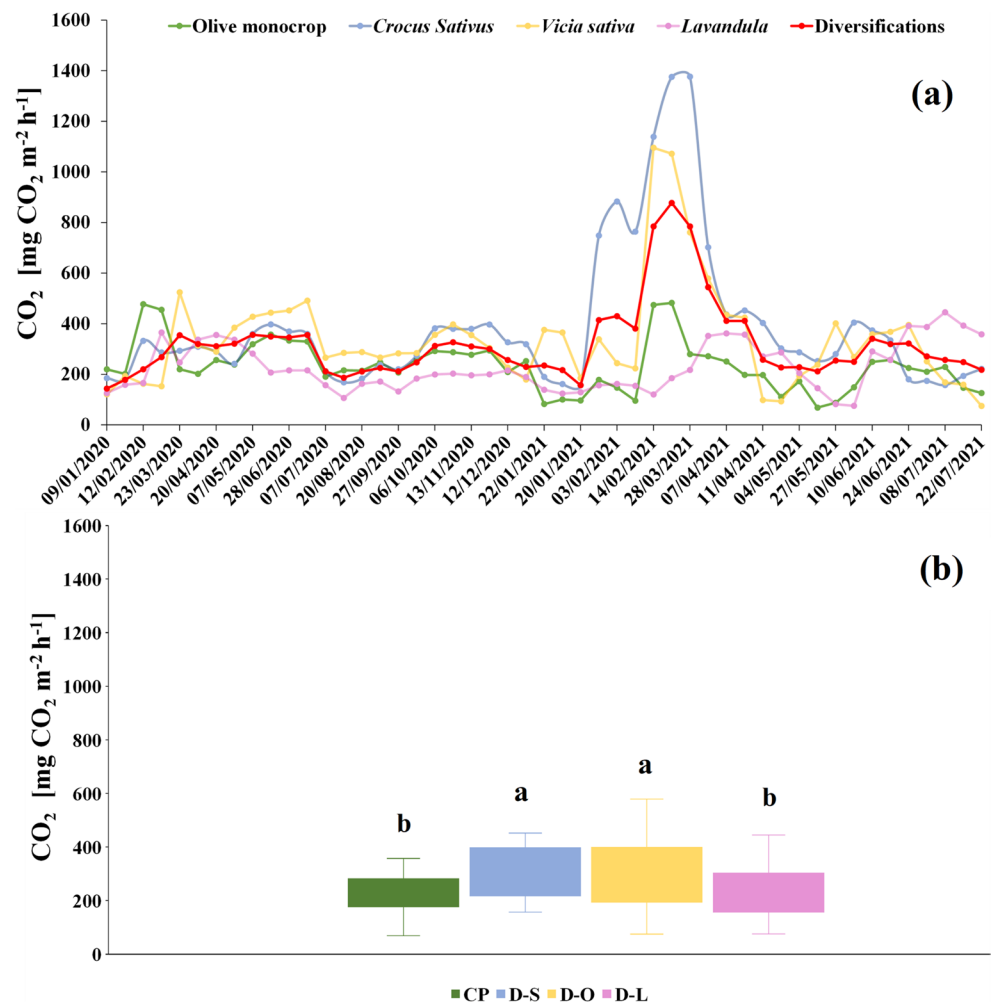
tion (Yellow color) and Olive intercropped with *Lavandula* (purple color) treatments over time (2020–2021)

of legumes (vetch and oats) that acidify the rhizosphere and alter Fe solubility (Zuo and Zhang 2011). The increase in TN in D-O could be attributed to the ability of legumes to fix nitrogen or improve SOM decomposition, thus enriching the soil TN pool (Tripolskaja et al. 2023).

As for exchangeable K, another essential nutrient, its higher levels in D-S and D-L may be related to the accelerated decomposition of SOM and increased root activity

in these diversified systems (Tripolskaja et al. 2023). TOC was also higher in D-S and D-L, reinforcing findings that associate plant diversity with higher TOC, as continuous plant inputs provide an energy source for microbial activity and improve soil structure (Cong et al. 2014; Lal 2015). The inclusion of diversified crops and the development of mixed cover also enhance root activity, rhizodeposition and microbial decomposition of new sources of

Fig. 3 Soil CO₂ emission rates under Olive monocrop (CP); Olive intercropped with *Crocus sativus* (D-S); Olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O) and Olive intercropped with *Lavandula* (D-L) treatments over time (2020–2021) (a) and CO₂ rates among the different treatments (CP, D-S, D-O and D-L) considering the whole study period (b). The boxes represent the treatment means $\pm$ standard error (SE) ($n=3$). The different lowercase letters above the error bars in Figure (b) indicate significant differences between the different treatments during the study period based on average CO₂ fluxes ($p < 0.05$)



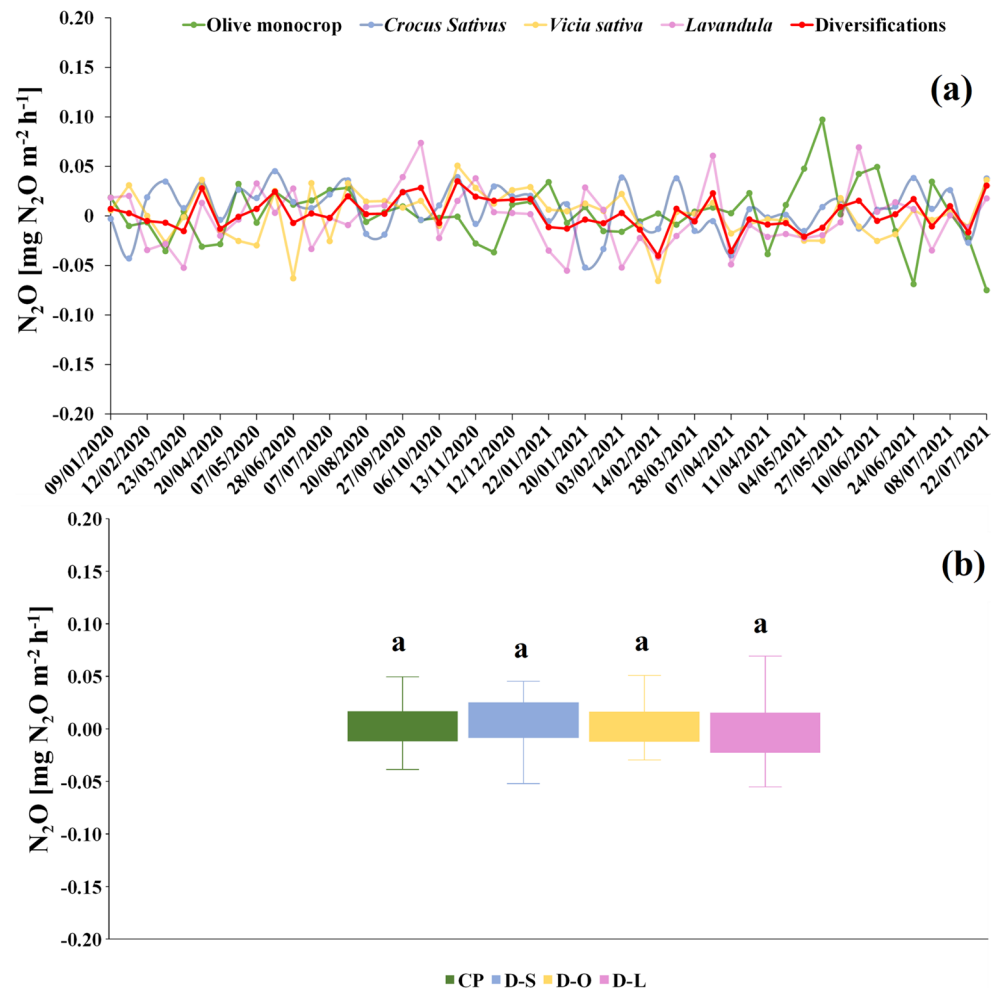
labile SOM (D'Acunto et al. 2018; Sánchez-Navarro et al. 2022), which is consistent with the higher TOC observed in diversified plots. Similar positive effects of cover crops and diversification on TOC and nutrient availability have been reported in Mediterranean perennial systems, including olive orchards, highlighting their potential to restore degraded soils and enhance ecosystem functioning. At the same time, this increase in TOC in diversified systems may contribute directly to higher CO₂ emissions in these treatments, as there is a greater reserve of labile carbon available for microbial respiration.

These changes in soil chemical properties were also accompanied by significant changes in soil enzyme activity, like it can be seen in the PCA (Fig. S1 (b)). Previous studies conducted at the same experimental site showed that diversification treatments, particularly D-S and D-O, significantly increased the activities of BG, NAG, LA, AS and AP, as well as DH activities compared to CP, while D-L showed intermediate values (Aguilera-Huertas et al. 2023). The higher DH activity in D-S and D-O reflects an intensification of overall microbial oxidative metabolism, while the

increase in BG, NAG, LA, and AS indicates a greater capacity to depolymerize organic substrates containing carbon and nitrogen, which improves nutrient turnover and favors more active microbial communities (Huang et al. 2023; Curtright and Tiemann 2021). In this context, extracellular enzyme activities are increasingly recognised as sensitive indicators of soil quality and management-induced changes in nutrient cycling, as they integrate microbial responses to shifts in organic matter inputs and environmental conditions.

From a functional perspective, these extracellular enzymes are key mediators of SOM decomposition and nutrient cycling, as they break down complex organic compounds into simpler forms that can be assimilated by soil microorganisms, releasing CO₂ as a byproduct of microbial respiration (Gómez et al. 2020; Li et al. 2023). Therefore, the higher TOC, POC, and nutrient availability observed in diversified plots, together with increased enzyme activity, indicates that crop diversification promotes a more dynamic soil biochemical environment. This improvement in nutrient cycling and microbial functioning increases soil fertility and ecological functioning but may also contribute to increased

Fig. 4 Soil N_2O emission rates under Olive intercropped with *Crocus sativus* (D-S); Olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O) and Olive intercropped with *Lavandula* (D-L) treatments over time (2020–2021) (a) and N_2O rates among the different treatments (CP, D-S, D-O and D-L) considering the whole study period (b). The boxes represent the treatment means $\pm$ standard error (SE) ($n=3$). The different lowercase letters above the error bars in Figure (b) indicate significant differences between the different treatments during the study period based on average N_2O fluxes ($p < 0.05$)



CO_2 emissions in the short term due to the stimulation of SOM mineralization (Beillouin et al. 2021; Liu et al. 2023).

3.2 Evolution of greenhouse gas fluxes and cumulative emissions under crop diversification

The assessment of GHG emission rates, their cumulative GHG emissions, and their expression in CO_2e are fundamental to evaluating the contribution of agricultural systems to climate change, as they determine the increase or decrease in the concentration of GHGs in the atmosphere and, therefore, their effect on the greenhouse effect and global warming (IPCC 2021). In addition, they allow the impact of different gases to be compared and added together, facilitating the design and evaluation of mitigation strategies (Myhre et al. 2013).

Regarding CO_2 emission rates, an increase in emissions was observed in D-S and D-O between January and April 2021 (Fig. 3 (a)). On average, emission rates were significantly higher in D-S ($385.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and D-O ($335.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) than in CP ($238.1 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$), whereas no significant differences were detected between

D-L and CP (Fig. 3 (b)). The main factor explaining this behaviour is the presence of a high percentage of mixed cover (spontaneous+diversification), which was greater in D-S and D-O and coincided with the highest CO_2 emission rates. In contrast, the lower development of spontaneous cover in D-L contributed to the absence of significant increases in CO_2 emission rates compared with CP. The greater cover in D-S and D-O implies higher carbon inputs to the soil through the establishment and development of plant biomass (Poeplau and Don 2015), increasing the metabolic activity of the soil microflora and root respiration, which are the main sources of CO_2 in the soil-plant system (Sanz-Cobena et al. 2014). In addition, this type of mulching indirectly enhances CO_2 emissions by increasing SOM, porosity and water content (Parkin et al. 2016).

Moreover, variations in soil T and M were observed in these plots (D-S and D-O) (Fig. 2). According to Zornoza et al. (2018), increases in soil M enhance CO_2 emissions in diversified plots by directly stimulating microbial activity and decomposition processes, while Dacal et al. (2019) indicate that, in arid soils, CO_2 emissions also increase when mean soil T decreases because these conditions favour the

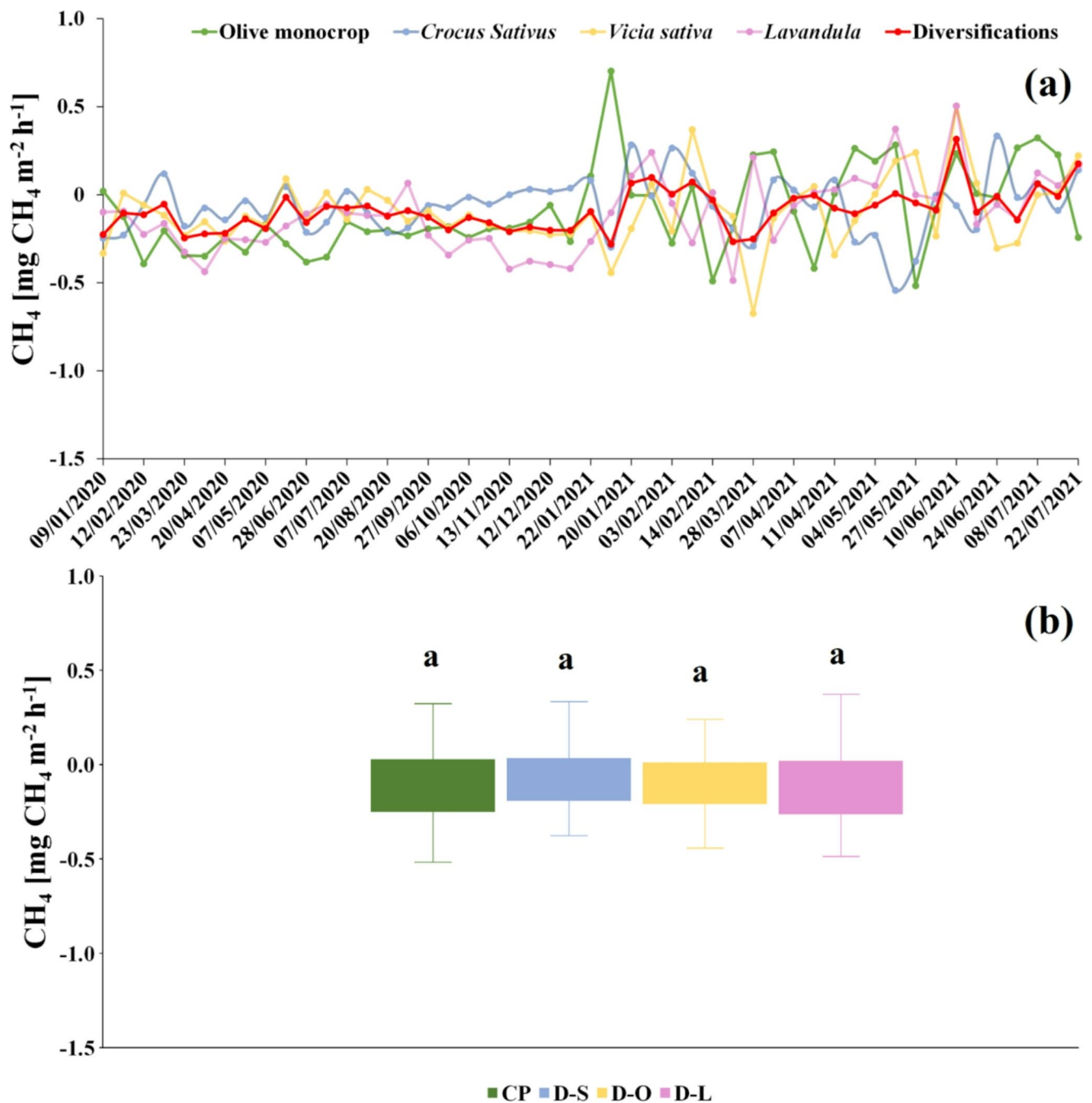


Fig. 5 Soil CH₄ emission rates under Olive monocrop (CP); Olive intercropped with *Crocus sativus* (D-S); Olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O) and Olive intercropped with *Lavandula* (D-L) treatments over time (2020–2021) (a) and CH₄ rates among the different treatments (CP, D-S, D-O and D-L)

considering the whole study period (b). The boxes represent the treatment means ± standard error (SE) (n=3). The different lowercase letters above the error bars in Figure (b) indicate significant differences between the different treatments during the study period based on average CH₄ fluxes (p < 0.05)

release of CO₂ accumulated during dry periods through the reactivation of microorganisms when M rises. In our case, between January and April, and in parallel with greater plant cover (diversification + spontaneous), the lowest T values (Fig. 2) and highest M values (Fig. 2) were recorded in D-S and D-O, coinciding with the highest CO₂ emission rates.

Importantly, interpreting N₂O and CH₄ within the same soil–microclimate framework helps explain why these gases remained negligible despite clear treatment effects on CO₂. Focusing on N₂O emission rates, fluxes followed a similar pattern in all managements throughout the study period (Fig. 4 (a)), with no significant differences between

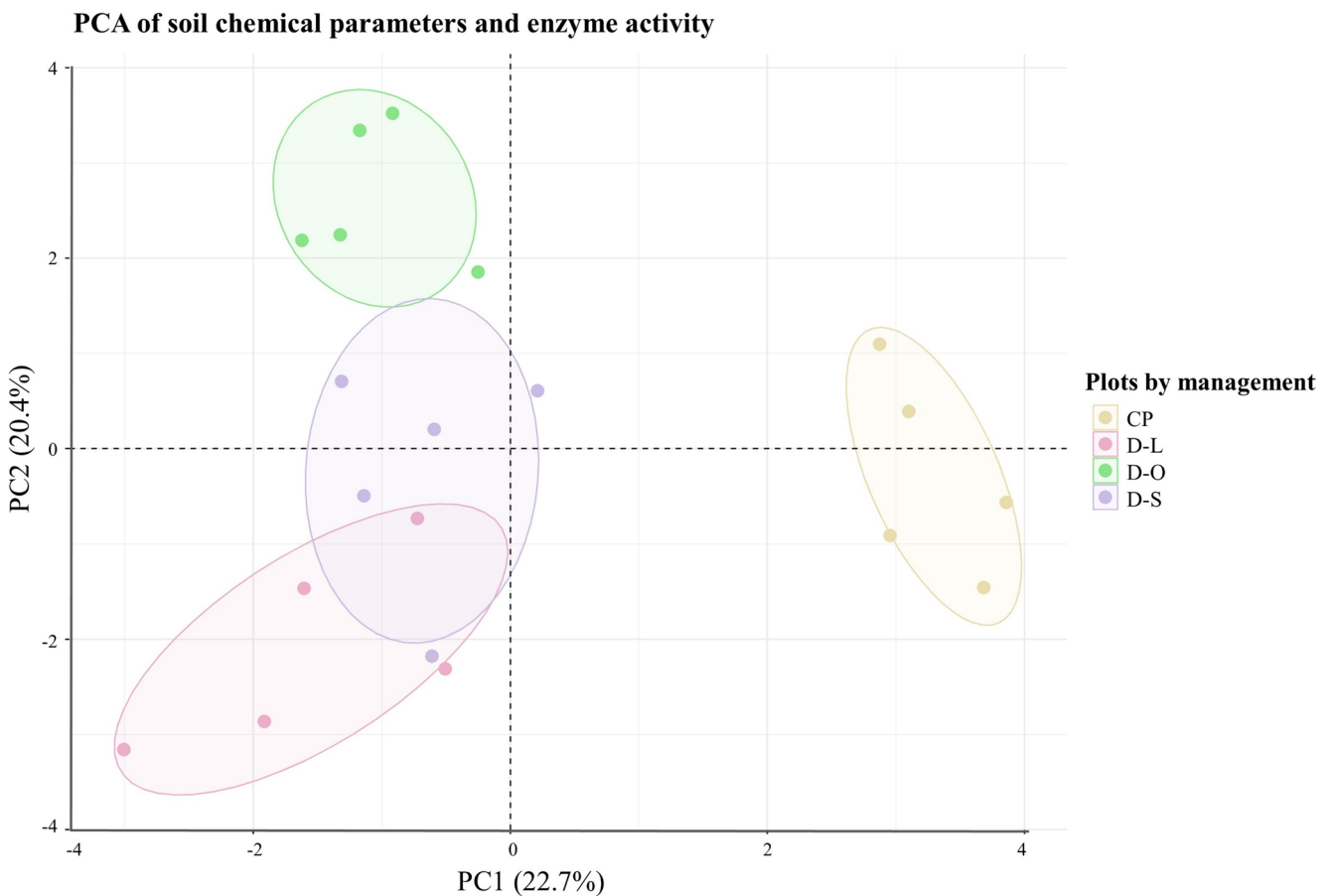


Fig. 6 Principal component analysis (PCA) of all data from Table S1, including soil chemical parameters and enzyme activities. Olive monocrop (CP) is shown in yellow, olive intercropped with *Lavandula* (D-L) in pink, olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O) in green, and olive intercropped with *Crocus sativus* (D-S) in purple. The first two principal components (PC1 and

PC2) explain the largest proportion of the total variance in the dataset, and their percentages indicate the amount of variance accounted for by each axis. Ellipses represent the dispersion of samples within each management treatment, encompassing the individuals associated with each group

treatments ($p > 0.05$) and mean emissions of $0.002 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ (CP), $0.007 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ (D-S), $0.001 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ (D-O) and $-0.002 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ (D-L) (Fig. 4 (b)). Although D-S and D-O showed higher soil M during part of the monitoring period (Fig. 2), N_2O production through nitrification and denitrification is mainly regulated by the availability of mineral N substrates (NH_4^+ and NO_3^-) and soil M/oxygen conditions that create anaerobic microsites and promote denitrification (Butterbach-Bahl et al. 2013; Krichels et al. 2022). In this study, the absence of fertilization implies low external inputs of mineral nitrogen and, together with semi-arid conditions, probably limited the formation of sustained anaerobic microsites, thus restricting denitrification and preventing higher N_2O emissions even when M increased transiently after rainfall events (Dusenbury et al. 2008; Ussiri et al. 2009). In addition, mixed covers can retain nitrogen in plant biomass and stimulate microbial immobilisation, thereby reducing the mineral nitrogen pool available for nitrification/denitrification (Sanz-Cobena et al.

2017; Wolz et al. 2018). Thus, enhanced plant uptake and microbial immobilisation under mixed covers likely buffered any potential increase in N_2O despite higher biological activity. Furthermore, the slightly negative average flux observed in D-L is consistent with evidence that net N_2O uptake can occur under some conditions in low-nitrogen soils, implying that near-zero/negative fluxes may reflect very low net exchange rather than significant treatment-driven differences (Chapuis-Lardy et al. 2007).

Regarding CH_4 emission rates also followed the same temporal pattern under all soil managements (Fig. 5 (a)), with no significant variations among treatments and mean fluxes of $-0.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ (CP), $0.1 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ (D-S), $0.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ (D-O) and $0.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ (D-L) (Fig. 5 (b)). Under upland and well-aerated conditions, agricultural soils tend to act as weak CH_4 sinks (negative fluxes) due to the predominance of methanotrophic oxidation, while net CH_4 emissions require sustained anaerobiosis to favor methanogenesis (Le Mer and Roger

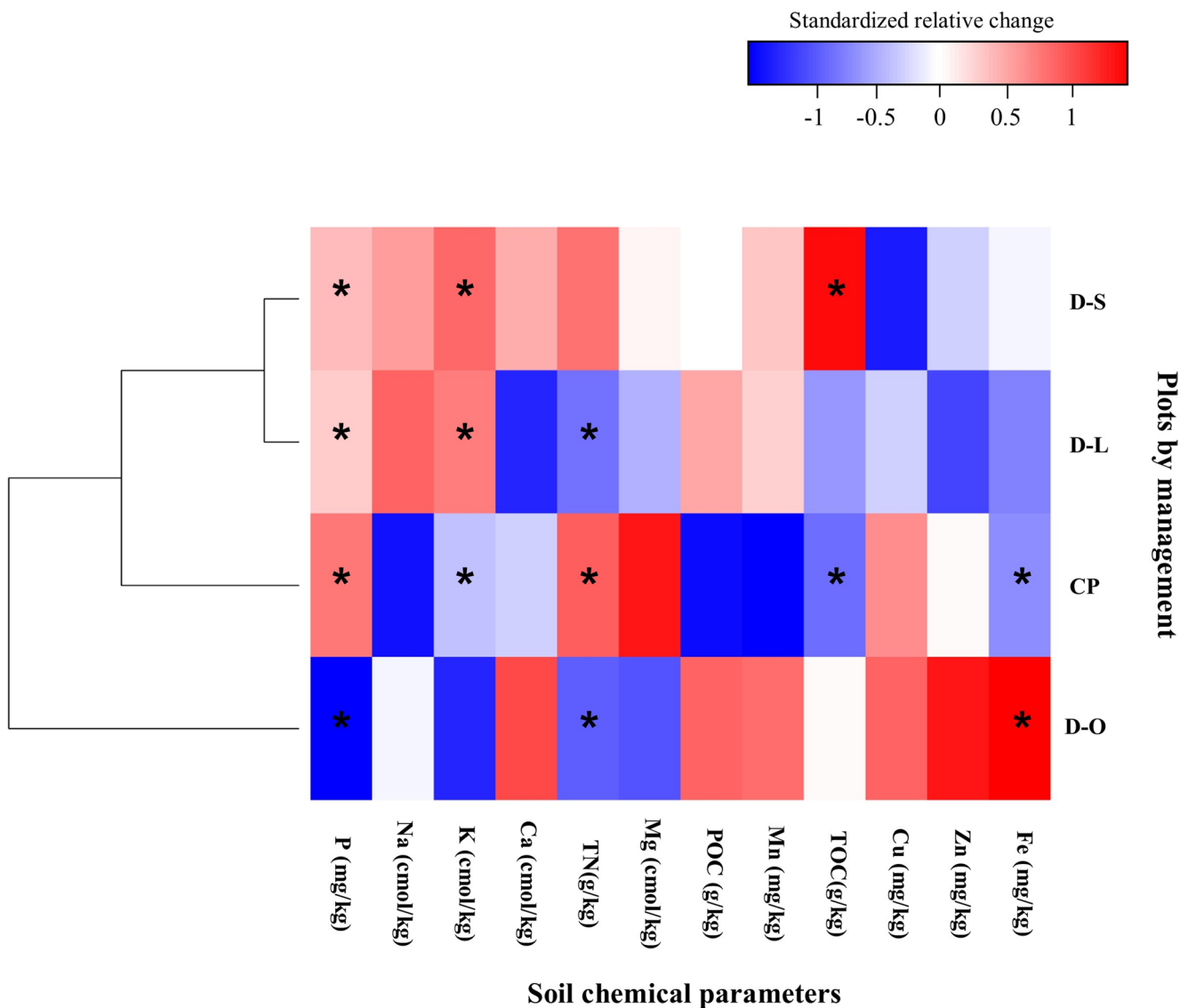


Fig. 7 Heatmap showing the relative changes of soil chemical parameters across the four management practices: olive intercropped with *Crocus sativus* (D-S), olive intercropped with *Lavandula* (D-L), olive monocrop (CP), and olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O). Values are expressed as standardized relative

changes, allowing comparison across soil variables. The x-axis represents the soil parameters and the y-axis represents the management practices. Asterisks indicate statistically significant differences among management practices based on Wilcoxon tests ($p < 0.05$)

2001; Dutaur and Verchot 2007). Therefore, the absence of clear treatment effects on CH_4 suggests that diversification did not cause prolonged oxygen limitation in the soils of our study; instead, any short-lived deviation from negative or near-zero CH_4 exchange is more plausibly related to transient M pulses affecting gas diffusivity and microbial activity (Castro et al. 1995). This interpretation is also consistent with the fact that vegetation cover can stimulate CH_4 oxidation by methanotrophic bacteria and thus keep CH_4 fluxes low under aerobic conditions (Bodelier and Laanbroek 2004).

Furthermore, these results are consistent with Abdalla et al. (2019), who reported that although cover crops tend to

reduce nitrogen leaching and increase carbon sequestration, they do not necessarily have a strong effect on N_2O and CH_4 emissions because vegetation covers can increase CH_4 oxidation by methanotrophic bacteria (Bodelier and Laanbroek 2004) and immobilise mobile nitrogen in plant biomass, using it more efficiently and reducing the concentration of mineral nitrogen available for N_2O production (Sanz-Cobena et al. 2017; Wolz et al. 2018). This mechanism could have helped retain N in the soil in our experiment while maintaining conditions favourable to CH_4 oxidation, thus explaining the low and weakly variable N_2O and CH_4 fluxes across treatments. Under these low-input conditions, the combination of low mineral nitrogen availability and

generally aerobic soils helps explain the weak N_2O and CH_4 responses (Dusenbury et al. 2008; Ussiri et al. 2009). Similar patterns have been observed in other Mediterranean perennial systems: Téllez-Río et al. (2015) reported no significant differences in N_2O and CH_4 emissions between conventional tillage and diversified crops in an olive grove, and Hüppi et al. (2022) also found no significant differences in CH_4 emissions in a vineyard with intercropping. In contrast, Grave et al. (2018) found higher N_2O emissions in untilled soils than in conventionally tilled soils, probably due to poorer aeration, highlighting the strong dependence of these gases on site-specific soil and management conditions.

In line with the patterns described for CO_2 fluxes, when emissions were integrated over time, the estimation of cumulative CO_2 emissions during the experimental period confirmed the results obtained previously, i.e., they were significantly higher in D-S and D-O, by 34% and 23%, respectively, compared with CP (Table 1), whereas CP and D-L showed no significant differences. These results can be interpreted in light of previous work carried out at the same experimental site (Aguilera-Huertas et al. 2023, 2024), where it was reported that *Crocus sativus* (D-S) loses all its shoots in June and resprouts in November, while the biomass of *Vicia sativa* and *Avena sativa* in rotation (D-O) is higher in winter due to greater water availability and lower water stress. In both cases, the diversified crops coexisted with a higher proportion of spontaneous cover, resulting in a greater annual biomass input. This high percentage of annual biomass increased soil microbiological and enzymatic activities and SOC concentrations in both plots (Aguilera-Huertas et al. 2023, 2024), which in turn led to the higher cumulative CO_2 values observed in D-S and D-O.

From a management perspective, these results contrast with the results derived from studies such as Pengthamkeerati et al. (2006) and Sánchez-Navarro et al. (2022), which suggest that reduced tillage could lead to soil compaction and lower GHG emissions in diversified plots, and that the highest cumulative emissions would be

expected under intensive conventional tillage (Lu et al. 2016; Almagro et al. 2017). In our study area, however, the opposite trend was observed, with higher cumulative CO_2 emissions in plots under minimum tillage combined with a dense alley cover, emphasising the key role of spontaneous and diversified vegetation in controlling soil GHG dynamics (de Lima et al. 2022).

Regarding the cumulative values of N_2O and CH_4 , the accumulated emissions were minimal and practically negligible, with values close to 0 g m^{-2} in this agroecosystem and no significant differences between treatments during the study period. This is probably due to the absence of fertilisation, which results in low mineral nitrogen availability and thus low cumulative emissions of these gases (Xu et al. 2004; Khalil et al. 2005). Authors such as Zornoza et al. (2018) and Sánchez-Navarro et al. (2022) also reported no significant differences in cumulative N_2O and CH_4 emissions in Mediterranean orchards. Other studies, however, have found higher N_2O emissions in minimally tilled soils than in conventionally tilled soils (Gregorich et al. 2008), attributing these increases to a higher availability of mineral nitrogen and reduced aeration under no-tillage, which favours the formation of anaerobic microsites for denitrification (Thomson et al. 2012). Nonetheless, the cumulative N_2O and CH_4 emissions obtained here are in line with previous research that monitored GHG in semi-arid Mediterranean soils (Téllez-Río et al. 2015; Fernández-Ortega et al. 2024).

With respect to CO_2e estimates derived from cumulative CO_2 , N_2O and CH_4 , the results showed the same pattern as for cumulative CO_2 , due to the negligible contribution of N_2O and CH_4 to total CO_2e . Significantly higher CO_2e values were observed in D-S and D-O compared with CP (Table 1), confirming that CO_2 is the main contributor to GHG emissions in the analysed context. The higher CO_2e in D-S and D-O highlights the importance of the specific activities of these diversified systems in contributing to climate forcing and underlines the need to focus mitigation efforts on agriculturally predominant CO_2 sources and on regions or management combinations with higher emissions in order to maximise the effectiveness of climate strategies (WRI 2023).

3.3 Relationships between GHG emissions and soil chemical and enzymatic parameters

Linear regression analysis provides insight into the relationships between soil variables and CO_2 emissions. In the study area, simple linear regression revealed significant associations between CO_2 accumulation and soil chemical parameters and enzymatic activities (Table S3). The bar plots (Fig. 8) summarize these results for each plot, highlighting variables

Table 1 Cumulative values of soil CO_2 , N_2O , CH_4 and total CO_2 equivalent (CO_2e) emissions under Olive monocrop (CP); Olive intercropped with *Crocus sativus* (D-S); Olive intercropped with *Vicia sativa* and *Avena sativa* in rotation (D-O) and Olive intercropped with *Lavandula x intermedia* (D-L) treatments over time (2020–2021). The values shown are mean $\pm$ standard error ($n=3$). Different lowercase letters indicate significant differences between the different treatments during the period considered at $p<0.05$

Treatments	Cumulative CO_2 (g m^{-2})	Cumulative N_2O (g m^{-2})	Cumulative CH_4 (g m^{-2})	CO_2e (g m^{-2})
CP	3111 $\pm$ 24a	-0.012 $\pm$ 0.097a	-1.35 $\pm$ 0.25a	3070 $\pm$ 43a
D-S	4708 $\pm$ 21b	-0.069 $\pm$ 0.025a	-1.43 $\pm$ 0.19a	4647 $\pm$ 25b
D-O	4004 $\pm$ 27c	0.085 $\pm$ 0.046a	-1.34 $\pm$ 0.24a	3991 $\pm$ 18c
D-L	3001 $\pm$ 24a	0.022 $\pm$ 0.018a	-1.68 $\pm$ 0.16a	2960 $\pm$ 15a

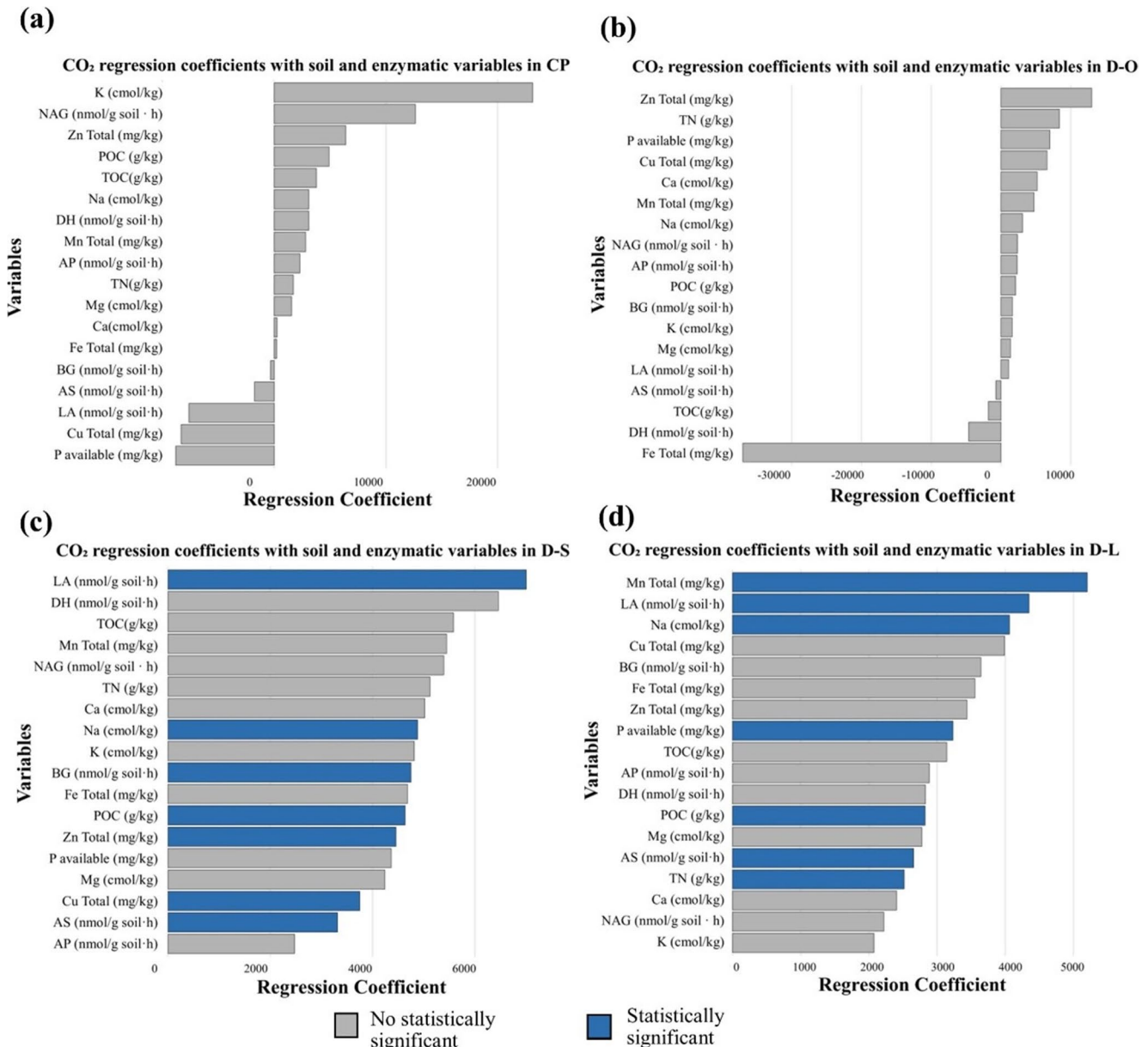


Fig. 8 Bar plots showing regression coefficients from simple linear regression models relating CO₂ concentration to individual soil chemical and enzymatic variables across the four management systems: CP (a), D-O (b), D-S (c), and D-L (d). For each soil variable, a separate model was fitted with CO₂ as the response variable (CO₂~variable).

with statistically significant regression coefficients and showing the magnitude and direction (positive or negative) of their effects on CO₂ accumulation in the different plots.

In the CP and D-O treatments, no significant relationships were found between CO₂ accumulation and the analysed soil variables based on the linear regression models (Figs. 8 (a) and 8 (b)). This suggests that, in CP, microbial activity and soil properties are not tightly coupled to the carbon cycle compared with the diversified treatments, likely because deep tillage reduces SOM inputs and disrupts soil

structure, so that the carbon entering the soil is similar to that mineralised and released as CO₂ (Hu et al. 2024). In D-O, the absence statistically of significant regression coefficients may be related to lower nutrient availability, which weakens the direct links between soil fertility, microbial processes and CO₂ emissions (Liu et al. 2022). Although D-O showed high CO₂ emissions and a substantial spontaneous vegetation cover, previous studies in the same area (Aguilera-Huertas et al. 2024) indicate that soil–plant–microbe interactions were less tightly coupled than in the

other diversified systems, and the correlations between soil chemical and enzymatic properties, while present, were not statistically significant.

By contrast, D-S and D-L were the treatments in which significant relationships were identified (Figs. 8-C and 8-D). In D-S, CO₂ accumulation was positively related to total Cu, exchangeable Na, POC, total Zn and the activities of BG, LA and AS. In D-L, statistical significant regression coefficients were found between CO₂ accumulation and exchangeable Na, total Mn, P available, POC, TN and the activities of LA and AS. Although D-L did not show the highest CO₂ emissions, its fluxes were closely linked to soil nutrient status and enzymatic activity, indicating a strong coupling between soil biochemical functioning and CO₂ dynamics under this diversification.

These patterns underline the role of specific chemical indicators. exchangeable Na, associated with soil salinity, can modify microbial community structure and SOM decomposition, thereby altering CO₂ emissions (Li et al. 2021). Mn is a key micronutrient in redox-related enzymatic reactions in the rhizosphere and participates in photosynthetic and respiratory processes; its strong correlation with CO₂ accumulation in D-L may therefore reflect enhanced microbial and root activity that favours SOM mineralisation (Mittal et al. 2023). POC, a labile fraction of organic carbon strongly associated with microbial turnover, showed positive correlations with CO₂ in both D-S and D-L, suggesting that POC acts as an active reservoir fuelling microbial respiration and CO₂ release. Similar relationships between higher POC content, increased carbon mineralisation and elevated CO₂ emissions have been reported by Hossain et al. (2017). Total Cu, an essential micronutrient for oxidative enzymes, also correlated positively with CO₂, consistent with its role in decomposition reactions and respiratory processes; Nunes et al. (2016) highlighted that Cu can affect microbial communities and SOM mineralisation, thereby modulating CO₂ emissions.

Enzymatic indicators further support this mechanistic interpretation. BG, LA and AS are involved in the degradation of carbohydrates, proteins and sulfur-containing compounds in soil. Their positive correlations with CO₂ suggest that microorganisms are actively processing SOM, with CO₂ as the end product of microbial respiration. Extracellular enzymes such as cellulases, chitinases and proteases depolymerise complex organic substrates into simpler forms that microbes can assimilate, a prerequisite for SOM mineralisation and CO₂ production (Gómez et al. 2020; Li et al. 2023). The strong associations between CO₂ accumulation and BG, LA and AS in D-S, and between LA and AS in D-L, therefore, indicate that these enzyme activities are key functional markers of enhanced SOM turnover in diversified systems.

Finally, TN and total Zn also emerged as relevant indicators in D-L and D-S, respectively. TN can stimulate microbial activity and accelerate SOM decomposition, releasing CO₂ as a metabolic by-product and intensifying carbon cycling (Hou 2021; Okolo et al. 2023). Total Zn has been linked to changes in soil respiration and the abundance of microbial genes associated with GHG production (Zhu et al. 2020), which is consistent with its positive relationship with CO₂ accumulation in D-S. Altogether, the correlations observed in D-S and D-L highlight that specific combinations of soil nutrients (Na, Mn, POC, Cu, Zn, TN) and enzyme activities (BG, LA, AS) are critical drivers of CO₂ dynamics under crop diversification, whereas in CP and D-O these linkages are weak or absent. This reinforces the idea that the capacity of diversified olive systems to modulate GHG emissions depends not only on changes in vegetation cover, but also on how management shapes soil biochemical functioning and its coupling to CO₂ fluxes.

In contrast to CO₂, regression analyses analogous to Fig. 8 are not statistically meaningful for N₂O and CH₄ in our study because fluxes were consistently near-zero, with low variance and no significant treatment effects (Figs. 4 and 5). Under these circumstances, linear models become strongly affected by the limited dynamic range (most observations clustered around zero), the measurement noise relative to signal (small absolute fluxes), and the presence of occasional negative values that reflect net uptake or near-zero net exchange rather than clear process-driven responses. Consequently, any apparent correlation between N₂O/CH₄ and soil chemical or enzymatic indicators would be unstable, highly sensitive to a few observations, and potentially misleading in mechanistic terms. Moreover, the soil indicators assessed here (nutrients and extracellular enzyme activities) are mechanistically closer to SOM depolymerisation and microbial respiration and therefore are expected to align more directly with CO₂ dynamics. In contrast, N₂O and CH₄ respond primarily to short-term shifts in mineral nitrogen substrates and aeration/redox conditions, which can vary rapidly in time and space and were not sufficiently expressed in this dataset to generate robust regression relationships; therefore, any apparent associations with N₂O/CH₄ would likely be driven by a few low-magnitude observations rather than reflecting stable, process-based relationships. Accordingly, any apparent associations with N₂O/CH₄ would likely be driven by a few low-magnitude observations rather than reflecting stable, process-based relationships. This does not imply that soil properties are irrelevant for N₂O and CH₄, but rather that the controlling factors were not sufficiently activated or variable during the monitoring period to produce a detectable statistical signal.

From a process perspective, the dominant controls on N₂O differ from those on CO₂. N₂O production is primarily

constrained by mineral nitrogen availability ($\text{NH}_4^+/\text{NO}_3^-$) and by oxygen limitation that promotes denitrification in anaerobic microsites (Butterbach-Bahl et al. 2013; Krichels et al. 2022). In our unfertilised rainfed system, low mineral nitrogen inputs likely limited substrate supply for both nitrification and denitrification, and semi-arid conditions likely prevented sustained anaerobiosis even when M increased transiently after rainfall (Dusenbury et al. 2008; Ussiri et al. 2009). Therefore, increases in TN and enzyme activities in diversified plots mainly reflect enhanced organic cycling and microbial functioning, but do not necessarily translate into higher mineral N pools at the timescales relevant for N_2O formation, particularly when mixed cover enhances plant uptake and microbial immobilisation of available nitrogen (Sanz-Cobena et al. 2017; Wolz et al. 2018). This explains why soil biochemical activation was clearly linked to CO_2 but not to N_2O .

Similarly, CH_4 exchange in upland Mediterranean soils is typically governed by the balance between methanotrophic oxidation (dominant under aerobic conditions) and methanogenesis (requiring sustained anaerobiosis) (Le Mer and Roger 2001; Dutaur and Verchot 2007). The absence of significant CH_4 responses indicates that diversification did not induce prolonged oxygen limitation, so the system remained close to neutral or a weak CH_4 sink. Under such conditions, soil structure improvements and vegetation cover may even favour CH_4 oxidation by maintaining aeration and supporting methanotrophic activity (Bodelier and Laanbroek 2004), thereby keeping CH_4 fluxes low and further limiting the variance required to detect robust statistical relationships with soil indicators. Overall, this framework clarifies why diversification strongly affected CO_2 highly responsive to labile carbon inputs and M pulses while leaving N_2O and CH_4 largely unchanged under low mineral nitrogen availability and predominantly aerobic conditions.

Accordingly, we interpret N_2O and CH_4 patterns using a process-based framework grounded in substrate availability and redox/aeration constraints, rather than regression outputs that would be statistically underpowered and potentially misleading given the near-zero fluxes and limited variance observed in this dataset.

3.4 Limitations and management implications

This study provides novel evidence that crop diversification in Mediterranean rainfed olive orchards, combined with minimum tillage, enhances soil fertility and biochemical functioning but increases CO_2 emissions in the short term, while N_2O and CH_4 remain negligible. These findings should, nevertheless, be interpreted in the context of several relevant features of experimental design. First, GHG fluxes were monitored over approximately one and a half years at a single experimental site, which is a realistic time frame for field trials but

still limits the representation of full interannual climatic variability and the diversity of Mediterranean olive systems. Second, the experiment was deliberately conducted under rainfed conditions without mineral fertilisation, in line with the low-input alternative management required by the European project and representative of many extensive olive groves; under fertilised or irrigated scenarios, GHG responses might differ and would warrant specific experiments.

Despite these limitations, the patterns observed have clear implications for the design of climate-smart management in traditional Mediterranean olive groves. Diversified systems (D-S and D-O) showed higher cumulative CO_2 emissions but also improved P available, total Fe, exchangeable K, TN, TOC and POC and stimulated key enzyme activities, moving soils towards higher fertility and more active biochemical functioning. In the medium to long term, these improvements in soil structure and carbon pools could contribute to greater carbon sequestration and resilience, potentially offsetting the initial rise in CO_2 emissions observed in the short term. At the same time, the very low cumulative N_2O and CH_4 emissions indicate that non- CO_2 gases are minor components of the GHG budget under unfertilised, rainfed semi-arid conditions, and that mitigation efforts in such systems should primarily focus on managing CO_2 fluxes associated with vegetation cover and soil biological activity.

Overall, our results suggest that the climate performance of diversified olive systems cannot be assessed solely on the basis of short-term CO_2 fluxes. Long-term monitoring of GHG balances and soil carbon stocks is needed to determine whether diversified alley crops ultimately lead to net emission reductions and sustained carbon sequestration. In this context, identifying and prioritising management combinations that simultaneously enhance soil health and limit excessive increases in CO_2 emissions will be crucial to maximise the effectiveness of mitigation strategies in Mediterranean perennial agroecosystems, in line with current recommendations to focus actions on major agricultural CO_2 sources and the most emission-intensive regions and practices (WRI 2023). This need for long-term monitoring of diversified systems is consistent with recent studies in Mediterranean orchards that emphasise the importance of multi-year evaluation of cover crops and diversification effects on soil functioning and GHG balances (Sánchez-Navarro et al. 2023).

4 Conclusions

Crop diversification with minimum tillage in Mediterranean olive groves increased CO_2 emissions in the short term. Average CO_2 fluxes were higher in saffron (D-S: $385.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) and in the oat-vetch rotation (D-O: $335.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) compared to the conventional

tillage control (CP: 238.1 mg CO₂ m⁻² h⁻¹), while D-L did not differ from CP. Consequently, cumulative CO₂ emissions were 34% (D-S) and 23% (D-O) higher than those of CP, suggesting increased root respiration and microbial decomposition under a denser alley cover. In contrast, N₂O and CH₄ remained close to zero with no treatment effects ($p > 0.05$), indicating that CO₂ dominated the GHG balance under unfertilized semi-arid conditions. Diversification also improved soil functioning: D-O increased P available, Fe, and TN, while D-S and D-L increased K, TOC, POC, and enzyme activities (BG, NAG, LA, AS, AP, and DH). CO₂ accumulation was positively correlated with key chemical and enzymatic indicators such as POC, TN, Zn, Mn, and BG, LA, AS, linking nutrient status and microbial activity to CO₂ dynamics. Overall, crop diversification improved soil fertility without increasing non-CO₂ GHGs, but it did intensify CO₂ emissions, justifying long-term assessments of net GHG balance and soil carbon stabilization.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11368-026-04259-z>.

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Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

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References

Abdalla M, Hastings A, Cheng K, Yue Q, Chadwick D, Espenberg M, Truu J, Rees RM, Smith P (2019) A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Glob Change Biol* 25:2530–2543. <https://doi.org/10.1111/gcb.14644>

- Abdeldaym EA, El-Sawy MBI, El-Helaly MA (2019) Combined application of different sources of nitrogen fertilizers for improvement of potato yield and quality. *Plant Arch* 19:09725210
- AEMET (Agencia Estatal de Meteorología) (2020) Standard climate values – period 1983–2010. Available at: <http://www.aemet.es/en/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=5270B&k=and>. Last accessed 24 November 2024.
- Aguilera-Huertas J, Parras-Alcántara L, González-Rosado M, Lozano-García B (2024) Intercropping in rainfed Mediterranean olive groves contributes to improving soil quality and soil organic carbon storage. *Agric Ecosyst Environ* 361:108826. <https://doi.org/10.1016/j.agee.2023.108826>
- Aguilera-Huertas J, Cuartero J, Ros M, Pascual JA, Parras-Alcántara L, González-Rosado M, Lozano-García B (2023) How binomial (traditional rainfed olive grove–*Crocus sativus*) crops impact the soil bacterial community and enhance microbial capacities. *J Environ Manage* 345:118572. <https://doi.org/10.1016/j.jenvman.2023.118572>
- Aguilera-Huertas J, Parras-Alcántara L, González-Rosado M, Lozano-García B (2022) Medium-term evaluation of the 4‰ initiative, soil organic carbon storage and stabilisation in a Mediterranean rainfed olive grove under conventional tillage: a case study. *Environ Res* 214:114382. <https://doi.org/10.1016/j.envres.2022.114382>
- Almagro M, Diaz-Pereira E, Boix-Fayos C, Zornoza R, Sánchez-Navarro V, Martínez-Mena M (2023) The combination of crop diversification and no tillage enhances key soil quality parameters related to soil functioning without compromising crop yields in a low-input rainfed almond orchard under semiarid Mediterranean conditions. *Agric Ecosyst Environ* 345:108320. <https://doi.org/10.1016/j.agee.2022.108320>
- Almagro M, García-Franco N, Martínez-Mena M (2017) The potential of reducing tillage frequency and incorporating plant residues as a strategy for climate change mitigation in semiarid Mediterranean agroecosystems. *Agric Ecosyst Environ* 246:210–220. <https://doi.org/10.1016/j.agee.2017.05.016>
- Álvaro-Fuentes J, Lóczy D, Thiele-Bruhn S, Zornoza R (2019) Handbook of plant and soil analysis for agricultural systems. CITA UPTC Editions, Cartagena, Spain, 389 pp.
- Bai X, Huang Y, Ren W, Coyne M, Jacinthe PA, Tao B, Hui D, Yang J, Matocha C (2019) Responses of soil carbon sequestration to climate-smart agriculture practices: a meta-analysis. *Glob Change Biol* 25:2591–2606. <https://doi.org/10.1111/gcb.14658>
- Beillouin D, Ben-Ari T, Malézieux E, Seufert V, Makowski D (2021) Positive but variable effects of crop diversification on biodiversity and ecosystem services. *bioRxiv*. <https://doi.org/10.1101/2020.09.30.320309>
- Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol Ecol* 47:265–277. [https://doi.org/10.1016/S0168-6496\(03\)00304-0](https://doi.org/10.1016/S0168-6496(03)00304-0)
- Bourlion N, Ferrer R (2018) The Mediterranean region's development and trends: framework aspects. In: FAO and Plan Bleu (eds) State of Mediterranean forests 2018. Food and Agriculture Organization of the United Nations, Rome, and Plan Bleu, Marseille, pp 2–15
- Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc Lond B Biol Sci* 368:20130122. <https://doi.org/10.1098/rstb.2013.0122>
- Cambardella CA, Elliott ET (1992) Particulate soil organic matter changes across a grassland cultivation sequence. *Soil Sci Soc Am J* 56:777–783. <https://doi.org/10.2136/sssaj1992.0361599500560030017x>
- Castro MS, Steudler PA, Melillo JM, Aber JD, Bowden RD (1995) Factors controlling atmospheric methane consumption by

- temperate forest soils. *Glob Biogeochem Cycles* 9:1–10. <https://doi.org/10.1029/94GB02651>
- Cerdà A, Lavee H, Romero-Díaz A, Hooke J, Montanarella L (2010) Soil erosion and degradation on Mediterranean type ecosystems. *Land Degrad Dev* 21:71–74. <https://doi.org/10.1002/ldr.968>
- Chabbi A, Lehmann J, Ciais P, Loescher HW, Cotrufo MF, Don A, SanClements M, Schipper L, Six J, Smith P, Rumpel C (2017) Aligning agriculture and climate policy. *Nat Clim Change* 7:307–309. <https://doi.org/10.1038/nclimate3286>
- Chapuis-Lardy L, Wrage N, Metay A, Chotte JL, Bernoux M (2007) Soils, a sink for N₂O? A review. *Glob Change Biol* 13:1–17. <http://doi.org/10.1111/j.1365-2486.2006.01280.x>
- Cong WF, van Ruijven J, van der Werf W (2014) Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *J Ecol* 103:1165–1174. <https://doi.org/10.1111/1365-2745.12280>
- Cuartero J, Pascual JA, Vivo JM, Özbolat O, Sánchez-Navarro V, Egea-Cortines M, Ros M et al (2022) A first-year melon/cowpea intercropping system improves soil nutrients and changes the soil microbial community. *Agric Ecosyst Environ* 328:107856. <https://doi.org/10.1016/j.agee.2022.107856>
- Curtright AJ, Tiemann LK (2021) Intercropping increases soil extracellular enzyme activity: a meta-analysis. *Agric Ecosyst Environ* 319:107489. <https://doi.org/10.1016/j.agee.2021.107489>
- R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- D'Acunzio L, Andrade JF, Poggio SL, Semmartin M (2018) Diversifying crop rotation increased metabolic soil diversity and activity of the microbial community. *Agric Ecosyst Environ* 257:159–164. <https://doi.org/10.1016/j.agee.2018.02.011>
- Dacal M, Bradford MA, Plaza C, Maestre FT, García-Palacios P (2019) Soil microbial respiration adapts to ambient temperature in global drylands. *Nat Ecol Evol* 3:232–238. <https://doi.org/10.1038/s41559-018-0770-5>
- Dahmani R, Borsali AH, Merzouk A, Zouidi M, Da Silva AMF (2023) Dynamics of chemical and microbial properties of Algerian forest soils: influence of natural and anthropogenic factors (northwest Tlemcen). *Forest Stud* 78:41–56. <https://doi.org/10.2478/fsmu-2023-0004>
- de Lima RP, Rolim MM, Toledo MPS, Tormena CA, da Silva AR, e Silva IAC, Pedrosa EMR (2022) Texture and degree of compactness effect on the pore size distribution in weathered tropical soils. *Soil Tillage Res* 215:105215. <https://doi.org/10.1016/j.still.2021.105215>
- Díaz-Pereira E, Martínez-Mena M, de Vente J, Almagro Bonmatí M, Boix-Fayos C (2019) Total carbon (organic and inorganic carbon) and nitrogen. In: Álvaro-Fuentes J, Lóczy D, Thiele-Bruhn S, Zornoza R (eds) *Handbook of plant and soil analysis for agricultural systems*. Crai UPTC Editions, Cartagena, Spain, pp 157–160
- Dusenbury MP, Engel RE, Miller PR, Lemke RL, Wallander R (2008) Nitrous oxide emissions from a northern Great Plains soil as influenced by nitrogen management and cropping systems. *J Environ Qual* 37:542–550. <https://doi.org/10.2134/jeq2006.0395>
- Dutaur L, Verchot LV (2007) A global inventory of the soil CH₄ sink. *Glob Biogeochem Cycles* 21(4):GB4013. <https://doi.org/10.1029/2006GB002734>
- EC (2020b) Caring for soil is caring for life. European Commission, Brussels. <https://doi.org/10.2777/918775>
- Eurostat (2019) The fruit and vegetable sector in the EU – a statistical overview. Available at: https://ec.europa.eu/eurostat/statisticsexplained/index.php/The_fruit_and_vegetable_sector_in_the_EU__a_statistical_overview. Accessed 3 February 2023
- FAO (2017) The future of food and agriculture: trends and challenges. Food and Agriculture Organization of the United Nations, Rome
- Fernández-Ortega J, Álvaro-Fuentes J, Cantero-Martínez C (2024) Double-cropping, tillage and nitrogen fertilization effects on soil CO₂ and CH₄ emissions. *Agric Ecosyst Environ* 359:108758. <https://doi.org/10.1016/j.agee.2023.108758>
- Ferreira CS, Seifollahi-Aghmiuni S, Destouni G, Ghajarnia N, Kalantari Z (2022) Soil degradation in the European Mediterranean region: processes, status and consequences. *Sci Total Environ* 805:150106. <https://doi.org/10.1016/j.scitotenv.2021.150106>
- García Azcárate T (2017) La PAC post 2020. ¿Qué PAC necesita el sector? Available at: <http://hdl.handle.net/10261/270650>
- Gómez EJ, Delgado JA, González JM (2020) Persistence of microbial extracellular enzymes in soils under different temperatures and water availabilities. *Ecol Evol* 10:10167–10176. <https://doi.org/10.1002/ece3.6677>
- González-Rosado M, Parras-Alcántara L, Aguilera-Huertas J, Lozano-García B (2022) Crop diversification effects on soil aggregation and aggregate-associated carbon and nitrogen in short-term rain-fed olive groves under semiarid Mediterranean conditions. *Horticulturae* 8:618. <https://doi.org/10.3390/horticulturae8070618>
- González-Rosado M, Parras-Alcántara L, Aguilera-Huertas J, Lozano-García B (2021) Long-term evaluation of the initiative 4% under different soil managements in Mediterranean olive groves. *Sci Total Environ* 793:143591. <https://doi.org/10.1016/j.scitotenv.2020.143591>
- Grave RA, da Silveira Nicoloso R, Cassol PC, da Silva MLB, Mezzari MP, Aita C, Wuaden CR (2018) Determining the effects of tillage and nitrogen sources on soil N₂O emission. *Soil Tillage Res* 175:1–12. <https://doi.org/10.1016/j.still.2017.08.011>
- Gregorich EG, Rochette P, St-Georges P, McKim UF, Chan C (2008) Tillage effects on N₂O emission from soils under corn and soybeans in eastern Canada. *Can J Soil Sci* 88:153–161. <https://doi.org/10.4141/CJSS06041>
- Guan X, Jiang J, Jing X, Feng W, Luo Z, Wang Y, Luo Y (2022) Optimizing duration of incubation experiments for understanding soil carbon decomposition. *Geoderma* 428:116225. <https://doi.org/10.1016/j.geoderma.2022.116225>
- Hossain MB, Rahman MM, Biswas JC (2017) Carbon mineralization and carbon dioxide emission from organic matter added soil under different temperature regimes. *Int J Recycl Org Waste Agricult* 6:311–319. <https://doi.org/10.1007/s40093-017-0179-1>
- Hou D (2021) Sustainable soil management and climate change mitigation. *Soil Use Manage* 37:2
- Hu M, Zhou S, Xiong X, Wang X, Sun Y, Meng Z, Deng Q (2024) Dynamics of soil microbial communities involved in carbon cycling along three successional forests in southern China. *Front Microbiol* 14:1326057. <https://doi.org/10.3389/fmicb.2023.1326057>
- Huang W, Kuzyakov Y, Niu S, Luo Y, Sun B, Zhang J, Liang Y (2023) Drivers of microbially and plant-derived carbon in topsoil and subsoil. *Glob Change Biol* 29(22):6188–6200. <https://doi.org/10.1111/gcb.16951>
- Hüppi R, Horváth L, Dezső J, Puhl-Rezsek M, Six J (2022) Soil nitrous oxide emission and methane exchange from diversified cropping systems in Pannonian region. *Front Environ Sci* 10:857625. <https://doi.org/10.3389/fenvs.2022.857625>
- IPCC (2019) Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. IPCC, Geneva
- IPCC (2021) AR6 Climate Change 2021: The physical science basis. Cambridge University Press, Cambridge
- Janzen HH, van Groenigen KJ, Powlson DS, Schwinghamer T, van Groenigen JW (2022) Photosynthetic limits on carbon sequestration in croplands. *Geoderma* 424:115810. <https://doi.org/10.1016/j.geoderma.2022.115810>

- Jiang M, Yang N, Zhao J, Shaaban M, Hu R (2021) Incorporation of crop straw mediates the impacts of soil aggregate size on greenhouse gas emissions. *Geoderma* 401:115342. <https://doi.org/10.1016/j.geoderma.2021.115342>
- Kassambara A, Mundt F (2016) factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Kay S, Rega C, Moreno G, den Herder M, Palma JHN, Borek R, Crous-Duran J, Freese D, Giannitsopoulos M, Graves A (2019) Agroforestry creates carbon sinks whilst enhancing the environment in agricultural landscapes in Europe. *Land Use Policy* 83:581–593. <https://doi.org/10.1016/j.landusepol.2019.02.025>
- Khalil MI, Baggs EM (2005) Oxidation of CH₄ and emissions of N₂O at various soil moisture contents and headspace CH₄ concentrations in soil. *Soil Biol Biochem* 37:1785–1794. <https://doi.org/10.1016/j.soilbio.2005.02.012>
- Krichels AH, Homyak PM, Aronson EL, Sickman JO, Botthoff J, Shulman H, Piper S, Andrews HM, Jenerette GD (2022) Rapid nitrate reduction produces pulsed NO and N₂O emissions following wetting of dryland soils. *Biogeochemistry* 158:233–250. <https://doi.org/10.1007/s10533-022-00896-x>
- Lahmar R, Ruellan A (2007) Soil degradation in the Mediterranean region and cooperative strategies. *Cah Agric* 16:318–323. <https://doi.org/10.1684/agr.2007.0119>
- Lal R (2015) Restoring soil quality to mitigate soil degradation. *Sustainability* 7:5875–5895. <https://doi.org/10.3390/su7055875>
- Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. *Eur J Soil Biol* 37:25–50. [https://doi.org/10.1016/S1164-5563\(01\)01067-6](https://doi.org/10.1016/S1164-5563(01)01067-6)
- Li X, Wang A, Wan W, Luo X, Zheng L, He G, Huang Q (2021) High salinity inhibits soil bacterial community mediating nitrogen cycling. *Appl Environ Microbiol* 87:e01366–21. <https://doi.org/10.1128/AEM.01366-21>
- Li J, Niu X, Wang P, Yang J, Liu J, Wu D, Guan P (2023) Soil degradation regulates the effects of litter decomposition on soil microbial nutrient limitation: evidence from soil enzymatic activity and stoichiometry. *Front Plant Sci* 13:1090954. <https://doi.org/10.3389/fpls.2022.1090954>
- Liu B, Schroeder J, Ahnemann H (2023) Crop diversification improves the diversity and network structure of the prokaryotic soil microbiome at conventional nitrogen fertilization. *Plant Soil* 489:259–276. <https://doi.org/10.1007/s11104-023-06011-9>
- Liu F, Yang J, Zhang Y, Yang S, Zhang Y, Chen Y, Zhang Y (2024) Mulches aid the recovery of degraded soil by stimulating biogeochemical cycling: metagenomic analysis. *Appl Microbiol Biotechnol* 108:20. <https://doi.org/10.1007/s00253-023-12824-6>
- Liu Z, Shi Z, Wei H, Zhang J (2022) Acid rain reduces soil CO₂ emission and promotes soil organic carbon accumulation in association with decreasing the biomass and biological activity of ecosystems: a meta-analysis. *Catena* 208:105714. <https://doi.org/10.1016/j.catena.2021.105714>
- Lu X, Lu X, Tanveer SK, Wen X, Liao Y (2016) Effects of tillage management on soil CO₂ emission and wheat yield under rain-fed conditions. *Soil Res* 54:38–48. <https://doi.org/10.1071/SR14300>
- Ma Q, Xu M, Liu M, Cao X, Hill PW, Chadwick DR, Jones DL (2022) Organic and inorganic sulfur and nitrogen uptake by co-existing grassland plant species competing with soil microorganisms. *Soil Biol Biochem* 168:108627. <https://doi.org/10.1016/j.soilbio.2022.108627>
- Marcos-Pérez M, Sánchez-Navarro V, Zornoza R (2023) Greenhouse gas emissions from soil in intercropping systems between melon and cowpea. *Rev Esp Edafol* 13:11368. <https://doi.org/10.3389/sjss.2023.11368>
- Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol Biochem* 33:1633–1640. [https://doi.org/10.1016/S0038-0717\(01\)00079-7](https://doi.org/10.1016/S0038-0717(01)00079-7)
- Mehlich A (1984) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Commun Soil Sci Plant Anal* 15(12):1409–1416. <https://doi.org/10.1080/00103628409367568>
- Mittal S, Saini SP, Meena HM, Singh P (2023) Soil manganese dynamics and performance of wheat cultivars in relation to soil and foliar manganese application with and without manures in north-western India. *J Soil Sci Plant Nutr* 23:1967–1980. <https://doi.org/10.1007/s42729-023-01151-4>
- Morugán-Coronado A, Linares C, Gómez-López MD, Faz Á, Zornoza R (2020) The impact of intercropping, tillage and fertiliser type on soil and crop yield in fruit orchards under Mediterranean conditions: a meta-analysis of field studies. *Agric Syst* 178:102736. <https://doi.org/10.1016/j.agry.2019.102736>
- Myhre G (2013) Anthropogenic and natural radiative forcing. *Climate change 2013: the physical science basis*. Cambridge University Press, Cambridge
- Nunes I, Jacquiod S, Brejnrod A, Holm PE, Johansen A, Brandt KK, Priemé A, Sørensen SJ (2016) Coping with copper: inherited effect of copper on the potential activity of soil bacteria after a century of exposure. *FEMS Microbiol Ecol* 92:fiw175. <https://doi.org/10.1093/femsec/fiw175>
- Ogle DH, Doll JC, Wheeler AP, Dinno A (2025) FSA: simple fisheries stock assessment methods. R package version 0.10.0. <https://CRAN.R-project.org/package=FSA>
- Okolo CC, Gebresamuel G, Zenebe A, Haile M, Orji JE, Okebalama CB, Eze PN (2023) Soil organic carbon, total nitrogen stocks and CO₂ emissions in top- and subsoils with contrasting management regimes in semi-arid environments. *Sci Rep* 13:1117. <https://doi.org/10.1038/s41598-023-28276-x>
- Panagos P, Ballabio C, Poesen J, Lugato E, Scarpa L, Montanarella L, Borrelli P (2020) A soil erosion indicator for supporting agricultural, environmental and climate policies in the European Union. *Remote Sens* 12:1365. <https://doi.org/10.3390/rs12091365>
- Paramesh V, Mohan Kumar R, Rajanna GA, Gowda S, Nath AJ, Madival Y, Toraskar S (2023) Integrated nutrient management for improving crop yields, soil properties, and reducing greenhouse gas emissions. *Front Sustain Food Syst* 7:1173258. <https://doi.org/10.3389/fsufs.2023.1173258>
- Parkin TB, Kaspar TC, Jaynes DB, Moorman TB (2016) Rye cover crop effects on direct and indirect nitrous oxide emissions. *Soil Sci Soc Am J*. <https://doi.org/10.2136/sssaj2016.04.0120>
- Peng H, Nijp JJ, Ratcliffe JL, Li C, Hong B, Lidberg W, Nilsson MB (2024) Climatic controls on the dynamic lateral expansion of northern peatlands and its potential implication for the ‘anomalous’ atmospheric CH₄ rise since the mid-Holocene. *Sci Total Environ* 908:168450. <https://doi.org/10.1016/j.scitotenv.2023.168450>
- Pengthamkeerati P, Motavalli PP, Kremer RJ, Anderson SH (2006) Soil compaction and poultry litter effects on factors affecting nitrogen availability in a claypan soil. *Soil Tillage Res* 91:109–119. <https://doi.org/10.1016/j.still.2005.11.008>
- Poeplau C, Don A (2015) Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. *Agric Ecosyst Environ* 200:33–41. <https://doi.org/10.1016/j.agee.2014.10.024>
- Ramzan S, Rasool T, Bhat RA, Ahmad P, Ashraf I, Rashid N, Mir IA (2020) Agricultural soils are a nitrous oxide trigger: a persuasive greenhouse gas and its management. *Environ Monit Assess* 192:84–10. <https://doi.org/10.1007/s10661-020-08410-2>
- Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. *Plants* 8:34. <https://doi.org/10.3390/plants8020034>
- Raza T, Qadir MF, Khan KS, Eash NS, Yousuf M, Chatterjee S, Oetting JN (2023) Unlocking the potential of microbes in organic matter decomposition and ecosystem carbon release. *J Environ Manag* 344:118529. <https://doi.org/10.1016/j.jenvman.2023.118529>

- Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability: update on microbial phosphorus. *Plant Physiol* 156:989–996. <https://doi.org/10.1104/pp.111.175448>
- Rocci KS, Lavalley JM, Stewart CE, Cotrufo MF (2021) The response of soil organic carbon to global environmental change depends on its distribution between mineral-associated and particulate organic matter: a meta-analysis. *Sci Total Environ* 793:148569. <https://doi.org/10.1016/j.scitotenv.2021.148569>
- Sánchez-Navarro V, Martínez-Martínez S, Acosta JA, Almagro M, Martínez-Mena M, Boix-Fayos C, Díaz-Pereira E, Temnani A, Berrios P, Pérez-Pastor A, Zornoza R (2023) Soil greenhouse gas emissions and crop production with implementation of alley cropping in a Mediterranean citrus orchard. *Eur J Agron* 142:126684. <https://doi.org/10.1016/j.eja.2022.126684>
- Sánchez-Navarro V, Shahrokh V, Martínez-Martínez S, Acosta JA, Almagro M, Martínez-Mena M, Boix-Fayos C, Díaz-Pereira E, Zornoza R (2022) Perennial alley cropping contributes to decrease soil CO₂ and N₂O emissions and increase soil carbon sequestration in a Mediterranean almond orchard. *Sci Total Environ* 845:157225. <https://doi.org/10.1016/j.scitotenv.2022.157225>
- Sanz-Cobena A, García-Marco S, Quemada M, Gabriel JL, Almendros P, Vallejo A (2014) Do cover crops enhance N₂O, CO₂ or CH₄ emissions from soil in Mediterranean arable systems? *Sci Total Environ* 466:164–174. <https://doi.org/10.1016/j.scitotenv.2013.07.023>
- Sanz-Cobena A, Lassaletta L, Aguilera E, del Prado A, Garnier J, Billen G, Smith P (2017) Strategies for greenhouse gas emissions mitigation in Mediterranean agriculture: a review. *Agric Ecosyst Environ* 238:5–24. <https://doi.org/10.1016/j.agee.2016.09.038>
- Tellez-Rio A, García-Marco S, Navas M, López-Solanilla E, Tenorio JL, Vallejo A (2015) N₂O and CH₄ emissions from a fallow–wheat rotation with low N input in conservation and conventional tillage under a Mediterranean agroecosystem. *Sci Total Environ* 508:85–94. <https://doi.org/10.1016/j.scitotenv.2014.11.041>
- Thomson AJ, Giannopoulos G, Pretty J, Baggs EM, Richardson DJ (2012) Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philos Trans R Soc Lond B Biol Sci* 367:1157–1168. <https://doi.org/10.1098/rstb.2011.0415>
- Tripolskaja L, Kazlauskaitė-Jadzevice A, Razukas A (2023) Organic carbon, nitrogen accumulation and nitrogen leaching as affected by legume crop residues on sandy loam in the eastern Baltic region. *Plants* 12:2478. <https://doi.org/10.3390/plants12132478>
- Ussiri D, Lal R, Jarecki K (2009) Nitrous oxide and methane emissions from long-term tillage under a continuous corn cropping system in Ohio. *Soil Tillage Res* 104:247–255. <https://doi.org/10.1016/j.still.2009.03.001>
- Velasco-Sánchez Á, Bennegadi-Laurent N, Trinsoutrot-Gattin I, van Groenigen JW, Moinet GYK (2024) Soil microorganisms increase Olsen phosphorus from poorly soluble organic phosphate: a soil incubation study. *Soil Use Manage* 40:e12960. <https://doi.org/10.1111/sum.12960>
- Volsi B, Higashi GE, Bordin I (2022) The diversification of species in crop rotation increases the profitability of grain production systems. *Sci Rep* 12:19849. <https://doi.org/10.1038/s41598-022-23718-4>
- von Mersi W, Schinner F (1991) An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol Fertil Soils* 11:216–220. <https://doi.org/10.1007/BF00335770>
- Warnes GR, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B, Galili T (2005) gplots: various R programming tools for plotting data. R package version 3.1.
- Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer, New York
- Wolz KJ, Branham BE, DeLucia EH (2018) Reduced nitrogen losses after conversion of row crop agriculture to alley cropping with mixed fruit and nut trees. *Agric Ecosyst Environ* 258:172–181. <https://doi.org/10.1016/j.agee.2018.02.024>
- WRI (World Resources Institute) (2023) Climate Watch: Historical GHG emissions. Available at: <https://www.wri.org>
- Xu YC, Shen QR, Li ML, Dittert K, Sattelmacher B (2004) Effect of soil water status and mulching on N₂O and CH₄ emission from lowland rice field in China. *Biol Fertil Soils* 39:215–217. <https://doi.org/10.1007/s00374-003-0692-4>
- Yu N, Liu J, Ren B, Zhao B, Liu P, Gao Z, Zhang J (2022) Long-term integrated soil-crop management improves soil microbial community structure to reduce GHG emission and increase yield. *Front Microbiol* 13:1024686. <https://doi.org/10.3389/fmicb.2022.1024686>
- Zhu X, Jackson RD, DeLucia EH, Tiedje JM, Liang C (2020) The soil microbial carbon pump: from conceptual insights to empirical assessments. *Glob Change Biol* 26:6032–6039. <https://doi.org/10.1111/gcb.15319>
- Zikeli S, Gruber S (2017) Reduced tillage and no-till in organic farming systems, Germany – status quo, potentials and challenges. *Agriculture* 7:35. <https://doi.org/10.3390/agriculture7040035>
- Zornoza R, Acosta JA, Gabarrón M, Gómez-Garrido M, Sánchez-Navarro V, Terrero A, Martínez-Martínez S, Faz Á, Pérez-Pastor A (2018) Greenhouse gas emissions and soil organic matter dynamics in woody crop orchards with different irrigation regimes. *Sci Total Environ* 644:1429–1438. <https://doi.org/10.1016/j.scitotenv.2018.06.398>
- Zou Y, Liu Z, Chen Y, Wang Y, Feng S (2024) Crop rotation and diversification in China: enhancing sustainable agriculture and resilience. *Agriculture* 14:1465. <https://doi.org/10.3390/agriculture14091465>
- Zuo Y, Zhang F (2011) Soil and crop management strategies to prevent iron deficiency in crops. *Plant Soil* 339:83–95. <https://doi.org/10.1007/s11104-010-0566-0>

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