Journal of Environmental Management 380 (2025) 125093

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Influence of the quality of organic amendments on Mediterranean agricultural soils following a drought episode

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A R T I C L E I N F O

Keywords:
Cover crop
Soil organic C
Compost
Soil DNA
qPCR
Climate change

A B S T R A C T

Soil organic matter loss is a major concern in agricultural Mediterranean soils due to its influence on soil water retention and hence increased susceptibility to drought conditions. An experiment was conducted to study the influence of organic inputs' quality on the resistance of a C-poor soil to drought. Four organic materials were added to the soil in a mesocosm experiment: 1) leonardite; 2) earthworm humus; 3) biosolid compost; and 4) dry biomass of *Vicia faba*, simulating the incorporation of a legume cover crop. Pots were sown with a forage mixture (*Lolium perenne* and *Medicago polymorpha*), and 45 days following C-addition a drought episode (30 % reduction in water inputs) was simulated in half of the replicates. Soil resistance was evaluated by measuring soil chemical properties, biological activity (enzyme activities and respiration rate), biomass and the fungi:bacteria ratio in the microbial community, and plant productivity. With the exception of earthworm humus, organic-C addition had positive effects on soil chemical properties and water retention. *V. faba* biomass was the most effective material for enhancing water retention (16.5 % higher than in the non-amended soil), microbial activity and abundance (2.5 and 8.4 times greater bacterial and fungal abundances than in the non-amended soil, respectively), and soil N and P availability. Soils enriched with *V. faba* biomass also had the highest fungi:bacteria ratio by the end of the experiment. Drought reduced plant productivity, but all soil function indices showed a high resistance to drought, regardless of the organic matter quality or the fungi:bacteria ratio.

1. Introduction

Climate change effects on soil functioning are a major concern in the Mediterranean basin. The region has been identified as one of the most vulnerable to climate change (Ali et al., 2022). Although climate change projections regarding rainfall reductions show a great variability among regions and emission scenarios (between 4 % and 22 %), drought events in the Mediterranean region are expected to become more frequent and intense (Ali et al., 2022). In this context, preservation of soil organic matter is a fundamental issue, given the importance of this soil component for water storage and balance (Wander, 2004; Lal, 2015). Soil organic matter largely determine soil structure and aggregate stability, increasing soil water infiltration and holding capacity (Bot and Benites, 2005). For instance, the content of organic matter has a positive influence on the abundance of soil micro and macrofauna, and soil biological activity, improving soil water retention capacity by enhancing porosity and aggregate cohesion (Bot and Benites, 2005). Soil organic matter can also promote the resistance of soil microbial communities to water stress (Griffiths and Philippot, 2013; Schimel et al., 2007). For all these reasons, soils with a low organic matter content, as agricultural soils with a long history of intensive use (involving intensive tillage practices), might be more vulnerable to drought conditions.

In the Mediterranean region a large fraction of soils (74 % of land surface) is affected by losses of organic matter, having very low levels (<2 %) of organic C (Jones et al., 2012). Indeed, the average content of organic carbon in Mediterranean agricultural soils ranges from 0.7 to 3.4 % (Grilli et al., 2021). In Spain, a study showed that agricultural soils from arid regions have very low levels of organic C (0.7–1 %) in the upper (0–30 cm) soil layer (Calvo de Anta et al., 2020). Besides different biophysical factors, these low levels are the result of historical cultivation with low use efficiency of inputs, intensive tillage and a limited return of crop residues to soil (Balesdent et al., 2000; Lal, 2009). Therefore, increasing soil organic carbon stocks should be a priority in order to promote a higher resistance of Mediterranean agricultural soils

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https://doi.org/10.1016/j.jenvman.2025.125093
Received 12 November 2024; Received in revised form 13 January 2025; Accepted 19 March 2025

to the projected changes in rainfall patterns.

The use of organic residues as soil amendments, previously composted or not, is a frequent technique in the management of degraded soils. Amendments can improve soil chemical properties by increasing the contents of organic C and macro and micronutrients (Fernández-Bayo et al., 2009; Madejón et al., 2016; Domínguez et al., 2020). In soils with a highly degraded physical structure the application of organic amendments can also have positive effects on hydraulic properties, improving soil water holding capacity (Singh and Agrawal, 2008; Madejón et al., 2019).

However, there is a limited amount of research concerning the influence of soil microbial communities on the resistance of degraded Mediterranean agricultural soils to drought conditions. The quality of the organic amendment may influence the soil microbial community structure and activity, which can also influence the response of soil functioning to drought events. Frequently the fungi:bacteria ratio is positively associated with the content of organic compounds derived from lignin or with the C:N ratio of the organic matter (Wardle et al., 2004; Fierer et al., 2009; Grandy et al., 2009), due to physiological and stoichiometric constraints that limit the growth of bacteria on substrates with a high C:N ratio (Keiblinger et al., 2010; Waring et al., 2013). A synthesis of studies analyzing the importance of the relative abundances of different functional groups for soil stability in the face of global change factors (De Vries and Shade, 2013) suggested that those communities dominated by organisms with a more conservative strategy in the use of resources (K strategists, such as fungi and Gram + bacteria) may present greater stability to drought, although less resilience (slower recovery), than those soils dominated by organisms with a more acquisitive strategy (r strategists). Some works have showed a tendency towards an increase in the dominance of fungi over bacteria in soils amended with materials with a high C:N ratio or that contain highly recalcitrant organic compounds (Ng et al., 2014; Lanza et al., 2016). As mentioned above, this could influence the stability of these communities in the face of disturbances, including drought episodes. For example, the addition of amendments with a high C:N ratio, such as biochar, can increase the resilience of certain soil functions to drought episodes, mediated by a greater stability of soil fungal communities (Liang et al., 2014). Indeed, microbial communities that exhibit enhanced tolerance are believed to possess superior capabilities for sustaining soil functions during environmental stressors such as drought, promoting soil resistance (Griffiths and Philippot, 2013). Within the framework of this study, soil resistance is defined as the absence of changes in soil biological activity in response to drought.

The main objective of this work was to evaluate the effect of the application of organic substrates with different quality (as indicated by their C:N ratios) on soil chemical properties, biological properties (enzyme activities, soil respiration rate, total DNA) and bacterial and fungal abundances, as well as on the resistance of soil functioning and plant productivity against simulated drought conditions. The resistance of soil functioning was expressed in terms of changes in microbial biomass, oxidative activity (respiration rates and dehydrogenase activity) and extracellular enzyme rates. For that we used four different organic amendments in a gradient of C:N ratios (leonardite, earthworm humus, *Vicia faba* dry biomass, and biosolid compost) applied to a Mediterranean agricultural soil poor in organic C. We expected that the addition of any of these organic amendments would increase the resistance of plant productivity and soil biological activity to a decrease in water inputs. We also expected that an organic substrate with a high C:N ratio would favour a fungal dominance within the soil microbial community, which is usually related to a higher stability of soil against disturbances, including drought.

2. Material and methods

2.1. Experimental design

To study the effect of the addition of the organic amendment, the experiment was carried out with a C-poor soil collected from an agricultural field with a long history of intensive agricultural use, from the Guadalquivir Valley (Coria del Río, Sevilla, Spain). Soil is classified as Typic Xerofluvent (Soil Survey Staff, 2014), has a sandy clay loam texture and alkaline pH (8.7). Soil carbonates content is 3.9 %, and the total content of organic C is 1 %.

Soil was collected with a hoe from the upper 20 cm, sieved and homogenised. Then it was amended with four different organic substrates, in a gradient of C:N ratios: 1) leonardite (HUMITA®40; LE); 2) earthworm humus (LOMBEC®; HU); 3) *Vicia faba* dry biomass (VF); 4) biosolid compost (made of sewage sludge and plant biomass from pruning supplied by EMASESA; BC). The *V. faba* treatment was included to represent a legume crop used to promote N-fixation in soils, which is receiving much attention as a cover crop in the last years (Brasier et al., 2023; Ghorbi et al., 2023; Dugger et al., 2024), and in order to compare this management practise with the application of externally produced organic subtrates. *Vicia faba* biomass was harvested from the same field from which soil was collected. Biomass was dried at 60 °C for 48 h and ground to a 2 mm particle size. These four types of amendments were compared to a non-amended control soil (NA) to make a total of five amendment treatments. These materials were selected because they have contrasted quality, as indicated by C content and the C:N ratio (Table 1) and because they are available in the region and frequently used in the remediation of degraded soils or in the management of agricultural soils to increase soil C content. For instance, the application of compost produced from sewage sludge is a common practice in Spain, promoted by the current waste-management and circular economy targets. The use of cover crops has increased a 15 % in Spain in the last decade, being used in a 25 % of the perennial woody crops the country in 2022 (Spanish Ministry of Agriculture, 2023). Biosolid compost and leonardite have been proved to be very useful for the long-term increase of soil organic matter in C-poor and degraded Mediterranean soils (Montiel-Rozas et al., 2018; Madejón et al., 2018).

Organic substrates were sterilized by autoclave (120 °C, 1 h) before addition to soil in order to minimize the addition of the microbes contained in the amendments. Total C, N and S contents of each amendment were analyzed by an external service with a TruMac CNS macro analyser. Total P was extracted with HCl after calcination (450 °C, 2 h) and determined by spectrophotometry at 880 nm (Murphy and Riley, 1962). Results are shown in Table 1.

The experiment was carried out under controlled greenhouse conditions using 2.5 L pots, filled with approximately 1.5 kg of the soil + amendment mixture. The application dose of each organic substrate was calculated in order to increase the initial soil organic C in a 1 % and reach a target value of 2 %. Therefore, based on the C content of each amendment different addition rates were used for each material (Table 1).

Pots were filled with the amended soil, having 20 pots for each treatment (100 pots in total). Pots were regularly watered and soil moisture was maintained at approximately 70 % of soil water holding

Table 1
Total C, N, P and S contents and application dose of the organic amendments.

	C (%)	N (%)	P (%)	S (%)	C:N	Application dose (g kg^{-1} soil)
Leonardite (LE)	12.0	0.2	0.04	2.8	58.6	86
Earthworm humus (HU)	18.5	1.2	0.01	0.2	15.6	56
Vicia faba (VF)	40.8	4.2	1.4	0.1	9.8	25
Biosolid compost (BC)	17.0	1.8	2.6	0.2	9.3	61

capacity (WHC). After a stabilization period of 15 days, a mixture of two forage species was sown: *Lolium perenne* (40 seeds per pot) and *Medicago polymorpha* (15 seeds per pot).

One month after sowing (45 days after soil amendment), a drought treatment was imposed. Drought conditions were simulated in half of the pots of each amendment treatment by reducing the water supply in a 30 % (DR), following the IPCC projections for the Mediterranean region for the growing season (springtime) by the end of this century (IPCC, 2013). The drought treatment was compared with a well-watered control treatment (CT). This resulted in a factorial experimental design of the organic amendment × water availability interaction, resulting in 10 treatments with 10 replicates each: LE-DR, LE-CT, HU-DR, HU-CT, BC-DR, BC-CT, VF-DR, VF-CT, NA-DR, NA-CT.

Dry conditions were maintained for one month and soil moisture in the upper 5 cm was measured with a sensor using the Time Domain Reflectometry (TDR) technique. Soil moisture measurements were taken 10, 13, 17, 24 and 27 days after the beginning of the simulated drought event. Soil moisture was measured just before pot watering.

2.2. Soil sampling and analysis

Two soil samplings were carried out: an initial soil sampling one month after seed sowing (45 days after soil amendment; sampling 1) and a second sampling at the end of the experiment (sampling 2), one month after the establishment of the drought treatment (75 days after soil amendment). For each sampling, one subsample was air-dried and used to analyze soil main chemical properties; a second subsample was stored at 4 °C to measure soil microbial activity; and a third subsample was frozen and stored at −80 °C to analyze soil microbial DNA.

Soil carbonate content was quantified by the volumetric method using a Bernard Calcimeter (Bernard, 1889). Soil organic C, analyzed by the Walkley-Black chromic acid wet oxidation method (Walkley and Black, 1934), was used to infer soil organic matter (SOM, %). Bioavailable phosphorus was extracted with a 0.5 N sodium hydrogen carbonate solution (pH 8.5) and determined by spectrophotometry at 880 nm (Murphy and Riley, 1962). Bioavailable Ca, Mg, K and Na were extracted with pH 7 ammonium acetate and measured with an atomic absorption spectrometer (Ojea and Carballas, 1976); Ca and Mg were determined by atomic absorption spectroscopy and K and Na by atomic emission spectroscopy (PerkinElmer AAnalyst 100). Bioavailable Fe, Mn, Cu, Zn, Pb and Cd were extracted with 0.05 M EDTA (Viro, 1955) and quantified by atomic absorption spectroscopy (PerkinElmer AAnalyst 100).

Soil dehydrogenase activity (DHA) was analyzed by absorbance spectroscopy at 490 nm using 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) as substrate, expressing the result as μg INTF g dry soil^{-1} h^{-1} (Mosher et al., 2003). β-glucosidase, aminopeptidase, phosphatase and N-acetyl-glucosaminidase activities were measured using a microplate fluorescence reader after the extraction in sodium acetate with a pH of 5.5 (Marx et al., 2001). 7-amino-4-methyl coumarin (AMC) and 4-methylumbelliferone (MUB) were used as fluorogenic substrates and enzyme activities were expressed as nmol AMC g dry soil^{-1} h^{-1} and nmol MUB g dry soil^{-1} h^{-1}. Soil respiration rate was determined with an infra-red gas analyser (IRGA) after incubating 1 g of soil for 24 h in a closed vial at 25 °C (Bekku et al., 1995) and expressed as μg C-CO_2 g dry soil^{-1} day^{-1}.

DNA was extracted from 0,25 g of each soil sample using the DNeasy PowerSoil Pro Kit (QIAGEN®). Soil DNA concentrations, quantified with a Qubit fluorometer, were used as an estimate of soil microbial biomass (Semenov et al., 2018). Subsequently, qPCRs (Takara Bio's SYBR® Premix Ex Taq) were carried out to study the relative abundances of fungi and bacteria within the soil microbial community. Primer pairs 341F/805R and ITS86F/ITS4 were selected for the amplification of the 16S (bacteria) and ITS2 (fungi) regions, respectively. The qPCR protocol was the following: 10 min at 95 °C, 40 amplification cycles (30 s at 95 °C, 30 s at 60 °C (16S) or 62 °C (ITS), and 30 s at 72 °C) and a final dissociation phase (1 min at 95 °C, 30 s at 55 °C and 30 s at 95 °C). To obtain samples of 16S and ITS2 amplicons in a concentration gradient and calibrate the qPCR method, PCRs were used to amplify both target regions (34 amplification cycles; annealing temperature of 60 °C or 62 °C for the 16S and ITS2 regions, respectively) from one random soil DNA extract. PCR products were loaded in an agarose gel, and 16S and ITS amplicons were purified using the GeneJET Kit (Thermo Scientific™). DNA concentrations were determined using a Qubit fluorometer (ThermoFisher), and seven serial dilutions were done (10^{-1} to 10^{-7}). Finally, the corresponding amplification cycles, used to draw the calibration curve, were determined by qPCRs (same protocol described above).

2.3. Plant cover development

Germination rate of each species was quantified one month after sowing, before the establishment of the drought treatment. At the end of the experiment the plant cover in every pot was harvested. Aboveground plant biomass of each species was sorted, dried at 60 °C for 48 h and plant dry biomass was recorded.

2.4. Statistical analysis

All statistical analyses were carried out in R version 4.1.3 (RCoreTeam, 2022). Effects of the application of the different organic materials, the simulated drought conditions and the interaction of both factors on soil moisture and chemical properties, on soil microbial activity and the abundance of soil fungi and bacteria, and on plant performance were studied using linear mixed models. The residuals of each model were evaluated in order to check the model assumptions and to validate them. When homogeneity of variance was not met, a variance coefficient was introduced in the model to account for heteroscedasticity among different factor levels, using the varIdent function of the nlme package (Pinheiro and Bates, 2000).

3. Results

3.1. Soil moisture

Mean values of soil moisture in the upper 5 cm recorded 10, 13, 17, 24 and 27 days after the establishment of the drought treatment are shown in Supplementary Table S1. Soil moisture was significantly influenced by the amendment treatment during the whole simulated drought event (Supplementary Table S1). The highest moisture values were observed in soils amended with BC, followed by the VF and LE treatments. However, soil moisture in pots amended with earthworm humus (HU) was not significantly different than soil moisture in the non-amended treatment (NA). Drought conditions only had a significant effect on soil moisture 13 days after the establishment of the drought treatment (7.3 ± 1.1 % v/v in CT and 6.8 ± 0.1 % v/v in DR, all amendment treatments pooled); in addition, 24 days after the beginning of the drought period the amendment × drought interaction had a significant effect (higher soil moisture in BC-DR than in HU-DR and NA-DR).

Besides, relative soil moisture, expressed as a ratio of soil moisture in drought (DR) over control conditions (CT) (Fig. 1), was higher for the VF treatment compared to LE and HU (day 10), and also compared to HU (day 13). 24 days after the beginning of the drought period soil moisture in BC pots showed a higher resistance against drought conditions than in LE and NA. However, no significant differences on the effect of the drought treatment among amendments were observed on day 17 and 27 (Supplementary Table S2). Considering soil moisture in relative values, VF and BC treatments were the most efficient in promoting water retention, been relative soil moisture 16.5 % and 9.2 % higher than in the non-amended control, respectively.

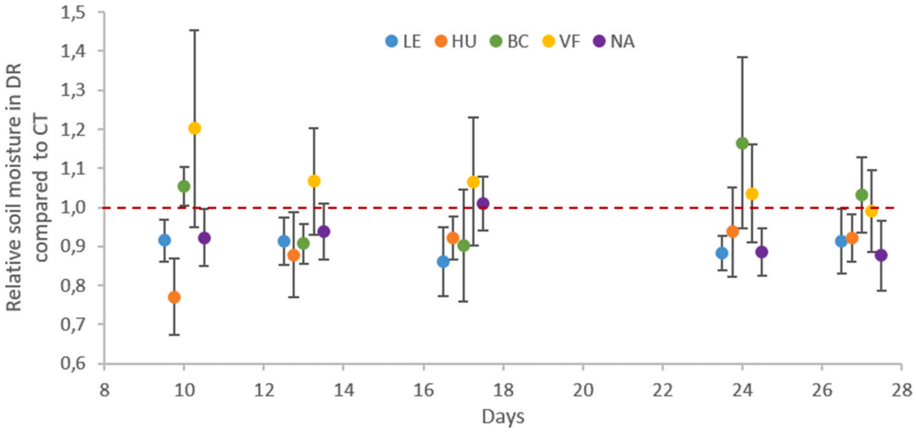

Fig. 1. Relative impact of the drought treatment on soil moisture at each amendment treatment. This relative impact represents the mean (± standard deviation) soil moisture recorded 10, 13, 17, 24 and 27 days after the beginning of the simulated drought event in pots with water restrictions (DR) divided by the mean soil moisture in pots without water restrictions (CT) for each amendment treatment at each date. LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control.

3.2. Plant cover development

Lolium perenne and *Medicago polymorpha* germination rates were significantly reduced in BC and VF treatments compared to the non-amended control (NA), while the other organic amendments did not have any significant effect on plant germination (Table 2, Supplementary Table S3). However, plant development showed a different pattern. *L. perenne* growth was enhanced by BC and VF, while *M. polymorpha* biomass was the highest in LE and the lowest in BC (Table 3, Supplementary Table S4).

Drought conditions significantly reduced *L. perenne* biomass (from 2.18 ± 1.30 g in CT to 1.75 ± 0.96 g in DR, all amendment treatments pooled), and the amendment × drought interaction had a significant effect, so that the reduction in *L. perenne* biomass due to drought was the highest in BC pots (Table 3, Supplementary Table S4). The growth of *M. polymorpha* was also significantly reduced by drought conditions (from 0.81 ± 0.46 g in CT to 0.53 ± 0.30 g in DR), while the amendment × drought interaction did not have a significant effect on this species (Table 3, Supplementary Table S4).

Table 2
Mean (± standard deviation) germination rate of *Lolium perenne* and *Medicago polymorpha*. LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control. Different letters indicate significant differences ($p < 0.05$) among the organic amendment treatments.

Amendment	Seed germination rate (%)	
	L. perenne	*M. polymorpha*
LE	90 ± 8 a	59 ± 11 a
HU	91 ± 7 a	50 ± 14 a
BC	55 ± 13 c	11 ± 8 c
VF	73 ± 9 b	23 ± 13 b
NA	91 ± 6 a	57 ± 16 a

Table 3
Mean (± standard deviation) aboveground dry biomass of *Lolium perenne* and *Medicago polymorpha* produced under drought conditions (DR; 30 % reduction of water supply) and in the control treatment (CT, 100 % water supply). LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control. Different letters indicate significant differences ($p < 0.05$) between the biomass produced in DR and in CT within each amendment treatment.

	Aboveground biomass (g)			
	L. perenne		*M. polymorpha*	
	DR	CT	DR	CT
LE	0.7 ± 0.1	0.78 ± 0.2	0.8 ± 0.2	1.2 ± 0.4
HU	1.7 ± 0.3	2.2 ± 0.5	0.6 ± 0.2	0.9 ± 0.4
BC	2.5 ± 0.4 b	3.6 ± 0.7 a	0.2 ± 0.2	0.3 ± 0.2
VF	2.9 ± 0.6	3.5 ± 0.7	0.5 ± 0.3	0.6 ± 0.4
NA	0.8 ± 0.1	0.9 ± 0.2	0.7 ± 0.2	1.1 ± 0.3

3.3. Soil chemical properties

Soil chemical properties were highly influenced by the amendment treatment (Fig. 2, Supplementary Tables S3, S4 and S5). At the first sampling soil pH was significantly reduced by all organic amendments, except for HU, and soil electrical conductivity (EC) was the highest in BC. As expected, soil organic C (TOC) was significantly increased in all the amended treatments compared to the non-amended control (NA, Fig. 2a). The amendment treatment also influenced the contents of soil macro and micronutrients available to plants. All organic amendments, except for LE (the amendment with the highest C:N ratio), significantly increased soil total N (Fig. 2b), and available P (Fig. 2c) and Mg (Fig. 2e) contents, compared to NA. Besides, available Ca (Fig. 2d) and K contents (Fig. 2f) were significantly increased by VF, while VF and HU treatments enhanced soil Na content. HU and VF treatments reduced the availability of Cu compared to NA; and bioavailable Fe was increased in LE. Bioavailable Mn content was highly increased by VF, and available Zn was higher in BC compared to LE and VF.

At the second soil sampling, one month after the establishment of the drought treatment, no effect of the drought treatment or the amendment × drought interaction on soil chemistry was observed (Supplementary Table S4). However, the positive effect of the organic amendment on soil properties was still patent, following a similar pattern than in the first soil sampling, (Fig. 2, Supplementary Table S5). 75 days after soil amendment a lower soil pH was observed in all amended treatments compared to the non-amended control (NA), but especially in LE. Soil EC was still higher in amended soils than in NA, although soil EC in BC was lower than in the first sampling, and the highest EC was recorded in LE. TOC in LE showed no significant differences compared to NA; while total N in BC and VF were reduced in comparison to the first sampling. However, available P content in all amended treatments was higher than in the previous sampling, although available P content in LE remained low. Available Ca, Mg and K contents were increased in all treatments compared to the first sampling, except for VF, and the opposite trend was observed for Na content. Regarding soil micronutrients, Cu showed the same pattern as in the first soil sampling; available Fe was reduced in all treatments and still showed the highest value in LE; Mn was increased, except for VF; and BC showed the

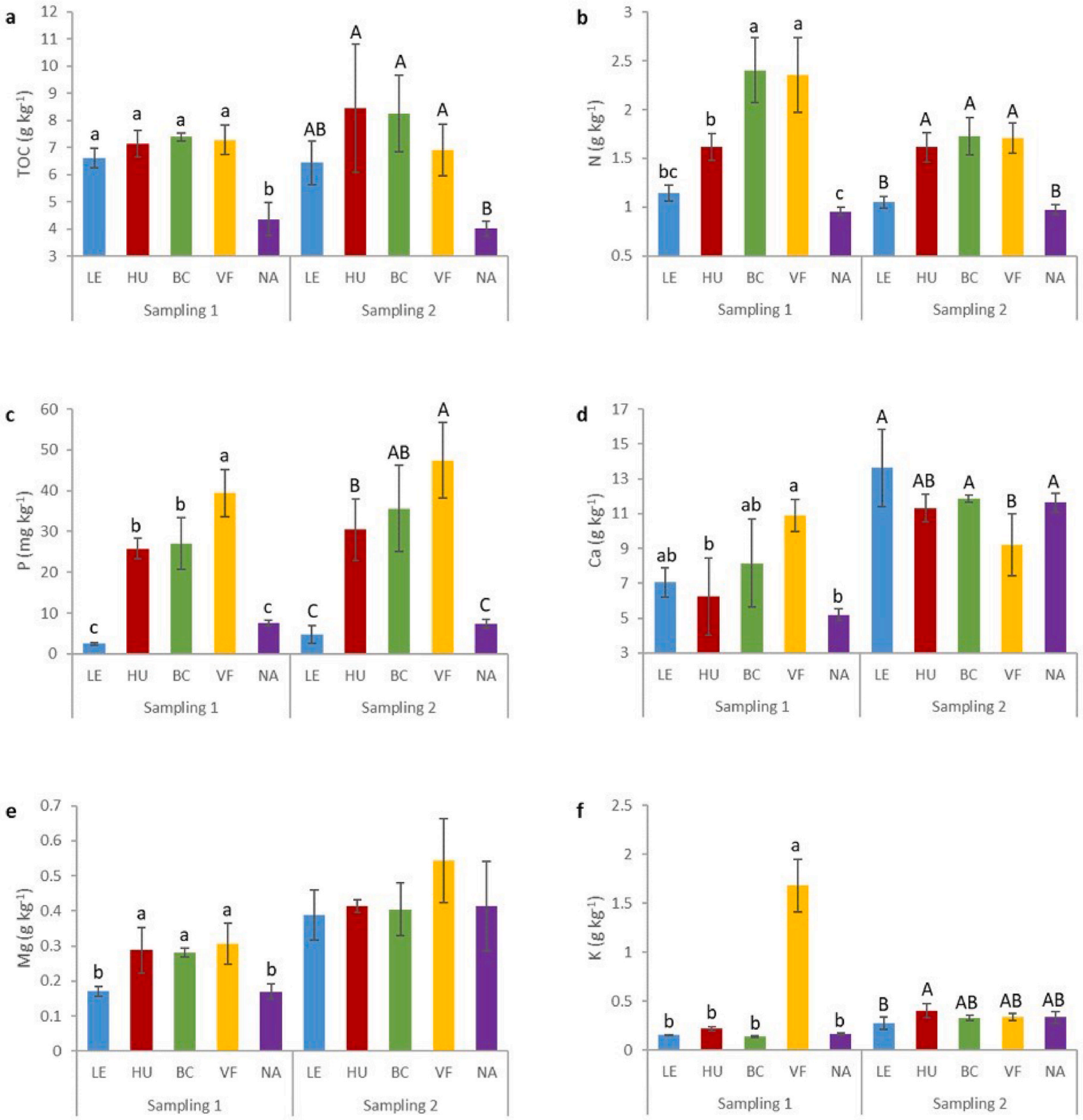

Fig. 2. Mean (bars) and standard deviation (lines) of soil total organic carbon (TOC) and macronutrients at samplings 1 (45 days after soil amendment and before the establishment of the drought treatment) and 2 (75 days after soil amendment and one month after the establishment of the drought treatment). LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control. Different letters (lower and uppercase letters for samplings 1 and 2, respectively) indicate significant differences ($p < 0.05$) among amendment treatments.

highest Zn content.

3.4. Soil enzyme activities and respiration rate

One month after sowing of the plant cover, significant effects of the amendment treatment were observed on soil dehydrogenase (DHA), phosphatase and β-N-acetyl-glucosaminidase activities, as well as on soil respiration rate (Fig. 3, Supplementary Table S3). DHA and soil respiration rate were greatly enhanced in VF, while phosphatase and N-acetyl-glucosaminidase activities were higher in the non-amended control (NA) compared to the amended treatments. Soil aminopeptidase and β-glucosidase activities showed no significant differences among treatments (Supplementary Table S3).

At the second soil sampling, soil DHA and N-acetyl-glucosaminidase activities, as well as soil respiration rate, were still enhanced by the addition of organic amendments compared to the non-amended control (Fig. 3, Supplementary Table S4). Soil aminopeptidase (167 ± 95 nmol AMC g^{-1} h^{-1}), phosphatase (588 ± 97 nmol MUB g^{-1} h^{-1}) and β-glucosidase (166 ± 88 nmol MUB g^{-1} h^{-1}) activities were not significantly affected by the amendment treatment. The drought treatment did not have a significant effect on the analyzed enzyme activities, but soil N-acetyl-glucosaminidase was affected by the amendment × drought interaction (higher activity in VF-DR than in VF-CT; Supplementary Table S4).

In contrast to enzyme activities, soil respiration rate was significantly lower in pots belonging to the drought treatment (2.1 ± 0.9 μg C-CO_2 g^{-1} day^{-1}) compared to the control (2.7 ± 0.9 μg C-CO_2 g^{-1} day^{-1}), although it was not influenced by the amendment × drought interaction (Supplementary Table S4).

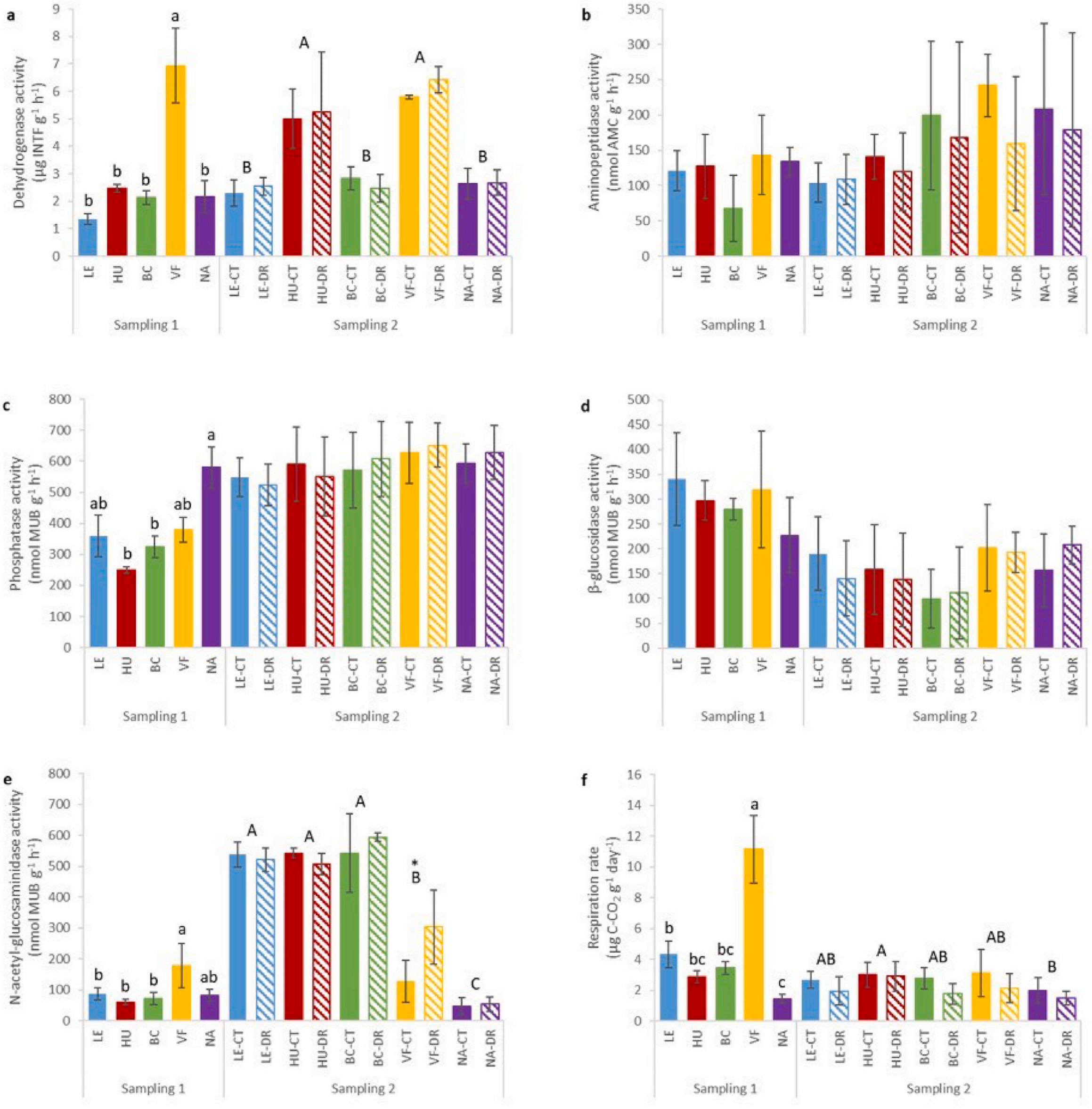

Fig. 3. Mean (bars) and standard deviation (lines) of soil dehydrogenase (a), aminopeptidase (b), phosphatase (c), β-glucosidase (d) and N-acetyl-glucosaminidase (e) activities, and soil respiration rate (f) measured at samplings 1 (45 days after soil amendment and before the establishment of the drought treatment) and 2 (75 days after soil amendment and one month after the establishment of the drought treatment). LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control. Different letters (lower and uppercase letters for samplings 1 and 2, respectively) indicate significant differences ($p < 0.05$) among amendment treatments. * indicates significant differences ($p < 0.05$) between control (CT) and drought (DR) pots within each amendment treatment.

3.5. Bacterial and fungal abundance

45 days after the application of the organic amendments, soil microbial biomass, estimated by the DNA content, as well as the abundance of bacteria and fungi, and the fungi:bacteria ratio (ITS:16S), were significantly enhanced by VF (Fig. 4, Supplementary Table S3). However, the application of LE reduced the abundance of bacteria compared to the non-amended control (NA).

By the second soil sampling (Fig. 4) all organic amendments enhanced soil microbial biomass compared to NA. The abundance of bacteria and fungi were still the highest in VF (Fig. 4b, c), while the highest fungal dominance (higher fungi:bacteria ratio) was again found in VF, followed by BC, the two organic amendments with the lowest C:N (Fig. 4d). In contrast, the lowest ITS:16S ratio was observed in LE, the substrate with the highest C:N (Fig. 4d). The simulated drought conditions or the amendment × drought interaction did not significantly affect total microbial biomass neither fungal or bacterial abundance (Supplementary Table S4).

4. Discussion

4.1. Soil moisture and plant cover performance

The aim of the study was to test whether the application of organic materials to a C-poor Mediterranean soil promotes soil resistance to drought conditions, and to investigate the relevance of the organic matter quality in this resistance. Four organic substrates were used to increase the initial soil organic C content, which according to previous knowledge and our initial hypothesis would enhance soil water retention and reduce the negative impact of drought conditions on soil functioning.

Except for the earthworm humus, our data demonstrated the positive effect of the application of organic amendments on soil moisture compared to the non-amended soil. Biosolid compost (BC), often applied

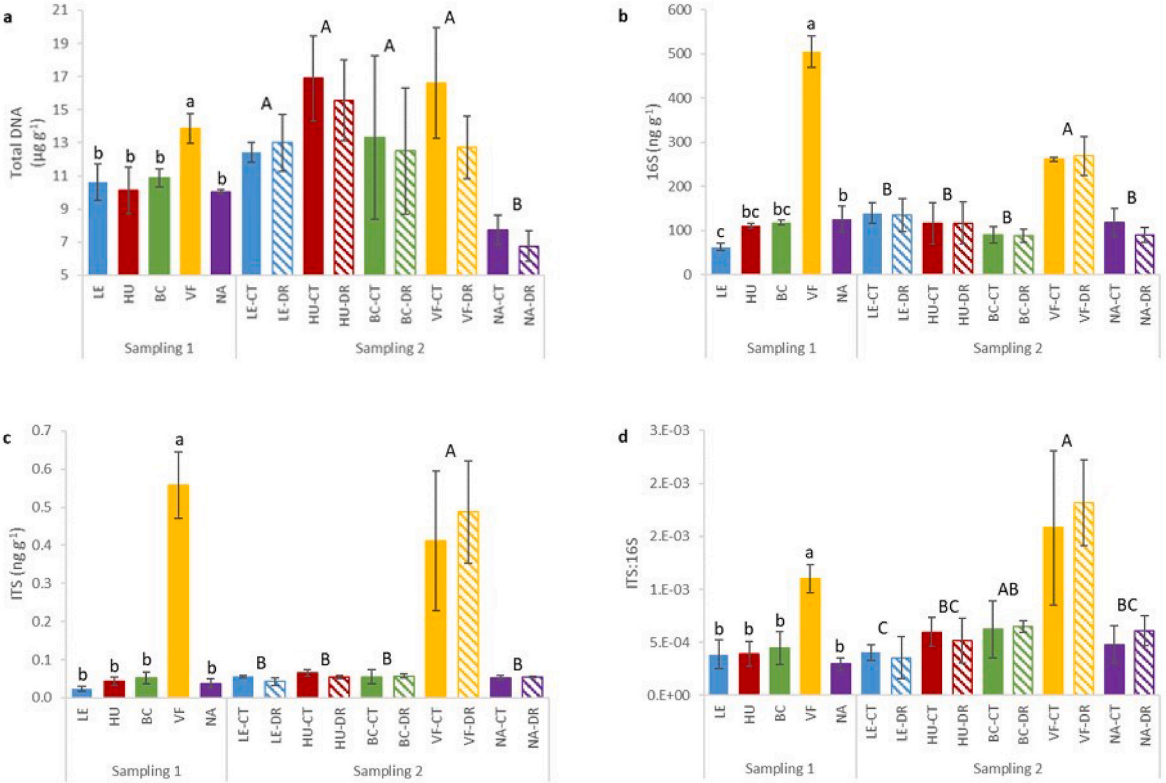

Fig. 4. Mean (bars) and standard deviation (lines) of soil total DNA (a), the abundance of bacteria (16S, b) and fungi (ITS2, c), and fungi:bacteria ratio (ITS:16S, d) measured in each amendment treatment at the first (45 days after soil amendment and before the establishment of the drought treatment) and second sampling (75 days after soil amendment and one month after the establishment of the drought treatment). LE: leonardite; HU: earthworm humus; BC: biosolid compost; VF: *Vicia faba* dry biomass; NA: non-amended control. Different letters (lower and uppercase letters for samplings 1 and 2, respectively) indicate significant differences ($p < 0.05$) among amendment treatments.

to increase soil organic matter and water holding capacity (Cooper and DeMarco, 2023), was the most efficient amendment in enhancing water retention. Besides possible differences in soil water retention among amendments, the effects of amendment addition on plant productivity could also explain differences in soil moisture among treatments. The higher development of the plant cover in BC and VF (discussed below) may have contributed to increase soil moisture retention since grasses are able to improve soil structure and promote water infiltration and conservation (Huang et al., 2017; Cerdà et al., 2021). However, a higher evapotranspiration rate in BC and VF would likely occur due to the increased aboveground plant biomass.

The reduction of the germination rate of both plant species in BC pots was previously observed in other experiments in which the same biosolid compost was applied (Morales-Salmerón et al., 2024; Morales-Salmerón et al., unpublished), and may be explained by the phytotoxicity of this organic amendment (Zubillaga and Lavado, 2006). In addition, the lower plant germination rate in VF (the amendment with the lowest C:N) compared to the germination registered in NA, especially in the case of *M. polymorpha*, could be the consequence of the supply of N content, that causes a toxic effect on the short-term and inhibits plant germination (Pérez-Fernández et al., 2006). Besides, the use of legume species residues as organic amendments can enhance fungal growth and the abundance of soil-borne fungal pathogens, that negatively affect seed germination and promote seedling damping-off (Bonanomi et al., 2011).

Despite the negative effects on seed germination, *L. perenne* growth was significantly favored by the addition of BC and VF, due to the higher soil moisture and the increased availability of N and P (Bai et al., 2014). Considering the application dose and total N and P contents of each organic amendment (Table 1), the addition of N and P in the BC treatment was equivalent to 1.6 t ha^{-1} and 2.3 t ha^{-1}, respectively; while with VF 1.5 t N ha^{-1} and 0.5 t P ha^{-1} would added to the soil. Oppositely, the addition of N and P in LE and HU treatments would be much lower: 0.2 t N ha^{-1} and 0.05 t P ha^{-1} in LE, and 1.0 t N ha^{-1} and 0.01 t P ha^{-1} in HU.

Additionally, it has been previously demonstrated that humic substances in composts have a biostimulant effect on plants, improving nutrient use efficiency and tolerance to abiotic stress (Canellas et al., 2015). Furthermore, *M. polymorpha* growth was limited by the interspecific competition and was higher in LE and NA than in BC and VF.

According to the initial hypothesis, the addition of an organic amendment would reduce the negative impact of dry conditions on plant production. Nevertheless, the simulated drought conditions significantly affected both species performance in BC pots, especially *M. polymorpha* (mean reduction of a 34.6 % in plant biomass), while *L. perenne* biomass was reduced in a 30.8 %. Although differences in soil moisture between drought and control pots were not statically significant for most dates, the high amount of total plant biomass in BC pots may have resulted in a higher inter and intraspecific competition for water resources, affecting *L. perenne* performance under drought conditions.

4.2. Soil chemical properties

Soil chemical properties, not affected by the simulated drought event, were highly influenced by the amendment type. Organic amendments tended to slightly reduce soil pH, especially LE. As expected, soil organic matter content was increased after the application of the organic amendments, and total N was increased in the short-term (45 days after the application) by the addition of BC and VF, the amendments with the lowest C:N ratio. However, soil N content decreased 75 days after the amendment addition (second soil sampling) probably related to plant consumption. Oppositely, the application of LE, the amendment with the highest C:N ratio, did not enhance soil total N content compared to the non-amended treatment.

Available P was also the highest in VF. The biomass of leguminous species, including *Vicia* spp., has a high content of orthophosphates (an available form of P) and is widely used in agriculture as green manure to increase soil available P and stimulate soil enzyme activities (Gao et al., 2016). On the other hand, LE did not increased soil available P compared to the non-amended control. Other studies have demonstrated that leonardite amendments reduce soil P fixation by reducing soil pH, but do not increase P content when used independently (Olego et al., 2022), what supports the low P content observed in LE.

Soil available macronutrients (Ca, Mg and K) and Na content were also increased after soil organic amendment, especially in VF, BC and HU, as it was observed in previous studies (Angelova et al., 2013). Furthermore, LE considerably increased soil extractable Fe, what is supported by the fact that humic acids are able to form complexes with Fe and increase its concentration in the soil solution (Chen, 1996). While the specific proportion of humic substances in the examined amendments was not assessed, the content of fulvic acids in the applied leonardite (100 mg kg^{-1}; data supplied by the provider) significantly exceeds the fulvic acid content expected in the other organic substrates.

4.3. Soil enzyme activities and respiration rate

As well as soil chemical properties, soil biological activity (enzyme activities and soil respiration rate) was improved by the organic amendments. The increase in soil dehydrogenase activity (DHA) and soil respiration rate in the VF treatment 45 days after the addition is likely linked to the increase in the abundance of fungi and bacteria. Soil DHA, involved in the biological oxidation of soil organic matter, is considered an indicator of microbial activity in degraded Mediterranean soils (generally poor in organic matter) and is often positively linked to soil moisture and N content (Garcia et al., 1997), as observed in VF. Furthermore, soil basal respiration is usually positively correlated with soil DHA, since it responds to microbial biomass and activity (Garcia et al., 1997).

In contrast, soil phosphatase and N-acetyl-glucosaminidase activities showed a very different trend, and 45 days after the application of the organic amendments the activity levels in the amended soils were not different or even lower than the activity recorded in the non-amended control. This effect has been observed in previous studies, in which soil N-acetyl-glucosaminidase activity in semiarid grasslands was reduced due to the supply of N sources, following the “microbial economics” hypothesis (Zhang et al., 2016). Once assimilable N forms were reduced by the second soil sampling (75 days after soil amendment in our case of study), N-acetyl-glucosaminidase activity was enhanced by the organic amendment, especially in LE, HU and BC (the substrates with a high C:N ratio).

Under our experimental conditions, soil enzyme activities showed resistance to the simulated drought event, while soil respiration rate was reduced by 23.7 % compared to the control treatment. This reduction in soil respiration was not influenced by the organic amendment. Recent studies have demonstrated that Mediterranean soils, frequently exposed to water stress, have acquired tolerance to drought, and that enzyme production is controlled by changes in microbial biomass, and not directly limited by soil moisture (Asensio et al., 2023; Morales-Salmerón et al., 2024). In our experiment, soil microbial biomass (estimated by the total DNA content) was not reduced by the drought treatment (discussed below), what could explain the stability of soil enzyme activities against changes in soil moisture.

4.4. Soil microbial community

According to our initial hypothesis, the application of an organic substrate with a high C:N ratio favours fungal dominance within the soil microbial community, enhancing soil resistance against environmental stresses. However, under our experimental conditions and 75 days after soil amendment, our results showed the opposite trend. While all amended treatments enhanced soil microbial biomass, the addition of *Vicia faba* biomass (VF) greatly increased both soil bacterial and fungal populations, even in the short-term (45 days after soil amendment). By the end of the experiment, the highest fungal dominance was recorded in VF followed by BC (the amendments with the lowest C:N ratio). Furthermore, it is essential to consider the distinct composition and properties of VF in comparison to the other amendments studied, noting that it was the only non-humified substrate. *V. faba* dry biomass is mainly composed of cellulose (46 %), with a relatively low lignin content (15 %) compared to other cover crops (Gómez et al., 2017), which makes it a more labile substrate. This chemical composition, along with its high total N content (4.2 %), may be responsible for the enhanced soil microbial populations, and particularly the increased fungal abundance, as fungi tend to be more responsive to the incorporation of plant residues into the soil (Muhammad et al., 2021).

Besides, since the simulated drought conditions had a limited effect on many of the studied indices of biological activity, and no differences were observed in the reduction of soil respiration rate among amendment treatments, it was not possible to confirm, under our experimental conditions, that C-poor soils with a higher fungi:bacteria ratio show a higher resistance to water stress.

In further studies it would be interesting to analyze the composition of the soil microbial community in order to determine what specific microbial taxa and functional traits were influenced by each amendment application. It was proved that some fungal taxa, like mycorrhizal and filamentous fungi, promote water infiltration and enhance soil water-retention capacity by improving soil structure and organic matter content (White et al., 2000; Querejeta, 2017). This may explain, together with the higher plant biomass (discussed above), the lower impact of the simulated drought event on soil moisture in VF and BC soils (those showing the highest fungal abundance) compared to the other treatments.

Furthermore, a more complex and recalcitrant organic matter favours the prevalence of Gram + bacteria in the soil bacterial community (Fanin et al., 2019). Therefore, the application of an amendment with a high C:N ratio, as leonardite, may increase the abundance of these bacterial taxa and, since Gram + bacteria are often reported to show a greater resistance to drought conditions than Gram – bacteria (De Vries and Shade, 2013), enhancing the resistance of soil biological activity to water stress. However, in the short-term, soil bacterial abundance was decreased in LE compared to the non-amended control (NA). In a previous study it was observed a negative effect of the application of humic acids (the main component of leonardite) on the abundance of most soil bacterial phyla (Li et al., 2019).

In summary, the incorporation of *V. faba* biomass was especially beneficial to soil in comparison to the addition of more humified organic materials, at least at the short-term. Faba bean biomass addition did not increase the resistance of plant production to drought in comparison to the other amendments, but it was clearly more effective than the other treatments in terms of promoting water retention, microbial abundance and activity, and in terms of improving soil N and P. This highlights the potential benefits of using this legume crop as cover crop. Much evidence suggests that the use of cover crops can be beneficial to face climate change in Mediterranean environments if properly managed (reviewed in Kaye and Quemada, 2017). In addition, the gradual release of nutrients trough litter decomposition plays a crucial role in regulating and aligning nutrient availability with the specific needs of the target crop, making cover crops a more sustainable nutrient source compared to inorganic fertilizers (Marañón-Jiménez et al., 2022). It would be interesting to conduct further works with faba bean as a cover crop under field conditions, evaluating whether the positive effects on soil found here translate into a better ability of the cash crop to deal with water stress.

Finally, some limitations of our study should be considered. Firstly, it is possible that the reduction of water inputs tested in this study was insufficient to detect changes in community structure and activity in response to drought, considering that native microbial communities are highly adapted to water stress conditions. Secondly, it is important to note that this is a short-term study that aims to simulate a reduction in water inputs during the growing season, however over the year microbial populations can be exposed to larger fluctuations in soil water content. Under field conditions during the summertime water content in the studied soil can be extremely low (<2 % v/v; Madejón et al., 2025). To have a complete understanding of the effect of different organic inputs on the resistance of soil to drought, long-term field experiments would be desirable. Finally, the analysis of the composition and functional guilds of soil microbial communities, which was not conducted in this study, could provide more information about which specific taxa were enhanced by each amendment, and would be useful to explain the observed resistance of soil to drought conditions.

5. Conclusions

The results of the study showed the positive effect of the addition of organic amendments, particularly those characterized by a low C:N ratio (*Vicia faba* dry biomass and biosolid compost), on the chemical properties of C-poor agricultural soils. These amendments were also the most efficient in improving soil water retention and plant productivity (especially *L. perenne*), although they had a negative impact on plant germination.

The addition of *V. faba* biomass had a remarkable effect on soil microbial populations and biological activity, particularly in terms of dehydrogenase activity and respiration rate, even in the short-term. Soils amended with *V. faba* exhibited the highest fungal dominance, which may be associated with enhanced soil water retention.

The simulated drought conditions did not alter soil chemical properties or enzyme activities; and drought effects on plant production and soil respiration were not clearly influenced by the amendment type. Consequently, the initial hypothesis suggesting that soils with a higher fungi-to-bacteria ratio would exhibit greater drought tolerance could not be confirmed.

CRediT authorship contribution statement

L. Morales-Salmerón: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **E. Fernández-Boy:** Writing – review & editing, Validation, Supervision, Investigation. **R. León:** Writing – review & editing, Supervision, Resources. **C.M. Navarro-Fernández:** Writing – review & editing, Investigation. **M.T. Domínguez:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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.

Acknowledgements

This study was funded by the Spanish Ministry of Science and Innovation (projects CGL2017-85891-R DEGRAMED and PID2021-122628OB-I00 WASTE4DROUGHT) and European funds. Laura Morales-Salmerón thanks the Ministry of Science and Innovation for the FPI fellowship (PRE2018-084467).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.125093.

Data availability

Data will be made available on request.

References

Ali, E., Cramer, W., Carnicer, J., Georgopoulou, E., Hilmi, N., Le Cozannet, G., Lionello, P., 2022. Mediterranean region. In: Pörtner, H.-O., Roberts, D.C., Tignor, M.M.B., Poloczanska, E., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B. (Eds.), Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York, pp. 2233–2272.

Angelova, V., Akova, V., Artinova, N.S., Ivanov, K., 2013. The effect of organic amendments on soil chemical characteristics. Bulg. J. Agric. Sci. 19, 958–971.

Asensio, D., Zuccarini, P., Sardans, J., Marañón-Jiménez, S., Mattana, S., Ogaya, R., Mu, Z., Llusià, J., Peñuelas, J., 2023. Soil biomass-related enzyme activity indicates minimal functional changes after 16 years of persistent drought treatment in a Mediterranean holm oak forest. Soil Biol. Biochem. 189, 109281. https://doi.org/10.1016/j.soilbio.2023.109281.

Bai, Y.-c., Gu, C.-h., Tao, T.-y., Zhu, X.-w., Xu, Y.-r., Shan, Y.-h., Feng, K., 2014. Responses of ryegrass (*Lolium perenne* L.) grown in mudflats to sewage sludge amendment. J. Integr. Agric. 13, 426–433. https://doi.org/10.1016/S2095-3119(13)60564-X.

Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Tillage Res. 53, 215–230. https://doi.org/10.1016/S0167-1987(99)00107-5.

Bekku, Y., Koizumi, H., Nakadai, T., Iwaki, H., 1995. Measurement of soil respiration using closed chamber method: an IRGA technique. Ecol. Res. 10, 369–373. https://doi.org/10.1007/BF02347863.

Bernard, A., 1889. Ein etwas modificirtes Calcimeter. Fresenius Z. F.Anal. Chem. 28, 86. https://doi.org/10.1007/BF01375878.

Bonanomi, G., Antignani, V., Barile, E., Lanzotti, V., Scala, F., 2011. Decomposition of *Medicago sativa* residues affects phytotoxicity, fungal growth and soil-borne pathogen diseases. J. Plant Pathol. 93, 57–69.

Bot, A., Benites, J., 2005. The importance of soil organic matter: key to drought-resistant soil and sustained food production. Food and Agriculture Organization of the United Nations. Soils Bulletin 80, 1-78. Rome.

Brasier, K., Zaragoza, I., Knecht, J., Munster, R., Coulter, H., Potter, A., Enke, E., Fox, A., Mosqueda, E., Zakeri, H., 2023. Potential of faba bean (*Vicia faba* L.) for dual-purpose vegetable production and cover cropping. Hortscience 58 (1), 23–31.

Calvo de Anta, R., Luís, E., Febrero-Bande, M., Galiñanes, J., Macías, F., Ortíz, R., Casás, F., 2020. Soil organic carbon in peninsular Spain: influence of environmental factors and spatial distribution. Geoderma 370, 114365. https://doi.org/10.1016/j.geoderma.2020.114365.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., Piccolo, A., 2015. Humic and fulvic acids as biostimulants in horticulture. Sci. Hortic. 196, 15–27. https://doi.org/10.1016/j.scienta.2015.09.013.

Cerdà, A., Terol, E., Daliakopoulos, I.N., 2021. Weed cover controls soil and water losses in rainfed olive groves in Sierra de Enguera, eastern Iberian Peninsula. J. Environ. Manag. 290, 112516. https://doi.org/10.1016/j.jenvman.2021.112516.

Chen, Y., 1996. Organic matter reactions involving micronutrients in soils and their effect on plants. In: Piccolo, A. (Ed.), Humic Substances in Terrestrial Ecosystems. Elsevier Science, Amsterdam, pp. 507–529.

Cooper, A., DeMarco, J., 2023. Composted biosolids amendments for enhanced soil organic carbon and water storage in perennial pastures in Colorado. Agric. Ecosyst. Environ. 347, 108401. https://doi.org/10.1016/j.agee.2023.108401.

De Vries, F., Shade, A., 2013. Controls on soil microbial community stability under climate change. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00265.

Domínguez, M.T., Panettieri, M., Madejón, E., Madejón, P., 2020. Thistle crops in marginal lands after compost addition: plant biomass and effect on soil physical, chemical and biological properties. Land Degrad. Dev. 31, 1167–1175. https://doi.org/10.1002/ldr.3510.

Dugger, C., Brasier, K.G., Pahlavani, M., Zakeri, H., 2024. Steps toward developing legume cover crop varieties: study of biomass and nitrogen partitioning and within-plant δ115N variation of faba bean at different growth stages. Crop Sci. 64, 1790–1801. https://doi.org/10.1002/csc2.21217.

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.-C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol. Biochem. 128, 111–114. https://doi.org/10.1016/j.soilbio.2018.10.010.

Fernández-Bayo, J.D., Nogales, R., Romero, E., 2009. Assessment of three vermicomposts as organic amendments used to enhance diuron sorption in soils with low organic carbon content. Eur. J. Soil Sci. 60, 935–944. https://doi.org/10.1111/j.1365-2389.2009.01176.x.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecol. Lett. 12, 1238–1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x.

Gao, X., Shi, D., Lv, A., Wang, S., Yuan, S., Zhou, P., An, Y., 2016. Increase phosphorus availability from the use of alfalfa (*Medicago sativa* L) green manure in rice (*Oryza sativa* L.) agroecosystem. Sci. Rep. 6, 36981. https://doi.org/10.1038/srep36981.

Garcia, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134. https://doi.org/10.1080/00103629709369777.

Ghorbi, S., Ebadi, A., Parmoon, G., Siller, A., Hashemi, M., 2023. The use of faba bean cover crop to enhance the sustainability and resiliency of No-till corn silage production and soil characteristics. Agronomy 13 (8), 2082. https://doi.org/10.3390/agronomy13082082.

Gómez, L.D., Amalfitano, C., Andolfi, A., Simister, R., Somma, S., Ercolano, M.R., Borrelli, C., McQueen-Mason, S.J., Frusciante, L., Cuciniello, A., Caruso, G., 2017. Valorising faba bean residual biomass: effect of farming system and planting time on the potential for biofuel production. Biomass Bioenergy 107, 227–232.

Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N., 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. Geoderma 150, 278–286. https://doi.org/10.1016/j.geoderma.2009.02.007.

Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37, 112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x.

Grilli, E., Carvalho, S., Chiti, T., Coppola, E., D'Ascoli, R., La Mantia, T., Rossana, M., Mastrocicco, M., Pulido, F., Rutigliano, F., Quatrini, P., Castaldi, S., 2021. Critical range of soil organic carbon in southern Europe lands under desertification risk. J. Environ. Manag. 287, 112285. https://doi.org/10.1016/j.jenvman.2021.112285.

Huang, Z., Tian, F.-P., Wu, G.-L., Liu, Y., Dang, Z.-Q., 2017. Legume grasslands promote precipitation infiltration better than gramineous grasslands in arid regions. Land Degrad. Dev. 28, 309–316. https://doi.org/10.1002/ldr.2635.

IPCC, 2013. In: Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.). Cambridge University Press, Cambridge and New York.

Jones, A., Panagos, P., Barcelo, S., Bouraoui, F., Bosco, C., Dewitte, O., Gardi, C., Hervás, J., Hiederer, R., Jeffery, S., 2012. The State of Soil in Europe. A Contribution of the JRC to the European Environment Agency's Environment State and Outlook Report—SOER 2010. Publications Office of the European Union, Luxembourg.

Kaye, J.P., Quemada, M., 2017. Using cover crops to mitigate and adapt to climate change. A Rev. Agron.Sustain. Dev. 37, 4. https://doi.org/10.1007/s13593-016-0410-x.

Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., Zechmeister-Boltenstern, S., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiol. Ecol. 73, 430–440. https://doi.org/10.1111/j.1574-6941.2010.00912.x.

Lal, R., 2009. Challenges and opportunities in soil organic matter research. Eur. J. Soil Sci. 60, 158–169. https://doi.org/10.1111/j.1365-2389.2008.01114.x.

Lal, R., 2015. Restoring soil quality to mitigate soil degradation. Sustainability 7, 5875–5895. https://doi.org/10.3390/su7055875.

Lanza, G., Rebensburg, P., Kern, J., Lentzsch, P., Wirth, S., 2016. Impact of chars and readily available carbon on soil microbial respiration and microbial community composition in a dynamic incubation experiment. Soil Tillage Res. 164, 18–24. https://doi.org/10.1016/j.still.2016.01.005.

Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G., Zheng, F., Tan, D., 2019. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment. Sci. Rep. 9, 12014. https://doi.org/10.1038/s41598-019-48620-4.

Liang, C., Zhu, X., Fu, S., Méndez, A., Gascó, G., Paz-Ferreiro, J., 2014. Biochar alters the resistance and resilience to drought in a tropical soil. Environ. Res. Lett. 9, 064013. https://doi.org/10.1088/1748-9326/9/6/064013.

Madejón, P., Alaejos, J., García-Álbala, J., Fernández, M., Madejón, E., 2016. Three-year study of fast-growing trees in degraded soils amended with composts: effects on soil fertility and productivity. J. Environ. Manag. 169, 18–26. https://doi.org/10.1016/j.jenvman.2015.11.050.

Madejón, P., Domínguez, M.T., Gil-Martínez, M., Navarro-Fernández, C.M., Montiel-Rozas, M.M., Madejón, E., Murillo, J.M., Cabrera, F., Marañón, T., 2018. Evaluation of amendment addition and tree planting as measures to remediate contaminated soils: the Guadiamar case study (SW Spain). Catena 166, 34–43. https://doi.org/10.1016/j.catena.2018.03.016.

Madejón, P., Domínguez, M.T., Fernández-Boy, E., Paneque, P., Girón, I., Madejón, E., 2019. Soil hydraulic properties as the main driver in the establishment of biomass crops in contaminated soils. J. Environ. Manag. 233, 812–822. https://doi.org/10.1016/j.jenvman.2018.10.008.

Madejón, P., Fernández-Boy, E., Madejón, E., Morales-Salmerón, L., Domínguez, M.T., 2025. Managing climate change impacts on crops: The influence of soil tillage on a triticale crop under water stress conditions. Ann. Appl. Biol. 186, 143–156. https://doi.org/10.1111/aab.12947.

Marañón-Jiménez, S., Serrano-Ortíz, P., Peñuelas, J., Meijide, A., Chamizo, S., López-Ballesteros, A., Vicente-Vicente, J.L., Fernández-Ondoño, E., 2022. Effects of herbaceous covers and mineral fertilizers on the nutrient stocks and fluxes in a Mediterranean olive grove. Eur. J. Agron. 140, 126597. https://doi.org/10.1016/j.eja.2022.126597.

Marx, M.C., Wood, M., Jarvis, S.C., 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.

Montiel-Rozas, M.M., Domínguez, M.T., Madejón, E., Madejón, P., Pastorelli, R., Renella, G., 2018. Long-term effects of organic amendments on bacterial and fungal communities in a degraded Mediterranean soil. Geoderma 332, 20–28. https://doi.org/10.1016/j.geoderma.2018.06.022.

Morales-Salmerón, L., Fernández-Boy, E., Madejón, E., Domínguez, M.T., 2024. Soil legacy and organic amendment role in promoting the resistance of contaminated soils to drought. Appl. Soil Ecol. 195, 105226. https://doi.org/10.1016/j.apsoil.2023.105226.

Mosher, J.J., Levison, B.S., Johnston, C.G., 2003. A simplified dehydrogenase enzyme assay in contaminated sediment using 2-(p-iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride. J. Microbiol. Methods 53, 411–415. https://doi.org/10.1016/S0167-7012(02)00251-8.

Muhammad, I., Wang, J., Sainju, U.M., Zhang, S., Zhao, F., Khan, A., 2021. Cover cropping enhances soil microbial biomass and affects microbial community structure: a meta-analysis. Geoderma 381, 114696.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Ng, E.L., Patti, A.F., Rose, M.T., Schefe, C.R., Wilkinson, K., Smernik, R.J., Cavagnaro, T.R., 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biol. Biochem. 70, 54–61. https://doi.org/10.1016/j.soilbio.2013.12.004.

Ojea, F.G., Carballas, T., 1976. Técnicas de Análisis de Suelos. Pico Sacro, Vigo.

Olego, M.Á., Cuesta Lasso, M., Quiroga, M.J., Visconti, F., López, R., Garzón-Jimeno, E., 2022. Effects of leonardite amendments on vineyard calcareous soil fertility, vine nutrition and grape quality. Plants 11, 356. https://doi.org/10.3390/plants11030356.

Pérez-Fernández, M.A., Calvo-Magro, E., Montanero-Fernández, J., Oyola-Velasco, J.A., 2006. Seed germination in response to chemicals: effect of nitrogen and pH in the media. J. Environ. Biol. 27, 13–20.

Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-PLUS. Springer, New York.

Querejeta, J.I., 2017. Soil water retention and availability as influenced by mycorrhizal symbiosis: consequences for individual plants, communities, and Ecosystems. In: Johnson, N.C., Gehring, C., Jansa, J. (Eds.), Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier, pp. 299–317.

RCoreTeam, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394. https://doi.org/10.1890/06-0219.

Semenov, M., Blagodatskaya, E., Stepanov, A., Kuzyakov, Y., 2018. DNA-based determination of soil microbial biomass in alkaline and carbonaceous soils of semi-arid climate. J. Arid Environ. 150, 54–61. https://doi.org/10.1016/j.jaridenv.2017.11.013.

Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. Waste Manag. 28, 347–358. https://doi.org/10.1016/j.wasman.2006.12.010.

Soil Survey Staff, 2014. Keys to Soil Taxonomy. USDA-Natural Resources Conservation Service, Washington DC.

Spanish Ministry of Agriculture, 2023. Agricultura de carbono en España: siembra directa y cubiertas vegetales. In: Ministerio de Agricultura, Pesca y Alimentación. NIPO:03190963. Available at: www.mapa.gob.es/es/ministerio/servicios/analisis-y-prospectiva/aypagriculturadecarbonon35_tcm30-645235.pdf.

Viro, P.J., 1955. Use of ethylenediaminetetraacetic acid in soil analysis: I. Experimental. Soil Sci. 79, 459–465. https://doi.org/10.1097/00010694-195506000-00005.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38. https://doi.org/10.1097/00010694-193401000-00003.

Wander, M., 2004. Soil organic matter fractions and their relevance to soil function. In: Magdoff, F., Weil, R.R. (Eds.), Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, pp. 67–102.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875.

Waring, B.G., Averill, C., Hawkes, C.V., 2013. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecol. Lett. 16, 887–894. https://doi.org/10.1111/ele.12125.

White, N.A., Hallett, P.D., Feeney, D., Palfreyman, J.W., Ritz, K., 2000. Changes to water repellence of soil caused by the growth of white-rot fungi: studies using a novel microcosm system. FEMS Microbiol. Lett. 184, 73–77. https://doi.org/10.1016/S0378-1097(00)00025-2.

Zhang, X., Tang, Y., Yao, S., He, N., Wen, X., Yu, Q., Zheng, C., Sun, X., Qiu, W., 2016. Responses of soil hydrolytic enzymes, ammonia-oxidizing bacteria and archaea to nitrogen applications in a temperate grassland in Inner Mongolia. Sci. Rep. 6, 32791. https://doi.org/10.1038/srep32791.

Zubillaga, M.S., Lavado, R.S., 2006. Phytotoxicity of biosolids compost at different degrees of maturity compared to biosolids and animal manures. Compost Sci. Util. 14, 267–270. https://doi.org/10.1080/1065657X.2006.10702295.