

Research paper

Soil legacy and organic amendment role in promoting the resistance of contaminated soils to drought

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ARTICLE INFO

Keywords:

Biosolid compost
Enzyme activity
Organic matter
Water stress
Soil bacteria
Soil fungi

ABSTRACT

The application of organic amendments is a common practice in the restoration of contaminated and degraded soils. We designed an experiment in pot mesocosms to study the effect of organic amendment (comparing a short-term addition of biosolid compost with soils amended 17 years ago) and soil legacy (comparing soils exposed or not to extensive grazing over a remediation process) on the resistance of a degraded Mediterranean soil against a simulated drought event. Pots were sown with a forage mixture (*Lolium rigidum* and *Medicago polymorpha*), and soil resistance was evaluated by measuring soil chemical properties, biological activity (soil enzyme activities and respiration rate), soil microbial community composition and plant production. Our results showed a positive effect of the organic amendment and the exposure to extensive grazing on soil properties, increasing soil water retention and the stability of plant production under drought conditions. The long-term amendment addition avoided the negative consequences of drought on *L. rigidum* production (the species with the lowest tolerance to water stress), while *M. polymorpha* biomass in soils exposed to grazing was 12 times the biomass in the non-amended and non-exposed soil. However, soil biological activity (enzyme activities and respiration rate) as well as microbial diversity were not limited by the simulated drought conditions under any type of soil management legacy, demonstrating the great adaptation of the microbial communities to water stress conditions in semiarid Mediterranean soils. Soils exposed to organic matter inputs in the long-term, through amendment addition or exposure to grazing, showed high similarities in terms of bacterial and fungal composition.

1. Introduction

Global climate change will impact the water cycle and promote changes in water flow and storage. Climate change projections include an increase of >50 % in precipitation in the tropics by the end of the 21st century, while in subtropical regions precipitations could decrease by 30 % or even more (IPCC, 2022). Drought conditions together with the increased air temperature could lead to increased water scarcity in already dry areas (Collins et al., 2013). Since the Mediterranean region will be strongly affected by climate change impacts, especially due to a higher occurrence and severity of drought periods in comparison to other regions (IPCC, 2022), enhancing the resistance and resilience of soils against drought episodes is a main challenge in this region. To address this concern, the application of organic amendments has been proposed in semiarid regions as a strategy to increase soil fertility and crop productivity, especially in contaminated and degraded soils, and to promote a higher stability against drought (Bastida et al., 2008; Bastida

et al., 2017). This is because organic matter has a direct effect on soil water dynamics through its influence on soil physical structure, which determines water storage capacity and the resistance of soil aggregates to desiccation (Wander, 2004; Lal, 2015). In addition, organic amendments reduce heavy metal availability in contaminated soils by raising soil pH and total organic C (Madejón et al., 2006).

The influence of organic matter on the resistance of soils to droughts can also be mediated by its effects on soil organisms. For instance, inputs of organic matter increase the abundance and activity of soil micro and macrofauna, which promote porosity and aggregate cohesion, with positive effects on soil water storage capacity (Bot and Benites, 2005). In addition, the amount of organic matter can modulate the response of soil microbial communities to several types of perturbations (Griffiths and Philippot, 2013), for example by supplying C substrates that can be used as osmolytes to resist desiccation (Schimel et al., 2007). Besides, soils with a high microbial diversity have a higher response diversity, defined as the range of microbial responses to a particular disturbance (Li et al.,

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2021a). Highly diverse soils usually also show a high functional redundancy, meaning that the same functionality is shared by different taxonomic groups within the soil microbial community (Li et al., 2021a). Both response diversity and functional diversity enhance soil resilience against disturbances (Schaeffer et al., 2016). In relation to C-inputs to soil, many studies have found a positive correlation between soil organic matter, nutrient availability, and microbial activity and soil resilience against several environmental factors, including water stress (Gregory et al., 2009; Hueso et al., 2011; Rivest et al., 2013; Lal, 2016).

Ecological legacy refers to the influence of past ecological dynamics and natural disturbance events (i.e. wildfires, floods), as well as land-use history and human impacts, that generate changes in ecosystem abiotic and biotic components that persist over time and have a significant effect on ecosystems contemporary dynamics (Cuddington, 2011). Consequently, legacies are considered the ecological inheritance that determines an ecosystem's current processes (Little et al., 1997; Vogt et al., 1997; Cuddington, 2011). Regarding soils, plant litter legacy and litter chemical composition have a strong effect on soil biota and organic matter decomposition, having an important influence on contemporary soil C and N dynamics (Carrillo et al., 2012). Past soil management can largely determine soil current soil functioning. For instance, a legacy of intensive soil management, including the use of fertilizers and intensive grazing, could lead to a reduction in soil total C content and an increase in bulk density (Ward et al., 2016), which could influence the response of soil to abiotic perturbations, such as extreme drought events. Extensive grazing, however, improves soil quality by increasing soil organic matter content and pH (Pérez-Esteban et al., 2014), and promotes plant growth and diversity (Renne and Tracy, 2007).

The aim of this study was to evaluate the effect of organic amendment addition (in the form of biosolid compost amendment) on soil resistance against simulated drought conditions in a C-poor, degraded and trace-element contaminated Mediterranean soil. For this, soils with a different legacy of soil management (exposed or not to a legacy of extensive grazing) undergoing soil remediation were studied. Changes in soil chemical properties, microbial community, and biological activity (enzyme activities and respiration rates) were measured during a drying episode. The effects on plant development were also studied. We hypothesized that the increase in organic C content to a degraded soil, via application of biosolid compost or through long-term inputs derived from extensive grazing enhances soil biodiversity and plant production, improves soil functioning, and increases soil resistance to drought periods.

2. Materials and methods

2.1. Study area

The soils under study were collected from the Guadiamar Green Corridor (SW Spain), an area affected by a toxic spill in 1998, when a failure in a tailings dam of a pyritic mine caused the release of about 6 million m³ of acid water and slurries (rich in Fe, Pb and Cu), covering 4630 ha of the Guadiamar riverbank (Arenas et al., 2001). During the following months the toxic sludge was removed, and the area was transformed into the currently protected Guadiamar Green Corridor. In 2002 an experimental site (50 m × 20 m) was established to study the effect of different amendments on trace element immobilization, related to a non-amended control soil (Madejón et al., 2006). Amendments were applied in 2002 and 2003 at 30 Tn ha⁻¹, respectively, in 7 m × 8 m plots (3 plots per treatment). Several studies concluded that, among the tested amendments, biosolid compost, made of sewage sludge and pruning residues, was the most efficient at raising soil pH, increasing total organic carbon, and immobilizing Cd, Cu and Zn (Burgos et al., 2006; Montiel-Rozas et al., 2018). The soil at the site is classified as Protocalcic Fluvisol (IUSS Working Group WRB, 2014).

2.2. Experimental design

The experiment was performed with soils collected from two contiguous locations (A and B) with the same topography in the described area. Site A corresponds to the above-mentioned experimental field where the long-term effect of soil amendment on soil properties was evaluated, while the 20-m-wide area surrounding site A was defined in this study as site B. Location B remained unamended after the toxic spill and has experienced a process of natural amelioration of pollution and colonization by vegetation. Both sites, A and B, are covered by grasses (dominated by *Heliotropium europaeum*, *Cynodon dactylon* and *Sonchus* spp.). It is worthwhile to note that while location A has been enclosed by a fence since 2002, location B was not fenced and thus has been exposed to extensive grazing by herbivores, mainly horses. This land use has been promoted by the landscape managers as way for weed control and fire risk reduction (Madejón et al., 2009). Therefore, soils from location A and B should not be considered simply as with a different history of amendment addition, but with a different legacy of soil management.

Regarding site A, soils were collected from the biosolid compost (BC) and the non-amended control (NA) plots. These soils were considered in this study as a long-term (LT) treatment, resulting in two soil types: LT-BC, amended with biosolid compost, and LT-NA, non-amended. The long-term effects of the application of biosolid compost in LT soils were compared to the short-term (ST) effects of the same amendment, using soil from site B.

At each location soil from the upper 20 cm was collected randomly from several points with a hoe to make a composite sample. After collection, sieving (removing rocks and leaf litter) and homogenization, ST soils were divided into two subsamples. The first subsample was subsequently amended with a biosolid compost (ST-BC) provided by the same supplier and at the same application rate as it was added to LT-BC in 2002 and 2003, but as a single dose (60 Tn ha⁻¹), while the second soil subsample remained as a non-amended control (ST-NA). The content of C, N, P and metallic elements (total and bioavailable fraction) in the applied biosolid compost is shown in Supplementary Table S1. This compost had a C:N ratio of 9.3, and high total contents of P, K, Ca, Mg and Fe. However, bioavailable forms of trace elements in the compost are in general low. For instance, Zn and Fe bioavailable fractions represented <2 % and 0.02 % of the total contents, respectively, while bioavailable Cu, Pb and Cd levels were below the detection limit.

This study was carried out under controlled greenhouse conditions using 2-l pots with a diameter of 13 cm. 12 pots were filled with each of the 4 soil treatments: LT-BC, LT-NA, ST-BC and ST-NA, as described above. Every pot was sowed with a forage mixture: *Lolium rigidum* (40 seeds per pot) and *Medicago polymorpha* (15 seeds per pot). Soil water holding capacity (WHC) was estimated for the non-amended soil, and all pots were watered twice a week with a constant volume of 80 ml, reaching 70 % of WHC.

One month after the establishment of the experiment, drought conditions were simulated in half of the replicates. Pots belonging to the drought treatment (DR) received 30 % less water supply (55 ml) compared to the control treatment (CT). This reduction was applied because, according to the IPCC's 2014 report (the latest report by the time this study was designed), this corresponds to the average rainfall reduction projected for both annual and growing season rainfall in the Mediterranean basin by the end of the century, under the A1B scenario. As a result, one month after the start of the study, the experiment was comprised of 8 treatments (2 soil management legacies × 2 amendment treatments × 2 water input treatments), each with 6 replicates. Drought conditions were kept for one month and soil moisture in the upper 5 cm of the soil surface was periodically measured before and after watering the pots with a ThetaProbe Soil Moisture Sensor (Delta-T Devices).

2.3. Plant cover and soil sampling

Germination was periodically (every 2–3 days) monitored and germination rate of each plant species was determined 20 days after sowing. During the experiment three soil samplings were carried out. Soils were first sampled (sampling 1; pre-seed) before the establishment of the experiment in order to characterize the soils of the four substrates used in the study (LT-BC, LT-NA, ST-BC and ST-NA). A second soil sampling (sampling 2; pre-drought) was performed one month later, before the beginning of the drought simulation. Finally, at the end of the experiment (two months after sowing and one month after the establishment of the drought treatment), the plant cover was manually mown and a final soil sampling was carried out (sampling 3; post-drought). Aerial plant biomass of both species was dried at 60 °C for 48 h and dry plant biomass from each species was separately measured.

At each sampling soil was carefully collected from the upper centimetres in several points of each pot using a lab spatula, and gently homogenised. Each soil sample was immediately divided into three subsamples. Subsequently, one subsample was air-dried and stored at room temperature to analyze soil chemical properties; 3 g of soil were immediately frozen and stored at –80 °C to analyze soil microbial DNA; and a third subsample was stored at 4 °C to study soil microbial activity (enzymatic activities and respiration rate). At each sampling main chemical properties (organic matter content, total C and N, and available P, K, Ca and Mg) as well as soil enzyme activities were analysed (see methods below). Total soil DNA and respiration rates were also determined at sampling 2 (pre-drought) and 3 (post-drought), as indexes of microbial biomass and activity. Available concentrations of micronutrients and other trace elements (Fe, Mn, Cu, Zn, Pb and Cd) were determined at sampling 1 (pre-seed) and 3 (post-drought). Soil samples were carefully and aseptically manipulated, especially the subsamples dedicated to the study of the microbial activity, to avoid sample contamination.

2.4. Analytical methods

2.4.1. Soil and biosolid compost chemical properties

Soil active and potential acidity were measured in a 1:2.5 soil:deionised water suspension (pHH₂O) and in 1:2.5 soil:0.1 M KCl suspension (pH_{KCl}), respectively, and soil electrical conductivity (EC) in a 1:5 soil:deionised water suspension. Soil organic matter (SOM) was estimated through loss on ignition at 550 °C for 3 h after drying the sample at 105 °C for 24 h (Hoogsteen et al., 2015). Total C and N were analysed using a micro elemental analyzer (Leco Truspect CHNS Micro). Available P was determined by Olsen method (Olsen et al., 1954). Ca, Mg, K and Na were extracted with pH 7.1 M ammonium acetate and measured using 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). Available Fe, Mn, Cu, Zn, Pb and Cd were extracted with 0.01 M CaCl₂ (Novozamsky et al., 1993) and quantified by atomic absorption spectroscopy. Total content of Ca, Mg, K, Na, Fe, Mn, Cu, Zn, Pb and Cd in the biosolid compost was measured by inductively coupled plasma-mass-spectrometry (ICP-MS, Agilent 7800), after a microwave digestion in a 1:3 HNO₃:HCl solution, and total C and N and bioavailable Fe, Mn, Cu, Zn, Pb and Cd were analysed in triplicate following the same methodology applied to soil samples.

2.4.2. Soil biological activity

Soil dehydrogenase activity (DHA) was measured by molecular absorption spectrometry using 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyl-tetrazolium chloride (INT) as substrate (Benefield et al., 1977). Aminopeptidase, β-glucosidase, phosphatase and *N*-acetyl-glucosaminidase activities were extracted in a 50 mM sodium acetate buffer solution at pH 5.5 and determined by fluorometry using 7-amino-4-methyl coumarin (AMC) and 4-methylumbelliferone (MUB) as fluorogenic

substrates (Marx et al., 2001). Soil DHA was expressed as μg INTF g⁻¹ dry soil⁻¹ h⁻¹ and the other enzymes as nmol AMC g⁻¹ dry soil⁻¹ h⁻¹ (aminopeptidase) and nmol MUB g⁻¹ dry soil⁻¹ h⁻¹.

Soil respiration rate was determined with an infra-red gas analyzer (EGM-4, PP Systems) after incubating 1 g of soil at 25 °C for 24 h in a closed glass vial, sealed with a septum (Bekku et al., 1995). Soil respiration rates were reported as g C-CO₂ g⁻¹ dry soil day⁻¹.

2.4.3. DNA extraction and characterization of soil microbial communities

Genomic DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil Kit (Quiagen) and DNA concentration was quantified with a Qubit fluorometer (ThermoFisher). Illumina high-throughput sequencing for 16S rDNA (341F/805R) and ITS rDNA (ITS86F/ITS4) were applied to DNA extracts from the last soil sampling (post-drought) to study soil bacterial and fungal communities, respectively, in a private sequencing service (Stab Vida, Portugal).

The Divisive Amplicon Denoising Algorithm (DADA2 R package, version 1.22.0) (Callahan et al., 2016) was applied to denoise raw sequences and infer the amplicon sequence variants (ASVs). The software FIGARO (Weinstein et al., 2019) was applied to 16S sequences to define the optimal trimming parameters. Cutadapt 4.0 (Martin, 2011) was used to remove the primers. After identifying and removing chimeric sequences, taxonomy was assigned to the resulting ASVs using the SILVA version 138.1 (Quast et al., 2012; Yilmaz et al., 2013; Glöckner et al., 2017) and UNITE (Abarenkov et al., 2022) databases for bacteria and fungi, respectively; and fungal taxa were classified into functional groups following the study of Pölme et al. (Pölme et al., 2020). Those ASVs which represented less than a 0.005 % of the sequences were filtered (Bokulich et al., 2013). The resulting ASVs were clustered using VSEARCH v2.21.1 (Rognes et al., 2016) and the LULU package (Froslev et al., 2017), and singletons were removed. Function rarefy in the vegan R package (Oksanen et al., 2022) was applied to compare the observed taxa richness with the expected richness in a theoretical sample with 1,000,000 reads. Rarefaction analysis showed that every sample curve from both data sets (bacterial and fungal) reached plateau, not being necessary to rarefy to a minimum number of reads per sample (McMurdie and Holmes, 2014). It was calculated that the observed ASV richness was over 96.53 % and 98.09 % of the expected bacterial and fungal ASV richness, respectively.

Fungal and bacterial indicator species associated with each soil type were determined using the Indicator Value (IndVal) index, that measures the association between a species and a specific site group (Dufrene and Legendre, 1997). Finally, Shannon and Whittaker indices were calculated to study bacterial and fungal alpha and beta-diversity among soil types.

2.5. Statistical analyses

All statistical analyses were carried out in R version 4.1.3 (RCoreTeam, 2022). Linear mixed models were used to study the effect of the biosolid compost application (LT and ST soils analysed separately), the drought conditions and the interaction of both factors on soil chemical properties, soil microbial activity and plant performance. Significant effects of the different experimental treatments on soil moisture were evaluated using mixed linear models (nlme package in R), with each experimental pot considered as a random factor to account for repeated measurements. For each model residuals were evaluated in order to check the model assumptions and to validate it. When homogeneity of variance was not met, a variance coefficient was introduced in the model to account for heteroskedasticity among different factor levels, using the varIdent function of the nlme package (Pinheiro and Bates, 2000). F statistic and *p*-value of each model are shown in Supplementary Tables S2, S3 and S4. In addition, correlation analyses between soil moisture and each plant species biomass were carried out.

Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities and permutational multivariate ANOVA (PERMANOVA)

analyses were carried out to study differences in the microbial community composition among treatments, using the vegan package in R.

3. Results

3.1. Soil moisture

Before the beginning of the simulated drought period soil moisture in LT pots was higher in LT-NA than in LT-BC (Fig. S1a), while in ST pots the biosolid compost increased soil moisture (Fig. S1b). This pattern continued during the whole simulated drought event in both LT and ST soils, so that in the LT soils moisture was significantly lower in the amended than in the non-amended treatment, while the opposite effect was observed in the ST soils (Fig. S1). The mixed linear model showed a significant effect of the organic amendment and the drought treatment, but not of the interaction, on soil moisture in both LT and ST soils.

Soil moisture measured just after pot watering showed that, during the drought period, maximum soil moisture in control pots was around 16 % in LT-BC, 15 % in LT-NA, 18 % in ST-BC and 16 % in ST-NA. However, maximum soil moisture in pots belonging to the drought treatment was around 14 % in ST-BC and 13 % in the others.

3.2. Plant cover performance

In LT pots, *L. rigidum* germination rate was significantly higher in LT-BC (43 ± 10 %) than in LT-NA (35 ± 9 %), while the germination of *M. polymorpha* was not significantly different in both treatments (average of 50 ± 14 % for both treatments pooled; Table S2). *L. rigidum* biomass was not affected by the amendment (1.7 ± 0.3 g on average considering both treatments; Table S3) but *M. polymorpha* biomass was highly increased in LT-BC (0.81 ± 0.41 g), compared to LT-NA (0.05 ± 0.02 g).

In ST pots, germination rates of *L. rigidum* (33 ± 8 %) and *M. polymorpha* (53 ± 12 %) were not affected by the application of compost (Table S2). However, for both species growth was enhanced by the BC application (Table S4). *L. rigidum* dry biomass was higher in ST-BC (2.2 ± 0.9 g) than in ST-NA (1.6 ± 0.7 g), while *M. polymorpha* had also a higher growth in ST-BC (0.9 ± 0.3 g) compared to ST-NA (0.60 ± 0.17 g). Biomass production in the two non-amended soils with a different legacy of management (LT-NA and ST-NA) was also different, in particular for *M. polymorpha*. In ST-NA pots, filled with soil collected from the no-fenced area and exposed to grazing, the biomass of this legume was 12 times higher than in LT-NA, belonging to soil collected from the experimental and fenced plot.

The impact of the drought treatment on plant growth depended on

the species and on soil management (Fig. 1; Tables S3 and S4). *L. rigidum* biomass was significantly affected by drought and by the amendment \times drought interaction, so that drought reduced plant growth in the LT-NA, ST-BC and ST-NA treatments, but not in the LT-BC soils. In contrast, *M. polymorpha* biomass was affected by drought conditions in the LT-BC soils only, but no differences were observed in LT-NA, ST-BC or ST-NA pots (Fig. 1).

A correlation analysis between soil water content at the end of the drought period and the plant cover biomass resulted in a positive Pearson correlation ($r = 0.32$, $p = 0.052$) between soil moisture and the *L. rigidum* biomass.

3.3. Soil chemical properties

The initial soil chemical characterization (pre-seed sampling; Table 1 and Table S2) showed that LT amended soils (LT-BC) had significantly higher values of soil organic matter (SOM), total C and N, and bioavailable P, K and Ca contents than non-amended soils (LT-NA). In contrast, LT-NA soils had higher electrical conductivity (EC) and available Fe, Mn and Zn contents compared to LT-BC. Soil $\text{pH}_{\text{H}_2\text{O}}$ in LT-BC was significantly higher than in LT-NA. ST-BC soils were also initially characterized by higher contents of SOM, total N, bioavailable P, K, Ca, as well as by a lower bioavailability of Fe and Zn compared to ST-NA soils. Higher contents of available Na and Mg were also found in ST-BC in comparison to ST-NA. However, soil $\text{pH}_{\text{H}_2\text{O}}$ was significantly lower in ST-BC compared to ST-NA, and the short-term biosolid compost application increased soil EC and bioavailable Mn in ST-BC. In all the four soil types, bioavailable Cu, Pb and Cd levels were below the detection limit.

In the pre-drought (one month after soil amendment; Table S5) and post-drought samplings (two months after soil amendment and one month after the establishment of the drought treatment; Table S6) soil chemical properties were still highly influenced by the biosolid compost application, showing the same pattern as in the initial sampling (Tables S2, S3 and S4). Measurements from the post-drought sampling showed that most of the analysed soil chemical properties were not significantly affected by the drought treatment or by the amendment \times drought interaction (Tables S3 and S4), and thus data were pooled. The only difference was the decrease in bioavailable Na under the simulated drought conditions (DR, 327 ± 20 mg kg^{-1}) compared to the control (CT, 403 ± 16 mg kg^{-1}) in the ST soils.

3.4. Soil biological activity

In general, compost addition had a positive effect on enzyme

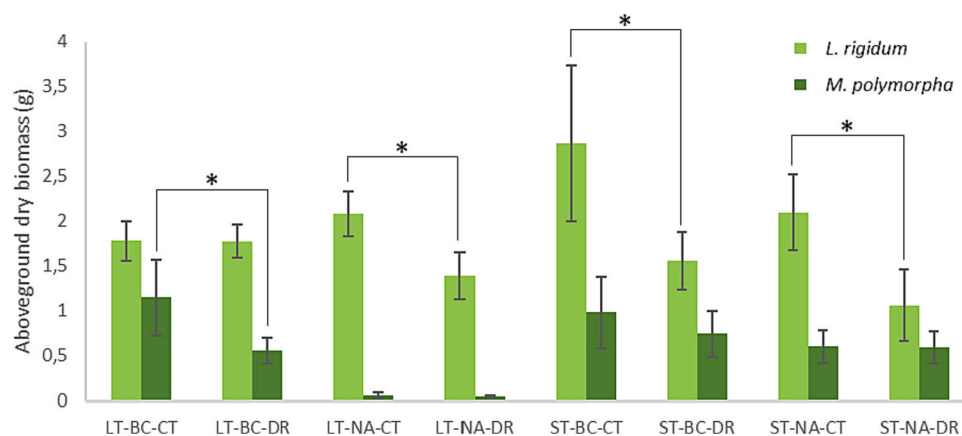


Fig. 1. Mean (bars) and standard deviation (lines) of the *Lolium rigidum* and *Medicago polymorpha* aboveground dry biomass produced in each treatment. * above bars indicates significant differences ($p < 0.05$) between the drought (DR, 30 % reduction of water supply) and control (CT, 100 % water supply) treatments within each soil type. LT: long-term amended soil; ST: short-term amended soil; BC: soil amended with biosolid compost; NA: non-amended soil.

Table 1

Initial chemical characterization (mean values \pm standard deviation) of the four soil types used in the experiment (pre-seed sampling). * Indicates significant differences between the amended and the non-amended soil at the 0.05 probability level, for LT and ST soils analysed separately. Detailed results of the statistical analysis are given in Supplementary Material, Table S2.

Parameters	Long-term experiment (LT) ^a		Short-term experiment (ST) ^b		
	BC ^c	NA ^d	BC ^c	NA ^d	
pH _{H2O}	7.12 \pm 0.08	4.56 \pm 0.04	* 6.59 \pm 0.09	6.78 \pm 0.05	*
pH _{KCl}	6.76 \pm 0.07	4.20 \pm 0.05	* 6.40 \pm 0.05	6.34 \pm 0.02	*
EC (dS m ⁻¹)	0.33 \pm 0.02	1.02 \pm 0.07	* 0.92 \pm 0.03	0.25 \pm 0.02	*
SOM (g kg ⁻¹)	91 \pm 1	61 \pm 1	* 97 \pm 12	62 \pm 4	*
C (g kg ⁻¹)	54 \pm 3	18 \pm 2	* 40 \pm 10	28 \pm 1	*
N (g kg ⁻¹)	4.03 \pm 0.29	0.92 \pm 0.17	* 4.45 \pm 0.69	2.57 \pm 0.04	*
P (mg kg ⁻¹)	129.8 \pm 6.4	20.1 \pm 0.9	* 97.3 \pm 6.1	21.2 \pm 0.3	*
K (mg kg ⁻¹)	241 \pm 41	138 \pm 11	* 422 \pm 12	157 \pm 5	*
Na (mg kg ⁻¹)	318 \pm 40	275 \pm 20	420 \pm 150	285 \pm 13	*
Ca (g kg ⁻¹)	5.5 \pm 1.0	2.5 \pm 0.4	* 4.7 \pm 0.8	3.2 \pm 0.3	*
Mg (mg kg ⁻¹)	147 \pm 50	168 \pm 30	285 \pm 60	147 \pm 50	*
Fe (mg kg ⁻¹)	2.13 \pm 0.15	6.27 \pm 0.81	* 1.60 \pm 0.26	3.37 \pm 0.55	*
Mn (mg kg ⁻¹)	8.83 \pm 0.06	69.33 \pm 1.15	* 7.90 \pm 0.40	5.13 \pm 0.29	*
Cu	nd	nd	nd	nd	
Zn (mg kg ⁻¹)	0.83 \pm 0.35	18.17 \pm 0.67	* 1.17 \pm 0.06	1.50 \pm 0.17	*
Pb	nd	nd	nd	nd	
Cd	nd	nd	nd	nd	

nd: Below detection limit.

^a Soils collected from the long-term experimental field (fenced 17 years ago and not exposed to grazing).

^b Soils collected from the unfenced area and exposed to extensive grazing for over 17 years.

^c Soil amended with biosolid compost.

^d Non-amended soil.

activities, in particular in the ST soils (Fig. 2; Tables S2, S3 and S4). Dehydrogenase activity in LT-BC soils was clearly greater than in LT-NA soils across the three samplings. In contrast, phosphatase activity was higher in the non-amended soils than in the LT-BC soils at the second (pre-drought) and third (post-drought) samplings. In the ST soils, compost addition had a clear positive effect on β -N-acetyl-glucosaminidase across the three samplings, and on phosphatase and β -glucosidase in the first (pre-seed) and second samplings. Dehydrogenase activity, in contrast, was higher in ST-NA than in ST-BC soils.

Without considering the amendment treatment, drought conditions significantly decreased soil β -glucosidase activity in DR (183 \pm 24 nmol MUB g⁻¹ h⁻¹) compared to CT (234 \pm 36 nmol MUB g⁻¹ h⁻¹) in the LT pots (Table S3). Soil dehydrogenase activity was also reduced in DR (5.4 \pm 0.6 μ g INTF g⁻¹ h⁻¹) compared to CT (6.1 \pm 0.9 μ g INTF g⁻¹ h⁻¹) in the ST pots (Table S4). No other significant effects of the drought treatment or the amendment \times drought interaction were observed in LT or ST soils (Tables S3 and S4). Fig. 3 shows the relative enzyme activities in DR pots compared to the activity in CT on each soil type, where values close to 1 indicate a high resistance of the enzyme activities to drought.

In LT, soil respiration rate was significantly increased by the biosolid compost application (3.9 \pm 1.6 μ g C-CO₂ g⁻¹ day⁻¹) compared to the non-amended control (1.7 \pm 1.4 μ g C-CO₂ g⁻¹ day⁻¹) at the pre-drought sampling (Table S2). This effect of the amendment treatment was also found at the post-drought sampling (3.6 \pm 1.2 μ g C-CO₂ g⁻¹ day⁻¹ in LT-BC and 2.3 \pm 1.4 μ g C-CO₂ g⁻¹ day⁻¹ in LT-NA; Table S3). No differences in soil respiration rates were found at the pre-seed sampling in ST soils (average of 4.9 \pm 2.1 μ g C-CO₂ g⁻¹ day⁻¹; Table S2), but at the final sampling amended soils had a slightly higher respiration rate (3.7 \pm 1.2 μ g C-CO₂ g⁻¹ day⁻¹) compared to the non-amended control (2.8 \pm 0.3

μ g C-CO₂ g⁻¹ day⁻¹; Table S4). Soil respiration rates in both long and short-term amended treatments were not significantly affected by the drought conditions or by the interaction amendment \times drought by the end of the simulated drought period (Tables S3 and S4).

3.5. Soil DNA concentration

Compost application had a clearer effect on soil DNA in the LT soils than in the ST soils (Tables S2, S3 and S4). LT-BC soils had 11.5 \pm 1.5 and 10.7 \pm 1.9 μ g DNA g⁻¹ soil at the pre and post-drought samplings, respectively, more than three times fold than LT-NA soils (3.2 \pm 0.8 and 2.7 \pm 0.6 μ g DNA g⁻¹ soil, respectively). However, after the short-term addition of compost (ST) total DNA did not significantly change (averages of 9.2 \pm 1.8 and 11.3 \pm 1.4 μ g DNA g⁻¹ soil for ST-BC and ST-NA, respectively). In addition, after the drought period total DNA in ST-BC (10.2 \pm 0.9 μ g DNA g⁻¹ soil) was significantly lower than in ST-NA (12.8 \pm 2.1 μ g DNA g⁻¹ soil). No significant effects of the drought conditions or the interaction between drought and compost addition were found for LT or ST soils (Tables S3 and S4).

3.6. Diversity and structure of soil bacteria and fungi

After the raw data purification, there were 2241 ASVs of bacteria belonging to 22 phyla, 47 classes, 100 orders, 143 families, and 252 genera. The NMDS analysis showed a high dissimilarity in bacterial composition due to compost addition in LT and in ST soils (Fig. 4a and b). Although the NMDS plots show an apparent separation between samples belonging to the drought (DR) and control (CT) treatments, PERMANOVA analyses determined a significant effect of the amendment treatment in both LT (F = 87.86; p < 0.001) and ST soils (F = 13.74; p < 0.001), but drought conditions and the amendment \times drought interaction did not significantly affect soil bacterial communities.

Bacterial community in LT-BC was dominated by the family WD2101 soil group (17 %), followed by *Chitinophagaceae* (5 %), *Pirellulaceae* (4 %) and *Vicinamibacteraceae* (4 %); while in LT-NA *Ktedonobacteraceae* (36 %), WD2101 soil group (6 %) and *Sphingomonadaceae* (5 %) were the most abundant families. ST-BC and ST-NA bacterial communities were dominated by WD2101 soil group (13 %), *Beijerinckiaceae* (5 %) and *Sphingomonadaceae* (5 %).

The indicator species analysis identified 561 ASVs in LT-BC, 366 in LT-NA, 332 in ST-BC and 510 ASVs in ST-NA for which the indicator value was significant (p < 0.05). From the total indicator species from each group, 71 bacterial taxa (mainly belonging to phylum *Acidobacteriota* and *Planctomycetota*) were specific and ubiquitous in LT-BC samples, 197 (mainly belonging to phylum *Actinobacteriota*, *Proteobacteria* and *Planctomycetota*) in LT-NA, 24 (mostly from phylum *Proteobacteria* and *Patescibacteria*) in ST-BC and 14 (mostly from phylum *Bacteroidota*, *Acidobacteriota* and *Myxococcota*) in ST-NA.

The Shannon Diversity Index (H') calculated for the soil bacterial community was significantly affected by the amendment treatment (Tables S3 and S4). A higher alpha diversity was found in LT-BC (6.33 \pm 0.007) than in LT-NA (4.91 \pm 0.12). No difference was found between ST-BC (6.17 \pm 0.04) and ST-NA (6.21 \pm 0.05) treatments, and no significant effects of the drought conditions and the interaction between the amendment and drought treatments were observed (Tables S3 and S4). Bacterial beta diversity, estimated by the Whittaker Index and expressed by mean distances to centroids, was also significantly higher in LT-BC (0.13 \pm 0.02) than in LT-NA (0.08 \pm 0.01); while no significant difference was found between ST-BC (0.14 \pm 0.02) and ST-NA (0.12 \pm 0.01), and no effects of the drought treatment or the interaction amendment \times drought were observed.

The fungal community analysis resulted in 594 ASVs, classified in 7 phyla, 24 classes, 53 orders, 118 families and 164 genera. The NMDS analysis showed more similarity of the fungal community in ST-BC and ST-NA soils, in comparison to bacteria (Fig. 4d), while fungal communities in LT-BC and LT-NA were very different (Fig. 4c). However, the

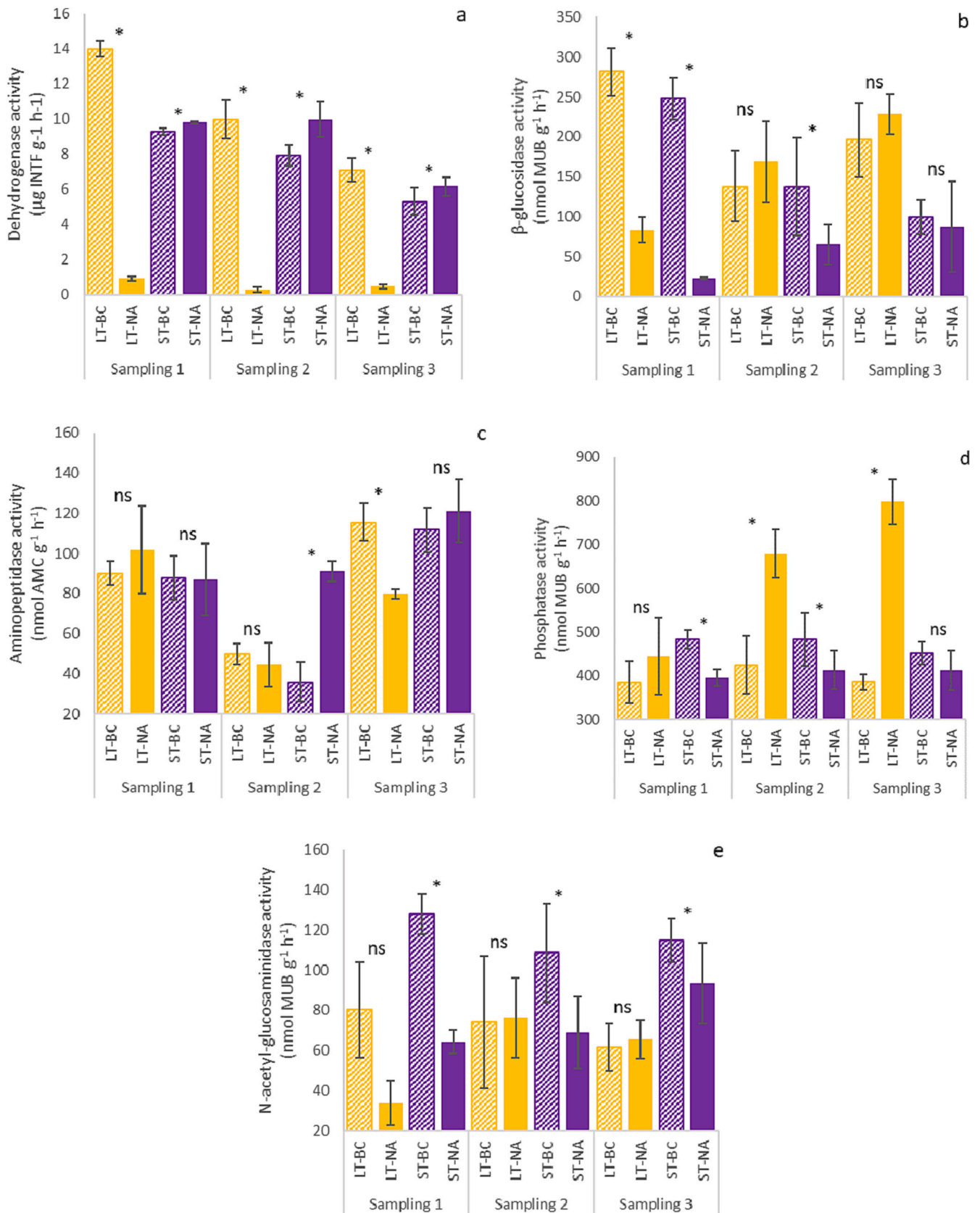


Fig. 2. Mean (bars) and standard deviation (lines) of soil dehydrogenase (a), β -glucosidase (b), aminopeptidase (c), phosphatase (d) and N-acetyl-glucosaminidase (e) activities at samplings 1 (pre-seed), 2 (pre-drought) and 3 (post-drought). * indicates significant differences ($p < 0.05$) between the amended (BC) and the non-amended control (NA) treatments within each soil type, with different legacies of soil management (long-term, LT- non-exposed to grazing; short-term, ST -exposed to grazing-). ns indicates no significant differences between treatments ($p > 0.05$).

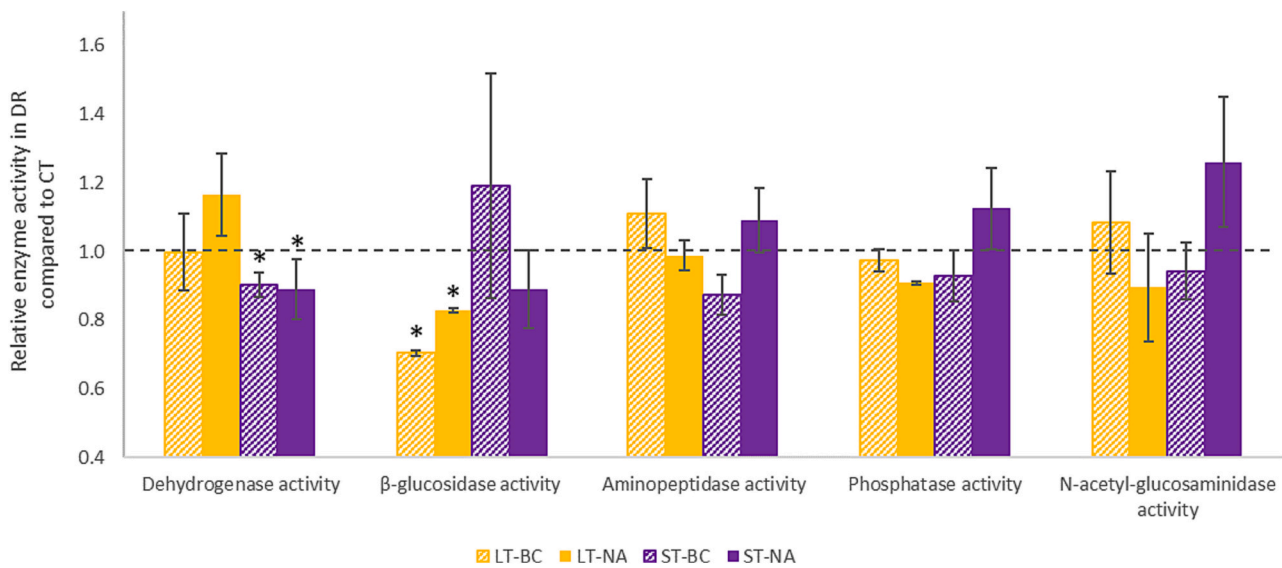


Fig. 3. Effect of drought conditions on soil enzyme activities. Mean (and standard deviation) enzyme activity measured in pots belonging to the drought treatment (DR) in relation to mean enzyme activity recorded under control conditions (CT) for each soil type. Values below and above 1 indicate a reduction and increase in enzyme activity in DR compared to CT, respectively. LT: long-term amended soil; ST: short-term amended soil; BC: soil amended with biosolid compost; NA: non-amended soil. Although β-glucosidase and dehydrogenase activities were reduced by drought conditions in LT and ST soils, respectively, no significant effects of the amendment × drought interaction were observed.

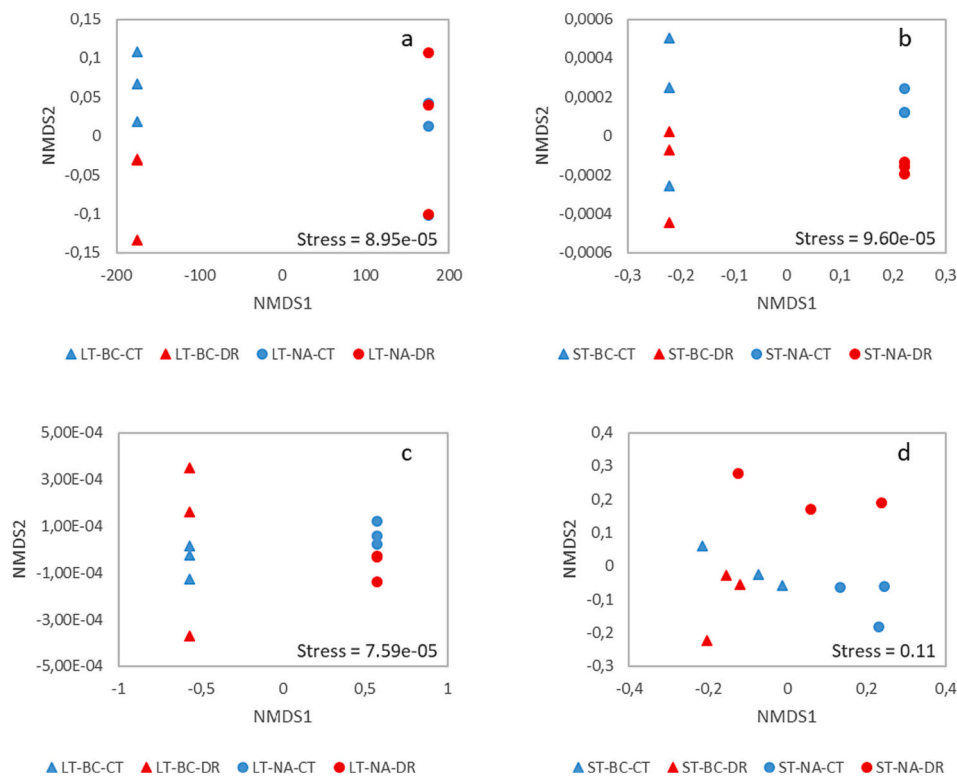


Fig. 4. NMDS analysis of the soil bacterial (a and b) and fungal (c and d) community in the four soil types at the end of the drought simulation. LT: long-term treatment; ST: short-term treatment; BC: soil amended with biosolid compost; NA: non-amended soil; CT: control treatment (100 % water supply); DR: drought treatment (30 % reduction of water supply).

separation of CT and DR samples within each group was less evident. The PERMANOVA analysis showed a clear effect of the organic amendment treatment in both LT ($F = 25.51; p < 0.001$) and ST soils ($F = 2.87; p < 0.001$) on the fungal community composition, while no significant effect of the drought treatment or the interaction was found.

In LT-BC the fungal community was dominated by the families

Nectriaceae (19 %), *Pleosporaceae* (10 %), *Phaeosphaeriaceae* (9 %) and *Cladosporiaceae* (8 %). In LT-NA the most abundant fungal families were *Pleosporaceae* (18 %), *Filobasidiaceae* (10 %), *Nectriaceae* (9 %) and *Didymosphaeriaceae* (7 %). Fungal communities in ST-BC and ST-NA soils, as well as in LT-BC, were dominated by the family *Nectriaceae* (15 %), followed by *Phaeosphaeriaceae* (9 %), *Pleosporaceae* (9 %) and

Cladosporiaceae (8 %).

The indicator species analysis identified 92 ASVs in LT-BC, 121 in LT-NA, 69 in ST-BC and 52 ASVs in ST-NA for which the indicator value was significant ($p < 0.05$). 4 fungal taxa (belonging to phylum *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Mortierellomycota*) were specific and ubiquitous in LT-BC samples, 24 (belonging to phylum *Ascomycota*, *Basidiomycota*, *Chytridiomycota* and *Mucoromycota*) in LT-NA, 1 (family *Powellomycetaceae*) in ST-BC and none in ST-NA.

Relative abundances of the main fungal traits are represented in Fig. 5. Plant pathogens (45 % in LT-BC, 32 % in LT-NA and 41 % in ST soils), litter saprotrophs (20 % in LT-BC, 16 % in LT-NA and 20 % in ST soils) and soil saprotrophs (11 % in LT-BC, 13 % in LT-NA and 11 % in ST soils) were the dominant fungal traits. Dung saprotrophs represented a 5 % in LT-BC and a 9 % in ST, but <1 % in LT-NA. Pollen saprotrophs were more abundant in LT-NA (7 %) than in LT-BC (2 %) and ST soils (1 %). The identified arbuscular mycorrhizal fungi (AMF) represented the 0.6 % in LT-BC, 0.05 % in LT-NA and 0.3 % in ST.

The Shannon Diversity Index of the soil fungal community showed a significantly (Table S3) higher alpha diversity in LT-BC (4.23 ± 0.16) than in LT-NA (4.04 ± 0.07). However, fungal alpha diversity in ST-BC (4.32 ± 0.07) and ST-NA (4.24 ± 0.21) was not significantly different (Table S4). Drought conditions and the interaction between the amendment and drought treatments did not have a significant effect on the Shannon Diversity Index (Tables S3 and S4). Fungal beta diversity was also significantly lower in LT-NA (0.135 ± 0.027) than in LT-BC (0.174 ± 0.003); while beta diversity in ST-BC (0.159 ± 0.011) and ST-NA (0.182 ± 0.020) was not significantly different. No effects of the drought treatment or the interaction were observed (Tables S3 and S4).

4. Discussion

4.1. Compost addition and legacy effects on soil moisture and plant productivity

In this work we have evaluated whether the addition of organic amendments confers a greater stability of soil functioning against reduction in water inputs in a degraded Mediterranean soil. We have used soils from adjacent locations (and thus of the same soil type) in a contaminated area; despite been located 20 m apart from each other,

these two soil groups that have been exposed to different conditions over a period of 17 years, namely the addition of biosolid compost and the exposure to extensive livestock grazing. The short-term application of biosolid compost, in accordance to previous studies (Hueso et al., 2011) and to our initial hypothesis, enhanced water retention and increased soil moisture in ST-BC pots compared to the non-amended ST-NA. Nevertheless, this positive effect of the organic amendment on soil moisture was not observed in the long-term amended treatment, despite the increase in organic matter in the LT-BC soils was still evident, 20 years after the start of the experiment and 17 years after the last compost addition. The greater biomass of *M. polymorpha* plants in LT-BC than in LT-NA may have contributed to a higher water loss through evapotranspiration and explain the lower soil moisture in LT-BC compared to LT-NA. Thus, *M. polymorpha* growth was particularly sensitive to the adverse soil chemical conditions in the LT-NA soils (discussed below).

In the soils from the long-term experiment the addition of biosolid compost increased *L. rigidum* germination rate, but this effect was not found in the short-term amended pots. Despite the positive effects of the biosolid compost application on soil properties, this organic amendment may contain chemical substances (ions, short chain fatty acids, phenolic compounds) with potentially phytotoxic properties that may reduce seed germination rates in the short term (Zubillaga and Lavado, 2006), and could explain the low germination rate of *L. rigidum* in ST-BC. The germination rate reduction in LT-NA and ST-BC may also be related to the high soil electrical conductivity of these soils, and to the low pH of LT-NA soils. This is consistent with a previous experiment that demonstrated the negative effect of soil salinity, due to osmotic potential, on the germination of two other species of ryegrass: *L. multiflorum* Lam. and *L. perenne* L. (Lin et al., 2018). *M. polymorpha* is known, however, for its adaptations and tolerance to soils with high salt concentrations (Nichols et al., 2009), and its germination rates, not affected by the different soil types of this study, reached the maximum expected under greenhouse controlled conditions (Wagner and Spira, 1994).

By the end of the experiment, probably related to the higher soil moisture and the higher availability of macronutrients (N, P, K and Ca), the growth of both species was favoured by the amendment application in both long and short-term scenarios (only excluding *L. rigidum* biomass in LT soils). Nevertheless, the scarce growth of *M. polymorpha* in LT-NA could be explained by the low tolerance of this species to soil acidity

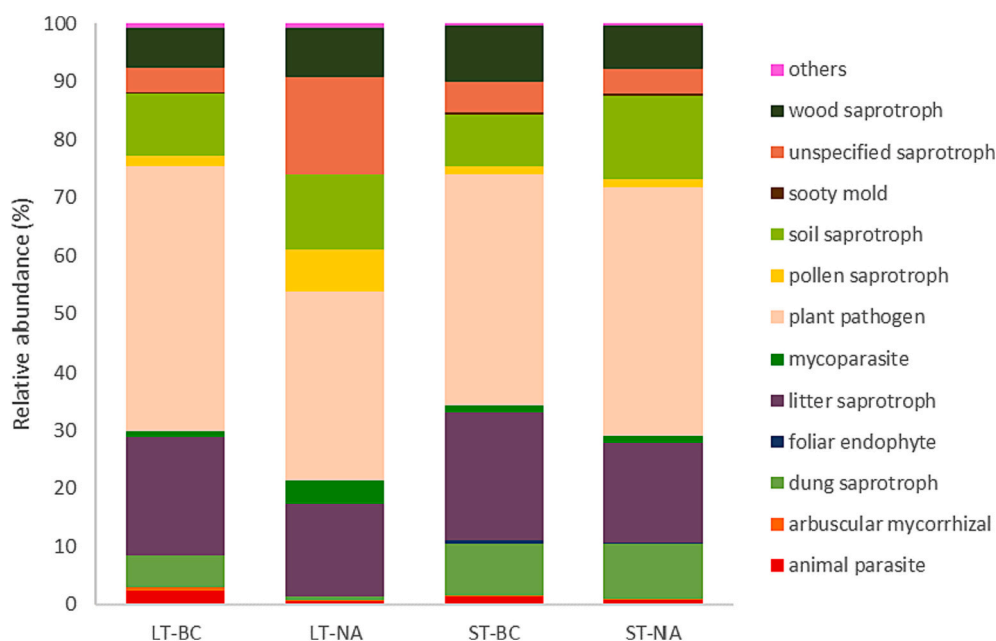


Fig. 5. Average relative abundances of the main fungal traits in each soil group. LT: long-term treatment; ST: short-term treatment; BC: soil amended with biosolid compost; NA: non-amended soil.

(Howieson et al., 1991), demonstrating the important influence of an adequate soil management after an acidification episode on plant productivity.

The simulated drought conditions negatively affected *L. rigidum* growth across all soil types except for the LT-BC treatment. Under our experimental conditions, *M. polymorpha* was more tolerant to water stress and its development was not impacted by the drought conditions, except in the LT-BC pots, where the measured biomass was significantly reduced by the drought treatment, probably due to a high interspecific competition with *L. rigidum*. In summary, biosolid compost had a long-lasting positive effect on the stability of plant productivity against a drought episode, but mainly for the species with the less tolerance to water stress.

4.2. Compost and drought effects on soil chemistry and biological activity

As expected, the long-term application of biosolid compost increased soil organic matter, total C and N, and macronutrient (P, K and Ca) availability. In the long-term experimental site several works have previously showed that the organic amendment also reduced soil salinity and increased soil pH, ameliorating soil acidity, what promotes trace element (Fe, Mn and Zn) immobilization (Burgos et al., 2006), and enhance soil biological activity (Montiel-Rozas et al., 2018). This work confirms that these effects lasted 17 years after the last BC application. In the short-term, biosolid compost had quick and positive effect on organic matter content, total N, and available macronutrients (P, K, Ca and Mg).

The strong influence of the different legacies of soil management is patent when the two non-amended soils are compared. ST-NA soils, exposed to a long-term legacy of extensive grazing, showed a much better soil quality than LT-NA soils, collected from the fenced experimental area. ST-NA soils had higher total content of C and N, as well as higher, neutral pH that promotes the immobilization of trace elements (Fe, Mn and Zn). Extensive grazing by horses has been promoted by the landscape managers as way for weed control and fire risk reduction (Madejón et al., 2009). It is possible that after more than a decade of grazing the organic matter inputs from horses has favoured the natural remediation of the soil. A previous study carried out with soils contaminated with heavy metals showed that the application of livestock manure raises soil organic C, increases soil pH and reduces metal bioavailability (Pérez-Esteban et al., 2014). In addition, it is possible that the movement of horses during grazing has promoted a higher colonization of bare soils by plants, by transporting seeds from other locations and alleviating soil compaction. It is known that a legacy of grazing exposure in disturbed grasslands enhances weed recruitment and diversity by mediating in below- and aboveground resource competition. Besides, cattle trampling promotes plant growth and germination, although this effect is limited by soil moisture and nutrient availability (Renne et al., 2006; Renne and Tracy, 2007).

Despite the great effect of the amendment treatment on soil chemical properties, the simulated drought event did not influence soil chemistry. However, drought conditions in ST-BC reduced soil available Na. It is known that, under water stress conditions, plant roots are able to absorb and accumulate salt ions, specially K^+ and Na^+ , to reduce their osmotic potential and promote water absorption (Huihui et al., 2021), what could explain the observed reduction in Na content in DR pots.

As a result of the positive effect of the long-term application of biosolid compost on soil chemical properties, soil biological activity was also enhanced. Total DNA and soil dehydrogenase activity, considered as a sensitive indicator of the overall microbial activity in degraded Mediterranean soils (García et al., 1997), were significantly higher in LT-BC soils than in LT-NA, and this effect was maintained until the end of the experiment. However, this effect was not observed in the short-term amended soils, probably due to the short period of time (two months) after the biosolid compost addition, and due to the better soil conditions in ST-NA compared to LT-NA soils. A different study carried

out using the same degraded soil and biosolid compost demonstrated that the amendment enhanced microbial biomass and, six months after the application, promoted soil dehydrogenase activity and plant cover development (Pérez de Mora et al., 2005).

Soil β -glucosidase and β -N-acetyl-glucosaminidase activities, positively correlated with the amount of soil organic C and total N (Deng and Tabatabai, 1996; Ekenler and Tabatabai, 2004), were positively affected by the organic amendment and immediately enhanced after the biosolid compost addition. β -glucosidase activity, considered as a good indicator of soil biological quality, quickly responds to the improvement in soil properties, especially with the increase in labile organic C (Turner et al., 2002). Therefore, the observed increase in soil β -glucosidase activity in both non-amended treatments may be explained by the development of the plant cover and the release of root exudates (Vives-Peris et al., 2020).

Previous studies reported that soil aminopeptidase activity is not influenced by the addition of inorganic N but it increases when organic N compounds are added to the soil (Norman et al., 2020). In addition, a higher availability of inorganic N in the soil suppresses plant dependency from organic N sources (Romero et al., 2023). In our study, there was a positive correlation between *M. polymorpha* biomass and the aminopeptidase activity by the end of the experiment ($\rho = 0.65$, $p = 0.007$), probably due to a substrate induction response as a result of the atmospheric N fixation.

Phosphatase activity is positively correlated with soil organic P, but also with soil microbial biomass and total N content (Margalef et al., 2017). P-rich organic amendments stimulate microbial biomass and phosphatase activity, especially in the short-term, although the enzyme activity gradually decreases with time (Criquet et al., 2007). These results are consistent with our results and explain the increase in phosphatase activity in ST-BC, but not in the soils from the long-term experiment. Indeed, in these soils phosphatase activity was much higher in LT-NA. These soils, with a low content of total N, were characterized by a very low pH. Other studies have demonstrated that microbial communities in N-limited acidic soils tend to be more dominated by fungi, what leads to a higher phosphatase activity (Margalef et al., 2017).

Biosolid compost application also increased soil respiration rate, as found in previous works (Montiel-Rozas et al., 2016). The low soil respiration in LT-NA is consistent with the lower soil quality and the lower microbial biomass under this treatment compared to LT-BC, but the development of the plant cover increased soil respiration by the end of the experiment, as found in other experiments with degraded soils (Oyonarte et al., 2012). The addition of the organic amendment at short-term increased soil respiration in ST-BC two months after the compost application. This result is supported by others obtained in a previous study which demonstrated that an increase in soil organic matter enhances microbial respiration in a degraded soil (Kowaljaw and Mazarino, 2007).

Under the experimental conditions, soil biological activity (total DNA, the analysed enzyme activities and soil respiration rate) was not limited by the simulated drought conditions, and no effects of the drought treatment (except for a light reduction in dehydrogenase and β -glucosidase activities, Fig. 3) or the amendment \times drought interaction were recorded. Our hypothesis that the application of organic amendments promotes a higher stability of soil functioning against drought was therefore only confirmed for the productivity of the plant species with a lower resistance to water stress (*L. perenne*), but not for *M. polymorpha* or soil biological activity. It must be considered that, according to data from the National Agency for Meteorology (AEMET), the area where soils were collected from has an average monthly temperature of 27 °C and average monthly precipitation of 5.6 mm during the summer season (June, July, and August; period 1981–2010), leading to severe water stress conditions. Soil microbial communities in such semi-arid Mediterranean regions are adapted to drought, and a previous study showed that soil biological activity in this kind of soils was only

reduced when soil moisture was below 2 % (Hueso et al., 2011). A previous experiment carried out in the same experimental area showed that mean soil moisture (calculated after drying the soil samples at 105 °C for 24 h) was around 7 % in March, while it was reduced to a 2.5 % in June, at the beginning of the dry season (Madejón et al., 2019). However, under our experimental conditions, mean soil moisture of samples belonging to the drought treatment was over 5.5 % at the third soil sampling. This fact could have limited the effect of the simulated drought conditions, as well as the potential positive effect of the organic amendment on soil biological activity resistance against water stress.

4.3. Effects on bacterial and fungal communities

The study of soil microbial community showed a great effect of the long-term compost addition, but also of the long-term exposure to extensive grazing in ST soils. Several studies have demonstrated that the application of different types of organic amendments can lead to a shift in soil bacterial community composition. In a previous experiment the long-term application of an organic manure stimulated the abundance of Chitinophagaceae, associated to the cellulose decomposition and Beijerinckiaceae families (Gautam et al., 2020). Pirellulaceae population was also increased by soil amendment with sewage sludge (Li et al., 2021b), and an increase in Vicinamibacteraceae abundance was observed after the application of an organic mulch (Xu et al., 2022). Vicinamibacteraceae and Sphingomonadaceae were also enhanced by the application of corn straw biochar and seaweed compost, respectively (Yin et al., 2022). The addition of biochar (Cheng et al., 2017) and a manure-based compost (Deng et al., 2022) increased Sphingomonadaceae abundance. These results support the dominance of these bacterial families in the long-term amended soil (LT-BC) and the soils that had been exposed to organic matter inputs through livestock activity (ST-BC and ST-NA).

The dominance of Ktedonobacteraceae and Sphingomonadaceae in the non-amended LT-NA treatment may be explained by the low soil pH. Both families are found in acidic soils (pH around 4), and their relative abundance was increased after the addition of biochar (Xu et al., 2014; Jutakanoke et al., 2023) or the use of cover crops (Shen and Lin, 2021). The WD2101 soil group, present in the four soil types, is known to have a wide distribution in terrestrial habitats and a special association with some grass species has been demonstrated (Florian et al., 2021; Lewin et al., 2021).

Fungal communities in LT-BC and both ST treatments were dominated by the same families and fungal traits. The observed fungal taxa belonging to Nectriaceae and Pleosporaceae families were mainly plant pathogens, taxa from family Phaeosporiaceae were plant pathogens and litter saprotrophs, and taxa belonging to family Cladosporiaceae were litter saprotrophs (Pölme et al., 2020). The higher abundance of dung saprotrophs in LT-BC and in soils exposed to grazing (ST-BC and ST-NA) compared to LT-NA was remarkable, which is consistent with the presence of organic matter inputs in these treatments. In LT-NA the lower relative abundance of plant pathogens and litter saprotrophs compared to the other soil types may be the result of the scarce development of the plant cover under this treatment. Fungal taxa belonging to family Filobasidiaceae were mainly unspecified saprotrophs and the family Didymosphaeriaceae was dominated by wood saprotrophs (Pölme et al., 2020).

Pollen constitutes a source of nutrient-rich fine particulate organic matter, with a special relevance in oligotrophic systems, and pollen grains are colonized by saprophytic fungi (Wurzbacher et al., 2014). This fact could explain the high abundance of pollen saprotrophs in LT-NA soils, poor in N and organic matter.

Arbuscular mycorrhizal fungi (AMF) relative abundances were also ten times lower in LT-NA soils than in the rest of the treatments. Nevertheless, the observed abundances of AMF were lower than the expected in a grassland (Honnay et al., 2017). The ITS86F/ITS4 primer pair is useful for the analysis of fungal communities from soil samples,

but it is biased for Ascomycota, Basidiomycota and Zygomycota clades, which may result in the underestimation of AMF abundance (Vancov and Keen, 2009).

The great number of indicator species (bacteria and fungi) exclusive and ubiquitous in LT-NA samples indicates the high difference in soil microbial community composition under this treatment, non-amended and excluded from the cattle effect, compared to the other soil types. Bacterial and fungal alpha and beta diversity were also lower in LT-NA than in LT-BC, probably due to the more adverse soil chemical properties.

The PERMANOVA analysis showed a significant effect of the amendment treatment on bacterial and fungal community compositions in both LT and ST soils. However, microbial communities in ST-BC and ST-NA were very similar and no effect of the biosolid compost application was observed on alpha and beta diversity, what indicates that effects of organic amendments on soil microbial communities may only be noticed in the long-term.

It is also very important to point out that the simulated drought conditions or the interaction amendment × drought, did not influence soil microbial community composition and diversity. Soil microbial communities from soils exposed to a recurrent drought conditions, as the Mediterranean soils, have developed strategies against water stress and are more resistant to drought events (Evans and Wallenstein, 2012). Long-term dry-rewetting episodes result in shifts in the composition of soil bacterial and fungal communities, increasing the abundance of tolerant taxa (Meisner et al., 2018).

5. Conclusion

The application of biosolid compost increased soil organic matter and enhanced water retention in the short-term. The organic amendment also had a positive effect on the plant cover, promoting plant production. It was remarkable the positive effect of the long-term application of biosolid compost on the stability of plant productivity under drought conditions, especially of species with a high vulnerability to water stress (*L. rigidum*).

The positive effects of biosolid compost on soil chemical properties (increases in soil pH, soil organic matter, total C and N content, and macronutrient bioavailability) were apparent 17 years after soil amendment and were also observed after the short-term application. However, the long-term legacy of extensive grazing also improved soil quality, promoting the natural remediation of the soil without the aid of the addition of amendments.

Regarding the soil microbial community, our results showed a clear effect of the long-term organic amendment, as well as the long-term exposure to livestock grazing, on soil microbial community, showing great similarities in bacterial and fungal composition. Furthermore, microbial community in the non-amended and non-grazed soil was driven by the high soil acidity and the low N and organic matter content.

Nevertheless, under our experimental conditions, soil biological activity, as well as microbial community composition and diversity, were not conditioned by the simulated drought event, probably due to microbial adaptations to water stress as consequence of a long-term exposure to natural dry-rewetting episodes. Once demonstrated the positive effect of organic C increase (via biosolid compost addition or a long-term exposure to extensive grazing) on soil chemical properties and microbial activity, further work that simulates harder drought conditions is necessary to evaluate the effect of these improvements on the resistance against drought events in semi-arid soils. Also, future studies using a higher number of soils with a gradient of organic matter content (via organic amendment application or taking advantage of different soil legacies) could be useful to infer the effect of soil chemical properties on the composition and diversity of the microbial community and the resistance to drought events.

CRedit authorship contribution statement

Laura Morales Salmerón: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **María Elena Fernández Boy:** Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Engracia María Madejón Rodríguez:** Resources, Supervision, Writing – review & editing. **María Teresa Domínguez Núñez:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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 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.apsoil.2023.105226>.

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