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ORIGINAL PAPER

Does an enhanced microbial diversity promote the resistance of soil multifunctionality against drought events in amended soils?

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Abstract

A large fraction of the Mediterranean soils is threatened by losses of organic matter and biodiversity, which could compromise the provision of soil ecosystem services and the stability of ecosystems in the face of climate change. In this work we explore several hypotheses related to the role of C inputs and microbial diversity on soil multifunctionality and its resistance to drought in degraded Mediterranean soils. We designed a factorial experiment to test the effect of the addition of an organic amendment and of microbial diversity (using four inoculants with different abundance and diversity of soil microbiota), on the resistance of soil functionality against drought in pot mesocosms. Pots were sown with a forage mixture (*Lolium rigidum* and *Medicago polymorpha*), and plant productivity, soil chemical properties, and microbial activity and diversity were measured before and after a simulated drought event. The amendment favored soil moisture, enhancing the stability of the productivity of *M. polymorpha*. In contrast, the manipulation of inoculation load had a limited effect on the resistance of microbiological activity. Indeed, microbial functioning was highly resistant to reduced water inputs, probably related to the prevalence of Gram positive bacteria. Besides, the effect of microbial diversity on soil multifunctionality was limited. Structural equation modelling confirmed that the enhancement of multifunctionality after soil amendment was attributed to the direct effect of organic C on soil moisture and chemical fertility. In these degraded soils, physico-chemical limitations are the major drivers of soil multifunctionality rather than bacterial or fungal diversity.

Keywords Water stress · SOM · Enzyme activity · Respiration rate · qPCR · DNA sequencing

Introduction

Although recent climate change projections indicate an increase in the frequency and intensity of heavy precipitation events in Northern Europe by the end of the century, countries in Southern Europe will face water scarcity due to heat and drought severity, that will affect food security (IPCC 2023). In Mediterranean countries, the annual mean moisture in the soil column, which is determined by the annual mean precipitation and evapotranspiration, is projected to decrease by 1-1.5 standard deviations of the interannual variability in soil moisture recorded between 1850 and 1900 (IPCC 2023).

There is much evidence that drought can have a negative impact on soil functions, including plant production and nutrient cycling, and that this impact can be more severe in croplands and grasslands than in shrublands and forests (Qu et al. 2023). One way to assess the impacts of abiotic factors or perturbation events on soil functioning is the multifunctionality approach. The concept of ecosystem multifunctionality attempts to represent an overview of all functions that perform simultaneously within an ecosystem (including biological, geochemical, and physical processes). It serves as an integrative tool to assess the overall functioning of an ecosystem (Manning et al. 2018).

Recent studies carried out in natural terrestrial ecosystems have described the key role of soil microbial diversity and richness on soil multifunctionality (Delgado-Baquerizo et al. 2016a, b, 2020), so that diversity and multifunctionality are positively related. Despite this fact is considered as

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a global trend, it has been also found that the effect of soil biodiversity on soil functioning is mediated by soil abiotic properties and climatic conditions (Delgado-Baquerizo et al. 2020), which may be especially relevant in degraded soils, given that these soils are often characterised by harsh chemical or physical conditions. For instance, Xue et al. (2023) observed how soil multifunctionality was conditioned by fungal diversity, and decreased by agricultural intensification, which provoked a decline in soil organic matter decomposition. However, they found that land degradation weakened the link between soil microbial community and soil individual functions (Xue et al. 2023), mainly due to the impacts of agricultural intensification on SOM decomposition gene and the abundance of saprotrophic and plant symbiotic fungi. A second study that compared soils across a gradient of ecological succession stages after agriculture abandonment (grassland, shrubland, forest) detected that, even though soil multifunctionality was the highest in forests, the positive correlation between soil multifunctionality and fungal diversity was only patent in grasslands, suggesting that the role of microbial diversity in driving soil functioning rely on the ecosystem properties (Chen et al. 2023). In addition, they highlighted the importance of soil properties (particularly soil pH) in controlling microbial diversity and its positive correlation with soil multifunctionality.

Soil microbial diversity might also have a critical role in the stability of soil functions against ecosystem perturbations, such as severe droughts or soil contamination. As could be expected following the positive relationship between microbial diversity and soil functioning, the loss in soil diversity would weaken soil functions and negatively affect soil stability. According to the insurance hypothesis proposed by Yachi and Loreau (1999), biodiversity serves as a safeguard for ecosystems, ensuring their functionality even in the face of a potential decline in diversity. The presence of numerous species increases the likelihood that certain species will continue to support ecosystem functions, even if others may not succeed. However, the relationship between diversity and stability in soil systems remains unclear. For instance, it has been noted that the primary factor influencing soil resistance is not the microbial diversity, but rather the different response of bacterial and fungal taxa to environmental fluctuations and perturbations (Loreau and de Mazancourt 2013). Other studies have demonstrated that the role of soil microbial diversity on soil stability is also conditioned by soil physico-chemical properties and the functional traits of the microbial species (Griffiths and Philippot 2013).

As exposed above, in degraded soils the role of microbial diversity on soil multifunctionality might rely on soil chemical properties. In these soils, organic amendments have been traditionally used to improve soil properties and enhance soil fertility by ameliorating adverse pH conditions, and increasing nutrient (N, P and K) and organic matter contents, what commonly leads to an important increment in soil microbial biomass and enzyme activity (Diacono and Montemurro 2010). In addition, from a deterministic perspective (Chase and Myers 2011), the addition of an organic amendment that significantly alters soil properties, especially in degraded soils, may in turn influence microbial community composition and function. Besides, since organic matter improves soil physical structure (through increasing the amount and stability of aggregates, and decreasing soil bulk density), organic amendments promote water retention and confer soils a higher resistance to drought conditions (Diacono and Montemurro 2010; Lal 2015).

The main objective of this study is to evaluate the effect of the application of an organic amendment on the stability of soil multifunctionality against a reduction in water inputs in a degraded soil. Unlike previous studies that focus on either soil biodiversity or soil chemical properties in isolation, the innovation of this research lies in its integrated approach to evaluating the stability of soil multifunctionality under drought conditions by discerning between the biological and physico-chemical effects of the amendment addition. For this purpose, a factorial design was implemented with mesocosms filled with a common autoclaved, degraded soil treated with two levels of an organic amendment and four microbial inoculums in a gradient of soil microbial biodiversity. We evaluated the influence of soil physico-chemical properties and microbial diversity on plant production, microbiological activity (enzyme activities and soil respiration rate), and on the stability of soil functioning to a simulated drought event. Because previous works have shown that in degraded soils the relationship between soil microbial diversity and multifunctionality is conditioned by soil chemical properties, we hypothesized that the addition of the biosolid compost will ameliorate soil abiotic conditions, increasing the positive influence of soil biodiversity on soil multifunctionality, compared to the non-amended control soil. Additionally, the study advances the understanding of the resistance of Mediterranean degraded soils to drought by linking soil biodiversity to multifunctionality in the presence and absence of organic amendments. We expected that the addition of the organic amendment will promote the stability of soil functioning against drought, and that this will be connected with both the improvement in physico-chemical properties and with increases in soil biodiversity due to the organic inputs. Along the experimental gradient of soil biodiversity in non-amended soils, we also expected a positive relationship between soil biodiversity and the stability of soil functioning against drought.

Materials and methods

Soil collection and experimental design

The experiment was performed with soil collected from the currently protected area Guadiamar Green Corridor (SW Spain). This area was affected by a toxic spill in 1998, when a tailings dam failure resulted in the release of acid water and toxic slurries with high contents of trace elements that covered over 4,600 hectares of the riverbank (Arenas et al. 2001). Soils were collected from the "Vicario" area within the Green Corridor (37° 26' 21" N; 6° 12' 59" W), an area that was affected by soil acidification and contamination due to the accident, but that was not treated after the cleaning-up operations. Since the mining accident the area has been exposed to extensive grazing by horses, and has experienced a process of natural regeneration. The current plant cover is dominated by grasses (mainly *Heliotropium europaeum*, *Cynodon dactylon* and *Sonchus* spp.). The climate at this location according to the Köppen-Geiger classification is Csa (temperate with hot and dry summers) (Peel et al. 2007), and soil at the site can be classified as Protocalcic Fluvisol (WRB 2014).

At this location 150 kg of soil were collected from the upper 20 cm of the soil surface. Once in the laboratory, soil was sieved and homogenized. In order to reduce soil microbial biomass and diversity drastically, different batches of the homogenized soil were autoclaved at 105 ºC for 1 h. Previous studies have demonstrated that a 1-cycle soil autoclaving drastically reduces bacterial and fungal abundances (over 85% and 99%, respectively) and soil respiration rate (Raducanu et al. 2016; Li et al. 2023). Under our experimental conditions, a preliminary trial showed a 90% reduction of soil total DNA after autoclaving soil at 105 ºC for 1 h. Soil DNA was extracted using the DNeasy PowerSoil Pro Kit (QUIAGEN) and the content of DNA was measured using a Qubit fluorometer (ThermoFisher).

After autoclaving, half of the soil was amended with autoclaved (105 ºC, 1 h) biosolid compost (BC) at dose equivalent to 60 000 kg ha^{-1}, while the other half remained un-amended (NA). Once established the amendment treatment, soil was used to fill 40 (BC) and 40 (NA) 2.5 L pots (diameter of 13 cm), where a gradient of soil microbial biodiversity was created by inoculation. Three different inoculums were added: (1) an inoculum with a potentially high biodiversity (A); (2) an inoculum with a medium biodiversity (B); and (3) an inoculum with a very low biodiversity (C). These inoculums were compared with a non-inoculated, autoclaved control soil (D). 8 different treatments resulted from the combination of the two treatments (2 amendment levels × 4 inoculums), each with 10 replicates: BC-A, BC-B, BC-C, BC-D, NA-A, NA-B, NA-C and NA-D (Supplementary Figure S1).

Inoculums A and B were made of soil collected from an experimental field located in the Vicario area where a long-term study that evaluated the application of organic amendments for soil remediation was carried out (Burgos et al. 2006). Inoculum A was obtained from plots amended with biosolid compost, while inoculum B was obtained from non-amended, control plots, where microbial communities had been exposed to low pH and a high availability of trace elements for more than a decade. The election of these specific soils was supported by previous studies that demonstrated the higher bacterial and fungal diversity in the amended soil compared to the non-amended one (Montiel-Rozas et al. 2018). In addition, in a recent experiment carried out with these soils, mean Shannon diversity indices calculated for bacterial and fungal communities were 6.33 and 4.23 in the amended soil, respectively, and 4.91 and 4.04 in the non-amended soil (Morales-Salmerón et al. 2024). To inoculate the pots with these two microbial communities, 160 g of the corresponding solid inoculum were incorporated into each pot belonging to the BC-A, BC-B, NA-A or NA-B, respectively. Soil and inoculum were thoroughly mixed by hand in a sterilized container to achieve homogeneity.

Inoculum C was the result of a dilution-to-extinction process. From a specific area in the Guadiamar Green Corridor where the toxic mud was not removed after the spill, material from the topsoil was collected. This material, from now on referred as "sludge", is mainly constituted by the remaining dry mud mixed with the topsoil, and is known to have a very low fungal biodiversity (Shannon diversity index of 2.95; Gil-Martínez et al. 2021). 500 g of sludge was mixed with 1 L of sterilized deionized water, and six serial dilutions were made. Twelve ml of the 10^{-6} dilution were added to each one of the BC-C and NA-C pots.

Finally, a forage mixture made of *Lolium rigidum* (80 seeds per pot) and *Medicago polymorpha* (30 seeds per pot) was sown in every pot. Pots were placed in a greenhouse under controlled environmental conditions. Soil water holding capacity (WHC) was estimated for the non-amended soil considering soil texture and organic matter content, and all pots were periodically watered with 80 ml of sterilized water (which was estimated to keep soil moisture around 70% of WHC).

One month after the soil inoculation (and one month after the plant cover sowing), drought conditions were simulated in half of the replicates. Pots were periodically watered with autoclaved water (105 ºC, 1 h), and those belonging to the drought treatment (DR) received 30% less water supply (55 ml) compared to the control treatment (CT). This represents a reduction of water inputs during the plant growing season within the range of climate change predictions for

the Mediterranean basin by the end of the century (IPCC 2023).

As a result, one month after the start of the study the experiment compared 16 treatments (2 amendment treatments × 4 inoculums × 2 water input treatments), each with 5 replicates and a total of 80 pots (Supplementary Figure S1). Drought conditions were kept for one month. Soil moisture in the upper 5 cm of the soil surface was periodically measured with a ThetaProbe Soil Moisture Sensor (Delta-T Devices).

Plant development measurements

The number of seedlings was periodically recorded and germination rate of each plant species was determined 40 days after sowing. One month after establishing the drought treatment, the plant cover was manually mown, and the aboveground biomass of each species was sorted. Dry biomass was determined by drying fresh biomass at 60 ºC for 48 h.

Soil sampling and analysis

Before the establishment of the experiment, soil samples were collected from the two initial soil mixtures (BC and NA), inoculums A and B, and the sludge. These samples were used for an initial characterization of soil chemical properties and microbiological activity. One month after the inoculation and before the drought simulation, a second soil sampling was carried out. Finally, a third soil sampling was done at the end of the experiment (two months after the start of the experiment and one month after the establishment of the drought treatment). Soil samples from the second and third samplings were divided into three subsamples. The first subsample was air-dried and later used to analyze soil chemical properties; the second subsample was stored at 4 ºC to study microbial activity (enzyme activities and respiration rate); finally, 3 g of soil were frozen and stored at -80 ºC to analyze soil microbial DNA.

Soil chemical properties

A 1:2.5 soil: water suspension was used to measure soil pH, and soil electrical conductivity (EC) was measured in a 1:5 soil: water suspension. Total C, N and S contents were analyzed with a micro elemental analyzer (Leco Truspect CHNS Micro). Bioavailable fractions of Ca, Mg, K and Na were extracted in ammonium acetate at pH 7 (Ojea and Carballas 1976). Ca and Mg contents were measured by atomic absorption spectroscopy, and K and Na by atomic emission spectroscopy (PerkinElmer AAnalyst 100). Olsen P was extracted in 0.5 N sodium bicarbonate and quantified by molecular absorption spectrophotometry (Murphy and Riley 1962).

Soil microbial activity

Soil dehydrogenase activity (DHA) was measured by molecular absorption spectrophotometry using 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) as substrate (Benefield et al. 1977). Leucine-aminopeptidase, β-glucosidase, acid phosphatase and N-acetyl-glucosaminidase activities were extracted in a 50 mM sodium acetate solution at pH 5.5 and determined by fluorometry using 7-amino-4-methyl coumarin (AMC) and 4-methylumbelliferone (MUB) as fluorogenic substrates, respectively (Marx et al. 2001). Soil respiration rate was determined with an infra-red gas analyzer (EGM-4, PP Systems) after incubating soil samples at 25 ºC for 24 h (Bekku et al. 1995).

Soil genomic DNA extraction and analysis

DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil Pro Kit (QUIAGEN). DNA concentration was measured with a Qubit fluorometer (ThermoFisher). qPCRs (Takara Bio's SYBR® Premix Ex Taq) of the 16 S rDNA (341 F/805R) (Muyzer et al. 1996; Watanabe et al. 2001) and ITS rDNA (ITS3/ITS4) (White et al. 1990) regions were carried out to determine the absolute abundance of bacteria and fungi within the soil microbial community. The qPCR experiments were performed on a Mx3000P Multiplex Quantitative PCR System (Stratagene). The amplification programme started with an initial denaturation stage of 10 min at 95 ºC; followed by 40 amplification cycles (30 s at 95 ºC, 30 s at 60 ºC (16 S) or 62 ºC (ITS), and 30 s at 72 ºC); and a final dissociation phase (1 min at 95 ºC, 30 s at 55 ºC and 30 s at 95 ºC). Each qPCR measurement was carried out in triplicate. To calibrate the qPCR method, samples of 16 S and ITS amplicons in serial concentration dilution gradient were used. PCRs were used to amplify both target regions (34 amplification cycles; annealing temperature of 60 ºC or 62 ºC for the 16 S and ITS regions, respectively) from one random soil DNA extract. PCR products were loaded in an agarose gel, and 16 S and ITS amplicons were purified using the GeneJET Kit (Thermo Scientific™). DNA concentrations were determined using a Qubit fluorometer (ThermoFisher). Subsequently, serial dilutions were done (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) and the corresponding Ct values used to draw the calibration curve, were determined by qPCRs (same protocol described above).

Finally, the same primer pairs were used in an Illumina high-throughput sequencing process to study the composition of bacterial and fungal communities. Raw sequences were submitted to the NCBI database (BioProject accession

number: PRJNA1233278; BioSample accession numbers: SAMN47264883-SAMN47264930). Amplicon sequence variants (ASVs) were inferred from the resulting raw sequences using the Divisive Amplicon Denoising Algorithm (DADA2 R package, version 1.22.0) (Callahan et al. 2016). Cutadapt 4.0 (Martin 2011) was used to find and remove adapter sequences and primers. Once paired-end reads were merged and chimeric sequences were removed, bacterial and fungal ASVs were assigned to the corresponding taxonomic information using SILVA (Yilmaz et al. 2013) and UNITE (Abarenkov et al. 2022) reference databases, respectively. Afterwards, fungal taxa were classified into functional groups following the database of Põlme et al. (2020).

ASVs that constituted less than 0.005% of the total sequences were excluded from the analysis (Bokulich et al. 2013). The remaining ASVs were then clustered utilizing VSEARCH v2.21.1 (Rognes et al. 2016) and the LULU package (Frøslev et al. 2017), and singletons were eliminated. The function rarefy in the vegan R package (Oksanen et al. 2022) was employed to compare the observed taxa richness with the expected richness in a theoretical sample containing 1,000,000 reads. The rarefaction analysis indicated that every sample curves from both datasets reached a plateau, thus negating the need to rarefy to a minimum number of reads per sample (McMurdie and Holmes 2014). Finally, Shannon and Whittaker indices were calculated to study bacterial and fungal alpha and beta-diversity among soil types.

Statistical analyses

All statistical analyses were performed in R version 4.1.3 (RCoreTeam 2022). Linear mixed models were used to study the effect of the biosolid compost application, the inoculation, the drought conditions and the interaction of these factors (amendment × inoculum × drought) on soil chemical properties, microbial activity, bacterial and fungal abundance and diversity, and plant development. Tukey tests were used as post hocs ($p<0.05$). The effect of the experimental treatments on soil moisture was evaluated using mixed linear models (nlme package in R), with each pot considered as a random factor to account for repeated measurements. The residuals of each model were studied before the model validation. In those cases where the model residuals showed heteroscedasticity among the different factor levels, a variance coefficient was introduced using the varIdent function of the nlme package (Pinheiro and Bates 2000).

Non-metric multidimensional scaling (NMDS) analyses based on Bray-Curtis dissimilarities were carried out to spatially visualize samples according to the composition of their microbial community. Differences among groups and the effect of the different treatments on the bacterial and fungal community composition were tested by permutational multivariate ANOVA (PERMANOVA) analyses and pairwise post-hoc tests were used to analyse differences among groups.

In order to integrate soil microbiological activity and plant productivity into a unified index, and to synthetize the influence of amendment addition, drought, and biodiversity on soil functioning, a soil multifunctionality index (Byrnes et al. 2014) was computed. This index included total plant production and soil microbiological activity (dehydrogenase, aminopeptidase, β-glucosidase, acid phosphatase and N-acetyl-glucosaminidase activities, and soil respiration rate). Among the different ways to measure multifunctionality, the averaging approach is calculated by averaging the standardized values of the multiple ecosystem functions (Byrnes et al. 2014). Data were log-transformed and subsequently z-transformed to standardize the seven soil functions included in this index. Pearson`s correlations between the resultant soil multifunctionality index and abundance, diversity and taxa richness of soil bacterial and fungal populations were evaluated.

Finally, structural equation modelling (SEM) was used to explore the structure of direct and/or indirect effects of the application of the organic amendment and microbial diversity on soil multifunctionality. Based on previous observations, main soil chemical properties (total C and N, and available P) were selected as expected relevant predictors of soil multifunctionality and were tested in separate models together with the bacterial and fungal diversity values to achieve the model with the best fit. Soil moisture was also included as predictor of soil multifunctionality since our study evaluates the effect of drought on soil functioning. Each model goodness-of-fit was evaluated according to three indexes. The Comparative Fit Index (CFI) and the Tucker-Lewis Index (TLI), that indicate the best fit of the postulated model compared to a baseline model, were tested for an acceptable fit (CFI ≥ 0.90 and TLI ≥ 0.95). In addition, the Root Mean Squared Error of Approximation (RMSEA) index was used to evaluate the badness-of-fit of the proposed model, with values < 0.05 considered as acceptable. Finally, the model with the best fit and the highest p-value in the chi-square test was selected. The chi-square test evaluates the null hypothesis that the predicted and the observed covariance patterns are equal, therefore a high p-value is desired. Analysis were carried out with the lavaan package (Rosseel 2012) and the obtained model was plotted using the package lavaanPlot (Lishinski 2021).

Results

Soil moisture

From the beginning of the experiment soil moisture was enhanced by the organic amendment. Before the drought period mean soil moisture before watering was 11.4 ± 2.8%v/v in BC pots and 9.7 ± 1.7%v/v in NA; and during the one-month drought period soil moisture was 13.5 ± 4.6% v/v in BC and 8.3 ± 1.3% v/v in NA. During the drought period significant effects of the amendment treatment (F = 77.68; $p < 0.001$), drought treatment (F = 15.22; $p = 0.002$) and the amendment × drought interaction (F = 8.41; $p = 0.005$) were observed, and, by the end of the experiment, soil moisture in the BC-CT and in the BC-DR treatment was 47% and 28% higher than in the non-amended NA-CT and NA-DR pots, respectively. Besides, the inoculum treatment had also a significant effect on soil moisture (F = 8.51; $p = 0.001$), being the highest in A pots in comparison to the rest of inoculums (12.7 ± 5.0% v/v, drought and control pooled). Nevertheless, no significant effect of the amendment × inoculum × drought interaction on soil moisture was recorded (F = 0.95; $p = 0.421$).

Plant germination and growth

The application of biosolid compost significantly decreased both species germination rates. *L. rigidum* germination was reduced from 14 ± 4% to 9 ± 4% (F = 32.73; $p < 0.001$), while *M. polymorpha* germination decreased from 31 ± 12% to 18 ± 9% (F = 40.17; $p < 0.001$) in BC and NA, respectively. In addition, the inoculum treatment (F = 3.66; $p = 0.016$) and the amendment × inoculum interaction (F = 4.38; $p = 0.007$) had a significant effect on *M. polymorpha* germination rate in NA pots (Supplementary Figure S2a). *L. rigidum* germination was not significantly influenced by the inoculum treatment (F = 1.93; $p = 0.133$) or the amendment × inoculum interaction (F = 0.72; $p = 0.543$).

L. rigidum final biomass was not significantly different in BC and NA treatments (pooled mean of 1.85 ± 0.65 g; F = 0.31; $p = 0.582$); but the inoculum (F = 9.94; $p < 0.001$) and the amendment × inoculum interaction (F = 8.09; $p < 0.001$) significantly influenced *L. rigidum* growth in BC pots, so that higher biomass was recorded with inoculum C and D than with A and B (Supplementary Figure S2b). Drought conditions negatively affected *L. rigidum* final biomass (F = 20.21; $p < 0.001$), reducing biomass by 23.5%, but the interaction amendment × inoculum × drought had no significant effect (F = 0.12; $p = 0.950$).

M. polymorpha growth was enhanced by the biosolid compost addition (mean of 0.30 ± 0.20 g per plot) compared to the non-amended treatment (0.16 ± 0.09 g; F = 16.21; $p < 0.001$), and was significantly higher in B than in D (F = 3.13; 0.032). In this species biomass production was not affected by drought (F = 0.45; $p = 0.507$) or by the interaction between the different factors (F = 0.12; $p = 0.948$; Supplementary Figure S2b).

In relative terms, when comparing the plant biomass in DR pots to the biomass measured in CT pots, it was observed that the negative effect of drought on *L. rigidum* production (Fig. 1a) was not influenced by the amendment (F = 0.03; $p = 0.867$), the inoculum (F = 0.55; $p = 0.654$) or the interaction amendment × inoculum (F = 0.29; $p = 0.836$). However, the relative impact of drought on *M. polymorpha* was ameliorated in amended soils in comparison to non-amended soils (F = 5.91; $p = 0.021$). The inoculum treatment also had a significant effect (F = 3.34; $p = 0.032$), and *M. polymorpha* biomass was less affected in soils inoculated with A compared to C (Fig. 1b), while the interaction amendment × inoculum had not a significant effect (F = 1.27; $p = 0.302$).

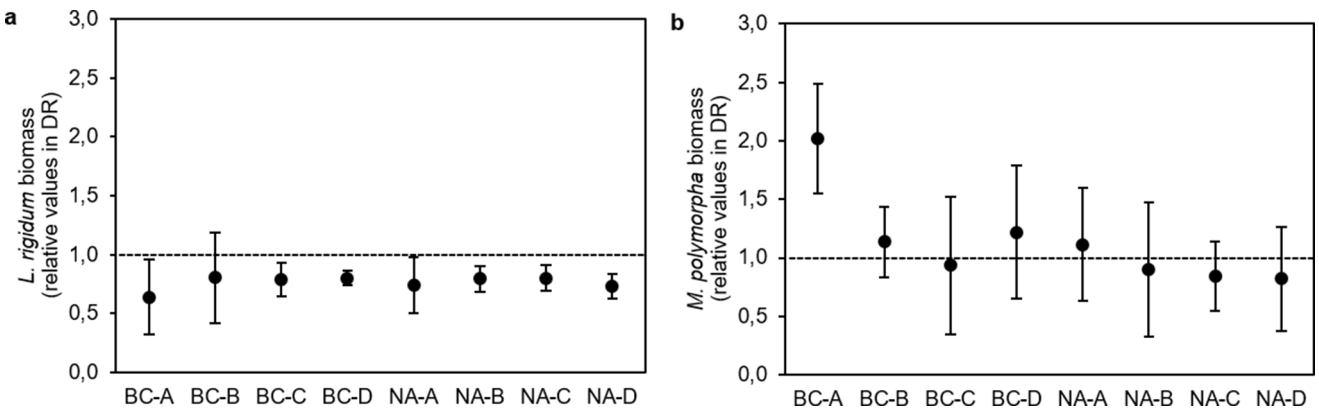

Fig. 1 Effect of drought conditions on *Lolium rigidum* (**a**) and *Medicago polymorpha* (**b**) biomass production. Values indicate mean (± standard deviation) biomass produced in pots belonging to the drought treatment (DR) divided by the mean plant biomass recorded under control conditions (CT) for each treatment. Values below and above 1 indicate a reduction and an increase in plant production in DR compared to CT, respectively

Soil chemical properties

At the beginning of the experiment the addition of the organic amendment significantly increased soil pH and electrical conductivity, total C and N contents, and the availability of Na and macronutrients (K, Mg, Ca and P). Inoculum A also had significantly higher pH, total C and N contents, and available K and P compared to inoculum B. In addition, the sludge was characterized by a very low pH, high content of S and available Ca, and very low content of available P. Mean values of the analyzed chemical properties in the initial soil and in the different inoculums are shown in Supplementary Tables S1 and S2.

Analyses performed at the second and third samplings provided very similar results regarding to the effects of biosolid compost addition on soil chemical properties: increases of pH, total C and N, available nutrients, and electrical conductivity (Table 1, Supplementary Tables S3, S4 and S5). Besides, the inoculum treatment had a significant effect on most of the analyzed soil chemical properties at both soil samplings, especially in NA soils, and the influence of the interaction amendment × inoculum on soil properties had a special relevance at the third soil sampling (Supplementary Table S5). In particular, NA soils inoculated with the A inoculum tended to have higher pH, organic C content, and P and Mg availability.

One month after the establishment of the drought treatment, soil pH and Na content were increased by the drought treatment and were influenced by the amendment × inoculum × drought interaction. Soil pH was significantly higher in BC-B-DR (5.89 ± 0.05) than in BC-B-CT (5.38 ± 0.12), and Na content was higher in BC-C-DR (0.27 ± 0.03 mg kg^{-1}) than in BC-C-CT (0.22 ± 0.01 mg kg^{-1}) pots. No effect of the simulated drought conditions, nor of the amendment × inoculum × drought interaction was found on the other analyzed soil chemical properties.

Soil microbial activity

As well as on soil chemical properties, the amendment treatment had a significant effect on soil microbiological activity at the first soil sampling, before soil inoculation. The addition of the biosolid compost enhanced soil dehydrogenase (DHA) and N-acetyl-glucosaminidase activities; while soil aminopeptidase and acid phosphatase activities, as well as soil respiration rate, were significantly higher in NA than in BC. Soil β-glucosidase activity was not significantly affected by the organic amendment. Comparing A and B inoculums, all microbiological parameters were significantly higher in A than in B, except for soil acid phosphatase activity which was higher in the inoculum B. The sludge showed the lowest values of N-acetyl-glucosaminidase, acid phosphatase and β-glucosidase activities. Mean values of the analyzed microbiological parameters from the first soil sampling, as well as the results of the statistical analyses, are shown in Supplementary Tables S1 and S2.

The positive effect of the organic amendment was observed at the second soil sampling on soil DHA, aminopeptidase and N-acetyl-glucosaminidase activities, as well as on soil respiration rate. However, no significant effect of the amendment treatment was observed for the other measured enzyme activities. The inoculation treatment had a significant influence on most of the indexes of microbiological activity (Supplementary Table S4), except for soil acid phosphatase activity, being soil DHA the highest in pots with inoculum A. However, N-acetyl-glucosaminidase activity was enhanced by inoculums C and D. Besides, the effect of the amendment × inoculum interaction was only significant for the aminopeptidase and N-acetyl-glucosaminidase activities (Supplementary Table S4).

Finally, at the third soil sampling, all enzyme activities, as well as soil respiration rate, were significantly different in BC and NA soils. Following the same pattern as in the previous soil sampling, DHA, aminopeptidase activity and soil respiration rate were increased in BC compared to NA. N-acetyl-glucosaminidase and β-glucosidase activities were also positively affected by the organic amendment, while soil acid phosphatase activity was higher in NA than in amended soils. Microbiological activity was also highly influenced by the inoculation treatment and the amendment × inoculum interaction (Table 1, Supplementary Table S5). In particular, the inoculum with the potentially highest levels of bacterial and fungal diversity (A) showed the highest respiration rates in both amended and unamended soils, and the highest dehydrogenase activity in NA soils (been the lowest in NA-D). Interestingly, soils inoculated with inoculums C (from the dilution-to extinction procedure) and D in the BC soils showed high values of N-acetyl-glucosaminidase, acid phosphatase and β-glucosidase activities. In addition, drought conditions significantly reduced soil N-acetyl-glucosaminidase (−13%) and acid phosphatase (−12%) activities in amended soils. However, no other effects of the drought treatment or the amendment × inoculum × drought interactions were found (Supplementary Table S5).

Soil microbial community: bacterial and fungal abundance

At the second soil sampling, one month after the inoculation and the beginning of the experiment, the amendment treatment increased the absolute abundance of soil bacteria (from 146 ± 47 ng g^{-1} in NA to 265 ± 99 ng g^{-1} in BC) while the absolute abundance of fungi was reduced (from 0.62 ± 0.25 ng g^{-1} in NA to 0.37 ± 0.15 ng g^{-1} in BC), significantly

Table 1 Mean (±standard deviation) soil chemical properties, enzyme activities, respiration rate and bacterial (16 S) and fungal (ITS) abundances at the third soil sampling (one month after the beginning of the simulated drought event)

	Amended soils (BC)				Non-amended soils (NA)			
Parameters	BC-A	BC-B	BC-C	BC-D	NA-A	NA-B	NA-C	NA-D
pH	5.70±0.12 b	5.63±0.29 b	6.02±0.04 a	5.71±0.07 b	5.28±0.14 A	4.78±0.11 B	4.92±0.12 B	4.66±0.35 B
EC (dS m^{-1})	2.9±0.6	2.9±0.3	2.5±0.3	2.6±0.4	1.7±0.4 B	2.5±0.1 A	1.6±0.7 B	1.4±0.4 B
Total C (g/kg^{-1})	50±5 b	46±6 b	56±8 b	65±14 a	42±2 A	32±3 B	39±5 AB	36±9 AB
Total N (g/kg^{-1})	4.1±0.5 b	3.6±0.5 b	4.3 ± 0.7 b	5.0±0.9 a	2.9±0.3	2.3±0.2	2.6±0.4	2.4±0.7
Total S (g/kg^{-1})	7.7±0.6 b	7.7±0.8 b	8.3±0.5 ab	9.6±1.5 a	8.4±0.9 AB	10.1±1.3 A	7.5±2.3 AB	6.6±2.4 B
K (mg kg^{-1})	665±52	607±44	625±86	625±135	850±126 A	527±147 B	532±140 B	481±73 B
Na (mg kg^{-1})	247±25	231±13	242±36	232±19	210±19	201±15	213±9	218±25
Mg (mg kg^{-1})	45±10 ab	40±5 b	43±7 b	75±33 a	75±14 A	45±20 B	27±4 B	29±7 B
Ca (mg kg^{-1})	644±144	660±221	708±190	1051 ± 420	1863±201 A	1346±994 A	303±110 B	242±97 B
P (mg kg^{-1})	79±2 a	68±6 b	56±3 c	58±2 c	29±2 A	18±1 C	23±5 BC	25±2 AB
Dehydrogenase activity (µg INTF $g_{dry\ soil}^{-1}$ h^{-1})	2.2±0.5	1.8±0.7	2.2±0.4	2.2±0.4	2.0±0.3 A	1.3±0.2 C	1.7±0.2 B	0.7±0.2 D
Aminopeptidase activity (nmol AMC $g_{dry\ soil}^{-1}$ h^{-1})	633±76	508±82	593±128	570±77	271±64 B	257±119 B	196±93 B	402±89 A
N-acetyl-glucosaminidase activity (nmol MUB $g_{dry\ soil}^{-1}$ h^{-1})	811±214 b	1193±249 a	1286±349 a	1427±252 a	640±133 B	653±107 B	1103±326 A	1061±330 A
Acid phosphatase activity (nmol MUB $g_{dry\ soil}^{-1}$ h^{-1})	1594±272 b	2130±264 a	2375±400 a	2204±446 a	2497±720 BC	3253±589 A	3069±676 AB	1880±335 C
β-glucosidase activity (nmol MUB $g_{dry\ soil}^{-1}$ h^{-1})	1495±447 b	1492±624 b	2086±1095 ab	2669±962 a	1268±282 B	1867±337 A	1431±224 AB	1585±739 AB
Respiration rate (g $C\text{-}CO_2$ $g_{dry\ soil}^{-1}$ day^{-1})	14.2±6.1 a	8.7±4.2 b	7.8±2.4 b	5.1±1.4 b	7.0±3.6 A	2.6±0.3 B	3.2±0.7 B	4.0±1.3 B
Total DNA (µg g^{-1})	13.8±1.5 ab	11.3±2.3 b	15.5±1.8 a	12.0±2.1 b	17.3±3.4 A	7.5±1.8 B	8.9±2.1 B	5.1±2.3 B
16 S abundance (ng g^{-1})	153±41 a	85±32 b	72±18 b	50±14 b	216±25 A	101±34 B	90±18 BC	51±19 C
ITS abundance (ng g^{-1})	0.48±0.14	0.24±0.10	0.48±0.12	0.47±0.16	0.61±0.28	0.39±0.10	0.66±0.25	0.65±0.19
ITS:16 S	0.0031±0.0002 b	0.0030±0.0009 b	0.0068±0.0016 a	0.0095±0.0020 a	0.0028±0.0011 C	0.0044±0.0024 BC	0.0072±0.0015 AB	0.0151±0.0044 A

Different letters indicate significant differences ($p<0.05$) among soils treated with the different inoculums (A, B, C and D), within amended (BC, lower-case letters) and non-amended soils (NA, upper-case letters)

Since no significant effect of the drought treatment was observed, data recorded in DR and CT treatments were pooled

decreasing the ITS:16 S ratio. However, the content of total DNA in BC (7.54 ± 1.44 μg g^{-1}) was slightly lower than in NA soils (9.13 ± 4.19 μg g^{-1}). At sampling two, the inoculation treatment also had a significant effect on soil DNA (being the highest in inoculum A with 12.24 ± 3.78 μg g^{-1}). Bacterial absolute abundance was higher in C and D (pooled mean of 254 ± 7 ng g^{-1}) compared to A and B (pooled mean of 157 ± 19 ng g^{-1}), while fungal absolute abundance was higher in D than in C, been the ratio ITS:16 S the lowest in C. The amendment × inoculum interaction also had a significant effect on total DNA (the highest in NA-A), the absolute abundance of bacteria (higher in BC-C and BC-D than in the other treatments), and the ratio ITS:16 S (Supplementary Tables S3 and S4).

After the simulated drought event a significant increase in total DNA in BC (13.15 ± 2.54 μg g^{-1}) in comparison to NA soils (9.71 ± 5.25 μg g^{-1}) was observed; however, the absolute abundances of bacteria and fungi were higher in the non-amended soil (114.31 ± 67.01 ng g^{-1} and 0.58 ± 0.23 ng g^{-1}, respectively) than in BC (89.84 ± 47.52 ng g^{-1} and 0.42 ± 0.16 ng g^{-1}, respectively). The ITS:16 S ratio was not significantly affected by the amendment treatment. The effects of the inoculum treatment on total DNA (the highest in A followed by C), absolute abundances of bacteria (the highest in A and the lowest in D) and fungi (the lowest in B), and the ratio ITS:16 S are shown in Supplementary Table S5. The amendment × inoculum interaction had a significant effect on total DNA, the absolute abundance of bacteria and the ratio ITS:16 S (Table 1), while drought conditions or the interaction amendment × inoculum × drought did not significantly influence soil microbial abundance (Supplementary Table S5).

Table 2 Mean (±standard deviation) bacterial and fungal richness (S) and diversity (Shannon index, H') at the third soil sampling (one month after the beginning of the simulated drought event)

		S (number of ASVs)	H'
Bacterial community	BC-A	569 ± 55 A	4.78 ± 0.21
	BC-B	508 ± 28 B	4.59 ± 0.08
	BC-C	535 ± 35 AB	4.71 ± 0.19
	BC-D	507 ± 32 B	4.79 ± 0.22
	NA-A	545 ± 23 a	4.54 ± 0.20 a
	NA-B	517 ± 12 a	4.68 ± 0.09 a
	NA-C	389 ± 56 b	4.05 ± 0.35 b
	NA-D	377 ± 26 b	4.37 ± 0.34 b
Fungal community	BC-A	163 ± 19 AB	3.05 ± 0.24
	BC-B	138 ± 23 B	3.25 ± 0.47
	BC-C	133 ± 31 B	3.18 ± 0.28
	BC-D	184 ± 19 A	3.36 ± 0.27
	NA-A	185 ± 15 ab	3.05 ± 0.39 ab
	NA-B	203 ± 15 a	3.80 ± 0.36 a
	NA-C	119 ± 21 c	2.67 ± 0.17 b
	NA-D	148 ± 33 bc	2.50 ± 0.85 b

Different lower and upper-case letters indicate significant differences ($p < 0.05$) among the four inoculums (A, B, C and D) within the amended (BC) and non-amended (NA) soils, respectively

Since no significant effect of the drought treatment was observed, data recorded in DR and CT treatments were pooled

Soil microbial community: diversity and functional groups

By the end of the experiment, bacterial richness (S) and diversity (indicated by Shannon diversity index, H') were significantly influenced by the amendment and the inoculum treatments, as well as by the amendment × inoculum interaction (Table 2, Supplementary Table S5), while bacterial richness was also influenced by the interaction inoculum × drought (significantly reduced by drought conditions in pots inoculated with C). Fungal richness and diversity were significantly affected by the inoculum treatment and by the interaction amendment × inoculum (Table 2, Supplementary Table S5).

Bacterial taxa richness and diversity were significantly increased in BC soils. Besides, the inoculum treatment had a significant effect, especially in non-amended soils, on bacterial and fungal richness and diversity. In non-amended soils bacterial and fungal richness and diversity decreased along the expected biodiversity gradient, being higher in A and B than in C and D. However, in BC soils microbial diversity was not influenced by the inoculum treatment, while bacterial and fungal richness were the highest in BC-A and BC-D, respectively.

Bacterial beta diversity, assessed using the Whittaker Index and expressed by mean distances to centroids, was marginally affected by the amendment treatment ($F = 4.58$; $p = 0.058$), being higher in NA (0.17 ± 0.04) than in BC soils (0.11 ± 0.05). However, no significant effects of the inoculum ($F = 0.20$; $p = 0.896$), drought treatment ($F = 0.05$; $p = 0.832$) or the interaction amendment × inoculum ($F = 0.55$; $p = 0.660$) on bacterial beta diversity were observed.

According to the PERMANOVA analysis, soil bacterial community was significantly influenced by the amendment ($F = 8.39$; $p < 0.001$), the applied inoculum ($F = 2.48$; $p = 0.003$), and the interaction amendment × inoculum ($F = 4.27$; $p < 0.001$), showing significant differences among the eight soil types (Fig. 2a). In general, samples from each soil type were closely grouped in the NMDS plot, indicating the common inoculant. Drought conditions ($F = 0.80$; $p = 0.604$) and the interaction amendment × inoculum × drought ($F = 1.10$; $p = 0.322$) did not significantly influenced soil bacterial community composition.

Considering the 10 most abundant bacterial genera (Supplementary Figure S3a), the genus *Pullulanibacillus* was more abundant in BC than in NA soils. On the contrary, the relative abundances of genera *Kitasatospora*, *Bacillus*, *Actinoallomurus* and *Alicyclobacillus* were higher in NA than in

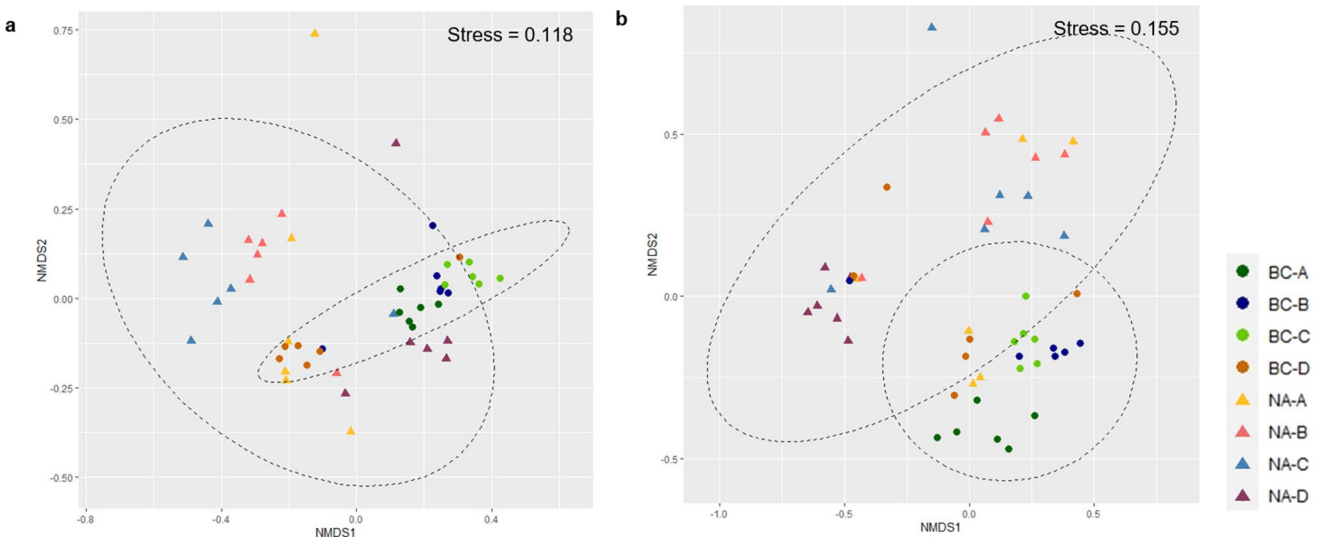

Fig. 2 NMDS analysis of the soil bacterial (**a**) and fungal (**b**) communities in the eight soil types at the end of the drought simulation. Circles and triangles represent samples belonging to the amended (BC) and non-amended (NA) treatments, respectively, while colours indicate the inoculum treatment. Ellipses draw 95% confidence intervals for amended and non-amended soils

BC. Besides, inoculums A and B enhanced the populations of *Kitasatospora* (especially in NA soils) and *Sphingomonas* compared to inoculums C and D. Oppositely, genus *Pullulanibacillus* had a higher relative abundance in soils, either amended or non-amended, inoculated with inoculums C and D; while *Bacillus* relative abundance was the highest in NA-C and NA-D and *Alicyclobacillus* dominated in NA-D.

Soil fungal community composition (Fig. 2b) was significantly influenced by the application of biosolid compost ($F=7.59$; $p<0.001$), the inoculum treatment ($F=4.00$; $p<0.001$) and the interaction amendment × inoculum ($F=2.32$; $p=0.002$). All soil types showed significantly different fungal communities. In contrast, the simulated drought conditions ($F=1.04$; $p=0.387$) and the interaction amendment × inoculum × drought ($F=0.74$; $p=0.825$) did not have a significant effect on soil fungal community composition. Besides, the Whittaker Index revealed no significant influence of the amendment ($F=2.68$; $p=0.133$), inoculum ($F=0.81$; $p=0.515$), drought ($F=0.54$; $p=0.480$) or the interaction amendment × inoculum ($F=1.17$; $p=0.381$) on the beta diversity of fungal communities.

Considering the relative abundance of the dominant fungal genera, it was remarkable the similarity of the fungal community of both soils, amended and non-amended, inoculated with the most diverse inoculum (A). Fungal communities of BC-A and NA-A were dominated by the genus *Penicillium*, while *Trichoderma*, *Coniochaeta* and *Spegazzinia* relative abundances were higher in NA-A compared to BC-A (Supplementary Figure S3b). Soils inoculated with inoculums C and D, especially NA-C and NA-D, shared a higher relative abundance of the genus *Chaetomium* compared to those soils where inoculums A and B were applied (Supplementary Figure S3b).

When fungal taxa were grouped according to their primary lifestyle (Fig. 3), a higher relative abundance of animal parasites (especially in BC-C and BC-D) in BC compared to NA soils was observed; while mycoparasites (enhanced in NA-C and NA-D) were relatively more abundant in NA soils. In both BC and NA plant pathogens were more abundant in soils treated with inoculums A and B, pollen saprotrophs were enhanced by inoculum B, and litter saprotrophs were more abundant in soils inoculated with C and D.

Soil multifunctionality

The calculated multifunctionality index was significantly increased by the amendment application (Fig. 4; $F=41.51$; $p<0.001$). Besides, a significant effect of the amendment × inoculum interaction was observed ($F=3.25$; $p=0.035$), so that in amended soils there were differences among inoculums; in these soils multifunctionality in BC-D was significantly higher than in BC-A and BC-B (Fig. 4). The inoculum treatment ($F=2.96$; $p=0.052$), the simulated drought conditions ($F=0.65$; $p=0.425$) and the interaction amendment × inoculum × drought ($F=0.26$; $p=0.852$) did not influence soil multifunctionality.

In addition, multifunctionality of the amended soils (BC) was not significantly correlated with bacterial or fungal diversity (Fig. 5a and b), while positive correlations between soil multifunctionality and the richness of fungal taxa and the fungi: bacteria ratio (indicated by ITS:16 S ratio) were observed (Fig. 5c and d). However, a slightly significant negative correlation between soil multifunctionality and

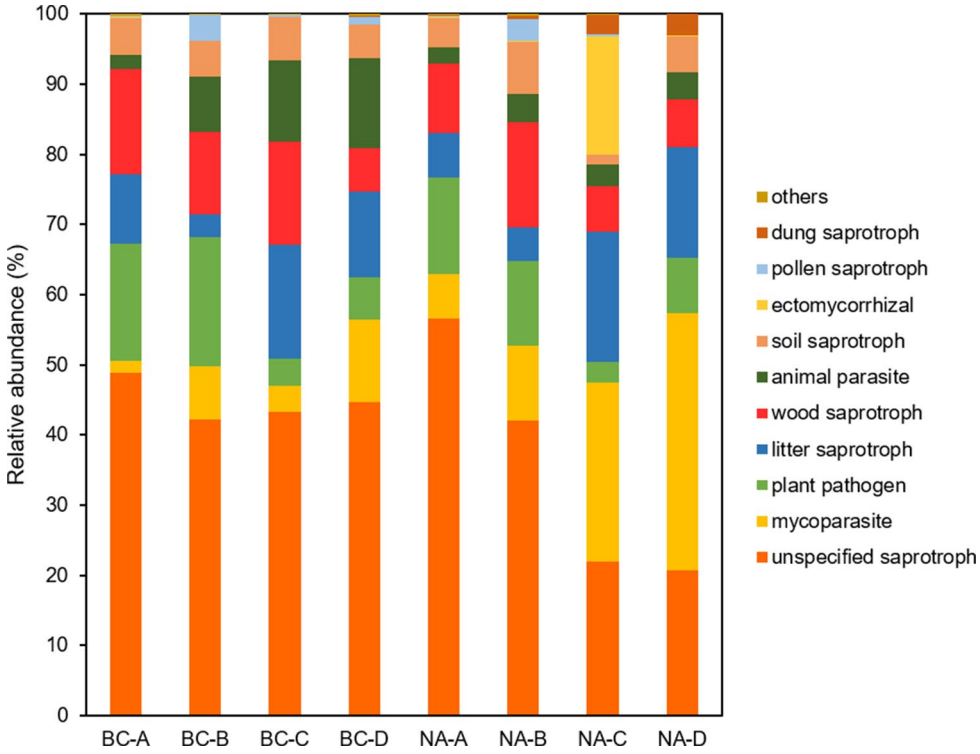

Fig. 3 Relative abundance of the main fungal guilds in the different soil types (means of 6 replicates per treatment for each fungal guild). BC: amended soil; NA: non-amended soil; letters **A**, **B**, **C** and **D** indicate the inoculum treatment

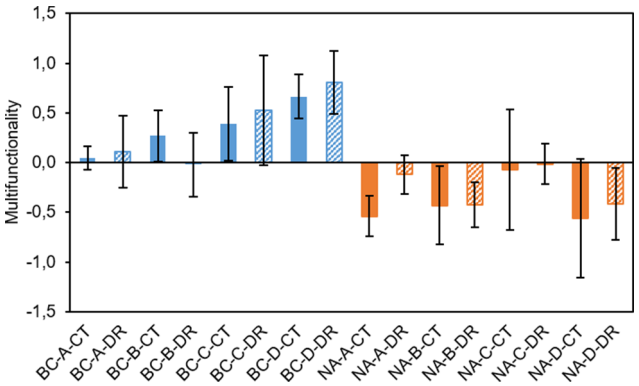

Fig. 4 Mean (bars)±standard deviation (lines) of the calculated soil multifunctionality index in each soil type. Full and striped bars correspond to samples belonging to the control (CT) and drought (DR) treatments, respectively. BC: amended soil; NA: non-amended soil; letters **A**, **B**, **C** and **D** indicate the inoculum treatment

bacterial diversity was observed in non-amended soils (NA, Fig. 5a). Correlation coefficients between multifunctionality and fungal and bacterial diversity indexes are shown in Supplementary Table S6.

The structure of correlations among the studied variables was explored with structural equation modelling (Fig. 6). The SEM model indicated a positive and direct effect of the biosolid compost application on bacterial and fungal diversity. However, soil multifunctionality was controlled by soil total C content and positively affected by the organic amendment, instead of by soil microbial diversity. The amendment had a positive and significant effect on soil total C content and soil moisture.

Discussion

Soil moisture, chemical properties and plant productivity

As expected and due to the increase in soil organic matter content (Cooper and DeMarco 2023), the application of the biosolid compost improved soil water retention capacity and helped to reduce water loss under the simulated drought conditions compared to the non-amended soil. Likewise, the positive effect of the inoculum A on soil moisture in both amended and non-amended soils could be partly explained by the supply of certain amount of organic matter, as inoculum A came from a long-term amended soil with a higher content of total C compared to inoculum B (Morales-Salmerón et al. 2024).

The analysis of soil chemical properties also revealed a positive effect of the organic amendment on soil pH, total C and N, and the availability of macronutrients, that were patent since the biosolid compost was applied. The effect of the inoculum treatment was especially important in non-amended soils, where soil chemical properties were improved, especially by inoculum A. Besides, the simulated drought conditions did not affect soil chemical properties except for slight increases in soil pH and available

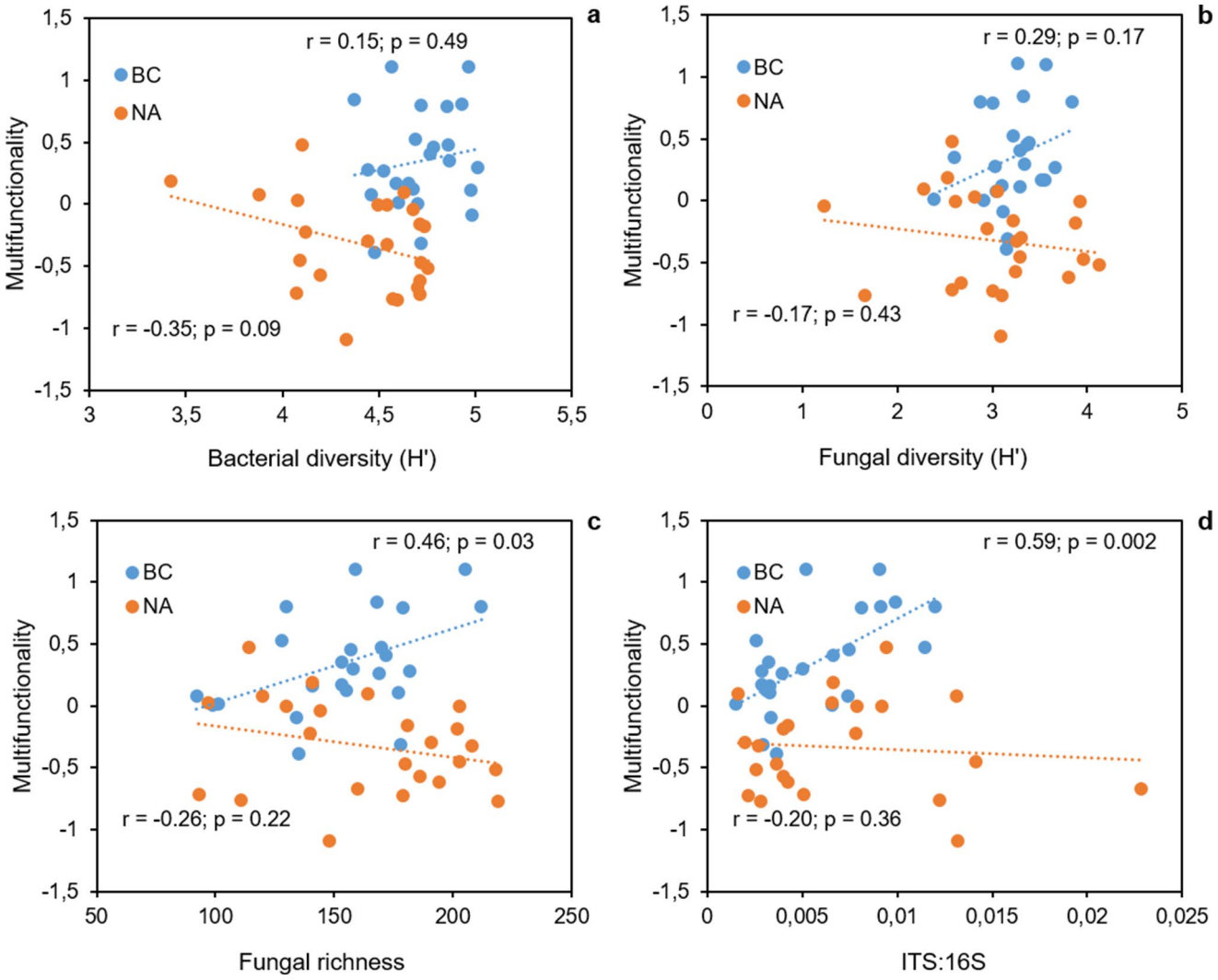

Fig. 5 Correlation analysis between soil multifunctionality and soil bacterial (**a**) and fungal (**b**) diversity (Shannon Diversity Index, H´), fungal richness (**c**), and fungi: bacteria ratio (ITS:16 S, **d**) in amended (BC) and non-amended (NA) soils. Pearson correlation coefficients and p-values are indicated next to the lines

Na content in amended soils. However, previous studies observed the opposite effect of drought on soil Na content, since the absorption of Na by plants is one of their strategies to face water stress (Sardans et al. 2008b; Huihui et al. 2021).

Thanks to the improvement in soil water retention capacity and the higher availability of macronutrients, the organic amendment also had a positive effect on the plant cover. Although in both species germination rate was reduced due to the toxicity of the biosolid compost on the short-term, as found in other works (Zubillaga and Lavado 2006), *L. rigidum* and *M. polymorpha* growth was promoted in amended soils. Drought conditions reduced *L. rigidum* biomass production in all cases, independently of the amendment or inoculum treatments. However, *M. polymorpha* showed a high tolerance to the simulated drought conditions and even had a higher growth under drought conditions in pots belonging to the amendment treatment (as well as in soils inoculated with inoculum A), probably explained by the lower competition with *L. rigidum* and the higher content of organic matter and nutrients supplied by the biosolid compost. Other works have also showed the high tolerance of *M. polymorpha* to drought (Nichols et al. 2009). In particular, a previous study also showed that *M. polymorpha* is more resistant to water stress than *L. rigidum* (Morales-Salmerón et al. 2024).

Soil microbial activity response to drought and compost addition.

Biosolid compost enhanced soil microbiological activity (enzyme activities and respiration rate), as it was observed in previous studies carried out with soils from the same area (Pérez de Mora et al. 2005; Montiel-Rozas et al. 2016).

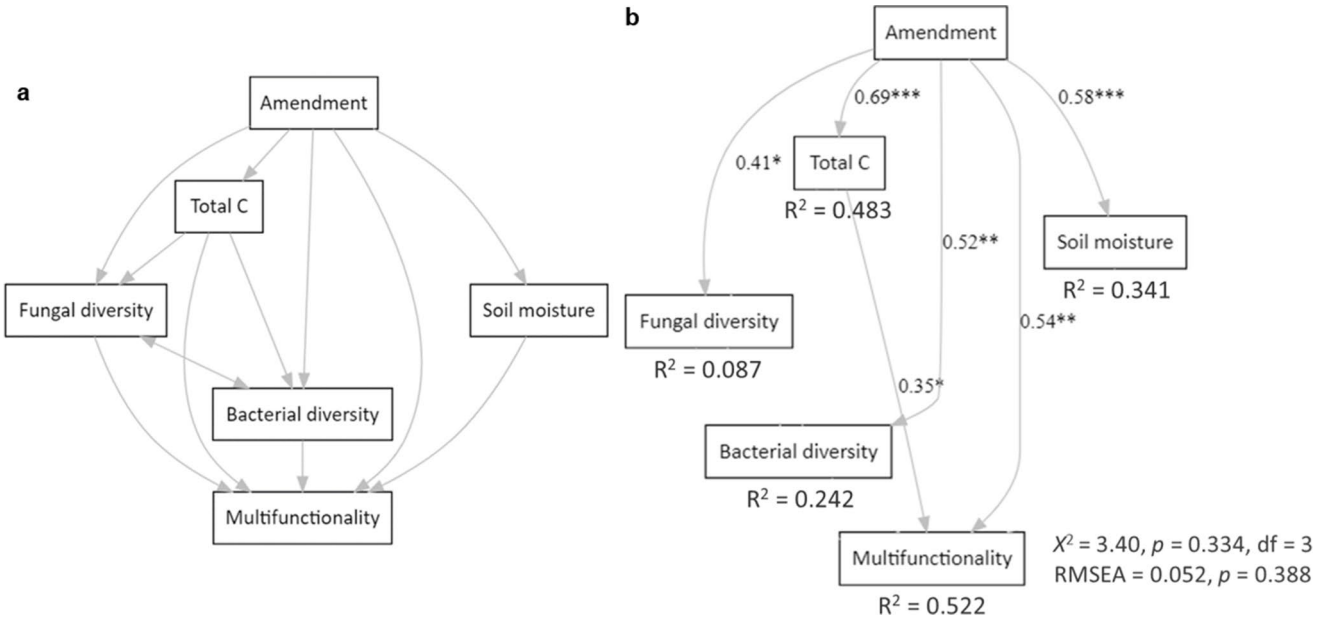

Fig. 6 Structural equation model (SEM) exploring relationships between soil multifunctionality, soil properties and microbial diversity. **a**) Proposed model and **b**) final full path diagram representing the hypothesized relationships among soil chemical fertility (indicated by soil **C**), soil moisture, fungal and bacterial diversity and soil multifunctionality. Regressions are indicated by simple arrows while double arrows represent correlations. Numbers next to arrows are the standardized coefficients of the significant relationships, and significance levels are indicated by the number of asterisks (*, ** and *** represent <0.05, < 0.01 and <0.001, respectively). R^2 represents the proportion of variance explained. The Root Mean Square Error of Approximation (RMSEA) index value indicates a good fit of the model

However, the higher content of inorganic P in amended soils, and especially in soils inoculated with inoculum A, resulted in the inhibition of soil acid phosphatase activity compared to the non-amended soils. Phosphatases, excreted by plant roots and soil microorganisms, are adaptive enzymes, so that the production of these enzymes is regulated by the content and availability of P (Silberbush et al. 1981). Previous research has observed a positive correlation between soil phosphatase activity and organic P content, increasing the activity level after the addition of organic fertilizers (Yadav and Tarafdar 2001). Oppositely, and as observed in our experiment, the application of inorganic P can result in an inhibition of the production and activity of soil phosphatase (Nannipieri et al. 1978).

The application of the organic amendment overshadowed the effect of the inoculation treatment on soil microbiological activity, explained by the higher relevance of soil chemical properties than of microbial diversity on soil functioning (discussed below). In non-amended soils there were some effects of the inoculation treatment on microbiological parameters; in particular dehydrogenase activity and soil respiration rates were the highest in soils where inoculum A, with the highest bacterial and fungal diversity, was applied, while soil aminopeptidase and N-acetyl-glucosaminidase activities were higher in inoculums C and D. The slight increases in organic C introduced with the A inoculum, rather than the highest levels microbial diversity, could be the reason for these observations; indeed, the SEM analysis confirmed that in the non-amended soils multifunctionality and bacterial/fungal diversity were disconnected (discussed below). In addition, the higher content of N and P in soils inoculated with A may be responsible of the observed reduction in soil enzyme activities (Xiao et al. 2018).

Regarding the impact of drought on microbiological activity, this experiment showed that under these experimental conditions soil functioning is quite resistant to decreases in water inputs, and that plant production is more sensitive to the decline in soil moisture than microbial activity in these soils. Water stress often reduces the rates of enzyme activity due to limited substrate supply and diffusion or due to altered enzyme hydration and conformation (Henry 2012). Indeed, reductions in soil enzyme activities have been reported in several field experiments with well-drained soils (Sardans and Peñuelas 2005; Sardans et al. 2008a; Steinweg et al. 2013). However, a meta-analysis of the effects of global change drivers of soil enzyme activities including data from 133 field experiments in natural ecosystems concluded that drought had a very limited effect on the activity of enzymes, in comparison to other global change drivers such as nutrient addition (Xiao et al. 2018), which is consistent with the results of this study, showing a much larger effect of compost addition than of drought on microbial activity.

As with soil enzyme activities, soil respiration was rather insensitive to the drought conditions simulated in this work. With our experimental design we aimed to simulate a reduction of water inputs during the plant growing season, which typically takes place over the spring months in the area where soils were collected. Mediterranean soils are naturally exposed to more severe water stress over the summer months (Madejón et al. 2023, 2024). Soils from semi-arid Mediterranean regions are adapted to drought, so that soil microbiological activity can be maintained even under extremely low moisture levels; for instance, in a previous work it was shown that microbiological activity was only reduced when soil moisture was below 2% (Hueso et al. 2011). Our results are in line with other works showing that the responses of soil respiration or multifunctionality to changes in soil moisture are less pronounced in soils that receive a low annual precipitation or that have a legacy of exposition to drought events (Hawkes et al. 2017; Canarini et al. 2021), likely due to adjustments in the composition or the abundance of microbial taxa promoting a greater drought resistance (Dacal et al. 2022).

Effect of treatments on soil microbial diversity

The application of the biosolid compost enhanced soil bacterial populations in the short-term (one month after soil amendment), while fungal populations were significantly decreased. However, two months after the biosolid compost addition, this effect was not observed and bacterial and fungal abundances were slightly higher in non-amended soils. Despite the similar abundance of soil bacteria and fungi in both amended and non-amended soils, microbial richness and diversity were positively influenced by the organic amendment by the end of the experiment (two months after the amendment application). The application of the biosolid compost, as occurred with soil microbiological activity, masked the effect of the inoculation treatment on bacterial and fungal diversity. Despite the inoculation treatment did not have a clear effect on soil bacterial and fungal abundances on the short-term, within the non-amended soils, those inoculated with inoculums A and B were characterized by higher bacterial and fungal richness and diversity, as expected given the diversity gradient established in the experimental design. It is noteworthy that the observed bacterial and fungal diversities accurately reflect the microbial diversity expected in an arid Mediterranean grassland (Castro et al. 2016; Gao et al. 2021; Bonanomi et al. 2022; Labouyrie et al. 2023).

The NMDS analysis revealed the role of the organic amendment, represented in the axis NMDS1, as the main driver of the composition of soil both bacterial and fungal communities. The inoculation treatment also had a significant effect on soil microbial communities. In both amended and non-amended soils, bacterial and fungal communities in soils inoculated with A and B were the most clearly distinguished, while microbial communities in C and D soils were more similar and showed higher variability among samples.

It is remarkable that soil bacterial communities, independently of the amendment and inoculation treatments, were dominated by genera belonging to phyla Actinobacteriota (genus *Nocardioides*), Firmicutes (genera *Pullulanibacillus and Bacillus*) and Actinomycetota (genera *Kitasatospora* and *Actinoallomurus*) all of them composed of Gram positive bacteria. Gram positive (monoderms) bacteria are characterized by a thick peptidoglycan cell wall layer, which contributes to enhance their tolerance to dessication (Schimel et al. 2007). This tolerance can also be related to the ability to form endospore of some Firmicutes groups (Schimel 2018). A dominance of the Gram positive phylum Actinobacteria is often reported in arid, nutrient-poor soils (Fierer et al. 2012; Maestre et al. 2015; Delgado-Baquerizo et al. 2016a, b). In addition, several experiments with soils from different biomes have shown that the relative abundances of Gram positive bacteria, including oligotrophic Actinomyces and Firmicutes, are increased in bulk and rhizospheric soils exposed to drought (Bastida et al. 2017; Canarini et al. 2021; Sun et al. 2023), as well in the root microbiome, which has been suggested to be an ubiquitous mechanism to promote drought resistance in plants (Naylor and Coleman-Derr 2018; Xu and Coleman-Derr 2019). In particular, in Mediterranean soils it has been suggested that Gram positive bacteria are underrepresented under optimal conditions of soil moisture (during the spring season) in relation to Gram negative lineages (Curiel Yuste et al. 2014). In arid soils key microbial taxa, rather than the richness, abundance or the ratio of bacteria and fungi, can control the resistance of soil multifunctionality to climate change drivers (Delgado-Baquerizo et al. 2017). This dominance of Gram positive bacteria could explain the limited effects of soil moisture reduction on microbial activity and diversity in our work. Under our experimental conditions the simulated drought event had a very limited impact on soil bacterial and fungal abundance, taxa richness and diversity. The most relevant effect of drought conditions on soil microbial communities was limited to the increment of the relative abundance of the genus *Trichoderma* in NA-C-DR and NA-D-DR soils. *Trichoderma*, a filamentous fungi (Põlme et al. 2020), shows a high tolerance to water stress and several *Trichoderma* species have been recently used as bio-inoculant to increase the plant capacity to absorb nutrients and water, enhancing crop resistance to drought conditions (Boorboori and Zhang 2023). This could explain

the higher relative abundance of this genus under drought conditions.

Soil multifunctionality

Our results showed the positive effect of soil organic amendment on soil multifunctionality, supporting the initial hypothesis that the improvement in soil chemical properties due to the biosolid compost application (organic matter and macronutrients) results in an increase in soil multifunctionality. However, the inoculum treatment only had an effect in amended soils, where, in contrast with our expectations, a higher multifunctionality index was observed in BC-D than in BC-A and BC-B. Although differences among the amended soils in fungal diversity were not significantly different, the higher dominance of fungi in BC-D may explain the higher multifunctionality, supported by the positive correlation between the ITS:16 S ratio and soil multifunctionality. In drylands, soil functionality is primarily influenced by the diversity of fungal taxa due to their higher tolerance to water stress when compared to bacteria (Delgado-Baquerizo et al. 2016a, b). This highlights the significant role played by soil fungal diversity in Mediterranean grasslands. Although plant pathogens may negatively affect soil multifunctionality, it was demonstrated that soil fungal richness and saprotrophic fungi are the main biotic factors in regulating soil functioning in semiarid environments (Li et al. 2022).

Under our experimental conditions, the results exhibited a high stability of soil multifunctionality to the simulated drought event, which could be explained by the high tolerance of the soil microbial community to water stress (discussed above). When inoculation and drought treatments were pooled together, the calculated soil multifunctionality tended to be correlated with bacterial and fungal diversity (estimated using the Shannon diversity index), what is consistent with the results obtained in previous studies (Delgado-Baquerizo et al. 2016a, b, 2020). However, different patters were observed for amended and non-amended soils. In amended soils multifunctionality was positively linked to fungal richness and abundance, while in non-amended soils a pattern of less multifunctionality in soils with higher bacterial and fungal diversity was observed. This may be related to the fact that higher levels of microbial diversity not always result in a higher soil multifunctionality, since the relationship depends on soil chemical properties and is affected by soil degradation (Xue et al. 2023).

When the role of bacterial and fungal diversity, and the effects of the organic amendment application, soil chemical properties (represented by the content of total C) and soil moisture on soil multifunctionality where simultaneously analysed in the structural equation model (SEM), it was demonstrated that the observed relation between microbial diversity and soil multifunctionality was an indirect effect. In our study the main driver of soil multifunctionality was the biosolid compost addition, followed by the total C content (also controlled by the organic amendment). The amendment application was also responsible for soil moisture, and bacterial and fungal diversity, what proves the positive effect of organic amendments in degraded soils. Our results show that inputs of organic matter largely determine the links between microbial diversity and soil functioning in these C-poor soils.

Conclusions

In summary, we could not confirm our initial hypothesis that a higher microbial diversity is linked to a higher stability of soil multifunctionality to drought in these degraded soils. Microbial functioning in these soils is quite resistant to reduced water inputs, at least at the levels of moderate moisture reductions tested in this work, which could be related to the dominance of Gram positive bacteria. According to our expectations, the addition of an organic amendment conferred a higher stability of plant production to drought. This was due to the direct effect of organic C on soil water retention and chemical fertility, rather than due to the direct effect of organic inputs on microbial richness or diversity. In these degraded soils physico-chemical limitations, rather than bacterial or fungal biodiversity, are the major drivers of soil multifunctionality. This was indicated by the lack of any significant relationship between biodiversity and multifunctionality in the most degraded, non-amended soils. These results highlight the importance of preventing C losses and increasing organic C levels in Mediterranean soils. Soil C was the main driver of soil multifunctionality and microbial diversity in these soils, and promoted a higher resistance of plant productivity to the reduction of water inputs. Given the climate change predictions for the Mediterranean region, where a large proportion of soils are threatened by C losses, it seems critical to increase the levels of organic C in these soils to promote a higher stability of soil productivity against droughts, even if microbial activity and diversity in these soils are highly resistant to fluctuations in soil moisture levels. However, it would be necessary to conduct further studies that replicate more severe drought conditions in order to better evaluate the resistance of Mediterranean soils to water stress. For instance, due to the extremely dry conditions that Mediterranean soils are exposed to in the summer, it would be interesting to test the impact of drought conditions on soil microbial activity in-situ trough a field experiment. Besides, an alternative way to inoculate soils must be considered in future experiments. The addition of organic matter through the solid inoculums, especially in the case

of inoculum A, had an unexpected effect on soil chemical properties in these C-poor soils, and made it difficult to discern between the chemical and the biological effect of the treatment on soil functions.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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