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Selective shifts in the rhizosphere microbiome during the drought season could explain the success of the invader *Nicotiana glauca* in semiarid ecosystems

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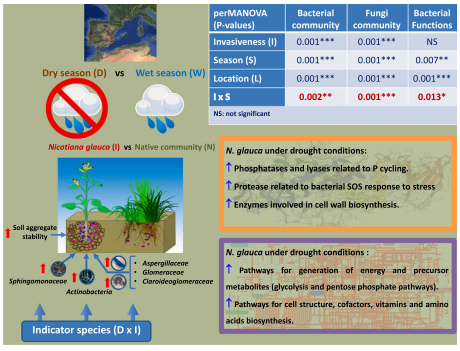
HIGHLIGHTS

- Drought resistant bacteria were prevalent in the invader rhizosphere.
- *Actinobacteriota* was an indicator phylum of the dry season and invader microbiome.
- Relative abundance of AMF was higher in invasive rhizosphere in dry season.
- Invader promoted specific bacterial functionality in response to drought.

GRAPHICAL ABSTRACT



perMANOVA (P-values)	Bacterial community	Fungi community	Bacterial Functions
Invasiveness (I)	0.001***	0.001***	NS
Season (S)	0.001***	0.001***	0.007**
Location (L)	0.001***	0.001***	0.001***
I x S	0.002**	0.001***	0.013*

NS: not significant

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ABSTRACT

The rhizosphere microbiome plays a crucial role in the ability of plants to colonize and thrive in stressful conditions such as drought, which could be decisive for the success of exotic plant invasion in the context of global climate change. The aim of this investigation was to examine differences in the composition, structure, and functional traits of the microbial community of the invader *Nicotiana glauca* R.C. Graham and native species growing at seven different Mediterranean semiarid locations under two distinct levels of water availability, corresponding to the wet and dry seasons. The results show that the phylum *Actinobacteriota* was an indicator phylum of the dry season as well as for the community of *N. glauca*. The dominant indicator bacterial families of the dry season were 67–14 (unclassified family), *Pseudonocardiaceae*, and *Sphingomonadaceae*, being relatively more abundant in the invasive rhizosphere. The relative abundances of the indicator fungal families *Aspergillaceae* (particularly the indicator genus *Aspergillus*), *Glomeraceae*, and *Claroideoglomeraceae* were higher in the invasive rhizosphere. The relative abundance of mycorrhizal fungi was higher in the invasive rhizosphere in the dry season (by about 40 % in comparison to that of native plants), without significant differences between invasive and native plants in the wet season. Bacterial potential functional traits related to energy and precursor metabolites production and also biosynthesis of cell wall, cofactors, vitamins, and amino acids as well as catabolic enzymes involved in the P cycle prevailed in the invasive rhizosphere under drought conditions. This study

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shows that the pronounced and beneficial shifts in the microbiome assembly and functions in the rhizosphere of *N. glauca* under conditions of low soil water availability can represent a clear advantage for its establishment.

1. Introduction

Some characteristics of a target ecosystem for plant invasion, such as soil water availability, can potentially affect the ability of invasive plants to compete with native species for environmental resources and, in consequence, control the success of exotic plant invasion (Zhang et al., 2023). Drought is anticipated to significantly affect the establishment and proliferation of exotic invasive plants owing to their higher phenotypic plasticity in acclimating to such adverse environmental conditions compared to native species (Davidson et al., 2011). Thus, experimental studies have shown evidence of the high resistance and resilience of invasive species to drought through shifts in morphological and ecophysiological traits (Díaz-Barradas et al., 2020; Leal et al., 2022). Climate change is triggering the occurrence of extended and more frequent drought episodes (the Intergovernmental Panel on Climate Change (IPCC), 2023), which would cause a greater decline in the growth and performance of native plant species with low or no tolerance of drought. In this context, the problem of invasive plants may worsen, especially in ecosystems where episodes of recurrent drought are already occurring such as those of the Mediterranean Basin (Pepe et al., 2022). Consequently, these stressful environmental conditions will result in increased loss of local biodiversity as well as substantial alteration of the functions and services of invaded ecosystems (Beaury et al., 2020).

In addition to the traits of exotic invasive plants, the success of plant invasions is related indirectly to changes induced by the invader in the plant-associated microbiome (Klironomos, 2002; Rodríguez-Caballero et al., 2020a). Host plant fitness, productivity, and adaptation to new environmental conditions are highly driven by the microbiome (Berendsen et al., 2012). The microbiome plays a crucial role in the ability of plants to colonize and thrive in stress conditions such as nutritional scarcity, drought, and soil disturbance (Kohler et al., 2016; Poudel et al., 2021; Ahmad et al., 2022). Within the microbiome, several beneficial microorganisms can help to improve plant performance by increasing the availability of nutrients to plants, suppressing soil-borne pathogens, and synthesizing phytohormones (Vessey, 2003). Associations between plants and their microbiomes can enhance plant resilience to drought directly, through modifications of root biomass and architecture as well as of plant physiology and biochemistry (Querejeta et al., 2007; Bahadur et al., 2019), or indirectly, by improving soil structure (Kohler et al., 2009). In fact, certain free-living bacteria and fungi included in the soil microbiome can retain soil moisture by producing extracellular polymeric substances, and thus increase water availability to plants (Guo et al., 2018). Some studies have indicated that plant invasions may enhance soil microbial diversity, promoting advantageous multifunctionality in the soil (Wagg et al., 2019), thereby benefiting plant growth (Lankau et al., 2022) and plant drought tolerance (Prudent et al., 2020).

Metagenomics studies have shown that exotic invasive plants are able to shape their own microbiome selectively by recruiting beneficial taxa from local bacterial and fungal communities under non-drought (Rodríguez-Caballero et al., 2017, 2020a, 2020b; LaForgia et al., 2022) and drought conditions (Fahey et al., 2020). Recently, the use of bioinformatics tools for functional metagenome data analysis of the rhizosphere microbial community has demonstrated that the invasion of grasses in combination with drought alters essential functions associated with nutrient stress and structure in the native rhizosphere community (Ettinger and LaForgia, 2024). In a previous study, we found that the functional characteristics of the microbiome harbored by the perennial woody invasive species *Nicotiana glauca* R.C. Graham were distinct from those reported for co-occurring native species assessed in Mediterranean ecosystems (Rodríguez-Caballero et al., 2020b). In a separate study, we revealed that CO_2 enrichment enhanced the bacterial functional potential of the rhizosphere microbiome of *N. glauca* as well as the growth of this highly invasive plant (Caravaca et al., 2022). However, there is a paucity of studies whether drought may influence differently the functional traits of the microbial communities of invasive and co-occurring native plant species, as well as how these shifts could be related to the growth advantage of the invader.

Thus, we hypothesize that the microbial composition and structure as well as the functional potential of the rhizosphere microbiome in invasive and native plant species will be differentially altered in drought seasons, and that these differences in the microbial taxa and functional traits of the microbiome can provide advantages to the invasive plant over native ones in dry periods. To corroborate this hypothesis, we compared the composition, structure, and functional traits of the microbial community of the invader *N. glauca* and native species growing at seven different Mediterranean semiarid locations under two distinct conditions of water availability, corresponding to the wet and dry seasons.

2. Materials and methods

2.1. The invasive plant species and invaded locations

The invader *N. glauca* (*Solanaceae*) is an evergreen shrub or small tree native to South America, introduced in many regions as an ornamental plant (Sanz Elorza et al., 2004) and widely naturalized in hot, dry climates throughout the world (GISD, 2024). The physiological and biological characteristics of *N. glauca* make it a very aggressive species with a high risk of becoming invasive (Florentine et al., 2016). This is due especially to its adaptation to dry and temperate climates and to the great diversity of soils and environments it occupies, its opportunistic role in the colonization of open spaces, its autogamous reproductive capacity and easy seed dispersion, its toxicity for all types of herbivores, its production of allelopathic compounds, and the lack of natural predators.

Seven semiarid Mediterranean locations where *N. glauca* has displaced the native vegetation, occupying >50 % of the surface area, were chosen in the province of Murcia, SE Spain. The climate of these locations is characterized by hot and long dry periods and scarce and irregular precipitations, which are concentrated during spring and autumn, as well as by high potential evapo-transpiration. The data of precipitation and temperature of the sampling seasons, geographical location, and composition of the native plant communities of the selected locations are shown in Table 1.

2.2. Experimental design and sampling

Two field samplings were conducted, one at the end of spring and the other at the end of summer in 2022, representing the wet and dry seasons, respectively. The experiment followed a three-factor factorial design with four replicates arranged randomly. The factors were "Location" with seven levels, the "Invasiveness" trait of the plant with two levels, and the "Season" of sampling with two levels. Within each location, four sampling plots invaded by the target invasive species, each measuring 3 m by 3 m and spaced 10 m apart, were established alongside areas where the invasive and native species coexisted. From each plot, one soil composite sample was collected from the rhizosphere of the invasive species *N. glauca*, and another composite sample was obtained from the rhizospheres of the most abundant native species. A total of 56 rhizosphere soil samples were collected for each sampling season.

Rhizosphere soil was collected by shaking each root system into a polyethylene bag. One portion of the rhizosphere soil was stored in a freezer at −20 °C for molecular analyses, while the remainder was divided into two parts: one was sieved to 0.25–4 mm to determine the percentage of water-stable aggregates, and the other was sieved at 2 mm for conducting the remaining soil analyses.

2.3. Analyses of rhizosphere soil

The pH and electrical conductivity of the soil were measured in a 1:5 (*w/v*) soil-to-water extract. Water-soluble carbon (WSC) was quantified in this extract using an automatic Carbon Analyser (Shimadzu TOC-5050A, Kyoto, Japan). Total organic carbon (TOC) and total nitrogen in the rhizospheric soil were determined by combustion followed by chromatographic separation in a LECO elemental analyzer (LECO CHN628 Series, LECO Corporation, St Joseph, MI USA). Phosphorus and potassium availability were assessed using a Thermo ICP/OE spectrometer (ICAP 6500 DUO; Thermo-Scientific, Waltham, MA, USA) following extraction with sodium bicarbonate and ammonium acetate, respectively.

The soil intracellular dehydrogenase enzyme activity was assessed by colorimetric measurement of the reduction product iodonitrotetrazolium formazan, following the method outlined in García et al. (1997). The activities of soil extracellular enzymes (β-glucosidase,

Table 1
Sampling points location, geographical coordinates, climatic parameters and native vegetation.

Location	Abbreviation wet (W) season (invasive = I, native = N)	Abbreviation dry (D) season (invasive = I, native = N)	Geographical coordinates	Spring 2022 total rainfall (mm)	Summer 2022 total rainfall (mm)	Spring 2022 mean temperature (°C)	Summer 2022 mean temperature (°C)	Dominant native plant species Wet season	Dominant native plant species Dry season
Guadalupe (G)	GWI, GWN	GDI, GDN	38° 00′ 12.8″ N/1° 10′ 01.4″ W	209.5	21.9	19.6	27.2	*Cardus bourgeanus* *Pallenis spinosa* *Piptatherum milliaceum* *Sonchus oleraceus*	*Carduus bourgeanus* *Pallenis spinosa* *Piphatherum miliaceum* *Zygophyllum fabago*
Murcia (M)	MWI, MWN	MDI, MDN	38° 01′ 28.8″ N/1° 08′ 43.9″ W	191.3	7.2	19.7	28.1	*Cardus bourgeanus* *Lavatera cretica* *Pallenis spinosa* *Piphatherum miliaceum*	*Cardus bourgeanus* *Pallenis spinosa* *Phagnalon saxatile* *Piphatherum miliaceum*
Molina de Segura (Mo)	MoWI, MoWN	MoDI, MoDN	38° 02′ 20.5″ N/1° 09′ 51.5″ W	74.9	7.2	19.2	27.3	*Cardus bourgeanus* *Helichrysum stoechas* *Lygeum spartum* *Phagnalon saxatile*	*Anthyllis cytisoides* *Helichrysum stoechas* *Lygeum spartum* *Phagnalon saxatile*
Santomera (S)	SWI, SWN	SDI, SDN	38° 05′ 33.7″ N/1° 03′ 21.0″ W	159.0	7.1	18.4	26.4	*Carduus bourgeanus* *Euphorbia helioscopia* *Phagnalon saxatile* *Piphatherum miliaceum*	*Euphorbia helioscopia* *Lavandula multifida* *Phagnalon saxatile* *Piphatherum miliaceum*
Churra (C)	CWI, CWN	CDI, CDN	38° 02′ 24.0″ N/1° 09′ 07.8″ W	191.3	7.2	19.7	28.1	*Carduus bourgeanus* *Echium vulgare* *Limonium cossonianum* *Piphatherum miliaceum*	*Carduus bourgeanus* *Limonium cossonianum* *Mesembryanthemum nodiflorum* *Setaria arvensis*
San Javier (J)	JWI, JWN	JDI, JDN	37° 57′ 55.8″ N/0° 58′ 09.5″ W	199.3	16.0	19.1	27.0	*Carduus bourgeanus* *Phagnalon saxatile* *Piphatherum miliaceum* *Sonchus oleraceus*	*Ballota hirsuta* *Echium vulgare* *Limonium echioides* *Piphatherum miliaceum*
Alcantarilla (A)	AWI, AWN	ADI, ADN	37° 57′ 11.0″ N/1° 12′ 12.1″ W	206.8	13.1	19.5	27.7	*Bromus rubens* *Diplotaxis erucoides* *Euphorbia helioscopia* *Sonchus oleraceus*	*Carduus bourgeanus* *Ecballium elaterium* *Salsola kali* *Setaria arvensis*

urease, protease, and alkaline phosphomonoesterase) were estimated according to Tabatabai (1994). In brief, these enzymatic activities were determined colorimetrically following soil incubation with the respective substrate at their optimal pH.

Microbial biomass carbon was quantified using the substrate-induced respiration (SIR) method, with glucose as the substrate, following the protocol of Anderson and Domsch (1978). The evolved CO_2 was measured using a μ-Trac 4200 analyzer (SY-LAB, GmbH, Neupurkersdorf, Austria). Basal soil respiration was assessed by monitoring the amount of CO_2 released from moist soil samples (at 60 % of their water retention capacity) using the aforementioned CO_2 analyzer.

2.4. Isolation of DNA, PCR amplification, and DNA sequencing

Total DNA was extracted from 0.25 g of frozen rhizosphere soil using the DNeasy PowerSoil DNA Isolation kit (Qiagen), following the protocol provided. Primers ITS86F and ITS4 were utilized to amplify the entire ITS2 region of the fungal ITS region for metabarcoding (Turenne et al., 1999; White et al., 1990), while primers 341F and 805R were used to target the V3-V4 region of the bacterial 16S SSU rRNA (Herlemann et al., 2011). These primers also included the Illumina sequencing primer sequences attached to their 5′ ends. Each PCR mixture contained 2.5 μL of the DNA template, 0.5 μL of the corresponding primers and 12.5 μL of Supreme NZYTaq 2× Green Master Mix (NZYTech) and was diluted to a total volume of 25 μL with ultrapure water. The PCR incubation program consisted of an initial denaturation at 98 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 10 min. To multiplex different libraries in the same sequencing pool, the barcodes identifiers were attached in a second PCR round with identical conditions but only five cycles and 60 °C as the annealing temperature. Each library was purified using the Mag-Bind RXNPure Plus magnetics beads (Omega Biotek Inc., Norcross, GA, USA), following the manufacturer indications. Finished libraries were pooled in equimolar amounts according to the results of a Qubit dsDNA HS Assay (Thermo Fisher Scientific) quantification. The pool was sequenced in a fraction (9/16) of a MiSeq PE300 flow cell (Illumina). The DNA metabarcoding analyses were conducted by AllGenetics & Biology SL (www.allgenetics.eu).

2.5. Sequencing data processing

The amplicon reads for 16S and ITS2 were processed using DADA2 (Callahan et al., 2016), implemented in QIIME2 (release 2020.8) (Bolyen et al., 2019). To ensure high quality, the DADA2 pipeline initially trims and filters data to remove amplification primers and low-quality bases. After assessing the read quality profiles, forward reads were truncated at position 299 and reverse reads at position 251. Denoising error rates were then calculated using the parametric error model integrated into DADA2. Prior to sequence variant inference, the dataset underwent dereplication, read merging, and removal of chimeras. As a result, the analysis provided a table with the frequency of each Amplicon Sequence Variant (ASV) in each sample.

Bacterial and fungal taxonomy were assigned to ASVs using pre-trained classifiers from the SILVA version 138.1 reference database (Quast et al., 2013, updated in August 2020) and the UNITE reference database (Abarenkov et al., 2020), respectively. The feature-classifier classify-sklearn method within QIIME2 was utilized for this purpose (Bokulich et al., 2018). Following this assignment, various filters were applied based on the results. For each ASV table, singletons, i.e. ASVs containing only one member sequence in the whole data set including all samples together, were removed. Likewise, ASVs occurring at a frequency below 0.01 % of the total number of sequences including all samples together were eliminated to correct for potential misidentification.

The metabarcoding analysis of rhizosphere bacterial communities from *N. glauca* and native plants resulted in a total of 1,489,763 reads during the spring wet season, averaging around 26,600 reads per sample. In the summer dry season, there were 1,762,461 reads across all samples, averaging approximately 31,500 reads per sample. These reads were categorized into 28,071 ASVs, where were assigned to a total of 39 phyla (Tables S1 and S2) and 562 distinct families (Tables S3 and S4).

For the ITS2 dataset, encompassing all samples, the ASV method yielded a combined total of 1,890,918 reads from all samples collected during the spring wet season (an average of 33,766 reads per sample) and 1,695,161 reads across all the samples of the summer dry season (an average of 30,271 reads per sample). A total of 5485 ASVs were identified, which were assigned to a total of 16 phyla (Tables S5 and S6) and 314 families, as detailed in Tables S7 and S8.

2.6. Functional analysis of the bacterial and fungal communities

Functional analysis of the bacterial communities identified through DNA metabarcoding was conducted using PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states, Douglas et al., 2020). The ASV sequences from the rhizosphere bacterial communities were aligned with reference sequences using HMMER (Eddy, 2011). Normalization of the data and prediction of the abundance of different gene families were performed using the Hidden State Prediction (HSP) algorithm from the "castor" R package (Louca and Doebeli, 2018). Predicted copy numbers of all Enzyme Commission (EC) numbers for each ASV were also generated. The contribution of each ASV to community-wide pathway abundance was inferred using pathway rules from the MetaCyc database (Caspi et al., 2019) and the MinPath (Minimal set of Pathways) tool (Ye and Doak, 2009). The effects of invasiveness and location as well as invasiveness and sampling season on bacterial functional profiles were assessed using a two-sided Welch's *t*-test. Benjamini-Hochberg FDR (Benjamini and Hochberg, 1995) and Storey FDR (Storey, 2011) correction methods were applied to the results, and features with a *P*-value ≥ 0.05 were removed. The statistical procedures were conducted using the STAMP package (Parks et al., 2014).

The ecological functions of fungal ASVs were assigned based on taxonomic assignments, using the FUNGuild bioinformatic tool (Nguyen et al., 2016) and its community-annotated database. Fungal functional groups (guilds) were classified according to three trophic modes (pathotrophs, saprotrophs, and symbiotrophs) and three confidence ranks (possible, probable, and highly probable).

2.7. Statistical procedures

The Kolmogorov-Smirnov test assessed the assumption of normal distribution for all soil and plant properties, while the Levene test examined variance homogeneity. Log transformation was applied to the data to make it conform to a normal distribution and to address variance heterogeneity. Three-way ANOVA was conducted to analyze the effects of three experimental factors - plant invasiveness, location of invasion, and sampling season - and their interactions on the measured variables. Differences among means were evaluated using Tukey's HSD (Honestly Significant Difference) test ($P < 0.05$).

Rarefaction curves of the rarefied dataset confirmed that the sequencing efforts adequately captured the diversity within microbial communities (see Figs. S1 and S2 in Supplementary material). Sequencing yielded 12,402 sequences per sample for bacterial communities and 13,766 sequences per sample for fungal communities, based on rarefaction analysis. These rarefied datasets were utilized in subsequent analyses.

Non-metric multidimensional scaling (NMDS) analysis, employing the Bray–Curtis distance, was conducted to visually compare the taxonomic composition and potential metabolic pathways across microbial communities among samples. The impact of plant invasiveness, invaded location, sampling season, and their interactions on the taxonomic composition of rhizosphere soil microbial communities was evaluated

using permutational multivariate analysis (perMANOVA) on the same Bray-Curtis distance matrix with 999 permutations. Additionally, a three-way ANOVA using NMDS axes scores was performed to compare the potential functions of native and invasive bacterial communities.

Canonical correspondence analysis (CCA) was utilized to assess the relative importance of soil and plant properties in driving changes in the bacterial or fungal community composition. The statistical significance of CCA relationships between explanatory variables and the microbial community composition was determined using 999 runs of Monte Carlo permutational procedures.

An indicator species analysis (ISA) was conducted to identify characteristic species for each group based on the three experimental factors. The indicator value index (IndVal) and its significance were computed for each indicator species using the "indicspecies" R package. All statistical analyses were performed using R software version 4.2.3 (R-Team-Core, 2023).

2.8. Data availability

The sequence files were submitted to the NCBI Sequence Read Archive repository (www.ncbi.nlm.nih.gov/sra) and are accessible in the BioProject PRJNA1107148.

3. Results

3.1. Description of bacterial communities in the rhizosphere soil of invasive and native plants during wet and dry seasons

The NMDS ordination plot and perMANOVA outcomes indicate significant differences in the bacterial community composition within the rhizosphere, as influenced by invasiveness (Fig. 1; F = 4.2, $P = 0.001$), sampling season (F = 3.6, $P = 0.001$), and location (F = 11.4, $P = 0.001$), along with the interaction between the factors invasiveness and sampling season (F = 2.2, $P = 0.002$).

The ISA revealed that the phylum *Actinobacteriota* was an indicator phylum for both the dry season and the community of *N. glauca* (Table 2). Meanwhile, the rhizosphere soil of *N. glauca* was associated with a slightly lower number of indicator families (37) compared to that of native species (43), as depicted in Table S9. Notably, *Micrococcaceae* (total relative abundance approximately 5 %) and *Beijerinckiaceae* (total relative abundance approximately 6 %) were the indicator families with

Table 2
Indicator value (IndVal) and associated significance value (*P*) of bacteria communities at phylum level for *Nicotiana glauca* (invasive) and the native plants and for the wet and dry seasons.

Bacterial phyla	IndVal	*P*-value
N. glauca		
Patescibacteria	0.815	0.001***
Actinobacteriota	0.728	0.007**
MBNT15	0.474	0.030*
Native plants		
Cyanobacteria	0.887	0.001***
Fibrobacterota	0.766	0.002**
Proteobacteria	0.721	0.013*
Chloroflexi	0.719	0.044*
Dry season		
Thermoplasmatota	0.619	0.002**
Hydrogenedentes	0.438	0.006**
Actinobacteriota	0.723	0.039*
Halobacterota	0.409	0.049*
Wet season		
Proteobacteria	0.724	0.002**
Gemmatimonadota	0.734	0.020*
Crenarchaeota	0.713	0.049*

*, **, *** significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

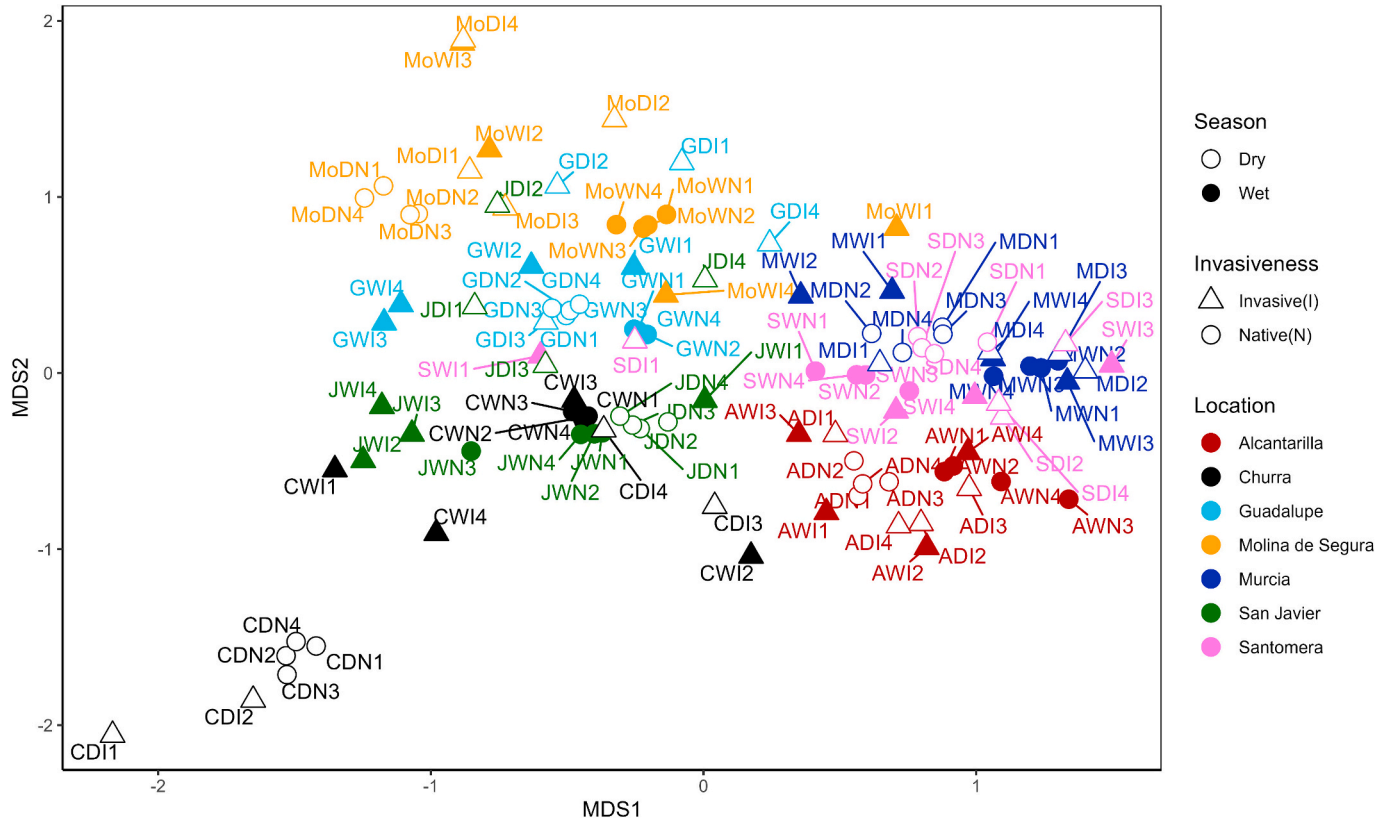

Fig. 1. NMDS ordination plot of the Bray-Curtis dissimilarity matrix based on the ASVs dataset retrieved from the bacterial communities of the rhizospheric soil of invasive (*N. glauca*) and native plants growing at seven different locations during the wet and dry seasons.

the highest relative abundances in the rhizosphere soils of *N. glauca* and native plants, respectively.

The ISA indicated that the number of indicator families in the rhizosphere soil of plants in the wet season (36) was lower than that of the rhizosphere soil of plants in the dry season (47), as shown in Table S10. The most abundant indicator taxa of the dry season with taxonomic assignation at the family level were 67–14, *Sphingomonadaceae*, and *Pseudonocardiaceae*. The relative abundances of these indicator families were higher in the rhizosphere soil of invasive plants than in that of native plants. The ISA indicated that the number of indicator genera of the rhizosphere soil of plants in the wet season (63) was lower than that of the rhizosphere soil of plants in the dry season (73), as shown in Table S11. The most abundant indicator taxa of the dry season were 67–14, *Sphingomonas*, 0319-7 L14, Subgroup.7, and Gitt-GS-136, the highest relative abundances being recorded in the rhizosphere soil of invasive plants.

3.2. Description of fungal communities in the rhizosphere of invasive and native plants during wet and dry seasons

The fungal communities residing in the rhizosphere soils demonstrated clustering based on the invaded location, sampling season, and the invasive nature of the plant, as evidenced in the NMDS ordination plot (Fig. 2). This visual observation was corroborated by the significance of the experimental factors in the perMANOVA analysis: the composition of the rhizosphere fungal communities was notably affected by location ($F = 10.1$, $P = 0.001$), invasiveness ($F = 5.3$, $P = 0.001$), and sampling season ($F = 5.3$, $P = 0.001$). Moreover, the interaction between these latter two factors was found to exert a strong influence on community composition ($F = 2.3$, $P = 0.001$).

Ten indicator families were identified in the rhizosphere soil of *N. glauca*, whereas 55 indicator families were found in the rhizosphere of native plants (Table S12). After applying the ISA for the factor sampling season, 30 families emerged as indicators for the rhizosphere soil of plants during the dry season (Table S13). Among those indicators, the most abundant families were *Aspergillaceae*, *Glomeraceae*, and *Claroideoglomeraceae*, particularly in the invasive rhizosphere. After applying the ISA for the factor sampling season, 58 genera appeared as indicators for the rhizosphere of plants during the dry season (Table S14). Among them, the most abundant genus was *Aspergillus*, particularly in the invasive rhizosphere.

3.3. Functional potential of bacterial and fungal communities in rhizosphere soil of invasive and native plants during wet and dry seasons

The PICRUSt2 analysis, utilizing the MetaCyc database, unveiled 2363 enzymes and 434 pathways predicted from 16S rRNA gene sequencing data for the bacterial communities, considering the rhizosphere of both *N. glauca* and native plants.

In the NMDS ordination plot, the predictive functional profile of bacterial communities linked to the rhizosphere of invasive plants was typically distinct from that of the corresponding native plants, and the functional profiles of the rhizospheres sampled in dry conditions differed from those sampled in wet conditions (Fig. 3). The effect of the experimental factors on the spatial ordination of bacterial community functional capabilities was determined by conducting a three-way ANOVA on the NMDS1 and NMDS2 axis scores. This statistical analysis confirmed a significant difference in microbial functional capabilities between native and invasive bacterial communities for the NMDS1 axis ($P = 0.003$).

Also, there was a significant difference in the functional profile of the rhizosphere between the dry and wet seasons ($P = 0.024$), and the functional potential of the bacterial community was strongly affected by the interaction of invasiveness and season ($P < 0.001$).

Out of the 2363 enzymes generated by PICRUSt2, 35 were unique to the rhizosphere of *N. glauca*, while 16 were exclusive to the rhizosphere of native plants. Among the enzymes shared by both types of plant species, the relative abundances of 376 enzymes differed significantly

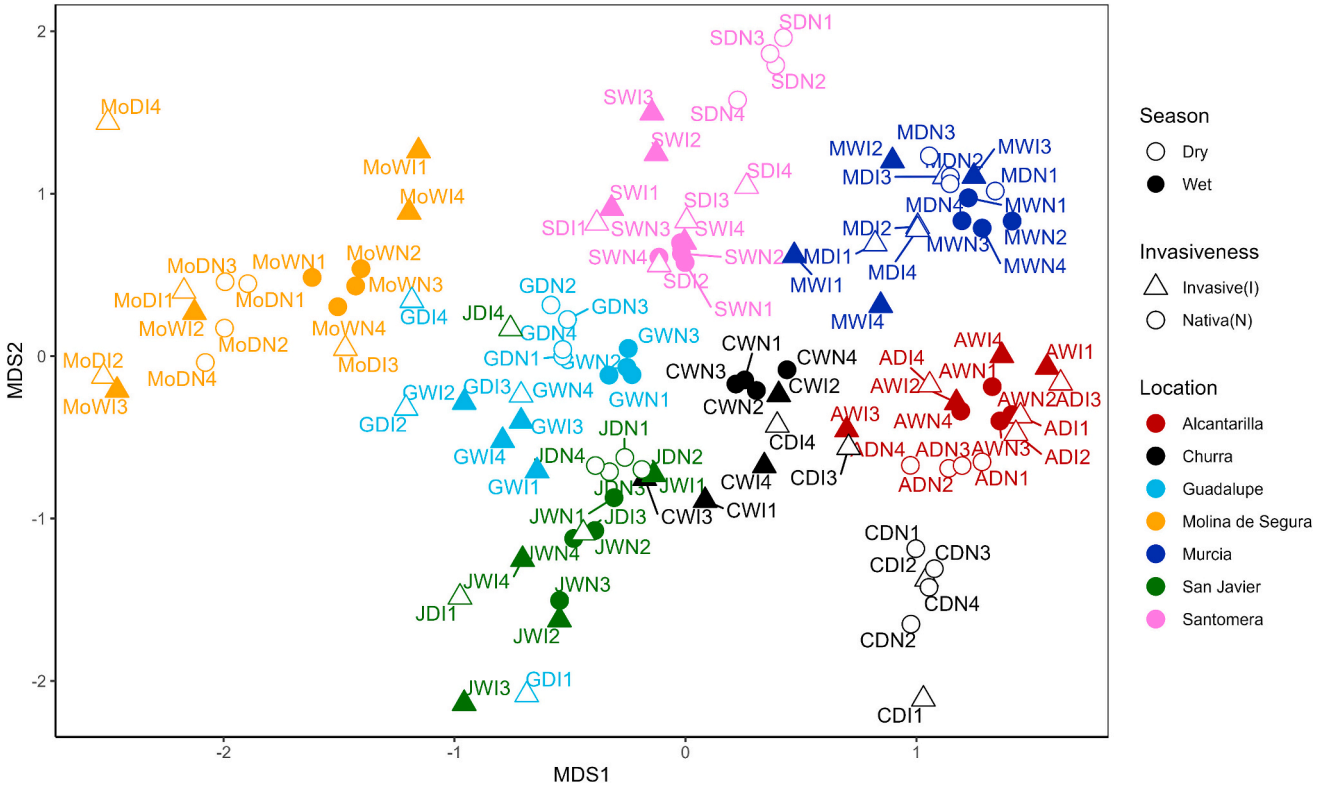

Fig. 2. NMDS ordination plot of the Bray-Curtis dissimilarity matrix based on the ASVs dataset retrieved from the fungal communities of the rhizospheric soil of invasive (*N. glauca*) and native plants growing at seven different locations during the wet and dry seasons.

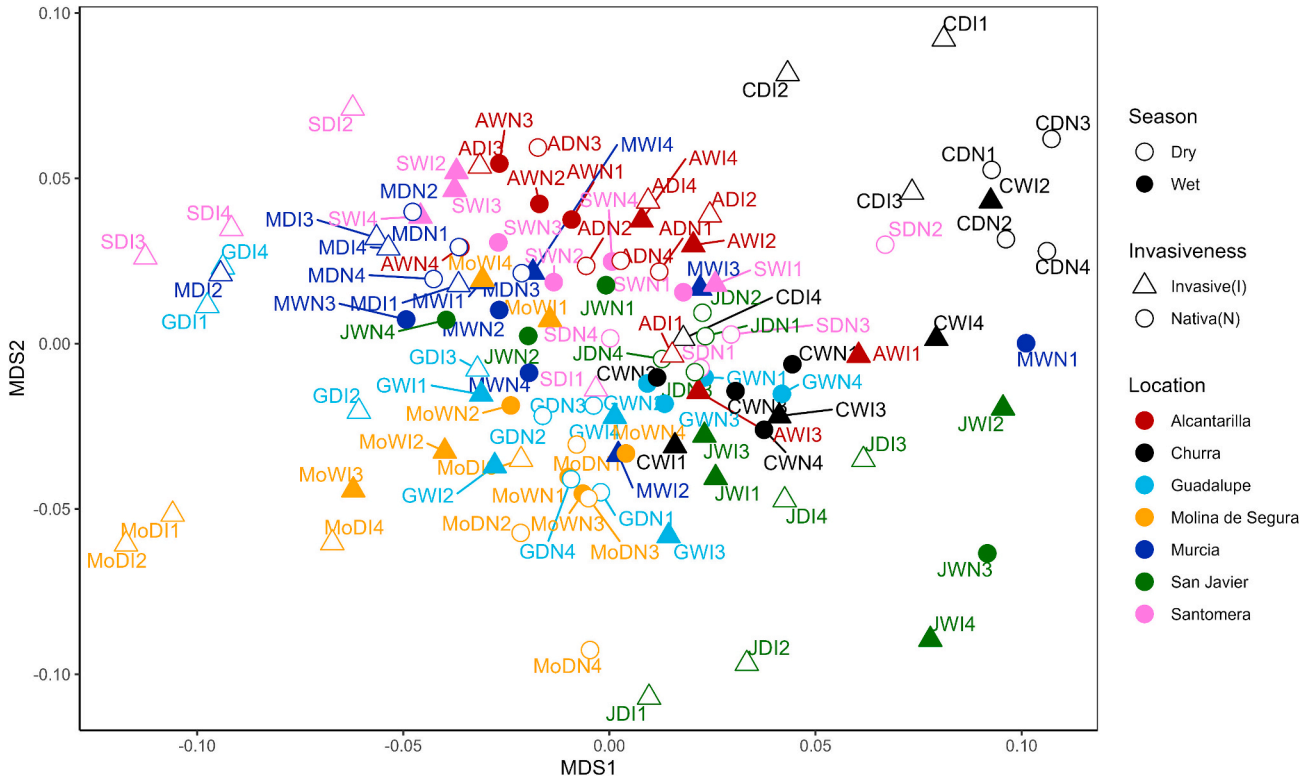

Fig. 3. NMDS ordination plot of the Bray-Curtis dissimilarity matrix based on the predicted functional pathway profiles associated with the rhizosphere bacterial communities of invasive (*N. glauca*) and native plants growing at seven different locations during the wet and dry seasons.

between them according to Welch's *t*-test (Benjamini-Hochberg FDR *q*-value <0.05) (Table S15). Regarding the sampling season, 37 enzymes were exclusive to the dry season and 13 enzymes to the wet season, with 351 enzymes differing significantly between the sampling seasons (Table S16). In particular, 190 enzymes were more abundant under wet conditions. Among the 161 enzymes with greater abundance under drought conditions, the relative abundance of 27 enzymes was greater in the rhizosphere of *N. glauca* (Table S17). Among the 190 enzymes whose relative abundance was greater under wet conditions, the relative abundance of 61 enzymes differed between the two types of plant. Specifically, 54 enzymes were more abundant in the rhizosphere of native plants and seven enzymes in the rhizosphere of *N. glauca*. Regarding the predicted MetaCyc pathways, 434 exhibited significant differences between the two plant types (Table S18), while 94 displayed significant differences between the two sampling seasons (Table S19), based on Welch's *t*-test (Benjamini-Hochberg FDR q-value < 0.05). In particular, 48 pathways were relatively more abundant under drought conditions than under wet conditions; the relative abundance of 12 of these was greater in the rhizosphere of *N. glauca* (Table S20). Most of the MetaCyc pathways that had a greater presence in the dry season belong to the primary superclass biosynthesis (10 pathways), mainly involved in cell structure (peptidoglycan, amino acid, cofactor, carrier, vitamin) and secondary metabolite biosynthesis, and the rest of the pathways are involved in the generation of precursor metabolites and energy. Among the 46 pathways that had a greater presence under wet conditions, 15 pathways differed between the two types of plant, their relative abundances mainly being higher in the rhizosphere of native plants. The rhizosphere bacterial community of native plants exhibited a relative enrichment of metabolic functions primarily related to the biosynthesis superclass (9 pathways) at MetaCyc level 1. These pathways were notably involved in fatty acid and lipid biosynthesis at MetaCyc level 2.

The FUNGuild analysis allocated approximately 33 % of fungal ASVs to the primary trophic modes: saprotrophs, symbiotrophs, and pathotrophs (Fig. 4). The relative abundances of these trophic modes were significantly affected by the sampling season ($F = 6.23$, $P = 0.015$ for saprotrophs; $F = 26.70$, $P < 0.001$ for symbiotrophs; $F = 11.91$, $P = 0.001$ for pathotrophs). The invasiveness had a significant effect on the relative abundances of symbiotrophs and pathotrophs ($F = 4.12$, $P = 0.040$; $F = 4.88$, $P = 0.030$, respectively), whereas the location influenced significantly the relative abundances of saprotrophs and pathotrophs ($F = 4.38$, $P = 0.001$; $F = 10.40$, $P < 0.001$, respectively). The relative abundances of saprotrophs and symbiotrophs were higher in the dry season than in the wet season, particularly in the rhizosphere of invasive plants (about 41 % higher with respect to the native rhizosphere). The symbiotrophs mainly comprised mycorrhizal fungi (on average, about 60 % of total symbiotrophs), without significant differences between the two types of plant in the wet season. Nevertheless, during the dry season, the relative abundance of mycorrhizal fungi in the rhizosphere of *N. glauca* was approximately 40 % higher than in that of native plants.

3.4. Influence of the experimental factors on rhizosphere properties

With the exception of TOC, soil physicochemical and chemical properties were influenced by invasiveness, with higher values observed in the rhizosphere soil of *N. glauca* compared to that of native plants (Tables 3 and 5). Conversely, soil electrical conductivity was greater in the rhizosphere of native plants. The factor sampling season, alone or in combination with invasiveness, had a significant effect on the soil chemical properties measured except for total N (Table 5). In particular, the percentage of stable aggregates was higher in the rhizosphere soil of plants sampled during the dry season (about 42 % higher than that of plants sampled during the wet season), particularly in the *N. glauca* rhizosphere (Table 5). Likewise, the levels of available P, extractable K, and WSC were higher in the rhizosphere soil of plants sampled during the dry season. The ANOVA also indicated that the sampling location significantly influenced all the chemical properties assessed.

Overall, the microbiological parameters displayed variability

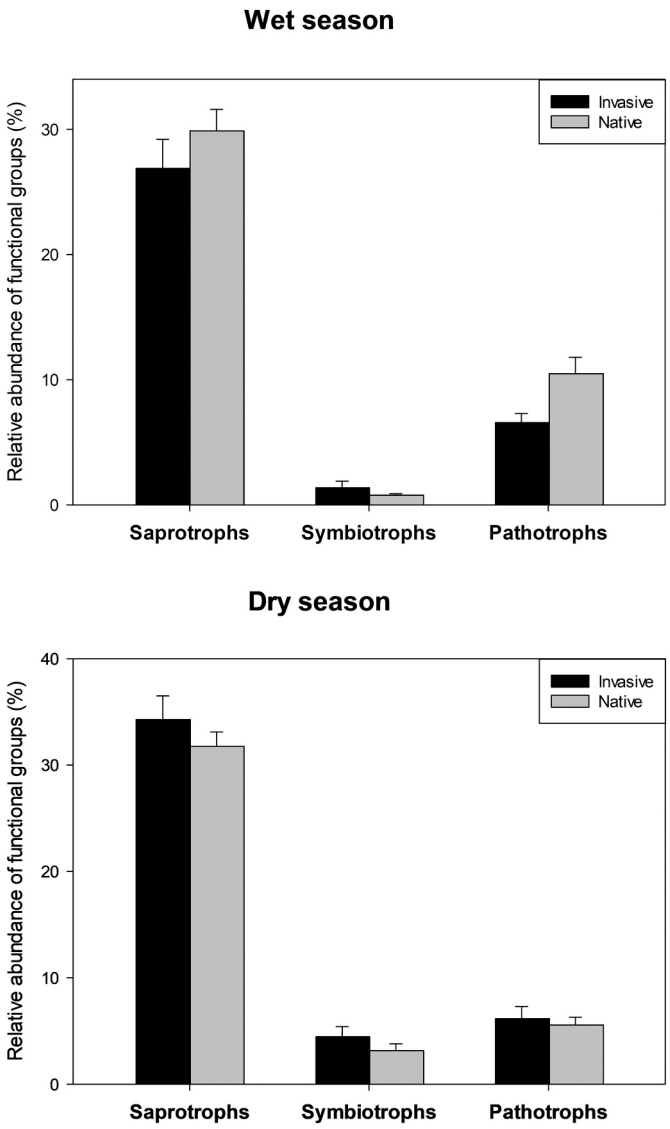

Fig. 4. Relative abundances of functional groups (saprotrophs, symbiotrophs and pathotrophs) predicted using FUNGuild for the fungal communities of the rhizospheric soil of invasive (*N. glauca*) and native plants growing at seven different locations during the wet and dry seasons.

depending on the factors invasiveness, location, and sampling season (Tables 4 and 5). The interaction invasiveness × sampling season significantly affected all soil enzyme activities. The activities of all soil enzymes were higher in the rhizosphere soil of *N. glauca* with respect to those of native plants (Table 4). The enzymatic activities recorded were higher in the rhizosphere soil of plants sampled during the wet season, compared to those sampled during the dry season. The differences in enzymatic activities between the seasons were more pronounced in the rhizosphere soil of native plants. In contrast, biomass C in the rhizosphere soil was higher in the dry season than in the wet season, for most locations.

3.5. Relationships between environmental variables and rhizosphere soil microbial communities

Based on the forward selection process, all environmental variables including the precipitation data of the invaded sites were incorporated in the final CCA model for the dataset of rhizosphere bacterial communities, as illustrated in Fig. 5.

The constrained axes of the CCA accounted for approximately 20 % of the total inertia. The cumulative percentage of constrained inertia for the first two axes was approximately 24 %. The first CCA axis was strongly associated with protease activity and extractable potassium, followed by total organic carbon and total N. Meanwhile, available phosphorus, water-soluble carbon, and soil respiration showed negative correlations with the second axis of the CCA.

The forward selection process in the CCA conducted for the rhizosphere fungal community dataset identified the same constraining variables as described above for the rhizosphere bacterial community (Fig. 6). The constrained axes of the CCA explained approximately 20 % of the total inertia, with the first two axes accounting for 22 % of the constrained inertia. The constraining variables that exhibited a negative correlation with the first CCA axis were extractable potassium and protease, whereas the second axis was associated with dehydrogenase activity and soil respiration.

4. Discussion

Shifts in the microbial community composition and structure of *N. glauca* and native plants rhizospheres in response to drought conditions.

The microbial community composition and structure were driven by edaphic properties as well as the climatological characteristics of the invaded locations such as precipitation, which might have been due to changes in soil moisture. This physical-chemical parameter fluctuated with the sampling season, being about 175 % higher during the wet season than during the dry season. Of special relevance is the fact that soil moisture during the dry season (about 2.5 %) is limiting for plant growth. The invasive plant *N. glauca* has colonized Mediterranean ecosystems that are frequently subjected to desiccation followed by relatively rapid rewetting events, competing with a large variety of native plant communities adapted to such stressful climatic events. Soil drying is a prevalent physiological stressor for soil microbial communities (Xie et al., 2021). In particular, soil drying can induce changes in living microbial biomass, activity, and community composition (Khan et al., 2019). The changes in soil microbiota caused by dry conditions in soil largely depend on either its physical stabilization within the soil matrix or its ability to withstand desiccation in soil. In this respect, dry conditions would increase the relative abundance of stress-tolerant microorganisms.

Through ASV metabarcoding analyses, we demonstrated that the presence of this invader in these ecosystems was correlated with changes in below-ground microbial communities under both dry and wet conditions. Indeed, the invasion by *N. glauca* has been accompanied by distinct rhizosphere bacterial and fungal communities, compared to those hosted by the native co-occurring plant species. Previous research conducted through Operational Taxonomic Unit (OTU) based metabarcoding analysis reported the shifts induced by the invasion of *N. glauca* growing during the spring rainy season in Mediterranean locations with different edaphic characteristics (Rodríguez-Caballero et al., 2020b). The invasive plant species may have induced an increase in bacterial taxa beneficial to its success under drought conditions of close to 3 %. The phylum *Actinobacteriota* (or *Actinobacteria*) was an indicator common to both the rhizosphere community of *N. glauca* and the rhizosphere communities under drought conditions. *Actinobacteria* are Gram-positive bacteria, which usually predominate over Gram-negative bacteria in moisture-limited soils (Naylor and Coleman-Derr, 2018). The members of Actinobacteria are recognized as a lineage resilient to drought stress (Jones et al., 2022; Hu et al., 2023) as well as for their ability to thrive in extreme environmental conditions; they are highly resistant to radiation, tolerant of high salt concentrations, and capable of withstanding high temperatures, even exhibiting thermophilic characteristics (Maisnam et al., 2023). The enrichment of the bacterial community with monoderms having thick cell walls, such as *Actinobacteria*, under drought conditions could be related to their fast drought response based on the large production of stress proteins (Xu

Table 3
Physico-chemical and chemical properties of the rhizospheres of invasive (I = *N. glauca*) and native plants (N = native) grown in seven different locations during the wet (W) and dry (D) seasons ($n = 4$, mean ± standard error). EC: electrical conductivity; Avail. P = available phosphorus; Ext. K = extractable potassium; AS: aggregate stability; TOC = total organic carbon; TN = total nitrogen; WSC = Water soluble carbon. For sampling season, values in column sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey HSD test.

Location	Season	Sample	pH (H_2O, 1:5)	EC (1:5, μS cm^{-1})	Avail. P (mg kg^{-1})	Ext. K (mg kg^{-1})	AS (%)	TOC (g kg^{-1})	TN (g kg^{-1})	WSC (mg kg^{-1})
Guadalupe	Wet	GWI, GWN	7.92 ± 0.15**ab**	830 ± 46**bc**	20 ± 1**b**	374 ± 35**cd**	18.6 ± 2.1**ab**	0.53 ± 0.07**ab**	0.05 ± 0.00**a**	38 ± 4**bc**
			8.06 ± 0.03**abc**	680 ± 32**abc**	17 ± 3**b**	347 ± 4**bcd**	15.9 ± 0.4**a**	0.64 ± 0.04**abcd**	0.09 ± 0.01**ab**	29 ± 2**ab**
Murcia		MWI, MWN	8.81 ± 0.04**fg**	153 ± 6**a**	41 ± 13**c**	454 ± 108**d**	19.6 ± 2.5**abc**	0.86 ± 0.95**bcd**	0.12 ± 0.03**bcd**	76 ± 8**gh**
			8.94 ± 0.02**g**	133 ± 1**a**	32 ± 2**bc**	430 ± 12**d**	25.5 ± 0.6**cde**	0.95 ± 0.03**de**	0.12 ± 0.01**bcd**	70 ± 1**fg**
Molina de Segura		MoWI, MoWN	8.52 ± 0.17**def**	657 ± 44**abc**	7 ± 4**a**	224 ± 43**ab**	23.2 ± 2.7**bcd**	0.56 ± 0.12**abc**	0.07 ± 0.02**a**	36 ± 5**b**
			8.21 ± 0.02**c**	909 ± 36**c**	4 ± 1**a**	176 ± 1**a**	20.3 ± 0.3**bcd**	0.41 ± 0.06**a**	0.06 ± 0.00**a**	22 ± 1**a**
Santomera		SWI, SWN	8.60 ± 0.07**def**	148 ± 14**a**	18 ± 2**b**	446 ± 3**d**	45.3 ± 2.9**g**	0.84 ± 0.19**bcd**	0.19 ± 0.01**e**	57 ± 6**de**
			7.87 ± 0.02**a**	1709 ± 62**d**	11 ± 3**a**	391 ± 84**cd**	43.5 ± 1.2**g**	1.58 ± 0.14**f**	0.15 ± 0.04**cd**	39 ± 0**bc**
Churra		CWI, CWN	8.17 ± 0.15**bc**	277 ± 13**abc**	17 ± 1**b**	281 ± 38**abc**	29.8 ± 3.0**ef**	0.71 ± 0.20**bcd**	0.12 ± 0.02**bcd**	56 ± 0**de**
			8.35 ± 0.04**cde**	255 ± 21**abc**	15 ± 1**ab**	278 ± 10**abc**	34.0 ± 1.6**f**	0.89 ± 0.09**cd**	0.10 ± 0.01**abc**	47 ± 1**cd**
San Javier		JWI, JWN	8.71 ± 0.06**fg**	165 ± 14**ab**	17 ± 5**b**	271 ± 36**ab**	17.8 ± 2.6**ab**	0.52 ± 0.09**ab**	0.10 ± 0.02**abc**	61 ± 1**ef**
			8.63 ± 0.01**ef**	283 ± 3**abc**	9 ± 1**a**	214 ± 5**ab**	22.0 ± 0.9**bcd**	0.57 ± 0.02**abc**	0.10 ± 0.01**abc**	47 ± 1**cd**
Alcantarilla		AWI, AWN	8.32 ± 0.17**cd**	704 ± 38**abc**	30 ± 10**bc**	669 ± 4**e**	26.4 ± 2.4**de**	1.23 ± 0.10**e**	0.16 ± 0.01**de**	88 ± 2**i**
			8.77 ± 0.01**fg**	170 ± 1**ab**	17 ± 2**b**	648 ± 30**e**	26.5 ± 0.3**de**	0.92 ± 0.04**de**	0.12 ± 0.01**bcd**	83 ± 1**hi**
Guadalupe	Dry	GDI, GDN	7.79 ± 0.17**a**	1347 ± 40**bc**	21 ± 7**b**	420 ± 31**e**	44.8 ± 3.9**e**	0.59 ± 0.12**ab**	0.07 ± 0.01**abc**	71 ± 5**b**
			7.80 ± 0.04**a**	1779 ± 20**cd**	18 ± 1**ab**	308 ± 5**cd**	35.6 ± 1.2**cd**	0.51 ± 0.07**ab**	0.07 ± 0.00**abc**	64 ± 2**b**
Murcia		MDI, MDN	8.81 ± 0.07**e**	194 ± 9**a**	59 ± 4**d**	771 ± 38**g**	32.9 ± 2.3**bc**	1.04 ± 0.13**bc**	0.12 ± 0.00**cde**	107 ± 2**d**
			8.71 ± 0.01**de**	160 ± 2**a**	35 ± 1**c**	558 ± 12**f**	33.3 ± 1.9**bc**	1.01 ± 0.05**bc**	0.11 ± 0.01**cde**	102 ± 6**cd**
Molina de Segura		MoDI, MoDN	8.35 ± 0.18**bc**	988 ± 42**b**	3 ± 1**a**	191 ± 15**ab**	35.8 ± 0.6**cd**	0.42 ± 0.08**ab**	0.05 ± 0.00**a**	62 ± 7**b**
			7.82 ± 0.01**a**	2078 ± 23**d**	2 ± 1**a**	188 ± 18**ab**	31.5 ± 0.9**bc**	0.26 ± 0.06**a**	0.04 ± 0.00**a**	57 ± 1**b**
Santomera		SDI, SDN	8.24 ± 0.14**b**	707 ± 50**ab**	32 ± 6**c**	490 ± 45**ef**	59.4 ± 5.9**f**	2.00 ± 0.66**d**	0.26 ± 0.07**f**	109 ± 9**d**
			8.37 ± 0.00**bc**	132 ± 1**a**	19 ± 1**b**	285 ± 6**bc**	43.5 ± 3.5**e**	1.00 ± 0.08**bc**	0.10 ± 0.01**bcd**	88 ± 1**c**
Churra		CDI, CDN	8.37 ± 0.06**bc**	353 ± 11**a**	31 ± 8**c**	525 ± 13**f**	42.5 ± 0.6**de**	1.58 ± 0.25**cd**	0.23 ± 0.01**f**	164 ± 10**e**
			8.32 ± 0.03**bc**	1005 ± 11**b**	18 ± 1**ab**	401 ± 87**de**	44.0 ± 0.9**e**	1.38 ± 0.32**cd**	0.13 ± 0.02**de**	112 ± 5**d**
San Javier		JDI, JDN	8.89 ± 0.01**e**	158 ± 6**a**	13 ± 4**ab**	244 ± 12**bc**	35.6 ± 0.8**cd**	0.48 ± 0.07ab	0.05 ± 0.01**ab**	34 ± 2**a**
			8.76 ± 0.01**de**	218 ± 3**a**	6 ± 0**a**	148 ± 5**a**	17.1 ± 1.3**a**	0.44 ± 0.09**ab**	0.05 ± 0.01**abc**	29 ± 1**a**
Alcantarilla		ADI, ADN	8.72 ± 0.07**de**	224 ± 7**a**	55 ± 2**d**	902 ± 56**h**	42.7 ± 2.1**de**	1.52 ± 0.10**cd**	0.17 ± 0.01**e**	104 ± 6**d**
			8.52 ± 0.03**cd**	268 ± 3**a**	39 ± 7**c**	737 ± 7**g**	26.6 ± 0.7**b**	1.39 ± 0.15**cd**	0.15 ± 0.01**de**	98 ± 0**cd**

et al., 2018). Likewise, the ability of *Actinobacteria* to form spores and enter into a dormant and resistant cellular state enables them to thrive under abiotic conditions unfavorable for plant growth, such as those in environments of low water availability. Members of the phylum *Actinobacteria* have been noted for their involvement in the degradation of organic matter, carbon fixation, and nitrogen metabolism (Zhang et al., 2019). In particular, actinobacterial taxa play an active role in processes involving nitrogen fixation, dissimilatory nitrate reduction, and denitrification (Yue et al., 2023).

The families 67–14 (of the order *Solirubrobacterales*) and *Pseudonocardiaceae* (of the order *Pseudonocardiales*) were among the most abundant indicator actinobacterial taxa of the bacterial community for the dry season prevailing in the rhizosphere of invasive plants. While members of the order *Solirubrobacterales* have not been extensively researched, recent studies have shown their capability to improve crop plant tolerance of abiotic stresses, such as water-limited conditions (Moore et al., 2023). Meanwhile, members of the family *Pseudonocardiaceae* have been found to be comparatively abundant in the rhizosphere and endosphere of desert plants subjected to abiotic stress and nutritional deficiency (Karray et al., 2020). Ebrahimi-Zarandi et al. (2023) described their capacity to stimulate plant growth through siderophore and phytohormones production and phosphate solubilization. Actinobacterial taxa with taxonomic assignation at the genus level, such as *Rubrobacter* and *Streptomyces*, were identified as prevailing in the rhizosphere soil of invasive plants subjected to dry conditions. These extremophilic actinobacteria are known for their ability to enhance the tolerance of plants to abiotic stress by producing phytohormone-like biomolecules (Acosta-Martínez et al., 2014; Yang et al., 2023).

Another abundant indicator taxon of the bacterial community for the dry season was the family *Sphingomonadaceae* (of the order *Sphingomonadales*). Under drought conditions, the invasive rhizosphere harbored a higher relative abundance of this family, in comparison with the native rhizosphere. The increases in *Sphingomonadaceae* were mainly attributable to members of the genus *Sphingomonas*. Indeed, certain studies have identified specific strains of *Sphingomonas* bacteria that exhibit the ability to enhance plant growth (Sukweenadhi et al., 2015) and mitigate

Table 4
Biochemical and biological properties of the rhizospheres of invasive (I = *N. glauca*) and native plants (N = native) grown in seven different locations during the wet (W) and dry (D) seasons (n = 4, mean ± standard error). DH = dehydrogenase activity; BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = soil respiration. For sampling season, values in column sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey HSD test.

Location	Season	Sample	DH (μg INTF g^{-1})	BGL (μmol PNF g^{-1} h^{-1})	ALP (μmol PNF g^{-1} h^{-1})	URE (μmol N-NH_4^+ g^{-1} h^{-1})	PRT (μmol N-NH_4^+ g^{-1} h^{-1})	SR (mg C-CO_2 h^{-1} kg^{-1})	Biomass C (μg g^{-1})
Guadalupe	Wet	GWI, GWN	125.9 ± 5.5**ef** 111.5 ± 2.2**de**	1.74 ± 0.30**cde** 2.06 ± 0.12**def**	1.74 ± 0.32**cd** 2.43 ± 0.05**de**	1.38 ± 0.04**e** 1.67 ± 0.10**f**	0.74 ± 0.07**ab** 0.97 ± 0.05**abc**	7.2 ± 0.3**cd** 4.8 ± 0.2**ab**	2402 ± 108**gh** 2052 ± 96**ef**
Murcia		MWI, MWN	86.7 ± 6.9**cd** 59.3 ± 0.5**b**	1.48 ± 0.20**bc** 1.39 ± 0.07**bc**	2.73 ± 0.58**def** 1.20 ± 0.29**abc**	1.20 ± 0.23**e** 0.24 ± 0.06**ab**	1.66 ± 0.09**def** 1.30 ± 0.15**bc**	6.2 ± 0.6**bc** 6.6 ± 0.4**c**	1869 ± 165**cde** 1502 ± 41**bc**
Molina de Segura		MoWI, MoWN	21.7 ± 0.5**a** 13.5 ± 0.7**a**	0.53 ± 0.11**a** 0.36 ± 0.03**a**	0.27 ± 0.05**a** 0.55 ± 0.07**ab**	0.10 ± 0.03**a** 0.03 ± 0.01**a**	0.46 ± 0.13**a** 0.33 ± 0.06**a**	4.6 ± 0.4**a** 4.0 ± 0.5**a**	543 ± 19**a** 464 ± 12**a**
Santomera		SWI, SWN	148.1 ± 8.2**fg** 99.5 ± 1.2**cde**	1.56 ± 0.37**cd** 2.40 ± 0.19**fg**	2.74 ± 0.60**def** 3.60 ± 0.07**fg**	0.10 ± 0.00**a** 0.66 ± 0.06**d**	1.95 ± 0.11**ef** 1.87 ± 0.19**def**	11.9 ± 0.7**gh** 9.5 ± 0.7**ef**	1476 ± 25**b** 1371 ± 23**bc**
Churra		CWI, CWN	162.6 ± 12.1**g** 94.4 ± 3.0**cd**	1.68 ± 0.26**cde** 2.76 ± 0.26**g**	3.10 ± 0.57**ef** 3.24 ± 0.22**ef**	0.70 ± 0.08**d** 0.62 ± 0.11**cd**	1.22 ± 0.23**bc** 1.60 ± 0.09**def**	10.2 ± 0.5**f** 10.4 ± 0.2**fg**	1852 ± 29**cde** 1602 ± 14**bc**
San Javier		JWI, JWN	274.8 ± 30.9**h** 73.6 ± 2.6**bc**	2.72 ± 0.29**g** 0.86 ± 0.11**ab**	2.60 ± 0.24**def** 1.35 ± 0.06**bc**	0.73 ± 0.05**d** 0.40 ± 0.01**bc**	1.96 ± 0.49**ef** 0.74 ± 0.11**ab**	12.7 ± 0.4**h** 8.6 ± 0.6**de**	2302 ± 29**fgh** 1276 ± 25**b**
Alcantarilla		AWI, AWN	70.1 ± 11.3**bc** 24.4 ± 1.3**a**	2.33 ± 0.27**efg** 2.20 ± 0.20**defg**	3.95 ± 0.30**g** 3.29 ± 0.04**ef**	1.14 ± 0.03**e** 0.87 ± 0.03**d**	1.39 ± 0.36**bcd** 2.19 ± 0.27**f**	13.3 ± 0.8**h** 11.0 ± 0.2**fg**	2528 ± 111**h** 1977 ± 25**de**
Guadalupe	Dry	GDI, GDN	91.7 ± 10.0**de** 81.2 ± 3.9**cde**	1.74 ± 0.06**de** 1.09 ± 0.21**bc**	1.96 ± 0.14**c** 1.65 ± 0.20**bc**	0.48 ± 0.04**c** 0.21 ± 0.04**ab**	0.62 ± 0.05**ab** 0.68 ± 0.01**ab**	2.7 ± 0.1**ab** 2.0 ± 0.2**a**	2503 ± 41**fg** 2002 ± 19**e**
Murcia		MDI, MDN	101.9 ± 6.6**e** 76.3 ± 1.6**bcd**	1.48 ± 0.16**cd** 0.76 ± 0.06**ab**	2.52 ± 0.20**d** 0.80 ± 0.17**a**	0.79 ± 0.04**efg** 0.57 ± 0.05**cd**	1.75 ± 0.13**de** 1.24 ± 0.04**c**	4.6 ± 0.6**c** 4.1 ± 0.3**c**	2528 ± 48**g** 2336 ± 24**fg**
Molina de Segura		MoDI, MoDN	23.1 ± 2.8**a** 15.8 ± 0.8**a**	0.30 ± 0.08**a** 0.28 ± 0.02**a**	1.49 ± 0.10**ab** 1.17 ± 0.12**ab**	0.31 ± 0.05**b** 0.13 ± 0.07**a**	0.35 ± 0.06**a** 0.36 ± 0.07**a**	3.6 ± 0.6**bc** 1.7 ± 0.1**a**	1168 ± 62**c** 898 ± 9**b**
Santomera		SDI, SDN	126.9 ± 13.7**f** 65.1 ± 3.2**bc**	2.38 ± 0.61**f** 1.97 ± 0.03**de**	3.39 ± 0.57**e** 1.85 ± 0.11**bc**	0.87 ± 0.09**fg** 0.31 ± 0.04**b**	2.48 ± 0.40**g** 0.93 ± 0.09**bc**	11.1 ± 0.9**efg** 7.2 ± 0.4**d**	2336 ± 16**fg** 1602 ± 41**d**
Churra		CDI, CDN	96.1 ± 8.7**de** 96.7 ± 3.8**de**	2.16 ± 0.19**ef** 1.72 ± 0.48**de**	2.44 ± 0.30**cd** 2.44 ± 0.34**cd**	0.73 ± 0.09**def** 0.67 ± 0.06**de**	1.84 ± 0.16**ef** 1.37 ± 0.13**cd**	12.3 ± 0.2**g** 11.0 ± 0.2**ef**	2436 ± 47**fg** 2327 ± 25**fg**
San Javier		JDI, JDN	179.2 ± 10.1**g** 60.1 ± 1.3**b**	0.80 ± 0.11**ab** 0.26 ± 0.04**a**	1.19 ± 0.20**ab** 0.70 ± 0.04**a**	0.18 ± 0.02**ab** 0.21 ± 0.05**ab**	0.43 ± 0.11**a** 0.20 ± 0.02**a**	3.8 ± 0.3**bc** 2.0 ± 0.2**a**	1326 ± 95**c** 563 ± 27**a**
Alcantarilla		ADI, ADN	35.3 ± 8.3**a** 32.1 ± 1.9**a**	2.31 ± 0.34**ef** 1.54 ± 0.15**cd**	3.61 ± 0.36**e** 2.57 ± 0.18**d**	0.91 ± 0.03**g** 0.60 ± 0.02**cd**	2.26 ± 0.26**fg** 2.05 ± 0.07**ef**	12.2 ± 0.3**fg** 10.7 ± 0.0**e**	2469 ± 47**fg** 2236 ± 13**ef**

abiotic stresses, including water stress, by driving developmental plasticity in the roots and regulating plant physiology and metabolism (Wang et al., 2022).

The rhizosphere communities harbored distinct fungal indicators under drought conditions, with the families *Aspergillaceae*, *Glomeraceae*, and *Claroideoglomeraceae* predominating. Under such environmental conditions, these important families showed a remarkable prevalence in the rhizosphere soil of invasive plants, in comparison with that of native plants. Among the members of the family *Aspergillaceae*, the filamentous fungus *Aspergillus* was found to be an abundant indicator in these rhizospheric communities. It has been reported that members of this fungal genus are able to secrete organic acids and solubilize insoluble phosphate, enhancing the bioavailability of phosphorus in soils and subsequent nutrient uptake by plants - as shown in semiarid agroecosystems (Kohler et al., 2007). The capabilities of these fungi for secreting indole-3-acetic acid (IAA) and siderophores have been described also, representing another way of promoting plant growth (Doilom et al., 2020). Our findings indicate that symbiotic fungi, particularly arbuscular mycorrhizal fungi (AMF) and endophytes, were relatively more prevalent in the rhizosphere of *N. glauca* during dry conditions. AMF improve the capacity of plants to survive under drought and low fertility conditions (Shi et al., 2023). In semiarid environments, indigenous AMF efficiently absorb water and nutrients in drying soil, thereby providing the host plant with enhanced drought resistance (Querejeta et al., 2007). Thus, the prevalence of AMF in the rhizosphere of *N. glauca* under drought conditions could counteract the negative effects of drought on nutrient and water uptake by the host plants, thus increasing their growth advantage with respect to the native plants. Also, the marked dominance of AMF in the rhizosphere of *N. glauca* could help to increase the stability of aggregates and in consequence, indirectly, to improve the survival of the microbiota of the invasive rhizosphere during such adverse conditions. The improved aggregate stability induced by mycorrhizal fungi may increase the water available to plants and facilitate their survival. It is worth noting that the presence of stable

Table 5
Three factors ANOVA (invasive character, sampling site, and sampling season) for plant and soil parameters studied. *P* significance values. EC: electrical conductivity; Avail. P = available phosphorus; Ext. K = extractable potassium; AS: aggregate stability; TOC = total organic carbon; TN = total nitrogen; WSC = Water soluble carbon; DH = dehydrogenase activity; BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = soil respiration.

	Invasiveness (I)	Location (L)	Season (S)	Interaction (I × L)	Interaction (I × S)	Interaction (L × S)	Interaction (I × L × S)
pH	0.022	<0.001	NS	<0.001	NS	0.005	<0.001
EC	0.010	<0.001	0.022	0.040	NS	<0.001	<0.001
Avail. P	<0.001	<0.001	0.003	0.009	NS	<0.001	NS
Ext. K	<0.001	<0.001	<0.001	NS	0.002	<0.001	NS
AS	<0.001	<0.001	<0.001	<0.001	<0.001	< 0.001	0.006
TOC	NS	<0.001	0.011	NS	0.013	0.016	0.005
TN	<0.001	<0.001	NS	<0.001	0.015	<0.001	NS
WSC	<0.001	<0.001	<0.001	<0.001	NS	<0.001	0.001
DH	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BGL	0.005	<0.001	<0.001	<0.001	0.006	0.003	<0.001
ALP	<0.001	<0.001	0.001	<0.001	0.012	<0.001	0.014
URE	<0.001	<0.001	<0.001	<0.001	0.050	<0.001	<0.001
PRT	0.001	<0.001	NS	<0.001	0.012	<0.001	<0.001
SR	<0.001	<0.001	<0.001	<0.001	NS	<0.001	0.009
Biomass C	<0.001	<0.001	<0.001	<0.001	NS	<0.001	0.007

NS: not significant.

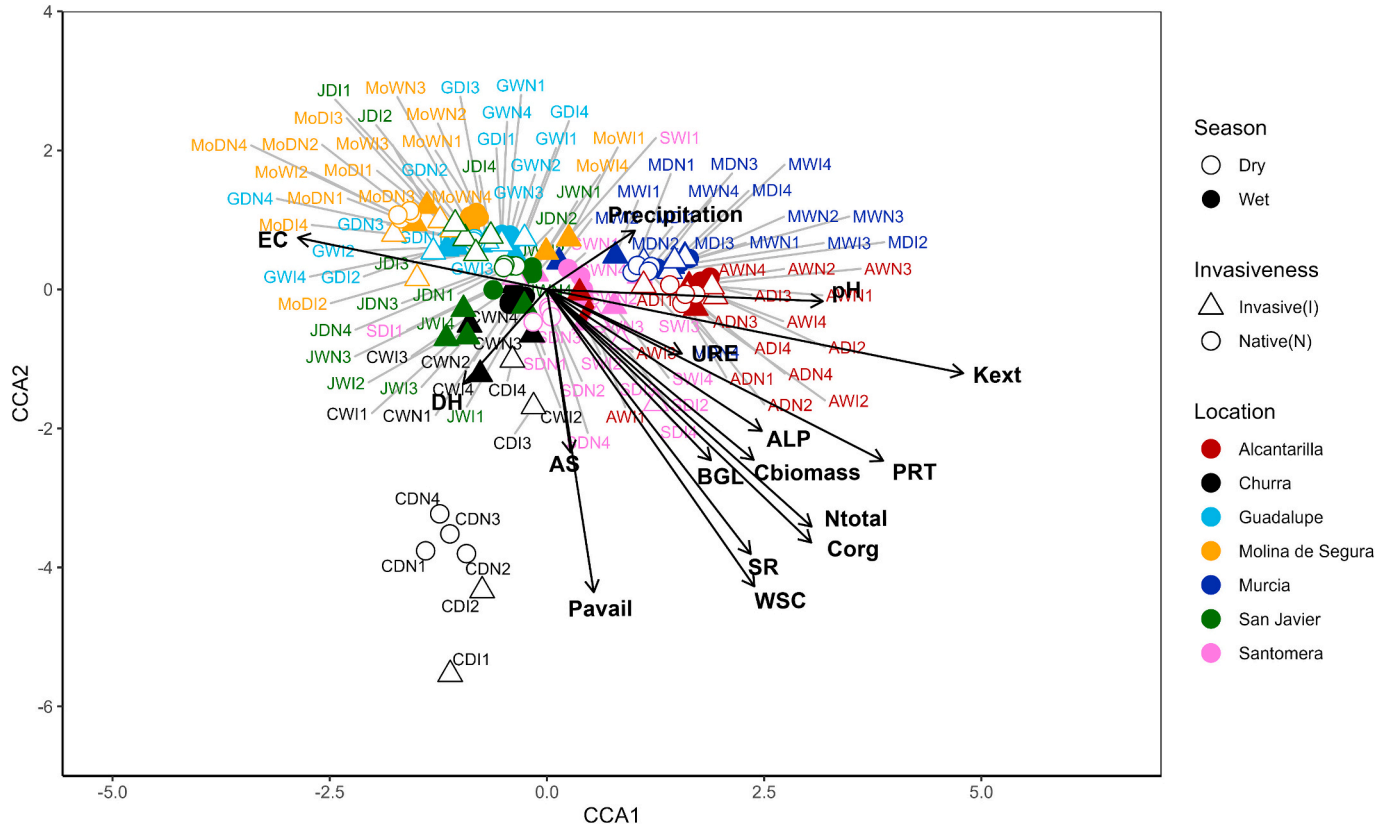

Fig. 5. Ordination plot generated by canonical correspondence analysis (CCA) on bacteria communities of the rhizospheric soil of *Nicotiana glauca* and native plants growing at seven different locations during the wet and dry seasons at ASV level. The environmental factors related to the rhizosphere bacteria communities were as follows: Precipitation; EC: electrical conductivity; Pavail = available phosphorus; Kext = extractable potassium; AS: aggregate stability; Corg = total organic carbon; Ntotal = total nitrogen; WSC = Water soluble carbon; DH = dehydrogenase activity; BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = soil respiration; Cbiomass = biomass carbon.

aggregates was significantly greater in the rhizosphere community of invasive plants under drought conditions. Likewise, mycorrhizal fungi can capture water for plants even when soil water potentials are negative and soils are close to absolute dryness. These assumptions are made based on relative abundance data in the rhizosphere of *N. glauca* in the dry season. As pointed out by some authors, the abundance of AMF in soil is strongly positively related to the abundance of AMF in host roots, measured as the total length of roots colonized by AMF (Barceló et al., 2020). For future studies, it is foreseen the assessment of changes induced by drought in the composition and abundance of AMF harbored in the root system of *N. glauca*, which could provide insights into the specific AMF species involved in the invasion success of invader under such environmental conditions and add support to our current findings.

Shifts in the microbial functionality of *N. glauca* and native plant

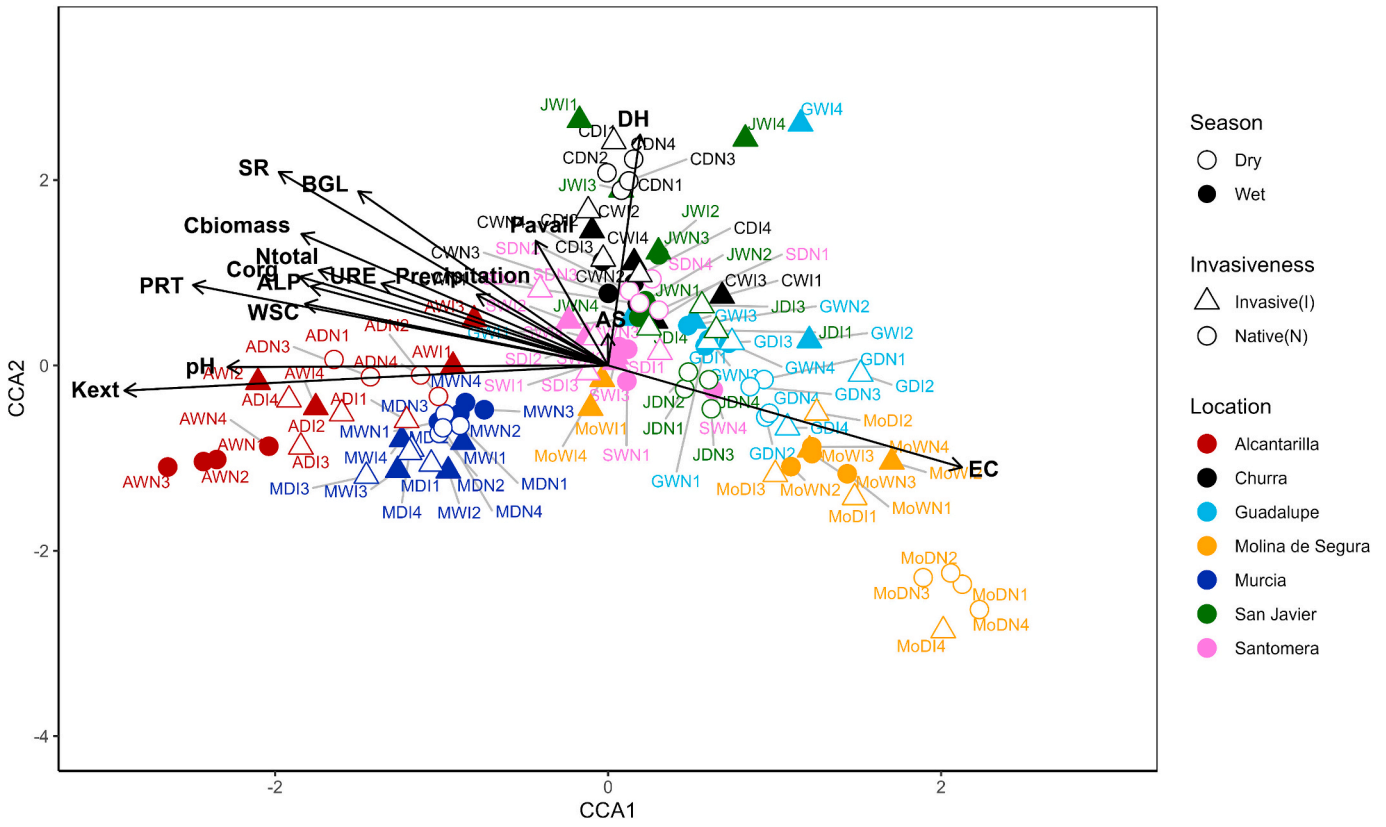

Fig. 6. Ordination plot generated by canonical correspondence analysis (CCA) on fungi communities of the rhizospheric soil of *Nicotiana glauca* and native plants at ASV level. The environmental factors related to the rhizosphere fungi communities were as follows: Precipitation; EC: electrical conductivity; Pavail = available phosphorus; Kext = extractable potassium; AS: aggregate stability; Corg = total organic carbon; Ntotal = total nitrogen; WSC = Water soluble carbon; DH = dehydrogenase activity; BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = soil respiration; Cbiomass = biomass carbon.

rhizospheres in response to drought conditions.

The establishment and proliferation of plant species in an ecosystem are greatly affected by the functional roles carried out by soil microbiota (Van der Putten et al., 2016). The findings of this study show that *N. glauca* did indeed shift soil microbial functionality under both dry and wet conditions. Specifically, we found several microbial functional traits overrepresented under drought conditions that predominated in the rhizosphere of invasive plants compared to the rhizosphere of native plants. The bacterial functions that were prevalent in the rhizosphere of invasive plants under drought conditions had annotations related to energy (ATP and NADPH) and precursor metabolites production, which are essential in order to sustain bacterial growth and multiplication. In this respect, the relative abundances of the glycolysis and pentose phosphate pathways were higher when *N. glauca* was exposed to drought. These pathways are vital because they produce precursor metabolites for the synthesis of building blocks required for macromolecules and other small molecules essential for all cellular biosynthesis. This finding is in accordance with the fact that the enzyme glucokinase or D-glucose 6-phosphotransferase (EC:2.7.1.2) that catalyzes the first step of glycolysis was more abundant in the invasive rhizosphere under drought conditions. The superior functional potential of the invasive rhizosphere under drought conditions with regard to bioenergetic processes crucial for cell life has been evidenced also by higher relative abundances of the EC:2.7.8.5 transferase enzyme associated with biosynthesis of the diphosphatidylglycerol lipid cardiolipin, crucial for the optimal functioning of numerous enzymes that participate in the mitochondrial processes of energy production (Mileykovskaya and Dowhan, 2009). Interestingly, the occurrence of *N. glauca* also resulted in increased relative abundances of four oxidoreductases: IMP dehydrogenase (EC:1.1.1.205), UDP-*N*-acetyl-D-mannosamine dehydrogenase (EC:1.1.1.336), malate dehydrogenase (EC:1.1.1.38), and succinate dehydrogenase (EC:1.3.5.1). These enzymes are involved in the biological oxidation of organic matter, enhancing energy provision to the rhizosphere microbiota and strengthening it (Nannipieri et al., 2003).

The occurrence of *N. glauca* under drought conditions resulted also in higher relative abundances of the methylerythritol phosphate I, methylerythritol phosphate II, and 1,4-dihydroxy-6-naphthoate I pathways related to isoprenoids biosynthesis, which play key metabolic, structural, and regulatory roles in all living organisms. In accordance with these findings, the synthases EC:2.5.1.10 and EC:2.5.1.1 and the transferase EC:2.5.1.1, which play a key regulatory role in the biosynthesis of isoprenoid precursors and isoprenoids, were more abundant in the invasive rhizosphere under drought conditions. Among the potential isoprenoids produced in the invasive rhizosphere, menaquinones (vitamin K2) are an essential cofactor necessary for sporulation in *Bacillus subtilis* and proper regulation of cytochrome formation in some Gram-positive bacteria (Farrand and Taber, 1974; Yassin et al., 1988).

Peptidoglycan biosynthesis was overrepresented in the rhizosphere bacterial microbiome of *N. glauca* under drought conditions. The prevalence of this pathway in the rhizosphere of invasive plants was due to the noteworthy presence of five enzymes (EC:2.4.1.129, EC:3.1.47.14, EC:3.6.1.27, EC:5.1.1.3, EC:5.4.2.10) essential for the biosynthesis of peptidoglycan. Among the enzymes involved in peptidoglycan biosynthesis, it has been demonstrated that the activity of the phosphatase EC:3.6.1.27 is crucial for normal sporulation of *B. subtilis* (Radeck et al., 2017). Peptidoglycan, also known as "basal structure", is the cell wall's main component in Gram-positive bacteria being more abundant in these organisms than in Gram-negative bacteria. The structure of this glycopeptide contributes to its remarkable tensile strength, which is

combined with an elasticity that enables it to expand or shrink. The elevated biosynthesis of peptidoglycan in the rhizospheric community of invasive plants in dry conditions coincided with the relative dominance of *Actinobacteria* in such rhizospheric communities.

Higher potential rates of bacterial activity associated with the autolytic protease activity (EC 3.4.21.88) of LexA were found in the invasive rhizosphere under drought conditions. This enzyme, helped by RecA protein, participates in the autocatalytic cleavage of repressor LexA, which is the key step in the initiation of the bacterial SOS response to DNA damage provoked by environmental stress. The putative functional annotations related largely to the bacterial SOS response can be particularly relevant in the soils of semiarid ecosystems subjected to abiotic and biotic stresses, where microbial activity is diminished due to the shortage of nutrients and water (Sardans et al., 2008).

Under drought conditions, the rhizosphere of *N. glauca* was also relatively enriched in three catalytic phosphatases and two lyases acting on phosphates: histidinol-phosphatase (EC:3.1.3.15), undecaprenyl-diphosphate phosphatase (EC:3.6.1.27), XTP/dITP diphosphatase (EC:3.6.1.66), 2-methylisoborneol synthase (EC:4.2.3.118), and DNA-(apurinic or apyrimidinic site) lyase (EC:4.2.99.18). These enzymes are involved in the P cycle, catalyzing the degradation of organophosphates (also known as phosphate esters) and providing available P to plants (Nannipieri et al., 2003).

5. Conclusions

The rhizosphere microbiomes of *N. glauca* and native plants exhibited different microbial communities and functional traits. These differences were significantly pronounced in the dry season with some evidence that leads us to the conclusion that the observed shifts in the rhizosphere of *N. glauca* under conditions of low soil water availability represent a clear advantage for its establishment. It is noteworthy that under drought conditions, certain bacterial and fungal indicator species predominate in the invasive rhizosphere, which exhibit plant growth-promoting and soil-improving potential traits. Moreover, there's a prevalence of bacterial functional traits linked to energy production, precursor metabolites, cell wall biosynthesis, cofactors, vitamins, amino acids, and catabolic enzymes crucial for the phosphorus cycle.

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Credit authorship contribution statement

F. Caravaca: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **P. Torres:** Resources, Investigation, Funding acquisition. **G. Díaz:** Resources, Investigation, Funding acquisition. **A. Roldán:** Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2020. UNITE QIIME release for Fungi. https://doi.org/10.15156/BIO/786385.

Acosta-Martínez, V., Cotton, J., Gardner, T., Moore-Kucera, J., Zak, J., Wester, D., Cox, S., 2014. Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. Appl. Soil Ecol. 84, 69–82. https://doi.org/10.1016/j.apsoil.2014.06.005.

Ahmad, H.M., Fiaz, S., Hafeez, S., Zahra, S., Shah, A.N., Gul, B., Aziz, O., Mahmood-Ur-Rahman, Fakhar, A., Rafique, M., Chen, Y., Yang, S.H., Wang, X., 2022. Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: a review. Front. Plant Sci. 13, 875774 https://doi.org/10.3389/fpls.2022.875774.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221. https://doi.org/10.1016/0038-0717(78)90099-8.

Bahadur, A., Batool, A., Nasir, F., Jiang, S., Mingsen, Q., Zhang, Q., et al., 2019. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. Int. J. Mol. Sci. 20, 4199. https://doi.org/10.3390/ijms20174199.

Barceló, M., van Bodegom, P.M., Tedersoo, L., den Haan, N., Veen, G.F.(Ciska), Ostonen, I., Trimbos, K., Soudzilovskaia, N.A., 2020. The abundance of arbuscular mycorrhiza in soils is linked to the total length of roots colonized at ecosystem level. PloS One 15, e0237256. https://doi.org/10.1371/journal.pone.0237256.

Beaury, E.M., Finn, J.T., Corbin, J.D., Barr, V., Bradley, B.A., 2020. Biotic resistance to invasion is ubiquitous across ecosystems of the United States. Ecol. Lett. 23, 476–482. https://doi.org/10.1111/ele.13446.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300. https://doi.org/10.2307/2346101.

Berendsen, R.L., Pieterse, C.M., Bakker, P.A., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486. https://doi.org/10.1016/j.tplants.2012.04.001.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., Caporaso, J.G., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581. https://doi.org/10.1038/nmeth.3869.

Caravaca, F., Torres, P., Díaz, G., Roldán, A., 2022. Elevated CO_2 affects the rhizosphere microbial community and the growth of two invader plant species differently in semiarid Mediterranean soils. Land Degrad. Dev. 33, 117–132. https://doi.org/10.1002/ldr.4133132.

Caspi, R., Billington, R., Keseler, I.M., Kothari, A., Krummenacker, M., Midford, P.E., Ong, W.K., Paley, S., Subhraveti, P., Karp, P.D., 2019. The MetaCyc database of metabolic pathways and enzymes - a 2019 update. Nucleic Acids Res. 48, D445–D453. https://doi.org/10.1093/nar/gkz862.

Davidson, A.M., Jennions, M., Nicotra, A.B., 2011. Do invasive species show higher phenotypic plasticity thannative species and, if so, is it adaptive? A meta-analysis. Ecol. Lett. 14, 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x.

Díaz-Barradas, M.C., Gallego-Fernández, J.B., Zunzunegui, M., 2020. Plant response to water stress of native and non-native *Oenothera drummondii* populations. Plant Physiol. Biochem. 154, 219–228. https://doi.org/10.1016/j.plaphy.2020.06.001.

Doilom, M., Guo, J.-W., Phookamsak, R., Mortimer, P.E., Karunarathna, S.C., Dong, W., Liao, C.-F., Yan, K., Pem, D., Suwannarach, N., Promputtha, I., Lumyong, S., Xu, J.-C., 2020. Screening of phosphate-solubilizing fungi from air and soil in Yunnan, China: four novel species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. Front. Microbiol. 11, 585215 https://doi.org/10.3389/fmicb.2020.585215.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688. https://doi.org/10.1038/s41587-020-0548-6.

Ebrahimi-Zarandi, M., Etesami, H., Glick, B.R., 2023. Fostering plant resilience to drought with Actinobacteria: unveiling perennial allies in drought stress tolerance. Plant Stress 10, 100242. https://doi.org/10.1016/j.stress.2023.100242.

Eddy, S.R., 2011. Accelerated profile hmm searches. PLoS Comput. Biol. 7, e1002195 https://doi.org/10.1371/journal.pcbi.1002195.

Ettinger, C.L., LaForgia, M.L., 2024. Invasive plant species interact with drought to shift key functions and families in the native rhizosphere. Plant and Soil 494, 567–588. https://doi.org/10.1007/s11104-023-06302-1.

Fahey, C., Koyama, A., Antunes, P.M., Dunfield, K., Flory, S.L., 2020. Plant communities mediate the interactive effects of invasion and drought on soil microbial communities. ISME J. 14, 1396–1409. https://doi.org/10.1038/s41396-020-0614-6.

Farrand, S.K., Taber, H.W., 1974. Changes in Menaquinone concentration during growth and early sporulation in *Bacillus subtilis*. J. Bacteriol. 117, 324–326. https://doi.org/10.1128/jb.117.1.324-326.1974.

Florentine, S.K., Weller, S., Graz, P.F., Westbrooke, M., Florentine, A., Javaid, M., Fernando, N., Chauhan, B.S., Dowling, K., 2016. Influence of selected environmental factors on seed germination and seedling survival of the arid zone invasive species tobacco bush (Nicotiana glauca R. Graham). Rangeland J. 38, 417–425. https://doi.org/10.1071/RJ16022.

García, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134. https://doi.org/10.1080/00103629709369777.
Global Invasive Species Database (GISD), 2024. Species profile *Nicotiana glauca*. Available from: https://www.iucngisd.org/gisd/species.php?sc=1453.
Guo, Y.-S., Furrer, J.M., Kadilak, A.L., Hinestroza, H.F., Gage, D.J., Cho, Y.K., Shor, L.M., 2018. Bacterial extracellular polymeric substances amplify water content variability at the pore scale. Front. Environ. Sci. 6, 93. https://doi.org/10.3389/fenvs.2018.00093.
Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5, 1571–1579. https://doi.org/10.1038/ismej.2011.41.
Hu, J., Cyle, K.T., Miller, G., Shi, W., 2023. Water deficits shape the microbiome of Bermudagrass roots to be Actinobacteria rich. FEMS Microbiol. Ecol. 99, fiad036 https://doi.org/10.1093/femsec/fiad036.
IPCC, 2023. Climate change 2023: synthesis report. In: Hourcade, J.-C., Johnson, F.X., Pachauri, S., Simpson, N.P., Singh, C., Thomas, A., Totin, E., Lee, H., Romero, J. (Eds.), Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland. https://doi.org/10.59327/IPCC/AR6-9789291691647, 184 p.
Jones, J.M., Boehm, E.L., Kahmark, K., Lau, J., Evans, S., 2022. Microbial community response to drought depends on crop. Elementa Sci. Anthropocene 10, 1. https://doi.org/10.1525/elementa.2021.00110.
Karray, F., Gargouri, M., Chebaane, A., Mhiri, N., Mliki, A., Sayadi, S., 2020. Climatic aridity gradient modulates the diversity of the rhizosphere and endosphere bacterial microbiomes of *Opuntia ficus-indica*. Front. Microbiol. 11, 1622. https://doi.org/10.3389/fmicb.2020.01622.
Khan, S.U., Hooda, P.S., Blackwell, M.S.A., Busquets, R., 2019. Microbial biomass responses to soil drying-rewetting and phosphorus leaching. Front. Environ. Sci. 7, 133. https://doi.org/10.3389/fenvs.2019.00133.
Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417, 67–70. https://doi.org/10.1038/417067a.
Kohler, J., Caravaca, F., Carrasco, L., Roldán, A., 2007. Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa*. Appl. Soil Ecol. 35, 480–487. https://doi.org/10.1016/j.apsoil.2006.10.006.
Kohler, J., Caravaca, F., Roldán, A., 2009. Effect of drought on the stability of rhizosphere soil aggregates of *Lactuca sativa* in a degraded soil inoculated with PGPR and AM fungi. Appl. Soil Ecol. 42, 160–165. https://doi.org/10.1016/S0929-1393(02)00136-1.
Kohler, J., Caravaca, F., Azcon, R., Díaz, G., Roldán, A., 2016. Suitability of the microbial community composition and function in a semiarid mine soil for assessing phytomanagement practices based on mycorrhizal inoculation and amendment addition. J. Environ. Manage. 169, 236–246. https://doi.org/10.1016/j.jenvman.2015.12.037.
LaForgia, M.L., Kang, H., Ettinger, C.L., 2022. Invasive grass dominance over native forbs is linked to shifts in the bacterial rhizosphere microbiome. Microb. Ecol. 84, 496–508. https://doi.org/10.1007/s00248-021-01853-1.
Lankau, R.A., George, I., Miao, M., 2022. Crop performance is predicted by soil microbial diversity across phylogenetic scales. Ecosphere 13, e4029. https://doi.org/10.1002/ecs2.402920 of 20.
Leal, R.P., Silveira, M.J., Petsch, D.K., Mormul, R.P., Thomaz, S.M., 2022. The success of an invasive Poaceae explained by drought resilience but not by higher competitive ability. Environ. Exp. Bot. 194, 104717 https://doi.org/10.1016/j.envexpbot.2021.104717.
Louca, S., Doebeli, M., 2018. Efficient comparative phylogenetics on large trees. Bioinformatics 34, 1053–1055. https://doi.org/10.1093/bioinformatics/btx701.
Maisnam, P., Jeffries, T.C., Szejgis, J., Bristol, D., Singh, B.K., Eldridge, D.J., Horn, S., Chieppa, J., Nielsen, U.N., 2023. Severe prolonged drought favours stress-tolerant microbes in Australian drylands. Microb. Ecol. 86, 3097–3110. https://doi.org/10.1007/s00248-023-02303-w.
Mileykovskaya, E., Dowhan, W., 2009. Cardiolipin membrane domains in prokaryotes and eukaryotes. Biochim. Biophys. Acta 1788, 2084–2091. https://doi.org/10.1016/j.bbamem.2009.04.003.
Moore, E.R., Carter, K.R., Heneghan, J.P., Steadman, C.R., Nachtsheim, A.C., Anderson-Cook, C., Dickman, L.T., Newman, B.D., Dunbar, J., Sevanto, S., Albright, M.B.N., 2023. Microbial drivers of plant performance during drought depend upon community composition and the greater soil environment. Microbiol. Spectrum 11, e0147622. https://doi.org/10.1128/spectrum.01476-22.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670. https://doi.org/10.1046/j.1351-0754.2003.0556.x.
Naylor, D., Coleman-Derr, D., 2018. Drought stress and root-associated bacterial communities. Front. Plant Sci. 8, 2223. https://doi.org/10.3389/fpls.2017.02223.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30, 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.
Pepe, M., Crescente, M.F., Varone, L., 2022. Effect of water stress on physiological and morphological leaf traits: a comparison among the three widely-spread invasive alien species *Ailanthus altissima*, *Phytolacca americana*, and *Robinia pseudoacacia*. Plants 11, 899. https://doi.org/10.3390/plants11070899.
Poudel, M., Mendes, R., Costa, L.A.S., Bueno, C.G., Meng, Y., Folimonova, S.Y., Garrett, K.A., Martins, S.J., 2021. The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. Front. Microbiol. 12, 743512 https://doi.org/10.3389/fmicb.2021.743512.
Prudent, M., Dequiedt, S., Sorin, C., et al., 2020. The diversity of soil microbial communities matters when legumes face drought. Plant, Cell Environ. 43, 1023–1035. https://doi.org/10.1111/pce.13712.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Querejeta, J.I., Allen, M.F., Alguacil, M.M., Roldán, A., 2007. Plant isotopic composition provides insight into mechanisms underlying growth stimulation by arbuscular mycorrhizal fungi. Funct. Plant Biol. 34, 683–691. https://doi.org/10.1071/FP07061.
Radeck, J., Lautenschläger, N., Mascher, T., 2017. The essential UPP phosphatase pair BcrC and UppP connects cell wall homeostasis during growth and sporulation with cell envelope stress response in *Bacillus subtilis*. Front. Microbiol. 8, 2403. https://doi.org/10.3389/fmicb.2017.02403.
Rodríguez-Caballero, G., Caravaca, F., Alguacil, M.M., Fernández-López, M., Fernández-González, A.J., Roldán, A., 2017. Striking alterations in the soil bacterial community structure and functioning of the biological N cycle induced by *Pennisetum setaceum* invasion in a semiarid environment. Soil Biol. Biochem. 109, 176–187. https://doi.org/10.1016/j.soilbio.2017.02.012.
Rodríguez-Caballero, G., Caravaca, F., Díaz, G., Torres, P., Roldán, A., 2020a. The invader *Carpobrotus edulis* promotes a specific rhizosphere microbiome across globally distributed coastal ecosystems. Sci. Total Environ. 719, 137347 https://doi.org/10.1016/j.scitotenv.2020.137347.
Rodríguez-Caballero, G., Roldán, A., Caravaca, F., 2020b. Invasive *Nicotiana glauca* shifts the soil microbial community composition and functioning of harsh and disturbed semiarid Mediterranean environments. Biol. Invasions 22, 2923–2940. https://doi.org/10.1007/s10530-020-02299-1.
R-Team-Core, 2023. R: A Language and Environment for Statistical Computing.
Sanz Elorza, M., Sobrino Vesperinas, E., Dana Sánchez, E.D., 2004. Atlas de las plantas alóctonas invasoras en España. Dirección General para la Biodiversidad, Madrid (Spain).
Sardans, J., Peñuelas, J., Estiarte, M., 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Appl. Soil Ecol. 39, 223–235. https://doi.org/10.1016/j.apsoil.2007.12.011.
Shi, J., Wang, X., Wang, E., 2023. Mycorrhizal symbiosis in plant growth and stress adaptation: from genes to ecosystems. Annu. Rev. Plant Biol. 74, 569–607. https://doi.org/10.1146/annurev-arplant-061722-090342.
Storey, J.D., 2011. False discovery rate. In: Lovric, M. (Ed.), International Encyclopedia of Statistical Science. Springer, Berlin, Heidelberg, pp. 504–508. https://doi.org/10.1007/978-3-642-04898-2_248.
Sukweenadhi, J., Kim, Y.-J., Kang, C.H., Farh, M.E.A., Nguyen, N.-L., Hoang, V.-A., Choi, E.-S., Yang, D.-C., 2015. *Sphingomonas panaciterrae* sp. nov., a plant growth-promoting bacterium isolated from soil of a ginseng field. Arch. Microbiol. 197, 973–981. https://doi.org/10.1007/s00203-015-1134-z.
Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, J.S., Botttomley, P.S. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. American Society of Agronomy, Soil Science Society of America, Inc., Madison, Wisconsi, pp. 775–833. https://doi.org/10.2136/sssabookser5.2.c37.
Turenne, C.Y., Sanche, S.E., Hoban, D.J., Karlowsky, J.A., Kabani, A.M., 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system. J. Clin. Microbiol. 37, 1846–1851. https://doi.org/10.1128/JCM.37.6.1846-1851.1999.
Van der Putten, W.H., Bradford, M.A., Brinkman, E.P., van de Voorde, T.F.J., Veen, G.F., 2016. Where, when and how plant–soil feedback matters in a changing world. Funct. Ecol. 30, 1109–1121. https://doi.org/10.1111/1365-2435.12657.
Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255, 571–586. https://doi.org/10.1023/A:1026037216893.
Wagg, C., Schlaeppi, K., Banerjee, S., et al., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat. Commun. 10, 4841. https://doi.org/10.1038/s41467-019-12798-y.
Wang, F., Wei, Y., Yan, T., Wang, C., Chao, Y., Jia, M., An, L., Sheng, H., 2022. *Sphingomonas* sp. Hbc-6 alters physiological metabolism and recruits beneficial rhizosphere bacteria to improve plant growth and drought tolerance. Front. Plant Sci. 13, 1002772 https://doi.org/10.3389/fpls.2022.1002772.
White, T.J., Bruns, T.D., Lee, S.B., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
Xie, J., Dawwam, G.E., Sehim, A.E., Li, X., Wu, J., Chen, S., Zhang, D., 2021. Drought stress triggers shifts in the root microbial community and alters functional categories in the microbial gene pool. Front. Microbiol. 12, 744897 https://doi.org/10.3389/fmicb.2021.744897.
Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K.K., et al., 2018. Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. Proc. Natl. Acad. Sci. U. S. A. 115, E4284–E4293. https://doi.org/10.1073/pnas.1717308115.
Yang, Z., Qiao, Y., Konakall, N.C., Strøbech, E., Harris, P., Peschel, G., Agler-Rosenbaum, M., Weber, T., Andreasson, E., Ding, L., 2023. *Streptomyces* alleviate

abiotic stress in plant by producing pteridic acids. Nat. Commun. 14, 7398. https://doi.org/10.1038/s41467-023-43177-3.

Yassin, A.F., Brzezinka, H., Schaal, K.P., Trüper, H.G., Pulverer, G., 1988. Menaquinone composition in the classification and identification of aerobic actinomycetes. Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A 267, 339–356. https://doi.org/10.1016/s0176-6724(88)80050-6.

Ye, Y., Doak, T.G., 2009. A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput. Biol. 5, e1000465 https://doi.org/10.1371/journal.pcbi.1000465.

Yue, Y., Rong, H., Yang, Z., et al., 2023. Microbial diversity and functional profiling in coastal tidal flat sediment with pollution of nutrients and potentially toxic elements. J. Soil. Sediment. 23, 2935–2950. https://doi.org/10.1007/s11368-023-03511-0.

Zhang, B., Wu, X., Tai, X., Sun, L., Wu, M., Zhang, W., Chen, X., Zhang, G., Chen, T., Liu, G., Dyson, P., 2019. Variation in actinobacterial community composition and potential function in different soil ecosystems belonging to the arid Heihe River Basin of Northwest China. Front. Microbiol. 10, 2209. https://doi.org/10.3389/fmicb.2019.02209.

Zhang, X., Oduor, A.M.O., Liu, Y., 2023. Invasive plants have greater growth than co-occurring natives in live soil subjected to a drought-rewetting treatment. Function. Ecol. 37, 513–522. https://doi.org/10.1111/1365-2435.14244.