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Arbuscular mycorrhizal fungi inoculation mediated changes in rhizosphere bacterial community structure while promoting revegetation in a semiarid ecosystem

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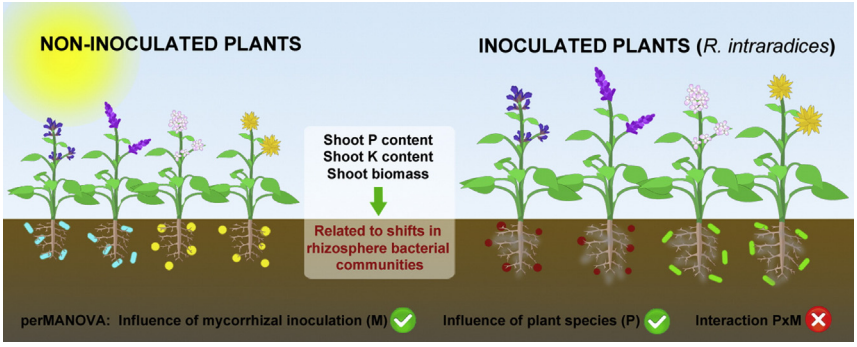
HIGHLIGHTS

- An AMF improved plant performance in the revegetation of a semiarid ecosystem.
- AMF and plant species altered the rhizosphere bacterial community structure.
- AMF-mediated bacterial community shifts were related to plant performance.
- *Anaerolineaceae* family was an indicator of AMF-inoculated rhizospheres.

GRAPHICAL ABSTRACT



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ABSTRACT

The main goal of this study was to assess the effect of the inoculation of four autochthonous shrub species with the arbuscular mycorrhizal (AM) fungus *Rhizophagus intraradices* on the rhizosphere bacterial community and to ascertain whether such an effect is dependent on the host plant species. Additionally, analysis of rhizosphere soil chemical and biochemical properties was performed to find relationships between them and the rhizosphere bacterial communities. Non-metric multidimensional scaling analysis and subsequent permutational multivariate analysis of variance revealed differences in bacterial community composition and structure between non-inoculated and inoculated rhizospheres. Moreover, an influence of the plant species was observed. Different bacterial groups were found to be indicator taxonomic groups of non-inoculated and inoculated rhizospheres, Gemmatimonadetes and *Anaerolineaceae*, respectively, being the most notable indicators. As shown by distance based redundancy analysis, the shifts in bacterial community composition and structure mediated by the inoculation with the AM fungus were mainly related to changes in plant nutrients and growth parameters, such as the shoot phosphorus content. Our findings suggest that the AM fungal inoculum was able to modify the rhizosphere bacterial community assemblage while improving the host plant performance.

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1. Introduction

Mediterranean semiarid ecosystems in Southeast Spain are frequently exposed to erosive processes and vegetation cover losses. This leads to degraded habitats which are especially fragile and endangered by environmental changes such as global warming. The harsh climate conditions and the nutrient scarcity that characterize these areas make the plant establishment more difficult, and thus reduce the effectiveness of restoration programs (Caravaca et al., 2005), which intend to prevent the loss of fertile soil and the desertification progression.

The introduction of autochthonous shrub species, adapted to semiarid environmental conditions and nutrient scarcity, is preferential in the revegetation of Mediterranean semiarid ecosystems (Requena et al., 2001; Caravaca et al., 2005). However, the progressive increase in aridity and land degradation has caused a severe loss of native microbial diversity and functioning (Maestre et al., 2015), which makes plant establishment more difficult in these areas. This is the case for the mycorrhizal potential, which is seriously depleted (Requena et al., 1996; Azcón-Aguilar et al., 2003).

Plant-microbe relationships are decisive for plant growth and survival (Van Der Heijden et al., 2008). In fact, plants are considered as holobionts, with the plant per se and all its associated microbiota comprising a "single dynamic entity" (Vandenkoornhuyse et al., 2015). The main source of these microorganisms is the surrounding rhizosphere soil - which works as a microbial "seed bank" that, together with the local environmental parameters, strongly influences the final composition of the rhizosphere microbial communities (Vandenkoornhuyse et al., 2015). The plant microbiome stimulates germination and plant growth, enhances nutrient acquisition, promotes direct and indirect mechanisms of defense against pathogens and against abiotic stress and influences plant phenology (Berg et al., 2016). Many of these functions might be indirectly influenced by the microbial interactions that occur in the rhizosphere because the resulting plant microbiome could be functionally different (Barea et al., 2005).

The application of microbial inoculants to the plant rhizosphere has been demonstrated to be a suitable strategy for revegetation purposes (Palenzuela et al., 2002; Armada et al., 2015; Mengual et al., 2016). Some native strains of actinobacteria have been successfully used in the revegetation with shrub species of semiarid ecosystems, whose effectiveness was related to increased NPK uptake and plant drought tolerance (Mengual et al., 2016). Meanwhile, arbuscular mycorrhizal fungi (AMF) are one of the best studied plant symbionts (Parniske, 2008) and the beneficial effect of these microbial inoculants on plant establishment - through improvement of plant adaptability and stress tolerance and enhancement of plant nutrient acquisition - has been reported in numerous studies (Jeffries et al., 2003; Van Der Heijden et al., 2008), including studies of revegetation in semiarid degraded areas (Alguacil et al., 2011; Caravaca et al., 2005; Barea et al., 2011). Many studies report that the beneficial effects of AMF may due to their interactions with rhizobacteria (Artursson et al., 2006). In this regard, AM mycelial exudates increased the growth and development of soil bacterial community, particularly the relative abundance of Gammaproteobacteria (Toljander et al., 2007). On the other hand, some bacteria can affect spore germination rate and mycelial growth modifying AMF functions on plants (Artursson et al., 2006). Previous findings suggest that microbial interactions involving AMF and nitrogen-fixing, phosphate-solubilizing and plant growth-promoting rhizobacteria, as well as AMF and rhizosphere native bacterial community, can have a beneficial effect on the growth and health of plants and on soil quality (Barea et al., 2005). This synergistic effect on plant growth could be related to enhancement of nutrient uptake to plants, suppression of plant pathogenic fungi and promotion of lateral root growth. Alteration of rhizosphere microbial community has also been proposed as a mechanism responsible of plant growth promotion by AMF (Artursson et al., 2006). However, a better understanding of the complex relationships in the rhizosphere is needed to design effective restoration programs.

It has been reported with a DGGE approach that the AM fungus *G. intraradices*, modified rhizosphere bacterial communities (Vestergård et al., 2008) and that this effect depended on the phenology of the plant (Wamberg et al., 2003). Marschner and Baumann (2003) also employed DGGE to describe changes in rhizosphere bacterial communities which were exposed to *G. intraradices* and *G. mosseae* inoculation, but they suggested that this effect may be partially mediated by the plant as well. In the same way, Marschner and Timonen (2005) concluded that a plant-fungus complex interaction may be affecting the bacterial communities, whereas a study conducted by Söderberg et al. (2002), showed that the AMF effect on the bacterial communities varied depending on the host plant species. More recently, different changes in particular bacterial groups, as a consequence of inoculation with AMF, have been described by the use of PLFA and NFLA (Mechri et al., 2014) and 16S rRNA gene microarrays (Nuccio et al., 2013).

The most commonly used techniques to address this question (DGGE and PLFA) have important resolution limitations and are confined to the most abundant bacterial groups (Zhang and Xu, 2008; Van Elsas and Boersma, 2011), resulting in just a general overview of changes in microbial communities. High-throughput sequencing methods provide more precise tools for the in depth study of soil microbial communities (Zhang and Xu, 2008; Van Elsas and Boersma, 2011). Despite the need to increase our knowledge of symbiosis-associated bacterial communities and the current development of these techniques, there are relatively few studies that deal with the effect of mycorrhizal inoculation on rhizosphere bacterial communities using such molecular tools (Cao et al., 2016; Qin et al., 2016). Besides, none of these studies was performed under natural field conditions or in a semiarid ecosystem.

We hypothesized that the inoculation of four autochthonous shrub species (*Salvia officinalis*, *Lavandula dentata*, *Thymus vulgaris*, and *Santolina chamaecyparissus*) with the AM fungus *Rhizophagus intraradices* would induce direct and/or indirect changes in rhizosphere bacterial community structure, regardless of the host plant species, and that such shifts could be linked to improvements in plant performance. Accordingly, this study aims to determine, through the use of pyrosequencing, whether the application of this mycorrhizal inoculum for revegetation purposes in a semiarid ecosystem mediates changes in the rhizosphere bacterial communities, as well as to analyze the influence of different host plant species on these alterations. Additionally, shifts in soil chemical and biochemical properties driven by the presence of the inoculum are also studied, as well as their relationship to the composition and structure of the resulting bacterial community.

2. Materials and methods

2.1. Study site, plant species and mycorrhizal inoculum

The study was carried out at an experimental site located in the Ecological Park "Vicente Blanes", El Rellano (province of Murcia, SE Spain; coordinates 38° 12′ 50.8″ N, 1° 13′ 30.9″ W). The climate of the area is Mediterranean semiarid, characterized by low and irregular rainfall rates. The annual precipitation recorded during the experimental period (from November 2014 to November 2015) was 315 mm, and the mean temperature was 17.9 °C. The soil in this area is classified as a Typic Torriorthent (SSS 2010) with very little development. It has a silty-clay texture and is poor in organic matter. Therefore, its structural stability is weak.

Four autochthonous shrubs species, representative from semiarid areas in Southeast Spain, were selected for the experiment. *Salvia officinalis* L., *Lavandula dentata* L., and *Thymus vulgaris* L. belong to the *Lamiaceae* family while *Santolina chamaecyparissus* L. belongs to the *Asteraceae*. They are all well adapted to water and nutrient scarcity and are potential candidates for revegetation of this kind of ecosystems (Azcón and Barea, 1997; Padgett et al., 2000; Bochet et al., 2010; Sánchez-Ormeño et al., 2016). Seedlings of each plant species were

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first grown in sterilized peat substrate and inoculated with *R. intraradices* inoculum at a rate of 5% (v:v). The non-inoculated seedlings received the same amount of autoclaved inoculum as well as 10 ml of a filtrate obtained by filtering an inoculum aqueous extract (1:20, w/v) through a 20-μm-pore-size nylon mesh, in order to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any fertiliser treatment. At planting, there were not significant differences in growth parameters between inoculated and non-inoculated plants in neither of plant species. The percentage of mycorrhizal root colonization ranged from 65 to 75%, without significant differences among plant species. It was estimated according to the gridline intersect method (Giovannetti and Mosse, 1980), after clearing with KOH and staining with trypan blue (Phillips and Hayman, 1970).

The mycorrhizal fungus used was *Rhizophagus intraradices* (BEG 121 isolate) (former *Glomus intraradices*). The inoculum was previously multiplied using trap cultures of *Sorghum bicolor* L. Moench, and consisted of rhizospheric soil, spores, hyphae and infected root fragments.

2.2. Experimental design and sampling

The experiment was setup in an area of 2000 m^2 of the experimental site and consisted of a two-factor factorial randomized block design with three replication blocks. The first factor was the mycorrhizal treatment and had two levels: inoculation (M) or non-inoculation (C) with *R. intraradices*; whereas the second factor was the host plant species and had four levels: *S. officinalis* (S), *L. dentata* (L), *T. vulgaris* (T) and *S. chamaecyparissus* (ST). In November 2014, the seedlings were transported to the experimental field, where were planted in individual planting holes (40 cm wide × 40 cm long × 30 cm deep) at least 1 m apart between holes, with 3 m between replication blocks. 40 seedlings per replication block were planted (5 seedlings × 4 plant species × 2 treatments in each block). One year after planting, one plant per replication block for each plant species and treatment was randomly selected and harvested, making a total of 24 plants. Soil firmly adhered to the roots (rhizosphere soil) and root systems were separated and preserved in polyethylene bags at 4 °C until they were prepared for analyzing. For pyrosequencing analyses, one portion of rhizosphere soil was stored at −20 °C. The remaining soil was divided into two subsamples: one soil subsample was sieved at 2 mm and stored at 4 °C for enzyme activities and basal respiration analyses and another subsample was allowed to dry at room temperature for chemical analyses.

2.3. Plant analyses

Dry weight of shoots and roots (60 °C, 48 h) were recorded. Shoot dry tissues were milled for nutrient analyses: total N was determined by dry combustion using a Tru-Spec CN analyzer (LECO, St. Joseph, MI, USA), whereas P and K foliar contents were determined by ICP/OES iCAP 6500-duo spectrometry (Thermo Elemental Co. Iris Intrepid II XDL).

The final level of root colonization by AMF was estimated as described in Section 2.1.

2.4. Soil chemical, biological and biochemical properties

Soil total nitrogen was determined by dry combustion using a Tru-Spec CN analyzer (LECO, St. Joseph, MI, USA). Available phosphorus was extracted with 0.5 M $NaHCO_3$ and then analyzed by ICP/OES iCAP 6500-duo spectrometry (Thermo Elemental Co. Iris Intrepid II XDL).

Soil basal respiration (SR) was measured as the amount of CO_2 emitted during the incubation of soil samples moistened at 45% of their water holding capacity. The SR was determined with an automatic analyzer μ-Trac 4200 (SY-LAB Microbiology, Wien, Austria).

Dehydrogenase activity was determined following the procedure described in García et al. (1997), which measures the reduction of INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) into INTF (iodonitrotetrazolium formazan). The estimation of urease and protease activities was based on the release of NH_4^+ from hydrolytic reactions where soil samples were exposed to the substrates urea or *N*-α-benzoyl-L-arginine amide (BAA), respectively (Nannipieri et al., 1980). Alkaline phosphomonoesterase and β-glucosidase activities were determined by measuring the formation of *p*-nitrophenol (PNP) through spectrophotometry as described by Tabatabai and Bremner (1969). *P*-nitrophenyl-β-D-glucopyranoside (PNG) was the substrate for β-glucosidase activity reaction (Tabatabai, 1982), whereas alkaline phosphomonoesterase determination used *p*-nitrophenyl phosphate disodium (PNPP) as substrate (Naseby and Lynch, 1997).

2.5. DNA extraction, PCR amplification and pyrosequencing

Total genomic DNA was extracted from 0.25 g of each individual rhizosphere soil sample employing the PowerSoil ™ DNA Extraction Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) and following the manufacturer's protocol. An electrophoresis in 1.5% (w/v) agarose gel, stained with Gel Red™ (Biotium, Hayward, CA, USA), was performed in order to check whether the DNA extraction had been successful. The extracted DNA was used as a template for the PCR amplification of the 16S rRNA hypervariable V3-V5 region, employing the U341F and U519R primers (Baker et al., 2003). These primers contain as well the pyrosequencing A/B adapters and the 8 bp multiplex identifiers (barcodes) (Binladen et al., 2007).

Each PCR mixture consisted of a total volume of 25 μl including 25 pmol of each primer, 1.8 mM of $MgCl_2$, 0.2 mM dNTPs, 1 X the corresponding Taq buffer, 1 U TaqMaster (5 Prime Inc., Gaithersburg, MD, USA) and 10 ng of the DNA template. The PCR programme consisted of an initial denaturation step at 94 °C for 4 min, 25 cycles of denaturation at 94 °C for 15 s, primer annealing at 55 °C for 45 s and extension at 72 °C for 1 min, followed by a final stage of heating at 72 °C for 10 min. PCR products were purified with Ultracentrifugal Filter Units Ultracel-100 K membranes (Amicon, Cork, Ireland). To measure the DNA concentration, a Quant-iT PicoGreen® ds DNA Assay Kit (Life Technologies, Carlsbad, CA, USA) was used. After that, the samples were pooled in equimolar amounts in two composite samples and submitted for pyrosequencing with the 454 FLX Junior system (Life Sciences, Brandford, CR, USA) at "Zaidin Experimental Station" (Spanish Council for Scientific Research) in Granada, Spain.

2.6. Pyrosequencing data processing

The obtained reads were processed using mothur software (Schloss et al., 2009) and the author's standard operating procedure for 454 data (Schloss et al., 2011). Raw sequences were denoised with the PyroNoise algorithm (Quince et al., 2011), implemented as a mothur component, which uses the flowgrams information. Reads with more than two mismatches to the barcodes and three mismatches to the primers were discarded, as well as those containing homopolymers >8 bp. Primers and barcodes were trimmed and a minimum sequence length of 200 bp was established. The alignment of the remaining unique sequences was carried out against the SILVA v.123 compatible alignment database (Schloss, 2009; Quast et al., 2013) in order to remove those reads which do not fit in the selected alignment range. They were, likewise, subjected to UCHIME algorithm (Edgar et al., 2011) to identify and eliminate chimeras. Chloroplast and mitochondria sequences were discarded too. The resulting filtered and trimmed sequences were clustered into operational taxonomic units (OTUs) applying the average neighbor algorithm with a similarity cutoff of 97%. Afterwards, the centroid sequence of each cluster was selected as the most representative of the OTU and was taxonomically classified, at a confidence level of 80%, according to the SILVA v.123 full-length sequences database. To

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avoid bias in some of the further analysis comparisons, the number of sequences of each sample was rarefied, if necessary, to the lowest one (1955 sequences of the LC1 sample).

2.7. Statistical analyses

The effects of the experimental factors, plant species (P) and mycorrhizal inoculation (M), and their interaction (P × M) on plant and soil parameters were assessed by a two-way ANOVA and comparisons among means were calculated using the Tukey's HSD (Honestly Significant Difference) test at $P < 0.05$. Both analyses were performed in R (R Development Core Team, 2015) and, to achieve the normality requirements, shoot and root dry weights data, shoot P and K contents, as well as protease activity and soil available P were previously log transformed.

The α-diversity estimators, including Chao1, Shannon-Weaver index (H′) and Pielou evenness index, were calculated by mothur using the resultants OTUs after rarefying the number of sequence per sample. The sampling effort and OTU richness across soil samples were represented by normalized rarefaction curves (1000 iterations, 1955 randomly chosen sequences per sample). Concerning these indices, a two-way ANOVA was employed to assess significant differences among groups. For rarefaction curves, we compared the number of observed OTUs at the same sampling effort (1955 sequences).

To analyze the bacterial communities' β-diversity, a phylip-formatted distance matrix was constructed by mothur using the Bray-Curtis dissimilarity estimator on the rarefied dataset. The resulting matrix was then imported to R, using the "phyloseq" package (McMurdie and Holmes, 2013), and a 3D non-metric multidimensional scaling (NMDS) was carried out at OTU level by employing the "vegan" package in R (Oksanen et al., 2016). With this ordination technique the spatial distribution of the communities was obtained according to their structure. In order to test for significant differences between communities, owing to the influence of the experimental factors, a permutational multivariate analysis of variance (perMANOVA) was conducted with the "adonis" function in "vegan" for R and subsequent post hoc pairwise comparisons (with Bonferroni correction method) have been performed on perMANOVA results. Prior to these analyses, the assumption of multivariate normality was assessed through the Henze-Zirkler's test implemented in "MVN" package for R (Korkmaz et al., 2014).

The relative abundance of each bacterial group was calculated with STAMP (Parks et al., 2014) at different taxonomic levels, and was used afterwards as the input data to perform an Indicator Species Analysis (ISA). This analysis allows us identifying species which are associated to a grouping factor by calculating an Indicator Value (IndVal) (Dufrêne and Legendre, 1997). The ISA was conducted with the "indicspecies" package (v. 1.7.5, (De Caceres and Legendre, 2009)), implemented in R. Since our main interest was to find bacterial taxonomic groups that can be indicators of the inoculation with *R. intraradices*, the mycorrhizal treatment was the only classifier factor to be considered. The analysis was run applying the original IndVal method, which avoids considering group combinations, and was performed at phylum, genus and OTU level including classified and unclassified OTUs.

Distance based redundancy analysis (dbRDA) was applied to assess the relationships between the measured soil properties, including mycorrhizal colonization, and the bacterial community structure. It was conducted with the "vegan" package in R, using the Bray-Curtis matrix at OTU level as the species dataset. First, all available soil properties were included in a global model which was subjected to a Monte-Carlo permutation test (999 permutations) to ascertain the significance of the variation in the communities structure explained by these environmental variables. Once significance of the global model was determined, a forward selection procedure was followed to reduce the number of variables to those which better explain the variance and then, a final dbRDA was constructed with the selected subset of variables.

2.8. Accession numbers

The sequence files were submitted to the NCBI Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) and are accessible in the BioProject PRJNA335713.

3. Results

3.1. Plant growth and nutrient content

Both experimental factors, mycorrhizal inoculation (M) and host plant species (P), as well as their interaction (P × M), had a significant effect on shoot and root biomass, as shown by the two-way ANOVA (Table 1). The inoculation with *R. intraradices* significantly increased the shoot and root biomass compared to non-inoculated plants (on average by 59% and 172%, respectively). Concerning the differences among plant species, the highest values of shoot biomass were recorded in *L. dentata* plants, which showed an increase of 100% compared to the respective non-inoculated plants, whereas the highest increase in root biomass (270%, compared to the respective non-inoculated plants) was observed in *T. vulgaris*.

Except for *S. officinalis*, the mycorrhizal inoculation led to significantly higher contents of shoot N and P (Table 1). On the other hand, only the shoot K content was influenced by the host plant species: *S. chamaecyparissus* differed significantly from the other species, exhibiting the lowest values for this nutrient.

The inoculation with *R. intraradices* caused a significant increase in the mycorrhizal colonization of the roots of the four host plant species, mainly in *T. vulgaris* and *L. dentata* (Table 1). The host plant species differed significantly in their degree of mycorrhizal colonization. The lowest colonization percentages were found in *S. chamaecyparissus* roots, in comparison to *S. officinalis* and *L. dentata*.

3.2. Composition and structure of the rhizosphere bacterial communities

The processing of the pyrosequencing reads obtained produced 91,927 useful sequences across the 24 samples. The number of sequences per sample varied from 1955 (LC1) to 5444 (STM2) and the average sequence length was 220 bp. After applying the clustering algorithm, 3840 OTUs were generated - of which 70.4% were classified at the phylum level. The mean coverage for the trimmed sequences dataset was 0.87 ± 0.01, being 0.81 ± 0.00 for the rarefied sequences dataset (Table 2).

As shown by the two-way ANOVA (Table 2), neither the inoculation nor the host plant species had a significant effect on the α-diversity estimators (number of OTUs, Chao1 estimator, Shannon-Weaver index, and Pielou evenness index). The two-way ANOVA results for the normalized rarefaction curves estimates (Fig. 1) are in accordance with those of α-diversity estimators indicating that, for the same sampling effort, the communities obtained did not differ in the number of OTUs with respect to host plant species and mycorrhizal inoculation.

After the classification of the OTUs, a total of 25 bacterial phyla and three archaeal phyla were identified across all rhizosphere soil samples. The most abundant phylum identified was Proteobacteria (23.8%), followed by Acidobacteria (18.6%), Actinobacteria (12.5%), Gemmatimonadetes (11.35%), Bacteroidetes (6.2%), Planctomycetes (5.13%), Chloroflexi (4.9%), Verrucomicrobia (1.6%), and Firmicutes (1.4%). The remaining identified phyla showed relative abundances below 1% and represented 1.7% of the total number of sequences. The results also show that an "Unclassified bacteria" group was the third most abundant phylum detected, with a total relative abundance of 12.7%.

At the genus level, 174 different bacterial groups were identified. All of them showed relative abundances below 1% - except *Bryobacter* (1.2%) and *Gemmatimonas*, which was the dominant genus with 6.3% of the total number of sequences.

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Table 1
Growth parameters, foliar nutrients and mycorrhizal colonization of four plant species (SO = *S. officinalis*; L = *L. dentata*; T = *T. vulgaris*; ST = *S. chamaecyparissus*) non-inoculated (C) and inoculated with *R. intraradices* (M), one year after planting. Mean ± standard error, $n = 3$. Significance of effects of plant species (P), mycorrhizal inoculation (M) and their interaction on the measured variables is also shown (F-values (*P*-values)). SDW = shoot dry weight; RDW = root dry weight. For log-transformed parameters, geometric mean and the confidence intervals (values in the parenthesis) are shown. When the interaction between factors was significant, values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey's HSD-test.

Plant	Treatment	SDW (g)	RDW (g)	Shoot N (mg plant^{-1})	Shoot K (mg plant^{-1})	Shoot P (mg plant^{-1})	Colonization (%)
SO	SOC	3.6 (3.5 to 3.8)^{ab}	2.1 (1.4 to 3.1)^{ab}	61 ± 2	93 (61 to 140)^{ab}	6 (3 to 12)	58 ± 1
	SOM	3.9 (2.8 to 5.3)^{ab}	3.1 (1.8 to 5.2)^{bc}	60 ± 4	92 (39 to 217)^{ab}	6 (5 to 7)	83 ± 4
L	LC	4.3 (3.0 to 6.2)^{ab}	1.7 (0.8 to 3.7)^{ab}	44 ± 3	68 (62 to 74)^{a}	5 (3 to 7)	50 ± 5
	LM	8.5 (4.1 to 17.6)^{c}	4.7 (2.8 to 7.9)^{c}	75 ± 12	162 (65 to 405)^{b}	8 (3 to 21)	89 ± 2
T	TC	4.0 (2.8 to 5.6)^{ab}	2.7 (1.6 to 4.5)^{abc}	55 ± 7	62 (51 to 75)^{a}	4 (3 to 7)	40 ± 6
	TM	6.4 (5.3 to 7.7)^{bc}	9.9 (6.8 to 14.5)^{d}	84 ± 4	88 (46 to 170)^{a}	7 (6 to 8)	77 ± 2
ST	STC	2.1 (1.5 to 2.9)^{a}	0.6 (0.3 to 1.3)^{a}	47 ± 5	29 (16 to 53)^{a}	4 (3 to 5)	41 ± 7
	STM	3.5 (3.2 to 3.9)^{a}	1.9 (1.4 to 2.5)^{ab}	70 ± 2	67 (56 to 81)^{a}	7 (6 to 8)	67 ± 3
ANOVA (*P* values)							
Plant (P)		35.89 (<0.001)	52.05 (<0.001)	1.47 (0.259)	16.63 (<0.001)	1.47 (0.259)	7.05 (0.003)
Mycorrhizal inoculation (M)		54.70 (<0.001)	113.51 (<0.001)	23.65 (<0.001)	29.99 (<0.001)	29.96 (<0.001)	109.47 (<0.001)
P × M		4.88 (0.013)	5.22 (0.010)	3.02 (0.060)	5.09 (0.011)	2.73 (0.078)	1.34 (0.297)

The indicator species analysis (ISA), used to identify bacterial indicators of non-inoculated and inoculated plants, revealed nine indicator genera for rhizospheres subjected to the *R. intraradices* treatment (Table 3). *Skermanella* was the most abundant indicator genus of communities from inoculated plants, with a total relative abundance of 0.7%. The ISA also revealed seven indicator genera for the control rhizosphere communities, of which *Nitrospira* and *Arenimonas* were the most abundant taxa. Both presented a total relative abundance of 0.14%. At the phylum level, three and two taxa were revealed as indicators of the bacterial communities from non-inoculated and inoculated plants, respectively (Table 3). The most abundant indicator phylum of inoculated rhizospheres was Chloroflexi, with a relative abundance of 6.2% versus 2.8% in non-inoculated plants. Concerning the phylum indicators of non-inoculated rhizosphere bacterial communities, Gemmatimonadetes was the most abundant (relative abundance of 12.5% in non-inoculated plants, 10.0% in inoculated plants). The analysis of the obtained indicator species at the OTU level (Table A.1) yielded 99 indicator OTUs for the non-inoculated rhizosphere bacterial communities (2.6% of the total number of OTUs) and 113 indicator OTUs for the inoculated rhizospheres (2.9%). It is worth mentioning that OTUs belonging to the *Anaerolineaceae* family (phylum Chloroflexi) were found exclusively as indicators of inoculated rhizosphere bacterial communities. In particular, this family was represented by a total of seven indicator OTUs, one of which showed the highest relative abundance of the ISA (2.5% in inoculated plants versus 0.6% in non-inoculated ones). Furthermore, the ISA provides the A and B values which respectively correspond to the specificity and fidelity components of the IndVal index (Table 3). Those indicator groups that showed higher relative abundances (such as Gemmatimonadetes, Chloroflexi, Nitrospirae, *Skermanella*, *Bacillus*, *Arenimonas* and *Sandaracinus*) presented the maximum score for the B component but relatively lower values for A. This means that these groups were always present in every sample from the category where they are indicators (non-inoculated or inoculated rhizospheres) but they could also be found in samples from the opposite category, although in significantly lower relative abundances.

When the 3D NMDS was performed at the OTU level to analyze the β-diversity, it produced a stress value of 0.11 and an R squared value of 0.988. The NMDS plots of the three axes showed that the rhizosphere bacterial communities clustered according to the mycorrhizal treatment but also according to the host plant species (Fig. 2). The perMANOVA confirmed these differences in community structure since it showed significance for both factors (inoculation and plant species, $P = 0.001$ in both cases), but no interaction was found between them. In addition, pairwise perMANOVA comparisons among plant species revealed that there were no significant differences in the rhizosphere bacterial community between L and SO plants or between ST and T plants for non-inoculated and inoculated plants. However, among both pairs of plants the differences in bacterial community were significant (L × ST, $P = 0.030$; L × T, $P = 0.025$; SO × ST, $P = 0.026$; SO × T, $P = 0.015$).

Table 2
Rarefied (at the lowest number of sequences per sample) values of bacterial diversity estimators of the rhizospheres of *S. officinalis* (SO), *L. dentata* (L), *T. vulgaris* (T) and *S. chamaecyparissus* (ST), non-inoculated (C) and inoculated with *R. intraradices* (M). Mean ± standard error, $n = 3$. Significance of effects of plant species (P), mycorrhizal inoculation (M) and their interaction on the measured variables is also shown (F-values (*P*-values)). R = rarefied; T = trimmed. When the interaction between factors was significant, values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey's HSD-test.

Plant	Treatment	OTUs	Coverage$_R$ (%)	Coverage$_T$ (%)	Chao1	H′	Pielou
SO	SOC	631 ± 31	81 ± 0.01	87 ± 0.00^{bc}	1214 ± 83	5.58 ± 0.09	0.87 ± 0.01
	SOM	631 ± 28	81 ± 0.01	85 ± 0.01^{ab}	1195 ± 89	5.60 ± 0.06	0.87 ± 0.00
L	LC	623 ± 4	82 ± 0.00	83 ± 0.01^{a}	1211 ± 40	5.64 ± 0.02	0.88 ± 0.00
	LM	646 ± 13	81 ± 0.01	86 ± 0.01^{bc}	1249 ± 43	5.67 ± 0.04	0.88 ± 0.00
T	TC	667 ± 4	80 ± 0.00	88 ± 0.00^{bcd}	1315 ± 28	5.74 ± 0.03	0.88 ± 0.01
	TM	659 ± 6	80 ± 0.00	89 ± 0.00^{cd}	1331 ± 26	5.69 ± 0.01	0.88 ± 0.00
ST	STC	654 ± 13	80 ± 0.00	90 ± 0.00^{d}	1301 ± 33	5.66 ± 0.05	0.87 ± 0.00
	STM	628 ± 13	81 ± 0.00	90 ± 0.00^{d}	1280 ± 25	5.61 ± 0.06	0.87 ± 0.01
ANOVA (*P* values)							
Plant (P)		1.39 (0.281)	1.50 (0.253)	21.22 (<0.001)	2.20 (0.127)	2.14 (0.136)	2.29 (0.117)
Mycorrhizal inoculation (M)		0.04 (0.838)	0.03 (0.873)	1.83 (0.195)	0.01 (0.921)	0.08 (0.784)	0.04 (0.847)
P × M		0.71 (0.559)	0.45 (0.723)	3.58 (0.037)	0.15 (0.927)	0.35 (0.787)	0.24 (0.865)

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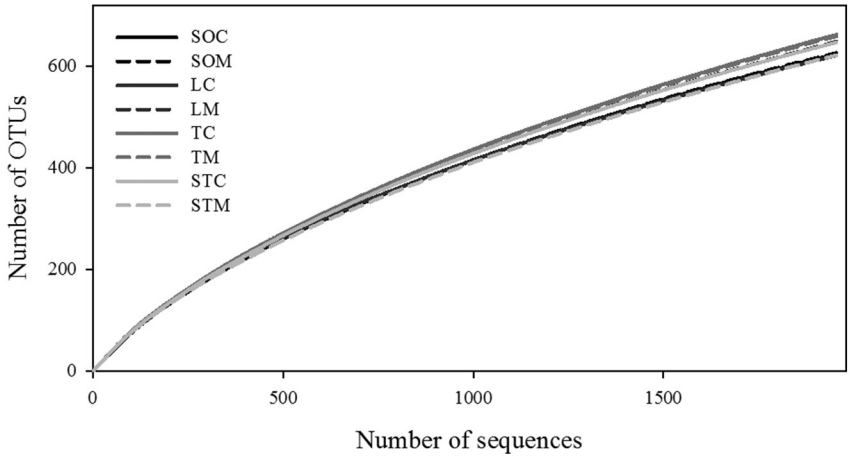

Fig. 1. Rarefaction curves (rarefied at 1955 sequences) along the number of sequences obtained from the rhizospheres of four plant species (SO = *S. officinalis*; L = *L. dentata*; T = *T. vulgaris*; ST = *S. chamaecyparissus*) non-inoculated (C) and inoculated with *R. intraradices* (M).

3.3. Soil microbiological and biochemical properties

All enzymatic activities were affected by the host plant species factor (Table 4). The *T. vulgaris* rhizosphere possessed the highest enzymatic activities, except for alkaline phosphomonoesterase. Only the dehydrogenase and urease activities were influenced by the *R. intraradices* inoculation. The two-way ANOVA also showed significance for the interaction of the two factors regarding all the enzymatic activities except that of protease. Additionally, the Tukey HSD test revealed that the inoculation involved higher values of dehydrogenase activity except for *S. officinalis*, whereas inoculated plants from this species, together with those of *T. vulgaris*, showed the highest values for urease activity. Regarding soil respiration, only significant differences due to the host plant species were detected (Table 4): the highest values were found in the rhizosphere of *T. vulgaris*.

Total N and available P were not significantly affected by the host plant species (Table 4) or inoculation with *R. intraradices*.

3.4. Relationship between soil properties and rhizosphere bacterial communities

A preliminary dbRDA was performed with the soil chemical, biochemical, and microbiological parameters measured, including mycorrhizal colonization. The significance of the global model generated was confirmed by the Monte-Carlo permutation test ($P = 0.007$). Then, the subsequent forward selection procedure generated a new model, which reduced the explanatory variables to shoot P, shoot K, shoot dry weight, and the urease and β-glucosidase activities. As a result, a final dbRDA (Fig. 3) was constructed using only these selected properties and the significance of the new model was tested ($P = 0.001$), as well

Table 3
Indicator species analysis (IndVal index) and relative abundances of bacterial genera and phyla in the rhizospheres of four plant species non-inoculated (RA_C) and inoculated with *R. intraradices* (RA_M).

Genus	IndVal	A	B	*P*-value	RA_C (%)	RA_M (%)
Non-inoculated plants						
Sandaracinus	0.876	0.77	1.00	0.0064	0.133	0.040
Nistrospira	0.826	0.68	1.00	0.0081	0.184	0.085
Nonomuraea	0.825	0.82	0.83	0.0089	0.055	0.012
Arenimonas	0.775	0.60	1.00	0.0494	0.163	0.108
Mizugakiibacter	0.744	0.83	0.67	0.0149	0.019	0.004
Thermincola	0.718	0.88	0.58	0.0246	0.033	0.004
Agromyces	0.707	0.75	0.67	0.0432	0.027	0.009
Inoculated plants						
Azoarcus	0.816	1.00	0.67	0.0020	0.000	0.071
Catellatospora	0.813	0.72	0.92	0.0236	0.036	0.094
Bacillus	0.809	0.65	1.00	0.0106	0.084	0.160
Skermanella	0.782	0.61	1.00	0.0183	0.532	0.837
Legionella	0.773	0.90	0.67	0.0036	0.003	0.032
Coxiella	0.764	1.00	0.58	0.0051	0.000	0.043
Microbacterium	0.759	0.77	0.75	0.0232	0.008	0.027
Pseudoxanthomonas	0.749	0.96	0.58	0.0044	0.002	0.042
Algoriphagus	0.722	0.89	0.58	0.0164	0.002	0.018
Phylum	IndVal	A	B	*P*-value	RA_C (%)	RA_M (%)
Non-inoculated plants						
Nitrospirae	0.826	0.68	1.00	0.0063	0.184	0.085
SHA.109	0.807	0.71	0.92	0.0153	0.061	0.025
Gemmatimonadetes	0.745	0.56	1.00	0.0053	12.525	10.021
Inoculated plants						
Chloroflexi	0.830	0.69	1.00	0.0004	2.800	6.201
Deinococcus-Thermus	0.731	0.92	0.58	0.0099	0.002	0.024

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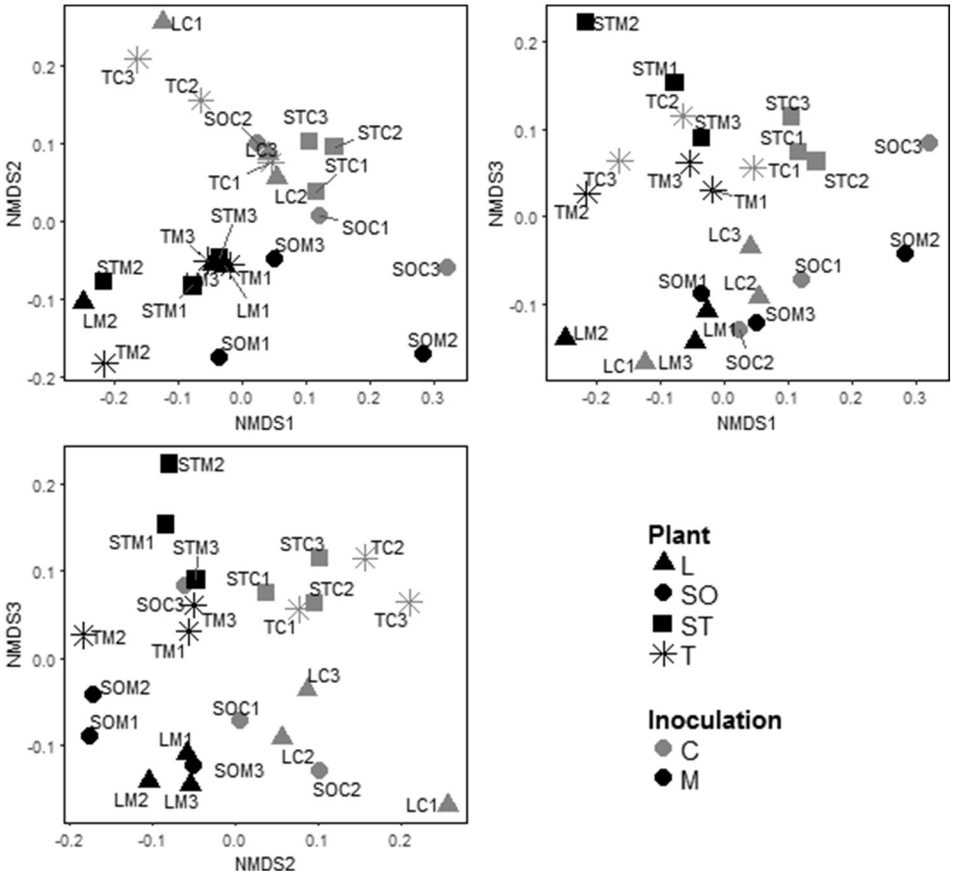

Fig. 2. Three-dimensional non-metric multidimensional scaling (NMDS) based on a lower-triangular distance matrix ("Bray-Curtis" similarity estimator) for bacterial communities at OTU level of the rhizospheres of four plant species (SO = *S. officinalis*; L = *L. dentata*; T = *T. vulgaris*; ST = *S. chamaecyparissus*) non-inoculated (C) and inoculated with *R. intraradices* (M).

as the significance of the dbRDA axes; the first three were found to be significant ($P < 0.05$).

The rhizosphere bacterial communities were distributed according to the AM fungal inoculation along the first axis of the dbRDA, which was strongly correlated with the shoot P content ($r = 0.88$), followed by the shoot dry weight ($r = 0.80$) and the shoot K content ($r = 0.70$). The communities from the inoculated plants clustered on the right-hand side of the plot as the host plants that harbored these rhizosphere communities exhibited higher values of these parameters than the non-inoculated plants (Fig. 3). The dbRDA plot also shows that the rhizosphere bacterial communities were ordinated according to the host plant species, along its second axis. The properties that contributed most to the second axis of the dbRDA were β-glucosidase activity ($r = -0.50$) and shoot K content ($r = 0.47$), whereas the third axis was mainly correlated with the urease ($r = -0.67$) and β-glucosidase ($r = -0.51$) activities.

Table 4
Biochemical, microbiological and chemical properties of the rhizospheres of four plant species (SO = *S. officinalis*; L = *L. dentata*; T = *T. vulgaris*; ST = *S. chamaecyparissus*) non-inoculated (C) and inoculated with *R. intraradices* (M) one year after planting. Mean ± standard error, $n = 3$. Significance of effects of plant species (P), mycorrhizal inoculation (M) and their interaction on the measured variables is also shown (F-values (*P*-values)). DH = dehydrogenase activity; BGL = β-glucosidase activity; ALP = alkaline phosphomonoesterase activity; URE = urease activity; PRT = protease activity; SR = Soil respiration; TN = soil total nitrogen; Avail. P = soil available phosphorus. For log-transformed parameters, geometric mean and the confidence intervals (values in the parenthesis) are shown. When the interaction between factors was significant, values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey's HSD-test.

Plant	Treatment	DH (μg g^{-1} INTF)	BGL (μmol PNP g^{-1} h^{-1})	ALP (μmol PNP 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})	TN (g kg^{-1})	Avail. P (mg kg^{-1})
SO	SOC	40.0 ± 2.0^{cd}	0.88 ± 0.05^{bcd}	1.20 ± 0.03^{b}	0.23 ± 0.05^{a}	0.42 (0.23 to 0.80)	15.5 ± 0.8	1.4 ± 0.2	6 ± 1
	SOM	39.3 ± 2.9^{cd}	0.72 ± 0.03^{ab}	1.03 ± 0.04^{ab}	0.45 ± 0.03^{bc}	0.41 (0.32 to 0.53)	16.2 ± 0.5	1.3 ± 0.1	6 ± 1
L	LC	31.2 ± 3.27^{bc}	0.84 ± 0.03^{abcd}	0.92 ± 0.05^{a}	0.21 ± 0.04^{a}	0.45 (0.35 to 0.57)	15.3 ± 0.7	1.8 ± 0.1	7 ± 1
	LM	45.6 ± 1.0^{de}	0.76 ± 0.02^{abc}	0.95 ± 0.04^{a}	0.33 ± 0.01^{abc}	0.43 (0.29 to 0.62)	14.9 ± 0.5	1.4 ± 0.1	5 ± 1
T	TC	33.5 ± 0.9^{bc}	0.92 ± 0.04^{d}	1.02 ± 0.02^{ab}	0.31 ± 0.04^{ab}	0.58 (0.44 to 0.78)	17.1 ± 0.7	1.4 ± 0.1	8 ± 1
	TM	52.3 ± 1.7^{e}	$0.91 \pm 0.03c^{d}$	1.09 ± 0.09^{ab}	0.50 ± 0.04^{c}	0.74 (0.41 to 1.35)	16.0 ± 0.4	1.6 ± 0.2	6 ± 0
ST	STC	18.3 ± 0.5^{a}	0.71 ± 0.03^{a}	0.95 ± 0.03^{a}	0.43 ± 0.03^{bc}	0.51 (0.41 to 0.96)	14.6 ± 0.7	1.2 ± 0.2	6 ± 1
	STM	28.7 ± 1.0^{b}	0.89 ± 0.02^{cd}	1.12 ± 0.03^{ab}	0.35 ± 0.03^{abc}	0.62 (0.40 to 0.96)	13.4 ± 0.3	1.4 ± 0.2	6 ± 0
ANOVA (*P* values)									
Plant (P)		42.71 (<0.001)	6.48 (0.004)	5.72 (0.007)	5.55 (0.008)	9.44 (<0.001)	6.46 (<0.004)	1.54 (0.243)	0.34 (0.793)
Mycorrhizal inoculation (M)		66.28 (<0.001)	0.55 (0.471)	0.63 (0.437)	19.17 (<0.001)	2.70 (0.120)	1.33 (0.266)	0.07 (0.791)	1.01 (0.331)
P × M		10.02 (<0.001)	9.35 (<0.001)	5.20 (0.011)	7.06 (0.003)	1.55 (0.241)	1.12 (0.371)	0.96 (0.433)	0.45 (0.721)

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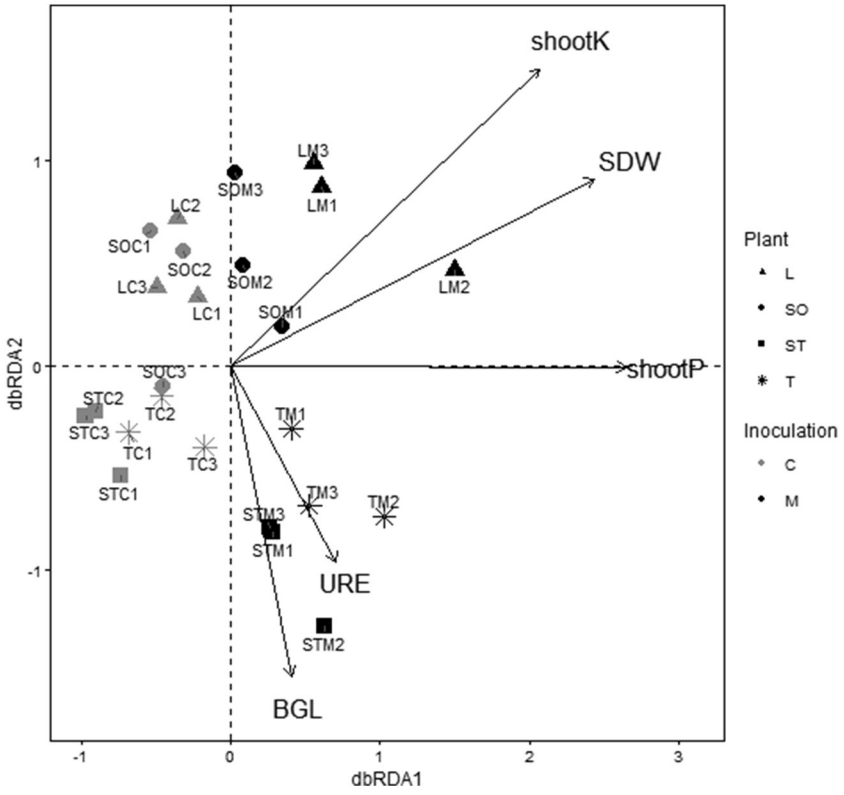

Fig. 3. Distance-based redundancy analysis (dbRDA) on rhizosphere bacterial communities at OTU level. The explanatory variables (BGL = β-glucosidase activity; URE = urease activity; shootP = shoot phosphorus content; SDW = shoot dry weight; shootK = shoot potassium content) are those selected by a previous forward selection procedure. The first two axes of the model explained 24% of the variation in the fitted model and 16% of the total variation between communities structure.

4. Discussion

4.1. Plant establishment

The inoculation with *R. intraradices* increased the AMF presence in roots and conducted to an improvement of growth parameters in the four host plant species. This indicated that roots colonization was successfully established. The beneficial influence of the AMF inoculant was found likewise on the shoot N, P and K contents indicating that, under severe semiarid conditions, the inoculated plants grew better than the non-inoculated ones. The improvement of plant growth and nutrient status after the inoculation with an AMF has already been reported in several studies with similar environmental characteristics. For instance, Caravaca et al. (2005) reported an increase in colonization, shoot biomass and foliar N, P and K contents when seedlings of native shrub species were inoculated with different AMF and grown in a field experiment under Mediterranean semiarid conditions. They also described an enhancement of soil structural stability and an increase in mycorrhizal propagules, both mediated by the AMF inoculation, which definitely are important goals to achieve in the restoration of these habitats. Similar results were reached by Alguacil et al. (2011) when they evaluated the success of an "in situ" *G. intraradices* inoculation of *O. europaea* plants in the same experimental area of the present study.

4.2. Composition and structure of the rhizosphere bacterial communities

Inoculation with an AM fungus modified the structure and composition of the bacterial communities associated with the rhizospheres of *S. officinalis*, *L. dentata*, *T. vulgaris*, and *S. chamaecyparissus*, while promoting the growth of these plant species in a semiarid soil. In fact, mycorrhizal plants, regardless of the plant species, supported distinct and characteristic rhizosphere bacterial communities, suggesting that the mycorrhizal inoculant selectively influenced the native bacterial population. The resulting bacterial community could have favored the establishment of mycorrhizal symbioses, an effect exerted by 'mycorrhiza-helper-bacteria' (Barea et al., 2005). Likewise, the altered bacterial community could have interacted synergistically with the AM fungal inoculum to improve host plant establishment. This latter assumption is supported by the synergistic effects on plant development resulting from the combined inoculation of specific bacterial functional groups and AMF (Barea et al., 2005). The inoculation with *R. intraradices* did not lead to significant changes in the species richness estimators or alpha-diversity indices, which were not affected by the host plant species either. The lack of influence of mycorrhizal inoculation on Shannon's diversity index for bacteria has been described already in research that assessed how inoculation with the AM fungus *Glomus hoi* altered bacterial communities during litter decomposition (Nuccio et al., 2013). Similarly, in a recent publication, Cao et al. (2016) did not find significant variations in the Chao1 estimator caused by inoculation with the AM fungus *Glomus caledonium*, in microcosms containing iron oxide nanoparticles.

On the other hand, the host plant species was a crucial factor that influenced the composition and structure of rhizosphere bacterial communities. This finding agrees with earlier studies (Zak et al., 2003; Rosenzweig et al., 2013) and may be explained by differences in the rhizodeposition patterns and decaying plant material among plant species (Marschner and Timonen, 2005). In the present investigation, the role of the AM fungus in shaping the rhizosphere bacterial community did not vary with the host plant species, coinciding with our initial hypothesis. In contrast, several reports showed that different mycorrhizal effects on rhizosphere bacterial communities were determined by the host plant species. This is the case of Söderberg et al. (2002), who used PLFA and Biolog techniques to investigate the influence of *Rhizophagus irregularis* colonization on bacterial communities from the rhizospheres of four different plant species (maize, cucumber, clover, and leek). They concluded that the effect of the AM fungal inoculation

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on the bacterial communities in the rhizosphere was dependent on the host plant species. In another study, Marschner and Timonen (2005), using the DGGE technique, also described distinct bacterial community structures for different plant species and genotypes inoculated with the same AM fungal strain.

In order to have an insight into the specific shifts in rhizosphere bacterial community in response to mycorrhizal inoculation, an indicator species analysis (ISA) was performed. The presence of bacterial indicators is consistent with the NMDS and perMANOVA results and suggests that *R. intraradices* was promoting or repressing the recruitment of some bacterial groups in the rhizosphere, regardless of the plant species. Seven indicator OTUs of the inoculated rhizospheres belong to the family *Anaerolineaceae*, which was one of the most abundant taxa. Its importance in the rhizosphere bacterial communities of inoculated plants is also reflected at the phylum level, as Chloroflexi, the phylum to which this family belongs, is an indicator of these rhizospheres too. *Anaerolineaceae* is a family of strictly anaerobic bacteria, so its presence under these habitat conditions would be expected to be marginal. However, in the already mentioned work by Cao et al. (2016) the Anaerolineae abundance was increased by *G. caledonium* inoculation in rhizospheres subjected to iron oxide nanoparticles. This bacterial family was also detected around the mycorrhizal hyphae of *G. mosseae* in a rhizobox experiment that tested the efficiency of AMF with respect to the dissipation of PCB contaminants (Qin et al., 2016). Members of the *Anaerolineaceae* are characterized by their ability to use complex carbohydrates and/or peptides (Yamada et al., 2006). They form multicellular filaments that degrade microbial products such as carbohydrates and cellular components (Miura et al., 2007; Miura and Okabe, 2008). Hence, in the present study the *Anaerolineaceae* bacteria may have metabolized fungal deposits of organic compounds such as chitin and glomalin, thereby increasing their presence in mycorrhizal rhizospheres.

At the genus level, *Bacillus* was an indicator of mycorrhizal rhizospheres. *Bacillus* is usually associated with AMF as "mycorrhiza helper bacteria" (Medina et al., 2003; Bonfante and Anca, 2009). *Skermanella*, which was the sixth most abundant genus of those identified, was likewise an indicator of mycorrhizal rhizospheres. We have not found previous evidence of the relationship between *Skermanella* and AMF, but its higher representation in inoculated rhizospheres might be due to the fact that its abundance increases with higher levels of gamma-aminobutyric acid (Badri et al., 2013), which is synthesized by plants and whose production is stimulated by the presence of AMF (Okada and Matsubara, 2012; Saia et al., 2015).

The introduction of the AM fungus may have affected negatively the abundance of Gemmatimonadetes, an indicator phylum of non-inoculated rhizospheres. The type and only described species of this mostly uncultured phylum is *Gemmatimonas aurantiaca*, which has been reported as a polyphosphate accumulating bacterium (Zhang et al., 2003) and has been associated with enhancement of durum wheat above-ground production (Yang et al., 2013). The AMF are known to be polyphosphate accumulating organisms as well, polyphosphate transport being an efficient mechanism for the P allocation from the extraradical to the intraradical hyphae and thus for plant nutrition (Rasmussen et al., 2000; Smith and Read, 2008; Yao et al., 2010). This finding suggests that, when functional redundancy occurs, AMF may outcompete and displace Gemmatimonadetes bacteria: in our work, the P metabolism of *R. intraradices* seemed to be more efficient - according to the shoot P content results. *Nitrospira* and *Arenimonas* were the most abundant indicators of control rhizosphere communities at the genus level. Contrary to our results, Cao et al. (2016) found that *Nitrospira* abundance was higher in maize roots colonized by *G. caledonium*. However, in their experiment soils were exposed to a pollutant whose negative impact on certain bacterial groups, such as *Nitrospira*, was alleviated by the AMF presence.

4.3. Relationships between rhizosphere microbial communities and soil and plant parameters

Soil enzymatic activities are useful tools for monitoring changes in microbial activity and soil functionality (Sinsabaugh, 1994; García Izquierdo, 2003). Different plant species have distinct effects on microbiota and microbial enzymatic activities according to the nature and quantity of their root exudates (Söderberg et al., 2002; Marschner and Timonen, 2005; Fuentes-Ponce et al., 2016). In line with the above discussion, we found that the activity of enzymes involved in the cycling of nutrients such as C, N, and P varied depending on the host plant species.

The inoculation with *R. intraradices* had a positive influence on dehydrogenase and urease activities. The AMF may stimulate microbial activity indirectly, by changing root exudation patterns, (Andrade et al., 1998) or directly via hyphodeposition. The enhancement of dehydrogenase activity, which provides a suitable estimate of the overall metabolic activity of microorganisms in soil, was accompanied by improved plant growth and nutrient status. Inoculated plants were favored by more active rhizosphere microbial communities that, at the same time, might be stimulated by a better physiological status of the plant in a reciprocal relationship (Vandenkoornhuyse et al., 2015). Urease is responsible for the hydrolysis of urea to ammonia. It has been demonstrated that AMF preferentially acquire ammonium over nitrate as the N source to transfer to the host plant (Govindarajulu et al., 2005; Tanaka and Yano, 2005). Therefore, in the present work, the higher availability of ammonium might be favoring the AMF growth.

The dbRDA allowed us to analyze the variation in rhizosphere bacterial communities explained by the measured soil and plant properties. Since it is not possible to ascertain if the changes mediated by the AM fungus were the result of direct effects on the rhizosphere bacterial communities, indirect effects related to an improvement of the plant physiological status, or a combination of these two mechanisms, we included both the soil and the plant properties in this multivariate analysis. As shown by the dbRDA, the effect of *R. intraradices* on the rhizosphere bacterial communities was related mainly to an improvement of the plant growth parameters and indicators of plant nutritional status. In particular, the shoot P content, whose values were only affected by the inoculation, was the principal variable explaining the bacterial communities variability mediated by the *R. intraradices* inoculation. It has been reported that the specific recruitment of the members of the rhizosphere bacterial communities is dependent on the quantity and quality of the root exudates (Marschner and Timonen, 2005; Vestergård et al., 2008), which in turn are influenced by the nutritional and physiological status of the plant (Yang and Crowley, 2000; Neumann and Römheld, 2007; Hunter et al., 2014). Furthermore, quantitative and qualitative changes in root exudation following colonization by AMF have been described (Mada and Bagyaraj, 1993; Jones et al., 2004). Therefore, our results support the hypothesis that AMF alter the bacterial communities composition indirectly, by modifying rhizodeposition patterns (Marschner and Baumann, 2003; Jones et al., 2004; Rillig, 2004; De Boer et al., 2005).

4.4. Conclusions

The inoculation of four different native shrubs with the AM fungus *R. intraradices* induced changes in the composition and structure of the rhizosphere bacterial communities as well as enhancing plant growth in a semiarid soil under field conditions. The ability of the AM fungus to shape the rhizosphere bacterial community structure was independent of the host plant species. Differences in bacterial community assemblage among the rhizospheres were mainly correlated to the improvement of plant performance provoked by the mycorrhizal inoculation. This indicates that the plant, AM fungus, and rhizosphere bacteria form a complex system in which each component and their relationships should be taken into account when designing effective

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restoration programs. Future investigations should include the analysis of functional bacterial groups associated with mycorrhizal symbiosis, which could contribute to the functions of AMF.

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