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ORIGINAL PAPER

Assessment of the potential role of *Streptomyces* strains in the revegetation of semiarid sites: the relative incidence of strain origin and plantation site on plant performance and soil quality indicators

Carmen Mengual[1] · **Mauricio Schoebitz**[2] · **Fuensanta Caravaca**[1] · **Antonio Roldán**[1]

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Abstract We performed a field assay to assess the efficacy of strains of actinobacteria belonging to the *Streptomyces* genus, isolated from two Mediterranean semiarid sites (Rellano and Calblanque) with different soil characteristics, with regard to the establishment of *Rhamnus lycioides* L. seedlings in both locations, as well as their effect on soil chemical and microbiological properties 1 year after planting. At the Calblanque site, the inoculation with native strains was more effective than that with allochthonous strains, with respect to increasing shoot dry weight (about 48 and 28 %, respectively, compared to control plants), primarily due to improvements in NPK uptake and plant drought tolerance. However, at Rellano, the efficacy of plant growth promotion was not influenced by the strain origin. The highest increases in the urease, protease, and dehydrogenase activities and in microbial biomass C in response to inoculation with actinobacteria occurred at the Rellano site (about 200, 28, 29, and 30 %, respectively, compared to the respective controls), regardless of the origin of the strain assayed. Strain origin and the biological fertility of the plantation site should be considered in the selection of strains of actinobacteria for use in the revegetation with shrub species in semiarid environments.

Keywords Actinobacteria · Allochthonous strain · Enzymatic activities · Mediterranean native shrub · Native strain · Revegetation

✉ Carmen Mengual
cmengual@cebas.csic.es

[1] Department of Soil and Water Conservation, CSIC—Centro de Edafología y Biología Aplicada del Segura, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain

[2] Department of Soils and Natural Resources, Faculty of Agronomy, University of Concepción, Casilla 160-C, Concepción, Chile

Introduction

Natural revegetation tends to be slow in arid and semiarid Mediterranean ecosystems, where the scarcity of water frequently limits plant establishment and growth (Caravaca et al. 2005a; Schoebitz et al. 2014). However, other environmental factors also could provoke major differences in the plant cover regeneration, including soil type and soil nutrient availability (Alegre et al. 2004; Caravaca et al. 2002). Several revegetation programs have been developed by using a plant cover based on autochthonous plant species, which seems the most appropriate strategy for reclaiming degraded lands (Caravaca et al. 2005a). The use of shrubs to recover dry areas has been encouraged by the Common Agricultural Policy of the European Union. The application of plant growth promoting microorganisms has been recorded as a successful tool in the reclamation of semiarid Mediterranean areas (Mengual et al. 2014; Schoebitz et al. 2014). Revegetation practices based on microbial inoculations require the development of an inoculum whose performance is optimum under specific environmental conditions (Bashan et al. 2014; Caravaca et al. 2003) in order to benefit the growth, nutrient uptake, and hydric status of the host plant (Ortiz et al. 2014). Cell immobilization is another biotechnological approach actively used in preparation and formulation of biofertilizers with partly unexploited potential (Vassilev et al. 2015) mainly in agricultural and environmental applications where the use of polysaccharide beads catching PGPR offers a great protection to cells against biotic and abiotic stresses (Vassilev et al. 2012, 2015). Plants and microorganisms co-existing in a soil are

often adapted to the same environmental conditions; so, presumably, native strains could be more effective for this purpose in semiarid Mediterranean sites (Armada et al. 2014), allowing the local biodiversity to be preserved without introducing new microbial species (Ortiz et al. 2014). Rhizobacteria are free-living bacteria which can colonize the rhizosphere and improve root system establishment (Antoun and Kloepper 2001), improving plant health and nutrition (Adesemoye and Kloepper 2009; Benabdellah et al. 2011; Mengual et al. 2014; Puente et al. 2004; Schoebitz et al. 2014; Pili et al. 2015). Furthermore, it has been shown that PGPR are able to synthesize some vitamins participating in physiological processes within plant-PGPR interactions (Palacios et al. 2014). Actinobacteria are one of the major components of soil microbial populations, comprising 10–50 % of the soil microfloral community over a broad range of soil conditions (Hamdali et al. 2008a). They are able to mineralize N and C, decompose organic material (Li et al. 2010), fix atmospheric N (Valdés et al. 2005), produce phytohormone-like compounds, and behave like biocontrol agents (Tarkka et al. 2008)—properties that benefit plant growth (Hamdali et al. 2008b). Due to their multiple traits, the use of actinobacteria to favor the establishment of plant species in semiarid environments is interesting. Actinobacteria have displayed their potential as plant growth promoting rhizobacteria (PGPR) under laboratory and greenhouse conditions (De Vasconcellos and Cardoso 2009; Franco-Correa et al. 2010; Shishido and Chanway 1998), but knowledge of their effectiveness under field conditions is scarce—being limited to assays in agricultural ecosystems (Jog et al. 2014). Meanwhile, no studies on the employment of actinobacteria to promote the establishment of plant species in revegetation programs have been conducted.

It has been proven that microorganisms that are native to a particular soil—such as arbuscular mycorrhizal fungi (Bashan et al. 2012; Caravaca et al. 2003; Ouahmane et al. 2007, 2006) or *Bacillus* strains (Armada et al. 2014; Ortiz et al. 2014)—are often successful inoculants in revegetation tasks, presumably as a result of their adaptation to specific edaphic and environmental conditions (Schreiner 2007). However, the efficacy of native strains of actinobacteria in comparison with allochthonous strains, regarding enhancement of plant growth, has not been investigated. We hypothesized that variations among actinobacterial strains from different sites could lead to distinct effects on plant growth and nutrient uptake. The aims of this study were (1) to ascertain if the positive effects of actinobacteria are maintained when the strains are inoculated in soils different from their isolation source, (2) to verify the relevance of the strain origin to the ability of actinobacteria to enhance plant growth under semiarid field conditions, and (3) to unravel the mechanisms which account for the differences in the ability of native actinobacterial isolates to influence growth and nutrient uptake. In this regard, we investigated whether the actinobacterial strains stimulate plant growth directly or indirectly, by improving soil properties. To address these questions, we assessed, in a field experiment, the efficacy of actinobacterial strains isolated from two Mediterranean semiarid sites with different soil characteristics on the establishment of *Rhamnus lycioides* L. seedlings in both locations, as well as their effect on soil chemical and microbiological properties. The information gained here will enable us to establish effective criteria for the selection of strains for use in the revegetation of semiarid environments.

Materials and methods

Study areas

The field assay was carried out in two different semiarid Mediterranean areas: Vicente Blanes Ecological Park in Rellano (coordinates 38°12′50.8″ N, 1°13′30.9″ W) and Calblanque Nature Reserve (coordinates 37°36′22.7″ N, 0°45′20.3″ W), both in the Province of Murcia, Spain. The soil is classified as a Typic Torriorthent (SSS 2010), very little developed with low organic matter content that facilitates the degradation of soil structure. This area supports vegetation dominated by *Piptatherum miliaceum* (L.) Cosson and some shrubs of *Thymus vulgaris* L., *Pistacia lentiscus* L., *Rhamnus lycioides* L., *Cistus clusii* Dunal, and *Rosmarinus officinalis* L. Calblanque Nature Reserve had a mean annual temperature of 18.7 °C and no frost period. The soil is a Lithic Torriorthent (SSS 2010). The vegetation in this study site is composed by several shrubs species such as *Tetraclinis articulata* (Vahl) Mast., *Rhamnus lycioides* L., *Maytenus senegalensis* (Lam.) Loes., *Periploca angustifolia* Labill., and *Calicotome intermedia* C. Presl in Abh., and some tree species like *Pinus halepensis* Mill. and *Chamaerops humilis* L. During the experimental period, the annual mean precipitation was 265 mm, being the rainfalls mainly concentrated in November 2012 and spring 2013. The annual mean temperature was 18.7 °C, reaching the maximum temperature of 26.7 °C in the summer months. Soil characteristics in both locations are reported in Table 1.

Plant material

The plant used was *Rhamnus lycioides* L. (*Rhamnaceae*), a perennial shrub which can reach a height up to 3 m, naturally occurring in the Western Mediterranean Basin, which can be found from sea level up to 1000 m (Gulías and Traveset 2012). This is a representative autochthonous species from semiarid areas in southeast Spain, well adapted to water stress conditions and high temperatures, used frequently in the revegetation of semiarid disturbed lands (Caravaca et al. 2003, 2005b; Alguacil et al. 2011). Prior to the experimental procedures,

Table 1 Soil physico-chemical and microbiological characteristics of Rellano and Calblanque sites

	Rellano	Calblanque
pH (H_2O)	8.5±0.0[a]	7.74±0.0
Electrical conductivity (1:5, μS cm^{-1})	176±3	250±3
Texture	Silty loam	Sandy loam
Total C (g kg^{-1})	98.5±1.5	41.4±1.3
Total organic C (g kg^{-1})	18.3±5.3	23.7±4.8
Water soluble C (mg kg^{-1})	76±3	328±3
Water soluble carbohydrates (mg kg^{-1})	11±1	75±2
Microbial biomass C (mg kg^{-1})	627±31	1229±49
Total N (g kg^{-1})	1.6±0.0	2.0±0.0
Available P (mg kg^{-1})	5±0	8±0
Extractable K (mg kg^{-1})	350±3	356±5
Aggregate stability (%)	43.0±1.0	71.4±2.1

[a] Mean±standard error (n=5)

R. lycioides seedlings were grown for 1 year in nursery conditions with peat as substrate. At planting, *R. lycioides* seedlings reached 27.9±1.8 cm high, with a shoot dry weight of 1.78±0.20 g and root dry weight of 2.45±0.50 g (n=5).

Microbial inoculants

Six strains coming from each study area were isolated from the rhizosphere of naturally established *R. lycioides* plants. The strains were isolated in oatmeal-agar medium supplemented with penicillin (25 mg ml^{-1}), nystatin (0.1 %), and cycloheximide (50 mg ml^{-1}) in order to inhibit growth of other bacteria and fungi (Franco-Correa et al. 2010). They were assayed in vitro for their abilities to solubilize phosphate from calcium, aluminum, and iron (III) phosphates (Premono et al. 1996; Bashan et al. 2013), to fix dinitrogen by measuring acetylene reduction activity (ARA) (Hardy et al. 1968), and to produce siderophores (SideroTec AssayTM, Emergen Bio) (Table 2). Four strains with similar capacities, two coming from Rellano RE1 and RE2 and two from Calblanque CA1 and CA2, were chosen to perform our experiment. The strains were identified using molecular methods based on polymerase chain reaction-denaturing gradient gel electrophoresis followed by 16S rDNA cloning and sequencing. The sequences were analyzed for the similarities using BLAST (NCBI). The strains RE1 and RE2 displayed 99 % similarity to sequences from *Streptomyces albospinus* (accession JN566023.1) and 100 % similarity to sequences from *Streptomyces* sp. (accession HM210306.1), respectively. The strains CA1 and CA2 showed a sequence similarity of 99 % to *Streptomyces* sp. (accession JN866719.1) and 99 % to *Streptomyces microsporus* (accession AB184459.2), respectively. The sequences of four strains were deposited in the GenBank with the accession numbers RE1=LN610452, RE2=LN610454, CA1=LN610453, and CA2=LN610455. For inoculum preparation, the selected isolates were grown into flaks containing 50 ml of liquid Yeast Extract Peptone (YEP) medium and subjected to shaking at 160 rpm during 15 days at 28 °C. Strains were immobilized by inverse gelation technique (Madene et al. 2006). Briefly, the suspension of cells was mixed with sodium alginate (3 %, *w*/*v*) and starch from potato (2 %, *w*/*v*) and subsequently stirred for 30 min for homogenization. Next, the mixture was transferred to a syringe (10 ml) and dropped into sterile calcium chloride. Gelling of alginate-starch beads was completed after 30 min in contact with the calcium solution, being the concentration of 1.2×10^8 CFU g^{-1}. The collected beads (about 0.5 cm in diameter) were used immediately after their preparation.

Experimental design

A full-factorial design was established with two factors and 5-fold replications in a split-plot design. The first factor was the origin of the strains (strains isolated from Rellano or isolated from Calblanque plus non-inoculated controls) and the second one was the planting site (Rellano or Calblanque). In late November of 2012, the seedlings were transported to the experimental sites, where planting holes 15×15 cm wide and 15 cm deep were dug manually. There, an amount of 7 g of microbial inoculant pellets was applied per plant. The same quantity of sterilized inoculant was applied to the non-inoculated plants. The seedlings were planted at least 1 m apart between holes, with 3 m between treatment levels.

Sampling procedures

Twelve months after planting, in November 2013, samples were collected from each experimental area. Five plants per treatment including root systems and soil firmly adhered to the

Table 2 Characterization in vitro of the four strains of *Streptomyces* sp. selected for the field experiment

Strain	Calcium phosphate solubilization index	Iron (III) phosphate solubilization index	Aluminum phosphate solubilization index	Ethylene (nm $ml^{-1}h$)	Siderophore excretion	Accession number
RE1	3.03±0.04 a	3.00±0.08 c	2.43±0.08 a	0.02±0.00 a	+	LN610452
RE2	3.66±0.09 b	1.73±0.03 a	2.21±0.06 a	0.01±0.00 a	+	LN610454
CA1	3.06±0.05 a	2.11±0.08 b	2.39±0.05 a	0.02±0.00 a	+	LN610453
CA2	3.80±0.07 b	1.76±0.06 a	2.22±0.10 a	0.01±0.00 a	+	LN610455

roots (rhizosphere soil) were harvested and introduced in polyethylene bags for transport to the laboratory. A total number of 50 plants and rhizosphere samples were collected. Rhizosphere soil samples were divided in two subsamples. One soil subsample was sieved at 2 mm and stored at 4 °C for microbiological and biochemical analyses and another soil subsample was allowed to dry at room temperature for chemical analyses.

Plant analyses

Dry weights of shoot and root (70 °C, 48 h), basal stem diameter, and plant height were recorded. Shoot P and K were determined by ICP/OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL) while shoot N was determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

Nitrate reductase activity was assayed in vivo by measuring NO_2^- production in tissue that had been vacuum-infiltrated with buffered NO_3^- solutions (Downs et al. 1993). The leaves of the shrubs were collected in the morning at 11:00 h solar time and were cut into 3-mm sections. Approximately 300 mg of leaf punches were placed in tubes containing 2 ml of an incubation medium consisting of 0.05 M Tris–HCl, pH 7.8 and 0.25 M KNO_3. The tubes were sealed and kept in the dark at 30 °C for 1 h. The nitrite released into the medium was determined after incubation by treating 1-ml aliquots with 1 ml of 1 % sulfanilamide in 1 M HCl and 1 ml of 0.01 % *N*-1-napthyl-ethylenediamine hydrochloride. After 15 min, the optical density was measured spectrophotometrically at 540 nm.

The level of lipid peroxidation was determined by the content of malondialdehyde (MDA) and a product of lipid peroxidation (Zhao et al. 1994) by the method of Minotti and Aust (1987). Lipid peroxides were extracted by grinding 0.5 g of fresh leaves in an ice-cold mortar and 6 ml of 100 mM potassium phosphate buffer (pH 7). Homogenates were filtered through one Miracloth layer and centrifuged at 15,000×*g* for 20 min. The chromogen was formed by mixing 200 ml of supernatants with 1 ml of a reaction mixture containing 15 % (*w*/*v*) trichloroacetic acid (TCA), 0.375 % (*w*/*v*) 2-thiobarbituric acid (TBA), 0.1 % (*w*/*v*) butyl hydroxytoluene, and 0.25 N HCl, and by incubating the mixture at 100 °C for 30 min. After cooling, it was centrifuged at 800×*g* for 5 min and the absorbance of the supernatant was recorded at 535 nm. The calibration curve was carried out with different concentrations of MDA.

The percentage of root length colonized by arbuscular mycorrhizal fungi (AMF) was calculated by the gridline intersect method (Giovannetti and Mosse 1980) after staining with Trypan blue (Phillips and Hayman 1970).

Soil chemical analyses

Soil texture was determined using the hydrometer method. Aggregate stability was measured according to the method described in Roldán et al. (1994). Total nitrogen (N) was determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). Available P, extracted with 0.5 M $NaHCO_3$, and K, extracted with ammonium acetate, were determined by ICP/OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). Water soluble C (WSC) was determined in water extracts (1:10 *w*/*v*) by using an automatic carbon analyzer for liquid samples (Shimadzu TOC-5050A). Water soluble carbohydrates (WSCH) were determined as reported by Brink et al. (1960).

Soil biochemical and microbiological analyses

Alkaline phosphomonoesterase activity was determined using *p*-nitrophenyl phosphate disodium as substrate (Naseby and Lynch 1997). Two milliliters of 0.5 M sodium acetate buffer at pH 11 and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 0 °C for 10 min. Then 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added and the mixture was centrifuged at 2287×*g* for 5 min. The *p*-nitrophenol (PNP) formed was determined at 398 nm (Tabatabai and Bremner 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase activity was determined using 0.05 M *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. For this assay, based on the release and detection of PNP, 2 ml of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethano (THAM) according to Tabatabai (1982). The amount of PNP was determined at 398 nm (Tabatabai and Bremner 1969).

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Aliquots of 2 ml of buffer and 0.5 ml of substrate were added to 0.5 g of sample followed by incubation for 90 min at 30 °C (urease) or 39 °C (protease). Both enzyme activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al. 1980).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60 % of its field capacity was exposed to 0.2 ml of 0.4 % INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodonitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and

filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Microbial biomass carbon was determined using the substrate induced respiration (SIR) method (Anderson and Domsch 1978). Moist (60 % of water holding capacity) soil samples were mixed with glucose at a rate of 0.5 % (*w*/*w*). Evolved CO_2 was monitored using the μ-Trac 4200 system (SY-LAB, GmbH, P.O. Box 47, A-3002 Neupurkersdorf, Austria). This system is based on the variation of electrical impedance of a KOH 0.2 % water solution (Fernández et al. 2004). Respiration rates were calculated in the linear phase of the respiration curves. Basal soil respiration was assessed with the same system described for microbial biomass C but in the absence of glucose.

Statistical analyses

Values were log transformed to achieve normality. The effects of the strains origin, planting site, and their interaction on measured variables were analyzed by a two-way ANOVA. The mean separation was performed by Duncan's multiple range test, calculated at $P < 0.05$. All statistical analyses were performed using the software SPSS version 19.0 for Windows.

Results

Growth parameters and shoot nutrients of *R. lycioides*

Both strain origin (SO) and planting site (PS) had a significant effect on *R. lycioides* shoot biomass and height, while root dry weight was only affected by PS (Fig. 1). One year after planting, the plants inoculated with *Streptomyces* sp. strains were taller at the Calblanque site than at the Rellano site, regardless of the SO. At Calblanque, the inoculation was more effective with native strains (CA1 and CA2) than with allochthonous strains (RE1 and RE2) for increasing shoot dry weight (by about 48 and 28 %, respectively, compared to control plants). However, at Rellano, the origin of the strain did not influence its efficacy regarding promotion of plant growth. Thus, in this soil, the native and allochthonous strains produced similar increases in the shoot biomass of *R. lycioides* (on average, about 44 % compared to control plants). Root biomass increased in response to the inoculation with both native and allochthonous strains at Calblanque (by 60 and 52 %, respectively, compared to control plants). In contrast, at Rellano, only the allochthonous strains (CA1 and CA2) provoked an increase in root biomass. The microbial inoculation hardly had an effect on *R. lycioides* height. A significant increase was observed only at Calblanque, after the inoculation with the native strains (Fig. 1).

There was a significant effect of SO and PS on NPK uptake (Fig. 2). The *Streptomyces*-inoculated plants grown at the Calblanque site possessed higher levels of nutrients than those grown at the Rellano site. As observed for the growth parameters, only the nutrient contents in shoots of *R. lycioides* seedlings grown at Calblanque were influenced by the SO. At Calblanque, the plants inoculated with native strains had higher NPK contents than those inoculated with allochthonous strains.

Plant stress parameters

The PS significantly affected the nitrate reductase activity, it being higher in the shoots of *Streptomyces*-inoculated plants grown at the Rellano site than in those at the Calblanque site (Fig. 3). The post hoc test showed that the actinobacteria enhanced the nitrate reductase activity, without significant differences between native and allochthonous isolates at both planting sites. There was a significant effect of SO and PS on the oxidative damage to lipids (Fig. 3). Oxidative damage was decreased significantly by the inoculations with actinobacteria, the greatest decrease being observed in the plants inoculated with native strains (CA1 and CA2) at the Calblanque site (50 %, compared to the corresponding control plants). The native and allochthonous strains provoked similar decreases in the levels of lipid peroxidation in plants grown at Rellano (Fig. 3).

AMF root colonization and soil chemical properties

None of the considered factors affected root colonization by AMF; indeed, there were no significant differences between the inoculated plants and the controls in both type of soils independently of the strains isolation site (Table 3). The SO, PS, and SO×PS interaction had significant effects on water soluble C (WSC) and water soluble carbohydrates (WSCH), while total N and extractable K were only influenced by the PS (Table 3). Neither SO nor PS affected available P. The highest contents of total N, WSC, and WSCH were recorded at Calblanque. The levels of WSC were increased by the inoculation with native and allochthonous strains, although the highest increases were recorded at Calblanque with native *Streptomyces* sp. strains (about 48 %, compared to the corresponding control). However, the native and allochthonous strains produced similar increases in WSC at Rellano. In both soils, the inoculation of *Streptomyces* sp. strains native to each PS was effective regarding increases in WSCH (3-fold at Rellano and 77 % at Calblanque).

Soil biochemical and microbiological properties

Except for urease activity, the type of inoculated soil affected significantly all enzymatic activities, microbial biomass C,

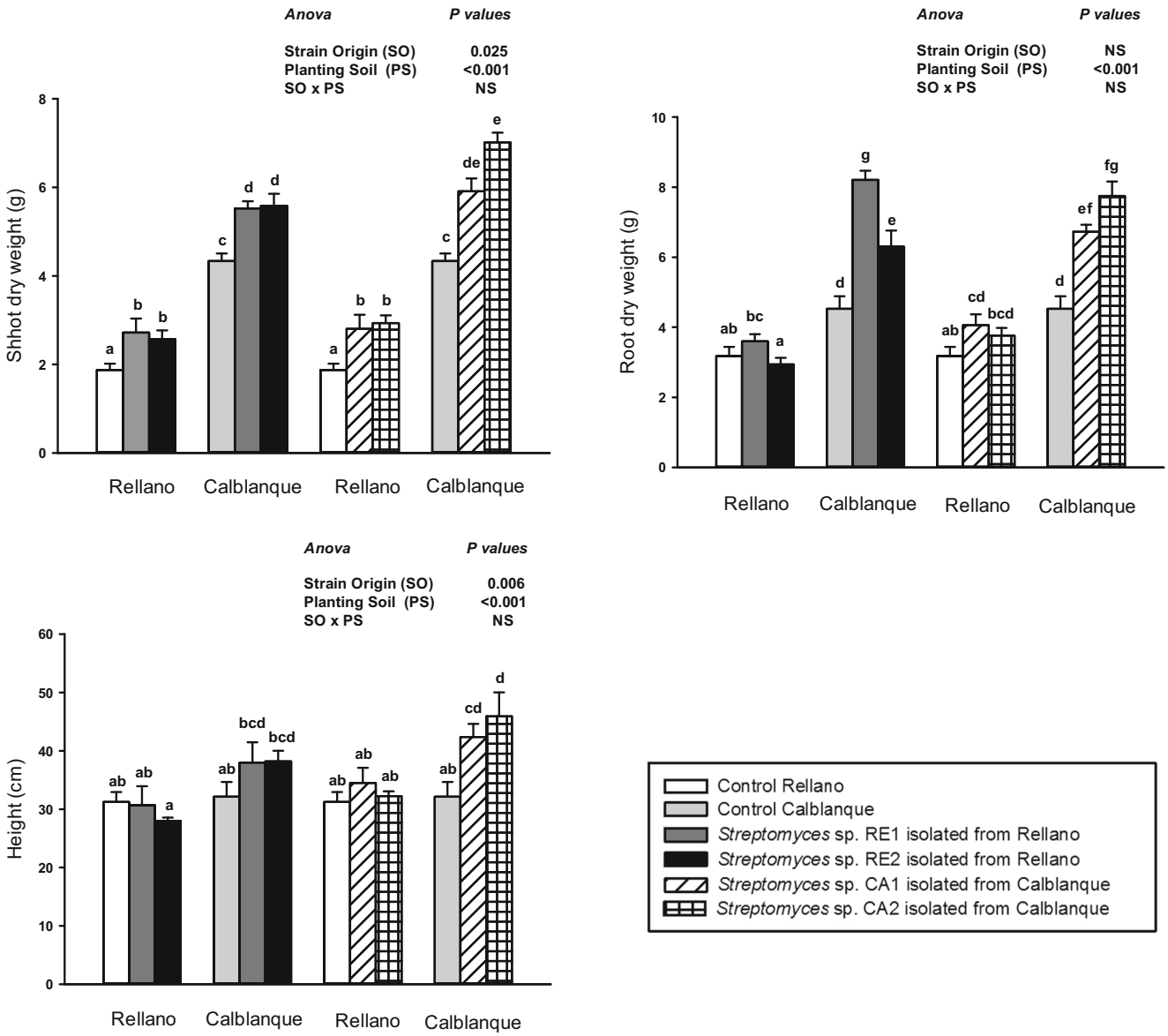

Fig. 1 Shoot and root biomass and height of *R. lycioides* seedlings in response to inoculation with immobilized *Streptomyces* sp. strains. Values are means of five replicates. Significant differences according to the Duncan test at $P < 0.05$ levels are indicated by different *letters*. Significance of effects of strain origin, planting soil, and their interaction on the measured variables is also shown. *NS* not significant

and soil basal respiration, the highest levels occurring at the Calblanque site (Table 4). The SO had a significant effect on all biochemical and microbiological parameters except protease and dehydrogenase activities and soil basal respiration. The Duncan test indicated that the highest increases in the urease, protease, and dehydrogenase activities and in microbial biomass C in response to the inoculations with actinobacteria were reached at the Rellano site (about 200, 28, 29, and 30 %, respectively, compared to their respective controls), regardless of the origin of the assayed strain (Table 4). The native and allochthonous strains produced similar increases in protease activity and microbial biomass C at Calblanque, while the allochthonous strains provoked a higher increase in such parameters at Rellano. The inoculation with native strains at Calblanque increased the urease and β-glucosidase activities to a greater extent than the inoculation with allochthonous strains. At Rellano, the actinobacteria increased the values of both of these biochemical parameters, compared to non-inoculated soil, although without significant differences between the native and allochthonous strains.

Discussion

The results of this study have revealed that inoculation with *Streptomyces* strains can be an effective tool for the revegetation of natural semiarid lands; this is a relevant result, bearing in mind that the PGPR character of actinobacteria had only

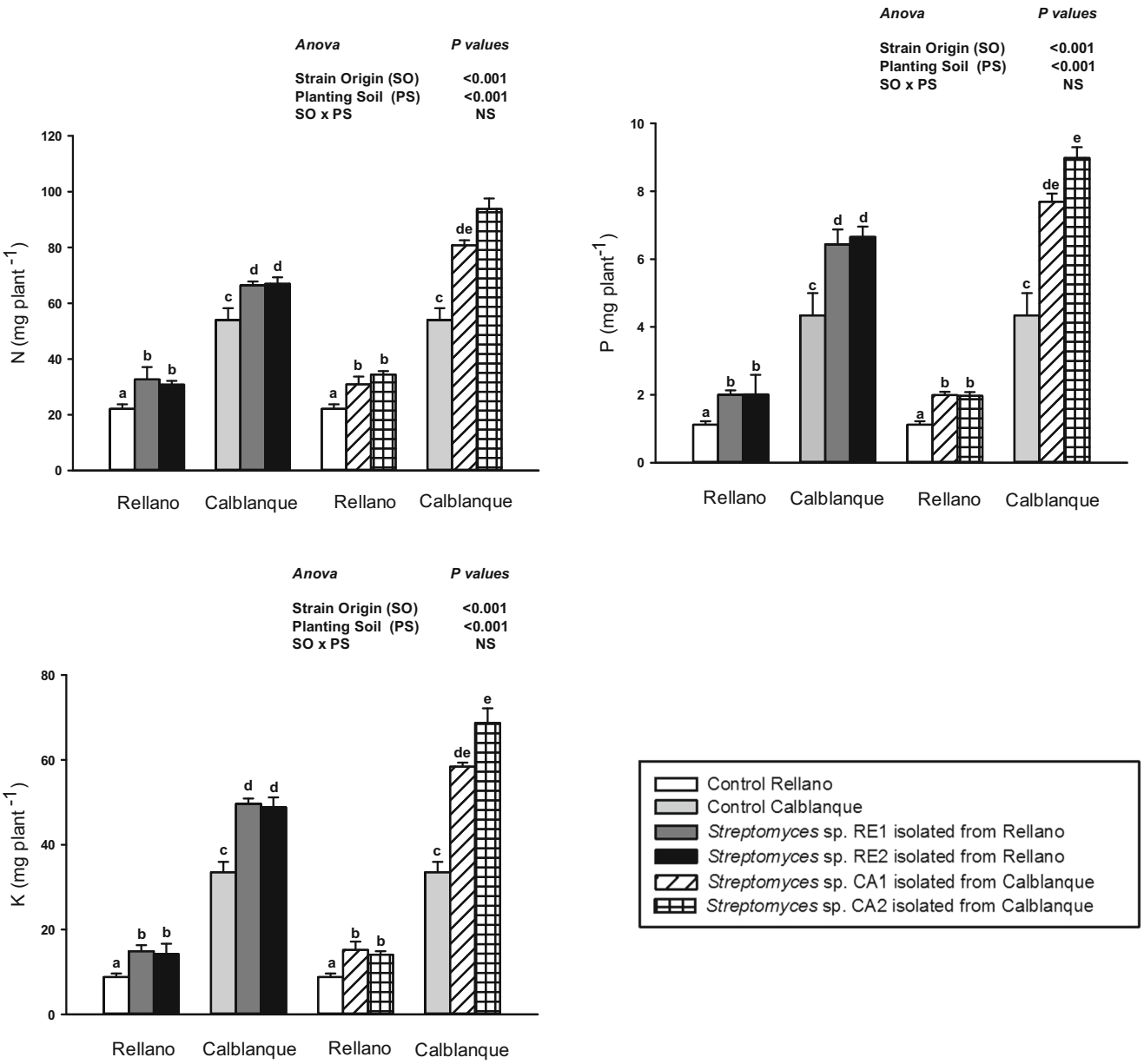

Fig. 2 Nutrient contents in shoot of *R. lycioides* seedlings in response to inoculation with immobilized *Streptomyces* sp. strains. Values are means of five replicates. Significant differences according to the Duncan test at *P* <0.05 levels are indicated by different *letters*. Significance of effects of strain origin, planting soil, and their interaction on the measured variables is also shown. *NS* not significant

been demonstrated previously in agricultural soils (Jog et al. 2014). We also found a number of different effects attributable to the experimental factors which deserve further explanation. The strains of actinobacteria differed in their ability to enhance nutrient uptake and growth of the *R. lycioides* seedlings, depending on the strain origin and planting site. It is known that some *Streptomyces* species can act as mycorrhiza helper bacteria, stimulating the mycorrhiza formation (Tarkka et al. 2015), but in our experiment the strains assayed did not have any effect on mycorrhizal root colonization. The importance of selecting suitable plant growth promoting microorganisms for successful biotechnological application in the field has been highlighted (Hrynkiewicz and Baum 2011). The use of native plant growth promoting microorganisms has been reported to be more effective (Ouahmane et al. 2007) or equally effective (Ortiz et al. 2014), in comparison to allochthonous strains. In our study, the efficacy of the actinobacterial strains native to a particular soil—as promoters of plant growth—was reliant on the fertility characteristics of the plantation site. Previous studies have shown the importance of soil characteristics such as OM content and soil structure in shaping the re-establishment of local microorganisms (Pereira e Silva et al. 2011; Lebron et al. 2012) even when the microorganisms originate from different soil sources (Nazir et al. 2013). In

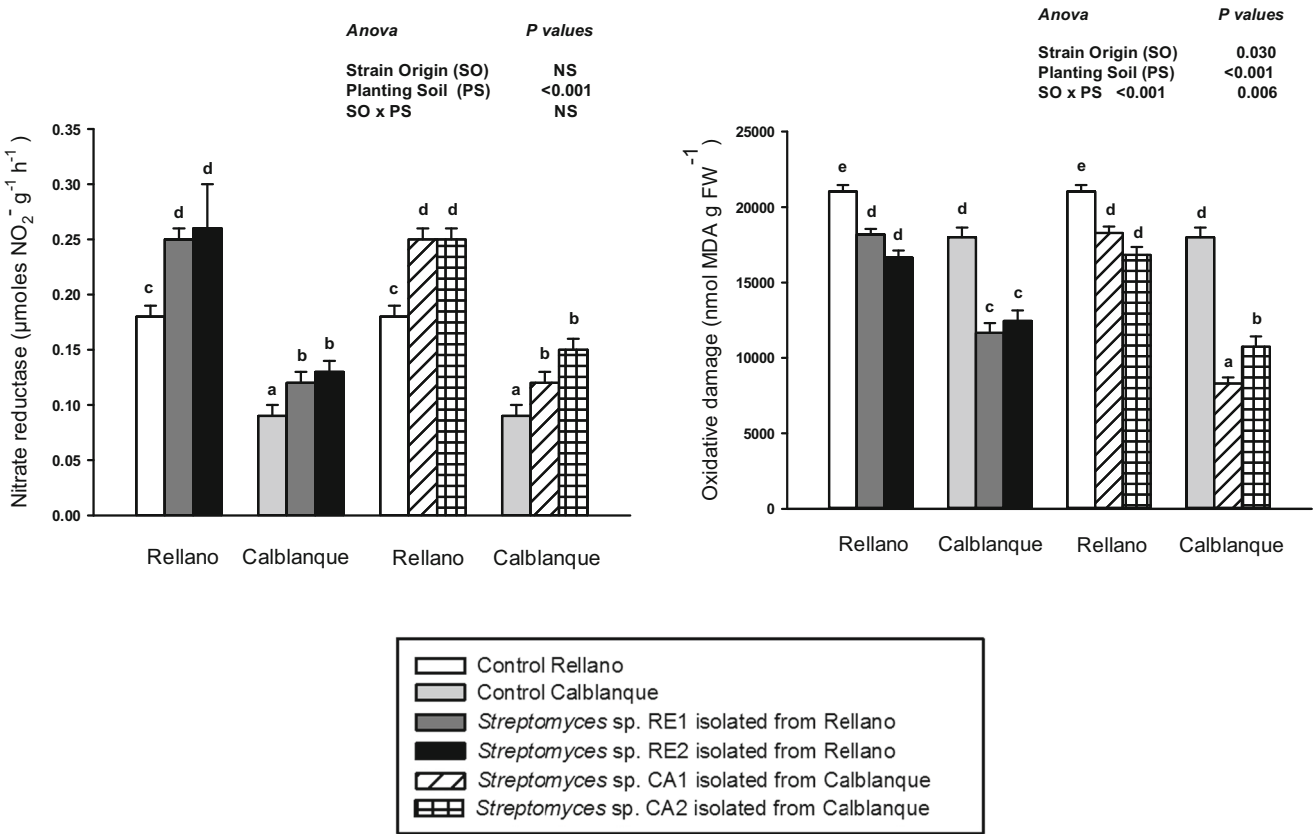

Fig. 3 Nitrate reductase activity and oxidative damage to lipids in leaves of *R. lycioides* seedlings in response to inoculation with immobilized *Streptomyces* sp. strains. Values are means of five replicates. Significant differences according to the Duncan test at $P < 0.05$ levels are indicated by different *letters*. Significance of effects of strain origin, planting soil, and their interaction on the measured variables is also shown. *NS* not significant

our study, the soils used for the revegetation experiment presented different levels of OM, microbiological activity, and structural stability. In the more fertile soil and with higher aggregate stability (Calblanque), the inoculation with native strains of *Streptomyces* sp. conferred a clear advantage, over inoculation with allochthonous strains, on *Rhamnus* growth. This result was expected as native strains of *Streptomyces* are presumably pre-adapted to the local conditions of the planting site and, probably, are more competitive colonizers of their original soil than allochthonous strains. Actinobacteria are ubiquitous inhabitants of soils, but they could show specificity with respect to the soil subjected to inoculation. Remarkably, in the less fertile soil and with lower structural stability (Rellano), *Rhamnus* shrubs inoculated with native strains had biomass yields comparable to those of shrubs inoculated with allochthonous strains. It is worth noting that the allochthonous strains, originating from a more fertile soil, were able to stimulate the growth of plants grown in a less fertile soil.

The characterization in vitro of the plant growth promotion abilities of the four strains revealed that they had similar effects with regard to solubilizing sparingly available inorganic P sources, producing siderophores, and fixing nitrogen. The ability of actinobacteria to synthesize siderophores might be especially important for the competitive abilities of rhizosphere microorganisms in soils with low nutrient concentrations (Franco-Correa et al. 2010). The increased nutrient uptake by both native- and allochthonous-inoculated plants, compared to their non-inoculated controls, indicates that the abilities of the actinobacteria manifested in vitro are preserved under field conditions. Release of low molecular mass organic acids by phosphate-solubilizing bacteria is a common mechanism to solubilize insoluble inorganic phosphates and make them available to plants, thus enhancing plant P uptake and growth (Kim et al. 1998). In this study, the organic acids produced by actinobacteria under environmental or in vitro conditions could have acted as chelating agent of mineral ions or decrease the pH to bring P into solution. In the more fertile soil, the greater improvement of shoot biomass in *R. lycioides* plants inoculated with the native *Streptomyces* strains than in the ones inoculated with non-native *Streptomyces* strains may have been partly due to differential enhancement of nutrients uptake.

Since the revegetation experiment was carried out in semiarid conditions, where water is by far the resource most limiting to plant growth, the increased shoot biomass of inoculated seedlings could also be related to the increase in the

Table 3 Root colonization and soil chemical properties in response to inoculation with immobilized *Streptomyces* sp. strains

Isolation site	Strain	Tested soil	AMF colonization (%)	Total N (g kg^{-1})	WSC (mg kg^{-1})	WSCH (mg kg^{-1})	Extractable K (mg kg^{-1})	Available P (mg kg^{-1})
Rellano	0	Rellano	53±8 a	1.2±0.0 a	29±1 a	5±0 a	179±4 c	5±1 a
	RE1		48±7 a	1.2±0.1 a	33±1 b	14±1 de	185±5 c	5±0 a
	RE2		61±12 a	1.1±0.0 a	32±1 b	12±1 d	187±2 c	5±0 a
	0	Calblanque	55±11 a	2.0±0.2 b	40±1 c	17±1 e	117±2 ab	6±0 a
	RE1		57±14 a	1.9±0.1 b	47±2 d	24±2 f	122±5 b	6±0 a
	RE2		46±8 a	1.8±0.1 b	45±2 d	28±1 fg	107±3 a	5±0 a
Calblanque	0	Rellano	48±8 a	1.2±0.0 a	29±1 a	5±0 a	179±4 c	5±1 a
	CA1		47±9 a	1.2±0.1 a	34±2 b	9±0 c	181±3 c	5±1 a
	CA2		55±9 a	1.3±0.1 a	34±2 b	8±1 b	187±4 c	5±0 a
	0	Calblanque	65±11 a	2.0±0.2 b	40±1 c	17±1 e	117±2 ab	6±0 a
	CA1		58±10 a	1.8±0.1 b	60±1 e	33±1 g	117±3 a	5±0 a
	CA2		66±12a	1.9±0.1 b	58±1 e	27±1 fg	111±6 a	5±0 a
ANOVA, *P* values								
Strain origin (SO)			NS	NS	<0.021	0.008	NS	NS
Planting soil (PS)			NS	<0.001	<0.001	<0.001	<0.001	NS
SO×PS			NS	NS	<0.001	<0.001	NS	NS

Mean±standard error (n=5). Significant differences according to the Duncan test at P<0.05 levels were indicated by different letters. Significance of effects of strain origin, soil type and their interaction on the measured variables is also shown

AMF arbuscular mycorrhizal fungi, *WSC* water soluble carbon, *WSCH* water soluble carbohydrates, *NS* not significant

resistance of plants to water stress induced by the actinobacterial strains. Nitrate reductase (NR) activity, which catalyzes the rate-limiting step in the nitrate assimilation pathway, has been proposed as a stress index since it is highly sensitive to the metabolic and physiological status of the plant (Ruiz-Lozano and Azcón 1996). In this study, we have found

Table 4 Soil biochemical and microbiological properties in response to inoculation with immobilized *Streptomyces* sp. strains

Isolation site	Strain	Tested soil	Phospho-monoesterase (μmol PNP g^{-1} h^{-1})	Urease (μmol N- NH_4^+ g^{-1} h^{-1})	Protease (μmol N- NH_4^+ g^{-1} h^{-1})	Dehydrogenase (μg INTF g^{-1})	β-Glucosidase (μmol PNP g^{-1} h^{-1})	Soil basal respiration (CO_2 h^{-1} kg^{-1})	Microbial biomass C (mg kg^{-1})
Rellano	0	Rellano	1.39±0.06 a	0.17±0.01 a	0.42±0.01 a	50.6±1.7 a	0.46±0.01 a	7.3±0.5 a	1045±48 a
	RE1		2.19±0.10 c	0.51±0.01 c	0.57±0.05 b	69.8±1.2 d	0.48±0.01 a	11.4±0.4 c	1202±41 b
	RE2		1.86±0.07 b	0.50±0.01 c	0.53±0.01 b	63.5±1.4 bc	0.48±0.00 a	12.0±0.1 cd	1344±61 c
	0	Calblanque	2.66±0.09 d	0.27±0.02 b	3.09±0.06 d	154.8±1.4 e	0.87±0.03 c	8.8±0.3 b	1635±39 de
	RE1		3.95±0.06 f	0.49±0.04 c	3.05±0.02 d	182.0±3.1 g	0.86±0.02 c	13.7±0.8 def	1761±47 ef
	RE2		3.78±0.09 f	0.47±0.02 c	3.00±0.03 d	173.3±1.2 fg	0.87±0.04 c	15.2±0.6 f	1943±49 g
Calblanque	0	Rellano	1.39±0.06 a	0.17±0.01 a	0.42±0.01 a	50.6±1.7 a	0.46±0.01 a	7.3±0.5 a	1045±48 a
	CA1		2.61±0.05 d	0.47±0.03 c	0.51±0.03 b	61.3±2.1 b	0.48±0.03 a	12.6±0.4 cde	1522±55 d
	CA2		2.83±0.06 d	0.55±0.02 cd	0.54±0.03 b	65.9±1.3 cd	0.55±0.03 b	13.4±0.3 def	1364±38 c
	0	Calblanque	2.66±0.09 d	0.27±0.02 b	3.09±0.06 d	154.8±1.4 e	0.87±0.03 c	8.8±0.3 b	1635±39 de
	CA1		3.96±0.12 f	0.61±0.02 d	3.22±0.07 d	177.1±2.4 fg	1.12±0.01 d	14.0±0.4 def	1881±48 fg
	CA2		3.66±0.06 e	0.54±0.02 cd	3.10±0.07 d	166.1±2.8 f	1.00±0.01 c	14.9±0.3 ef	1758±49ef
ANOVA, *P* values									
Strain origin (SO)			<0.001	0.004	NS	NS	<0.001	NS	0.049
Planting soil (PS)			<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001
SO×PS			<0.001	NS	NS	NS	0.045	NS	0.012

Mean±standard error (n=5). Significant differences according to the Duncan test at P <0.05 levels are indicated by different letters. Significance of effects of strain origin, soil type, and their interaction on the measured variables is also shown

NS not significant

that the inoculation with actinobacteria induced an increase in NR activity, regardless of strain origin. Improvements in plant drought tolerance induced by rhizobacteria other than actinobacteria have been previously recorded (de-Bashan et al. 2012; Mengual et al. 2014). The oxidation of membrane lipids is a reliable indication of oxidative stress (Porcel et al. 2004). In the shoot, lipid peroxidation was decreased in the *Streptomyces*-inoculated plants, compared to non-inoculated plants, which could be explained partially if the former were submitted to less oxidative stress under field conditions. In the more fertile soil, the plants inoculated with the native strains displayed oxidative stress to a lesser extent than the plants inoculated with the allochthonous strains. This could also have contributed to the superior performance of plants inoculated with native *Streptomyces* under semiarid field conditions.

The effect of inoculants on microbial activity in the rhizosphere is decisive for maximizing plant nutrient availability since the soil microbial community in the rhizosphere plays a key role in plant nutrition and thus in plant growth. A direct measurement of the reactivation of microbial populations is the C-biomass. Also, certain C fractions, namely water soluble C and water soluble carbohydrates, are used as C and energy sources by soil-borne microflora (Ghani et al. 2003; Roldán et al. 2006). In this study, these fractions of C were enhanced to a greater extent by the inoculation with native *Streptomyces* strains at both planting sites. Enzyme activities are sufficiently sensitive to indicate changes in ecosystem function resulting from microbial inoculations (Naseby and Lynch 1997). Oxidoreductases, such as dehydrogenase, are involved in oxidative processes in soils, and their activity mainly depends on the metabolic state of soil biota; thus, they are considered as good indicators of the soil microbial activity (García et al. 1997). The increase in microbial activity was also reflected by the increase in dehydrogenase activity in the rhizosphere soil of inoculated plants. The measurement of hydrolase activities provides an early indication of changes in soil fertility since they are related to the mineralization of important nutrient elements required for both plant and microbial growth (Alguacil et al. 2005). The increases observed in the hydrolases alkaline phosphomonoesterase, urease, protease-BAA, and β-glucosidase activities may be related to shifts in the rhizosphere microbial population, as a consequence of the inoculation treatments with actinobacteria (Conn and Franco 2004; Trabelsi et al. 2011). It is worth noting that the greatest improvement in microbial activity in response to the inoculation with *Streptomyces* strains was recorded in the less fertile soil. The stimulation of microbial activity by the native and allochthonous isolates varied with both the planting soil and biochemical parameter. The highest increases in alkaline phosphomonoesterase activity were recorded in the rhizosphere soil of plants inoculated with allochthonous *Streptomyces* sp. strains and grown in the less fertile soil, which may indicate direct bacterial secretion of this enzyme. It has previously been reported that bacteria can mineralize organic P through the action of phosphomonoesterase enzymes (Abd-Alla 1994).

In conclusion, the actinobacterial strains were able to promote the establishment of *R. lycioides* in soils different from that of their isolation source, indicating that their plant growth promoting abilities are preserved under different field conditions. The efficacy of native strains, with respect to allochthonous strains, was conditioned by the characteristics of the soil subjected to revegetation. For the more fertile soil, the high growth rate of shrubs inoculated with native *Streptomyces* was attributable mostly to a direct nutritional enhancement mediated by the inoculum, as well as to a concomitant improvement in plant drought tolerance. In the less fertile soil, the superior increases in soil microbial functionality suggest that the proliferation of introduced and/or native microflora could also have contributed to the improvement in plant growth, but the character (native or non-native) of the strains was not a key factor for plant establishment. The strain origin and biological fertility of the plantation site should be considered in the selection of actinobacterial strains for use in the revegetation with shrub species in semiarid environments.

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