Science of the Total Environment 466–467 (2014) 67–73

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Combined effects of clay immobilized *Azospirillum brasilense* and *Pantoea dispersa* and organic olive residue on plant performance and soil properties in the revegetation of a semiarid area

Mauricio Schoebitz *, Carmen Mengual, Antonio Roldán

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

HIGHLIGHTS

- Nutrient uptake is a representative parameter of PGPR immobilized effectiveness.
- Immobilized PGPR can be used as a partial substitute for chemical fertilization.
- PGPR and amendments to aid in the restoration of plant cover and soil quality

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 19 June 2013
Accepted 3 July 2013
Available online 27 July 2013

Editor: Charlotte Poschenrieder

Keywords:
Rhizobacteria
PGPR/PGPB
Microbial inoculant
Nutrient uptake
Enzyme activities
Soil restoration

ABSTRACT

The reestablishment of autochthonous shrubs species is an essential strategy for recovering degraded soils under semiarid Mediterranean areas. A field experiment was carried out to assess the effectiveness of an immobilized microbial inoculant (*Azospirillum brasilense* and *Pantoea dispersa*) and the addition of organic olive residue (alperujo), for plant growth promotion of *Cistus albidus* L. and enhancement of soil properties. Sixteen months after planting, the microbial inoculant and organic residue combined treatment was the most effective for stimulating the root dry weight of *C. albidus* (by 133% with respect to control plants) and microbial inoculant was the most effective treatment for increasing the shoot dry weight of plants (by 106% with respect to control plants). Available phosphorus and potassium content in the amended soils was about 100 and 70% respectively higher than the non-amended soil. Total C, total organic C and microbial biomass C content and enzyme activities (dehydrogenase, urease and protease) of the rhizosphere of *C. albidus* were increased by microbial inoculant and organic residue combined, but not by the microbial inoculation and organic residue applied independently. The combined treatment, involving microbial inoculant and the addition of the organic residue, had an additive effect improving the biochemical and microbiological quality of the soil.

1. Introduction

Establishment of native plant species is a widely used practice for reclaiming degraded lands and constitutes the most effective strategy in semiarid Mediterranean areas (Alguacil et al., 2003) and other arid ecosystems (de-Bashan et al., 2012). Shrub species such as *Cistus albidus* L. associated with other small woody plants are indigenous of the plant communities in these semiarid ecosystems. In semiarid Mediterranean areas, the establishment of shrubs is made difficult by low soil fertility and the severe climate, characterized by low precipitation and frequent drought periods. Therefore, it is necessary to apply methods that improve soil quality and the ability of the seedlings to resist semiarid environmental conditions (Caravaca et al., 2005).

The term rhizosphere was coined by Hiltner (1904) and describes the volume of soil affected by plant roots (Hartmann et al., 2008). Soil microbial populations undergo a proliferation, process known as rhizosphere effect, induced by the roots, due to the excretion of organic compounds in several forms (Benizri et al., 2002). Among all the components of soil microbiota, rhizobacteria are of great interest. They are free-living bacteria often labeled as plant growth-promoting rhizobacteria (PGPR), which can colonize the surface or intercellular spaces of the host plant roots, frequently improving root system establishment. In this regard PGPR has a potential role in establishment of plant species in arid environmental conditions (Puente et al., 2004), where these mechanisms lead to plant growth promotion of diverse nature such as non-symbiotic nitrogen fixation, phosphate solubilization and production of various phytohormones improving root growth, water absorption and nutrients uptake (Bashan et al., 2004). Nevertheless, colonization around plants roots by direct

* Corresponding author. Tel.: +34 968 396337; fax: +34 968 396200.
E-mail address: mschoebitz@gmail.com (M. Schoebitz).

0048-9697/$ – see front matter
http://dx.doi.org/10.1016/j.scitotenv.2013.07.012

inoculation of free PGPR cells into soil is not easy because this process is very susceptible to environmental variations such as UV radiation, temperature fluctuation, protozoa depredation and salt stress (Wu et al., 2012). This unpredictability of the PGPR inoculation success on plants is mainly due to the quality in the inoculants formulations containing an effective bacterial strain and determines the success or failure of a plant growth promotion.

Immobilization of microbial inoculants has been used to improve their effectiveness by supplying nutrients, protection from desiccation and slow cells release (Bashan, 1998; Kim et al., 2012). The success of using microbial inoculants introduced into soil requires the survival of adequate numbers of bacteria reaching suitable habitats where they can stay alive (Heijnen and Van Veen, 1991). The principle of immobilization of rhizobacteria is to protect the microorganisms (Schoebitz et al., 2012, 2013) and to ensure a gradual and prolonged release into the soil (Bashan et al., 2002; Wu et al., 2011). The use of clay in inoculant formulation can increase cell survival in the soil, due to the formation of a protective clay envelope around rhizobacteria which modifies the rates of water flow into and out of the cells during drying and rewetting (Cassidy et al., 1996).

Several studies about the application of organic amendments, for example, alperujo (organic olive residue) (Kohler et al., 2008), urban refuse (Alguacil et al., 2009), sugar beet residue (Caravaca et al., 2005) and leftover material from wastewater treatment (Trejo et al., 2012) have reported a beneficial effect on semiarid and arid soils, increasing the proliferation and development of natural populations of soil microorganisms and improving soil properties. In addition, it is known that immobilized rhizobia and rhizobacteria play a very important role in growth promotion of plants in crop systems (Vassileva et al., 1999; Albareda et al., 2008; Wu et al., 2012). Recent studies have also reported that the inoculation with rhizobacteria and organic residue addition can improve the revegetation with cacti and leguminous trees in the Sonoran Desert (Bashan et al., 2009, 2012). However, to the best of our knowledge, no studies have been reported on the effects of the inoculation of clay immobilized rhizobacteria and organic olive residues evaluated to facilitate the revegetation in a degraded Mediterranean area. We hypothesize that the combined effect of clay immobilized rhizobacteria and organic addition can improve both the plant performance and soil properties in the revegetation of a degraded area. The objectives of this study were to assess the effect of these management techniques on *C. albidus* growth and nutrient uptake and to evaluate the influence of the rhizosphere effect on the physicochemical and biological soil properties.

2. Materials and Methods

2.1. Study Site

The experimental area was located in Vicente Blanes Ecological Park in Molina de Segura, Province of Murcia, Spain (Lat. 38° 12′ N, Long. 1° 13′ W, Elev. 392 m). The climate is semiarid Mediterranean, with an average annual rainfall about 300 mm, and a potential evapotranspiration reaching out to 1000 mm per year. The mean annual temperature is 17.5 °C with no frost period. The soil in the experimental area is a Typic Torriorthent (Soil-Survey-Staff, 2006), and very little developed with low organic matter content and a silty loam texture (Table 1) that facilitates the degradation of soil structure. The vegetation in the zone was dominated by the invasive grass *Piptatherum miliaceum* L. Cosson and some native shrubs of *Thymus vulgaris* L., *Pistacia lentiscus L., Cistus clusii* Dunal and *Rosmarinus officinalis* L.

2.2. Plants

The target plant species used in our experiment (*Cistus albidus* L.) is a representative autochthonous shrub from semiarid shrublands in southeast Spain, well adapted to water stress conditions and, therefore, frequently used in the revegetation of semiarid disturbed lands (Alguacil et al., 2011). The initial biomass characteristics of the *C. albidus* plants used were 2.11 g (shoot dry weight); 1.23 g (root dry weight); 2.80 mm (basal stem diameter) and 31 cm (height of plants). The initial foliar concentrations were: total C, 438.08; N, 11.87; P, 0.78 and K, 7.23 (values are expressed in mg g plant^{-1}).

Table 1
Physico-chemical, biochemical and microbiological characteristics of the soil used in the experiment.

pH (H_2O)	8.5 ± 0.02[a]
Electrical conductivity (1:5, μS cm^{-1})	176.1 ± 2.55
Texture	Silty loam
Total C (g kg^{-1})	98.5 ± 1.54
Total organic C (g kg^{-1})	18.3 ± 5.3
Water soluble C (mg kg^{-1})	76.6 ± 2.58
Total carbohydrates (μg g^{-1})	2254 ± 235
Water soluble carbohydrates (μg g^{-1})	10.86 ± 0.59
Microbial biomass C (mg kg^{-1})	627.1 ± 31.2
Total N (g kg^{-1})	1.62 ± 0.03
Available P (mg kg^{-1})	4.85 ± 0.13
Extractable K (mg kg^{-1})	350.1 ± 3.1
Dehydrogenase (mg INTF g^{-1})	101 ± 16
Urease (μmol NH_3 g^{-1} h^{-1})	0.5 ± 0.2
Protease-BAA (μmol NH_3 g^{-1} h^{-1})	1.3 ± 0.3
Phosphatase (μmol PNP g^{-1} h^{-1})	19.8 ± 2.3
β-Glucosidase (μmol PNP g^{-1} h^{-1})	0.4 ± 0.1
Glomalin-related soil protein (μg g^{-1})	493 ± 35
Aggregate stability (%)	43.0 ± 1.01

[a] Mean ± standard error (n = 5).

2.3. Microbial inoculants and organic residues

The microbial inoculant used for replanting was developed by Probelte, S.A., Murcia. This inoculant contained two beneficial rhizobacterias identified as *Azospirillum brasilense* and *Pantoea dispersa* immobilized on clay pellets to allow bacterial survival for longer periods of time. These strains were deposited in the Spanish Type Culture Collection (CECT) with the numbers CECT-5801 (*P. dispersa*) and CECT-5802 (*A. brasilense)*. The cells concentrations of both rhizobacteria present in the inoculant were 10^9 CFU g^{-1}. The amendment used was the organic fraction extracted with KOH from composted "alperujo". The raw material was collected from an olive-mill and mixed with fresh cow bedding as bulking agent for composting (Kohler et al., 2008). The analytical characteristics of the organic residue are shown in Table 2.

Table 2
Chemical characteristics of the olive organic residue "alperujo" used in the experiment.

pH (H_2O)	8.8
EC[1] (1:5, μS m^{-1})	6.12
OM[2] (%)	73.1
Lignin (%)	38.7
TOC[3] (%)	43.9
Total carbohydrates (%)	0.81
Total N (%)	3.2
N-NH_4^+(mg kg^{-1})	119
N-NO_3^-(mg kg^{-1})	31
C/N	13.8
C_{OH} (%)	2.3
N_{OH} (%)	2.6
C_{OH}/ N_{OH}	8,8
K (%)	4.3
Ca (%)	2.97
Mg (%)	0.57

Data expressed on dry matter. EC[1]: electrical conductivity; OM[2]: organic matter; TOC[3]: total organic carbon.

2.4. Experimental Design

A complete aleatorized factorial was established with two factors and five-fold replication in a split plot design The first factor was the inoculation of *C. albidus* seedlings with microbial inoculant (*A. brasilense* and *P. dispersa*) and the second was the addition of olive residue into the soil. The experimental design was performed as follows: treatment 1, *C. albidus* without rhizobacteria treatment and soil without organic residue addition (Control); treatment 2, *C. albidus* inoculated with microbial inoculant and soil without organic residue addition (MI); treatment 3, *C. albidus* without microbial inoculant treatment and soil with organic residue addition (OR) and treatment 4, *C. albidus* inoculated with microbial inoculant and soil with organic olive residue addition (MI + OR). Planting holes 15 × 15 cm wide and 15 cm deep were dug manually. In early February 2011, microbial inoculant was applied at a rate of 30 g per plant. The same amount of sterilized inoculant was applied to the non-inoculated plants. Organic olive residue was added to the holes (0 ± 15 cm depth) corresponding with a rate of 2% by weight (186 g of organic olive residue per plant). Microbial inoculant and organic olive residue were manually mixed into 2 kg of soil in plastic bags an introduced in the plantation holes. The seedlings were planted at least 1 m apart between holes, with 3 m between treatment levels in five 40 m^2 replicated plots. At least 10 seedlings per treatment level were planted.

2.5. Sampling Procedures

Sixteen months after planting, soil samples of each treatment and replicate were collected. Twenty rhizosphere soil samples were collected at 0–15 cm depth from planting holes. The same number of bulk soil samples was simultaneously taken from outside the canopy of the seedlings (at a distance of 20 cm from the planting holes). The sampling was carried out in early June 2012. At the same time, five plants per treatment were harvested.

2.6. Plant Analyses

To evaluate the response to rhizobacteria inoculation and organic residues application the following growth parameters were evaluated: dry weights of shoots and roots (70 °C, 48 h), basal stem diameter and plant height were recorded before chemical analysis. The foliar concentrations of C, N, P and K were determined by ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). Proline accumulation was determined by the method described by Paquin and Lechasseur (1979). Proline was determined after extraction with sulphosalicylic acid and reaction with ninhydrin. A standard curve of L-proline (PRO) was used for calibration. Free proline was extracted from 0.5 g of fresh leaves. The methanolic phase was used for the quantification of proline. Proline was estimated by spectrophotometric analysis at 515 nm of the ninhydrin reaction, according to Bates et al. (1973).

2.7. Soil Physical, Chemical, and Biochemical Analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. Total nitrogen (N), total carbon (C), total organic C, available phosphorus (P) and extractable potassium (K) were determined by ICP-OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). Water-soluble carbohydrates and total carbohydrates were determined by the method of Brink et al. (1960). Soil respiration was calculated as the amount of CO_2 emitted during a 24 h incubation period: 10 g of dry soil were placed in a incubation vessel, the moisture was adjusted to 45% of water-holding capacity and a vial containing 2 mL of KOH (0.1 g of KOH in 50 mL distilled water) was placed inside the incubation vessel for retention of the evolved CO_2. Soil microbial biomass C was evaluated by the Substrate Induced Respiration (SIR) method following addition of glucose to soil. The transformation of the amount of CO_2 emitted to microbial biomass C was done with the equation developed by Anderson and Domsch (1978). Soil respiration and soil microbial biomass C were determined with an automatic analyzer (μ-TRAC 4200, SY-LAB). Glomalin related soil protein (GRSP) was determined in the easily extractable glomalin form according to Wright and Anderson (2000). It was extracted from soil samples sieved between 0.2 and 4 mm with 20 mM sodium citrate (Panreac) (pH 7.0) at a rate of 250 mg of aggregates in 2 mL of buffer and autoclaving at 121 °C for 30 min. The supernatant was removed and two additional sequential 1-h extractions were performed. All supernatants from a sample were combined, the volume was measured, an aliquot was centrifuged at 10,000 *g* for 15 min to remove soil particles and Bradford-reactive (Bio Rad) total protein was measured.

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-iod-ophenyl-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. Dehydrogenase activity was defined as: μg g^{-1} INTF.

Urease and N-a-benzoyl-L-arginine amide (BAA) hydrolysing protease 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 activities were determined as the NH_4^+ released in the hydrolysis reaction (Nannipieri et al., 1980). Urease and protease activities were defined as: μmol NH_3 g^{-1} h^{-1}.

Alkaline phosphatase activity was determined using p-nitrophenyl phosphate disodium (PNPP 0.115 M) as substrate. For the assay, 2 mL of 0.5 M sodium acetate buffer adjusted to pH 11 using acetic acid (Naseby and Lynch, 1997) 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 centrifuged at 4000 rev min^{-1} for 5 min. The p-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremmer, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH. Phosphatase activity was defined as: μmol PNF g^{-1} h^{-1}.

β-glucosidase was determined using 0.05 M p-nitrophenyl-b-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 by spectrophotometry at 398 nm (Tabatabai and Bremmer, 1969). β-glucosidase activity was defined as: μmol PNF g^{-1} h^{-1}.

2.8. Statistical Analysis

Values were log-transformed to compensate for variance heterogeneity. The effects on plants and soil of organic residue and microbial inoculation and their interactions were analyzed by a two-way ANOVA and post-hoc mean separation was performed by Duncan's multiple range test at $P \leq 0.05$ by using the software SPSS version 19.0.

3. Results

3.1. Effects of the Inoculation with Microbial Inoculant and Organic Residue on Growth of C. albidus

Dry weights of roots and shoots, basal stem diameter and plant height were evaluated. The inoculation with the microbial inoculant

Table 3
Effect of inoculation with immobilized rhizobacteria and addition of olive organic residue on *C. albidus* growth parameters.

Treatments	Shoot (g dw)[a]	Root (g dw)	Root/shoot ratio	BSD[b] (mm)	Plant height (cm)
Control	6.3 ± 0.71 a	2.1 ± 0.35 a	0.33 ± 0.03 a	4.8 ± 0.10 a	51.2 ± 1.83 a
Microbial inoculant (MI)	13.0 ± 0.63 c	3.0 ± 0.25 a	0.23 ± 0.02 a	5.4 ± 0.25 a	51.8 ± 0.73 a
Organic residue (OR)	8.9 ± 0.29 b	2.4 ± 0.30 a	0.26 ± 0.02 a	5.4 ± 0.30 a	50.4 ± 2.94 a
MI + OR	9.7 ± 0.82 b	4.9 ± 0.35 b	0.50 ± 0.03 b	5.1 ± 0.31 a	53.0 ± 2.49 a

[a] Grams dry weight; [b]BSD basal stem diameter. Values are means of five replicates.
Significant difference according to the Duncan test at $p < 0.05$ levels were indicated by different letters.

promoted an increase in shoots dry weight higher than 100% (Table 3) and the MI + OR treatment increased the roots dry weight and the root/shoot ratio compared with control plants (133% and 51% respectively). The experimental factors tested did not significantly affect the basal stem diameter and plant height.

3.2. Nutrients Uptake

Experimental treatments did not significantly affect the total C and N shoot content (Table 4). However, the total P concentration in the plants was significantly improved by the addition of the organic amendment (around 100%) and microbial inoculation (68%). The combination of these two factors increased P content only by 34%. The total K concentration in shoots was increased significantly by the microbial inoculant (40%) and the addition of the organic residues also showed a significant increase (68% greater than control plants). For K and P plant uptake, a negative interaction between the two factors considered was recorded (Table S1).

The comparative effects of microbial inoculant, organic residue and MI + OR treatment regarding accumulation of proline, demonstrated that this osmoregulatory compound was accumulated to a greater extent in control plants, microbial inoculant and organic residue treatment (Table 4) Otherwise, the combined treatment of microbial inoculant and organic residue showed a statistically significant decrease of around 30% in the shoot proline content.

3.3. Soil Physicochemical and biochemical analyses

Microbial inoculant and organic residue treatments significantly increased pH values in the rhizosphere soil. The microbial inoculant and MI + OR treatments also significantly increased the electrical conductivity in the soil, this increase being more pronounced in bulk soil than in rhizosphere soil of *C. albidus*. The MI + OR treatment significantly increased the total C in the rhizosphere soil and the total organic C in both rhizosphere and bulk soil. A significant decrease was found for total C, total organic C and total N in rhizosphere soil, for microbial inoculant respect to the control. Besides, a significant decrease was observed in total organic C and total N in bulk soil (Table 5).

The addition of the amendment and MI + OR treatment significantly increased the available P in rhizosphere soil. The available P content levels in rhizosphere soil mediated by the microbial inoculant, organic amendment and their combination were about 35, 43 and 47% higher than in controls. Regarding the factorial analysis the organic residue addition, soil sampling position and MI + OR + S interactions were significant (Table S2). Extractable K content in rhizosphere soil showed a statistically significant difference (<0.05) twofold increase for the microbial inoculant and organic residue and approximately threefold increase for the MI + OR treatment. Extractable K in bulk soil significantly increased in organic amendment and MI + OR treatments (Table 5). Thus, the greatest increase in response to the inoculation, addition of the amendment, soil sampling position and their interactions (except OR + S) were observed in the soil extractable K content (Table S2).

The organic amendment increased the total carbohydrates values in the rhizosphere soil (Table 5). The MI + OR treatment significantly increased the total carbohydrates concentration of the rhizosphere soil. However, the microbial inoculant decreased significantly the total carbohydrates in bulk soil. No significant differences between microbial inoculant, organic residue and MI + OR treatment were found for water-soluble carbohydrates. Concerning the factorial analysis (Table S2), among all the interactions analyzed, microbial inoculant, organic amendment, soil sampling position and their interaction were significant for total carbohydrates contents (except MI + S and MI + OR + S interactions).

Microbial biomass C and glomalin-related protein were significantly increased in rhizosphere soil in comparison with bulk soil (Table 6). The MI + OR treatment significantly increased the microbial biomass of the rhizosphere and bulk soil. However, the microbial inoculant decreased significantly the microbial biomass in rhizosphere and bulk soil respect to control samples. The glomalin-related protein was increased significantly by the organic residue, about 80% and MI + OR treatment showed also a significant increase (100% greater than the control rhizosphere soil). Respect to soil respiration rates, a significant decrease mediated by the microbial inoculant treatment was found when compared to control rhizosphere soil, whereas in bulk soil the soil respiration rates significantly decreased with the microbial inoculant and MI + OR treatments.

The application of MI + OR treatment significantly increased enzyme activities in rhizosphere soil (urease, protease and dehydrogenase), but the microbial inoculant and organic residue independently failed to do that (Table 6). The greatest increase on enzyme activities was observed in rhizosphere soil in comparison to bulk soil; in this sense the soil factor was significant for enzyme activities (except β-glucosidase; Table S2).

4. Discussion

Information about the beneficial effects of rhizobacteria inoculation on the growth of plants is well documented under greenhouse and

Table 4
Nutrients uptake on *C. albidus* in response to immobilized rhizobacteria and addition of organic residue.

Treatments	Total C (mg g plant^{-1})	N (mg g plant^{-1})	P (mg g plant^{-1})	K (mg g plant^{-1})	Proline (μmol PRO g fw^{-1})
Control	473.0 ± 2.06 a	12.8 ± 0.23 a	0.75 ± 0.10 a	7.6 ± 1.04 a	8.14 ± 0.50 b
Microbial inoculant (MI)	472.4 ± 1.36 a	13.0 ± 0.66 a	1.26 ± 0.09 bc	10.7 ± 0.44 bc	8.36 ± 0.33 b
Organic residue (OR)	473.8 ± 0.85 a	13.5 ± 0.35 a	1.49 ± 0.10 c	12.8 ± 1.74 c	7.22 ± 0.53 b
MI + OR	471.4 ± 1.58 a	13.6 ± 0.79 a	1.01 ± 0.13 ab	8.7 ± 0.34 ab	5.87 ± 0.30 a

Values are means of five replicates. Significant difference according to the Duncan test at $p < 0.05$ levels were indicated by different letters.

Table 5
Changes in physicochemical properties, of rhizosphere soil and bulk soil of *C. albidus* in response to immobilized rhizobacteria and organic residue addition.

Treatments	Soil Type	Control	Microbial inoculant (MI)	Organic residue (OR)	MI + OR
pH (H_2O)	Rs[1]	8.4 ± 0.02 ab	8.5 ± 0.02 c	8.5 ± 0.04 c	8.4 ± 0.02 ab
	Bs[2]	8.3 ± 0.01 a	8.4 ± 0.01 ab	8.4 ± 0.02 ab	8.4 ± 0.03 ab
EC[3] (1:5; μS cm^{-1})	Rs	309 ± 5.7 a	346 ± 13.6 b	342 ± 19.0 ab	435 ± 16.0 c
	Bs	405 ± 17.1 c	330 ± 7.6 ab	358 ± 9.6 b	560 ± 10.7 d
Total C (g kg^{-1})	Rs	101 ± 1.2 b	94 ± 0.8 a	102 ± 0.7 b	108 ± 2.5 c
	Bs	98 ± 0.7 ab	95 ± 1.1 a	97 ± 1.3 ab	99 ± 1.2 b
TOC[4] (g kg^{-1})	Rs	14.4 ± 0.65 cd	12.1 ± 0.63 b	13.9 ± 0.44 c	19.3 ± 0.48 e
	Bs	13.4 ± 0.37 bc	10.1 ± 0.44 a	13.3 ± 0.60 bc	15.9 ± 0.69 d
Total N (g kg^{-1})	Rs	2.1 ± 0.07 cd	1.1 ± 0.10 a	2.3 ± 0.08 d	2.4 ± 0.11 d
	Bs	2.2 ± 0.11 cd	1.7 ± 0.08 b	2.1 ± 0.04 cd	1.9 ± 0.10 c
P available (mg kg^{-1})	Rs	13.2 ± 1.67 bc	17.9 ± 1.54 cde	18.9 ± 1.19 de	19.4 ± 1.62 e
	Bs	10.2 ± 1.23 ab	8.2 ± 0.87 a	12.4 ± 1.69 b	14.2 ± 1.91 bcd
K extractable (mg kg^{-1})	Rs	310 ± 14.7 a	618 ± 29.4 c	662 ± 14.6 c	840 ± 34.0 d
	Bs	292 ± 17.0 a	294 ± 13.8 a	469 ± 12.7 b	466 ± 18.3 b
Total CH[5] (μg g^{-1})	Rs	2025 ± 66 b	2057 ± 158 b	2357 ± 129 c	2763 ± 75 d
	Bs	2149 ± 69 bc	1531 ± 61 a	2206 ± 76 bc	2376 ± 74 c
Water-soluble CH[5] (μg g^{-1})	Rs	10.7 ± 1.40 a	12.8 ± 1.36 a	9.9 ± 0.71 a	11.9 ± 1.41 a
	Bs	10.7 ± 1.23 a	13.6 ± 2.67 a	13.6 ± 2.49 a	16.3 ± 2.94 a

Rs[1]: Rhizosphere soil; Bs[2]: Bulk soil; EC[3]: electrical conductivity; TOC[4]: total organic carbon; CH[5]: carbohydrates;. Values are means of five replicates. Mean ± standard error. For each species, values in columns followed by the same letter do not differ significantly ($p < 0.05$) as determined by Duncan test.

laboratory conditions (Vessey, 2003; Bashan et al., 2004; Schoebitz et al., 2009). Nevertheless, reports of rhizobacteria inoculation and/or organic residues addition evaluated in field experiments are scarce, even more in non-agricultural systems (Bashan et al., 1999, 2009, 2012; Carrillo-Garcia et al., 2000). Sixteen months was considered as an enough period of time (one drought period and two growth periods) to ascertain the combined effects of immobilized rhizobacteria and organic amendment on plant performance and soil properties in the revegetation of a degraded area. Plants showed different levels of response to the microbial inoculant and the addition of the organic residue. Thus, it was observed an increase in shoot biomass when microbial inoculant was applied. It is well documented that PGPR exert a beneficial effect on plant growth and development, and many different rhizobacteria have been commercialized for using in agriculture (Bashan et al., 2004; Adesemoye et al., 2009; Adesemoye and Kloepper, 2009). Assuming that root/shoot ratio could reflect the degree of microorganism effectiveness (Tobar et al., 1999), *C. albidus* did not respond to inoculation and organic amendment applied independently. MI + OR was the treatment with the greatest response, reaching the most significant increases in root/shoot ratio. Decreased root/shoot ratio in the inoculated treatment with respect to control plants indicates low microorganisms activity in relation to root biomass production. And in this sense we can partially conclude that the combined treatment was the most effective for improving seedling performance. However, root biomass and nutrient uptake, as affected by microbial inoculant, must all be considered together.

Immobilized microbial inoculants have solved many problems associated with traditional liquid inoculants finding numerous advantages such as increasing cell survival and controlling the release of rhizobacteria into the soil, as well as protecting the microorganisms against drought stress, especially in semiarid regions. Our results are in agreement with those found by other authors, who reported that the use of immobilized *A. brasilense* and *P. dispersa* had a pronounced beneficial effect on pepper biomass and N uptake in greenhouse conditions (Flores et al., 2010). Nevertheless, in our revegetation experiment with *C. albidus* seedlings, the microbial inoculants, organic residue and MI + OR did not increase the total N, but appeared effective for improving the uptake of other nutrients such as P and K, which might explain why the microbial inoculant yielded the highest shoot biomass of *C. albidus*. Higher P and K uptake may be attributable to the mobilization of nutrients from soil because of the secretion of organic acids mediated by rhizobacteria (Basak and Biswas, 2010) and also to the addition of the organic olive residues due to the P and K amounts incorporated with the amendment, in comparison with previous field experiment in a degraded semiarid Mediterranean area adding composted urban residue to soil, Caravaca et al. (2003) found an increase of plant

Table 6
Changes on biological properties and enzymatic activities in rizhosphere soil and bulk soil of *C. albidus* in response to inoculation with immobilized rhizobacteria and addition of organic residue.

Treatments	Soil Type	Control	Microbial inoculant (MI)	Organic residue (OR)	MI + OR
Urease (μmol NH_3 g^{-1} h^{-1})	Rs[1]	0.8 ± 0.09[3] bc	0.6 ± 0.08ab	1.0 ± 0.09c	1.4 ± 0.20d
	Bs[2]	0.5 ± 0.09 a	0.3 ± 0.08 a	0.5 ± 0.08 ab	0.6 ± 0.09 ab
β-glucosidase (μmol PNF g^{-1} h^{-1})	Rs	0.4 ± 0.02 abc	0.3 ± 0.03 a	0.4 ± 0.02 abc	0.4 ± 0.07 c
	Bs	0.4 ± 0.03 abc	0.3 ± 0.03 ab	0.4 ± 0.04 bc	0.4 ± 0.04 abc
Phosphatase (μmol PNF g^{-1} h^{-1})	Rs	2.1 ± 0.12 bc	1.7 ± 0.16 ab	2.3 ± 0.16 c	2.4 ± 0.11 c
	Bs	2.0 ± 0.10 abc	1.7 ± 0.11 a	1.9 ± 0.11 ab	1.7 ± 0.15 ab
Protease (μmol NH_3 g^{-1} h^{-1})	Rs	1.9 ± 0.08 bc	1.7 ± 0.14ab	2.2 ± 0.11 cd	2.5 ± 0.14 d
	Bs	1.3 ± 0.13a	1.2 ± 0.15a	1.6 ± 0.17ab	1.6 ± 0.17ab
Dehydrogenase (μg g^{-1} INTF)	Rs	102 ± 3.5 bc	103 ± 7.2 bc	110 ± 5.6 c	148 ± 8.0 d
	Bs	101 ± 7.3 bc	80 ± 4.3 a	86 ± 6.7 ab	106 ± 8.2 c
Microbial biomass C (mg kg^{-1})	Rs	1296 ± 30 d	670 ± 18 b	1201 ± 10 d	2080 ± 24 e
	Bs	662 ± 31 b	536 ± 22 a	756 ± 20 c	820 ± 32 c
Soil respiration (CO_2 h^{-1} kg^{-1})	Rs	11.1 ± 0.17 de	9.6 ± 0.75 bc	10.5 ± 0.19 cd	12.5 ± 0.52 e
	Bs	10.5 ± 0.53 cd	5.0 ± 0.29 a	10.4 ± 0.33 cd	8.7 ± 0.46 b
GRSP[3] (μg g^{-1})	Rs	487 ± 23.9 bc	447 ± 7.7 b	892 ± 29.4 f	995 ± 23.7 f
	Bs	510 ± 10.8 c	297 ± 20.0 a	660 ± 27.3 d	769 ± 7.8 e

Rs[1]: Rhizosphere soil; Bs[2]: Bulk soil. GRSP[3]: glomalin-related protein. Values are means of five replicates. Mean ± standard error, values in columns followed by the same letter does not differ significantly ($p < 0.05$) as determined by Duncan test.

nutrient content (NPK) 18 months after the organic residue addition. Rhizobacteria are rhizosphere competent bacteria that colonize plant roots; they are able to colonize all the ecological niches found on the rhizosphere (Antoun and Kloepper, 2001) and consequently can explore a wider range for nutrients mobilization. In this sense, total nutrient content can be taken as a representative parameter of rhizobacteria immobilized effectiveness. In addition it has been reported that rhizobacteria could help plants to compensate for deficiencies of immobile nutrients such as P and K, by the increase of root biomass due to the production of phytohormones which can mediate the extent of a greater root surface area for nutrients uptake (Bashan et al., 2004). It can be concluded that the activity of the inoculated bacteria and the fertilizers added with the olive residue can improve the nutrient uptake by the seedlings with the exception of the N.

Most plant species can accumulate proline, which plays a major role in the process of osmotic adjustment decreasing the cell osmotic potential, thus allowing higher water retention during drought (Medina et al., 2010). Our results suggest the role of the proline in the protection of plants against drought stress. Here, control plants and those grown with microbial inoculant and organic residue independently having high proline contents were also the most affected by drought. We observed that the MI + OR treatment presented lower proline content suggesting that this combined treatment was more effective to induce resistance to drought conditions in this semiarid area.

The rhizosphere effect is an area of intensive interactions between plant roots and soil. The rhizosphere effect is mainly based on the microbiota activity influenced by root growth (Berg and Smalla, 2009). Physicochemical and biological properties of the soil samples were measured and we observed that the rhizosphere effects did not influence the bulk soil in the majority of the evaluated soil properties. Nevertheless, we found that the combined treatment increased the electrical conductivity and organic C in bulk soil. Besides, the application of the organic residue, independently and combined, also increased extractable K, glomalin and biomass C.

In rhizosphere soil, supplemented with organic residue and microbial inoculant combined treatment, it was produced a significant increase in the amounts of total C, total organic C, total carbohydrates and glomalin, which can be used as carbon and energy sources for soil microflora. Wright and Anderson (2000) have indicated that arbuscular mycorrhizal fungi produce glomalin, a glycoprotein able to increase the hydrophobicity of soil particles and to promote soil aggregation. Total N levels in the rhizosphere were not increased by the treatments tested, even a significant decrease was observed in the microbial inoculant treatment and this may be explained through the assimilation of N by the soil microorganisms. In the treatments involving organic amendment it is assumed that the addition of the amendment mediates an input on N levels, even so the total N levels in soil were not modified. MI + OR treatment increased microbial biomass and in particular enhanced the protease and urease activity, which is involved in the N cycle and could reveal a shift in microbial populations mediated by an increase in N assimilation by the soil microorganisms.

Soil microorganisms are able to excrete organic acids and phosphatases, which increase the concentration of P in rhizosphere soil (Rodriguez et al., 2006; Vassilev et al., 2006). The application of the organic residue with microorganisms has been used for increasing soil available P and improvement of plant growth and nutrients uptake. Caravaca et al. (2005) demonstrated the effectiveness of these biosystems for increasing the growth and nutrient uptake of *C. albidus* using an amendment with sugar beet, phosphate rock and *Aspergillus niger*. In our experiment, available P in the rhizosphere was increased by the use of both organic residue and MI + OR treatments but not by the inoculant, assuming that the rhizobacteria inoculated was able to solubilize P from the organic residue applied and not from the soil.

All the treatments tested increased K contents in rhizosphere soil with regard to the control. Microbial inoculant and organic olive residue helped plants to compensate for deficiencies of immobile nutrients such as potassium, which may be attributed to the mobilization of K by the soil rhizobacteria and also by the input of K provided with the addition of organic residue. The inoculation of soil with immobilized rhizobacteria can be considered an effective tool for the development of biotechnologycal products than can be used as a partial substitute for chemical fertilization. In that way, introduction of a microbial inoculant can improve nutrients availability for plants and thereby to increase the efficiency of the applied manures (Adesemoye and Kloepper, 2009).

In this assay, the effect of the microbial inoculant combined with organic residue on soil properties might also be attributed to a greater promotion of biological activity in the rhizosphere soil of the *C. albidus* plants. In fact, microbial biomass C, glomalin, dehydrogenase, protease and urease activities were higher in the rhizosphere soil of MI + OR treatment in comparison to control plants and these parameters have frequently been used as indicators of soil microbial activity (Caravaca et al., 2002). Alguacil et al. (2003) also reported an increase in enzyme activities when measured in the rhizosphere soil of *C. albidus* after amendment with fermented sugar beet residue in a semiarid Mediterranean area. Enzyme activities are sufficiently sensitive to indicate changes caused by microbial inoculation (Naseby and Lynch, 1997). Furthermore, *A. brasilense* and *P. dispersa* in combination with organic amendment may release enzymes involved in the mineralization of organic matter. Thus, a positive correlation in MI + OR treatment has been reported between enzyme activity and microbial biomass C. However, we observed a significant decrease on microbial biomass C and soil respiration when the microbial inoculant treatment was applied independently, suggesting than the introduced microbiota presented a lower biological activity in comparison to the autochthonous soil microorganisms. In general, the combined treatment was the most effective for increasing microbial activities in the soil of our experiment.

In conclusion, the microbial inoculation of the seedlings with *A. brasilense* and *P. dispersa* immobilized in clay, increased the performance of *C. albidus*, even more when the microbial inoculation was combined with the addition of organic olive residue. When considering the improvement of soil quality, the combination of both treatments was the most effective, yielding a significant increase in C fractions, microbial biomass and enzyme activities, but microbial inoculation and organic residue applied independently failed to restore soil properties. Therefore, the application of combined treatments involving immobilized PGPR inoculation and organic amendments appear to be the most suitable tool to aid in the restoration of both plant cover and soil quality in semiarid degraded lands.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.07.012.

Acknowledgements

This research was supported by “Plan Nacional” Spain (Project numbers AGL2009-12530-C02-01 and AGL2012-39057-CO2-01). C. Mengual was supported by the “Formación de Personal Investigador” programme (Ministerio de Economía y Competitividad, Spain). M. Schoebitz would like to thank the National Commission for Scientific and Technological Research of Chile (CONICYT) for the postdoctoral fellowship.

References

Adesemoye AO, Kloepper JW. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 2009;85:1–12.

Adesemoye AO, Torbert HA, Kloepper JW. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 2009;58:921–9.

Albareda M, Rodríguez-Navarro DN, Camacho M, Temprano FJ. Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 2008;40:2771–9.

Alguacil MM, Caravaca F, Azcón R, Pera J, Díaz G, Roldán A. Improvements in soil quality and performance of mycorrhizal *Cistus albidus* L. seedlings resulting from addition of microbially treated sugar beet residue to a degraded semiarid Mediterranean soil. Soil Use Manag 2003;19:277–83.
Alguacil MM, Diaz-Pereira E, Caravaca F, Fernandez DA, Roldán A. Increased diversity of arbuscular mycorrhizal fungi in a long-term field experiment via application of organic amendments to a semiarid degraded soil. Appl Environ Microbiol 2009;75:4254–63.
Alguacil MM, Torrecillas E, Kohler J, Roldán A. A molecular approach to ascertain the success of "in situ" AM fungi inoculation in the revegetation of a semiarid, degraded land. Sci Total Environ 2011;409:2874–80.
Anderson JPE, Domsch KH. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 1978;10:215–21.
Antoun H, Kloepper JW. Plant growth-promoting rhizobacteria (PGPR). In: Brenner S, Miller JH, editors. Encyclopedia of Genetics. New York: Academic Press; 2001. p. 1477–80.
Basak BB, Biswas DR. Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. Biol Fertil Soils 2010;46:641–8.
Bashan Y. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 1998;16:729–70.
Bashan Y, Rojas A, Puente ME. Improved establishment and development of three cacti species inoculated with Azospirillum brasilense transplanted into disturbed urban desert soil. Can J Microbiol 1999;45:441–51.
Bashan Y, Hernandez JP, Leyva LA, Bacilio M. Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol Fertil Soils 2002;35:359–68.
Bashan Y, Holguin G, de-Bashan LE. Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Can J Microbiol 2004;50:521–77.
Bashan Y, Salazar B, Puente ME, Bacilio M, Linderman R. Enhanced establishment and growth of giant cardon cactus in an eroded field in the Sonoran Desert using native legume trees as nurse plants aided by plant growth-promoting microorganisms and compost. Biol Fertil Soils 2009;45:585–94.
Bashan Y, Salazar BG, Moreno M, Lopez BR, Linderman RG. Restoration of eroded soil in the Sonoran Desert with native leguminous trees using plant growth-promoting microorganisms and limited amounts of compost and water. J Environ Manage 2012;102:26–36.
Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205–7.
Benizri I, Dedourge O, Dibattista-Leboeuf C, Piutti S, Nguyen C, Guckert A. Effect of maize rhizodeposits on soil microbial community structure. Appl Soil Ecol 2002;21:261–5.
Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 2009;68:1–13.
Brink RH, Dubar P, Lynch DL. Measurement of carbohydrates in soil hydrolysates with anthrone. Soil Sci 1960;89:157–66.
Caravaca F, Masciandaro G, Ceccanti B. Land use in relation to soil chemical and biochemical properties in semi-arid Mediterranean environment. Soil Tillage Res 2002;68:23–30.
Caravaca F, Figueroa D, Alguacil MM, Roldán A. Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. Bioresour Technol 2003;90:65–70.
Caravaca F, Alguacil MM, Azcón R, Parlade J, Torres P, Roldán A. Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate, and *Aspergillus niger*. Microb Ecol 2005;49:73–82.
Carrillo-Garcia A, Bashan Y, Diaz-Rivera E, Bethlenfalvay GJ. Effects of resource — island soils, competition, and inoculation with Azospirillum on survival and growth of Pachycereus pringlei, the giant cactus of the Sonoran Desert. Restor Ecol 2000;8:65–73.
Cassidy MB, Lee H, Trevors JT. Environmental applications of immobilized microbial cells: a review. J Ind Microbiol 1996;16:79–101.
de-Bashan LE, Hernandez JP, Bashan Y. The potential contribution of plant growth-promoting bacteria to reduce environmental degradation — a comprehensive evaluation. Appl Soil Ecol 2012;61:171–89.
Flores P, Fenoll J, Hellin P, Aparicio-Tejo P. Isotopic evidence of significant assimilation of atmospheric-derived nitrogen fixed by *Azospirillum brasilense* co-inoculated with phosphate-solubilising *Pantoea dispersa* in pepper seedling. Appl Soil Ecol 2010;46:335–40.
García C, Hernandez MT, Costa F. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun Soil Sci Plant Anal 1997;28:123–34.
Hartmann A, Rothballer M, Schmid M. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 2008;312:7–14.
Heijnen CE, Van Veen JA. A determination of protective microhabitats for bacteria introduced into soil. FEMS Microbiol Ecol 1991;85:73–80.
Hiltner L. Uber neuer Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Soil Biol Biochem 1904;32:1405–17.
Kim IY, Pusey PL, Zhao Y, Korban SS, Choi H, Kim KK. Controlled release of *Pantoea agglomerans* E325 for biocontrol of fire blight disease of apple. J Control Release 2012;161:109–15.
Kohler J, Tortosa G, Cegarra J, Caravaca F, Roldán A. Impact of DOM from composted "alperujo" on soil structure, AM fungi, microbial activity and growth of *Medicago sativa*. Waste Manag 2008;28:1423–31.
Medina A, Roldán A, Azcón R. The effectiveness of arbuscular-mycorrhizal fungi and *Aspergillus niger* or *Phanerochaete chrysosporium* treated organic amendments from olive residues upon plant growth in a semi-arid degraded soil. J Environ Manage 2010;91:2547–53.
Nannipieri P, Ceccanti B, Cervelli S, Matarese E. Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Sci Soc Am J 1980;44:1011–6.
Naseby DC, Lynch JM. Rhizosphere soil enzymes as indicators of perturbation caused by a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol Biochem 1997;29:1353–62.
Paquin R, Lechasseur P. Observations sur une méthode de dosage de la proline libre dans les extraits de plantes. Can J Bot 1979;57:1851–4.
Puente ME, Bashan Y, Li CY, Lebsky VK. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. Plant Biol (Stuttg) 2004;6:629–42.
Rodriguez H, Fraga R, Gonzalez T, Bashan Y. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 2006;287:15–21.
Schoebitz M, Ribaudo C, Pardo M, Cantore M, Ciampi L, Cura JA. Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. Soil Biol Biochem 2009;41:1768–74.
Schoebitz M, Simonin H, Poncelet D. Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. J Microencapsul 2012;29:532–8.
Schoebitz M, López MD, Roldán A. Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. Agron Sustain Dev 2013. http://dx.doi.org/10.1007/s13593-013-0142-0.
Soil-Survey-Staff. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. Washington, DC: US Government Printing Office; 2006.
Tabatabai MA. Soil enzymes. In: Page AL, Miller EM, Keeney DR, editors. Methods of soil analysis. Madison: ASA and SSSA; 1982. p. 501–38.
Tabatabai MA, Bremmer JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1969;1:301–7.
Tobar RM, Azcon R, Barea JM. The improvement of plant N acquisition from an ammonium treated drought stressed soil by the fungal symbionts in *Arbuscular mycorrhizae*. Biol Fertil Soils 1999;9:1–8.
Trejo A, de-Bashan LE, Hartmann A, Hernandez JP, Rothballer M, Schmid M, et al. Recycling waste debris of immobilized microalgae and plant growth-promoting bacteria from wastewater treatment as a resource to improve fertility of eroded desert soil. Environ Exp Bot 2012;75:65–73.
Vassilev N, Vassileva M, Nikolaeva I. Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 2006;71:137–44.
Vassileva M, Azcon R, Barea JM, Vassilev N. Effect of encapsulated cells of *Enterobacter* sp on plant growth and phosphate uptake. Bioresour Technol 1999;67:229–32.
Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 2003;255:571–86.
Wright SF, Anderson RL. Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biol Fertil Soils 2000;31:249–53.
Wu Z, Zhao Y, Kaleem I, Li C. Preparation of calcium alginate microcapsuled microbial fertilizer coating *Klebsiella oxytoca* Rs-5 and its performance under salinity stress. Eur J Soil Biol 2011;47:152–9.
Wu Z, Guo L, Qin S, Li C. Encapsulation of *R. planticola* Rs-2 from alginate–starch–bentonite and its controlled release and swelling behavior under simulated soil conditions. J Ind Microbiol Biotechnol 2012;39:317–27.