Journal of Environmental Management 134 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions

Carmen Mengual [a,*], Mauricio Schoebitz [a], Rosario Azcón [b], Antonio Roldán [a]

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

[b] Estación Experimental del Zaidín (CSIC), Department of Soil Microbiology and Symbiotic Systems, Profesor Albareda, 1, 18008 Granada, Spain

ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form
12 December 2013
Accepted 2 January 2014
Available online 22 January 2014

Keywords:
Degraded soil
Enzymatic activity
Revegetation
PGPR
Microbial biomass
Sugar beet

ABSTRACT

The re-establishment of autochthonous shrub species is an essential strategy for recovering degraded soils under semiarid Mediterranean conditions. A field assay was carried out to determine the combined effects of the inoculation with native rhizobacteria (*Bacillus megaterium*, *Enterobacter* sp, *Bacillus thuringiensis* and *Bacillus* sp) and the addition of composted sugar beet (SB) residue on physicochemical soil properties and *Lavandula dentata* L. establishment. One year after planting, *Bacillus* sp. and *B. megaterium* + SB were the most effective treatments for increasing shoot dry biomass (by 5-fold with respect to control) and *Enterobacter* sp + SB was the most effective treatments for increasing dry root biomass. All the treatments evaluated significantly increased the foliar nutrient content (NPK) compared to control values (except *B. thuringiensis* + SB). The organic amendment had significantly increased available phosphorus content in rhizosphere soil by 29% respect to the control. *Enterobacter* sp combined with sugar beet residue improved total N content in soil (by 46% respect to the control) as well as microbiological and biochemical properties. The selection of the most efficient rhizobacteria strains and their combined effect with organic residue seems to be a critical point that drives the effectiveness of using these biotechnological tools for the revegetation and rehabilitation of degraded soils under semiarid conditions.

1. Introduction

The introduction of plants and shrubs species in degraded Mediterranean soils is a difficult task due to low soil fertility, low and irregular precipitations and a severe drought period (Caravaca et al., 2002a). Under these conditions, it is necessary to assess new methodologies to facilitate the revegetation and improve physicochemical and biological soil properties. Thus, the establishment of a plant cover with shrub species in revegetation programs has been successfully used in revegetation activities in degraded soils, principally those developed under Mediterranean ecosystems (Alguacil et al., 2003; Caravaca et al., 2003b).

Microbial inoculations and organic amendments are widely used tools to aid in the restoration of plant cover and soil quality in degraded Mediterranean areas (Alguacil et al., 2003; Azcon et al., 2009; Caravaca et al., 2003a; Medina et al., 2004). Rhizobacteria, as an important part of the soil microbiota, are known for their ability to increase the root surface area and improve nutrient uptake, biological nitrogen fixation and phosphate solubilization (Bashan et al., 2004). Rhizobacteria may enhance plant growth by improving the supply of nutrients of low mobility from soil, such as phosphorous (Caravaca et al., 2003c) and potassium. In this regard, rhizobacteria may have a potential role in the establishment of plant species in arid environmental conditions (Benabdellah et al., 2011), although their use is quite more frequent in agricultural lands (Kohler et al., 2006, 2007).

Some recent studies showed the beneficial effects of the application of organic amendments in reclamation of semiarid soils, for example, alperujo (Kohler et al., 2008), urban refuse (Alguacil et al., 2009a) and sugar beet residue (Caravaca et al., 2005). They have reported beneficial effects on soil quality by increasing the proliferation and development of natural populations of soil microorganisms and improving soil properties. These effects could be extended to the enhancement of the soil enzyme activities, which are considered as key factors contributing to soil activity (Caravaca et al., 2005), fertility of soil and availability of nutrients to plants. Sugar beet containing cellulose, lignocellulose and other polysaccharides, can be used by the inoculated microorganisms as a

* Corresponding author. Tel.: +34 968 396337; fax: +34 968 396200.
E-mail address: cmengual@cebas.csic.es (C. Mengual).

0301-4797/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jenvman.2014.01.008

substrate and as carbon and energy source (Vassileva et al., 2010). Additionally, the application of the sugar beet interacts positively with some microorganisms inoculated, like arbuscular mycorrhizal fungi, and can improve both the soil quality and plant performance in degraded soils (Caravaca et al., 2004).

The main objective of this assay was to investigate the role of native rhizobacteria strains in the revegetation of a Mediterranean semiarid area and to prove whether the application of an organic residue could have a synergistic effect with the studied microorganisms. We hypothesize that the combined effects of native rhizobacteria and sugar beet residue addition can increase the plant establishment and soil properties in a degraded semiarid area.

2. Material and methods

2.1. Study site

The study area was located in Vicente Blanes Ecological Park in Molina de Segura, (southeast Spain) (Lat. 38° 12′ N, Long. 1° 13′ W, Elev. 392 m). The climate is semiarid with a potential evapotranspiration reaching up to 1000 mm per year and an average annual rainfall around 300 mm. The mean annual temperature is 17.5 °C with no frost period. The soil is a Typic Torriorthent (SSS, 2010), with low organic matter content and a silty loam texture (Table 1). The vegetation in the zone was dominated by the invasive *Piptatherum miliaceum* L. Cosson and some native shrubs of *Thymus vulgaris* L., *Pistacia lentiscus* L., *Cistus clusii* Dunal and *Rosmarinus officinalis* L.

The plant used for the revegetation experiment was *Lavandula dentata* L., which is a small shrub that reaches a height of 30 cm, widely distributed in the Mediterranean area. It is also well adapted to water stress conditions and, therefore, could be potentially used in the reafforestation of semiarid disturbed lands (Ouahmane et al., 2006).

2.2. Microbial inoculants and organic residues

The plant growth promoting rhizobacteria *Bacillus megaterium*, *Enterobacter* sp, *Bacillus thuringiensis* and *Bacillus* sp were isolated in the experimental area and cultivated in Estación Experimental del Zaidín (EEZ). The rhizobacteria were grown in a liquid nutrient medium composed of yeast extract, peptone and sodium chloride (Yeast extract peptone - YEP) for 2 days at room temperature on a Heidolph Unimax 1010 shaker. The bacterial culture was centrifuged at 2287 *g* for 5 min at 2 °C, and the sediment was resuspended in sterilized tap water (Alguacil et al., 2009b). The cells concentration of the bacterial suspension was 10^7 CFU ml^{-1}.

The organic residue used in this assay was a lignocellulosic material called sugar beet (SB). Its analytical characteristics are cellulose, 29%; hemicellulose, 23%; lignin, 5%; (total C, 55%); total N, 1.7%; total P, 2.4%, total K, 0.8% and pH 3.0. This amendment was dried in a 60 °C oven and then ground in an electrical grinder to 2 mm fragments (Caravaca et al., 2005).

Table 1
Physicochemical, biochemical and microbiological characteristics of the soil present in the experimental site.

pH (H_2O)	8.5 ± 0.02[a]
Electrical conductivity (1:5, μS cm^{-1})	176.1 ± 2.55
Texture	Silty loam
Sand	31.9%
Loam	61.1%
Clay	7%
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})	1.98 ± 0.23
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. Experimental design

A complete aleatorized factorial assay was established with two factors and five fold replication in a split plot design. The first factor was the inoculation of *L. dentata* seedlings with microbial inoculant (*B. megaterium*, *Enterobacter* sp, *B. thuringiensis* and *Bacillus* sp) and the second one was the addition of sugar beet residue into the soil. The experimental design was performed as follows: treatment 1, *L. dentata* without rhizobacteria treatment and soil without organic residue addition (Control); treatments 2, 3, 4 and 5, *L. dentata* inoculated with *B. megaterium*, *Enterobacter* sp, *B. thuringiensis* and *Bacillus* sp, respectively, and soil without organic residue addition; treatment 6, *L. dentata* without microbial inoculant treatment and soil with sugar beet residue (SB) addition; treatments 7, 8, 9 and 10, *L. dentata* inoculated with *B. megaterium*, *Enterobacter* sp, *B. thuringiensis* and *Bacillus* sp soil with sugar beet residue addition. In later March of 2011, pots of 500 ml containing 500 g of soil from the experimental area were used for planting *L. dentata* seedlings. Sugar beet residue was added to the pots at a rate of 2% by weight (10 g of sugar beet residue) and the rhizobacteria dose per inoculation corresponded to 10^{10} CFU $plant^{-1}$. After fifteen days, a new inoculation was carried out for each treatment. Plants were allowed to establish in the pots for two months, and in later May, the plants were carried to the experimental field, where planting holes 15 × 15 cm wide and 15 cm deep were dug manually. The seedlings were planted at least 1 m apart between holes, with 3 m between treatment levels. At least 5 seedlings per treatment level were planted.

2.4. Sampling procedures

Samples were collected twelve months after planting, in early June 2012. Five plants per treatment, including root systems and rizhosphere soil, were collected between 0 and 15 cm depth from planting holes, and placed in polyethylene bags for transport to the laboratory. Rhizosphere soil samples were divided into two subsamples before physicochemical and biochemical analyses: one subsample sieved to <2 mm and other subsample sieved between 4 and 0.25 mm.

2.5. Plant analyses

Fresh and dry weights of shoots and roots (70 °C, 48 h) were recorded before chemical analysis. The shoot contents of N, P and K were determined by ICP/OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL).

2.6. Soil physicochemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. Total organic carbon (C), total nitrogen (N),

available phosphorus (P) and extractable potassium (K) were determined by ICP/OES spectrometry (Thermo Elemental Co. Iris Intrepid II XDL). Water soluble carbohydrates were determined by the method of Brink et al. (1960). In all these analysis soil sieved to <2 mm was used.

2.7. Soil biochemical analyses

Soil respiration was calculated as the amount of CO_2 emitted during a 24 h incubation period: 10 g of dry soil sieved to <2 mm were placed in an incubation vessel. The moisture was adjusted to 45% of water holding capacity. After the incubation, 4 g of the wet soil were placed in an internal vial and this one, inside an external vial containing 2 ml of KOH (0.1 g of KOH in 50 ml distilled water) for retention of the evolved CO_2 (Carbonell-Martin et al., 2011). Soil microbial biomass C was evaluated by the Substrate Induced Respiration (SIR) method following addition of glucose to soil (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 (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 sample sieved to <2 mm 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.

Urease and N-a-benzoyl-L-arginine amide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used, respectively, as substrates. 2 ml of buffer and 0.5 ml of substrate were added to 0.5 g of soil sieved to <2 mm and next it was incubated 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).

Alkaline phosphatase activity was determined using p-nitrophenyl phosphate disodium (PNPP 0.115 M) as substrate. 2 ml of 0.5 M sodium acetate buffer at pH 11 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil sieved to <2 mm 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 4000 rpm for 5 min. The p-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, but the substrate was added before the $CaCl_2$ and NaOH.

β-glucosidase determination is based on the release and detection of PNP. For that, 2 ml of 0.1 M maleate buffer at pH 6.5 and 0.5 ml of p-nitrophenyl-b-D-glucopyranoside (PNG 0.05 M) using as substrate were added to 0.5 g of soil sieved to <2 mm. Straightaway, sample was 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 Bremner, 1969).

2.8. Statistical analyses

Values were log transformed to achieve normality. The effects of amendment addition, microbial inoculation and their interaction on measured variables were analyzed by a two way ANOVA and post hoc mean separation was performed by the Tukey honestly significant difference (HSD) test, calculated at $P < 0.05$. All statistical analysis was performed using the software SPSS version 19.0 for Windows.

3. Results

3.1. Growth parameters of L. dentata after microbial inoculation and amendment with organic residue

Both experimental factors, microbial inoculation (MI) and the addition of organic amendment (OA), as well as the MI × OA interaction were significant on *L. dentata* shoot and root growth (Table 2). One year after planting, all the treatments: microbial inoculations, addition of SB and the combined treatments (MI + SB) significantly increased shoots fresh weight (except *B. thuringiensis* + SB) and shoots dry weight compared to control values. The greatest improvement was observed in plants inoculated with *Bacillus* sp (which increased the shoots fresh weight by 7 fold), in plants inoculated with *Bacillus* sp and in plants subjected to the combined treatment *B. megaterium* + SB (both increasing shoots dry weight by 5 fold; Table 2). Roots dry weight showed a statistically significant increase mediated by almost all the treatments with respect to the control plants. Only the combined treatments with *B. thuringiensis* and *Bacillus* sp produced a significant decrease while the inoculation with *Enterobacter* sp did not show any significant change. The inoculation with *B. thuringiensis* and *Bacillus* sp and the mixed treatment with *Enterobacter* sp promoted an increase in roots fresh weight, while it was showed a decrease with *B. thuringiensis* + SB and *Bacillus* sp + SB mixed treatments, compared with the control plants (Table 2).

Table 2
Effect of microbial inoculants and sugar beet addition on *Lavandula dentata* growth parameters ($n = 5$).

	Shoot (g fw)[a]	Root (g fw)	Shoot (g dw)[b]	Root (g dw)
Treatments				
Control	1.2 ± 0.03 a	0.6 ± 0.10 ab	0.8 ± 0.02 a	0.2 ± 0.02 b
B. megaterium	6.1 ± 0.17 de	1.0 ± 0.09 bc	3.6 ± 0.03 e	0.4 ± 0.05 cd
Enterobacter sp	2.7 ± 0.23 c	0.8 ± 0.09 bc	1.1 ± 0.03 b	0.3 ± 0.04 bc
B. thuringiensis	5.0 ± 0.15 d	2.1 ± 0.22 d	2.8 ± 0.03 d	0.7 ± 0.03 ef
Bacillus sp	8.8 ± 0.14 g	1.4 ± 0.22 cd	4.7 ± 0.04 f	0.6 ± 0.02 de
Sugar beet (SB)	5.6 ± 0.24 de	1.0 ± 0.15 bc	3.3 ± 0.05 e	0.4 ± 0.03 cd
B. megaterium + SB	6.8 ± 0.18 ef	1.0 ± 0.12 bc	4.4 ± 0.04 f	0.4 ± 0.02 cde
Enterobacter sp + SB	5.8 ± 0.19 de	2.5 ± 0.21 d	3.6 ± 0.03 e	1.1 ± 0.06 f
B. thuringiensis + SB	1.5 ± 0.12 ab	0.3 ± 0.03 a	1.0 ± 0.04 b	0.1 ± 0.01 a
Bacillus sp + SB	1.8 ± 0.18 b	0.4 ± 0.11 a	1.8 ± 0.04 c	0.1 ± 0.03 a
ANOVA, *P* values				
Microbial Inoculant (MI)	89.85 (<0.001)	8.06 (<0.001)	819.18 (<0.001)	23.35 (<0.001)
Organic Amendment (OA)	5.66 (0.027)	14.52 (0.001)	215.45 (<0.001)	20.64 (<0.001)
MI × OA	286.44 (<0.001)	37.75 (<0.001)	1886.14 (<0.001)	83.69 (<0.001)

[a] Grams fresh weight.
[b] grams dry weight. Significant difference according to the HSD Tukey test at $P < 0.05$ levels were indicated by different letters. Significance of effects of microbial inoculant, organic amendment and their interaction on the measured variables is also shown.

3.2. Shoot nutrients

The ANOVA revealed that the microbial inoculation, organic amendment and MI × OA interaction affected significantly N, P and K uptake (Table 3). All the treatments mediated an improvement on N, P and K contents, except the combined treatment *B. thuringiensis* + SB which did not produce any change on shoot N content (Table 3). The greatest values for these nutrients were recorded in plants subjected to the inoculation with *Bacillus* sp and the mixed treatment *B. megaterium* + SB. N and P contents were increased by 6 fold with respect to their controls, whereas shoot K values were improved by 8 fold by both treatments.

3.3. Soil physicochemical analyses

The microbial inoculation, organic amendment and MI × OA interaction significantly affected the soil electrical conductivity and total organic C (TOC), while only organic amendment affected pH values (Table 4). The post hoc test showed that the addition of SB treatment and the combined treatment *Enterobacter* sp + SB increased the electrical conductivity values (by 10% respect to the control). An improvement in TOC contents was mediated by all the treatments with the exception of the inoculation with *Enterobacter* sp and *B. thuringiensis* (Table 4). With regard to pH values, a significant decrease with respect to the control was observed with the use of any treatment that contained sugar beet residue.

The ANOVA showed that both microbial inoculation and organic amendment factors and their interaction affected significantly N values (Table 4). Thus, the combined treatments *B. megaterium* + SB and *Enterobacter* sp + SB showed an improvement in total N content (by 38% and 46% respectively, Table 4). For available P, both microbial inoculation and organic amendment factors were significant (Table 4). All the microbial inoculation treatments, except the inoculation with *Bacillus* sp, produced a decrease on available P content, while the addition of SB increased it by 29% (Table 4). With regard to extractable K, significant effects were recorded for microbial inoculation and MI × OA interaction (Table 4). The inoculation with *B. megaterium* and the combined treatment *B. thuringiensis* + SB mediated a decrease in K extractable content compared to the control (Table 4).

The ANOVA showed that microbial inoculation significantly affected water soluble CH values (Table 4). The post hoc test recorded that the inoculation with *Enterobacter* sp, *Bacillus* sp, and the combined treatments *B. megaterium* + SB and *Enterobacter* sp + SB increased water soluble CH values. The inoculation with *B. thuringiensis* produced a significant decrease in this parameter respect to the control (Table 4).

Table 3
Nutrients content in *Lavandula dentata* as a result of microbial inoculants and sugar beet addition ($n = 5$).

	N	P	K
	(mg plant^{-1})	(mg plant^{-1})	(mg plant^{-1})
Treatments			
Control	7.98 ± 0.30 a	0.59 ± 0.02 a	12.37 ± 0.80 a
B. megaterium	36.02 ± 0.66 ef	2.60 ± 0.15 d	84.92 ± 2.86 ef
Enterobacter sp	11.14 ± 0.89 b	0.95 ± 0.04 b	22.85 ± 1.03 b
B. thuringiensis	32.05 ± 0.35 de	2.37 ± 0.03 d	65.01 ± 2.82 d
Bacillus sp	48.53 ± 1.17 g	3.70 ± 0.21 e	107.03 ± 4.48 g
Sugar beet (SB)	30.08 ± 0.78 d	2.66 ± 0.03 d	70.50 ± 1.02 de
B. megaterium + SB	51.06 ± 1.68 g	4.01 ± 0.16 e	98.27 ± 3.13 fg
Enterobacter sp + SB	40.61 ± 0,51 f	2.82 ± 0.13 d	74.94 ± 2.17 de
B. thuringiensis + SB	9.42 ± 0.50 a	0.81 ± 0.02 b	19.57 ± 1.18 b
Bacillus sp + SB	17.06 ± 0.85 c	1.63 ± 0.10 c	35.87 ± 1.28 c
ANOVA, *P* values			
Microbial Inoculant (MI)	303.64 (<0.001)	174.95 (<0.001)	174.95 (<0.001)
Organic Amendment (OA)	34.83 (<0.001)	43.51 (<0.001)	71.47 (<0.001)
MI × OA	495.53 (<0.001)	679.71 (<0.001)	355.99 (<0.001)

Significant difference according to the HSD Tukey test at $P < 0.05$ levels were indicated by different letters. Significance of effects of microbial inoculant, organic amendment and their interaction on the measured variables is also shown.

3.4. Soil biochemical properties

There was a significant effect of microbial inoculant, organic amendment and MI × OA interaction on soil respiration and microbial biomass (Table 5). The Tukey HSD test confirmed a significant increase of these properties in many of these treatments compared with the control (Table 5). The greatest value for soil respiration was observed with the mixed treatment *Enterobacter* sp + SB which improved it by 48%. This parameter underwent a significant decrease when plants were inoculated with *Bacillus* sp and no change with the combined treatment *B. thuringiensis* + SB respect to the control. Microbial biomass significantly decreased after the inoculation with *B. thuringiensis* and *Bacillus* sp and with the mixed treatment *B. thuringiensis* + SB. It did not experiment any change after *Enterobacter* sp inoculation (Table 5). Glomalin related soil protein significantly increased with organic amendment and MI × OA interaction (Table 5). Thus, the concentration of this protein increased mainly after the addition of SB and the combined treatments *Enterobacter* sp + SB and *B. thuringiensis* + SB (by one fold in each case; Table 5).

The MI × OA interaction significantly affected enzyme activities (urease, β-glucosidase, protease and dehydrogenase). Microbial inoculation had a significant effect on urease, protease and dehydrogenase, while the addition of organic amendment significantly affected urease, β-glucosidase, and dehydrogenase activities. None of the factors or MI × OA interaction had a significant effect on phosphatase (Table 5). Thus, phosphatase did not undergo any change with respect to the control (Table 5). β-glucosidase activity increased after the addition of SB and with almost all the combined treatments, except *B. thuringiensis* + SB, which did not differ with respect to the control. Although the microbial inoculation factor did not affect significantly this activity, an improvement was observed after the inoculation with *Bacillus* sp (Table 5). Urease values showed an increase mediated by the inoculation with *Bacillus* sp and all the combined treatments (Table 5). Protease activity was improved by the inoculation with *Bacillus* sp and the mixed treatment *Bacillus* sp + SB. The addition of SB also increased the protease activity values (Table 5). Dehydrogenase activity was increased with the inoculation with *Bacillus* sp, the amendment with organic residue and the application of the combined treatments *B. thuringiensis* + SB and *Bacillus* sp + SB (Table 5).

4. Discussion

The amendment of degraded soils with organic residues improves plant performance under semiarid conditions (Alguacil et al., 2009a; Caravaca et al., 2005; Fernández et al., 2012; Kohler et al., 2008). It is well documented that plant growth promoting rhizobacteria (PGPR), under greenhouse and laboratory conditions, exert beneficial effects on plant growth and development (Adesemoye and Kloepper, 2009; Adesemoye et al., 2009; Schoebitz et al., 2009). In this field experiment, the addition of sugar beet residue and the inoculation with the native rhizobacteria, applied separately or in combined treatments, were very efficient to promote the shoot growth of *L. dentata*. Caravaca et al. (2002a) reported a synergistic effect of combining mycorrhizal fungi inoculation and organic amendments on plant performance in semiarid soils; nevertheless, in our, case the observed effects of combining rhizobacteria and sugar beet showed a function merely additive. The combination of *Enterobacter* sp and sugar beet was

Table 4
Changes in physicochemical properties in rhizosphere soil of *L. dentata* as a result of microbial inoculant and organic amendment addition ($n = 5$).

	pH	EC[1]	TOC[2]	Total N	P available	K extractable	Water soluble
	(H_2O)	($\mu S\ cm^{-1}$)	($g\ kg^{-1}$)	($g\ kg^{-1}$)	($mg\ kg^{-1}$)	($mg\ kg^{-1}$)	CH[3] ($\mu g\ g^{-1}$)
Treatments							
Control	8.6 ± 0.2 b	484 ± 3 a	11.5 ± 0.3 a	1.3 ± 0.0 ab	10.4 ± 0.6 de	208 ± 12 cd	9.9 ± 0.66 b
B. megaterium	8.4 ± 0.0 ab	490 ± 11 ab	15 ± 1.4 bc	1.6 ± 0.2 bcd	8.5 ± 0.5 abc	171 ± 5 ab	12.1 ± 0.6 bcd
Enterobacter sp	8.4 ± 0.0 ab	495 ± 6 ab	14 ± 0.9 abc	1.6 ± 0.2 bcd	8.0 ± 0.1 ab	202 ± 1 c	25.7 ± 2.76 f
B. thuringiensis	8.4 ± 0.0 ab	480 ± 11 a	13 ± 0.3 ab	1.1 ± 0.1 a	7.4 ± 0.3 a	202 ± 5 c	6.6 ± 0.38 a
Bacillus sp	8.4 ± 0.0 ab	484 ± 4 a	15 ± 1.0 bc	1.3 ± 0.1 abc	9.0 ± 0.0 bcd	191 ± 5 bc	15.8 ± 0.93 de
Sugar beet (SB)	8.3 ± 0.0 a	535 ± 14 c	17 ± 0.7 c	1.6 ± 0.1 bcd	13.5 ± 0.3 f	234 ± 3 d	11.8 ± 0.31 bcd
B. megaterium + SB	8.3 ± 0.0 a	503 ± 5 abc	16 ± 0.2 bc	1.8 ± 0.0 d	10.6 ± 0.1 de	195 ± 5 bc	19.1 ± 1.16 ef
Enterobacter sp + SB	8.3 ± 0.0 a	525 ± 1 bc	16 ± 0.4 c	1.9 ± 0.1 d	9.9 ± 0.2 cde	197 ± 4 bc	13.9 ± 1.10 cde
B. thuringiensis + SB	8.3 ± 0.0 a	501 ± 1 abc	17 ± 0.4 c	1.3 ± 0.0 abc	10.5 ± 0.2 de	160 ± 4 a	9.8 ± 0.71 b
Bacillus sp + SB	8.3 ± 0.02 a	486 ± 4 a	15 ± 0.1 bc	1.7 ± 0.03 bcd	10.8 ± 0.4 d	195.6 ± bc	10.3 ± 0.29 bc
ANOVA, *P* values							
Microbial Inoculant (MI)	0.843 (0.515)	3.40 (0.028)	4.18 (0.013)	11.38 (<0.001)	21.73 (<0.001)	6.23 (0.002)	3.33 (0.030)
Organic Amendment (OA)	6.64 (0.018)	29.61 (<0.001)	34.52 (<0.001)	27.00 (<0.001)	110.52 (<0.001)	0.01 (0.938)	0.11 (0.744)
MI × OA	1.15 (0.363)	5.50 (0.004)	7.20 (0.001)	3.57 (0.023)	0.58 (0.682)	6.77 (0.001)	0.985 (0.438)

EC[1]: electrical conductivity; TOC[2]: total organic carbon; CH[3]: carbohydrates. Mean ± standard error. For each species, values in columns followed by the same letter do not differ significantly ($P < 0.05$) as determined by HSD Tukey test. Significance of effects of microbial inoculant, organic amendment and their interaction on the measured variables is also shown.

the best treatment to improve root biomass. However, roots dry weight underwent a significant decrease with the combined treatments *B. thuringiensis* + SB and *Bacillus* sp + SB. Population dynamics within the rhizosphere of the plant host and ability to colonize roots and shoots by endophytic *Enterobacter* and *Bacillus* species have been reported in crops, increasing their vegetative growth under greenhouse conditions (Rekha et al., 2007; Schoebitz et al., 2009). Nevertheless, true root colonists are those bacteria that colonize roots in competitive conditions, i.e., natural field soils (Kennedy et al., 2004). It could be considered that *B. thuringiensis* and *Bacillus* sp were not capable to compete with autochthonous soil microbiota to colonize plant roots when the organic amendment was added. *Enterobacter* sp seemed to be a more efficient colonist with the addition of sugar beet under field conditions.

Rhizobacteria can play a fundamental role on nutrient uptake and therefore on the biomass promotion (Adesemoye and Kloepper, 2009; Bashan et al., 2004). The increase observed in shoot K and P contents may be attributable to the mobilization of nutrients from soil due to the secretion of organic acids mediated by the inoculants acting as plant growth promoting rhizobacteria (Basak and Biswas, 2010). The improvement observed on foliar N concentration may be explained by the fact that some *Bacillus* species, i.e. *Bacillus sphaericus*, are diazotrophic bacteria able to increase the uptake of N derived from N_2 biological fixation (Zakry et al., 2012) although, in our study, the application of the organic amendment seemed to inhibit this characteristic in *B. thuringiensis*.

With regard to changes in soil properties, the improvements observed in total organic C, water soluble CH and glomalin are quite relevant because these compounds can be used as carbon and energy sources for soilborne microflora. Glomalin is a glycoprotein, produced by arbuscular mycorrhizal fungi, capable to increase the hydrophobicity of soil particles and to form soil aggregates (Rillig et al., 2001; Wright and Anderson, 2000). These results of total organic C, water soluble CH and glomalin are in agreement with the β-glucosidase and dehydrogenase activity values recorded. Enzyme activities are properties sufficiently sensitive to indicate changes caused by microbial inoculation (Schoebitz et al., 2014). β-glucosidase results in the treatments with residue application are in

Table 5
Changes in biological properties and enzymatic activities in rizhosphere soil of *L. dentata* in response to inoculation with microbial inoculant and sugar beet addition ($n = 5$).

	Dehydrogenase	β-glucosidase	Urease	Phosphatase	Protease	Respiration	Biomass C	GRSP[1]
	($\mu g\ g^{-1}$ INTF)	($\mu mol\ PNF\ g^{-1}\ h^{-1}$)	($\mu mol\ NH_3\ g^{-1}\ h^{-1}$)	($\mu mol\ PNF\ g^{1}\ h^{-1}$)	($\mu mol\ NH_3\ g^{1}\ h^{-1}$)	($CO_2\ h^{-1}\ kg^{-1}$)	($mg\ kg^{-1}$)	($\mu g\ g^{-1}$)
Treatments								
Control	113 ± 2.74 a	0.2 ± 0.02 a	0.4 ± 0.01 ab	1.6 ± 0.03 abc	1.19 ± 0.01 ab	7.7 ± 0.04 b	1154 ± 7 b	378 ± 11.92 a
B. megaterium	128 ± 4.9 abc	0.3 ± 0.01 abc	0.5 ± 0.05 bc	1.7 ± 0.03 abc	1.4 ± 0.04 bc	9.9 ± 0.03 f	1761 ± 12 d	615 ± 27.76 de
Enterobacter sp	120 ± 8.6 ab	0.3 ± 0.02 ab	0.5 ± 0.04 abc	1.2 ± 0.02 a	1.2 ± 0.06 ab	9.0 ± 0.03 d	1230 ± 11 b	555 ± 5.41 cd
B. thuringiensis	115 ± 10.7 a	0.3 ± 0.02 abc	0.5 ± 0.01 bcd	1.4 ± 0.05 ab	1.2 ± 0.02 abc	8.5 ± 0.03 c	1056 ± 21 a	479 ± 2.71 b
Bacillus sp	150 ± 7.6 bc	0.5 ± 0.01 d	0.6 ± 0.04 cde	1.7 ± 0.01 abc	1.8 ± 0.06 d	6.8 ± 0.05 a	988 ± 18 a	692 ± 11.44 ef
Sugar beet (SB)	161 ± 4.9 c	0.5 ± 0.04 d	0.4 ± 0.02 a	2.0 ± 0.06 bc	1.8 ± 0.05 d	10.9 ± 0.05 g	1903 ± 16 d	778 ± 14.86 fg
B. megaterium + SB	126 ± 4.2 ab	0.4 ± 0.03 bcd	0.7 ± 0.04 de	1.2 ± 0.01 a	1.2 ± 0.07 abc	9.5 ± 0.03 e	1880 ± 15 d	529 ± 15.78 bc
Enterobacter sp + SB	131 ± 5.1 abc	0.4 ± 0.03 cd	0.8 ± 0.07 e	2 ± 0.01 bcd	1.2 ± 0.07 a	11.4 ± 0.04 h	1895 ± 39 d	787 ± 2.47 g
B. thuringiensis + SB	151 ± 2.33 bc	0.4 ± 0.01 abcd	0.6 ± 0.04 cde	1.8 ± 0.03 abc	1.2 ± 0.06 abc	7.8 ± 0.04 b	1049 ± 36 a	769 ± 13.86 fg
Bacillus sp + SB	151 ± 1.76 bc	0.4 ± 0.04 bcd	0.6 ± 0.02 cde	1.3 ± 0.06 a	1.5 ± 0.06 c	9.5 ± 0.04 e	1558 ± 14 c	692 ± 24.18 ef
ANOVA, *P* values								
Microbial Inoculant (MI)	7.491 (0.001)	2.16 (0.115)	36.95 (<0.001)	2.08 (0.123)	7.07 (0.001)	13.44 (<0.001)	19.56 (<0.001)	1.88 (0.154)
Organic Amendment (OA)	19.29 (<0.001)	13.97 (0.001)	21.71 (<0.001)	3.25 (0.085)	0.061 (0.808)	39.58 (<0.001)	52.66 (<0.001)	26.76 (<0.001)
MI × OA	5.741 (0.003)	5.73 (0.003)	14.09 (<0.001)	4.05 (0.092)	7.79 (0.001)	6.48 (0.002)	4.21 (0.012)	6.83 (0.001)

GRSP[1]: Glomalin related soil protein. Mean ± standard error, values in columns followed by the same letter does not differ significantly ($P < 0.05$) as determined by HSD Tukey test. Significance of effects of microbial inoculant, organic amendment and their interaction on the measured variables is also shown.

accordance with microbial biomass and soil respiration values, which have frequently been used as indicators of soil microbial activity (Caravaca et al., 2002b).

It is remarkable the low values for total N in soil recorded in this assay. When the treatments including organic residue were applied, it could be assumed that the addition of the amendment would mediate an input on N levels. However, this only occurred after the application of sugar beet with *B. megaterium* or *Enterobacter* sp. This may be explained through the assimilation by both plant and soil microbiota of the N provided with the amendment. Besides, it could be assumed that *B. megaterium* and *Enterobacter* sp were able to fix more N_2 than the other strains in presence of the organic residue. Actually, these two treatments yielded the highest values recorded for the urease activity, which is involved in the N cycle. In general, protease activity was low, but with the treatments including *Bacillus* sp underwent a significant increase. It could be attributable to a greater capacity of *Bacillus* sp to produce this type of enzyme. In fact, the proteases obtained from certain strains of *Bacillus* have a great applicative significance on global biotechnology (Chu, 2007).

Available P content in rizhosphere soil was increased only after the amendment with sugar beet. This could suggest that the microbial strains used in this assay did not solubilize P. Furthermore, this nutrient suffered a significant decrease mediated by almost all the microbial strains when they were applied independently. However, it was observed that shoot P content increased with all the treatments, and this would indicate that, somehow, these bacteria strains were able to solubilize P in soil. To solubilize P in soil, microorganisms have to excrete organic acids or phosphatases (Rodríguez et al., 2006; Vassilev et al., 2006) and supporting in our results, the low phosphatase values recorded could point that these rhizobacteria only were capable to solubilize P by excreting organic acids (Basak and Biswas, 2010). In this sense, pH values in soil decreased in all the treatments tested; in the case of those including the organic amendment, it could be attributable to the low pH of the residue (3.0), and in the case of microbial inoculation to a limited excretion of organic acids. Similar mechanisms could be involved in the high K assimilation recorded for the established plants.

In conclusion, the microbial inoculation of the seedlings with native rhizobacteria strains clearly improved plant performance in the revegetation of a degraded semiarid soil. The combined effect of the organic addition can exert an additive effect with some strains, but also can diminish the positive effect of rhizobacteria. When considering the improvement of soil quality, addition of sugar beet residue and *Enterobacter* sp combined with sugar beet residue were the treatments that enhanced it to a greater extent. Based on these data, the application of combined treatments involving rhizobacteria inoculation and organic amendments seem to be the most appropriate method to aid in the restoration of both plant cover and soil quality in semiarid degraded areas, although an adequate selection of the rhizobacteria strains must be considered the critical point when developing this restoration technology.

Acknowledgments

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

References

Adesemoye, A.O., Kloepper, J.W., 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85, 1–12.
Adesemoye, A.O., Torbert, H.A., Kloepper, J.W., 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb. Ecol. 58, 921–929.
Alguacil, M.M., Caravaca, F., Azcón, R., Pera, J., Díaz, G., Roldán, A., 2003. 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. 19, 277–283.
Alguacil, M.M., Díaz-Pereira, E., Caravaca, F., Fernández, D.A., Roldán, A., 2009a. 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. 75, 4254–4263.
Alguacil, M.M., Kohler, J., Caravaca, F., Roldán, A., 2009b. Differential effects of *Pseudomonas mendocina* and *Glomus intraradices* on lettuce plants physiological response and aquaporin PIP2 gene expression under elevated atmospheric CO_2 and drought. Microb. Ecol. 58, 942–951.
Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.
Azcon, R., Medina, A., Roldan, A., Biro, B., Vivas, A., 2009. Significance of treated agrowaste residue and autochthonous inoculates (Arbuscular mycorrhizal fungi and *Bacillus cereus*) on bacterial community structure and phytoextraction to remediate soils contaminated with heavy metals. Chemosphere 75, 327–334.
Basak, B.B., Biswas, D.R., 2010. 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 46, 641–648.
Bashan, Y., Holguin, G., de-Bashan, L.E., 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Can. J. Microbiol. 50, 521–577.
Benabdellah, K., Abbas, Y., Abourouh, M., Aroca, R., Azcón, R., 2011. Influence of two bacterial isolates from degraded and non-degraded soils and arbuscular mycorrhizae fungi isolated from semi-arid zone on the growth of *Trifolium repens* under drought conditions: mechanisms related to bacterial effectiveness. Eur. J. Soil Biol. 47, 303–309.
Brink, R.H.J., Dubach, P., Lynch, D.L., 1960. Measurement of carbohydrates in soil hydrolyzates with anthrone. Soil Sci. 89, 157–166.
Caravaca, F., Barea, J.M., Roldán, A., 2002a. Synergistic influence of an arbuscular mycorrhizal fungus and organic amendment on *Pistacia lentiscus* L. seedlings afforested in a degraded semiarid soil. Soil Biol. Biochem. 34, 1139–1145.
Caravaca, F., Masciandaro, G., Ceccanti, B., 2002b. Land use in relation to soil chemical and biochemical properties in a semiarid Mediterranean environment. Soil Tillage Res. 68, 23–30.
Caravaca, F., Barea, J.M., Palenzuela, J., Figueroa, D., Alguacil, M.M., Roldán, A., 2003a. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. Appl. Soil Ecol. 22, 103–111.
Caravaca, F., Figueroa, D., Alguacil, M.M., Roldan, A., 2003b. Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. Bioresour. Technol. 90, 65–70.
Caravaca, F., Figueroa, D., Roldán, A., Azcón-Aguilar, C., 2003c. Alteration in rhizosphere soil properties of afforested *Rhamnus lycioides* seedlings in short-term response to mycorrhizal inoculation with *Glomus intraradices* and organic amendment. Environ. Manag. 31, 412–420.
Caravaca, F., Alguacil, M.M., Azcon, R., Diaz, G., Roldan, A., 2004. Comparing the effectiveness of mycorrhizal inoculation and amendment with sugar beet, rock phosphate and *Aspergillus niger* to enhance field performance of the leguminous shrub *Dorycnium pentaphyllum* L. Appl. Soil Ecol. 25, 169–180.
Caravaca, F., Alguacil, M.M., Azcón, R., Parladé, J., Torres, P., Roldán, A., 2005. Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate, and *Aspergillus niger*. Microb. Ecol. 49, 73–82.
Carbonell-Martin, G., Pro-Gonzalez, J., Aragonese-Grunert, P., Babin-Vich, M.M., Fernandez-Torija, C., Tarazona-Lafarga, J.V., 2011. Targeting the environmental assessment of veterinary drugs with the multi-species-soil system (MS·3) agricultural soil microcosms: the ivermectin case study. Span. J. Agric. Res. 9 (2), 433–443.
Chu, W.H., 2007. Optimization of extracellular alkaline protease production from species of Bacillus. J. Ind. Microbiol. Biotechnol. 34, 241–245.
Fernández, D.A., Roldán, A., Azcón, R., Caravaca, F., Baath, E., 2012. Effects of water stress, organic amendment and mycorrhizal inoculation on soil microbial community structure and activity during the establishment of two heavy Metal-Tolerant native plant species. Microb. Ecol. 63, 794–803.
García, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134.
Kennedy, I.R., Choudhury, A.T.M.A., Kecskés, M.L., 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Biol. Biochem. 36 (8), 1229–1244.
Kohler, J., Caravaca, F., Carrasco, L., Roldan, A., 2006. Contribution of *Pseudomonas mendocina* and *Glomus intraradices* to aggregate stabilization and promotion of biological fertility in rhizosphere soil of lettuce plants under field conditions. Soil Use Manag. 22, 298–304.
Kohler, J., Caravaca, F., Carrasco, L., Roldan, A., 2007. Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa*. Appl. Soil Ecol. 35, 480–487.

Kohler, J., Tortosa, G., Cegarra, J., Caravaca, F., Roldán, A., 2008. Impact of DOM from composted "alperujo" on soil structure, AM fungi, microbial activity and growth of *Medicago sativa*. Waste Manag. 28, 1423–1431.

Medina, A., Vassileva, M., Caravaca, F., Roldán, A., Azcón, R., 2004. Improvement of soil characteristics and growth of *Dorycnium pentaphyllum* by amendment with agrowastes and inoculation with AM fungi and/or the yeast *Yarowia lipolytica*. Chemosphere 56, 449–456.

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase, urease, proteases, organic-carbon, and nitrogen from soil. Soil Sci. Soc. Am. J. 44, 1011–1016.

Naseby, D.C., Lynch, J.M., 1997. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol. Biochem. 29, 1353–1362.

Ouahmane, L., Hafidi, M., Plenchette, C., Kisa, M., Boumezzough, A., Thioulouse, J., Duponnois, R., 2006. *Lavandula* species as accompanying plants in *Cupressus* replanting strategies: effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. Appl. Soil Ecol. 34, 190–199.

Rekha, P.D., Lai, W.A., Arun, A.B., Young, C.C., 2007. Effect of free and encapsulated Pseudomonas putida CC-FR2-4 and Bacillus subtilis CC-pg104 on plant growth under gnotobiotic conditions. Bioresour. Technol. 98, 447–451.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., Torn, M.S., 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant Soil 233, 167–177.

Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287, 15–21.

Schoebitz, M., Ribaudo, C.M., Pardo, M.A., Cantore, M.L., Ciampi, L., Curá, J.A., 2009. Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. Soil Biol. Biochem. 41, 1768–1774.

Schoebitz, M., Mengual, C., Roldan, A., 2014. 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. Sci. Total Environ. 466-467, 67–73.

SSS, 2010. Keys to Soil Taxonomy, 11th ed. USDA. Natural Resources Conservation Service, Washington DC.

Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (Eds.), Methods of Soil Analysis. ASA and SSSA, Madison, pp. 501–538.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem 1, 301–307.

Vassilev, N., Vassileva, M., Nikolaeva, I., 2006. Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl. Microbiol. Biotechnol. 71, 137–144.

Vassileva, M., Serrano, M., Bravo, V., Jurado, E., Nikolaeva, I., Martos, V., Vassilev, N., 2010. Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl. Microbiol. Biotechnol. 85, 1287–1299.

Wright, S.F., Anderson, R.L., 2000. Aggregate stability and glomalin in alternative crop rotations for the central. Gt. Plains Biol. Fertil. Soils 31, 249–253.

Zakry, F.A.A., Shamsuddin, Z.H., Rahim, K.A., Zakaria, Z.Z., Rahim, A.A., 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the N-15 isotope Dilution Technique. Microb. Environ. 27, 257–262.