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

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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.

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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 physico-chemical 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

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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),

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