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REGULAR ARTICLE

Success of long-term restoration of degraded arid land using native trees planted 11 years earlier

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Abstract
Background and aims Restoration of degraded desert soil with three species of legume trees and the giant cardon cactus was evaluated 11 years after planting in the southern Sonora Desert.
Methods The trees in six independent field experiments were grown individually or in combination of a legume tree and cardon cactus and were originally treated with plant growth-promoting bacteria, arbuscular mycorrhizal fungi, or small amounts of cattle compost or a combination of all treatments. Survival and height of trees and cacti and cactus biovolume were measured.
Results When data were combined from all experiments and analyzed together, the best survivor was the cardon cacti and, to a lesser extent, the legume tree mesquite amargo. Over a decade later, a combination of a legume tree with cardon cactus, while detrimental to the legume, significantly increased the chances of the cactus to survive and grow in degraded soil. The biotic and compost treatments, while enhancing the initial establishment of the plants in 2004, had only marginal benefit on the growth of cactus 11 years later.
Conclusions Long-term desert restoration with native trees is possible. Because this cactus is the native, long term soil stabilizer, a combination cactus-legume tree is recommended for long term desert restorations.

Keywords Cardon cactus · Desert · Mesquite · Plant growth-promoting bacteria · Restoration · Re-vegetation

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Introduction

Restoration of arid lands with native plants without frequent irrigation and based mainly on the natural precipitation is a prolonged process, which takes years. Success is measured in decades (Aronson et al. 1993; Le Houérou 2000, 2002; Bainbridge 2007; Bashan and de-Bashan 2010). Restoration of arid lands that is not used for agriculture, using plant growth-promoting bacteria (PGPB) alone or combined with mycorrhizae fungi (AM-fungi) was done experimentally on a relatively small scale in studies conducted mainly in pots under controlled or semi-controlled conditions (de-Bashan et al. 2012; Medina and Azcón 2012). Studies were mainly concentrated in southern Spain (Requena et al. 1997, 2001; Valdenegro et al. 2001; Medina et al. 2004a; b; Marulanda et al. 2009; Benabdellah et al. 2011; Armada et al. 2014a; b; Mengual et al. 2014a; b; Ortiz et al. 2015), Mexico (Puente and Bashan 1993; Bashan et al. 1999, 2009a, b, 2012; Carrillo-Garcia et al. 2000a; Toledo et al. 2001; Carrillo et al. 2002; Puente et al. 2004, 2009; Bacilio et al. 2006, 2011; Leyva and Bashan 2008; Lopez et al. 2012), west Africa (Founoune et al. 2002; Duponnois and Plenchette 2003), USA (Grandlic et al. 2008; de-Bashan et al. 2010a, b), Argentina (Felker et al. 2005) and India (Ramachandran and Radhapriya 2016). Because evaluation of success of field studies is a long-term task, there are fewer studies (Requena et al. 2001; Gao et al. 2002; Vovides et al. 2011; Lopez-Lozano et al. 2016).

In 2004, an arid degraded area (a road that was never built) failed to naturally re-vegetate for 25 years was chosen. It was restored using three common native legume trees and the giant cardon cactus, the dominant cacti of the Baja California Peninsula (Medel-Narváez et al. 2006). This was done with the help of two PGPB, *Azospirillum brasilense* (a non-specific PGPB, Pereg et al. 2016) and the desert PGPB *Paenibacillus* sp., along with unidentified consortium of desert mycorrhizae and small amounts of cattle compost that greenhouse experiments had shown improvements in growth of these plants (Bacilio et al. 2006; Bashan et al. 2009b). The six field experiments intended to reveal if, during the restoration program, any of these amendment helped establishment of legume trees (medium-term restoration) and for long term restoration by columnar cacti when the amendments were added alone or in combinations. Additionally, two planting configurations were tested, plants grown individually as single species and the same plant species growing as nurse plant of cardon cactus (~20 cm apart). The experiments were maintained for 61 months. The development of all plants was monitored every six months for 30 months. Inoculation with PGPB and compost amendment differentially supported growth of these plants but not all plant parameters are supported equally (Bashan et al. 2009a, 2012). Since 2009, the experimental field was unattended, except maintaining fencing against large grazers.

All four species are dominant plants in the southern Sonoran Desert. The three legume trees serve as nurse trees in natural revegetation in desert resource islands (McAuliffe 1984; Carrillo-Garcia et al. 1999). Resource islands serve as a hub of life, creating numerous micro-habitats that allow other plants to grow under their canopy; they are critical to the structure and function of the Sonoran Desert fauna community. For prevention of soil erosion and soil stabilization, the giant cardon cactus has a central role because it is widely distributed (Bashan et al. 2000; Medel-Narváez et al. 2006). It is probably the most massive cactus species, tree-shaped with up to 70 ascending branches, height of up to 20 m, and can weigh >25,000 kg. Its finely branched, shallow root system stabilizes desert soil at large distance from the plant (Nobel 1996; 1988). They are very long-lived (Delgado-Fernández et al. 2016), often several centuries, without significant natural afflictions (Bashan et al. 1995). This cactus and the mesquite trees are also known to positively respond to inoculation with a variety of PGPB, including *A. brasilense* and phosphate-solubilizing bacteria (Puente and Bashan 1993; Carrillo-Garcia et al. 2000a; Carrillo et al. 2002; Puente et al. 2004, 2009; Leyva and Bashan 2008) and to application of compost (Bacilio et al. 2006).

Periodic visual surveys of the experimental site over the years showed differential responses and survival among the four species (data not presented). Therefore, our hypothesis was that the long-term response and survival of the plants is affected by the inoculation/amendment treatments or planting configuration all performed over a decade earlier. The objectives of this study were: 1) to evaluate if desert restoration with native trees is possible and, 2) what are the most important parameters affecting such restoration over long period of time. As the underlying long-term goal of all experiments was restoration of degraded arid soils, only parameters relevant to this goal (survival of all species and size of the cactus, the long-term restorer), were done. Consequently, re-analysis of the six experiments regarding these parameters was done in 2015 and is presented here.

Materials and methods

Location

The restored area is 17 km northwest of the city of La Paz, Baja California Sur, Mexico at the southern limit of the Sonoran Desert (24°07′36″, 110°25′48″W). The climate is arid with mean annual precipitation of 180 mm, and in exceptionally rainy years, rainfall can reach over 300 mm, mainly from infrequent hurricanes and tropical storms during late summer. After greater rainfall, perennial vegetation is normally established in the Sonoran Desert (Drezner 2006). This alluvial coastal plain, derived from weathered granite, is a transition between xerophilic scrubland and dry tropical forest (León de la Luz et al. 2000). Soil physical-chemical characteristics and biological nitrogen fixation communities and properties were determined recently (Lopez-Lozano et al. 2016). The degraded area was cleared around 1980 for a road that was never paved. This area never recovered naturally.

Organisms, cultivation, planting, and inoculation

Four species of plants were originally used: the legume trees, mesquite amargo *Prosopis articulata* (S. Watson), yellow palo verde or foothill palo verde *Parkinsonia microphylla* (Torr.), blue palo verde or palo junco *Parkinsonia florida* (Benth. Ex A. Gray; S. Wats), and the giant cardon cactus *Pachycereus pringlei* (S. Wats Britt. & Ross). Three types of microorganisms were used for inoculation: the non-specific plant growth-promoting bacteria (PGPB) *Azospirillum brasilense* Cd (DSM 1843, Leibniz-Institut DMSZ, Braunschweig, Germany, Bashan 1990; Bashan et al. 2006), the phosphate-solubilizing bacteria (PSB) *Paenibacillus* sp. strain RIZO1 (FJ032016, GenBank of NCBI, later re-classified as *Bacillus pumilus* (de-Bashan et al. 2010a)) and AM fungi. The fungi were a consortium, mostly *Glomus* sp. and several unidentified native species found in resource islands under mesquite trees in the southern Sonoran Desert (Carrillo-Garcia et al. 1999; Bashan et al. 2000).

Production of AM fungi and compost, plant propagation in nurseries, preparation of the field, and special protections against herbivores were described in previous publications (Bashan et al. 2009a, b; 2012).

Microbial cultivation, inoculation and planting procedures in the field

In 2004, the two PGPB *A. brasilense* and *Paenibacillus* sp. were cultivated on tryptone–yeast extract–glucose medium supplemented with microelements (described in: Bashan et al. 2011, Bashan and de-Bashan 2015) for 24 h at 30 °C on a shaker at 120 rpm. The two bacterial species were formulated into dry microbead inoculants preparation made of alginate using specialized equipment (Bashan et al. 2002). In the nursery, this bacterial inoculant was attached to the seeds of wild trees as described for wheat plants (Bashan et al. 2002) at a level of 1.2×10^6 cfu·g^{-1} soil of *Paenibacillus* sp. and 1×10^6 cfu·g^{-1} soil of *A. brasilense*. Inoculants and compost for the field experiments were manually mixed into the local soil and maintained in 30 kg sacks at ambient temperature for about 2 weeks until used. In the field, each tree was transplanted into identical holes (25 cm deep, 40 cm in dia.) excavated by commercial garden excavator. About 80% of the soil removed from the hole was mixed with the bacterial and AM fungal inoculants and the compost and returned to the hole. Each planting hole received 6 kg of this inoculated soil at the day of planting (total 180 kg per treatment, 900 kg per experiment) (Bashan et al. 2009a; 2012). No additional inoculation treatment was given.

Experimental plan and field maintenance

The experimental plan of the six field trials was published in Bashan et al. (2012), (Supplementary material, Fig. S1). All experiments had a randomized block design with five blocks each with five replicates of each treatment. Planting was during a drought year (~50 mm rainfall). Therefore, rainfall was supplemented in that year and up to 2007 by irrigation to achieve an annual 300 mm in amounts and frequencies similar to natural rainfall and according to the multi-year average of monthly precipitation equivalent to 10–20 mm rain per month. The amount of water that the trees received in any given year, either from rainfall or irrigation, did not exceed 300 mm per year. The inoculation treatments were (1) inoculation with *A. brasilense* combined with the phosphate-solubilizing bacteria *Paenibacillus* sp., (2) inoculation with AM fungi, (3) application of a small amount of cattle compost at planting, (4) the three treatments combined, and (5) no inoculation. Two planting configurations were done; plants grown individually (1 m between plants) and plant grown in combination of legume tree and cactus seedling planted in the same hole (Fig. 1), as commonly occurs under natural conditions (Carrillo-Garcia et al. 1999; Suzán-Azpiri and Sosa 2006).

Measurements of survival of trees and cacti and cactus biovolume

During the rainy season (August–October) when trees are in leaf, and using the original metal rods installed at planting as markers for the different treatments, we measured survival of the plants, where plants with

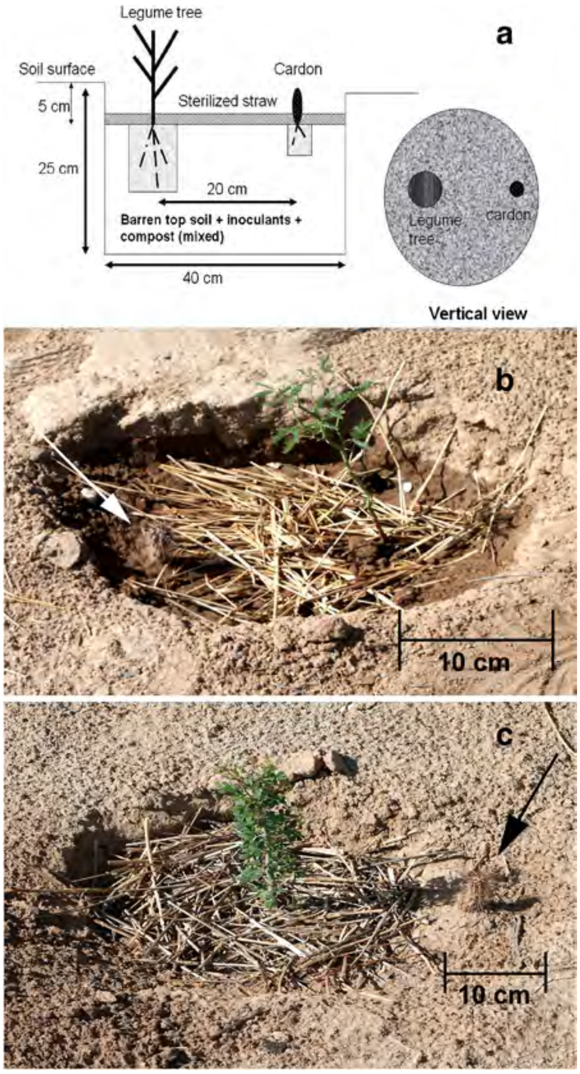


Fig. 1 The original planting hole design used for all trees in 2004. **a** schematic design; **b** after planting with mesquite amargo and cardon cactus (arrow); **c** the same with yellow palo verde

leaves or green cactus were considered surviving plants. Because cardon cacti are very slow growers (<10 cm·y^{-1}; Delgado-Fernández et al. 2016), the volume of a young columnar cactus is considered a true representation of plant size. Since it was impossible to uproot the plants for accurate volume determinations, an indirect calculation of volume was done, as described in an earlier study of this cactus (Bashan et al. 1999).

Statistical analysis

Plant survival for all experiments was analysed by one-way ANOVA and Tukey's post hoc analysis at $P < 0.05$, using Statistica 10 (Tibco Statistica, Palo Alto, CA) and presented as an average with standard error (SE). Effect of the inoculation treatments on plant survival across two planting configurations (alone and legume and cactus together) were analysed by principal component analysis (PCA), using Statistica 8.

Results

Survival of legume trees and cardon cactus 11 years after restoration

Of over 1500 trees that were planted in 2004 (Figs. 1 and 3a, b), survival largely depended on the plant species. Of the three legumes, mesquite amargo grown alone survived the best (>60% still growing in 2015). When planted with cardon, survival of mesquite amargo significantly declined to >20%. About 20% of yellow palo verde trees survived and grew when planted alone. Planted with cardon reduced their survival to ~15%. Blue palo verde did not survive, either alone or when planted with cardon. The cardon survived well when planted alone, where >70% of the original population was growing 11 years later. However, when planted with any of the legume trees, survival of cardons significantly increased to >90% (Fig. 2), along with a significant increase in growth (Fig. 3c–f).

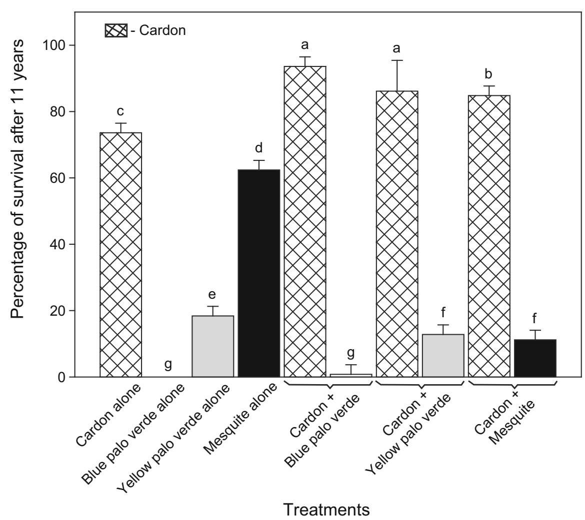


Fig. 2 Percentage of survival after 11 years of mesquite amargo, yellow palo verde, blue palo verde, and cardon cactus. Columns denoted by a different letter differ significantly by one-way ANOVA and Tukey's post hoc analysis at $P < 0.05$. Whiskers indicate SE

Fig. 3 Views of the field experiments taken during the rainy season. **a** Area abandoned for 25 years; **b** Planting in 2004; **c** Single planting blocks in 2006; **d** A block contains yellow palo verde and cardon cactus at five years after planting; **e** Mesquite population at seven years after planting; **f** Cardon plants growing alone at 11 years after planting

PCA showed a general pattern of survival, differentiating three groups: (1) legume trees, (2) mesquite, and (3) cardon (Fig. 4). The grouping was defined by two principal components (95.8% of explained variance) and by the effect of compost treatment (PCA loading = 0.75 in PC-1). The legume trees group (blue palo verde, yellow palo verde, and a few mesquites) had the lowest survival, whether planted alone or with cardon. The mesquite group (amargo mesquite) had moderate survival, whether planted alone or with cardon. The cardon group (cardon) had the highest survival, whether planted alone or with other plants. This analysis confirmed that survival of cardon was positively influenced by the nearby legume tree, specifically when planted with blue palo verde that had poor survival (Fig. 4).

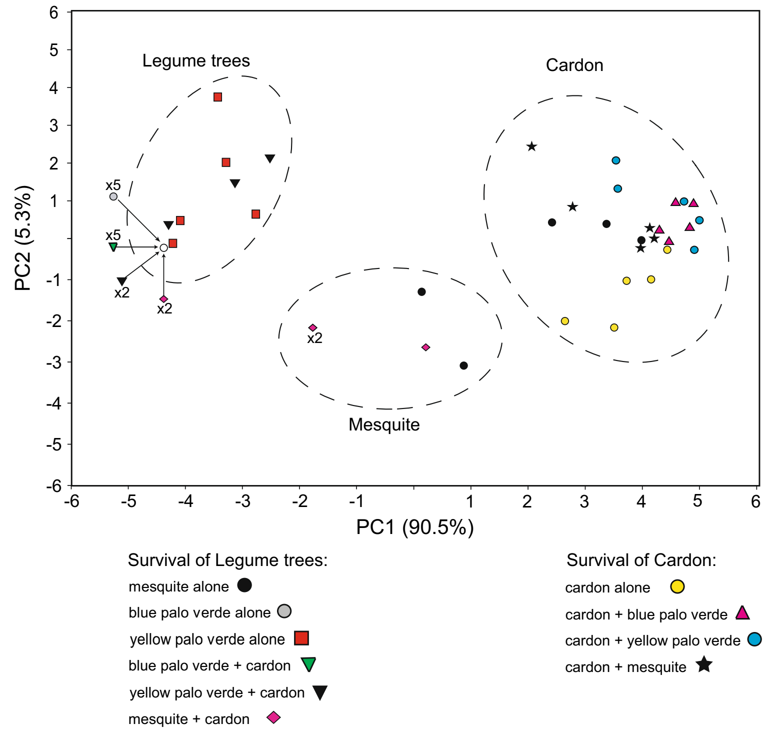
Effects of original biotic and abiotic treatments at planting on development of cardon cacti

In 2004, some of the plants were inoculated with PGPB + PSB, or AM fungi, or augmented with a small amount of cattle compost, or a mix of these. Analysis of the volume of the cacti 11 years after planting revealed that all cacti grew well (3000–12,000 $cm^3 \cdot plant^{-1}$). Few treatment combinations showed a lasting effect after 11 years. The most successful treatments were when cardon were planted with compost or inoculated with PGPB + PSB and growing alone (Fig. 5a) and when cardon were planted with blue palo verde and compost (Fig. 5d).

Discussion

Land clearing for seasonal agriculture, overgrazing, and wide spread rural urban development are major afflictions in the Mexican part of the Sonoran Desert. Combined, they cause land degradation, desertification, reduced soil fertility, and local climate changes (Bryant et al. 1990; Balling et al. 1998). Additionally, these negative impacts lead to invasive buffelgrass, which lowers net primary productivity of the land and reduces biodiversity of the desert (Franklin et al. 2006), and caused severe soil erosion (Bashan et al. 1999).

Fig. 4 Principal Component Analysis of survival of mesquite amargo, yellow palo verde, blue palo verde, and cardon cactus. Numbers indicate the number of plants contained in the corresponding spot



In the southern Sonoran Desert, mesquite trees and cardon cactus are the climax dominant plants (Carrillo-Garcia et al. 1999; León de la Luz et al. 2000). Soil erosion and bare land surfaces create dust pollution and are a major affliction in urban areas (Ortega-Rubio et al. 1998; Carrillo-Garcia et al. 2000b). The original restoration study (2004) addressed soil erosion using legume trees for medium-term restoration (30–50 years) and cardon cactus as long-term restoration (Bashan et al. 2009a, b; 2012).

We demonstrated that if fields were protected from grazing, without any maintenance or replacing of plants, and over more than a decade, two species distinguished themselves, the mesquite amargo and the cardon cactus. While most of the mesquite that we planted survived and thrived, most of the cardon performed outstandingly, only a small percentage died. Two patterns emerged: (1) It was possible to establish these two climax species with a variety of single treatments performed only at planting (Bashan et al. 2009a, b, 2012). The plants survived and grew for years to come. (2) The combination of a small nurse legume and a cactus seedling significantly increased the number of surviving cacti. While this combination seems detrimental to the nurse plant (the three species of legume), the long-term benefit of establishing a "forest" of giant cacti is greatly reduced soil erosion. Elimination of soil erosion by a population of several species of cacti was previously shown when cacti were inoculated with the PGPB *A. brasilense* over 3.5 years (Bashan et al. 1999).

We did not expect that the planting treatments (biotic and abiotic) would have lasting effects over a decade later. Surprisingly, when the cardon was planted alone in 2004, application of small amount of compost and inoculation with PGPB had a significant lasting effect a decade later. This was not observed with the nurse plant-cactus planting configuration that was tested in these field experiments. This configuration shows a plausible contribution of mineral and plant material from the nurse plant to the nursling. Such contribution was proposed earlier for nurse tree-nursling interaction (Carrillo-Garcia et al. 1999; Bashan and de Bashan 2010) and plant interactions in deserts (Cross and Schlesinger 1999; Li et al. 2008b). Increased volume of cardon cactus resulting from inoculation with the PGPB *A. brasilense* was observed for one year (Bashan et al. 1999); this study provided field evidence that a restoration program starting with larger plants as a result of PGPB inoculation has an advantage for survival of the plant over a prolonged period.

A recent analysis of functional restoration of this experimental area, 10 years after planting, showed that

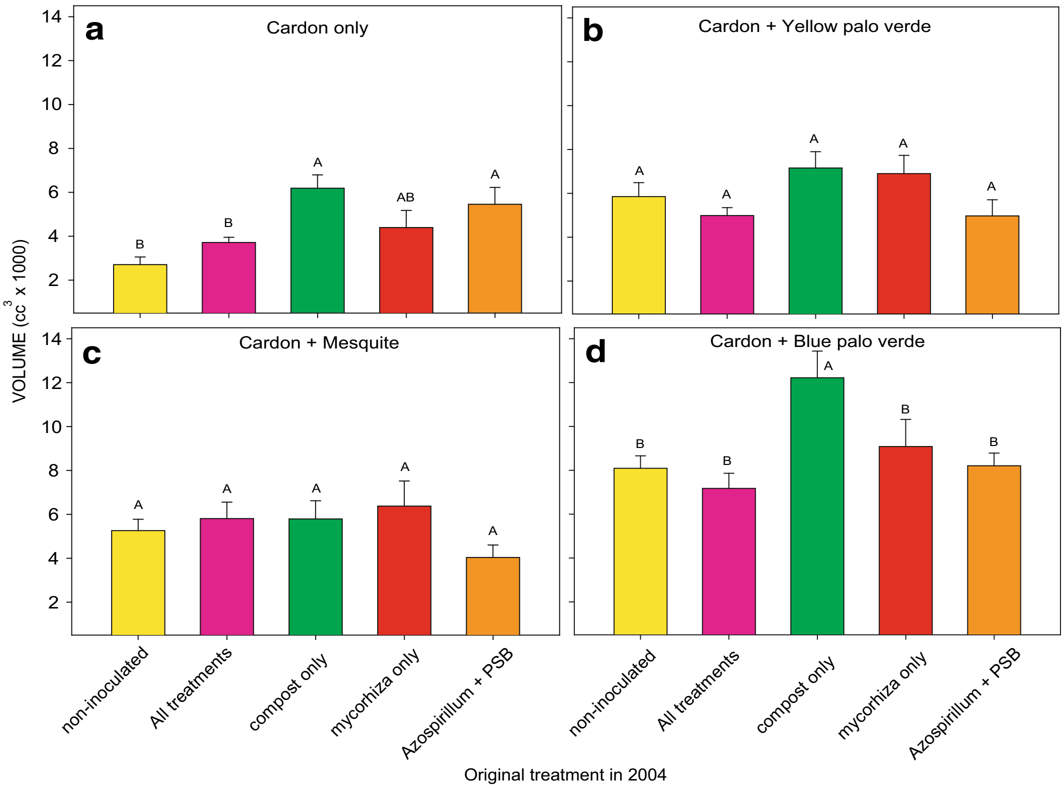


Fig. 5 Long-term effect of biotic and abiotic treatments applied in 2004 on the volume of cardon cactus in 2015. **a** Cardon growing alone; **b** Cardon growing with yellow palo verde; **c** Cardon growing with mesquite amargo; **d** Cardon growing with blue palo verde. Columns, in each subfigure, denoted by a different letter, differ significantly by one-way ANOVA and Tukey's post hoc analysis at $P < 0.05$. Whiskers indicate SE

surviving trees have a N_2-fixation potential similar to plants in undisturbed desert land, compared to highly disturbed land that remained untreated (Lopez-Lozano et al. 2016). It provides evidence that a reforestation of arid lands with native plants have the potential to restore, with time, the functional properties of the land. This functionality study is supported by a few field studies. (1) Arid marine mangroves trees in the southern Sonoran Desert showed functional recovery of N_2-fixation 12 years after reforestation. This time was sufficient to establish the community of functioning diazotrophic bacteria at the same levels as nearby preserved sites (Vovides et al. 2011). (2) In two long-term experiments (5 years) in a desertified semiarid ecosystem in southern Spain, inoculation of a native, key legume species with indigenous AM fungi and *Rhizobium* sp. enhanced the establishment of these plants over time, but also improved soil fertility (Requena et al. 2001). (3) In a shorter experiment (28 months) in southern Spain, inoculation of pine trees with a consortium of two PGPB and olive residue, one PGPB similar to the PGPB used in our study, significantly improved plant growth and improved soil fertility (Mengual et al. 2014a). (4) In the same area of Spain, inoculation of a native shrub with several PGPB and composted sugar beet had similar positive effects on plant and soil (Mengual et al. 2014b).

Considering the short terms field studies of 2.5 years (Bashan et al. 2009a; b; 2012), the restoration of nitrogen fixation functionality after 10 years (Lopez-Lozano et al. 2016), and the current study of 11 years, we propose three avenues for soil restoration in this desert region. (1) Protection against large grazing animals (low metal fence in our study) is paramount for long-term success. All previous experiments done in our institute over decades that lacked fencing failed. (2) Climax plants can be established directly with the aid of several amendments at planting time. Once a plant population is established, they will continue to grow, based solely on natural precipitation, and minerals deposited by common dust storms (Li et al. 2008a). (3) If long-term restoration of eroded soil by long-lived plants is the main goal, combining a legume tree that will not survive the restoration period with a cardon cactus provides the optimum solution.

In summary, long-term evaluations of six field experiments to restore degraded desert soil showed that soil restoration with native legume trees and cacti is possible. Because this giant cactus is the native, long term soil stabilizer in the southern Sonoran Desert, a combination cactus-legume tree is recommended for long term desert restorations.

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Compliance with ethical standards

Dedication This study is dedicated for the memory of the German/Spanish mycorrhizae researcher, Dr. Horst Vierheilig (1960–2011) of CSIC-Granada, Spain.

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