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The Role of Relict Vegetation in Maintaining Physical, Chemical, and Biological Properties in an Abandoned *Stipa*-Grass Agroecosystem

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A study of rhizosphere physical-chemical and biological properties for dominant vegetation, including Stipa tenacissima *and* Rosmarinus officinalis*, and for relict natural vegetation, namely* Olea europaea *subsp.* sylvestris, Pistacia lentiscus, Retama sphaerocarpa*, and* Rhamnus lycioides*, was carried out in an abandoned agricultural soil from a semiarid Mediterranean area. Rhizospheres of* R. sphaerocarpa *and* S. tenacissima *had the highest concentration of total N. Rhizospheres of* R. sphaerocarpa *and* R. lycioides *showed the highest soluble C-fraction values (water soluble C and water soluble carbohydrates). The highest percentages of stable aggregates were recorded in the rhizospheres of* P. lentiscus *(about 69%) and* S. tenacissima *(about 79%)*. O. europaea *and* R. sphaerocarpa *had the highest capacity to enhance the development of mycorrhizal propagules in their rhizospheres.*

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The mycorrhizal potential of S. tenacissima *was on average 3.2-fold lower than that of* O. europaea *and* R. sphaerocarpa*, which points to the necessity of reconstituting it prior to carrying out revegetation processes.*

Keywords aggregate stability, AM fungi, labile C fractions, mycorrhizal potential, semiarid Mediterranean areas

Stipa tenacissima L. (esparto grass) cropping, based on the removal of natural vegetation, was a common agricultural practice in southeastern Spain. After abandonment of these lands, dwarf shrubs and tall grass species grow, following a patchy distribution, with a sparse plant cover. The vegetation patches commonly constitute "fertility islands" or "resource islands" (Schlesinger et al., 1996), where facilitation among plants may be widespread. Recent studies have described the beneficial effect of *S. tenacissima* tussocks on their own microenvironment through self-promoting changes in microclimate, soil structure, water infiltration, and organic matter inputs in relation to between-tussock areas (Cerdà, 1997; Bochet et al., 1999). In addition, it has been recently reported that *S. tenacissima* tussocks can facilitate the introduction of tall shrub species (Maestre et al., 2001), thus it can act as a "nurse plant," as described by Carrillo-García et al. (1999). Several authors have also demonstrated that shrub legume species such as *Retama sphaerocarpa* (L.) Boiss., which is common in semiarid environments of southeastern Spain, are able to increase soil fertility (Moro et al., 1997). Reestablishment of the natural shrubland in revegetation program for abandoned agricultural lands has recently been encouraged by the agricultural policies of the European Union as a means for regenerating the biodiversity of these areas. The presence, during several decades, of *Stipa* croppings has led to changes in soil physical-chemical and biological properties, which need to be assessed prior to revegetation practices.

Mycorrhizal symbioses are key ecosystem factors governing the cycles of major plant nutrients and hence in sustaining a vegetation cover in natural habitats (Jeffries & Barea, 2000). There is evidence that mycorrhizae help plants to thrive in arid conditions (Nelson & Safir, 1982) by increasing the supply of nutrients to the plant (particularly P) (Querejeta et al., 1998), improving soil aggregation in eroded soils (Caravaca et al., 2002) and reducing water stress (Augé, 2001). In degraded soils from desertification threatened areas in typical Mediterranean ecosystems, the inoculum potential of mycorrhizal mutualistic microbial symbionts may disappear or, at least, be severely depleted (Requena et al., 1996). The loss or depletion of the mycorrhizal potential in degraded areas can preclude any natural or tailored process of revegetation. Thus, it may be necessary to reinforce or replace it by appropriate mycorrhizal inoculation technologies in order to guarantee success in the establishment of plants under marginal growth conditions (Requena et al., 1996). A rehabilitation approach for revegetation of these areas must begin with the evaluation of the mycorrhizal status of the soil (Requena et al., 1996). However, there is no previous assessment for *S. tenacissima* regarding its potential for providing mycorrhizal propagules or as a basis to produce arbuscular mycorrhiza (AMF) inoculum for selected plant species to be used in the revegetation strategies.

Soil structural stability plays an important role in the control of erosion in semiarid areas and in the implementation of vegetation cover restoration programs, since it greatly affects plant growth and development (Caravaca et al., 2002). Likewise, plant cover contributes to the formation and stability of soil aggregates by supplying organic matter from plant remains. In this way, it is necessary to ascertain the changes in the stability of aggregates in relation to vegetation degradation.

The objective of this study was to assess structural stability, nutrient concentration, water soluble carbon fractions and mycorrhizal status changes in the rhizosphere of *S. tenacissima* and *Rosmarinus officinalis* L., these species being the dominant vegetation of an agricultural semiarid Mediterranean area after its abandonment. This was done by comparison with the rhizosphere physical-chemical and biological properties of the relict natural vegetation from this area, selecting as target species four indigenous Mediterranean shrub species, namely *Olea europaea* L. subsp. *sylvestris* L., *Pistacia lentiscus* L., *R. sphaerocarpa*, and *Rhamnus lycioides* L. Also, it was determined whether the mycorrhizal potential of the target species could be exploited as a source of AMF inoculum in revegetation programs.

Materials and Methods

Study Area and Target Plant Species

The study area was located on the El Picarcho range in the Province of Murcia (southeastern Spain) (1°10′W and 38′23′N, 320 m elevation). The predominant soils are Petrocalcic Xerosol, Petric Calcisol, and Haplic Calcisol types (FAO, 1988) developed from limestones with a silt loam texture. The climate is semiarid Mediterranean with an average annual rainfall of 312 mm and a mean annual temperature of 15.3°C; the potential evapotranspiration reaches 813 mm y^{-1}. The topography of the area is mainly flat, and slopes do not exceed 6%. The climax vegetation was dominated by shrublands of *O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa*, and *R. lycioides*, which were selected as target species. The plant cover is sparse (less than 20% canopy cover) and degraded due to ancient grazing and logging. In this area, dwarf shrubs (<1 m high) such as *R. officinalis* and *S. tenacissima* grass are very common (constituting more than 98% of plant cover in this area). Bare soil surfaces are abundant between the patches of plants.

Sampling and Laboratory Procedures

A field sampling survey was carried out in spring 1999. For this, 30 individual plants similar in size (five replicates for each of the four target shrub species and for the *Stipa* and *Rosmarinus* plants) were randomly chosen in a homogenous area from the El Picarcho site, measuring approximately 4 ha. One rhizosphere soil sample per individual plant was collected, each sample consisting of five bulked subsamples (200 cm^3 soil cores) randomly collected at 0 to 20 cm depth. Samples of bare soil were also taken in the same way.

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous extract. Total nitrogen was determined by the Kjeldhal method (Jackson, 1960), and the total organic C according to Yeomans and Bremner (1989). Available P, extracted with Na-bicarbonate, was determined by spectrophotometry by using Kontron Uvikon 940, according to Murphy and Riley (1962). Extractable (with NH_4^+ -acetate) K^+ was determined by flame photometry.

In aqueous soil extracts, water soluble carbon (WSC) was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm (Sims & Haby, 1971). Water soluble carbohydrates were determined by the method of Brink et al. (1960).

The percentage of stable aggregates was determined according to Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.250 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 mL with an energy of 270 Jm^{-2}. The remaining soil on the sieve was put in a previously weighed capsule (T), dried at 105°C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.250 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105°C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by $(P1 - P2) \times 100/(4 - P2 + T)$.

For measurement of the mycorrhizal potential in these soil samples, a dilution technique (Sieverding, 1991) was followed. This method allows calculation of the most probable number (MPN) of mycorrhizal propagules able to develop colonization units on the root of a test plant.

Statistical Analysis

The data were tested for normality and subjected to analysis of variance, and comparisons among means were made using the Least Significant Difference (LSD) test, calculated at $P < 0.05$. Statistical procedures were carried out with the software package Statgraphics (1993).

Results and Discussion

Physical-Chemical Parameters

The pH of the soil under the different types of vegetation and bare soil ranged from 7.78 to 8.08 (Table 1), which is typical for calcareous soils. The highest electrical conductivity value was observed in soil beneath *R. sphaerocarpa* ($0.046\,S\ m^{-1}$), although this value would not limit its growth.

Soil beneath *R. sphaerocarpa* and *S. tenacissima* had the highest concentration of total N (Table 1). The beneficial effect of *R. sphaerocarpa* on soil fertility, mainly on total N, is related to the ability of leguminous species for fixing nitrogen. The highest available phosphorous concentration was found in soil beneath *R. sphaerocarpa* and *R. lycioides* shrubs. Leguminous species are important because their associated rhizobial symbioses constitute a source of N input to the ecosystem. Recently, Requena et al. (2001) have described an improved N status of nonleguminous plants grown in association with legumes, for natural plant communities in a semiarid

TABLE 1 Physical-Chemical Parameters and Nutrients Content in the Rhizosphere Soil of the Four Target Shrub Species (*O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa*, and *R. lycioides*), in the Rhizosphere Soil of *S. tenacissima* and *R. officinalis* and in the Bare Soil

Plant species	pH (H_2O)	EC ($S\ m^{-1}$)	Total N ($g\ kg^{-1}$)	P avail ($mg\ kg^{-1}$)	K extr ($mg\ kg^{-1}$)
Olea europaea subsp. *sylvestris*	7.98bc*	0.030bc	2.5b	3a	657ab
Pistacia lentiscus	8.04c	0.034cd	1.8a	2a	579ab
Retama sphaerocarpa	7.78a	0.046e	3.6c	4b	672ab
Rhamnus lycioides	7.86ab	0.038d	2.2ab	5b	876b
Rosmarinus officinalis	8.08c	0.020a	2.8b	3a	766ab
Stipa tenacissima	7.98bc	0.026ab	3.7c	3a	751ab
Bare soil	8.08c	0.026b	2.8b	3a	313a

*Values sharing the same letter in vertical columns are not significantly different ($P < 0.05$) by the LSD test.

EC = electrical conductivity; P avail = P available; K extr = K extractable.

TABLE 2 Total Organic Carbon and Carbon Fractions in the Rhizosphere Soil of the Four Target Shrub Species (*O. europaea* subsp. *sylvestris*, *P. lentiscus*, *R. sphaerocarpa*, and *R. lycioides*), in the Rhizosphere Soil of *S. tenacissima* and *R. officinalis* and in the Bare Soil

Plant species	TOC (g kg^{-1})	WSC (mg kg^{-1})	WSCH (mg kg^{-1})
Olea europaea subsp. *sylvestris*	27.4b*	92a	7a
Pistacia lentiscus	19.9a	86a	7a
Retama sphaerocarpa	26.5b	151b	25c
Rhamnus lycioides	34.2c	216c	23c
Rosmarinus officinalis	18.2a	65a	16b
Stipa tenacissima	26.5b	104a	18b
Bare soil	18.4a	62a	17b

*Values sharing the same letter in vertical columns are not significantly different ($P < 0.05$) by the LSD test.

TOC = total organic carbon; WSC = water-soluble carbon; WSCH = water-soluble carbohydrates.

ecosystem. The legumes also form a symbiosis with arbuscular mycorrhizal fungi (Requena et al., 1996), which increases the supply of nutrients to the plant, particularly N and P. Increased soil fertility, due to a canopy of *Retama* shrubs, has been also reported by Moro et al. (1997). Thus, the reestablishment of leguminous plants should be a key step in revegetation strategies. The extractable K^+ did not vary with the type of vegetation. There were only differences in the extractable K^+ between the soil under *R. lycioides* and bare soil, the lowest concentration being in the bare soil.

The main input of organic matter to soil is plant litter, decaying aerial parts and roots and rhizomes that remain in the soil. Other sources are polysaccharides, excreted by roots or resulting from microbial and animal activities. The highest total organic carbon (TOC) concentration was found in the soil under *R. lycioides*, followed by the soil under *O. europaea*, *R. sphaerocarpa,* and *S. tenacissima* (Table 2).

The water-soluble organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins, and low molecular weight organic acids. This fraction can be used as carbon and energy sources for soil microflora and may also have a structural function (Roldán et al., 1994). The soil under *R. sphaerocarpa* and *R. lycioides* shrubs showed the highest soluble C-fraction values (WSC and water-soluble carbohydrates, WSCH), suggesting a high biological activity associated with the rhizosphere of such shrubs (De Luca & Keeney, 1993).

Soil Aggregate Stability

The revegetation of degraded ecosystems must be carried out with plants selected on the basis of their ability to survive and regenerate in the local environment, and on their ability to stabilize the soil structure (Caravaca et al., 2002). The agents responsible for aggregate stability are mainly organic, and hence biological, in origin and are usually developed in the rhizosphere (Oades, 1993). The mechanisms involved in stabilizing aggregates are based on the enmeshment of soil particles by hyphae and roots, and the exudation of polysaccharides (Bearden & Petersen, 2000). According to Roldán et al. (1994), the binding effect of polysaccharides is short-lived and the maintenance and increase of aggregate stability is attributable to the

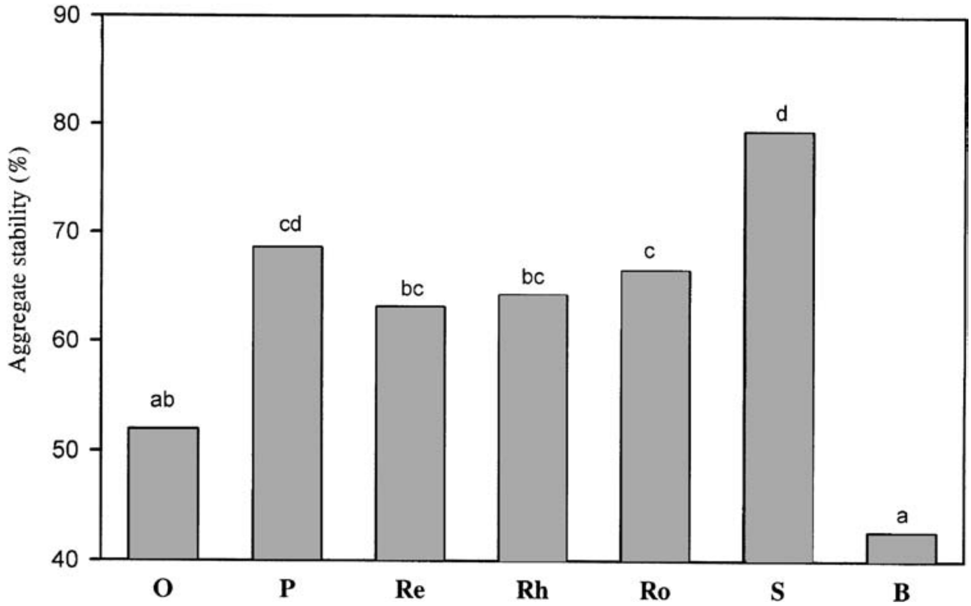

FIGURE 1 Percentage of stable aggregates in the rhizosphere soil of the four target shrub species (O = *Olea europaea* subsp. *sylvestris*; P = *Pistacia lentiscus*; Re = *Retama sphaerocarpa*; Rh = *Rhamnus lycioides*), in the rhizosphere soil of *Stipa tenacissima* (S) and *Rosmarinus officinalis* (Ro) and in the bare soil (B). Values with the same letter are not significantly different at $P < 0.05$, according to the LSD test.

increases in microbial populations, and particularly to the proliferation of fungal mycelia. The symbiosis between AMF and plants has been shown to increase the stability of soil aggregates (Bearden & Petersen, 2000). Mycorrhizae primarily influence the stability of macroaggregates (> 250 μm) (Bearden & Petersen, 2000). Recent studies have also indicated that AMF produce a glycoprotein, glomalin, that acts as an insoluble glue to stabilize aggregates (Wright & Anderson, 2000).

The present study also confirmed the influence of plant cover on soil aggregate stability as shown by Cerdà (1998). *S. tenacissima* and *P. lentiscus* had the highest percentage of stable aggregates, while *O. europaea* developed soils with aggregates less stable than the *Rosmarinus officinalis* dwarf shrub (Figure 1). The most unstable soil was the bare soil. *S. tenacissima* develops soils with high aggregate stability because it is a plant species with a graminoid type, very dense root system (Cerdà, 1997).

Mycorrhizal Potential

The four test shrub species, growing in patches in the target ecosystem, maintained a higher population of infective mycorrhizal propagules in their rhizosphere with respect to bare soil (Figure 2). However, these four species differed in their capabilities to enrich the soil with infective mycorrhizal propagules, with *R. sphaerocarpa* and *O. europaea* having a higher capacity to enhance the development of AMF propagules in their rhizospheres than *R. lycioides* and *P. lentiscus*. This can be explained by considering that the first two species form *Arum*-type mycorrhizae, while *R. lycioides* and *P. lentiscus* form those of the *Paris*-type. As is well known, *Paris* mycorrhizae, which are common in Mediterranean ecosystems (Bedini et al.,

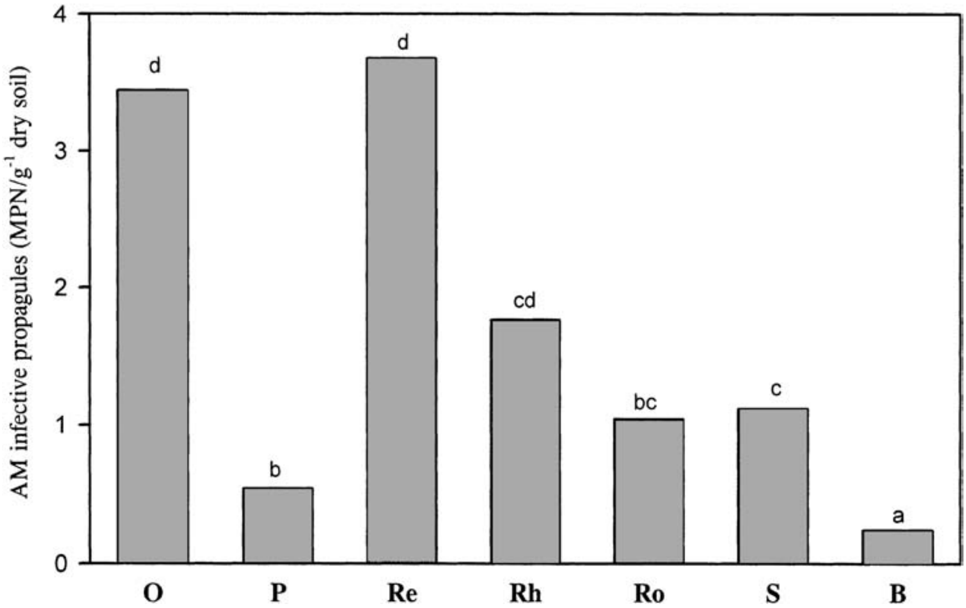

FIGURE 2 Most probable number (MPN) of mycorrhizal propagules in the rhizosphere soil of the four target shrub species (O = *Olea europaea* subsp. *sylvestris*; P = *Pistacia lentiscus*; Re = *Retama sphaerocarpa*; Rh = *Rhamnus lycioides*), in the rhizosphere soil of *Stipa tenacissima* (S) and *Rosmarinus officinalis* (Ro) and in the bare soil (B). Values with the same letter are not significantly different at $P < 0.05$, according to the LSD test.

2000), use most of their available carbon sources to support their particular intraradical development, to the detriment of the extraradical development and sporulation. Results from the present study suggest an important ecological function of the mycotrophic *O. europaea* and *R. sphaerocarpa* species in providing available AMF propagules as a source of inoculum. The AMF strains isolated from the rhizospheres of these shrub species can be considered "ecotypes" (Jeffries & Barea, 2000), which are physiologically and genetically adapted to the whole environment of the desertification-threatened Mediterranean ecosystems in southeastern Spain. These native AM fungal species could be more effective than introduced species, with respect to improving plant growth and nutrition (Requena et al., 2001).

The rhizosphere of *Stipa* shows a low mycotrophic habit (Figure 2), suggesting that its inoculum potential of AM fungi is inadequate to support either natural or directed revegetation processes.

In conclusion, our results confirm that the abandonment of agricultural soils from Mediterranean ecosystems has caused a change in the soil physical-chemical and biological properties, and a clear decrease in mycorrhizal activity. The mycorrhizal potential of some shrub species from the natural succession in these areas could be exploited as a source of inoculum for plant seedlings in revegetation programs.

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