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Effects of mycorrhizal inoculation of shrubs from Mediterranean ecosystems and composted residue application on transplant performance and mycorrhizal developments in a desertified soil

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Abstract Arbuscular mycorrhizal inoculation and composted residue application are being assayed to aid restoration of desertified areas under Mediterranean climate. The particular objective of the present study was to assess the short-term (8 months) effects on the initial stages of plant performance and on mycorrhizal propagule release, key factors to decide further developments in the restoration process. Mycorrhizal inoculation, with *Glomus intraradices*, was practised during nursery production of representative shrub species from Mediterranean ecosystems and composted residues were added to soil before transplanting to a desertified area in southern Spain. *Pistacia lentiscus, Rhamnus lycioides, Olea europaea* subsp. *sylvestris* and *Retama sphaerocarpa,* key species from the natural succession in the target area, were the test plants. Mycorrhizal inoculation, and in some cases compost addition, improved the ability for nutrient acquisition by plants upon transplanting in the field. The number of "infective" mycorrhizal propagules was higher in soil around mycorrhiza-inoculated shrubs than that around the corresponding non-inoculated controls. The organic amendment significantly increased propagule production in the rhizosphere of mycorrhiza-inoculated plants. The number of mycorrhizal spores was relatively low in soil around transplants, being hardly affected by treatments. Only three distinguishable glomalean spore morphotypes were found, belonging to the species *Glomus geosporum, G. contrictum* and *Scutellospora calospora*, with very few unidentified spores, corroborating the low diversity in degraded ecosystems. An increased development of the extramatrical AM mycelium was found in soil around the roots of the four mycorrhiza-inoculated test plants, probably the main source of AM fungal propagules in the ecosystem at this stage of plant development. In conclusion, the tailored AM inoculation assayed was functioning under field conditions to enhance nutrient acquisition by the target indigenous shrubs and, in interaction with organic amendments, promoted mycorrhizal propagule production in soil, critical factors to benefit further stages of the revegetation process.

Keywords Arbuscular mycorrhiza · Organic amendments · Degraded Mediterranean ecosystems · Restoration · Revegetation strategies

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Introduction

Mediterranean ecosystems are defined by a set of characteristic climate conditions, such as a scarce and irregular rainfall and a long dry and hot summer. This multiple stress situation, together with uncontrolled man-mediated activities, is a constraint for plant development and may promote desertification in these ecosystems, as for example in southern Spain (Francis and Thornes 1990; Albaladejo et al. 1996). Losses in natural plant communities are known to occur concomitantly with generalised damages of physical-chemical and biological soil properties (Kennedy and Smith 1995; García et al. 1997; Albaladejo et al. 1998). In particular, soil organic matter losses and reductions in the belowground microbial diversity and/or activity are usually affected by the degradation of plant cover due to desertification (Jeffries and Barea 2001). Among other microbial groups, mycorrhizal fungi suffer from soil disturbance (Jasper et al. 1991). This has been demonstrated to occur in degraded Mediterranean ecosystems (Requena et al. 1996). Because mycorrhizal symbioses are known to enhance the ability of plants to establish and cope with stress situations and organic mat-

ter is a key factor for quality and productivity, losses or diminution of these two soil components may limit the successful reestablishment of native plants (Requena et al. 2001). Therefore, mycorrhizal inoculation and the addition of organic amendments to soil must be considered for the restoration of degraded areas.

In the context of revegetation programmes currently being developed for the restoration of desertification-threatened areas in southeast Spain (Francis and Thornes 1990), mycorrhizal inoculation and organic amendments are being assayed to improve transplant performance (Roldán et al. 1997; Vallejo et al. 1999; Requena et al. 2001). Drought-tolerant, deep-rooting shrub species are used in restoration strategies for degraded areas in these environments and, as Requena et al. (1996) have shown, most of the native shrub species in these communities form arbuscular mycorrhiza (AM). Because the effectiveness of organic amendments greatly depends on their stability, it has been recommended that organic matter should be composted before being applied to soil. This will facilitate biological transformations of this material and avoid the presence of low molecular weight compounds able to display phyto-toxic activities (Gliotti et al. 1997).

As any restoration approach, this research programme is aimed at long-term objectives, which include the achievement of beneficial effects not only on plant development but also on the fertility and quality of the degraded soil. However, and because the realisation of some short-term benefits is critical to assure further developments in the revegetation process, early effects must be first ascertained. The particular objective of the present study was, therefore, to assess the short-term (8 months) effects of mycorrhizal inoculation, practised during nursery production of representative shrub species from Mediterranean ecosystems, together with the addition of composted residues at transplanting to a desertified area. The response variables to be tested were growth and nutrient acquisition by the field-grown plants, and the release of mycorrhizal propagules in soil around their root system.

Materials and methods

Study areas and target plant species

A representative area within a desertified semi-arid ecosystem was chosen at the El Picarcho site, Murcia, southeastern Spain. This experimental field site is located at 320 m above sea level and its co-ordinates are 1°10′W, 38°23′N. The predominant soils in the area are Haplic Calcisols and Petric Calcisols developed from limestone with a sandy loam texture (FAO 1988). The average annual precipitation is 280 mm, the mean annual temperature is 16.5°C, and the potential evapotranspiration reaches 900 mm y^{-1}. The topography of the area is mainly flat and slopes do not exceed 6%. The plant cover is sparse and degraded, not only because of the climatic conditions but also because of uncontrolled grazing.

Four representative shrub species from the experimental environment, and in general from semi-arid shrublands in southeastern Spain, namely *Pistacia lentiscus* L., *Rhamnus lycioides* L., *Olea europaea* L. subsp. *Sylvestris* L. and *Retama sphaerocarpa* (L.) Boiss, were selected as target shrub species. These are well adapted to water stress conditions and, therefore, frequently used for revegetation of semi-arid disturbed lands under a Mediterranean climate.

Nursery production of mycorrhizal plantlets

Nursery procedures were conducted at a commercial nursery company (Paisajes del Sur, Granada, Spain). The mycorrhizal fungus used was *Glomus intraradices* (BEG no. under registration process) and the AM inocula consisted of a mixture of rhizospheric soil from a pure pot culture containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1:1, v:v) mixed (5%) with *G. intraradices* inoculum. The same amount of the autoclaved mixture of the inocula was used for control plants, supplemented with a filtrate (<20 μm) of culture to provide the microbial populations accompanying the mycorrhizal fungi. Inoculated and non-inoculated seedlings were grown for 8 months under nursery conditions without any fertilisation treatment, before field transplanting.

Organic amendments

The organic residue used was the organic fraction of a municipal solid waste obtained from a municipal waste treatment plant in Murcia, Spain. The composted residue was mechanically produced by fast fermentation (60 days), mixing the waste heap daily under aerobic conditions. The analytical characteristics of the composted residue, determined by standard methods (Page et al. 1982), are shown in Table 1.

Experimental design and layout

The experiment consisted of a randomised block design with two factors and four replicate blocks. The first factor was the addition or not of composted organic residue to the soil, and the second factor was mycorrhizal inoculation or not of the test seedlings in the nursery. Thus, plants were grown in the field as affected by four experimental variables: (1) control soil (C); (2) composted residue (R) addition to soil before transplanting; (3) mycorrhiza (M) inoculation in the nursery; and (4) composted residue application and mycorrhizal inoculation (RM). An area of 1,200 m^2 was mechanically prepared with a sub-soiler. Eight rows (1 m wide, 25 m long, 3 m apart) were established. Half of the rows were amended following the randomised design with the composted residue (0–20 cm depth) at a rate of 6.7 kg m^{-2}, which is sufficient

Table 1 Analytical characteristics of the composted residue used in the experiment

Ash (%)	44.8
pH (1:10)	6.7
Electrical conductivity EC (1:5, mS cm^{-1})	4,700
Total organic C (g kg^{-1})	276
Water-soluble C (mg g^{-1})	1,950
Water-soluble carbohydrates (mg g^{-1})	76
Total N (g kg^{-1})	14.5
$N\text{-}NH_3$ (mg g^{-1})	3,350
Total P (g kg^{-1})	3.8
Total K (g kg^{-1})	12
Cu (mg g^{-1})	146
Zn (mg g^{-1})	261
Ni (mg g^{-1})	25
Cr (mg g^{-1})	63
Cd (mg g^{-1})	5
Pb (mg g^{-1})	98

to raise the total soil organic carbon content by 1%. Three weeks after the addition of the compost, plantlets from the four shrub species selected, either inoculated or non-inoculated, were planted in individual holes, at least 1 m apart in a single row and 3 m between blocks. At least, 40 plantlets per shrub species and replication block were planted (10 plants from each species × 4 treatments in each block).

Sampling procedures

Eights months after transplanting, individual plants similar in size (five replicates per each target shrub species, block and treatment) were randomly chosen. Once plants were harvested, shoot biomass was determined after drying for 48 h at 60°C and shoot tissues analysed for N, P and K (Lachica et al. 1973). Representative samples of soil associated to feeder roots developed at a 5–20 cm depth were taken for each individual plant.

Mycorrhiza determinations

The percentage of root length that became mycorrhizal was microscopically assessed in a representative root aliquot by using the gridline intersect method of Giovannetti and Mosse (1980), after clearing and staining the root samples with trypan blue (Phillips and Hayman 1970).

The mycorrhizal potential in soil samples from around the root systems was measured by a dilution technique (Brundrett 1966). This method involves calculation of the most probable number (MPN) of mycorrhizal propagules able to develop colonisation units on the root of a test plant.

The extent of the extraradical AM mycelium was measured by the combination of the filtration and gridline methods of Jones and Mollison (1948), Newman (1966) and Miller et al. (1995), using trypan blue for staining the AM hyphae.

AM fungal spores were extracted from soil by wet sieving and decanting, followed by sucrose centrifugation (del Val et al. 1999). After centrifugation, the supernatant was poured through a 50-µm mesh and quickly rinsed with tap water. Spores were counted using a Doncaster dish under the dissecting microscope, and grouped according to morphological characteristics. Permanent slides were prepared for each different spore morphotype using polyvinyl-alcohol and polyvinyl-alcohol plus Melzer's solution (1:1). After confirming the uniformity of the morphological groups under the optical microscope, the different morphotypes were identified to genus and, when possible, to species level. Spore identification was mainly based on spore size and colour, wall structure and hyphal attachment (Walker 1983; Morton and Benny 1990; Schenk and Perez 1990; INVAM 1997).

Statistical analysis

Analysis of variance (ANOVA) were used to test differences and, when appropriate, Fisher's Protected Least Significant Differences Test was used for comparison of means.

Results

At the end of the nursery period mycorrhiza-inoculated seedlings were slightly larger than non-inoculated ones, although differences in size were not statistically significant. Most of the plantlets established with survival rates ranging from 90–100%, independently from the mycorrhizal or compost amendment treatments applied.

As Table 2 shows, plants inoculated in the nursery, display a considerable degree of mycorrhizal colonisation after 8 months growing in the field, while non-mycorrhizal transplants show low levels of mycorrhization. Addition of the composted residue did not change the mycorrhizal colonisation level in plants whether or not these were inoculated in the nursery before transplanting. Plant growth was improved by mycorrhizal inoculation but this effect was statistically significant only for *O. europea* and *R. lycioides.* Compost application tended to increase plant growth but not significantly (Table 2).

Table 2 Effect of mycorrhizal (*M*) inoculation during nursery production of representative plant species from a Mediterranean ecosystem, and composted residue (*R*) application on transplanting to a desertified area, on mycorrhiza formation and plant growth after 8 months under field conditions. For each plant species and parameter, *values sharing a letter in common* do not differ significantly ($P < 0.05$) by the test of the Least Significant Difference of Fisher

Plant/Treatment	Mycorrhizal root length (%)	Shoot height (cm)	Shoot dry weight (g)
Pistacia lentiscus			
Control	7a	23.9a	2.0a
M	37b	27.8a	2.4a
R	8a	23.9a	2.0a
RM	47b	33.0a	2.9a
Retama sphaerocarpa			
Control	13ab	30.0a	0.7a
M	33bc	41.9a	0.8a
R	6a	35.1a	0.6a
RM	37c	44.4a	1.0a
Olea europeae			
Control	15a	29.1a	1.5a
M	65b	45.2b	4.0b
R	14a	35.9ab	1.7a
RM	65b	59.5c	4.2b
Rhamnus lycioides			
Control	1a	22.9a	0.9a
M	48b	32.0ab	1.9b
R	2a	24.0a	0.8a
RM	38b	44.8b	2.3b

Mycorrhizal inoculation significantly increased shoot content of major nutrients for the four shrubs (Table 3). In most of the cases, compost addition increased nutrient acquisition by mycorrhiza-inoculated plants. In particular, this effect was statistically significant for N in *R. sphaerocarpa* and *R. lycioides*; for P in *R. sphaerocarpa*, *O. europeae* and *R. lycioides*, and for K in *R. lycioides* (Table 3).

Data from analysis of the amount and type of AM fungal propagules released into soil around the root system of the target plants are shown in Table 4. Mycorrhizal inoculation significantly increased the number of "infective" mycorrhizal propagules in the soil around the four target shrub species, as shown by the MPN measurements. The addition of composted residue significantly increased the effect of mycorrhizal inoculation on propagule production. Mycorrhizal inoculation also increased the development of the extramatrical AM mycelium developing in soil around the roots of the four test

Table 3 Effect of mycorrhizal (*M*) inoculation during nursery production of representative plant species from a Mediterranean ecosystem, and composted residue (*R*) application on transplanting to a desertified area, on nutrient acquisition by plants after 8 months under field conditions. For each plant species and parameter, *values sharing a letter in common* do not differ significantly ($P < 0.05$) by the test of the Least Significant Difference of Fisher

Plant/Treatment	Total nutrient content in plant shoots (mg/plant)		
	N	P	K
Pistacia lentiscus			
Control	12.4a	2.6a	17.2a
R	29.0bc	4.8b	25.4bc
M	23.0b	2.9a	22.2b
RM	33.6c	4.2b	31.3c
Retama sphaerocarpa			
Control	6.7a	0.6a	6.4a
M	11.7b	1.0b	9.4b
R	7.1a	0.7ab	5.2a
RM	13.0c	2.4c	11.9b
Olea europeae			
Control	16.3a	1.9	17.6a
M	46.8b	8.4	64.4c
R	17.2a	1.5	23.1b
RM	42.4b	10.1	63.4c
Rhamnus lycioides			
Control	8.7a	0.6	9.3a
M	27.7b	2.7	24.9b
R	10.0a	0.8	9.9a
RM	35.9c	3.7	40.0c

plants. This effect was enhanced by the addition of compost in the case of *R. sphaerocarpa* and *O. europeae*. Neither mycorrhizal inoculation nor compost addition affected AM fungal spore production in soil associated with *R. sphaerocarpa* and *R. lycioides* but a higher number of spores was detected in soil around mycorrhiza-inoculated *P. lentiscus* and *O. europaea* (Table 4).

Only three distinguishable glomalean spore morphotypes, with some unidentified types, were characterised, belonging to the species *Glomus geosporum*, *G. contrictum* and *Scutellospora calospora*.

Discussion

Because of the quality of the nursery production procedures, most of the plantlets established after field transplanting, independent of the mycorrhizal or compost treatments applied. However, the effects of these treatments on plant growth were evident after 8 months of field growth, thought not statistically significantly for *P. lentiscus* and *R. sphaerocarpa*. ANOVA tests did not evidence any type of interaction between treatments. Nevertheless, the most rewarding effects of mycorrhizal inoculation and organic amendments on transplant performance were those exerted on the nutritional status of the plantlets, as ascertained by using the total nutrient content in plant tissues. As has been demonstrated (Jarrel and Beverley 1981) this is the most useful response variable to evaluate the effect of any particular treatment on plant nutrient acquisition since it takes into account effects on mineral concentration in plant tissues and biomass production. In this context, increases in the major

Table 4 Effect of mycorrhizal (*M*) inoculation during nursery production of representative plant species from a Mediterranean ecosystem, and composted residue (*R*) application on transplanting to a desertified area, on the development of arbuscular mycorrhiza (*AM*) propagules in soil as affected by plants growing for 8 months under field conditions. For each plant species and parameter, *values sharing a letter in common* do not differ significantly ($P < 0.05$) by the test of the Least Significant Difference of Fisher

Plant/treatment	MPN of AM propagules (100 g dry soil)	Length of extramatrical AM mycelium (m/100 g dry soil)	Number of AM spores (100 g dry soil)
Pistacia lentiscus			
Control	5a	149a	14a
M	126b	197b	28b
R	8a	184ab	22ab
RM	297c	211b	26b
Retama sphaerocarpa			
Control	5a	173a	16a
M	74b	303c	18a
R	23ab	239ab	18a
RM	147c	386d	20a
Olea europeae			
Control	5a	172a	18a
M	206b	305b	22b
R	19a	214a	14a
RM	649c	375c	20ab
Rhamnus lycioides			
Control	5a	129a	14a
M	138b	230c	26a
R	8a	185b	22a
RM	280c	224c	28a

nutrients N, P and K support the beneficial effects of mycorrhizal inoculation in increasing plant ability for nutrient acquisition, effects which were enhanced, in some cases, by compost residue application. These results corroborated, under field conditions, the well-known role of AM inoculation on plant nutrition. ANOVA tests showed positive interaction between residue application and mycorrhizal inoculation in some cases.

It is generally accepted that the number of mycorrhizal propagules able to develop colonisation units on plant roots is the most realistic response variable to express the mycorrhizal potential of a soil (Brundrett 1996). As was expected (Eom et al. 2000) the four target shrub species differed in their capabilities to enrich the soil in mycorrhizal propagules, i.e. to enhance the mycorrhizal potential of the soil. In all cases, mycorrhiza inoculation increased the number of propagules in the field soil around the transplants and the organic amendment enhanced such an effect. ANOVA tests showed positive interaction between these two treatments.

In relation to these observations, the following three points deserve discussion: (1) which type and origin of propagules are contributing to the increased micorrhizal potential in mycorrhiza-inoculated plants; (2) how the organic amendments enhanced propagule release; and (3) the meaning of these facts in further stages of the revegetation process.

Because results are self-evident no proper correlation analyses were carried out to show that the numbers of spores were similar in soil around differentially treated plants where the numbers of mycorrhizal propagules were quite different. Thus the AM fungal spore is not the main influencing propagule source. Instead, since mycorrhiza-inoculated plants showed an increased length of extramatrical AM mycelium around their roots and released a higher number of AM propagules it seems that the extraradical AM mycelium is the propagule source more related to the total mycorrhizal potential in the rhizosphere of the target plant species. It is clear that such a relationship is more qualitative than quantitative. These findings agree with previous observations showing that the mycelium extending from mycorrhizal roots is usually the main source of inoculum in semi-arid and arid ecosystems, while the importance of soil-borne spores is less recognised (Requena et al. 1996; Bashan et al. 2000).

The number of AM fungal spores is relatively low in the target ecosystem and only three identified and about two unidentified AM fungal spore morphotypes were consistently detected in the rhizosphere of the target plant species. Diversity of AM fungi present in the test area seems therefore rather low, indicating the high degree of degradation of the ecosystem (Krebs 1985). No spores of *G. intraradices*, the species inoculated, were recovered in the soil, thus the AM fungal spores detected will probably be coming from the natural mycelium network usually present in these ecosystems (Requena et al. 1996). Because these species have a preferential sporulation inside the root, it seems that no release of their spores was detected even by harvest time. Instead, the extraradical mycelium from *G. intraradices*-inoculated plants largely contributed to the mycorrhizal potential of the soil.

The effect of organic matter addition on the increase of AM propagules in soil around mycorrhiza-inoculated roots is consistent and significant. Previous studies are contradictory because the results reached are different depending on the type, origin and regime of the application of the organic amendment (Sainz et al. 1998). Several reports support composted residues addition increasing the AM “infectivity” of the soil (Joner and Jakobsen 1995; Noyd et al. 1996). Organic agriculture approaches also give an enhanced mycorrhizal potential to the system (Mader et al. 2000; Muthukumar and Udaiyan 2000). Information on specific experiments to ascertain the mechanisms involved is lacking. It can be argued that changes in the rhizobacteria population, which are know to greatly affect mycorrhiza developments (Barea et al. 2002), are promoted by composted organic amendments (Kim et al. 1997; Crecchio et al 2001). The beneficial effect of organic amendments on soil structure (Garcia et al. 1998) can also account for the improvement of AM mycelial development.

At this stage of the research, discussion on the repercussion of an enhanced micorrhizal potential on further development of the restoration process is rather speculative but it is logical to expect that aspects such as improvement of plant performance, aggregate formation, and facilitation of natural revegetation, would be improved by the enhanced mycorrhizal propagule availability (Requena et al. 2001).

In conclusion, the reported results support the view that the tailored AM inoculation was functioning under field conditions to enhance nutrient acquisition by the target indigenous shrubs and, in interaction with organic amendments, promoted mycorrhizal propagule production in the soil, critical factors to benefit further stages of the revegetation process.

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