Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain

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A B S T R A C T

Mycorrhizas are worldwide symbiotic associations established between certain soil fungi and most vascular plants and are fundamental in optimizing plant fitness and soil quality. Mycorrhizal symbioses improve the resilience of plant communities against environment stresses, including nutrient deficiency, drought and soil disturbance. Since these stresses are paramount in the degradation of semi-arid ecosystems in the SE Spain, a series of basic, strategic and applied studies have been made to ascertain how the activity and diversity of mycorrhizal fungi affect plant community composition, structure and dynamics in this region. These investigations are reviewed here in terms of: (i) analysing the diversity of mycorrhizal fungi; (ii) assessing the ecological and functional interactions among plant communities and their associated mycorrhizal fungal populations; and (iii) using mycorrhizal inoculation technology for the restoration of degraded semi-arid areas in Southeast Spain. Disturbance of the target semi-arid ecosystems decreases the density and diversity of mycorrhizal fungust populations. Nevertheless, the mycorrhizal propagules do not disappear completely suggesting a certain degree of stress adaptation, and these remaining, resilient ecotypes are being used as plant inoculants. Numerous field experiments, using plant species from the natural succession inoculated with a community of indigenous mycorrhizal fungi, have been carried out in revegetation projects in the semi-arid Iberian Southeast. This management strategy improved both plant development and soil quality, and is a successful biotechnological tool to aid the restoration of self-sustaining ecosystems. However, despite a 20-year history of this work, we lack a comprehensive view of the mycorrhizal potential to improve the composition, diversity, structure and functionality of drought-adapted plant communities in the Region.

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1. Mycorrhizas: general concepts, types and significance in the soil-plant system

Maintaining the quality and sustainability of soil resources is a key issue, not only for optimizing the stability and productivity of natural ecosystems, but also to prevent erosion and minimize negative environmental stresses (Buscot, 2005). Many chemical, physical and biological factors are involved in the framework of interactions involved in ecosystem functioning (Barea et al., 2005a). The biological components are based on diverse genetic and functional groups of soil microbial populations (Chaudhary et al., 2009). Soil microbes are responsible for critical ecosystem functions such as the biogeochemical cycling of nutrients and matter, and the maintenance of plant health and soil quality (Avis et al., 2008; Barea et al., 2005a,b; Richardson et al., 2009). Microbial activities are particularly relevant at the root-soil interface, the rhizosphere, where microorganisms interact with plants and soil constituents (Dessaux et al., 2010; Giri et al., 2005; Lambers et al., 2009).

Among the most influential members of the soil microbiota are the mycorrhizal fungi, responsible for establishing mycorrhizas with most vascular plant species on Earth (Barea et al., 2008). Mycorrhizas are symbiotic associations established between soil fungi and most vascular plants, where both partners exchange nutrients and energy (Brundrett, 2002). It is now universally accepted that mycorrhizal symbioses are fundamental for good plant nutrition and health, and soil quality (Azcón-Aguilar el al., 2009; Smith and Read, 2008). The mycorrhizal fungi colonize the root cortex and develop an extraradical mycelium which permeates the soil surrounding the plant roots. This mycelium forms a network specialized for the acquisition of water and mineral nutrients from soil, particularly those whose ionic forms have poor mobility or are present in low

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concentration in the soil solution, such as phosphate and ammonium (Barea et al., 2005a). Thus, mycorrhizal formation is an adaptive strategy which provides the plant with an increased ability for nutrient capture and cycling in soils with low nutrient availability, particularly in arid and semi-arid ecosystems (Allen, 2007). In addition, the mycorrhizal symbiosis improves plant health through increased protection against environmental stresses, either biotic (e.g. pathogen attack) or abiotic (e.g. drought, salinity, heavy metals, organic pollutants), and enhances soil structure through the formation of hydro-stable aggregates necessary for good soil tilth (Azcón and Barea, 2010; Barea et al., 2005a,b; Rillig and Mummey, 2006; Ruíz-Lozano et al., 2008).

The two main types of mycorrhizas are ecto- and endomycorrhizas, which differ considerably in their structure and physiological relationships with symbionts (Barea and Honrubia, 2004; Smith and Read, 2008). In ectomycorrhizas, the fungus develops a sheath or mantle around the feeder roots. The mycelium penetrates between the cells of the root forming the Hartig net but not forming intracellular penetrations. About 3% of vascular plants, mainly forest trees (Fagaceae, Betulaceae, Pinaceae, *Eucalyptus,* and some woody legumes) form ectomycorrhizas. In spite of the relatively low number of plant species forming ectomycorrhizas, these mycorrhizal associations and the tree species involved play a key role in forest ecosystems and are widely distributed. The fungi involved are mostly Basidiomycota and Ascomycota. In endomycorrhizas, no sheath is formed and the fungi colonize the root cortex both intercellularly and intracellularly. A few endomycorrhizal types are restricted to species in the Ericaceae ("ericoid" mycorrhiza) or Orchidaceae ("orchid" mycorrhiza), but the arbuscular mycorrhizal (AM) type is the commonest being widely distributed throughout the plant kingdom. This ubiquitous mycorrhizal type is characterized by the tree-like symbiotic structures, the arbuscules, which are formed by the fungus within the root cortical cells. It is here where most of the nutrient exchange between the fungus and the plant is thought to occur (Smith and Read, 2008). The AM fungi were formerly included in the Zygomycota, order Glomales (see Redecker et al., 2000b), but they now form a new phylum, the Glomeromycota (Schüßler et al., 2001). One further mycorrhizal type, the ectendomycorrhiza, shares characteristics of both groups and is formed by hardy plant species in the Ericales, and in the Monotropaceae and Cistaceae. In ectendomycorrhizas the fungi form both a sheath and intracellular penetrations.

The discovery of well-preserved fossil plants in the Early Devonian Rhynie Chert, revealed the existence of mycorrhizal associations (Kenrick and Crane, 1997; Remy et al., 1994; Stubblefield et al., 1987) in the early evolution of land plants. Fungal structures, such as hyphae and spores, resembling those of extant AM fungi, were found in fossil records of small 400-million-year-old plants (Honrubia, 2009; Kenrick, 2003). Thus AM fungi played a key role during land colonization by plants (Pirozynski and Malloch, 1975; Redecker et al., 2000a; Simon et al., 1993). These pioneering observations were followed by morphological and phylogenetic (molecular) studies which demonstrated that the primitive roots developed in association with AM fungi and co-evolved with them to build up the mycorrhizal root systems of extant vascular plants (Brundrett, 2002). The other types of mycorrhizal associations emerged later in evolution (Honrubia, 2009).

2. Purpose of review: prospecting and applying mycorrhizal fungi to improve functioning of plant communities in the semi-arid Southeast (SE) Spain

Given their important role in plant evolution, it is now well accepted that mycorrhizas currently continue to help plants to develop in stressed environments (Barea et al., 2008; Honrubia, 2009) such as those of the Semi-arid Mediterranean ecosystem in SE Spain. In this particular region, mycorrhizal fungi have been shown to help plants to establish and cope with nutrient deficiency, drought, soil disturbance and other environmental stresses characteristically involved in soil degradation (Barea et al., 2007; Barea and Honrubia, 2004; Martínez-García, 2010; Martínez-García and Pugnaire, 2009; Morte et al., 2009; Palenzuela and Barea, 2009). This ability has promoted investigations of the impact of mycorrhizas in maintaining diversity and functioning of plant communities in arid/semiarid ecosystems in this region.

Applied mycorrhizal research in the SE of Spain began at the end of the 1980s, in the framework of the LUCDEME Project (www.mma.es/portal/secciones/…/lucdeme). Many basic, strategic and/or applied studies have been carried out since aimed to use mycorrhizal biotechnology to improve the performance of the regional plant communities. These investigations are reviewed here in terms of: (i) analysing the diversity of mycorrhizal fungi (section 3); (ii) assessing the ecological and functional interactions among plant communities and their associated mycorrhizal fungal populations (section 4); and (iii) using mycorrhizal inoculation technology for the restoration of degraded areas in SE Spain (section 5). The main conclusions and future trends on this research will then be outlined.

3. Analyzing the diversity of mycorrhizal fungi in SE Spain semi-arid ecosystems

Presence and diversity of Basidiomycota, Ascomycota or Glomeromycota have been investigated in several surveys (Barea, In press). Fungal characterization has been done using morphological and/or molecular techniques usually used in mycorrhiza research (Dahlberg et al., 1997; El Karkouri et al., 2005; Gamper et al., 2010; Hempel et al., 2007; Horton and Bruns, 2001; Morton, 2009; Oehl et al., 2009; Öpik et al., 2008; Perotto et al., 2002; Pickles et al., 2009; Sanon et al., 2009). Although molecular techniques have gained prominence, identification approaches based on morphological criteria are still important and complement molecular methods (El Karkouri et al., 2005; Morton, 2009; Oehl et al., 2009; Taylor, 2002).

Apart from a few surveys involving ectomycorrhizal (Torres and Honrubia, 1997; Díez et al., 2001) or ectendomycorrhizal (Díez et al., 2002; Morte et al., 2009) fungi, most diversity studies on mycorrhizal fungi in the SE Spain have been focused on AM fungi (Barea, In press). For ecto- and ectendo-mycorrizal fungi, restriction fragment length polymorphism (RFLP) comparisons and internal transcribed spacer (ITS) sequencing of the nuclear rDNA have been used to characterize, for example, morphotypes of the genera Terfezia or Pisolithus. In contrast, the studies of AM fungal communities diversity in SE Spain have been based on the morphological characterization of their large multinucleate spores or on sequence analysis of the small-subunit (18S) ribosomal DNA of the spores and/or the mycelia from these fungi or a combination of both approaches (Barea, In press). Other surveys have used molecular tools to identify the AM fungi actually colonizing plant roots. Table 1 summarizes the surveys of AM fungi associated with plant communities from semi-arid SE Spain, as a basis for diversity analysis.

As a result of this analysis, a germ-plasm bank of mono-specific cultures of Glomeromycota from SE Spain has been established in the Estación Experimental del Zaidín CSIC, Granada. This comprises almost 200 cultures of ecotypes from diverse morphotypes within eleven genera, with *Glomus* being the most prevalent. Almost all ecotypes have been sequenced and phylogenetically defined as operational taxonomic units (OTUs).

Table 1
Analyzing the diversity of AM fungi in semi-arid SE Spain ecosystems.

Target AM fungal propagule	Host plant/region	Methodology	AM fungal taxa	References
Soil-borne spores	Several shrub species, Murcia	Spore morphology	*Glomus* sp., *Sclerocystis* sp.and *Entrophospora* sp.	Diaz and Honrubia (1993)
	Retama, Granada	Spore morphology	*Glomus* sp.	Herrera et al. (1993)
	P. lentiscus, R. lycioides, O. europaea var. *sylvestris* and *R. sphaerocarpa*, Murcia	Spore morphology	*Glomus coronatum, Gl. constrictum, Scutellospora calospora* and *Entrophospora* sp.	Azcón-Aguilar et al. (2003)
	A. cytisoides, Sierra de los Filabres, Almería	Spore morphology	*Glomus* sp., *Scutelospora* sp., *Acaulospora* sp.	Requena et al. (1996)
	Endangered/endemic flora, Sierra Nevada National Park, Granada	Spore morphology	Species from *Glomus, Acaulospora, Gigaspora, Entrophospora, Ambispora, Scutellospora, Pacispora,* and *Diversispora*	Azcón-Aguilar et al. (2010)
	Asteriscus maritimus (L.) Less., *Ononis talaverae* Devesa & G. López, *Medicago marina* L., *Ammophila arenaria* (L.) Link, *Lotus creticus* L., *Teucrium dunense* Sennen, *Erodium chium* (L.) Willd. Cabo de Gata, Almería	Spore morphology	*Rococetra persica, Glomus intraradices, Gl. etunicatum, Gl. constrictum, Gl. coronatum, Gl. geosporum* and *Gl. mosseae*	Estrada Personal communication
	A *P. lentiscus* dominated shrub community, Murcia	Spore morphology and genetic characterization by PCR analysis of the NS31-AM 1 region of the 18S rDNA	*Gl. mosseae, Gl. claroideum, Gl. viscosum, Gl. constrictum* and *Paraglomus occultum*	Ferrol et al. (2004)
	Shrub communities from Sierra de Baza Natural Park, Granada,including a dolomitic thyme-shrub, a rosemary grove, a broom shrub and a sage shrub	Spore morphology and genetic characterization by PCR-TGGE analysis of the NS31-Glo1 region of the 18S rDNA gene	26 AM fungal morphotypes, assigned to phylogenetical defined as OTUs.	Palenzuela and Barea, 2006, 2009
	A dolomitic shrubland in Sierra de Baza Natural Park, Granada	Spore morphology and genetic characterization by PCR-TGGE analysis of the NS31-AM1 region of the 18S rDNA gene	*Otospora bareai* A new species for science	Palenzuela et al. (2008)
	Endangered/endemic flora from Sierra Nevada National Park, Granada	Spore morphology and genetic characterization as above	*Entrophospora nevadensis* A new species for science	Palenzuela et al. (2010)
	A. maritimus Cabo de Gata Natural Park, Almería	Spore morphology and genetic characterization as above	*Diversispora* sp. *Glomus eburneum Paraglomus occultum*	Beltrán Personal communication
Root colonizing AM fungi (mycelium)	*L. latifolia, T. mastichina, R. officinalis* and *R. sphaerocarpa*	TTGE fingerprinting of PCR-amplified 18S rDNA fragments	A managed community of *Glomus* spp. for testing the technique	Cornejo et al. (2004)
	G. cinerea, L. latifolia, T. mastichina and *T. zygis*	Nested PCR, SSCP fingerprinting of the NS31-AM1 and NS8-ARCH1311 regions of the 18S rDNA	Ten AM fungal phylotypes 8 from Glomeraceae, with a dominance of *Glomus* type sequences, and 2 from Diversisporaceae	Sanchez-Castro et al. (2008, 2009)
	Phagnalon rupestre L. DC., *Piptatherum miliaceum* L. Coss., *Stipa parviflora* Desf., and *Plantago lagopus* L., Murcia	SSCP fingerprinting analysis of PCR-amplified 18S rDNA fragments	Nine AM fungal phylotypes belonging to *Glomus* groups A and B	Alguacil et al. (2009a)
	Gypsum area in Murcia: *Gypsophila* struthium, *Teucrium* libanitis, *Ononis* tridentata and *Helianthemum* squamatum	SSCP fingerprinting analysis of PCR-amplified 18S rDNA fragments	21 AM phylotypes: 19 *Glomus*, 1 *Diversispora* and 1 *Scutellospora* with *Gl. constrictum, Gl. intraradices, Gl. clarum, Scutellospora* sp., and *Otospora bareai*.	Alguacil et al. (2009b)
	Artemisia barrelieri Besser, *Hammada articulata* (Moq.) O. Bolòs & Vigo, *Launaea arborescens* (Batt.) Murb., *Salsola genistoides* Juss. ex Poir. in Lam., *Salsola oppositifolia* Desf. and *Thymus hyemalis* Lange	T-RFLP fingerprinting analysis of PCR-amplified 18S rDNA fragments	Not identified	Martínez-García (2010) and Martínez-García et al. (2011)
	Taxus baccata L. a endangered plant species in Sierra de Baza Natural Park, Granada	T-RFLP fingerprinting analysis of PCR-amplified 18S rDNA fragments	Glomeraceae,Diversisporaceae, Acaulosporaceae and Paraglomaceae.	López-García, Personal communication

Recent studies (Sanchez-Castro et al., 2008, 2009; Alguacil et al., 2009a, 2009b, 2009c) have revealed a hidden diversity, with many AM fungal sequences detected in plant roots that cannot be related to known AM taxa present in the surrounding soil as spores, These cryptic colonists have also been found for other ecosystems in Europe (Hempel et al., 2007; Öpik et al., 2008). These molecular studies clearly show that populations of spores in soil do not reflect completely the fungi present in roots and vice versa. Thus, both methodological approaches have to be implemented to investigate the AM populations associated with target plants/communities in Mediterranean ecosystems in SE Spain.

4. Ecological and functional interactions between plant communities and their associated mycorrhizal fungal populations in SE Spain semi-arid ecosystems

The interactions occurring around the plant-mycorrhizal fungus relationship have been the subject of many studies which investigate linking between biodiversity and ecosystem functioning (Bever, 1999; van der Heijden and Sanders, 2002). Research concerning the impact of mycorrhizal fungi on plant community composition and functioning concludes that the diversity and activity of mycorrhizal fungi is a key mechanism for ecosystem functioning Hart and Klironomos, 2002; Kennedy et al., 2007; Martínez-García and Pugnaire, 2009; Read, 1998). Conversely, diversity and structure of plant cover affects diversity of AM fungal populations (Bever et al., 2002; Read, 2002; Wolfe et al., 2005). The nature of the community feedbacks in mycorrhizal associations has been investigated with special emphasis on the several mechanisms/factors responsible for the ecological interactions involved. These mechanisms/factors include: (i) the functional specificity of the different plant-fungus combinations (Klironomos, 2002; van der Heijden et al., 1998); (ii) the mycorrhizal dependency of the plant species involved (Hart and Klironomos, 2002; Kennedy et al., 2007; Read, 1998); and (iii) the structure of the individual plant species within the community (O'Connor et al., 2002; van der Heijden, 2002; van der Heijden et al., 2006).

Some key concepts can be drawn from the consolidated knowledge on these aspects of mycorrhizal ecology:

(i) although specificity *sensu strictum* does not exist in mycorrhizal associations (as almost all plants in a community can be colonized simultaneously by several species of mycorrhizal fungi), different mycorrhizal ecotypes are more beneficial to some plant species than others (Sanders, 2002). It also seems that not every fungus can colonize every plant in the community (Barea et al., 2008).

(ii) in addition to its role in carbon allocation, the establishment of a mycelial web around the roots from the plant community constitutes a diverse inoculum source for the different plant species (Read, 1998) and

(iii) the intermingling and extensive extra-radical mycelium allows a more efficient exploitation of soil nutrients and water, thus benefiting the nutrient flow through the soil-fungus-plant system - particularly relevant in arid ecosystems (Allen, 2007).

Research investigating these aspects of mycorrhizal ecology and function in the semi-arid SE Spain is summarised below:

4.1. Impact of mycorrhizas on the dynamics and functioning of plant communities

Much information on the role of mycorrhizas in SE Spain semi-arid ecosystems has been generated during the last two decades (see section 5). However, most studies concern the mycorrhizal impact on the establishment and development of individual plant species rather than the functioning of plant communities. However, some experiments can be considered in this context, and are discussed here.

The effect of mycorrhizas at the plant community level was studied by Requena et al. (2001) in a degraded area within the Sierra de los Filabres in Almería. The existing natural vegetation was shrubland, where *Anthyllis cytisoides* L., a drought-tolerant legume able to form symbioses with both rhizobial and mycorrhizal micro-symbionts, was the dominant species. This experiment (further discussed in Subsection 5.2) demonstrated the long-term benefits of inoculation not only on plant establishment but also on P acquisition and N_2 fixation by the target legume. The benefits also included increased available P, N and organic matter, and the number of hydro-stable aggregates in the soil supporting the community. Studies using the stable isotope ^{15}N evaluated the amount of N_2 fixed by the shrub legume and showed how this improved N nutrition, via N-transfer to non-N-fixing vegetation grown in association with the inoculated legume. In addition, the mycorrhizal, nodulated *Anthyllis* plants behaved as a source of mycorrhizal inoculum for the surrounding area, where new seedlings flourished and accelerated the natural succession. This study showed that the introduction of target indigenous plant species, associated with a managed community of microbial symbionts, could be a successful biotechnological tool to aid the integral recovery of degraded ecosystems.

Navarro-Fernández et al. (2011) studied a dolomitic "thyme-shrub" plant community in Sierra de Baza Natural Park, which comprised endemic species dominated by *Thymus granatensis* Boiss. Efficient functioning of the community was dependent on a dolomite-adapted AM fungal community, particularly under the drought stress conditions characteristic of the target area. The presence of AM fungal ecotypes from the high-dolomite environment appeared fundamental for the development of the endemic plant community, as they were involved in the adaptation mechanisms that enable the plant to grow.

4.2. Influence of the plant species on the structure and composition of their associated mycorrhizal fungal populations

The influence of the plant species on production of mycorrhizal propagules has been investigated in SE Spain. For example, Azcón-Aguilar et al. (2003) studied typical shrubs from semi-arid areas of Murcia and found that *Olea europaea* var. *sylvestris* (Mill.) Lehr and *Retama sphaerocarpa* (L.) Boiss, have a higher capacity to enhance the development of AM propagules in their rhizospheres than *Pistacia lentiscus* L.or *Rhamnus lycioides* L.

Further studies (Sánchez-Castro et al., 2008) analysed the genetic diversity of the AM fungal community that colonized the roots in a shrubland species community (*Genista cinerea* (Vill) DC. in Lam. & DC., *Lavandula latifolia* Medicus, *Thymus mastichina* L., *Rosmarinus officinalis* L. and *Thymus zygis* L.). The different co-occurring plant species were colonized by AM fungal communities of different composition. These findings support the earlier contention that there is some level of specificity in mycorrhizal associations. For methodological approaches and results from this AM fungal diversity analysis see Table 1.The genetic diversity of AM fungi colonizing the root, the rhizosphere soil and the root-free soil was investigated (Martínez-García et al., 2011). Differences in AM fungal communities were found in soils under shrubs and in gaps among them, whereas no differences were detected among AM fungal communities colonizing roots. Soil nutrient content drove most of the spatial variations and genetic diversity in the AM fungal community. Consequently, it was suggested that different shrub

species generate resource islands (Allen, 1988), which differ in nutrient content and, therefore, support different AM fungal communities, at least in their associated rhizosphere soil. This increases AM fungal diversity at the landscape level. Using the same genetic approach Martínez-García (2011) characterized the AM fungal community colonizing roots of two plant species, *Ballota hirsuta* Bentham and *Lobularia maritime* (L.) Desv., growing under shrubs and in open areas. Differences between AM fungal genetic diversity of the communities associated with the two species were found. These data represent new information on the specificity of AM fungus-plant interactions in patchy environments, and suggest a control of AM fungi on plant population and community dynamics in arid ecosystems.

4.3. Impact of plant cover degradation on mycorrhizal fungal diversity

Disturbance of natural plant communities, the first symptom of ecosystem degradation, is often accompanied or preceded by loss of key physical-chemical and biological soil properties such as soil structure, plant nutrient availability, organic matter content and microbial activity (Francis and Thornes, 1990). In particular, plant community degradation causes disturbance of mycorrhizal inoculum potential, which is a critical ecological factor to help further plant development in degraded habitats (Requena et al., 2001). The impact of plant cover and/or soil degradation on mycorrhizal fungal diversity has been investigated in SE Spain where drivers of ecosystem degradation include climatic change, mining activities, wild fire, flooding and salinity and land abandonment. For example, in the Sierra de Baza Natural Park, Granada, Palenzuela and Barea (2006, 2009; see Table 1) selected five plant community plots where the vegetation cover was optimal and compared these with plots where diversity and structure of the optimal vegetation cover were degraded. Rhizosphere samples were taken from all the target species and AM spores isolated and morphologically characterized. The main conclusion was that the degradation of the vegetation cover negatively affected the density (number of spores per 100 g of soil), richness and diversity of AM fungi.

To assess the impact of drought on AM associations in semi-arid plant communities from SE Spain, Martínez-García (2010) measured root length colonization in *Stipa tenacissima* L. and *A. cytisoides* along a natural environmental precipitation gradient, and in *Artemisia barrelieri* Besser growing in several plots subjected to different precipitation regimes. The results showed that the response of the AM associations to precipitation patterns depended on the host species. Natural drought stimulated AM colonization, but artificially-induced drier conditions lowered the AM colonization. It was suggested that arid ecosystems caused by climate change could alter AM interactions in different ways depending both on the host plant and the intensity of the drought, which might then lead to changes in plant communities.

In a study by Díaz and Honrubia (1994), sampling sites with differing degrees of disturbance were established in an area of the SE coast of Murcia that had been severely degraded by mining activities and was covered by waste sediments. The mycorrhizal population level (mycorrhizal root colonization and the number of spores in the rhizosphere) was adversely affected by soil degradation. However, mycorrhizal propagules did not disappear completely, suggesting a certain degree of adaptation to the soil disturbance suffered by the test area.

The effects of a wild fire on mycorrhizal inoculum potential of soil were investigated in a *Pinus halepensis* Mill. forest (Torres and Honrubia, 1997). For two years after the fire episode, the fruiting of fungal species, the number of sclerotia in soil, and the percentages and types of ectomycorrhizas present were determined.

Diversity of mycorrhizal fungi was differentially affected in burned stands: the Ascomycota populations appeared resistant to fire while the Basidiomycota were strongly reduced.

The influence of flooding and salinity gradients on the AM fungal spore counts and root colonization levels of *Inula crithmoides* L. was studied in a transect spanning a gradient from shoreline to interior in La Mata lagoon, Alicante. The plots with high salinity levels and flooding showed decreased spore numbers in soil, a decrease in the percentage of mycorrhization, and a very low number of mycorrhizal fungal propagules as measured by the most probable number test (Roda et al., 2008).

Roldán et al. (1997) studied the AM fungal population in agricultural soils abandoned for different lengths of time (3–45 years) in a semi-arid area of Murcia and found that agricultural use reduced soil fertility and lowered AM fungal populations compared to the soil kept in its natural state. After abandonment, there was a 5-year period when the soils underwent further degradation of their AM potential. After that the AM propagules slowly recovered, reaching values similar to those of the virgin soil after 45 years.

5. Applying mycorrhizal inoculation technology for the restoration of degraded areas in SE Spain

Loss of mycorrhizal propagules following degradation of vegetation cover in Mediterranean ecosystems can inhibit either natural or artificial processes of revegetation. Augmentation of the inoculum potential may be needed (Requena et al., 1996). In some cases, mycorrhiza-inoculated shrubs act as a "resource islands" (Allen, 1988; Palenzuela et al., 2002; Azcón-Aguilar et al., 2003; Caravaca et al., 2005a), serving as a source of inoculum for the surrounding area thus stimulating revegetation.

A key strategy for mycorrhizal application in revegetation strategies is the nursery production of quality native seedlings with a tailored mycorrhizal status. This is a pre-requisite to help transplant performance, as demonstrated in nursery production of *P. halepensis* seedlings mycorrhizal with *Pisolithus tinctorius* or *Lactarius deliciosus* (Díaz et al., 2009, 2010). The biotechnological procedures followed to produce ectendomycorrhizal *Helianthemum* plants with *Terfezia* species (desert truffles) have also been recently discussed (Morte et al., 2009). The different ways of producing mycorrhizal synthesis between desert truffles and the *Helianthemum* species were analysed, considering the type of fungal inoculum, plant source (sometimes micropropagated) and culture conditions. This enabled the formulation of a management protocol for establishing desert truffle plantations (Morte et al., 2009). Several companies which produce either AM inoculum or native woody plants with a tailored AM status, under commercial nursery conditions, have been established in Spain (Barea, In press).

Here we review several field inoculation experiments carried out in the semi-arid SE Spain using mycorrhizal inoculation technology for the restoration (by revegetation) of areas suffering disturbance of their plant cover.

5.1. The importance of using native plant species

Mycorrhizal inoculation in revegetation strategies for degraded areas from SE Spain was first investigated in a semi-arid desertification-threatened environment south of the Sierra Nevada, Granada (Herrera et al., 1993). This assay used woody legumes as plant species symbiotic with both N_2-fixing rhizobial bacteria and AM fungi, associations which enable the plant to develop in water-deficient and low nutrient environments (Azcón and Barea, 2010). The target legumes included two native shrubs (*A. cytisoides* and *Spartium junceum* L.), and four non-native tree legumes (*Robinia pseudoacacia* L., *Acacia caven* (Mol.) Mol. and *Prosopis chilensis*

(Mol.) Stuntz.and *Medicago arborea* L.). The results of this four-year-old trial showed that: (i) only the native shrub legumes were able to establish and develop under the local environmental conditions; (ii) the biotechnological manipulation of the seedlings, by inoculation with selected rhizobia and mycorrhizal fungi, improved outplanting performance, plant survival, and biomass production. A revegetation strategy based on plant species was proposed, selecting *A. cytisoides,* a particularly drought-tolerant, highly mycotrophic legume species, for the next stage in development. A technique for accelerating the process of natural revegetation was proposed by replanting randomly-spaced groups of mycorrhizal shrubs following the natural pattern and structure of the undisturbed ecosystem (Francis and Thornes, 1990). The results are described in Subsection 5.2.

For revegetation programmes in the water-stressed regions, it is important to evaluate the influence of mycorrhizal inoculation on the water use efficiency (WUE) of the target native plant species. Querejeta et al. (2003) used *O. europaea* var. *sylvestris* and *R. lycioides* to evaluate whether two eco-physiological response variables (foliar carbon isotope ratios (δ^{13}C) and leaf gas exchange) were affected by inoculation with *Glomus intraradices.* They found that the WUE was enhanced under drought conditions by inoculation in *O. europaea* but not in *R. lycioides. O. europaea* plants are long-lived, slow-growing evergreen trees with a conservative strategy for water use, while *R. lycioides* is a drought-deciduous shrub with a shorter lifespan. These differences may explain their dissimilar patterns of response to inoculation with the same AM fungus. The results suggested that some of the interspecific variability in δ^{13}C observed for arid land plant communities may be due to different physiological responses to mycorrhization.

Additional examples referring to inoculation with ectomycorrhizas and ectendomycorrhizas are given by Honrubia et al. (1992); Barea and Honrubia, (2004); Morte et al. (2009). The information reviewed in these publications emphasizes the importance of using plants from the natural succession in revegetation strategies.

5.2. Comparing the use of autochthonous vs. non-native mycorrhizal fungi as inoculants

Several field experiments have compared the use of autochthonous vs. exotic (and generalist) mycorrhizal fungi for revegetation programmes in the Region. For example, Requena et al. (2001) tested two mycorrhizal inoculation protocols for rhizobium-inoculated *A. cytisoides* seedlings that were to be transplanted to a degraded area: (i) an exotic mycorrhizal fungus (*Glomus intraradices*), (ii) a mixture of five taxa of indigenous mycorrhizal fungi representing the natural abundance and diversity in the target site (Sierra de los Filabres, Almería). A long-term improvement of both plant performance and the physicochemical properties in the soil around *A. cytisoides* plants inoculated with indigenous AM taxa was evidenced over a 5-year period, while the exotic fungi were effective only during the first year after transplanting.

The effectiveness of mycorrhizal inoculation with either a mixture of native AM fungi or an exotic AM fungus (*Glomus claroideum*) was tested in a degraded area in Murcia province (Alguacil et al., 2005) using *O. europaea* subsp. *sylvestris* and *R. sphaerocarpa* as the test plants. Eighteen months after planting, the mixture of native AM fungi was the most effective treatment for stimulating the growth of both plants and for increasing the aggregate stability in the rhizosphere soil of *R. sphaerocarpa.* Increases in enzyme activities (dehydrogenase, urease, protease-BAA, acid phosphatase and P-glucosidase) were recorded in the rhizosphere of both shrub species colonised by indigenous AM fungi. In further experiments, Caravaca et al. (2003b, 2005a) compared the effectiveness of inoculation with similar combinations of fungi in increasing growth and nutrient acquisition of target shrub species, and in improving soil structural stability. The mixture of native endophytes was more effective than *G.claroideum* for enhancing growth and foliar NPK contents of *O. europaea* var. *sylvestris, R. sphaerocarpa* and *R. lycioides*, and in improving soil structural stability. Thus it appears that native, drought-adapted AM fungi often improve host-plant performance to a greater extent in the target dry environments than non-native AM fungi.

Little is known, however, about the physiological basis for the differential plant responses that are affected by the origin of the test AM fungi. To investigate this, Querejeta et al. (2006) inoculated seedlings of *O. europaea* var. *sylvestris* or *R. lycioides* with either a mixture of eight native *Glomus* species or with the non-native *Glomus claroideum*, before field transplanting in a semi-arid area in Murcia province. Inoculation with native AM fungi produced the greatest improvement in nutrient and water status as well as in long-term growth for both *O. europaea* and *R. lycioides.* Foliar δ^{18}O measurements indicated that native AM fungi enhanced stomatal conductance to a greater extent than the non-native AM fungus in both target plants. The δ^{13}C data showed that intrinsic water-use efficiency in *Olea* was stimulated by native AM fungi. The results suggested that modulation of leaf gas exchange by native, drought-adapted, AM fungi is critical to the long-term performance of host plants in semi-arid environments. In further work, Querejeta et al. (2007) hypothesised that enhanced transpiration as well as an improved plant water status were key mechanisms involved in plant growth stimulation by native AM fungi in semi-arid soils. These authors pre-inoculated seedlings of the dry-land shrubs *P. lentiscus* and *R. sphaerocarpa* L. before transplanting them into a degraded site in Murcia. Pre-inoculation with native AM fungi enhanced shoot water content and shoot δ^{15}N in both shrub species. Increased K uptake and improved water relations were considered key to growth stimulation by native AM fungi in *P. lentiscus.* Shoot δ^{18}O was significantly lower in AM-inoculated than in non-inoculated *P. lentiscus* seedlings, indicating enhanced cumulative transpiration. Growth stimulation by native AM fungi in *R. sphaerocarpa* was attributed to increased P uptake, enhanced N_2 fixation and a largely nutrient-mediated improvement of plant water status.

The importance of using native ectomycorrhizal fungi had been suggested earlier by Maestre et al. (2002) who found no effect of nursery inoculation on seedling survival. They attributed the lack of effectiveness to the fact that they used an exotic fungal strain which did not survive the drought summer conditions in the study area (Alicante).

5.3. Effect of mycorrhizal inoculation, and interaction with organic amendments, on transplant performance and soil quality

Mycorrhizal seedlings of plant species from natural communities, produced in commercial nursery conditions, have been used for a series of field experiments testing the impact of organic amendment additions on plant performance and soil quality. These assays involved ecto-, ectendo- or AM-mycorrhizal inoculation.

Ectomycorrhizal inoculation in interaction with organic amendments was investigated in field experiments using *P. halepensis* seedlings inoculated with *Pisolithus arhizus* and planted in a terraced rangeland amended with urban solid refuse (Roldán et al., 1996; Querejeta et al., 1998). The application of the organic amendment increased soil fertility and soil water content, whilstthe combination of soil terracing, refuse amendment and mycorrhizal inoculation significantly improved the performance of *P. halepensis.*

A multi-factorial field experiment was designed to evaluate the influence of a fresh organic residue addition, and mycorrhizal inoculation with *P. arhizus* on *P. halepensis* in a semi-arid rangeland in Murcia (Caravaca et al., 2002c). Both treatments increased C-fractions and enzyme activities (dehydrogenase and phosphatase) and aggregate stability in the mycorrhizospheric soil around the roots of this tree. Aggregate stability in the rhizosphere was strongly correlated with the microbial biomass carbon and soluble C-fractions (water soluble carbon and water soluble carbohydrates) as well as with dehydrogenase and phosphatase activities.

Caravaca et al. (2005b) then used seedlings of *Cistus albidus* L. and *Quercus coccifera* L., inoculated with the ectomycorrhizal fungus *Scleroderma verrucosum*, to assess the effect of the addition of sugar beet, rock phosphate, and *Aspergillus niger* directly into the planting hole, in a target, degraded, semi-arid area in Murcia. Mycorrhizal inoculation resulted in promotion of plant growth of both plant species and an enhancement of soil physicochemical, biochemical, and biological properties.

Field experiments involving inoculation with AM fungi and addition of composted urban residues have been carried out using indigenous shrub species such as *O. europaea* var. *sylvestris* (Caravaca et al., 2002a); *P. lentiscus* (Caravaca et al., 2002b); *R. sphaerocarpa* (Caravaca et al., 2003a); or *R. lycioides* (Caravaca et al., 2003d). Water-soluble carbon, water-soluble carbohydrates, microbial biomass carbon content and enzyme activities (dehydrogenase, urease, protease-N-alpha-benzoyl-L-argininamide, and acid phosphatase) were increased in the mycorrhizosphere of the target shrubs. Subsequent integrative studies corroborated these findings (Caravaca et al., 2003b, 2003c; Alguacil et al., 2005), whilst similar synergistic effects were found by adding other organic amendments such as sewage sludge (Alguacil et al., 2004; Caravaca et al., 2005a and b).

The impact of AM inoculation in combination with organic amendments has also been tested for *Dorycnium pentaphyllum* Scop., an autochthonous legume from semi-arid areas in SE Spain used for restoration purposes. The tailored AM-seedlings were transplanted to a degraded semi-arid area in Murcia, in combination with several *A. niger*-treated organic amendments and rock phosphate additions (Alguacil et al., 2008). These treatments produced beneficial effects on the physical, chemical and biological properties of the mycorrhizosphere soil of the transplanted target legume. Similar benefits on *D. pentaphyllum* performance were obtained using other type of residues: sugar beet residues (Caravaca et al., 2004a) or dry olive cake residues (Caravaca et al., 2004b; Medina et al., 2004).

6. Conclusions

Morphological and molecular approaches have been applied successfully to analyze the diversity of mycorrhizal fungi in several semi-arid ecosystems of SE Spain. A germplasm bank of semi-arid zone-adapted ecotypes of Glomeromycota fungi has been established, and these are available for revegetation programmes in the Region. Disturbance of the target semi-arid ecosystems caused by global climate change or other stresses decreases the density and diversity of mycorrhizal fungus populations, yet some mycorrhizal propagules remain, suggesting adaptation to the situation within the stressed area.

Many field experiments have demonstrated that using native plant species, inoculated with a managed community of indigenous mycorrhizal fungi, is a successful biotechnological tool to aid the integral recovery of degraded semi-arid SE Iberian ecosystems. Improvements of both plant development and soil quality were reported, thus initiating the restoration and maintenance of a self-sustaining ecosystem. Inoculation with indigenous mycorrhizal fungi has resulted in improvements of both plant performance and the physicochemical properties in the soil around native shrub species, whereas exotic fungi were effective only during the first year after transplanting. New information on the specificity of mycorrhizal fungus-plant interactions in patchy environments has been reported, indicating a strong influence of mycorrhizal fungi on plant populations and community dynamics in arid ecosystems.

Isotopic techniques based on approaches using $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$ measurements have proved useful in determining how mycorrhizal inoculation affects some eco-physiological responses related to water-use efficiency and nutrient acquisition by plants. Results show that tailored mycorrhizal inoculation improves certain physiological processes in plant communities in semi-arid SE Spain. Native, drought-adapted, mycorrhizal fungi appear critical to the long-term performance of host plants in semi-arid environments. Field experiments have shown that tailored inoculation with mycorrhizal fungi in combination with composted organic residues, using indigenous shrub/tree species as test plants, improved plant establishment, enhanced enzymatic activities involved in C, N and P cycling and increased soil aggregate stability.

7. Future trends

Despite the advances in our knowledge of mycorrhizal presence and functioning in SE Spain over the last two decades, further basic, strategic and applied studies are needed to better understand the significance of mycorrhizas in determining biodiversity and function in the semi-arid ecosystems in this region. A key aim would be to use current molecular approaches in the integral analysis of the diversity of all types of mycorrhizal propagules associated with target plant communities. This is particularly critical to detect the "hidden diversity" of AM populations. A thorough knowledge of this diversity is needed to produce mycorrhizal fungal inoculants representing the integral diversity of the target area. Seedlings with a tailored mycorrhizal status will act as a "resource islands" of inoculum for the surrounding area to benefit plant cover development and for improving soil quality in degraded ecosystems. This would maximize the mycorrhizal benefit in revegetation/conservation programmes facilitating nutrient and water recycling and capture.

An increased knowledge of the role of mycorrhizas in stress adaptation will be fundamental to improve the eco-physiological gains in plant resistance to drought stress induced by a tailored mycorrhization. It will be important to assess how an optimized mycorrhizal status of transplanted seedlings can be used in an adaptive management strategy to improve the resilience of the target endangered plant/community against global climate change.

To carry out field studies to investigate how the diversity and activity of mycorrhizal fungi affect the composition, diversity, structure and functionality of plant communities in semi-arid SE Spain ecosystems will constitute a research priority in the next future.

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