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Arbuscular Mycorrhizas and their Significance in Promoting Soil-Plant System Sustainability against Environmental Stresses

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Introduction

The stability and productivity of agro-ecosystems is largely dependent on maintaining the quality of soil resources through a sustainable soil management, which aims to conserve its productive capacity, minimizing energy and resource use by optimizing the rate of turnover and recycling of nutrients (Altieri 2004). These are fundamental issues not only for a sustainable production of healthy crops, or to ensure ecosystem self-sustainability, but also to prevent erosion and to minimize negative environmental impacts (Buscot 2005).

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Sustainability of the plant-soil systems can be achieved when utilization of mineral resources by plants is balanced by an efficient biogeochemical cycling, thereby avoiding nutrients to be rapidly depleted. This means that agricultural productivity can be maintained with a reduction of fertilizer inputs and that natural plant communities can exist in a stable form for prolonged periods of time (Jeffries and Barea 2012). To achieve sustainability both environmental pollution and depletion of agricultural and forestry resources have to be prevented, the structure and diversity of natural plant communities should be maintained, and the susceptibility of soils to erosion reduced (Klironomos 2002).

Diverse chemical, physical and biological factors are involved in the framework of plant-soil interactions responsible for a sustainable ecosystem functioning (Barea et al. 2005a). The biological components are based on diverse genetic and functional groups of soil microbial populations able to carry out critical ecosystem functions such as the biogeochemical cycling of mineral nutrients, organic matter decomposition and the formation and maintenance of soil structure, key issues in a sustainable production scenario (Barea et al. 2005b, Chaudhary et al. 2009, Richardson et al. 2009). Among the beneficial microbes, mycorrhizal fungi are recognized as one of the most influential group of soil biota in the context of ecosystem sustainability once they establish mutualistic plant-fungus symbioses, so-called mycorrhizas (Jeffries and Barea 2012). Mycorrhizal associations are formed by most vascular plant species on Earth and can be found in almost all terrestrial ecosystems worldwide (Smith and Read 2008, Brundrett 2009), being universally accepted that they are fundamental to improve plant performance and soil quality (Jeffries et al. 2003).

Mycorrhizal functioning is based on the exchange of nutrients and energy between both the plant and fungal partners (Brundrett 2002). A variety of mycorrhizal types are formed, depending on the plant and fungal taxa involved. However, arbuscular mycorrhizal (AM) symbiosis is the most common and over 70 percent of plant species are capable of forming these associations (Smith and Read 2008, Brundrett 2009). This chapter will focus on AM symbiosis but the importance and ecological meaning of other mycorrhizal types are reviewed in this book (Chapter 17).

AM fungi, included in the phylum Glomeromycota (Schüßler et al. 2001), are ubiquitous soil-borne fungi, whose origin and divergence have been dated back over 450 million years (Redecker et al. 2000, Honrubia 2009, Schübler and Walker 2011). Pioneering observations, followed by morphological and phylogenetic (molecular) studies, suggested that AM symbioses played a key role in land colonization by plants (Pirozynski and Malloch 1975), and that 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). As a consequence of this co-evolution the AM relationship became an integral component of plant ecology in both natural and agricultural ecosystems (Brundrett 2002).

Nowadays it is well accepted that AM associations, which helped plants to thrive in hostile environments such as those prior to their origin and during their evolution, continue helping plants to develop in stressed environments (Barea et al. 2011). This is fundamental because adverse conditions of different origin, particularly exacerbated in the current scenario of global change, generate a great array of stress situations which interferes with the stability of both natural and agricultural systems. Plants must be able to cope with these stresses. Consequently, adaptive strategies able to increase their resilience to overcome negative impacts should be developed. AM establishment can be considered as one of these adaptive strategies, crucial in maintaining ecosystem sustainability, through activities able to confer the plant an increased tolerance to environmental constraints (Barea et al. 2011, Jeffries and Barea 2012).

In this chapter we describe how the AM symbiosis results fundamentally in maintaining ecosystem sustainability. Accordingly, we first summarize key information on the establishment, functioning and significance of AM symbiosis in the soil-plant system. Then the physiological and molecular mechanisms responsible for the increased tolerance of mycorrhizal plants to a variety of environmental stresses, either biotic or abiotic, and their implications for plant performance are analyzed. We later discuss how to explore and manage the diversity of AM fungal populations from different habitats. Finally, examples where the appropriate management of the AM symbiosis can help restore disturbed environments as an adaptive strategy to improve their resilience against adversity are given. The trends of this thematic area will be outlined to suggest what research is needed in the future.

Arbuscular Mycorrhiza: Establishment and Functioning

The information generated during the last years on the cellular and molecular events that took place during AM establishment and the eco-physiological and molecular components of AM functioning have recently been reviewed (Bonfante and Genre 2008, Parniske 2008, Smith and Read 2008; Gianinazzi-Pearson et al. 2009). Accordingly, only the main well-established conclusions from these review articles are considered and critically summarized here.

AM Establishment

AM fungi can colonize plant roots from three main types of soil-based propagules: spores, fragments of mycorrhizal roots and extraradical hyphae, all of them producing more or less a well-developed mycelial network expanding in the soil. Establishment and functioning of the AM symbiosis requires a high degree of coordination between the two partners based on a finely regulated molecular dialogue (Hause et al. 2007, López-Ráez et al. 2010). The AM fungi-plant communication starts in the rhizosphere with the production and exudation of certain signaling molecules—strigolactones—by the host plant that are recognized by AM fungi (Bouwmeester et al. 2007, López-Ráez et al. 2011b). As signaling cues in the rhizosphere, strigolactones are perceived by the AM fungus engaging its metabolism and giving rise to the so-called pre-symbiotic stage. This stage is characterized by a profuse hyphal branching, thus increasing the probability to contact the root and establish symbiosis (Akiyama et al. 2005). It has been suggested that they might also act as the chemo attractant that directs the hyphal growth to the roots (Sbrana and Giovannetti 2005). Conversely, when the AM fungus starts to branch in the vicinity of the root, plants perceive diffusible fungal signals, called "Myc factors", that induce symbiosis-specific responses in the host root, even in the absence of any physical contact (Parniske 2008, Genre and Bonfante 2010). The chemical structure of these Myc factors was identified as lipochito-oligosaccharides with structural similarities with rhizobial Nod factors (Maillet et al. 2011).

When finally a hypha contacts the plant root, it adheres to epidermal cells forming a characteristic fungal structure called appressorium (also called hyphopodium). This event marks the initiation of the symbiotic phase. From the appressorium a penetrating hypha is formed, which reaches the root cortex by following, in most cases, an intracellular route across epidermal cells. The plant cell actively prepares a pre-penetration apparatus to guide the fungus into the cell (Genre et al. 2005, 2008). Colonization then proceeds to produce the characteristic tree-like structures, called "arbuscules" that the fungus develops within the root cortical cells. They consist of highly ramified hyphae with very fine terminal tips. The arbuscules, which give the name to the symbiosis, are structures where most nutrient exchange between the fungus and the plant is thought to occur (Smith and Read 2008). Vesicles, potentially important fungal storage structures, are also developed by some AM fungal species.

Following root colonization AM fungi form extensive mycelial networks outside the root—the extraradical mycelium, ERM—where spores are developed completing their life cycle. The ERM result in a tri-dimensional structure specialized in the acquisition of mineral nutrients from soil, particularly those whose ionic forms have poor mobility or are present in low concentration in the soil solution, as it is the case with phosphate and ammonia (Barea et al. 2005a). The ERM produced by different fungi have quite varied characteristics, in terms of size and architecture. For example, there exist important differences in hyphal diameter (usually in the range of 2–20 µm) and extend from the roots and able to absorb and translocate nutrients (at a distance up to 25 cm) among individual AM fungi (Smith and Smith 2011). Hyphal length densities in field soils are also variable and range from 3 to 14 m/g depending at least in part on the identity of the AM fungus. They are, in any case, much higher than the root length densities of associated plants (Li et al. 2008), emphasizing how effectively AM fungi can explore soil.

AM Functioning

Through the activities of the interlinked and extensive soil ERM, AM fungi affect the distribution and movement of nutrients within the soil ecosystem (Richardson et al. 2009). The major flux is the transfer of carbon from plant to fungus (and thereby to the soil) and the reciprocal movement of phosphate and ammonium from fungus to plant. In addition to the uptake of nutrients, the AM symbiosis improves plant performance through increased protection against environmental stresses, whether they be biotic (e.g., pathogen attack) or abiotic (e.g., drought, salinity, heavy metals toxicity or presence of organic pollutants), and also enhances soil structure through the formation of hydro-stable aggregates necessary for good soil tilth (Barea et al. 2005a,b, Turnau et al. 2006, Ferrol and Pérez-Tienda 2009, González-Guerrero et al. 2009, Ruíz-Lozano and Aroca 2010, Pozo et al. 2010, Azcón and Barea 2010, López-Ráez et al. 2012). The role of AM fungi in water uptake, and at improving plant tolerance to environmental stresses is discussed later in this chapter. Here we focus on the effects of extraradical AM hyphae on plant nutrient acquisition and in the formation of stable soil aggregates.

Phosphorus (P) availability is the most limiting factor for crop yield in 30–40 percent of the world's arable soils (Barea et al. 2008). The role of AM fungi in phosphate uptake has been well documented for a long time (see Smith and Read 2008). The use of ^{32}P-based isotope dilution approaches has allowed to ascertain and to quantify the AM fungal contribution to plant P acquisition (Barea 2010) and it has been shown that the majority of P taken up by plants comes via the fungal partner (Smith and Smith 2012). The AM fungi take up P from the same pool of soluble ions as do roots, and thus act as an extension to the root system. There is evidence that phosphatase activity is higher in the rhizosphere soil around AM-plants than around non-mycorrhizal roots but there is no clear evidence if this is a fungal-mediated phenomenon that allows alternative P sources to be accessed (Jeffries and Barea 2012).

The acquisition of N via the fungal partner can also be considerable (Leigh et al. 2009), despite the high N demand by the fungi themselves and that the global pool of N in AM mycelia is at least as big as that in fine roots (Hodge and Fitter 2010). While the mycorrhizal activity at improving

N_2-fixation represents a considerable contribution to N inputs in legume species (Azcón and Barea 2010), the possibilities that the mycorrhizal hyphae uses N sources less available to non-mycorrhizal plants was suggested sometime ago (Hodge et al. 2001). To investigate such an AM contribution to N acquisition by plants, the isotope ^{15}N was used to measure the apparent plant available N pool size, i.e., the A_N value of the soil for AM and non-mycorrhizal plants. Several studies, as reviewed by Barea et al. (2005a), demonstrated that the A_N value for mycorrhizal plants is higher than that for non-AM controls. This suggests that the AM mycelium is accessing N forms which are sparingly available for non-mycorrhizal plants. In spite of this, the role played by AM fungi in N acquisition from organic N sources, the dominant form of N in most soils remains controversial (Hodge et al. 2010).

In addition to P and N, AM fungi are able to improve assimilation of other nutrients. When plants are growing in soils deficient in low mobility micronutrients such as Zn, Cu or Fe, their uptake by plants is often increased by the formation of AM symbioses (Liu et al. 2000). As in the case of P and N, this has been attributed to the capacity of the external mycelium to exploit larger volumes of soil. By contrast, it has also been observed that AM fungi are able to confer increased metal tolerance to host plants when they are exposed to supra-optimal concentrations of metals. Therefore, it has been postulated that AM function as a "buffer" that protects the plant against damaging alterations in metal levels in the soil (González-Guerrero et al. 2009).

The analysis of the biochemical and molecular mechanisms involved in nutrient transport processes in AM associations, and in the bidirectional nutrient exchange between symbionts is a matter of recent and current interest but will not be considered here. The interested reader is referred to some of the reviews on the topic (see Barea et al. 2008, Ferrol and Pérez-Tienda 2009, Franken 2010, Harrison et al. 2010, Pérez-Tienda et al. 2011 for details and references).

The AM hyphal network is thus vital for plant nutrition in natural ecosystems and for sustainable crop production in low-input agricultural situations. Many studies have also shown that inter-plant bridges formed by AM fungi can provide channels for direct nutrient transfer between AM fungi associated with different plants (Azcón and Barea 2010). It is clear that the AM hyphal network is essential for the continued cycling of nutrients within the plant community, and once it is lost, nutrient sequestration or leaching will occur at a faster rate than in its presence (Jeffries and Barea 2012).

In addition to the content in available nutrients, physical properties are fundamental for soil quality, with soil structure being one of the most influential factors (Buscot 2005). Soil structure depends on the aggregation status of soil particles and a well-aggregated soil ensures appropriate soil–plant water relations, good aeration, root penetrability and organic matter accumulation (Miller and Jastrow 2000). AM hyphae are involved in the formation of stable soil aggregates and, in consequence, in soil conservation and in promoting good soil tilth (Rillig and Mummey 2006, Wright et al. 2007, Kohler et al. 2010, Barea et al. 2011). In addition to the ERM effect entangling soil particles, glomalin-related glyco-proteins produced by AM fungi seem to be involved in the initiation and stabilization of water-stable soil aggregates due to its glue-like hydrophobic nature (Miller and Jastrow 2000, Rillig and Mummey 2006, Wright et al. 2007, Bedini et al. 2009, Curaqueo et al. 2011).

Physiological and Molecular Basis of AM Functioning, and their Implications in Promoting Soil-Plant System Sustainability against Environmental Stresses

The ecological, physiological and molecular components of AM functioning and their implications at enabling the plant to cope more effectively with cultural or environmental stress, either biotic or abiotic, have been the subject of diverse experimental and review studies during the last decade (Barea et al. 2013). The main conclusions from recent articles specialized in the different thematic areas involved are considered and critically summarized here. The selected topics of interest include:

Priming Plant Defenses against Pathogens

Root colonization by AM fungi can improve plant resistance/tolerance to biotic stresses. Although this bioprotection has been widely described in different plant systems, some of the underlying mechanisms remain largely unknown but experimental evidence supports the activation of plant defenses in the AM-mediated plant protection (Azcón-Aguilar and Barea 1996, Goicoechea et al. 2010). During AM establishment modulation of plant defense responses occurs upon recognition of AM fungi to achieve a functional symbiosis and a mild but effective activation of the plant immune responses may occur, both in local and in systemic tissues. This leads to a primed state of the plant that allows a more efficient activation of defense mechanisms in response to attacks by potential enemies (Pozo et al. 2009, 2010, Jung et al. 2012).

Modulation of Plant Defense Responses in Mycorrhizal Plants

The establishment of mycorrhizas implies an alteration in the roots in the level of plant hormones related to defense. For example, changes in jasmonates (JA), salicylic acid (SA) and ethylene (ET) have been described in tomato roots colonized by different *Glomus* species (López-Ráez et al. 2010). As a consequence of this altered balance, the regulation of plant defenses may have an impact on potential attackers. Accumulation of reactive oxygen species and transcripts of certain defense related genes, activation of phenylpropanoid metabolism and accumulation of specific isoforms of hydrolytic enzymes such as chitinases and glucanases has been reported in mycorrhizal roots. Although these reactions are mostly localized, suggesting a role in AM establishment or control of the symbiosis (Pozo et al. 2009), they may contribute to the induction of resistance against certain root pathogens.

Concerning above-ground effects, transcriptional regulation of defense-related genes and accumulation of insect anti-feedant compounds have been reported in shoots of mycorrhizal plants (Gange 2006, Liu et al. 2007). Defense-related genes were among those with altered expression levels, and the authors correlated this finding with increased resistance to shoot pathogens. Furthermore, the volatile blends released by AM plants can be more attractive to aphid parasitoids than those from non-mycorrhizal, as shown in tomato plants (Guerrieri et al. 2004). These results indicate that not only direct, but also indirect plant defense mechanisms may be modulated in mycorrhizal plants. All together, experimental evidences confirm systemic modulation of plant defenses in AM. This modulation may explain the pattern of enhanced resistance/susceptibility of AM plants to diverse enemies on the basis of the different signaling pathways involved in the plant response to particular attackers. In addition, it would explain the fact that AM can modulate the effectiveness of chemically induced plant resistance (Sonnemann et al. 2005).

Priming of Defense Mechanisms in Mycorrhizal Plants

With the exception of limited activation of plant defenses discussed above, a direct activation of defenses has not been observed in AM plants. Despite the vital character of defense responses, constitutive expression of defenses is too costly for the plant in the absence of challenging attackers. Thus, beneficial microorganisms have developed the ability of enhancing resistance not through a direct activation of defenses but through priming of the defense mechanisms (Pozo et al. 2009). A rapid and strong activation of defense mechanisms is crucial for success in controlling attackers. Accordingly, preconditioning of plant tissues for a quick and more effective activation of defenses upon attack has important ecological fitness benefits and seems to be a common feature of the plant´s immune system (Conrath et al. 2006). This boost of basal defenses, known as priming, seems to be successfully triggered by certain beneficial microorganisms including AM fungi (Pozo and Azcón-Aguilar 2007).

Several studies point to priming as a main mechanism operating in Micorrhiza Induced Resistance (MIR), as indicated by the stronger defense reactions triggered in the mycorrhizal plant upon attack (Pozo et al. 2009, 2010). To investigate whether mycorrhizal colonization leads to priming of defenses in aerial tissues, the response of non-mycorrhizal plants or plants colonized by either *Glomus mosseae* or *Glomus intraradices* to the application in the shoots of different defense-related stimuli were compared. Gene expression and enzymatic activities were monitored in a time-course experiment after shoot treatment with JA, ET and SA. Transcript profiling of leaves of mycorrhizal and non-mycorrhizal plants 24 hr upon treatment with JA indicated a stronger induction in mycorrhizal plants of JA regulated genes, including typical defense-related JA responsive genes supporting a prominent role of priming for JA-dependent responses in AM-induced resistance (Pozo et al. 2009, 2010).

Signaling Pathways Involved

Plant defense mechanisms are tightly regulated through an interconnected network of signaling pathways in which JA, ET and SA play major roles. Priming is often manifested as a sensitization of the tissues to one or some of the signaling molecules (Conrath et al. 2006). SA coordinates defense mechanisms that are generally effective against biotrophic pathogens, whereas JA regulates wounding responses and resistance against necrotrophs (Glazebrook 2005). Nevertheless, there is some overlap in their spectrum of efficiency, especially on pathogens with intermediate lifestyles (Pozo et al. 2004). Insect feeding guilds also determine the response they trigger in the plant. Generalist chewing insects, but not phloem-feeding ones, cause wounding and trigger JA-regulated responses (De Vos et al. 2005). These signaling pathways are not independent, intensive interactions ranging from synergism to antagonism shape a complex regulation network, in which trade-offs between SA and JA pathways are well documented (Koornneef and Pieterse 2008). Mycorrhizal roots are related with increased levels of endogenous JA (Hause et al. 2007). The increase probably occurs when connected to a fully AM establishment. Elevated levels of basal JA production could be related to the increased resistance of mycorrhizal plants to certain pathogens and insects. In shoots, increased sensitivity to the hormone, rather than an increase in its level, seems to underlay the primed defense capacity observed in AM plants. Thereby, a partial suppression of some SA-dependent defense responses in the plant is compensated by an enhancement of these JA-regulated. This would result in priming of JA-dependent defense mechanisms (Pozo and Azcón-Aguilar 2007), a defense regulation model which is coherent with the spectrum of effectiveness described for MIR: increased susceptibility to biotrophs, and increased resistance to necrotrophs and generalist chewing insects (Pozo et al. 2009, 2010, Jung et al. 2012).

Agricultural Weed Control

In addition to the already mentioned role of strigolactones as signaling molecules for AM symbiosis establishment, they also act in the rhizosphere as detection signals for root parasitic plants of the *Orobanchaceae*, including the genera *Striga*, *Orobanche* and *Phelipanche* (Bouwmeester et al. 2007, López-Ráez et al. 2011a). These parasitic weeds are some of the most damaging agricultural pests worldwide affecting important crops such as rice, maize, legumes and tomato, causing up to 70 percent of yield losses (Parker 2009). Their seeds surrounding the roots perceive the strigolactones produced by the host plant and germinate, thus starting their lifecycle (López-Ráez et al. 2009). The fact that most of the lifecycle of these weeds occurs belowground, causes great difficulty in diagnosing the infection and usually only after irreversible damage has already been caused to the crop.

Currently, several approaches such as hand weeding, crop rotation, sanitation, fumigation, solarization and improvement of soil fertility are being used to control root parasites without desirable success. Moreover, the most efficient control method—fumigation—is environmentally hazardous. Therefore, new and more environmentally-friendly methods for a more effective control against these agricultural pests should focus on the initial steps in the host-parasite interaction, particularly based on the germination stage induced by strigolactones (López-Ráez et al. 2009).

The fact that strigolactones play a dual role in the rhizosphere as signaling molecules for both AM fungi and root parasitic plants opens the possibility of potential interactions in both biosystems and the use of AM symbiosis as a strategy for pest management. In this sense, it was shown that AM colonization in maize and sorghum induced a lower infection by the parasitic weed *Striga hermonthica* in the field (Lendzemo et al. 2005). These authors proposed that this reduced infection was caused, at least partially, by a reduction in the production and/or exudation of strigolactones in the mycorrhizal plants. Similarly, AM-colonized pea plants showed less stimulatory capacity of germination for seeds of *Orobanche* and *Phelipanche* species than non-colonized plants (Fernández-Aparicio et al. 2010), again suggesting a reduction in strigolactones. More recently, this AM-induced reduction in strigolactone production has been confirmed in tomato and showed that a well established colonization is required for such a reduction. AM symbiosis led to a reduction in the germination of seeds of the root parasite *Phelipanche ramosa*. When the root exudates were analyzed by liquid chromatography-tandem mass spectrometry, a correlation between the germination activity and the levels of strigolactones was analytically demonstrated (López-Ráez et al. 2011a). These results observed in maize, sorghum, pea and tomato indicate that reduction in strigolactone production induced by AM symbiosis is a conserved phenomenon across the plant kingdom. Since this beneficial association is established by most plant species worldwide, it can potentially be used as an environmentally-friendly biocontrol strategy for economically important crops that suffer from these root parasites worldwide. Interestingly, these crops could take advantage of all the other well-known benefits of the AM symbiosis, such as positive effect on plant fitness and tolerance/resistance against biotic and abiotic stresses. However, since strigolactones are also AM hyphal branching factors and are involved in plant architecture, the consequences for the AM fungal community in the soil and possible unwanted side-effects on plant architecture should be carefully evaluated before following this approach.

In addition to the root parasitic plants, it has been suggested that certain AM fungi may suppress growth of other aggressive agricultural weeds such as *Chenopodium album* and *Echinocloa crus-galli*, which cause between 10 to 30 percent of crop yield losses every year (Rinaudo et al. 2010). The authors reported that the presence of AM fungi reduced total weed biomass, while sunflower benefited from AM symbiosis via enhanced phosphorus nutrition. Overall, these observations indicate that the use or stimulation of AM fungi in agro-ecosystems may suppress some aggressive weeds and a possible applicability of the AM symbiosis in weed control is suggested, an agricultural practice in the context of sustainability issues.

Plant Response to Osmotic Stresses

In the last decades the land areas of the world becoming arid or semi-arid have increased progressively, rising drought and salinity events that plants need to cope with (Porcel et al. 2012, see also Chapters 4 and 5 in this volume). Both stresses (drought and salinity) have an osmotic component which causes dehydration of plant tissues. Indeed, both stresses cause a diminution of the soil water potential by increasing solutes concentration (salinity) or by diminishing water availability (drought). Both stresses also cause stomatal closure in order to limit water loss (Aroca et al. 2012). So, drought and salinity stresses share a common osmotic component and some of the plant responses are common, besides salinity also causes ionic imbalance and toxic effects (Porcel et al. 2012), which will not be discussed here. Plant mechanisms to cope with osmotic stresses include fine regulation of water uptake capacity and transpiration rate and activation of the antioxidant machinery to overcome the overproduction of reactive oxygen species (ROS) caused by the stress (Aroca et al. 2012, Porcel et al. 2012). These two mechanisms (keeping water and ROS balance) may be ameliorated by the establishment of the AM symbiosis (Ruiz-Lozano 2003, Porcel et al. 2012).

AM Ameliorates Plant Water Balance

It has been extensively found that AM plants showed better leaf water status than non-AM plants under osmotic stress conditions (Porcel and Ruiz-Lozano 2004, Aroca et al. 2007, Porcel et al. 2012). However, when AM plants are bigger than non-AM ones and the growing substrate is limited, the former may have worse leaf water status because the limited water availability in a pot is depleted faster (Ruiz-Sánchez et al. 2011). The better water status of AM plants under osmotic stress conditions is mainly caused by the direct transfer of water from the fungal hyphae to the plant roots. Recently, Ruth et al. (2011) estimated that about 20 percent of root water uptake taken by roots of AM plants is caused by the presence of mycorrhizal mycelium. The higher root water uptake capacity of AM plants is caused by more soil volume explored by the AM roots plus fungal mycelium, and because AM mycelium can reach water reservoirs in the soil not accessible to plant roots because of their bigger size (Ruth et al. 2011). Consequently, AM plants have more chances to take water from soil under osmotic stress conditions. However, to take water efficiently from soils affected by osmotic stresses it is also necessary that the roots (and the mycelium) decrease their osmotic potential below the osmotic potential of the soil. The AM symbiosis has been shown to improve the plant osmotic adjustment by accumulation of different compounds such as proline, sugars, free amino acids, etc., although this effect may differ according to the plant tissue considered (Bheemareddy and Lakshman 2011, Sheng et al. 2011). In this context, Porcel and Ruiz-Lozano (2004) and Ruiz-Lozano et al. (2011) found that non-AM plants accumulated more proline in shoots than AM plants. In contrast, in roots, AM plants subjected to drought accumulated more proline than non-AM plants. This suggests that in root tissues AM plants accumulate more proline in order to cope with the low water potential of drying soil and to keep a water potential gradient favoring water flow into the roots.

Root water uptake depends on root hydraulic conductivity (L) values, which depend ultimately on aquaporin functioning (Aroca et al. 2012). Aquaporins are membrane intrinsic proteins that allow water and other small neutral molecules to pass through them following an osmotic gradient (see Maurel et al. 2008). Thus, *Arabidopsis* plants lacking the expression of one aquaporin gene showed less L than wild type plants (Postaire et al. 2010). Both L and aquaporins are regulated by AM symbiosis under osmotic stress conditions. Indeed, L decreased less or even remained unchanged in AM plants under osmotic stress conditions, together with a different regulation of the expression of aquaporin genes (Aroca et al. 2007). The modulation of particular aquaporins by the AM symbiosis results in a better regulation of plant water status and contributes to global plant resistance to the stressful conditions, as evidenced by better growth and water status of AM plants under conditions of water deficit. As a result, the better water uptake capacity of AM plants allows them to also have higher transpiration rates and hence higher photosynthetic rates under osmotic stress conditions (Porcel et al. 2012).

AM Ameliorates Plant ROS Balance

Since osmotic stresses induce stomatal closure, they also cause an oxidative stress because of the enhancement of ROS (Porcel et al. 2012). This ROS over-production is caused when the stomata are closed there is not enough CO_2 available to be fixed by the enzyme Rubisco, and reduction power is accumulated in excess. Such an excess of reduction power causes an inhibition of the electron transport from photosystem II to photosystem I, and the excess of electrons are trapped by oxygen molecules generating ROS (Miller et al. 2010). Plants have several enzymatic and non-enzymatic mechanisms to eliminate ROS (for details see Miller et al. 2010, Porcel et al. 2012). Several studies found that AM plants show less oxidative damage than non-AM plants under osmotic stress conditions (Porcel and Ruiz-Lozano 2004, Ruiz-Sánchez et al. 2010, Porcel et al. 2012), in some cases because of a higher activity of antioxidant enzymes (Porcel et al. 2012), or a higher concentration of antioxidant compounds (Ruiz-Sánchez et al. 2010). However, since AM plants are able to overcome water deficit induced by osmotic stresses and show higher stomatal conductance than non-AM plants, they also generate less ROS under osmotic stress conditions (Porcel and Ruiz-Lozano 2004). Hence, AM fungi enhance osmotic tolerance of the host plants by several mechanisms or by avoiding water deficit induced by osmotic stresses and/or by increasing antioxidant capacity. It is evident that more research is needed in order to fully understand the involvement of AM symbiosis in tolerating osmotic stresses.

Mechanisms Underlying Tolerance to Heavy Metals

Soil heavy metal (HM) pollution is one of the major problems that negatively affect environmental health. Excessive accumulation of metals in the soil threatens ecosystem sustainability by altering above- and below-ground community structure, often through severe loss of floristic diversity due to removal of the more HM sensitive components of the community. Although considerable variability in plant responses to AM inoculation has been observed in contaminated soils, the potential of AM fungi to buffer HM-stress has been demonstrated in a number of studies (reviewed by Göhre and Paszkowski 2006 and Hildebrandt et al. 2007). This alleviative influence can be attributed not only to AM-mediated nutritional effects, but also to the impact of AM fungi on metal distribution at the soil–fungus–plant interface. Immobilization of metals on both extra- and intraradical fungal structures has been shown (Kaldorf et al. 1999, Joner et al. 2000), thus providing a plausible explanation for repeated enhancement of the barrier for metal translocation from the roots to the shoots of inoculated plants as reported.

HM have been reported to reduce or delay AM colonization. However, even in highly contaminated soils, AM fungal propagules never disappear completely, and whenever colonization occurs, even to a small extent, it induces beneficial effects on the host plant (Gamalero et al. 2009). Heavy metal-tolerant AM fungi have been isolated from polluted soils and these indigenous AM fungal populations have been reported to cope better with HM-toxicity than those not exposed to such long-term selection pressure (del Val et al. 1999). In order to persist in environments with high metal content, AM fungi should have evolved a series of strategies to restrict entry of toxic metal species and to keep intracellular metal homeostasis.

In general, mechanisms of HM tolerance in fungi include reduction of metal uptake and/or increased efflux, metal immobilization (e.g., cell-wall adsorption), extracellular metal sequestration by, e.g., exo-polysaccharides or other extracellular metabolites, intracellular chelation by, e.g., metallothioneins or phytochelatins, and metal localization/sequestration within vacuoles (Gadd 2007). These mechanisms seem to be used in AM fungi as well (reviewed by Ferrol et al. 2009 and González-Guerrero et al. 2009). Accumulation of metal ions on the cell wall has been shown to be an important mechanism leading to metal immobilization by AM fungi (Joner et al. 2000, González-Guerrero et al. 2008). Chitin, the main component of the fungal cell wall, displays free amino and hydroxyl groups that can be very efficient in binding toxic metals. Moreover, AM fungi produce glomalin (glomalin-related soil protein, GRSP), first believed to be a hydrophobin, later identified as likely a 60-kDa heat shock protein homolog (Gadkar and Rillig 2006) that strongly and irreversibly sequesters metal such as Cu, Cd and Zn (González-Chávez et al. 2004, Cornejo et al. 2008) therefore contributing to metal stabilization in the soil.

The fungal vacuoles also seem to play an important role in the regulation of cytosolic metal ion concentrations and the detoxification of potentially toxic metals. Through the activity of specific metal transporters, such as the vacuolar Zn transporter GintZnT1 or the ABC transporter GintABC1 (González-Guerrero et al. 2005, 2010), excess metal is translocated to the vacuoles, where it would cause less damage. Simultaneously, excess metal would be sequestered in the cytosol by the metallothioneins GmarMT1 or GintMT1, which are cysteine-rich polypeptides that confer increased tolerance to Cd and Cu in *Gigaspora margarita* and *Glomus intraradices*, respectively (Lanfranco et al. 2002, González-Guerrero et al. 2007). Additionally, the fungus struggles to reduce the free radicals produced by heavy metals by activating its enzymatic and non-enzymatic antioxidant systems (reviewed by Ferrol et al. 2009).

In addition to metal immobilization by the fungus, increased HM tolerance of mycorrhizal plants may be related to extensive changes in gene expression and protein synthesis induced by the symbiosis itself. Some studies suggest that plant mechanisms responsible for HM tolerance are down-regulated in mycorrhizal plants. For example, while Cd induced up-regulation of a metallothionein gene and induction of gluthation reductase activity in roots of non-mycorrhizal pea plants, no effect was observed in mycorrhizal roots (Rivera-Becerril et al. 2005). Down-regulation of some heavy metal transporters was observed in mycorrhizal roots of several plant species when grown under toxic metal conditions (Burleigh et al. 2003, Ouziad et al. 2005). Additionally, it has been found that AM fungi modulate the leaf transcriptome of a poplar clone grown on a HM-contaminated soil (Cicatelli et al. 2012) and that changes induced by the AM symbiosis on the shoot proteome of *Medicago* are modulated during Cd stress (Aloui et al. 2011). The proteomic suggests that metal toxicity escape in shoots of mycorrhizotrophic plants is supported by a mobilization of the defense mechanisms at the expense of the photosynthesis-dependent symbiotic sucrose sink.

Exploring and Managing the Diversity of AM Fungal Populations in Agro-ecosystems

In AM ecological research it is fundamental to have information on the individual fungi that are actually functional within an ecosystem since differential benefits can be conferred to each plant host by different AM fungi. Even more, different isolates of the same species differ in their effectiveness in conferring benefits to similar host plants (Jeffries and Barea 2012). Because of the necessity to identify and discriminate particular individuals within any AM fungal population, analyzing this sub-set of soil biodiversity is a key issue in rhizosphere ecology. Exploring AM fungal diversity is important per se and is used as a marker for assessing the impact of perturbations over a target agro-ecosystem. The reasons for maintaining AM diversity in agriculture is that mixed crops will benefit more from a mixed population of AM symbionts. In non-agricultural situations, as plant diversity has been related to AM diversity, maintenance of a sustainable mixed plant population depends on the maintenance of a diverse AM fungi population and vice versa (Maherali and Klironomos 2007). Thus, it is important to recognize the impact of mycorrhizal fungi on plant community composition and functioning is a key issue in ecosystem dynamic and that the ad hoc research over the last few decades has led to the conclusion that the activity and diversity of mycorrhizal fungi is a key mechanism in linking biodiversity and ecosystem functioning (Read 1998).

On the other hand, and because of an increasing demand for low-input agriculture has resulted in a greater interest in the manipulation and use of beneficial soil microorganisms, management of the native population of AM fungi is recognized as a sustainable strategy in agriculture or for the restoration of natural ecosystems, because it can reduce the use of chemicals and energy in agriculture leading to a more economical and sustainable production, while minimizing environmental degradation (Jeffries and Barea 2012). These biological interventions are becoming more attractive as the use of chemicals for fumigation and disease control is progressively discouraged and fertilizers have become more expensive (Atkinson 2009).

Accordingly, recent knowledge on the analysis of AM fungal diversity and on AM technological developments is summarized here.

Exploring AM Fungus Diversity and Inoculum and Inoculation Technologies

Diversity Analysis of AM Fungal Populations

Earlier studies concerning the analysis of the diversity of AM fungal communities were based on the morphology, wall characters and ontogeny of their large multinucleated spores. However, molecular tools are now available for a challenging dissection of AM fungal population dynamics (Robinson-Boyer et al. 2009). For molecular identification, the PCR-amplified rRNA gene fragments of the spores and/or the mycelia from AM fungi are usually subjected to cloning, fingerprinting and sequencing (Hempel et al. 2007, Öpik et al. 2008, Toljander et al. 2008, Rosendahl et al. 2009, Alguacil et al. 2009, Sonjak et al. 2009, Sánchez-Castro et al. 2012a,b). Alternative molecular tools now exist to quantitatively analyze the effect of environment, management or inoculation of soils on more diverse AM fungal communities. For example, q-PCR can be used for simultaneous specific and quantitative investigations of particular taxa of AM fungi in roots and soils colonized by several taxa (Gamper et al. 2008, König et al. 2010). In addition, new techniques of high-throughput sequencing (e.g., pyrosequencing) are now being used for AM fungi (Lumini et al. 2010). Despite the advancement in molecular techniques, the identification approaches employed for AM fungi based on morphological characteristics are still valid and used, being considered complementary to the molecular methods (Morton 2009). A lack of relationship between genetic and functional diversity has been shown (Munkvold et al. 2004, Ehinger et al. 2009).

Molecular techniques able to discriminate individual AM fungi within roots have allowed us to corroborate that AM fungal taxa exhibit little host specificity, whilst a single plant can be colonized by many different AM fungal taxa within the same root. These molecular approaches also help to corroborate that a certain degree of host preference (functional compatibility) occurs and this has been shown to play an important role in regulating diversity, stability and productivity of natural ecosystems (Smith and Read 2008).

AM Inoculants' Production and Inoculation Techniques

Once the importance of the AM symbiosis in sustainable strategies of agriculture or restoration of natural ecosystems is recognized, a decision must be made as to whether the native population of AM fungi suffices as the starting material from which to develop a sustainable system. If not, it will be necessary to augment the native species with inoculum of exotic or indigenous isolates, as it is the case with other beneficial soil microorganisms.

Accordingly, novel agro-biotechnological approaches include the use of microbial inoculants but while technology for the production of inexpensive rhizobial and free-living beneficial bacteria is commercially available, constraints on the production of AM inocula and the development of inoculation techniques have limited the exploitation of the AM potential. This is a key issue for sustainable management of either agricultural or natural ecosystems (Azcón and Barea 2010).

The difficulty in culturing obligate symbionts such as AM fungi in the absence of their host plant is a major obstacle for massive inoculum productions (Baar 2008). Despite these problems, the beneficial effects of AM fungi on plant growth have led to their development as bioinoculants for forestry, agriculture and horticulture (Ijdo et al. 2011) and several companies worldwide are producing AM inoculum products which are now commercially available (Gianinazzi and Vosátka 2004, Vosátka et al. 2008, Ijdo et al. 2011). Selection of the appropriate AM fungi is however a key step (Estaún et al. 2002), while specific procedures are required to multiply AM-fungi and to produce high quality inocula (von Alten et al. 2002). Recent developments in AM-inoculum production systems range from nursery plots (Koltai et al. 2008, Cuenca et al. 2008) to *in vitro* monoxenic root organ cultures (Bago and Cano 2005). The resulting materials (spores, hyphae, root fragments, etc.) are added to different carriers, resulting in a wide range of formulations, including encapsulation, to be applied at an agronomical scale using different application methods (Jaizme-Vega et al. 2003, Cuenca et al. 2008, Vosátka et al. 2008), including hydro-seeding (Estaún et al. 2007). It is indeed a matter of discussion whether "generic products" , containing several AM fungi, potentially suitable for a range of applications, are more appropriate for the market than those with precise formulations and AM fungi specifically tuned to particular end-uses (Smith and Read 2008).

Among other companies producing AM inocula in Spain, two *spin-off*, related to the Spanish Thematic Network on Beneficial Plant-Microbial Interactions, have emerged to produce AM inocula. One of them, MYCOVITRO (www.mycovitro.com), from EEZ-CSIC, produces (*in vitro* as monoxenics root cultures) and commercializes inoculants globally known as "glomigel", specifics for each cropping system/ecosystem. The other, Thader-Biotechnology (www.thaderbiotechnology.com), from University of Murcia, which produce and commercialize several microbial (mainly fungi)-based formulated, known as AGROMIC BASE, as an AM fungal inoculants for horticulture and fruit-culture.

Inoculation at broad scale in highly developed farming systems, however has many constraints. To overcome this, management of indigenous populations is a currently viable option (Brito et al. 2008). However, at relatively small-scale (nursery production), AM inoculation is feasible and advantageous. Inoculation of seedlings is potentially a good method for establishing selected fungi in roots before potting on or planting-out into the field. Inoculation is appropriate where transplanting is part of the normal production system, as is the case with horticulture, including plantation crops. If possible it is best to use indigenous isolates that are already adapted to the prevailing conditions at the field site (Requena et al. 2001, Pelligrino et al. 2011), thus, the concept of using native inoculants rather than exotic ones is now widely accepted. Introduction of exotic inoculum may only be necessary if a native population is either absent or has a low inoculum potential, or if the native fungi are ineffective for the crop species that are being planted (Jeffries and Barea 2012).

Managing AM Fungi for the Restoration of Degraded Ecosystems

Ecosystem degradation is one of the most serious problems in the world, affecting plant community structure and functionality and in some geographic areas can have a high risk of desertization, a complex and dynamic process which is claiming several hundred million hectares annually (Jeffries and Barea 2012). Human activities can cause or accelerate desertification and the loss of most plant species and their corresponding symbionts (Barea et al. 2007).

As a result of degradation/desertification processes, disturbance of natural plant communities is often accompanied, or preceded by, loss of physical, chemical and biological soil properties, such as soil structure, plant nutrient availability, organic matter content, microbial activity, etc. (Jeffries and Barea 2012). These traits are fundamental for soil quality, particularly soil structure stabilization (Miller and Jastrow 2000, Buscot 2005), and thus limit re-establishment of the natural plant cover. In particular, desertification causes a sharp decrease in the numbers and diversity of AM fungal spores while soil disturbance is also responsible for the destruction of the AM hyphal network, thereby affecting inoculum potential (Cuenca et al. 1998, Requena et al. 2001, Allen 2007, Barea et al. 2011). Thus, consideration of the AM symbiosis is usually recommended in the establishment of plants in degraded soils, particularly in arid and semi-arid ecosystems where they would enable greater plant tolerance of environmental stresses characteristic of these ecosystems including nutrient deficiency, drought and soil disturbance (Goicoechea et al. 2004, Allen 2007, Barea et al. 2011).

The semiarid Southeast Spain could be a representative scenario for prospecting and applying AM fungi to improve functioning of plant communities, the most arid region of continental Europe. The area is characterized by a warm dry Mediterranean climate where scarce and irregular rainfall, a long dry and hot summer and man-mediated degradation activities may synergistically act as driving-forces able to promote desertification processes (Requena et al. 2001). The ecological and functional impact of mycorrhizas in semiarid ecosystems of Southeast Spain have been investigated for more than 20 yr through basic, strategic and applied studies addressed at ascertaining how the activity and diversity of mycorrhizal fungi affect plant community composition, structure and dynamics in the region. These studies have recently been reviewed (Barea et al. 2011) and the main conclusions can be summarized as follows.

Disturbance of the target semi-arid ecosystems caused by global climate change or other stresses decreases the density and diversity of AM fungal 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 AM fungi, is a successful biotechnological tool to aid the integral recovery of degraded semi-arid Southeast Iberian ecosystems. Inoculation with indigenous AM fungi has resulted in improvement of both plant performance and the physicochemical properties in the soil around native plant species, whereas exotic fungi were effective only during the first year after transplanting. This is the way for restoration and maintenance of a self-sustaining ecosystem.

Field experiments have shown that tailored inoculation with AM 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.

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.

Recovering Endangered Flora

Ecosystems worldwide can include large numbers of endangered plant species, some of them threatened with extinction (Bothe et al. 2010). Although extinction is a natural biological process, it is reckoned that the current rate of plant species extinction is between a hundred and a thousand times faster than the average historical extinction rate for the Earth (Pimm et al. 1995). As it is widely accepted, this accelerated extinction rate is linked almost exclusively to the activities of human beings including overexploitation of species, alterations in their natural habitats or environmental changes, threats which are exacerbated in the current scenario of global warming and the subsequent climate change (Stork 2010). According to the International Union for Conservation of Nature (IUCN) Red List, these constraints are particularly relevant for tropical forests and high mountain ecosystems, where the numbers of plant species included in the catalogues of endangered flora are currently increasing.

In the context of managing mycorrhizal symbioses to exploit their role in promoting soil-plant systems sustainability against environmental stresses, special attention has been devoted to ascertain their potential significance in protecting endangered plants and habitats in high mountain ecosystems. This is an important issue because high mountains, which often bear plant diversities richer than those in their surrounding lowlands, usually include large numbers of threatened species (Körner 2003). Plants growing in high mountain habitats have developed numerous adaptations imposed by the harsh conditions derived from the increase in altitude (low temperatures, intense winds, short growing season, nutrient-poor soils, etc.). These adaptations are manifested in different life strategies and physiological processes (Grabherr et al. 2003). As most plants depend on mycorrhizas to thrive, particularly in fragile and stressed environments, the establishment of mycorrhizal associations was suggested to be one of these adaptive strategies for endangered plants to survive and reproduce in high mountain ecosystems (Fuchs and Haselwandter 2004). Accordingly, diverse studies have been carried out trying to corroborate whether mycorrhiza formation help endangered plants to perform in degraded high mountain ecosystems and contribute to the regeneration of their natural habitats (see Azcón-Aguilar et al. 2012 for references).

Since many endangered species in high mountains form AM associations (Zubek et al. 2008), the potential role of AM fungi in protecting endangered plants in these environments has been especially investigated (Zubek et al. 2009, Bothe et al. 2010, Navarro-Fernández et al. 2011, Azcón-Aguilar et al. 2012). In one of these studies (Bothe et al. 2010) it was shown that AM colonization affects vegetative growth and sexual reproduction of endangered plant species by increasing the number of inflorescences, fruit and seed production, and offspring vigor. In another study, the response of endangered plant species to inoculation with AM fungi was investigated with the aim of improving the *ex situ* propagation of the threatened taxa and the success of their reintroduction in their natural habitats (Zubek et al. 2009). They concluded that AM fungal application can improve performance of the target species and suggested a beneficial influence of AM fungi on the acclimatization of the reintroduced endangered species in nature. In a series of studies carried out in Sierra de Baza Natural Park in Granada, Spain, Navarro-Fernández et al. (2011) found that the AM symbiosis was critical for improving the growth of *Thymus granatensis*, an endemic and threatened plant species from dolomitic soils. These soils have supra-optimal Mg and Ca concentrations and a low water holding capacity, characteristics which restrict the number of plants able to survive in these environments and their reproduction. The AM fungal community adapted to these dolomitic soils was able to contribute to the tolerance mechanisms of *T. granatensis* plants to high Mg concentrations under the prevalent drought conditions.

The significance of AM fungi on the conservation of endangered flora is also being investigated in the high Mediterranean mountains of the Sierra Nevada National Park, Granada, Spain (Azcón-Aguilar et al. 2012). Sierra Nevada constitutes an exceptional refuge for flora and one of the enclaves with a higher biodiversity level of the European continent. Seedlings from a number of selected endangered species were nursery produced and inoculated with specific mixes of suitable autochthonous AM fungi previously isolated from the target environments.

In conclusion, research carried out in the field of AM conservational management supports that native AM fungi could be used as a tool for the propagation of endangered species for restoration purposes, and for the *in situ* and *ex situ* conservation of these plant species and their natural habitats. Additionally, this research also aims at the conservation of AM fungal population diversity in threatened areas worldwide. In this context, Turrini and Giovannetti (2012) suggest the use of protected areas (national parks and other nature reserves) as a basis for a strategic perspective for the *in situ* conservation of AM fungi, alongwith the conservation of their endangered plant species.

Mycorrhizosphere Interactions to Improve Plant Development under Stress Situations

Managing AM fungi and their interactions with ecosystem beneficial microorganisms (mycorrhizosphere tailoring) is nowadays recognized as a feasible biotechnological tool to improve plant growth and health, and soil quality as a sustainable practice in agriculture or restoration of natural ecosystems (Barea et al. 2005b). Many co-inoculation experiments using selected AM fungi and rhizosphere microorganisms and their ecological impact have recently been reported (Barea et al. 2013). The main conclusions from some of these experiments are summarized below with emphasis on the ecological impacts of interactions related to: (1) biofertilization in nutrient deficient soils; (2) abiotic stress alleviation; and (3) restoration of degraded ecosystems.

Mycorrhizosphere Interactions to Improve Plant Growth in Nutrient Deficient Soils

Soil microorganisms that increase the amount of nutrients available to plants (biofertilizers) include rhizobial bacteria and phosphate-solubilizing bacteria and fungi. The interactions of these beneficial rhizosphere microorganisms with AM fungi in the so-called mycorrhizosphere are relevant in stressed soils with a low availablity of nutrients.

The widespread presence of the AM symbiosis in legumes and its role in improving nodulation and N_2 fixation by legume-rhizobia associations are both universally recognized processes, as based on the supply of P by the AM fungi to satisfy the high P-demand of symbiotic N_2 fixation. This information has recently been reviewed (Azcón and Barea 2010, Barea et al. 2013), and only a summarized analysis is given here. Methodologies based on the use of ^{15}N-enriched inorganic fertilizer allow to ascertain and quantify the amount of N which is actually fixed by legume-rhizobia consortia in a particular situation and to measure the contribution of the AM symbiosis to the process. A lower $^{15}N/^{14}N$ ratio in the shoots of rhizobia-inoculated AM plants with respect to those achieved by the same rhizobial strain in non-mycorrhizal plants was found. This indicated an enhancement of the N_2 fixation rates (an increase in ^{14}N from the atmosphere), as induced by the AM activity (Barea et al. 2005a). The ecological impact and benefit from using two biological systems to supply the plant with the two major nutrients, N and P, in nutrient-deficient, stressed soils, is self-evident. In addition, the ^{15}N-based isotopic techniques have been also used to measure N-transfer in mixed cropping where legumes are usually involved. Since the AM mycelia can link different plant species growing nearby, and help overlap the pool of available nutrients for the intercropped plant species, the N released into the overlapping mycorrhizospheres by legume root exudation, or by nodule decay, can result in nitrogen available for non-fixing plants (Azcón and Barea 2010).

The interactions between AM fungi and phosphate-solubilizing-microorganisms (PSM) are relevant to P cycling and plant nutrition, particularly in P-deficient soils. Because the Pi made available by PSM, acting on sparingly-soluble P sources, has limited diffusion in soil solution, the already available Pi may not reach the root surface. However, AM fungi could tap the phosphate ions solubilized by the PSM and translocate them into plant roots (Barea 2010). The microbial interaction of AM fungi and PSM has been tested in experiments using ^{32}P-tracer methodologies (Barea et al. 2007). Upon adding a small amount of ^{32}P to label the exchangeable soil P pool, the isotopic composition, or "specific activity" ($SA = {}^{32}P/{}^{31}P$ quotient), was determined in plant tissues. It was found that dual inoculation reduced the SA of the host plant, indicating that these plants acquired P from sources which were not directly available to non-inoculated or singly-inoculated plants. Microbial inoculation improved biomass production and P accumulation in plants, demonstrating the interactive effects of PSM and AM-fungi on P capture, cycling and supply in a tailored mycorrhizosphere (Barea et al. 2008).

Multi-microbial interactions, including those between locally isolated AM fungi, PSB, and *Azospirillum*, have also been reported, which indicate that microorganisms can act synergistically when co-inoculated (Azcón and Barea 2010).

Mycorrhizosphere Interactions in Phytoremediation of Heavy Metal Contaminated Soils

AM fungi improve phytoremediation of soils contaminated with heavy metals, radionuclides or polycyclic aromatic hydrocarbons (Leyval et al. 2002). Most phytoremediation assays involving mycorrhizosphere interactions concern HM and different strategies of phytoremediation, have been investigated (Turnau et al. 2006, Ruíz-Lozano and Azcón 2011). These studies mostly concentrated on Zn, Cu, Cd, Pb or Ni.

Interactions between rhizobacteria and AM-fungi have been investigated in diverse experiments to ascertain whether they are able to benefit phytoremediation (Azcón et al. 2009a,b, 2010). The main achievements resulting from these experiments using HM-multiple contaminated soils and *Trifolium* as test plants were: (i) a number of bacteria and the AM-fungi were isolated from a HM-contaminated soil, and identified by 16S rRNA or 18S rRNA gene sequencing, respectively; (ii) the target bacteria were able to accumulate large amounts of metals; (iii) co-inoculation with a HM-adapted autochthonous bacteria and AM fungi increased biomass, N and P content as compared to non-inoculated plants, and also enhanced the establishment of symbiotic structures (nodule number and AM colonization), which were negatively affected as the level of HM in soil increased; (iv) dual inoculation lowered HM concentrations in *Trifolium* plants, inferring a phytostabilization-based activity, however, as the total HM content in plant shoots was higher in dually-inoculated plants, due to the effect on biomass accumulation, a possible phytoextraction activity was suggested; and (v) inoculated HM-adapted bacteria increased dehydrogenase, phosphatase and β-gluconase activities, and auxin production, in the mycorrhizosphere, indicating an enhancement of microbial activities related to plant development. The physiological/biochemical mechanisms by which the tested bacterial isolates enhanced phytoremediation activity in AM plants include: (i) improved rooting, and AM formation and functioning; (ii) enhanced microbial activity in the mycorrhizosphere; and (iii) accumulation of metals in the root-soil environment, thus avoiding their transfer to the trophic chain, or to aquifers (Ruíz-Lozano and Azcón 2011).

Inoculation of autochthonous AM fungi and bacteria, together with the application of treated agrowaste residue, changed the bacterial community structure and enhanced phytoextraction to remediate heavy metals contaminated soils (Azcón et al. 2009a). An enhancement of antioxidant activities in plants inoculated with AM fungi and bacteria, and agrowaste residue, was observed (Azcón et al. 2009b). Such a mycorrhizosphere effect seems to help plants to limit oxidative damage to bio-molecules in response to metal stress.

Mycorrhizosphere Interaction for Restoring Degraded Ecosystems

As desertification causes disturbance of plant-microbe symbioses, the recovery of populations of AM fungi and rhizobial bacteria is essential to the integral restoration of a degraded area (Barea et al. 2011, Jeffries and Barea 2012). Accordingly, management of AM fungi, together with rhizosphere bacteria, was proposed for the integral restoration of degraded ecosystems. A model experiment in this context was carried out in a desertified semi-arid ecosystem with *Anthyllis cytisoides*, a drought-tolerant legume, as the test plant (Requena et al. 2001). *Anthyllis* seedlings inoculated with indigenous rhizobia and AM fungi were transplanted to field plots for a five-year-trial. The tailored mycorrhizosphere enhanced seedling survival and growth, P-acquisition, N-fixation, and N-transfer from N-fixing to associated non-fixing species in the natural succession. The improvement in the physical-chemical properties in the soil around the *Anthyllis* plants was shown by the increased levels of N, organic matter and number of hydro-stable soil aggregates. Glomalin-related glycol-proteins, produced by the external hyphae of AM fungi, seen to be involved in the initiation and stabilization of water-stable soil aggregates, due to its glue-like hydrophobic nature (Miller and Jastrow 2000, Rillig and Mummey 2006, Bedini et al. 2009).

Future Trends

As the current scenario of climate change is threatening the stability of both natural and agricultural systems, research efforts must be addressed to potentiate the AM role as an adaptive strategy to confer plants an increased tolerance to environmental stresses. Therefore, both basic and applied research needs to be implemented. On the one hand, we need to increase our knowledge on the underlying mechanisms involved in an increased tolerance of mycorrhizal plants to either biotic or abiotic stresses. Future functional genomics (including transcriptomics, proteomics and metabolomics) developments will be useful to identify the genes expressed in AM plants that play key roles in processes such as nutrient mobilization, abiotic stress alleviation or suppression of plant diseases. On the other hand, the specific management of AM fungi should be one of the main objectives of applied studies in the future and the use of AM inoculants must take into account the importance of retaining AM diversity in the rhizosphere to achieve realistic and effective biotechnological applications in agricultural and natural systems.

Accordingly, mycorrhizal technology should be a component in sustainable agricultural strategies in the future, since application of AM fungi can reduce fertilizer and energy inputs yet promoting healthy plant growth, particularly in a world of depleting non-renewable resources. The application of AM inoculants is likely to become even more important due to the agro-ecological threats of agrochemicals, which are urgently required to be reduced, and even avoided, to increase food quality, sustainable food production and environmental protection. Further studies must address the consequences of the co-operation between microbes in the mycorhizosphere under field conditions to assess their ecological impacts as a viable alternative for agriculture, horticulture, and revegetation of degraded ecosystems.

With regard to natural ecosystem conservation, in spite of many achievements that have been reached investigating the ecological and functional role of mycorrhizas in the restoration of degraded areas, we still lack a comprehensive view of the mycorrhizal potentiality in propelling ecosystem dynamics and activities by improving the composition, diversity, structure and functionality of stress-adapted plant communities.

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