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## The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility

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**Abstract** Beneficial plant–microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility. Arbuscular mycorrhizas are the most important microbial symbioses for the majority of plants and, under conditions of P-limitation, influence plant community development, nutrient uptake, water relations and above-ground productivity. They also act as bioprotectants against pathogens and toxic stresses. This review discusses the mechanism by which these benefits are conferred through abiotic and biotic interactions in the rhizosphere. Attention is paid to the conservation of biodiversity in arbuscular mycorrhizal fungi (AMF). Examples are provided in which the ecology of AMF has been taken into account and has had an impact in landscape regeneration, horticulture, alleviation of desertification and in the bioremediation of contaminated soils. It is vital that soil scientists and agriculturalists pay due attention to the management of AMF in any schemes to increase, restore or maintain soil fertility.

**Keywords** Biodiversity · Bioremediation ·  
Desertification · Horticulture · Mycorrhizas ·  
Rhizosphere · Sustainability

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### Introduction

Soil microorganisms are paramount in the biogeochemical cycling of both inorganic and organic nutrients in the soil and in the maintenance of soil quality. In particular, microbial activity in the rhizosphere is a major factor that determines the availability of nutrients to plants and has a significant influence on plant health and productivity. An understanding of the basic principles of *rhizosphere* microbial ecology, including the function and diversity of the microorganisms that reside there, is necessary before soil microbial technologies can be applied (Bolton et al. 1992). In this context, it is important to use a broad definition of the rhizosphere to include the rhizosphere soil, the volume of soil adjacent to and influenced by the root, the root surface or *rhizoplane*, and the root itself, which includes the cells of the root cortex where invasion and colonisation by endophytic microorganisms has occurred. Soil–plant–microbe interactions are complex and there are many ways in which the outcomes can influence plant health and productivity (Kennedy 1998). The results may be harmful, neutral or beneficial to the plants. In this review, we concentrate on the beneficial interactions between plants and rhizosphere microorganisms. In particular, focus will be placed on the arbuscular mycorrhizal fungi (AMF), as these organisms have been the subject of several successful EU COST Actions (8.10, 8.21, 8.38) over the last few years. The review ends with some practical examples of where AMF have been shown to have considerable significance in the maintenance of soil health and fertility.

There are several groups of beneficial rhizosphere microorganisms. Some engage in well-developed symbiotic interactions in which particular organs are formed, such as mycorrhizas and root nodules, whilst others develop from fairly loose associations with the root. The former arise from a series of developmental events programmed as a result of molecular cross-talk between plant roots and their respective symbionts. The interaction between rhizobial bacteria and the roots of leguminous plants has been well researched (e.g. Brockwell et al.

1995), but for the mycorrhizal relationship it has only recently become a significant topic of research (Smith and Read 1997). Other plant root–microbe interactions arise from specific interactions between groups of bacteria or fungi that are adapted to live in the rhizosphere. These *rhizobacteria* or *rhizofungi* are adapted to exploit this niche and proliferate in the rhizosphere. These organisms often act synergistically in combination with AMF. Of particular importance in plant health and soil fertility are the plant-growth-promoting rhizobacteria (PGPR) and plant-growth-promoting fungi (PGPF). Their effects are mediated through a variety of mechanisms. Some rely on interactions with potential phytopathogens such that ‘bioprotection’ results (Azcón-Aguilar and Barea 1996). Others produce compounds that directly stimulate plant growth, such as vitamins or plant hormones (Barea 1997, 2000). Others, such as the fungus *Trichoderma*, may stimulate plant growth in more than one way (Ousley et al. 1994).

In terms of ubiquity and partnerships throughout the plant kingdom, mycorrhizal relationships are the most significant plant–microbe symbiosis. There are several types of mycorrhizal relationship. For example, ectomycorrhizal fungi (ECMF) form a particular, extracellular morphological complex with the roots of many temperate forest trees. Many thousands of fungi can form these relationships, in contrast to the restricted range of plant species that are involved. Even more specific are the unique mycorrhizal relationships formed by the arbutoid, orchid and ericoid plant families. However, it is the arbuscular mycorrhizal fungi which form the most ancient and widespread mycorrhizal relationship. More than 80% of plant species can form arbuscular mycorrhizas (AM), yet relatively few fungal species (~120) from a restricted taxon, the Glomales, are involved. This reflects the evolutionary history of the relationship. Both fossil (Remy et al. 1994) and molecular phylogenetic (Simon et al. 1993) evidence supports the hypothesis that terrestrial plants evolved with the aid of existing arbuscular mycorrhizal relationships. Thus many plants have co-evolved with this symbiosis – a significant factor in explaining the dependency and stability of this relationship. The mycorrhizal symbiosis is a keystone to the productivity and diversity of natural plant ecosystems and it is rare to find a situation where AM do not have a significant ecological presence. As a consequence, loss or perturbation of this relationship can have serious consequences in terms of plant community degradation, health or productivity. Loss of propagules of AMF will result in a decrease in the capacity of plants to take up nutrients, thus lowering soil fertility. The stability of the ecosystem then becomes threatened.

As a consequence, there are a number of situations where manipulation or management of the mycorrhizal symbiosis is necessary to restore plant cover, improve plant health or increase plant productivity. These situations usually arise from anthropogenic sources, but natural events may also contribute (e.g. volcanic activity, climate drift). Soils with an absence of appropriate

mycorrhizal propagules can be found where subsurface soils are brought to surface (e.g. mine spoils, tunnelling activities, volcanic depositions). In contrast, other soils may contain a mycorrhizal flora compatible with an established plant ecosystem, but may be subjected to a drastic change in plant community as a result of human intervention (e.g. deforestation, shifting agriculture, reforestation or revegetation). Finally, the soil microorganisms may be depleted as a result of agricultural practices that reduce the inoculum potential of the beneficial components (Jeffries and Barea 2001). It is well documented, for example, that elevation of soil P concentrations as a result of intensive agriculture can decrease the soil populations of AMF (Smith and Read 1997). Thus mycorrhizal technology becomes an important consideration in low-input, organic or soil-less agriculture. These situations are reviewed here in the context of soil biotechnology. Plant growth can also be inhibited as a result of the accumulation of phytotoxic levels of heavy metals and organic xenobiotics. In these situations, the mycorrhizal relationship becomes important in ameliorating the toxic effects. However, the selection of appropriate adapted strains of AMF is important. Application of mycorrhizal technologies then requires a knowledge of the biodiversity across and within the species involved. This then needs to be interpreted in terms of a predictor of potential mycorrhizal effectiveness. Recent research has highlighted the complexity and novelty of population biology of AMF and will be considered later in this review. The individuality and plasticity of specific isolates needs to be evaluated. Not all isolates of the same species have identical properties, and it is now clear that multiple genomes can co-exist within individual AMF (Kuhn et al. 2001; Rodriguez et al. 2001).

Mycorrhizal technology is aimed at restoring the inoculum potential of AMF in problem soils. This may be achieved through bioaugmentation, by inoculating soils with AMF or by using transplanted seedlings that already have the appropriate AMF in their roots. Alternatively, indigenous but depleted populations of AMF may be restored by the use of a mycotrophic cover crop that stimulates the build-up of inoculum such that subsequent crops or plant communities gain the benefits (Dodd et al. 1990). Here, we describe how the arbuscular mycorrhizal symbiosis can be affected by a variety of abiotic and biotic factors, and how genetic diversity can be evaluated. We then illustrate the application of the technology with a series of representative examples. In terms of cost–benefit analyses of the AM symbiosis, it is well known that the beneficial effect of the AM symbiosis is multifaceted, and does not result solely from improved P uptake. Thus it is not appropriate to compare the costs of inoculation directly with the costs of P fertiliser additives that achieve an equivalent growth response in target plants. Nevertheless, comprehensive data on the relative effects of both in tropical agrosystems is given by Sieverding (1991). Indeed, the benefits of soil additives are usually short-lived, unless slow-release formulations

are used, and hence are not sustainable in low-input or natural ecosystems. If the bare economics of the relative costs of fertilisers versus inoculum are compared, the latter will be more costly in the short term. However, in terms of overall plant health and sustainability, the benefits of establishing an effective AM symbiosis are much wider and more long-lasting, as the examples quoted in this review will illustrate.

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### Contribution of AMF to soil health

Soil is a limited resource and its health is critical for any sustainable development. Many anthropogenic practices have resulted in soil degradation and in the case of agricultural soils, in the progressive loss of soil fertility. The quality of soil depends not only on its physical or chemical properties, but also on the diversity and activity of its biota (Doran and Linn 1994). Arbuscular mycorrhizal fungi are essential components of soil biota; they can be found in nearly all ecological situations, both in natural ecosystems, particularly in those supporting plant communities with a high species diversity, and also in normal cropping systems, especially if managed with sustainable practices (Gianinazzi and Schüepp 1994). The impact of AMF on the reduction of soil-borne diseases has mainly been evaluated in studies on soil fungal pathogens such as *Phytophthora*, *Aphanomyces*, *Fusarium* and *Verticillium* (Azcon-Aguilar and Barea 1996) and nematodes causing, respectively, root rots and lesions and galls (Guillemin et al. 1993; Pinochet et al. 1996). Some studies have shown that mycorrhizal protection could also occur against *Erwinia carotovora* and *Pseudomonas syringae* (Garcia-Garrido and Ocampo 1989). Arbuscular mycorrhizal fungi are obligate symbionts and their life cycle depends on plant roots, and in return they decrease disease in the latter and reduce population levels of pathogenic microorganisms in the soil, especially where the supply of P is limiting (Linderman 1994). This raises the question of how AMF can be manipulated to harness the benefits for plant health that can be derived from these microorganisms, via management practices that enhance their activity in reducing soil-borne diseases. However, this will depend heavily on our understanding of the functional processes carried out by AMF as well as the development of strategies to enhance their activity in soils.

Arbuscular mycorrhizal fungi develop intensively inside roots and within the soil by forming an extensive extraradical network and this helps plants considerably in exploiting mineral nutrients and water from the soil. Phosphorus is the key element obtained by plants through the symbiosis and the evidence to support this is extensive (Smith and Read 1997). In exchange, mycorrhizal plants provide the fungus with photosynthetic C, which in turn is delivered to the soil via fungal hyphae. The extraradical hyphae of AMF therefore act as a direct conduit for host C into the soil and contribute directly to its C pools, bypassing the decomposition process. As a consequence of

this, the amount and activity of other soil biota are stimulated; however, this seems to be a selective phenomenon, since it stimulates in particular the microbes having antagonistic activity against soil-borne pathogens (Linderman 2000). The reason for this phenomenon is unknown, but this observation clearly indicates that AMF could be useful biological tools for maintaining healthy soil systems.

The important role of the soil mycelium of mycorrhizal fungal mycelium in the formation of water-stable soil aggregates is well documented (Andrade et al. 1998; Bethlenfalvai et al. 1999; Miller and Jastrow 2000). Indeed, AMF produce a very stable hydrophobic glycoprotein, glomalin, which is deposited on the outer hyphal walls of the extraradical mycelium and on adjacent soil particles, and which appears to act as a long-term soil binding agent (Wright and Upadhyaya 1998, 1999). As a consequence, the extraradical hyphae, together with the fibrous roots, can form a “sticky-string bag that contributes to the entanglement and enmeshment of soil particles to form macroaggregates” (Miller and Jastrow 2000), a basic building block of soil structure. This underlines once more that AMF are essential components of ecosystems and that their use could be crucial, not only for revegetation of spoiled lands, but more importantly for maintaining soil structure in agricultural soils. Many biotic and abiotic interactions around roots are probably mediated by AMF and, as suggested by Bethlenfalvai and Lindermann (1992), “the role of AMF may be critical if agriculture is to return to the state where luxury levels of farm inputs of fertilisers, pesticides and or chemicals are decreased to levels that are still economic, yet do not pollute the environment or pose health risks to consumers or handlers”. However, a successful shift in emphasis from chemicals to natural methods, such as crop rotation and the rational use of beneficial soil microorganisms, such as AMF, requires better knowledge on the dynamic relationships between agricultural practices and spatiotemporal interactions between cultivated crops, AMF and other soil biota.

This need for knowledge, together with the fact that AMF are obligate symbionts, which has hindered inoculum production, has limited the exploitation of AMF in farming systems. In spite of this, evidence exists that the modification of some agricultural practices could favour the beneficial influence of AMF in crop productivity. For example, the large changes in populations of AMF associated with different crop rotations under the same soil and climatic conditions (Hendrix et al. 1995), suggest that plants are dominant factors affecting communities of AMF. Therefore an appropriate crop rotation may represent a useful tool for managing AMF in the field. Pre-cropping with mycorrhizal-dependent plant species has been successfully used in the field to bolster indigenous populations of beneficial AMF (Dodd et al. 1990). The ability of arbuscular mycorrhizal plants to utilise organic forms of P (Kahiluoto and Vestberg 1998) and N (Hawking et al. 2000) may also be an important factor for favouring development of AMF where organic

sources of nutrients are used. This should be balanced with the type of soil and the amount and the content of farm and urban wastes used (Jacquot-Plumey et al. 2001). A shift towards more sustainable forms of agriculture may also permit a reduction in the amount of herbicides used. The result would be the persistence of an acceptable amount of weeds within crops, which could provide a reservoir of mycorrhizal inoculum, particularly advantageous when non-mycorrhizal crops are part of the rotation (J.P. Caussanel and S. Gianinazzi, unpublished results).

### Interactions of AMF and abiotic factors

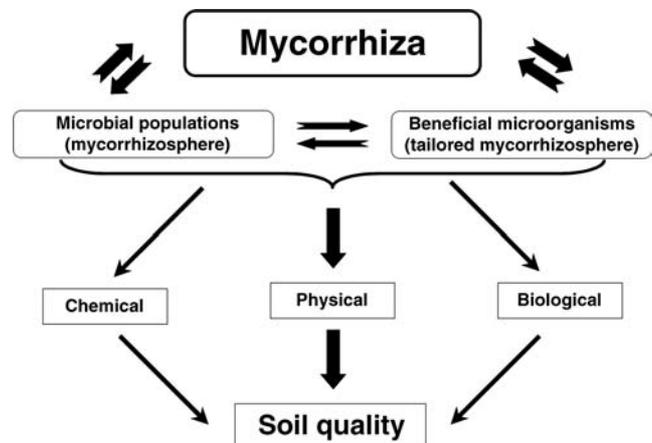
A number of abiotic factors such as climate change, drought stress, pollution and heavy metal contamination can influence the development of mycorrhizal relationships. In almost all cases, their presence can alleviate the stresses to plants caused by these external influences. Here we concentrate on excess levels of chemical components in the soil. Inefficient mining processes, treatment of soil with sewage sludge or industrial effluents, overuse of heavy-metal-containing fertilisers or gas exhausts have, among other factors, contributed to the creation of large areas contaminated by heavy metals, radionuclides and persistent organic pollutants. Such areas may jeopardise human health and ecology (Adriano et al. 1998) and, therefore, should not only be under strict control, but should also be managed in a way which attenuates the possibility of any risk concerning the contamination of the food chain and the ground water or air by wind erosion.

The stimulation of microorganisms by the plant root system has now attracted attention. Microbial activity has been found to be an important factor influencing metal solubility and an immobiliser of soil metals due to precipitation of sulphides and hydrated ferric oxides or by exudation of polysaccharides (Lodenius and Autio 1989; Ernst 1996). Organic functional groups on the surface of bacterial cell walls play an important role in adsorption of metals from the soil solution (Fein et al. 1997). Under other conditions Pb, Zn and Cu may be mobilised from the carbonates and oxides by microbial activity (Bloomfield 1981). Biological methods to remove pollutants have mainly employed bacteria and saprobic fungi, while the role of mycorrhizal fungi has been almost completely neglected. A well-developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by better nutrient acquisition, water relations, pathogenic resistance, phytohormone production, contribution to soil aggregation, amelioration of soil structure, and thus improved success of all kinds of bioremediation. For example, AMF have been found to decrease caesium uptake by plants (Berreck and Haselwandter 2001), and thus could be used in the establishment of plant vegetation on soil contaminated with radionuclides, and therefore have the potential to reduce environmental risks. Effective mycorrhiza may also attenuate deleterious soil conditions (Haselwandter and Bowen 1996).

Arbuscular mycorrhizal fungi also have a potential role in the monitoring of site toxicity (Weissenhorn et al. 1993, 1995; Gucwa-Przepióra and Turnau 2001) or the efficiency of restoration techniques (Orłowska et al. 2002). The use of mycorrhizal parameters as an indicator of changes occurring during soil restoration and a tool for biomonitoring of soil quality has already been addressed by Lovera and Cuenca (1996), Haselwandter (1997) and Jacquot et al. (2000). Levels of colonisation of grasses in polluted field soils have been shown to correlate with heavy metal contamination (Mikanová et al. 2001). Plants such as *Plantago lanceolata* might be of special value for biomonitoring (Orłowska et al. 2002). This species is strongly mycorrhizal, suitable for use in growth chambers and greenhouses where it easily forms mycorrhizal associations (Walker and Vestberg 1994), and can be vegetatively propagated (Wu and Antonovics 1975), facilitating the avoidance of genetic variability in response to toxic substances. In addition it is widespread and tolerant to a broad range of soil types and pollutants (Wu and Antonovics 1976; Baroni et al. 2000; Bakker et al. 1999).

### Interactions of AMF and biotic factors

Arbuscular mycorrhizal fungi are key components of the soil microbiota and obviously interact with other microorganisms in the rhizosphere (Bowen and Rovira 1999). In this context, formation of AM changes plant physiology and certain nutritional and physical properties of the rhizosphere soil. This, in turn, affects colonisation patterns of this region by soil microorganisms by the so-called *mycorrhizosphere* effect (Gryndler 2000). Arbuscular mycorrhizal fungi thus interact with natural and introduced microorganisms in the mycorrhizosphere, hence affecting soil properties and quality (Fig. 1). Conversely, soil organisms are known to affect AM formation and functioning markedly (Barea et al. 2002).



**Fig. 1** Arbuscular mycorrhizal fungi interact with natural and introduced microorganisms in the mycorrhizosphere, thus affecting soil properties and quality

Here, we summarise the main effects of the rhizosphere microbiota on the development of AM, specifically on: (1) AM formation, (2) nutrient cycling and plant growth and (3) biological control of plant pathogens. In addition, it is known that the soil micro-fauna can also affect development of AM, but the evidence is fragmentary (Gange 2000; Gryndler 2000).

Microbial populations in the rhizosphere can either interfere with or benefit establishment of AM (Vosátka and Gryndler 1999). Deleterious rhizosphere bacteria (Nehl et al. 1996) and mycoparasitic relationships (Jeffries 1997) have been found to interfere with AM development, while many microorganisms can stimulate AM formation and/or functioning (Gryndler 2000; Barea et al. 2002). Soil microorganisms can produce compounds that increase root cell permeability, thereby increasing the rates of root exudation. This, in turn, stimulates the growth of hyphae of AMF in the rhizosphere and facilitates root penetration by the fungus. In addition, polysaccharide-producing bacteria may have a synergistic effect on hydrostable aggregate formation by AMF as discussed by Miller and Jastrow (2000). This aspect will also be considered in the later section regarding the role of AMF in alleviating the effects of desertification.

Rhizosphere microorganisms are also known to affect the pre-symbiotic stages of AM development (Giovannetti 2000), such as spore germination and germ tube growth (Azcón-Aguilar and Barea 1992, 1995). Biologically active substances such as amino acids, plant hormones, vitamins, other organic compounds and volatile substances (CO<sub>2</sub>), produced by soil microorganisms, can stimulate the growth rates of AMF (Azcón-Aguilar and Barea 1995; Barea 1997, 2000). Detrimental effects of soil microorganisms on spore germination and hyphal growth in soil have also been reported (Linderman 1992; Azcón-Aguilar and Barea 1992). It is noteworthy that antifungal compounds produced by *Pseudomonas* spp. did not interfere with AM formation or functioning (Barea et al. 1998; Vázquez et al. 2000), nor did a similar molecule produced by *Paenibacillus* sp. (Budi et al. 1999).

The well-known activities of dinitrogen-fixing bacteria and phosphate-solubilising microorganisms in improving the bioavailability of the major plant nutrients, N and P, contribute to the AM role in nutrient acquisition (Barea et al. 2002). Management of such interactions is a promising approach either for low-input agricultural technologies (Bethlenfalvay and Linderman 1992; Gianinazzi and Schüepp 1994; Jeffries and Barea 2001), or for the re-establishment of natural vegetation in a degraded area (Miller and Jastrow 2000; Jeffries and Barea 2001). It is known that certain rhizobial strains improve processes involved in AM formation by *Glomus mosseae*, i.e. spore germination, mycelial growth from the mycorrhizal propagules and “entry point” formation on the developing root system of the common host legume plant (Barea et al. 1996). Measurements of the <sup>15</sup>N/<sup>14</sup>N ratio in plant shoots indicated enhancement of the N<sub>2</sub> fixation rates in *Rhizobium*-inoculated mycorrhizal plants, relative to that

achieved by the same *Rhizobium* strain in non-mycorrhizal plants (Toro et al. 1998).

Multi-microbial interactions including AMF, *Rhizobium* spp. and PGPR have also been investigated (Requena et al. 1997). In general, the results demonstrate the importance of physiological and genetic adaptation of microbes to the environment, and thus local isolates must be used. Several microbial combinations were effective in improving plant development, nutrient uptake, N<sub>2</sub>-fixation (<sup>15</sup>N) or root system quality, showing that selective and specific functional compatibility relationships among the microbial inoculants were evident with respect to plant response. Interactions between AMF and *Azospirillum* may also enhance mycorrhiza formation (Volpin and Kapulnik 1994).

The interactive effect of phosphate solubilising rhizobacteria (PSB) and AMF on plant use of soil-P sources of low bioavailability (endogenous or added as rock phosphate) has been evaluated in soil microcosms using a <sup>32</sup>P isotopic dilution approach (Toro et al. 1997). The rhizobacteria behaved as mycorrhiza-helper bacteria, promoting AM establishment by either the indigenous or inoculated AMF, while AM formation increased the size of the PSB population. Because the bacteria did not change root weight, length or specific root length, they probably acted by improving the pre-colonisation stages of AM establishment. The dual inoculation treatment significantly increased microbial biomass and N and P accumulation in plant tissues and these dual-inoculated plants displayed lower specific activity (<sup>32</sup>P/<sup>31</sup>P) than their comparable controls, suggesting that the mycorrhizal and bacterized plants were using P sources otherwise unavailable to the plant. It therefore appears that these rhizosphere/mycorrhizosphere interactions contributed to the biogeochemical cycling of P, thus promoting plant fitness. These effects were further validated under field conditions (Barea et al. 2002).

### Evaluation of biodiversity and conservation of AMF

The diversity of AMF has significant ecological consequences because individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors. Thus, the composition and dynamics of populations of AMF have a marked impact on the structure and diversity of the associated plant communities, both in natural and agricultural ecosystems (Grime et al. 1987; Gange et al. 1990; van der Heijden et al. 1999). An important prerequisite to the analysis of populations of AMF in ecological studies is the correct identification of individual isolates. In addition, physiological studies as well as field inoculation experiments may benefit from genetic and functional analysis of selected isolates.

Until recently, the markers identifying AMF for population and phylogenetic studies were solely morphological or biochemical (Giovannetti and Gianinazzi-

Pearson 1994). Studies of the macro- and micro-anatomy of fungi yield characters that “form the historical bedrock in fungal taxonomy” (Kohn 1992), and have been used to build the taxonomy of AMF (Morton and Benny 1990). However, many structures needed for morphological identification and species differentiation are lost during the symbiosis, and the arbuscules produced by AMF in the plant, for example, are very similar from one species to another. Allozymes have been helpful in providing diagnostic biochemical markers to identify species of AMF, even in colonised roots (Sen and Hepper 1996). However, the most powerful tools to study the evolution and population genetics of AMF are molecular techniques that analyse DNA sequences. These techniques have been used in combination with morphological or biochemical data to investigate specific groups. For example, combined morphological and molecular data were recently used to define relationships among ancient species within Glomales (Redecker et al. 2000) as well as to provide diagnostic primers important in the classification of these species (Morton and Redecker 2001). With a similar approach, isolates of uncertain taxonomic position within the family Gigasporaceae could be assigned to known species (Lanfranco et al. 2001). The combination of isozyme profiles and ribosomal gene sequences has also proved useful in defining groups in the genus *Gigaspora* (Bago et al. 1998).

A wide variety of techniques can be employed to detect DNA sequence variation in populations of AMF (Lanfranco et al. 1998). PCR amplification of targeted genomic sequences followed by RFLP, allele-specific hybridisation, direct sequencing, or single-strand conformation polymorphisms are increasingly used to detect AMF in natural ecosystems (Sanders et al. 1996; Redecker et al. 1997; Helgason et al. 1998, 1999). PCR primers based on highly conserved regions of nuclear and mitochondrial ribosomal DNA have been designed (White et al. 1990; Simon et al. 1992; Sanders et al. 1996; Lanfranco et al. 1999; Redecker 2000; Schüssler et al. 2001) to amplify two variable non-coding regions, namely the internal transcribed spacers (ITS) and the intergenic spacers (IGS). Microsatellite-primed PCR, RAPD and repeated DNA probes are highly efficient approaches for the identification of distinct genotypes (Wyss and Bonfante 1993; Longato and Bonfante 1997) and have been employed to determine the genetic structure of populations of AMF.

DNA markers have been successfully employed to track specific AMF from agricultural and natural ecosystems (Helgason et al. 1998, 1999; Antonioli et al. 2000; Pringle et al. 2000; Jacquot-Plumey et al. 2001; Turnau et al. 2001a). DNA markers have also revealed considerable diversity in a single root system: several types of AMF were associated with a single *Hyacinthus* root (Merryweather and Fitter 1998), and with individual small root fragments of leek or onion (van Tuinen et al. 1998) and with two *Prunella* species (Streitwolfengel et al. 1997).

One aspect that has recently received attention in the evaluation of diversity of AMF is their unusual associ-

ation with endosymbiotic bacteria and the degree of their intraspecific diversity. Bacteria-like organisms in the cytoplasm of AMF were first observed by transmission electron microscopy in the early 1970s (Scannerini and Bonfante 1991), but confirmation of their prokaryotic nature was impeded by their inability to grow on cell-free media. A combined morphological and molecular approach has now shown that the cytoplasm of *Gigaspora margarita* spores harbours a homogeneous population of bacteria identified, from the sequence of their 16S ribosomal RNA gene, as close to the genus *Burkholderia* (Bianciotto et al. 1996). More recent phylogenetic studies suggest that these endobacteria probably represent a new bacterial taxon (Bianciotto et al. 2002). PCR assays with oligonucleotides specific for this 16S sequence have revealed these bacteria in all stages of the fungal life cycle (spores and symbiotic mycelia). In addition, isolates of different origin from the three Glomalean families (Glomaceae, Gigasporaceae and Acaulosporaceae) display bacteria when observed by confocal microscopy using a fluorescent dye specific for bacterial staining. The endobacteria of Gigasporaceae seem to be distinct from those found in other fungal taxa in terms of density, morphology and PCR amplification with specific primers (Bianciotto et al. 2000). It should be noted, however, that different situations exist within this genus: *Gigaspora rosea* was the only species to be completely devoid of endobacteria, an observation supported both by morphological observation of several isolates and by PCR experiments, whereas *Gigaspora gigantea* harboured genetically and morphologically distinct bacteria (Bianciotto et al. 2000). These intracellular bacteria seem therefore to be a general feature of spores of AMF and not a sporadic component. Additional evidence of the association of endobacteria with this group of fungi is their occurrence in *Geosiphon pyriforme*, a free-living Zygomycete closely related to Glomales (Gherig et al. 1996).

The evaluation of genetic diversity in Glomales is complicated by the presence of several different ribosomal variants, both in the conserved regions (large subunit) and in the more variable internal transcribed spacers (ITS). The ITS spacers are generally considered to be appropriate for discriminating at species level, provided that genetic variability among isolates of the same species is lower than variability among species. In AMF, a peculiar feature is that ITS variants have been described not only among different isolates of the same species, but also within the same isolate (Sanders et al. 1995; Lloyd-MacGilp et al. 1996; Lanfranco et al. 2001). Three different species of *Gigaspora* were investigated, and within each species the degree of intra-isolate ITS variation was as great as the variation found for geographically distinct isolates (Lanfranco et al. 2001). Similar patterns have been described within and among isolates of *Glomus mosseae* (Lloyd-MacGilp et al. 1996). In some cases it was demonstrated that the ITS variants occur within single spores (Sanders et al. 1995; Lloyd-MacGilp et al. 1996; Hijri et al. 1999; Lanfranco et al.

1999; Antonioli et al. 2000; Pringle et al. 2000). The origin of this genetic heterogeneity remains to be established, although various different hypotheses have been proposed (Sanders 1999; Gianinazzi-Pearson et al. 2001). ITS sequence heterogeneity in Glomales has been correlated with its multinucleated spores (Sanders et al. 1995; Lloyd-MacGilp et al. 1996; Lanfranco et al. 1999). The presence of 1,000–5,000 nuclei in the same spore (Bécard and Pfeffer 1993), together with the presence of about 75 copies of ribosomal genes for each nucleus (Hosny et al. 1999), may lead to a sequence variability because (1) nuclei of the same spore are genetically different (intraspore polymorphism) or (2) ribosomal gene sequences are different in a single nucleus (intra-organelle polymorphism). The latter hypothesis requires mutation and rearrangement events within a single nucleus. On the other hand, in sexual organisms, the occurrence of concerted evolution (via unequal crossing over or gene conversion during recombination processes) maintains sequence homogeneity in multicopy genes (Li 1997). The heterogeneity of ribosomal sequences in AMF, thought to be asexual organisms, could be explained by the absence of these mechanisms (Sanders et al. 1996; Sanders 1999). Fluorescent DNA–DNA hybridisation studies suggest that ITS2 variation does occur across individual nuclei within single Glomalean spores (Kuhn et al. 2001), thus supporting the hypothesis that genetically distinct nuclei do co-exist within a single individual. This phenomenon requires a heterokaryotic status as the result of hyphal anastomosis, which has been observed in AMF (Giovannetti et al. 1999, 2001). Morphological studies, flow and static cytometry, and bromodeoxyuridine uptake experiments (Bécard and Pfeffer 1993; Bianciotto et al. 1995), have shown that only half the 2,500 nuclei present in *G. margarita* spores migrate towards the germinating hypha. Only 10–15% then enter the cycle, and move along the hyphae that contact the root and produce an infection unit, where new mitoses occur. Given that the nuclei of the originating spore have a different genotype, the fingerprint of the colonising mycelium (and the newly produced spores) will be different from the originating spore. On the other hand, the same root is usually colonised by mycelia from different spores, resulting in infection units with dissimilar genetic fingerprints, and the possibility of fresh heterokaryotic events.

DNA sequence variants have also been found in more conserved ribosomal regions. Clapp et al. (2001) estimated the magnitude of sequence variation in the large subunit rDNA gene, which should contain regions with interspecies polymorphisms sufficient to discriminate AMF (van Tuinen et al. 1998). The analysis on isolates from three species (*Glomus mosseae*, *G. coronatum* and *G. constrictum*) revealed several sequence variants within the same isolate in all species, and cluster analysis showed that some sequences from *G. mosseae* and *G. constrictum* clustered with *G. coronatum*. Sequence variability among isolates therefore obscured species level resolution. A similar study of *Entrophospora infrequens* (Rodríguez et al. 2001) demonstrated that this

species contained large subunit rDNA gene sequences that clustered with sequences from two different families of Glomalean fungi. Whatever the origin of this genetic heterogeneity, wider investigations are an important prerequisite for developing specific molecular tools to be used for diagnostic purposes.

Given their importance in ecosystems, conservation of AMF both in situ and ex situ must be a major objective. Germplasm collections have been developed to acquire, characterise and maintain AMF in living cultures. The two main collections, the BEG (Dodd et al. 1994; <http://www.ukc.ac.uk/bio/beg/>) and INVAM (Morton et al. 1993; <http://invam.caf.wvu.edu/>) play a crucial role in the preservation and distribution of fungal isolates for research. It is again important to stress that an accurate identification of reference fungal isolates deposited in public collections is required for correct conservation of this biodiversity as well as for physiological and phylogenetic studies.

## The impact of AMF in management of disturbed ecosystems

### 1. In landscape development

Arbuscular mycorrhizal fungi have been shown to rapidly invade virgin soils at Samphire Hoe in the UK (Dodd et al. 2002) and hence accelerate the natural process of plant community development. Samphire Hoe is a newly created land platform comprising the sub-seabed material excavated during the construction of the Channel Tunnel (Fig. 2). It represents a unique resource where the arrival and establishment of AMF within a sown plant community on a low-nutrient substrate can be monitored. Invasion by AMF was monitored in three ways: by assessing the degree of root colonisation within the roots of plants on the site; by using a successive trap culture technique to determine species richness of AMF; and by



Fig. 2 Aerial view of Samphire Hoe

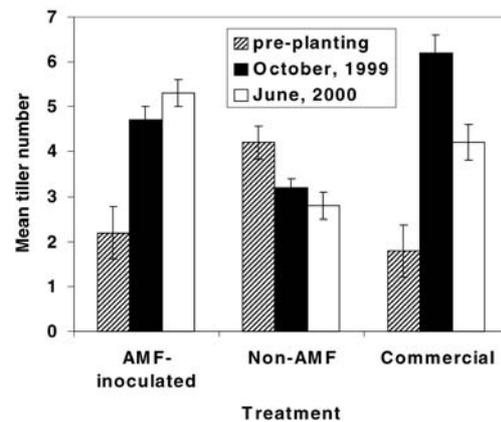


**Fig. 3** Field trial at Samphire Hoe where growth of AMF-inoculated *Elymus* is being evaluated against fertilised controls

using sterile substrate bins to determine the extent of wind-borne and rain-dispersed immigration of propagules of AMF. Levels of colonisation of indigenous plants by AMF were high in May–June (the pre-flowering phase of growth for many plants) reflecting the important role of the mycorrhizal symbiosis in dry, low-nutrient soils. Twelve species of AMF were identified, representing a relatively high diversity for a recently deposited subsoil. An on-site experiment indicated that inoculum of AMF could enter the site within 8 months and that wind dispersal and rain were possible vectors. A field experiment compared the outplanting performance of commercially-produced *Elymus pycnanthus* seedlings (in a commercial compost with added nutrients) with seedlings produced in a low-nutrient substrate and inoculated with AMF isolated from the site (a mixture of five species of *Glomus*) or left uninoculated (Fig. 3). After 14 months in the field, seedlings inoculated with the indigenous AMF had the same tiller production as the commercially produced plants, despite poorer initial growth. In contrast, non-mycorrhizal controls grew very poorly, with a greater frequency of plant mortality relative to the other treatments. *Elymus* seedlings inoculated with the indigenous AMF ultimately produced approximately seven times the mean number of seed spikes per surviving plant as the commercially produced seedlings and five times greater weight of seed spike (Fig. 4). A phyto-microbial approach to the revegetation of nutrient-poor soils has been proposed to stimulate plant successional processes as an economically viable sustainable input for landscaping anthropogenic sites (Dodd et al. 2002). A similar conclusion was reached by Greipson and El-Mayas (2001) during soil reclamation in Iceland.

## 2. In horticulture and agriculture

The use of AMF in agriculture could lead to a considerable decrease in the amount of chemical pollution in

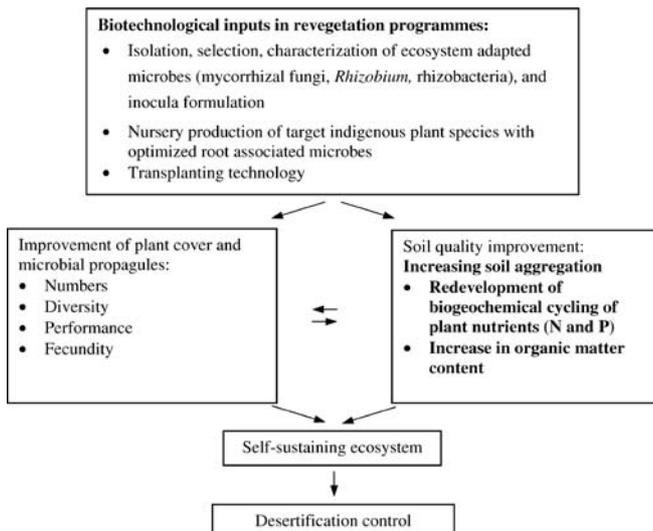


**Fig. 4** Mean seed spike numbers of *Elymus* plants inoculated with AMF compared with uninoculated control plants and a sample of commercially produced plants which have been fertilised. Error bars = SEM

soil water, as recently demonstrated for maize (Giovannetti 2001). This clearly indicates the potential of AMF for promoting a low chemical input agriculture (for a more exhaustive review, see Atkinson et al. 2002). The recent development of molecular probes able to differentiate AMF within roots and soils (van Tuinen et al. 1998; Jacquot et al. 2000; Jacquot-Plumey et al. 2001) opens new biotechnological perspectives for defining their population biology and therefore management strategies in the use of these symbiotic microbes in agriculture.

The difficulty in producing a large amount of inoculum of AMF for agricultural practices is less of a problem in horticultural crops, where inoculation could take place in seedling or cutting beds, over a relatively small surface area. Furthermore, the intensive use of artificial substrates where AMF are absent facilitate their introduction. The main difficulties relate to the choice of substrates and of horticultural practices compatible with the development of mycelium of AMF. The literature regarding AMF and their application in horticulture is extensive (Gianinazzi et al. 2001). Successful inoculation is usually achieved when AMF are introduced very early in the plant developmental process followed by the use of low amount of phosphate fertilisers and selective use of pesticides (Guillemin et al. 1993; B. Blal, unpublished results). By doing so, colonisation by AMF will follow root development of the inoculated seedlings or cuttings, with the consequence that plants will already be extensively mycorrhizal when transplanted into the field (Gianinazzi et al. 1995; Lovato et al. 1995). In this respect, the case of micropropagated plants is particularly interesting in that inoculation with AMF can (1) reduce plant losses during the acclimatisation phase, (2) subsequently stimulate plant development (including flowering) and (3) increase productivity after transplantation to the field (Lovato et al. 1996; Estaun et al. 1999).

Following field transplantation, the rhizosphere of a micropropagated plant is usually colonised with a variety of other soil microorganisms, some of which are syner-

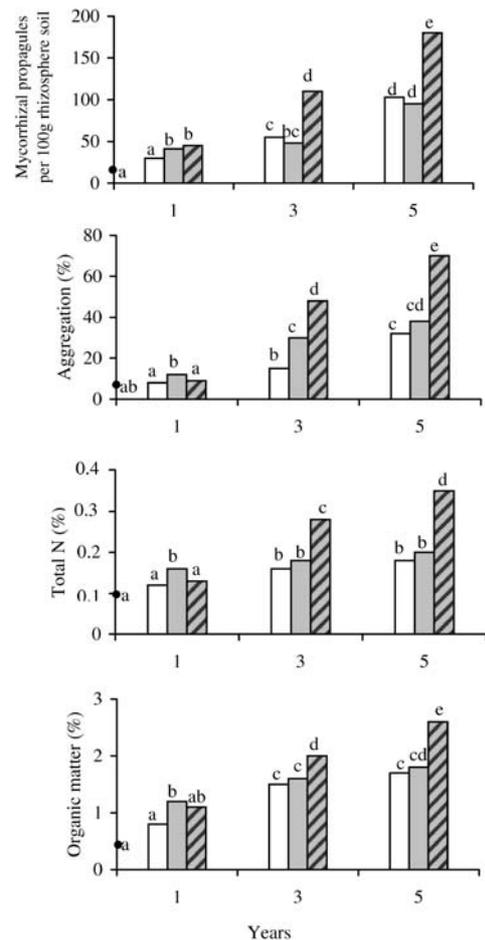


**Fig. 5** Proposed approaches to help plant establishment and to improve physical, chemical and biological soil properties essential to redevelop a self-sustaining ecosystem and combat desertification

gistic but some of which may be antagonistic to AMF development. Therefore the study of multi-microbial interactions in the rhizosphere of microplants may be a very useful approach for developing our understanding of managing AMF in plant production systems (Cordier et al. 1999).

### 3. In alleviating desertification

As a result of the ecosystem degradation processes in desertification-threatened areas, disturbance of natural plant communities is often accompanied or preceded by loss of physico-chemical and biological soil properties, such as soil structure, plant nutrient availability, organic matter content and microbial activity. Therefore, it is becoming critical to recover not only the vegetation but also these biological and physico-chemical soil qualities (Miller and Jastrow 2000; Jeffries and Barea 2001). There is an increasing interest in using AMF to improve revegetation processes for desertified ecosystems, particularly those developed under Mediterranean environments, based on the use of shrub plants belonging to the natural succession (Herrera et al. 1993). A proposed approach to combat desertification is shown (Fig. 5), which includes inoculation with symbiotic microorganisms including AMF. Experiments have been carried out aimed at assessing the long-term benefits of inoculation of shrub legumes with rhizobia and AMF. This has included improving the establishment of target legume species as well as the benefits induced by the symbiotically tailored seedlings in key physico-chemical soil properties (Requena et al. 2001). In this context, a representative area within a desertified semi-arid ecosystem in southeastern Spain was chosen for field studies on this topic. The existing natural vegetation was a degraded shrubland



**Fig. 6** Changes in soil quality parameters with time in the rhizosphere of field-established *Anthyllis* in a desertified ecosystem either uninoculated (first column), inoculated with an single exotic AMF species (second column) or inoculated with a mixture of indigenous AMF (third column). Mean values not sharing a letter differ significantly at  $P < 0.05$ . Data points on the y axis represent background levels in bare soil

where *Anthyllis cytisoides* was the dominant species (Requena et al. 1997). This is a drought-tolerant legume able to form symbioses with both rhizobia and AMF. The interaction of these microsymbionts on seedling establishment, survival rates, growth, N-fixation, and N-transfer from N-fixing to non-fixing species associated in the natural succession was studied (Requena et al. 2001). In addition, the possible improvement of soil quality in terms of N content, levels of organic matter, and hydrostable soil aggregates in the rhizosphere of the target plants were also evaluated. A long-term improvement in the physico-chemical properties was evident in the soil around the *Anthyllis* plants inoculated with an inoculum of AMF based on indigenous taxa (Fig. 6). The benefits included an increased N content, and higher amounts of organic matter and soil aggregation in the soil around the roots (Requena et al. 2001). It can be assumed that the increase in N content in the rhizosphere of the legume can be accounted for by an improvement in

nodulation and N-fixation rates resulting from inoculation of nodulated plants with AMF (Barea et al. 1992). The improvement of soil aggregation will maintain good water infiltration rates, good tilth and adequate aeration for plant growth, thus improving soil quality (Wright and Upadhyaya 1998).

Inoculation with native AMF also benefited plant growth, N fixation and P acquisition by plants. Improved N status of non-leguminous plants grown in association with legumes has previously been described for agricultural crops (Azcón-Aguilar et al. 1979), but this was the first demonstration of this phenomenon for natural plant communities in a semi-arid ecosystem. The results support the general conclusion that the introduction of target indigenous species of plants, associated with a managed community of microbial symbionts, is a successful biotechnological tool to aid the recovery of desertified ecosystems, suggesting that this represents the initial steps in the restoration of a self-sustaining ecosystem (Requena et al. 2001).

#### 4. In the bioremediation of soils containing pollutants

Several remediation technologies exist to treat soils containing pollutants. Physico-chemical extraction is the main technique used, though it is extremely expensive and usually destroys all soil organisms existing within the substratum. Bioremediation is an alternative. Three important approaches can be considered in the use of plants to clean up polluted soil. *Phytostabilisation* is a process in which pollutants are immobilised by plant activity, resulting in attenuation of the wind and soil erosion and run-off processes into the ground water or air (Losi et al. 1994). *Phytodegradation* covers the whole range of metabolic processes in which plants, usually assisted by microorganisms, degrade organic compounds such as hydrocarbons, pesticides, explosives etc. *Phytoextraction* involves metal hyperaccumulating plants, which can contain more than 1% of metals in harvestable tissues. The technique consists of growing the plants on polluted soil, and then collecting, drying and finally incinerating the plant material (Kumar et al. 1995; Brooks 1997).

Originally, attention was focused on the potential use of the plant rhizosphere to remediate contaminated soil. Plants were considered technically by engineers as solar-driven pumps (Adriano et al. 1998) without considering the associated microbiota. Remediation attempts were directed towards the application of soil amendments to increase the availability of the toxic substances, thus ameliorating the efficiency of phytoremediation (Blaylock et al. 1995; Salt et al. 1995; Chłopecka and Adriano 1996). Optimisation of the technology included improvement of biomass yield via nutrient supply (Baker et al., 1994), selection of the most efficient plant varieties, and genetic engineering (Levebre et al. 1987; Baker and Brooks 1989; Misra and Gedamu 1989; Maiti et al. 1991). The capability of plants to produce acid exudates

such as  $H_2CO_3$  and organic substances altering the plant rhizosphere and solubilisation of the adsorbed metals were also considered (Rao Gadde and Laitinen 1974; Ernst 1996; Krishnamurti et al. 1997). This raised the possibility of using plants effective in organic acid production, such as *Lupinus* spp., as an alternative for chemical amendments, stimulating the uptake of metals by plants used in soil cleaning technologies.

Irrespective of these early successes, it is now evident that the mycorrhizal symbiosis must be taken into account in phytostabilisation. Although initial colonisers of heavily polluted soils tend to be non-mycorrhizal (Shetty et al. 1994), the successful restoration and increase in plant community production, as well as improvement of soil structure, strongly depend upon the appearance of fungal symbionts. Attenuation of stresses is extremely important in sites such as post-flotation wastes, which are often devoid of basic nutrients such as N or P, have poor water-holding capacity, and are vulnerable to wind erosion (Turnau 1998; Gucwa-Przepióra and Turnau 2001). In places lacking AMF, the introduction of inoculum offers an interesting perspective for phytostabilisation techniques. The process may be stimulated by appropriately selected fungal strains. The source of fungi for inoculum production is very important, as they should be adapted not only to toxicity but also to climatic conditions. Fungal ecotypes from polluted places appear to be more successful than those from uncontaminated sites (Galli et al. 1994; Leyval et al. 1995). Extramatrical hyphae developed within the soil may play a role in heavy metal sequestration and thus in alleviation of their toxicity. The mycelium of metal-tolerant *Glomus mosseae* was shown to be several times more efficient in heavy metal adsorption than non-tolerant fungi and over ten times more effective than *Rhizopus arrhizus* used as a biosorption organism (Joner et al. 2000). Although the *G. mosseae* strain was shown to transport Cd from the soil into the fungal structures within the roots of clover, Cd was immobilised there and its transfer to the plant tissues was restricted (Joner and Leyval 1997). Similar results were obtained on the Cd- and Zn-binding capacity of a fungal strain colonising clover roots isolated from an absolute metallophyte plant, *Viola calaminaria* (Tonin et al. 2001). Inoculation of maize, lucerne (alfalfa), barley and others by the fungal isolate obtained from *V. calaminaria* roots has also been shown to attenuate heavy metal stress (Hildebrandt et al. 1999). This demonstrates the contribution of AMF to the accumulation of heavy metals in a non-toxic form within plant roots (Tonin et al. 2001). Differences in metal binding capacity exist not only between metal-tolerant and non-tolerant fungi, but possibly also between different species or isolates from the same site. This was shown for native *G. mosseae* in mycorrhizal roots from Polish zinc wastes. Mycorrhizal fungi of *Fragaria vesca* were identified using molecular tools (nested PCR with taxon-specific primers performed on spores and trypan blue stained roots) and cytochemical staining was used to visualise the distribution of heavy metals (Turnau et al. 2001b). The diversity in tolerance

mechanisms towards heavy metals transferred by the fungus into the root cortical cells also exists between plant species/varieties/ecotypes. The plants alone react differently to heavy metals (Antosiewicz 1992) and may also regulate the effectiveness of mycorrhizal colonisation (Koide and Schreiner 1992).

Biological methods of removal/degradation of organic pollutants from the soil have mainly employed bacteria and saprobic fungi (Wilson and Bradley 1996; Bezalel et al. 1997; Schützendübel et al. 1999). The positive effect of plants on the degradation of polycyclic aromatic hydrocarbons (PAHs) has also been reported (Schwab and Banks 1994; Reilley et al. 1996). In such cases plants producing a high root surface area have been the most extensively used. Well-developed associations with soil microbiota are an alternative for using chemicals to increase the availability of toxic substances (Schwab and Banks 1994; Shann and Boyle 1994). Among the organisms that could effectively increase the area of activity are mycorrhizal fungi. A range of mycorrhizal fungi were shown to be involved in the degradation of organic pollutants and thus they may be also potentially useful in phytodegradation. Although colonisation by AM was negatively affected by increasing PAH levels in soil (Leyval and Binet 1998), it can still enhance plant survival and growth by decreasing phosphorus deficiency (Joner and Leyval 2001) and water stress (Sanchez-Diaz and Honrubia 1994), improving membrane integrity (Graham et al. 1981) or by stimulation of oxidative enzyme production (Salzer et al. 1999). These phenomena are all responsible for the attenuation of stress due to pollution. It should be noted that the mechanisms involved in pollutant degradation may be indirect, and mediated through stimulation of the associated rhizosphere microflora (Joner et al. 2001).

Arbuscular mycorrhizal fungi can also be helpful in the management of constructed wetlands used for detoxification of a broad range of toxic substances. The importance of mycorrhiza of plants such as *Phragmites communis*, widely used to treat effluents, e.g. containing nitrophenols (Dias 1998), has been neglected. Oliveira et al. (2001) reported, however, the presence of the symbiosis when soils had reduced water content. This might play an important role in the initial steps of the establishment of wetland places, and subsequently could influence plant biodiversity in later stages, encouraging the re-appearance of mycorrhizal species (Vangronsveld et al. 1996).

Recently the presence of an arbuscular mycorrhizal symbiosis was also demonstrated in hyperaccumulating plants, which are being used in phytoextraction. Preliminary research (K. Turnau and J. Mesjasz-Przybyłowicz, unpublished data) has shown this for members of Compositae that hyperaccumulate Ni. Although the fungi are not expected to strongly increase heavy metal uptake of these plants, they certainly improve plant survival and health, stabilise the soil and attenuate soil erosion. Large areas in central and eastern Europe, which are strongly contaminated, await restoration. Despite the high con-

centration of elements such as Cd, Pb or As, vegetables are being cultivated there by inhabitants who ignore warnings concerning health risks. These areas cannot be chemically cleaned. They could be more easily restored by growing selected plant cultivars, such as maize, inoculated with metal-resistant fungi (Hildebrandt et al. 1999). For successful bioremediation, symbionts must be selected that can withstand the hostile environment of polluted sites. While introduction of new isolates is promising in areas devoid of AMF, the proper management of microbial resources, including mycorrhizal fungi and a broad range of rhizosphere bacteria, could be more appropriate where they are already present.

## Conclusions

This review has highlighted the practical situations where AMF have a significant impact in restoring or maintaining soil health and fertility. They are not unusual situations, but are commonplace throughout the world. Although they probably represent the most significant plant-microbe symbiosis, arbuscular mycorrhizal relationships are just one facet of the complex microbial interactions that occur in the rhizosphere. It is vital that soil scientists and agriculturalists pay them due attention in any schemes to increase, restore or manage soil fertility.

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