

Manzoor Ahmad Shah

Mycorrhizas: Novel Dimensions in the Changing World

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Preface

We are presently a part of the fast-changing world wherein our perceptions about various environmental concerns, and actions to address them, must change accordingly. Anthropogenic climate change, biological invasions, deforestation, desertification and frequent droughts are just some of the major elements of current global environmental change. In-depth knowledge and proper understanding of mycorrhizal mutualism, and all it entails in the global change context, is vitally important. I believe that ecologists and mycorrhizologists must rethink basic notions of how we set and accomplish our objectives in mycorrhizal research in a complex and changing environment. Contemporary researchers, especially in the field of ecology, are beset with a challenge to make sense of the traditional disciplines in the context of global environmental change and using new approaches to solve old problems. This challenge became a driving force for my motivation to write this book and provide students and budding researchers some hopefully useful insights into the changing roles of mycorrhizal symbioses in the rapidly changing world. How far I succeed in doing so can be best judged by the readers of the book.

In fact, more than a decade ago when I started working on mycorrhizas at the University of Kashmir with my supervisor Professor Z.A. Reshi, I could realise the dynamism of plant–mycorrhiza interactions, notwithstanding the methodological difficulties to manipulate such interactions in the field or laboratory for experimental purposes. A number of books have been written on various aspects of mycorrhizas. How this book is different from previously published books is something that readers might be eager to know about. In this book, I attempt to provide a global perspective of mycorrhizal diversity and distribution followed by providing some insights into the impact of various global change elements such as climate change, plant invasion and extreme environmental conditions on mycorrhizas and the role of these mutualists in turn to help their host plants to withstand such changing selection pressures. Special attention here is given to the interesting, but largely neglected topics, such as the role of mycorrhizas in ecological restoration of degraded environments and mycorrhizal status of aquatic plants. Though most of the aforementioned topics and processes are taught and researched within the academic discipline of mycorrhizal ecology independently, this book attempts to unify these topical areas in a common integrated framework.

This book can be used by the undergraduate- and graduate-level students studying mycorrhizal symbioses in the context of current ecological applications. The materials in this book will benefit biological scientists actively involved in research on mycorrhizal ecology and global environmental change. Besides, the contents of the book make sense to restoration ecologists and biodiversity managers. It is important to mention that this book was never intended as a review of the literature on any subject pertaining to the title and chapters of this book. I render apology to my readers and solicit forgiveness for my error of omission if their seminal work is not cited. The limitations of time, space and my brain are to blame.

Srinagar, India

Manzoor Ahmad Shah

Acknowledgements

The writing of this book has been a mammoth task. However, I have been very fortunate to get the opportunity to work for various mycorrhiza-related projects during the formative phase of my research with competent mentors like Prof. Zafar A. Reshi and Damase P. Khasa, whose wisdom, knowledge and experience shine through. I therefore thank them for their superb support and hard work for enabling me to handle the nuts and bolts of mycorrhizal research. I would certainly have not been able to complete this task had there not been a huge support from my family, especially my wife, Dr. Sameera, who all through took care of the kids – Muqadas, Ahmad and Mohammad. I dedicate this book to my mother who is an embodiment of wisdom, love and affection.

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About the Author



Dr. Manzoor Ahmad Shah holds master's degree in Botany from the University of Kashmir and worked for his Ph.D. jointly in the University Laval, Quebec, Canada, and University of Kashmir. He currently teaches at the Department of Botany, University of Kashmir, and has been a faculty affiliate in the Division of Biological Science, the University of Montana, USA. His basic research interests include plant–soil microbial feedback interactions with a major focus on the biogeography and molecular ecology of plant invasions. He has published more than 40 high-impact research papers and book chapters, in recognition of which he has been awarded several national and international fellowships and prestigious awards, such as the Paryavaran Mitra Award, Gold Medal from Indian Academy of Environmental Sciences, the DBT-CREST Award 2012, the Canadian Commonwealth Exchange Fellowship (Asia-Pacific) in 2010, Doctoral Internship Award by the Canadian Bureau of International Education (CBIE) in 2008 and Young Scientist Grant Award in 2009 by the Federation of European Microbiological Societies (FEMS). Dr. Shah is on the editorial board of some prestigious journals and has been an invited speaker at various scientific meetings/conferences within India and abroad. He is currently a part of many national network projects on ecological niche modelling, biological invasions, species conservation and mycorrhizal symbioses.

Mycorrhizas, the symbiotic association between plant roots and nonpathogenic fungi, facilitate movement of nutrients to host plants in exchange of carbon. About 80–90 % of terrestrial plants are mycorrhizal, and only few higher plants do not form mycorrhizal associations, including some aquatic vascular plants and members of the conventional non-host families, such as Brassicaceae, Chenopodiaceae, Cyperaceae and Juncaceae. A wealth of valuable literature has emerged over the years on various aspects of the plant–mycorrhizal interactions from different perspectives across the globe. Particularly, in recent years, mycorrhizal research has undergone rapid expansion, and the intervention of various novel molecular tools and genomic approaches have led to some path-breaking insights into the evolution, physiology, function, community patterns and biogeography of mycorrhizal fungi. To make sense of these novel and ground-breaking dimensions of mycorrhizal research in concert with classical theories and applied aspects of mycorrhizas is a challenging discourse. Hence, the present book is a humble effort in this direction and is basically aimed at linking the past and present strides in mycorrhizal research to various global change elements, such as climate change, biological invasions, environmental extremism and ecological restoration. From a mycorrhizal perspective, some of these selective forces will tend to strengthen this symbiotic relationship while others may act to diffuse it. But from the host plant standpoint, mycorrhizal symbiosis is likely to play a pivotal

role in their growth and performance under the various forces of global change. It is pertinent to mention that, though there are seven different types of mycorrhizas, the main focus of this book is on arbuscular mycorrhizas because of being most dominant and widespread association with more than 80 % of higher plants. The second chapter of this book provides a brief historical account of arbuscular mycorrhizas, followed by an overview of their evolution, biology and development. A brief account of identification and classification is also given in this chapter.

Explaining the global distribution pattern and community dynamics of mycorrhizas in light of the established ecological paradigms assumes crucial importance in mycorrhizal and plant ecology. What determines the global distribution of mycorrhizas? Does the mycorrhizal diversity pattern follow the known latitudinal biodiversity gradient? How does distribution of mycorrhizas along different gradients and at different spatial scales influence distribution and diversity of the host plant? Can mycorrhizas become invasive? These are some of the fundamental, yet largely unanswered, questions in mycorrhizal ecology. In fact, understanding the suite of environmental factors influencing the global distribution of AM fungi is fundamental to understanding and interpretation of the local dynamics of these fungi. Some recent global surveys of AM fungal taxa have indicated different distribution patterns, with some taxa showing a global range and others being limited to a few ecosystems only. Some AM taxa are, however, rarely reported.

In view of the rapid expansion and major breakthroughs in mycorrhizal research during recent years, novel insights have been obtained in understanding the community patterns and biogeography of mycorrhizal fungi. In fact, advances in mycorrhizal ecology, especially during the past decade, have turned our attention to even more novel dimensions, such as the possible consequences of mycorrhizal introduction and likelihood of these mutualists becoming invasive. Given the potential utility of fungal inoculations in agricultural, horticultural and ecological management, concerns have been expressed regarding the potential for negative ecological consequences of invasions by mycorrhizal fungi. The third chapter of the book therefore attempts to address these aforementioned fundamental issues related to global patterns of mycorrhizal distribution in light of handful studies conducted so far.

The global climate change potentially influences most of the ecological processes and biotic interactions directly or indirectly, mycorrhizal symbiosis being no exception. Hence, understanding how mycorrhizal association of plants has responded to the factors and rate of climate change over time and how it is expected to change in future in response to such changes are some interesting and challenging directions of research for mycorrhizologists. Since a multitude of factors and drivers of climate often interact in complex and nonadditive ways to influence plant–mycorrhiza feedback relationships, different components of global climate change need to be understood together vis-a-vis mycorrhizal mutualism in a broad conceptual framework. The fourth chapter highlights dynamism of mycorrhizal mutualism in the context of global climate change. In view of the unequivocal empirical evidence for the fact that increasing atmospheric concentration of carbon dioxide is one of the most significant factors of global change, the two fundamental aspects addressed herein include the role of mycorrhizas in offsetting the climate change due to elevated CO₂ and the impact of climate change on mycorrhizas.

Anthropogenic climate change is believed to be one of the major driving factors to promote

plant invasion and biotic homogenisation. The interactive effects of climate change and invasive species with other anthropogenic disturbances can potentially bring endemic species of different biogeographic regions to the brink of extinction. Observational and manipulative studies have revealed that exotic plant invasions can draw ubiquitous AM fungi to their advantage at the cost of native species. However, studies exploring the role of such symbionts in invasiveness of exotic species and invasibility of communities are limited. The studies on AM-mediated plant invasion so far have yielded contradictory results with some indicating facilitation of invasion by AM fungi and others its inhibition. Invasive plants may also potentially impact mycorrhizal community structure and functions in the invaded habitats in different ways. The fifth chapter of this book addresses these paradoxically conflicting observations in the context of mutualism–commensalism–parasitism gradient that characterises the relationship between AM fungi and their exotic vs. native hosts.

In view of burgeoning invasions exacerbated by climate change and other anthropogenic perturbations, many ecosystems call for eco-restoration measures. A restoration project is, however, doomed to fail if one does not take into account fundamental factors and processes underlying ecosystem functioning. Restoration of plant communities in degraded ecosystems requires explicit understanding of the role of soil symbionts such as mycorrhizas in functioning of plant communities and vegetation patterns. Recent evidence suggests that mycorrhizal association could be one of the potential factors that may lead to plant rarity or abundance or differential performance in different communities. Therefore, mycorrhizal symbionts, in view of their versatile roles in seedling establishment and plant growth in nutrient poor systems, could be the key players in ecological restoration of disturbed communities. In Chap. 6, an attempt is made to draw attention to various applications of mycorrhiza-mediated ecological restoration.

The waves of global change have rendered most of the environments more and more

stressful. Amongst the various biotic interactions that promote stress tolerance and avoidance of plants, arbuscular mycorrhizas are of exceptional importance. These fungi are known to improve protection of host plants against a range of environmental stresses, such as drought, cold, salinity and pollution. In the seventh chapter, an overview of the mycorrhizal status of plants in various stressful environments is presented, and the role of mycorrhizas in stress tolerance or avoidance of host plants is highlighted. The potential applications of mycorrhizal technology to overcome the challenges of stressful environments in future are discussed.

Besides terrestrial ecosystems, aquatic systems are equally or even more stressed by myriad of anthropogenic pressures. Whether mycorrhizal symbioses can play any role in aquatic systems is an open-ended question. In contrast to widespread occurrence of AM in terrestrial plants, aquatic plants are considered to be relatively less mycorrhizal, mainly because the benefits of mycorrhizal association to plants under aquatic conditions are expected to be relatively lesser. It is, however, hard to accept these perceptions, partly because only a few studies have been hitherto carried out on mycorrhizal symbioses of aquatic plants thereby leaving huge information gaps and paucity of quantitative data. Chapter 8 focusses on mycorrhizal association of aquatic plant and identifies key knowledge gaps in this area of research. Accordingly, some future directions that mycorrhizologists may follow to get better insights into mycorrhizal symbioses in aquatic habitats are highlighted, and a conceptual framework to address such challenges is also provided.

In view of various roles that mycorrhizas can play in the context of global environmental change, if a researcher is convinced to pursue studies related to any of the aforementioned discourses, it is imperative to provide some basic methodological insights. There is a reasonably good basket available, and a number of procedures can be chosen and followed for mycorrhizal studies, depending upon the objective of the researcher. Therefore, the concluding chapter deals with methods that can be followed

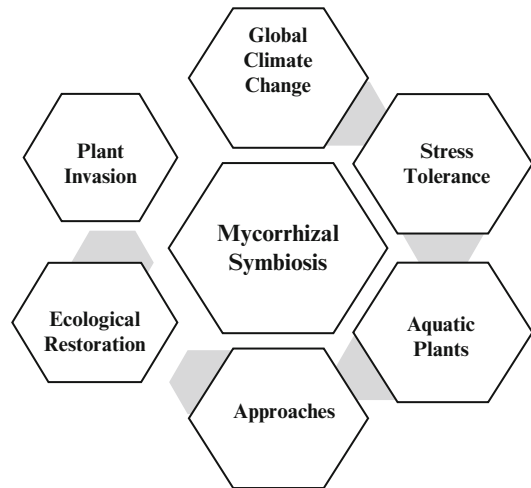


Fig. 1.1 Spectrum of issues and topics specifically focussed in the present book

for detection of mycorrhizas in living roots through various staining techniques, different approaches that can be used for spore extraction, and various standard morphological and molecular methods for identification of AM fungi (Fig. 1.1).

Peeping into the Past of Mycorrhizology

The term 'symbiotismus' (symbiosis) is said to have been probably first used by Frank (1877), who later (1885) gave the name 'mycorrhiza' to the peculiar association between tree roots and ectomycorrhizal fungi, though de Bary (1887) is often credited with the introduction of the term for the organisms that mutually help each other. The discovery of the arbuscular mycorrhiza was made more than 100 years ago when Janse (1897) called the intramatrical spores 'vesicules' and Gallaud (1905) made very accurate observations of the arbuscules. Gallaud (1905) further distinguished between the *Arum* and *Paris* types of arbuscules. Phillips and Hayman (1970) made a pioneering contribution in clearing of the roots of cytoplasm by heating in KOH and staining fungal cell walls,

(continued)

(continued)

vesicles, hyphae and arbuscules with trypan blue in lactophenol. However, McGonigle et al. (1990) standardised quantification of these structures (hyphae, arbuscules and vesicles). Schenck created INVAM in 1985, though since 1990 the collection has been curated by Morton at West Virginia University (<http://invam.caf.wvu.edu/>). Subsequently, the BEG/IBG was established as an international collaborative effort to provide registration of individual isolates of fungi for research purposes (<http://www.kent.ac.uk/bio/beg/>). *Transactions of the British Mycological Society*, published between 1896 and 1989, is amongst the oldest journals to regularly publish research on mycorrhizas, though *New Phytologist*, founded in 1902, also published some pioneering papers on mycorrhizas. Of late, *Mycorrhiza*, established in 1991, is the only international journal devoted entirely to the publishing of research on mycorrhizas.

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Introduction

Mycorrhizas (Greek – ‘fungus + root’) represent by and large mutualistic associations between plant roots and nonpathogenic, obligate symbiotic fungi that colonise the cortical tissue of roots during periods of active plant growth. The mutualistic relationship is usually characterised by the movement of nutrients – carbon flows to the fungus and inorganic nutrients move to the plant – thereby providing a critical linkage between the plant root and soil system. About 90 % of terrestrial plants, including angiosperms, gymnosperms and pteridophytes, are mycorrhizal (Read et al. 2000). Only few higher plants do not form mycorrhizal associations, including some aquatic vascular plants and members of the conventional non-host families, such as Brassicaceae, Chenopodiaceae, Cyperaceae and Juncaceae.

The arbuscular mycorrhizas derive their name from the treelike structures within the cortical cells of plant roots called ‘arbuscules’ that play a key role in bidirectional exchange of nutrients, carbon from plants to fungus and other nutrients, especially phosphorus, in reverse direction from fungus to plant. Though the storage structures called vesicles, located within or between the root cortical cells, are also a characteristic feature of AM fungi, the arbuscules are an evolutionary conserved feature in all lineages of Glomales (Morton 1990b). It is pertinent to mention that besides characteristic vesicles and arbuscules, other intraradical structures such as intracellular

hyphal coils are also formed by AM fungi, which sometimes occur in the absence of any arbuscules (Dickson 2004). Though earlier the term vesicular-arbuscular mycorrhizal (VAM) fungi was commonly used for these symbiotic fungi, of late the ‘vesicular’ part was deleted, and only the ‘arbuscular’ part has been retained and is more commonly used. In rhizosphere of the host plants, glomalean fungi produce a large number of spores, ranging in size from about 20 to 500 μm (or 50–800 μm according to some estimates) in diameter. The spore types, hyphal attachments and wall characteristics are some of the important attributes for morphological distinction of various AM taxa, at species, generic or even family level.

There are seven types of mycorrhizal associations recognised, including vesicular-arbuscular mycorrhizas, ectomycorrhizas, ectendomycorrhizas, ericoid mycorrhizas, orchid mycorrhizas, arbutoid mycorrhizas and monotropoid mycorrhizas. The vesicular-arbuscular mycorrhizas are now more appropriately termed ‘arbuscular’ because the fungus produces specialised treelike structures known as arbuscules in root cortical cells that are involved in bidirectional exchange of nutrients, especially carbon, phosphorus and other physiologically important molecules. These arbuscules are considered as a key structural evolutionary innovation because they are a conserved feature in all lineages of Glomales (Morton 1990a). Most common association amongst various types is the arbuscular mycorrhizal (AM)

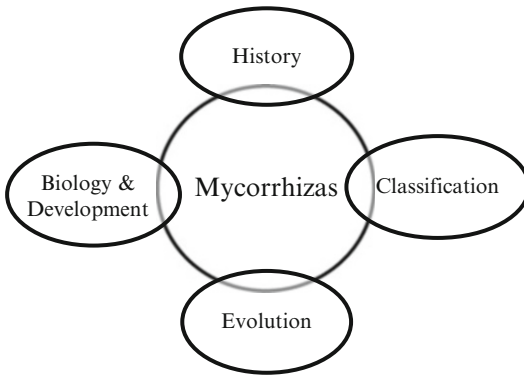


Fig. 2.1 Depiction of mycorrhizal history, biology, development, evolution and classification as a connected loop. Developmental biology and classification of historically primitive, taxonomically not so diverse but functionally diverse mycorrhizas have some inherent connections in an evolutionary perspective

symbiosis followed by ectomycorrhizas as probably the next most common type of symbiosis. The AM fungi form a monophyletic group of obligate plant symbiotic fungi previously classified in phylum Zygomycota, but now grouped into their own phylum Glomeromycota, order Glomales (Schußler et al. 2001). In this chapter, a brief overview of the history of AM fungi is given followed by a concise account of their biology and development. Finally, some light is thrown on the evolution and classification of mycorrhizas (Fig. 2.1).

Arbuscular Mycorrhizas: A Brief Historical Account

The earlier observations of arbuscular mycorrhizas date back to the drawings of Nageli (1842), though his drawings remotely resembled the real AM association. The other early records of the AM symbiosis can be found in Schlicht (1889), Dangeard (1896), Janse (1897), Trappe and Berch (1985), Petri (1903), Rayner (1926–1927), Gallaud (1905), Peyronel (1924), Jones (1924) and Lohman (1927). In fact, after the recognition of the arbuscular mycorrhizas in the late nineteenth century, Janse (1897) and Gallaud (1905) played a pioneering role in reporting their widespread occurrence in various plants across different

phyla and families. Jones (1924) was the first to describe the appressorium, and Gallaud (1905) earlier on provided some useful insights into the structural organisation of the AM fungi and distinguished between *Arum* and *Paris* types of arbuscules. The classical contributions in the history of arbuscular mycorrhizal research include the observations by Peyronel (1923) that hyphae of the endophyte could be traced to the sporocarps of fungal species and their classification in the Endogonaceae, followed by the convincing work of Mosse (1953) showing that mycorrhizal strawberry plants were colonised by a species of *Endogone*. It is pertinent to mention that Frank (1885) gave the name ‘mycorrhiza’ to the peculiar association between tree roots and ectomycorrhizal fungi. Until very recent times (late 1970s), the names such ‘*Endogone*’ or ‘*Rhizophagus*’ were used for AM fungi.

The earliest microscopic studies on AM fungi were facilitated through founding of the Centro di Studio sulla Micologia del Terreno as early as 1950 by Peyronel in Torino, Italy (Bonfante 1991), where Scannerini and Bellando (1968) first noted that a space between the host membrane and the fungal wall contained materials of host origin. A detailed historical analysis of the mycorrhizal symbioses is beyond the scope of the present book, and the interested readers are referred for more information about the history of AM research to Koide and Mosse (2004). However, despite the aforementioned early strides, there was no significant progress made in the direction of further understanding the structural and functional attributes of AM fungi until 1950s, mainly due to lack of appropriate tools and techniques to deal with these obligate symbionts.

Evolution of Mycorrhizas

The ancient AM symbiosis since its origin around 1,000 Ma ago has not changed substantially over time, and AM fungi have not taxonomically diversified the way other organisms have. Such surprisingly little diversification over very long evolutionary history is still an unresolved

paradox, though they are believed to be functionally far more diverse. In fact, AM fungi are said to have facilitated the transition of early plants from aquatic to terrestrial habitats, mainly through their role in nutrient uptake (Redecker et al. 2000; Heckman et al. 2001). The AM symbiosis has monophyletic origin as evidenced by the cladistic analysis of morphological (Morton 1990b) and molecular (18S rRNA gene sequence divergence) analysis (Simon 1996; Simon et al. 1993). There are different views regarding the nature of progenitor mycobiont (common ancestor) in mycorrhizal symbiosis. The suggestions of Pirozynski and Malloch (1975) that progenitor mycobiont was a semiaquatic alga and aquatic oomycete ‘fungus’ were refuted by Morton (1990a) who suggested that it was a saprobic zygomycetous fungus with perhaps a more terrestrial habitat. Morton’s view is corroborated by affinities of a zygomycetous fungus, *Geosiphon pyriforme*, which has morphological and molecular affinities with the *Glomus*

lineage in Glomales, though it does not form associations with higher plants. Relatively more recent molecular evidence based on 18S rRNA gene sequence also aligns *G. pyriforme* closely with those of glomalean than non-glomalean fungi (Gehrig et al. 1996). The symbiosis between mycobiont and phytobiont is generally mutualistic and characterised by the exchange of nutrients against carbon. The mycobiont can capture as much as 20 % of the fixed carbon in the phytobiont (Jakobsen and Rosendahl 1990), mostly by converting glucose to trehalose (Shachar-Hill et al. 1995).

Biology and Development

Based on their morphological attributes, two types of AM, namely, *Arum* and *Paris* (Plate 2.1), first described by Gallaud (1905) and subsequently

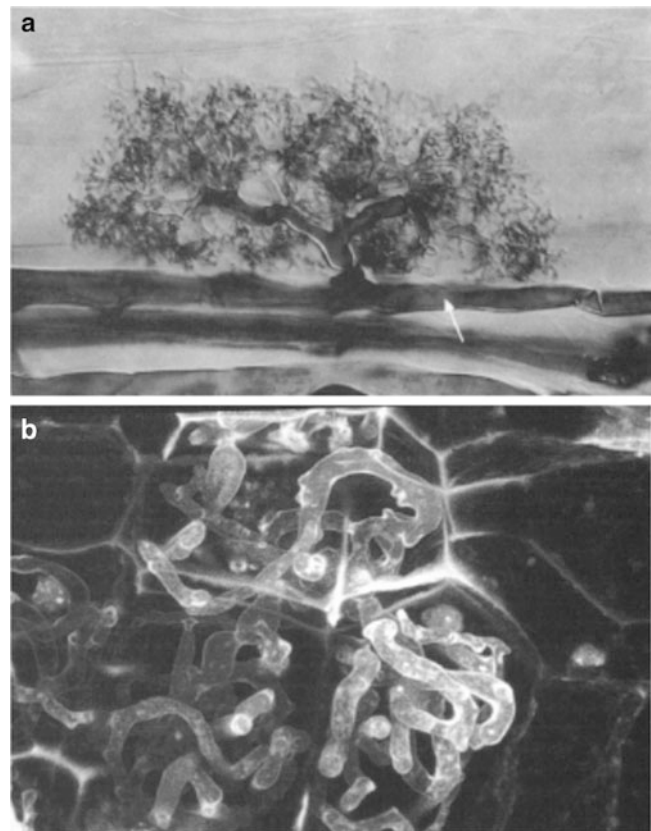


Plate 2.1 (a) A mature *Arum*-type arbuscule of *Glomus mosseae* within a cortical cell of *Allium porrum* grown from a well-developed intercellular hypha (arrow) (From Brundrett et al. 1984). (b) *Paris*-type intracellular coils of *Glomus intraradices* in cortical cells of a root of *Panax quinquefolius* with no intercellular hyphae (Reproduced from Peterson et al. 2004)

reviewed by Smith and Smith (1997) and Dickson et al. (2007), are recognised. In the *Arum*-type colonisation, AM fungi form extensive intercellular hyphae in well-developed air spaces between cortical cells and invaginate the cells as short side branches to form arbuscules. Contrastingly, in the *Paris* type, colonisation spreads directly from cell to cell in the root. This is further characterised by the absence of intercellular hyphae and the development of intracellular hyphal coils that frequently have intercalary arbuscules. The AM morphological type, according to some studies (Brundrett and Kendrick 1990; Smith and Smith 1997), is largely dependent on the plant species and according to the others (e.g. Cavagnaro et al. 2001) is influenced by the fungal identity. While both the types are known to result in phosphorus transfer to the host plant, yet the higher proportion of *Arum*- than *Paris*-type colonisation in weedy plants (Yamoto 2004) and decrease in the ratio of *Arum*- to *Paris*-type AM colonisation from pioneer to late successional stages (Ahulu et al. 2005) are indicative of some functional differences between them.

Over the years some important strides have been made to understand the biology and development of AM fungi. The AM fungi produce large (up to 500 µm diameter), thick-walled and multinucleate spores, rich in storage lipids, some carbohydrates and chitin (Gianinazzi-Pearson et al. 1994a; Lemoine et al. 1995). The spores not only vary in the number of nuclei (ranging from 800 to about 35,000 haploid nuclei) but also in the DNA content (ranging from 1.7 to 3.4 pg) and genome size (ranging from 16.54 to 1,058.4 Mb).

The life cycle begins with germination of spores, in response to some signal molecules from the roots of host plant, and production of limited amounts of branching and coenocytic mycelium, capable of anastomosis (Akiyama et al. 2005; Besserer et al. 2006). Spore germination and subsequent hyphal growth cannot take place without the living host, and such an arrested growth in the absence of host makes AM fungi unculturable on artificial media. But with the onset of symbiosis with a host, mycelial growth continues both within roots and in the soil, thereby ultimately leading to the formation of new multinucleate spores terminally on the hyphae. The efforts of culturing AM fungi have been a focus of research for many years without much success, though the reports of production of a small number of viable and infective spores by a *Glomus intraradices* isolate cocultured with two particular bacteria (Hildebrandt et al. 2006) have been encouraging to some extent.

Identification and Classification

The identification and classification of AM fungi (Fig. 2.2), until very recent use of molecular tools, was based mainly on the development and wall structure of the spores. There are various impediments in the identification of AM fungi, such as small and microscopic dimensions, difficulties in culturing, many overlaps in morphological traits, obligate mutualistic nature and so on. As against many of the mushroom-producing

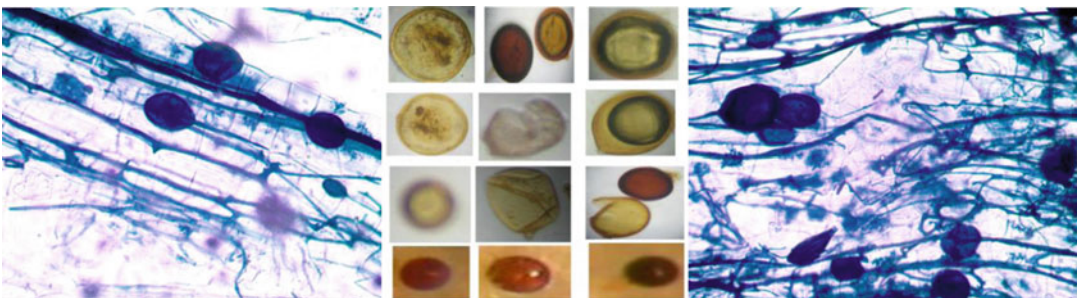


Fig. 2.2 A view of root colonisation by AM fungi in some host plants. In between there are some mycorrhizal spores usually found in soil

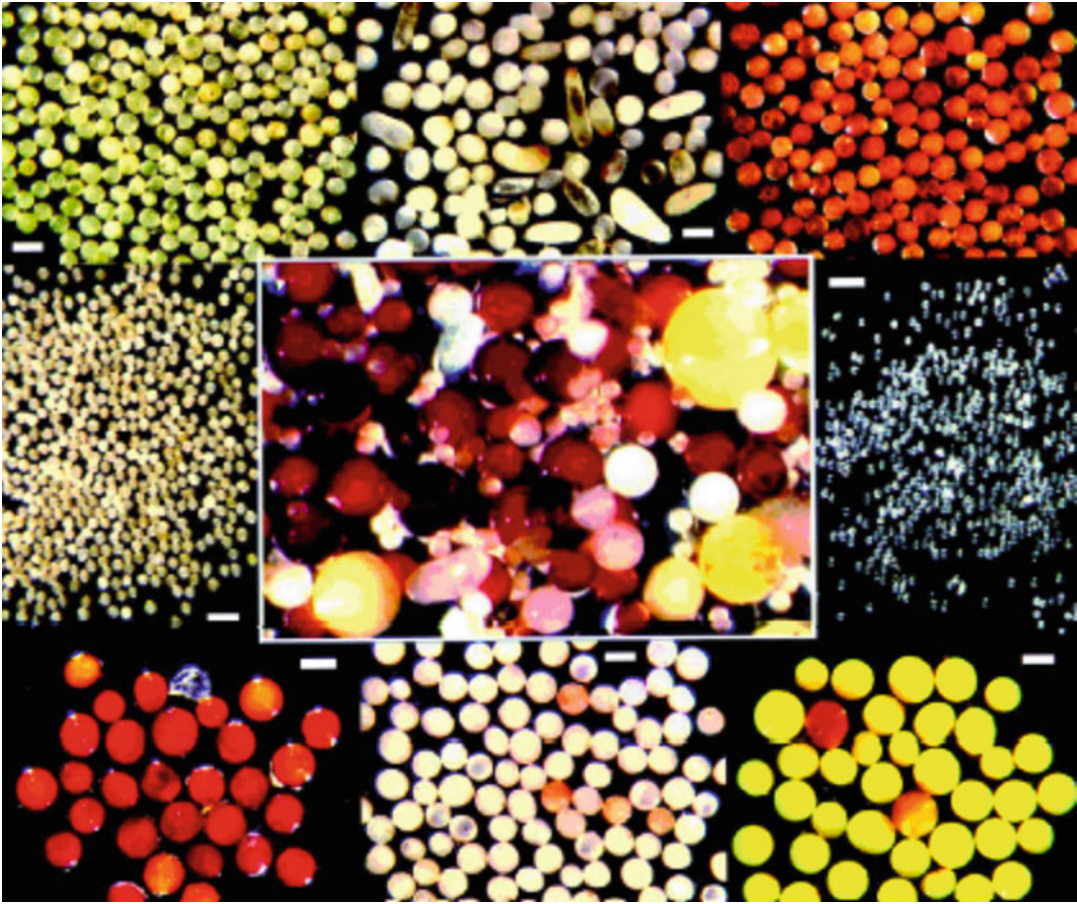


Plate 2.2 A glimpse of spores of AM fungi. Starting from upper left corner moving clockwise around the central composite picture of mycorrhizal spores, the species represented are *Scutellospora calospora*, *S. pellucida*,

S. heterogama, *Archaeospora trappei*, *Gigaspora gigantea*, *G. rosea*, *Acaulospora collosica* and *A. morrowiae* (Source: Bever et al. 2001, *Bioscience*, 51 (11), 1–9)

members of Basidiomycota can be often identified to a reasonably low taxonomic level with good keys and field guides and knowledge of how to make and interpret spore prints. The morphological identification of mycorrhizas is mainly based on the spore characteristics (Plate 2.2), and only a few traits can be used to help identify the fungal species that produce these spores (Redecker 2002). However, at times it is very difficult to distinguish on morphological grounds between species in distantly related genera. For instance, *Glomus* and *Paraglomus* cannot necessarily be distinguished from one another based on spore morphology (Redecker et al. 2003). This necessitates the use of more precise molecular

approaches for AM fungal identification. A glance at the development of methodical approaches used by taxonomists reveals that they have become increasingly sophisticated over time, from those based upon morphological and anatomical characteristics, through serological approaches (Aldwell and Hall 1987), isozyme variation analysis (Hepper 1987) and fatty acid variation pattern (Bentivenga and Morton 1994), to more DNA-based methods (Cummings 1990; Davidson and Geringer 1990; Simon et al. 1992, 1993; Redecker 2000).

The notion that AM fungi were most closely related to the Zygomycota due to their aseptate hyphae was reevaluated through the use of DNA sequences. In fact, the classification system of AM

fungi based on morphology (Gerdemann and Trappe 1974) is being gradually replaced by molecular-based classification schemes. The first approach to a phylogeny-based classification as an attempt to consider AM fungi as a separate group was made by Morton (1990a) using cladistic tools, wherein a new order (Glomales) was separated from the Endogonales, though still included in the Zygomycota. Using cladistic analysis to determine evolutionary relationships resulted in the phylogenetic tree, which reflects separation of the *Glomus/Sclerocystis* group from *Gigasporal/Scutellospora* and the existence of *Acaulosporal/Entrophospora* as a line apparently diverging from *Glomus*. The subsequent DNA sequence analysis and lipid analysis confirmed the division of AM fungi into the same three families as done with the cladistic approach and established 'glomalean' fungi as true fungi of monophyletic origin (Gianinazzi-Pearson et al. 1994b; Lemoine et al. 1995). Various approaches that can be followed for morphological or molecular identification and characterization of AM fungi are discussed in Chap. 9 of this book. The details of mycorrhizal diversity and global distribution pattern can be seen in Chap. 3.

Arbuscular Mycorrhizas on Web INVAM

<http://invam.caf.wvu.edu/>

BEG (La Banque Européenne des Glomales)

<http://www.kent.ac.uk/bio/beg/englishhomepage.htm>

Mark Brundrett's website (www. <http://mycorrhizas.info/>)

The Fifth Kingdom

<http://www.mycology.com/fifthtoc.html>

International Directory of Mycorrhizologists

<http://mycorrhiza.ag.utk.edu/searchMycorr.asp>

Mycorrhizal Information Exchange

<http://mycorrhiza.ag.utk.edu/>

MycorWeb

<http://mycor.nancy.inra.fr/index.html>

Practical Guide to Mycorrhiza

<http://www.mycorrhiza.org/>

Working with Mycorrhizas in Agriculture and Forestry

<http://www.ffp.csiro.au/research/mycorrhiza/>

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Introduction

Explaining the global distribution pattern and community dynamics of mycorrhizas in light of the established ecological paradigms assumes crucial importance, both in mycorrhizal studies and invasion ecology. What determines the global distribution of mycorrhizas? Does mycorrhizal diversity pattern follow the known latitudinal biodiversity gradient? How does distribution of mycorrhizas along different gradients and at different spatial scales influence distribution and diversity of host plant? Can mycorrhizas become invasive? These are some of the fundamental, yet completely unanswered, questions in mycorrhizal ecology. In fact, understanding the suite of environmental factors influencing the global distribution of AM fungi is fundamental to understanding and interpretation of the local dynamics of these fungi (Allen et al. 1995). Some recent global surveys of AM fungal taxa have indicated different distribution patterns, with some taxa showing a global range and others being limited to a few ecosystems only (Öpik et al. 2013). Some AM taxa are, however, rarely reported. In view of the rapid expansion and major breakthroughs in mycorrhizal research during recent years, novel insights have been obtained in understanding the community patterns and biogeography of mycorrhizal fungi. In fact, advances in mycorrhizal ecology, especially during the past decade, have turned our attention to even more novel dimensions,

such as the possible consequences of mycorrhizal introduction and likelihood of these mutualists becoming invasive. Given the potential utility of fungal inoculations in agricultural, horticultural and ecological management, concerns have been expressed regarding the potential for negative ecological consequences of invasions by mycorrhizal fungi. In this chapter, an attempt is made to address these fundamental issues in light of handful studies conducted so far.

Global Patterns in the Distribution of Mycorrhizas

There have been very few attempts to understand global distribution pattern of mycorrhizas and the underlying mechanisms and drivers of such patterns. Some recent global surveys of AM fungal taxa have indicated different distribution patterns, with some taxa showing a global range and others being limited to a few ecosystems only (Öpik et al. 2006). Öpik et al. (2006) surveyed 26 publications that used rDNA region sequences for reporting the occurrence of natural root-colonising AM fungi on 52 host plant species involving 95 fungal taxa. The number of AM fungal taxa per host plant species was found to differ between habitat types, reflecting a relatively higher richness in tropical forests (18.2 fungal taxa per plant species), followed by grasslands (8.3), temperate forests (5.6) and habitats under anthropogenic influence (arable

fields and polluted sites, 5.2). Not only did the number of AM fungal taxa per host plant species vary, but also AM fungal communities differed in compositions in broadly defined habitat types, such as tropical forests, temperate forests and habitats under anthropogenic influence. This review of molecular-based studies by Öpik et al. (2006) found that a number of AM fungi exhibit global distribution. Overall the picture emerging out from this review indicated that mycorrhizas may exhibit different distribution patterns with high degree of variability in AM taxon richness and composition between particular ecosystems. Öpik et al. (2006) made another attempt to bridge the gaps in understanding the molecular diversity of AM fungi by specifically targeting previously unstudied geographical areas and building a new and more complete global dataset. In a cross-continental study, they sampled 96 plant species from 25 sites and detected AMF in plant roots by sequencing the nuclear SSU rRNA gene fragment. A total of 204 AMF phylogroups were recorded, including a number of new records, increasing the described number of Glomeromycota from 308 to 341 globally. The largest increases in phylogroups were recorded in previously little-studied regions, and differences in AM communities between different continents and climatic zones indicated that both biogeographic history and environmental conditions underlie these global variation patterns in AM communities. From these studies it seems that AM richness can be expected to significantly increase by taking into account the remaining unstudied areas.

Global plant diversity patterns are believed to be, amongst other factors, an outcome of the accumulation pattern of soil organic matter and nutrient availability. Mycorrhizas of different types confer specific advantages to hosts for nutrient uptake from specific soil types under a particular set of ecological conditions. For instance, more than about two decades ago, Read (1984, 1991) hypothesised that the mycorrhizal community composition is a function of the accumulation of organic matter in soils, thereby indicating that AM plants should be more abundant in ecosystems with smaller soil

organic nutrient pools because of the limited ability of these fungi to degrade organic matter. As opposed to AM, ectomycorrhizas (ECM) can decompose labile organic nutrients, and their plant hosts should proliferate in areas with moderate organic accumulation. The ericoid mycorrhizas, on the other hand, have a special capability to break down more recalcitrant compounds and characteristically occur in ecosystems with large standing stocks of humified material. Treseder and Cross (2006) examined the potential large-scale controls over the distribution of AM fungi and their host plants by testing the hypothesis that AM fungi should be more prevalent in biomes where nutrients are primarily present in mineral, and not organic, forms. They reviewed various studies representing 151 geographic locations and nine biomes to show that percentage root length colonised (% RLC) by AM fungi differed slightly but significantly amongst biomes and was greatest in savannas. On the other hand, the AM abundance (defined as total standing root length colonised by AM fungi) was found to vary by a magnitude of 63-fold, with lowest values in boreal forests and highest values in temperate grasslands. Interestingly, they also found that biomes did not differ significantly in the percentage of AM host plant species. So the overall results, in contrary to the Treseder and Cross (2006) hypothesis, showed that % RLC, AM abundance and host plant availability were not related to the size, influx or turnover rate of soil organic matter pools, though AM abundance was positively correlated with standing stocks of fine roots. The authors further noted that regions with largest stocks of AM fungi are also particularly vulnerable to anthropogenic nitrogen deposition, which could potentially alter global distributions of AM fungi in the near future.

There are several other factors, in addition to soil organic matter content, that influence distribution of AM fungi at large scales. Since AM fungi are estimated to receive 37–47 % of C delivered belowground by host plants (Harris and Paul 1987; Jakobsen and Rosendahl 1990; Johnson et al. 2002), AM fungal abundance may consequently vary in proportion to belowground

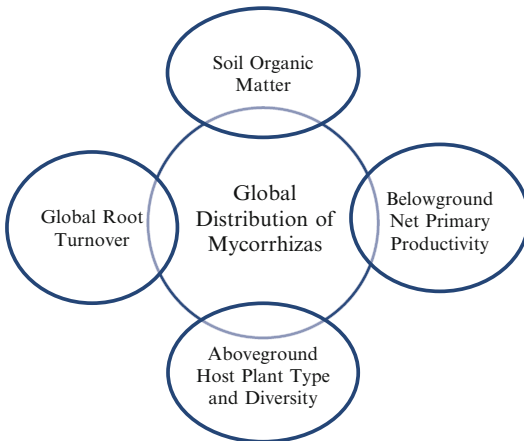


Fig. 3.1 Global diversity and distribution of mycorrhizas as a function of soil organic matter, root turnover rate, above-ground host plant diversity and belowground net primary productivity

net primary productivity of AM plants (Harley 1971). Moreover, since fine roots provide a substrate for AM colonisation, fine root length is also most likely to influence AM biomass. In view of the significant role of AM fungi in P acquisition, AM abundance is likely to be greater where plants are more limited by P. As the P availability varies in nature along various environmental gradients, the variability in the extent of mycorrhizal intensity along such gradients seems to be an inevitable outcome. Testing the relative importance of various regulating factors such as SOM, nutrient status and host types at larger spatial scales (ecosystem or biome) in influencing the diversity and distribution of mycorrhizas is a promising discourse. This is because it seems likely that the global distribution of mycorrhizal types is an indirect strategy of plants to obtain nutrients along a comprehensive gradient of varying soil conditions, which in turn has significant implications for large-scale fluxes of CO₂ between the soil and the atmosphere (Treseder and Allen 2000). The details of the role of mycorrhizas in carbon sequestration and in offsetting the impacts of global climate change are discussed in Chap. 4 of this book.

As mycorrhizas are root-associated fungi, the global patterns of root turnover may potentially influence mycorrhizal dynamics (Fig. 3.1). Gill

and Jackson (2000) attempted to assess the global patterns of root turnover for terrestrial ecosystems by testing global controls on root turnover across climatic gradients and for plant functional groups by using a database of 190 published studies. They interestingly found that root turnover rates increased exponentially with mean annual temperature for fine roots of grasslands and forests and for total root biomass in shrublands. However, when the authors accounted for temperature, the global relationship between precipitation and root turnover did seem no longer to hold true. Though root turnover rates seemed to vary between different studied systems, the turnover decreased from tropical to high-latitude systems for all plant functional groups (Gill and Jackson 2000). In conclusion, global patterns in rates of root turnover between plant groups and across climatic gradients were established. How fascinating it would be to correlate these global patterns of variability in root turnover with variation patterns in types and extent of mycorrhizal association. Theory suggests that high fine root extent and turnover may not necessarily be correlated with high mycorrhizality because higher network of finer roots will do the job that otherwise mycorrhizas are supposed to do. But from another perspective, more number of finer roots can provide more colonising surfaces, thereby encouraging higher rates of colonisation. Which of the two situations is really true would be answered in terms of the hard data collected through wide-ranging field studies at a global scale.

Mycorrhizal Introductions and Likely Fallouts

In view of the growing transport and introduction of mycorrhizal fungi from one region to the other, special attention need to be paid towards the potential fallouts of such introductions as they are poorly understood. The main reasons for the AM introductions include their utility potential as inoculants for a wide range of agricultural and horticultural crops. Whether there are chances of AM introduction along with their

hosts and whether or not these introduced fungi can turn out as invasive are some open questions. It is pertinent to mention that AM fungi have been found to play an important role in facilitating invasive plant in their non-native range (see Chap. 5 for details). Since the invasive species are often detrimental to the structural organisation and functional integrity of the invaded ecosystems (Pimentel et al. 2005), whether or not mycorrhizal introductions have a similar effect on introduced systems is yet another interesting discourse to be undertaken. On the contrary, since the transition of man from a food gatherer to food cultivator, there are many instances of intentional introductions, especially of agricultural crops, for various economic benefits and to support human societies. Since mycorrhizal inoculants act as natural bio-fertilisers to improve crop productivity, the rate and volume of the intentional movement of non-indigenous mycorrhizal fungi has significantly increased over time to harness maximum possible benefits of this association not only for improved agriculture (Gianinazzi et al. 2002) and horticulture (Azcon-Aguilar and Barea 1997) but also for habitat restoration (Miller and Jastrow 1992), bioremediation (Leyval et al. 2002) and forestry (Brundrett et al. 1996; Duponnois et al. 2005). In this context, the assessment of mycorrhizal dynamics along with general patterns of plant invasions vis-à-vis cost–benefit analysis assumes central importance in mycorrhizal ecology in an era of globalisation.

Attempts have been made to assess the degree to which intended introductions of mycorrhizal fungi for various positive purposes may end up in some unintended negative consequences with significant ecological and economic implications. The objective of such attempts was to recommend appropriate management guidelines and highlight priority research needs in the direction of mycorrhizal invasion (Fig. 3.2). Some of the recommendations emerging from these attempts for mycorrhizal invasion management include the following: (a) conduct of careful assessment and evaluation trials for the effectiveness of AM inoculation, because inoculations are not always beneficial; (b) preferable use of local

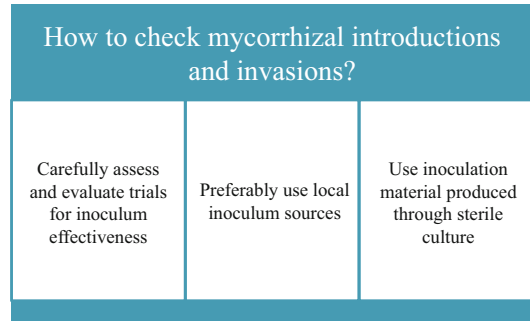


Fig. 3.2 Three major steps for checking the mycorrhizal invasions

inoculum sources whenever and wherever possible, because if mycorrhizas may become invasive, it would be very difficult to manage them by the time they are recognised as invasive; (c) use of inoculation material that has been produced through sterile culture when local inoculum is not available because non-sterile cultures of inoculum can result in the movement of saprobes and pathogens as well as mutualists; and (d) choosing fungal taxa, when using nonlocal fungal inocula, that carry life-history traits that may reduce the possibility of harmful invasive species problems. Notwithstanding the preliminary efforts and very sporadic information available in the area of mycorrhizal invasions, this offers an important opportunity and a new window of research for mycorrhizal ecologists that promise to deliver novel insights.

Mycorrhizas: Solution to Global Issues

Environmental degradation, biodiversity depletion, deforestation, growing stress, decreasing soil fertility, reducing cultivable area for agriculture and resultant food insecurity are some of the real challenges of the contemporary world. Mycorrhizas can be potentially a part of the solution for most of these issues. The need is, however, to put these highly beneficial symbionts to their best possible use and accentuate the applied aspects of mycorrhizal research. The mass inoculum production and its application for the promotion of sustainable agriculture and rebuild

of soil biota assume special significance in this direction. Isolating mycorrhizal strains associated with plants of varied utility and their characterisation for effectiveness, growth-promoting traits and compatibility with conventional agricultural practices merit special attention. There is indeed a need to explore, document, screen and characterise the mycorrhizal species associated with prized crops of different regions across the globe. Examining the interactions between some commonly occurring mycorrhizal symbionts and yield/quality of the target crops so as to identify and develop novel bio-inoculants for their large-scale use in the cultivation of such crops is equally important. These novel bio-inoculants could be investigated not only for their performance on host crops but also for persistence as part of the inoculum when applied to natural soils with the existing AMF community. It is important to note that the impact of agricultural practices on the native mycorrhizal community also needs to be understood so as to obtain the elite strains that can be successfully used under varied land management practices.

Mycorrhizology: A Discipline in its Own Right

Mycorrhizal studies were earlier considered as a part of overarching discipline of mycology, though over time it emerged as a major discipline in its own right. The discipline of mycorrhizology owes its genesis and emergence to the dedicated work of a network of mycorrhizologists across the globe. Existence of various mycorrhizologist societies, refereed and high-impact journal such as *Mycorrhiza*, global conference series specifically on mycorrhizas and many national and international directories of mycorrhizologists stand testimony to the distinction and strength of this discipline. The International Mycorrhiza Society (IMS) is a transnational scientific society exclusively dedicated to the advancement of education, research and development in the area of

mycorrhizal symbiosis. 'Mycorrhiza' is the official scientific journal of the IMS that provides an excellent outlet for mycorrhizologists to publish their contributions in rapidly expanding area of mycorrhizal research and report major breakthroughs in the understanding of structural details, functional attributes, evolutionary trends, physiological mechanisms, community types and biogeographic patterns of mycorrhizal fungi. The 'International Conference on Mycorrhiza (ICOM)' as the official conference of IMS is the most reputed conference series focusing on the research and developments in the field of mycorrhiza. The conference takes place after every four years, and generally the programme comprises of plenary sessions, symposia and workshops on relevant themes aimed at exploring the developmental, functional and environmental genomics of mycorrhiza. The delegates profit from an international forum that provides up-to-date distribution of valuable information concerning scientific and industrial developments throughout the world, as literature reviews, recent published books, training education, employment and relevant news from related fields.

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Introduction

Climate broadly refers to average weather over a long time period resulting from subtle interactions of a number of factors operative at many spatio-temporal stages and scales. Climate is inherently dynamic and has been changing historically due to very natural causes, yet there is now a broad scientific consensus that the rate and extent of global climate change has witnessed a multifold increase over the last few decades, mainly due to anthropogenic causes. The global climate change potentially influences most of the ecological processes and biotic interactions directly or indirectly, mycorrhizal symbiosis being no exception. Hence, understanding how mycorrhizal association of plants has responded to the factors and rate of climate change over time and how it is expected to change in future in response to such changes are some interesting and challenging discourses for mycorrhizologists. Since a multitude of factors and drivers of climate often interact in complex and nonadditive ways to influence plant–mycorrhiza feedback relationships, different components of global climate change need to be understood together vis-a-vis mycorrhizal mutualism in a broad conceptual and integrated framework. In view of the unequivocal empirical evidence for the fact that increasing atmospheric concentration of carbon dioxide is one of the most significant factors of global change (e.g. Keeling et al. 1995), the two fundamental aspects of mycorrhizal symbioses (Fig. 4.1) merit

attention: (a) the role of mycorrhizas in offsetting the climate change caused due to elevated CO₂ and (b) the impact of climate change on mycorrhizas. This chapter begins with an overview of the role of mycorrhizas in carbon sequestration, followed by a synopsis of the impact of elevated CO₂ on root turnover and mycorrhizal colonisation of host plants. Finally the role of mycorrhizas in the adaptation of host plants to withstand various elements of global climate change is briefly discussed, and some future directions in these areas of research are identified.

The Role of Mycorrhizas in Soil Carbon Sequestration

In the context of global climate change, understanding the carbon sequestration potential of soils has assumed renewed importance. Soil carbon sequestration refers to the process of transfer of CO₂ from the atmosphere into the soil through various sources, such as plant residues, organic solids and so on. The soil carbon sequestration significantly helps offset emissions from fossil fuel combustion and other carbon-emitting activities while enhancing soil quality and long-term crop productivity. While in atmosphere the enhanced levels of carbon are highly undesirable due to its contribution to global warming and greenhouse effect, in soil systems higher amounts of carbon are very much desirable. This is because in soils higher amounts of organic carbon

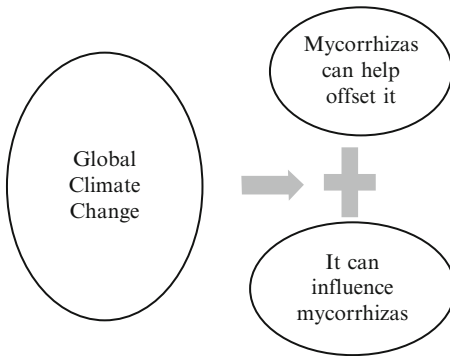


Fig. 4.1 Twofold relationship between global climate change and mycorrhizas. Mycorrhizas, though likely to be influenced by climate change per se, can significantly help offsetting climate change as well

contribute significantly to soil health in terms of its influence on soil structure, biotic composition and microbial activity. In view of the sizeable pool of mycorrhizal fungi in soil compartment of many terrestrial ecosystems (Allen 1991), these fungi play a phenomenal role in soil carbon sequestration.

There is a huge and extensive wood wide web of underground hyphae in soils, and there are variable estimates of the extent of hyphal network in soil systems. The variation in extraradical hyphal lengths in the fields can reportedly range from 0.02 m to 111 m ml⁻¹ of soil. The below-ground hyphal network stores substantial amounts of carbon in biomass, besides constantly draining large amounts of carbon from their hosts against the exchange of other benefits. In addition to hyphae, there is a significant pool of AM-fungus-related carbon in soil in the form of a glycoprotein called Glomalin (Wright and Upadhyaya 1996). However, there is very little information regarding the amount and turnover of this protein in different soils. In view of preferential feeding of soil microarthropods on saprophytic hyphae rather than on AM fungal hyphae (Klironomos and Kendrick 1996), the mycorrhizal carbon pool may be considered as a relatively slow-turnover pool. Though some attempts have been made to estimate the soil carbon pool and the relative contribution of different soil biotic elements in the same, a real challenge for soil ecologists would be to assess the potential change in the nutritional value of AM fungal hyphae in the current global change

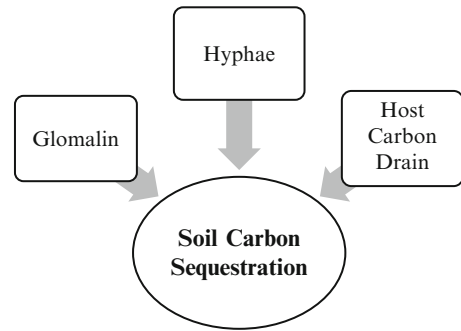


Fig. 4.2 Soil carbon sequestration as a function of mycorrhizal hyphal turnover, Glomalin production and carbon drainage from host plants

scenario. All the studies aiming at assessing the soil carbon sequestration potential need to take into account the contribution of three important components, including mycorrhizal hyphae, Glomalin and the host carbon drain (Fig. 4.2).

Elevated Atmospheric CO₂ and Root Turnover

Arbuscular mycorrhizas preferably colonise fine roots; hence the density of AM in the rhizosphere is linked to turnover of fine roots. Rate of turnover of fine roots is believed to increase under elevated atmospheric CO₂, thereby augmenting flux of organic substrates and associated nutrients from the root system for belowground mycorrhizal growth and proliferation. It is pertinent to mention that fine root turnover refers to the flux of carbon and nutrients from plants into soil per unit area per unit time. In view of the short lifespan of fine roots, the turnover of carbon and nutrients from them is high, but how fast the carbon stored in mycorrhizas is turned over is not clearly known. Preliminary indications show that turnover of carbon from mycorrhizas is very slow. Increase in the rate of root growth under elevated CO₂ translates into increased turnover of roots, which in turn connotes increased rates of C flux from the root system to the soil. But, under continuous levels of CO₂ elevation over longer periods of time in the projected global change scenario, how long increased rates of

root growth and absolute turnover will be sustained by ecosystems is still an open question. It is very likely that the response of plant fine root growth and turnover to elevated atmospheric CO₂ may decline if other factors such as nutrient or water eventually turn limiting (Oren et al. 2001). The contribution of root–rhizosphere interactions to biogeochemical cycles in a changing world has been reviewed by Pregitzer et al. (2007). Some of the potential links between global root turnover rates and mycorrhizal dynamics and distribution are discussed in Chap. 3 of this book.

Impact of Elevated Atmospheric CO₂ on Mycorrhizal Colonisation

The impact of elevated CO₂ on mycorrhizas is intricately linked to its impact on the host plant physiology and ecology. Different plant species are expected to be influenced differently by increased CO₂ levels. Theoretically, for instance, C₃ plants are expected to be favoured more under a high CO₂ regime than C₄ plants due to more likely action of RUBISCO as carboxylase than oxygenase. Hence C₃ plants should be able to channelise relatively more carbon to associated mycorrhizal symbionts under a higher CO₂ regime. The increased mycorrhizal association in turn is expected to reciprocate with a positive feedback to host plants. It is, however, pertinent to mention that host-mediated impact of elevated CO₂ on mycorrhizas can be confounded by many other factors under field conditions. Nevertheless, the impact of elevated atmospheric CO₂ can be best understood by growing the mycorrhizal plants along a gradient of CO₂ levels and recording their performance. Many attempts have been made in this direction to grow plants at ambient and elevated atmospheric CO₂ concentrations. The studies that have been hitherto carried out range from single plant-based pot experiments to whole community-level analyses (see Staddon et al. 1998 for review). The results of these studies are, however, inconclusive. While some studies reveal decrease in the extent of mycorrhizal intensity under elevated CO₂ levels, most others show exactly the opposite.

Inconsistency in the outcome of aforementioned studies has been partly attributed to experimental flaws (Staddon et al. 1998). Though there is a need to overcome the shortcomings in the future experimental studies, it is important to note that extrapolation of the results obtained from controlled greenhouse experiments to what exactly happens under field conditions will be not so easy.

Role of Mycorrhizas in Plant Adaptations to Global Climate Change

Mycorrhizas can play a potentially significant role in assisting host plants to withstand climate change. In response to climate change, range shifts are expected to take place for a large chunk of species. In the new or extended ranges, mycorrhizas can extend a helping hand to such new colonisers, if at all they occur in soil system. Managing migration of soil symbionts so that they are available to migrating plants in their new locations could be an alternative, though not feasible and cost-effective at larger scales.

An elevated CO₂ level would demand more photosynthesis, which in turn would need more supply of nutrients. In view of the important role of AM fungi in mineral nutrition, they can overcome the nutrient limitation of their hosts for increased photosynthesis rates concomitant with elevated CO₂. Elevated atmospheric CO₂ would also demand higher plant water-use efficiency (e.g. Bazzaz 1990), and AM fungi can substantially help in this regard through improved plant water relations, especially in water-limited ecosystems. While mycorrhizas primarily do it for their own self because increased water throughput maximises plant carbon uptake for subsequent translocation to the fungus itself, by doing so they benefit hosts as well. Furthermore, AM fungi can help plants to withstand elevated CO₂ levels by providing a physiologically important belowground carbon sink (Wright et al. 1998). It has been found that mycorrhizal roots receive about 4–20 % more photosynthate than comparable non-mycorrhizal roots (Smith and Read 1997). Estimates show that AM fungi could use up to 20 % of the total fixed 14CO₂

in young plants (Jakobsen and Rosendahl 1990). So it is obvious that plants may respond to elevated CO₂ through increased photosynthesis, mainly if there are no sink limitations. However, there is much more than meets the eye in mycorrhiza-mediated plant response to elevated CO₂. For instance, upon measuring total non-structural carbon pools of mycorrhizal and NM plants in ambient and elevated CO₂, no change in the C-sink was found (Jongen et al. 1996).

The role of soilborne plant pathogens vis-à-vis mycorrhizal symbioses cannot be underestimated in the context of climate change. In the elevated CO₂ regime as more photosynthate is channelised to roots, length and specific area of roots are expected to respond in a positive feedback manner. Increased surface area of roots and richer sources of carbon per root length (Rouhier et al. 1996; Paterson et al. 1997) may provide potential zones for attack by non-mycorrhizal pathogenic fungi. However, mycorrhizal association has been found to significantly reduce disease incidence in plants and root infection by parasitic fungi (St-Arnaud et al. 1995). How pathogenic and symbiotic fungi may interact under the climate change scenario would be an interesting discourse for future research. To how much extent the root-inhabiting parasitic fungi contribute to carbon drain on the plant and soil carbon sequestration potential, besides AM fungi, also merits attention for lending precision to our estimates of carbon pool in different soil compartments.

To conclude with observational studies reveal that mycorrhizal colonisation can either increase, decrease or remain unaffected in response to climate change, depending upon the species conditions, stage of growth and specific context. Not only this, experimental studies also have reveal uneven response in terms of benefits accrued to host plants from mycorrhizas in an elevated CO₂ scenario.

Future Directions

The science of mycorrhizal behaviour in relation to elements of global climate change is still in its infancy. Despite the pivotal contributors of mycorrhizas in the soil carbon sequestration

and facilitation of host plants to withstand the impacts of climate change, a long road is still to be travelled to understand in detail the functioning and role of these mutualistic fungi in an era of global climate change. It is appropriate to suggest that further studies on changing land-cover patterns, shifting vegetation distribution and plant water relations in elevated CO₂ atmospheres should not ignore mycorrhizal aspects. The future challenge is to ask appropriate questions, devise suitable approaches to seek answers for those questions, overcome shortcomings of the past studies and get some widely acceptable generalisations in this exciting area of what could be termed as 'climate change mycorrhizology'. This new research field can potentially allow integration of related disciplines from the level of genes to ecosystems through a systems ecology approach. The advances in this new field of research will be driven by exponentially expanding information base on mycorrhizal biology by high-throughput techniques, aided by rapidly developing computational and bioinformatics tools and resources, in a broader framework of climate change impacts and responses to these soil symbionts.

Mycorrhizal Network: Supreme Soil Symbionts Significantly Sink C

There is a huge mycorrhizal web that networks the above-ground plants below-ground, popularly known as wood wide web. The extraradical hyphal lengths in the field range very widely, from 0.02 m g⁻¹ soil to about 111 m ml⁻¹ of soil, depending upon the type of host and above-ground community. As a significant carbon drain, this belowground mycorrhizal hyphal network stores a sizeable pool of carbon, though its turnover is relatively slow. Mycorrhizal roots receive about 4–20 % more photosynthate than comparable non-mycorrhizal roots, and AM fungi use up to 20 % of the total fixed 14CO₂ in young plants. Glomalin, a glycoprotein produced by AM fungi that occurs in soils in the order of several mg per g of dry soil, is by far one of the most

(continued)

(continued)

significant pools (approximately 20–30 % C) of carbon in soil. Notwithstanding these approximations, understanding the global potential of SOC sequestration through mycorrhizas well and evolving correct global figures would be potentially a win-win strategy because soil carbon restores degraded soils, enhances biomass production, purifies surface and ground waters and reduces the rate of enrichment of atmospheric CO₂ by offsetting emissions through various anthropogenic sources.

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Introduction

The growing anthropogenic impacts on natural environments have brought many prized species to different degrees of rarity and endangerment. Whether mycorrhizal interactions, in terms of colonisation intensity and impact, contribute towards intrinsic or human-mediated rarity in plants and/or whether or not these mutualists play some role in invasiveness of plants comprises new and challenging dimensions of mycorrhizal research. In fact, understanding the factors that contribute to plant rarity and invasiveness per se is an interesting discourse.

The enormous ecological and socio-economic damage inflicted by plant invasions (Pimentel et al. 2005) has stimulated great interest in elucidating how invasive plant species influence and are influenced by above- and belowground mutualistic and antagonistic organisms. Like most native plants, many exotic plant species depend upon mutualistic facilitation by soil microbes and arbuscular mycorrhizas (Smith and Read 1997; Richardson et al. 2000). But exotic plants, unlike native plants, have been reported to typically drive the mycorrhizal associations in their introduced range to their utmost benefit for release from native enemies (Shah and Reshi 2007), avoidance of herbivores (Abigail et al. 2005) and alteration of competitive interactions with native plant species (Shah et al. 2008a, b). Being poor AMF hosts, naturalised exotic plants have been found to respond less to native AMF

than native plants (Vogelsang et al. 2004), but more recent studies (Shah et al. 2008a, b) have shown exactly the opposite. Moreover, whereas proliferation of plants with low mycorrhizal dependency may degrade AMF density in soils (Vogelsang et al. 2004), some non-mycorrhizal species of invasive plants have been reported to proliferate in ecosystems with high AMF density (Mooney and Hobbs 2000; Stinson et al. 2006). This may be detrimental to native plant productivity and diversity which is mainly determined by the belowground AMF diversity (van der Heijden et al. 1998).

This chapter evaluates, in light of the recent findings, soil mycorrhizal mechanism behind plant invasion and changes in soil conditions created by dominance of a habitat by invasive plants in relation to growth and fitness of native plant species. An overview of positive and negative AM fungal feedbacks with invasive plants vis-à-vis likely influence and implications of AM-mediated invasiveness on structure and diversity of native plant communities is discussed. But, at the outset, the role of mycorrhizas in rarity of plants or restoring population status of rare and endangered species is briefly described.

Mycorrhizas and Rarity of Plants

One of the fundamental challenges in ecology is to understand the factors that determine the rarity and invasiveness of plants. Whether mycorrhizal

interactions contribute towards intrinsic or human-mediated rarity in plants is a new dimension in mycorrhizal research. In fact, the rarity of species has been linked to a wide range of ecological traits (Schemske et al. 1994; Bevill and Louda 1999). The role of mycorrhizal fungi in causing rarity of the host has been rarely tested (see Swarts et al. 2010). While some attention has recently been paid to the role of mycorrhizas in plant invasiveness, just two recent studies (Swarts et al. 2010; Phillips et al. 2011), to my knowledge, have so far evaluated whether mycorrhizas do limit the distribution and abundance of plants, thereby contributing to their rarity.

Some plant species may, at least theoretically, be supposed to get rare either by not finding mycorrhizal symbionts in their typical natural habitat, such as rock crevices in case of myriad of the Kashmir Himalayan rare species, or by not being able to form association with whatsoever mycorrhizas are available in the rhizosphere. So, mycorrhizas can lead to rarity of host plant species through limiting distribution, restricting the potential range of usable habitats and being per se scarce within habitats suitable for rare species. Whether plant rarity occurs due to the absence of a mycorrhiza or the inability to form a symbiosis in certain environments by rare species are indeed open and quite stimulating research questions. Some related questions have been asked recently on some orchid species (Huynh et al. 2009; Jacquemyn et al. 2010; Roche et al. 2010; Swarts et al. 2010). The author's personal observations revealed most of the native, rare and endemic plant species in Kashmir Himalaya as mycorrhizal (*unpublished data*). It is pertinent to mention that many of the rare and threatened species in the Kashmir Himalaya, and probably elsewhere as well, are the Orchids. Terrestrial orchids are notable for their obligate, often specific relationship with mycorrhizal fungi. Therefore, Orchids are suitable candidate taxa to test the hypothesis that mycorrhiza are involved in plant species rarity. In fact, previous studies using DNA sequencing analysis of orchid mycorrhizas have shown that some orchid species use a small number of mycorrhizal fungal

species (e.g. McCormick et al. 2004; Irwin et al. 2007; Huynh et al. 2009; Jacquemyn et al. 2010; Roche et al. 2010; Swarts et al. 2010). However, the caution has been alarmed that distinction must be made between phylogenetic specificity and ecological specificity. Several orchid species have been shown to use numerous closely related fungal species and to have narrow phylogenetic specificity (e.g. Taylor and Bruns 1997; Shefferson et al. 2007; Ogura-Tsujita and Yukawa 2008), though the use of several fungal species may still provide ample opportunity to exploit different edaphic environments and geographical regions.

Regardless of whether mycorrhizas contribute to plant rarity by one or the other reason, more important would be to evaluate whether mycorrhizas can really help in reestablishment and restoration of the status of rare species. Field experiments can be designed wherein some rare plant species can be reintroduced along a habitat suitability gradient and inoculated with mycorrhizas to evaluate the role of these mutualists in species reintroduction, reestablishment and recovery. Even the growth performance of rare plant species can be compared under inoculated vs. un-inoculated conditions to quantify the role of mycorrhizas. Accordingly, soil-based management and restoration strategies can be devised for such rare plant species.

Mycorrhizas and Plant Invasion

Biological invasions are being increasingly implicated as the second most pervasive threat, after habitat degradation, to biodiversity at local, regional and global scales. This threat has been exacerbated by increasing globalisation of markets, explosive rise in world trade, travel, tourism and exchange of goods. Thus, elucidation of all the factors that facilitate and mediate such invasions is of paramount significance in formulating effective strategies for management of such invasions. In view of unprecedented increase in transport, introduction and spread of invasive plants in areas well outside their potential range as defined by their natural dispersal

mechanisms and biogeographic barriers, the extent to which mycorrhizas mediate exotic species invasions merits special attention. Despite the established role of ubiquitous arbuscular mycorrhizal (AM) fungi in plant interactions, studies exploring the role of such symbionts in invasiveness of exotic species and invasibility of communities are limited, in part because of the difficult culturability of AM fungi on artificial media and apparent complexities in manipulations of AM–plant interactions in field and laboratory experiments. Moreover, analysis of the AM–plant invasion studies conducted so far has yielded contradictory results with some indicating facilitation of invasion by AM fungi and others its inhibition. Other studies have indicated that arbuscular mycorrhizal symbiosis has no effect on invasiveness of exotic plants. Two excellent reviews on the role of mycorrhizas in plant invasion have been provided by Shah et al. (2009a) and Pringle et al. (2009). While arbuscular mycorrhizas may facilitate invasiveness of some exotic plants, such plants may also potentially impact mycorrhizal community structure and functions in the invaded habitats in different ways. This chapter addresses these paradoxically conflicting observations in the context of mutualism–commensalism–parasitism gradient that characterises the relationship between AM fungi and their exotic vs. native hosts and also discusses the influence of exotic invasive plants on mycorrhizal community structure of invaded ecosystems. Through critical analysis of costs and benefits for invasive plants that associate with AM fungi in their introduced range, invasion-induced shifts in AM mutualism are evaluated in the context of their impact on native biodiversity. Underlining limitations of methodologies and experimental designs usually employed to understand AM-mediated plant invasiveness, we propose herein some alternative frameworks and experimental approaches to overcome these limitations.

Despite the need for an urgently required unifying framework for understanding exotic plant invasions, a number of partially overlapping hypotheses (reviewed by Hierro et al. 2005), to explain how exotic species change from being

minor components of their native communities to dominant components of invaded communities, have been advanced. While these hypotheses implicate one or the other attribute, trait or set of traits in promoting invasions, AM mutualism in relation to exotic plant invasions needs more detailed examination. This is because the AM–plant relationship may possibly change from a completely mutualistic to one of parasitism during different stages of invasion. A meta-analysis of the several relevant studies by Levine et al. (2004) pointed out that soil microbes, particularly mycorrhizal mutualists, play a critical role in determining patterns of abundance and invasiveness of certain species. Despite such indications, carefully planned experimental studies that specifically and objectively examine the role of AMF in plant invasion are lacking, presumably due to their difficult cultivability on artificial media and apparent complexities in manipulations of mycorrhiza–plant interactions in field and laboratory experiments. Building upon the understanding of AM–host interaction, the present chapter brings out that the role of AM fungi in plant invasion needs to be investigated in light of following three paradigms: (a) is invasiveness of introduced species in non-native habitats due to their establishment of new symbiotic relationships with AM fungi that are native to the invaded region which contributes to fitness of exotic species, (b) do AM fungal symbionts get transported and introduced along with propagules of exotic plant species and whether the AM fungi act as pathogens to native species or as superior mutualists in non-native habitats and confer additional benefits and (c) whether cessation of symbiotic associations in the non-native habitats decrease the invasiveness of introduced species.

Conceivable Roles of Mycorrhizas in Plant Invasion

The role of mycorrhizal symbiosis in plant invasions could be better examined and understood in the light of Resistance Hypothesis (indirect effect of not having appropriate mutualists is that the invader is repelled from areas)

(Mack 1996), Enhanced Mutualisms Hypothesis (invasion at a biogeographical scale is facilitated by mutualists with strong beneficial effects) (Reinhart and Callaway 2006), Mutualisms Hypothesis (Richardson et al. 2000) and Degraded Mutualisms Hypothesis (invasion by non-mycorrhizal species reduces the abundance of AMF thereby negatively affecting strongly mycorrhizal native plant species) (Vogelsang et al. 2004). There are a number of ways in which AM fungi can facilitate or constrain the establishment and spread of exotic plants in invaded communities, and mechanisms behind these impacts can be identified or inferred from the published works. A critical analysis of the research works so far on different species in different ecosystems worldwide (Table 5.1) brings out a bias towards some life forms, probably for the sake of convenience, such as annual or perennial forbs in grasslands ecosystems in comparison to the other growth forms and habitats such as forests and wetlands. Besides, the evidence for AM feedback interactions with invasive plants predominantly comes from greenhouse experiments and pot trials, rather than from field studies (Table 5.1), notwithstanding the fact that under field conditions a number of factors operate in combination.

Evidence shows that the exotic invasive plants can potentially modify soil environment thereby influencing the AMF community composition and abundance, which in turn influences invasiveness of many exotic plants in the introduced range (Shah et al. 2008a, b). The possible interactive feedback between invasive plants, soil properties and AMF is presented in the form of a successional loop in Fig. 5.1. For the present review, invasive plant–mycorrhizal feedback (Fig. 5.2) is considered as negative if performance of the plant species decreases relative to other native species and positive if the opposite is true. While most of the studies indicate by and large positive effect of AM fungi in plant invasions, some studies also suggest the opposite. The mutualistic facilitation of invasive plants by AM fungi is most likely through their influence on competitive interactions of these plants with native species. The altered interactions between native and invasive

species are an outcome of differential impact of AMF on nutrient uptake and exchange, stage-specific spatio-temporal successional changes or mediation of plant–herbivore interactions. Occasionally, AM can suppress invasive plants to specially favour the native species. The invaders in turn may impact mycorrhizal community structure and functional dynamics in the invaded habitats in different ways. While critically evaluating evidences for different possible roles of AM in plant invasion (Fig. 5.3), their implications for prediction, prevention and management of plant invasions are also discussed. To begin with, we briefly highlight the importance of developing exhaustive checklists of mycorrhizal status of invasive plants to act as baseline information for the subsequent studies.

Mycorrhizal Status of Invasive Plants

In view of the recently reported multifaceted role of arbuscular mycorrhizas (AM) in plant invasions, large-scale exploration of invasive plants from different habitat types across biogeographical regions for determining the extent and type of their colonisation by AMF assumes special importance. Such baseline information would be highly helpful in further elucidating the role of AMF in exotic plant invasions. Notwithstanding the importance of such studies, no major survey exploring the mycorrhizal status of exotic invasive plants has yet been carried out except a recent attempt by Shah et al. (2009b). The study was conducted to evaluate the extent and type of AM occurrence in exotic plant species at different stages of invasion in the Kashmir Himalaya and revealed high incidence of AM symbiosis both at species (92 %) and family (96 %) level. However, the extent and type of AM colonisation were variable. In fact, about 78 % of the species investigated by Shah et al. (2009b) belonged to the highest three frequency classes C, D and E, based on percent root length colonised.

As regards morphological AM types (Gallaud 1905; Dickson et al. 2007), *Arum* type is more common in weedy plants (Yamamoto 2004), and

Table 5.1 Major studies depicting the role of AM in plant invasions

Exotic invasive species	Growth form	Invaded habitat/region	Study type	AM effect/ response variable	References
<i>Centaurea stoebe</i> , <i>Euphorbia esula</i> , <i>Bromus tectorum</i>	Annual forbs and grass	Grasslands – North America	Field experiments	Invasive plants increase AM abundance and diversity	Lekberg et al. (2013)
<i>Carduus pynoccephalus</i>	Herb	Grasslands – California	Mesocosm	Invasive plant grows better in soils lacking AMF	Vogelsand and Bever (2009)
<i>Ageratina adenophora</i>	Annual herb	Forest understories – China	Greenhouse experiment	Invasive plant increased AM abundance	Hong-bang et al. (2007)
<i>Anthemis cotula</i>	Annual herb	Disturbed ecosystems – Kashmir Himalaya	Field studies and pot experiments	Positive effect on growth, fitness and enemy release	Shah and Reshi (2007) and Shah et al. (2008a, b)
<i>Ambrosia artemisiifolia</i>	Annual or perennial herb	Disturbed areas and crop fields – France	Field studies and greenhouse experiment	Positive on invasive spread	Fumanal et al. (2006)
<i>Centaurea maculosa</i>	Annual forb	Grasslands – North America	Defoliation effects on AM in competition	Negative on competitive ability	Walling and Zabinski (2006)
<i>C. maculosa</i>	Annual forb	Grassland – USA	Using field inocula in greenhouse experiments	Positive on biomass	Callaway et al. (2004a)
<i>C. maculosa</i>	Annual forb	Grassland – USA	Using field inocula in greenhouse experiments	Positive overall	Callaway et al. (2004b)
<i>Alliaria petiolata</i>	Biennial herb	Hardwood forest – North America	Field studies and pot trials	Native AM suppressed by the invader	Stinson et al. (2006)
<i>Oenothera laciniata</i>	Annual herb	Coastal sand dune – Japan	Field and culture experiments	Nonsignificant effect on establishment	Funatsu et al. (2005)
<i>Sapium sebiferum</i>	Perennial tree	Hyric forest – USA	AM inoculation of invasive in competition with five native species in greenhouse experiments	Positive on growth of invasive and negative on native species	Nijjer et al. (2004)
<i>Solidago canadensis</i>	Perennial herb	Chongming Island – China	Evaluating mycorrhizal association as a function of time	Positive on colonisation in reclaimed lands	Liang et al. (2004)
<i>Acer negundo</i> + <i>Acer platanoides</i>	Tree	Riparian sites and mesic forests – North America	Field studies and greenhouse experiments	Positive on height and biomass	Reinhart and Callaway (2004)
<i>Centaurea maculosa</i>	Annual forb	Grassland – USA	Grown with native neighbours with and without AM inoculum	Positive on C transfer	Carey et al. (2004)

(continued)

Table 5.1 (continued)

Exotic invasive species	Growth form	Invaded habitat/region	Study type	AM effect/ response variable	References
<i>Centaurea melitensis</i> + <i>Avena barbata</i>	Annual forb + annual grass	Grassland – USA	Grown with native neighbours with and without AM inocula	Positive on biomass	Callaway et al. (2003)
<i>Ardisia crenata</i>	Shrub	Forest – Japan	Field inocula in greenhouse house experiments	Differential effect on growth, physiology and competitive ability	Bray et al. (2003)
<i>Prunus seroti</i>	Tree	Forest – northwestern Europe	Field studies and greenhouse experiments	Positive on neighbouring conspecific establishment and seedling performance	Reinhart et al. (2003)
<i>Bidens pilosa</i> L.	Annual herb	Natural ecosystems – Hawaii, USA	Microcosm study	Positive or negative depending upon AM species identity m	Stampe and Daehler (2003)
<i>Centaurea maculosa</i>	Perennial forb	Grassland – USA	Field trials	Positive on P uptake	Zabinski et al. (2002)
<i>Centaurea melitensis</i>	Annual forb	Grassland – USA	Greenhouse experiments in inter- and intraspecific competition	Positive on compensatory growth and competitive ability	Callaway et al. (2001)
<i>Bromus madritensis</i>	Annual grass	Coastal scrub – southern California	Field inoculum in greenhouse experiments	Positive on number of leaves	Yoshida and Allen (2001)
<i>Pinus elliottii</i>	Tree	Fynbos – Africa	Mycorrhizal distribution and competitive interactions	Positive or negative	Allsopp and Holmes (2001)
<i>C. maculosa</i>	Annual forb	Grassland – USA	Grown with various neighbours using field inocula in greenhouse experiments	Positive on biomass	Marler et al. (1999)
<i>Andropogon gerardii</i>	Grass	Grassland/ prairies – North America	Radiolabelled P-transfer	Positive on P-transfer	Francis and Read (1994)

Modified from Shah et al. (2010)

decrease in the ratio of *Arum*- to *Paris*-type AM colonisation from pioneer to late successional stages (Ahlu et al. 2006) is indicative of some functional differences between them. Of late, not only some invasive plants have been

reported to harbour the *Arum*-type AM (Fumanal et al. 2006; Shah et al. 2008a, b) but also this morphological type has been linked to the rate of spread of some weedy plant species (Yamoto 2004).

Fig. 5.1 Interactive feedback between invasive plants, soil properties and AMF. Invasive plants modify the soil environment that determines the AMF community structure and abundance which in turn influences the invasiveness of exotic plants in the introduced range

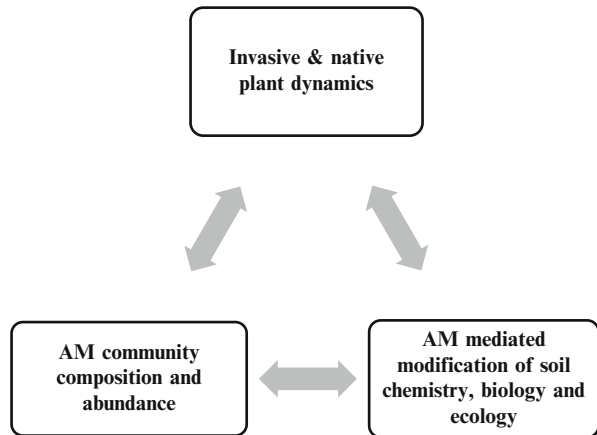
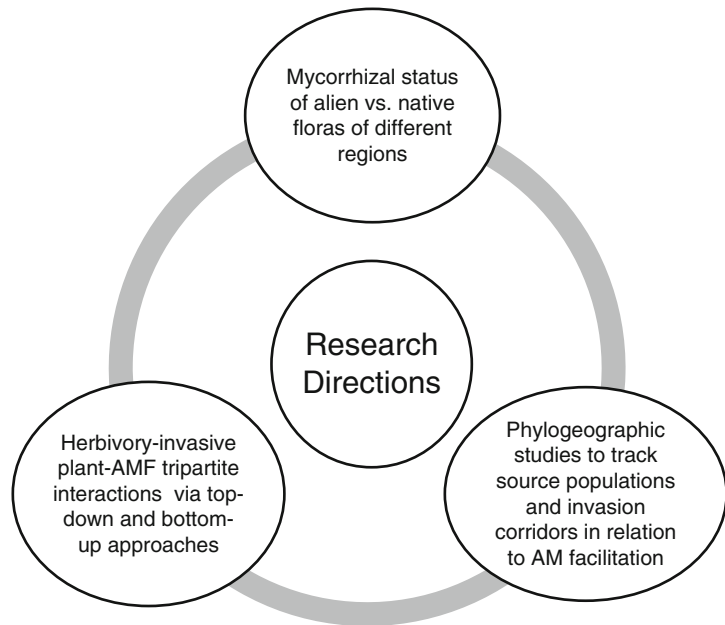


Fig. 5.2 Important research directions proposed to overcome the limitations to understand interactions between AMF and invasive plants



Since 80–90 % of the land plant species and families are mycorrhizal (Wang and Qui 2006), mere association of arbuscular mycorrhizal symbionts with exotic plants cannot be taken as an indication of their role in promotion of exotic plant invasions. But in view of the promotion of invasiveness of some exotic plant species by their associated AMF mutualists (Fumanal et al. 2006; Shah and Reshi 2007; Shah et al. 2008a, b), information about the mycorrhizal status of invasive plants in different biogeographical regions and habitat types may help to better understand the role of AMF in exotic plant invasions.

Mycorrhizas Influence Competitive Interactions

The importance of soil nutrients in plant invasions has been highlighted by many studies. With respect to invasive plants, some correlations have been drawn between nutrient availability and enemy release (Blumenthal 2005), invasiveness and disturbance (Davis et al. 2000) and invasion facilitation upon experimental resource enrichment (Davis and Pelsor 2001; Daehler 2003). In view of the well-established

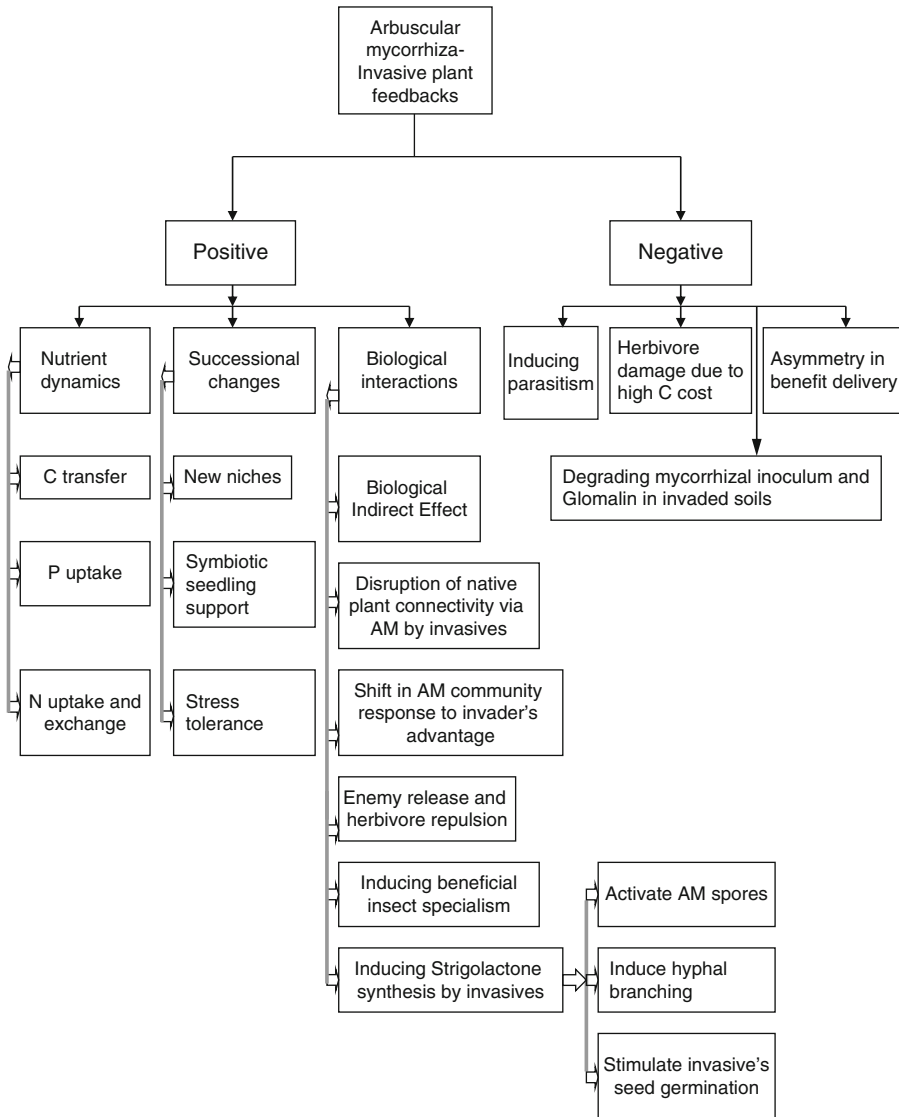


Fig. 5.3 Possible ways in which AMF may influence invasiveness of alien plants and invasive plants may affect AMF community dynamics (Reproduced from Shah et al. 2009a, b)

role of AMF in nutrient uptake for host plants in different forms and from different sources, plant-wide mycorrhizal web is likely to influence competitive interactions of invasive and native plant species through differential exchange of nutrients between them. The competitive relationships of plants have been shown to be influenced by both the presence and identity of AMF (Scheublin et al. 2007), though such relationships have seen a renewed interest in the context of plant invasions

(Hetrick et al. 1990; Hartnett et al. 1993; Bever et al. 1996; Moora and Zobel 1996, 1998). Has seen a renewed interest in the context of plant invasions. This surge in the interest has been especially due to the potential of AM to acquire nutrients at a lower carbon cost than roots because of their smaller diameter and greater surface/volume ratio. In addition, extensive mycorrhizal colonisation substitutes for the main root function of nutrient uptake, thereby reducing resource

allocation to roots (Berta et al. 1993; Vance et al. 2003). This helps most of the invasive plants to allocate the available resources more towards defence than growth, a strategy of particular significance for successful invasion as suggested by the evolution of increased competitive ability (EICA) proposition (Blossey and Nötzold 1995). Whether such increased competitive ability due to AMF emerges only from the growth and defence trade-off or due to differential response of native and invasive species to geographical source and taxonomic or functional identity of mycorrhizal species are still open questions.

Arbuscular mycorrhizas have been reported to indirectly enhance competitive effects of an invasive forb *Centaurea maculosa* over a native bunch grass *Festuca idahoensis* to invade native grasslands of western North America (Marler et al. 1999). While mycorrhizal mediated inter-plant carbon transfer was reported earlier by Francis and Read (1984), subsequently Carey et al. (2004) provided a direct isotopic and physiological evidence for transfer of carbon from the native species of *F. idahoensis* to invasive *C. maculosa*. The mycorrhizal mediated carbon theft by exotics from native neighbouring species thereby tilting the balance of competition in their favour is supported also by Giovannetti et al. (2006). However, Zabinski et al. (2002) showed that phosphorus uptake, not carbon transfer, is responsible for arbuscular mycorrhizal enhancement of *C. maculosa* in presence of native grassland species. The apparent contradiction of such studies, some showing C transfer and others P uptake but not C transfer as the means of mycorrhizal favour to invasive plants, needs comprehensive field studies and laboratory testing to elucidate whether they are the alternative mechanisms operating under different situations to favour invasives. Studying the role of AMF in facilitating neighbour recognition by invasive species in invaded ranges through altered resource availability and molecular crosstalk would be quite interesting.

The findings that AMF facilitate N uptake by host plants (He et al. 2003) in different forms and even aid in N transfer from one plant to another

(Govindarajalu et al. 2005) need to be put in the right perspective in respect of invasive plants, the establishment and subsequent spread of which is usually nitrogen limited (Wolf et al. 2004). Mycorrhizal mediated enhanced invasiveness is discernible from a North American grassland invader, *Centaurea diffusa*, which competed best under low N conditions but lost its competitive ability under low P conditions (Kathrine et al. 2004) under which AM symbiosis might turn out of critical importance for this species. Arbuscular mycorrhizas may be especially important in regions with NH_4^+ -dominated soils (Ames et al. 1983; Johansen et al. 1996) due to the fact that some allelopathic compounds released by invasive plants inhibit nitrification (Lodhi and Killingbeck 1980; Thibault et al. 1982), thus limiting growth of native plants by inducing nitrate deficiency. This hypothesis, however, needs to be validated by further investigations, which also need to determine the relative mycorrhizal dependency and species sensitivity of invasive vs. native plant species to determine the precise outcome of AM association in relation to invasiveness.

Invasive plants generally prefer disturbed habitats because disturbance promotes invasion by increasing resource availability and causing nutrient flushes. However, the way different disturbance regimes affect AM communities merits due attention in invasion ecology. It has been reported that the soil disturbance affects AM communities by breaking up AM extraradical mycelium both in pots (McGonigle and Miller 1996, 2000) and in the field (Kabir et al. 1997). This may result in not only delayed root colonisation but also reduced nutrient uptake. Yet paradoxically many invasive plant species have been reported to be highly mycorrhizal (Fumanal et al. 2006; Shah et al. 2008a, b). This indicates that the facilitative role of AM in plant invasions may not necessarily be through improved nutrient uptake but via some other mechanisms. Establishing the mycorrhizal responsiveness and mycorrhizal dependency of invasive plants, both in their invaded and home ranges, under different disturbance regimes is suggested as a useful approach in this direction.

Mycorrhizas Facilitate Plant Invasion via Succession

Plant invasion, being a multistage process like succession, is characterised by conspicuous spatio-temporal dynamics along the introduction–establishment–naturalisation–invasion continuum. Mycorrhizal symbioses may contribute to plant invasiveness from the initial stage of their introduction to the final stage of widespread occurrence and abundance, possibly with changing roles during different stages along a mutualism–commensalism–parasitism gradient. AMF can potentially integrate the emerging seedlings into the introduced communities with the extensive hyphal networks to nourish them (van der Heijden 2004) and act as a symbiotic support system to overcome their recruitment limitation in the invaded habitats. Many introduced plant species have been shown to rely on mutualisms in their new habitats to overcome barriers to establishment and to become naturalised and, in some cases, invasive (Richardson et al. 2000).

Working out the invasion history of an exotic plant, *Solidago canadensis*, on the Chinese Chongming Island, Liang et al. (2004) found a significant positive correlation between the time of invasion and rate of AM colonisation. They showed that the total number of AM species increased with increasing invasion time and was positively related to the number of plant species occurring in plant communities. This suggests that invasion time and plant diversity can influence AM species diversity. Further studies, however, need to explore the spatio-temporal variations in AM communities as a function of invasive spread of exotic species. Linking the process of invasion to the development pattern of mycorrhizal symbiosis during primary and secondary successions may give some insights into this complex relationship because the invasive plant species are often the primary colonisers in secondary succession. Such an approach of integrating habitat characteristics and invasive attributes into invasion dynamics may be helpful in the prediction and prevention of plant invasions.

Response of Native and Invasive Plants to Mycorrhizas

The role of mycorrhizal fungi in plant invasions, though empirically tested hitherto by few studies only, needs to be viewed in light of the established ecological theories and principles in order to have restoration and management implications. Extensive field studies and subsequent greenhouse experiments (Fumanal et al. 2006) with *Ambrosia artemisiifolia*, a North American invader in Europe, showed positive impacts of AM on growth, development and spread of this invasive plant species thus underlining the need to integrate symbiotic interactions in future work on invasive plant processes. Earlier mycorrhizas have been reported to be associated with invasion of *Erechtites glomerata* on Californian San Miguel Island (Halvorson and Koske 1987). In a recent greenhouse study (Nijjer et al. 2004), mycorrhizal inoculation unusually increased growth of Chinese tallow (*Sapium sebiferum*), an invasive tree in the southeastern United States, but caused zero to negative growth changes of its five co-occurring native tree species (*Liquidambar styraciflua*, *Nyssa sylvatica*, *Pinus taeda*, *Quercus alba* and *Q. nigra*). The study, however, indicated that the potential advantage *Sapium* gets from mycorrhizal associations may vary with native species and soil fertility. This is fully supported by our recent studies (Shah et al. 2008a, b) on the influence of resident and foreign AM on growth invasiveness of exotic *Anthemis cotula* in Kashmir Himalaya vis-à-vis the effect of four common co-occurring neighbours, *Conyza canadensis*, *Galinsoga parviflora*, *Sisymbrium loeselii* and *Daucus carota*. The field studies revealed high incidence of Arum-type mycorrhizal colonisation in natural populations of *A. cotula*, and the pot trials confirmed reliance of its invasiveness on AM with more favourable effect of resident than foreign AMF. The mycorrhizal colonisation intensity in field populations of *A. cotula* was, however, strongly influenced by neighbour identity with major reduction recorded in the presence of *Sisymbrium loeselii* (a cruciferous non-host) in

comparison to other con-familial neighbours. Pot experiments confirmed the differential effect of co-occurring species on *A. cotula*'s invasive traits. Such studies on tripartite, invader-AM-neighbour, interactions provide a conceptual framework for future studies to analyse soil biota feedback and competition as interlinked processes influencing exotic plant invasions. However, we suggest further studies to screen most effective native plant species that could deprive the invasive species of the benefits obtained from mycorrhizal association in invaded ranges and advocate use of such native species in ecological restoration of invaded habitats. Testing whether different AM taxa from native and invaded ranges of exotic species differ in the rate, extent and location (root or soil) of colonisation of invasive species and their non-native noninvasive congeners would help in determining the taxonomic and origin basis of AM functional diversity in relation to plant invasion. Native mycorrhizal isolates from invaded habitats also need to be screened for their comparative influence on growth promotion of invasive vs. native plant species. The AM isolates that favour growth of native species more than invasive species can be used as effective bio-inoculants to restore native plant communities in invaded habitats.

AM inoculation not only promotes growth of exotic plants but can also influence plant and microbial community structure associated with them. The influence of *Gmelina arborea*, a potentially invasive tree in West Africa and native to India, on resident herbaceous plant community structure and microbial community function was shown to be significantly modified by the massive AM inoculation (Sanon et al. 2006). However, AM species identity may influence the invader's success, and some invaders specifically increase abundance of their selectively cultivated AM species possibly to the detriment of native neighbours (Stampe and Daehler 2003). Therefore, simple comparisons of plant growth with and without mycorrhizas, overlooking the identity of naturally associated AM species, may be of limited relevance from an invasion standpoint. Hence, the upcoming studies need to work out the effect of AM

identity on invasive as well as co-occurring native species and compare the mycorrhizal status of invasive species in their native and invaded ranges to correlate AMF with invasiveness. More importantly, attention to the effects of native soil mycorrhizas on non-native plants that do not successfully invade will be crucial if we are to assess the relative importance of AMF in invasions. Furthermore, the role of AM in plant invasion needs to be viewed in light of the often overlooked biotic indirect effect, how one species alters the effect that another species has on a third (White et al. 2006). Moreover, the complexity of biotic interactions, influencing or getting influenced by plant invasion, underlines the need for further studies to shift from a single-factor to a multifactorial approach to be truly reflective of natural communities. Better understanding of mycorrhizology through cross-fertilisation of empirical data with the concepts of ecology, mycology and plant pathology will help us not only to predict but possibly prevent exotic invasions.

Tripartite Interactions of Mycorrhizas, Invasive Plants and Herbivores

Mycorrhizas and herbivores both have been shown to influence plant invasions at different ecological scales. While their effect has been hitherto studied independently, interactions between herbivores and mycorrhizal fungi are expected because both depend upon and influence important plant resources. Herbivores, being aboveground foliage consumers, may reduce photosynthate translocated to the root system and available to mycorrhizal fungi, resulting in a reduction in mycorrhizal colonisation and reduced development of the symbiosis (Gehring et al. 1997; Hetrick et al. 1990; Trent et al. 1988). Mycorrhizas, in turn, can have many potential effects on plant-herbivore interactions. Under certain conditions, up to 40–50 % of a plant's net production may be allocated to its fungal symbiont (Fogel and Hunt 1979; Harris and Paul 1987). Because mycorrhizal fungi both consume photosynthate and at the same time enhance mineral

nutrient acquisition and growth capacity, the cost–benefit relationships among mycorrhizal fungi, herbivores and host plants are likely to be complex. Mycorrhizas may affect herbivores through alteration of plant growth or foliar chemistry (e.g. Goverde et al. 2000; Koide 2000), and they may have large effects on plant responses to herbivores by influencing anti-herbivore defences and/or herbivory tolerance (regrowth capacity). Klironomos et al. (2004) while studying the response of AMF to simulated herbivory suggested that it is difficult to generalise on the effects of herbivory on plant and fungal responses, even when dealing with the same plant species.

In view of the significance of enemy release and biotic resistance in plant invasions (Mitchell and Power 2003; Klironomos 2003; Shah and Reshi 2007), developing AM association in the invaded ranges might help invasives overcome this biotic resistance. Reinhart et al. (2003) provided experimental evidence for escape from specific pathogens by *Prunus serotinus*, a native to North America and invasive in Europe, where it harvests the maximum benefits of interacting with generalist mutualists such as AMF. This is in concurrence with our findings of exotic *A. cotula*, an annual herbaceous plant native to southern Europe–west Siberia, where the species is attacked by about 68 insect pathogens, and invasive in Kashmir Himalaya where it has escaped all the native herbivores and pathogens (Shah and Reshi 2007) due to characteristically very high (>84 %) AM root length colonisation. The indirect role of high mycorrhizal colonisation in keeping herbivores at bay by exotic invasives in their invaded areas is of specific significance because it is too costly for plants in terms of carbon economy to harbour mycorrhizal association at home where their foliage is under intense insect herbivory. A path-breaking study supporting this case (Abigail et al. 2005) showed that mycorrhizas, to a great extent, may benefit plants subjected to herbivory by stimulating compensatory growth and herbivores, in turn, may increase the development of the mycorrhizal symbiosis. However, their results indicate strong interspecific differences among tallgrass prairie plant species in their responses to the interaction

of aboveground herbivores and mycorrhizal symbionts. More studies on AM-mediated invasive plant–herbivore interactions need to be specifically carried out with invasive plants to draw robust conclusions. Furthermore, AMF can induce insect specialism in host plants by altering their chemistry (Gange et al. 2002) thereby preventing the generalist insects from attacking the host plants (Gange et al. 2005). Thus, if fewer specialist insects are absent in invaded habitats, the mutualism can be drawn to the best advantage by the invaders to avoid generalist insects. In order to have an inclusive picture of the outcome of plant–herbivore interactions, developing comprehensive mycorrhizal status-wise checklists of invasive plants, as reported recently by Shah et al. (2009a, b), together with associated specific herbivores, parasites and pathogens on a local, regional and global scale is important. Despite the fact that invasiveness can be affected both by mycorrhizal fungi and herbivores, very few studies have hitherto examined the interactive effects of these factors on exotic plants. While most of the available data suggest reduction in AM root colonisation by severe herbivory (Gehring and Whitham 1994), the reverse interactions have also been documented. Although consistent patterns and mechanistic explanations are yet to emerge, it is likely that herbivore–AM interactions have important implications for plant invasions.

Negative Feedback Interactions of Mycorrhizas and Invasive Plants

Mycorrhiza–plant interactions may vary along a mutualism–commensalism–parasitism gradient depending upon several factors such as the host species, soil fertility status and other environmental conditions (Lovelock et al. 2003). Arbuscular mycorrhizas may not always confer benefits to their host species but may also reduce their competitive abilities due to high carbon costs (Walling and Zabinski 2006). A negative mycorrhizal feedback on plant growth can be attributed to asymmetries in the delivery of benefit between

plants and AM species (Bever 2002), and this may result in community dynamics where competing plant species can coexist. This reduces the possibility of competitive exclusion of native species by invasives. Landis et al. (2004, 2005) corroborated their field data with controlled experimentations to show that AM can induce parasitism on susceptible hosts and non-mycorrhizal plants may not only persist in and successfully compete with mycorrhizal plants in well-established species-rich communities but can even invade and dominate them. However, studies on variation in plant response to native and exotic AMF (Klironomos 2003) have shown that extreme responses are more common in case of locally adapted plants and fungi. Though exotic AM may not function any differently from native AM, the former offer less variation in plant response than later (Klironomos 2003) thus having relatively lesser positive or negative feedback with exotics than native plant species. In addition, a negative correlation between AM density and invasive plant (knapweed) cover was recorded (Lutgen and Rillig 2004) by demonstrating that areas with high knapweed density generally had lower glomalin concentration and AM hyphal length compared with areas having no or less knapweed cover. Through floral examinations and experimental tests, naturalised plants are reported not only to be less dependent on and poor hosts of AMF but also their initial establishment and dominance of invaded habitats can inhibit the reestablishment of effective mycorrhizal mutualists (Bever et al. 2003). Alteration of soil biotic characteristics in such a way by invasive species may negatively feedback to change their performance relative to co-occurring native plant species. On the other hand, even a small increase in growth of native species from mycorrhizal mutualists has been shown to help them to compete effectively with exotic species (Gillespie and Allen 2005) despite the fact that the exotic invasion may cause changes in the mycorrhizal community. While Goodwin (1992) indicated a negative role of fungal mutualisms in maximising fitness of invasive species, Bever et al. (2003) suggested that dominance of naturalised plant

species in Southern California is facilitated by degradation of mycorrhizal mutualisms. It appears from such findings that communities with rich AM diversity may be more resistant to invasion by exotic plants. Also because of their low host specificity, AMF may not necessarily play a major role to specifically facilitate or hinder the growth of exotic plants. The potential of exotic invasive species to improve phosphorus dynamics and bio-availability (Chapuis-Lardy et al. 2006) indicates their pervasive influence on AM communities. However, exhaustive field observations and controlled experiment, including different permutations and combinations to incorporate most of the variables affecting or getting affected by invasive plant-AM interactions, need to firmly establish whether exotic invaders outcompete more easily the mycorrhizal or non-mycorrhizal native plants and which of them can resist invasion more strongly. These findings will be most useful in identification and selection of the effective native mycorrhizal species/isolates conferring more benefits to native than exotic plants that can be used to restore invaded habitats. In situations where exotics rely more on mycorrhizal symbiosis, the non-mycorrhizal native plants with suppressive influence on AM inocula could be used for restoration purposes.

A basic ecological attribute of successful invaders is to be least or nondependent on mutualists such as AMF, which if indispensable or obligate may hamper their introduction and successful colonisation in usually disturbed and AM poor habitats in their invaded range. Whether invasive plants have obligate or facultative dependence on AMF in invaded communities need to be ascertained through convincing evidence. Although development of negative plant-soil feedback in the root zone of invasive plants has been reported (van der Stoep et al. 2002), there can be a shift in the organisms causing this feedback during subsequent stages of invasion. The mycorrhizal association may turn from mutualistic to parasitic during subsequent stages in the life cycle of invasive host species or even during different stages of invasion. Detecting these stage-specific changes in the

nature of AM–invasive plant interaction along the introduction–establishment–naturalisation–invasion continuum may help in devising effective soil-based management strategies for some plant invasions.

Impact of Plant Invasions on Mycorrhizal Communities

How exotic plants affect the soil microbial communities in their invaded habitats is an exciting aspect of contemporary invasion biological studies. A study by Hong-bang et al. (2007) elucidated that soil biota alteration after *Ageratina adenophora* establishment may be an important part of its invasion process in Chinese forest understories to facilitate it and inhibit native plants. Furthermore, invasion by *A. adenophora* was found to strongly increase the abundance of soil AMF and the fungi/bacteria ratio. Earlier Mummey and Rillig (2006) indicated significant AM community alterations and considerable reduction in their diversity in response to invasion by *Centaurea maculosa* invasion. A major shift in composition and function of soil microbial community, of which AMF comprise an important part, due to exotic invasion in numerous ecosystems has been reported (reviewed by Wolfe and Klironomos 2005). Allelochemistry of invasive plants, depending upon whether they are mycorrhizal or non-mycorrhizal, may differently influence AMF communities in native soils of the introduced range. For instance, *Alliaria petiolata* (a noxious invader of eastern North American hardwood forests) is non-mycorrhizal but produces allelochemicals that directly degrade AM fungi (Roberts and Anderson 2001; Stinson et al. 2006). Through such positive feedback mechanisms, *A. petiolata* alters the mycorrhizal soil environment to one that is more conducive to its own growth and development rather than mycorrhizal-dependent native plants. Such degradation of local mycorrhizal fungi has also been noted for a variety of other invasive plants of disturbed ecosystems (although through other indirect mechanisms), leading to a new hypothesis for exotic plant invasion – the Mycorrhizal

Degradation Hypothesis (Vogelsang et al. 2004). Nevertheless, some allelochemicals secreted by some plants like sesquiterpenes may induce proliferation of hyphal branching in AMF (Akiyama et al. 2005) and cause improved germination of seeds of such invasive plants. While reviewing recently the role of allelopathy and mycorrhizas in plant invasions, Weir (2007) pointed out that allelochemicals play a much larger role in plant invasion than reflected by current literature. In fact, exotic plants may alter soil chemistry and soil ecology, probably creating conditions that favour their invasion at the cost of native species, as reported in case of *Halogeton glomeratus* (Duda et al. 2003). Species shift and significant reduction in abundance of soil biota, which may include AMF, has been attributed to the response of native species to soil nutrients like N, P and K present before invasion which were elevated in the soils that produced the greatest native species biomass (Belnap et al. 2005). Allsopp and Holmes (2001) also showed that following a single cycle of dense exotic vegetation, mycorrhizal plant species are not negatively affected, but other effects of exotic vegetation on nutrient cycling may change the balance between different mycorrhizal–plant guilds. Exotic invasion through their profound influence on soil properties and elemental cycling (Blank and Young 2002) may indirectly impact AM diversity and distribution which in turn can translate into the success or failure of invasive species. This can be related to altered soil quality and textural properties due to plant invasions as indicated in case of invasion by *Parthenium hysterophorus* (Annapurna and Singh 2003). The self-altered soil conditions by this exotic species may potentially promote its invasiveness over a broad range of habitat conditions. Since AM can notably influence soil quality and texture (Landis et al. 2004), their role in plant invasion needs further investigations in light of this perspective as well. The differential abilities of plants to influence their abundance by changing the structure of their soil communities (Klironomos 2002) are considered to regulate plant community structure which in turn determines community invasibility. We argue that soil aggregation should be included

in a more complete ‘multifunctional’ perspective and that in-depth understanding of tripartite, mycorrhiza–soil process–invasion, relationships will require analyses emphasising feedbacks between soil structure and mycorrhizas vis-à-vis plant invasion, rather than a unidirectional approach simply addressing mycorrhizal effects on soils.

Stinson et al. (2006) presented novel evidence that antifungal phytochemistry of the invasive plant, *Alliaria petiolata*, a European invader of North American forests, suppresses native plant growth by disrupting mutualistic associations between native canopy tree seedlings and below-ground AM. The pervasive influence of an invasive plant (*Centaurea maculosa*) on AM communities in roots of its competitors such as *Dactylis glomerata* was indeed an interesting proposition (Mummey et al. 2005) adding a biological spatial component to controls on root colonisation. An insight into the possible mechanism of how invasive plants drive mycorrhizal symbionts to their advantage is discernible from Parniske (2005) and Akiyama et al. (2005). Their studies showed that roots of some parasitic weeds release potent molecules such as strigolactones that activate symbiotic fungi at very low concentrations by providing cue for the hyphal branching connections and triggering seed germination, thus facilitating plant roots to enter into symbiosis with AM. However, it is currently unclear precisely which phytochemicals produced by invasive plants have the antifungal properties, whether and how they interact with other functionally important soil microbes. In addition, within the home range, it is important to know if evolutionary natural resistance of co-occurring native plant species buffers the effects of invasive plant’s anti-mycorrhizal properties. Further research in these directions is needed to better understand the effects of invasives on natural ecosystems and the mechanisms involved.

Summary

While many studies suggest driving influence of AM fungi on plant invasiveness (Table 5.1) by facilitating competitive dominance of exotic plants

over the native species (positive feedback), some studies also indicate the opposite showing that AM may contribute to the coexistence of competing plant species (negative feedback). Reciprocally, the exotic plant species may impact mycorrhizal community structure and function in the invaded habitats in different ways (Fig. 5.2). Elucidating the facilitative as well as suppressive role of AM fungi in plant invasions, the gaps and limitations in the field studies and experimental designs of complex AM–invasive plant interactions research identified hereby call for alternative strategies in future studies (Fig. 5.3). Understanding the stage-specific transition of mycorrhizal associations, from mutualism to parasitism or vice versa, along the introduction–establishment–naturalisation–invasion continuum would be an interesting discourse for future research. This, however, needs a unified top-down and bottom-up approach targeting both biotic and abiotic factors under field and laboratory conditions.

Invasive Plants: Problems, Prospects and Paradoxes

Invasive species refer to the alien species that spread rapidly in non-native ranges and generally inflict economic and ecological damage to varying degrees, besides causing evolutionary and human health problems. The annual economic losses due to invasive alien species worldwide run in \$ trillions per annum. Given the threat to native diversity due to exotic invasions, nations are obligated to have in place definitive strategies and action plans for the management of invasive species. In fact, according to Target 9 of Strategic Goal B of the Strategic Plan for Biodiversity – 2011–2020, countries need to ensure that by 2020 invasive alien species and their pathways are identified and prioritised, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment. Notwithstanding this urgency and importance for managing exotic invasions, invasive species also offer novel model systems to understand the process of rapid evolution.

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What role does shift in mutualistic and antagonistic interactions of invasive species upon introduction from native to non-native regions play in such rapid evolution is a challenging discourse. Whether rapid coevolution of invasive plants and their symbiotic partners corresponds to the fast evolutionary transitions of invasive species comprises an important subset of the aforementioned major question. Recently, there have been some attempts to challenge the entire premise of invasion biology, and invasions have been perceived as a positive rather than negative phenomenon. However, prompt rebuttals have followed with stronger arguments supporting the very basis of invasion biology and its importance. Since invasive species are introduced from their native to non-native regions, the global coordinated experiments promise to help decipher interesting biogeographic patterns in invasion biology.

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Introduction

Huge ecological and economic costs associated with wanton degradation of natural ecosystems underscore the urgency and importance of ecological restoration. A large number of restoration projects are undertaken for achieving various targets, but there are only very few success stories. A restoration project is doomed to fail if it does not take into account fundamental factors and processes underlying ecosystem functioning. The restoration of plant communities in degraded ecosystems requires explicit understanding of the functioning of natural communities and the driving ecological forces that produce different vegetation patterns. Understanding the fundamental question as to what makes certain species absent in most communities and occur frequently elsewhere has significant implications for restoration projects.

Recent evidence suggests that mycorrhizal association could be one of the potential factors that may lead to rarity, abundance or differential performance of plants in different communities (see Chap. 5 for details). In fact, mutualistic facilitation has been found to be highly important, though largely neglected, aspect of the functioning of plant populations and communities. Mycorrhizal symbionts, in view of their versatile role in maintaining soil structure, influencing successional dynamics and facilitating seedling establishment and plant growth in nutrient-poor systems, could be the key players in ecological

restoration of disturbed communities (Fig. 6.1). In this chapter, an attempt is made to give an overview of the handful of studies carried out so far in the area of mycorrhiza-mediated restoration ecology. The knowledge gaps are identified with the objective to draw attention of mycorrhizologists and ecologists to this applied area of research so that appropriate future directions are provided with highly valuable implications. If well explored and better understood, mycorrhizal technology promises to be an integral part of most of the ecological restoration projects.

Mycorrhizas and Soil Structure

In ecological restoration of plant communities, the role of soil structure is of central importance. Mycorrhizas play a significant role in determining the soil structure, though this area of research has received relatively little attention. Mycorrhizas can influence the soil structure in different ways using different mechanisms. An in-depth understanding of the role of mycorrhizas in soil structure is partly limited by the lack of suitable tools and apparent complexities involved in manipulating plant–AM interactions under field conditions. Different mechanisms by which mycorrhizas can influence soil structure at macroaggregate and microaggregate scales have been reviewed by Rillig et al. (2006). Mycorrhizal fungi can influence soil aggregation in a hierarchical manner at the

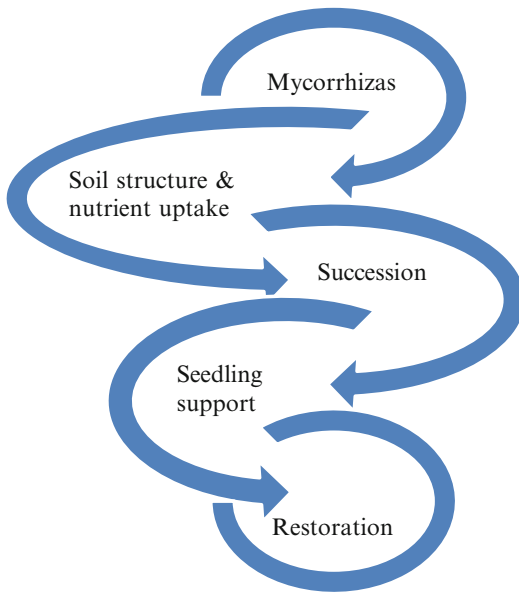


Fig. 6.1 A linked spiral of steps and stages in mycorrhiza-mediated ecological restoration. Mycorrhizas through their influence on soil structure and nutrient uptake support plant seedlings in a successional dynamics, thereby facilitating restoration

scale of plant community, individual plant and fungal mycelium.

Belowground mycorrhizas are believed to be determinants of the composition and productivity of above-ground plant communities (Grime et al. 1987; van der Heijden et al. 1998; Klironomos 2000). Mycorrhizal network connects plants belowground and causes differential benefits to different host plants, depending partly upon the nature of the plant species. In fact, plants apparently have variable effects on soil aggregation depending upon the type of exudation, root architecture, habitat preference and even ecosystem type. The differential role of mycorrhizas has been demonstrated for plants growing in agricultural and natural ecosystems (Eviner and Chapin 2002; Rillig et al. 2002; Piotrowski et al. 2004). In nature, there supposedly exists a connected loop wherein mycorrhizas influence the composition and productivity of plant communities, which through their root architecture, exudations and nutrient additions do influence soil structure, which in turn affect mycorrhizal density and

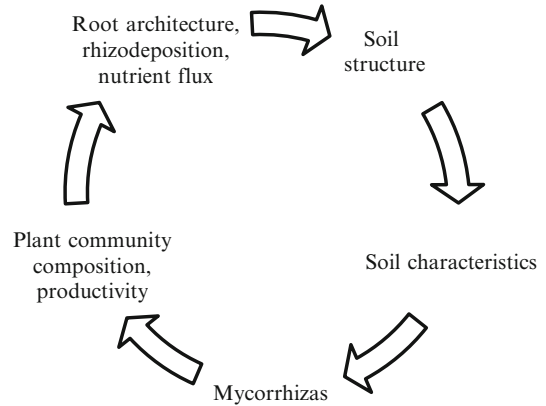


Fig. 6.2 Mycorrhizas through their influence on composition, productivity and diversity of plant communities determine the kind of exudations and rhizodepositions, which together with the root architecture strongly influence soil structure. The soil structure and other characteristics are intricately linked to mutually influence the rhizospheric mycorrhizal communities

diversity in soil system (Fig. 6.2). As a consequence, changes in any of the components of this connected loop at any level may directly or indirectly translate into effects on soil structure or vice versa.

Plant roots can influence soil structure through physical penetration, altering soil water regime, exudations and nutrient release through decomposition (Six et al. 2004). So, how mycorrhizas influence plant root morphology and architecture is important to understand as to how these fungi do impact soil structure. Observational and experimental studies have shown that mycorrhizal colonisation has a strong impact on root: shoot ratios (Shah et al. 2008a, b, 2009a, b). Mycorrhizal association can either improve or reduce plant root development and proliferation. Some studies have shown that mycorrhizal plants may need to allocate relatively lesser resources towards roots than non-mycorrhizal plants, partly because mycorrhizas take over the function of roots through an extended mycelial network. This facilitates uptake of nutrients by host plants from otherwise inaccessible areas of soil systems. Under such circumstances plants can allocate their resources more towards shoots and reproductive structures, thereby improving fitness attributes. On the other hand, mycorrhizas through

their positive influence on overall growth and performance of the plant may help them having more robust root system that can be used for better uptake of nutrient, water and other related things. Soil aggregation and stability of aggregates is directly influenced by soil water content and its water holding capacity, which in turn has a significant bearing on plant growth. Mycorrhizas can influence plant growth and dampen the effect of variable water regimes under different wet and dry cycles, and mycorrhizal plants can generally withstand a wider range of water-level fluctuations than non-mycorrhizal plants (Augé 2001, 2004). Root exudates and mycorrhizal Glomalin act as natural glues to bind soil particles and influence aggregation process and contribute to short-term aggregate stability. It is pertinent to mention that Glomalin is a class of fungal proteins that, on the basis of correlative evidence, has been found to tightly bind soil particles, thereby linking fungal physiology with soil aggregation. It seems that different soil-binding factors function synergistically involving different plant and fungal processes, especially carbon metabolism and soil microbial activities.

In the complex soil web, dissecting the role of mycorrhizas in ecological processes such as soil aggregation from other soil microbiota is not realistic. Evidence suggests that mycorrhizas can potentially influence soil microbial communities (Artursson and Jansson 2003; Artursson et al. 2005; Rillig et al. 2006), though different facets of this interactive influence are poorly understood. While mycorrhizas exert a strong influence at the macroaggregate scale, other soil microbes are likely to influence the formation and stabilisation at microaggregate level. But, these influences are not mutually exclusive because mycorrhiza-mediated soil microbial alterations or possible even vice versa may lead to the alteration of soil aggregate distributions and turnover. It has been found that mycorrhizal mycelium products can serve as substrates for bacterial growth (Filion et al. 1999). This is evidenced by the isolation of bacteria (such as *Paenibacillus* spp.) from AM fungal mycelia (Bezzate et al. 2000; Hildebrandt et al. 2002; Mansfeld-Giese et al. 2002). Ravnskov et al.

(1999) reported that mycorrhizal deposition products may likely contain bacteriostatic or fungistatic agents, which can affect the pattern and type of other rhizodeposition products, thereby having important implications for soil aggregation. An interesting study by Rillig et al. (2005), using heat-inactivated AMF inoculum and phospholipid fatty acid patterns, indicated that symbiosis-influenced microbial communities could influence soil aggregate water stability in an AMF-species-dependent manner.

Based on their synthesis, Rillig et al. (2006) argued that different species or communities of mycorrhizal fungi can promote soil aggregation to different degrees. They furthermore highlighted new tools and directions that could potentially contribute to better understanding of the role of mycorrhizas in soil aggregation. Different aspects of the fungal-mediated soil aggregation have also been documented. The abundance and architecture of AM fungal mycelia do influence the rate of both formation and stabilisation of soils. Though mechanistic understanding of fungal-mediated soil aggregation is still poor, novel field-based studies and controlled experimental approaches need to be devised to lend more precision to our understanding of AM-influenced soil structural attributes.

Mycorrhiza-Mediated Seedling Support Systems

For the restoration of native plant communities in an invaded or disturbed ecosystem, recruitment of seedlings of desirable plant species is essential for maintaining species-rich plant communities. One of the important factors that ensures successful establishment and survival of recruited seedlings is mycorrhizal symbiosis. This is partly because mycorrhizas facilitate integration of seedlings into the community through below-ground mycelial network and also help these newly recruited seedlings to overcome nutrient and other limitations. Notwithstanding the established role of mycorrhizas in seedling establishment and performance through pot experiments with juvenile plants (Smith and

Read 1997 for an overview), such pot experiments with single plants are critiqued to be a far simplified version of the conditions encountered actually in natural plant communities where seedlings need to establish between adult plants. To how much extent do mycorrhizas help newly recruited seedlings to be able to compete with adult plants in existing vegetation is poorly understood.

Through microcosm studies van der Heijden (2004) tested whether AM fungi promote seedling recruitment in perennial grassland communities by adding seeds of four plant species (two grasses and two forbs) to patches within 1-year-old grassland microcosms that were inoculated with different AMF taxa or to control microcosms that were not inoculated. He found better seedling growth and phosphorus uptake in AMF-inoculated microcosm plants, though seedlings obtained different amounts of P in microcosms inoculated with different AMF taxa. The study demonstrated that AMF promote seedling establishment by integrating emerging seedlings into extensive mycelial networks and by improving nutrient supply to the seedlings. An overview of handful studies that have dealt with the role of AMF as a symbiotic support system to promote seedling establishment in natural communities yields inconclusive results, with some showing positive and others negative impacts. For instance, two earlier studies that monitored seedling establishment between 6- and 8-week-old plants (Grime et al. 1987; Francis and Read 1995) showed positive as well as negative effects of AMF on seedling establishment. Kytoviita et al. (2003) also suggested that AMF do not provide much benefit to seedling that established near 14-week-old plants.

Notwithstanding the paradoxical results obtained from earlier studies, ecological theory suggests that mycorrhizas may potentially benefit seedling establishment after germination by linking them with the mycorrhizal hyphal networks already maintained by the surrounding vegetation (Read and Birch 1988), thereby making seedlings accessible to a cheaper nutrient pool. Otherwise, it is unlikely that the roots of juvenile seedlings are able to compete effectively

with adult plants for nutrients. It is pertinent to mention that mycorrhiza-mediated interplant transport of nutrients and carbon from one plant to another through mycorrhizal mycelium has been reported on several occasions (Grime et al. 1987; Newman 1988; Simard et al. 1997). Moreover, the role of mycorrhizas in plant protection against soil pathogens (Newsham et al. 1995) and the lack of host specificity are also valid reasons for AMF to be supportive to seedlings. So it is imperative that restoration ecologists should not underestimate the importance of mycorrhizal symbionts as possible seedling support systems in different restoration projects, at least when the aim is to restore native plants in established natural communities.

The Role of Mycorrhizas in Ecological Succession

The classical ecological theory suggests that succession is a series of predictable processes, the trajectories of which are primarily influenced by nutritional constraints (Clements 1916; Odum 1971; MacMahon 1981). Under such a scenario, mycorrhizal colonisation is likely to play a significant role in determining both the rate and direction of the processes. Moreover, the vegetation patterns emerge from combined and interactive influence of the local biotic and abiotic components of the environment on differential propagule dispersal and establishment. Though both biotic and abiotic factors were earlier thought to equally shape the course of succession, emphasis has shifted over time more towards biotic interactions. Of the various biotic interactions, mycorrhizas have been found to be of crucial importance in both primary and secondary succession. This is mainly because of the critical role that mycorrhizas can play under the conditions of nutrient scarcity to facilitate the process of primary succession (Gorham et al. 1979) and supportive role for juvenile seedlings in secondary succession.

A reasonably good account of how mycorrhizas facilitate development and progression of plant communities during succession can be

found in different works (see, for instance, Nara et al. 2003a, b; Nara and Hogetsu 2004; Cazares et al. 2005). One of the pertinent case studies to support the facilitative role of mycorrhizas in successional dynamics of plant communities was conducted on the North American sagebrush steppe by Allen and Allen (1988), where one of the dominant plant species over decades is an exotic introduced weed, *Salsola kali* L., that was introduced in the late 1800s from the Asian steppes. Restoration efforts to control *S. kali* lead to the establishment of many other species, some of which are desirable (e.g. *Elymus smithii* and *Bouteloua gracilis*) and some undesirable (e.g. *Artemisia tridentate*). This desirability, however, is governed by different perspectives. For instance, the dominant shrub, *Artemisia tridentate*, though increases with grazing and has to be often removed in rangelands managed for cattle, is important for wildlife including pronghorns, birds and invertebrates. During the successional dynamics in sagebrush steppe communities, the soils tend to be relatively nutrient-rich, though bound organic nutrients later on tend to decline (Allen and MacMahon 1988). All late seral plants in these systems form arbuscular mycorrhiza relationships that facilitate them in extracting nutrients from bound organics.

The relationships between soil quality, disturbance and mycorrhizal status can be better appreciated during a more predictable process of primary succession on sand-dune ecosystems (Read 1989), where it has been found that non-mycorrhizal species predominate in the disturbed and nutrient-enriched conditions of the drift line, and late seral species following successional shift from drift line to stable back are more responsive to AM colonisation. In this sand-dune succession model, earlier conditions seem more favourable for ectomycorrhizal and ericoid mycorrhizal hosts, which subsequently create better conditions conducive for dual colonising or only AM plants (van der Heijden 2001).

In contrast to primary successions, which often commences under nutrient-impoverished barren soils, secondary succession is normally initiated in an environment of relatively

nutrient-rich conditions (Walker and Syers 1976). Weedy annuals, especially belonging to non-mycorrhizal families (Chenopodiaceae, Brassicaceae and Polygonaceae), are the pioneers of secondary succession (Stahl 1900). One of the possible reasons for this is the loss of mycorrhizal inocula during the devastation phase after primary succession that favours non-mycorrhizal plants. These early colonisers of secondary succession are also coincidentally ruderals or 'r' strategists with high fecundity, short generation time and an ability to rapidly exploit pulses of nutrient availability (Grime 1979). However, fast decline of nutrient availability after the initial flush of minerals, either due to rapid utilisation by ruderals or excessive leaching losses, paves way for mycorrhizal host plant species which can thrive better under relatively nutrient-poor conditions. In fact, the sensitivity of many non-mycorrhizal plants to the presence of AM to competition from associated AM plants raises the possibility that the reduction of inoculum potential of these fungi is an essential prerequisite for their success. The typical microclimatic and soil nutrient conditions produced at different successional stages may influence the type and extent of mycorrhizality in plants. For instance, seedlings of the *Hyacinthoides non-scripta* germinating in the organic matter in ECM *Quercus* woodland are largely non-mycorrhizal, but with time, the developing bulb and the roots produced from it descend into mineral soil where they develop arbuscular mycorrhizas in isolation from the largely surface-rooting trees (Merryweather and Fitter 1995). Similarly, *Eucalyptus marginata* plants have been found to form AM associations when rooted in mineral soil, but are ectomycorrhiza if grown in litter (Reddell and Malajczuk 1984). Such type of plasticity gives added advantages to the host plants to thrive better in different ecological scenarios.

Though the aforementioned discussions highlight the role of mycorrhizas in ecological succession, the need is to support the generalisations through quantitative data generated through more field-based experiments manipulating the mycorrhizal status and species interactions at different

stages of the succession. Such type of approaches can lend significant precision to our understanding of the role of the mycorrhizal symbiosis in the successional dynamics of plant communities.

Art of Eco-restoration

The fast-sweeping waves of unsustainable activities across the globe have considerably damaged the aquatic, marine and terrestrial environments, thereby underpinning the need for serious and dedicated efforts to reverse this process of degradation and putting in place effective ecological restoration measures. The successes and failures in restoration ecology largely depend on how science is informing these efforts, though the science and art of restoration are inextricably linked. While in art, restoration involves recapturing aesthetic value of ecosystems and developing conceptual clarity of the kind of world and environment we want to be a part of, in science it generally implies regeneration of economic goods and ecosystem services provided by ecosystems. Nevertheless, the ultimate goal of restoration ecology is not necessarily to restore an ecosystem to a pristine, pre-disturbance ideal. Notwithstanding that restoration ecology is a relatively new science, over a short span of just more than two decades, it has grown as an emerging area with a major role in sustainable development efforts across the globe. In fact, restoration has become an integral part of the environmental policy with novel opportunities, probably greater than ever before. One of the opportunities is exploration and exploitation of mycorrhizas for restoration of rare and threatened plant species either in their original or novel habitats. How effective the role mycorrhizas can play in ecological restoration and enhancing ecosystem services and increasing resilience to future change need to be evaluated through some forward-looking research paradigms. Since the success stories of restoration ecology are grounded in persistence of species and abiotic

processes that permit natural regeneration, belowground mycorrhizal network viability and functionality largely determines the diversity and distribution pattern of above-ground plant community structure and dynamics.

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Introduction

Plant life in extreme environments is of special importance because it is potentially helpful in understanding of how plants can adapt to these extreme environments. Before discussing extreme environments and the plants that exist in them, it is necessary to be familiar with the types of these environments. Extreme cold environments, hydrothermal vents, sulphuric springs, extremely acidic or alkaline environments and highly saline aquatic and terrestrial systems exemplify major extreme environments on the planet Earth. There have been very few studies conducted so far in the context of mycorrhizas in extreme environments. Since the waves of global change have rendered most of the environments more and more stressful, the biotic interactions that promote stress tolerance and avoidance of plants attain renewed importance. How mycorrhizas can help host plants to withstand increasing droughts, salinity, cold and heat stresses is therefore becoming an interesting discourse. A challenging area for mycorrhizologists would be to understand what kind of morphological and physiological adaptations plants need to undertake to get maximum benefits in order to survive in extreme environments.

Arbuscular mycorrhizas, as depicted in Fig. 7.1, are known to improve protection of host plants against a range of these environmental stresses (Sylvia and Williams 1992), such as drought (Augé et al. 2007, 2008), cold (Charest et al. 1993), salinity and pollution (Leyval et al. 1997).

Since mycorrhizal plants are better nourished, well adapted to environmental conditions and more vigorous and healthy, such plants tend to have relatively fewer incidences of root diseases and reduced association with other pathogenic agents (Dehne 1982; St-Arnaud et al. 1995). In this chapter an overview of the mycorrhizal status of plants in various stressful environments is presented, and the role of mycorrhizas in stress tolerance or avoidance of host plants is also discussed. The potential applications of mycorrhizal technology to overcome the challenges of stressful environments in the contemporary world are highlighted.

Mycorrhiza-Mediated Tolerance of Drought Stress

Since the time of simple observations by Mosse and Hayman (1971) more than four decades ago that mycorrhizal *Allium cepa* did not wilt upon transplantation and the non-mycorrhizal plants did, many studies (for instance, Busse and Ellis 1985; Huang et al. 1985; Augé 2001; Ruiz-Lozano 2003) have focussed on the role of mycorrhizal symbiosis in plant water relations. Mycorrhizas have been found to have a profound role in maintaining plant water relations. Sufficient evidence has accumulated over the past few decades for the role of AM fungi in amelioration of drought stress by host plants (Sanchez-Diaz and Honorubia 1994; Allen and

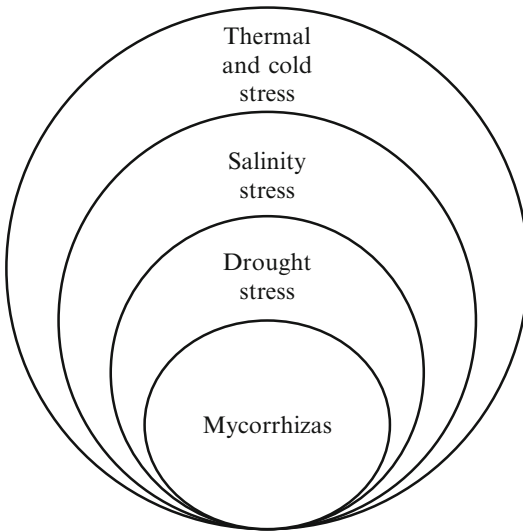


Fig. 7.1 Illustration of the centrality of the role of mycorrhizas in plant resistance against major stresses

Bosalis 1983; Nelson and Safir 1982; Augé 2000, 2001). However, the mechanistic understanding of how exactly mycorrhizas facilitate plants to withstand drought stress is of fundamental importance. It is suggested that mycorrhizal plants may avoid drought to some extent through enhanced water uptake at low soil moisture levels. Mycorrhizas may also influence water uptake through improved nutrient uptake as a result of which water uptake has to follow for osmotic adjustments. An overview of the studies carried out on mycorrhiza-mediated plant water uptake, however, yields various context-specific or species-specific situations. For instance, in onion, resistance of AM plants to wilt under low moisture levels appears to be conferred through improved phosphorus nutrition (Nelson and Safir 1982). While in wheat AM fungi are believed to influence the host osmotic potential (Allen and Bosalis 1983), in *Bromus* and rose, some other mechanisms are expected to prevail (Bildusan et al. 1986).

Mycorrhizas can significantly contribute to the ability of plants to extract waters from soils with very low moisture content. Studies along changing soil moisture characteristic curves in mycorrhizal versus non-mycorrhizal soils have shown that nutrients become less and less available as soil

dries up because of the increasing tortuosity of the diffusion path (Augé 2001, 2004). Hence, the growth of non-mycorrhizal plants is likely to be more limited by nutrient availability under drought conditions, and reduced root growth would limit the accessibility of water. Under such circumstances, contribution of mycorrhizas to take up nutrients in mycorrhizal plants lends considerable competitive advantage to them. This has been demonstrated in case of sorghum by Neumann and George (2004) who showed that AM plants were much better able to access P from dry soil than non-mycorrhizal plants. They attributed these results to higher soil moisture as a result of hydraulic lift in which both hyphae and roots are involved in soil water redistribution along water potential gradients in the soil.

In his comprehensive review on AM-influenced plant water relations, Augé (2001) came across some contradictory results and admitted that little knowledge about variations in water relations on different plant–fungus combinations exists. He concluded that we should distinguish direct effects of fungal colonisation from indirect effects resulting from changes in plant size or P status. However, the work carried out after Augé’s review (e.g. Vivas et al. 2003) has added some important dimensions to our understanding of AM-mediated plant responses to water stress and to some extent can help explaining apparently contradictory results in earlier investigations. Notwithstanding this added information a lot still needs to be done in this complex subject to overcome many inconsistencies in the literature.

Mycorrhizas in Thermal and Cold Environments

Despite the ubiquity of arbuscular mycorrhizas (AM) and their likely importance in extreme environments, very little is known about the distribution of these symbiotic fungi in extreme environments, especially thermal environments. These environments not only promise to be the novel niches for novel isolates of AM fungi but also offer opportunities to explore the newer

roles that thermal resistant strains or species of mycorrhizas can play under such environments. The Yellowstone National Park (YNP) is one of the classical sites that offer a variety of thermal environments and where soils are characterised by extreme pH, elevated temperatures and toxic element concentrations. Bunn and Zabinski (2003) surveyed various thermal sites in YNP for the presence of AM and found that plants at five sites, growing in soils with rooting-zone temperatures up to 48 °C and soil pH values as low as 3.4, were mycorrhizal with colonisation levels varying from 4 to 34 %. They further found significant variations in rooting-zone temperature of soils from a sparsely vegetated thermal area and an adjacent, continuously vegetated transition area with mycorrhizal infectivity potential (MIP) about 77 % greater in later than former soils. Furthermore, mycorrhizal colonisation of some plant species, such as *Agrostis scabra*, *Dichanthelium lanuginosum* and *Mimulus guttatus*, was found to be consistently high throughout the growing season (from 48 to 72 %), implying that AM are possibly essential for plant life on the edge of thermal areas. Recently, Lekberg et al. (2011) investigated the community assembly of the AM fungi in geothermal and nonthermal grasslands in YNP using sequencing and RFLP of root extracted AMF ribosomal DNA. They unexpectedly found that the AMF community composition, except for generalist *Glomus intraradices*, correlated with soil pH or pH-driven changes in soil chemistry. They also carried out controlled greenhouse experiment to confirm the direct effect of the soil chemical environment on the distribution of two AMF morphospecies, *Paraglomus occultum* and *Scutellospora pellucida*. An interesting finding of this study was the isolation of several viable AMF taxa from bison faeces, thereby indicating bison as a possible vector for some AMF within YNP. In nutshell, the soil environment appeared to be the primary factor affecting community composition and distribution in YNP.

It is difficult to draw a clear picture of diversity and distribution of AM fungi in extreme thermal environments, and the role these fungi

can play in such environments from just a few studies available till date. There is obviously a need for undertaking more detailed studies on the extent and type of AM root colonisation in plants growing in different extreme environments and characterising the AM spore communities in rhizospheres of such plants. Furthermore, appropriately designed controlled studies need to be devised using different AM isolates as inoculum, individually and in combination, on single host and multiple hosts in isolation, and competition under a regime of soil temperatures can lend more precision to our understanding of the role that AM can play in extreme thermal environments.

Like in thermal environments, a reason of equal curiosity is extremely cold environments for the diversity and role of AM fungi. There have been only a few attempts to survey and assess mycorrhizal associations of plants in very cold environments, such as Antarctic and sub-Antarctic environments. Since Williams et al. (1994) detected the presence of mycorrhizas in the Antarctic liverwort, *Cephaloziella exoliflora*, some surveys have been conducted on sub-Antarctic islands. Laursen et al. (1997) surveyed the vascular plants on Macquarie Island for mycorrhizal status and found that out of the 40 taxa examined, the majority (28) were mycorrhizal. Of the examined taxa, only three had vesicles and arbuscules, 15 had vesicles only with 11 of these taxa also having dark septate fungi (DSFM) and 10 species had DSFM only. In Marion Island's flora, Smith and Newton (1986) recorded arbuscules from 23 of the 24 species examined, while at Iles Kerguelen 9 of 17 plant species surveyed had some form of mycorrhization (Strullu et al. 1999). In Kerguelen archipelago, *Glomus kerguelense*, a new glomalean species, was found associated with the rhizosphere of *Agrostis stolonifera*, an introduced Poaceae (Dalpé et al. 2002). Finding such a new species in these novel environments gives more strength to the expectations of the existence of more novel species in Arctic and Antarctic conditions. Frenot et al. (2005) examined nine of the 12 vascular plant species present on sub-Antarctic Heard Island for mycorrhizal

status and found all of them mycorrhizal, associated either with AM or dark septate fungi, with site-specific variations in the degree of mycorrhization.

Mycorrhizality is an important attribute of plants in extreme environments, and mycorrhizas can potentially improve the capacities of plants to colonise in extreme thermal or cold environments. In the context of climate change as the vegetation boundaries are shifting and community distribution is changing in ecologically sensitive areas like Arctic and Antarctic, the role of mycorrhizas becomes even more important. So, in an era of climate change, studies on predictive modelling of plant niche shifts aided or constrained by mycorrhizal support assume special importance to see if and how AM-plant interactions can change under environmental change.

Mycorrhizas in Saline Environments

Growing soil salinisation is a severe threat to crop yield in the contemporary world as the crop production is low in saline soil. Not only more than 7 % of the global land surface is covered with saline soils (Ruiz-Lozano et al. 1996), a sizeable fraction of the cultivated land (about 77 million ha or 5 %) is affected by excess salt content and resulting salt toxicity. Salt toxicity in plants leads to osmotic imbalances, decrease in plant water holding capacity, discrepancy of nutrient uptake and decline in photosynthetic ability (van Hoorn et al. 2001). Plants respond to salt stress through an array of adaptations at morphological, molecular, biochemical and physiological levels (Garg and Manchanda 2008). Several workers have shown that AM fungi can significantly reduce detrimental effects of salinity and improve the ability of plants to cope up with salt stress (Gupta and Krishnamurthy 1996; Ruiz-Lozano et al. 1996; Al-Karaki and Hammad 2001; Feng et al. 2002; Yano-melo et al. 2003; Rabie 2005; Jahromi et al. 2008). The inoculation of maize plants with *Glomus mosseae* by Sheng et al. (2008) under five levels of salt stress showed that mycorrhizal plants grow better than

non-mycorrhizal plants under saline conditions as reflected by higher shoot and root dry weights of mycorrhizal than non-mycorrhizal plants. Similar results have been reported by earlier studies on other plants, such as tomato (Al-Karaki and Hammad 2001), cotton (Feng et al. 2002) and barley (Mohammed et al. 2003). This improved salt tolerance in mycorrhizal plants could be an independent or synergistic function of AM fungi-mediated enhanced plant nutrient uptake (Canterall and Linderman 2001; Asghari et al. 2005), better ion balance (Zandavalli et al. 2004; Giri et al. 2007), well protection of enzyme activity (Rabie and Almadini 2005; Giri and Mukerji 2004) and facilitation of water uptake (Berta et al. 2002; Ruiz-Lozano and Azcon 1995). A comparative overview of the role of AM symbiosis in drought and salt stress resistance shows that AM-induced increased resilience of host plants to salinity stress occurs perhaps with greater consistency than host plant resilience to drought stress (Cho et al. 2006). Since developing salt stress-tolerant plants or identifying genes for salt tolerance and transferring them into intolerant or less tolerant crop plants are a mandate for various research institutions world over due to growing salinity, it is suggested that the role of mycorrhizas should not be underestimated in this direction. Understanding the molecular crosstalk between mycorrhizal symbionts and genetic controls of salt tolerance in host plants promises to yield useful insights into AM-induced salt resistance of plants. Moreover, using mycorrhiza-based biofertilisers in saline agricultural lands can potentially alleviate salt stress tolerance of plants.

Mycorrhizas and Environmental Toxicity

Mycorrhizal fungi have been for long thought to help host plants to adapt to toxic environmental conditions through various benefits (Mosse et al. 1981). A glance at the literature reveals that successful adaptation of plant life under toxic environmental conditions created by industrial effluents (Oliveira et al. 2001), heavy

metal toxicity (Chaudhry et al. 1999; Leyval et al. 1997), biocide treatment (Heggo et al. 1990), slurry application (Chistie and Kilpatrick 1992), sulphur dioxide fumigation (Clappert et al. 1990) and wildfire recovery (Puppi and Tartnlini 1991) involves the use of AM fungi (Barea et al. 1993). One of the main culprits of soil toxicity is higher levels of heavy metals. Notwithstanding that some of the metals, such as Zn, Cu, Fe, Mn, Ni, Mo and Co, are essentially required for plant growth, higher content and long-term persistence of some heavy metals (such as Cd, Pb) and metalloids (As), especially in surface horizon of agricultural soils, are a serious cause of concern (Keller et al. 2002; Voegelin et al. 2003; Kabata-Pendias and Mukherjee 2007). It has been found that certain metals at toxic levels become less toxic to plants in the presence of AM, while at non-toxic or optimal level, acquisition of such metals is enhanced by AM symbioses (Bethlenfalvai and Lindcann 1992; Sylvia and Williams 1992; Barea et al. 1993; Khan et al. 2000). In heavy metal-contaminated soils, AMF adapted to the high toxic metal concentrations can restore the biomass and vegetation of the sites through rhizo-remediation. AM fungi, having been reported to have evolved arsenate resistance, are also reported to confer enhanced resistance on *Holcus lanatus* (Gonzalez-Chavez et al. 2002a, b). Since many metals cause drastic changes in pH of the rhizospheric soils, AM have also been found to play a positive role in protecting plants from pH extremes (Sylvia and Williams 1992). Overall, mycorrhizostabilisation can potentially reduce the mobility, bioavailability or toxicity of the pollutant in the rhizosphere, mainly through glistering action of glomalin.

Metal tolerance of AM fungi has been assessed on the basis of several observational patterns of AM spore numbers and root colonisation in contaminated versus non-contaminated sites. But many questions related to the actual role of AM in metal tolerance are not answered through such observation-based procedures. Since AMF coexist with other microbial communities and plant roots that can also tolerate and accumulate metals, it is very hard to delimit the role of mycorrhizas due to such confounding

factors. It is also hard to work out through such methods the limitations and threshold values ensuring the survival and growth of AMF, or about the genetic basis for multimetal resistance and tolerance.

It is pertinent to mention that we still lack a mechanistic understanding of the fundamental interaction at the cellular and molecular level between AMF and metals (Martin et al. 2007). Nevertheless, some insights about the involvement of certain metal transporters and plant-encoded transporters in the tolerance and uptake of heavy metals from extracellular media (Göhre and Paszkowski 2006; Hildebrandt et al. 2007) or in their mobilisation from intracellular stores (Gaither and Eide 2001) have been obtained. In fact, more than one mechanism may underlie the ability of AM to tolerate or resist metal toxicity, including fungal gene expression, extracellular metal sequestration and precipitation and production of metallothioneins (Gadd 1993, 2005; Leyval and Joner 2001; Lux and Cumming 2001; Ouziad et al. 2005). It is postulated that metals could be released at the pre-arbuscular interface and then taken up by plant-encoded transporters (Göhre and Paszkowski 2006).

The information generated so far by various works on AM fungi in relation to metal stress suggests that certain AM strains are well adapted to metal toxicity (Weissenhorn et al. 1993; Del Val et al. 1999a; Leyval and Joner 2001; Toler et al. 2005; Sudova et al. 2007), and these fungi evolve rapidly to metal stress. Studies have shown that tolerant strains of some AM fungi can develop within a span of just 1 or 2 years (Tullio et al. 2003; Sudova et al. 2007). Gonzalez-Chavez et al. (2002a, b) reported that arbuscular mycorrhizal fungi have evolved arsenate resistance and conferred enhanced metal resistance on *Holcus lanatus*. It is important to note that high metal toxicity may whether decrease the numbers and vitality of AMF (Dixon et al. 1988; Dixon and Buschena 1988) or may have no effect on mycorrhizal colonisation (Wilkins 1991; Leyval et al. 1997). The experimental studies by Biró et al. (2005) on AM-*Hordeum vulgare* interaction under varying dosages of heavy metals found a strong dose dependency at the arbuscular richness in general.

They also found sporulation of the AMF as the most sensitive parameter to long-term metal (loid) stress. Their results showed that Al, As, Ba, Cd, Cr, Cu, Pb, Se, Sr and Zn reduced significantly the spore numbers of the AMF, while the Ni loadings (at 36 g/soil) increased mycorrhizal sporulation.

Extreme Environments: Novel Niches for Novel Mycorrhizas

The most celebrated examples of extreme environments on Earth include sea ice (extreme cold), hydrothermal vents (extreme heat and high metal content), sulphuric springs (extreme heat and highly acidic), salt lake (extreme salt concentrations) and soda lake (extreme salt concentration and highly alkaline). Studying life in extreme environments has always been exciting, yet mycorrhizas have largely escaped attention of researchers. There has been a significant neglect in understanding the costs and benefits of mycorrhizas in host plants existing in extreme environments. This is despite the likelihood of the existence of some novel strains or isolates in such environments and potentially novel role that they might play in facilitating plant life in extreme environments. A global coordinated effort is needed to explore, document and characterise the mycorrhizal diversity in extreme environments so as to identify novel applications of such 'extremophilic mycorrhizas' in the changing world. If explored well in different types of extreme environments, mycorrhizas can also be probably grouped into various categories the way the rest of microbes are classified into extremophiles, such as halophiles, thermophiles, alkaliphiles, acidophiles and psychrophiles. Since the likelihood of obtaining novel mycorrhizal species from extreme environments with unimaginably novel applications cannot, and should not, be ruled out, why not think on taking our next step in this direction.

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Introduction

In contrast to widespread occurrence of arbuscular mycorrhizas (AM) in terrestrial plants, aquatic plants are considered to be relatively less mycorrhizal. It is mainly because the benefits of mycorrhizal association to plants under aquatic conditions are expected to be relatively lesser. It is, however, hard to accept these generalised perceptions, partly because only a few studies have been hitherto carried out on mycorrhizal symbioses of aquatic plants, thereby leaving huge information gaps and paucity of quantitative data in this area of research. The cost–benefit analysis of mycorrhizal associations with aquatic plants has been relatively much less explored mainly because of more challenging methodological issues. The present chapter begins with a brief outline of aquatic habitats, followed by an overview of the studies conducted so far on mycorrhizal association of aquatic plant species with the main objective to identify key knowledge gaps in this area of research. Finally, the important questions that merit attention by future researchers to get better insights into mycorrhizal symbioses in aquatic habitats are highlighted, and a conceptual framework to address these questions is also provided (Fig. 8.1).

Why Study Mycorrhizas in Aquatic Habitats

Aquatic systems on a global scale are by far more diverse and extensive than terrestrial habitats.

Freshwater ecosystems support almost 6 % of the described species (Hawksworth and Kalin-Arroyo 1995), despite comprising only a tiny fraction of the world’s water (0.01 %) and the Earth’s (0.8 %) surface (Gleick 1996). It is important to note that aquatic habitats are highly diverse, especially in plant species richness. Plants in aquatic systems provide a wide variety of valuable economic goods and contribute to irreplaceable ecosystem services provided by these ecosystems. The importance of aquatic biodiversity and threats to this valuable diversity by problems, such as overexploitation, pollution, water flow changes, habitat degradation and invasion by exotic species, made the United Nation to declare 2005–2015 as the International Decade for Action ‘*Water for Life*’ (Dudgeon et al. 2006).

The diversity of plant species in aquatic ecosystems is amazing. The aquatic plants are characterised by a variety of life forms and functional groups, such as emergents, rooted- and floating-leaf types and submersed types that adapt to diverse habitat conditions, depending upon the level, quality and availability of water during the growing season. In view of the relationship of these life forms to the ecological milieu in and around aquatic habitats, the role of mycorrhizas is expected to be variable. Theoretically, mycorrhizas are supposed to have a stronger role in emergents and rooted aquatic plants than in free floating types. However, a better idea of the importance of mycorrhizas in aquatic plants can be obtained through quantitative estimation of the magnitude of variation in mycorrhizal association

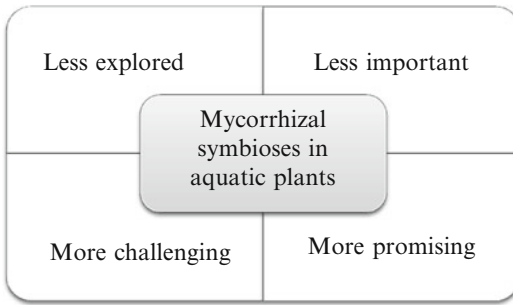


Fig. 8.1 Paradoxical dimensions of mycorrhizal symbioses in relation to aquatic plants. In comparison to terrestrial habitats, mycorrhizality is considered to be less important in aquatic habitats, hence less explored. Though it is relatively more challenging to study mycorrhizas in aquatic plants, it could be more promising in terms of potential new insights into this symbiosis

of different types of aquatic plants along a gradient of inundation and trophic status. A range of interesting ecological questions can be asked along these lines to get useful insights into the role of mycorrhizal mutualists in aquatic habitats.

Mycorrhizal Status of Aquatic Plants

Until 1970s it was claimed that hydrophytes are not infected by arbuscular mycorrhizas (AM) (Harley 1969; Khan 1974). But, since the first observation of AM in aquatic plants made by Søndergaard and Laegaard (1977), many other studies have recorded AM association in freshwater macrophytes. These studies mainly include Bagyaraj et al. (1979), Chaubal et al. (1982), Clayton and Bagyaraj (1984), Farmer (1985), Khan and Belik (1995) and Beck-Nielsen and Madsen (2001). Very earlier Mejsstrik (1965, 1976) reported mycorrhizal association with some common emergent plants growing in peat bogs in Czechoslovakia and related it to the water-level fluctuations, tending to develop only when the water table dropped belowground level. Manjunath et al. (1981) observed AM association with rice under semiaquatic conditions with an increasing tendency under non-flooded conditions. Very low levels of AM colonisation were found in some emergent plants from a eutrophic marsh by Read et al. (1976).

Chaubal et al. (1982) recorded AM colonisation in five aquatic species and related infections to temporary drying following low water levels and oligotrophic conditions. While Bagyaraj et al. (1979) studied the occurrence of AM fungi in some tropical aquatic plants in India, Clayton and Bagyaraj (1984) documented the association of AM fungi in 22 submerged water-plant species in New Zealand with records down to 6.0 m depth of water in one lake and at 2–3 m depth in two other lakes. The authors reported a general decline in colonisation levels with increasing water depth and unexpectedly found no relationship between mycorrhizal intensity and the trophic status of the lakes sampled. High levels of AM colonisation were often found in low-growing, shallow-rooted water plants with noticeably sparse root hair growth, in contrast to no or very low levels of colonisation in tall-growing, deeper-rooted plants with abundant root hairs. It is believed that high absorptive shoot surfaces for tall plants make them less dependent on roots for nutrients, and aerobic substrates exist in shallow water sandy substrates of the wave wash zone in lakes which could cause soluble phosphorus levels to be low in the rhizosphere of plants growing in this zone. Khan (1993) and Khan and Belik (1995) evaluated the occurrence and ecological importance of mycorrhizas in aquatic plants, especially in aquatic trees of New South Wales, Australia. Concurrently, Hussain et al. (1995) reported higher incidence of AM fungi in hydrophytes growing in and around Rawalpindi and Islamabad regions of Pakistan and related it to the availability of dissolved oxygen, effect of running water or radial release from aerenchymatous tissues of the hydrophytes. More recently, Bohrer et al. (2004) studied seasonal dynamics of AM fungi in differing wetland habitats. Recently, Radhika and Rodrigues (2007) screened a bunch of aquatic plant species for mycorrhizal association and found AM fungal root colonisation in 10 out of 14 hydrophyte species screened and five out of six marshy species screened. Their results revealed vesicular colonisation in 12 plant species and arbuscular colonisation restricted to only three plant species.

A rooted submerged pteridophyte, *Isoetes coromandelina*, was also found to be mycorrhizal showing vesicular colonisation. In this study, the AM associates were found to be dominated by *Glomus* followed by *Scutellospora*, with *Glomus claroideum* recovered from 14 plant species.

So it seems from the aforementioned review of available literature that mycorrhizal association in aquatic plants is not uncommon, as generally perceived. Whether mycorrhizas accrue similar benefits to aquatic plants as they provide for terrestrial plants still remains, however, to be fully understood.

The Role of Mycorrhizas in Aquatic Plants

The aquatic plants are considered to be relatively less mycorrhizal, as stated above, and the benefits of mycorrhizal association to plants under aquatic conditions are also expected to be relatively lesser. These sweeping generalisations are, however, less supported by the quantitative data-based studies. In fact, the lack of sufficient studies hampers a complete understanding of the role of mycorrhizas in aquatic plants, though some useful insights have been obliquely obtained through a handful of studies carried out in this direction. For instance, under experimental conditions, Reid and Bowen (1979) reported a decrease in AM colonisation with decreasing soil moisture availability. It has been found that mycorrhizal

association can potentially benefit aquatic plants under the conditions of low nutrient availability, especially low plant tissue P concentrations (Chaubal et al. 1982; Christensen and Wigand 1998; Wigand et al. 1998) and dissolved oxygen deficiency (Tanner and Clayton 1985; Beck-Nielsen and Madsen 2001). As the aquatic system transforms from mesotrophic to eutrophic status, the role of mycorrhizas supposedly becomes less important. This is evidenced indirectly by a high degree of positive correlation between AM colonisation and low nutrient concentrations in the sediments (Christensen and Wigand 1998).

The role of mycorrhizas in aquatic plants (Fig. 8.2) can be more appropriately assessed through some controlled experiments manipulating moisture regimes. Keeley (1980) was the first to provide experimental confirmation of tolerance of submersion by AM fungi using blackgum seedlings (*Nyssa sylvatica*, Marsh). This was further confirmed by Crush and Hay (1981) by growing mycorrhizal clover (*Trifolium repens* L.) in an aerated nutrient solution, who found that mycorrhizas failed to establish with normal nutrient solutions and only grew when the concentration of phosphorus was equivalent to that of phosphorus-deficient soils. Keeley (1980) also found that the waterlogged roots remained infected for 1 year, with highest infection near main roots and decreasing outwards. He attributed this to limited oxygen transport to distal roots under flooded conditions. Anderson et al. (1986) examined mycorrhizal colonisation

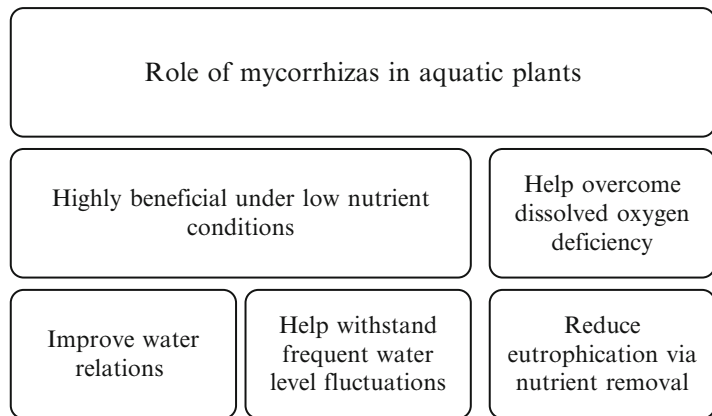


Fig. 8.2 Depiction of the possible roles of mycorrhizas in aquatic plants

in prairie cordgrass (*Spartina pectinata* Lind.) under varied soil moisture conditions ranging from continuous saturation to allowing the soil to dry to -15 bars. The results of their experiments indicated that the plant host and AM colonisers may act independently of one another with regard to moisture treatments. On the other hand, Nelson and Safir (1982) demonstrated that drought-stressed mycorrhizal plants had higher fresh and dry weights than drought-stressed non-mycorrhizal plants. It is pertinent to mention that mycorrhizas can potentially improve water relations by lowering resistance to water transport in host plants (Safir et al. 1971; Hardie and Leyton 1981; Allen 1982).

Future Directions

Whether or not mycorrhizal symbioses hold any primary significance in aquatic plants is still an open question. The area offers unique opportunities for researchers to ask interesting and yet unanswered questions. For instance, some of the pertinent questions that could be addressed in the field of aquatic mycorrhizology may include:

- (a) How does the extent of mycorrhizal colonisation of aquatic plants vary along a moisture gradient?
- (b) How mycorrhizal symbioses is expected to behave with aquatic plants in the scenario of global climate change.
- (c) What help, if any, can mycorrhizas extend in the restoration of the threatened and rare aquatic plant species in human-disturbed aquatic habitats?
- (d) Do mycorrhizas have any relationship with burgeoning invasion of aquatic habitats by exotic macrophytes?
- (e) How are the trophic status and water-level fluctuations of aquatic ecosystems related to mycorrhizality of aquatic plants?

Understanding variation in the extent of mycorrhizal colonisation of aquatic plants along a moisture gradient will provide useful clues into whether or not diversity and distribution pattern

of plant communities in surrounding land areas has some relationship with mycorrhizal association of aquatic habitats. Since freshwater ecosystems are expected to undergo many changes due to global climate change, the role of mycorrhizal symbioses in aquatic plant dynamics in the context of such global change would indeed be an interesting discourse. Theoretically, mycorrhizas are expected to lend considerable support for some aquatic plant species to withstand water level below their critical requirement. The climate change is also expected to exacerbate the process of invasion by exotic species in aquatic systems. Evaluating the role of mycorrhizas in invasiveness and range expansion of aquatic plants, not only in aquatic but also in semiaquatic and terrestrial habitats, merits special attention. In fact, distinguishing between the exotic invasive plant species and exotic non-invasive aquatic species for their mycorrhizal status could provide some useful insights into the likely role of mycorrhizas in invasiveness of aquatic plant species. To explore the extent and type of mycorrhizal colonisation in aquatic plants growing in water bodies of differing trophic status merits more detailed investigations, though conventional wisdom says that mycorrhizas could be more beneficial under oligotrophic conditions. Use of mycorrhizal inoculants is an important part of the restoration projects aimed at re-establishing desired plants at disturbed sites in terrestrial system (see Chap. 6). Whether mycorrhizal associations can be of some help in the restoration of the threatened and rare aquatic plant species in anthropogenically disturbed aquatic habitats merits further investigations.

Freshwaters: A Global Focus

Freshwater ecosystems support almost 6 % of the described species despite comprising only a tiny fraction of the world's water (0.01 %) and the Earth's (0.8 %) surface. Though freshwater biodiversity provides a wide variety of valuable economic goods and irreplaceable ecosystem services for humanity, it is increasingly threatened by

(continued)

(continued)

overexploitation, pollution, water flow changes, habitat degradation and invasion by alien species. Of these threats, spread of invasive species appears most severe and causes considerable damage with cascading effects on structural organisation and functional integrity of freshwater ecosystems. There is relatively more decline and extinction of species in freshwater ecosystems than in terrestrial or marine environments, mainly due to hydrologic alterations and biological invasions. It is for these reasons that freshwater biodiversity comprises a priority conservation concern during the United Nation's International Decade for Action 'Water for Life' and UN General Assembly declared 2013 as the United Nations International Year of Water Cooperation and dedicated the 22nd of March 2013 as World Water Day for water cooperation. In this context, understanding the role of mycorrhizas in growth and fitness of aquatic plants, especially their rarity or invasiveness, assumes pivotal importance. Whether or not mycorrhizas could be a part of the solution towards conservation and management of aquatic plant resources promises to make sense if it is proved beyond doubt that these mutualists have some role in growth and establishment of these plants. Besides, whether freshwater systems act as real habitats for mycorrhizas, or just arbitrary and transient places where they are washed off from nearby terrestrial ecosystems, needs special attention to ascertain if mycorrhizas are a part of the aquatic biodiversity.

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Introduction

For a researcher convinced to undertake any of the challenging discourses in mycorrhizal research in the context of global change, it is imperative to have a basic and mechanistic understanding of different approaches and methods to be followed. A number of procedures can be followed for mycorrhizal studies, depending upon the objective of the researcher. This chapter deals with an array of approaches that can be used for detection of mycorrhizas in living roots through various staining techniques and extraction, identification and characterisation of spores from rhizospheric soils (Fig. 9.1). In fact, an overview of both the classical methods and modern approaches to analyse mycorrhizas in plants under natural environments is provided. The objective is to provide the readers some basic methodological insights to deal with new challenging discourses on mycorrhizas in the light of various global change elements. The limitations of morphological approaches and specific advantages of molecular methods to overcome such limitations in identification and characterisation of AM fungi are briefly outlined. Since our understanding of the plant–AM interactions and factors that drive these associations is still hampered by many methodological limitations and inability to culture these obligate host-associated mutualists, the implications of overcoming such limitations are also briefly discussed. It is important to note that though there are a number of books, practical

guides and manuals available on methodology of mycorrhizal studies, the aim of giving an overview here is to facilitate readers with handy basic information without troubling them initially to look for the alternative sources.

Methods for the Detection of AM Fungi in Plant Roots

Assessment of the type and extent of mycorrhizal associations in plant roots through appropriate techniques is fundamental to mycorrhizal research. A number of staining techniques can be used to detect and quantify AMF in plant roots. An excellent review of methods for the detection and observation of arbuscular mycorrhizal fungi in roots has been provided by Vierheilig et al. (2005). Since Phillips and Hayman (1970) described an easy but classical and standard method to stain AM fungi (AMF) in roots, a number of other methods to visualise AMF in roots have been developed. The review by Vierheilig et al. (2005) can be helpful in the choice of appropriate method(s) to visualise AMF in roots for specific experimental set-ups. In addition to light microscopy-based methods, alternative biochemical methods are also used to measure AM intensity (Bothe et al. 1994; Frey et al. 1992; Schmitz et al. 1991). However, recently developed molecular approaches (Alkan et al. 2004; Sanders 2002) are more reliable for quantitative and qualitative studies on mycorrhizas.

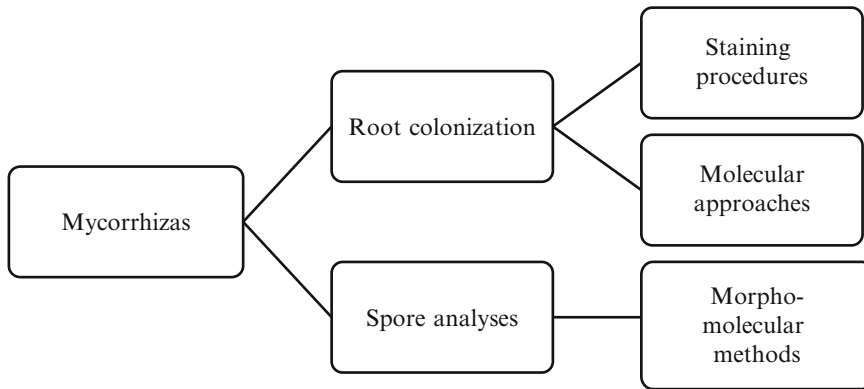


Fig. 9.1 Different approaches for the assessment of mycorrhizal association with host plants

Staining of the roots using appropriate stains and the counting of the stained fungal structures such as arbuscules, vesicles and hyphae in the root by routine light microscopy procedure still remains the standard technique for the quantification of root colonisation by AMF. This is because staining provides important clues about the intensity of root colonisation through quantifiable visualisation of the key mycorrhizal features, especially arbuscules (Brundrett 2004). It is, however, important to select finer and more fibrous roots at the time of sampling for detection of mycorrhizal colonisation. More stiff, older and darkly pigmented roots should be avoided because they harbour relatively lesser, or often times no, mycorrhizal colonisation and need additional bleaching and incubation steps while processing through staining procedure. The various stains used in mycorrhizal studies have subtly different properties and as such vary in the quality of colouration produced. For instance, aniline blue and chlorazol black E stain all fungal tissue very darkly and intensely, while as the quality of colouration by acid fuchsin varies according to root tissue and fungal species. This sometimes helps to distinguish between different types of AM fungal mutualists, though their taxonomy is more based on spore morphology. The techniques such as fluorescence microscopy can considerably increase the effectiveness of such

staining-based distinctions (Merryweather and Fitter 1991).

After collection of roots from the field, they should be thoroughly washed to remove all rhizospheric particulates, cut into small fragments and cleared using hot 10 % KOH to remove cytoplasmic contents from cells in a water bath at 90 °C. Clearing leaves root structure and fungal elements intact. Darkly pigmented roots may need to be bleached with hydrogen peroxide. Cleared roots are acidified by immersion in 1 % HCl before staining with trypan blue or acid fuchsin for about 25–30 min in a water bath at 90 °C. Then the stain needs to be drained followed by destaining overnight using 50 % lactic acid to remove colouration from empty root cells. For examination of AM fungal colonisation and various fungal structures, destained roots should be mounted on a microscope slide beneath cover slip. Further details of this hot staining procedure can be found in Kormanik and McGraw (1982) and Brundrett et al. (1994).

It is important to note that there is an alternative of cold staining as well (see Grace and Stribley 1991; Koske and Gemma 1989; Walker and Vestberg 1994 for details) which takes relatively longer to finish but requires less attention. Staining mycorrhizal roots with ink (hot or cold) remains, however, the safest alternative, the details of which can be found in Vierheilig et al. (1998) (Fig. 9.2).

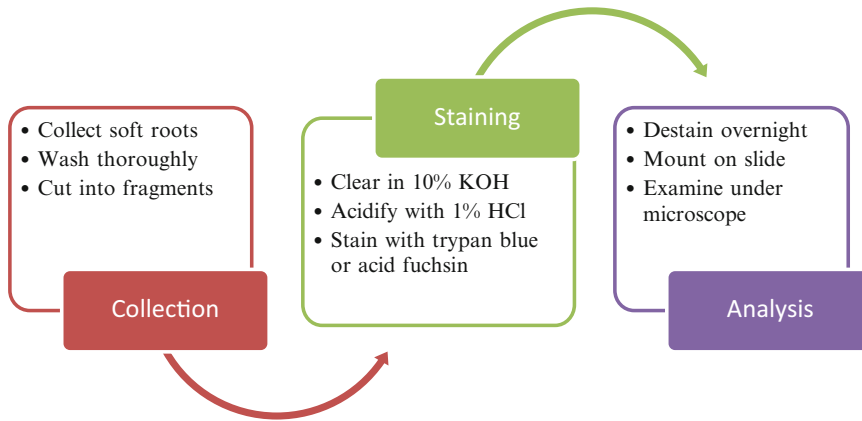


Fig. 9.2 Scheme of steps in the processing of plant roots for staining for the examination of mycorrhizal colonisation

Quantification of Intraradical Colonisation by AM Fungi

After clearing and staining roots, the type and extent of root length colonisation can be estimated by different procedures. One of the simple methods for the assessment of root length colonisation was proposed by Biermann and Linderman (1983), which is also called as frequency distribution method. In this method, the colonisation is assessed as proportion of root length colonised by AM fungi using a compound microscope.

Out of the mass of stained roots, a randomly selected aliquot of root segments of 1 cm length suspended in lactoglycerol is spread in a petri dish. The ocular micrometre is calibrated with the stage micrometre by placing it on the eyepiece of the microscope. Five to ten root pieces are mounted on the glass slide, and the ocular micrometre is calibrated with the stage micrometre at the particular x of microscope to observe the roots. The proportion of the length of each root segment consisting of vesicles, arbuscules or hyphae is estimated to the nearest 10 %. The percentage of root length colonised in the sample is calculated from frequency distribution.

The alternative method, known as the magnified intersection method proposed by McGonigle et al. (1990), provides relatively more reliable quantitative estimate of arbuscular mycorrhizas. In this method the slide is placed on the stage of a

compound microscope equipped with a cross-line eyepiece graticule, scanned methodically while aligning one axis of the graticule with the long axis of each root encountered. For assessing the percentage of root length colonised, presence or absence of AM fungi is scored touched by graticule axis which crosses the root each time a root is encountered. A reasonable estimate of % RLC can be obtained from 25 intersections, though accuracy is achieved only if more counts (100) are recorded. The percentage root length colonised is recorded as follows:

$$\%RLC = \frac{\text{Number of intersections with AM hyphae}}{\text{Total number of intersections counted}} \times 100$$

The estimates can be refined by counting intersections ‘hits’ on hyphae, entry points, arbuscules, vesicles or other structures considered individually.

Culture of Mycorrhizas

Being obligate symbionts, AM fungi cannot be cultured without a living host plant. The AM cultures can be produced if inocula in the form of soil hyphae, colonised roots and/or spores are presented to the suitable host plants. The pure and most desirable culture, however, contains a single fungal taxon raised from a single spore.

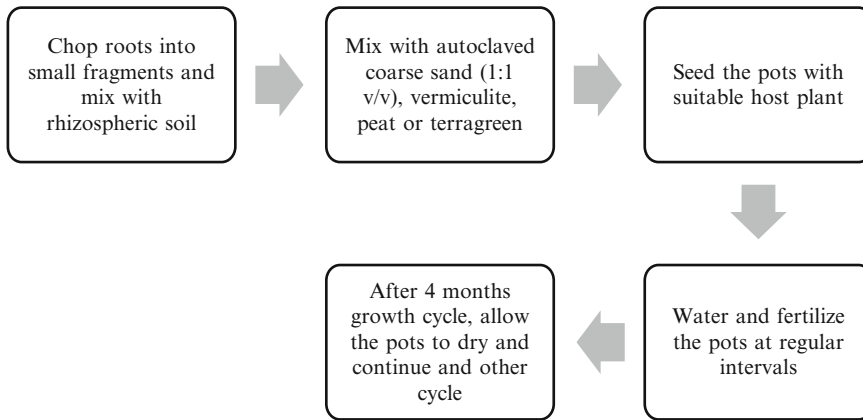


Fig. 9.3 Steps involved in raising of trap cultures of arbuscular mycorrhizas

These pure cultures are often termed as ‘isolates’ which can be given an identification code or even a conventional scientific name after following the appropriate taxonomic procedure. The field-gathered spores, which could be damaged while extraction, do not yield good results for viable single spore isolation for culture. Hence, an alternative and widely used method of obtaining viable AM isolates is ‘trap’ culture.

In trap culture a suitable host plant species, such as *Allium sativa*, is selected on the basis of some ideal characteristics including production of not much vegetation and formation of mycorrhizal association with a wide range of glomalean species (see Morton 1995 for details). The trap culture is generally used for the purpose of trapping as many AM fungal organisms or taxa as possible that are indigenous to field soil, establishing each organism of a species as unique culture and increasing inoculum of each organism for wider use and distribution. Mixed species may be trapped from living plant roots, excised mycorrhizal roots and mycelial fragments, roots and spores, etc. When roots are well colonised, a small number of spores may be extracted for subculturing.

For raising trap cultures (Fig. 9.3), roots are chopped into small fragments and mixed with the rhizospheric soil. The blend/inoculum is then mixed in pots with autoclaved coarse sand (1:1 v/v) or vermiculite, peat or terragreen.

The pots are seeded with a variety of host plants, such as *Allium cepa*, *Sorghum vulgare*, *Medicago sativa*, etc., and allowed to grow in a greenhouse at 20–25 °C with 60 % relative humidity. Pots need to be watered at regular intervals and fertilised with half-strength Hoagland’s solution. Usually after 4 months of growth cycle, the pots are left to drying slowly, after which the dried shoots are cut at the ground level to start the next growth cycle in the same aforementioned manner.

For raising the monosporal cultures, the micropipette tip is filled with the substrate (terragreen and sand used in the ratio of 1:1), and few seeds of a suitable host plant are placed over the substrate. Subsequently, a carefully chosen healthy AM spore is placed over the host seeds to ensure colonisation of the germinated spore with the host. The cultures are kept in the tray containing water to ensure continuous moisture available for seeds and seedlings in the micropipette tips and incubated in a growth chamber. As the seedlings emerge out of the tip, the tips are taken out of the water-containing tray for 1 day for drying. This cycle of drying is repeated three times in order to stimulate vigorous root production. Subsequently, roots are chopped off from the tip region, and the seedlings are transferred to larger pots. More seeds can be placed in these pots and regular watering needs to be done to allow the plants to complete their life cycle, after which the above-ground parts of the plant are chopped off

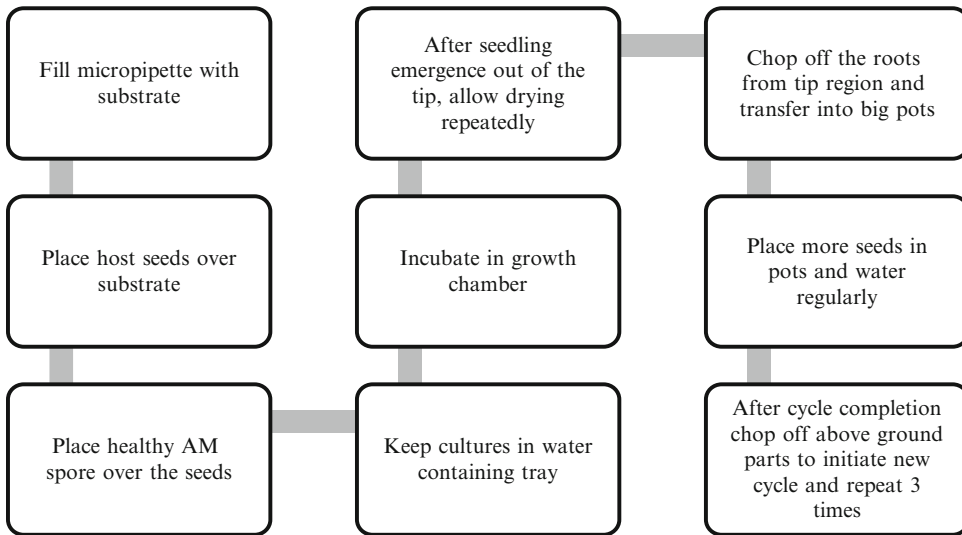


Fig. 9.4 Steps involved in raising of monosporal cultures of arbuscular mycorrhizas

to initiate new cycle of different host plant. This culture cycle needs to be repeated three times (Fig. 9.4).

Extraction of Glomalean Spores from Soil

Routine extraction of spores of arbuscular mycorrhizal fungi from soil was made possible by wet-sieving and decanting method (Gerdemann 1955; Gerdemann and Nicolson 1963). Glomalean spores are multinucleate and typically larger in size, ranging from about 20 to 500 μm (or 50–800 μm according to some estimates) in diameter. In natural soils a large proportion of spores are mixed with high organic matter content and other soil debris. Therefore, extraction of spores from a hue of debris and dirt requires specific methods of wet-sieving, decanting, floatation and separation. The sucrose floatation method is considered as one of the best methods wherein about 100 g of fresh rhizospheric soil collected from fields is suspended in water, shaken vigorously and passed through a series of sieves of varying mesh size (ranging from 32 to 710 μm), followed by passing the solid matter to 50 ml centrifuge tubes for centrifugation at 1,800 rpm for about 5 min. The supernatant containing floating

organic matter and dead spores is discarded, and the pellet is resuspended in 60 % sucrose solution and centrifuged at 1,800 rpm and stopped by applying brakes. The supernatant is rapidly sieved and washed thoroughly before transferring into gridded petri dishes for counting and further examination under the microscope. Though a relatively rapid spore extraction method which skips the sucrose floatation and centrifugation can be used, the quality of purification of spores is compromised to a large extent.

The spores extracted can be preserved by freeze-drying or freezing with liquid nitrogen and storing at $-80\text{ }^{\circ}\text{C}$. The other solutions, such as 5 % formaldehyde, 3.5 % glyceraldehyde and 0.025 % sodium azide, are also very effective in spore preservation. Though lactophenol and FAA were used in the past, they are known to cause unacceptable damage to AM spores (Fig. 9.5).

Molecular Methods

There are a number of issues related to morphological diagnosis of arbuscular mycorrhizal fungi, and these can be resolved only through molecular approaches. In fact, the use of various molecular tools and techniques during the last more than a decade significantly improved

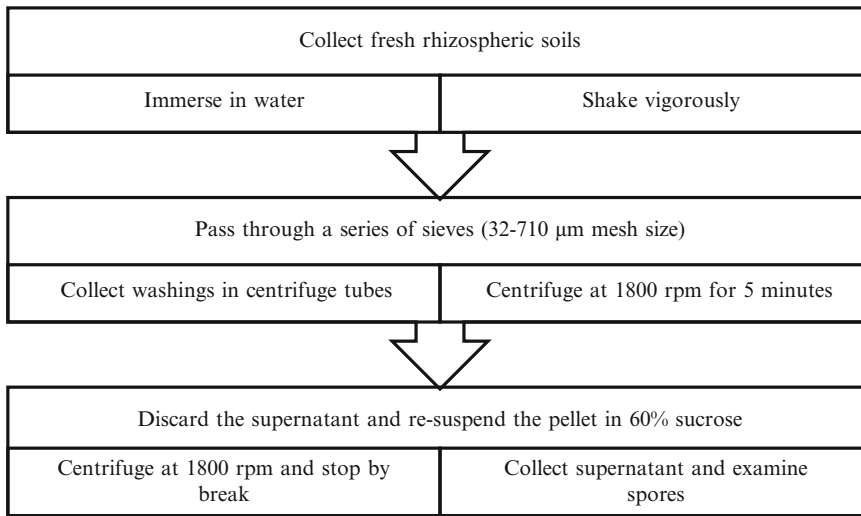


Fig. 9.5 Schematic representation of the steps involved in extraction of glomeralean spores from rhizospheric soils

the understanding of taxonomy, evolution and phylogenetic affiliations of AMF. In 2001 Schußler et al. used molecular data to establish the relationships among AMF and between AMF and other fungi and elevated this group to the level of phylum (Glomeromycota), which was shown to be as distinct from other fungi as the Ascomycota are from the Basidiomycota. Here we discuss some of the major steps involved in routine molecular analysis of AMF.

Extraction of DNA from AM Spores and Colonised Roots

DNA from AMF spores can be extracted either by using manual CTAB-based procedure or through various kits supplied by standard manufacturers following their protocol. I, together with Marie-Eve, standardised the Chelex method as described by Simon (1996) with some modifications and found it easier. Spores representing the whole range of morphological diversity in the target rhizospheric soils, after extraction through aforementioned wet-sieving–decanting procedure, need to be placed in the Eppendorf tube with 50 µL of distilled water and crushed with blue pestles before and after adding 50 µL of 20 %

Chelex 100. Then the samples are to be incubated at 85 °C for 3 min followed by a short vortex and incubation in ice for 5 min. This heating–cooling cycle should be repeated twice, and subsequently the samples are to be incubated at 60 °C for 90 min followed by centrifugation at 13,000 rpm for 5 min. The supernatant be collected and immediately processed for PCR amplification. The samples can be stored at –80 °C until used in subsequent molecular analysis. The alternative methods with details for DNA extraction, amplification and cloning from single AM fungal spores can be found in Schwarzott et al. (2001).

So far as the extraction of DNA from AM-colonised roots is concerned, depending on root thickness, 0.5–1 cm colonised root pieces need to be crushed in 40 µl TE buffer in a PCR tube of appropriate capacity (see, for instance, Van Tuinen et al. 1998) with the help of a flamed glass pestle. Crushed root samples should then be kept on ice followed by addition of about 10 µl 20 % Chelex 100. The samples are then denatured for about 10 min at 95 °C (Di Bonito et al. 1995; Van Tuinen et al. 1998). Samples are then centrifuged for about 5 min at 12,000 g and the supernatant is recovered, generally followed by dilution for use in the amplification by PCR.

DNA Amplification

The precise protocol for amplification and the choice of primers for amplification may vary from lab to lab or person to person, depending upon the target gene and purpose of research. According to my personal experience, a portion of the SSU rRNA gene from the total AMF DNA extracted may be amplified using the universal eukaryotic primer NS31 (Simon et al. 1992) and the AMF-specific primer AM1 (Helgason et al. 1998). The master mix composition may vary, depending upon the choice and purpose of the researcher, though I have used for each reaction a master mix composed of Feldan Bio kit (Feldan Bio, Canada) with 1 unit of taq DNA polymerase, 0.5 μ M of each primer, 0.2 mM of dNTP and 1 μ l of total AM DNA. The PCR reaction be performed in a thermal cycler. I have used MJ Research PTC-225 Peltier Thermal Cycler as follows: (1) initial denaturation step (4 min at 94 °C), (2) 35 cycles of denaturation (50 s at 94 °C) followed by annealing (1 min at 59 °C) and elongation (2 min at 72 °C) and (3) final elongation step (10 min at 72 °C). The PCR products should usually be purified using easily available kits, such as QIAquick PCR Purification kit (QIAGEN, Canada) following the manufacturer's protocol. The purified PCR products can be quantified in 1 % agarose gel against the mass ladder.

Cloning and Restriction Digestion

Although after PCR amplification one can proceed directly for sequencing and avoid restriction digestion step, the RFLP pattern may give a first-hand idea about the diversity profile. If the objective is obtaining diversity patterns of AM fungal communities, about 75 ng of purified PCR products should be cloned using, for instance, the pGEM®-T Easy Vector Systems (Promega, Corporation, USA) according to the manufacturer's instructions. For each sample, sufficient number (usually 100 or above) of

cloned products be randomly selected, and then PCR be performed for each of the cloned products using the M13 universal primer set. PCR amplification for transformant DNA should be performed, as above, with appropriate modification of about 30 cycles, with 1 step consisting of denaturation (50 s at 94 °C), annealing (50 s at 55 °C) and elongation (1 min at 72 °C). The PCR products are digested independently with a number of restriction enzymes according to the manufacturer's instructions. The pattern of restriction fragment length polymorphism (RFLP) can be observed by running 2 % agarose plus 1 % Synergel. After analysing the restriction fragment length polymorphism (RFLP) pattern manually or mechanically (with Genetools), the clones with similar pattern in RFLP type or restriction groups be grouped together. Then representative clones of each RFLP type (RGs) are sequenced. As mentioned earlier, one can, however, avoid the restriction digestion step and directly proceed for sequencing step after DNA extraction and amplification step (Fig. 9.6).

Grouping and Phylogenetic Analysis

DNA sequences can be edited with BioEdit (latest version <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>; Hall 1999). The BLASTn algorithm (<http://www.ncbi.nlm.nih.gov/BLAST/>) can be used to query GenBank (NCBI) for highly similar sequences. The sequences not corresponding to the mycorrhizal species should be discarded, and the sequences retained can be used to precisely analyse the restriction grouping. Many restriction groups can yield the same sequence, so they need to be grouped together to give a new grouping pattern, the sequence groups. Sequences can be used for phylogenetic analyses using appropriate programmes followed by bootstrap analysis. The sequences should be deposited in the GenBank vide proper voucher numbers before processing the data for publication. A flow chart of the aforementioned steps is given in Fig. 9.7.

Fig. 9.6 RFLP pattern of arbuscular mycorrhizal communities associated with the rhizosphere of the Canadian horseweed, *Conyza canadensis*

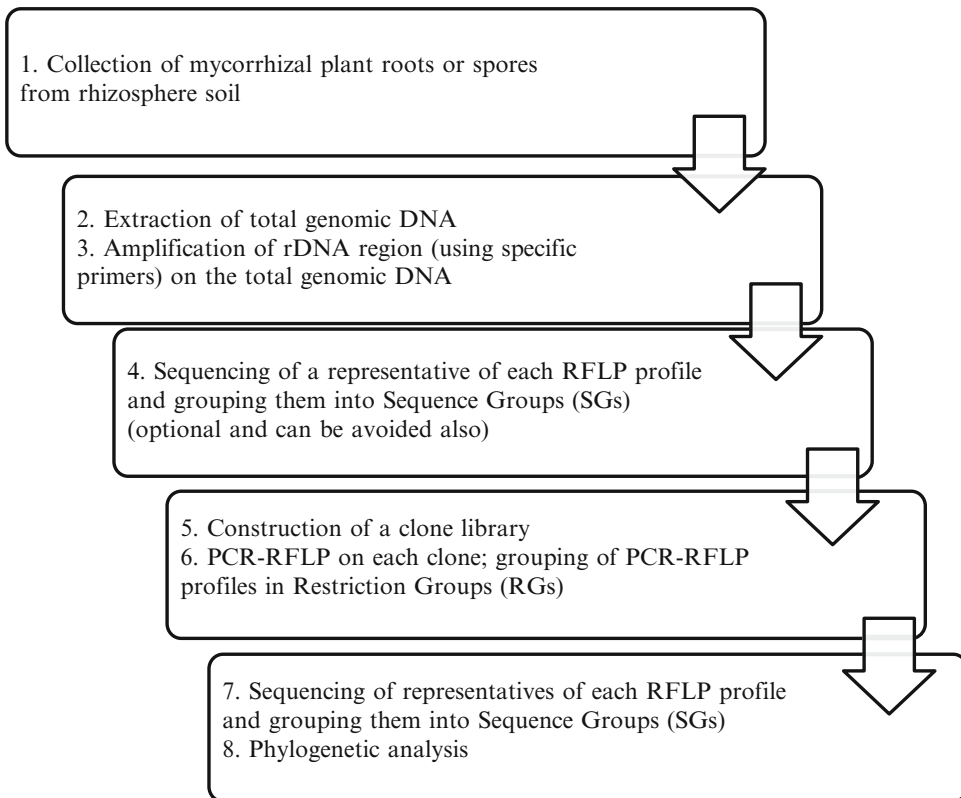
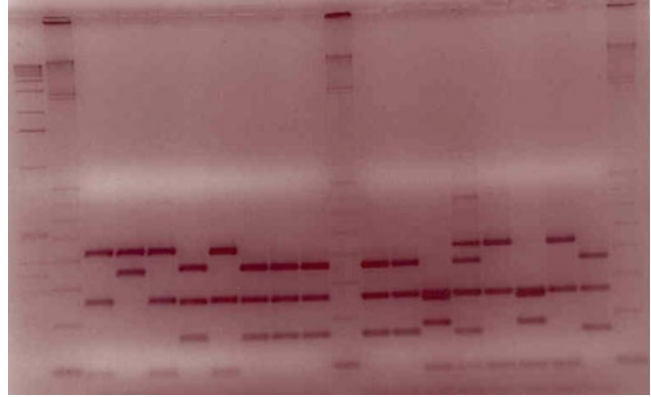


Fig. 9.7 Sequence of steps involved in molecular analysis of arbuscular mycorrhizal communities

Glomalin Extraction

The AM fungi produce a characteristic carbon-containing gluing protein, known as Glomalin, which upon deposition on soil particles increases the aggregate stability and improves soil structure. It is important to note that the aggregate stability

and soil structure are pivotal ecological attributes and important prerequisite for better crop production. The details of the procedures to be followed for Glomalin extraction can be found in Wright et al. (1996) and Wright and Upadhyaya (1996, 1998). For the removal of Glomalin from field soil, roots, mesh strips or bags or pot culture media, either the total protein or easily extractable protein

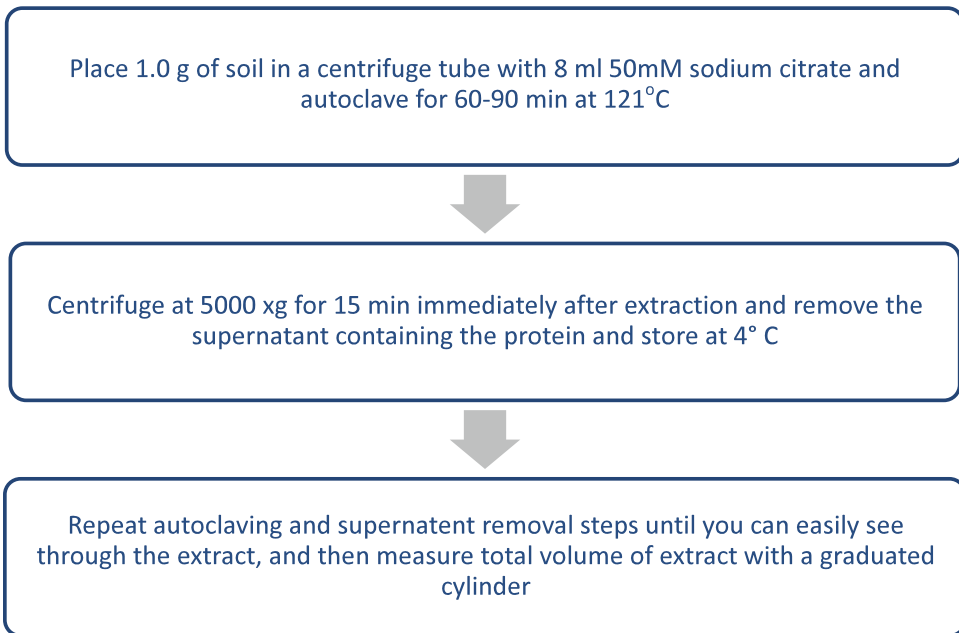


Fig. 9.8 Procedure for total glomalin protein extraction

procedure may be used, depending on the objective. The former extraction procedure gives a total protein concentration, which may have reduced immunoreactivity, while the later extraction procedure yields the most immunoreactive fraction but is not necessarily a measure of the total protein concentration in the sample (Fig. 9.8).

Nuts and Bolts for Handling AMF

Various modern tools, in concert with some classical and/or recent taxonomic keys, can be effectively used, in isolation or combination, to overcome the identification problems and limitation in the knowledge of arbuscular mycorrhizal biodiversity in a commonly usable format. For instance, an Expert System, i.e. a sophisticated computer program that manipulates knowledge to solve problems efficiently in a narrow problem area, linked to the European Bank of Glomales, BEG (La Banque Européenne des Glomales), is available on a CD-ROM to provide a multimedia identification system for AM fungi. This multimedia can be used together with the detailed taxonomic descriptions provided by the INVAM

for correct identification of AM fungi. In fact, the Expert System was put together using Linnaeus II software (Expert Centre for Taxonomic Identification – ETI, University of Amsterdam) for biodiversity documentation and species identification, and the CD-ROM contains approximately 120 images to be used for reference for identification. Besides, MYCOLIT, a bibliography of the mycorrhizal literature, is one of the most complete compiled source containing almost 12,000 references with publications spanning over a long time period. The need of the hour is not only to enrich databases such as MYCOLIT but also to apply different classical theoretical ecological perspectives to AM fungi, in addition to exploring new ways to combine different tools and techniques to better understand and describe AM fungal taxonomic and functional diversity. Since there are yet many breakthroughs awaiting in the field of mycorrhizology and small innovative interventions through creative use of nuts and bolts can potentially help make these happen.

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Glossary

- Adaptive evolution** Changes in the genetic composition of populations due to natural selection that improve their reproductive fitness in a particular environment.
- Aggregate stability** A measure of resistance of soil to break down upon wetting or immersing in water.
- Aquatic systems** Ecosystems with water as an important abiotic component, such as lakes, wetlands, ponds and rivers.
- Arbuscular mycorrhizal fungi** A group of soil fungi that grow in symbiotic association with roots of the host plants.
- Arbuscule** A treelike structure found in cortical cells of roots where exchange of nutrients between the plant and the fungus takes place. These play a key role in bidirectional exchange of nutrients, carbon from plants to fungus and other nutrients, especially phosphorus, in reverse direction from fungus to plant.
- Arbutoid mycorrhizas** Arbutoid mycorrhizas are Basidiomycetes that form ectomycorrhizal associations with the plant genera, such as *Arctostaphylos* and *Arbutus*.
- Arum** In this type of colonisation, AM fungi form extensive intercellular hyphae in well-developed air spaces between cortical cells and invaginate the cells as short side branches to form arbuscules.
- Biodiversity** The variety and variability of ecosystems, species, populations within species and genetic diversity within living organisms.
- Biogeography** Refers to the study of the distribution of species and ecosystems in geographic space and through geological time.
- Classification** The systematic arrangement of organisms, based on different attributes, morphological or molecular.
- Climate change** Change in average weather over a period of long time, generally more than a decade.
- Cloning** Producing organisms all of which contain copies of the same gene: the desired gene is removed from the donor and inserted into a vector (usually a plasmid); the vector is used to transform a host culture, and then those hosts which have taken up the vector are selectively cultured.
- Colonisation** Establishment of the association between plant roots and arbuscular mycorrhizal fungi that allows the fungus to grow.
- DNA** Stands for deoxyribonucleic acid and refers to the molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.
- Drought stress** Stress induced by non-availability or less availability of water than required amounts which in turn induces different other types of stress.
- Ecological restoration** Reinstatement of a naturally or anthropogenically disturbed ecological system in its original state or alternative state of stability and composition.
- Ectomycorrhizas** Mycorrhizal association commonly occurring in forest trees and is characterised by a mycelium that ramifies through the soil and forms a mantle around individual rootlets growing between cells of the root cortex and forming a Hartig net (the interface between the symbionts).
- Ericoid mycorrhizas** Refers to a specialised symbiotic relationship between fungi and the

- roots of plants that belong to the order Ericales that confers special advantages to the family Ericaceae to thrive in a variety of edaphically stressful environments worldwide.
- Evolution** The change in the characteristics of living organisms over successive generations driven by various selection pressures that promotes diversification at various levels.
- Extraradical** Refers to ‘beyond root’ phase of mycorrhiza and is comprised of hyphae which extend in typical mycelial fashion into the soil surrounding the root.
- Extreme environments** Harsh environments characterised by conditions, such as extreme temperature regimes, excessive.
- Fungi** Any group of plants, including mildews, moulds, mushrooms, rusts and toadstools that have no leaves, flowers or green colour and reproduce by means of spores.
- Global climate change** Change in average weather regime (temperature, precipitation, sea level, etc.) on a longer time scale at the global level (see also climate change above).
- Glomalin** A glycoprotein with glue-like properties produced by arbuscular mycorrhizal fungi.
- Glomeromycota** A phylum within the kingdom Fungi, with about 200 described species, generally belonging to arbuscular mycorrhizas (AM) with the roots of host plants.
- Glycoprotein** A protein, containing both carbon and nitrogen, with attached carbohydrates.
- Host** An organism in which a parasitic, necrotrophic or symbiotic fungus lives, for instance, plants act as hosts for AM fungi.
- Hyphae** Threadlike filaments of an AM fungi through which they colonise living roots.
- Inoculate** To put a microorganism into an organism or a substratum.
- Inoculum** A small amount of a fungus used to inoculate fresh culture medium or to infect a host organism.
- Intraradical** Refers to ‘within root’ phase of mycorrhizas and is comprised of simple hyphae, with or without additional structures, that occur within the root epidermis and cortex, either between and within individual cells or just between them.
- Melzer’s reagent** A reagent (with composition, chloral hydrate, 100 g; potassium iodide, 5 g; iodine, 1.5 g; distilled water, 100 mL) used to elicit amyloid or dextrinoid reactions in spores, asci, hymenial tissues, etc.
- Meta-analysis** A statistical analysis that uses the combined information from several different studies, or several different species.
- Microorganism** Any microscopic or ultramicroscopic organism generally not visible to a naked eye, such as bacteria and fungi.
- Molecule** The smallest particle of a compound that can exist in the free state and still retain the characteristics of the compound.
- Monophyletic origin** Refers to taxa or group of organisms consisting of an ancestral species and all its descendants.
- Monotropoid mycorrhizas** *Mycorrhizal association* formed by plants of the Monotropaceae and characterised by mantle, Hartig net and cellular penetration.
- Mutualism** A kind of symbiosis in which both or all partners gain from the association, e.g. mycorrhizas.
- Mycorrhizal** Refers to plants that form mycorrhizal association.
- Mycorrhizas** The symbiotic association between plant roots and nonpathogenic fungi that facilitate movement of nutrients to host plants in exchange of carbon.
- Mycotrophic** Refers to a group of plants (species, genus, family, etc.) whose members can or must enter into mycorrhizal relationships.
- Net Primary Productivity** Rate of photosynthesis or production of biomass in a given area per unit time excluding the respiratory losses.
- Nutrient uptake** Taking up of nutrients by host plants as they are made available by mycorrhizas.
- Orchid mycorrhizas** Type of mycorrhizas specifically associated with plants of the family Orchidaceae.
- Paris** This type of colonisation spreads directly from cell to cell in the root and is further characterised by the absence of intercellular hyphae and the development of intracellular hyphal coils that frequently have intercalary arbuscules.

- pH** A symbol for hydrogen ion concentration in a solution that stands for potential hydrogen and values range from 0 to 14 on a logarithmic scale with lower values indicating acidity and higher values alkalinity.
- Phylogenetic analysis** Working out evolutionary relationships between species and lineages mainly using sequencing data.
- Plant invasion** Fast spread by introduced alien plants in non-native ranges with significant ecological and economic impacts.
- Polypyletic origin** Taxa or group of species characterised by one or more character states that have converged or reverted over time so as to appear to be the same but which have not been inherited from common ancestors.
- Primer** A short nucleotide sequence that pairs with one strand of DNA and provides a free end at which DNA polymerase begins synthesis of a complementary segment of DNA.
- Restriction digestion** Digestion of the genomic DNA or its specific parts by using some restriction enzymes that act as molecular scissors to yield a fragment length polymorphism as an indicator of genetic diversity.
- Restriction enzyme** An enzyme that cuts DNA at points determined by specific DNA recognition sequences of various lengths.
- Restriction fragment length polymorphism (RFLP)** Genetic diversity detected by cutting DNA with restriction enzymes, resulting in different-sized fragments of DNA from different alleles.
- Rhizodeposition** Various types of depositions originating from plant roots in the soil system.
- Rhizosphere** The biologically active part of the soil that is under the influence of living roots of the plants.
- Root length colonisation** Average length of living roots colonised by mycorrhizal fungi which is used as a measure of the intensity of mycorrhizal infection.
- Salinity stress** Stress induced by excessive concentration of salts in the environment.
- Seedling support** Sustenance received by seedlings in terms of nutrient uptake through different agencies, such as mycorrhizas.
- Soil carbon sequestration** The process of transfer of CO₂ from the atmosphere into the soil through various sources, such as plant residues, organic solids and so on.
- Soil organic matter** Refers to the organic matter component of soil, generally consisting of plant, animal and microbial residues at various stages of decomposition.
- Soil stability** Resistance of a soil to erosion and degradation by different forces, such as wind and water.
- Soil structure** Describes the organisation of the soil mainly determined by how individual soil granules aggregate, thereby leaving a characteristic arrangement of soil pores between them.
- Solubilisation** The process of dissolving Glomalin, an insoluble chemical compound.
- Species** The most easily recognisable unit of biodiversity that comprises individuals very similar in all major respects, often used for organisms that are normally capable of interbreeding.
- Spore** Specialised microscopic propagules produced by AM fungi in rhizospheric soils that act as agents of dispersal and propagation in these fungi.
- Spore extraction** Taking out the mycorrhizal spores from soils through procedures such as wet-sieving and decanting.
- Sporulation** The production of spores by AM fungi.
- Sterilisation** The process whereby all microorganisms and their propagules are killed by exposure to various killing agents such as heat, radiation, chemicals or filtration.
- Substrate** Refers to the food of a fungus or the material from which a fungus is fruiting.
- Succession** The process of development of biotic communities in either barren areas (primary succession) or previously vegetated but later disturbed areas (secondary succession).
- Symbiosis** Positive interaction between two different living systems that mutually benefits both the associated partners.

Systemic Describes a fungicide or pathogen which enters and becomes widely distributed within the body of a plant or animal.

Taxonomy The classification of organisms on the basis of their structural similarity and evolutionary relationship.

Thermal stress Stress caused due to high temperatures and resulting heat.

Threatened An IUCN category that refers to population or species that has a finite risk of extinction within a relatively short time frame

and generally include the combination of critically endangered, endangered and vulnerable categories.

Vesicle Sac-like structure formed by some AM fungi in plant roots as ephemeral storage structures.

Vesicular-arbuscular mycorrhizas (VAM) Vesicular-arbuscular mycorrhizal fungi, an earlier expression for what are now more correctly called as *AM* fungi (see arbuscular mycorrhizas).

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