11 Mycorrhizosphere: Strategies and Functions

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1 Introduction

Hiltner recognized the rhizosphere as the volume of soil in the immediate vicinity of the roots, which is predominantly affected by the activity of plants. The rhizosphere differs from the surrounding soil in most of the physico-chemical factors and a wide range of microorganisms colonizes this rhizosphere soil along with the rhizoplane (i. e., the root surface; Phillips et al. 2003). The number of these microorganisms per gram of soil is much larger in the rhizosphere compared to bulk soil. This increased microbial activity in the vicinity of roots can be ascribed to root exudates, sloughed senescent root cells and mucigel, which have been described as rhizodeposition (Mukerji et al. 1997; Bansal et al. 2000).

In nature, most of the actively absorbing rootlets form a symbiotic association with mycorrhizal fungi, which are ubiquitous soil inhabitants. The formation of symbiotic associations with mycorrhizae significantly changes the physiology and/or morphology of roots and plants in general, leading to altered root exudation (Bansal and Mukerji 1994). The changes in root exudates affect the microbial communities around the roots, leading to the formation of the "mycorrhizosphere" (Mukerji et al. 1997; Varma et al. 1999). The mycorrhizosphere is the zone of soil where the physical, chemical and microbiological processes are influenced by plant roots and their associated mycorrhizal fungi. A major difference in the rhizosphere around the nonmycorrhizal roots and mycorrhizosphere effect is the presence of extramatrical hyphae of mycorrhizal fungi. These extramatrical hyphae extend well beyond the roots into the bulk soil and are an impor-

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tant source of carbon to the soil organisms (Schreiner and Bethlenfalvay 1995). The mycorrhizal hyphae increase the soil aggregation and in root association increase exudation, which favors the microbial growth (Schreiner and Bethlenfalvay 1995; Bansal and Mukerji 1996).

The mycorrhizosphere microbiota differs qualitatively as well as quantitatively from the rhizosphere of nonmycorrhizal plants. The soil microfauna influences the mycorrhiza formation as well as the host growth response (Fitter and Garbaye 1994). Many kinds of interactions occur between these microbial communities in the mycorrhizosphere and mycorrhizae. The interactions between the mycorrhizae and soil microorganisms may be mutualistic or competitive and they affect the establishment and functions of mycorrhizal symbionts as well as modify the interactions of the plant with other symbionts or pathogens in soil.

2 The Rhizosphere

The rhizosphere is the region in which materials released from the root, and root metabolic activities such as respiration, affect microbes (Table 1). Roots in the process of rhizodeposition release volatile, soluble, and particulate materials. The rhizosphere microbes, after their growth on these materials

Table 1. Various spheres and materials released in the soil

Terms	Definition
Rhizosphere	Region around the plant root where materials released from the root modify microbial populations and their activities
Endorhizosphere	Regions of the various cell layers of the root itself where microorganisms also colonize
Ectorhizosphere	An area surrounding the root and containing root hairs, plant and bacterial mucilage
Rhizoplane	Root surface that can be colonized by microorganisms
Mycorrhizosphere	The ectorhizosphere extends a substantial distance from the root with the development of mycorrhizal fungal associations. Materials released from the fungus increase the microbial populations and their activities around the fungal hyphae
Spermosphere	The region around the germinating seed
Rhizodeposition	Release of materials from roots
Exudates	Compounds of low molecular weight produced by plant cells and released into the root environment
Mucilages	Gelatinous organic materials released by the plant in the root cap region derived from the Golgi apparatus, polysaccharides hydrolysis, and epidermal materials

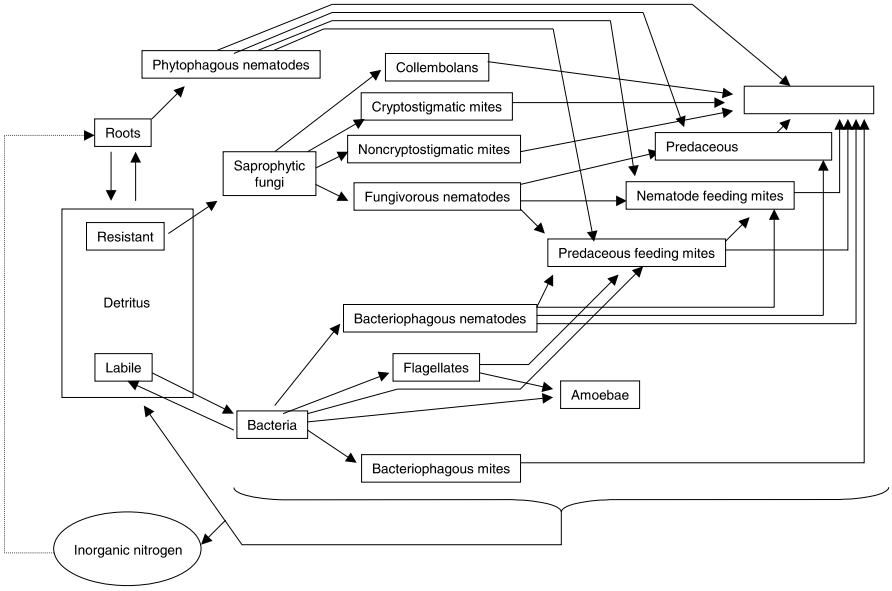

Fig. 1. Components of the rhizosphere food web. (Modified after Moore et al. 2003)

and their cellular turnover, release nutrients in forms which can be utilized by plants. Plants and their rhizospheres are found in soils in which the environment is primarily aerobic, and in many marine and freshwater environments in which oxygen is often limited. The rhizosphere encompasses not only the region of nutrient uptake by the roots, but also extends into the soil by the action of root products and the trophic interactions that are affected by these products (van der Putten et al. 2001). A growing root can reach the regions from the root tip to the crown, where different populations of soil biota have access to a continuous flow of organic substrates derived from the root. This infusion of organic substrates into the rhizosphere by plants explains why the biomass and activity of microbes and soil fauna are greater in the rhizosphere than the bulk soil (Parmelle et al. 1993; Bardgett et al. 1998). The root tip is the site of root growth and is characterized by rapidly dividing cells and secretions or exudates that lubricate the tip as it passes through the soil. The exudates and sloughed root cells provide carbon for bacteria and fungi, which in turn immobilize nitrogen and phosphorus. Further up the root is the region of nutrient exchange, characterized by root hairs and lower rates of exudation which stimulate additional microbial growth (Bringhurst et al. 2001).

The food web that develops within the rhizosphere is complex (Fig. 1), consisting of multiple assemblages of species that are supported by roots and their by-products. These assemblages are dubbed as the root, bacterial, and fungal energy channels. Live roots form the basis of the root energy

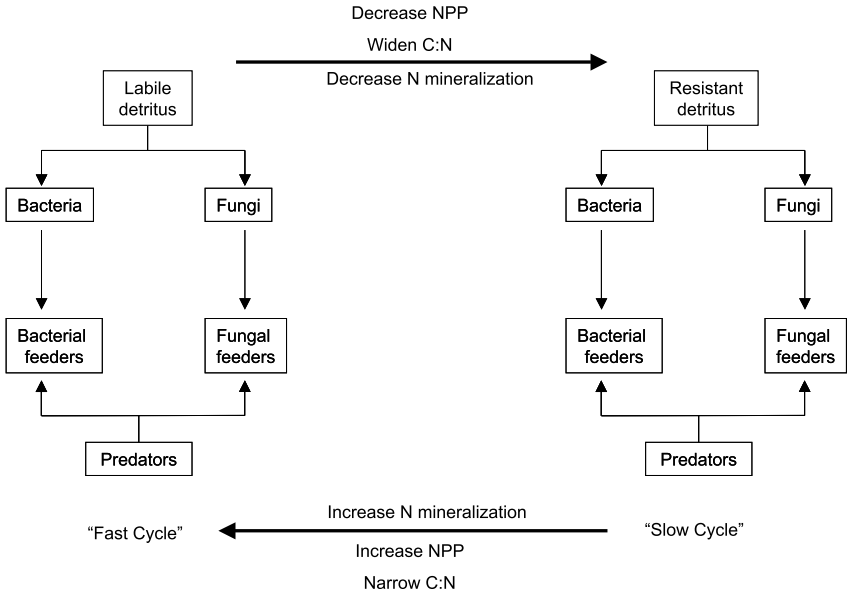

Fig. 2. Events in the evolution of the rhizosphere. (Modified after Phillips et al. 2003)

channels. The root energy channel consists of root-feeding insects and nematodes, and microbes that engage in symbiotic relationships with plant roots (mycorrhizal fungi, rhizobia, *Frankia*). Detritus forms the basis of the bacterial and fungal channels. The bacterial energy channel is composed of bacteria, protozoa, rotifers, nematodes, and a few arthropods. The fungal energy channel largely consists of saprophytic fungi, arthropods, and nematodes.

Soil saprophytic bacteria that compose most of the microbial biomass in the rhizosphere are aquatic organisms and are more efficient in using the more labile root exudates than saprophytic fungi. In contrast, fungi are better adapted to utilize more resistant root cells and substrates than are bacteria (Lynch 1990). The bacterial energy channel represents a "fast cycle", while the fungal energy channel represents a "slow cycle" (Fig. 2).

A common suite of nematode and arthropod predators links the root, bacterial, and fungal energy channels. The linkages between the energy channels tend to be weak at the trophic levels occupied by roots, bacteria and fungi, and strongest at the trophic levels occupied by predatory mites (Moore et al. 2003). The strength of the linkages between energy channels and the dominance of a given energy channel vary with the type of ecosystem, changes with disturbance, and affects nutrient turnover rates (Fig. 2). The fungal energy channel tends to be more dominant in systems where the ratio of carbon to nitrogen (C:N) is high while the bacterial channel is more dominant in systems with narrow C:N ratios (Moore et al. 2003).

3
Evolution of the Rhizosphere

Plants surely encountered microorganisms in primordial soil as they moved from aquatic to terrestrial environments (Fig. 3). Geochemical evidence for microorganisms exists from 2600 million years ago (m.y.a.) and bacterial fossils dating back 1200 m.y.a. are known (Horodyski and Knauth 1994; Watanabe et al. 2000). Although true roots with vascular tissue appeared

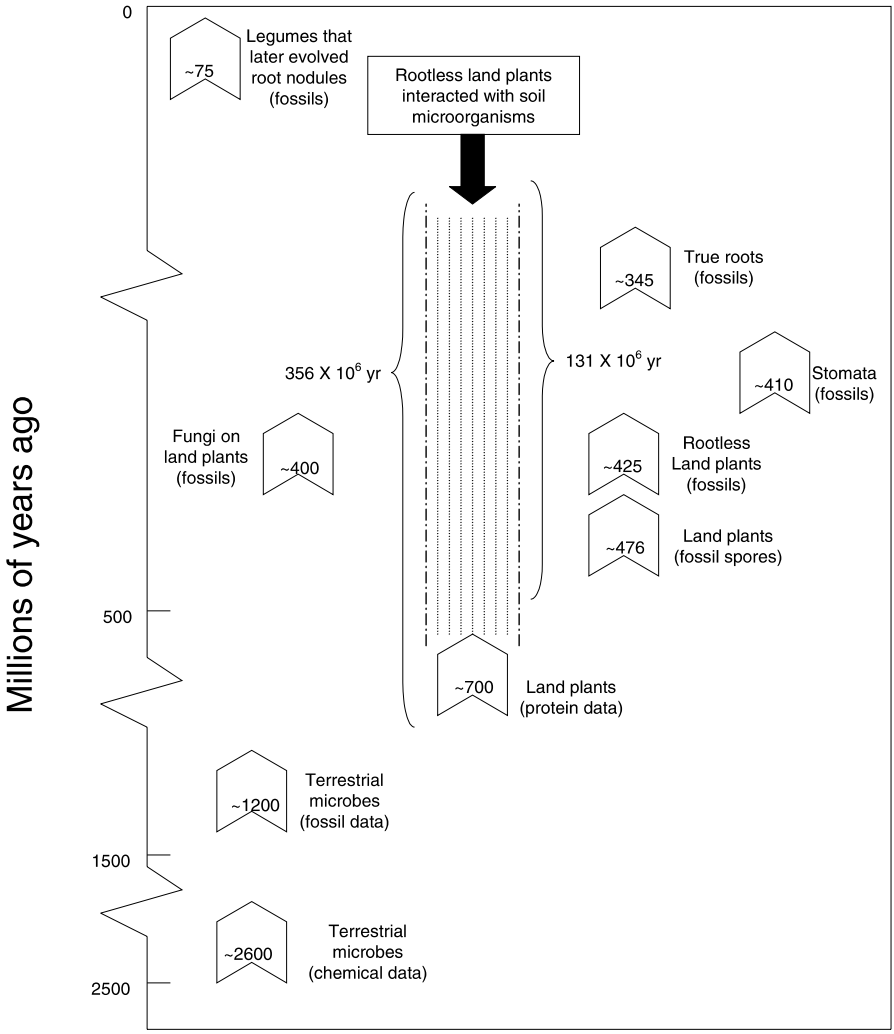

Fig. 3. Bacterial energy channels representing fast slow cycles. (Modified after Moore et al. 2003)

perhaps 345 m.y.a. (Stewart and Rothwell 1993), early terrestrial plants had a variety of underground structures, including stems and rhizoidal appendages (Raven and Edwards 2001), which were beset early on by bacteria and from at least 400 m.y.a. by fungi (Taylor et al. 1995). Stomata were present approximately 410 m.y.a. (Edwards et al. 1998), and thus water movement through the evolving soil food web towards early terrestrial plant tissues probably predated roots.

Phillips et al. (2003) pointed out that all plants in the early terrestrial environment interacted with microorganisms. Those relationships in primordial soil predated vascular roots by 131–355 million years, depending on whether one documents the beginning of interactions by plant microfossils (Kenrick and Crane 1997), or by estimates based on protein data (Heckman et al. 2001). It is often thought that the complex rhizobial symbiosis with legumes evolved a mere 75 million years after the Caesalpiniodeae group of legumes appeared; either estimate offers sufficient time for simpler mutualisms to develop (Phillips et al. 2003).

One cannot assess the extent to which primitive plants resisted microbial attacks, but the presence of their reasonably intact, fossilized remains shows that some protective mechanisms existed. Thus, it is reasonable to suggest that populations of epiphytic and endophytic microorganisms were an accepted fact of life for early land plants. The chemical residues of those microbial populations, as well as any signals released among the microorganisms must have been in close contact with early land plants. Under such conditions, a sifting of water-soluble microbial products for potentially important data on the water and mineral content of nearby environments probably occurred (Phillips et al. 2003).

4
Anatomy of the Root Through the Eyes of a Microbiologist

Vascular plants are widely distributed over the world. They are one of the most important links which humans have to nature. The vast majority of our food and fiber are directly derived from plants. Although it often is not evident, plant roots and their surrounding microbes (the rhizosphere) are important wherever plants are found: forests, grasslands, tundra, deserts, and wet areas such as marshes and mangrove swamps. The root of these plants is divided into three zones: (1) zone of cell division or meristematic activity; (2) zone of cell elongation and (3) zone of cell maturation. Roots grow by the activity of apical meristems, which also form a root cap distally. The root cap is a dynamic, specialized organ that facilitates root penetration of soil, senses threats as well as bounty, and responds by transmitting signals that alter growth patterns. The root supports a unique modified

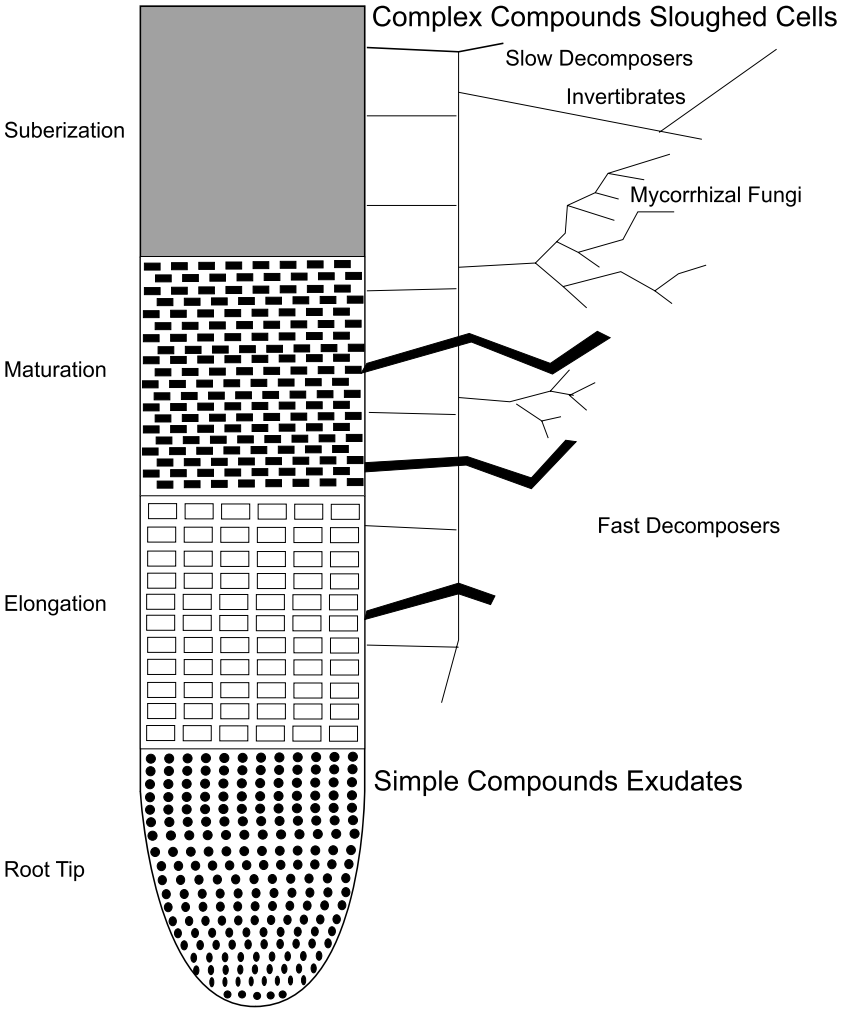

Fig. 4. Structure of mycorrhizal fungi in relationship to the structure of the root and associated root organisms

microbial community in an environment termed the rhizosphere, the region influenced by the root and its activities. Microbes directly colonize root surfaces and are also found under them, creating additional unique environments for microbes (Fig. 4).

The term rhizosphere, which has been used for 100 years, is critical to understanding how plants interact with their environment. In essence, the microbes in the rhizosphere provide the critical link between plants, which require inorganic nutrients, and the environment, which contains the nutrients, but often in organic and largely inaccessible forms.

Microbes also colonize the plant root surface (the rhizoplane). The colonization of the rhizoplane by microbes can involve specific attachment mechanisms. For *Agrobacterium thaliana*, which forms tumors in susceptible plants, this involves a two-step process of (1) loose binding to the cell surface and (2) the synthesis of cellulose fibrils by the bacterium. This results in binding of the bacteria to the plant root surface. If mutant bacteria are used which do not have these attachment characteristics, they will not bind to the root surface.

Plants also have other microbes with which they develop unique relationships in the root environment, including the symbiotic nitrogen-fixing bacteria such as *Rhizobium*. These bacteria form nodules on susceptible legumes, and the fixation of nitrogen by filamentous bacteria of the genus *Frankia*, an association that occurs with a wide range of shrubs and woody plants. Another important group of microbes, which form direct associations with plants, includes the mycorrhizae or "fungus roots", which occur in a wide variety of plants, considered to be one of the oldest plant-microbe associations. The nitrogen-fixing bacteria and the mycorrhizae form structures within the plant root, indicating these physiologically active relationships. The mycorrhizal hyphal network, supported by carbon derived from the plant, also releases organic carbon. Microbes grow around the mycorrhizal hyphae.

5
Production of Chemical Compounds in the Rhizosphere by Plant Roots

A general thought is that aerial parts (stem and leaves) contain greater biomass than root. This impression is misleading. For many plants the root:shoot ratio is such that more of the plant mass is in the roots than in stems and leaves. The materials released by the plants include a wide variety of organic compounds (Table 2). The types of these substances are constantly changing due to a wide range of plant and environment-related factors. These factors can include temperature and moisture stress, fertilizer additions, herbage removal (both above- and below-ground) changes in sunlight, herbicide additions, plant age, and other changes in the plant's environment. The materials lost from plant roots can be 30–40% of the carbon fixed through photosynthesis.

The fine hairs are a critical part of the root system (Fig. 5). These can be rapidly shed when environmental conditions become less suitable for plant growth. Cortical and epidermal cells, called mucilages, and soluble metabolic products (amino acids, sugars, organic acids, etc.), described as exudates, are also released. In addition, a variety of gaseous metabolites flow

Table 2. Compounds released by plant roots in the process of rhizodeposition

Compound	Exudate components
Sugars	Glucose, fructose, sucrose, maltose, galactose, rhamnose, ribose, xylose, arabinose, raffinose, oligosaccharide
Amino compounds	Asparagine, α-alanine, glutamine, aspartic acid, leucine/isoleucine, serine, γ-aminobutyric acid, glycine, cystine/cysteine, methionine, phenylalanine, tyrosine, threonine, lysine, proline, tryptophane, β-alanine, arginine, homoserine, cystathionine
Organic acids	Tartaric, oxalic, citric, malic, proponic, butyric, succinic, fumaric, glycolic, valeric, malonic
Fatty acids and sterols	Palmitic, stearic, oleic, linoleic, linolenic acids, cholestrol, campesterol, stigmasterol, sitosterol
Growth factors	Biotin, thiamine, niacin, pantothenate, choline, inositol, pyridoxine, ϱ-aminobenzoic acid, *N*-methyl nicotinic acid
Nucleotides, flavonines and enzymes	Flavonine, adenine, guanine, uridine/cytidine, phosphatase, invertase, amylase, protease, polygalacturonase
Miscellaneous compounds	Auxins, scopoletin, fluorescent substances, hydrocyanic acid, glycosides, saponin (glucosides), organic phosphorus compounds, nematode-cyst or egg-hatching factors, nematode attractants, fungal mycelium growth stimulants and inhibitors, zoospore attractants

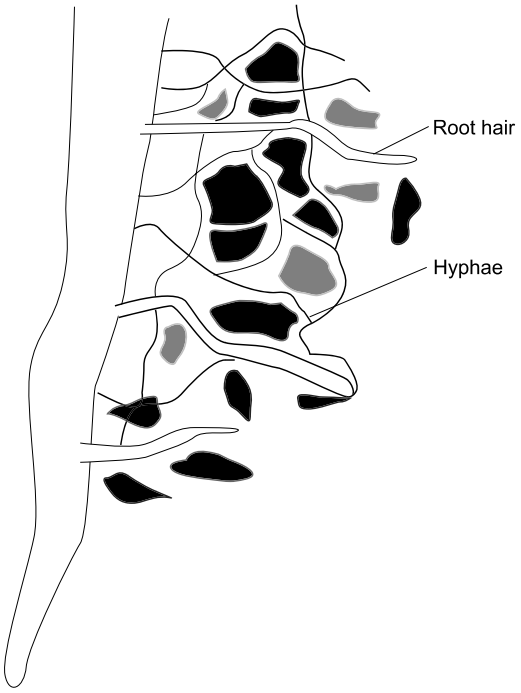

Fig. 5. Root hairs that assist the plant in exploring resources present in soils

from the roots. The release of these different materials is described as the process of rhizodeposition. When the mucilages combine with microbes, soil colloids, and soil organic matter, mucigels are formed which cover and protect the root tip.

6 Microbial Diversity in the Rhizosphere

The rhizosphere is a "cloud" of microbes which literally surrounds plant roots and is vital for the plant's survival and growth. Plant roots create new environments for microbes due to the increased levels of nutrients; microbial populations increase, often by 1000–10,000-fold, and marked changes in the composition of the microbial community will also occur (Table 3), as indicated by the rhizosphere:soil (R:S) ratio for a soil. The number and types of microbes often increase along the root away from the tip of the plant root. The plant roots also respire (use oxygen), which changes the environment of the rhizosphere microbes.

The microbial community, which develops in this changed rhizospheric environment will face additional challenges; many of the materials released from roots do not contain sufficient nitrogen, and sometimes phosphorus, to allow rapid microbial growth. This situation limits both the plant and the associated rhizosphere microbes.

The plant has an increasing demand for inorganic nutrients, which are often not available at a sufficient rate. The rhizosphere contains a wide variety of free-living and symbiotic nitrogen-fixing bacteria (Table 3), which make a major contribution to meet this demand, but at a high energetic cost for the plant. The filamentous fungi, including the free-living and mycorrhizal types, also play a unique role in making nutrients available to the plant which cannot be provided by most bacteria. The filamentous fungi in the rhizosphere have an extensive hyphal network. With this hyphal network, they can utilize carbon derived from the plant while obtaining their nitrogen and other limiting resources from outside the immediate root zone.

Table 3. Microbial diversity of major groups in the rhizospheric and nonrhizospheric soils

Organism	Rhizosphere soil (microbes/g dry soil)	Nonrhizosphere soil (microbes/g dry soil)	R:S ratio
Bacteria	1200×10^6	53×10^6	23
Actinomycetes	46×10^6	7×10^6	7
Fungi	12×10^5	1×10^5	12
Algae	5×10^3	27×10^3	0.2

Free-living nitrogen-fixing bacteria, including the genera *Azotobactor*, *Azospirillum*, and *Azoarcus* are abundant in the rhizosphere. In the presence of nitrogen-free or lower nitrogen-content substrates released from the root, these bacteria play an important role. They carry out associative nitrogen fixation and thus provide nutrient for the plant. The rhizosphere community not only has bacteria and fungi but also contains protozoans and nematodes. These consumers feed on the nutrient-rich bacteria and fungi, leading to more rapid turnover of the microbes, which leads to an accelerated release of nutrients for plant use.

7
What Are Mycorrhizal Fungi?

Mycorrhizae provide an intimate link between the soil environment and the functional nutrient-absorbing system of the plant. The modification of plant roots by symbiotic fungi into the distinct structures characteristic of mycorrhizae results in a unique and intriguing component of the rhizosphere (Fig. 6). Since the first published description of a mycorrhizal

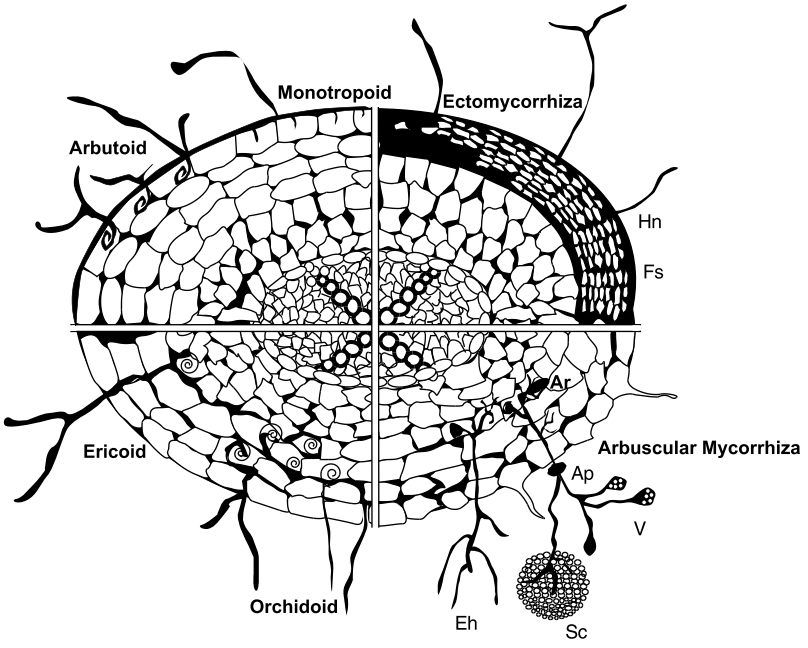

Fig. 6. Various kinds of mycorrhizal colonization in the root of the vascular plant (*Fs* fungal sheath, *Eh* extramatrical hyphae, *Hn* Hartig's , *V* vesicle, *Ar* arbuscule, *Sc* sporocarp, *Ap* appresorium)

association by Frank, scientists continue to be challenged by the role of mycorrhizae in the ecological and physiological context of plants. Although most research and observations on mycorrhizae have been concerned with nutrient uptake by mycorrhizae, especially immobile elements such as phosphorous, there is an increasing awareness of their potential importance in many diverse aspects of a plant's ability to grow and survive in natural and man-altered environments.

8
Types of Mycorrhizal Fungi

Over the years, seven types of mycorrhizae have come into general use on the basis of morphology and anatomy, but also of either host plant taxonomy or fungal taxonomy (Srivastava et al. 1996; Smith and Read 1997). These are: ectomycorrhiza, endomycorrhiza or arbuscular mycorrhiza, ericoid mycorrhiza, arbutoid mycorrhiza, monotropoid mycorrhiza, ect-endomycorrhiza and orchidaceous mycorrhiza.

8.1
Ectomycorrhiza

The ectomycorrhizae (ECM) are sometimes referred to as "sheathing" mycorrhizae because of the distinct presence of a sheath or mantle of fungal mycelium that covers the absorbing root. ECM are found almost exclusively on woody perennials. The plant symbionts include both Gymnosperms and Angiosperms. There is no hyphal penetration of cells. Fungal hypha is generally separate. A distinct Hartig's net is present between the cells. Hartig's net is a plexus of fungal hyphae between epidermal and cortical cells. It provides a large surface area for the interchange of nutrients between the host and the fungi.

8.2
Arbuscular Mycorrhiza

The term refers to the presence of intracellular structures – vesicles and arbuscules – that form in the root during various phases of development (Fig. 7). These mycorrhizae are the most commonly occurring group since they occur on a vast taxonomic range of plants, both herbaceous and woody. The plant symbionts range from Bryophytes to Angiosperms. There is no fungal sheath. Aseptate hyphae enter the root cortical cells and form

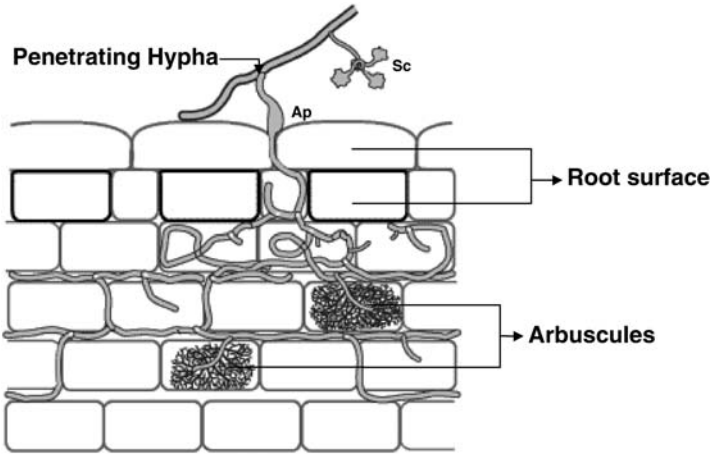

Fig. 7. Colonization of AM fungi in the root of the vascular plant. Arbuscules are the sites for bidirectional flux. Transfer of photosynthates from root to soil and nutrients from soil to root (*Ap* appresorium, *Sc* sporocarp)

characteristic vesicles and arbuscules. The plasmalemma of the host cell invaginates and encloses the arbuscules.

8.3 Ericoid Mycorrhiza

The ericoid mycorrhizae are endomycorrhizae in the general sense, since the fungal symbiont penetrates the cortical cell wall and invaginates the plasmalemma. Infection of each cortical cell takes place from the outer cortical wall; lateral spread from cell to cell does not occur. Infected cells appear to be fully packed with fungal hyphae. The mycorrhizae do not form a sheath although a loose weft of hyphae around the root can sometimes be observed. The functional life of the association in epidermal cells may be short-lived, being only a matter of weeks in *Rhododendron*. In the ericoid mycorrhizae, the host cell dies as the association disintegrates, thereby restricting the functional life (i. e., nutrient absorption) of these epidermal cells to the period prior to breakdown of the infected cell.

8.4 Arbutoid Mycorrhiza

The arbutoid mycorrhizae have characteristics found in both ECM and other endomycorrhizae. Intracellular penetration of cortical cells and for-

mation of a sheath can occur, and a Hartig's net is present. A feature distinguishing them from ericoid mycorrhizae is the presence of the dolipore septate in internal hyphae. It appears from most reports that the fungal associate in arbutoid mycorrhizae is a basidiomycete.

8.5 Monotropoid Mycorrhiza

This term is applied specifically to mycorrhizae that are observed on the achlorophyllous plants in the family Monotropaceae. These mycorrhizae are very similar to the ECM and form a distinct sheath and Hartig's net. However, they exhibit a distinctive type of intracellular penetration in cortical cells that is unlike other endomycorrhizal types. The fungus forms a fungal peg, which invaginates the cell wall.

8.6 Ect-endomycorrhiza

Ect-endomycorrhiza are only formed with genera in the Pinaceae. These mycorrhizae form a Hartig's net in the cortex of the root, but develop little or no sheath. Intracellular penetration of cortical cells takes place, and thus they are similar to the arbutoid type. Ect-endomycorrhizae in Pinaceae seem to be limited to forest nurseries and are formed by a group of fungi called E-strain. These fungi are most likely to be the imperfect stage of ascomycetes; they may cause ect-endomycorrhizae in some tree species and ECM in other tree species.

8.7 Orchidaceous Mycorrhiza

The fungal association is of the endomycorrhizal type, where the fungus penetrates the cell wall and invaginates the plasmalemma and forms hyphal coil within the cell. Once the plant is invaded, spread of the fungus may occur from cell to cell internally. The internal hyphae eventually collapse or are digested by the host cell. Since the symbiosis forms an external network of hyphae, it would seem probable that the fungal hyphae function in nutrient uptake as with other mycorrhizae and that the coarse root system of orchids would be supplemented by the increased absorbing surface area of the hyphae (Smith and Read 1997). A number of basidiomycetes genera have been shown to be involved in the symbiosis, although many reports on the isolation of the symbiotic fungus from the roots of orchids have

placed the symbionts in the form genus *Rhizoctonia* when the perfect stage was not known or the isolate was not induced to fruit in culture.

9
Functions of Mycorrhizal Fungi

In terrestrial ecosystems, arbuscular mycorrhizal (AM) fungi make various promises (Table 4) where the organic detritus–decomposer pathway accounts for the majority of energy flow and nutrient turnover. Microflora coupled with microfauna in the soil are the major components of both biomass and activity affecting nutrient availability. Although soil bacteria and fungi generally immobilize mineral nutrients as carbon is consumed and thereby compete with plants for macronutrients, mycorrhizal fungi,

Table 4. Advantages of AM fungi

Promotes plant growth	Maintain plant and soil health
Bio-protection against root diseases (bacteria, fungi and nematodes)	Plant production with reduced fertilizers and pesticides
Nutrient acquisition	Plant size or biomass
Improved soil-root contact	Influence population dynamics of soil flora
Symbiosis alters host water relations	Revegetation of landscape or contaminated soils
Symbiosis alters root length, root architecture and root/shoot ratio	Biological hardening of tissue culture-raised plants
Alters rate of water movement into, through and out of host plants	Effects on tissue hydration and leaf physiology
Postpones leaf dehydration	Alters leaf osmotic potential
Alters the number of photosynthetic units	Photosynthetic storage and export rates
Dissimilar symplastic solute pools	More effective scavenging of soil water
Effects on osmotic adjustment	Drought responses
Altered transpiration rates	Stomatal conductance to water vapor
Intrinsic leaf hydraulic or biochemical properties	Osmoprotection of enzymes
Altered nodule number and their activity	Enhanced P acquisition
Altered total protein	Altered morphological and phenological effects
Altered leaf abscission	Altered leaf drop, necrosis and senescence
Altered leaf movements	Altered wilting of leaves
Altered recovery from wilting	Provide salt tolerance to plant

because of their unique carbon strategies, can efficiently couple soil mineralization and nutrient uptake by the plant roots. In many cases, the mycorrhizal system actually "bridges" across the rhizosphere, and provides an organic link between the root and the bulk soil. In addition, AM fungi help the plant to cope with various kinds of stress such as soil pH, heavy metals, soil salinity, and water and drought stresses. A brief account of the functioning of AM fungi under these stresses is described in this chapter.

9.1 Arbuscular Mycorrhizal Fungi in Relation to Soil pH

Soil pH crucially affects development of mycorrhizal fungi by affecting the solubility of several compounds. Most of the phosphate exists as insoluble complexes of Al and Fe at acidic pH and at alkaline pH, phosphate exists as insoluble complexes of Ca and Mg while maximum solubility of phosphate occurs at neutral pH. However, inorganic forms are still largely insoluble. Many metals are insoluble under alkaline edaphic conditions, but highly soluble at acidic conditions.

It has been well demonstrated that mycorrhizal fungi vary in their tolerance of soil pH. Some grow only in low pH soils, whereas others grow after modifying the soil pH with a certain amount of lime (Giri et al. 2003a). A few mycorrhizae have a tendency to grow at the pH from which they have been isolated (Giri and Mukerji 2003).

Soil pH is an important factor in studying the ecology of endomycorrhizal fungi. Low soil pH has a profound effect on the movement and uptake of P. Rhizosphere acidification affects fungal soil plant nutrient supply mechanisms (Giri et al. 2003a). Gillespie and Pope (1991) reported that the P diffusion rate increased with an increase in the acidity of the soil. *Glomus* sp. extracted from a neutral soil grew best at pH 7, while its growth was less at acidic and alkaline pH. Relative tolerance of *Glomus mosseae*, *G. fasciculatum*, and *G. macrocarpum* to graded pH levels (7.8–10.5) and their influence on P uptake in *Prosopis juliflora* were evaluated by Sidhu and Behl (1997). They found that an increase in pH adversely affected growth, biomass and P concentration in seedlings. Chlamydospore formation by all three AM fungi decreased with an increase in the rhizosphere soil pH. However, application of AM resulted in a significant increase in seedling root and shoot length, collar diameter and biomass production at high pH levels. Biomass production of mycorrhizal seedlings grown at pH 10.5 was equivalent to that of uninoculated plants at pH 7.8. In AM-colonized roots P concentration increased by 68% at pH 7.8 and 40% at pH 10.5. *Glomus fasciculatun* originally isolated from a site having a high pH (9.2) showed relatively high tolerance to a pH ranging between 8.5–10.5 compared to

other species as exhibited by a higher degree for chlamydospore formation and root colonization (Sidhu and Behl 1997).

Soil pH and available soil nutrients have a cumulative effect on the efficiency of AM fungi on plant growth. Habte and Soedarjo (1996) reported that increased P concentration leads to high soil pH and reduced available Mn concentration in soil. This is probably due to the precipitation of cations directly by an excess of phosphate, which induced an elevation in pH that increased plant growth.

9.2 Arbuscular Mycorrhizal Fungi in Relation to Heavy Metal Stress

Metal toxicity in soil may be induced by the discharge of sewage or industrial pollutants into the soil, or due to the presence of excessive quantities of metals in certain categories of soils. In soil they are present as free ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble complexes such as oxides, carbonate and hydroxides or they may form part of the structure of silicate minerals (indigenous soil content). The toxicity of metals in the soil depends on their bioavailability, which may be defined as the availability of metals to be transferred from a soil compartment to living organisms. High metal concentration in soil is not only toxic to plants, but also affects germination and growth of soil microorganisms. Amongst the myriad of soil microorganisms, mycorrhizal fungi are considered as integral functioning parts of plant roots and the fungi involved provide a direct link between soil and plant roots. The influence of mycorrhizal fungi on plant nutrition is greater for elements with narrow diffusion zones around plant roots such as P (Smith and Read 1997; Giri et al. 1999).

There are only a few reports concerning the interaction of arbuscular mycorrhiza with metals. Nonetheless, it is apparent that soils high in available metals do provide a habitat for specific AM fungi, which do provide some degree of protection to the host plant from toxic metals by restricting the uptake of metals to the plant or tolerating these themselves. *Medicago sativa* grown in mine spoils and waste sediments containing high amount of Zn and Cd showed significant mycorrhizal colonization although the number of AM fungal spores was lower than in an adjacent soil not altered by mining activity (Diaz and Honrubia 1993). *Agropyron trachycaulum* showed considerable mycorrhizal colonization in a subalpine coal mine spoil and on oil sand tailings amended with peat containing AM fungal propagules, but colonization was absent in the same species cultivated in the unamended oil sand tailings, revealing them to be devoid of arbuscular mycorrhizal fungi. These results indicated that poor or absent mycorrhizal propagules

in some of the mine spoils may have resulted in nonmycorrhizal colonization. However, mycorrhizal rather than nonmycorrhizal grasses could colonize polluted mining sites, suggesting that heavy metal tolerance was due to mycorrhizal association (Shetty et al. 1994).

In *Festuca* and *Calamagrostis epigejos*, mycorrhizal colonization was observed when grown in coastal dunes contaminated by atmospheric deposition from a blast furnace (Duke et al. 1986). Similarly, extensive colonization of mycorrhizal fungi was observed in *Agrostis capillaris* in a Zn- and Cd-contaminated site (Griffioen et al. 1994). Experiments conducted on three populations of *A. capillaris* using a sandy soil contaminated with smelter and limestone-derived clay with or without metals of natural origin, however, did not show a significant difference in mycorrhizal root colonization between these populations.

In *Albizzia amara* a high level of colonization by AM fungi was observed in agricultural soil and fly ash severely contaminated with heavy metals (Giri 2001). *Oxalis acetosella* grown in low pH soil polluted with Cd, Zn, and Pb showed even higher mycorrhizal colonization (Turnau et al. 1996). These results further substantiate the fact that mycorrhizal fungal colonization has the potential to tolerate heavy metals particularly in the case of those AM fungi which originated from metal-contaminated sites. In contrast, there are some reports demonstrating inhibition of mycorrhizal colonization in the presence of metals of different origin (Chao and Wang 1991; Weissenhorn et al. 1995; Vidal et al. 1996; Joner et al. 2000).

Experiments conducted on maize in the metal-contaminated soil showed that mycorrhizal colonization either increased plant biomass and decreased Cd, Cu, Zn and Mn concentrations in shoot- and root-tissues, or had no effect on growth and metal uptake depending on root density, plant growth conditions, and mycorrhizal inoculum (Weissenhorn et al. 1995). Loth and Hofner (1995) observed that mycorrhizal colonization increased uptake of Cu, Zn and Cd in *Avena sativa* roots from a highly contaminated soil, but reduced translocation to the aerial part. Weissenhorn and Leyval (1995) reported a higher uptake of metal by mycorrhizal plants under high metal concentration. It was found that *G. fasciculatum* reduced the negative effect of Zn on plant growth. However, they did not report on the effect of mycorrhiza on the Zn concentration in shoot and root tissues.

The effect of AM fungi on plant metal uptake also depends on soil pH. With increasing soil pH, (diethylenetriaminepentaacetate (DTPA)-extractable metals decrease, but at the same time AM fungi increased Cd, Zn and Mn uptake in the shoots of *Medicago sativa*. At a lower soil pH, mycorrhizal colonization decreased metal uptake. In both cases, mycorrhizal colonization enhanced alfalfa growth. It is unfortunate that most of the studies on heavy metal tolerance/uptake by mycorrhizal fungi have been performed in pots where it is not possible to separate the effect of

the fungus and of the host plant on the mobilization and uptake of metals (Leyval et al. 1997). To differentiate between fungus and host plant effects, plant containers with different compartments separating root and extraradical hyphae of *G. mosseae* from a sandy loam to clover was undertaken. In mycorrhizal clover, uptake of Cd increased by 55% in comparison to nonmycorrhizal plants. In the same plant, Burkert and Robson (1994) also reported transport of Zn in extraradical hyphae from a sandy soil. In the bean plants, a higher amount of Cd, Zn and Cu was transferred by mycorrhizal extraradical hyphae.

The experiments carried out on the capacities of extramatrical hyphae to bind Cd and Zn have shown that AM fungal mycelium has a high metal sorption capacity and a cation exchange capacity (CEC) comparable to other microorganisms. Metal sorption by AM fungi was rapid and appeared mainly to be due to passive adsorption. It was also noticed that the highest adsorption took place in a metal-tolerant *G. mosseae* isolate and intermediate for fungus isolated from a soil treated with metal contaminated waste. *G. mosseae* absorbed ten times more metals than the commonly used biosorption organism *Rhizopus arrhizus* (Joner et al. 2000). These results confirm the AM involvement in plant protection against excess heavy metal uptake and more binding capacity of mycorrhizal fungi than others.

9.3
Arbuscular Mycorrhizal Fungi in Relation to Soil Salinity

Soil salinity is a problem of great concern. About one third of the world's irrigated land and half of the land in arid, semi-arid and tropical regions is not in use due to salinity (Juniper and Abbott 1993; Briccoli-Bati et al. 1994; Giri and Chamola 1999; Giri et al. 2002, 2003a, b; Giri and Mukerji 2003). Ten million hectares of irrigated agricultural land is abandoned annually. In India alone, 75 Mh of land has lost its fertility because of the deposition of salts. Most of the areas of Uttar Pradesh (Indo-gangatic plain), Rajasthan and Haryana are adversely affected due to a high salt concentration and have lost their fertility (Giri et al. 2000). Thus, there is an urgent need for improving such degraded wastelands to combat the increasing pressure of the alarming rise in population on agriculture by low input technologies. Several microorganisms are known to have the ability to tolerate high salt concentrations. They can survive under a wide salinity range. Among these microorganisms, mycorrhizal fungi are of growing concern (Table 5). Several field experiments have demonstrated the impacts of high salt concentration on AM fungi. Arbuscular mycorrhizal fungi-colonized plants established and survived better in soils with an electrical conductivity of 10 dS/m or higher (Al-Karaki et al. 2001; Giri et al. 2003a).

Table 5. Plants and AM fungi that tolerate high salinity levels

Plants	AM fungi	EC	References
Bell pepper	*Glomus fasciculatum*	1–12	Hirrel and Gerdemann (1980)
Bell pepper	*Gigaspora margarita*	1–12	Hirrel and Gerdemann (1980)
Onion	*G. fasciculatum*	1–12	Hirrel and Gerdemann (1980)
Onion	*Gigaspora margarita*	1–12	Hirrel and Gerdemann (1980)
Indian ricegrass	*Entrophospora infrequences*	1.6–2.0	Stahl and Williams (1986)
Indian ricegrass	*G. fasciculatum*	1.6–2.0	Stahl and Williams (1986)
Indian ricegrass	*Glomus microcarpum*	1.6–2.0	Stahl and Williams (1986)
Indian ricegrass	*Glomus mosseae*	1.6–2.0	Stahl and Williams (1986)
Yellow sweetclover	*E. infrequences*	1.6–2.0	Stahl and Williams (1986)
Yellow sweetclover	*G. fasciculatum*	1.6–2.0	Stahl and Williams (1986)
Yellow sweetclover	*G. microcarpum*	1.6–2.0	Stahl and Williams (1986)
Yellow sweetclover	*G. mosseae*	1.6–2.0	Stahl and Williams (1986)
Big sagebrush	*G. fasciculatum*	0.6	Stahl et al. (1988)
Big sagebrush	*G. microcarpum*	0.6	Stahl et al. (1988)
Big sagebrush	*E. infrequences*	2.6–3.8	Stahl et al. (1988)
Big sagebrush	*G. fasciculatum*	2.6–3.8	Stahl et al. (1988)
Big sagebrush	*G. macrocarpum*	2.6–3.8	Stahl et al. (1988)
Big sagebrush	*G. microcarpum*	2.6–3.8	Stahl et al. (1988)
Big sagebrush	*G. mosseae*	2.6–3.8	Stahl et al. (1988)
Acacia auriculiformis	*G. fasciculatum*	1–10	Giri et al. (2003b)
Acacia auriculiformis	*G. macrocarpum*	1–10	Giri et al. (2003b)
Sesbania aegyptiaca	*G. macrocarpum*	1–5	Giri and Mukerji (2003)
Sesbania grandiflora	*G. macrocarpum*	1–5	Giri and Mukerji (2003)

A high salt concentration inhibits the germination of AM fungal spores as well as the growth of hyphae, resulting in decreased growth and development of AM fungal density in soil (Juniper and Abbott 1993; Al-Karaki and Clark 1998; Al-Karaki et al. 2001). McMillen et al. (1998) found that an increasing concentration of NaCl inhibits either the hyphal growth or the infectivity of hyphae and AM colonization of plant roots. This may be due to the adverse effect of NaCl on the hyphal growth as well as the altered supply of carbohydrates from the plant to the fungus.

In our laboratory, the effect of AM fungi *Glomus fasciculatum* and *G. macrocarpum* was investigated on growth, development and nutritional responses of *Acacia auriculiformis*, under nursery and field conditions (Giri et al. 2003a). Plants were grown under different salinity levels imposed by 3, 5 and 10 dS/m solutions of 1 N NaCl. Both AM fungi protected the host plant against the detrimental effects of salinity. The extent of AM response on growth as well as root colonization varied with their species and salinity levels. Mycorrhiza-inoculated plants accumulated greater amounts of P

and K, while Na uptake was lowered as salinity increased. Greater nutrient acquisition, change in root morphology and electrical conductivity of soil in response to AM colonization were observed during the course of the study and may be the possible mechanisms protecting the plant from salt stress (Giri et al. 2003a). Inoculation of *Sesbania grandiflora* and *Sesbania aegyptiaca* with AM fungus *Glomus macrocarpum* had a significant increase in growth and biomass production (Giri and Mukerji 2003). Under saline conditions, *Sesbania* spp. had a higher amount of Mg and reduced Na content in shoot tissues; the increased Mg uptake and reduced sodium uptake helped in chlorophyll synthesis. AM fungus also increased the establishment and survival of tree plants. Both the tree species were highly dependent on *G. macrocarpum* (Giri and Mukerji 1999; Giri et al. 1999).

The response of mycorrhizal and nonmycorrhizal *Olea europaea* under saline conditions with or without supplemental Ca resulted in less depolarization, at the cellular level (cell transmembrane electropotential), in roots of mycorrhizal than nonmycorrhizal plants. Supplemental Ca in the saline treatments had a protective effect on membrane integrity canceling or reducing the differences in depolarization between mycorrhizal and nonmycorrhizal plants. Mycorrhizal roots accumulated greater quantities of Na, K, and Ca and exhibited a lower K:Na ratio, but in leaves, mycorrhizal plants had a greater K:Na ratio than nonmycorrhizal plants (Rinaldelli and Mancuso 1996).

Mycorrhizal colonization brought about a noticeable improvement in salt-tolerance in olive plants, which was clearly demonstrated by trends in impedance parameters (Mancuso and Rinaldelli 1996). Uncer saline conditions, the electrical impedance parameters in shoots and leaves of olive plants were studied to understand variations in extracellular resistance, intracellular resistance and the state of the membrane in mycorrhizal and nonmycorrhizal plants. There was a reduction in extra- and intracellular resistance for nonmycorrhizal plants with increased NaCl concentration (Mancuso and Rinaldelli 1996). Ezz and Nawar (1994) found that sour orange seedlings irrigated with water containing 450 ppm salt reduced total leaf chlorophyll, peroxidase activity, starch and total carbohydrate concentration in leaves and roots. Inoculation with *Glomus intraradices* increased total chlorophyll, polyphenol activity, leaf and root sugars, and carbohydrate concentrations, but peroxidase activity was not altered.

9.4 Arbuscular Mycorrhizal Fungi in Relation to Water and Drought Stress

Arbuscular mycorrhizal fungi often results in altered rates of water movement into, through and out of host plants, with consequent effects on tissue hydration and leaf physiology (Smith and Read 1997; Auge 2000, 2001). The notation that AM effects on water relations were mainly nutritional in nature was prevalent for several years, i. e., the behavior of mycorrhizal and nonmycorrhizal plants altered because plants differed in size or tissue P concentrations. Various reports indicated that water relations and gas exchange of soybean could be affected by AM symbiosis independently of P nutrition (Bethlenfalvay et al. 1988; Auge 2001).

A few studies have shown important AM effects on stomata conductance and water potential of host plants (Gupta 1991; Koide 1993; Auge 2000). These studies suggested that such alterations in mycorrhizal plants are due to hormonal involvement, more effective scavenging of soil water, possibly through improved soil/root contact, stimulation of gas exchange through increased sink strength with possible effects on osmotic adjustment, and contributions of soil hyphae to water absorption (for more details, see Auge 2001). Many workers have studied water transport in terms of hydraulic conductivity of the root. Koide (1993) suggested that hydraulic conductivity is generally not improved by AM symbiosis in the absence of AM-induced growth or P effects. In fact, hydraulic conductivity was lower in mycorrhizal than in nonmycorrhizal roots when plants of similar size were examined (Graham et al. 1987; Auge 2001). In studies comparing AM and non-AM plants of either dissimilar size or tissue P concentrations, hydraulic conductivity was usually higher in AM than in non-AM roots (Cui and Nobel 1992), but not always (Syvertsen and Graham 1990). *Glomus fasciculatum* colonization increased whole plant, soil-to-root and root-to-leaf hydraulic conductance in *Bouteloua* (Allen et al. 1981; Allen 1982) and decreased soil-to-plant hydraulic conductance in *Bromus* relative to similarly sized nonmycorrhizal plants. AM hyphae were reported to enhance water uptake in sunflower and cowpea and lettuce, but not in clover or couchgrass or wheat (Faber et al. 1990; Tarafdar 1995; Auge 2001).

Arbuscular mycorrhiza symbiosis usually increased host growth rates during drought by affecting nutrient acquisition and possibly hydration. AM fungi have also typically increased water use efficiency and colonization by different fungi has affected water use efficiency differently (Simpson and Daft 1990). AM effects on host growth during drought are often related to improved P acquisition, as the availability of P in soils is reduced by soil drying. Copper and zinc concentrations were each higher in leaves of

drought-affected mycorrhizal than nonmycorrhizal plants (Subramanian and Charest 1995; Giri and Chamola 1999).

Under drought conditions, inoculation of soybean plants with the AM fungus *Glomus mosseae* enhanced nodule dry weight and increased its leghemoglobin and protein contents as well as the nodule activity, thus helping to alleviate drought-induced nodule senescence in legume plants (Porcel et al. 2003). Drought considerably enhanced oxidative damage to lipids and proteins in nodules of nonmycorrhizal plants, whereas mycorrhizal treatments were protected against oxidative damage. Therefore, the alleviation of oxidative damage in nodules of AM plants has been suggested as an important mechanism involved in the protective effects of the AM symbiosis against premature nodule senescence (Ruiz-Lozano et al. 2001).

10
The Mycorrhizosphere

The rhizosphere is defined as the narrow zone (1–2 mm) of soil around the plant roots which is influenced by the presence and activity of the root. This area has the largest microbial activity of soil since it represents an important source of nutrients and physical support for many microorganisms (Weller and Thomashow 1994; Varma et al. 1999). The constant release of exudate, cell debris, mucilage or lysates provides the nutrient requirements of most saprophytic microbes. The root itself is a perfect niche for some symbiotic microorganisms such as *Rhizobium* or mycorrhizal fungi, and for other microorganisms intimately associated with the roots such as the so-called plant growth promoting rhizobacteria (PGPRs). The rhizosphere is a dynamic environment where microbial interactions take place constantly and may significantly affect plant growth and health. The microbial impact on plant growth is called the rhizosphere effect and is due to the microbial production of plant hormones, enzymes, or changes in the nutrient availability for the plant (Azcon-Aguilar and Barea 1992).

The rhizosphere of mycorrhized plants is very different from the rhizosphere of the same plant when nonmycorrhized. First, the prolongation of the root absorption ability in the form of fungal external mycelium increases the nutrient depletion area surrounding the roots. Secondly, the pattern of root exudation of mycorrhizal plants is altered and consequently, the physico-chemical soil properties, such as pH, moisture, nutrient content, organic matter or soil aggregation are normally modified around the mycorrhizal roots. The mycorrhizae act in modifying the nutrient availability for the rest of the rhizospheric microorganisms, and also in providing an additional ecological niche for these microorganisms. All these crucial changes due to mycorrhizal formation have caused some authors rename

Table 6. Synergistic interactions between mycorrhizal fungi and other rhizosphere microorganisms. (Modified from Bansal et al. 2000)

Mycorrhizal fungi	Rhizosphere microorganisms
Glomus fasciculatum	*Frankia*
G. fasciculatum	*Streptomyces cinnamomeous*
Glomus mosseae	*Rhizobium leguminosarum*
G. versiforme	*R. loli*
Glomus fasciculatum	*Beijerinckia*
G. fasciculatum	*Azotobacter chroococcum*
G. fasciculatum	*Azospirillium brasilense*
G. versiforme	*Corynebacterium*
Glomus versiforme	*Pseudomonas* sp.
Endogone sp.	*Agrobacterium* sp.
	Pseudomonas sp.
G. macrocarpum	*Bacillus megaterium*
	Pseudomonas fluorescence
Glomus macrocarpum	*Cladosporium* sp.
	Gliocladium virens
G. intraradices	*Fusarium oxysporium* f.sp. *chrysanthemi*

Table 7. Antagonistic interactions between mycorrhizal fungi and rhizosphere microorganisms. (Modified after Bansal et al. 2000)

Mycorrhizal fungi	Soil microorganisms
Glomus mosseae	*Phytophthora nicotianae* var. *parasitica*
G. macrocarpum	*Fusarium* sp.
G. intraradices	*Fusarium oxysporium* f.sp. *radicic-lycopersici*
G. intraradices	*Ordium lini*
G. fasciculatum	*Pythium ultimum*
G. fasciculatum	*Aphanomyces euteiches*
Glomus sp.	*Verticillium albo-atrium*
Glomus sp.	*Rhizoctonia solani*
G. fasciculatum	*Phytophthora perasitica*
G. fasciculatum	*P. fragariae*
G. etunicatum	*P. fragariae*
G. fistulosum	*Meloidogyne hapla*
Gigaspora margarita	*Pratylenchus vulnus*
G. intraradices	*Pratylenchus vulnus*
G. etunicatum	*Rhodopholus similis*
G. manihotis	*M. incognita*
G. mosseae	*Azotobacter chrococcum*

this area as the mycorrhizosphere effect (Linderman 1988). The microbial components of this mycorrhizosphere have normally an increased activity which, in turn, may affect plant growth and health, and also modify the

behavior of its fungal counterpart. These complex interactions are crucial to understanding the hidden world under the soil surface. Interactions among mycorrhizal fungi and other soil microorganisms are reciprocal, i. e., mycorrhizal fungi affect the other microbes and other microbes in turn affect the mycorrhizal association. The interaction can be synergistic and antagonistic, and is summarized in Tables 6 and 7, respectively.

11
Interactions in the Mycorrhizosphere

11.1
Interactions at the Pre-Symbiotic Stage

During the pre-symbiotic phase, the AM fungi do not interact actively with the rest of the soil microbiota since their saprophytic growth is very limited and mainly supported by the endogenous lipid reserve of the spore. Nevertheless, the positive influence of certain microorganisms on AM fungal germination has been reported. The presence of certain contaminant bacteria in the germination media accelerated the germination of AM fungal spores (Tylka et al. 1991; Carpenter-Boggs et al. 1995), although there are very few reports showing a net increase in the final number of germinated spores due to any microbial interaction. The addition of antibiotics to prevent bacterial contamination of the spores also inhibits germination of *Glomus versiforme*. Some of the bacteria involved in this effect are *Pseudomonas* and *Cornybacterium*. Soil fungi have also been shown to exert a beneficial effect on AM fungal spore germination. *Trichoderma* sp. hastened spore germination of *Glomus mosseae* in water agar (Calvet et al. 1992). In addition to the stimulatory effect on germination, most microorganisms tested have been shown to exert a stimulatory effect on hyphal growth, branching pattern, vegetative spore or auxiliary cell formation of AM. These effects are species-specific since not all bacterial or fungal treatments can induce them, and the magnitude of the effects is also dependent on the specific interaction.

Soil microorganisms also have detrimental effects on AM fungi. Thus, it has been observed that two AM fungi, *Glomus etunicatum* and *Glomus mosseae*, were only able to germinate in a certain soil after disinfection, showing a fungistatic ability exerted by some soil microbes. Moreover, the fungistasis was restored when the original nonsterile sieved soil was added to the pasteurized soil. Wilson et al. (1989) showed that this fungistasis could be due to the competition for P because it was reverted by addition of P to the soil. In addition, the spores and the emerging hyphae can contribute to microbial nutrition since they are sometimes observed to be

parasitized by other fungi, actinomycetes or amoeboid organisms (Paulitz and Linderman 1991; Requena et al. 1996).

A very novel and attractive topic in the context of mycorrhiza-bacterial interactions was raised with the recent disclosure of the existence of an endosymbiotic bacteria living inside AM fungal spores. The discovery of the presence of these bacteria-like organisms (BLOs) in the cytoplasm of the spore was already reported in 1973, but the impossibility of cultivating them hampered their study (Perotto and Bonfante 1997). Recently, by using the PCR technique, it has been possible to amplify bacterial DNA from spores of *Gigaspora margarita*, and to determine their taxonomic position inside the genus *Burkholderia* using 16S rDNA primers (Bianciotto et al. 1996). So far, the role of BLOs is unknown, but current research on the topic seems to indicate a possible implication in the nitrogen metabolism (Perotto and Bonfante 1997).

11.2 Interactions at the Post-Symbiotic Stage

Given the obligate biotrophism of AM fungi, it is logical that most of the interactions between this group of fungi and the rest of the soil microbiota take place during their symbiotic phase. This fact hinders the analysis of such interactions in detail, because many of the effects and also the causes involved are mediated through the plant. It has been described that the root colonization rates of AM fungi can be improved by the presence of certain microorganisms, such as *Azospirillum*, *Rhizobium*, *Acetobacter, Pseudomonas* (Staley et al. 1992; Barea et al. 2002a). Some microbes have been shown to induce specifically the arbuscular formation, while having a slight, or no effect on the total percentage of root colonization (arbuscules + internal mycelium; Gryndler et al. 1995). It is not well established, however, whether these effects take place through a direct stimulation of AM fungi or via the stimulation of the root exudate production, which is strongly correlated to mycorrhiza colonization (Barea et al. 2002a). The extracellular polysaccharides (EPS) from *Rhizobium meliloti* were able to increase the formation of mycorrhizae in alfalfa plants, probably due to the ability of the EPS to increase exudation rates of their specific legume. Nevertheless, there are also many other beneficial microbial effects on the symbiosis with AM fungi, which do not necessarily correlate with an increase in the root colonization rate (Requena et al. 1997, 2001).

Moreover, in the rhizosphere AM fungi interact with various types of other soil bacteria. These interactions can be considered as: (1) interaction with plant growth-promoting rhizobacteria (PGPRs); (2) interaction with

plant symbiotic N_2-fixing rhizobacteria; (3) interaction with phosphate-solubilizing bacteria and (4) interaction with soil-borne pathogens.

11.3 Interactions Between Arbuscular Mycorrhizal Fungi and Plant Growth-Promoting Rhizobacteria

The plant growth-promoting rhizobacteria (PGPRs) are involved in the nutrient cycling and the protection of the plant against plant diseases (Dobbelaere et al. 2001; Barea et al. 2002a; Probanza et al. 2002). After the biotrophic colonization of the root cortex, the arbuscular mycorrhizae (AM) develop an external mycelium which is a bridge connecting the root with the surrounding soil microhabitats. Mycorrhizal fungi in the mycorrhizosphere interact with PGPRs. Their activity in soil affects the populations of PGPRs in the rhizosphere both quantitatively and qualitatively. AM fungus, *Glomus fasciculatum*, inoculation showed a positive influence on the population of actinomycetes in the tomato rhizosphere. The same effect was observed after inoculation with *Azotobacter chroococcum*. The population of fluorescent pseudomonads was reduced significantly after inoculation of cucumber seedlings with *Glomus intraradices*, but not after inoculation with *Glomus etunicatum*. *Glomus fasciculatum* in the rhizosphere of sweet corn and clover reduced the population of *Streptomyces* sp. and chitinase-producing actinomycetes. Interaction studies with bacteria have indicated the longer survival of bacteria in the rhizosphere of the mycorrhizal root. Mycorrhizal fungi positively influence the survival of *Azotobacter paspali* in the rhizosphere of *Paspalum notatum* (Barea et al. 1998, 2002b). Secilia and Bagyaraj (1987) reported a higher bacterial population and number of nitrogen fixers, *Streptomyces* and *Pseudomonas solanacearum* in the rhizosphere of AM fungal-colonized plants. Moreover, it has been reported that plants in the presence of bacteria and AM fungi produced more phytohormones. These hormones and growth-promoting substances have a direct effect on the root biomass of the plant and germination, penetration, and establishment of AM fungi (Barea et al. 2002b).

11.4 Interactions Between Arbuscular Mycorrhizal Fungi and N_2-Fixing Bacteria

Nitrogen fixation is a key factor in biological productivity, it being accepted that more than 60% of the N-input to the plant community has a biological origin, with half of this input due to the symbiotic plant–bacteria

systems, particularly those involving legumes (Postgate 1998). The bacterial partner in the symbiotic relationship with legume species belongs to the genera *Rhizobium*, *SinoRhizobium*, *Bradyrhizobium*, *MesoRhizobium*, and *AzoRhizobium*, collectively known as *Rhizobium* or rhizobia, which interact with legume roots, leading to the formation of N_2-fixing nodules (Spaink et al. 1998).

Arbuscular mycorrhiza is one of the most efficient ecological factors in improving growth and N content in legumes (Barea et al. 2002b). *Rhizobium*-associated plants are usually mycorrhizal. The mycorrhizal and *Rhizobium* symbiosis usually acts synergistically on infection rate, mineral nutrition and growth of the plant. AM fungi improve P uptake in conditions where N and P are limited. The higher P concentration in the plant benefits the bacterial symbiont and nitrogenase functioning, leading to increased nitrogen fixation, which in turn promotes root and mycorrhizal development. The AM fungus-mediated enhancement of N_2 fixation can be reduced, but either or both their mean weight and specific nitrogenase activity may increase in legumes. Symbiotic N_2 fixation depends on an adequate steady supply of P to the root nodules. AM fungi play important roles in improving growth, nodulation and N_2 fixation by legume crops symbiotic with *Rhizobium* spp. (Barea et al. 1993). Certain rhizobial strains improve processes involved in AM formation, i. e., spore germination, mycelial growth from the mycorrhizal propagules and "entry point" formation on the developing root system of the common host legume plant (Barea et al. 1996).

The use of ^{15}N-labeled soils has ascertained the effect of microbial interactions on N_2 cycling (Barea et al. 2002b). This methodology confirmed that mycorrhizae improve nitrogen nutrition in crop plants by facilitating the use of certain nitrogen forms that are difficult for nonmycorrhizal plants to exploit (Barea et al. 1993). Measurements of the $^{15}N/^{14}N$ ratio in plant shoots indicated enhancement of the N_2-fixation rates in *Rhizobium*-inoculated mycorrhizal plants, relative to that achieved by the same *Rhizobium* strain in nonmycorrhizal plants (Toro et al. 1997). In addition, mycorrhizae can indirectly enhance biological N_2 fixation, hence facilitating nitrogen input into the plant–soil system, thereby participating in N_2 cycling.

11.5 Interactions Between Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Bacteria

The synergistic interaction of phosphate-solubilizing bacteria (PSB) and mycorrhizal fungi is well reported (Toro et al. 1997; Jeffries and Barea 2001; Barea et al. 2002a; Jeffries et al. 2003). Phosphate-solubilizing bacteria sur-

vived longer around mycorrhizal than nonmycorrhizal roots and acted synergistically with the mycorrhiza to increase plant growth, especially where rock phosphate was added to the soil. Two general types of microbiologically mediated processes for increasing the phosphate availability in soil have been described: those known to promote solubilization of nonavailable P sources in soil, yielding available phosphate ions, and those known to improve plant uptake of the already solubilized phosphate. The first type of activity is carried out by a great number of both saprophyte bacteria and fungi acting on sparingly soluble phosphates in soil, mainly by chelation-mediated mechanisms (Whitelaw 2000), while activity to improve the phosphate uptake properties of the host plants is typically carried out by mycorrhizal fungi (Smith and Read 1997). The external mycelium of mycorrhizal fungi acts as a bridge connecting the root with the surrounding soil microhabitats to extract phosphate ions from soil solution beyond the phosphate-depletion zone surrounding the roots, and transfer these ions to the plant. Thus, by linking the biotic and geochemical proportions of the ecosystem, the mycorrhizal fungi can contribute to P capture and supply, and P cycling (Jeffries and Barea 2001).

The interaction of PSB and AM fungi on plant use of soil P sources having low bioavailability has been evaluated in the soil microcosm using ^{32}P isotope (Toro et al. 1997). The rhizobacteria acted as 'Mycorrhiza-Helper Bacteria', promoting AM establishment by either the indigenous or inoculated AM fungi, while AM formation increased the size of the PSB population. The dual inoculation of PSB and AM fungi significantly increased microbial biomass and N and P accumulation in plant tissues. It was observed from the isotope dilution approach that the mycorrhizal and bacterized plants were using P sources otherwise unavailable to the plant (Barea et al. 2002b). Sharif (1999) studied the interactions among *Bacillus megaterium* var *phosphaticum* and *Glomus manihot* and their effects on growth and N and P uptake of pearl millet. P uptake by plants was significantly increased by the combined inoculation of *G. manihot* and *B. megaterium* var *phosphaticum*. Seed inoculation with *Pseudomonas striata* and *Glomus fasciculatum*, *G. mosseae* and *Gigaspora margarita* resulted in increased root biomass and P uptake in soybean. In a dual inoculation of PSB and AM fungi, the PSB rendered more P soluble, while mycorrhizae enhanced P uptake. The combined inoculation of *Glomus macrocarpum* and *Bacillus polymyxa* resulted in more fruit production due to their synergistic effect of P supply (Chandraghatgi and Sreenivasa 1995).

11.6 Interactions Between Arbuscular Mycorrhizal Fungi and Soil-Borne Pathogens

Many studies have demonstrated that AM fungi inhibit growth of the soil-borne pathogens (Al-Raddad and Adhmad 1995; Azcon-Aguilar and Barea 1996; Mukerji et al. 1997; Sharma et al. 1998; Sharma and Mukerji 1999; Mukerji 1999; Joseph and Sivaprasad 2000; Singh et al. 2000). Since AM fungi are established in the roots of host plants, research on the mycorrhizae and disease incidence has been concentrated on disease caused by soil-borne pathogens only. In the rhizosphere AM fungi occupy a unique ecological position as they are partly inside and partly outside the host thus, root-borne pathogens could directly interact with AM fungi in the mycorrhizosphere. A summary of the AM fungi and soil-borne pathogens is given in the Tables 8 and 9.

12 Conclusion

Microbial survival and reproductive success in many systems require colonization of a surface and/or integration into a biofilm community. Success in a community context requires morphological, physiological, and genetic attributes that have only recently been explored. The development of multicellular biofilm communities represents the interplay of many factors including specific cell–cell interactions and, in many cases, metabolic communications. Microbial interactions enable a variety of microorganisms to coexist in environments in which individual organisms cannot survive. Typically, these communities consist of various microbial aspects with different metabolic activities and nutritional requirements. Particularly within a biofilm, temporal and spatial formation of chemical microzones, positioning of syntrophic partners and establishment of complimentary metabolic pathways may all occur. Interaction between different species and populations is often characterized by close but, in general, poorly understood interdependencies.

Predation may also affect microbial activity. The role of protozoa in regulating population numbers in the microbial community is well recognized. This leads to increased mineralization of carbon, phosphorus, and nitrogen as a result of predation. Collembola are established mycophagous and after mites and nematodes they are among the soil's most abundant microfauna. They distribute the mycorrhizosphere flora.

Currently, there is considerable resistance to the use of chemical insecticides, pesticides, herbicides, weedicides and fungicides and fertilizers, be-

Table 8. Influence of VAM fungi on the control of nematodes. (Modified after Singh et al. 2000)

Host	Nematodes	AM fungi	Effect on host
Allium cepa	*Meloidogyne hapla*, *Meloidogyne incognita*	*Glomus etunicatum*	Tolerance of plants against nematodes increased
Avena sativa	*Meloidogyne incognita*	*Glomus mosseae*	Inhibitory effect on the disease incidence and development
Citrus limon	*Radopholus citrophilus*	*Glomus intraradices*	Larger shoot and root weights, lower nematode population densities
Citus sp.	*Radopholus citrophilus*	*Glomus etunicatum*	Increased host tolerance to nematodes
Citus sp.	*Radopholus citrophilus*	*Glomus intraradices*	Increased host tolerance to nematodes
Citus sp.	*Tylenchulus semipenetrans*	*Glomus fasciculatum*	Growth enhanced
Citus sp.	*Tylenchulus semipenetrans*	*Glomus mosseae*	Growth enhanced
Cydonia oblonga	*Pratylenchus vulnus*	*Glomus intraradices*	Growth favored and protection against nematodes
Daucus carota	*Meloidogyne hapla*	*Glomus mosseae*	Inhibitory effect on disease development
Elettaria cardomomum	*Meloidogyne incognita*	*Glomus fasciculatum*, *Gigaspora margarita*	Improved plant growth and reduced nematode population
Glycine max	*Heterodera glycines*	*Gigaspora margarita*	Growth enhanced
Glycine max	*Meloidogyne incognita*	*Gigaspora heteogama*	Growth enhanced
Glycine max	*Meloidogyne incognita*	*Gigaspora margarita*	Growth enhanced
Glycine max	*Meloidogyne incognita*	*Gigaspora margarita*	Growth enhanced
Gossypium hirsutum	*Aphelenchus avenae*	*Gigaspora margarita*	Growth stimulated
Gossypium hirsutum	*Meloidogyne incognita*	*Glomus fasciculatum*	Reduction in egg and nematode number/g of root
Lycopersicon esculentum	*Meloidogyne incognita*	*Glomus fasciculatum*	Reduction in number and size of root galls produced by nematodes
Lycopersicon esculentum	*Meloidogyne incognita*	*Gigaspora margarita*	Growth inhibited
Lycopersicon esculentum	*Meloidogyne javanica*	*Glomus mosseae*	Suppression of gall index and the average number of galls per root system
Lycopersicon esculentum	*Rotylenchus reniformis*	*Glomus fasciculatum*	Reduced juvenile penetration and development of nematode
Musa acuminata	*Radopholus similis*	*Glomus fasciculatum*	Increased length, dry and fresh weight
Nicotiana tabacum	*Meloidogyne incognita*	*Glomus mosseae*	Improved plant growth with lower incidence of disease
Nicotiana tabacum	*Meloidogyne incognita*	*Glomus fasciculatum*	

Table 9. Influence of AM fungi on the control of fungal pathogens. (Modified after Singh et al. 2000)

Host	Disease	Pathogen	AM fungi	Effect on host
Allium cepa	White rot	*Sclerotium cepivorum*	*Glomus* sp.	Delayed disease epidemic and increased the yield by 22%
Asparagus officinalis	Root rot	*Fusarium oxysporum*	*Glomus fasciculatum*	Lower incidence of disease
Cajanus cajan	Pigeon pea blight	*Phytophthora drechsleri* f.sp. *cajani*	*Gigaspora calospora*	Inhibitory effect on the development of disease and improved plant growth
Capsicum frutescens	Wilt	*Fusarium oxysporum*	*Glomus mosseae*	No effect
Chamaecyparis lawsoniana	Root rot	*Phytophthora cinnamomi*	*G. mosseae*	Delayed onset of disease incidence and the population of pathogens plant growth
Cicer arietinum	Wilt	*Fusarium oxysporum*	*G. mosseae*	No effect on wilt incidence
Citus sp.	Wilt	*Phytopthora parasitica*	*Gi. margarita, Glomus constrictum*	Significant increase in root dry weight and better growth of plants
Cuminum cyminum	Wilt	*Fusarium oxysporum* f.sp. *cumini*	*Acaulospora laevis, Gigaspora calospora*	Increased nutrient uptake and reduced disease severity
Glycine max	Root rot	*Fusarium solani, Macrophomina phaseolina, Rhizoctonia solani*	*Glomus mosseae*	Reduced disease symptoms
Glycine max	Root rot	*Phytopthora megasperma* var. *sojae*	*Glomus macrocarpum* var. *geosporum*	Increased severity of disease, internal stem discoloration
Hevea brasiliensis	Leaf blight	*Microcyclus ulei*	*Glomus etunicatum*	Increased resistance to leaf blight, lesion size and production of spores of the pathogen significantly lower
Hordeum vulgare	Root rot	*Bipolaris sorokiniana*	*Glomus* sp.	Severity of disease reduced

Linum usitatissimum	Wilt	*Fusarium oxysporum*, f.sp. *oidium lini*	*Glomus intraradices*	AM plants showed increased tolerance to *F. oxysporum*
Lucerne sp.	Wilt	*Fusarium oxysporum* f.sp. *medicagnisis*, *Verticilium aldoatrrum*	*Glomus fasciculatum*, *Glomus mosseae*	Lower incidence of wilt and increase in plant growth
Lycopersicon esculentum	Root rot	*Fusarium oxysporum* f.sp. *radices-lycopersici*	*Glomus intraradices*	Reduction in disease incidence and the population of pathogen and improvement in plant growth
Lycopersicon esculentum	Wilt	*Fusarium* sp.	*Glomus mosseae*	Reduced wilt to 11% as against 45% in nonmycorrhizal plants
Lycopersicon esculentum	Corky root disease	*Pyrenochaeta lycipersici*	*Glomus caledonium*	Disease index lower and root growth better
Lycopersicon esculentum	Root rot	*Phytopthora nicotiana* var. *parasitica*	*Glomus mosseae*	Decreased root necrosis
Nicotiana tabacum	Root rot	*Thielaviopsis basicola*	*Glomus microcarpum*	Reduction in the number of pathogen propagules
Nicotiana tabacum	Root rot	*Thielaviopsis basicola*	*Glomus monosporum*	Better tolerance to pathogen, higher root and leaf dry weight
Phaseolus aureus	Root rot	*Macrophomina phaseolina*	*G. mosseae*	Disease incidence reduced
Pisum sativum	Root rot	*Aphanomyces euteiches*	*G. fasciculatum*	Infection suppressed
Vicia faba	Wilt	*Fusarium oxysporum*	*G. mosseae*, *Gigaspora calospora*	More incidence of disease when plants has more AM infection
Vigna unguiculata	Root rot	*Macrophomina phaseolina*	*Glomus etunicatum*	Disease incidence reduced

cause of their hazardous influence on the environment, and on soil, plant, animal, and human health. Hence, the use of biofertilizers and biocontrol agents is recommended in practical agriculture, forestry, horticulture and flori-culture. There are a large number of bacteria and fungi that solubilize unavailable forms of phosphate and make it available for plant growth. Among them, mycorrhizae form symbiotic associations with the roots of plants and help in the uptake of phosphorus from the labile pool.

Colonization by mycorrhizal fungi alters the physiology, morphology, and nutritional status of the host–soil biota and structure. There is no host–plant or host–soil specificity, but some plant–fungus and some soil–fungus combinations are more effective than others. In all ecosystems, mycorrhizae link plants and soil, and that coupling influences most of the dynamics that occur in the mycorrhizosphere. Research efforts on mycorrhizosphere are fragmented, data synthesis and modeling on the ecosystem level are lacking. There is no information on the genetic potential of mycorrhizal fungi and very little on the associated plant growth promoting rhizobacteria (PGPRs) to tolerate environmental or cultural conditions to modulate host–plant and soil responses. In damaged or disturbed coupling, there is a need to develop an understanding and technology to recouple plants and soil with mycorrhizae, emulating the balance that occurs in undisturbed ecosystems and returning our crop production systems to a level of sustainability that allows for reduced inputs of chemicals.

Our knowledge of the regulation of a specific process may be detailed, but our understanding of its role in microbial survival and proliferation in natural systems is limited. Interpreting how heterotrophic microorganisms respond to and benefit from community growth in the natural environment, as well as the underlying molecular biological mechanisms, awaits application of the range of techniques now available. The science of the mycorrhizosphere is expanding fast and will soon connect the mycorrhizae with other sciences of plant systematics, ecology, and physiology, molecular biology, horticulture, agronomy, soil science, climatology, and certainly, plant pathology.

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