CHAPTER 12

Mycorrhizal Symbioses

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> *To survive and flourish in the long run, we usually need to develop mutualistic relationships. Nonmutualistic members will eventually disappear or lose influence in the community.*
>
> —J. M. Trappe

Mycorrhiza (*plural* -zae or -zas) refers to a variety of symbiotic associations between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth. The association is characterized by the movement of (1) plant-produced carbon to the fungus and (2) fungal-acquired nutrients to the plant. The term **mycorrhiza**, which literally means *fungus-root*, was first applied to fungus-tree associations described in 1885 by the German forest pathologist A. D. Frank. Since then we have learned that more than 80% of all land plant species form symbiotic associations with fungi. Indeed, the mycorrhizal condition is the rule among plants, not the exception!

The benefits afforded to plants from mycorrhizal symbioses can be characterized either agronomically by increased growth and yield, or ecologically by improved fitness or reproductive ability. In either case, the benefits accrue primarily because mycorrhizae form a critical linkage between plant roots and the soil. Mycorrhizae usually have a proliferation of fungal biomass both in the root and in the soil. The soilborne or **extramatrical hyphae** (outside the root) take up nutrients from the soil solution and transport them to the root. Mycorrhizae thus increase the effective absorptive surface area of the plant. In nutrient-poor or moisture-deficient soils, nutrients taken up by the extramatrical hyphae lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are nonmycorrhizal plants.

The objective of this chapter is to review the biology of mycorrhizal associations and to evaluate their roles in natural and managed ecosystems. Because ecto- and arbuscular mycorrhizae are the most widespread, these types of associations are emphasized.

GLOBAL PERSPECTIVE

Mycorrhizal associations vary widely in structure and function. Despite the many exceptions, it is possible to state broad generalizations about latitude (or altitude), soil properties, and the structure and function of the different mycorrhizal types (Table 12–1) that colonize the dominant vegetation in a gradient of climatic zones (Fig. 12–1). Ericaceous plants, which dominate the high-organic heathland soils of subarctic and subalpine regions, are colonized by a group of ascomycetous fungi (Chapter 6), giving rise to the **ericoid** type of mycorrhiza. This mycorrhizal type is characterized by extensive growth *within* (i.e., intracellular) cortical cells, but little extension into the soil. The fungus produces extracellular enzymes that mineralize organic matter, allowing the plant to access nutrients tied up in the organic materials surrounding the root. Moving along the environmental gradient, coniferous trees replace ericaceous shrubs as dominants. These trees are colonized by a wide range of mostly basidiomycetous fungi (Chapter 6) that grow *between* (i.e., intercellular) root cortical cells forming the **ectomycorrhizal** type of mycorrhiza. Ectomycorrhizal fungi may produce large quantities of hyphae on the root and in soil. These hyphae function in the absorption and translocation of inorganic nutri-

TABLE 12–1   Plant Host, Fungi, and Important Characteristics of Major Types of Mycorrhizal Associations

| Mycorrhizal Type	Hosts Involved	Fungi Involved	Characteristic Structures	Characteristic Functions
Ectomycorrhizae	Mostly gymnosperms Some angiosperms Restricted to woody plants	Mostly basidiomycetes Some ascomycetes Few zygomycetes	Hartig net Mantle Rhizomorphs	Nutrient uptake Mineralization of organic matter Soil aggregation
Arbuscular	Bryophytes Pteridophytes Some gymnosperms Many angiosperms	Zygomycetes/ Glomeromycota	Arbuscules Vesicles Auxiliary cells	Nutrient uptake Soil aggregation
Ericaceous	Ericales Monotropaceae	Ascomycetes Basidiomycetes	Some with hyphae in cell, some with mantle and net	Mineralization of organic matter Transfer between plants
Orchidaceous	Orchidaceae	Basidiomycetes	Hyphal coils	Supply carbon and vitamins to embryo
Ectendomycorrhizae	Mostly gymnosperms	Ascomycetes	Hartig net with some cell penetration Thin mantle	Nutrient uptake Mineralization of organic matter
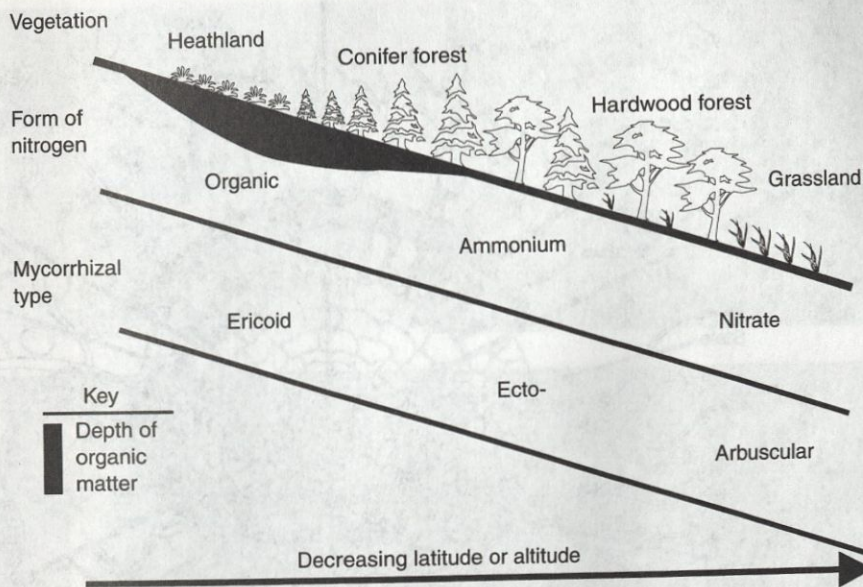
FIGURE 12–1
Changes in dominant vegetation, nitrogen form, and mycorrhizal types with decreasing latitude or altitude. Members of the Ericaceae, which grow in the organic soils of subarctic and subalpine regions, have a characteristic internal fungal colonization termed ericoid mycorrhiza. In contrast, on the mineral soil of warmer and drier climates, grasses and herbs often predominate and these plants are colonized by arbuscular mycorrhiza. Between these zones are found the boreal and temperate forests that often have ectomycorrhiza associated with the dominant trees (e.g., Pinaceae, Fagaceae, and Myrtaceae). *Adapted from Read (1984). Used with permission.*

ents and water, but also release nutrients from litter layers by production of enzymes involved in mineralization of organic matter. At the warmer and drier end of the environmental gradient, grasslands often form the dominant vegetation. In these ecosystems nutrient use is high and phosphorus is frequently a limiting nutrient for growth. Grasses and a wide variety of other plants are colonized by fungi that form arbuscules (highly branched structures—the term literally means little trees; see Fig. 12–4) *within* (i.e., intracellular) root cortical cells, giving rise to the **arbuscular** type of mycorrhiza. These fungi may produce extensive extramatrical hyphae and can significantly increase phosphorus uptake by the plants they colonize. The diversity of these mycorrhizal associations affords plants a range of strategies for efficient functioning in an array of plant-soil systems.

KEY FEATURES OF MYCORRHIZAL TYPES

Ectomycorrhizae

The diagnostic feature of ectomycorrhizae is the presence of hyphae between the root cortical cells producing a net-like structure termed the **Hartig net**, after Robert Hartig who is considered the "founder of forest biology" (Fig. 12–2 a,b). Many
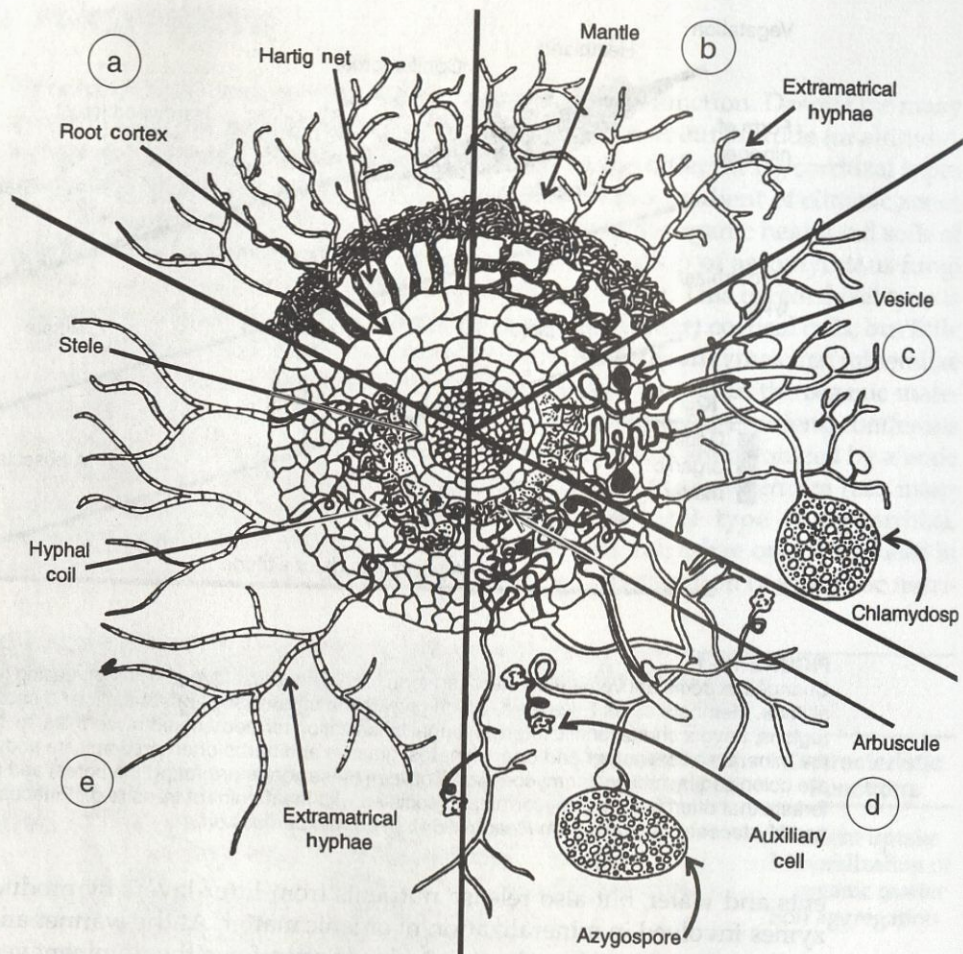
FIGURE 12–2
Diagrammatic cross section of a fine root illustrating key features of several types of mycorrhizae. Ectomycorrhiza typically formed on (a) Fagaceae and (b) Pinaceae hosts. Arbuscular mycorrhizae typically formed by fungi classified in the (c) Glomineae and (d) Gigasporineae. (e) Orchidaceous type of mycorrhiza. *Modified from original drawing by V. Furlan. Used with permission.*

ectomycorrhizae also have a sheath or **mantle** of fungal tissue that may completely cover the absorbing root (usually the fine-feeder roots). The mantle can vary widely in thickness, color, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often fine-root morphology is affected, resulting in root bifurcation and clustering (Fig. 12–3). Contiguous with the mantle are hyphal strands that extend into the soil. Often the hyphal strands will aggregate to form **rhizomorphs** that may be visible to the unaided eye. The internal portion of rhizomorphs can differentiate into tube-like structures specialized for long-distance transport of nutrients and water.

Ectomycorrhizae are found on woody plants ranging from shrubs to forest trees. Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae,
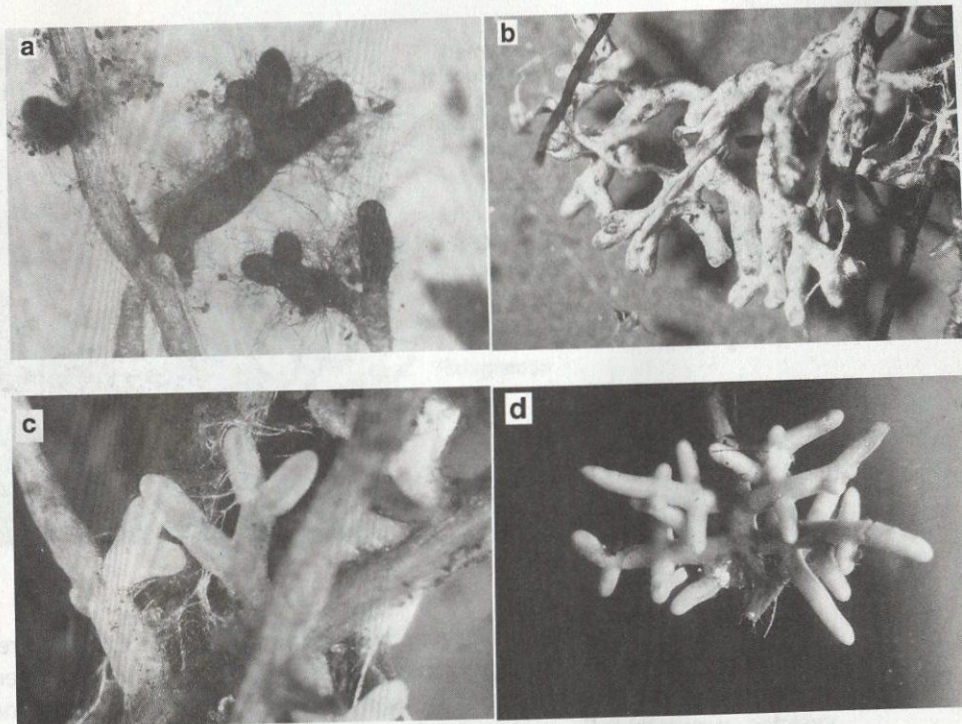
FIGURE 12–3
Examples of ectomycorrhizal short roots of conifers formed by various fungi. (a) *Cenococcum geophilium* (b) *Pisolithus arhizus* = *P. tinctorius*, (c) *Thelephora terrestris* and (d) *Amanita* sp. Root tips range from 1 to 2 mm in diam. *Figure (d) is courtesy of the Department of Plant Pathology, Cornell University. Used with permission*

and Myrtaceae. Over 4,000 fungal species, belonging primarily to the Basidiomycota and fewer to the Ascomycota, are known to form ectomycorrhiza. Many of these fungi produce mushrooms and puffballs on the forest floor. Some fungi have a narrow host range such as *Boletus betulicola* on *Betula* spp., whereas others have a very broad host range such as *Pisolithus tinctorius*, which forms ectomycorrhiza with more than 46 tree species belonging to at least eight genera.

Arbuscular Mycorrhizae

The diagnostic feature of an arbuscular mycorrhiza is the development of a highly branched **arbuscule** within the root cortical cell (Fig. 12–4). The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is **endomycorrhiza**. In this association neither the fungal cell wall nor the host cell membrane is breached. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. This **apoplastic** space prevents direct contact between the plant and fungal cytoplasm and allows for

FIGURE 12–4
Photomicrograph of an arbuscule formed
by *Glomus mosseae* within a cortical cell of
Liriodendron tulipifera (yellow poplar).
*From Kinden and Brown (1975). Used with
permission.*

10μm

efficient transfer of nutrients between the symbionts. The arbuscules are relatively short lived, less than 15 days, and may be difficult to see in field-collected samples.

Other structures produced by some arbuscular mycorrhizal fungi include vesicles, auxiliary cells, and asexual spores (Fig. 12–2 c,d). **Vesicles** are thin-wall, lipid-filled structures that usually form in intercellular spaces. Their primary function is thought to be for storage; however, vesicles can also serve as reproductive propagules for the fungus. **Auxiliary cells** are formed in the soil and can be coiled or knobby. The function of these structures is not known. Reproductive spores can be formed either in the root or more commonly in the soil. Spores produced by fungi forming arbuscular mycorrhizal associations are asexual, forming by the differentiation of vegetative hyphae. For some fungi (e.g., *Glomus intraradices*) vesicles in the root undergo secondary thickening and a septum (cross wall) is laid down across the hyphal attachment leading to spore formation, but more often spores are borne from hyphal swellings in the soil.

The fungi that form arbuscular mycorrhizal are classified in the order Glomales (Fig. 12–5), phylum Zygomycota (Morton, 1988), though a recent study places them in a new phylum, Glomeromycota (Chapter 6). The taxonomy is further divided into suborders based on:

- presence of vesicles in the root and formation of **chlamydospores** (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae, or
- absence of vesicles in the root and formation of auxiliary cells and **azygospores** (spores resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae.

The term **vesicular-arbuscular mycorrhiza** was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder
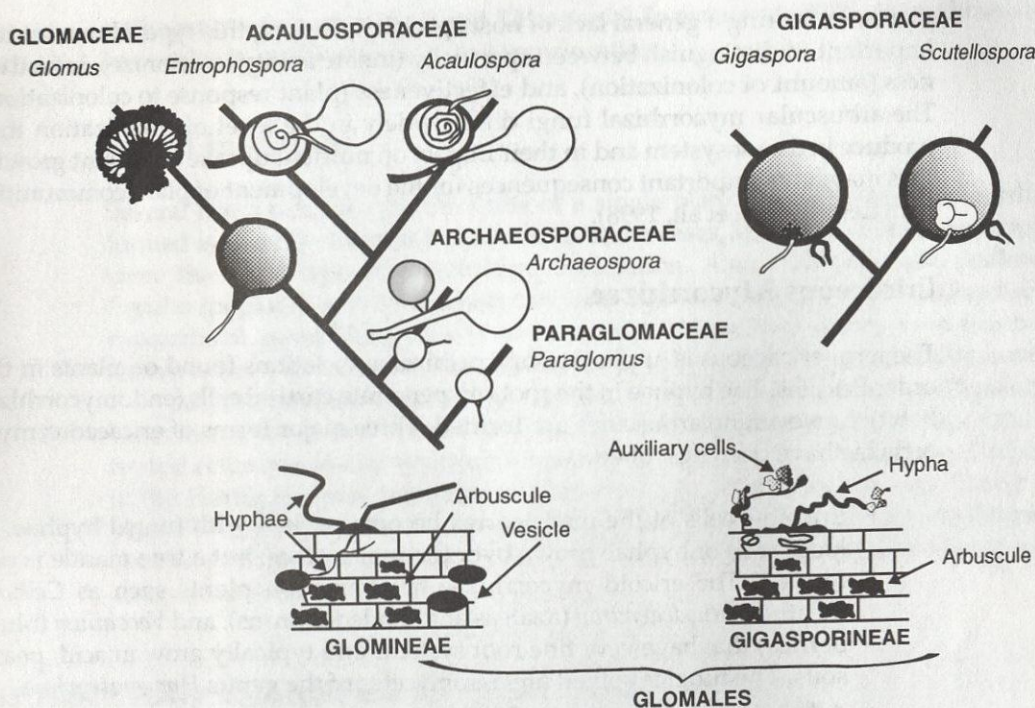
FIGURE 12–5
Taxonomic relationships among arbuscular mycorrhizal fungi. This figure arranges all taxonomic groups hierarchically according to their phylogenetic relationships. It is based on structure and development of the whole organism. All species are grouped together in the Glomales because they form mycorrhizae with arbuscules in root cortical cells. Members of each suborder (Glomineae and Gigasporineae) differ in kinds of mycorrhizal structures and the sequence in which they form. Families (names ending in *aceae*) and genera (names in italics) differ in mode of spore formation, organization, and germination processes. Species are distinguished by differences in subcellular properties of the spore wall or internal flexible walls usually associated with germination (not shown). *Original drawing by J. B. Morton. Used with permission.*

lacks the ability to form vesicles in roots, arbuscular mycorrhizal is now the preferred term. The Glomineae is further divided into families and genera according to the method of spore formation. The spores of arbuscular mycorrhizal fungi are very distinctive. They range in diameter from 10 μm for *Glomus tenue* to greater than 1000 μm for some *Scutellospora* spp. The spores can vary in color from hyaline (clear) to black, and in surface texture from smooth to highly ornamented. *Glomus* forms spores on the ends of hyphae, *Acaulospora* forms spores *laterally* from the neck of a swollen hyphal terminus, and *Entrophospora* forms spores *within* the neck of the hyphal terminus. The Gigasporineae are divided into two genera based upon the presence of inner membranous walls and a **germination shield** (wall structure from which a germ tube can arise) for *Scutellospora* or the absence of these structures for *Gigaspora*. Based upon molecular evidence, two new families (*Archaeosporacea* and *Paraglomus*) have been added to the Glomales, illustrating that the taxonomy of this group is still in a state of flux (Morton and Redecker, 2001).

The arbuscular mycorrhizal type of symbiosis is very common as the fungi involved can colonize a vast taxonomic range of both herbaceous and woody

plants, indicating a general lack of host **specificity** among this type. However, it is important to distinguish between specificity (innate ability to colonize), **infectiveness** (amount of colonization), and **effectiveness** (plant response to colonization). The arbuscular mycorrhizal fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth. This may have important consequences for the development of plant communities (van der Heijden et al., 1998).

Ericaceous Mycorrhizae

The term ericaceous is applied to mycorrhizal associations found on plants in the order Ericales. The hyphae in the root can penetrate cortical cells (endomycorrhizal habit); however, no arbuscules are formed. Three major forms of ericaceous mycorrhizae have been described.

- Ericoid—cells of the inner cortex become packed with fungal hyphae. A loose welt of hyphae grows over the root surface, but a true mantle is not formed. The ericoid mycorrhizae are found on plants such as *Calluna* (heather), *Rhododendron* (azaleas and rhodedendrons), and *Vaccinium* (blueberries) that have very fine root systems and typically grow in acid, peaty soils. The fungi involved are ascomycetes of the genus *Hymenoscyphus*.
- Arbutoid—characteristics of both ectomycorrhizae and endomycorrhizae are found. Intracellular penetration can occur, a mantle forms, and a Hartig net is present. These associations are found on *Arbutus* (Pacific madrone), *Arctostaphylos* (Bearberry, Hairy Manzanita), and several species of the Pyrolaceae. The fungi involved in the association are basidiomycetes and may be the same fungi that colonize ectomycorrhizal tree hosts in the same region.
- Monotropoid—the fungi colonize achlorophyllous (lacking chlorophyll) plants in the Monotropaceae (*Monotropa* species; Indian Pipes), producing a Hartig net and mantle. The same fungi also form ectomycorrhizal associations with trees and thereby form a link through which carbon and other nutrients can flow from the autotrophic host plant to the heterotrophic, parasitic plant.

Orchidaceous Mycorrhizae

Mycorrhizal fungi have a unique role in the life cycle of plants in the Orchidaceae. Orchids typically have very small seed with little nutrient reserve. The plant becomes colonized shortly after germination and the mycorrhizal fungus supplies carbon and vitamins to the developing embryo. For achlorophyllous species, the plant depends on the fungal partner to supply carbon throughout its life. The fungus grows into the plant cell, invaginating the cell membrane and forming hyphal coils within the cell (Fig. 12–2e). These coils are active for only a few days, after which they lose turgor and degenerate, and the developing orchid absorbs the nutrient contents. The fungi participating in the symbiosis are basidiomycetes similar to those involved in decaying wood (e.g., *Coriolus*, *Fomes*, *Marasmius*) and

pathogenesis (e.g., *Armillaria* and *Rhizoctonia*). In mature orchids, mycorrhizae also have roles in nutrient uptake and translocation.

Mixed Infections

Several fungi can colonize the roots of a single plant, but the type of mycorrhiza formed is usually uniform for a host. In some cases, however, a host can support more than one type of mycorrhizal association. *Alnus* (alders), *Salix* (willows), *Populus* (poplars), and *Eucalyuptus* can have both arbuscular mycorrhizal and ecto-mycorrhizal associations on the same plant. Some ericoid plants have also been shown to have occasional ectomycorrhizal and arbuscular mycorrhizal colonization.

An intermediate mycorrhizal type can be found on coniferous and deciduous hosts in nurseries and burned forest sites. The **ectendomycorrhiza** type forms a typical ectomycorrhizal structure, except the mantle is thin or lacking, and hyphae in the Hartig net may penetrate root cortical cells. The ectendomycorrhiza is re-placed by ectomycorrhiza as the seedling matures. The fungi involved in the association were initially designated "E-strain" but were later shown to be ascomycetes and placed in the genus *Wilcoxina*.

UPTAKE AND TRANSFER OF SOIL NUTRIENTS

When a nutrient is deficient in the soil solution, the critical root parameter con-trolling its uptake is surface area. Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of the root. For example, Rousseau, Sylvia, and Fox (1994) found that although extramatrical hyphae accounted for less than 20% of the total nutrient absorbing surface mass, they contributed nearly 80% of the absorbing surface area of pine seedlings (Fig. 12–6). It is also important to consider the distribution and function of the extramatrical hyphae. If the mycor-rhiza is to be effective in nutrient uptake, the hyphae must be distributed beyond the **nutrient depletion zone** that develops around the root. A nutrient depletion zone develops when nutrients are removed from the soil solution more rapidly than they can be replaced by diffusion. For a poorly mobile ion, such as phosphate, a sharp and narrow depletion zone develops close to the root (Fig. 12–7). Hyphae can readily bridge this depletion zone and grow into soil with an adequate supply of phosphorus. Uptake of micronutrients such as zinc and copper is also improved by mycorrhizae because these elements are also diffusion-limited in many soils. For more mobile nutrients such as nitrate, the depletion zone is wide and it is less likely that hyphae grow extensively into the zone that is not influenced by the root alone. Another factor contributing to the effective absorption of nutrients by mycorrhizae is their narrow diameter relative to that of roots. The steepness of the diffusion gradient for a nutrient is inversely related to the radius of absorbing unit; therefore, the soil solution will be less depleted at the surface of a narrow absorb-ing unit such as a hypha. Furthermore, narrow hyphae can grow into small soil pores not accessible to roots or even root hairs.
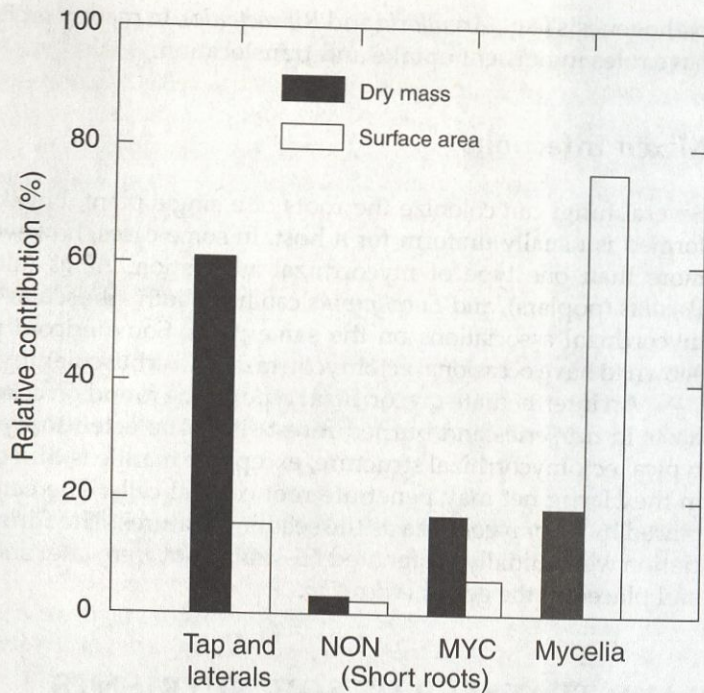
FIGURE 12–6
Relative distribution of dry mass and surface area among root system components of mycorrhizal seedlings colonized by *Pisolithus arhizus*. NON is nonmycorrhizal short root, MYC is mycorrhizal short root. *Modified from Rousseau, Sylvia, and Fox (1994). Used with permission.*
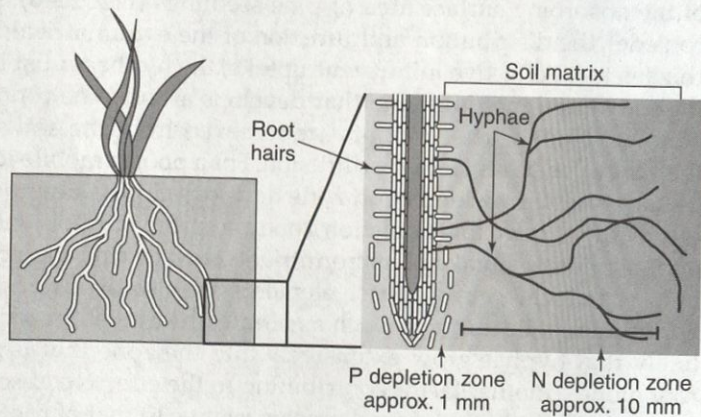

FIGURE 12–7
Diagrammatic representation of the development of a depletion zone adjacent to plant roots. A narrow depletion zone (e.g., 1 mm) usually develops for P (i.e., phosphate) and a wide depletion zone (e.g., 10 mm) develops for N (i.e., nitrate). Mycorrhizal hyphae can generally grow beyond the P-depletion zone, but not beyond the N-depletion zone. *Original drawing by D. A. Zuberer. Used with permission.*

Another advantage attributed to mycorrhizal fungi is access to pools of phosphorus not readily available to the plant (Miyasaka and Habte, 2001). One mechanism for this access is the physiochemical release of inorganic and organic phosphorus by organic acids. Some ectomycorrhizal fungi have been shown to produce large quantities of oxalic acid and this may partially explain enhanced nutrient uptake by ectomycorrhizal roots. A second mechanism by which mycorrhizal fungi release inorganic phosphorus is through mineralization of organic matter. This occurs by phosphatase-mediated hydrolysis of organic phosphate (C-O-P) ester bonds.

Ericoid mycorrhizae and ectomycorrhizae have a special role in the mineralization of nitrogen (Martin, Cliquet, and Stewart, 2001). Most plant litter entering the soil has a high C:N ratio and is rich in lignin and tannins. Only a few mycorrhizal fungi can mobilize nutrients from these primary sources. However, a wide range of ericoid and ectomycorrhizal fungi can obtain nitrogen and other nutrients from secondary sources of organic matter such as dead microbial biomass because they produce hydrolytic and oxidative enzymes capable of depolymerizing organic nitrogenous polymers such as proteins and chitin.

CARBON FLUXES IN MYCORRHIZAL PLANTS

Mycorrhizal fungi range from **obligate symbionts**, which can only obtain carbon from the plant host, as in the case of arbuscular mycorrhizal fungi, to **facultative symbionts**, which can also mineralize organic carbon, as in the case of some ectomycorrhizal species. In nature, however, the heterotrophic mycorrhizal fungi obtain all or most of their carbon from the autotrophic host plant. Ectomycorrhizae and ericoid mycorrhizae transform host carbohydrates into fungal-specific storage carbohydrates, such as mannitol and trehalose that may produce a sink for photosynthate that favors transport of carbohydrate to the fungal partner. In arbuscular mycorrhizal, lipids accumulate in vesicles and other fungal structures and provide an analogous sink for host photosynthate.

As much as 20% of the total carbon assimilated by plants may be transferred to the fungal partner. This transfer of carbon to the fungus has sometimes been considered a drain on the host. However, the host plant may increase photosynthetic activity following mycorrhizal colonization, thereby compensating for carbon "lost" to the soil. Occasionally plant growth suppression has been attributed to mycorrhizal colonization, but usually this occurs only under low-light (photosynthate limiting) or high-phosphorus conditions.

In an ecosystem context, the flow of carbon to the soil mediated by mycorrhizae serves several important functions. For some mycorrhizae, the extramatrical hyphae produce hydrolytic enzymes, such as proteases and phosphatases, which can have an important impact on organic matter mineralization and nutrient availability. Extramatrical hyphae of mycorrhizae also bind soil particles together and thereby improve soil aggregation. Typically there are between 1 to 20 meters of arbuscular mycorrhizal hyphae g^{-1} of soil. Furthermore, a high-molecular-weight glycoprotein (glomalin) is produced in abundance by arbuscular mycorrhizal fungi (Box 12–1). This material accumulates in soil and is positively

BOX 12–1

Contribution of Arbuscular Mycorrhizae to Soil Carbon and Aggregate Stability

Arbuscular mycorrhizae make a significant contribution to soil organic matter by producing a unique glycoprotein called **glomalin**. This material can contribute up to 5% of the total soil carbon in some tropical soils (Rillig, et al., 2001). Glomalin accumulation has a direct effect on soil aggregation that may in turn improve water infiltration rates, aeration, and tilth of the soil.
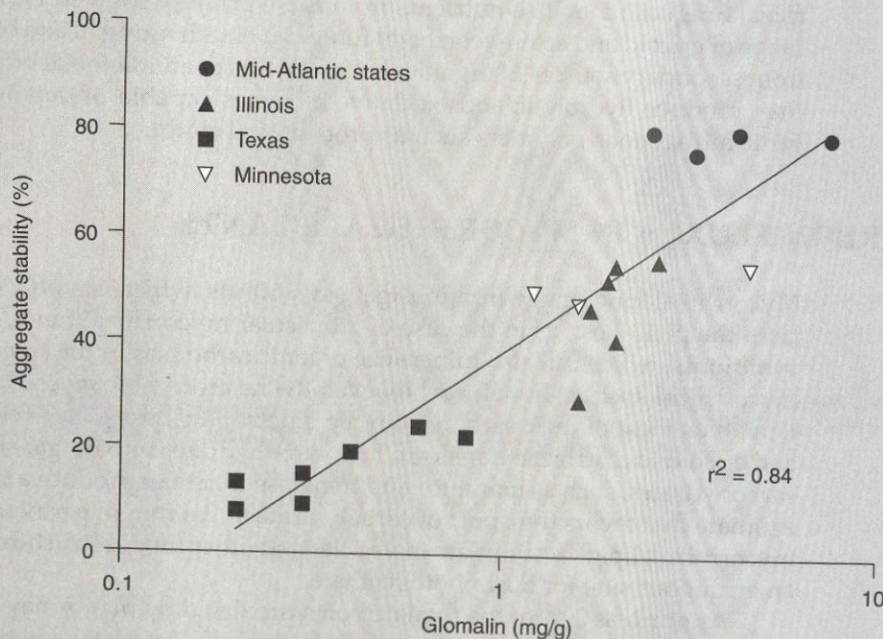
Relationship between stability of 1- to 2-mm-size aggregates and the concentration ($\log_{10}$) of extractable glomalin from soils in four regions of the United States. *From Wright and Upadhyaya (1998). Used with permission.*

correlated with aggregate stability. Soil scientists now realize that carbon flow to the soil is critical for the maintenance of a healthy plant–soil system and should be considered an important benefit of mycorrhizal colonization.

INTERACTIONS WITH OTHER SOIL ORGANISMS

Mycorrhizal fungi interact with a wide assortment of organisms in the **rhizosphere** (Chapter 11). The effect on the mycorrhizal association or a particular component of the rhizosphere can be positive, neutral, or negative. An important consequence of

the carbon flow to the fungal partner previously described is that it results in the development of a unique rhizosphere microbial community called the **mycorrhizosphere** (Hodge, 2000). This altered community may greatly impact nutrient uptake (Box 12–2) and phytoremediation (Joner et al., 2001).

Furthermore, specific bacteria have been shown to associate with ectomycorrhizae and stimulate mycorrhizal formation in conifer nurseries. These "mycorrhization helper bacteria" may even preclude the need for soil fumigation (Garbaye, 1994).

The interaction between rhizobia (Chapter 16) and arbuscular mycorrhizal fungi has received considerable attention because of the relatively high phosphorus demand of N_2-fixation. The two symbioses typically act synergistically, resulting in greater nitrogen and phosphorus content in combination than when each is inoculated onto the legume alone. Legumes are typically coarse-rooted and therefore not efficient in extracting phosphorus from the soil. The arbuscular mycorrhizal fungi

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BOX 12–2

Why Do Mycorrhizae Reduce Manganese Uptake?

Though the uptake of many elements is increased by mycorrhizae, Mn uptake is often reduced in mycorrhizal plants. Manganese is predominantly taken up in the reduced form (Mn^{2+}) and its redox state (Chapter 3) is altered by rhizosphere microorganisms (Marschner, 1988). Posta, Marschner, and Römheld (1994) reported that the proportion of Mn-reducing bacteria was fivefold higher in the rhizosphere of nonmycorrhizal plants compared to the mycorrhizosphere of mycorrhizal plants (as shown below), resulting in lower acquisition of Mn in mycorrhizal plants. This example clearly illustrates that mycorrhizae have a substantial qualitative impact on root-associated microorganisms.
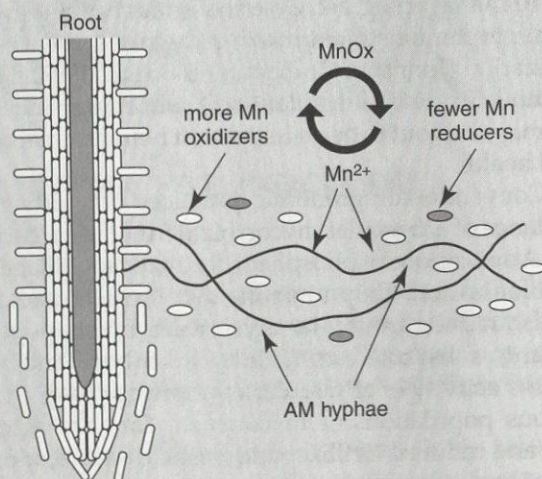
associated with legumes are an essential link for adequate phosphorus nutrition, leading to enhanced nitrogenase activity (Chapter 15) that in turn promotes root and mycorrhizal growth.

Mycorrhizal fungi colonize feeder roots and thereby interact with root pathogens (Chapter 22) that parasitize this same tissue. Therefore, it is not surprising that they have been shown to reduce the incidence and severity of root diseases. Mycorrhizae may stimulate root colonization by selected biocontrol agents, but our understanding of these interactions is meager. Much more research has been conducted on the direct effects of mycorrhizal colonization on root pathogens. The mechanisms proposed to explain this protective effect include:

- development of a mechanical barrier—especially the mantle of the ectomycorrhizae—to resist infection by pathogens,
- production of antibiotic compounds by ectomycorrhizae that suppress the pathogen,
- competition for nutrients in the rhizosphere, including production of siderophores, and
- induction of generalized host defense mechanisms.

FUNCTION OF MYCORRHIZAE IN ECOSYSTEMS

By this point it should be apparent that mycorrhizae have important multifunctional roles in natural ecosystems. Besides improving uptake of poorly mobile nutrients, mycorrhizae impact drought tolerance (Augé, 2001), pathogen interactions (Borowicz, 2001), and contribute to soil quality by channeling carbon to the soil (Jastrow, Miller, and Lussenhop, 1998). There is also mounting evidence that mycorrhizal fungi are important determinants of plant community structure and plant succession (Hart, Reader, and Klironomos, 2001). The function of mycorrhizal fungi in managed agroecosystems is less certain. In some high-input systems it is possible to demonstrate growth reductions due to arbuscular mycorrhiza fungal colonization (Johnson, Graham, and Smith, 1997). Nonetheless, when one takes a more holistic view of the plant-soil continuum, the "cost" of the symbiotic association may turn out to be an important benefit as carbon addition to soil contributes to soil health.

Conventional agronomic practices may adversely affect the diversity and abundance of arbuscular mycorrhizal fungi in agroecosystems (Douds and Millner, 1999). Applications of phosphatic fertilizer and tillage generally reduce mycorrhizal root colonization. Crop rotations that include nonhost plants or long-term fallow may also reduce arbuscular mycorrhiza fungal populations. Pesticides, especially fumigants, substituted aromatic hydrocarbons, and benzimidazoles may adversely affect the activity of arbuscular mycorrhizal fungi in soil. Strategies to enhance indigenous populations of mycorrhizal fungi in agroecosystems include reduced tillage and reduced fertilizer and pesticide applications, as well as careful management of cropping sequences.

BOX 12–3

Factors to Consider When Assessing the Potential Role of Mycorrhizae in an Agroecosystem

- Mycorrhizal dependency of the host crop—**Mycorrhizal dependency (MD)** is usually defined as the growth response of mycorrhizal (M) versus nonmycorrhizal (NM) plants at a given phosphorus level.

$$MD = ((M - NM)/NM) \times 100$$

  Although most agricultural crops form mycorrhizae, not all benefit equally from the symbiosis. Generally coarse-rooted plants benefit more than fine-rooted plants.
- Nutrient status of the soil—Assuming that the major benefit of the mycorrhizal symbiosis is improved P-uptake, the management of mycorrhizal fungi will be most critical when soil phosphorus is limiting. Many tropical soils fix phosphorus and proper mycorrhization of plants is essential to obtain adequate phosphorus nutrition. In temperate zones, phosphorus is sometimes applied in excess of crop demand. However, with increased concerns about environmental quality, phosphorus use in developed countries may be reduced, resulting in increased dependence on native mycorrhizae for nutrient uptake. Another factor to consider is the interaction of water stress with nutrient availability. As soils dry down, phosphorus becomes limiting even in soils that test high in available phosphorus.
- Inoculum potential of the indigenous mycorrhizal fungi—**Inoculum potential** is a product of the abundance and vigor of the propagules in the soil and can be quantified by determining the rate of colonization of a susceptible host under a standard set of conditions. Inoculum potential can be adversely affected by management practices such as fertilizer and lime application, pesticide use, crop rotation, fallowing, tillage, and topsoil removal.

PROBLEMS AND POTENTIAL FOR INOCULUM PRODUCTION AND USE

In situations where native mycorrhizal inoculum potential is low or ineffective, consideration should be given to introducing appropriate fungi into the plant production system (Box 12–4). With the current state of technology, inoculation is most feasible for transplanted crops and in areas where soil disturbance has greatly reduced native inoculum potential.

The first step in any inoculation program is to obtain an isolate that is both **infective**, able to penetrate and spread in the root, and **effective**, able to enhance

BOX 12–4

Inoculation

Two examples are presented where inoculating with either ectomycorrhizal or arbuscular mycorrhizal fungi is beneficial when planting a mycorrhizal-dependent plant in an area where native inoculum potential is low:

Pines are not native to Puerto Rico and their fungal symbionts were absent from the soil (Vozzo and Hacskaylo, 1971). As far back as the 1930s, attempts to establish pine on the island were unsuccessful. Typically, the pines germinated well and grew to heights of 8 to 10 cm in a relatively short time, but then rapidly declined. Phosphorus fertilizers did not substantially improve plant vigor. In 1955, soil from under pine stands in North Carolina was transported to Puerto Rico where it was incorporated into soil around 1-year-old "scrawny" pine seedlings growing at Maricao in the western mountains. Within one year, inoculated plants had abundant mycorrhizal colonization and had achieved heights of up to 1.5 m, whereas most of the noninoculated plants had died. Further trials with mixtures of duff soil containing mycorrhizal fungi and with pure inocula, consisting of fungi growing in a peat-based medium, confirmed that inoculated seedlings were consistently more vigorous and larger than nonmycorrhizal ones. Subsequent surveys more than 15 years after inoculation indicated that the inoculated fungi continued to grow and sporulate in the pine plantations.

Beach erosion is a problem in many coastal areas and replenishing the beaches with sand dredged from offshore is often the method of choice for restoring them. Native grasses are planted in the back beach to reduce further erosion and to initiate the dune-building process. In native dunes, beach grasses are colonized by a wide array of arbuscular mycorrhizal fungi. However, when these grasses are propagated in nurseries they do not form mycorrhizae. Furthermore, the replenishment sand is typically devoid of arbuscular mycorrhizal propagules. In a series of studies (Sylvia, 1989), arbuscular mycorrhizal fungi were isolated from grasses growing in native dunes. The fungi were screened for effectiveness with the given host/soil combination and for compatibility with the nursery production system, and the effect of inoculation was documented on transplants placed on newly restored beaches. In the nursery, moderate amounts of colonization were achieved, even with relatively high levels of pesticide and fertilizer use. After transfer of these plants to a low-nutrient beach environment, arbuscular mycorrhizal colonization spread rapidly and enhanced plant growth compared to non-inoculated control plants even though the plants were equal size when they left the intensively managed nursery. In most cases the objective of nursery inoculation is not to achieve a growth response, but rather to establish the symbiosis with the plant so that it can be effectively transferred to the field.

growth or stress tolerance of the host. Individual isolates of mycorrhizal fungi vary widely in these properties, so screening trials are important to select isolates that will perform successfully. Screening under actual cropping conditions is best because indigenous mycorrhizal fungi, pathogens, and chemical and physical soil properties will influence the result.

Isolation and inoculum production of ectomycorrhizal and arbuscular mycorrhizal fungi present very different problems. Many ectomycorrhizal fungi can be cultured on artificial media; therefore, isolates of ectomycorrhizal fungi can be obtained by placing surface-disinfested portions of sporocarps or mycorrhizal short roots on an agar growth medium. The resulting fungal biomass can be used directly as inoculum but, for ease of use, inoculum often consists of the fungal material mixed with a carrier or bulking material such as peat. Obtaining isolates of arbuscular mycorrhizal fungi is more difficult because they will not grow apart from their host. Spores can be sieved out of soil, surface disinfested, and used to initiate "pot cultures" on a susceptible host plant in sterile soil or an artificial plant growth medium. Inoculum is typically produced in scaled-up pot cultures. Alternatively, hydroponic or aeroponic culture systems are possible; a benefit of these systems is that plants can be grown without a supporting substrate, allowing colonized roots to be sheared into an inoculum of high propagule number. Sylvia (1994) summarizes the methods for working with arbuscular mycorrhizal fungi.

The goal of inoculation is to introduce propagules of selected mycorrhizal fungi into the rhizosphere of the target plant. The most common method is to place inoculum below the seed or seedling prior to planting. Alternative strategies include coating seed or encasing of somatic embryos with inoculum. Such technologies should allow for more widespread use of inoculum in the future.

SUMMARY

Mycorrhizal associations are symbiotic relationships that form between plant roots and fungi. Bidirectional movement of nutrients characterizes these symbioses where carbon flows to the fungus and inorganic nutrients move to the plant. Mycorrhizae occur on most plant species where they provide a critical linkage between the plant root and soil. In infertile soils, nutrients taken up by the mycorrhizal fungi can lead to improved plant growth and reproduction. As a result, mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are nonmycorrhizal plants.

Mycorrhizal associations vary widely in form and function. Ectomycorrhizal fungi are mostly basidiomycetes that grow between root cortical cells of many tree species, forming a Hartig net. Arbuscular mycorrhizal fungi belong to the order Glomales and form highly branched structures (arbuscules) within root cortical cells of many herbaceous and woody plant species.

Plant responses to colonization by mycorrhizal fungi can range from dramatic growth promotion to growth depression. Factors affecting this response include the mycorrhizal dependency of the host crop, the nutrient status of the soil,

and the inoculum potential of the mycorrhizal fungi. Mycorrhizae add significant quantities of carbon to the soil and improve soil aggregation. Management practices such as tillage, crop rotation, and fallowing may adversely affect populations of mycorrhizal fungi in the field. Where native inoculum potential is low or ineffective, inoculation strategies may be helpful. With the current state of technology, inoculation is most feasible for transplanted crops and in areas where soil disturbance has greatly reduced the native inoculum potential.

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