

Mutualistic Symbiosis Between Fungi and Autotrophs

Sarah C. Watkinson

University of Oxford, Oxford, UK

Introduction

All plants are hosts to fungi that are specialised to establish close contact with living plant cells and feed on the products of photosynthesis. This mode of fungal nutrition is termed **biotrophy**. Some biotrophic fungi are parasites, including the pathogenic rusts and smuts described in Chapter 8. Others confer advantage on their hosts and are said to be **mutualistic**, and these are the subject of this chapter. We first consider the **mycorrhizal** fungi, which sustain all terrestrial ecosystems by mutualism with roots. In **lichens**, multicellular tissues of the fungal partner accommodate unicellular green algae and/or photosynthetic prokaryotes that provide them with carbon and energy. Lichens can therefore grow on bare illuminated surfaces and include species that are the main primary producers in environments too harsh for vascular plants, on mountains, in deserts, and the Arctic and Antarctic. Fungi that grow microscopically inside the tissues of plants are termed **endophytes**. Toxic secondary metabolites of some endophytes can protect the host from insect attack. These molecules are of pharmacological interest because they target specific enzymes common to human and animal metabolism.

MYCORRHIZA

The mycorrhizal mode of nutrition, in which the development and physiology of the fungus are integrated with plant roots to form a joint nutrient-absorbing structure, has evolved separately in different clades of the fungal kingdom. In all cases, the mutualism is based on the exchange of photosynthate from the plant with nutrients scavenged from the soil by the fungal mycelium. The interface for nutrient exchange forms during development of the mycelium in the root. In the following sections we consider arbuscular mycorrhizal fungi (AMF) formed by the monophyletic Glomeromycota (Chapter 1, p.25); Ectomycorrhiza (ECM) formed mainly between forest trees and basidiomycetes but a few ascomycetes; and ericoid mycorrhiza (ERM) formed by ascomycetes with Ericaceae and other plants of acid

nutrient-poor soils. In all cases the plant supplies carbon and energy to the fungus, and the fungus assists plant nutrition by scavenging mineral nutrients from the soil and from organic remains.

Figures 7.1 and 7.2 show the structures of AMF, ECM, and ERM mycorrhiza. Ectomycorrhiza and ERM, being formed mainly by basidiomycetes and ascomycetes, respectively, produce mycelium capable of persistent growth and tissue formation. They can give rise to persistent and extensive networks of **extraradical mycelium** – mycelium extending into the soil beyond the root. They may form multicellular fruiting bodies and decompose substantial plant remains. The extraradical mycelium of AMF, formed by the relatively ephemeral Glomeromycota, extends only short distances from the root and forms no multicellular tissues.

Arbuscular Mycorrhizal Fungi

All AMF are monophyletic, belonging to the Glomeromycota, a basal clade of the Kingdom Fungi. They are the most universally distributed of mycorrhizal fungi, both geographically and in host range, the simplest in form, and the most ancient. They are present in over 70% of land plant species including liverworts, ferns, gymnosperms, and angiosperms. Important food and forest crop species, such as wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize

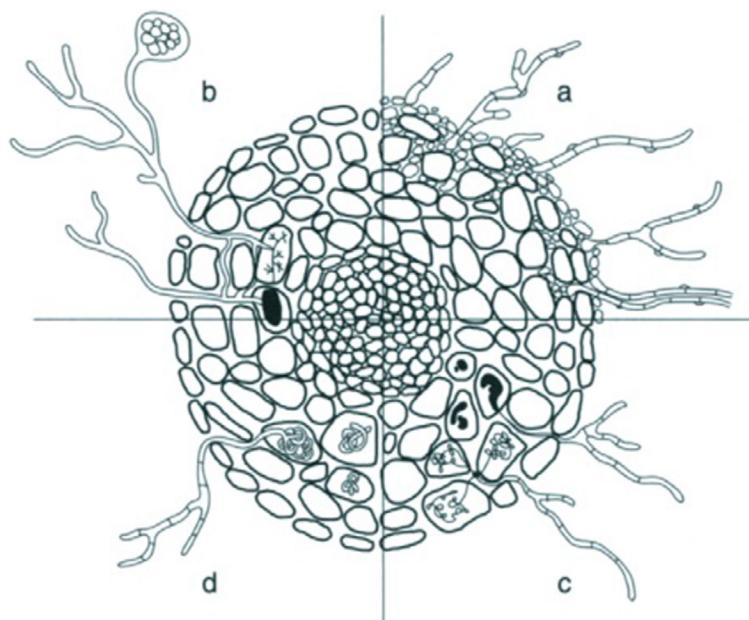


FIGURE 7.1 Types of interaction between plant roots and different kinds of mycorrhizal fungi: (a) ectomycorrhiza, with fungal mantle outside the root epidermis connected to mycelium in the soil, and to intercellular tissue of the Hartig net in the root cortex; (b) arbuscular mycorrhiza, infecting the root from a large spore in the soil, growing intercellularly in the cortex and forming intracellular vesicles and branched arbuscules in cells of the inner root cortex; (c) orchid mycorrhiza, forming intracellular hyphae which live briefly in each invaded cortical cell before being digested; (d) ericoid mycorrhiza forming bundles of hyphae in root cells, which are connected to hyphae in the soil. Source: Carlile, M.J., Watkinson S.C., Gooday, G.W., *The Fungi*, second edition: Figure 7.12b.

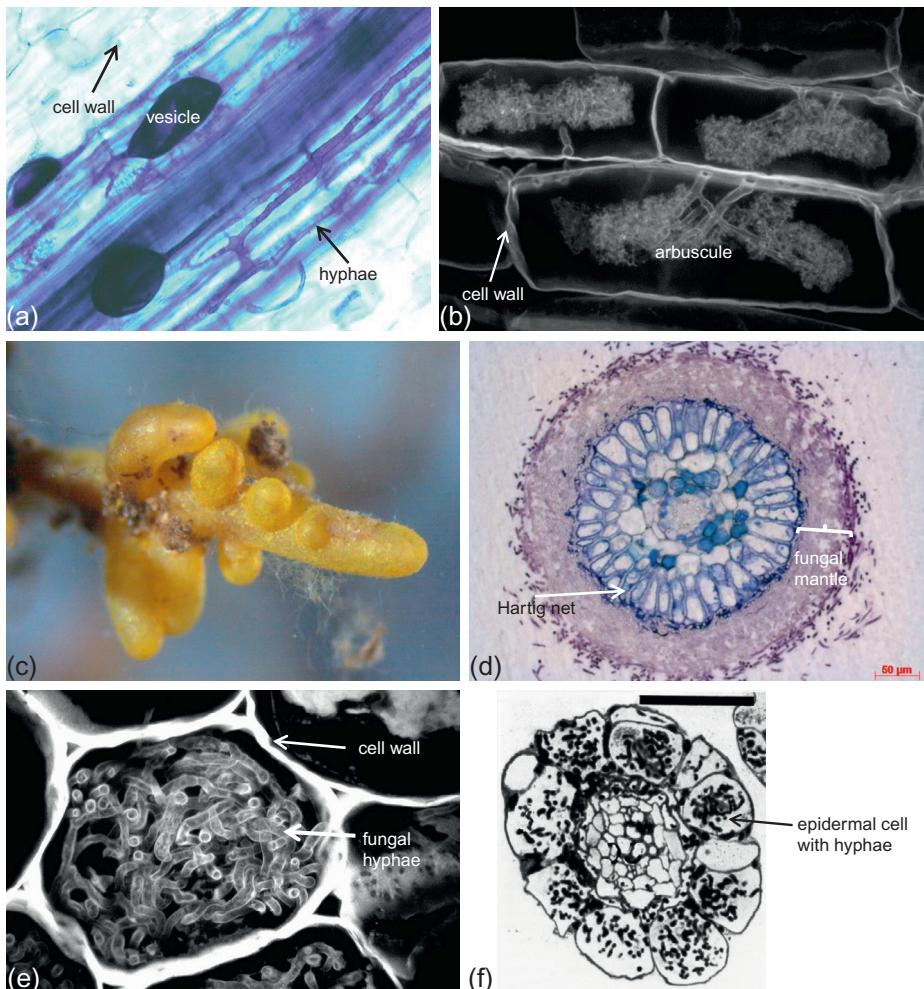


FIGURE 7.2 Typical structures of arbuscular mycorrhizas (a, b), ectomycorrhizas (c, d), and ericoid mycorrhizas (e, f). Source: van der Heijden et al. (2015).

(*Zea mays*), poplar (*Populus* spp.), and soybean (*Glycine max*) are mycorrhizal, and symbiosis with AM fungi is now recognised as the default mineral nutrient acquisition strategy of land plants. Fossilised intracellular fungi identifiable as AMF have been found in the rhizomes of petrified early land plants in rocks from the Ordovician era laid down 460 Mya. Arbuscular mycorrhiza thus pre-date vascular plants with roots and would have associated with the first green plants to colonise the land. Colonisation of plants by AM fungi can result in a 20% net increase in photosynthesis, a major contribution to the global carbon cycling budget of ecosystems. Any soil where plants grow will contain spores of AMF, the largest of which, at up to 0.5 mm diameter, are visible to the naked eye and can be separated from soil by sieving and filtration of soil suspensions. The spores have thickened and resistant walls coloured white,

yellow, orange, or brown with carotenoids. They contain reserves which on germination support the outgrowth of a short hypha. Because AMF fungi are all obligately biotrophic, they cannot continue to grow after exhausting their reserves without colonising the roots of a host plant, so culture collections of AMF fungi have to be maintained on pot-grown hosts.

Five genera, *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, and *Scutellospora*, are distinguished on morphotype. About 230 morphospecies can be distinguished by experts on the basis of limited morphological variations. The advent of molecular techniques has revealed a much greater diversity of AMF than was known even a few years ago. Molecular methods for identification are much needed in AMF studies because their simple morphology gives insufficient information to distinguish the full range of diversity. For the usual reasons of plasticity within strains, and convergence between strains not closely related, morphology is not a reliable indicator of relatedness between types. Molecular studies of field diversity are difficult because the ITS region of the ribosomal DNA small subunit is too variable within AMF strains to identify them at species level, and multiple nuclear genotypes are usually present in a single individual. However, a public database, MaarjAM (<http://www.maarjam.botany.ut.ee>) now allows grouping into taxonomic hierarchies equivalent to families and genera, and environmental OTU's with resolution equivalent to species, are recognised.

Glomeromycota have recently been found to contain intracellular Gram-positive endobacteria in their cytoplasm, revealed by fluorescent *in situ* hybridization. These bacteria are vertically inherited and globally distributed, with sequence similarity to bacterial endosymbionts of insect cells. Their contribution to the biology of the fungus has yet to be discovered. Two kinds are known: rod-shaped Gram-negative cells limited to members of the *Gigasporaceae* family, and coccoid cells related to Mollicutes that are widely distributed across different lineages of AMF. Both types may exist in a single fungal cell.

The life cycle of Glomeromycota is obscure. No sexual morphology has been reported, and until recently they were assumed to be asexual. However, hyphae of different individuals will fuse to form heterokaryons with nuclei of more than one genotype, with segregation of genetic markers. Meiosis-associated genes conserved and expressed in four different *Glomus* species suggest that recombination can occur, though it has never been seen. This has important implications for any future application of AMF for crop productivity, since inoculant strain improvement might be possible by breeding. On the other hand, any improved inoculum might lose its superiority by interbreeding with the pre-existing soil AMF population.

The association begins when a spore germinates close to a root (Figure 7.3) in the rhizosphere (the region of soil in the vicinity of a root that is influenced by root exudates and abraded cells that support a distinctive microbiota). When the outgrowing hypha meets the root close behind the advancing root tip, it forms pads termed **hyphopodia**, that adhere like the appressoria of pathogenic fungi (Chapter 8). From the hyphopodium, an infection peg enters the root, either between two rhizodermal cells, or through a rhizodermal cell. Once in the cortex of the root, hyphae spread in the apoplast along its longitudinal axis (Figure 7.1b). AMF vary in the development and structure of the interface between fungal and plant cells. In some, hyphae grow mainly as intracellular coils inside the host's root cells, while in others extensive intercellular hyphae give rise to finely branched intracellular **arbuscules** that provide an interface for developmental and metabolic interactions between host and fungus (Figure 7.2b). The common genus *Rhizophagus* develops storage vesicles (Figures 7.1b and 7.2) as well as arbuscules within host tissue. In a root cell invaded by a hypha, the nucleus moves from the periphery to the centre of the cell, the vacuole becomes fragmented and plastid



FIGURE 7.3 Growing hyphae and spores of *Glomus* sp. colonising chick pea roots *in vitro*. Extraradical mycelium, ERM spores, S. Bar 300 micrometres. Source: Lanfranco and Young (2012).

organisation alters. Both plant and fungal cell membranes remain intact, with the plant cell membrane expanding and invaginating around the branching hypha and retaining a thin layer of plant cell wall materials between fungal and plant cell. The fungus connects to the soil outside the root via intercellular hyphae that traverse the plant cortex and epidermis and extend short branches from the root surface into the rhizosphere.

Each AMF mycelium in a root results from a separate colonisation by a spore present in the soil (Figure 7.3). As the root tip grows, the root surface behind becomes thickened and inaccessible for live-cell colonisation. Thus, colonisation is dynamic and continuous, and maintenance of the association depends on abundant inoculum pre-existing in the soil. As each root becomes colonised, it develops a weft of outgrowing hyphae. Roots can extend at the rate of several hundred millimetres a day, and every millimetre of new root growth can support over a thousand millimetres of hyphae. The combined root-fungus structure thus expands rapidly through the soil, although AMF do not penetrate deeply and are found mostly in the top 20 cm of the soil horizon.

Development of the symbiosis depends on recognition between partners. Recognition between AMF and plant host begins with an exchange of chemical signals in the soil. Plant roots exude strigolactones which initiate the interaction by inducing tropic growth in AM germ tubes. Concentrations as low as 10^{-13} M can induce this response. Strigolactones also stimulate germination of the seeds of parasitic plants, suggesting that these chemicals are a reliable and sensitive indicator of the presence of plant roots. Germinating AMF fungi release diffusible low-molecular-weight chitin derivatives, lipo-oligosaccharides and chito-oligosaccharides, which act as the 'myc factors' that induce symbiotic development in root cells. Genomic investigations are revealing the reciprocal molecular responses of AMF and host during mycorrhiza

development. The plant genes involved in recognising and responding to the presence of AMF prove to have ancient origins. A putative signalling pathway, deduced from the successive up-regulation of the genes during colonisation, starts with recognition of fungal chitooligosaccharides by **LysM** domain receptor kinases of the plant. This elicits calcium spiking in the root cell nucleus, which activates a calcium- and calmodulin-dependent protein kinase, leading to transcriptional activation of symbiosis-associated plant genes. Some of the genes involved in AMF symbiont recognition are functionally conserved across all vascular plants, and have homologues in Bryophytes and in Charales (green algae). Others, found only in leguminous plants, are involved in symbiotic nodule formation with Rhizobium bacteria. Since leguminous plants diverged only 60 million years ago, compared with the 400 million year history of AMF, legumes presumably recruited these AMF symbiosis genes for the development of nitrogen-fixing nodules with bacteria. Entry into the root alters the patterns of gene expression in the fungus. [Figure 7.4](#) shows a comparison between the sets of genes expressed in extraradical (ERM) and intraradical (IRM) mycelium during development of the association. In the fungus growing outside the root, transport functions predominate, but once nutrient exchange is established within the root, the pattern of expression changes in favour of genes associated with metabolism. Transcriptional analysis at successive phases of spore germination, rhizosphere interaction, and subsequent symbiotic hyphal growth of *Rhizophagus irregularis* inside the root of *Medicago truncatula* have shown different sets of genes are preferentially expressed at each stage. In some cases the proteins they encode have been visualised *in vivo*. Once inside the host tissue, AMF hyphae exude multiple small secreted proteins – termed mycorrhizal small secreted proteins (MiSSP) – into the apoplastic space. This is the space inside the root but accessible to the soil solution, which includes both plant cell walls and intercellular spaces, but which lies outside the plant cell membranes. One such protein, SP7, inhibits a plant transcription factor responsible for up-regulating plant host defence genes. Transgenic expression of *SP7* in a transformed host plant resulted in greater mycorrhizal colonisation, confirming the role of this gene in suppressing host resistance. The SP7 protein enters the plant cell from the apoplast and localises to the plant cell nucleus where it binds to an ethylene-responsive transcription factor regulating the expression of several defence-related genes. This protein shares homology with MiSSPs from ectomycorrhizal fungi and also with the protein effector molecules that enable plant pathogenic fungi to overcome their hosts' resistance (Chapter 8, p.255). Thus, mutualistic symbiotic fungi share fundamental mechanisms with parasitic biotrophs, using similar components of signal and response pathways to achieve entry into the plant cell.

Once an interface is established, nutrient exchange occurs between plant and fungus. Fungal sugar transporters that facilitate carbon supply to the fungus are among the first host genes to show increased expression, a sequence of events which is presumably adaptive, because the obligate AMF requires a carbon supply once the spore reserves have been exhausted. One of these up-regulated transporters, **MST2**, has affinity and specificity to xylose. Since no invertase was found in the AMF genome, this suggests that symbiosis may depend on apoplastic plant invertases. Plant cell wall formation is inhibited in the part of the plant plasmalemma forming the arbuscule, but wall precursor sugars may still be produced in this region and assimilated by the fungus instead of being used for host cell wall synthesis.

When symbiosis has been established, further physiological changes occur that are associated with the role of the fungal partner in host mineral nutrient uptake. From physiological

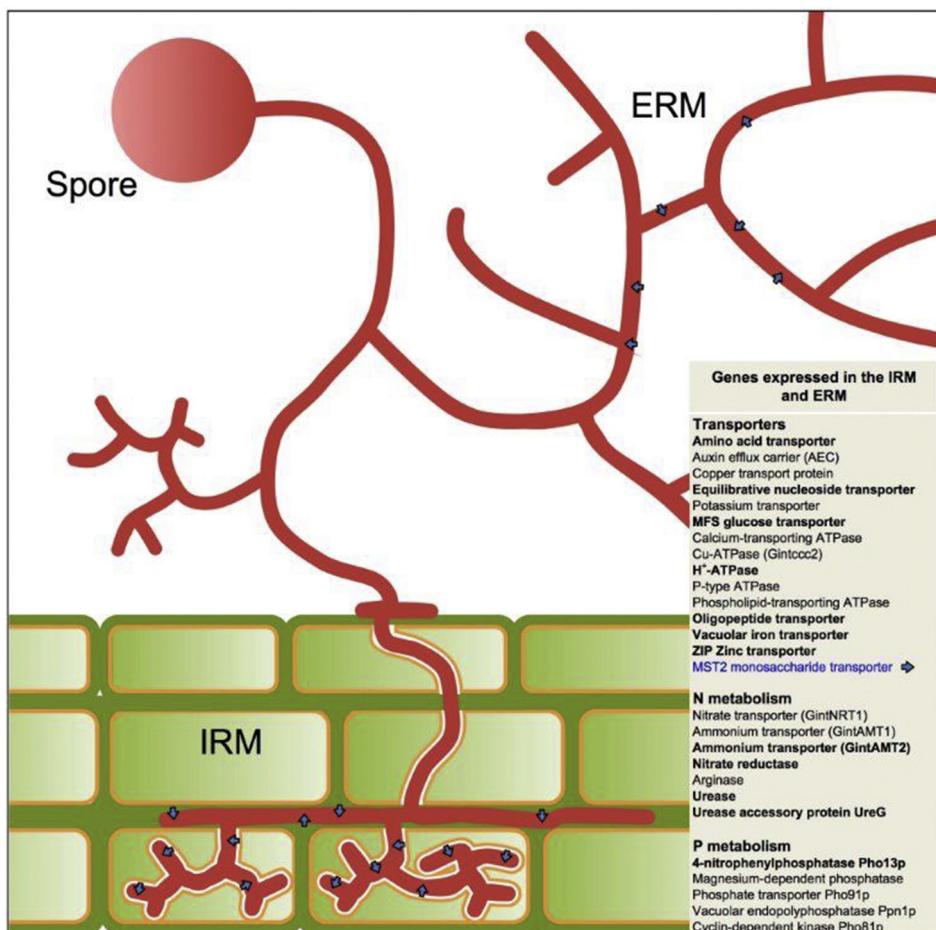


FIGURE 7.4 Gene expression during different life stages of AMF. Comparison of genes expressed in extraradical (ERM) and intraradical IRM mycelium, shows a switch from the expression of genes encoding proteins with transport functions to those involved in metabolism as the host–fungus nutrient exchange interfaces become established. Source: Lanfranco and Young (2012).

experiments, uptake by the plant of both phosphate and nitrogen is enhanced following AMF colonisation. Cell membrane transporters, including those involved in transport of inorganic phosphate, nitrate, and cations including iron and zinc, are all up-regulated. A unique clade of plant phosphate transporters (**Pht1**, subfamily I), includes members expressed exclusively in the AM symbiosis, for example, **MtPT4** of *Medicago trunculata*, which is essential not only for the acquisition of phosphate from the fungus, but also for development of the fungal symbiotic structures within the root. Other genes expressed at this stage include proteins involved in the interconversion of metabolites shuttled between partners, and those for phosphate acquisition, such as secreted phosphatases able to act on phosphate esters. Free soluble phosphate is low in soil because soil cations – calcium,

iron, and aluminium – form poorly-soluble salts. Roots become surrounded by a diffusion-limited zone depleted in phosphate, nitrogen, nitrate, and other nutrients. AMF can relieve this limitation as they can grow beyond the depletion zone, but this is not their only contribution to phosphorus acquisition. Not only do fungi take up soluble soil phosphate more efficiently than unaided roots, they can also dissolve poorly-soluble phosphate esters present in acid soils, as well as dissolving phosphate from rock minerals (Chapter 5, p.181). Phosphate taken up by extraradical hyphae is accumulated as polyphosphate, in which form it is translocated in vacuoles towards the interfacial apoplast separating host and fungal cells. Several proteins involved in this process have been identified from the transcriptome of the fungus growing inside the root. Lipid metabolism is up-regulated here, presumably to facilitate export of carbon from lipid vesicles of AMF and to provide precursors for the plasma membrane synthesis demanded by arbuscule formation.

AMF import soil nitrogen into the root by means of spatially differentiated metabolism. Fungi accumulate amino acids by combining soil-acquired nitrogen compounds with carbon skeletons from respiratory metabolism, in amination reactions catalysed by glutamine dehydrogenase, and glutamine synthase/glutamine aminotransferase. Base-rich amino acids such as arginine are accumulated, stored, and transported in vacuoles (Chapter 5, p.155). Arginine acts as a vehicle for transporting amino nitrogen by translocation through hyphae into the plant tissues, where it is deaminated to release amino groups for plant uptake. In effect, the steps of the urea cycle are spatially separated, being divided between amination in the fungus and deamination in the plant. Sources of nitrogen in the soil utilised by AMF include not only soluble nitrogen compounds but also nitrogen-rich plant litter, as demonstrated by ^{15}N tracer experiments detailed below.

Different AMF strains and species vary in their contribution to plant nutrition, with some promoting phosphate uptake far more than others. From experiments in which separate AMF infections on a single root were manipulated to supply varied amounts of phosphate or none to the root, a subtle balance seems to be maintained between plant and fungus. Only those colonising fungi that earn their carbon and energy supply, in terms of minerals returned to the root, are retained on the plant and supplied with carbon for growth. In turn, the fungus provides more phosphorus to the plant in response to an increased sugar supply from it.

High-throughput transcriptional analysis reveals that the effect of AMF symbiosis on the plant is not limited to the root, but modifies the entire plant transcriptome systemically to an extent that was not previously appreciated. Experimental AMF colonisation of the root was found to affect the expression of hundreds of plant genes in rice, *Oryza sativa*, and in tomato, *Solanum lycopersicum*, including many that confer important phenotypic characteristics, such as lycopene synthesis in tomatoes. The results point to the potential of AMF inoculation in crop nutrition and protection.

AMF in Ecosystems

In natural and semi-natural ecosystems, AMF fungi play vital, but still poorly-understood parts in plant productivity and community diversity ([Figure 7.5](#)) as well as making essential contributions to soil structure and carbon sequestration. The ability to connect to an AMF network can affect nutrient capture by roots. Plants colonised by AMF may be more efficient at extracting soil phosphate and/or nitrogen than plants grown in sterile soil. Roots typically form mycorrhizal associations under nutrient limitation. Plant roots containing abundant

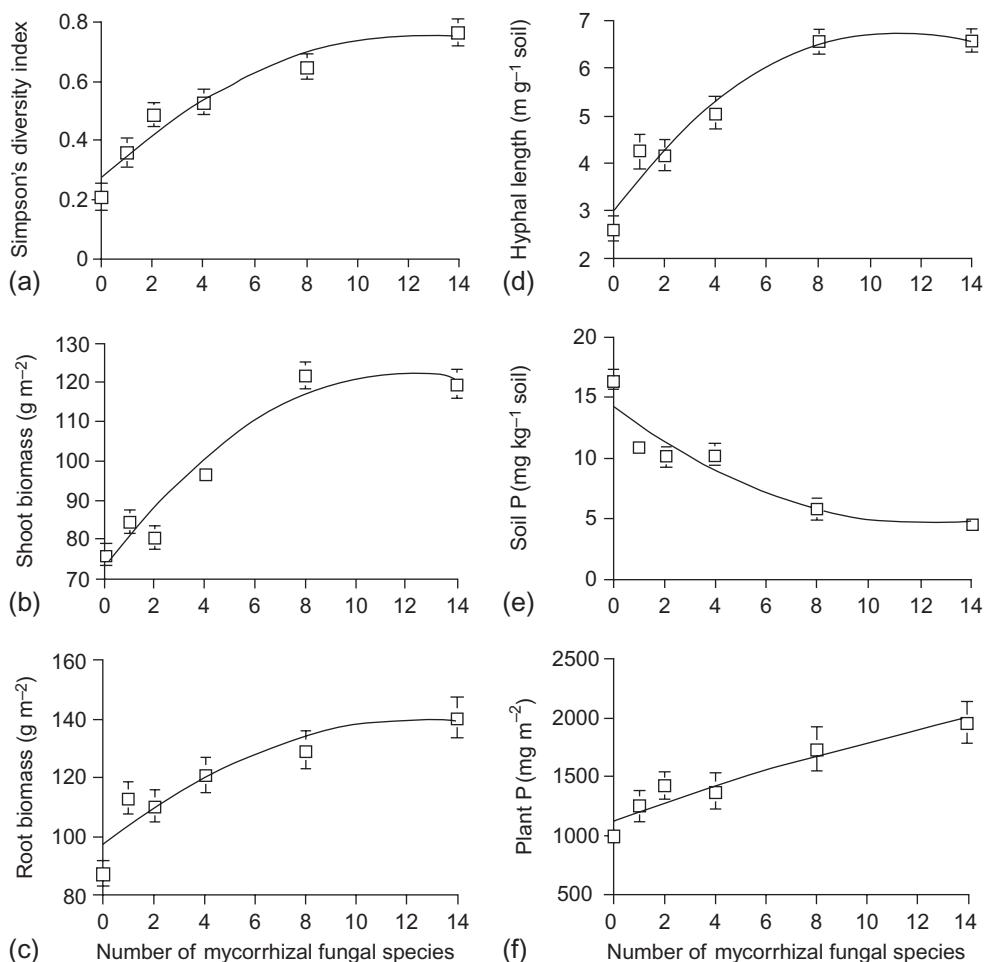


FIGURE 7.5 The effect of AMF species-richness on plant and fungal growth. Sets of replicate trays of sterilised soil, placed out of doors in a field site, were inoculated with soil containing 1, 2, 4, 8, or 14 different strains of species of AMF. The inoculated trays were then sown with 100 seeds from a mixture of 15 plant species. After one growing season, the plants growing in each tray were assessed for diversity of species (a), shoot and root biomass (b and c, respectively) and the total length of hyphae of mycorrhizal fungi in the soil (d). The levels of phosphate were also measured in the soil (e) and in plants (f). Plants grew better, and the plant community was more diverse, when 8 or 14 different AMF were present. There were accompanying increases in hyphal growth and phosphate uptake from soil to plants. *Source:* van der Heijden *et al.* (1998).

phosphate inhibit the formation of AMF arbuscules, the plant thereby avoiding an unnecessary drain on its carbon resources. Soil minerals accumulated by the extraradical hyphae are partitioned between the fungus and the plant.

AMF hyphae translocate nutrients not only from soil to root, but also between roots of adjacent plants. Using growth chambers divided by partitions either permeable or non-permeable by growing hyphae, it was shown that a plant can acquire nitrogen from dead leaf litter via

its AMF hyphae, when these were allowed to colonise plant litter enriched with the stable isotope ^{15}N (Figure 7.6). Nitrogen import through AMF hyphal connections enhanced host plant growth as well as supplying the fungus. Some aspects of the mechanism by which AMF acquire soil nitrogen remain unclear. Readily-available forms of nitrogen are scarce in natural soils, in particular those of boreal forest, where most nitrogen is in the form of complex organic compounds resistant to microbial attack. Mycorrhizal fungi are probably the main route by which trees acquire nitrogen from these compounds in the soil. The ability of AM fungi to take up both labile and recalcitrant organic nitrogen compounds under field conditions in boreal forest has been recently demonstrated, using an ingenious technique involving test substances (glycine and chitosan as labile and recalcitrant nitrogen compounds, respectively) bound to the surface of nanoparticles called 'quantum dots'.

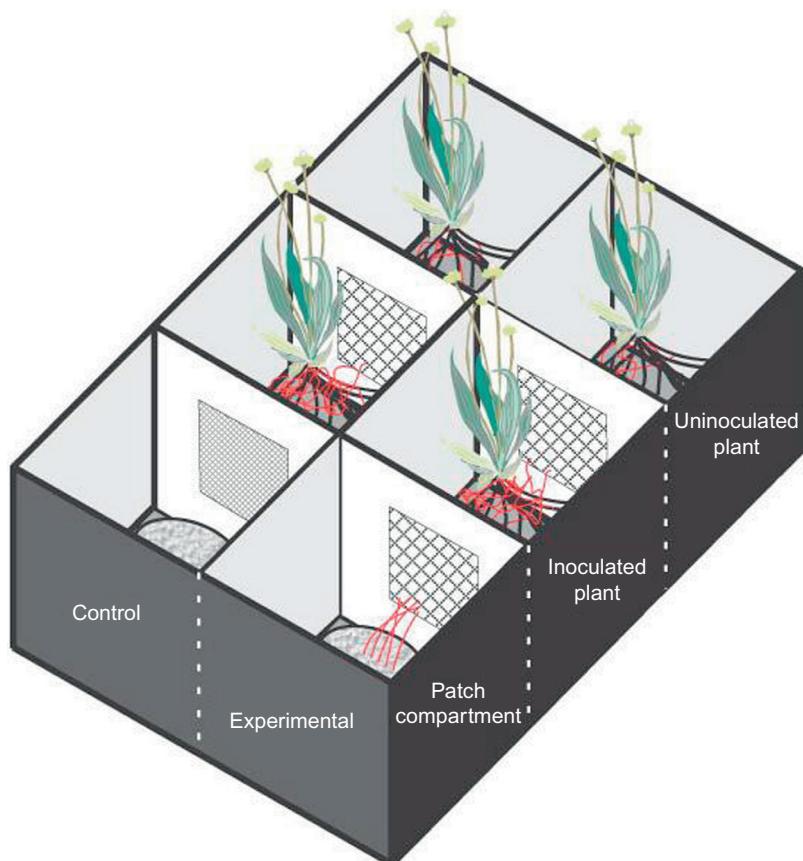


FIGURE 7.6 Microcosm used to demonstrate the ability of AMF mycelium to translocate nitrogen from plant litter into roots of living host plants. Hyphae from experimentally AMF-inoculated plants in the centre compartments of the experimental row grew into the patch of ^{15}N isotope-labelled grass litter in the 'Patch' compartment. In the control row, intervening mesh prevented hyphae growing between compartments but allowed solute diffusion. It was found that AMF-colonised plants allowed to access the grass litter acquired three times as much nitrogen as those where AMF hyphae were prevented from reaching the litter. *Source: Hodge et al. (2001).*

Natural and semi-natural plant communities host a much wider diversity of AMF fungi than arable soils. Ploughing, fertilising, and monoculture reduced AMF diversity in a UK arable wheat field compared to that in an adjacent woodland on the same type of soil. In field experiments, the diversity of AMF fungi added to sterile soil influenced the productivity and diversity of a plant community subsequently developing from a standard seed mix sown in the soil (Figure 7.5). The presence of AMF relaxes competition between plant species, allowing different species to flourish alongside each other. Several independent parameters of plant growth and phosphate uptake were enhanced in the mixed plant community when a variety of AMF types were present, suggesting that different plants might require different types of mycorrhiza. AMF composition may thus affect the capacity of a soil to support a diverse plant community or a particular type of plant. The invasiveness of introduced exotic plant species may also be influenced by specific interactions with local AMF. Those AMF strains or species which are found widely may have a wide range of habitat tolerances, while it is likely that those strains or species more specialised to host or habitat are the ones most under-represented in collections and databases. We still know little about the extent of host specificity, and whether AMF include rare, keystone or threatened species.

AMF have potential as inoculants to agricultural soil to improve crop productivity. An advantage of AMF is that they are biologically targeted to roots, compared with fertiliser that is quickly leached out by rain. Other potential benefits include enhanced nutrient uptake, drought resistance and protection against pathogens and parasites. A 2-year trial with two bioinoculants, *Glomus fasciculatum*, together with the mycorrhizal helper bacterium *Pseudomonas monteilii*, demonstrated significant protection of the medicinal crop plant *Coleus forskohlii* against attack by the soil fungus pathogen *Fusarium chlamydosporum*.

Although it is not yet clear that AMF could be developed for sustainable management in industrialised agriculture, it has been suggested that, rather than attempt inoculation, simply reducing tillage could preserve the integrity of AMF networks already present in all soils, and thus enhance crop growth and soil carbon sequestration. AMF not only aid nutrient uptake by plants but also channel carbon from plant photosynthesis into the soil rapidly and in significant amounts, with estimates ranging from 50 to 900 kg ha⁻¹. Fungi may add carbon to soil in the form of extraradical hyphae or as exudates such as **glomalin**, an extracellular mycelium-bound substance, believed to be a glycoprotein, which is sticky and hydrophilic. Glomalin contributes to the moist, cohesive character of soils under natural vegetation compared with the friable crumb structure of cultivated arable soils. In a 16-year-long field monitoring study, treatments that increased soil AMF hyphae also increased glomalin-related soil protein (GRSP) pools and water-stable macro-aggregates, while all three were reduced by fungicide application. Conserving the resident AMF population could thus be seen as a valid goal in soil management for carbon sequestration as well as plant productivity.

Ectomycorrhizal Fungi

Ectomycorrhiza (ECM) are so-called because the fungus is extracellular throughout the association, forming no intracellular structures. These fungi associate mainly with woody perennial plants, but some shrubs that occur in early stages of plant successions are ectomycorrhizal, for example, *Salix herbacea* and *Dryas octopetala*. About 3% of plant species form ECM with thousands of fungi, mainly basidiomycetes although some common types are formed

by ascomycetes. Although relatively few plant species are involved, the importance of ECM fungi on a global scale cannot be overestimated, because they partner with tree hosts that form the most important terrestrial carbon sinks on the planet: Pinaceae, the vast northern conifer forests of the world, Fagaceae, temperate deciduous woodlands, and Dipterocarpaceae, the South East Asian rainforests. In ECM the fungal partner receives carbon and energy as photosynthate from the host tree via an extracellular interface within the root cortex described in detail below. However, many ectomycorrhizal species of fungi can also feed on plant remains (Figure 7.9), an ability they retain from saprotrophic ancestry (Figure 7.10).

The main clades of ectomycorrhizal basidiomycetes are the Agaricomycetes *Amanita*, *Boletus*, *Cortinarius*, *Laccaria*, *Lactarius*, *Russula*, and *Suillus*, the wood decomposing basidiomycetes *Tomentella* and *Thelephora* in Polyporales, the ascomycete *Coenococcum*, and the Sebacinales which have relatively poorly differentiated sporophores and the capacity to form symbioses with a wide variety of plants including ericaceous species and non-woody orchids. Molecular analysis is revealing much greater infraspecific variation in ECM fungi than was once supposed. For example, *Pisolithus tinctorius*, used as an inoculant in forestry, is represented worldwide by many separate species each with different host plant associations. Because the diversity of natural populations is correlated with resilience under disturbance, infraspecific diversity in mycorrhizal fungi is likely to be an important factor in the resilience of forest ecosystems under climate change, and is being intensively investigated now that sequence data representative of natural ecosystems are accessible.

Morphology and Anatomy

In the surface soil horizons of most temperate forests, fine ectomycorrhizal rootlets (Figure 7.7) will be found running through surface layers of soil rich in decomposing plant litter. Their short lateral branches, about a millimetre wide or less, differ in colour and surface characteristics from their parent axis, and are typically thicker. Their distinctive appearance is due to their surface coating of fungal tissue, the ectomycorrhizal mantle or sheath. Within the root, living root cells interface with fungal hyphae that penetrate between them forming the Hartig net, named after the German forest biologist Robert Hartig.

The mantle (Figure 7.2d) is the living tissue barrier between absorbing rootlet and soil. It develops continuously as the root grows, suppressing root hair development and forming a sock-like sheath several cells thick encasing the entire root tip. As new roots arise they are colonised by hyphae which may originate from ECM already existing on the root, from spores, or from mycelium already growing on other roots or plant litter. Some species readily colonise from spores, others require an already-established base on a living root in order to establish associations with further roots. In the initial stages of root infection, hyphae grow between epidermal cells to establish the nutrient exchange interface. Angiosperm and gymnosperm ECM differ in the radial extent of root tissue colonised. In angiosperms, only the epidermal layer is colonised, but in gymnosperms hyphae penetrate between the outer cortical cells as well. In all cases hyphae are limited to the apoplastic space outside host cell plasma membrane and thus remain outside the root endodermis. The characteristics of the mantle are the result of alterations in growth form of both partners as the association develops. The plant may be induced by colonisation to form new lateral rootlets, and the pattern of these is typical of the fungal species, so that different fungi form different characteristic ECM morphotypes, even on the same species of tree (Figure 7.7). Fungal association may stimulate radial elongation

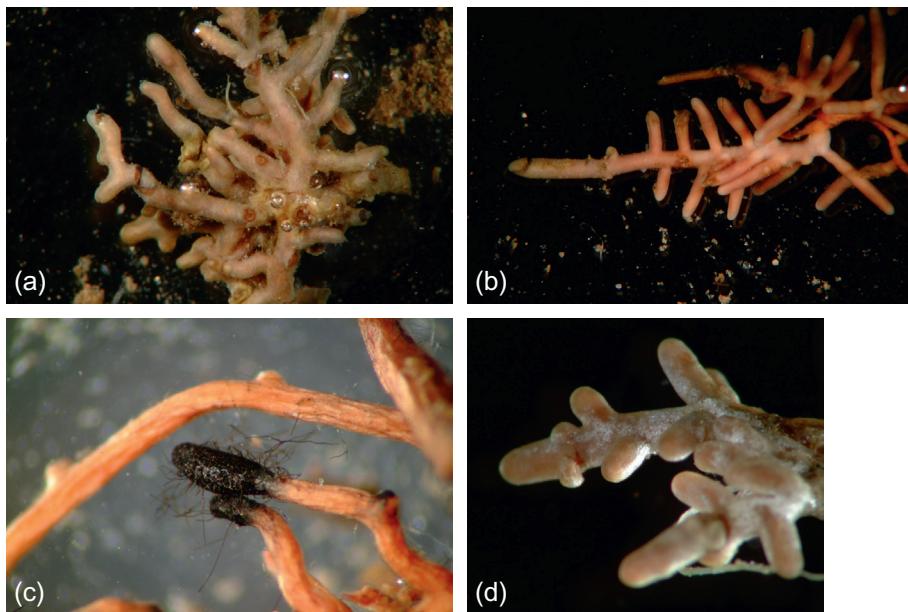


FIGURE 7.7 Ectomycorrhizal morphotypes from beech (*Fagus sylvatica*) woodland soil; (a) *Laccaria* sp., (b) *Lactarius* sp., (c) *Coenococcum* sp., and (d) *Russula* sp. Source: John Baker.

of the cortical cells, resulting in a greater potential surface contact area for nutrient exchange and producing the root thickening characteristic of some ECM morphotypes. Fungal mantle tissue forms either by extensive interweaving of hyphal filaments, or by cell division to produce isodiametric cells. Differentiated cell types characteristic of the species may arise, such as latex-producing cells in *Lactarius*. Pigments give each morphotype its characteristic colour: purple, black, white, and shades between dark brown to pale yellow. Morphotypes also vary in whether they produce mycelial outgrowths from the mantle into the soil, and the extent of such outgrowth. Species vary widely in the nature and extent of their exploratory mycelium. Some, like members of the Boletales genus *Suillus*, form extensive networks, with translocating systems of mycelial cords (Chapter 5, p.176), while others, like the common ECM genus *Russula*, simply encase the rootlet in a smooth sheath with no visible emanating mycelium. The widespread black ECM formed by the ascomycete *Coenococcum geophilum* is recognisable by its hairy-looking surface bristling with melanized hyphae.

The life span of ECM rootlets is of interest in the context of forest soil carbon sequestration via ectomycorrhiza, but difficult to measure. The residence time of carbon (from ^{14}C data) in ECM of several species (identified from morphotype and RFLPs) was investigated in woodland of mixed pine and juniper. The turnover time of 4–5 years in the carbon of ECM rootlets suggested that they were relatively long-lived compared with unassociated mycelium and with mushrooms. Many variables are likely to affect longevity of ectomycorrhizal fungi on rootlets, including the species of fungus, the growth rate of roots, grazing of fungi by soil invertebrates and soil nutrient status. The contribution of dead ectomycorrhizal fungi to soil carbon is significant and is further discussed below.

The Hartig net is the interface between fungal hyphae and plant root cells, where the development and physiology of both partners becomes integrated into a functional unit. Here, fine hyphae ramify as finger-like processes over the plant cell surfaces and through the intercellular middle lamellae. Only in relatively few types of association, or in senescence of the mycorrhiza, do these hyphae invade the plant cell with intracellular growths. Instead, exchange occurs across both hyphal and plant cell walls, though plant cell walls are thinner at the Hartig net interface. Fungal growth is confined to the apoplastic space between root cells, freely accessible to the soil solution. Hyphae do not cross the endodermis, the cylinder of tissue that contains the central conducting tissue of the tree. From comparative genomic studies of closely related ectomycorrhizal and saprotrophic basidiomycetes, it appears that evolution of the ECM habit is accompanied by expansion and refinement of gene families of pectinases and glucanases active in hydrolysing components of the intracellular middle lamella, suggesting that enzymic lysis by the fungus may facilitate contact between plant and fungal cells in the Hartig net.

The sequence of root-fungus interactions leading to ectomycorrhiza formation starts when the fungus is chemotropically attracted to grow towards a nearby root, stimulated by root exudates including flavonoids and strigolactones. The exchange of signals between host and fungus during establishment and maintenance of ectomycorrhizal symbiosis has been dissected at the molecular level in the model interaction between *Laccaria bicolor* and *Populus* sp. As the partners come into contact, the most highly up-regulated fungal protein is, **MiSSP7**, one of a constellation of small secreted proteins (**MiSSP's**) exuded by the fungus. This protein can be visualised *in situ* in the region of the developing Hartig net by immuno-localisation (Figure 7.8). **MiSSP7** enters the plant cell via active phosphatidylinositol 3-phosphate-mediated endocytosis, and is targeted to the nucleus, where it acts as a transcription factor affecting the expression of genes involved in defence, cell wall remodelling and signalling. **MiSSP7** shares some features with the protein **SP7** of AMF, described above, which prepares the root for symbiosis even before physical contact between partners. Both are effector proteins similar to the widely conserved effectors of plant pathogens (Chapter 8, p.252), and share the same role of suppressing resistance and setting up biotrophic interaction between cells of plant and fungus. *Laccaria bicolor* transformants with reduced expression of **MiSSP7** do not enter into symbiosis with poplar roots.

Following the initial burst of fungal **MiSSP** release, protein markers for plant resistance, elicited on first contact, decrease, and the plant's auxin responses change, leading to lateral root induction forming the typical ECM morphotype. As the root accepts fungal colonisation and starts to form joint tissues with the fungus, transport proteins involved in nutrient exchange start to be up-regulated. The genome of *Laccaria bicolor* has 15 genes characterised as high affinity H⁺/glucose transporters, which are expressed as mycorrhiza develop and are probably involved in the uptake of glucose from host cells. As fungal glucose metabolism accelerates, fed by sugars synthesised by the leaves and translocated to the Hartig nets of the roots, the ECM root system becomes a strong carbohydrate sink for the tree. Between 10% and 20% of the plant's photosynthate is estimated to be allocated to the ECM root system.

Genomic studies are revealing additional functional groups of genes up-regulated in ECM symbiosis that are considered to have roles in setting up and maintaining the plant-fungal interface. Cell wall remodelling enzymes, including pectinases, glucanases, and peptidases, presumably assist the hyphae to penetrate between cells in the apoplastic middle lamellar

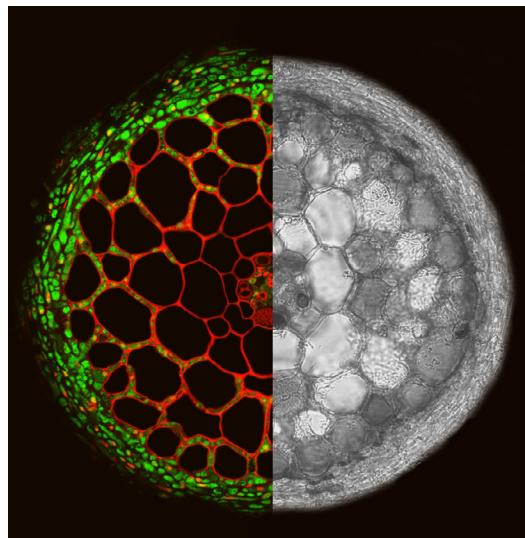


FIGURE 7.8 Immuno-localisation of a highly expressed fungal effector-like protein in a *Populus trichocarpa*-*Laccaria bicolor* ectomycorrhizal root tip. A transverse cross section of a poplar root colonised by the symbiotic ectomycorrhizal fungus *L. bicolor*. The green signal is an immuno-localisation of the fungal effector protein MiSSP7 highly expressed in the hyphae of *L. bicolor* while staining with propidium iodide highlights the cell walls of the root cells. Source: Jonathan Plett and Francis Martin.

spaces of the root cortex. Symbiosis-related acidic polypeptides released by the fungus during ECM formation share features with adhesins of fungal pathogens of animals and plants so might help attachment of the hypha to the host cell. A third group of up-regulated proteins are hydrophobins, secreted proteins that form an amphipathic monolayer on surfaces with one hydrophilic and one hydrophobic side. These could be involved in covering the mantle (and extraradical cords), making the mycorrhizal root tip a sealed system. Genomic research such as the Agaricomycete sequencing programme of the Joint Genome Institute can be expected to reveal a diversity of function among the ECM fungi that populate forest soils.

The association between ECM partners is facilitated by soil bacteria of genera including *Pseudomonas*, *Rhodococcus*, *Streptomyces*, *Burkholderia*, and *Bacillus*, known as **mycorrhiza helper bacteria**. These colonise the surface of fungal hyphae, stimulate hyphal growth, and may even live within hyphae as endobacteria. They assist ECM nutrient acquisition, protect their host plants from pathogens, and are probably important for tree growth in forests and nurseries, but we know little about the mechanism by which they promote the formation and function of mycorrhiza. The first genome sequence of a helper bacterium, *Pseudomonas fluorescens*, a Gram-negative rod-shaped bacterium isolated from a sporocarp of the ectomycorrhizal fungus *Laccaria bicolor*, was published in 2014.

Basidiomycete mycelium growing from long-lasting food sources such as dead wood and tree roots can persist for decades in the forest floor (unlike the ephemeral mycelia of AMF that turn over in a few days). Some species of ectomycorrhizal fungi can develop extensive and persistent foraging networks fuelled by tree hosts that in turn scavenge mineral nutrients

for the host, from the soil, leaf litter, and even from pollen (Figure 7.9). The lignin/humus degrading capacity of ectomycorrhizal fungi varies between genera. Comparative genomics suggests that *Laccaria* has lost much of its wood decomposing ability, *Paxillus* decomposes complex organic matter in a way similar to brown rotters, and *Cortinarius* species which are typically found in well-established woodland have a full set of Mn-peroxidase genes and are likely to act as mycorrhizal white-rotters. Interesting parallels have been discovered between the evolution of saprotrophic brown rot and ectomycorrhizal nutritional modes in basidiomycetes. The clade Boletales, for example, includes both the wood-destroying dry rot fungus *Serpula lacrymans* and its closest known relative, the ectomycorrhizal *Austropaxillus*, both of which have lost many wood degrading enzymes in the course of evolution.

Evolution

Unlike AMF, ECM symbiosis is polyphyletic, having arisen in at least ten clades of fungi (Figure 7.10). Phylogenies of ECM basidiomycetes, such as species in the common ECM genus *Laccaria*, show many short branches indicating recent radiations. Molecular clock data, calibrated with fossil evidence, point to the earliest occurrence of ECM symbiosis around a hundred million years ago, alongside the origin and diversification of angiosperm and gymnosperm plants (Figure 7.11). Subsequently, ECM fungi appear, from present patterns of biogeographical distribution, to have accompanied their tree hosts, perhaps even enabling them to spread into new regions. Pine ECM fungi probably accompanied the earliest pine ancestors on the former southern hemisphere continent of Gondwanaland, and radiated along with the spread of pine forests across the northern hemisphere following the break-up of the older continents.

ECM associations continue to evolve dynamically today, with repeated acquisitions and some possible losses of symbiosis evident in basidiomycete phylogeny. This is not a stable mutualism like that of the *Neotyphodium* endophytes of grasses where the fungus has lost independent sexuality and is effectively part of the host (below, p. 235). Both partners retain the capacity for ecologically independent life. Sexual reproduction in each species occurs independently of the symbiotic partnership, retaining the potential for independent variation and evolution by natural selection. This is consistent with phylogenies that show both losses and acquisitions of the ectomycorrhizal mode of nutrition. While ECM symbionts are generally nested within mainly saprotrophic clades, there is some evidence, notably in Boletales, that the reverse step may have occurred, with loss of ECM-forming capability and reversion to saprotrophy.

ECM in Ecosystems

Mycelium of both ECM and plant litter saprotrophs mingles in the organic soil horizons of forests, and their interactions with each other and with other soil biota are of major significance in ecosystem nutrient dynamics. Basidiomycete mycelium dominates the microbiota of forest soils, and can amount to several tonnes per hectare. Living mycelium accumulates, stores, and redistributes carbon, nitrogen, phosphorus, and other nutrients. Mycorrhizal fungi not only cycle soil nutrients, but may deposit large amounts of recently fixed carbon in soils, building large pools of carbon in the form of complex molecules that contribute to long-term ecosystem carbon sequestration. Until recently it was assumed that soil carbon came mainly from plant remains accumulated aboveground and gradually incorporated into the upper soil

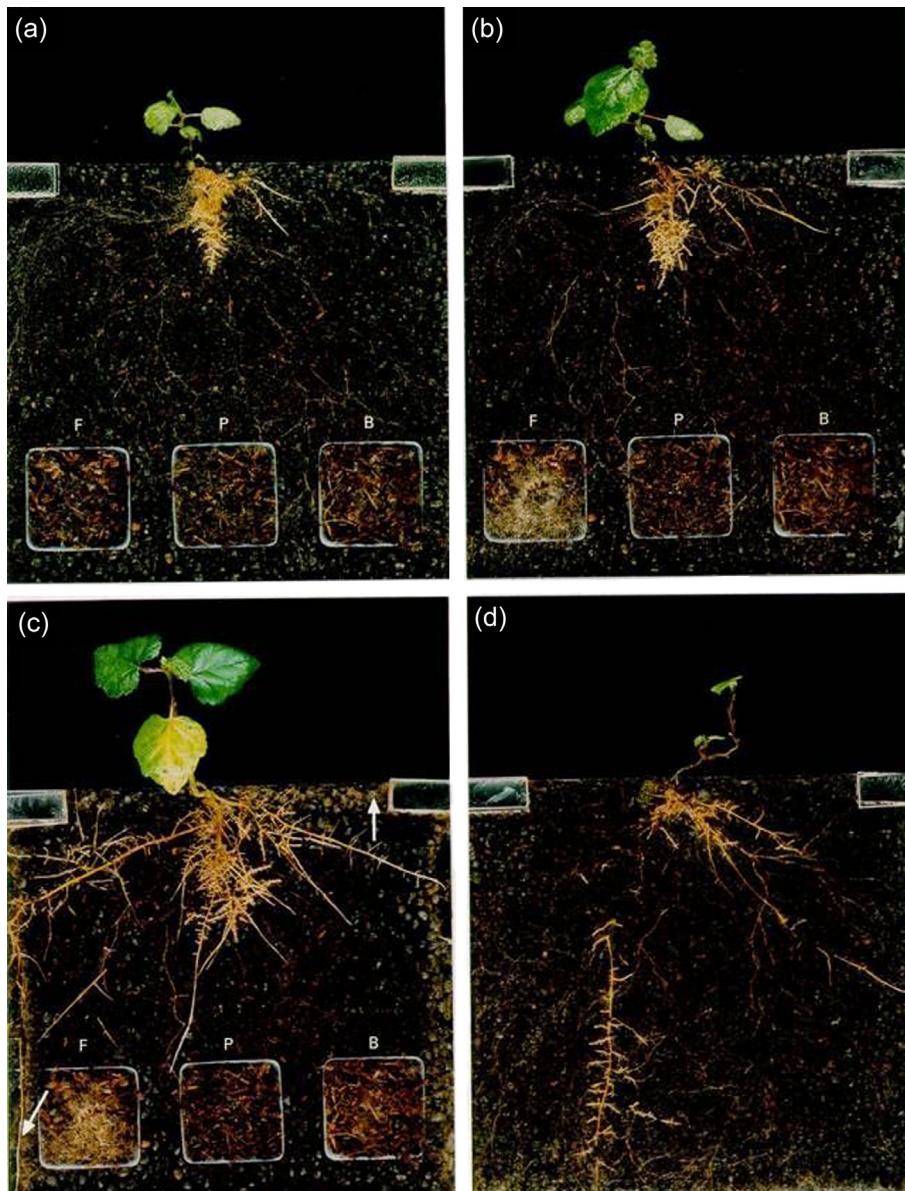


FIGURE 7.9 Photographs (a)–(c) show sequential development of ectomycorrhizal seedlings of birch, *Betula pendula*, associated with *Paxillus involutus* ectomycorrhiza in observation chambers containing trays of litter of beech (F), pine (P), and birch (B) at 8, 35, and 90 days after litter placement. Initial colonisation of litter is followed by increased plant growth, compared with d, a mycorrhizal control plant without litter addition. Source: Perez-Moreno and Read (2000).

AGARICOMYCOTINA

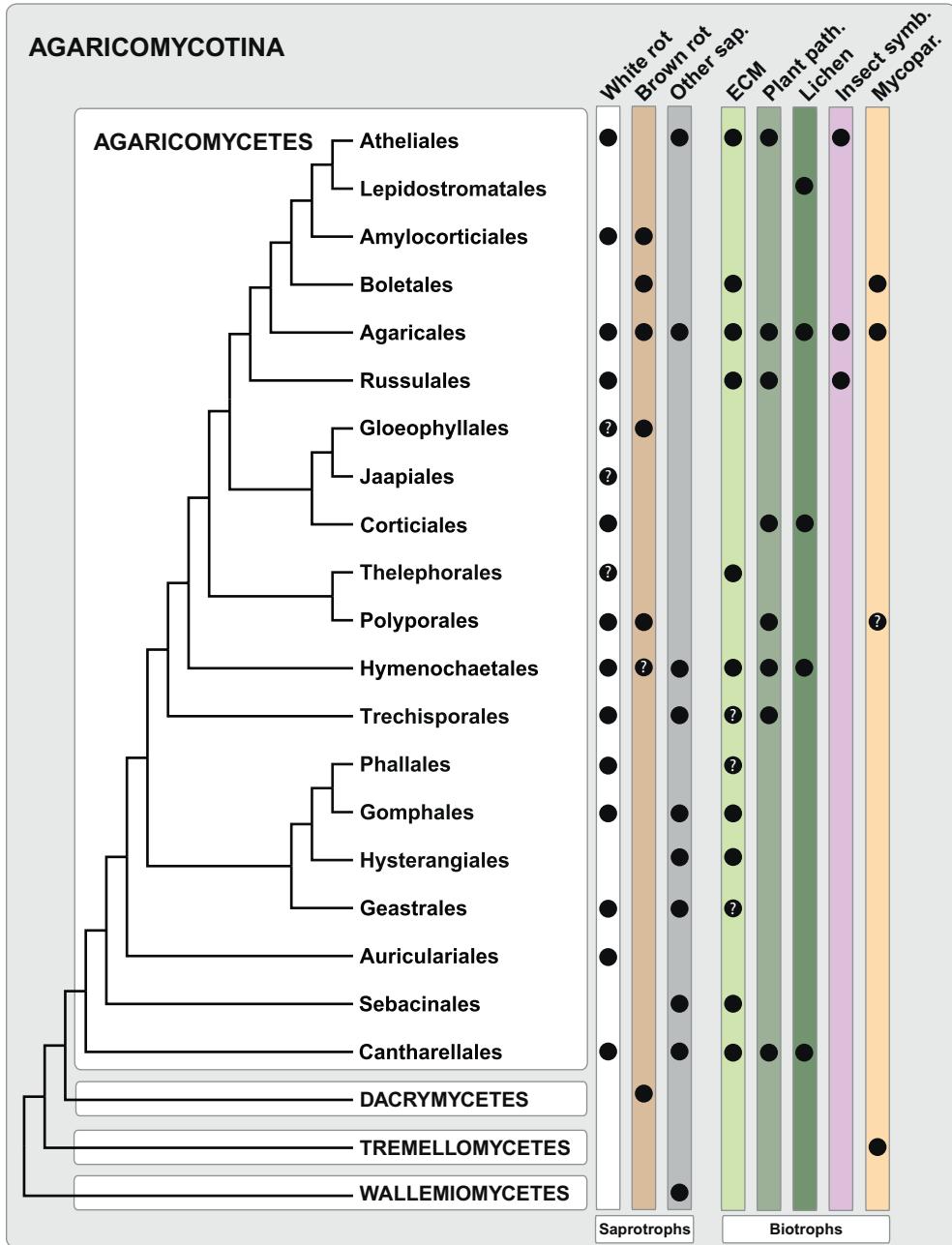


FIGURE 7.10 Phylogenetic distribution of major nutritional modes across the Agaricomycotina. The tree summarises recent phylogenomic and multi-gene phylogenetic studies. Saprotrhops include white rot and brown rot wood decay fungi, and a broad category of 'other' saprotrophs, such as litter, dung, and keratin-degrading fungi. White rot is very widespread and is probably the ancestral condition of the Agaricomycetes, but not the Agaricomycotina as a whole (note that it is absent from Dacrymycetes, Tremellomycetes, and Wallemiomycetes). Brown rot has evolved independently in at least five orders of Agaricomycetes, as well as Dacrymycetes. Biotrphs include ectomycorrhizal symbionts (ECM), plant pathogens, lichen-forming basidiomycetes, insect symbionts and mycoparasites, all of which are ultimately derived from saprotrophic ancestors. Agaricomycotina also include other biotrophs that are not shown, including endophytes, nematode-trapping fungi, bacteriovores, parasites of algae and bryophytes, and animal pathogens. Question marks indicate uncertainty. Source: See James et al. (2006). Figure David Hibbett.

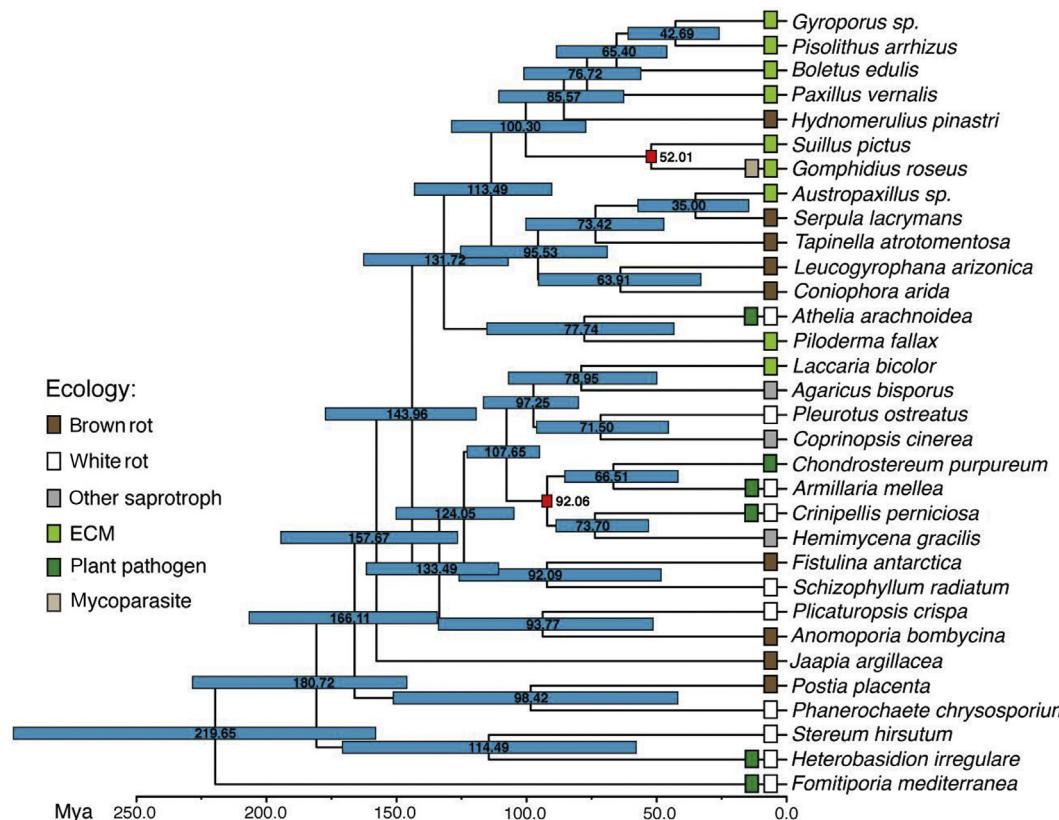


FIGURE 7.11 Molecular phylogeny and the evolution of Agaricomycete ecology. The chronogram, inferred from a combined six-gene data set by molecular clock analysis, illustrates the divergences of nutritional mode in Agaricomycetes in relation to the likely time of divergences in angiosperm and gymnosperm plants. The estimated times of divergence are shown as blue bars, with the mean node ages in the bars. Calibration points with fossil ages are shown in red (dark gray in the print version). Source: D. Floudas, in Eastwood et al. (2011) Chapter 5, Further Reading.

horizons as litter fragments and humus. However, recent evidence suggests that organic soil layers may also grow from below, through continuous additions of carbon compounds from roots and their mycorrhiza. A chronosequence of soils under boreal forest that had developed over periods between centuries and millennia was investigated on a cluster of Scandinavian lake islands. The smaller the island, the older the soil carbon, because smaller islands are less frequently burned from lightning strikes, being statistically less likely to be struck. On these small islands, unburned for over 2000 years, the deeper soil layers harbouring predominantly mycorrhizal fungi and roots had accumulated proportionately larger amounts of persistent organic compounds of root and fungal origin, which was associated with tightly-bound nitrogen, leaving little available nitrogen to support plant growth. In other studies, forest fungi have been found to have a range of enzymes able to liberate nitrogen and phosphorus from such complexes. However, because of the variety of chemical bonds, they cannot be easily targeted by most soil microbes and so decompose only slowly.

Extraradical mycelium facilitates acquisition of nutrients from poorly-soluble minerals. Organic acid secretion and hyphal intrusion into rock enables ectomycorrhizal trees growing on nutrient-poor rocky ground to acquire mineral nutrients via mycelium, from underlying rock and from insoluble mineral particles in the soil, as described in Chapter 5, p.181. This is a key process in the establishment and maintenance of boreal forest, since ultimately these inorganic materials are the only source of mineral nutrients for the entire overground biota. Several common ectomycorrhizal species of *Suillus*, *Lactarius*, and *Piloderma* penetrate at least half a metre down into mineral horizons, with some species present exclusively in these layers. Typically the acid, nutrient-poor soils of these forests are strongly layered **podsols**, with a dark organic horizon overlying a pale, highly-leached eluvial horizon containing mineral particles such as the phosphorus-containing mineral apatite. Other minerals utilised by mycorrhiza include silicates containing calcium, magnesium, and potassium. Hyphae have been found growing into apatite particles, using both hyphal pressure and exudation of citric and oxalate acids, which act both by protonation and chelation to release soluble phosphate. In this way, ECM roots have direct access to phosphorus, bypassing competing biota in the soil and avoiding toxic metal ions such as aluminium that are often present in the acid soil solution of podsols. Other essential cations acquired by ECM from rock include iron, captured by exuded iron-chelating siderophore molecules (Chapter 5, p.159) such as ferricrocin, secreted by the common symbionts *Coenococcum geophilum* and *Hebeloma crustuliniforme*. Mineral solubilisation is not confined to mineral particles in soil. Rock surfaces can also be dissolved by fungal **bioweathering**, and the ability of fungal root symbionts to utilise solid rock as a nutrient source presumably underlies the ability of some pine trees to root directly into the bedrock.

The processes by which a diverse ECM community becomes established is of interest because of the interaction between plant community diversity and that of mycorrhizal fungi. A fungal foray through woodland will typically find scores of sporophores of species belonging to mycorrhizal genera such as *Amanita*, *Boletus*, *Laccaria*, *Lactarius*, *Cortinarius*, *Tricholoma*, and more. Excavating tree rootlets will reveal a similar number of ectomycorrhizal morphotypes, while molecular sampling will reveal the presence of even more species. Most of the diversity can be ascribed to a few common taxa, while intensive sampling will reveal an almost inexhaustible tally of rarities. How do these fungi arrive?

In temperate climates *Laccaria amethystina*, with distinctive purple sporophores and purple-tipped pale ectomycorrhizal roots, are among the first colonisers of new tree seedlings. They occur on a variety of host trees and colonise readily from easily-germinated spores, so behaving as widespread ruderal members of the ECM community. Others, including, for example, the *Cortinarius* species typical of ancient woodland, are later colonists of roots. They colonise roots more readily by mycelial growth from already-established ECM, and their spores are slow to germinate.

Arrival and colonisation is limited by the dispersal capacity of fungal species. While airborne spores are produced in staggering abundance by many ECM species, their concentration falls away exponentially with distance from the source, and root colonisation is dose-dependent. Analysis of species and infraspecies diversity across landscape and geographical scales shows that ECM communities are dispersal-limited. It is not true of mycorrhizal fungi that ‘everything is everywhere’ and ECM fungi do not show cosmopolitan distributions. Large geographical areas such as neotropical and palaeotropical rainforest have largely endemic ECM populations. Within a species range, airborne spores serve to facilitate gene flow throughout the population.

Ericoid Mycorrhizal Fungi

The Ericaceae are dominant plants of acid heathland and upland soils including the genera *Calluna*, *Erica*, *Vaccinium*, *Azalea*, *Rhododendron*, and the Epacrids of Australasia which grow in dry sandy soils. Ericaceae are enabled to grow in acid and upland soils too poor in mineral nutrients for other plants, by forming ERM, mainly with Helotiales (ascomycetes). The plants provide the fungi with sugars from photosynthesis, while partnership with fungi enables plants to acquire mineral nutrients from insoluble organic residues, through enzymes and siderophores deployed for nutrient acquisition by the fungus. The Helotiales appear to have arisen and diversified during the Cretaceous era in Gondwanaland, which might therefore be a site of origin of the ericoid mycorrhizal association.

Ericaceous plants have distinctive root morphology. Instead of the single-celled root hairs of other plants, they have very fine multicellular roots only 100–750 µm in diameter, termed hair roots, in which the outermost epidermal layer immediately behind the advancing root tip meristem consists of cells packed with fungal hyphae (Figures 7.1d and 7.2e & f). The central vascular strand and cortex are highly reduced so that as much as 80% of the hair root volume is composed of intracellular fungus. Each epidermal cell is separately colonised from the soil by hyphae which invade by penetrating the cell wall and proliferating inside, with the plant cell membrane invaginated around the hyphae. This symbiotic epidermal tissue of the hair root lasts only a few weeks until overtaken by root secondary thickening. An endemic population of suitable soil fungi is thus essential to maintain the continual root recolonisation which is a feature of ERM. Electron micrographs of established partnerships show a clear matrix between the host and symbiont membranes, with surrounding plant cytoplasm enriched in rough endoplasmic reticulum and mitochondria, indicating active physiology likely to be associated with uptake of fungus-acquired nutrients. Helotiales include many well described species of readily-cultured saprotrophic soil ascomycetes identifiable by morphology of ascocarps and spores. Those isolated from roots of Ericaceae grow slowly in culture producing dark mycelium that very rarely sporulates.

Molecular phylogeny indicates the existence of a much wider taxonomic group of ericoid mycorrhizal fungi than previously known from direct observation and culture of fungi on colonised roots. *Rhizoscyphus* (=*Hymenoscyphus*) *ericae* consists not of a single species but of a broad clade of Helotiales. Culture experiments have suggested a functional as well as genetic distinction between symbiotic and saprotrophic members of the clade. Many other soil fungi are emerging from environmental genomic analyses as root symbionts, although to be classed as true ERM fungi it is necessary to determine that they form the characteristic morphological association described above. The previously overlooked basidiomycete group Sebacinales appear to be ubiquitous as root symbionts and include members that form ERM, as well as ECM, mycoheterotrophic, and saprotrophic fungi. Members of the Chaetothyriales (black yeasts) are particularly important formers of ERM. They are the source of most fungal DNA extracted from ericoid roots, and in axenic culture they have been shown to associate with roots to form the diagnostic intracellular coils.

The enzymes of ericoid mycorrhizal fungi acquire nitrogen and phosphorus for their plant hosts by breaking down organic residues of plants and animals in which these elements are bound. In the cold, acid conditions of ecosystems where ERM predominate, microbial decomposition is slower than photosynthetic production. Organic material, such as woody plant

and fungal remains and insect exoskeletons, accumulate as deep layers of brown peat forming the surface horizons of **mor** soils. Phenolic lignin residues bind proteins and form complex and long-lasting compounds with nitrogen, making it unavailable to roots. Phosphorus similarly becomes immobilised by covalent linkage with polysaccharides in the form of phosphomonoesters (**phytate**). Mycelium emanating from ERM-colonised hair roots can decompose these materials with secreted and wall-bound enzymes, including phytases and phosphodiesterases that respectively hydrolyse phytate and nucleic acids, liberating phosphate. Microcosm experiments demonstrate the capacity of the ERM fungus to accumulate and import liberated mineral nutrients into its host plant.

Mycoheterotrophic Associations

Mycoheterotrophy is a form of plant nutrition in which a plant species that has lost its chlorophyll in the course of evolution depends on the mycelium of a mycorrhizal fungus to supplement or replace photosynthesis as a source of carbon/energy. In effect, a mycoheterotrophic plant is a parasite on the mycorrhizal symbiosis, cheating it of the carbon resources shared in the mycorrhizal mutualism. Around 400 plant species in 87 genera and 10 families have lost all chlorophyll and receive all their carbon from green plant hosts via fungal connections. There are also about 20,000 species of partially mycoheterotrophic plants (**mixotrophs**), most of which depend on fungi only during seedling establishment. Mycoheterotrophic plants are commonly found in forest understorey habitats where low light limits photosynthesis. AMF mycoheterotrophs include representatives from many different plant phyla including mosses, liverworts and ferns, and many non-green flowering plants. Mycoheterotrophic plants exploiting ECM fungi include representatives of several plant families, but many are Orchidaceae, all of which require a fungal symbiont to germinate, some being only transiently mycoheterotrophic, and others achlorophyllous and wholly dependent on fungal supply. There is even an orchid which is subterranean throughout its life cycle, the Australian species *Rhizanthella gardneri*. Of the 400 fully mycoheterotrophic angiosperm plant species, 35% are orchids. The dust-like seeds of orchids carry insufficient food reserves for germination and the embryo must be colonised by hyphae of the appropriate fungus which imports sugars and stimulates development of root and shoot primordia. Orchid seeds placed in forest soils were found to need colonisation by mycelium of local ectomycorrhizal fungi. For example, seeds of a *Neottia nidus-avis* orchid would only germinate within 5 m of a beech tree, *Fagus sylvatica*, where they were colonised and supplied with nutrients by the beech ectomycorrhizal basidiomycete *Sebacina* sp. Mycoheterotrophic plants show a remarkable degree of specificity for their fungal partners compared with ECM or ERM plants. The conservation of such plant species thus depends upon the conservation of habitats where their specific mycorrhizal fungus is present in the soil.

Common Mycorrhizal Networks

Mycelium of mycorrhizal fungi that can extend into the soil beyond the host root may colonise adjacent host plants. Separate individual plants may thus become linked into an underground mycorrhizal network – the so-called wood-wide web. Nutrient exchange can occur between plants of different species linked by a mycelial network where plant and fungal partners are compatible. Fungi that form mycelial cords have been found to redistribute

significant amounts of nutrients between plants. In nature, some plant species regularly occur together in some habitats, prompting speculation that they might be mutually supportive in this way. In the north of Scotland, pine (*Pinus*) trees commonly grow with an understorey of cowberry, *Vaccinium vitis-idaea*. Molecular analysis identified a fungal ECM of pine roots as *Meliomyces*, in the ascomycete *Hymenoscyphus ericae* clade, which also formed ERM with cowberry roots. The fungus formed mycelial connections between plants in microcosms, and was found to mediate reciprocal carbon and nitrogen nutrient exchange between pine and cowberry, indicating that *Meliomyces* mycelium may also channel a mutualistic exchange of resources in the field. The ERM association presumably helps the partners acquire nitrogen from the peaty, N-poor soil, while the ECM-pine connection supplies the shaded cowberry with photosynthate from the sunlit pine canopy.

The physical extent of fungal webs in soil must, at least in part, depend on specificity between plant and fungus, and on genetic compatibility between fungi. Where all the plants are compatible with predominant mycorrhizal fungi, and the fungi themselves are compatible and capable of fusing with adjacent mycelia, a mycelial web encompassing many plants is theoretically possible. A community composed of different plant species may be connected via a single genetic type of mycelium provided that the fungus is sufficiently generalist in its plant specificity. There may, however, be greater specificity at sub-specific taxonomic levels than has been recognised. Interactions between mycorrhizal fungi and plants in communities have been investigated using network analysis to discern pattern in multiple interactions. Instead of conventional pair-wise analysis, network theory deals with patterns of multiple interactions, such as those between the whole fungal population of a habitat and the community of plants which potentially interact with them. The topology of interaction networks can predict previously undetected host specificity. 'Nested' network structures, where specialists interact with symbionts that also interact with generalists, are common in mutualistic associations. For example, when Glomeromycota sequences from 450 plants in 100 square metres of woodland were analysed, the pattern of interaction predicted the existence of both generalist and specialist AMF fungi. This had been expected from previous findings, for example, the fact that natural plant communities host a more diverse AMF population than agricultural monoculture. However, network analysis has the additional power of potentially identifying the interacting species via their sequences, providing a basis for *in situ* investigation of the taxa of interest.

Habitat factors can be as influential as phylogeny in the composition of mycorrhizal assemblages. An intriguing result was obtained in a network analysis of 430 orchid plants and their mycorrhizal fungi on the island of Reunion in the Indian Ocean. The orchids are either epiphytic, living in the branches of trees, or terrestrial, with roots in the soil. The fungi all belong to the basidiomycete anamorphic group *Rhizoctonia*. However, there was little phylogenetic overlap between fungal populations in terrestrial and epiphytic orchids. The two different fungal populations had apparently assembled from varied phylogenetic origins through shared ecology. The analysis suggested a difference in fungal niche adaptation. Both terrestrial and epiphytic plants utilise fungi to supplement the tiny reserves carried in their powder-like seeds, but the terrestrial orchids continue to employ fungi to acquire carbon resources from soil, and so it might be predicted that these would be selected for an additional set of attributes such as cellulolytic ability.

Common mycorrhizal networks pre-existing in a habitat can help newly-arrived plants to become established by nurturing their seedlings, providing photosynthate from established mature plants. For example, mycorrhizal networks play a key role in the gradual establishment

of vegetation on the volcanic desert slopes of Mount Fuji. Here, the first vegetation consists of scattered clumps of willow, whose ectomycorrhiza, acquired from airborne fungal spores, then fosters the growth of other plants which are compatible with the mycorrhizal fungus. This 'nurse' function of common mycorrhizal networks is exploited in forestry to regenerate forest ecosystems by planting pre-colonised saplings which host suitable ECM fungi. For example, nursery plants of *Arbutus menziesii*, which hosts a wide range of mycorrhizal taxa that develop extramatrical networks into surrounding soil, is planted to help re-establish mixed evergreen forests in Oregon.

In mature woodland, the pattern of tree colonisation by ectomycorrhizal fungal individuals was mapped in relation to host trees of various sizes. Different fungal species, *Rhizopogon vinicolor*, and *Rhizopogon vesiculosus*, each formed 13–14 genets (see Chapter 4, p.100) within a 30 m × 30 m plot of Douglas fir (*Picea abies*) forest, each genet colonising up to 19 trees. *Rhizopogon vesiculosus* genets were larger and connected more trees than *Rhizopogon vinicolor*. Large trees provided dominant nodes in spatial networks, forming centres of mycelial systems which extended over an area of several metres, containing other trees of various ages which were also colonised.

Fungal connections in common mycorrhizal networks are dynamic and variable. The spatial extent and duration of connectivity can be expected to vary as hyphal connections are continually made and broken under the influence of physical changes and biotic interactions. Mapping the area of underground distribution of ECM fungi of several common species in woodland showed that the relative size of the area occupied by each species changes. Some, but not all, ECM fungal species are patchily distributed, and the size of patches differs between species and seasons.

LICHENS

Lichens are fungi that have evolved to house a population of unicellular or filamentous photosynthetic cells (**photobionts**) that provide the fungus host (**mycobiont**) with carbon compounds. Unlike the mycorrhizal associations described above, in lichens it is the fungal partner that forms the main structural component. This takes the form of a differentiated multicellular body termed a **thallus**, with defined tissues including a specialised layer housing the photobiont cells. The association is traditionally considered to be mutualistic because of its stability, with the captive photobiont persisting unharmed, closely integrated in form and function with its fungal host. Having its own internal photosynthetic carbon supply frees a lichenised fungus from the need to grow through soil and organic material to forage for carbon and energy sources. Instead, lichens grow aboveground, exposed to the light, attached to solid surfaces: stable soil, rocks, trees, and even manmade surfaces, including concrete, rubber, and plastic. Lichens show a variety of phenotypes (Figure 7.12). Some form leafy (foliose) or branching (fruticose) three-dimensional structures that grow from defined areas at the base or margin. In humid environments these can grow into gelatinous plates or tangles of branches, tens of centimetres across, although, having no internal long-distance water conduction they cannot grow tall enough to compete with vascular plants for light. Others make hard, dry crusts so closely applied to rock (crustose), and so slow-growing, that they may look more like paint marks than anything living, and others produce a pebble-like growth close to the

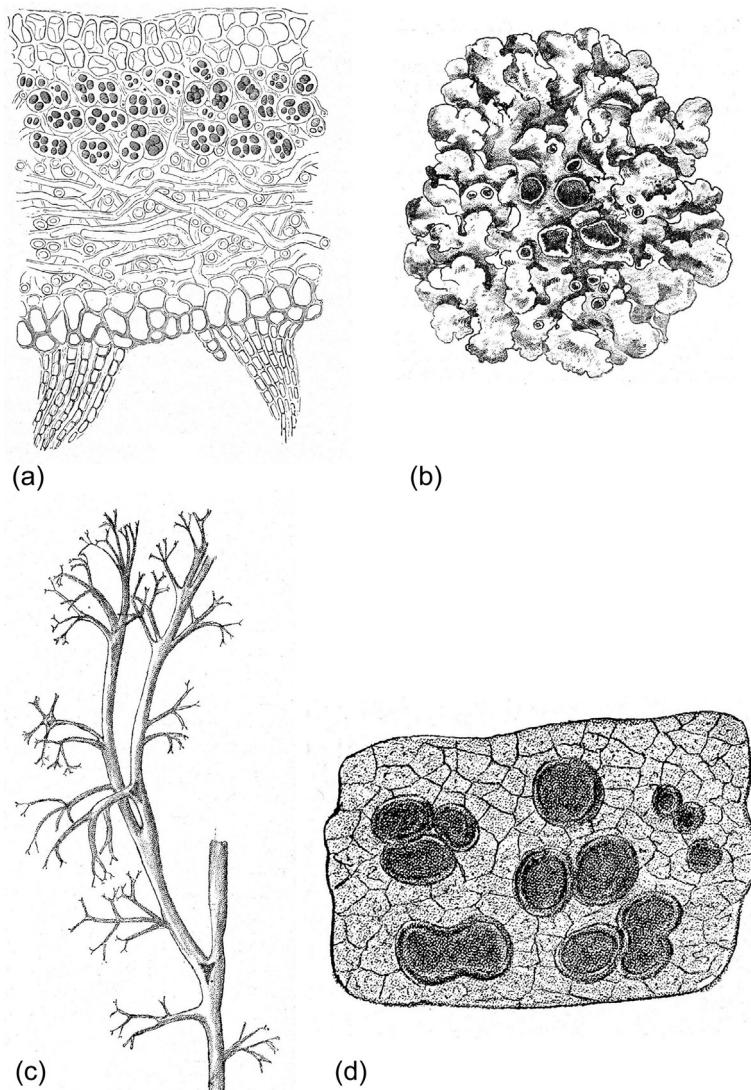


FIGURE 7.12 Anatomy and range of form in lichens: (a) vertical section of *Sticta fuliginosa*, a foliose lichen, showing the position of photobionts in the upper cortical layer of the thallus; (b) Foliose form, *Parmelia acetabulum*; (c) fruticose form, *Cladonia rangiferina*, (d) crustose form, *Lecidea confluens*. Source: The Bodleian Library, University of Oxford, from Engler and Prantl (1907).

substrate. Some are beautifully coloured by secondary metabolites produced by the combined metabolism of both partners. Form is determined by the fungal species, with the photobiont retaining its free-living form. Grown in isolation by culturing spores of the fungal partner, a lichen-forming fungus typically produces only slow and weak hyphal growth which is not ecologically viable. Some lichenised fungi form associations with two, or rarely, three different

photobionts, producing different phenotypes with each. A lichen is identified as a partnership between a named mycobiont and named photobiont(s). In spite of the intimacy and complementarity of function between the mycobiont and photobiont partners, sexual reproduction is confined to the ascospores (rarely, basidiospores) produced by the mycobiont. New individuals may be produced when clumps of combined myco- and photobiont tissues (**soredia**) are dispersed, enabling vegetative spread of the association. Sexual reproduction involves recombination of only the fungal partner, via ascospores. To form a new lichen individual, these must encounter and combine with suitable photobionts, for example, when an ascospore lands on a damp surface already inhabited by green algae or cyanobacteria.

Classification and Nomenclature

Even though lichen symbiotic phenotypes are identified and referred to as species, the biological species concept does not apply and lichens are classified by phylogeny of the mycobiont. The species name refers to the fungal partner. There are between 17,500 and 20,000 described species, of which 99% are Ascomycetes, with over 40% of known Ascomycete species lichenised, all in Pezizomycotina. A relatively very small number of lichens, around 150, belong to four families of Basidiomycota. In contrast, fewer than 150 lichen photobionts are known, mostly only by genera. Few have been assigned to species, but it appears that photobiont species diversity is much less than that of lichen fungi, with an estimated 85% belonging to the green algae, mainly in the genus *Trebouxia*. There appears to be little selectivity for species within the *Trebouxia* genus. For example, the lichen *Lecanora rupicola* is a crustose species found on siliceous rocks worldwide. *Trebouxia* samples of *L. rupicola* from geographically widespread sites were found to belong to numerous distantly related lineages. About 10% of lichens have cyanobacterial rather than green algal photobionts, and around 4% of lichens have both. In many lichens the Cyanobacterium is partitioned within structures called **cephalodia**, where microaerophilic conditions are maintained, promoting the activity of nitrogenase which catalyses reduction of molecular nitrogen to ammonia. Cyanobacterial photobionts contribute combined nitrogen as well as carbon to the symbiosis, by virtue of their nitrogen-fixing capability. New lichen photobionts continue to be discovered, particularly in simpler forms and less-studied habitats. No differences have yet been discovered between the photosynthetic green algae and cyanobacteria isolated from lichen associations and those of the same taxonomic group found as free-living microorganisms.

An enormous diversity of small organisms lives in or on lichens. It has been said that a lichen is not an individual, but rather a consortium with an unknown number of participants. The metagenome of the lichen *Lobaria pulmonaria*, a foliose epiphytic lichen found in European montane woodland, has recently revealed an extensive microbiome including hundreds of bacterial types with the genetic potential to contribute to the viability and productivity of the symbiosis. Rhizobia are the most abundant, and grow both on the surface and within the thallus.

Endophytic fungi, not directly involved in the symbiotic partnership, are represented by more types than in any other habitat. They associate preferentially with the green algal partner and it has been hypothesised that lichen endophytes might have been evolutionary breeding grounds for fungal parasites of vascular plants. Other lichen inhabitants include bacteria, parasitic fungi, and lichen-eating invertebrates including specialised mites.

Physiology and Adaptation

Lichens are **poikilohydric**, their water potential equilibrating with that of their environment, like mosses. The lichen grows as a fungal tissue within which photosynthesising photobiont cells occupy a relatively small volume in particular positions within the thallus. Many lichen species can grow, though slowly, under conditions of intermittent water supply. The unique cellular structure and physiology that makes this possible has been analysed in detail in the large structurally complex lichen *Sticta sylvatica* (Figure 7.13) where the hyphae of the outer top and bottom layers (upper and lower cortex) are more or less isodiametric, embedded and stuck together in a hydrophilic matrix. When wet, the upper cortex transmits light to the

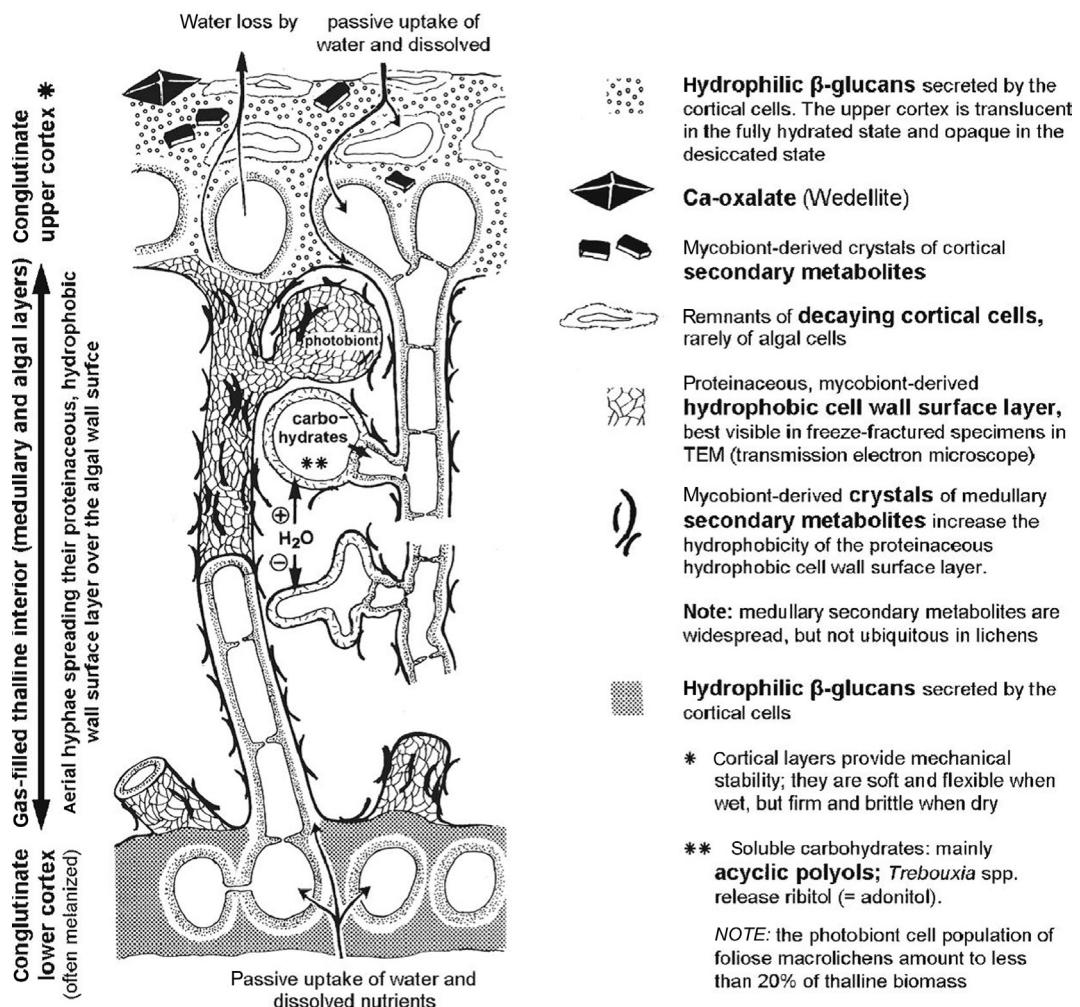


FIGURE 7.13 The functional anatomy of internally stratified thalli of lichenized ascomycetes. Source: Honegger (2009).

underlying photobiont cells, which form a layer at its lower side. The central part (medulla) consists of loosely interwoven filamentous hyphae with gas-filled spaces between them, which are prevented from becoming waterlogged by layers of **hydrophobin** (Chapter 2, p.53) coating their walls. Differentiation of the lichen thallus involves a remarkable localisation of hydrophilic and hydrophobic cells and tissues. Mass flow of water over short distances occurs in the tissues (thalli) of some large lichens such as *Peltigera* spp., through vein-like ribs of thickened tissue. These contain cells with highly hydrophilic walls, insulated by outer hydrophobic layers, which channel passive but rapid capillary flows of water and solutes. In dry weather the cortical layers can lose so much water that they shrink and become brittle. Remarkably, cell damage is minimal even though air spaces can occur within fungal cells, and on rewetting the thallus rehydrates and cell structure is regained. There is, however, a cost in photosynthetic assimilation on each drying episode, because on rehydration photosynthesis takes longer to resume than respiration.

The interface for nutrient exchange between myco- and photobiont in lichens is unlike that in many biotrophic associations, in that no intracellular haustorial structures are formed. Instead, the medullary hyphae grow into the gelatinous sheath that surrounds the photobiont cells and the associated photobiont and hyphal cells become sealed together within the hydrophobic coating material. The carbon compounds transferred from the photobiont to the fungus are polyols from green algae, and glucose from cyanobacteria. When the lichen is moistened by rain or humid air, photosynthate is released from the photobiont and taken up by the closely-associated hyphae. The mechanism and regulation of carbon transfer is not well understood, but C flux from photobiont to fungus has been found to depend on thallus water content. The interaction between partners has proved less amenable than mycorrhizal partnerships to molecular analysis. Changes in gene expression induced by mixing cultures of the model lichen species *Cladonia grayi* with its green alga partner *Astrochloris* sp. include up-regulation of cell recognition in the fungus and metabolic changes in both organisms. A similar experiment in the cyanobacterial lichen *Pseudocyphellaria crocata* showed that in early thallus development the cyanobacterial symbiont, *Nostoc punctiforme*, shows up-regulation of genes concerned with nitrogen fixation in the heterocyst.

The close interaction of the partners in the lichen symbiosis is expressed not only in their integrated morphology and primary metabolism but also in their secondary metabolites (Chapter 5, p.161), including the 'lichen acids' that are produced by the fungus and crystallise on the surfaces of the hyphae. However, in pure culture the quantities and sometimes the nature of the products differ from those in the lichenized state. Over a thousand compounds have been characterised, including some with biological activities that include photoprotection, as well as allelochemical, antibacterial, anti-tumour, anti-herbivore, and antioxidant action. Usnic acid has activity against bacteria, including clinical isolates of vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*. Traditionally lichen products have a variety of folk uses. They have been used to produce a range of pleasing muted colours when used to dye cloth, and can also be used in the identification of species. Vulpinic acid, a mycotoxin produced in *Letharia vulpina*, has been used as a poison for wolves and foxes. Atranorin is used in men's cosmetics, imbuing products with a refreshing smell of the outdoors.

Many lichens have a remarkable ability to survive drought, freezing, high temperatures, and scarcity of key nutrients, and dominate terrestrial ecosystems too harsh for vascular plants, including Arctic and Antarctic, high alpine, desert, and steppe. Ecosystems covering

more than 12% of Earth's land mass are lichen-dominated. Endolithic and epilithic lichens that grow in or on rocks are important agents in eroding and solubilising minerals at both cell and landscape scales. Crust-forming lichens that bind the surface soil in arid zones play an important part in stabilising soils against wind erosion and preventing desertification. In the boreal Arctic, poikilohydry, and the nitrogen-fixing capacities of lichens such as *Stereocaulon* and *Peltigera*, enable lichens to colonise bare and dry ground. Lichens that tolerate freezing have been shown to do so by virtue of ice nucleation sites in wall surfaces that ensure that ice crystallises in the intercellular spaces, and not within cells. The photobiont partner may affect stress tolerance, for example, different clades of the alga *Trebouxia* have been found in Arctic lichens, with one clade preferentially associated with lichens of extremely cold habitats.

In boreal Arctic regions the vegetation can be dominated by species of *Cladonia*, *Cetraria*, *Stereocaulon*, and *Alectoria*, which form closed mats loosely attached to the soil (Chapter 12, pp. 383–386). These lichen mats can provide as much as 60% of the winter food of caribou and reindeer. Disastrously, they have been affected by radioactive pollution. Lichens bind metal cations and accumulate metal-rich particles within the thallus, absorbing them over the surface. Radionuclides from fallout from H-bomb testing and accidents such as those at Chernobyl and Fukushima accumulate in lichens and constitute a health hazard for Scandinavian Sami people and North American Eskimo, who depend on meat of the caribou and reindeer that graze lichen and accumulate high concentrations of radionuclides in their bodies.

Lichens are valuable indicators of several forms of atmospheric pollution. Assay of lichen-bound lead has provided longitudinal data on global atmospheric lead pollution, and showed a decline in levels following the introduction of catalytic converters on car exhausts. They are sensitive to sulphur dioxide and nitrogen pollution (e.g. ammonia, oxides of nitrogen and elevated deposition in rainfall) but different species are affected to varying degrees, so that lichen diversity can be used to monitor air pollution. Changes in the physiology of arctic lichens can indicate atmospheric pollution from nitrogen oxides originating from fossil fuel combustion in distant industrialised regions. When their environment becomes nitrogen-sufficient, lichen metabolic profiles indicate a change from nitrogen-limited to phosphorus-limited metabolism.

Evolution

The earliest fossils that have been identified as undeniably lichens, with photobiont partners and stratified tissues comparable to extant lichen species, have been found in 415 million year old rock from the Lower Devonian. Internal anatomy was intact and could be examined by scanning electron microscopy. It appeared to consist of septate hyphae forming tissue containing cyanobacterial and a unicellular, presumably green algal photobiont, and asexual spores in a pycnidium typical of Pezizomycotina (Ascomycota).

Molecular genetics has revolutionised our understanding of the processes that have led to lichenisation in fungi. We now know that the lichen symbiosis is polyphyletic, having evolved convergently in separate fungal lineages, and in some, including those of well-known and widespread saprotrophs such as *Aspergillus* and *Penicillium* in the Eurotiales, there is evidence that a formerly lichenised mode of nutrition has been lost. This is of particular interest because a lichenised past may have endowed some of these saprotrophs with important biological attributes such as their pathways for the synthesis of bioactive secondary metabolites including penicillin.

ENDOPHYTES

Microscopic fungi of remarkable diversity inhabit the intercellular and apoplastic spaces of plant tissues, and are collectively termed **endophytes**. Plants provide the habitat for thousands of endophytic fungi, as well as bacteria and viruses. Some cause host disease and produce reproductive structures at the hosts' expense. However, many endophyte fungi produce no visible structures on the plant surface and may only be found when searched for by microscopic inspection, isolation into culture or from the presence of their DNA. Environmental genomic sampling is now revealing microbiomes associated with every larger organism examined, and plants are no exception. Endophytic fungi are ubiquitous in plants throughout natural ecosystems. Some, described in detail below, are known to confer fitness benefits on their hosts, but tantalisingly little is known of the biology and host interactions of the majority. Discerning the effects of this hyperdiverse normal microbiota on plant physiology and ecology is a current challenge. Most are Ascomycota, the majority belonging to Hypocreales. They fall into four broad functional types according to the range of hosts colonised, the extent of colonisation, mode of transmission between hosts, taxonomic diversity and known effects on host fitness (Table 7.1).

Clavicipitaceus endophytes Fungi belonging to the Ascomycete group Hypocreales (Clavicipitaceae) occur as endophytes in Gramineae (grasses, including cereal crops) (Figure 7.14). They show a range of plant interactions from parasitism to mutualism. Mutualistic endophytes confer selective advantage on their hosts by producing anti-insect and anti-vertebrate toxins that protect their host plants from insect attack and herbivore grazing. The genus *Epichloë* has given rise to an exclusively asexual form, known as *Neotyphodium*, which differs in life cycle on the host grass (Figure 7.15). *Epichloë* species reproduce both asexually by conidia, and also through a sexual cycle where ascospores are formed on a stroma produced in place of the inflorescence. In cereal crops this is known as 'Choke' disease. Because both sexually produced ascospores and asexual conidiospores are released, the fungus can spread horizontally through the cereal population from one individual plant to another. However, asymptomatic endophytes evolved from *Epichloë* have lost the capacity for release of ascospores, and grow exclusively as the asexual anamorphic form within host tissues (Figure 7.15), and

TABLE 7.1 Criteria Used to Characterise Classes of Fungal Endophytes

Criteria	Clavicipitaceous		Nonclavicipitaceous	
	Class 1	Class 2	Class 3	Class 4
Host range	Narrow	Broad	Broad	Broad
Tissue(s) colonised	Shoot and rhizome	Shoot, root, and rhizome	Shoot	Root
<i>In planta</i> colonisation	Extensive	Extensive	Limited	Extensive
<i>In planta</i> biodiversity	Low	Low	High	Unknown
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
Fitness benefits ^a	NHA	NHA and HA	NHA	NHA

^aNonhabitat-adapted (NHA) benefits such as drought tolerance and growth enhancement are common among endophytes regardless of the habitat of origin. Habitat-adapted (HA) benefits result from habitat-specific selective pressures such as pH, temperature, and salinity. Adapted from Rodriguez et al. (2009).

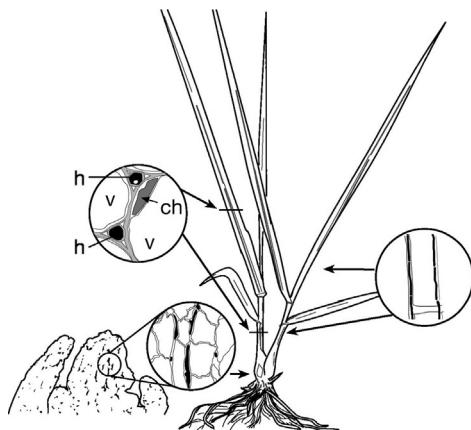


FIGURE 7.14 Endophyte growth in a grass plant. Bottom left: fungal growth in the stem and leaf primordia. The fungal hyphae are shown darkly stained with osmium as they would appear in transmission electron microscopy. Upper left: a cross section of leaf or leaf sheath reveals hyphae (h) of the *Epichloë* endophyte between host cells. Also shown are a chloroplast (ch) and vacuoles (v). Right: the endophyte as it appears in a leaf epidermal peel, stained for hyphae, which are arranged mainly along the longitudinal axis of plant cells. Unstained septa separate individual fungal cells, each of which bears a single haploid nucleus (not shown). Source: Selosse and Schardl (2007).

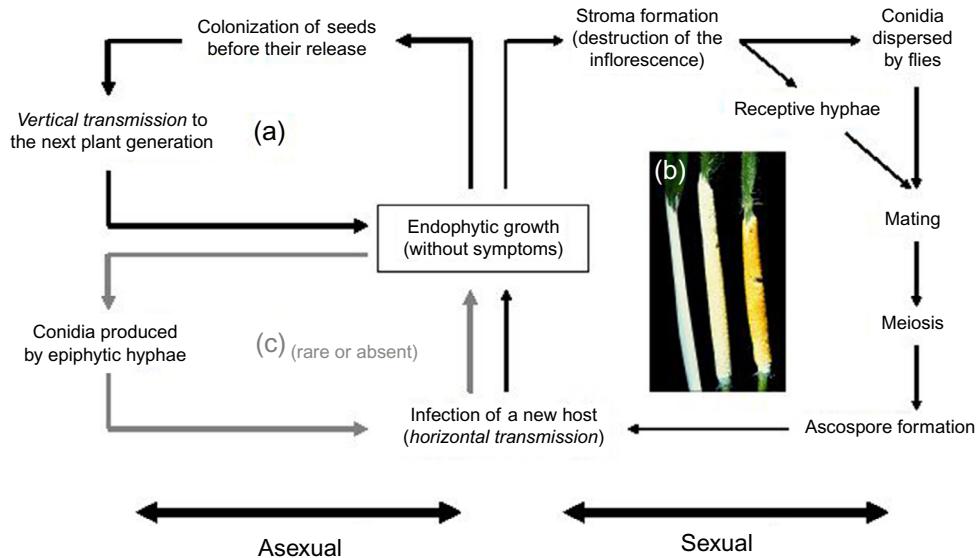


FIGURE 7.15 Life cycles of *Epichloë* endophytes of Gramineae. *Epichloë* can reproduce asexually by invading the host's seeds (a), by sexual cycles where ascospores are formed (b) on a stroma or 'choke' that destroys the inflorescence, and more rarely through asexual spores (c). Transmission is thus vertical (a) or horizontal, (b, c). Neotyphodium species, endophytes derived from *Epichloë*, mostly carry out asexual reproduction through host seeds (a), resulting in mainly vertical transmission. Source: Selosse and Schardl (2007).

these anamorphic forms are classified as *Neotyphodium*. Transmission in the host population is predominantly vertical, from one generation to the next, via seedborne mycelium. Mycelium enters the ovule from tissues of the shoot in which it is systemic, and colonises the seed itself. No independent sexual reproduction occurs and the evolutionary fate of the fungus thus becomes indissolubly linked to that of its host. From population studies of *Neotyphodium* species in grasses of natural ecosystems, it has been found that individuals consist mainly of multiple asexual hybrid genotypes, probably arising from genetic exchange between individuals within a single plant, each arising from separate conidial infections. Because the hybrids are only transmitted through host reproduction, they are likely to be selected for traits that improve host fitness. The selective advantage the mutualism confers on both partners explains the stability of the partnership. As a result, the plant carries the endophyte throughout its geographical range and most of the population is infected. The characteristics conferred by infection, such as increased vigour of vegetative growth, and the presence of secondary metabolites antagonistic to herbivores, become general features of the grass species. Many species of the common temperate grass family *Poaceae* are affected in this way by *Neotyphodium* endophytes. The toxins produced by the endophyte are alkaloids: indole-deterpenes and ergot alkaloids poisonous to vertebrates, and peramines and lolines that act mainly against insects. Alkaloids produced by *Epichloe* or *Neotyphodium* endophytes across a range of grass species were analysed. The production of anti-insect alkaloids peramine and loline was found to be greater than anti-vertebrate ergot alkaloids in the asexual *Neotyphodium*, perhaps because, having lost sexual reproduction, it no longer depends on insects to transmit male gametes in fertilisation. Mutualism based on the anti-insect properties of alkaloids appears to represent some cost to the plant. The grass *Bromus setifolius* is used by leaf cutting ants. Where the ants are abundant, for example, in Argentinian desert, 80–100% of sampled plants contained the endophyte *Neotyphodium tembladerae*, but in places with no ants, where the pressure of herbivory was relaxed, endophyte levels were down to 20–0%.

Alkaloids of Clavicipitaceae have a potent effect on humans and domestic animals. *Claviceps purpurea* is a plant pathogen which produces black sclerotia (ergots) instead of grain in the ear of cereal crops. In the past, local outbreaks of ergotism were caused by this mycotoxin which causes vasoconstriction and hallucinations. Ergot alkaloids have been well characterised in terms of biosynthetic pathways and gene clusters encoding them and are important in pharmacology. Various syndromes occur among farm animals due to endophyte alkaloid consumption. Ergot alkaloid poisoning of cattle causes vasoconstriction resulting in sloughing off of hoofs and abortion. Horses in the western United States who eat 'sleepy grass', *Achnatherum robustum* colonised by an endophyte producing lysergic acid, may sleep for days, then gradually recover, but the experience leaves its mark and they do not eat the grass again. Similar phenomena are reported from Asia (drunken horse grass) and South Africa (drunk grass).

Even endophytes that are not acutely toxic to animals can deter feeding, and a variety of turf grass cultivars are now identified as 'endophyte enhanced'. Some Class 1 endophytes confer resistance to disease, for example, *Epichloë festucae*-infected turf grass is resistant to some leaf spot diseases (e.g. dollar spot caused by *Sclerotinia homeocarpa* and red thread disease caused by *Laetisaria fuciformis*). Systemic endophytes can affect not only individual plants but also whole ecosystems. *Neotyphodium coenophialum* in tall fescue (*Lolium arundinaceum*) not only affects plant–herbivore interactions and plant productivity but also plant–plant competition, decomposition rates, and grassland species diversity.

Class 2 endophytes mostly belong to Pezizomycotina (Ascomycota) with some Basidiomycota. They are an ecologically distinct class as they colonise the roots, stems, and leaves of monocotyledonous and dicotyledonous plants, often forming extensive infections, with especially high infection frequencies (90–100%) in plants growing in stressful environments. They can be transmitted vertically via seed coats and rhizomes, and also horizontally, some (e.g. species of *Phoma* and *Arthrobotrys*) being abundant in soil, others having low abundance in soil. The latter are probably unable to compete outside the host, whereas those abundant in soil may have several lifestyles – symbiotic and saprotrophic. As with other endophytes, they colonise plant tissues by direct penetration or using infection structures such as appressoria, and grow mainly between plant cells, upon which they have little or no obvious impact. In healthy plants, sporulation or appressorial formation is low, but the fungi rapidly emerge and sporulate when plants senesce. Species of *Alternaria*, *Cladosporium*, *Epicoccum*, and *Phaeosphaeria* are dominant endophytes of *Dactylis glomerata* and other grasses and sporulate over a significant area of the aerial plant when it senesces.

In most cases Class 2 endophytes are found by culture and/or molecular sampling, rather than through altered host characteristics, and their effect on plant fitness is not known. However, a few species have been demonstrated to have a remarkable effect in allowing their hosts to grow in habitats that are too stressful for either plant or fungus alone (Rodriguez et al., 2009). For example, *Curvularia protuberata*, which colonises all tissues of the geothermal plant *Dichanthelium lanuginosum*, appears to be confer thermotolerance on its host. The effect is reciprocal since neither host nor endophyte can tolerate temperatures above 40 °C when growing alone, but the colonised plant can grow in places where the temperature is up to 65 °C. Similarly, the coastal dune grass *Leymus mollis* can only grow at the salinity of sea water when colonised by its endophyte *Fusarium culmorum*. *Colletotrichum* spp. confer disease resistance on their hosts. These habitat-adapted fitness benefits are specific to the fungal isolates concerned, but can be conferred on a taxonomically wide range of host plants. Clearly these fungi are of great agronomic interest and the physiological mechanisms of their effects await investigation.

Class 3 endophytes are characterised by ubiquity in plants, and by their hyperdiversity. They occur in the aboveground herbaceous and woody tissues of an extremely wide range of plants and can be extremely diverse even within a single plant, for example, over 80 species have been isolated from juniper (*Juniperus communis*) and from oak (*Quercus petraea*). Diversity is related to latitude, with much higher diversity in the tropics than in boreal forest and arctic tundra, though local abiotic conditions can increase endophyte diversity, for example, wet microclimates in temperate regions. Plants with long-lived evergreen foliage may harbour a greater diversity of endophytes than shorter-lived foliage. Unlike fungi in Classes 1 and 2, they form highly localised infections, involve a wide range of species in Ascomycota (especially Pezizomycotina but also Saccharomycotina), and some Basidiomycota belonging to Agaricomycotina, Pucciniomycotina, and Ustilaginomycotina. Unlike Class 1 and 2 endophytes, Class 3 endophytes are transmitted horizontally via spores and/or hyphal fragments and are major contributors to the air spora at leaf fall in temperate regions (Chapter 3, p. 92). Sterile seedlings and newly-emerged leaves become colonised rapidly in the field. Over 80% of leaves of endophyte-free cocoa tree (*Theobroma cacao*) seedlings contained endophytes within 2 weeks of leaf emergence in a tropical forest, in the early wet season.

Hyperdiversity makes it almost impossible to distinguish single ecological roles for Type 3 endophytes. Some protect host from parasites, for example, bark endophytes may have a role in protection against Dutch elm disease and some endophyte assemblages decrease lesion formation and leaf death by *Phytophthora* in cocoa trees (*Theobroma cacao*), and might be thus exploited as inoculants in biological control of disease. Endophyte infection can damage host plants, for example, by making seedlings wilt faster under drought. The endophyte flora includes latent saprotrophs and parasites that become active once tissues start to senesce. Early stages in the decomposition of fallen leaves are dominated by endophytes such as *Alternaria* that live asymptotically in and on healthy leaves in summer, but start to decompose cellulose and sporulate when the leaf falls.

Wood decay fungi (typically Basidiomycota and xylariaceae in the Ascomycota), present as latent endophytes in the functional sapwood of angiosperm trees, can start to grow once loss of the xylem water column results in aerobic conditions required for hyphal growth. Decay columns of single fungal genotypes develop very rapidly – sometimes in less than a single growing season, probably by the growth of genetically identical mycelia from many different foci. These xylem-inhabiting endophytes are broadly species-specific in the development of decay columns, even though a wide range of species is revealed to be latent in most tree species. For example, in the UK, *Daldinia concentrica* fruit bodies are usually found on ash (*Fraxinus excelsior*) and occasionally on beech (*Fagus sylvatica*) in the south and on birch (*Betula* spp.) in the north; *Eutypa spinosa* and *Hypoxylon fragiforme* are usually found on beech and *Fomes fomentarius* on birch (*Betula* spp.) *Hypoxylon fuscum* and *Stereum rugosum* on hazel (*Corylus avellana*), and *Stereum gausapatum* and *Vullemia comedens* on oak (*Quercus* spp.) and beech. However, all of these fungal species and many more are found by molecular sampling in a wide range of asymptomatic angiosperm tree xylem, suggesting a narrower range of conditions for mycelial development than for endophytic survival.

Class 4 endophytes consist of a poorly-understood group of fungal root associates characterised by melanised hyphae and melanised septa, and known as Dark Septate Endophytes (DSE). They have been found in over 600 plant species in more than 100 plant families. They appear to have evolved repeatedly in Ascomycota, and are found in the genera *Cadophora*, *Microdochium*, *Trichocladium*, *Phialophora*, *Leptodontidium*, and *Phialocephala*. Root colonisation by *Phialocephala fortinii* begins by superficial colonisation of the root surface by a loose network of hyphae. Subsequently, individual hyphae grow along the main axis of the root, between root cortex cells and within depressions between epidermal cells, ultimately penetrating some root cells. Some DSE form a structure similar to the Hartig net of ectomycorrhiza, and may be mycorrhizal. *Cadophora* includes both DSE and ectomycorrhizal taxa, and some DSE form ericoid mycorrhiza *in vitro*. Ubiquitous worldwide, they are particularly prevalent in environments with high abiotic stress, including arid sandy soils, the Arctic and Antarctic, and heavy metal contaminated sites. They may confer lead tolerance on plants, believed to be mediated by their intracellular antioxidant systems, including the melanin in their mycelium. DSE can precede plants in early succession because they can grow and form propagules in the absence of a host plant, and have been sampled from the barren forefront of glaciers where they are assumed to arrive as airborne propagules. Their transmission is likely to be horizontal, via conidia or microsclerotia. They probably have low host specificity. Groups of DSE from arid sandy plains

in Hungary were characterised by sequence and tested for root association by inoculation, and were found to be generalists able to colonise both native and invasive host species.

Bioactive Compounds Produced by Endophytes

Interaction with other organisms is believed to accelerate selection of microbial secondary metabolites for bioactivity (Chapter 5). Endophytes, with their long-term association with hosts and multitude of interactions with other plant inhabitants including fungi, bacteria, nematodes, and insects, provide a fertile area for exploring fungal biosynthetic potential. Since the discovery that taxol is produced by endophytes of the yew tree (*Taxus baccata*), there has been an explosion of endophyte screening studies, and over 4000 biologically active secondary metabolites have already been found with varied structural groups. These are investigated for potential antibacterial, anti-viral, antifungal, anti-cancer, anti-inflammatory activity, as insulin receptor activators, acetylcholinesterase inhibitors, β -glucuronidase inhibitors, eosinophil inhibitors, insecticides, and root growth accelerators. Novel compounds from endophytes include new lactones with potential as leads for anti-malarial drugs, (+)-ascochin and (+)-ascodiketone, apiosporic acid, chaetocyclinones, colletotrichic acid, cyclopentanoids, enalin derivative, isofusidienols, myrocin A, naphthoquinone, pestalotheols A–D, phomopsilactone, and spiroketals. As well as producing novel bioactive chemicals, endophytes can affect biotransformations of chemicals that can be used in drug modifications, and offer the potential for conversions relevant to biofuel production. **Bioprospecting**, the search for useful genes in environmental sequences, can identify gene clusters encoding biosynthetic pathways in unculturable or poorly culturable fungi such as lichens and endophytes, and promising genes can be transformed into more tractable heterologous hosts for expression.

With the advent of metagenomics, including expression analyses linked to taxa, new knowledge is certain to elucidate the huge taxonomic and functional diversity of fungal symbionts that populate plant tissues and microenvironments. Symbiosis, once considered a specialised way of life, is being revealed as an engine of plant evolution that enabled plants to colonise the land and continues to maintain the productivity and diversity of all Earth's terrestrial biomes.

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