

1 • *Introduction*

Travel to your nearest nature reserve. It could be anywhere, within a human habitation or wildland, on continents or islands around the globe. It could be a forest, shrubland, grassland, forbland, or even desert. Across your view is a diverse array of plants, complete with a complex architecture with several levels from the ground surface to the highest leaf, be it a redwood tree extending hundreds of meters into the sky, or a diminutive, but still complex, structure only 10 cm high of mosses, liverworts, and cryptogams. As you separate the stems and look down, you see roots: tens of meters of roots or rhizoids for every square meter of the soil surface. Take your hand lens and pull up some of those roots. There will be soil hanging from those roots, held together by tens to hundreds of meters of threads of fungal hyphae per cubic centimeter. The vast majority of these hyphae form mycelia from many species of fungi, directly connecting fine roots to the larger soil matrix, serving as a living pathway interconnecting the plant, which is actively fixing carbon, within the micro patches of nutrients and water necessary to fix that carbon. This is the macro-microscopic world of mycorrhizae; the space where many plants interface with many fungi, interweaving a matrix of roots, soil particles, and decomposing organic debris from once living tissue, extending thousands of kilometers across continents. But there are holes in this web. It is dynamic. Individual hyphae may die within hours or live for decades. Mortality may come from a grazing collembolan, an ant colony, an agricultural field, a volcano, or an ice age. What connects all of the organisms comprising this landscape? The first primitive proto-eukaryote established a mutualism with an endosymbiotic energy machine (mitochondrion), a symbiosis that resulted in a creature capable of far more efficient growth and adaptation. Somewhere among these primitive proto-eukaryotes, one cell ingested a photosynthetic cyanobacterium, forming a group of autotrophic, eukaryotic microorganisms with organelles, thereby changing the globe. Today, symbiosis plays a similar critical global role. The mutualistic arbuscular mycorrhizae form

glycoproteins and other complex organic recalcitrant compounds that sequester atmospheric CO₂ and regulate global greenhouse gases. This is the complex, interconnected web of mycorrhizal symbiosis. It is comprised of a mutualism between the vast majority of plants, and fungi from all phyla of the true fungi.

Mycorrhizae are mutualistic symbioses between plants and fungi localized in the roots or rhizoids, in which the fungi obtain carbon from the plant in exchange for resources that the fungus extracts from the surrounding substrate. This definition, which I outlined three decades ago (36), extends the concept of a complex suite of relationships that go well beyond the single plant–fungus symbiosis focus, reasonably well understood, to a *network* connecting many plants and many fungal mycelia and comprising an ecosystem. This view encompasses complexity resulting from two advances in evolutionary and ecological thinking. Before the 1990s, the predominant idea was that mutualisms were abnormal, and generally unimportant in determining the evolutionary history of an organism and ecological functioning. Since then, the study of mutualisms in general and for mycorrhizae specifically have entered mainstream journals and thinking in both ecology and evolution. The second was that the functioning of mycorrhizae could be understood in the context of enhancing the fitness of a plant and a fungus through the supply of external resources. We know that a single plant lives in an environment in which it is colonized by many mycorrhizal fungi, and a single mycorrhizal fungus invades many plants. This connectedness view from new observations complicates and enriches the picture that was the focus for many of us a quarter century ago.

The formal study of mycorrhizae (Latinized plural of mycorrhiza) is well over a century old. The term “mycorrhiza,” coined by A. B. Frank in 1885 (256), refers to *μύκητας-ρίζα*, *mykitas-riza*, a fungus–root. He described the symbiosis as a mutualism in that it is comprised of a distinctive morphological structure in which the fungus encases a root and acts as a “wet-nurse” to the plant, “. . . er funktioniert im Bezug auf diese Ernährung als die Amme des Baumes.” This description followed the remarkable observations of F. Kamienski (398) on *Monotropa* in which he classified the fungus–*Monotropa* relationship as a mutualistic type of symbiosis. These were notable advances conceptually. Theodore Hartig (324) had illustrated the Hartig net, which formed in conifer roots. However, he did not recognize this structure as fungal in origin. His son, Robert Hartig, the “father of forest pathology,” studied *Agaricus* (= *Armillaria*) *melleus*, the honey fungus, and his observations on the

invasion and structure of this disease postulated that it resembled the Hartig net and rhizomorphs of ectomycorrhizae (323), and the Tulasne brothers, in their detailed descriptions of *Elaphomyces granulatus*, noted that the mature sporocarps enclosed the root tips and opined that this fungus parasitized the plant (727). Indeed, research on the importance of fungi as plant parasites had made noteworthy progress during this period, as evidenced by Tillet's (709) experimental demonstration of the disease of wheat kernels (smuts and bunts), Berkeley's (117) discovery of *Botrytis* (= *Phytophthora*) *infestans* as the causal agent of potato blight as opposed to lightning or electricity, and DeBary's detailed studies of rusts and smuts (197).

The evidence for mutualism was initially quite shaky and viewed by many with skepticism. Kamienski (398) described the functioning of the monotropoid mycorrhizae as a symbiotic relationship existing along a parasitism-to-mutualism gradient, concluding that this relationship was probably more mutualistic than parasitic. His hypothesis was based, in part, on the evidence that the fungus invaded a root, but no necrosis of that root was observed and the root remained healthy. In the absence of experimental growth response data, this approach remains a useful observation that at least sets the stage for further study. This evidence formed the basis of Janse's (381) remarkable study of arbuscular mycorrhizae in Indonesia, Lohman's (465) survey of mycorrhizal types in Iowa woodlands, and even more recent work such as our observations of dark-septate fungi found in epiphytic bromeliad roots in the seasonal forests of Mexico (72). These observations contrasted with the views of early researchers such as McDougall and Liebtog (493), who envisioned mycorrhizae as a "mutual reciprocal parasitism," in that both would gain some nutrients (such as organic N) but independently the plant would still grow better without the fungus.

DeBary (198; 199) formally defined a symbiosis as two organisms living intimately together. These observations were based initially on his observations of the cyanobacteria-*Azolla*, and lichens; mutualistic associations as opposed to the known parasitism of many smuts, rusts, and blights. He envisioned symbiotic relationships in a neutral context as comprising interactions ranging from mutualism to parasitism. From his work we can develop a simple but powerful +/0/- suite of relationships between the two symbionts, plant and fungus, that differentiates between a mycorrhiza and a parasite, and other symbiotic relationships. These include parasitism (+/+), amensalism (0/+), antagonism or competition (-/-), neutralism (0/0), commensalism (+/0), and mutualism (+/+) (36).

Frank (256) defined a “mycorrhiza” as a functional relationship whereby the underlying carbon needs of the fungus are provided by the tree through its leaves, “*Nahrungsbedurfniss des Pilzes wird sich hauptsächlich auf die assimilirten, kohlenstoffhaltigen Nahrungstoffe beziehen welche der Baum durch seine chlorophyllhaltigen Organs bereitet,*” and a significant quantity of water and nutrients required by the tree from soil can only be supplied via the fungus, “*das ganz für den Baum erforderliche Quantum von Wasser und Nährstoffen aus dem Boden nur durch Vermittelung des Pilzes demselben zugeführt wird.*” This means structural elements that are both internal to the plant for accessing C and extramatrical or external in the soil to access water and nutrients.

Crucially, fungi provide external resources (from the soil, or other growth medium) to the hosts and, in exchange, plants provide carbon to the fungi. This exchange, in which both symbionts are evolutionarily adapted to acquire, provides positive benefits for both (450). Thus, a mycorrhiza has a defined structural aspect. While the nature of a mutualism–parasitism relationship of this symbiosis is sometimes difficult to measure, and fluctuates temporally depending on resource availability, mutualism is a defining character. A mycorrhiza is evolutionarily, a mutualistic symbiosis.

Up to the 1980s, the widespread view of mycorrhizae, and mutualisms in general, was illustrated in Williamson’s paradigm (782) that [mutualism] is interesting, but unimportant. He stated that most examples of mutualism are tropical (see discussion in (36)). May (491) further stated that mutualisms are mathematically unstable. Indeed, most mathematical community models suggest that, while parasitism confers stability, mutualisms result in unstable dominance by a limited suite of hosts (e.g., Bever (119)). But experimental evidence (303; 406) continues to show that mycorrhizae increase diversity by supporting lower density species. Molecular evidence now points to both widespread and stable mutualisms going back as far as eukaryotic organisms (480), and mycorrhizal relationships go back to the early invasion of land by plants.

Somewhere between 10 and 85 percent of the net CO₂ fixed in terrestrial ecosystems travels through the mycorrhizal fungal/root interface (239). This simple loss of large amounts of C by a plant requires compensatory procurement of large amounts of soil resources. In many situations, a majority of resources like N and P taken up by the plant may come from the mycorrhizal fungus. But water transport, pathogen protection, and other resources like cations can be just as crucial, under

limiting circumstances. Acquisition and exchange of resources creates the complexity that is the core of this book.

A mycorrhiza is a co-evolved mutualistic relationship between plants and fungi in which the fungus extends from the plant into the surrounding substrate, extracting soil resources in exchange for carbon resources fixed by the plant. In this definition, the fungus must extend into the substrate differentiating a mycorrhiza from endophytic fungi, which are important ecologically, but appear to have very different modes of physiological interaction. I also note that the fungus uses carbon directly transferred by the plant. While some mycorrhizal fungi access soil organic C, the majority come from a host plant. This is crucial, as mycorrhizal fungal carbon can be measured isotopically as autotrophic, not saprotrophic carbon, allowing it to be tracked in ecosystem models by age and function (44; 410; 723).

One problem with this definition is that mycorrhizae are also formed by achlorophyllous plants that receive their carbon from the fungus (called mycoheterotrophs). This issue will be discussed in greater detail in Chapters 3, 4, and 6. Especially relevant to this discussion, again, is Kamienski's (398) description of monotropoid mycorrhizae. At this point, we still do not understand what resources the plant provides to the fungus. Is this a case of fungus as host and plant as parasite, or does the achlorophyllous plant provide an unknown resource? Or is the fungus a conduit between the mycoheterotroph and a nearby community of chlorophyllous plants?

Another view is that almost any plant–fungus interaction that appears mutualistic is a mycorrhiza. For example, there were reports early in the twentieth century of *Fusarium* as a mycorrhizal fungus (89). Wilde et al. (780) and Iyer (374) described the “epirhizal” mycorrhizal type, in which almost any fungus can sometimes increase plant growth. Many fungi can be found localized in roots, including numerous facultative parasites, or saprotrophic fungi living on dying roots. Recent versions of the same arguments have emerged in describing members of the Sebaciales, fungi whose DNA are often found in EM tissue, as forming a mycorrhiza. However, the mechanisms of interaction, soil–fungus–plant, may not fit my definition of a mycorrhiza (769). Endophytes are mutualists found in almost every plant part. The fact that a group of fungi, such as the Sebaciales, are often an element of the mycorrhizosphere microbiome, is a very interesting topic for research of a potential fungal symbiotic mutualism (sensu (199)). Moreover, there is a report that these interesting fungi produce pelotons in ericoid plants (651). But at this stage, the mycorrhizal and physiological status needs more work.

Historical Types of Mycorrhizae

Research differentiating the types of mycorrhizal associations goes back well over a century. The origins of the study of mycorrhizae lie in the type called an ectotrophic mycorrhiza and, more recently, with its relative, the arbutoid mycorrhiza. Ectomycorrhizae (or EM) are characterized by an extensive hyphal network growing in the interstitial boundaries between the walls of cortical cells (the Hartig net), and a mantle that covers individual short roots, and whose hyphae extend outward into the soil, often for many meters! This mycorrhiza is the original one described by Frank (256) and Kamienski (398), and occurs between some woody plants and fungi in the Endogonaceae and members of the Ascomycota and Basidiomycota. The ectomycorrhiza evolved independently in many plant and fungal lineages. Arbutoid mycorrhizae were often originally described as ericaceous ectendomycorrhizae. Although they penetrate cortical cell walls, they are comprised of fungi that form EM. These arbutoid mycorrhizae may be mostly Basidiomycota, many of which also form ectomycorrhizae with neighboring plants, and with Ericaceae in the Vaccinioideae, Arbutoideae, and Monotropoideae families.

Frank (257) followed his 1887 descriptions of types of EM with the observation that there were also mycorrhizae that penetrated cortical cells, but did not lead to cell necrosis. He called these endomycorrhizae. In this work, he described what we now call ericoid and arbutoid mycorrhizae. His student, Schlicht (639), carefully described another type of endomycorrhiza associated with herbs now called arbuscular mycorrhizae (or AM), in which arbuscules form a structure capable of resource exchange. Dangeard (189; 190) illustrated both arbuscules and vesicles, also showing the multinucleate status of the fungi forming AM. Janse (381), in studying the endophytic fungi in Java, illustrated AM in many tropical plants, and orchid mycorrhizae from arboreal and ground orchids. Just as interesting, he also reported that achlorophyllous orchids also formed mycorrhizae, just as Kamienski (398) noted for ericaceous achlorophyllous plants. Gallaud (272) clearly described and illustrated the “Arum” type of AM, forming arbuscules independently within cortical cells from intercellular hyphae running between cells, and the “Paris” type of AM, in which the hyphae run from cell to cell, forming intracellular hyphae, arbuscules, and coils, or pelotons.

Only a few of the early studies actually demonstrated a growth promotion of mycorrhizae. Frank (255) showed a growth enhancement

in pine growing in organic soil, limited by N. In other studies, a lack of necrosis in the root tissue following fungal penetration was observed (see (381; 639)) and a mutualistic interaction was postulated. A number of studies subsequently demonstrated the growth enhancements by ectomycorrhizae (e.g., see (305; 319)). In the 1950s, the groundbreaking studies by Mosse (520) and Gerdemann (282) demonstrated that glomalean fungi, classified as zygomycetes and not pythiaceous fungi, transferred nutrients and enhanced growth, characteristics of a mycorrhiza. For an early history, I highly recommend reading the historical section of the Proceedings from the 6th NACOM (509).

As a practical means of organizing mycorrhizal types, two initial foci can be utilized. The first is based on the type of interface between the host and fungus, while the second is phylogenetically-related associations. The first focus is characterized by the location of fungal structures within or outside root cortical cells. In an endotrophic mycorrhiza, or endomycorrhiza, the fungus penetrates the cell wall, forming an extended fungus membrane–interspace–plant membrane interface creating an enlarged surface area between plant and fungus. In the ectotrophic mycorrhiza, or ectomycorrhizae, the fungus forms a network of hyphae between the cortical cells (the Hartig net) and an external mantle (of varying coverage and thickness). The cell membranes of both symbionts remain intact. Because these are morphological characteristics, and often overlap, I prefer to think of these as gradients in types, rather than absolutes.

The second focus is based on phylogenic interactions between plants and fungi. I will explore these in greater detail in Chapters 2 and 3, but I establish some basic relationships here to form consistent terminology. Four primary categories stand out, three of which are endomycorrhizal and one is ectomycorrhizal. The most common is the *endotrophic mycorrhiza* (or endomycorrhizae), which is comprised of arbuscular mycorrhizal (AM) or Glomalean mycorrhizae (also called vesicular–arbuscular mycorrhizae (VAM) or, in some older literature, phycomycetoid mycorrhizae). These occur in some 70 to 80 percent of plants, from basal “primitive” plants through some of the most advanced clades, in symbiosis with the Glomeromycotina of the Mucoromycota. This association appears to have evolved a single time as primitive plants emerged onto land, and all Glomeromycotina fungi are symbionts.

A second category of AM is called the “fine-endophyte,” initially identified as *Glomus tenue* (299), which appears to have evolved about the same time, and is another endomycorrhiza. These fine endophytes appear to be a polyphyletic group of Mucoromycota (614) that can be

found in plants ranging from ancient liverworts to modern grasses and saltbush. Little is really known about the group, but they are repeatedly observed in many habitats, especially those under drought stress or under highly acidic or highly eutrophic conditions.

A third category, also of *endomycorrhizae*, is formed by the septate fungi (Ascomycota and Basidiomycota). This is a diverse group, including the orchid mycorrhizae and the ericoid mycorrhizae. Orchid mycorrhizae form associations with fungi in the Cantharellales [note: in the older literature, the fungi were known by their imperfect name, *Rhizoctonia*]. The ericoid mycorrhiza type is found between plants in the Ericaceae, subfamilies Ericoideae, and ascomycetous fungi in the Eurotiales and Onygenales (in the Eurotiomycetidae) and Helotiales (in the Leotiomycetes). There are suggestions that other Ascomycota in the Hypocreales, and potentially even Basidiomycota in the Sebaciales, form orchid or ericoid mycorrhizae, but these are not yet resolved. A type of mycorrhiza, called an ectendotrophic or “E-strain” mycorrhiza, is formed between conifers and Ascomycota, especially in the genus *Wilcoxina*, in the Pyrenomataceae. Finally, there are “dark-septate” mycorrhizae. This is a diffusely defined relationship. An early study by Haselwandter and Read (328) characterized this mycorrhiza between sedges and Ascomycota in alpine ecosystems. But subsequently many “dark-septate mycorrhizae” have been delineated simply through the presence of saprotrophic or weakly parasitic fungal presence within roots. I will discuss this more in Chapter 3.

Many plants form dual mycorrhizal types, in time or space. Many plants form dual mycorrhizae for a variety of ecological and phylogenetic reasons (705).

Often any fungi within roots are described as mycorrhizal (epirrhizal, dark-septate, and fungi in the Sebaciales). These relationships will be discussed in more detail as we go through further chapters. Some may be mycorrhizal, some other mutualistic symbioses, some simply present, and there are many that we simply do not understand. Because mycorrhizae have evolved independently several times, and because the interface structure exists as a gradient, communities of mycorrhizae form more of an array of functioning entities than of any single function. For example, one fungus may secrete high levels of lignocellulases releasing and taking up organic N, where a second largely searches out NH_4^+ bound to organic surfaces. A community with multiple mycorrhizal types and functions is an attribute of complexity that is essential to forming a basic understanding of what mycorrhizae are and how they work.

Resource Dynamics and Mycorrhizae

At its most basic, the importance of a mycorrhiza is to increase the interface between the root and the external growth medium. This interface is comprised of a root cortical cell–fungal wall interstitial zone in which materials are exchanged between the host and fungus. Frank (256) observed that the fungus often completely encased the host short roots, requiring that a large fraction of the nutrients and water, especially N, must go through the fungus to be taken up. The fungus extends outward from the root into the soil, forming a second interface between the fungus and growth medium, generally soil. The fungus provides an increasing surface area for uptake and transport of limiting nutrients. Stahl (678) noted greater throughput of water in a mycorrhizal plant. His postulated mechanism was that more water moving brought more nutrients to the interface, increasing plant nutrient uptake. Hatch (334), in an elegant suite of studies, demonstrated that those resources, such as orthophosphate, that are not dissolved and carried by mass flow, are especially reliant on mycorrhizal relationships for their uptake. Hyphal transport from the soil to root cells is the basic structure that forms mycorrhizal functioning. This basic structure also differentiates a mycorrhiza from other mutualisms, such as leaf endophytes, in which the fungus does not infiltrate the surrounding growth matrix.

The extramatrical fungal hypha is the external functional feature of the mycorrhiza. Individual hyphae range in size from 2 to 20 μm in diameter. This is smaller than fine roots (20 μm in fine grasses to 300 μm in tree roots), root hairs (as thin as 7 μm in grasses), or even cluster roots (8 to 250 μm). This size difference allows the fungus to penetrate soil pores that roots cannot. Many fungi also form cords of multiple hyphal strands, allowing them to grow long distances across the soil. There can be up to a kilometer of hyphae per square centimeter of soil. This small diameter and high density allow the fungi to reach nutrients bound to organic or clay particles and penetrate soil micro- and even ultramicropores in search of nutrients and water. These hyphal tips can pick up nutrients and water away from the host (up to several meters) and, wrapping into cords, transport these resources between and along hyphae apoplastically as well as within (symplastically) hyphae to the mycorrhizal interface. This movement can occur through complex “vessel-like” elements (213) in some EM fungi. Even AM fungi can form arterial hyphae that will wrap together, creating an interstitial space within a small network.

Although mycorrhizal fungi do not carry out photosynthesis, by entering the root, they have direct access to C fixed by the plant. As a means to obtain C, the fungus exchanges nutrients for C with the host. This provides a much more direct pathway to plant C than for pathogens or saprotrophs. This basic structure also creates a unique feature, differentiating it from all other symbioses, in that a mycorrhiza is the only mutualist that can directly and simultaneously connect two hosts. A plant may have multiple rhizobia, or leaf endophytes, but only a mycorrhiza can physically connect two plants at the same space and time.

The formation of hyphal connections has led to a controversial hypothesis that mycorrhizal fungi redistribute resources among plants (e.g., (567)). Alternatively, Fitter (246) argued that this merely means that a fungus can maximize its own C gain by taking advantage of many plants in its environment. I will explore these arguments later in the book.

The nutrient sink of the plant and C sink of the fungus represents a mycorrhiza at its most basic (36). But nutrient and carbon allocation do not necessarily coincide (Figure 1.1). In general, remember that if a mycorrhizal fungus is present, it will try to invade any potential host. From the plant perspective, infection depends upon whether the plant rejects the fungus or not. We do not know if the fungus has selective mechanisms, other than C transport to pre-select the plant. At the

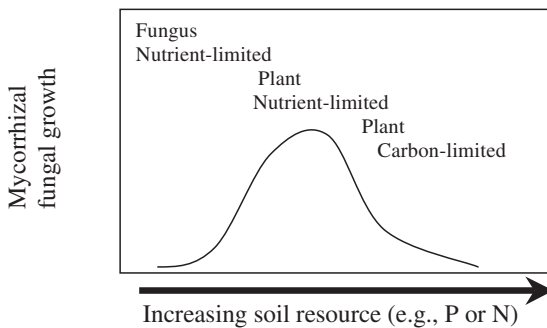


Figure 1.1 The interaction of plant and fungal nutrient limitation on the biomass of mycorrhizal fungi. At high nutrient levels, fungi will receive little carbon from plants and will be C-limited. At lower nutrient levels, plants will be N- or phosphorus P-limited and will allocate C to mycorrhizal fungi. At the same time, if N or P concentrations are sufficient for fungal growth, mycorrhizal fungi will proliferate. At the lowest nutrient levels, both fungi and plants should be nutrient limited, and fungal biomass will be low or lost regardless of C allocation to the fungi by plants. Derived from Treseder and Allen (719).

extreme low end of nutrient availability, mycorrhizae may not facilitate nutrient acquisition, and do not form. I have observed mycotrophic plants with no mycorrhizae growing among recently deposited pumice particles with no organic matter and extremely low measurable nutrients, although presumably there are enough diffusing in the water to support these scattered individuals. This condition is also the case for proteoid cluster roots that grow in low-nutrient sandy soils. As nutrients become limiting to the plant, but available to the mycelial network, the exchange of C and nutrients mediated by the mycorrhiza is maximized. On the opposite extreme, as nutrients become more available the plant can access them readily, reducing the transfer of carbon and, consequently, mycorrhizal fungal growth. Importantly, nutrients and carbon have different transfer proteins at the membrane level. What this means is that the C deficiency of the fungus and P (or other nutrient) deficiency of the plant must cross-talk, but how remains unknown.

A critical point to understanding mycorrhizal complexity is that the Treseder and Allen model is dynamic. For example, at the upper end of the soil resource curve, when soil nutrients are readily available and plant densities are low, mycorrhizal fungal growth is reduced as the plant allocates less C to the fungus. At that point, plant densities increase, driving the curve toward a lower nutrient availability per plant. Mycorrhizae will increase as each plant attempts to maximize its share, and plants will allocate more C for fungi to that end. I will explore this relationship in more detail in Chapter 4.

The mycorrhizal relationship is comprised of organisms with unique, individual physiologies, not just pipes. For instance, the fungi do not function well in saturated, O₂-limited soils, and often the plant rejects the symbiosis under high nutrient conditions. Organisms have growth, metabolic, and reproductive requirements and have many means to acquire these requirements. Water flow, mineralization, transfer of growth-regulating chemicals, and complex enzymatic dynamics all regulate mycorrhizal associations. These all contribute to a diverse array of interactions within a mycorrhizal symbiosis.

Distribution of Mycorrhizae

Mycorrhizae may be the most widespread type of terrestrial mutualistic symbiosis. They can be found across the globe. They can be found in nearly every terrestrial habitat, including many wetlands (see (36)). Mycorrhizae also exist in extremely deficient to extremely rich growth

media. The tropical forests are often regarded as optimally conditioned to support mycorrhizae (high rates of production, low soil nutrients), but plants at high altitudes and high latitudes form mycorrhizae as well.

Three conditions in which mycorrhizae are limited stand out. One is in aquatic environments when a lack of O₂ inhibits aerobic respiration. A second is under extremely high fertility in which the plant can readily obtain all nutrient and water conditions necessary for growth. Under these conditions, CO₂ is the limiting factor for growth, and the plant initiates rejection mechanisms to reduce C loss (the extreme right end of the Figure 1.1 curve (24). These conditions today appear to exist largely in glasshouse pot culture and hydroponic conditions, and in sandy, heavily fertilized, wet (or irrigated) agricultural soils. Importantly, these are also highly leaky conditions and can lead to groundwater pollution (e.g., nitrates) and are an undesirable extreme.

The third is severe disturbance. Extreme cases include newly-formed, almost sterile substrates such as volcanic eruption materials and retreating glaciers. This can also include extreme conditions such as the Antarctic Dry Valleys, where there are no plants, and habitats dominated by extremophiles without plant growth, such as caves, hot springs, and highly contaminated, heavy-metal soils. While many of these are natural, human disturbances often reduce or eliminate mycorrhizae through soil loss caused by erosion, strip-mining in which the topsoil is discarded, and high-intensity agriculture. Mycorrhizae become an essential element in the restoration of a self-sustaining, desirable ecosystem.

Hierarchy and Complexity

Just as a mycorrhiza became more complex when multiple fungi and plants became involved, understanding the basic functioning of mycorrhizae in communities or ecosystems also became more than simply a nutrient uptake mechanism for individual plants. One idea is that groups of organisms, growing and reproducing independently, nevertheless interact to create additive ecosystem processes – that is they exhibit “emergent properties” (495), where the interaction is greater than the sum of the parts. Previously, I proposed a simple hierarchical structure for studying the ecology of mycorrhizae based on the “individualistic” concept (36). This idea was based on the predication that an individual is the entity selected against (471). Rillig and Allen (609) realigned community and ecosystem hierarchies dependent on relationships to changing environments. More recent ideas have complicated this view

even further. Organisms can exist in “metapopulations” that are relatively isolated, but occasionally interactive. This concept has been extended to “metacommunities,” repeating groups of organisms that interact and support one another (783). It remains an important theoretical topic as to whether the interactions among organisms under shifting conditions represent true “emergent properties,” but these have the potential to create complexity. For this reason, an alternative is to envision a more complex hierarchy that cross-talks both up and down within a hierarchy, and across hierarchies at co-existing temporal and spatial scales (Figure 1.2).

Read and Perez-Moreno (599) portrayed another hierarchical perspective, focusing on scaling as a trade-off between precision of measurement and relevance of process. As one measures the individual physiological interaction between a mycorrhizal plant and fungus, accurate determinations of elemental exchange of elements can be made, largely in the lab or glasshouse (occasionally in the field). However, as one moves to the scale of the ecosystem, measurements of exchange rates become less precise, but more accurately reflect conditions in the field or forest. They make the point that most studies have been done at short-time intervals, across small spatial scales. This is especially true for measuring “ecosystem processes” in glasshouse pots or growth chambers. But how representative are these studies to understanding how mycorrhizae affect forest production across a landscape where perturbations are applied?

One result of complexity is that the highly relevant end of the Read and Perez-Moreno curve, may not actually be a reduced precision, but may simply reflect a family of individually-precise outcomes, rather than a single outcome that results from a small-scale study. This represents adding multiple variables, rather than a single-variable experiment. For example, in a pinõn–juniper stand (49), water and N were the limiting factors to production. When NH_4NO_3 was added, the EM pinõn pine shifted C allocation from EM to increased needle production to enhance even more production. The juniper simply increased both root growth (no change in AM) and shoot growth. However, P then became limiting. The AM juniper was quite able to continue taking up P; but without the EM fungi, P declined in the pinõn pine leaves. When seasonal drought hit, the AM juniper was quite able to tolerate. But because the pinõn now had larger leaves (and thus greater evapotranspirational area), and reduced EM to search for water, the pinõn died whereas the juniper increased productivity. These interacting

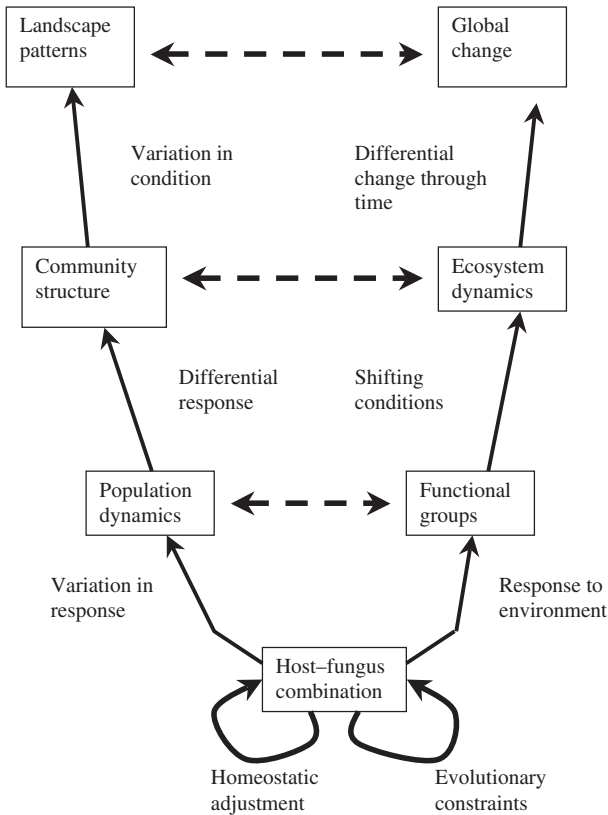


Figure 1.2 Hierarchical interactions among levels of mycorrhizal interaction. For the purposes of this text, I envision two distinct hierarchies that interact between similar levels creating a complex suite of mycorrhizal controls. Mycorrhizae themselves are sensitive to homeostatic adjustment by the environment, and constraints based on the individual lineages. From Rillig and Allen (609).

mycorrhizal and resource factors created a complex web of interactions, the understanding of which required past laboratory–glasshouse studies of nutrients and water, coupled with newer technologies of isotope signatures and continuous monitoring of changing environmental (soil and atmosphere) conditions.

Networking Topology and Connectivity Dynamics

One of the more important recent developments in ecology resides in studying networking topology to study interactions across a community.

Network theory links complexity, because of the multiplicity of entities involved, and connectivity, because of the interconnected exchange of a unit between them. One example is in the application of network theory to evaluating power grids, and directionality and susceptibility to disruption. A power grid exchanges energy between a power generation hub (say a tree in a mycorrhiza), across a power grid cable link (a mycorrhizal fungal hypha), and to a metropolitan area (maybe a mycoheterotrophic plant). Increasing use of network theory to understand vulnerability holds interesting clues to describing mycorrhiza behavior (98; 511; 659). In the case of mycorrhizae, we have traditionally focused on mycorrhizal fungus and host plant; this sets up a conceptual hierarchical relationship, a small parasitic or mutualistic symbiont, and the larger (and more important) host. However, mycorrhizae consist of extremely complex networks of small and large fungi, and small and large plants. Carbon preferentially moves from plant to fungus (except for mycoheterotrophic plants), but other elements, such as N and P, primarily move from fungus to plant. Fungi range from small individuals of *Cenococcum graniforme* to a *Leccinum scabrum* that may extend across a plant stand. The plants can be large, such as a clone of a single aspen (*Populus tremuloides*) that will have connections with thousands of individual stems and of both EM and AM fungi and hundreds of taxa of mycorrhizal fungi, down to a small, herbaceous columbine (*Aquilegia formosa*) located within the clone and tapping the *Scutellospora calospora* that is connected into the aspen. Networking theory provides an interesting perspective looking at both the number and structure of linkages to assess the stability of systems (e.g., (102)).

This approach is just beginning to be applied in mycorrhizal research (674), but provides a unique opportunity to tease apart complex interactions of mycorrhizal communities. For example, Southworth et al. (674) proposed that, for a mycorrhizal network, the node is actually the fungus, whereas linkages are the plants. This perspective reverses the usual thought and research process wherein the plant is studied as the focal organism contributed to by an array of symbiotic fungi.

What we do know, at this beginning point, is that stability comes not from a single mycorrhiza, but from a complex network of hubs, nodes, and linkages across a community. This will become clearer as we examine topics ranging from mycorrhizal communities to mathematical ideas of stability.

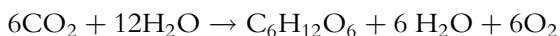
Complexity and Stoichiometry

Complexity itself is a complex topic. What comprises complexity, and how does it relate to understanding mycorrhizal dynamics? Complex derives from *complexus*, literally a weaving or twining together. Webster's dictionary defines complex as that which is made up of many elaborately interconnected parts. Complexity is then the condition or quality of being complex.

Researchers working in cybematics developed a view that derives from Schrödinger's famous cat, where elements of a complex cannot be examined without destroying it (351). Biologists have not been quite so pessimistic, but ecological theory is clearly as diverse, disjointed, and fragmented. Heylighen (351) goes on to organize complexity into two dimensions that may help to study mycorrhizae: distinction, which implies variety, encompassing disorder, chaos, heterogeneity, and entropy; and connection, which implies constraint, encompassing order and negentropy.

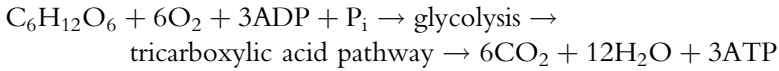
How can we utilize this approach to better understand mycorrhizal dynamics? One way to focus our thinking is to visualize complexity by building on the original framework of Mosse (521) of a mycorrhiza as an integrating overlap of soil, fungus, and plant forming a series of interacting resource exchanges. Complexity derives from the intertwining of distinction and connection. For example, each of the three spheres is comprised of many distinct players, each of whom is competing internally and externally. A patch of seasonal tropical forest 6 meters in diameter may contain 18 species of plants, all capable of tapping into the hyphal networks of some or all of the 15 taxa of AM fungi present (21). In another analysis, out of 129 EM root tips extending from a single tree, 42 taxa were differentiated, the same morphotypes found in the surrounding trees (695). Just as importantly, the "soil" is comprised of an almost infinite number of patches of physical, chemical, and biological entities, each of which affect the mycorrhiza.

All of these entities are interconnected, transporting materials and energy. Thus, the connectivity of complexity derives from the chemical reactions that must occur for each component to persist. Ecological complexity can be simplified conceptually to a suite of well-known chemical reactions comprising a living plant and a living fungus. For example, photosynthesis is well described:



In order to take up 6 molecules of CO_2 through the plant's stomata, approximately 300 times 6, or 1,800 molecules of H_2O are transpired.

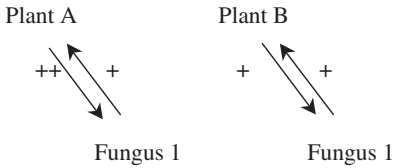
The fixed C is respired by the leaf, or translocated to the roots and respired by the fungus, again by a well-known suite of pathways:



The complex part is that to fix the CO_2 requires a large amount of N, in the form of enzymes, particularly RuBPCarboxylase, and Fe and Mg forming the core of the chlorophyll molecule, and P to serve as the recipient and carrier of the energy fixed to convert the CO_2 and H_2O into $\text{C}_6\text{H}_{12}\text{O}_6$ (potential energy) and O_2 . All of these elements must be present in appropriate amounts and they form a distinct stoichiometry. These processes create the famous “Redfield” ratio, wherein the optimal values of C:N:P of 106:16:1 typify the values necessary for growth in an autotroph algal cell. Plant cells normally have more C because of the need for structural compounds. If we focus only on the seven critical macronutrients, our C:N:K:Ca:P:Mg:Fe ratio is approximately 512:17:11:2.7:2.4:2.2:1 (using table 6.1 of Salisbury and Ross (631)). If one of these elements is in short supply, photosynthetic rates decline to a level based on the lowest common denominator – Leibig’s law of the minimum. For example, in nature, N-limited plant leaves can have a C:N ratio as high as 50, whereas fungi may have a C:N ratio of 7–10. If the fungal N uptake is constrained to a concentration below 7–10, then the ability to transport to the plant element declines. There is a feedback then to the plant to reduce C production and allocation, and the overall symbiosis is affected. We know that the mycorrhizal fungus and plant interact intimately to acquire adequate amounts from their respective sources. The fungus acquires N, P, K, Ca, Fe, and Mg from the soil, providing those to the plant. The plant fixes C, critical for energy and building structure, and provides energy-rich C compounds to the fungus in exchange. Complexity, therefore, arises from the incredible number of requirements of each with a different source, sink, and exchange process and rate across spatial and temporal patches.

A tertiary level of complexity in mycorrhizal relationships comes at the inter-species level. This perspective is based on the co-evolutionary, mutualistic relationship as initially envisioned by DeBary (199). However, instead of an endpoint, take the +/0/- interactions described by DeBary and place these relationships into a web instead of a single linear exchange. We can build the web using two axes. First, add a second plant. This simple action creates a conceptually complex view of mycorrhizae – a plural view of symbioses (Figure 1.3). In this case, the

In separate environments, i.e., glasshouse pots



In an interactive environment, i.e., same pot or in the field

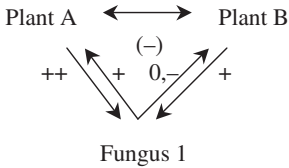


Figure 1.3 Changing relationships between symbionts as a function simply of adding species.

mycorrhizal fungus in separate environments or locations forms a mutualistic relationship with both host plants. However, plant A provides somewhat more C to the fungus in exchange. The fungus still simultaneously infects both hosts to enhance its own C gain. Consequently, when both plants are in the same location, the fungus preferentially sends resources to plant A versus plant B. Over the life of the plant, plant A gains even further competitive capability over plant B because of the mycorrhiza. The relationship between the mycorrhizal fungus and plant B begins to become less mutualistic. As more plants and fungi are added, varying outcomes can occur.

Both the environment and the organisms are dynamic, not static. Temperature may initially be limiting, then N to increase the photosynthetic machinery, then P for energy storage and use, and finally water as a dry season begins. If the form of N available is NO_3^- , which can move by mass flow, then most of the N may bypass the fungus. If the dominant form is NH_4^+ , which binds to clay and organic matter particles, mycorrhizae will play a large role. If organic N predominates, EM will serve better than AM, and so on. Growth of roots and the fungal network can be very rapid (38) or very slow (723) depending on the organisms involved and the dynamic nature of the environment. The simple +/0/- becomes an intricate interplay of biotic and abiotic forces that underlie a great deal of the complexity inherent in shaping plant communities and the ecosystems they comprise.

In 1991, when the *Ecology of Mycorrhizae* was published, it was rare that ecologists considered mutualisms important. They were considered fascinating oddities. May (491; 492) calculated that mutualisms are mathematically unstable. For this reason, he postulated that there are few natural examples of importance. Great strides have been made since then, such that most general textbooks acknowledge the importance of both mycorrhizae and mutualisms in general. Mycorrhizal ecologists no longer have to justify the importance of studying our favorite subject! I do not believe that particular issue could have been adequately addressed in 1991. However, I believe that our newer understanding of complexity in mycorrhizal associations specifically addresses this hypothesis. Specifically, what is stable is far more challenging than the mathematics used by May. We will address the specifics in Chapter 3, to show that, evolutionarily, mutualisms are not only stable mathematically, but also stable ecologically and evolutionarily.

Natural History, Theory, and Complexity

In studying mycorrhizae, like all of the sciences, there is a search for universals. Organisms, connectivity, and complexity exist in an intricate dance, both structurally and temporally. But in the end, scientists search for simple, definable universals, preferring those that are elegant, as outlined in Occum's Razor (726).

Alternatively, Natural History, defined broadly, is scientifically those observations that link the multitude of organisms, chemistry, and physics, as organized into communities and ecosystems (see (106)). Natural history often reflects complexity even when initially viewed as simple interactions. Natural History is messy!

A search for universals is an appropriate approach, especially at the cell or molecular level for how exchanges occur and are regulated. However, highly complex behavior emerges at the organismal scale as the dynamics play out. While AM appear to be a monophyletic group of fungi (Glomeromycotina), other mycorrhizal groups such as EM are clearly polyphyletic, and very different morphologically. Different plant and fungal taxa have very different nutrient, water, and light requirements that shift, in both time and space. Mycorrhizal fungi are not microorganisms, despite the necessity to use microscopes to observe structure and behavior. While individual hyphae can be only a few micrometers in diameter, individual organisms can extend many meters in length. I estimated that a single organism, such as a fairy ring, 10 m across with

a highly branched mycelium, could weigh 3 g (dry mass). Many can be far larger, extending through many different environments, and sometimes living to a great age. The very size of both organisms results in a high degree of connectivity, well beyond a simple 1:1 relationship. Thus, we are dealing with two highly complex symbionts mutually dependent upon each other. Because a plant has many roots, with many mycorrhizal fungi, and a fungus attaches to all compatible roots encountered, there is a potential for many types of relationships in the field. *Here, I use a natural history approach, with as many independent field observations, compiled over a human lifetime, to describe the mycorrhizal symbioses living around us.* Natural History observations fundamentally result to increase complexity. However, with enough observations, they provide meaning that can form basic relationships leading to theoretical universals, which in turn, reduce complexities.

Summary

- Prior to the 1990s, studies of mycorrhizae focused on the physiology and morphology of the mycorrhizae of individual organisms.
- During the 1990s, the advent of molecular technologies allowed us to identify the associations in the field, and also to realign phylogenies of fungi, recognizing the relationships that characterize different mycorrhizae. We also developed the ability to differentiate different functional groups, which indicate that mycorrhizae represent a range of responses, not just a single function.
- Connectivity is not 1-to-1, it is networks of nodes and links with networks comprised of multiple fungi interacting with multiple plants. With this understanding models can then begin to scale to global level processes.
- Mycorrhizal fungi sequester and respire CO₂, release methyl bromides that affect the Antarctic ozone hole, immobilize and sequester carbon that can make global contributions to the carbon budget, and determine the relative efficiencies of different element immobilization and mineralization. However, the relative contributions depend on the composition of plant and fungus and the rapid shifts between them in an ever-changing environment.
- Connectivity and complexity are the reality, not just an interesting sideline.