Nexus of the Underground: A Tale of Mycorrhizae

David W. Wolfe

"In the days when the 'information superhighway' is the buzz phrase, we would do well to look at our inventive fungal predecessors who, for four hundred million years, have already been leading the communication network of life on land."—Lynn Margulis (1994), in the Foreword to *Hypersea* by Mark and Dianna McMenamin

Over the centuries, naturalists and poets have often attempted to describe their intuitive sense of a "connection" between living things. Science is now revealing how very real and intimate some of these connections are. In many of our forests, grasslands, and other natural ecosystems, if it were possible to gaze down and witness all that is below, one of the most striking things we would see is the vast network of gossamer-like fungal threads linking the roots of plants of different species. We have only recently learned that the expansion of life up and out of both the sea and the deep Earth was founded on this very important symbiosis between plant and soil fungus. It has been just as fundamental to the evolution of life on our planet as the relationship between nitrogen-fixing bacteria and legume plants. The roots of almost all plant species in the world today are joined with these specialized fungi that help them obtain water and nutrients from the soil (and sometimes from neighboring plants), in exchange for the carbon- and energy-rich sugars produced by the plant during photosynthesis. It's a connection that began long ago, when life on the land surface was just getting started.

The colonization of Earth's land surface did not begin in earnest until the early Devonian period, a little over 400 million years ago. This land invasion was more than three billion years in the making, during which time the subterranean creatures that would be required to support surface life had become established. The first photosynthetic organisms to attempt life on land came ashore from marine environments as rootless, green algae-like creatures. Needless to say, these sea dwellers were in for quite a shock when they tried their luck on land. Most quickly shriveled up and died. Over the course of millions of years, however, some managed to survive by being the first to establish a successful partnership with soil fungi. The fungi functioned as surrogate roots, supplying their algal partners with water and nutrients mined from the underground, while the algae collected solar energy at the surface and supplied the fungi with the products of photosynthesis.

The descendants of those first photosynthetic species gradually evolved into primitive plants with roots of their own. The symbiosis with fungi did not end, however. Today, more than 400 million years after the first tentative union between alga and fungus, you are likely to find descendants of those beneficial fungi growing on almost any plant you yank up by the roots, regardless of where on Earth you might be. In many cases you would need to use a microscope and special staining procedures to spot the delicate fragments of threadlike hyphae dangling from the roots, but they would be there. These fungi-root associations are referred to as "mycorrhizae" (from the Greek, *mykos* = fungi, *rhiza* = root). More than 90 percent of the approximate 230,000 species of vascular plants on our planet today have found it advantageous to continue this mutually beneficial partnership. In fact, the

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The aboveground fruiting bodies—or mushrooms—of the genus Amanita, *common types of ectomycorrhizal fungi.*

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Many fungi in the Boletus genus are ectomycorrhizal, forming a mutually beneficial partnership with plants in which the plants provide the fungi with the products of photosynthesis (carbon-rich sugars) while the fungi help the plants acquire water and nutrients.

majority of these plant species could not survive in nature without it.

Many of the mycorrhizal fungi are just as dependent on their plant partners as the plants are on them. We are unable to culture some of the most common types in the laboratory, even with our most sophisticated concoctions of sugars and nutrients. Scientists have speculated that these fungi depend on plants not only for a share of the products of photosynthesis, but also for some as yet unidentified essential growth hormones.

The fossil record confirms that the first mycorrhizal fungi evolved at just about the same time as land plants. Viewed through a microscope, one can see their unique hyphal branching structures, resembling miniature tree shapes, that form just within the root tissues. These structures, called "arbuscles" (from the Latin, *arbor* = tree), are the interface for exchange of nutrients with the plant host. In 1994, fossils from an important archaeological site called the Rhynie Chert, near Aberdeen, Scotland, were re-examined and found to contain evidence of arbuscles. The rocky layers in which these fossils were discovered are just over 400 million years old, from the early Devonian period. Along with the fungi, fossil evidence of primitive, pioneer land plants was also found.

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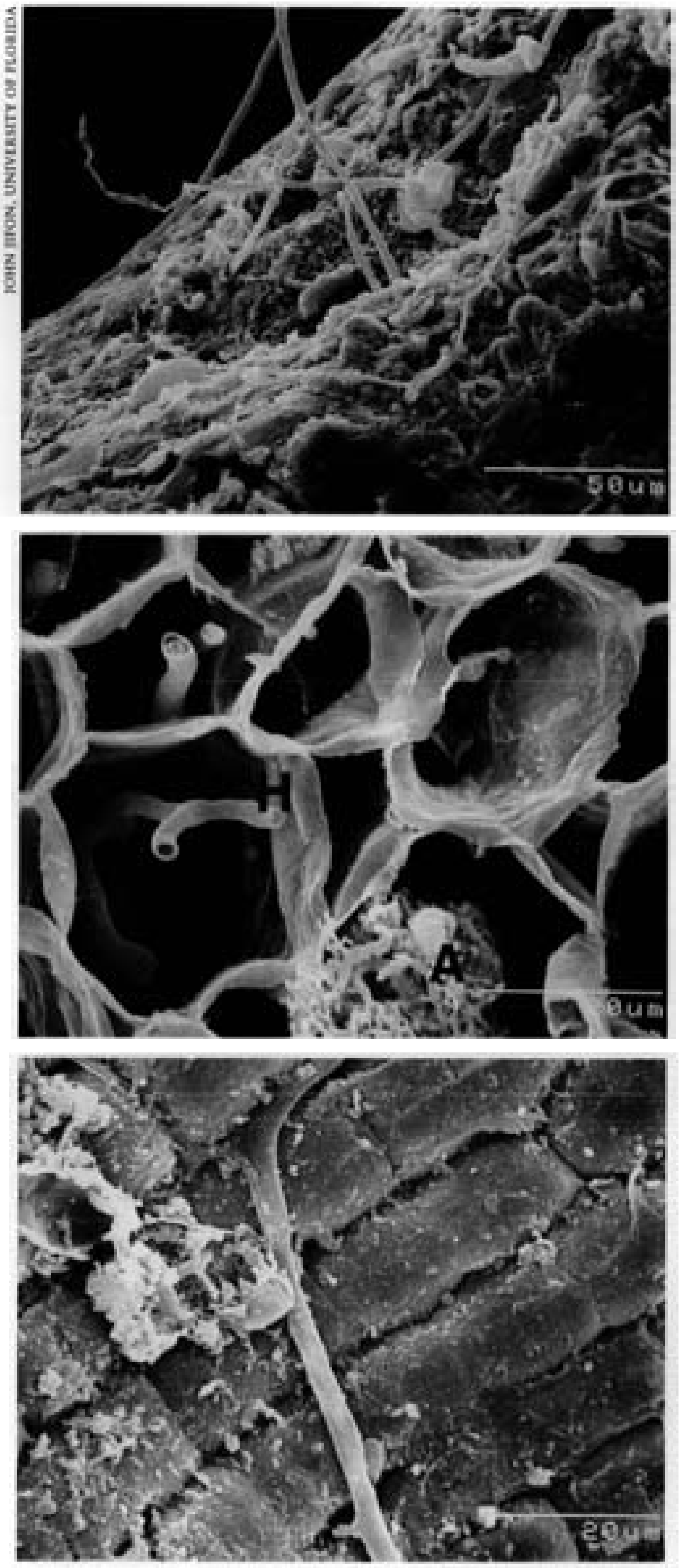
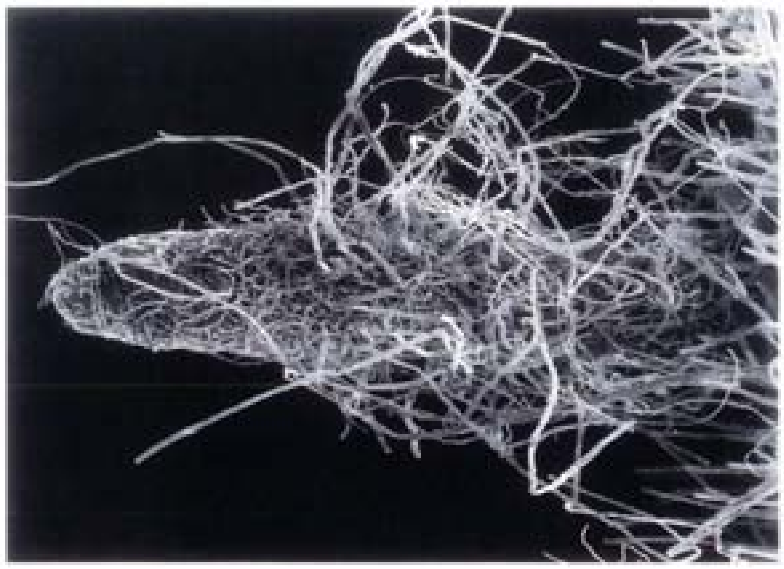


Genetic analyses have provided additional evidence of the long history of this symbiosis. In the early 1990s, the first sequencing and evolutionary classification of ribosomal DNA collected from arbuscular mycorrhizal fungi confirmed that they originated between 350 to 460 million years ago, coincident with the estimated time of origin of the first land plants. Today, arbuscular mycorrhizae are found almost everywhere except in some arctic regions, and the list of plant species involved as hosts is a very long one. Most temperate and tropical nonwoody plants, such as grasses, wildflowers, and our most important crop plants are host to this type of mycorrhizae, as well as woody perennials such as azalea, apple, grape, cedar, maple, ash, and many tropical trees. We have not yet found a single arbuscular mycorrhizal fungus that grows independently of a plant host.

The fossil record as well as other DNA studies reveal that a second major group of mycorrhizal fungi, called "ectomycorrhizae," evolved about 160 million years ago. The ectomycorrhizae are unique in that they do not form arbuscles inside the root tissues, and their silken hyphal filaments extend out farther from the roots (often several yards) compared to arbuscular mycorrhizae. The ectomycorrhizal fungi release a plant hormone that causes the growth of short, stubby, branching rootlets here and there along the main roots of the plants they

The scanning electron micrograph at top shows an arbuscular mycorrhizal fungus (Glomus intraradices) *on the surface of a root from a grapefruit plant* (Citrus paradisi). *Penetration of the threadlike hyphae (H) and arbuscles (A) into the root cells is shown at center At bottom, note the points where the hyphae branch and penetrate between rhizodermic cells. In this symbiosis, the fungus helps the plant roots gather water and nutrients from the soil or adjacent plants, and the plant provides the fungus with carbon and energy captured via photosynthesis* inhabit. These characteristic multi-branched rootlets are about one-eighth of an inch long (about three millimeters), so can usually be identified in the field without the aid of a microscope. The mycorrhizal roots are composed of a central core of plant tissue, completely encased by a dense mat of fungal hyphae. The ectomycorrhizae are particularly important in many temperate and arctic forests. They form symbioses that are crucial to many economically important timber trees such as pine and other conifers, oak, beech, chestnut, and birch. Their list of plant hosts is not as long as the more ancient arbuscular mycorrhizae, but they have a wide geographic range and are found growing with woody perennials and even a few nonwoody species from the tropics to the arctic.

There is a great deal of overlap between the ectomycorrhizae and the more ancient arbuscular mycorrhizae with regard to their geographic range and plant hosts. Both types of root fungi can often be found in the same forest, meadow, or crop field, and even growing on the same plant. Maples and poplar trees, for example, are sometimes simultaneously a host to representatives of both major groups of mycorrhizal fungi.



At top, ectomycorrhizae on pine with the characteristic shortened, thick roots covered by fungal hyphae; and at bottom, a magnified view of the thick hyphal mat surrounding a modified root hair

The mycorrhizal fungi are so common and abundant in nature that it is very difficult to find or create an environment without them. Those doing research with mycorrhizae have sometimes found it necessary to not only sterilize the soil to create a "no mycorrhizae" control treatment, but they also must filter the air entering the greenhouse to prevent free-floating volunteer spores of mycorrhizae from contaminating their experiments.

The discovery of mycorrhizae was, as is often the case, serendipitous. The road to discovery began in the early 1880s, when the king of Prussia commissioned one of the world's leading forest biologists, Professor A. B. Frank of the Landwirtschaftlichen Hochschule in Berlin, to study, of all things, truffles. These highly prized delicacies are the belowground fruiting (spore-forming) body of a rare type of aromatic fungus (*Tuber melanosporum* and *T. magnatum* species) found in some hardwood forests of western Europe. Then, as now, truffles sold at incredibly high prices because of their rarity and their unique aroma and delicate flavor. The hope of the Prussian government was that Frank would develop a way to produce truffles on a commercial scale, like the common (and much less expensive) grocery store mushroom, which is the aboveground fruiting body of another type of fungus (*Agaricus brunnescens*).

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Chanterelles (shown above) and truffles have a unique and delightful flavor prized by gourmets around the world. They also play an important ecological role: the belowground hyphae of these mycorrhizal fungi attach to the roots of nearby plants and assist the plants in mining the soil for water and nutrients.

Professor Frank failed rather miserably at coming up with a commercial method for cultivation of truffles. (Incidentally, so have all others who followed him, which is why, if I wanted to buy a pound of truffles today, I would have to be willing to pay close to one thousand dollars per pound!) There are two edible species—black (*Tuber melanosporum*), native to Germany, France, and Spain, and white (*T. magnatum*), found in northern Italy. Both types are still extremely rare. They have been sought after for their culinary qualities since Roman times. The black "Perigord" truffle is reported to have been hunted down in fifteenth-century France with the use of trained, muzzled pigs that could sniff out the smelly fungi. The highly competitive "truffle hunters" of today, or "truffiers" as they are called in France, roam their secret forest haunts with trained dogs instead of pigs, but little else has changed. Humans and their trained animals aren't the only truffle hunters—voles and other rodents seek them out by smell, and spread the spores as they take the truffles back to their underground burrows.

What Frank did discover launched a century's worth of research, the results of which we are only now beginning to fully appreciate. "We should all fail so nobly," as Michael Allen, one of today's leading mycorrhizal researchers put it. Frank was a meticulous and very observant scientist, and fairly quickly came to realize that truffles were never found growing independently, but were always in the vicinity of oaks, filberts, and certain other forest trees. At first he suspected the truffles of being weak parasites, but eventually, through well-designed and carefully conducted experiments, he was able to prove that the belowground hyphae of the truffles formed a very important mutually beneficial symbiosis with the roots of the trees they inhabited. It was Frank who coined the term "mycorrhizae" to describe this fungus-root partnership in a classic paper published in 1885. In his own words, he concluded that the mycorrhiza "functions in a nutritional capacity as a wet nurse of the tree."

Although Professor Frank is given credit for the discovery of mycorrhizae, scientific historians have found that others had studied other kinds of mycorrhizae before him. Frank was aware of this earlier work, and it undoubtedly laid the groundwork for his more conclusive experiments. In the mid 1800s scientists had identified a fungus growing on the roots of small nongreen plants in the genus *Monotropa*, and another type associated with orchids, both of which we now know to be mycorrhizal. Several of these researchers suspected some type of reciprocal relationship between the fungi and their plant hosts, but their experiments were inconclusive, and the subject remained controversial until Frank came along. In addition to studying truffles, Frank also spent a great deal of

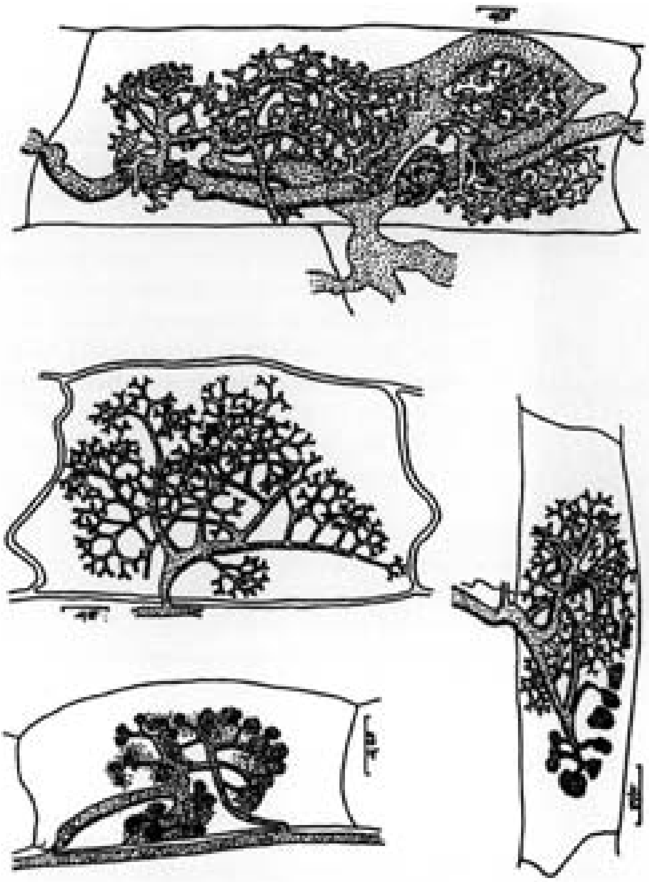
Some of the first mycorrhizal fungi were discovered growing on the roots of plants in the genus Monotropa *(also known as Indian pipes). These plants lack chlorophyll and a well-developed root system. They rely on their belowground partners not only for water and nutrients, but also for carbon-rich sugars that the fungus obtains by tapping into the root systems of adjacent trees or other photosynthetic plants.*

time studying *Monotropa* plants and their associated fungi, which helped him elucidate the function of mycorrhizae.

The mutually beneficial root fungi that Frank and the other nineteenth-century biologists discovered were all ectomycorrhizal. Although these were the latest to evolve, it is not surprising that they would be the first to be discovered since the short stubby roots and thick hyphal covering they produce are visible to the naked eye. Also, their reproductive structures are quite large and obvious, many even more so than truffles, because they form as aboveground mushrooms.

It is impressive that within just a decade of Frank's pioneering work with ectomycorrhizae, scientists also discovered the arbuscular mycorrhizae, which can only be seen with a microscope. However, the latter were initially thought to be parasitic. In 1905, a French researcher named I. Gallaud published a set of outstanding drawings of his microscopic observations of this fungal-root interaction, which he referred to as "endomycorrhizae." These drawings were our first view of this most ancient type of mycorrhizae, and Gallaud's drawings are still used in some modern textbooks to depict them.

Some paleobiologists have made the intriguing speculation that plant roots may have actually evolved from the earliest fungal symbionts of rootless green algae. The evolutionary progression from an intimate symbiosis between two species to their complete integration into a single organism has been documented in other cases. The biologist Lynn Margulis was one of the early proponents of this form of evolution, and argued for many years that the green chloroplast organelles (where photosynthesis takes place) found in the cells of green plants originated from an ancient symbiosis between a photosynthesizing cyanobacteria and a larger, single-celled (eukaryotic) organism. Many were skeptical of Margulis' "endosymbiosis" theory, even when it was discovered that chloroplasts contain their own genetic material that is separate from the genes contained in the nuclei of the plant cells they inhabit. Recent comparative analyses of the nucleotide sequences of chloroplast and cyanobacteria genetic material have revealed a remarkable similarity. This more or less settled the matter, and most now accept the endosymbiotic pathway for the evolution of photosynthesizing plant cells. However, in the case of plant roots, no trace of genetic material to prove a fungal heritage can be found, and few plant biologists are convinced that roots evolved from fungi.



Microscopic images of arbuscular mycorrhizae as drawn by the pioneer researcher I Gallaud and first published in Revue Generale de Botanique *17 (1905).*

It is only in the relatively recent evolutionary past that a handful of plant families have gained independence from the symbiosis with fungi. The exceptions to the rule can literally be counted on one hand, and include Chenopodiaceae, common species being spinach and the weed known as lambsquarters; Brassicaceae, such as cabbage, broccoli, and wild mustard; and Amaranthaceae, such as edible amaranth and pigweed. Even among these plant families, some particular species are mycorrhizal.

Next time you are walking among the mighty giants of a temperate or tropical forest, hiking through a grassy meadow, mowing your lawn, or puttering in the flower or vegetable garden, take a look around. Most of the plants you see, if they don't fall into one of those three exceptional groups, are probably thriving through the good graces of their subsurface symbiosis with root fungi. Mycorrhizae are particularly essential for survival during those periods of water and nutrient stress that almost every plant must occasionally face—the "ecological crunch," as mycorrhizal researcher, Michael Allen, calls it. Without the fragile, gossamer-like net of subterranean fungal hyphae at their base, the towering redwoods, oaks, pines, and eucalyptus of our forests would collapse during hard times. Beneath every great tree there is a fungus, you could say. And the same can be said of most other plants as well. Mycorrhizal fungi form the foundation of most terrestrial ecosystems on the planet, from our orchards, vineyards, and other farmlands, to the vast savannas of Africa, the heathlands of Scotland, the tropical rainforests of South America, and the deserts of the American Southwest.

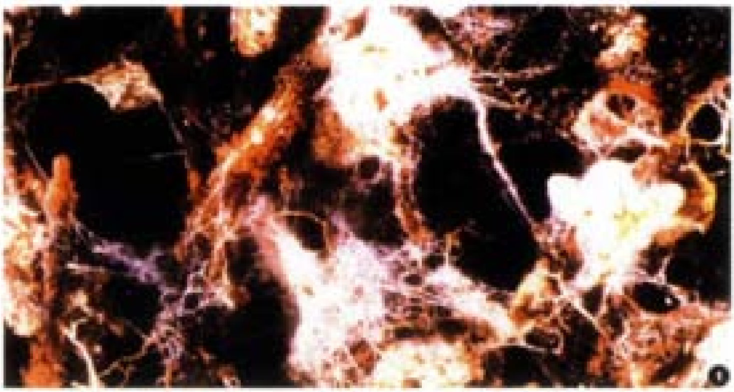
So just what is it about mycorrhizae that make plants willing to devote as much as 20 to 30 percent of their carbon and energy to support them? What are they doing that plant roots alone cannot? The secret lies in the unique structure of the fungi themselves: the hyphal threads are an order of magnitude finer than the finest of root hairs and thereby provide access to nooks and crannies in the soil that could not otherwise be penetrated. This is especially helpful in acquiring certain nutrients, such as phosphorus, potassium, copper, and zinc, that do not move freely with the flow of water being taken up by roots. The finest of root hairs will have a diameter of 20 to 30 micrometers (about the diameter of a hair pulled from your arm), while the diameter of a strand of mycorrhizal hyphae is only one to two micrometers.

The capacity for a plant to exploit a given patch of soil expands tremendously with the prolific growth of their subterranean fungal partners. If one were to take a cubic centimeter of soil (about a teaspoonful) from the root zone of a mycorrhizal plant and spread all of the bits and pieces of root and root hairs end-to-end, the total length might measure a few inches. In that same volume, the length of mycorrhizal hyphae, if completely unraveled, might range from 60 to 120 *feet* (20 to 40 meters)! It is primarily the superiority of mycorrhizae at mining the soil for water and nutrients that makes it worth the cost to the plant. Some have speculated that in some mycorrhizal associations the roots are doing little more for the plant than serving as a vehicle to transport the attached fungi to deeper soil layers.

In some ecosystems the mycorrhizal fungi do not just function as passive absorbers of nutrients but also as active decomposers. Like many other fungi involved in decomposition, they are capable of releasing powerful enzymes that can externally "digest" wood and other organic matter. Before the liberated nutrients have a chance to float away in the soil environment, they are immediately snapped up by the fungus and directly transmitted to the plant hosts. This short-circuiting of the nutrient cycle is particularly valuable to plants in tropical ecosystems, where heavy rains often wash free-floating soil nutrients below the root zone before they can be absorbed.

Evidence has been accumulating over the past several decades that, in addition to enhancing the function of the individual roots they inhabit, mycorrhizae also often serve as a living subterranean connection between plants of different species through which water, nutrients, and possibly other substances can be transferred. Mycorrhizal fungi are not nearly so host-specific as the nitrogen-fixing bacteria discussed earlier, and because of this they often spread from plant to plant and species to species. The fungi do this for purely selfish reasons, of course, not with the objective of creating a pipeline between plants. For the fungi it is to their advantage to attach to any plant that will have them, as a means of maximizing their intake of the products of photosynthesis.

The movement of nutrients from plant to plant via mycorrhizae was first clearly demonstrated in a field experiment conducted in the mid 1960s. Researchers applied radioactively labeled calcium and phosphorus to the cut stump of a maple tree and then tracked the movement of the calcium and phosphorus into attached mycorrhizae and eventually into adjacent plants. Since then, the movement of calcium, phosphorus, carbon, and nitrogen from plant to plant has been demonstrated among many plant species and in many ecosystems.



RANDY MOLINA, U.S. FOREST SERVICE

The threadlike hyphae of mycorrhizal fungi sometimes connect roots to each other and can serve as a belowground conduit of water and nutrients from one plant species to another.

In several studies scientists have documented the movement of atmospheric nitrogen fixed by legume plants to adjacent nonlegume plants via a mycorrhizal conduit. Nitrogen transfer from clover and soybean to maize has been demonstrated, and in one study, as much as 15 percent of the nitrogen fixed by a species of alder tree was transferred to nearby pine trees through a fungal connection. This is an amazing phenomenon of unwitting cooperation. It requires the symbiosis between nitrogen-fixing bacteria and their legume host plant, as well as a willing mycorrhizal fungus attached simultaneously to the legume and nonlegume plant to act as the pipeline for transport of the nitrogen.

The importance of the underground plant-to-plant mycorrhizal conduits remains a matter of debate, but many are convinced that in some ecosystems the sharing of resources through such networks is so great that the plant communities function as a unified "guild" and the distinction between individual plants becomes blurred.

Like many soil fungi, the aboveground fruiting structures (mushrooms) of ectomycorrhizal fungi often appear in a "fairy-ring" pattern. This pattern is formed as the fungus grows slowly outward from a central point, with fruiting bodies produced only at the active growing points along the perimeter. The pen at center gives scale.

How large are these underground networks? No one knows for sure. It is quite possible that plants and mycorrhizae of many species could be loosely linked together over tracts of land measuring many acres. The maximum distance nutrients or other substances can be transported via mycorrhizae, and to what extent this might lead to a sharing of resources among plants, is still poorly understood. What we do know is that the spread of an individual fungus organism can be substantial—for example, "fairy rings" of ectomycorrhizal mushrooms several meters in diameter are commonly observed surrounding pine, oak, or other host trees. A fairy ring is the aboveground manifestation of an individual subterranean fungus that may be several hundred years old. The fairy ring pattern is formed as the fungi grow slowly outward from a central point, and the reproductive structures (mushrooms) pop up from the perimeter where the most active, healthy parts of the fungus are.

Some nonmycorrhizal types of soil fungi are among the oldest and largest living creatures on Earth. For example, in 1992, genetic analysis of samples of the wood-eating fungus *Armillaria bulbosa*, collected in a Michigan hardwood forest over an area equivalent to several football fields, showed that it was a single organism that had been alive and had remained genetically stable for more than 1,500 years. The estimated weight of this individual fungus was 220,000 pounds (100,000 kilograms), equivalent to the weight of a blue whale! So far, no beneficial mycorrhizal fungus has yet been found that is as large as this hefty monster, but undoubtedly a series of mycorrhizae, networking from root to root and plant to plant, could encompass a very large area.

Even in the absence of physical plant-to-plant hyphal connections, the ubiquitous mycorrhizal symbiosis between individual plants and fungi plays a key role in linking the activities of subterranean creatures with life at the surface. In the broadest terms, it provides aboveground life with greater access to water and nutrients stored in the soil, while supplying life in the underground with greater access to the carbon and solar energy collected by plants. The productivity of virtually all terrestrial ecosystems relies on this exchange of energy, water, and nutrients between the surface and subsurface.

Although mycorrhizae have been known for over a hundred years, it is only very recently that their essential role in the functioning of most terrestrial ecosystems, and in the evolution of land plants, has come to be fully appreciated. For much of the twentieth century, most scientists adopted a very skeptical view of cooperation between species. The reports of mycorrhizae were seen as isolated incidents. They were considered intriguing but of ecological significance only in very special environmental circumstances. When I was in graduate school in the early 1980s, a small number of ecologists had begun to recognize that we had underestimated the prevalence and importance of mycorrhizae, but it is only in the past ten years or so that the textbooks have caught up.

For a long time scientists had trouble reconciling the coevolution of mutually beneficial symbioses with twentieth-century discoveries of the "selfish" mode of gene action. Also, mathematical models, which were new to ecology in the 1970s and 80s, "proved" that mutually beneficial symbioses between two species were inherently unstable. The outcome of these computer simulations led to the conclusion that successful cooperation between species would seldom persist because "cheating" was usually too advantageous, at least at the individual level and in the short term, which is the level at which evolutionary forces operate.

Today, less than twenty years later, there has been a complete turnaround in our thinking. This has been due in part to the sheer weight of empirical evidence that has demonstrated the essential function and ubiquity of mycorrhizal associations with plants. This research was carried out primarily by a handful of dedicated soil ecologists during the 1970s and 80s who were not dissuaded by the majority opinion that their work with root fungi was esoteric and insignificant. Over and over again they showed that as long as samples were handled correctly to preserve their integrity, wherever there were active plant roots, there were active mycorrhizal fungi collaborating with them.

The "icing on the cake"—the genetic evidence—has only been attainable in recent years. With today's technology, we can grind up a tiny sample of root or soil, isolate the fragments of DNA released, and if mycorrhizal DNA is present, it will pair up with "fingerprint" nucleotide sequences of known mycorrhizal DNA that are added to the mix. This sounds complex, and it is, but with today's automated genetic analysis methods, it is much faster than tedious microscopic examination. Most important, it allows positive identification even in samples where most of the fungi have been lost or killed by mishandling, and would never be identified otherwise.

Today it is widely accepted that nearly 90 percent of plant species enter into mycorrhizal associations with fungi. This recognition of the prevalence and profound importance of this subterranean symbiosis has had an impact beyond soil ecology. Our understanding of the forces of evolution and our mathematical models used to depict species interactions have matured as a result of this work. Evolutionary biologists now recognize that "even with selfish genes at the helm, nice guys can finish first," as Richard Dawkins, author of *The Selfish Gene*, so aptly put it.

The mathematical population models used by ecologists have been improved to better reflect this reality. The early models were mostly "equilibrium" models, which assumed that nature reached a more or less steady-state situation with regard to population levels of species. Today we better appreciate the fact that in the real world there are simply too many natural disturbances—invasions by new species, dramatic weather events, forest fires, plagues—for equilibrium to ever be reached. And it turns out that when disturbance is commonplace, cooperation, both in our mathematical models and in the natural world, can persist and often flourish. We have also learned that dominance of one partner over another can be prevented when there are "third party" species that are predators of species involved in the symbiosis. For example, in nature, small soil arthropods such as springtails often feed on mycorrhizal fungi, perhaps keeping them in check so that they do not become pathogenic to their plant hosts. The newer models have also been reconfigured to take into account the fact that a little bit of "cheating" is tolerable when the partners are as distinct in size and morphology as are plants and fungi. All of these changes have led to a better match between the real world, where symbiosis clearly thrives, and the simulated world predicted by mathematical models.

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