Roots and their fungal partners

A chapter in: *Trees of the People*, by Alan R. Walker

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A young seedling of birch grew from between small fragments of rock where a load of road-stone had been dumped beside a forestry road then neglected for several years. During that time a thin growth of seedlings appeared on the pile: birch and Scots pine, also New Zealand willow-herb. The herb was a long way from its original home, but the trees were in a forest that had been flourishing naturally in northern Europe for hundreds of years. Little resembling soil could be found within the pile of recently quarried stone, just finer gravel, mineral grains and a trace of decaying vegetation. Yet the seedlings were healthy, with little competition from grass or moss, and well lit beside this wide road.

Seedling of birch, *Betula*, on stone pile, and at left an older seedling from same pile, showing roots and mycorrhizal colonisation.

The birch seedling grew at an unusual place: a pile of road gravel. I pulled it out and shook off mineral grains of soil. Under a hand-lens appeared the fine roots branching off the main root, and numerous short root tips. Most of these tips bore bulging sheaths, each like the finger of a glove. Later, when I submerged the seedling under water in a white dish, fine contrasting threads became just visible as they spread away from the sheaths. The roots of the seedlings bore some companion organism down there in that inhospitable ground. A species of fungus was firmly attached to the seedling's roots. Clearly the fungal sheaths and threads were of the mycorrhizal type: a fungus-root.

Roots of the birch seedling as shown above. Most root tips are sheathed with mycorrhizas, as arrowed in black.

Spores from mushrooms of a species typical of this sort of forest, possibly a poisonpie mushroom, *Hebeloma crustiliniforme*, had been shed into the air near the stone pile and here they developed and spread sufficiently to contact the roots of a potential useful partner. Once there the fungal threads wrapped around the growing tips of roots and penetrated between the outer cells. Here the fungus started to extract simple sugars from the seedling, sugars coming from the photosynthetic activity of the seedling's few leaves. To establish and grow on that barren soil it seemed that the seedling must have gained something in exchange.

Seeds of trees germinate where they land after being blown on the wind, washed away in floods, or carried by animals. If their parent population is to continue to flourish, the germinating seeds will need space for their leaves to spread into the sunlight and capture the energy of photons. Their roots will need to provide a system of tubes for water and nutrients to be sucked up by transpiration to supply the leaves. A forest fire supplies both space and nutrients after it has devastated a stand of trees. Trees wind-thrown after a gale make space and disturbed ground, so

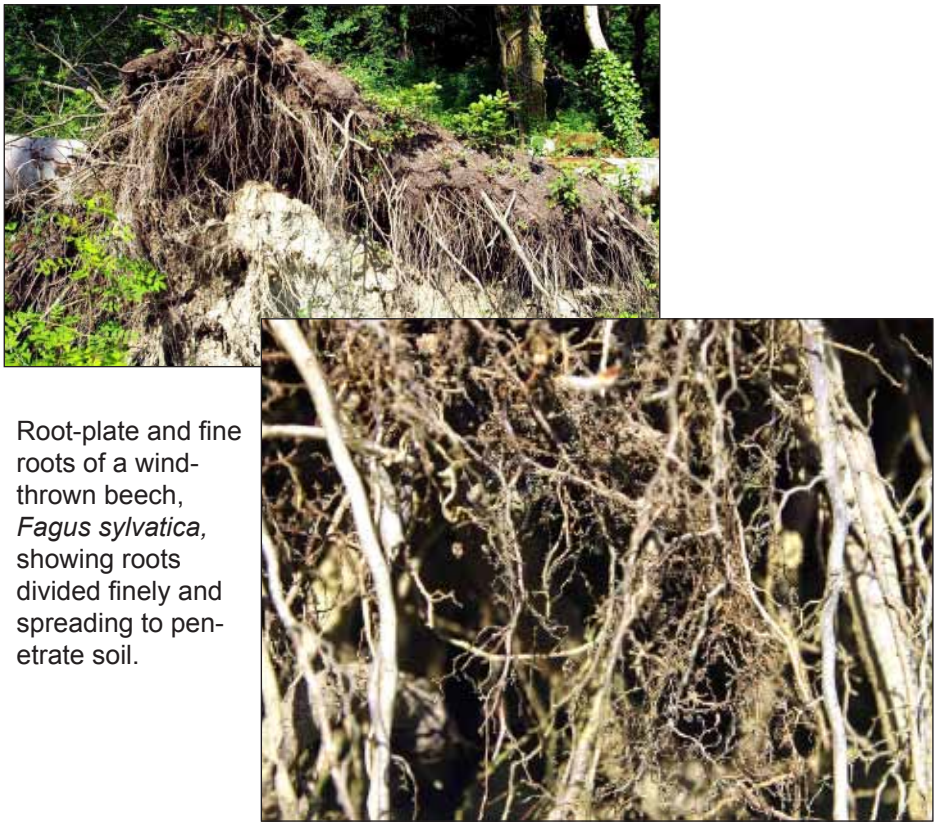
within a year seedlings will thrive on the new light and more accessible soil. Then mycorrhizal fungi that grew with the old trees discover the new roots of seedlings to colonize as a source of their energy from the plant's sugars.

Even ground as bleakly barren as the slopes of a volcano, or rock and till dumped by a retreating glacier, becomes vegetated with tree seedlings, along with lichens and mosses. Species of trees adapted to life on mountains or lands near the poles, dwarf birch (*Betula nana*) and alpine dwarf willow (*Salix reinii*) can establish a few plants that slowly mature. Soon enough threads of mycorrhizal fungi spread through the stony soil in search of nutrients and water and become partners with these pioneer trees. Then any new seedling of the same tree species will encounter an established network of fungal threads. Rapid fungal colonization of these newly arriving seedlings boosts their growth and enables them to grow and spread faster than their parents. However, in the development of these trees, before these fungi come the roots

Root structure and growth.
A germinating seed of a tree soon uses up its own resources to grow a primary root, its radicle. This active tissue rapidly penetrates the soil to start providing the shoot with water and nutrients. Then lateral roots emerge from the radicle and these in turn form branches, many of which spread close to the soil surface rather than penetrating deeply. At the tips of the roots a clump of cells, a meristem, continually produces new cells both behind and forwards. The lower new cells develop forward into a cap of loosely bound cells. These are fat with vesicles of a watery, gelatinous, material: a mucigel. As a growing root pushes through the soil it discards these cap cells, which disintegrate to form a blob of gel around the root tip, lubricating and easing its passage through the soil.

Behind a root's meristem new cells grow and differentiate into layers for separate tasks. Outermost is the epidermis, just one cell thick. From each epidermal cell projects a thin hair as a simple extension of the cell surface. Root-hairs are active at the tips of advancing roots, taking up water

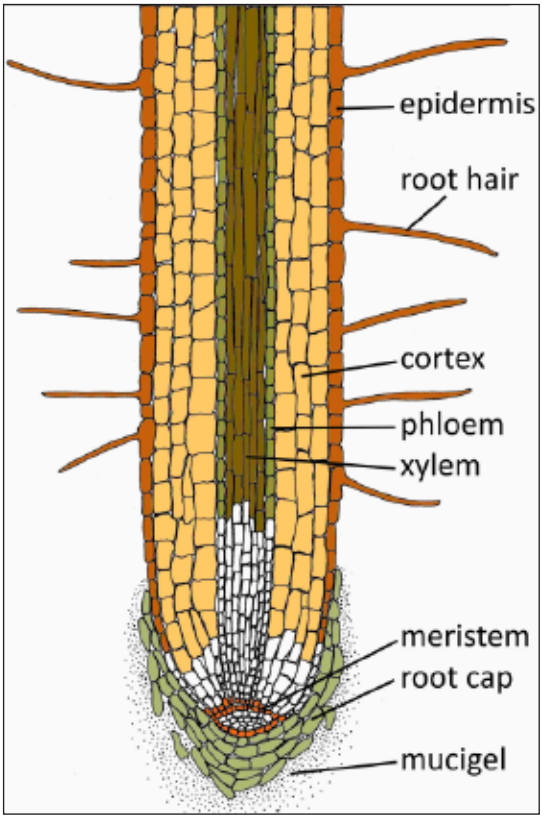
with its dissolved nutrients through this array of large surface area. As the root continues to branch, each meristem at the root tip provides cells for the bulk of the root, its cortex layer. Cells at the centre of the cortex specialize further into transport tubes (xylem) allowing water and nutrients to reach the shoot. There is a contra-flow: down phloem tubes to conduct nourishing sap down from the highest leaves to the tips of roots.



Root-plate and fine roots of a wind-thrown beech, *Fagus sylvatica,* showing roots divided finely and spreading to penetrate soil.

Branching growth of the roots will never cease in the life of the tree. As the shoot grows the main roots need to radiate in search of nutrients and to support the shoot. But older roots also continue to produce branches close to the centre of the root system so that the soil here continues to be exploited. So spread of roots away from the shoot is limited, rarely more than twice the diameter of the mature leafy canopy. The roots branch, extend, do their work, die back and regrow within this perimeter.

A researcher studying roots of a stand of seventy year old northern red oaks (*Quercus rubra*) in eastern USA examined samples of soil of one cubic centimetre. He found there one thousand root tips with a calculated total length of 2.5 metres. Although these fine roots occupied only three percent of the soil cube their surface area was about six square centimetres, without counting the huge surface area of all the root-hairs.



Life in soil is physically harsh and the demands of the shoot are great, so new fine roots must be replaced rapidly. As the root tip advances it leaves behind a trail of spent mucilage and cells. Redundant root branches rot where they ceased to function. Under a typical forest in a temperate climate between one and five tonnes of dead roots are left each year in the soil under an area of one hectare. All this dead plant material can be counted as a store of carbon in soil.

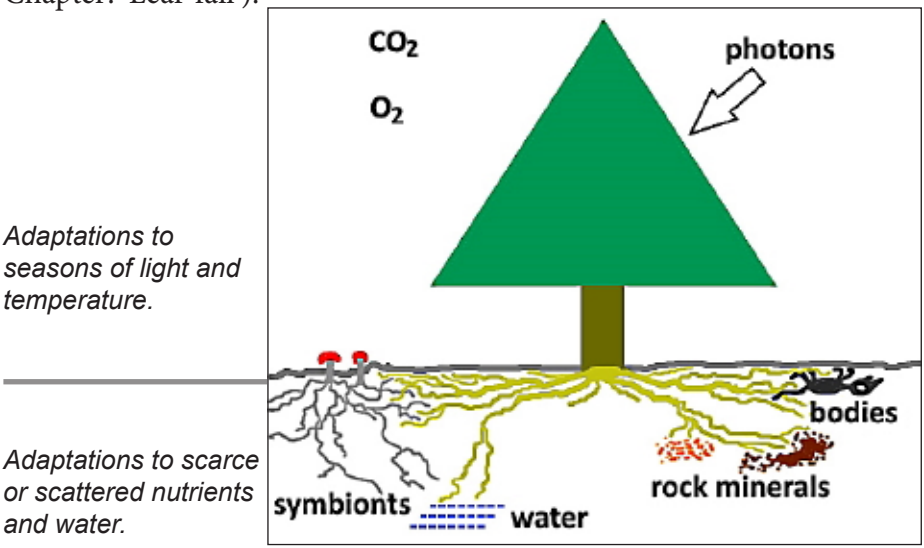
The growing shoot of a young tree needs support against the force of gravity as it rises. Most of the water and nutrients required are found near ground level where rain and leafy detritus land. So most forest trees stand on a superficial base plate where they thrive on adequate nourishment but at risk of wind-throw during every gale. Hundreds of trees may be toppled in a fierce storm, but for the reproductive success of any one mature tree the fate of being wind-thrown increases its chances of leaving successful offspring. Its own seedlings may establish and grow fast in the newly sunlit space and on disturbed soils.

Root hairs on a fine root that is isolated from soil as it explores a void. Credit: Wikimedia.

Roots compared to leaves.

All rooted plants live strangely compared to other organisms: half up in the air, half down in the ground. This split life demands continual compromise from seedling to senescent tree. The branches of a tree expand freely into the air, constrained only by gravity, their supply of nutrients, and competition for sunlight by nearby trees. Air provides a uniform supply of carbon dioxide for photosynthesis and oxygen for respiration. Only the amount of water vapour in air varies significantly. Every individual tree in one area of forest is exposed to the same overall supply of

the sun's photons during one season. A stand of trees of the same species receives similar supply of nutrients to their leaves. Out in the open air risks to leaves are herbivores, blasting by wind, abrasion from particles of ice, and drought. The biological characteristics of leaves, needle-leaf or broad-leaf, evergreen or seasonal-green, can be described in terms of well defined physical components of their environment. This ecological analysis of leaves has been termed the leaf economics spectrum (see Chapter: 'Leaf-fall').



Constraints on roots compared to leaves.

Roots conform poorly to a similar analysis. They are impeded by the solidity of soil. Within a mass of soil, such as one metre deep by one hectare wide, the nutrients may be fairly evenly distributed in a soil of wind blown dust such as loess. In soil made from mountains ground down by glaciers and rivers there may be patches of volcanic origin, rich in mineral nutrients, whilst other patches are of sandstone made of grains that long ago were leached bare of nutrients. A small patch of soil may contain the decomposing remains of an animal, a mouse to a moose. Here the bones and soft tissues are a concentrated source of phosphorus, calcium, nitrogen . . . The list of essential nutrients for trees contains about fifteen elements: boron to zinc. The best that roots can do

is grope through the soil at random in search of patches of high concentration of a wide variety of nutrient types. A constant expense of carbon and nutrients to the ever growing and dying roots must be borne by tree's leaves.

At least roots are protected from the browsing of large herbivores and massive infestations of insects, but soil is not just mineral dirt and some organic humus. The sort of soil that will support the growth of a tree will be heaving with life. Bacteria, protozoa, fungi, tiny mites and insects, nematode worms, earth-worms and moles. Some of the larger animals will eat fresh roots and there are wood-rotting fungi that roots need defences against. So researchers who aimed to analyse roots in terms similar to the leaf economics spectrum have instead demonstrated how different is the economic ecology of roots compared to leafy branches. Obviously soil is crucial to most plant life but also it contains much carbon that remains underground like a store. An estimate has been made of seventy four percent more carbon in the soil than in above-ground biomass of carbon in trunks, branches and leaves . There may be about eighty tonnes per hectare of carbon in the soil under a forest. Then there are the mycorrhizal fungi to consider.

Oyster mushrooms, *Pleurotus ostreatus*. The fruiting bodies, sporocarps, of a fungus that feeds on wood, usually on stumps of dead trees but may also feed as a parasite on live trees. Credit: Wikimedia, H. Krisp.

Soil and fungi.

A sample of fertile soil typically has, by volume, about forty five percent as inorganic grains of rock. Five percent is the organic component: live roots, plus all the other forms of life, all mixed in with the decaying

remains of these organisms, plus leaf litter and stems of trees, herbs and grasses. The remaining half of the volume comprises air spaces and

Beech tree with continuing growth of new foliage but the damaged area now invaded by wood-rotting fungi.

water. Most of this living component of the soil is unseen by silviculturist or gardener, unless they come equipped with core-borers, sieves, extractors, and a microscope. There is however one of the living components of soil that regularly makes itself conspicuous: mushrooms. These are the reproductive bodies of fungi, their fruiting bodies or sporocarps. These create and disperse potential offspring as minute spores. For every sporocarp rising above-ground there spreads a network of fungal threads in all directions of the upper layers of soil. Here most nutrients can be found. Not all soil fungi produce mushroom type sporocarps, and those that do may only expend such energy and material during conditions are suitable, not on a regular seasonal schedule.

Fungi are classified as an entire Kingdom of life forms. Other Kingdoms are: Plants, Animals, Bacteria, plus another three of lesser known organisms. Fungi are only plants in popular speech – technically they are no

more plants than are animals or bacteria. Our familiarity with mushrooms tends to obscure the strangeness of fungi in all their vast variety and ways of living, mostly hidden from our view. The more that we people discover about them the weirder they seem. But the less we think of them as some kind of underground-living plants and the more their astonishing versatility of ways of living become known, the easier it is to come to some understanding about these fungi.

Woodlands and forests are home to many thousands of species of fungi. None of them possess chlorophyll and no fungus ever evolved into a relationship with the cyanobacteria that became the chloroplasts of all green plants (see 'Photosynthesis'). So fungi cannot directly obtain energy from sunlight. Instead, very early in the history of life, they evolved a suite of powerful enzymes that digest all manner of the most abundant food: dead organisms of every kind. Fungi are the chemists of the living world – they mostly feed by dissolving their food with enzymes they secrete. This method is called saprotrophy: these fungi are *saprotrophs* (from sapro- for decaying matter; also called saprophytes, saprobes or saprophages).

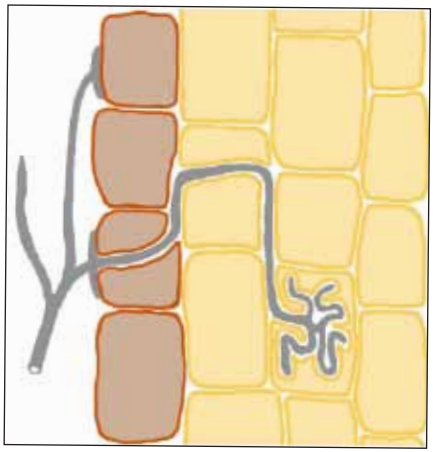
A typical saprotrophic fungus is the oyster mushroom, *Pleurotus ostreatus*, which naturally feeds on dead trees and cut timber. However, the enzymes that the fungal threads secrete onto potential food are powerful enough to enable this fungus sometimes to feed on living trees, as a parasite rather than a saprotroph.

Other species of fungi can attack and feed on live cells and organisms. These are *parasites,* their effect on their hosts is pathogenic, causing harm that is often defined as a particular disease of plants or animals. If we get a problem with athlete's-foot infection, or a ringworm infection, these diseases are caused by the sort of parasitic fungi that infect animals by contact or by air-borne spores.

Under most stands of trees there will be scores of species of fungi in mycorrhizal partnership with the trees, herbs and grasses. The sporocarps

of some of these species, as they emerge at the end of the plant's growing season, are a splendid sight. Amongst the best is the fly agaric *Amanita muscaria*, often under stands of birch trees, *Betula* species. Although the combination of live plant and fungal material and their decaying remains in the soil is small in terms of the volume of soil, their biomass in the soil as kilograms per hectare of forest ground can be a high proportion of the total biomass within a forest, above and below the ground.

A study of stands of Pacific silver fir, *Abies amabilis*, found that of this total biomass (non-animal) eighty eight percent was in the soil. Although only six percent of the below-ground biomass was living and just a small fraction of the live matter was identified as mycorrhizal fungi, these fungi were responsible for fifteen percent of the combined net primary production of the assemblage of trees plus fungi. Most of the carbon that supported this growth was from the photosynthate of the fir trees. A significant proportion of the carbon captured by photosynthesis by needle-leaves went to support respiration and growth of fungi.

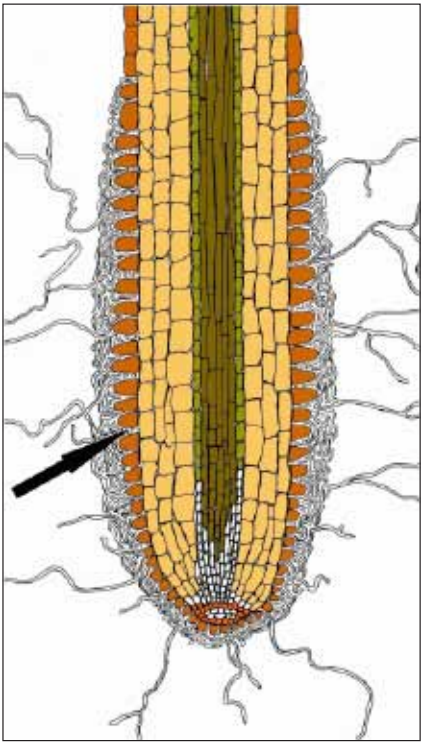


Simplified representation of an arbuscular mycorrhiza colonizing a root. Hypha (grey) penetrates an epidermal cell then grows through or around cells of the cortex, and finally forms an arbuscule inside a cortex cell.

Types of mycorrhizal fungi.

There is a common type of mycorrhizal fungus described as *arbuscular* (from a tree-like part of their structure). They are in the large group Ascomycota (a phylum or division, depending on which taxonomist you ask). The arbuscular mycorrhizal fungi partner with many types of plant. Their fine spreading threads, their hyphae, advance through the

soil, contact the roots of a suitable plant then squeeze through the spaces between cells of the epidermis and cortex. The hyphae also penetrate through the cellulosic outer wall of the plant's cells and out to the other side. They achieve this without piercing the plasma membrane of the plant cell. Instead the plasma membrane forms an invagination, it folds in on itself, so these violated cells remain alive and with functional cytoplasm and nucleus.



Representation of a root tip in cross section showing colonization by an ectomycorrhizal fungus.

The root is of a broad-leaf tree (as shown on page 5). The fungal threads from the mycelium penetrate between the epidermal cells to form a Hartig net, arrowed. These fungal hyphae do not penetrate inside epidermal or cortical cells.

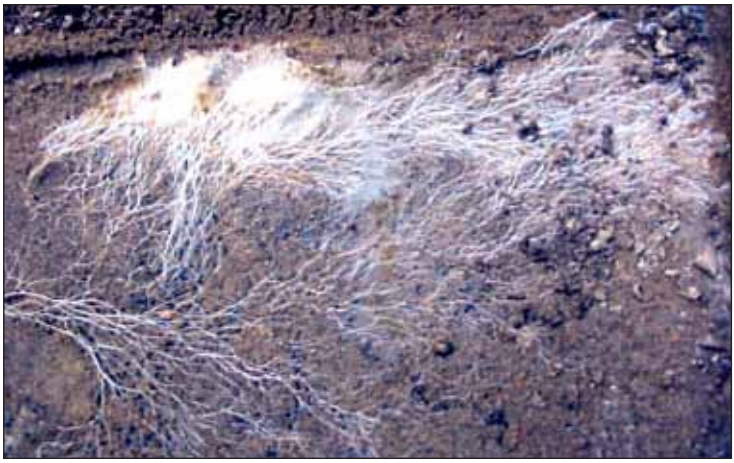
Once in a suitable cortex cell the tip of a hypha will branch and curl into a tangled mass. This develops within the boundary of the cell whilst remaining outside the living plasma membrane of the cell. This structure, the arbuscle, sits in intimate contact with the metabolic apparatus of the cell, absorbing the plant's sugars. After four or five days the arbuscle withers and the colonized cell slowly recovers its normal appearance and function Meanwhile nearby root cells are being newly colonized.

Another group of mycorrhizal fungi belong to the group called Basidiomycota. They commonly live in woods and forests of temperate climates in north and south hemispheres. These fungi are the ones that wrap around as tight sheaths on the root tips of many species of trees. Hyphal threads push down between the outer cells of the root, but do not penetrate the cells. These are the *ectomycorrhizal* fungi, always external to the cells of their plant partner. Ectomycorrhizal fungi are of many species that can be found under woodlands, a count of 1406 species has been made for forests of Europe. Sixty two percent of these fungi reproduce by means of above ground sporocarps, the rest are far less obvious.

LEFT: Roots of Sitka spruce seedling extending left and downwards, and with tangled mass of mycorrhizal hyphae associated with the seedling.
RIGHT: Root of silver birch seedling with associated mycorrhizal hyphae.
NOTE: Different scales of magnification for these two photographs

Ectomycorrhizas.
Ectomycorrhizal fungi become familiar once their connection with characteristic woodland mushrooms is known and this type will remain the focus of this chapter, for simplicity and because they are the commonest type of mycorrhizas on forest trees in much of the world.

A hypha is a single thread, and masses of hyphae spread through soil as a network called a mycelium. Hyphae construct their outer walls mainly of chitin, combined with other materials including cellulose. Chitin is the same organic material that animals such as insects use for their external skeleton – tough and flexible. Hyphae may form with walls of separate cells or they may form into exceedingly fine and long open tubes. Within the hyphae are always true nuclei, membrane bound structures of the type called eukaryotic. This is one reason why the many species of single-celled fungi are distinguished from bacteria. Bacteria always have a type of nucleus that is diffusely spread within the cell, as a prokaryote.



Hyphae of a fungus spreading across soil. These fine structures make up the main mass of many species of fungi that live in soil. From such hyphae the fruiting bodies, sporocarps, develop. Credit: Wikimedia.

Hyphae may be as narrow as 0.5 micrometres diameter, visible only under a microscope at high magnification, or as wide as 100 micrometres, which is twice the thickness of a human hair. Hyphae grow rapidly from

their origin as a single spore, lengthening from their tips as they spread in all directions. The mycelium that develops is a genetically identical organism, a genet, either an individual or a clone.

Ectomycorrhizal fungi tend to predominate in the soil under a forest. This can be estimated by comparing stands of trees of different average age of trees during the natural process of woodland succession. The older the woodland the proportion of ectomycorrhizal fungi increases from about forty percent to eighty percent.

Mature sporocarp of *Amanita muscaria*, the fly agaric mushroom, showing the spore forming gills on the underside of the cap. These fruiting bodies of the mycorrhiza developed directly from the underground mycelial network.

Ectomycorrhizal fungi reproduce sexually, using a genetically varied and complex method to produce spores for dispersal. These individual fungi need to disperse their offspring far away from their mycelial network in the soil. Offspring need fresh soils to forage in, new plants to colonize and new fungi to reproduce with. Mycelial hyphae bind together to construct a sporocarp as a stiff body that pushes out into open air. Minute spores are produced by the trillion from various sporocarp structures.

The ectomycorrhizal *Amanita muscaria* is typical, with the underside of its mushroom cap grown into a dense array of thin white gills that hang vertically. The dark gills of the commercial edible mushroom sold by greengrocers, *Agaricus bisporus*, are easily seen. At the surface of gills the tips of hyphae protrude as sexually maturing basidia. These bud off repeatedly as oval spores, about the size of a red blood cell. On a calm day many spores will land on soil occupied by their parent fungus, to no effect, but a wind will carry millions of spores up to several kilometres away. Here there may be a gap of disturbed ground with few fungal competitors and some seedling trees to colonize. You may have seen fungal spores like dark smoke when pumped out of a mature puffball sporocarp (*Lycoperdon* species and others) as heavy raindrops land, or by gently poking a mature puffball that has its opening on top.

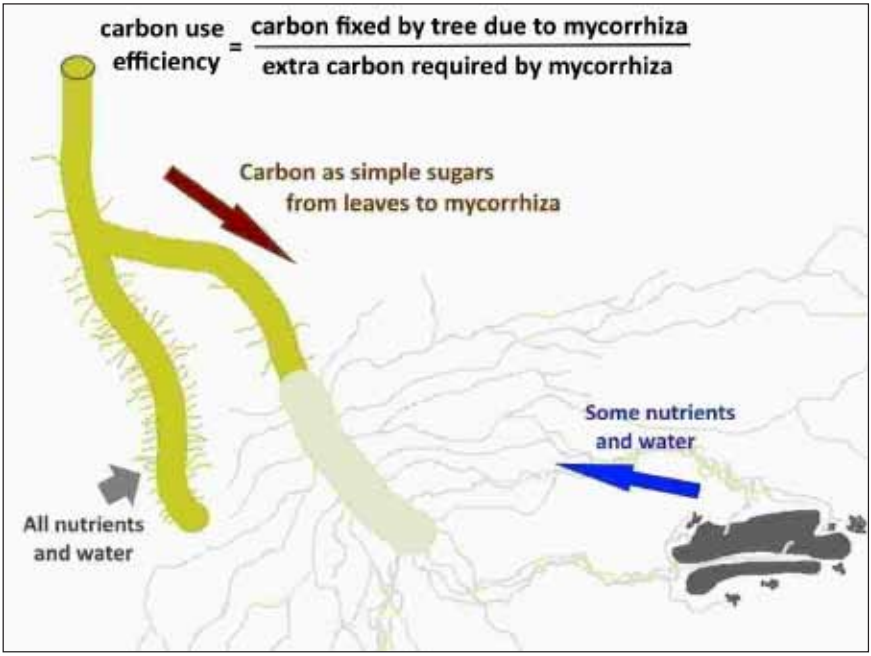
Spores of *Pleurotus ostreatus,* oyster mushroom. Scale bar is 10 micrometres. Fungal spores are minute, similar to a red-blood cell, and can travel far on the wind. Credit: Wikimedia, Alan Rockefeller.

Symbiosis: the losses and gains of living together.

Mycologists have found and named one hundred thousand species of fungi and reckon there are as many still waiting discovery. At one thousand new species formally described in journals of taxonomy each year, and the undiscovered ones being more cryptic, that could take a while. Fungi are a profuse, abundant, life form because their basic method of obtaining food is to rot down dead organic material. This rotting by fungi, this *saprotrophy*, must have been earliest method of feeding to evolve in fungi based on the concept that to become a fungus partnered with a plant it is necessary to start as a fully independent organism of

a particular species. Then a partnership, a *symbiosis*, with some other organism may evolve, such as fungi living together with algae as the life-form called lichen. Both partner species must change. The mycorrhizal fungi of both arbuscular and ectomycorrhizal types have been coevolving with their plant partners for sufficient millions of years to have lost some of their ability to feed as saprotrophs.

A symbiosis is between separate species reproducing independently whilst living close together in some distinct relationship. Symbiosis refers to the physical location of the species, without implying gain or loss. The type of mycorrhizal fungi and trees of this chapter have a relationship that is fairly specific: one species of fungus may partner up with several species of tree, similarly for one species of tree and other species of fungal partners. Always both partners have evolved modifications of form and physiology that enable their symbiosis to work.



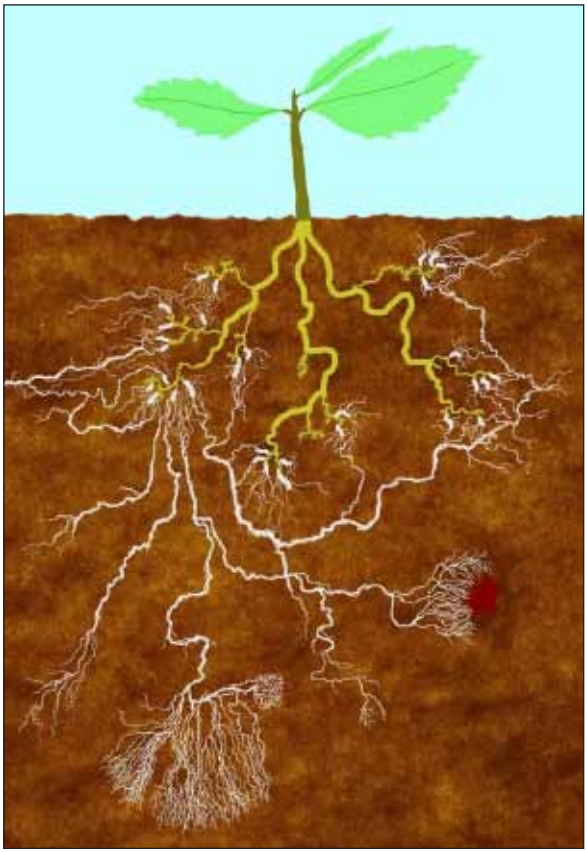
Representation of two root tips and an ectomycorrhizal mycelium. Left: root tip with functioning absorptive hairs and not colonized. Right: root tip colonized and mainly absorbs nutrients from the fungal mantle.

Whilst trees are capable of becoming partners with ectomycorrhizal fungi there is field evidence that they are not dependent on the fungi. Seed from trees dispersed far from the margin of a stand of trees onto open grassy land will germinate, produce roots and a shoot with cotyledons, then full leaves, and thus grow from seedling to sapling. Seedlings grown in sterile conditions of a nursery then planted to regenerate woodland on former pasture land that has been treeless for hundreds of years establish readily and mature into seed-producing trees at a similar time that the same species in an established wood will develop.

Seedling of Scots pine (*Pinus sylvestris*) from same stony site as the birch seedling shown in page 1. Its roots were colonized by an ectomycorrhizal fungus which might have colonized seedlings of these two tree species.

Symbiosis varies from orchids on a tree branch without harm or benefit to the tree (*commensal* symbiosis) through to fungi that invade trees and harms it by causing a disease (*parasitic* symbiosis). Furthermore, there

are relationships where both partners gain some mutual benefit from each other (*mutualistic* symbiosis). There is a great range of symbiotic relationships between different organisms that are mutualistic. Flowers and insects have evolved together over millions of years in mutualistic symbiosis. This has developed as a structure of the flower with its reproductive apparatus of pollen and nectar to attract insects. These have some adaptations for feeding on nectar. They need sucking mouthparts that can reach the nectar, and to ensure pollination some plants hide their nectar deep within their flowers. Some moths have very long sucking mouthparts. These types of symbiosis have evolved over vast spans of time and are usually so effective to both partners that they are described as an *obligate* mutualistic symbiosis.



Representation of a tree seedling colonized by an ectomycorrhizal fungus, all within a laboratory microcosm.

Roots (**yellow**) spread and forage for nutrients. Fungal mycelium (**white**) spreads from a site of inoculation and forms mantles around many of the root tips.

In the case of the more flexible mycorrhizal fungi the resource they have to offer to plants is mineral nutrients. The connection is by a mycelial network of threads of a genetically single fungus that can connect to individual plants of several species. These connections form a *mycelial network* and can be an important component of how a forest works naturally.

Nutrients for plants are usually in short supply. If the plant can extract nutrients from the fungus without harming the fungus then the balance of mutual benefit may be maintained. But how to compare sugars that travel from tree to fungus with nutrients such as nitrogen that travel from fungus to tree? The economic value of a tree's sugars to the fungus can be measured directly in units of energy available (as joules, or kilowatt hours), or units of mass of construction carbon (grams). The efficiency of use of carbon by the mycorrhizal plant can be calculated by dividing the mass of carbon gained by the plant by the mass of carbon provided by the plant as sugars to the mycorrhizas. Nitrogen however is an element that enables respiration and construction indirectly. Similarly for other crucial nutrients such as phosphorus that come in extra amounts to the plant through the mycorrhizas. The problem is how to relate metabolic effects of nitrogen and phosphorus to gains of carbon.

Remains of a red deer in a pine forest: a concentrated pool of nutrients will form in the soil below.

The criterion for balance of benefits that works from an evolutionary perspective is the fitness of both partners. This biological fitness is defined as the ability of a particular genetic or physical type (genotype or phenotype) of organism to produce more offspring relative to other organisms of the same species. Natural selection will probably then lead, eventually, to more of that new type of organism. The variants arise from random mutations that change the information transmitted from parent to offspring by DNA. The mutations arise either spontaneously within the organism or are induced by external influences such as natural ionizing radiation. Those variants most likely to prosper are an infinitesimally small subset of all possible variants, but because the mutations arise randomly and frequently there is within nature a constant bubbling up of slightly new designs of potentially fitter organisms – fit in this evolutionary sense. Living things flourish through their relentless drive to grow and reproduce themselves, by being adaptable in how they survive and reproduce.

Researchers have studied growth rates in many combinations of tree and mycorrhizal fungus, grown together in simple apparatus in a laboratory. They construct a chamber, a microcosm, from a pair of small transparent plastic plates fixed five millimetres apart. Bigger kit of this sort is called rhizotron. After filling the chamber with artificial soil and planting a seedling, and either inoculation with mycorrhizal fungus, or without the fungus as negative control, then seedling growth is compared. Growth rate as gain of seedling weight during a fixed time can be measured for many combinations of tree and fungus species. By comparison of many studies (a meta-analysis) researchers showed that in forty eight percent of such experiments there was a positive effect of mycorrhiza on seedling growth, whilst twenty seven percent showed no effect and twenty five percent showed a negative effect.

Were these negative effects caused by mycorrhizas on those seedlings behaving as parasites? Possibly they did, but to prove that researchers would need to expand their studies to measure both fitness of the trees and fitness of the fungi. For the trees these seedlings would need to be

nurtured to reproductive maturity at twenty years or more, whilst the control trees would need to be kept free of mycorrhizas for that time. Only then could fitness be compared by testing viability and vigour of the new seeds and trees. Soil biology must be a tough way to make a living as a researcher. Nevertheless, this analysis shows that the concept of mycorrhizal symbiosis may be most productive if it includes the potential for both parasitic and saprotrophic behaviour by the fungi. Life thrives by its endless spontaneous variation in ways of reproducing itself – indifferent to how we humans try to define and categorize it.

Foraging for nutrients.
Viewed under a stereo-microscope a sample of a tree's roots will probably show a few root tips freshly pale, bearing root-hairs. The remaining root tips will be covered with a dark dense matt of hyphae, as a sheathing mantle. Spreading from the mantle will be hyphae and further from the mantle are hyphae from other mantles that combine as a mycelium. Nothing of the root tip can be seen: the fungus dominates by its colonization. On a flowering tree, an angiosperm plant, the hyphae squeeze between the cells of the epidermis and no further, but on a coniferous tree, a gymnosperm, the hyphae push in amongst the cells of the root's cortex. In both cases the resulting mesh of hyphae is named the Hartig net. No evidence of defensive modifications by the tree roots can be seen, no thickenings or suberization of the epidermis or cortex cells.

For any one symbiosis of fungus species and plant species this is a dynamic and variable relationship. Ordinary non-colonized root tips emerge, grow, absorb nutrients and water, all in few weeks then they die. For colonized roots the same tempo proceeds, three to ten weeks is typical for the life of a mycorrhizal mantle. From the mantles the mycelial hyphae grow at two to three millimetres daily. This is visible when seedlings are grown in microcosms. Hyphae intertwine and thicken into strands, braided like a white rope.

Under a forest floor these hyphal strands, growing like roots, are called rhizomorphs. They radiate out metre after metre, with fans of separate fine hyphae at their periphery, foraging at random for nutrients and water. Once a good patch of resources is found the hyphae concentrate around it densely. Roots continually grow from their fine tips, become colonized and engage in short-lived mutualist exchanges, to be replaced with more root tips that continue to seek nutrients and water. Growth, movement, temporary exchanges, senescence, death and renewal by fresh growth is the life of roots and mycorrhizas – a dynamism hidden under the soil surface.

Three sporocarps of fly agaric at different stages of maturation in a stand of birch trees: a mycorrhizal symbiosis easy to see at this level.

Saplings from a nursery had been planted on abandoned pasture for livestock. The nearest mature birch wood is 1km distant. These trees probably were first colonized by mycorrhiza from windblown spores from sporocarps in the distant birch wood.

Estimates of the mass of mycelium in the soil under a forest vary from the high hundreds to several thousands of kilograms per hectare. To that must be added the seasonal crop of sporocarps above-ground. Typical data from one study, in a Swedish forest of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) colonized with ectomycorrhizal fungi, showed a mycelium turnover of 125 to 200 kilograms of per hectare

per year, and similarly 6 kilograms of sporocarps. The total biomass of these mycelia in the soil was 600 to 900 kilograms per hectare and this estimate did not include the mass of fungal mantles on roots or above-ground sporocarps. These estimates of biomass of mycelium are in the same range as estimates of the biomass of roots. Walking through a forest we find it difficult to comprehend these comparisons of biomass of trees and their fungal partners – too counter intuitive. We people of the woods, where sunlight shines through green leaves and dapples the ground, can only dimly imagine life from the perspective of a mole burrowing in search of worms.

Of the total respiration of a symbiotic root system of a colonized tree thirty percent can occur in the fungal partner. All of the energy used by the fungus comes from the photons captured by leaves. Energy flows from leaves as sugary sap down the fluid transport system of phloem tubes and out to the root's cortex and epidermal cells. There the sucrose in the sap diffuses to the fungal hyphae. The plant is the *source* of the sucrose whilst the fungus is the *sink*.

The benefit to the trees from these below-ground fungi comes as nutrient minerals, borne in watery solution as ionic compounds such as ammonium ion bearing nitrogen, and phosphate ion bearing phosphorus. There are about thirteen other elemental nutrients required but in smaller amounts and some may be easily available. These forms of nitrogen and phosphorus are naturally absorbed through the root-hairs but are frequently in short supply to the tree because they are scarce in the soil, or the roots cannot forage sufficiently to meet the demands of shoot and leaves. Nitrogen is the most important for plants by quantity, essential to construct amino acids, proteins and nucleic acids. Ammonium ion is usually the major source of nitrogen in forests. The mycorrhiza *Paxillus involutus,* on conifer and birch trees, contains ammonium ion in its mycelium. Trees in symbiosis with *P.involutus*, being short of nitrogen, act as a sink of this resource, drawing it in by diffusion and differences in concentration gradients across cell membranes. Alternatively, nitrogen is actively transferred across cell membranes of cortex and epidermal cells

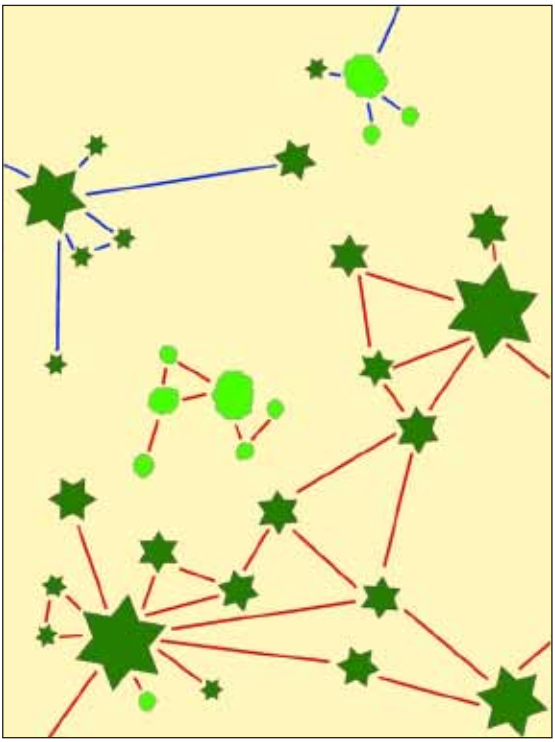
by specific transporter molecules.

If a nutrient such as nitrogen or phosphorus is scarce then the plant may have a relative excess of carbon available to its mycorrhizal partner. In contrast, if the plant happens to be on soil rich in the nutrient element then its potential for growth may be diminished by loss of too much of its carbon to the mycorrhiza. The precise ecological context of both soil and above ground climate conditions can strongly influence the balance between loss and gain in this mutualism. Phosphorus is often in severely short supply for plants. The hyphae of ectomycorrhizal fungi secrete phosphatase enzymes that digest organic debris and absorb soluble phosphate ions. The tree's roots absorb the phosphate in ways similar to those for nitrogen. This occurs at the other end of the mycelial network from metres away where concentrated patches of nutrient may occur.

Researchers using a radioactive isotope of phosphorus, ^{32}P, can trace the movement of phosphate within individual hyphae from a concentrated spot in artificial soil all the way into a tree seedling. Radiation from the isotope is detected on photographic film exposed to mycelia and roots of laboratory microcosms. Phosphorus concentrates around the roots of *Pinus* seedlings colonized by *Suillus bovinus* mycorrhiza. These experiments show an increase in transfer of phosphorus from soil to colonized plant at x25 to x40 the level of control seedlings without mycorrhizas.

Ecology of trees and mycorrhizas.
The term 'Mycorhiza' was invented by the German botanist Albert Frank in 1885, but now this field of research has been modernized by application of molecular genetic techniques. These enable researchers to follow fungal activity in soil as it happens in the forest in addition to what they can observe in the laboratory. Molecular markers have been developed for many uses in biology. These can be adapted in the lab for use on known species of fungus in pure isolation, or in culture, or from their sporocarps. Now researchers can plot the distribution and activity of an individual fungus that grew from a spore. This genetically defined organism, or clone, is called a genet. The trees the fungus is mutualistic with

are surveyed as they grow in a forest stand. With sufficient number of samples from soil-borers, researchers make maps of the natural distribution patterns of mycelia of different fungal genets.

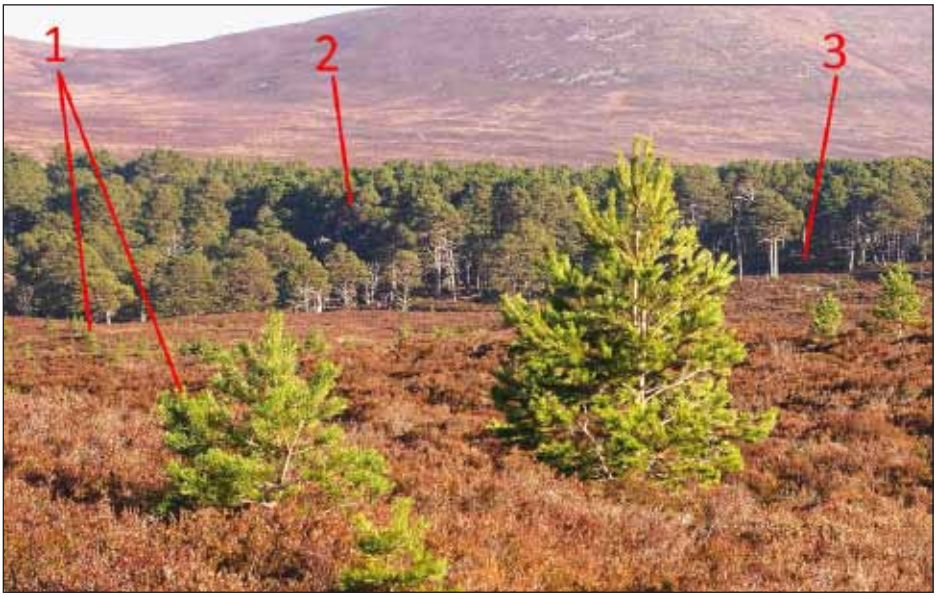


Simplified representation of a mycelial network of hyphae and rhizomorphs that interconnects trees of two species in a forest. Fungi are all of one species but of two genetically different populations (genets) shown as red and blue inter-connections.

Of the 33 trees shown the large conifer at bottom left is the dominant hub tree of this group. (Based on: Beiler, 2010.)

One study of a stand of sixty seven Douglas-firs, *Pseudotsuga menziesii*, in south west Canada was sampled intensively for mycelia containing the genets of thirteen individuals of the mycorrhiza *Rhizopogon vesiculosus* and fourteen individuals of *R.vinicolor*. These species develop rhizomorphs growing tens of metres through forest soil. Their individual mycelia formed symbioses with many individual trees. The complex interconnections created a common mycelial network. The greater the age of any single tree the better connected it was, often to trees many metres away.

The type of information from such studies of networks of mycorrhizas and trees has become a controversial topic about the ecology of mycorrhizal mutualisms. Studies of the functional connections within assemblages of species are difficult out in the open air and far more so underground. For this chapter I instead use a simpler perspective: what is in plain sight as trees on the ground.



1: Scots pine saplings established naturally on heather moorland beyond edge of a stand of mature pines; these saplings are likely to acquire mycorrhizal mutualisms from spores dispersed from sporocarps within the stand. **2:** dense canopy formed by mature pines. **3:** Dark interior of mature stand; a bleak site for pine seed to land on.

Within a mature stand of beech trees, *Fagus sylvatica*, or Scots pines, *Pinus sylvestris,* as examples, there will be few or no seedlings of the same species. Sunlight within the stand will be diffuse or at brightest only in patches. The vast production of seed by these mature trees will only have any future as growing trees when some of it disperses away into an open gap in the forest or beyond the edge of the forest. A pattern of the population of trees in a natural or planted stand of beeches or pines can be seen at a boundary between the stand and naturally open

land; or at a forestry clear-cut and the remaining stand of trees. At such edges natural regeneration of the trees shows a pattern where close to the mature trees there is little to no regeneration and further away regeneration is vigorous as number and size of saplings. Seedlings close to the stand may have sufficient light but they will be in direct competition with roots of the mature trees at the open edge of the stand. Possibly in competition with their parent tree.

All the trees in a stand are potential sinks for scarce nutrients that the mycelia contain. The faster the tree is growing the stronger is its relative strength as a sink toward which nutrients travel. These trees have evolved to have seedlings adapted to grow fast to establish themselves in competition for light down there at ground level and in competition with herbs and grasses that need the same space and nutrients. The more rapidly and robustly a seedling can gain its own mutualism with a common mycelial network the greater its chances of survival and ultimately its reproductive success.

Many species of ectomycorrhizal fungi are flexible about what species of tree they partner with. Where there is open ground that both mycorrhizal spores and tree seeds may by chance land, this low mutualistic specificity is an adaptive advantage to both partners. The probability of rapid establishment of mutualism is increased. The higher the local diversity of species of fungi and of trees the better it will be for the benefits of mycorrhizas. These symbiotic interactions on seedlings are readily accessible to study, as the introductory photographs of this chapter show.

Mycorrhizas as an aid to forestry.
This knowledge of mycorrhizas is being used for forest management and silviculture. The commercial value of the largest and straightest tree trunks in an area for harvesting is obvious. Felling such trees in preference to lesser trees during harvest of forest will remove the ecologically most important trees: hubs of the common mycelial networks. So for the long-term productivity of wide areas of forest sufficient hub trees should be left as a source of mycorrhizal fungi into replanted areas nearby.

This is similar to the forestry method known as continuous cover forestry. The potential here is to maintain thriving mycelial networks in the soil whilst such a semi-natural woodland goes through its succession from early growth phase, or stand-initiation, through to old-growth phase over several hundred years. During such woodland development the proportion of ectomycorrhizal fungi in the soil will increase to about eighty percent of the fungal biota.

New plantations might contain a range of species of conifers and broad-leaves so that a wide diversity of mycorrhizal species can be supported. A typical replanting scheme for a commercial plantation of conifers will include a proportion of ten percent or more of a mixture of broad-leaf species.

LEFT: Mycorrhizal pellets to be applied one per seedling as it is planted. RIGHT: Granules of mycorrhizal treatment for application to soil. Granules are approx. 3mm across and consist of fungal hyphae and spores in an inert carrier. This is a small packet sold to gardeners; similar formulations are manufactured and sold in 25kg bags for commercial forestry use.

Mycelia remaining in the soil after clear felling survive for no more than about six months without their tree partners. They do not fruit as mushrooms without their trees and their saprotrophic capacities are insufficient to support continued independent growth or reproduction. Trees remaining beyond the clear-felling coupe will continue to support mycelial networks and these will produce sporocarps and fungal spores that will disperse widely and tend to re-establish new mycelial networks as the replanting develops. But this natural regeneration of the mycelial network may be too slow relative to commercial constraints.

Another solution to the lack of desired fungi in the soil of a plantation is to artificially introduce fresh fungi that have been cultured by people using microbiological methods in factories. These methods have been used since the 1950s and are now thoroughly commercialized.

Nursery for conifer seedlings to supply forestry companies for commercial plantations. Credit: Wikimedia, Mick Garratt.

Cultures of relevant species of fungi are isolated from forests, grown in artificial culture conditions where they have ample nutrients, then prepared as dry granule or pellet formulations for use during replanting. Alternatively formulated as liquid formulation to apply to forestry seedlings in a nursery. Genetic analysis of isolates of potentially useful fungi enables isolation of known useful species.

Use of these fungal cultures as living fertilizer is well commercialized, with many competing claims and marketing prices and methods. The high cost of some inoculants can be offset by the direct relevance of the species and local strains of fungi that comes from prior survey of the plantation where the inoculant will be used, or the treated tree seedling planted.

Additionally the seedlings can be cultivated in nurseries where mycorrhizas are introduced as inoculants. The fungus *Thelophora terrestris* forms symbioses with species of pine, spruce and oak when in nurseries. Inocula of *Pisolithus tinctorius*, *Rhizopogon vinicolor, R.colossus, Hebeloma crustuliniforme, Laccaria bicolor* and others are also used. With these ranges of fungi suitable for cultivation the best effect will be where the species of fungus matches what is usually found with the plantation trees. However, the relatively low specificity of some mycorrhizas for their guild of partner trees provides useful flexibility.

From the liquid of fungal cultivation media masses of hyphal fragments and spores are combined with an inert carrier material such as vermiculite to form small granules. In this form manufactured and packaged inoculant can be spread broadly over the soil. Many commercial companies supply these products, often packaged in large bags for forestry and horticultural use. An alternative method of application is to combine the inoculant as a watery suspension of the fungi with an inert and porous pellet designed to be placed, one per seedling, during planting.

Mycorrhizal fungi are a topic full of mysterious connections and the complexities of studying theories of coevolution. As if office-bound

theory was not problem enough, out in the forests these diffuse, protean, and dynamic organisms live underground with their tree root partners in soil all dark, dirty and teeming with other life competing for nutrients, water and space. Despite all these impediments research on mycorrhizas is thriving as it contributes to both understanding of basic biology and to improving silvicultural practice.

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