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# How Mycorrhizae Structure, Function, and Role are Associated with Micorestoration in Forest Ecosystems

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## Abstract

Mycorrhizae may be the keystone to forest health and restoration practices. This research explores the impact of mycorrhizae on commercial, economic and environmental systems, examines relationships among types of mycorrhizae, and depicts the process of human assistance in the carbon cycle and regeneration of polluted land through micoremediation practices. These practices provide regenerative habitats utilizing wood chips to create fungal spores or promote applications of spent mushroom substrate to regenerate land victimized by toxic waste spills or other pollutants. The function, structure, and role of each mychorrizal or saprophytic fungal type are detailed with an exploration of the manner in which enzymes and nutrients break down and recycle materials such as leaf litter, herbicides, pesticides, oil spills, etc.

The incentives essential to maintain and promote successful restoration are also detailed. Principles and management practices of micorestoration and Pleurotusostreatus (oyster mushrooms) are the main focus of this research. Manipulating mycelium and using these methods, the scientific community can work toward eradication of hunger and poverty worldwide via soil renewal and revegetation processes. This paper concludes by promoting coexistence, sustainability, and new policies requiring the implementation of micoremediation frontier science because micoremediation will protect future generations from suffering the consequences of our generation's wanton disregard for the environment.

**Keywords**:micorrhizae, mycellium, micorestoration, micoremediation, mycology, mycoforestry, mycosphere, afforestation, revegetation

## Introduction

There are four phyla in the kingdom of fungi according to B. V. Barnes, D. R. Zak, S.R. Denton, and S.H. Spurr -in their text entitled Forest Ecology: Zygomycota, Chytridiomycota, Ascomycota, and Basidiomycota. Basidiomycetes produce the most fruiting structures, usually in the form of mushrooms, on trees or fallen logs in damp forests. They suggest that many basidiomycetes assist plants and partnerships in mychorrhizae associations of the root of the plant with mycelium of a fungus and "...it increases the volume of soil exploited by an individual plant tree" (Barnes, Zak, Denton, Spurr, 1980, p. 539). The term mycorrhizae means fungal root in German, and it was coined by pathologist A. B. Frank in 1885. He described this relationship as the union of two different organisms that form a single morphological organ in which the plant nourishes the fungus and the fungus does the same for the plant. Basidiospores give rise to haploid hyphae (discussed later) and this produces a dikaryotic hyphae/mycelium that eventually grows into a fruiting body on a tree or log. These and Ascomycotina contain thousands of species that are capable of forming mycorrhizae. The feeding body of the fungus is the mycelia -it is composed of a mass of hyphae, which contain quickly growing tubular filaments made of singular cells that can extend great distances into the soil of forest floors. Without help, the root hairs of plants and trees fail to take up water and minerals to achieve supreme growth, so a relationship has evolved among nearly all tracheophytes where roots are infected by fungi and form the mycorrhizae association, and this is their role in nature.

Based on the extent of dependency from plant species on mycorrhizae, plants are often grouped into three categories: Non-Mycorrhizal, Facultative Mycorrhizal and Obligate Mycorrhizal. Nearly "...6,500 species of angiosperms have been studied for the occurrence of mycorrhizae, and the roots of 70 percent are consistently associated with these fungi ... and another 12 percent are facultatively mycorrhizal - sometimes forming the association and sometimes not" (Barnes et al.,1980,p. 384). Integrating mycorrhizal, endophytic, and saprophytic (usually facultative and discussed further later) fungi can be of great significance in mycorestoration processes. All three types interact in order to decompose wood at different stages and eventually turn that wood back into soil. "Soils are generated and replenished by saprophytic fungi upon germination, seeds seek mycorrhizal germination, and endophytic fungi rain down from the sky to associate with accepting plant hosts" (Stamets, 2006, p.23). Humans have the ability to synergize the populations of these three fungal groups by providing habitats and fueling their carbon cycles in soils with wood chips and fungal spores (processes are also explained later). Managing and protecting soil connections among forest organisms through these microbial fungal links below ground will protect the health of forest ecosystems and their constituents.

### Types of Mycorrhizae

It is universally recognized that nearly all trees are colonized by some version of micorrhizal fungi. Mycorrhizae were classified into two groups: endomycorrhizae or ectomycorrhizae for several decades after their initial discovery and they were later renamed endotrophic and ectotrophic. Ectotrophic mycorrhiza exploit mineral ions released from decaying leaves in the litter layer of forest floors. In all types of mycorrhizae, the association affects the host and the fungi receive photosynthates. They usually associate with a specific type of vegetation and their most recent classification was divided into seven groups: "...Vasicular Arbiscular Mycorrhizae (VAM),

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Ectendomycorrhizae, Ectomycorrhizae (EMF), Arbutoid Mycorrhizae, Monotropoid Mycorrhizae, Ericoid mycorrhizae, and Orchid mycorrhizae" (Harley and Smith, 1983, p.483).

Belonging to the fungal order Endogonales of the Zygomycetes with lower fungi/aseptate hyphae, is Vascular Arbuscular Mycorrhizae (VAM). "There are six VAM genera: Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis and Scutellospora, contaning -200 species that belong to one fungal order (Glomales) in the division of Zygomycota "(Morton & Benny, 1990, p.471-491; He, Xu, Qiu, Zhou, 2009, p.108). Endogenaceae VAM are usually nonspecific and occur in germinaceous or herbaceous species; they predominate most grasses, bryophytes, forbs, pteridophytes, and most tropical tree genera -they are also usually associated with agricultural crops. They associate with long lived woody perennials and other diverse plant groups, such as the Sequiadendrongigantum and Sequoia sempervirens. Studies confirm that inoculated giant redwood seedlings were two to three times larger than noninoculated control seedlings, when fungal spores were added to the soil (Molina, 1994, p.80; Adams, Tidwell, Ritchey, Wells, 1990, p.7-11). Thus, mycorrhizae also aid in the successful growth of trees -similar to a natural fertilizer.

All the remaining forms of mycorrhizae, have endophytes with higher fungi/septate hyphae, and they belong to the Ascomycetes or Basidiomycetes- other subdivisions of the fungal kingdom. Temperate species that form VAM are redwood and many hardwoods such as maples, tulip tree, ashes, sweet gum, black walnut, black cherry, and sycamore (Barnes et al,1980,p. 540). Most plants produce VAM, -most frequently in western forests, such as those from the Pacific yew (Taxusbrevifolia) and Cupressaceae Family (Calocedrus, Chamaecyparis, Juniperus, and Thuja) and in tropical forests. Common VAM broadleaf trees include: genera Cornus(dogwoods), Fraxinus(ashes), Acer (maples),Aesculus (buckeyes), Plataus(sycamores) Prunus(cherries), Sambucus (elderberries), and Ulmus (elms). The festuca species is located in the western United States and is found in 11 different species of Endgonaceae (Molina, 1979,p.1223-1228). Most plants have VAM, but most temperate regions have ectomycorrhizal fungi (EMF -discussed more later). These examples show how common mycorrhizal associations are in nature and where they originate.

VAM exhibit no saprobic functions so they reproduce asexually, usually producing large spores not large fruiting bodies, and there are only a few hundred discovered thus far. "Microscopically, VAM form no sheath, and the arbuscules, literally meaning 'little tree" are finely branched hyphal structures that proliferate within a single cortical cell and function as the exchange site between fungus and host" (Molina, 1994, p.80). These hyphae grow inside cortical cells of both roots and epidermal cells. "Vesicles are balloon shaped and function as storage organs for VA micorrhizae; they also proliferate the soil but their mycelium is typically colorless and thus not visible to the unaided eye" -so hyphal roots must be stained to reveal internal fungus colonization (Molina, 1994, p.80). "Repeated failures to isolate and grow aexenic cultures of VAM fungi indicate the obligate nature of VAM fungi" (Agarwol and Sah, 2009,p.108). More importantly, without VAM, afforestation efforts would be unsuccessful, hence proving the necessity of VAM existence in forests.

Ericoidmycorrhizae are septate fungal symbionts of the Ascomycetes and are restricted to Angiosperms of the species Ericaceae such as Ericoideae, Gaultheria, Vacciniodeae, and Rhodedendroidae (Agarwal &Sah, 2009, p.108). They can also form on Epacridaceae and Empetraceae. They are abundant and often dominant components of the understory, and consequently widespread. Ericoid mycorrhiza is restricted to the epidermal cells and forms intracellular coils (Agarwol and Sah 2009, p.108). The cells are then colonized with little change in fine hair-like root morphology and the fungi have a broad range of hosts within the aforementioned family. "Ericoid mycorrhizal fungi differ enzymatically from EMF and VAM fungi because they are able to breakdown and mobilize nitrogen from organic sources" (Molina, 1994,p.108). Ericoid mycorrhizae are important for nitrogen recycling in forests, and have been put into a separate category as a result of these unique abilities.

Ectendomycorrhizae are an intermediate mycorrhizal association type found on coniferous and deciduous trees, in their nurseries, and in burned forests. They form an unusual structure, because the mantle is missing or very slight and the hyphae/hartig net can infiltrate root cortical cells. The EMF replaces the ectendomycorrhizae while the seed matures. These fungi are formerly known as "E-strain" but later were discovered to be Ascomycetes and were then moved to the Wilcoxina genus. Another type called monotropoid mycorrhizae are members of the Monotropoideae Ericaceae family and its fungi have a narrow host range, the hosts share wide receptivity, and there is dependency of achlorophyllous plants on carbon mycorrhizally associated EMF plants and trees (Agarwol and Sah, 2009, p. 109). Achlorophyllous plants will always have mycorrhizae they share with the roots of green plants that photosynthesize, because without the energy they receive from those plants that perform photosynthesis, they would have no energy themselves and thus parish on the forest floor.

Arbutoidmycorrhizae have two different plant family genera -Ericaceae which has Arbutus and Arctostaphylosgenera -the fungi that form them also form EMF on different hosts. Arbutoids are similar to ectendomycorrhizae because their hyphae are both intracellular and intercellular colonizers and ericoid mycorrhizae may also occur on arbutoid hosts (Agarwal &Sah, 2009, p.109). Regarding structure of endomycorrhizae, the internal root is infected and no hyphae can be seen at the root surface. Concomitantly, Orchids have seeds that will not germinate unless they are infected by the fungus that forms their Orchid mycorrhizae and it is part of endotrophic associations-they later colonize the whole plant (Agarwal & Sah, 2009, p.109). This commensalistic relationship is found among various trees and plants called epiphytes (such as those of bromeliads) that attach themselves to the trunks or branches of large trees in tropical and subtropical forests. Orchids root in the fork of the tree rather than the soil without penetrating or harming the tree, and thus gain access to water and nutrient debris that falls from the tree's upper leaves and limbs. Two types of colonization can occur in Orchid mycorrhizae: primary -which includes the germination of the seed or seedlings, and secondary -which relates to new roots. These processes explain the role and function of these types of mycorrhizae within forests.

Ectomycorrhizal fungi (EMF) are facultative biotrophs with nearly `7,750 species, making them more common than other types of mycorrhizae; they have the most important associations to forests, most belonging to Basidiomycetes and some to Ascomycetes, in the division of Dikaryomycota (Agarwal &Sah, 2009, p.109). They are usually found in northern temperate forests (where soils are used as storage compartments) and sub-temperate forests, but also in boreal, and sub-tropical forests, where Myrtaceae can be found, as well as tropical forest trees (a. Molina et al., 1992, p.357-423; Wang & Qui, 2006, p.299-363; a. He et al., 2009,p.143-151). "... Fungi belonging to Basidiomycetes, species of Hymenomycetesinclude Boletus, Corinarius, Suillus, Russula, Gomphredries, Hebelema, Tricholoma, Laccaria, and Lactarius and species of Gasteromycetes, e.g. Rhizopogen, Scleroderma, Alpara, and Pisolithus all form ectomycorrhizae"(Agarwol & Sah, 2009,p.111). Their abundance consequently makes EMF very important in forest ecosystems.

EMF occurs in the following types of trees: Pinus, Picea, Abies, Populus, Salix, Fagas, Betula, Quercus -and in southern hemisphere trees such as: Eucalyptus, Northofagus , and Shorearobusta of the Dipterocarpaceae (usually forming Obligatory mycorrhizae) family -in the monsoon forests of Southeast Asia (Moore, 2011). Arctostaphylos, Cercocarpus, create EMF -and all Pinaceae species have EMF (Agarwol & Sah, 2009, p.111). Over 400 taxa of EMF, (generally belonging to Russulaceae, Boletaceae, Canterallaceae, Amanitaceae and Cortinariaceae -with partners typically limited to woody trees) have been described from African forests and woodlands, and similar numbers are likely to be reported in the future (Barnes et al. p. 384). "It has been estimated that between 5,000 and 6,000 species on about 2,000 plants of fungi form ecto- or ectoendo-mycorrhizas," (Agarwol&Sah, 2009,p.111). "Around 4,500 of these are epigeous (have above-ground fruiting bodies), but up to a quarter are hypogeous (with underground fruiting bodies -usually associated with Ascomycota)" (Moore, 2011).Over 2,400 species of fungi have been shown to form EMF in North American trees alone (Marx & Beatie, 1977, p.6-9). Each set of EMF thus has its own set of physiological characteristics -some are active in cool or moist areas while others are active in warm or dry locations, and some thrive on coarse woody debris while others prefer humus or other substrate components (Amaranthus, 1998, p.2; a.Trappe, 1977, p.203-222). These nonspecific characteristics also make EMF globally significant to forest ecosystems.

Regarding additional structure, function, and role of EMF -"... they start to develop when hyphae infect the secondary or tertiary roots of woody species and they seem to prefer the short feeder roots on trees" (Moore, 2011). Their hyphae grow back up the root from just behind the root cap and meristem, forming a weft, that may later become a bulky sheath or mantle '...that serves as storage tissue and protects the fine roots from desiccation;' hyphae grow between the epidermal and cortical cells by excreting pectinases (an enzyme) and then form the 'hartig net' -but fail to puncture the individual root cells (Moore, 2011, Marx &Beatie, 1977,p.6-9).

EMF envelope the root and proliferate between the cells of the root. The mass can be as great as the total root and the hyphae increase the absorption of minerals, water, and the size of the mychorriza -holding the water nearby (similar to a sponge). This makes the roots of the tree swollen. "Sheathing reduces the rate of cell division (so all nutrients must enter via the sheath) at the root tip, slowing cell division, and reducing the root growth lengthways; they are also affected radially... hence the name short root" (Moore,2011). "Such sheathing mycorrhizae forming fungi are bio-trophic in their host relationship and ecologically obligate parasites in their mode of carbon (energy) nutrition" (Agarwol&Sah, 2009,p.109). EMF also modify their environments "through acidification around the hyphae and by exuding metal-complexing weathering agents such as organic acids, and play a central role in mineral weathering of boreal forest soils" (Moore, 2011). The fungal sheath extends with growing roots and provides a cover so colonization does not occur with other fungi -even though the fungi has the possibility of being replaced by other colonizers after dormancy thus making the roots vulnerable to other symbionts. This process can be beneficial to other organisms within a forest -allowing for nutritional exchange.

These processes allow the fungus to grow into the host plant roots and hyphae to breach between the outer cortical cells, forming the "hartig net" -usually a single cell labyrinth within the epidermis. The "Hartig net constitutes the plant fungus interface,""...where solutes are translocated between the partners;""...carbohydrates from plant to fungus and inorganic nutrients from fungus to plant "(Smith & Read 2008; He et al., 2009, p.107-118). Host responses to the hartig net may include "...polyphenols (tannins) production in cells and the deposition of secondary metabolites in walls" (Moore, 2011). Through this exchange role the roots gain access to a greater volume of soil -as opposed to trees without such associations, and this makes the surface area increased for nutrient uptake and helps trees tap further into soils for moisture and nutrients -making the forest thrive.

EMF also have roles of protecting plant roots from pathogens, moderating the effects of heavy metal toxins, and promoting soil structure -in harsh sites such as: glacial moraines, fresh volcanic deposits, and mine spoils (Harley & Smith, 1983,p.334). EMF protect trees from rotting root pathogens by using antibiotic exudates (a. Marx, 1969, p.411-417.; b. Marx, 1973, p.288-297, Barnes et al,1980,p. 386). The "...exudates and hyphae of EMF form a major link between aboveground producers and soil food webs, while providing photosynthetically fixed carbon to rhizosphere consumers such as bacteria, protozoa, arthropods, and micro fungi"(Amaranthus, 1998,p. 3). The sheath of the fungi bars parasites from invading, and the antibiotics deter the pathogens from the roots and attack the root aphids, while "...other mycorrhizae can extend the lifetime of small roots and contribute to soil aggregate formation"(Zak, 1965, p.410-411). EMF also contributes to root development, antibiotics, hormones, and vitamins useful to the plant, tree, and ultimately -the health of the entire forest ecosystem (Amaranthus, 1998, p.1, Trappe & Fogel, 1977).

The consumption of micorrhizae in forests has a process which includes the passage of fungal spores through the mammals that devour them. These are dispersed via defecation and the spores then re-enter the soil, germinate, and form additional mycorrhizae with tree roots. Having favorable tastes or smells draw the animals to eat them and spread their spores. Some fungi depend solely on this form of spore-spreading. They include morels and truffles -which are often found in the ground -on the roots of oak trees and in more mutualistic associations. These cycles nourish both animals and plants in forest ecosystems, and are thus a necessity.

Unfortunately, EMF are directly affected by pollution (the acidification and nitrification of soils).Application of artificial fertilizers, organic dung, acid precipitation from coal burning, and clearing Northofagus forests -as well as introducing Pinusradiata -decrease EMF fruiting. Some ways to conserve EMF include retaining assemblages of native host species, habitats, and structures across a landscape, as well as decreasing levels of pollution (Amaranthus, 1998, p.5). EMF diversity is usually higher among older forest stands, rather than younger ones. Scientists can diversify EMF by using stand-level silviculture -by "leaving large trees after regeneration harvests ('green tree retention') -while maintaining the energy source for certain EMF species and providing for more diverse age-class distribution"(Amaranthus, 1998, p.7). Implementing such prevention and action where necessary will aid in overall forest health.

EMF promotes different relationship via its associations of different levels of importance -depending on the tree or forest surroundings. "Dual AM and EM associations have been found in some plant species, including: Abies, Acacia, Adenostoma, Alnus, Cauarina, Dicymbe, Eucalyptus, Pinus, Pseudotsuga, Populus, Quercus, Salix, Tsuga, Uapacaand certain legumes (He et al. 2003, He et al 2009). Both EM and VAM increase the ability of tree roots to absorb and this allows the plant or tree to forage for its nutrients in a larger volume of soil. However, acccording to Agarwal and Sah, EM fungi may require more photosynthetic products (carbohydrates) from their hosts than VAM because they have larger biomass of fruiting bodies, hyphae, and rhizomorphs in EM than in VAM, yielding a higher carbon cost -if equal hyphae respiration rates of EM and VAM fungi are assumed. They also suggest that, "...EM fungi are stronger sinks for photosynthetic products than VAM because EM produce plant hormones that influence translocation of carbon compounds, and convert the host sugars into storage sugars" (Agarwal&Sah, 2009,p.112). Thus, when EM predominates (as in dipterocarp forests), the role of EM in the carbon allocation of the ecosystem is expected to be of primary importance.

Neither VAM nor EM networks have been observed in natural ecosystems, due to their cryptic, fragile, and microscopic nature, but there is evidence that plants from different populations can share a common hyphal network (He et al., 2006,531-567; Kennedy et al., 2003, p.1071-1080, He et al,. 2009,p.108). Furthermore, "there is no known example of gene-for-gene level of host fungus specificity" and little "data exist(s) on the diversity, abundance, synecology, or autecology of these fungi and thus the role of EMF is largely unexplored -especially regarding ...resilience to environmental challenges" (Amaranthus, 1998,p. 1). These associations advocate that mycorrhizae is important to the success of forest ecosystems all over the world, but not enough is known. What we do know is that"...a diverse mosaic of host species, habitats, and structures promote EMF diversity -which also reflects the different major forest types, different successional stages within a given forest type, and distinctive communities and microhabitats that encompass a forest landscape"(Amaranthus, 1998,p. 1). These explanations epitomize how mycorrhizae, structure, function, and roles are associated with forest health.

## Roles of Mycorrhizae

Since, the earth is closed to significant inputs of matter from space; all nutrients used by organisms are already present on earth and must be recycled again and again for life to continue. "The cycling of matter includes atoms, ions, or molecules needed for survival by living organisms and they are taken up by different parts of the biosphere" (Miller, 2002,p. 74). Fungi act as our planet's recyclers, disassembling large molecules into simpler forms by breaking down lignin and cellulose, the primary structural components of wood. This in turn nourishes ecological forest communities by creating soils and their contribution to the decomposition cycle mobilizes recalcitrant organic compounds found in dead wood (Molina, 1994, p.79). The forest's vigor is directly correlated to the variety, abundance, and presence of mycelial (fungal) companions and the richness of soil and its depth correlates with centuries of mycelial recycling.

Mycelium is a web of fungal cells that may "...generate fruiting mushrooms called sporocarps" at some point in its life cycle; it has the ability to build soils and cycle nutrients as well as retain them and reduce leaching through the food chain (Molina, 1994, p.78; Stamets, 2006, p.1). "Mushrooms can be placed in 4 basic categories: saprophytic (around 8,000), parasitic, mycorrhizal (around 2,000 to 3,000), and endophytic, depending on how they nourish themselves" (Stamets, 2006, p. 19, 55). "Total species richness is greatest in the soil: functions of soil microbes power the complex biochemistry of nutrient cycling that yields (this) soil fertility and ultimately, plant and animal productivity" (Molina, 1994, p.78). This level of categorization explains the importance of the various types of fungi and their connections in forest ecosystems.

Different kinds of soils will spawn varieties of trees and mychorrizal associations. ""Podzols(for instance) are typical under coniferous, boreal, and forests in the northern hemisphere and eucalyptus forests and heathlands in the southern; in podzols organic material and soluble minerals are leached from the upper layers (horizons) to the lower" (Moore, 2011, Fig. 4, para. 2). The E horizon is composed of heavily leached soil and is largely composed of insoluble materials. The high abundance of mycelium in this type of soil with high mineral content proves it as an important substrate paired with EMF in forests.

The role of nutrient exchange in mycorrhizae occurs by "…sugars and vitamins moving into the fungus, while water and nutrients move into the plant" (Molina, 1994,p.79) Important mineral nutrients include: N, P, S, Ca, K, Fe, Cu, and Zn -which are transferred to the root cells (Smith & Read, 1997, p. 78). "Michorrhizae are associations between specific fungi and the roots of plants and in such associations the fungus obtains organic compounds from its photosynthetic partner, but provides it with minerals and water so that the partner's nutrition is also promoted" (Purves, Sadava, Orians, Heller, 2001, p. 532). This association is mutually significant because the fungus obtains the organic compounds it needs such as sugars, amino acids, etc., from the plant or tree and the fungus increases the absorption of minerals, like Phosphorous for ATP and Nitrogen (NO3 and NO4), copper, phosphorous, zinc, and water. Mycorrhizae are responsible for advanced tolerance of hostile conditions, as well as the control or attempted regulation of most root disease; they "…are strongly aerobic and need ample oxygen for efficient physiological functioning" (Molina, 1994, p.80). Therefore, forest compaction via bulldozing or clear-cutting can be a serious hindrance and interrupt the cycles of mycorrhizae in forest ecosystems.

Mycorrhizae produce extracellular enzymes such as cellulases and phosphatases , which assist in the decomposition of organic matter and they produce organic acids that release tree nutrients from soil minerals (Barnes et al.,1980,p.540). However, mycorrhizae "…have limited saprophytic ability -meaning they possess few of the enzymes needed to break down complex organic matter in the soil" (Molina, 1994, p.79).The amount of sugars transferred is directly proportional to the amount of shading" (Simard, Perry, Jones, Myrold, Durrall, Molina, 1997, p. 579-582). "Fungi benefit from the relationship because it gives them access to these plant-secreted sugars, mostly hexoses that the fungi convert to mannitols, arabitols, and erythritols" (Stamets, 2006, p.24).This symbiosis is essential for both partners and "…the difference in vegetation and climate influence the mycorrhizal diversity within (any given) region" (Agarwal &Sah, 2009, p. 112). The fungus can also protect the plant from microorganisms via growth hormones and "…protect the roots from fungal and bacteria pathogens in the soil" (Barnes et al., 1980,p.384). Thus, the role and function of mycorrrhizae is extremely diverse and essential for forest ecosystems in these ways, as well as many others.

One of many studies via Manuela Giovannetti suggests measuring and calculating the hyphal length per total root length and the mean growth rate per day instead of obstructively extracting soil, which has much more variability in hyphal length per millimeter of root (Giovannetti et al, 2006, p.3). "Anastomosis is the complete fusion of hyphal walls" (characterized by protoplasmic continuity via diamidinophenylindole staining), cytoplasmic flow and migration of nuclei through hyphal bridges -and for the detection of incompatibility responses -i.e., protoplasm retraction from hyphal tips and septum formation in approaching hyphae -even before physical contact, as revealed by time-lapse, video-enhanced and epifluorescence microscopy -as well as image analysis" (Giovannetti 2006)."Hyphae may fuse due to the widespread occurrence of anastomoses, whose formation depends on a highly regulated mechanism of self-recognition; and the root systems of plants belonging to different species, genera, and families may be connected by anastomosis formation between extra radical (symbiotic) mycorrhizal networks, which can create indefinitely large numbers of belowground fungal linkages within plant communities" (Giovennetti et al., 2006, p.1). Thus allowing ecologists to observe hyphae and anastomoses in their natural states within forest ecosystems will allow for true measurements and understanding of associations between mycorrhizae and forests.

The anastomosis process facilitates the fusion and exchange of nuclei, vacuoles, mitochondria, and fat droplets -according to Giovannetti . "Anastomoses success occurs between hyphae belonging to the same individual and to different individuals of the same isolate, during the presymbiotic growth of VAM, while hyphae of different individuals of the same isolate can show rejection responses and are unable to fuse -which reveals arbutoid mycorrhizae hyphal ability to discriminate against non-self" (Giovannetti 2006). This study proposes that the "…viability of the mycorrhizal networks was 100% (in their research) and all anastomoses showed protoplasmic continuity and nuclear occurrence in hyphal bridges (using formazan salt depositions for staining), confirming the occurrence of nuclear exchange during fusions between symbiotic hyphae" (Giovannetti et al, 2006, p.4). These results (as well as many other studies) propose that hyphae can attach underground and exchange nutrients to multiple host plants or trees throughout the forest. Many nutrients translocate from leaves to roots, but many also move from roots to leaves, and they move amongst themselves through their hyphae-and the hyphae of other plants or trees belowground.

New experiments show that different species, genera, and families can fuse via mycorrhizal networks, but there is little measurable data on the frequency of hyphal amalgamations. "A common mycelial net could unite 3 species of trees and mycorrhizae can keep diverse species of trees in forests fed, -particularly younger trees struggling for sunlight" (Stamets, 2006, p. 26). This explains how young trees survive in the shadows of older trees that block out light. A single mycorrhizal mushroom can support two different types of trees nutritionally- one deciduous and the other coniferous -which proves that the overall health of forest ecosystems are sustained via mycelium. However, interactions between hyphae belonging to the same germling of VAM fungal species of the genera Gigasporaand Scutellospora never led to anastomosis formation (Giovannetti et al, 2006, p.2). The most common connections were made by wound healings between broken hyphae -described by Gerdemann in 1955. Therefore, fungi are capable of discriminating and thus suggesting genetic isolation, as well as fusion among different species at will. More studies are necessary to determine exactly how and why this self-selection among species occurs, but fungal fusions have proven themselves to be both beneficial and harmful in many ways.

Whether trees are successful in drought-prone and nutrient-poor soils may be correlated with mycorrhizal fungi presence. Mycorrhizae infrequently form on tree roots in nutrient-rich soil, regardless of whether the fungus is inoculated. Pines often grow on nutrient-poor ground and require EMF, rarely succeeding when lacking the association and in Oregon, white fir harvesting also depends on micorrhiza presence (Barnes et al., 1980, p. 384). Afforestation attempts have been failures in many areas, including the United States and India until EMF were introduced (Barnes et al,1980, p.384). In the United States, mine spoils may only hold successful pines if roots are successfully inoculated with EMF. More importantly, "... attempts to introduce some plant or tree species to new areas have failed until a bit of soil from the native area (hardiness zone) that usually contains the fungus necessary to establish mycorrhizae was provided" (Purves et al, 2001, p. 540). When clear-cutting a forest, afforestation attempts may prove to be unsuccessful because of changes in the microbial composition and soil structure. These studies are important examples that show the significance of micorrhizae in trees, forest nutrition, and nutrient cycling success.

In short, fungi break down organic matter using the aforementioned enzymes, then absorb the nutrients for growth, aiding in a cannibalistic recycling pattern, which is present in all successful forest ecosystems. This process explains the beneficial role and importance of mycelium and micorrhizae in forests, thus making them a keystone species that allows future flora and fauna to flourish. Fungal succession is a response to tree age, tree composition, and soil qualities such as accumulation of organic matter, even though there are large knowledge gaps regarding forest community succession and disturbance events (Molina, 1994, p.79). By viewing trees of a certain area, ecologists can usually predict what types of mycorrhizae are below the ground and how to restore the land that surrounds them -simply by viewing the landscape. Observation and mapping aid in efforts to restore forests by providing the required native fungi for mycoforestry success.

## Mycorestoration and Mycoforestry

Humans have the ability to synergize mycorrhizae (specifically mushroom mycelium and saprophytic spawn) and use them for healing forest habitats that may suffer from stress, toxic waste, or poor nutrition -making them critical to our mutual evolutionary survival (Stamets, 2006,p.23). By custom pairing native fungal species with plant communities, because native species allow for adaptability in designed habitats, humans can act responsibly and restore our shared environment. Matching the appropriate fungi is the key to successful restoration. Introducing new species to unfamiliar environments usually leads to unsuccessful restoration efforts. Therefore, mapping out the land and its familiar fungi should be the first step in mycorestoration efforts.

One part of mycorestoration is mycoforestry, which " is the use of fungi to sustain forest communities," including goals such as "...preservation of native forests, recovery and recycling of woodland debris, enhancement of replanted trees, strengthening sustainability of ecosystems, and economic diversity" (Stamets, 2006,p.69). Sustaining forests thus sustain animal populations that breathe in $CO_2$. These cycles are all interconnected, such as the idea that mushrooms have "...a preselecting influence on the bacteria sharing their habitat" (Tornberg, Baath, & Olsson, 2003, p.190-197). Bacteria regulate nitrogen cycles and are limited in phosphorous. Mycelium absorbs phosphorous and moves mineral salts over distances. They later release them when the mycelium dies or when they rot; then the fungal bacteria mushrooms decompose the mycelium and absorbs the phosphorous; cycling from rotting mushrooms occurs among phosphorous, potassium, zinc, and other minerals, as they are deposited back into the food bank(Stamets, 2006, p.69-70). Humans assist in this recycling effort in forests by manipulating and applying their knowledge of mycorrhizae associations -thus creating mycoforestry.

The guiding principles of mycoforestry include: "...using native species, amplifying saprophytic fungi, using proper species for reestablishment (based on interactions with bacteria) -that attract insects whose larvae attract fish or birds, choosing economically valuable mushrooms to aid in preservation goals, promoting ground contact, using spored oils in chain saws, chippers, and cutting tools (so wood debris is in contact with fungi immediately after cutting and thus speeding up decomposition), retaining wood debris on the site or where erosion control is needed, saving burning for a final measure, using mycorrhizal spore inoculum when replanting forestlands (seedlings cultivated in pasteurized soils or constructed soils on tree nurseries typically lack mycorrhizae), and choosing species that compete with disease rot fungi (like Armillaria or Heterobasidionannosum) for mycorestorative saprophytes like Hypholoma, Psilocybe, Trametes, Ganoderma, Sparassis, etc." (Stamets, 2006,p.73-74).

Mycologist Paul Stamets suggests, "the bottom line is that we need to focus on carbon cycles and raise the nutritional plateau in timberlands by accelerating decomposition of wood debris and restarting plant cycles" (2006,p.72). These cycles influence organism succession. In clear-cut forests, mycorrhizal communities die back once the trees are killed. To stimulate habitat recovery and decomposition, scientists must introduce keystone species of mushrooms -such as the first species to feed on dead wood: saprophytic fungi.

The most important notion is that saprophytic fungi are not the same as mycorrhizal. Saprophytic fungi are primary, secondary, or tertiary in their ability to break down wood and are usually found above ground, intermingling, and depending on one another -with regard to their successional stages (Stamets, 2006, p.19). Without mycorrhizal fungi to start the recycling process, there would be no saprophytic fungi on top of wood chips or for any other means of mycoforestry or restoration. Thus, recycling is performed by many types of fungi in forests because each type of fungus has a different function and ability on the forest floor -such as saprophytes breaking down large molecules and reassembling them into polysaccharides. Saprophytes that "…take advantage of dying trees are termed facultative parasites" and are usually found "…on dying cottonwood, oak, poplar, birch, maple, and alder trees" (e.g. oyster mushrooms or Pleurotusostreatus) (Stamets, 2006,p.23). Some parasitic fungi, like the honey mushroom, can destroy miles of forests, and are usually known as blights; when blights arrive, nurse logs occur, soil depth increases, and richer habitats result (Stamets, 2006,p.23).Moreover, some species can be both saprophytic and parasitic by changing their nature depending on the degradation of the host. Thus the forests and fungi change depending on the needs of their components and knowing these processes guide ecologists in mycoforestry efforts.

Traditional forestry involves burning debris and clear-cutting land in order to limit fuel for fires in the future and removing obstructive brush. Introducing mycorrhizae with a top layer of woodchips, for delayed nutrients and moisture retention help reestablish forests for future generations. More specifically, 1 gallon of wood chips (4 pounds) per tree will usually enhance moisture retention and cool the soil, as well as "… slow the streaming of nutrients to the root zone as saprophytic fungi decompose the wood chips"(Stamets, 2006,p.77). One foot deep wood chip beds decompose into one to two inches of rich soil in 2-3 years when they are inoculated with mycelium (Stamets, 2006,p.77). Adding saprophytic and mycorrhizal spores to oil to lubricate the teeth of chainsaws or wood chipping mechanisms is recommended. Any remaining wood chips can be used to reduce silt flow and erosion in depressions near roads. This process is very useful for mycoforestry success and can be utilized to revegetate land where impoverished people reside -providing basic resources to those forced into deforestation.

Furthermore, wood debris does not fuel fires if mycelium is applied (the risk may be increased initially but reduced later due to moisture retention). Fungal decomposition of trees releases cool carbon dioxide slowly over the years. "Plant cells absorb carbon dioxide and use it as raw material for creating cellulose, lignin, carbohydrates, proteins, sterols, and outgas oxygen" (Stamets, 2006,p. 80). This slow decomposition of the fungi buffers carbon dioxide emission and creates cycles that put much of the gas right back into the ecosystem. This benefits the plants -especially in harsh or polluted atmospheres. They are then less reliant on outside sources for their nutrients. Therefore creating more nutrients for greater periods of time through natural mycoforestry processes that aid in long term soil and water retention are more beneficial, than periodic burning. Other benefits of leaving wood chips on the forest floor include: "… supporting mycofiltration membranes that reduce erosion and siltation, providing cavity habitats for diverse populations of bacteria, fungi, plants, insects, and animals;"this process also provides"…protection against forest fires (via long term moisture laden wood chips), and substrates for decommissioned logging roads" (Stamets, 2006,p.81). Hence, the benefits of mycoforestry are endless.

After logging, the mycosphere lives underground, so we must spread fungi amongst wood debris or wood chips immediately after cutting. Mycelia reach toward the wood to form bonds and "…whether the wood is whole or fragmented affects the rate at which nutrients return to the soil; wood chips of varying sizes are quickly consumed by fungal mycelium"(Stamets, 2006,p.89). This decreases the need for fertilizers, pesticides, and herbicides-thus saving our hurting economy financial resources. However, if the wood chips are too deep (more than 1 foot) or too finely chipped (smaller than 1/8 of an inch), it can smother fungi and anaerobic organisms may thrive. Using a Hydro-Ax will thin forests and chip wood on site, while reducing fuel costs (usually required for transporting wood from a thinning site). A wood chipper or chip blower can be used to spread spawn while making a layer of sheet mulch, and this leads to faster colonization. Another method is to rake the spawn or lay it on the polluted site (1 to 1.5 feet deep). After the residual levels decline, trees can be planted with mycorrhizal infusions and this steers the site on a path toward healing itself. Stamets observed an 8 percent increase in height and girth of 700 trees inoculated with mycorrhizal spores over just 10 months (2006, p.78). Therefore, using mycorrhizae to replenish soils and aid in tree growth is less expensive, more successful, and more natural than traditional methods of forest regeneration.

One school of thought believes the root zone of a tree should be pasteurized soils and then inoculated with micorrhizae. Another school believes that seeds (spores dusted over the forest floor) should be connected prior to germination. This is because "…fungal propagules including spores and hyphae do not remain viable for long periods" due to "…predation, germination under unsuitable conditions, and weathering" (Amaranthus, 1998,p.7). Both methods are more successful than the "do-nothing" approach and Stamets' measurements for inoculation of mycorrhizae and dusting wood with spores while chipping on-site have proved to be greatly successful -in regard to mycoforestry efforts. Straight recommends that buffers like wood chips lessen the impact of nitrates, pesticides, and hydrocarbons, in order to control pollution vectors channeling from roads, ditches, and other sources (2001). These barriers would also help to preserve the livelihood of mycorrhizae - if chemical leaching and anthropogenic changes to the landscape were of no concern. All of these processes will aid in forest health and recovery from both manmade and natural disasters (including pollutants) without excessive costs or new adaptation requirements.

Combining Regreen, a non-seeding wheat approved for erosion control, with a broadcast seeder stocked with 1 pound of mycogrow (a mycorrhizal inoculum available via www.fungiperfecti.com-usually 'early stage' fungi is used), with native grasses over commercial varieties, handfuls of oyster mushroom spawn (Pleurotusostreatus) on top of woodchips, bark, and fir needles will aid in micoforestry efforts that prevent and remedy forest lands. The first stages of restoration will occur in one week with seeds sprouting. The few species available on the market for mycorrhizal spore inoculum are based on 1.) the method required for collection (separation prior to rot)2.) economic feasibility of extraction and 3.) the market value of mushrooms. (Stamets, 2006,p.75). Therefore, gaining access to different types of mychorrizae for restoration purposes can be difficult depending on the required native match.

Mycoremediation is another form of mycorestoration. Mycoremediation is the use of fungi to degrade or remove toxins from the environment by removing heavy metals from the land and channeling them into the fruiting bodies of mushrooms for removal (Stamets, 2006,p.86). This method is useful because mushrooms can be hyper accumulators and intensify the amount of toxins in their fruiting bodies. Mycoremediation can be achieved by mixing mycelium into contaminated soil (oil or toxic waste spills), placing mycelial mats over toxic sites, a combination of these techniques, by a single inoculation or successive treatments. This too can be applied to forests because not all oil spills and toxic waste spills occur in aquatic environments. If these spills are to occur on (or near) land, plants are destined to be harmed, and mycoremediation methods could be utilized in restoration processes.

Oyster mushrooms (white rots) are being seen as a keystone species because of their adaptability and vigor -regardless of climate. Introducing oyster mushrooms (from a mushroom farm -not pure culture spores) is extremely helpful. White rots are unique for creating manganese dependent peroxidase, an enzyme that mineralizes wood and is particularly efficient in breaking hydrogen-carbon bonds -and the primary nonsolid byproducts are water (50 percent of mass cleave) and carbon dioxide (10 to 20 percent)(Stamets, 2006,p.92). This results in a maturing shrinking waste pile.

Many mycelium bonds and their abilities to dismantle bonds that hold plants together are similar to that of petroleum products, such as oil, diesel, herbicides, and pesticides. More microbes will grow on straw as oyster mycelium ages and bacteria create their own toxin-ingesting enzymes. According to Chui, Eggen, and Sasek, spent mushroom substrate (compost) performs better than mushroom mycelia when attempting to remove biocide penatachlorophenol (PHA toxins), because it has a higher degradative capacity (Chui et al., 1998,p.; Eggen and Sasek, 2002,). Hence many experiments for restoring polluted lands using mushrooms as the remedy have taken place. Some are successful, while others are not and at times, the skills of the mycologist may be equally important to the method used for forest ecosystem restoration.

Regarding cycles, as mushrooms mature, spores are released, and this creates more mycelium, which colonizes the substrate, and as the mycelium infiltrates the wood, more moisture is retained. New mushrooms draw insects and rotting mushrooms become grounds for breeding larvae and grubs, which then attract lizards, birds, and animals. As wheat grass dies and wood chips decompose- new soil is created (assisting other species). "For every 12 inches of wood chips, we estimate that 1 to 2 inches of soil are created after 4 years of decomposition by oyster mushrooms"(Stamets, 2006,p.85). After two or more likely three years, gravelly sandy soils will connect a mantle of newly formed mycelium and this sheath overlay will begin to hold the gravel together by adding structural resilience . This management strategy assists in forest sustainability and erosion prevention. Consequently, spore inoculation leads to natural breakdowns in toxic waste and oil spills that remedy the land back to its natural state -all through natural processes.

Amaranthus and Molina (1989 & 1991) "stress that although soil microorganisms are adapted to disturbance, those adaptations fall within the limits of natural phenomena and intensity" (Molina, 1994,p.36-52). Subsequently, they suggest "…strategies that minimize disturbance to soil structure, maintain organic matter and woody debris, and promote rapid recovery of vegetation" (Molina 1994). Furthermore, of all the information studied, each mycologist agrees that "…the lack of convincing data underlines the need for creative, careful experimental manipulations" and understanding these associations could have great benefits for agriculture and natural ecosystems (such as additional mycorestoration efforts) in the future (He et al,. 2009,p.531-567).

## Conclusion

The evolution of this symbiotic association (fungi and host) has been suggested to be the most significant step that led to the colonization of the terrestrial environment by living things dating back -via fossil spore records, nearly 460 million years ago(Pyronzinski and Mallock, 1975,p.153-164). Today, fungi are important economically and commercially because they create yeasts for wine and beer -and antibiotics like penicillin (Penicilliumchrysogenum -discovered by Fleming). They are gathered as food for their edible bodies and "…for production of metabolites in an emerging biotechnical industry" (Amaranthus, 1998, p. 4). Fungal metabolites are byproducts (sought after by the pharmaceutical industry) used in the agrochemical industry, fine chemical, and in industrial services ranging from oil recovery to effluent treatment to bioplastics and biocontrol (Amaranthus, 1998,p. 4).Fungi are also socially valuable for those that study them as a recreational pastime. Exploration of fungi for medicinal purposes has yet to be entirely investigated -thus providing greater reason for forest habitat preservation and micorestoration techniques. Secondary products (sustaining profits), climate issues, and deforestation also provide strong economic incentive to restore or preserve forests. Therefore, short term economic gains do not exceed natural capital, because the values of the aforementioned natural and societal components correlate with milestones and enormity of knowledge as humans progress. In short, the more we discover about forest fungi, the more useful they become to human populations -as well as to environmental regenerative purposes.

These methods can be applied to help reduce hunger and poverty worldwide via soil renewal and revegetation for food purposes and contribute to the "Green Revolution." "Unlike typical fertilizers that farmers must apply every few weeks, one application of mushroom fungi lasts all year and costs just pennies per plant"(Miller, 2004,p.225). Therefore, fungi are imperative to natural and anthropogenic cycles in numerous ways. Our hurdles to date are patents and legal restrictions on science that could make our world a better place. The key is using science to combat nature and the effects of human actions, without worrying about who owns which method and how much compensation they should receive.

As mentioned, forest mycorrhizae have an important role in soil fertility, nutrient cycling, and ecosystem productivity.

Without fungi, the earth would soon be buried under a thick covering of dead animal and plant remains (Wilkins, 1988,p 126-127). Ultimately, all ecosystems would fail without fungi and thus without fungi, there would be no forests -and without $CO^2$ emitted from trees, there would be no way to sustain human life on Earth. Mycological restoration efforts should be the main focus of frontier science; since reactionary policy, rather than preventative policy, dominates activities within the United States and many other nations (a.k.a. there needs to be a mycological cleanup crew to restore the land). Growth based economies such as ours will always lead to further exploitation of natural resources, such as deforestation which inhibits natural recycling patterns. And since timber is regenerated on a longer time frame than the average human life-span, it is unlikely that conservation and preservation efforts will be successful (compared to current degradation rates). Increasing the soil supply to sustain mycorrhizae, and ultimately forests, will have a chance at resolving the issues we have created relating to our need to produce secondary products and coexist with nature in generations to come.

Photographer: Mr. David Wilson

Forest Fungus

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