EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 5/ August 2016

ISSN 2286-4822
www.euacademic.org

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

Ectomycorrhizae: Activity and Growth

SUMIRA TYUB[1]
AZRA N KAMILI
Centre of Research for Development, University of Kashmir
Department of Environmental Science, University of Kashmir
ZAFAR A RESHI
SYED SANA MEARAJ
Department of Botany, University of Kashmir
TASEEM A MOKHDOMI
SHOIAB BUKHARI
ASRAR H WAFAI
ASIF AMIN
RAIES A QADRI
Department of Biotechnology, University of Kashmir

Abstract:

Ectomycorrhizal associations (ECM, or EM) are mutualistic associations between higher fungi and Gymnosperms or Angiosperms. ECM associations consist of a soil mycelium system, linking mycorrhizal roots and storage or reproductive structures. Ectomycorrhizal roots (formerly known as ectotrophic or sheathing mycorrhizas) are characterized by the presence of a mantle and Hartig net. Ectomycorrhizal (ECM) associations involve the most diverse category of myocrrhizae. The diversity derives from the fungal partners; more than 5,000 species of fungi, mainly Basidiomycetes, Ascomycetes and a few Zygomycetes. On the contrary relatively few families of plants such as Fagaceae, Pinaceae, Betulaceae, and Dipterocarpaceae are involved in the ECM associations. These plants, however, are distributed over wide areas of temperate and boreal forests, and are therefore economically important. ECM makes a significant contribution to forest ecosystems by increasing biomass and

---

[1] Corresponding author: tyubsumira@gmail.com

creating a network among trees through which nutrients are transported. ECM fungi also improve the growth of host plants at the seedling stage.

Key words: Ectomycorhizae, Morphology, Sporocarp, Nutrient acquisition.

INTRODUCTION

Most land plants form associations with mycorrhizal fungi. Mycorrhizae are mutualistic associations between fungi and plant roots (Dell, 2002; Brundrett, 2009) and regulate elemental and energy flows in terrestrial ecosystems (Allen *et al.*, 2003). This interaction occurs in and on the root tips involving a bidirectional exchange of nutrients (Smith and Read, 1997). In mycorrhizal associations, the term symbiosis is often used to describe highly interdependent mutualistic relationships where the host plant receives mineral nutrients while the fungus obtains photosynthetically derived carbon compounds (Harley, 1989; Harley and Smith, 1983). They are described as symbiotic because the fungus receives photosynthetically derived carbon compounds and the plant has increased access to mineral nutrients and sometimes water. Mycorrhizal associations predominate in most natural terrestrial ecosystems (Brundrett, 1991). Whereas the AM fungi are widespread geographically and have a very extensive host range, the ECM fungi are more restricted, forming associations predominantly with genera of important woody plants. Nevertheless, ECM fungi are dominant components of the ground-dwelling macro-fungi in ecosystems where members of the following plant families abound: Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, Pinaceae, Ulmaceae, Salicaceae (Dell, 2002). Because of their ability to acquire water and nutrients at low concentrations in the soil, ECM have been cited as having been crucial for the colonization of land by plants (Cairney, 2000; Blackwell, 2000; Brundrett, 2002: Pirozynski and Malloch, 1975). They are considered to be of importance in nutrient stressed infertile environments (Gao and Yang, 2010), hence believed to be vital in restoration of degraded forest ecosystems.

CLASSIFICATION OF MYCORRHIZAE:

Mycorrhizae are multifaceted associations comprising diverse morphological, functional and evolutionary categories (Smith and Read, 1997; Brundrett, 2002). Some types of mycorrhizae are similar and share plant lineages while others have highly distinct anatomical features and separate evolutionary histories (Brundrett, 2002). Frank (1885) distinguished two main types of mycorrhiza: ectotrophic and endotrophic. Early morphological classifications separated mycorrhizas into endomycorrhizal, ectomycorrhizal and ectendomycorrhizal associations based on the relative location of fungi in roots (Peyronel *et al.*, 1969).

i) Ectomycorrhizae, where absorbing organ is entirely surrounded by a well developed compact mantle of fungal material from which hyphae arise that pass into the absorbing organ and grow between the absorbing cells.

ii) Endomycorrhizae, where the hyphae external to absorbing organ are not aggregated by any extent, but inter and intracellular penetration by hyphae is characteristic

iii) Ectendomycorrhizae which have an external mantle of some sort although it may not be always be very well developed and the hyphae within the host penetrate its cells as well as grow between them.

On the basis of morphological characteristics, mycorrhizal types can be divided into seven main groups namely arbuscular mycorrhiza, arbutoid mycorrhiza, ectendomycorrhiza, ectomycorrhizae, ericoid mycorrhiza, monotropoid mycorrhiza and orchid mycorrhiza (Harley and Smith, 1983; Brundrett, 2004 and Finlay, 2008). The mycorrhizal types contribute differentially to functional traits of plant carbon (C) cycling (Cornelissen *et al.*, 2001).

2. ECTOMYCORRHIZAE (ECM):

Ectomycorrhizae are the dominating mycorrhizal type in temperate and boreal forests as 80%–90% trees in temperate and boreal forest ecosystems live in symbiosis with ectomycorrhizal (ECM) fungi (Read, 1992; Valtanen, 2012; Deckmyn *et al.*, 2014). Fossil records of ECM originate from 50-52 million years ago (Beimforde *et al.,* 2011, LePage *et al.* 1997), but presumably ECM evolved together with gymnosperms and exist since 190 million years (Taylor *et al.,* 2009). ECM associations are formed by estimated 7,000-10,000 fungal taxa and by 8,000 plant taxa (Taylor and Alexander, 2005). Even though only a small fraction of terrestrial plants form ECM symbioses, they include numerous ecologically and economically important tree species (Taylor and Alexander, 2005).

In the terrestrial ecosystems ectomycorrhizal association is one of the most ecologically important symbiotic associations (Smith and Read, 1997; Cairney and Chambers, 1999; Rinaldi *et al.*, 2008). This association is supposed to be valuable for alleviating nutritional stress for both plants and fungi (Gao and Yang, 2010). Ectomycorrhizal fungi cover the finest root branches of the tree and provide nutrients from the soil in exchange for phytosynthetically derived carbon (Tedersoo *et al.*, 2012). These symbionts contribute up to 39% of microbial biomass and 10–35% of respiration in boreal forest soils (Smith and Read, 2008; Hogberg *et al.,* 2010). The partners in this association are members of the fungus kingdom (Zygomycetes, Ascomycetes and Basidiomycetes, but not protistan fungi such as Oomycetes) and most vascular plants both gymnosperms (Pinaceae, Cupressaceae) and angiosperms (Juglandaceae, Fagaceae, Betulaceae, Tiliaceae, Salicaceae, Ulmaceae, Rosaceae, Leguminosae, Sapindacea, Aceraceae, Myrtaceae, Ericaceae and Dipterocarpaceae) (Harley and Smith, 1983; Newman and Reddell, 1987; Smith and Read, 1997; Brundrett, 2009). The fungus partner mainly belongs to higher fungi, which produce mushrooms and puffballs (Trappe, 1977). Fungal members of the Basidiomycota dominate this type of mycorrhiza (45 genera) with members of Ascomycota occasionally forming ectomycorrhizas (18 genera), and Zygomycota very rarely forming ectomycorrhizas (members of the genus *Endogone*) (Carlile *et al.*, 2001, Brundrett *et al.*, 1996, Smith and Read, 1997). Genera such as *Amanita*, *Boletus*, *Cantharellus*, *Cortinarius*, *Inocybe*, *Laccaria*, *Lactarius*, *Rhizopogon*, *Russula*, *Suillu*s and *Xerocomus* are common and well recognized ectomycorrhizal (ECM) Basidiomycota genera (Cairney and Chambers, 1999). ECM fungi are considered to form more species specific associations as compared to AM fungi which mostly are generalists. However, some ECM fungal species associate with a wide range of hosts, for example *Pisolithus* spp. that associate with 20 confirmed host plant genera (Cairney and Chambers, 1999), as opposed to members of the genus *Suillus* that associate exclusively with plant hosts within the family Pinaceae (Dahlberg and Finlay, 1999). ECM fungi generally, but not without exception, associate with perennial woody tree species belonging to plant genera that commonly include: *Abies*, *Betula*, *Fagus*, *Picea*, *Pinus*, *Populus*, *Quercus* and *Salix*, (Europe and America), and *Acacia*, *Eucalyptus*, *Nothofagus* in Australia (Brundrett *et al.*, 1996), and members of Caesalpiniaceae and Dipterocarpaceae in Southern Africa (Hogberg, 1986). Many of the plant species naturally found in the Northern Hemisphere are planted in managed forest plantations in the Southern Hemisphere, such as *Pinus*. Although ECM plant host diversity appears to be limited, plant hosts from 130 genera in 43 plant families have been recorded (Carlile *et al.*, 2001).

2.1 Morphology of ECM

Morphologically, ectomycorrhizae have been characterized by three structural traits: a mantle of fungal tissue that encases the root tip, secondly, a net of hyphae penetrating the root and thereby surrounding epidermal and cortical root cells, which is called the Hartig's net, and thirdly, a system of emanating hyphae, the extra-radical mycelium. This extra radical mycelium is the collection of filamentous hydrophilic fungal hyphae emananting from ectomycorrhizae and act as the direct connection between the symbiotic plant and the microsites in the soil environment (Bodeker, 2012).

The early phase of interaction between the roots and ectomycorrhizal fungi prior to symbiosis establishment is accompanied by stimulation of lateral root development in the host (Felten *et al.*, 2009). Infected short roots become swollen and variously coloured due to the colour of fungal symbionts. Each such short root, regardless of branching pattern is an ectomycorrhiza. The formation and functioning of ectomycorrhizas lead to complex morphological and physiological changes in both the plant and the fungus (Martin and Nehls, 2009; Courty *et al.*, 2010). Root hairs probably play a role during the initial stages of ectomycorrhizal development due to their sensitivity to diffusible factors released by the symbiotic fungi and act as a preferential anchorage site. Root hairs respond to the fungal presence with nuclear movements, although fungal penetration most often occurs through atrichoblasts or occasionally at the base of trichoblasts.

Upon ectomycorrhizal colonization a fungal sheath called the mantle covers the short roots of the host tree. To maximize the contact between the plant and the fungi, hyphae colonize the intercellular space between cortical root cells, by excreting pectinases forming the Hartig net (Moore, 2011, Marx and Beatie, 1977; Kottke and Oberwinkler, 1987). From the mantle, hyphae extend out into the surrounding substrate. The mantle of fungal tissue surrounding the host lateral roots varies from pseudoparenchymatous tissue to a weft arrangement of hyphae on the root surface (Graham and Miller, 2005). Development of the mantle proceeds through a programmed series of events (Martin *et al.*, 1997). Fungal hyphae, originating from a soil propagule or an existing mycorrhizal root tip, penetrate into the root cap cells and grow between them. The root cap tissue is progressively transformed by intercellular colonization into the inner layers of the mantle. A high level of structural and physiological heterogeneity exists within the mantle and between the mantle and the fungal networks (Cairney and Burke, 1996).

The mycorrhizal short root is the functional unit of the symbiosis where exchange of nutrients, carbon and water between the symbiotic partners takes place (Smith and Read, 1997). Saprotrophic and mycorrhizal fungi are not separate groups from an evolutionary perspective, indicating that the ability of fungi to form symbiotic associations with plants is a life strategy that has appeared from ancestral saprotrophic life strategies several times during evolutionary history (Hibbet *et al.*, 1997). The mycorrhizal strategy to derive carbon from its living host releases them from competition with other soil fungi for deriving carbon from sources of dead organic material (Rosling *et al.*, 2003).

2.2 Sporocarp formation and sporulation

Sporocarp formation is the sexual reproductive stage of a fungus, the function of which is spore dispersal of the species. Sporocarps are the result of two monokaryon hyphae that meet to form dikaryon hyphae with a pair of sexually compatible nuclei. The cell is now technically diploid, but contains two haploid nuclei (Smith and Read, 1997). Occasionally, the hyphae may contain nuclei from more than two individuals and are then termed polypoid (Carlile *et al.*, 2001). Compatibility of strains and nuclear migration are controlled too by unlinked genetic factors, A and B. Factor A encodes transcription factors that regulate the development of the dikaryotic mycelium. Factor B is thought to encode for pheromones and receptors that control interactions between the nuclei of the two strains (Smith and Read, 1997). Dikaryotic hyphae then either form ECM associations or sporocarps (Smith and Read, 1997). In case of the latter, the hyphae elongate up towards the soil surface. A specialized hyphal layer called the hymenium undergoes meiosis to produce spores (Carlile *et al.*, 2001). The hymenial hyphae may be exposed or enclosed and are known as basidia, asci or sporangia, according to the ontogeny of the spore (Carlile *et al.*, 2001) and are borne in corresponding sporocarps known as basioma and ascoma. The position of the hymenial layer may be exposed, forming along gills, within pores or along spines, or enclosed within the sporocarp. According to Hibbett *et al.*, (1997) gilled sporocarp morphology has evolved at least six times, and enclosed ball-like morphology, at least four times, based on the sequencing and phylogenetic analysis of nuclear and ribosomal DNA. Sporocarp morphology is therefore not monophyletic, i.e. descended from a single origin. The fruiting bodies of Basidiomycota ECM, known as sporocarps (or basidioma), are often presented with a stipe, pileus (cap), and lamellae (gills) or pores, although the variety of sporocarp morphology is large. Fruiting bodies develop only from the secondary mycelia, initially forming a primordium, after which, the hyphae undergo elongation, extending a stipe to a suitable position to release spores borne on the fertile hymenium (Kendrick, 1992). The hyphae in the secondary mycelium characteristically have clamp connections, which are necessary to maintain the dikaryotic state of the mycelium. The spores develop on basidia, which develop four protrusions, known as sterigmata, the tip of which swells. The nuclei undergo fusion and meiosis and the four resulting haploid nuclei move into the four sterigmata. The tip of the sterigmata is then separated by a cell wall from the basidium, forming a spore and is dispersed actively using a surface-tension catapult mechanism (Carlile *et al.*, 2001).

2.3 Nutrient acquisition and assimilation

ECM fungi have been shown to absorb and exchange a number of nutrients with their host plants (Cairney, 2011; Plassard and Dell, 2010). Marschner and Dell (1994) summarized that phosphorus (P), ammonium (NH_4^+), nitrate (NO_3^-) and potassium (K^+) are involved in fungus-plant transfers, but probably the most important and the most widely investigated, is the uptake and transfer of P. Because of the high uptake efficiency of mineral P and the low mobility in the soil in a forest, P is generally derived from organic compounds that are less available as a source of P to plants (Deckmyn *et al.,* 2014). Extracellular enzymes released by fungal hyphae tips degrade organically bound compounds. Organic P compounds, often in the form inositol phosphate, are degraded by extracellular phosphatases and organic acids, such as oxalic acid, that release P from organic compounds (Marschner and Dell, 1994). The P is then assimilated as polyphosphate, which reduces the P concentration within the hyphae.

Similarly, extracellular acid proteinases are involved in the breakdown of organic nitrogen (N) compounds (Finlay *et al.*,

1992) such as amino acids and proteins. In general, plant N uptake is improved by association with ECM fungi through four possible mechanisms: (i) efficiency of the uptake mechanisms, (ii) exploration of bigger soil volume, (iii) uptake of organic forms (e.g., amino acids), and (iv) release of nutrients from nonplant available sources (e.g., complex organic matter) (Plassard, *et al.*, 1991; Deckmyn *et al.,* 2014).

Although it is assumed that ectomycorrhizal fungi have some capacity to degrade complex organic compounds (Smith and Read, 2008; Durall *et al.*, 1994; Hobbie and Hobbie, 2006; Rineau *et al.,* 2012), the majority of species seem to rely on the host plants' C supplies to a very high degree (Nehls, 2008; Courty *et al.,* 2010).The litter layer is a rich source of organic nutrients, which fungi break down, assimilate and transfer to the plant. Complex carbon compounds, such as lignin and cellulose, present in soil are primarily found in the litter and organic layers. ECM has a limited ability to degrade and use these complex carbon compounds and it is therefore assumed that ECM could only make use of simple carbohydrates released into the soil in the form of exudates from plants and micro-organisms (Melin, 1925; Smith and Read, 1997). Cairney and Burke (1994) emphasize that, although evidence of extracellular endo- and exoglucanase has been documented from *Pisolithus tinctorius*, the level of cellulytic enzyme activity is much lower than some saprotrophic wood decomposers, e.g. *Trichoderma reesei*. Hydrolytic activity of carbon compounds, from complex starch and cellulose to more simple sugars, such as glucose and fructose, varies within and between species and it is thought that this enzyme activity is functional only at the colonization stage, where plant cell wall softening is needed for hyphal penetration (Cairney and Burke, 1994).

Most ECM fungi can assimilate forms of complex inorganic P and N, such as phosphates, ammonium and nitrates. Oxalic acid produced by the fungus is a strong solubilizer of mineral phosphates, for instance calcium phosphate that can then readily be assimilated (Zohlen and Tyler, 2004). Mineral nitrogen, in the form of ammonium and nitrate, is available for uptake in the soil. Martin *et al.*, (1986) suggested that fungi utilize a combination of glutamate dehydrogenase and glutamine synthetase pathways to assimilate N. The N is then transported to the plant in the form of glutamine or glutamate. Finlay *et al.*, (1988) used ^{15}N-labelled ammonium to follow the N uptake and assimilation by *Rhizopogon roseolus*, *Suillus bovinus*, *Pisolithus tinctorius* and *Paxillus involutus* associated with *Pinus sylvestris*. They found that glutamine / glutamic acid, alanine and aspartate / asparagines are important nitrogen sinks and that amino acids were used for N storage. Also, Finlay *et al.* (1988) supplied ^{15}N-labelled ammonium and nitrate to a *Paxillus involutus*/*Fagus sylvatica* symbiosis. N was incorporated into a wide range of free amino acids and the study, once again, identified Glutamic acid, Aspartic acid and Alanine as important N sinks. All fungi are known to readily assimilate ammonium but studies show that nitrate is less readily mobilized. Utilizing different nitrogen sources as substrates for three strains of *Cantharellus cibarius*, Rangel- Castro *et al.* (2002) experimentally determined that nitrate is assimilated less readily than ammonium and that there exists a variation between the strains in their ability to assimilate the different nitrogen sources. Similarly, Finlay *et al.* (1988) showed that although assimilation of nitrate was lower than ammonium, there was significant uptake by the fungus. It has also been reported that ECM readily utilize inorganic than organic nutrients (Smith and Read, 1997).

2.4. Factors affecting ECM fungal growth

2.4.1 ECM growth, species composition and diversity

Although seasonal moisture and temperature changes affect fungal growth, soil pH is the major determining factor influencing fungal growth and fungal species diversity. Culture experiments on fungal ECM species show sensitivity to a pH of 7 (Hung and Trappe, 1983). Generally ECM fungi are acidophilic (pH 3-5) but optimal pH ranges differ between and within species. In contrast to cultural conditions Erland *et al.* (1990) showed that effect of pH dependant was not evident when *Piloderma croceum* was associated with plant hosts. The study demonstrated that although the optimum pH growth range of *P. croceum* occurred at pH 4-5 in culture, an extensive hyphal network developed on peat, buffered at pH 7.3. Even though the mycelial network was not as dense as at pH 3.8, there was still significant growth. Therefore, in vitro growth experiments may not accurately reflect in vivo processes (Erland *et al.*, 1990).

At the international level, mycorrhizal research has been undertaken for decades and has contributed to the understanding of physiological function and factors that affect fungal behaviour. Of particular interest are the effects of pollution, liming, acid rain and fertilization of forests on the diversity of ECM fungi, as these factors change the soil nutrient composition (Finlay, 1995). Many studies produced contrasting community structure results in response to the afore-mentioned disturbances. Lilleskov *et al.*, (2002) investigated an area comprising a gradient from high ammonia deposition to low deposition and found that species richness decreased from 29 species in the low N to 7 species in the high N sites. In addition to decrease in ECM fungal diversity, a shift in dominant species from *Piloderma byssinum*, *Amphinema byssoides*, *Thelephoroid*, *Tricholoma inamoenum* and *Lactarius theiogalus* in the low N sites to *Paxillus involutus*, *Lactarius theiogalus*, *Basidiomycete*, *Tomentella sublilacina* and *Cenococcum geophilum* in the high N sites was also found. The enhanced nutrient concentrations in the soil resulting from intensive stock farming, industrial and vehicular emissions (acid rain) and managed plantations result in decreased pH and solubility of nitrogen and phosphorus compounds and increased solubility of aluminium (Arnolds, 1988).

In managed plantations a number of disturbance factors may affect ECM abundance and species composition. Mahmood *et al.*, (1999) found that repeated harvesting of forest residues lead to decrease in ECM fungal colonization from 295 roots/metre root lengths to 181 ECM roots per metre root length. Clear-felling and slash burning not only change the litter dynamics, but have also been shown to remove some ECM fungal species (Bruns *et al.*, 2002) especially from the upper soil layer. The fertilization of managed plantations to enhance plant growth including the addition of nutrient supplements such as nitrogen (N), phosphorus (P) and potassium (K) affected ECM fungal populations. These studies have concentrated on short-term effects, but lack sufficient information regarding long-term effects (Erland and Taylor, 2002). Erland and Soderstrom (1990) studied the effects of pH on ECM communities and reported an increase in root colonization of up to 100% at pH 5, but colonization decreased to 40% at pH 7. Chalot *et al.* (1995) reported that amino acid uptake, including alanine and aspartate of the ECM fungus, *Paxillus involutus*, is reduced at pH 4.5 to 6.4 and concluded that this may have consequences regarding the ability of the fungus to colonize host plants.

Nutrient addition of P has a direct effect on ECM fungi and inhibits both mycelia growth and effective colonization of host plant roots. Treseder (2004) conducted a meta-analysis study in a range of biomes and recorded a 32% decline of mycorrhizal abundance resulting from P fertilization. The application of N fertilizers also have a considerable short-term effect on both the species richness and abundance, although some studies have shown that instead of decrease there is a shift in species composition. Fransson *et al.*, (2000) studied the ECM root tip community in a Norway spruce forest fertilized with ammonium and nitrate. The results indicated that there was no overall effect of N fertilizer on the species richness or abundance, but change in the relative abundance of dominant species occurred.

There is considerable variation between different ECM fungal species in their ability to breakdown, assimilate and transfer nutrients to the host plant. Therefore, shifts in the species diversity could have direct consequences on host plant nutrition. The function of individual ECM fungal species in the field is largely unknown, hence the effect that shifts in ECM fungal communities have on plant nutrition is uncertain (Erland and Taylor, 2002).

2.4.2 Sporocarp formation

Factors that affect sporocarp production include a number of environmental factors such as nutrient availability, light, moisture and temperature. Natural gradients of rainfall correlated positively with ECM sporocarp biomass and species richness in a study conducted by O'Dell *et al.* (1999). The production of large sporocarps, such as those produced by ECM fungi, requires a large nutrient input (Kendrick, 1992). Fungi that produce these sporocarps normally have a large mycelial network accessing nutrients, in particular carbon, needed for the production of the spores (Carlile *et al.*, 2001). ECM fungi primarily rely on host plant carbon products to meet the energy requirements for sporulation. The dependence of fungi on host-plant carbon to produce sporocarps was demonstrated by Hogberg *et al.* (2001). The study involved the tree girdling of *Pinus sylvestris*, inhibiting carbon allocation to the roots. This resulted in a decrease of species richness from 11 species in the control plots to one in the early girdled plots and a reduction in abundance from 252 sporocarps in the control plots to four in the early girdled plots. Light, particularly in the blue and UV spectrum, may have an effect on some fungi (Kendrick, 1992), by influencing the development of phototropic sporocarps. Light may determine the position of the sporocarp and the release of the spores. Some fungi show no response to light and will sporulate in darkness (Carlile *et al.*, 2001).

Added to these factors are those of nutrient additions to the soil through fertilization treatments, acid rain pollution and liming that may have positive or negative effects on species abundance and richness (Smith and Read, 1997). It is has been suggested that the response of sporocarp production to fertilization techniques, particularly N, is more acute than that of ECM roots in terms of species diversity and abundance (Wallanda and Kottke, 1998). Lilleskov *et al.*, (2001) noted a large decrease in species diversity from 144 to 14 in response to increased N application and Pampolina *et al.* (2002) recorded a 14% reduction in sporulation in response to high P application. Wallanda and Kottke (1998) summarized that N applications may have considerable effects, usually a reduction of sensitive species, on both species richness and abundance, although some generalist ECM fungal species may be unaffected.

On a more localized scale ECM fungal species composition and diversity is driven by biotic factors such as host plant species composition and abiotic factors such as season and soil quality and composition (Smith and Read, 1997). Additionally, a theory of successional dynamics also suggests that stand age may also determine the composition of ECM fungal species present (Smith *et al.*, 2002, Bigg, 2000, Jumpponen *et al.*, 2002). Tedersoo *et al.* (2012) used meta-analysis to disentangle the global determinants of diversity and community composition for ectomycorrhizal fungi and revealed that richness of these root symbionts has a unimodal relation both with temperature and latitude. Fungal richness peaks at temperate and boreal latitudes, but there is large variation among sites indicating the importance of habitat heterogeneity and local processes. The low richness in subalpine environments of temperate latitudes shows that temperature rather than distance from the equator per se have the strongest impact on ECM fungal richness (Rahbek, 2004). In addition, both the subarctic and lowland tropical environments have also lower phylogenetic diversity, that is, fewer lineages of EcM fungi (Bjorbækmo *et al.* 2010; Tedersoo and Nara, 2009), indicating that environmental filtering may occur at both ends of the temperature gradient and it operates at both species and higher taxonomic levels.

REFERENCES:

1. Allen, M. F., Swenson, W., Querejeta, J. I., Egerton-Warburton, L. M., & Treseder, K. K. (2003). Ecology of mycorrhizae : A conceptual framework for complex interactions among plants and fungi. Annual Review of Phytopathology, 41(1), 271–303. doi:10.1146/annurev.phyto.41.052002.095518
2. Arnolds, E. (1988). The changing macromycete flora in the Netherlands. Transactions of the British mvcological Society 90, 391-406.
3. Beimforde, C., Schäfer, N., Dörfelt, H., Nascimbene, P. C., Singh, H., Heinrichs, J., Reitner, J., Rana, R.S. & Schmidt, A. R. (2011). Ectomycorrhizas from a Lower eocene angiosperm forest. New Phytologist, 192(4), 988–996. doi:10.1111/j.1469-8137.2011.03868.x
4. Bigg, W.L. (2000). Fungal succession and diversity in ectomycorrhizal associations: A Case Study Approach USDA Forest Service Gen. Tech. Rep. PSW-GTR,178.

5. Bjorbækmo, M., Carlsen, T., Brysting, A., Vrålstad, T., Høiland, K., Ugland, K., Geml, J., Schumacher, T., & Kauserud, H. (2010). High diversity of root associated fungi in both alpine and arctic Dryas octopetala. BMC Plant Biol, 10(1), 244-256. doi:10.1186/1471-2229-10-244
6. Blackwell, M. (2000). EVOLUTION: Enhanced: Terrestrial Life--Fungal from the Start? Science, 289(5486), 1884–1885. doi:10.1126/science.289.5486.1884
7. Bödeker, I.T.M. (2012). Functional ecology of ectomycorrhizal fungi. Diss. Uppsala, Sweden: Swedish University of Agricultural Sciences.
8. Brundrett, M. C (2004) Diversity and classification of mycorrhizal associations. Biol Rev Camb Philos Soc 79:473–495. doi:10.1017/ S1464793103006316
9. Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. New Phytol, 154(2), 275–304. doi:10.1046/j.1469-8137.2002.00397.x
10. Brundrett, M. C. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: Understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant and Soil, 320(1-2), 37–77. doi:10.1007/s11104-008-9877-9
11. Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1996). Working with Mycorrhizas in Forestry and Agriculture. ACIAR, Canberra.
12. Bruns, T. D. Bidartondo, M.I., & Taylor, D.L. (2002). Host specificity in ectomycorrhizal communities: What do the exceptions tell us? Integrative and Comparative Biology, 42(2), 352–359. doi:10.1093/icb/42.2.352
13. Cairney, J. W. G. & Burke, R. M. (1996). Plant cell wall degrading enzymes in ericoid and ectomycorrhizal fungi. In: Mycorrhizas in Integral Systems, C. Azcon- Aguilar and J.M. Barea, Eds. European commission, Brussels Belgium pp 218-221.
14. Cairney, J. W. G. (2000). Evolution of mycorrhiza systems. Naturwissenschaften, 87(11), 467–475. doi:10.1007/s001140050762

15. Cairney, J. W. G. (2011). Ectomycorrhizal fungi: the symbiotic route to the root for phosphorus in forest soils. Plant and Soil, 344(1-2), 51–71. doi:10.1007/s11104-011-0731-0
16. Cairney, J. W. G., & Burke, R. M. (1994). Fungal enzymes degrading plant cell walls: Their possible significance in the ectomycorrhizal symbiosis. Mycological Research, 98(12), 1345–1356. doi:10.1016/s0953-7562(09)81062-9
17. Cairney, J.W.G & Chambers, S.M. (1999). Ectomycorrhizal Fungi: Key Genera in Profile. Springer-Verlag Berlin Heidelberg Germany
18. Carlile, M.J., Watkinson, S.C. & Gooday, G.W. (2001). The fungi, 2nd edn. San Diego, CA, USA: Academic Press.
19. Chalot, M., Kytöviita, M. M., Brun, A., Finlay, R. D., & Söderström, B. (1995). Factors affecting amino acid uptake by the ectomycorrhizal fungus *Paxillus involutus*. Mycological Research, 99(9), 1131–1138. doi:10.1016/s0953-7562(09)80784-3
20. Cornelissen, J., Aerts, R., Cerabolini, B., Werger, M., & van der Heijden, M. (2001). Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia, 129(4), 611–619. doi:10.1007/s004420100752
21. Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., … Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry, 42(5), 679–698. doi:10.1016/j.soilbio.2009.12.006
22. Dahlberg, A. & Finlay, R.D. (1999). *Suillus* In: Ectomycorrhizal Fungi: Key Genera in Profile. Ed. by J.G.W Cairney & S. Chambers. Springer, Heidelberg. Pp.33-64.
23. Deckmyn, G., Meyer, A., Smits, M. M., Ekblad, A., Grebenc, T., Komarov, A., & Kraigher, H. (2014). Simulating ectomycorrhizal fungi and their role in carbon and nitrogen cycling in forest ecosystems. Canadian Journal of Forest Research, 44(6), 535–553. doi:10.1139/cjfr-2013-0496
24. Dell, B. (2002). Role of Mycorrhizal Fungi in Ecosystems CMU. Journal, 1(1), 47- 60.

25. Durall, D. M., Todd, A. W., & Trappe, J. M. (1994). Decomposition of ^{14}C-labelled substrates by ectomycorrhizal fungi in association with Douglas fir. New Phytol, 127(4), 725–729. doi:10.1111/j.1469-8137.1994.tb02976.x
26. Erland, S. & Taylor, A.F.S. (2002). Diversity of ecto-mycorrhizal communities in relation to the abiotic environment. In: Mycorrhizal Ecology. Ecological Studies, vol. 157. van der Heijden, M., Sanders, I. (Eds.), Springer, Berlin, pp. 163–200
27. Erland, S., & Soderstrom, B. (1990). Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L.. I. Mycorrhizal infection in limed humus in the laboratory and isolation of fungi from mycorrhizal roots. New Phytol, 115(4), 675–682. doi:10.1111/j.1469-8137.1990.tb00499.x
28. Erland, S., Soderstrom, B., & Andersson, S. (1990). Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L.. II. Growth rates in pure culture at different pH values compared to growth rates in symbiosis with the host plant. New Phytol, 115(4), 683–688. doi:10.1111/j.1469-8137.1990.tb00500.x
29. Felten, J., Kohler, A., Morin, E., Bhalerao, R. P., Palme, K., Martin, F., Ditengou, F.A, Legue, V. (2009). The ectomycorrhizal fungus *Laccaria bicolor* Stimulates lateral root formation in Poplar and *Arabidopsis* through auxin transport and signaling. Plant Physiology, 151(4), 1991–2005. doi:10.1104/pp.109.147231
30. Finlay, R. D. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. Journal of Experimental Botany, 59(5), 1115–1126. doi:10.1093/jxb/ern059
31. Finlay, R. D., Ek, H., Odham, G., & Soderstrom, B. (1988). Mycelial uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium by *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. New Phytol, 110(1), 59–66. doi:10.1111/j.1469-8137.1988.tb00237.x
32. Finlay, R. D., Frostegard, A., & Sonnerfeldt, A.-M. (1992). Utilization of organic and inorganic nitrogen sources by

ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. New Phytol, 120(1), 105–115. doi:10.1111/j.1469-8137.1992.tb01063.x

33. Finlay, R.D. (1995). Interactions between soil acidification, plant growth and nutrient uptake in ectomycorrhizal associations of forest trees. Ecol Bull., 44, 197-214.
34. Frank, A. B. (1885). Über die auf Wurzelsymbiose beruhendc Ernahrung gewisser Baume duren unterirdische Pilzc. Ber. dtsch. Bot. Ges., 3, 128-145
35. Fransson, P. M. A., Taylor, A. F. S., & Finlay, R. D. (2000). Effects of continuous optimal fertilization on belowground ectomycorrhizal community structure in a Norway spruce forest. Tree Physiology, 20(9), 599–606. doi:10.1093/treephys/20.9.599
36. Gao, Q., & Yang, Z. L. (2010). Ectomycorrhizal fungi associated with two species of Kobresia in an alpine meadow in the eastern Himalaya. Mycorrhiza, 20(4), 281–287. doi:10.1007/s00572-009-0287-5
37. Graham, J. H., & Miller, R. M. (2005). Mycorrhizas: Gene to Function. Plant and Soil, 274(1-2), 79–100. doi:10.1007/s11104-004-1419-5
38. Harley, J. L. & Smith, S. E. (1983). Mycorrhizal Symbiosis. Academic Press, Toronto.
39. Harley, J. L. (1989). The significance of mycorrhiza. Mycological Research, 92(2), 129–139. doi:10.1016/s0953-7562(89)80001-2
40. Hibbett, D. S., Grimaldi, D., & Donoghue, M. J. (1997). Fossil Mushrooms from Miocene and Cretaceous Ambers and the Evolution of Homobasidiomycetes. American Journal of Botany, 84(7), 981. doi:10.2307/2446289
41. Hobbie, J.E. & Hobbie, E.A. (2006). ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. Ecology, 87,816–22.
42. Högberg, P., 1986. Soil nutrient availability, root symbioses and tree species composition in tropical Africa: a review. Journal of Tropical Ecology, 2(04), p.359. doi. 10.1017/s026646740000099

43. Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D. D., & Högberg, M. N. (2010). Recovery of ectomycorrhiza after "nitrogen saturation" of a conifer forest. New Phytologist, 189(2), 515–525. doi:10.1111/j.1469-8137.2010.03485.x
44. Högberg, P., Nordgren, A., Buchmann, N., Taylor, A. F. S., Ekblad, A., Högberg, M. N., Nyberg, G., Ottosson-Lo¨fvenius, M. & Read, D. J. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature, 411(6839), 789–792. doi:10.1038/35081058
45. Hung, L.-L. & Trappe, J.M., 1983. Growth Variation between and within species of ectomycorrhizal fungi in response to pH in vitro. Mycologia, 75(2), p.234. doi 10.2307/3792807.
46. Jumpponen, A., Trappe, J. M., & Cázares, E. (2002). Occurrence of ectomycorrhizal fungi on the forefront of retreating Lyman Glacier (Washington, USA) in relation to time since deglaciation. Mycorrhiza, 12(1), 43–49. doi:10.1007/s00572-001-0152-7
47. Kendricks, B. (1992). The fifth kingdom. Second edition. Mycologue Publications, Waterloo, Canada
48. Kottke, I., & Oberwinkler, F. (1987). The cellular structure of the Hartig net: coenocytic and transfer cell-like organization. Nordic Journal of Botany, 7(1), 85–95. doi:10.1111/j.1756-1051.1987.tb00919.x
49. LePage, B. A., Currah, R. S., Stockey, R. A., & Rothwell, G. W. (1997). Fossil Ectomycorrhizae from the Middle Eocene. American Journal of Botany, 84(3), 410. doi:10.2307/2446014
50. Lilleskov, E. A., Fahey, T. J., Horton, T. R., & Lovett, G. M. (2002). Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology, 83(1), 104. doi:10.2307/2680124
51. Lilleskov, E.A., Fahey, T.J. & Lovett, G.M. (2001). Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. Ecol. Appl., 11, 397–410.
52. Mahmood, S., Finlay, R. D., & Erland, S. (1999). Effects of repeated harvesting of forest residues on the ectomycorrhizal

community in a Swedish spruce forest. New Phytol, 142(3), 577–585. doi:10.1046/j.1469-8137.1999.00414.x

53. Marschner, H. & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil, 159, 89–102.
54. Martin, F., & Nehls, U. (2009). Harnessing ectomycorrhizal genomics for ecological insights. Current Opinion in Plant Biology, 12(4), 508–515. doi:10.1016/j.pbi.2009.05.007
55. Martin, F., Stewart, G. R., Genetet, I., & Tacon, F. (1986). Assimilation of 15nh4+by beech (*Fagus sylvatica* l.) Ectomycorrhizas. New Phytol, 102(1), 85–94. doi:10.1111/j.1469-8137.1986.tb00800.x
56. Martin, F., Tagu, D. & Lapeyrie, F. (1997). Altered gene expression during ectomycorrhiza development. In P Lemke, G Caroll, Eds, The Mycota, Vol VI: Plant Relationships. Springer-Verlag, Berlin, pp 223–242.
57. Marx, D. H. & D.J. Beattie. (1977). Mycorrhizae -promising aid to timber growers. Farmer, 36, 6-9.
58. Melin E (1925) Untersuchungen über die Bedeutung der Baummykorrhize. Fischer, Jena.
59. Moore, D. 2011. Internet Source. Ectomycorrhizae, http://www.world-of-fungi.org/Moore.
60. Nehls, U. (2008). Mastering ectomycorrhizal symbiosis: the impact of carbohydrates. Journal of Experimental Botany, 59(5), 1097–1108. doi:10.1093/jxb/erm334
61. Newman, E. I., & Reddell, P. (1987). The distribution of mycorrhizas among families of vascular plants. New Phytol, 106(4), 745–751. doi:10.1111/j.1469-8137.1987.tb00175.x
62. O'Dell, T. E., Ammirati, J. F., & Schreiner, E. G. (1999). Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the Tsuga heterophylla zone. Rev. Can. Bot., 77(12), 1699–1711. doi:10.1139/cjb-77-12-1699
63. Pampolina, N. ., Dell, B., & Malajczuk, N. (2002). Dynamics of ectomycorrhizal fungi in an Eucalyptus globulus plantation: effect of phosphorus fertilization. Forest Ecology and Management, 158(1-3), 291–304. doi:10.1016/s0378-1127(00)00721-0

64. Peyronel, B., Fassi, B., Fontana, A., & Trappe, J. M. (1969). Terminology of Mycorrhizae. Mycologia, 61(2), 410. doi:10.2307/3757138
65. Pirozynski, K. A., & Malloch, D. W. (1975). The origin of land plants: A matter of mycotrophism. Biosystems, 6(3), 153–164. doi:10.1016/0303-2647(75)90023-4
66. Plassard, C., & Dell, B. (2010). Phosphorus nutrition of mycorrhizal trees. Tree Physiology, 30(9), 1129–1139. doi:10.1093/treephys/tpq063
67. Plassard, C., Scheromm, P., Mousain, D., & Salsac, L. (1991). Assimilation of mineral nitrogen and ion balance in the two partners of ectomycorrhizal symbiosis: Data and hypothesis. Experientia, 47(4), 340–349. doi:10.1007/bf01972075
68. Rahbek, C. (2004). The role of spatial scale and the perception of large-scale species-richness patterns. Ecology Letters, 8(2), 224–239. doi:10.1111/j.1461-0248.2004.00701.x
69. Rangel-Castro, I., Danell, E., & Taylor, A. (2002). Use of different nitrogen sources by the edible ectomycorrhizal mushroom Cantharellus cibarius. Mycorrhiza, 12(3), 131–137. doi:10.1007/s00572-002-0160-2
70. Read, D. J. (1992). The mycorrhizal mycelium. In Mycorrhizal Functioning. An integrative plant-fungat process. Ed. M Allen. pp 102-133. Chapman and Hall, New York.
71. Rinaldi, A.C., Comandini & Kuyper, T.W. (2008). Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity, 33, 1-45.
72. Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B., Olsen, P. B., Persson, P., Grell, M. N., Lindquist, E., Grigoriev, I.V., Lange, L. & Tunlid, A. (2012). The ectomycorrhizal fungus *Paxillus involutus* converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. Environmental Microbiology, 14(6), 1477–1487. doi:10.1111/j.1462-2920.2012.02736.x
73. Rosling, A., Landeweert, R., Lindahl, B. D., Larsson, K.-H., Kuyper, T. W., Taylor, A. F. S., & Finlay, R. D. (2003). Vertical distribution of ectomycorrhizal fungal taxa in a

podzol soil profile. New Phytologist, 159(3), 775–783. doi:10.1046/j.1469-8137.2003.00829.x

74. Smith, J. E., Molina, R., Huso, M. M., Luoma, D. L., McKay, D., Castellano, M. A., Lebel, T. & Valachovic, Y. (2002). Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. Canadian Journal of Botany, 80(2), 186–204. doi:10.1139/b02-003
75. Smith, S. & Read, D. (2008). Mycorrhizal symbiosis, 3rd edn. London, UK: Academic Press.
76. Smith, S.E. & Read, D.J. (1997). Mycorrhizal symbiosis, Academic Press, London
77. Taylor, A. F. S., & Alexander, I. (2005). The ectomycorrhizal symbiosis: life in the real world. Mycologist, 19(3), 102–112. doi:10.1017/s0269-915x(05)00303-4
78. Taylor, T. N., E. L. Taylor, and M. Krings. (2009). Paleobotany: The Biology and Evolution of Fossil Plants, Second Edition. Burlington: Elsevier Academic Press, 1230 pp
79. Tedersoo, L., & Nara, K. (2009). General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. New Phytologist, 185(2), 351–354. doi:10.1111/j.1469-8137.2009.03134.x
80. Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A. G., Henkel, T. W., Kjøller, R., Morris, M.H., Nara, K., Nouhra, E., Peay, K.G., Polme, S., Ryberg, M., Smith, M.E. & Kõljalg, U. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology, 21(17), 4160–4170. doi:10.1111/j.1365-294x.2012.05602.x
81. Trappe, J. M. (1977). Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu. Rev. Phytopathol., 15(1), 203–222. doi:10.1146/ annurev.py. 15.090177.001223
82. Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in

field studies. New Phytologist, 164(2), 347–355. doi:10.1111/j.1469-8137.2004.01159.x

83. Valtanen, K. (2012). Functional diversity of mycorrhizal fungi with regard to nutrient transfer Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultäten der Georg-August-Universität Göttingen
84. Wallenda, T., & Kottke, I. (1998). Nitrogen deposition and ectomycorrhizas. New Phytol, 139(1), 169–187. doi:10.1046/j.1469-8137.1998.00176.x
85. Zohlen, A. & Tyler, G. (2004). Soluble inorganic tissue phosphorus and calcicole-calcifuge behaviour of plants. Annals of Botany, 94(3), 427–432. doi:10.1093/aob/mch162