Chapter 3
The Importance of Ectomycorrhizal Networks for Nutrient Retention and Carbon Sequestration in Forest Ecosystems

Håkan Wallander and Alf Ekblad

Abstract Extramatrical mycelium (EMM) of mycorrhizal fungi have a fundamental role in carbon (C) cycling in forest ecosystems. This carbon is used for building extensive mycelial networks in the soil as well as for metabolic activity related to nutrient uptake. Here we discuss the factors that regulate the production and turnover of EMM and its role in soil C dynamics and nitrogen retention. C availability seems to be the key factor determining EMM production and possibly its standing biomass in forests but direct effects of mineral nutrient availability on the EMM can also be important. There is great uncertainty about the rate of turnover of EMM, and the increasing evidence that residues of EM fungi play a major role in the formation of stable N and C in soil organic matter highlights the need to include mycorrhizal effects in models of global soil C stores.

Keywords Carbon · Nitrogen · Nutrient retention · Extramatrical mycorrhizal mycelium · Ingrowth mesh bags

3.1 Introduction

Mycorrhizal fungi form extensive mycelial networks in the soils of boreal and temperate forests (Smith and Read 2008). Most of the trees form symbioses with ectomycorrhizal fungi (EMF), while shrubs and herbaceous plants are colonized by arbuscular mycorrhizal fungi, ericoid or orchid mycorrhizal fungi. Here we will

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focus on the EMF mycelial networks formed by many forest trees, especially in Pinaceae, Fagaceae and Betulaceae.

Trees invest large amounts of carbon (C) to facilitate nutrient uptake by the EMF networks, especially in nutrient-poor sites, and the growth of these networks is regulated by the C flux from the trees (Smith and Read 2008). Network functionality diminishes shortly after termination of the current photosynthate flux from the trees, as has been shown both in microcosms after severing the connection between the fungus and the host (Söderström and Read 1987), and in field experiments when the belowground C flux has been terminated by girdling the trees (Högberg et al. 2001). When uptake is terminated, leaching of nutrients can follow. This is commonly seen after clear-cutting forests, especially in nutrient-rich sites, but nutrient retention usually recovers after a few years when field layer vegetation has been established (Futter et al. 2010). Nitrogen (N) can also leach from standing forests when N input has been continuously high and the forests are subjected to N saturation (Emmett 2007). This leaching is possibly an effect of impaired EMF function (Aber et al. 1998; Högberg et al. 2011).

C sequestration in forest soils is dependent on N availability since the C:N ratio of stable soil organic matter (SOM) in deeper soil layers is rather constant around 10–15. This value is similar to the ratio of EMF biomass (Wallander et al. 2003) and it has been suggested that the SOM in boreal forests to a large extent is composed of EMF residues (Högberg et al. 2011; Clemmensen et al. 2013; Fernandez et al. 2013; Fernandez and Kennedy 2015). However, the enhanced C sequestration that occurs after N fertilization (Franklin et al. 2003; Hyvönen et al. 2007) is difficult to attribute to EMF since many of these fungi decline under elevated N conditions (Wallenda and Kottke 1998; Nilsson and Wallander 2003; Högberg et al. 2011; Bahr et al. 2013). This makes assessing the role of EMF networks in C sequestration complicated. The enhanced tree growth and litter production, and the reduced decomposition of SOM (Nohrstedt et al. 1989; Franklin et al. 2003), usually found after N fertilization are possible reasons for enhanced C sequestration. But it is also possible that changes in ectomycorrhizal (EM) community composition that occurs after N fertilization plays a significant role since different EM species decompose at different rates (Langley and Hungate 2003; Koide and Malcolm 2009; Koide et al. 2011; Fernandez et al. 2013). Clemmensen et al. (2013) found larger C sequestration in old compared to young successional stages of boreal forests in northern Sweden and attributed this, at least in part, to different mycorrhizal communities (Clemmensen et al. 2015). Furthermore, certain species of EMF may reduce SOM accumulation by degrading recalcitrant compounds to obtain N that is delivered to the host trees (Talbot et al. 2008). Thus EMF appears to have a central role in C sequestration, although the overall effect is difficult to predict because of these opposing processes.

In this chapter we will discuss (1) how the production of EMF networks can be measured under field conditions, (2) how the production is regulated, and (3) to what extent EMF networks are important for ecosystem processes such as nutrient retention and C sequestration.

3.2 Methods to Study Production of EMF in the Field

3.2.1 Observational Methods

One of the problems with quantifying standing biomass or production of EM networks in the field is difficulties in separating mycorrhizal mycelia from saprotrophic mycelia. There is no biochemical or DNA-based marker to distinguish extramatrical mycorrhizal mycelia (EMM) from the complex fungal soil community since EM fungi do not form a monophyletic clade (Tedersoo et al. 2010), but there are various ways to overcome this problem. Production of EMM can be studied in the field through direct observation in root windows or minirhizotrons (Coutts and Nicoll 1990; Treseder et al. 2005; Pritchard et al. 2008), although the resolution is too low to observe individual hyphae. Coutts and Nicoll (1990) followed the growth rate of the advancing hyphal front of two EM species observed through observation windows installed on 2 m large tubes placed outside. The estimated growth rate of *Thelephora terrestris* was 1–3 mm day^{-1} during the plant growing season which is similar to what has been found for EMF in laboratory microcosms (Read 1992) as well as under axenic growth (Gafur et al. 2004). Furthermore, the mycelium continued to grow over the winter, although at a slower rate (0.3 mm day^{-1}). *Laccaria proxima* on the other hand grew slower and the mycelium disappeared during the autumn. In this way observation methods are useful for determining longevity of EMM and Pritchard et al. (2008) found that EM rhizomorphs observed in minirhizotrons lived much longer than mycorrhizal root tips (mean longevity 532 and 104 days respectively, Fig. 3.1). Observation methods are useful to study seasonal dynamics of EMF networks in the field but it is more difficult to quantify the production in terms of biomass.

3.2.2 Mesh Bags

The most common approach to quantify EMM production is the use of ingrowth mesh bags (Wallander et al. 2001; Fig. 3.2) or in-growth cores (Godbold et al. 2006; Hendricks et al. 2006). Such techniques have so far been used to estimate EMM production at $\sim$140 different sites (Ekblad et al. 2013). The mesh bags or cores are usually filled with sand, free of fungal material, and incubated in the soil for various periods of time. The amount of fungal biomass detected at harvest is used as an estimate of EMM production. However, colonization by saprophytic fungi can also occur, although to a smaller extent, and to account for this, trenched plots can be used to measure production of the saprophytic mycelium only (Wallander et al. 2001). It should be noted that trenching by forcing down tubes into the soil will only last a limited amount of time since EMM may enter the tubes from below. In studies in Sweden such trenched plots were free of EMM for one growing season but EM fungi entered some of the tubes after two growing seasons

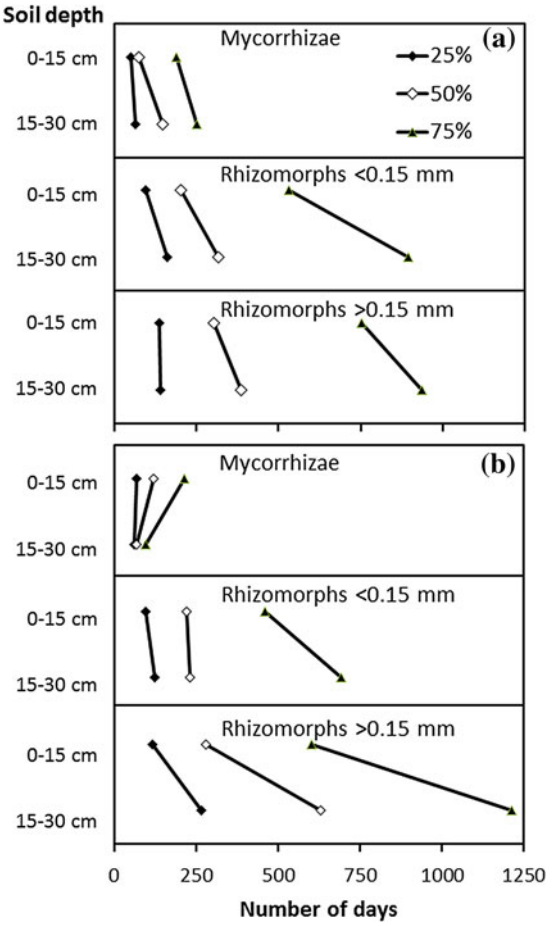

Fig. 3.1 Number of days until 25, 50 or 75 % mortality of mycorrhizal tips and of rhizomorphs of two diameter classes; **a** ambient CO_2, **b** elevated CO_2. Data from minirhizotrons installed in a loblolly pine forest in North Carolina (from data in Pritchard et al. 2008)

(Wallander et al. 2001, 2011). Molecular analysis of the fungal communities of ingrowth mesh bags has revealed that between 70 and 90 % of the sequences obtained from the bags originate from fungi known to form ectomycorrhizal symbiosis (Parrent and Vilgalys 2007; Wallander et al. 2010; Berner et al. 2012). Another approach to check for saprophytic ingrowth is to analyse the C isotopic signature of mycelia extracted from mesh bags (Wallander et al. 2001). Fruitbodies of wood—and litter decomposing fungi usually have a $\delta^{13}C$ value that is 2–3 ‰ higher than values for EMF fruitbodies (Högberg et al. 1999; Taylor et al. 2003; Hobbie et al. 2012) and the $\delta^{13}C$ of mycelia in mesh bags usually resemble values for EMF fruitbodies (Wallander et al. 2001; Hagerberg et al. 2003; Mikusinska et al. 2013). However, with time, it is possible that fungi that decompose the EMF mycelium will establish in the bags; whether the mycelium formed by these fungi differs in $\delta^{13}C$ from EMF is unknown.

Fig. 3.2 A harvested mesh bag after 2 years incubation in a Norway spruce forest in Sweden has been opened in the lab. Abundant mycelia and rhizomorphs of EM fungi are clearly visible. Photo: Adam Bahr

Studies using natural soil in mesh bags or cores have shown higher production rates of EMM than studies using sand (Hendricks et al. 2006). The reason for this is probably that soil is a more natural substrate for the fungi, but problems arise when using soil since it contains background fungal material that needs to be subtracted before EMM production can be calculated. This seems to work when the soil has low SOM content (Hendricks et al. 2006; Sims et al. 2007) but under other circumstances fungal biomass background values are too high to make ingrowth measurements reliable (Wallander personal observation). An alternative approach to estimate EMM production in mesh bags or cores filled with soil is to use the different C isotopic signatures of C_4 and C_3 plant material (Godbold et al. 2006; Wallander et al. 2011). In this case mesh bags or cores are filled with ^{13}C-enriched C_4 material (soil or plant material) and the change in isotopic composition that occurs when the bags/cores are colonized by ^{13}C depleted EMM is followed and used to calculate C flux into the bags. This approach was used by Godbold et al. (2006) who estimated a C flux to EMF of around 1000 g C m^{-2} during a period of 2.5 years in a poplar plantation in Italy. Wallander et al. (2011) used a mixture of sand and compost made of maize leaves (a C_4 plant) in mesh bags and estimated a C flux of around 100 g C m^{-2} over a three year period in Norway spruce forests in Sweden.

One of the problems of using ingrowth mesh bags or cores to quantify EMM is that the fungal community that colonizes the bags/cores may not be representative of the soil community. The reason for this is the use of artificial substrate (sand) and the fact that fungal-free bags or cores select for fast-growing EMF species. Fungi that

proliferate in the mineral soil may be overrepresented in the bags when a sandy substrate is used. One way to overcome some of these problems may be to incubate mesh bags for longer time periods and analyse annual production and turnover of EMM in the mesh bags after colonization by fast-growing EMF species has terminated. However, it is not known whether the contribution of saprotrophic mycelium increases over time, and this needs to be tested. Production of specific species can probably be measured by qPCR or other molecular techniques, and the C flux into the bags or cores over time can be followed by isotopic techniques as discussed above. The mesh bag method is best suited for relative comparisons between different forest management practices or treatments and for comparing how different mesh bag amendments influence EMM production. Estimations of absolute amounts produced must be interpreted with caution. For a more detailed review on this subject see Wallander et al. (2013).

3.2.3 Exploration Types

EMF communities can be extremely diverse (Dahlberg 2001) and the composition of the EM community is probably of large importance for ecosystem processes such as nutrient retention and C sequestration. One approach to handle this diversity of EMF in functional terms has been to classify the species into exploration types based on the amount of hyphae emanating from the root tips and the presence and differentiation of rhizomorphs (Agerer 2001, 2006). The different C demand among the explorations types will most likely have profound effects on their ecological roles in terms of nutrient uptake/retention and their potential to sequester C. When more physiological data have been collected about the different exploration types it might be possible to incorporate them into ecosystem models with the aim to increase predictions of key ecological processes such as nutrient uptake, leaching of nutrients and SOM cycling. Work along this line has been started by Weigt et al. (2011, 2012a, b) who have quantified the amount of mycelium produced by representatives of a few different exploration types in laboratory experiments. If these values are applied to EM communities that have been identified on root tips in the field, it might be possible to extrapolate potential EMM production in field sites from EM community composition estimated from analysis of root tips.

3.3 Regulation of EM Growth by C Supplied from the Host Trees

3.3.1 Tree Growth

Since the EMM depends on C delivered from the host trees, higher photosynthetic rates can potentially result in higher EMM production. Support for this view was found by Korkama et al. (2007) who studied EMM production related to fast- and

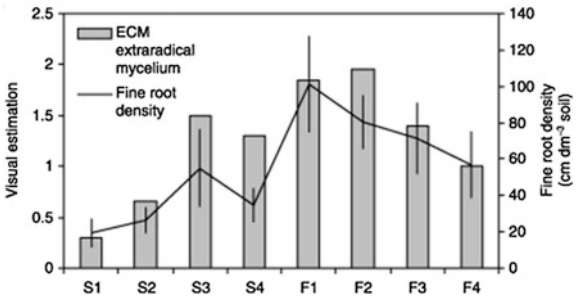

Fig. 3.3 The biomass of extraradical mycelia developing in mesh bags (bars) is related to the fine root density (lines) under slow (S1–S4) and fast-growing Norway spruce (*Picea abies*) clones (F1–F4). Fungal biomass was estimated visually under a dissecting microscope and according to aggregation of the sand. It was classified into four categories: (0) no mycelial strands and sand aggregation; (1) a few mycelial strands but no sand aggregation; (2) moderate number of mycelial strands and some sand aggregation; (3) considerable extraradical mycelium and sand aggregation (from Korkama et al. 2007)

slow-growing spruce clones. Significantly higher EMM production was found in the fast-growing compared to slow-growing ones (Fig. 3.3). Furthermore, EMM production was correlated to fine root biomass suggesting that enhanced belowground allocation of C was necessary to sustain the better growth of fast-growing clones. In a larger data set of Scandinavian Norway spruce forests (data from Ekblad et al. 2013), EMM growth and spruce productivity were positively correlated (Fig. 3.4), but other factors such as nutrient availability are important for the relative allocation above and belowground (see below) which complicates the picture.

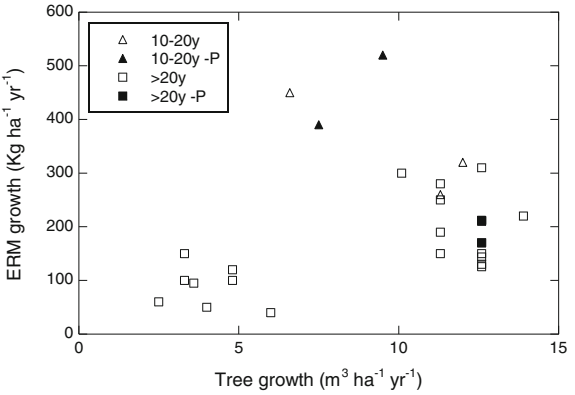

Fig. 3.4 Relationship between growth of extramatrical mycelium (EMM; Kg ha^{-1} $year^{-1}$) in the top 10 cm and wood production (m^3 ha^{-1} $year^{-1}$) in the Norway spruce stands from Table 2 in Ekblad et al. (2013). *Open triangles* (young stands 10–20 year), *closed triangles* (young stands with P deficiency, needle P < 1.3 mg P g^{-1}), *open squares* (stands older than 20 year), *closed squares* (older stands with P deficiency)

3.3.2 Tree Age

Trees usually peak in nutrient uptake during canopy closure when nutrient demand is highest. When the trees mature more nutrients are supplied through internal cycling (Kimmins 2004). EMM growth in the soils shows similar pattern (Kalliokoski et al. 2010; Wallander et al. 2010), and its peak in production seems to be close to that of the usual canopy closure stage of coniferous forests in southern Scandinavia (25–40 years, Schmalholz and Hylander 2009).

3.3.3 Seasonality

Winter is obviously a season with poor EMM growth in boreal and boreo-nemoral regions but might be a period of active growth in warmer climates. Thus, in pine forests of northeastern Spain the living EMM biomass, quantified by specific primers and qPCR, peaked in February for *Boletus edulis* and in December for *Lactarius deliciosus* (De la Varga et al. 2013). Also in cooler temperate regions some species are able to continue growing at a low rate during winter, at least in the study in UK by Coutts and Nicoll (1990). But EMM growth is probably not directly related to temperature in the same way as growth of saprotrophs in soil, since EMM growth depends on C allocated from the trees. In northern temperate and boreal regions maximal growth can be expected in the second half of the growing season when the above ground C sink in terms of tree growth has ceased. Support for this view was found by Nilsson et al. (2007) who found better EMM growth in oak forests during the colder fall period compared to the warmer summer period. Another factor that might be confounding in these studies is soil humidity which usually is higher in the fall than in the summer.

3.3.4 Elevated CO_2

Elevated CO_2 could potentially increase the photosynthetic rate and thereby increase EMM growth. However this will also depend on other factors since nutrient availability may limit photosynthesis resulting in less or no increased growth rate after elevated CO_2. Several studies in the laboratory have demonstrated increased EMM growth after elevated CO_2 (e.g. Rouhier and Read 1998), but although an increased rhizomorph production and longevity was observed in a *Pinus taeda* plantation (Pritchard et al. 2008), these results have been difficult to repeat in the field (Godbold et al. 2006; Parrent and Vilgalys 2007; Dawes et al. 2013). The effect of elevated CO_2 on EMF was recently reviewed by Fransson (2012).

3.3.5 *Herbivory*

Herbivory of tree foliage could potentially result in reduced EMM growth if photosynthetic capacity declines, but few studies have tested this. However, herbivory from scale insects reduced colonization of pinyon pines by EMF (Del Vecchio et al. 1993) and suilloid fungi have shown reduced growth more than other species after artificial herbivory (Kuikka et al. 2003). These species appear to have higher C demands when tested in the laboratory (Fransson et al. 2007) which may be one explanation for their decline after herbivory.

3.4 How Nutrient Availability Influence C Allocation and EMM Growth

Nutrient availability strongly affects C allocation pattern in the host trees. Work by Ericsson (1995; Fig. 3.5) demonstrated that allocation belowground increased when N, P, S and Fe was limiting growth, while limitation of K, Mg, and Mn resulted in reduced belowground allocation. This was explained by impaired photosynthesis at K, Mg and Mn limitation resulting in lower carbohydrate production. In contrast limitation of N, P, S and Fe resulted in impaired growth but lower direct effects on photosynthesis, leading to accumulation of carbohydrates in the leaves. These carbohydrates could be loaded into the phloem and transported belowground (Ericsson 1995). Much less is known about how EMM is influenced by nutrient

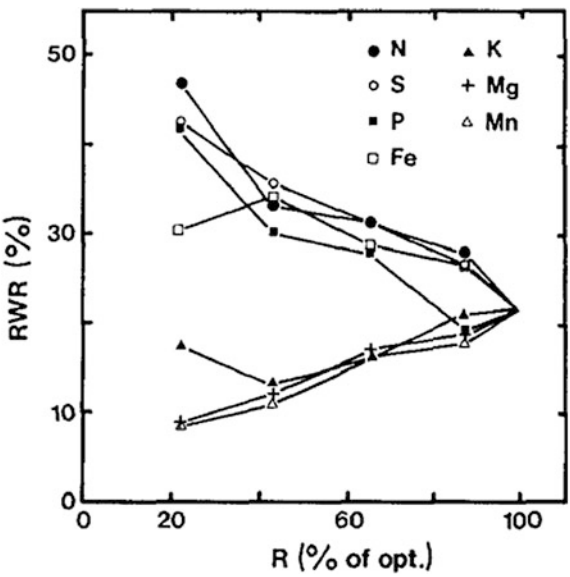

Fig. 3.5 Root weight ratio (RWR) in *Betula pendula* seedlings grown at different nutrient regimes. The indicated nutrients were added to give plant growth rates between 20 and 100 % of optimum. Reduced availability of N, S, P and Fe resulted in increased RWR while reduced availability of K, Mg and Mn resulted in increased RWR (data from Ericsson 1995)

limitation and excessive amounts of nutrients, but as demonstrated in the following section, the current knowledge appear in many respects to follow the results found by Ericsson (1995) for roots.

3.4.1 Nitrogen

The large production of EMM in boreal forests is attributed to N commonly being the limiting nutrient in these ecosystems. EMF are well adapted to cope with N limitation by producing large mycelia that can take up and store N during periods of high N availability (Mikusinska et al. 2013). In addition, many species can mobilize and take up organic forms of N (Perez-Moreno and Read 2001). Thus it is not surprising that the activity of EMF is reduced when N input to the system increases. This can be seen both in terms of fruitbody formation and in the number of mycorrhizal root tips (Wallenda and Kottke 1998; Peter et al. 2001; Lilleskov et al. 2002; Lilleskov et al. 2011). EMF species more efficient in taking up organic N are usually the ones that become less frequent in response to inorganic N loads (Taylor et al. 2000; Lilleskov et al. 2011).

Growth of EMM is strongly reduced after N addition in laboratory-grown seedlings (Wallander and Nylund 1992; Arnebrant 1994) and recent work using ingrowth mesh bags have confirmed this also for EMM growth in natural forests (Nilsson and Wallander 2003; Hendricks et al. 2006; Parrent and Vilgalys 2007; Kjøller et al. 2012). It should however be noted that this negative effect is reduced when N is balanced by other nutrients (Wallander et al. 2011). The number of mycorrhizal root tips or the fungal biomass on the root tips may remain similar after several years of annual N addition (Kåren and Nylund 1997), while the growth of the EMM was severely reduced in the same forest (Nilsson and Wallander 2003). This suggest that C demanding fungi (e.g. suilloid spp., Fransson et al. 2007), decline after N input. This was also found along a N deposition gradient in Alaska (Lilleskov et al. 2002) where the contact type *Lactarius theiogalus* (presumably low C demand) dominated (68.5 % of colonized root tips) in the most N-polluted site, while it decreased to only 7.4 % of root tips in the least N-polluted site. In contrast, medium-distance types like *Amphinema byssoides* and *Piloderma byssinum* (presumably high C demand) became more abundant in the least N-polluted site (40 %), while they were totally absent at higher N input sites. Along another short-distance N deposition gradient in a Norway spruce forest in Denmark, Kjøller et al. (2012) found that *Lactarius quietus* (contact type) dominated the root community at the forest edge (56 %) with the highest inorganic N deposition (43 kg N ha^{-1}) while short distance types (89 %, mainly *Tylospora fibrillosa* and *Cenococcum geophilum*) dominated further into the forest (N deposition 27 kg N ha^{-1}). Very few medium and long-distance types were formed in this forest (<5 % in the forest, 0 % at the edge), probably because of the rather high deposition of inorganic N. EM communities dominated by contact types, with less well developed mycorrhizal networks, may result in vulnerability to N leaching. This aspect will be further elaborated below.

3.4.2 Phosphorus

P deficiency is less common in boreal and temperate regions than in tropical regions, but mass balance calculations suggest that intensive harvesting of forest residues in combination with high N deposition will lead to P deficiencies in many forests in temperate and boreal regions (Akselsson et al. 2006). In laboratory grown seedlings, P deficiency resulted in strong enhancement of EMM production (Wallander and Nylund 1992; Ekblad et al. 1995). Phosphorus effects on the EMM has not been much studied in the field but Wallander and Thelin (2008) found that EMM ingrowth into sand-filled bags amended with apatite was higher than in bags filled with sand only, but this effect disappeared when the forests were fertilized with P and K. The magnitude of the EMM ingrowth response to apatite was negatively correlated to needle P status which supports the view that P availability in the soil is of great importance for the regulation of EMM production (Wallander and Thelin 2008). Since P is rarely limiting tree growth in temperate and boreal forests, the needle P status is probably not low enough to stimulate growth of EMM under field situations. However, Bahr et al. (2013) found a negative correlation between EMM growth and needle P status also when needle P levels were above 1.3 mg P g^{-1}, which was the threshold value where apatite stimulated EMM growth in the study by Wallander and Thelin (2008). Furthermore, Blum et al. (2002) found apatite to be an important calcium source for ectomycorrhizal trees in base-poor forest ecosystems in the US.

3.4.3 Other Nutrients

Under laboratory conditions, both K (Ekblad et al. 1995) and Mg (Wallander and Nylund 1992) deficiency has resulted in reduced EMM growth, supporting the finding by Ericsson (1995) of reduced belowground C allocations during such conditions (Fig. 3.5). These findings have however not been confirmed under field conditions, since Hagerberg et al. (2003) found no difference in EMM growth in Norway spruce forests with varying K availability. Some indications that Mg deficiency may impair EMM growth was found in a study in the Czech Republic by Berner (2013). EMM was much lower in Norway spruce forest growing on Mg-poor granite soil compared to similar forests growing on more Mg-rich amphibolite or serpentinite bedrock. Furthermore, a positive correlation between needle Mg concentration and EMM growth was found. It should however be noted that many other factors also varied among these sites and a causal relationship between Mg availability and EMM growth could not be established in this study. Mg deficiency can have severe effects on belowground C allocation since carbohydrate loading of the phloem can be impaired (Cakmak and Kirkby 2008). The forest die-back that occurred in central Europe during 1980–1990 was suggested to

be caused by Mg deficiency as a result of acid rain (Schulze 1989). The Mg deficiency was proposed to be the result of dysfunctional mycorrhizal associations when belowground C allocation was impaired (Mejstrik 1989).

3.5 Ecological Consequences of Altered EMM Production

3.5.1 Nitrogen Leaching

Boreal forest soils have large retention capacity for N, especially when the C:N ratio of the organic layer (O horizon) is above 30. Nitrate leaching can be induced when ratios drop below 25, especially if N deposition exceeds 10 kg N ha^{-1} (Gundersen et al. 1998). Increased N retention was correlated to enhanced fungal proportion of the microbial biomass in a gradient of C:N ratios of Norway spruce forests in southern Scandinavia (Nilsson et al. 2012) and this may be attributed to the high capacity of EMF networks to assimilate N (Read et al. 2004). Wallander et al. (2004) estimated that EMM contained between 100 and 200 kg N ha^{-1} in Norway spruce and mixed oak/Norway spruce forests in southern Sweden. This amount is higher than what is found in the tree stems in the same forests (Thelin et al. 2002). The high N retention capacity of EMF is an effect of the large flux of C to the EMM in forest ecosystems, resulting in a well-developed EMM network in the soil that efficiently captures available N. Some of the N that is taken up is allocated to the host trees but significant amounts are also retained in the EMF biomass in the soil (Näsholm et al. 2013). Aber et al. (1998) suggested that N was exuded from EMF as enzymes that formed stable complexes with humus material in the soil, while Högberg et al. (2011) and Fernandez et al. (2013) proposed that the EMF mycelia itself could be a precursor for stable N. This view is supported by the fact that recalcitrant SOM deeper in the soil becomes more and more similar to EMF in terms of C:N ratio and N isotopic signatures (Boström et al. 2007; Lindahl et al. 2007; Högberg et al. 2011; Clemmensen et al. 2013). Furthermore, Näsholm et al. (2013) concluded that EMF immobilize large amounts of N in boreal forests to restrict establishment of species that are more N demanding. Along these lines, Franklin et al. (2014) developed a model that could explain why ectomycorrhizal symbiosis does not alleviate nitrogen limitation in boreal forests.

The efficiency by which forest trees and their EMF networks capture N to avoid leaching of N may depend on availability of other nutrients. Stevens et al. (1993) found enhanced N leaching from Sitka spruce stands which had developed K and P deficiency, while leaching was repressed upon fertilization with K and P. In the French Ardennes increased N leaching has been correlated with reduced availability of nutrients such as K, Mg and P (Jonard et al. 2012). Root (Gress et al. 2007) and EMM (Wallander and Thelin 2008) growth were enhanced in P-rich microsites in P-poor Norway spruce forests with high N input. This enhanced growth may

explain the reduced N leaching after P fertilization. Slow release P fertilizer has been used in Finland (Aarnio et al. 2003) and this approach may reduce N leaching from forests with low P availability.

In another recent paper, Blanes et al. (2012) was able to separate the effect of autotrophs (roots + EMF) and saprotrophs on N retention by combining fertilization treatments with root-exclusion and isotope labeling. They found enhanced N retention in N-saturated forests after P fertilization, mainly due to a better N uptake by EM roots as shown by a ^{15}N labeling experiment. Trenching verified that N retention was also enhanced among saprotrophic organisms after P fertilization, probably by fungi since short-lived bacteria with much lower C:N ratios are less likely to be important for N retention (Högberg et al. 2011). Interestingly the P effect on the N retention by saprotrophs was only found in trenched plots, suggesting superior N retention capacity by the autotrophic (root + EMF) compared to the saprotrophic organisms.

N leaching from standing forest can be an effect of impairment of EMM growth as discussed above. But it can also be an effect of a changed EM community to species with lower capacity to take up N. Kjøller et al. (2012) found enhanced N leaching and a drastically changed EMF community when moving towards a forest edge that is more exposed to N deposition compared to more protected areas within the forest. The EMF community at the forest edge was dominated by smooth root tips with low capacity to form extensive mycelia networks and presumably low capacity to retain N. Lilleskov et al. (2002) suggested a shift in the EMF community of N-saturated forests to species more efficient in P uptake, which could lead to less efficient N uptake and more N leaching. Although smooth root tips probably are inefficient in P uptake, other N tolerant species such as *Paxillus involutus* are known to have extremely high P uptake rates (Colpaert et al. 1999), and it would be interesting to see if the N uptake rates from such species are reduced under conditions of high N input, which would allow more N to leach. Gorissen and Kuyper (2000) have demonstrated that nitrophilic (N tolerant) species such as *Laccaria bicolor* retain more N in the fungal biomass while nitrophobic (N sensitive) *Suillus bovinus* deliver more N to the host plant when studied in a pot experiment. If nitrophilic species can reduce N uptake by retaining it in their biomass rather than transferring it to the host plant, they may tolerate N better by spending less C on N assimilation. This would allow them to spend more C on EMM growth under excess N, as suggested in the hypothesis presented by Wallander (1995). In support for this hypothesis, Gruffman et al. (2012) found recently that, contrary to inorganic N, organic N fertilizer (based on amino acids) C did not impair ectomycorrhizal colonization of Norway spruce roots. The reason is probably a lower C cost for the fungus when amino acids are taken up instead of inorganic N, and this will result in more C available for fungal growth. Organic N fertilizers were as efficient as inorganic fertilizers and were recommended in nurseries to improve mycorrhiza formation (Gruffman et al. 2012).

3.5.2 The Importance of EMM for C Sequestration

Recent results suggest that EMM contribute significantly to SOM formation. Godbold et al. (2006) used ingrowth cores with soil that had a different C isotopic signature than the colonizing EMM, and found that EMM accounted for an accumulated input of 1000 g C m^{-2} over a period of ~2.5 years, which corresponded to 62 % of new soil C in a poplar plantation in Italy. Wallander et al. (2011) used a similar approach by amending ^{13}C-enriched maize compost material to mesh bags and found a lower (100 g C m^{-2}), but still significant, C input to a Norway spruce forest soil through EMM over a period of two years. Furthermore, by using ^{14}C dating of SOM at different soil depth and a modeling approach in boreal forests in northern Sweden, Clemmensen et al. (2013) concluded that the majority (70 %) of the C in the upper 20 cm of the soil in later successional forests originated from roots and associated EMM while this figure declined to 47 % in stands at earlier stages of successions. This was explained by impaired degradation of fungal residues in later successional forests. It is possible that different EM and ericoid mycorrhizal species contribute differently to SOM formation by producing compounds that are more or less recalcitrant (Clemmensen et al. 2015). Fernandez et al. (2013) demonstrated recently that root tips formed by *Cenococum geophilum* persisted 4–10 times longer than other EMF species in the soil which suggest that this species is resistant to decay and may contribute significantly to C sequestration. In support for this Dahlberg et al. (1997) found that sclerotia formed by this fungus could make up 400 kg ha^{-1} in a Swedish Norway spruce forest soil. Some recent results highlight the capacity of many EM species to degrade or modify SOM, which may lead to enhanced decomposition and reduced C sequestration (Chapela et al. 2001; Talbot et al. 2008; Courty et al. 2010). These effects are however debated. Treseder et al. (2006) could not demonstrate any C uptake by EM fungi from ^{14}C-labelled litter added to an oak forest but work at natural ^{14}C abundance of fungal proteins suggested uptake of C (as amino acids or oligopeptides) by several taxa of EMF (Hobbie et al. 2013). Talbot et al. (2008) suggested that EM fungi released C as a side effect when removing N-rich compounds and Lindahl et al. (2007) demonstrated an increasing C:N ratio of SOM in the lower part of the organic horizon where EMF dominate, indicating preferential uptake of N-rich compounds. Old SOM may also be released through priming when labile C is exuded by roots and associated EMF (Dijkstra and Cheng 2007). Peroxidase-encoding genes have been identified among a wide range of EMF suggesting that these fungi degrade SOM in a similar way as white rot fungi (Bödecker et al. 2009) and other EMF seem to use the Fenton reaction to modify SOM to obtain N-rich compounds in a similar way as brown rot fungi (Rineau et al. 2012; Lindahl and Tunlid 2015).

The contribution of EM fungi to SOM formation depends on production, turnover and recalcitrance of EMM in the soil. When studied in microcosm systems, the mycelium of several long-distance types (e.g. *Suillus* spp., *Paxillus involutus*) are known to spread rapidly to colonize nutrient-rich organic patches, but disappear

after a couple of weeks when the nutrients are exhausted (Bending and Read 1995). Other types form perennial mycelial mats in the soil (Ingham et al. 1991; Kluber et al. 2010) that presumably affect SOM formation differently than more short-lived types. Many short-distance and contact types produce very little mycelium but contribute to accumulation of SOM since they decompose much more slowly than non-mycorrhizal roots (Langley and Hungate 2003). In contrast, Koide et al. (2011) found that non-mycorrhizal roots decomposed faster than EM roots in a similar experiment. The reason for this controversy is not known but could be related to differences in species composition. Mycorrhizal mycelia decomposed fast when incubated in forest soil, 40–80 % of the mass remained after 1 month (Fernandez and Koide 2012). Bahr et al. (2015) found that most of the mycelium (90 %) produced in mesh bags incubated in forest soil degraded within a year. Although the rate of decomposition is of relevance for SOM formation, the most important aspect for long-term C sequestration is the proportion of the litter material that remain in the soil for longer time periods. For pine needle litter, Berg et al. (2010) demonstrated that up to 17–53 % of the mass remained after 3–5 years of incubation in litter bags. At the time of harvest decomposition rate had approached zero. If such high amounts of remaining material also exist among EM fungi, and if different species vary in this respect, the composition of the EM community could have a fundamental role in SOM formation. Another aspect recently highlighted is that the molecular structure of SOM does not alone control the long-term decomposition of SOM (Schmidt et al. 2011). Instead, the degree of protection from decomposition in the soil was suggested to be a more important regulator (Schmidt et al. 2011). Molecules can be protected inside soil aggregates and on mineral surfaces (Sollins et al. 2009), and one challenge for future research is to sort out the role of mycorrhizal fungi in this respect. The interactions between EMF and minerals have been reviewed recently (Hoffland et al. 2004; Finlay et al. 2009), but the possible role of these processes in C sequestration has been largely neglected. On the other hand, the decomposition of forest humus, with low amounts of mineral surfaces, was extremely slow in late successional forests in boreal forest chronosequence (Clemmensen et al. 2013), suggesting the molecular structure of SOC to be the most important factor in determining decomposition rates in these forests.

3.6 Conclusions

Accurate data on production, biomass and turnover of ectomycorrhzial mycelium are essential for improving the ability of ecosystem models to predict nutrient leaching and C sequestration in forest ecosystems. The composition of the EM community appears to have a fundamental role on N retention and turnover of EMM and more research on the influence of individual species on these processes is urgently needed. A possible way forward could be to classify EMF into functional groups along the lines of Agerer (2001). Apart from morphological characters, other criteria's such as nitrophilic/nitrophobic, enzyme production, sensitivity to

disturbance etc. could be useful when defining the groups. There is increasing evidence that the EMM of mycorrhizal fungi play a key role in C cycling in ecosystems. This was highlighted in a recent paper from a boreal forest chronosequence in Sweden which suggests that belowground litter of roots and rhizosphere fungi, with EMF being the most prominent, contributed up to 70 % of the C sequestered in SOM (Clemmensen et al. 2013). The importance of EMF for the C cycling in forests has been the topic of two other recent reviews (Cairney 2012; Ekblad et al. 2013). Having other focuses than the present review, we therefore recommend the reading of these for a more complete cover of the subject.

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