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ORIGINAL PAPER

Long-term effect of apatite on ectomycorrhizal growth and community structure

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Abstract Ectomycorrhizal (ECM) fungi are efficient at taking up phosphorus (P) from mineral sources, such as apatite, which are not easily available to the host trees. Since ECM fungal species differ in P uptake rates, it can be expected that the composition of the ECM fungal community will change upon exposure to apatite, provided that the P transfer is rewarded by more carbon being transferred to the fungal symbiont. Control and apatite-amended mesh bags were buried in pairs in the humus layer of a P-poor Norway spruce forest. The ECM fungal community that colonized these bags was analyzed by DNA extraction, PCR amplification of the internal transcribed spacer (ITS) region, cloning, and random sequencing. Fungal biomass was estimated by ergosterol analysis. No change in the ECM fungal community structure was seen after 5 years of apatite exposure, although the fungal biomass increased threefold upon apatite amendment. Our results indicate that host trees enhance carbon allocation to ECM fungi colonizing P sources in P-poor forests but the lack of change in the composition of the ECM fungal community suggests that P transfer rates were similar among the species. Alternatively, higher P transfer among certain species was not rewarded with higher carbon transfer from the host.

Keywords Apatite · Community structure · Ectomycorrhiza · External mycelium · Norway spruce · Weathering

Introduction

Most trees in coniferous forests live in symbiosis with ectomycorrhizal (ECM) fungi. ECM fungi increase the soil volume that can be exploited for nutrients and thereby increase the trees ability to take up nutrients from the soil. In return, ECM fungi receive up to 20 % of the photosynthetic production by the trees (Simard et al. 2002), and this flux is regulated by various factors, such as nutrient status, management, and climate. Plants are able to allocate carbon to specific parts of the ECM mycelium, especially those parts that are in contact with areas of the soil containing nutrients limiting plant growth (Bending and Read 1995; Leake et al. 2001).

To estimate growth of ECM mycelium in the field, one can use in-growth mesh bags developed by Wallander et al. (2001). With these bags it is possible to quantify the ECM fungal growth during the time period when the bags have been in the soil, which is not possible by taking soil samples. This method has been used in previous studies for both quantifying ECM fungal growth and analysis of community structure (Boström et al. 2007; Hagerberg et al. 2003; Hedh et al. 2008; Hendricks et al. 2006; Kjøller 2006; Nilsson and Wallander 2003; Parrent and Vilgalys 2007; Potila et al. 2009; Wallander et al. 2010; Wallander and Thelin 2008; Wallander et al. 2001). This method has been especially useful for studying how forest management practices, such as fertilization, influence ECM fungal growth (Nilsson and Wallander 2003) and how the ECM mycelium responds to locally applied nutrient sources (Hagerberg et al. 2003; Hagerberg and Wallander 2002).

In the soil, phosphorus (P) is usually taken up from organic sources found in the surface horizon but over time the most important input of P comes from weathering processes in the mineral horizon. ECM fungi can obtain P from mineral sources (Smith and Read 2008). In the soil there are many different primary phosphate minerals; however, the most abundant is apatite and ECM fungi are able to obtain P from apatite (Wallander et al. 1997). Addition of apatite has been shown to enhance ECM fungal growth (Hagerberg et al. 2003). This effect disappears if P is added as fertilizer suggesting that apatite stimulates the ECM fungal growth

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only in P-poor forests. The stimulatory effect of apatite on ECM fungal growth is thus dependent on the P status of the tree (Wallander and Thelin 2008).

There is a limited understanding about how the ECM community structure and species richness are influenced by variation in carbon flux belowground induced by forest management practices and anthropogenic factors (Anderson and Cairney 2007; Byrd et al. 2000; Dahlberg 2001; Smith et al. 2005). Shifts in the community structure can have consequences for ecosystem services such as carbon sequestration and nutrient uptake for trees.

Representative sampling of the fungal community can be difficult due to the spatial distribution of fungi in the soil (Martin 2007). Different sampling methods will give different pictures of the community, and using only one method cannot give a complete picture of the whole community (Tóth and Barta 2010). Community analysis is now performed more frequently at the hyphal level and less frequently at the root tip and fruit body levels (Koide et al. 2007). This increased focus towards the hyphae recognizes the hyphae's importance in acquisition of nutrients, especially nitrogen (N) (Smith and Read 2008) and also phosphorus (P) (Jentschke et al. 2001). The analysis of hyphae has also been made possible with the development of new molecular techniques such as PCR and sequencing (Anderson and Cairney 2004; Dahlberg 2001; Horton and Bruns 2001; Hynes et al. 2010; Martin 2007).

Although it is well established that apatite stimulates the overall growth of ECM fungi under P-limiting conditions, less is known about how the stimulatory effect can influence the ECM community composition. A change in community composition can be expected since different ECM fungal species vary in their C demand (Alberton and Kuyper 2009) and P uptake capacity (Colpaert et al. 1999). Manipulation of carbon allocation belowground altered the composition of the ECM fungal community in beech forests (Pena et al. 2010). Furthermore, Bidartondo et al. (2001) found variable effects of apatite amendment on growth rate as well as on respiration rate among five different ECM fungal species grown in microcosms. Although P uptake was not measured in that study, the enhanced respiration rate was probably related to P mobilization and uptake. Högberg et al. (2008) showed that mycorrhizal roots that provided the host with most N from the soil were also the largest sinks for photosynthates, indicating that more nutrients provided by fungus could result in more carbon being transferred from the host. Therefore, one can assume that increased carbon allocation to the ECM fungi under P limitation will alter the community, favoring those species that acquire more P.

The influence of apatite addition on the ECM community composition has previously been studied in a P-poor forest by Hedh et al. (2008), where mesh bags were incubated in the soil for 8 months between April and November. The experiment did not show any difference in species composition between bags amended with apatite and non-amended control bags (Hedh et al. 2008). The reason for this lack of effect may be that longer time is needed for changes in the community to occur. The mesh bags are first colonized by fast-growing mycelia and fungi specialized on utilizing apatite as a P source might need more time to colonize the bags. Furthermore, if some of the early colonizers are benefitted by apatite amendment, more time than 8 months may be needed for this to be expressed in the community composition.

In the present study, we examined the long-term (5 years) effect of apatite on the fungal biomass as well as on the community composition in a P-poor spruce forest. Mesh bags were buried in pairs in the lower part of the organic horizon. After harvest, the fungal species composition was analyzed using DNA extraction, PCR of the ITS region, cloning, and random sequencing. Sequences were compared to the UNITE database (Koljalg et al. 2005) and GenBank. The fungal biomass was measured by analyzing the ergosterol content in the mesh bags.

The following hypothesis were tested:

1. Apatite will enhance ECM fungal biomass in mesh bags incubated in the soil since the forest is deficient in P.
2. The ECM fungal community in the mesh bags amended with apatite will differ from that in non-amended control bags.

Materials and methods

Experimental site

Dyneboda forest, located close to lake Immeln in the southern part of Sweden, was used for this study (56 °14′15 N 14 °22′26 E). The soil is a podzol on sandy till with a developed mor layer. The pH of the organic horizon was 2.6 after extraction of 25 g of fresh soil in 100 ml 1 M KCl in a rotary shaker. The P status of the forest is below average indicated by a suboptimal P concentration in the needles (0.82 mg g^{-1}, Thelin et al. 2002). Optimal concentration for P is 1.3 mg g^{-1} (Linder 1995; Thelin et al. 2002).

Experimental design

Mesh bags were made from nylon mesh (mesh size 50 μm) and filled with 40 g of acid-washed sand. The mesh size allowed fungal hyphae but not roots to grow into the mesh bag, as described by Wallander et al. (2001). Five milliliter

of perlite was also added to the mesh bags to increase the aeration. Each apatite-amended bag was amended with 200 mg (0.5 %) fluoroapatite ($Ca_5(PO_4)_3(OH,F,Cl)$) from Madagascar (Krantz, Germany). The rock material was crushed and sieved to obtain a fraction of 50–250 μm. This fraction was further cleaned by sonication in distilled water. The water was removed and the process repeated until the water was clear.

Ten bags with and ten bags without apatite amendment were buried pairwise in the experimental site in the fall of 2008. The bags were placed at the interface between the organic horizon and the mineral soil at an approximate depth of 5 cm. At the time of the harvest in October 2008 only six pairs were recovered. Each pair consisted of one apatite-amended and one non-amended mesh bag. Selected apatite grains from the mesh bags were analyzed by taking backscatter electron images using a Hitachi S-3400 SEM (acceleration voltage 10 kV, working distance 9 mm, vacuum 110 Pa).

Mycelium sampling and DNA extraction

Mesh bags were cut open and the contents were divided into subsamples of 10 g each and frozen to later be used for DNA extraction. Each subsample was gently shaken in a glass flask to loosen the fungal mycelia from the sand and mineral grains. This procedure was repeated until the water appeared clear and no apparent mycelia were longer visible. Collected mycelial suspensions were filtered through a nylon mesh and collected into Eppendorf tubes, freeze-dried, and stored at −80 °C.

The mycelium samples were mixed with crystalline Al_2O_3 to a total dry volume of approximately 200 μl and then ground together with glass beads in a Fast Prep® shaker (FP120, MP Biomedicals, Irvine, CA, USA). Between 50 and 100 mg of the mix was then used for DNA extraction using a CTAB buffer (3 % cetyltrimetylammonium bromide, 2 mM EDTA, and 150 mM Tris–HCl, pH 8) at 65 °C for 1 h followed by precipitation with 1.5 volume of 100 % isopropanol. The pellet was resuspended in 50 μl of MiliQ-water (Millipore).

PCR and cloning

PCR was carried out using the fungal-specific primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Templates from the DNA extraction were diluted 1:100–1:150 in MilliQ-water (Millipore). Each 50-μl PCR reaction consisted of 1× PCR buffer (Fermentas), 200 μM dNTPs, 200 nM of each primer, 2.75 mM $MgCl_2$. and 1.25 U *Dream Taq* DNA polymerase (Fermentas). The PCR was run on a Gene Amp PCR System 9700 (Applied BioSystems, Foster City, CA) and cycling conditions were 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. A final 40-min elongation step at 72 °C was added for downstream TA cloning. PCR products were purified using UltraClean PCR Clean-up Kit (MoBio Laboratories, Carlsbad, CA) and eluted in 50 μl MilliQ-water (Millipore), and the concentration was assessed using a spectrophotometer. Five out of the six pairs, ten bags in total, gave good enough PCR products to continue cloning and sequencing.

Purified PCR products were ligated into a plasmid vector pGEM-T Easy Vector kit (Promega, Madison, WI) according to the manufacturer followed by transformation into J109 competent cells (Promega, Madison, WI). Cloning products were plated on LB plates according to the manufacturer. Ninety-six positive colonies were collected from each mesh bag library sample and transferred to a 96-well Falcon ™ plate containing 150 μl 0.1× TE buffer in each well. Plates were microwaved at 800 W for 2.5 min to lyse the bacterial cells.

To sequence the cloned fragments, inserts were PCR amplified in a 10-μl PCR reaction containing 5 μl of bacterial lysate. Primers used were the universal primer SP6 (Yates-Siilata et al. 1995) (5′-TATTTAGGTGACACTTA TAG-3′) and T7 (5′-TAATACGACTCACTATAGGG-3′). The PCR chemistry and cycling conditions were identical to the above with the exception of the annealing temperature changed from 57 to 50 °C to meet Tm of the primers. As a clean-up step, PCR products were treated with a mixture containing EXO (Exonuclease I) and FastAP (FastAP Thermosensitive Alkaline Phosphatase) to remove primers and nucleotides. From this, 2 μl were used in a 10-μl sequencing reaction.

The sequencing was performed using the BigDye terminator (Applied Biosystems, Foster City, CA) according to manufacturer. The products were purified by ethanol precipitation and sequenced on an ABI3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Sequence analysis

Sequences were analyzed and manually curated using the 4Peaks software (v. 1.7) (http://mekentosj.com/science/4peaks). First, primers were removed from the sequences; second low quality ends were removed; and third sequences with low quality regions were removed. Lastly, sequences shorter than 400 bp were discarded. Sequences were checked for chimers using the UCHIME module in UCLUST v2.1 (http://drive5.com/usearch/usearch2.1.html) using the default settings. The search for sequence identities were performed using the BLAST algorithm in the UNITE database (Koljalg et al. 2005) and GenBank (ww.ncbi.nlm.nih.gov). A sequence was assorted to a

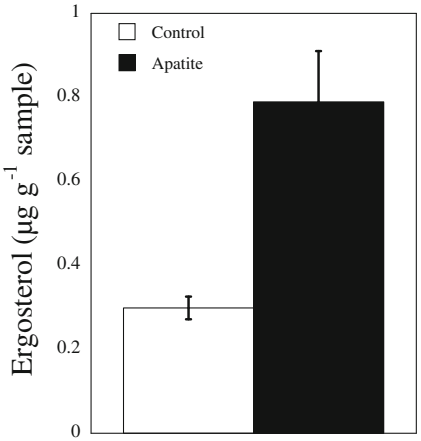

Fig. 1 Ergosterol concentration in the bags collected after 5 years. To convert ergosterol to fungal biomass conversion factors between 3 and 6 μg ergosterol per mg fungal biomass have been used (e.g., Nylund and Wallander 1992)

species when there were more than 95 % identity between query and top hit. Only sequences assigned to ECM fungi in the databases were further used in the analysis.

Ergosterol analysis

Ergosterol analysis was used to estimate the ECM fungal biomass. A subsample (5 g sand) was used to estimate the ergosterol content in each mesh bag. Ergosterol was extracted using 5 ml of 10 % KOH in methanol. The samples were sonicated for 15 min followed by incubation for 60 min at 70 °C. After cooling, 1 ml of H_2O and 2 ml of cyclohexane were added. Samples were vortexed for 1 min followed by a centrifugation for 5 min at 3,000 rpm. The hydrophobic phase was transferred to a new tube and the methanol was extracted with an additional 2 ml cyclohexane. The cyclohexane was evaporated under N_2 and samples were dissolved in 200 μl ethanol. Before the quantification of ergosterol, the samples were filtered through a 0.45-μm Teflon syringe filter (Millex LCR-4; Millipore). The chromatographic system consisted of a high-performance liquid chromatograph (Hitachi model L2130, Japan), a UV detector (Hitachi model L2400, Japan), and a C_{18} reversed phase column (Chromolith, Merck) preceded by a C_{18} reversed phase guard column (Elite LaChrome, Hitachi). Extracts were eluted with methanol at a flow rate of 1 ml min^{-1} followed by absorbance measurements at 282 nm.

Statistical analysis

Pairwise *t* tests were performed using SPSS version 19 (SPSS Inc, Chicago, IL). Each bag was considered as a replicate for each treatment. Pairwise comparisons were made both for the number of sequences and the fungal biomass by comparing apatite-amended bags against non-amended bags. Differences were considered significant when $P<0.05$.

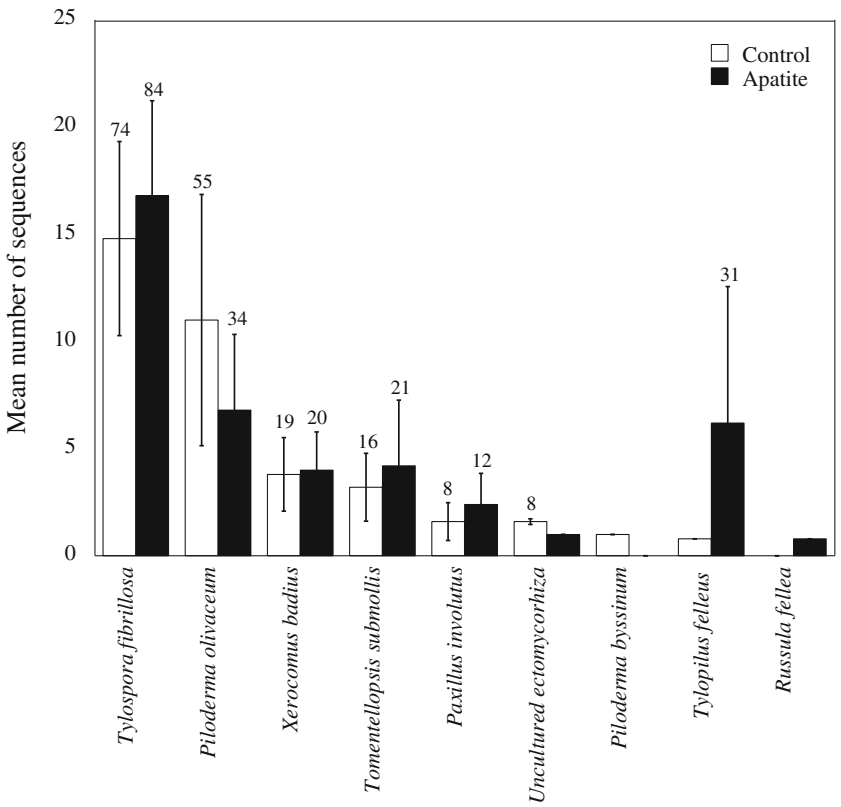

Fig. 2 Mean number of sequences from mycorrhizal species found in the apatite-amended and non-amended mesh bags if total number of sequences ≥4. The *bars* show standard error (1 S.E.). *Numbers* indicate total number of sequences if ≥6

Results

The concentration of ergosterol differed significantly between the amended and the non-amended bags ($P=0.02$) (Fig. 1); the concentration in apatite-amended bags was three times higher than in non-amended bags.

We obtained 623 sequences from cloning that met our quality criteria and could be matched to sequences from the database, on average 62±9 (mean±S.E.) sequences per bag. Out of these, 445 sequences were matched to ectomycorrhizal fungi. One hundred twenty-one sequences were matched to unknown fungal species. Fifty-seven sequences were identified as non-ECM fungal species. This means that at least 71 % of the sequences were of known ECM fungal origin. ECM fungal species belonged to the genera *Tylospora*, *Piloderma*, *Xerocomus*, *Tomentellopsis*, *Paxillus*, *Tylopilus*, and *Russula*. The most abundant species found in both control and apatite-amended bags were *Tylospora fibrillosa* and *Piloderma olivaceum*. A paired *t* test was performed to compare differences in overall species composition, as well as fungal biomass, between amended and non-amended mesh bags. Overall there was no significant difference in community composition between apatite-amended bags and non-amended bags ($P=0.70$). Apatite-amended bags showed a higher amount of sequences from *Tylopilus felleus* compared with non-amended bags (Fig. 2), although the difference was not significant.

Some apatite grains were severely weathered while others looked more or less intact, even in the same mesh bags. It was therefore not possible to determine the role of the fungus in the process, but close contact between fungal hyphae and apatite grains was commonly found (Fig. 3).

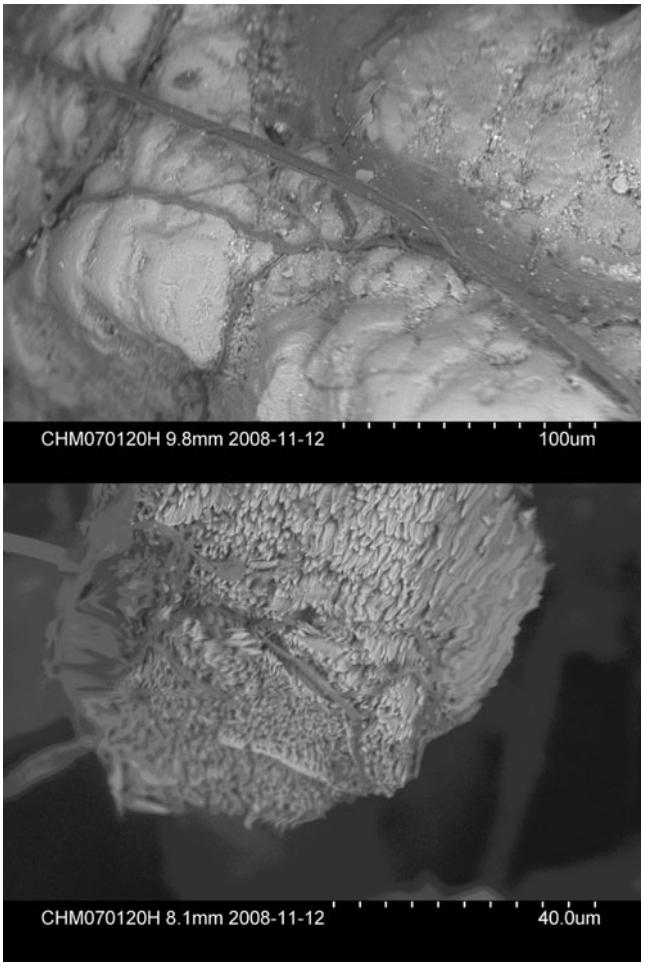

Fig. 3 Electron microscope images showing apatite grains with attached hyphae

Discussion

Our first hypothesis that apatite-amended bags would have a higher fungal in-growth than non-amended bags in P-poor forests was confirmed since apatite-amended bags showed a significantly higher fungal biomass compared to non-amended control bags (Fig. 1). Hagerberg et al. (2003) found a similar effect of apatite amendment after 1 or 2 years in the same site. Our values are however considerably higher than the ones in Hagerberg et al. (2003) which suggests that longer incubation time results in higher fungal biomass, especially when the mesh bags are amended with apatite. To get stimulation of ECM fungal growth by apatite, a certain amount of time seems to be necessary. Potila et al. (2009), found a higher fungal biomass in apatite-amended mesh bags after 16 months but not after 4 months (Potila et al. 2009), while Hedh et al. (2008) did not find increased ECM fungal growth in apatite-amended mesh bags after 8 months, though in their case the forest was probably not P limited.

As for our second hypothesis that addition of apatite would cause a change in species composition, there was no significant difference in the overall species composition between amended and non-amended mesh bags. This suggests that even though the fungal growth increased threefold, no specific species seemed to benefit from this at the expense of other species. In a recent paper by Turpault et al. (2009), dissolution of apatite was not stimulated by the presence of roots and their associated fungi when the apatite was placed in the topsoil (2–10 cm). However, when the apatite was placed deeper in the soil (20 cm), a small but significant enhancement of apatite dissolution was found when mycorrhizal roots had access to the apatite (Turpault et al. 2009). It is possible that a different result would have been obtained, also in our study, if the mesh bags were placed deeper in the soil. ECM fungi active in apatite weathering may proliferate especially in the B horizon where the weathering of minerals has been less intense than in the E horizon and consequently more apatite remains.

In this study, we see increased fungal biomass in apatite-amended bags but no effect on the ECM fungal community. This is somewhat surprising given that Bidartondo et al. (2001) found 2.5-fold increases in growth of extramatrical mycelium of some ECM fungal species (*Rhizopogon*) after apatite amendment while other species did not respond at all (*Suillus pungens*).

It is important to consider that mesh bags provide a non-exploited area in the soil, which will select for explorative types of ECM fungi that form large mycelia. It is not known to what extent these ECM fungal species respond more or less to apatite amendment compared to other species. Species belonging to, e.g., *Russula* and *Lactarius* for instance are less likely to colonize the bags, although such species can still be important for P uptake from apatite. The most common species found by Hedh et al. (2008) and Wallander et al. (2010) were also found in our mesh bags. However, *Piloderma* were detected in much higher abundance in our study compared to previous studies (Hedh et al. 2008; Wallander et al. 2010). This is probably because *Piloderma* needs longer time to colonize an area already occupied by other fungal species and in turn outcompete these fungal species (Erland and Finlay 1992; Wu et al. 1999). In contrast, *Cortinarius* species which are also in low abundance in mesh bags compared to root tips (Kjøller 2006) were not detected in our study, probably because many *Cortinarius* species avoid mineral substrates since they are more involved in utilizing nutrients from organic sources (Taylor et al. 2000).

In conclusion, the addition of apatite to mesh bags in a P-poor forest for 5 years was associated with a threefold increase of ECM fungal biomass (Fig. 1) but no difference in species composition (Fig. 2). Some of the mineral grains showed strong signs of weathering (Fig. 3), but it was not possible to relate these signs to the extent of fungal colonization. All ECM fungal species benefitted from the apatite amendment probably because of the enhanced P availability, but it was not possible to determine to what extent ECM fungi stimulated dissolution of apatite.

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References

Alberton O, Kuyper TW (2009) Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: implications for ecosystem responses to global change. Global Change Biol 15(1):166–175

Anderson IC, Cairney JWG (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ Microbiol 6(8):769–779

Anderson IC, Cairney JWG (2007) Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS Microbiol Rev 31(4):388–406

Bending GD, Read DJ (1995) The structure and function of the vegetative mycelium of ectomycorrhizal plants. 5. Foraging behavior and translocation of nutrients from exploited litter. New Phytol 130(3):401–409

Bidartondo MI, Ek H, Wallander H, Söderström B (2001) Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? New Phytol 151(2):543–550

Boström B, Comstedt D, Ekblad A (2007) Isotope fractionation and C-13 enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153(1):89–98

Byrd KB, Parker VT, Vogler DR, Cullings KW (2000) The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. Canadian Journal of Botany-Revue Canadienne De Botanique 78(2):149–156

Colpaert JV, van Tichelen KK, van Assche JA, Van Laere A (1999) Short-term phosphorus uptake rates in mycorrhizal and non-mycorrhizal roots of intact *Pinus sylvestris* seedlings. New Phytol 143(3):589–597

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytol 150(3):555–562

Erland S, Finlay R (1992) Effects of temperature and incubation time on the ability of 3 ectomycorrhizal fungi to colonize *Pinus sylvestris* roots. Mycol Res 96:270–272

Gardes M, Bruns TD (1993) Its primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2(2):113–118

Hagerberg D, Wallander H (2002) The impact of forest residue removal and wood ash amendment on the growth of the ectomycorrhizal external mycelium. FEMS Microbiol Ecol 39(2):139–146

Hagerberg D, Thelin G, Wallander H (2003) The production of ectomycorrhizal mycelium in forests: relation between forest nutrient status and local mineral sources. Plant Soil 252(2):279–290

Hedh J, Wallander H, Erland S (2008) Ectomycorrhizal mycelial species composition in apatite amended and non-amended mesh bags buried in a phosphorus-poor spruce forest. Mycol Res 112:681–688

Hendricks JJ, Mitchell RJ, Kuehn KA, Pecot SD, Sims SE (2006) Measuring external mycelia production of ectomycorrhizal fungi in the field: the soil matrix matters. New Phytol 171(1):179–186

Högberg P, Högberg MN, Gottlicher SG, Betson NR, Keel SG, Metcalfe DB, Campbell C, Schindlbacher A, Hurry V, Lundmark T, Linder S, Nasholm T (2008) High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. New Phytol 177(1):220–228

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10 (8):1855–1871

Hynes MM, Smith ME, Zasoski RJ, Bledsoe CS (2010) A molecular survey of ectomycorrhizal hyphae in a California *Quercus–Pinus* woodland. Mycorrhiza 20(4):265–274

Jentschke G, Brandes B, Kuhn AJ, Schröder WH, Godbold DL (2001) Interdependence of phosphorus, nitrogen, potassium and magnesium translocation by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol 149(2):327–337

Kjøller R (2006) Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia. FEMS Microbiol Ecol 58 (2):214–224

Koide RT, Courty PE, Garbaye J (2007) Research perspectives on functional diversity in ectomycorrhizal fungi. New Phytol 174 (2):240–243

Koljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166(3):1063–1068

Leake JR, Donnelly DP, Saunders EM, Boddy L, Read DJ (2001) Rates and quantities of carbon flux to ectomycorrhizal mycelium following C-14 pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. Tree Physiol 21(2–3):71–82

Linder S (1995) Foliar analysis for detecting and correcting nutrient imbalances in Norway spruce. Ecol Bull 44:178–190

Martin KJ (2007) Introduction to molecular analysis of ectomycorrhizal communities. Soil Sci Soc of Am J 71(2):601–610

Nilsson LO, Wallander H (2003) Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. New Phytol 158 (2):409–416

Nylund JE, Wallander H (1992) Ergosterol analysis as a means of quantifying mycorrhizal biomass. Methods Mircrobiol 24:77–88

Parrent JL, Vilgalys R (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. New Phytol 176(1):164–174

Pena R, Offermann C, Simon J, Naumann PS, Gessler A, Holst J, Dannenmann M, Mayer H, Kögel-Knabner I, Rennenberg H, Polle A (2010) Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl Environ Microb 76(6):1831–1841

Potila H, Wallander H, Sarjala T (2009) Growth of ectomycorrhizal fungi in drained peatland forests with variable P and K availability. Plant Soil 316(1–2):139–150

Simard S, Jones M, Durall D (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijed M, Sander I (eds) Mycorrhizal ecology. Springer, Berlin, pp 33–74

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Smith JE, McKay D, Brenner G, McIver J, Spatafora JW (2005) Early impacts of forest restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a mixed conifer forest. J Appl Ecol 42(3):526–535

Taylor AFS, Martin F, Read DJ (2000) Fungal diversity in ectomycorrhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along North-South transects in Europe. In: Schulze E-D (ed) Ecological Studies, vol 142. Springer-Verlag, Heidelberg, pp 343–365

Thelin G, Rosengren U, Nihlgård B (2002) Barrkemi på Skånska Gran- och Tallprovytor. Rapport 20. County administrative board of Skåne:1-35

Tóth BB, Barta Z (2010) Ecological studies of ectomycorrhizal fungi: an analysis of survey methods. Fungal Diversity 45 (1):3–19

Turpault M-P, Nys C, Calvaruso C (2009) Rhizosphere impact on the dissolution of test minerals in a forest ecosystem. Geoderma 153 (1–2):147–154

Wallander H, Thelin G (2008) The stimulating effect of apatite on ectomycorrhizal growth diminishes after PK fertilization. Soil Biol & Biochem 40(10):2517–2522

Wallander H, Wickman T, Jacks G (1997) Apatite as a P source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Plant Soil 196(1):123–131

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol 151(3):753–760

Wallander H, Johansson U, Sterkenburg E, Durling MB, Lindahl BD (2010) Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. New Phytol 187 (4):1124–1134

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Innis, M A, et al. (ed) PCR protocols: a guide to methods and applications XVIII+482p Academic, San Diego: 315-322

Wu B, Nara K, Hogetsu T (1999) Competition between ectomycorrhizal fungi colonizing *Pinus densiflora*. Mycorrhiza 9 (3):151–159

Yates-Siilata KE, Sander DM, Keath EJ (1995) Genetic diversity in clinical isolates of the dimorphic fungus blastomyces-dermatitidis detected by a PCR-based random amplified polymorphic DNA assay. J Clin Microbiol 33(8):2171–2175