Ann. For. Sci. 67 (2010) 200
DOI: 10.1051/forest/2009107
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Letter to the editor

A leap forward in geographic scale for forest ectomycorrhizal fungi

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(Received 30 August 2009; accepted 18 November 2009)

Keywords: biogeography / ICP Forests / function / mycorrhizas / symbiosis

In this letter we propose a first large-scale assessment of mycorrhizas with a European-wide network of intensively monitored forest plots as a research platform. This effort would create a qualitative and quantitative shift in mycorrhizal research by delivering the first continental-scale map of mycorrhizal fungi. Readers may note that several excellent detailed accounts of ectomycorrhizal biology, including its methodological and conceptual strengths and weaknesses, are available elsewhere (e.g., Peay et al., 2008; Smith and Read, 2008; Taylor, 2002). Although we focus on a specific group of fungi in this letter, many of the methods and ideas discussed could be extended to include communities of other groups of forest fungi, such as saprobes and pathogens.

Ectomycorrhizal (ECM) fungi form intimate symbioses with most temperate and boreal tree species, playing a pivotal functional role in the uptake of nutrients, as well as providing protection against pathogens and drought (Baxter and Dighton, 2005). In exchange, the tree host supplies ca. 15% of its current photoassimilate to the fungi (Hobbie and Hobbie, 2006; Smith and Read, 2008). The diversity of ECM fungi is high, with estimates of over 7 000 species worldwide, associating with approximately 8 000 plant species (Rinaldi et al., 2008; Wang and Qiu, 2006), leading to research efforts into the degree of functional diversity within ECM fungi. It is known, for example, that different species of ECM fungi can access alternative nitrogen sources (Lilleskov et al., 2002b), and that certain ECM species may play a role in the decomposition of organic carbon (Chapela et al., 2001); some fungi confer more benefit to their host than others under certain environmental conditions (Gorissen and Kuyper, 2000; Johnson et al., 1997), and different species of fungi can have different carbon requirements from the host (Bidartondo et al., 2001; Fransson et al., 2007). Therefore, the biodiversity of ECM fungi in any given forest is likely to have direct implications for host tree health, carbon and nitrogen cycling, and the resiliency of forest functional biodiversity. Because forests are major players in the sequestration of atmospheric carbon, it is critical to understand the processes acting to maintain forest ecosystem function. To gain an understanding of factors that influence the mycorrhizal communities of forests, studies have used both natural gradients and manipulative experiments, demonstrating that pH, soil type, moisture, host tree species and nitrogen availability, amongst others, are determinants of mycorrhizal communities at local scales (e.g. Børja and Nilsen, 2009; Gehring et al., 1998; Kauserud et al., 2008; Lilleskov et al., 2002a).

Given the observed responsiveness of mycorrhizal fungi to shifts in environmental variables and global environmental change, it is of immediate concern to know how mycorrhizal communities will respond to environmental shifts at the regional and continental scale, and what effects such shifts will have on forest health and function. This task requires knowledge about current mycorrhizal distributions, both to provide a baseline against which to measure changes in communities, and to understand the effect of broad-scale environmental conditions (e.g., annual temperature, latitude, rainfall) on mycorrhizal biogeography. Whilst it would be convenient to extract these data from the numerous investigations of local ECM fungal communities carried out so far, the diversity of sampling methodologies employed makes this an impossible task. A primary disparity is that much research has relied upon morphology of reproductive structures (fruitbodies) and/or mycorrhizal roots to establish fungal identity, whilst more recent studies tend to apply various DNA techniques to mycorrhizas to identify fungi, posing problems when comparing datasets

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based on the two different methodologies (Gardes and Bruns, 1996; Horton and Bruns, 2001). In addition, most studies do not collect environmental data in a consistent fashion, making incorporation of data from multiple studies into a single predictive model extremely challenging.

Methodological inconsistencies aside, the responses of mycorrhizal communities to changes in environmental conditions appear to be complex, and it is difficult to confidently identify trends across studies. This is probably due to the localized nature and constrained time-scale at which most studies have been carried out, making results highly context-dependent. Particular site characteristics, pre-existing ECM fungi, and the scale of environmental change being studied, may all play a part in generating the complex picture of ECM fungal responses to specific environmental changes we see when comparing studies. It was in this context that Lilleskov and Parrent (2007) recently called for the development of a large-scale, unified approach to ectomycorrhizal research, which would allow the distributions of individual species of ECM fungi to be mapped. Here we identify a specific research platform and strategy aimed at meeting this challenge, through the utilisation of one of the largest biomonitoring networks on Earth.

ICP FORESTS – AN INVALUABLE PLATFORM FOR LARGE-SCALE RESEARCH

The International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests, http://www.icp-forests.org) was launched in 1985. The programme is operated under the United Nations Economic Commission for Europe Convention on Long-Range Transboundary Air Pollution that is ratified by 51 parties. The over-arching aim of ICP Forests is to understand the links between forest health, air pollution, climate change and biodiversity, through the establishment and monitoring of two complementary networks of forest plots. The first of these networks (called Level I) includes over 6 000 forest plots, set-up on a 16 × 16 km grid across Europe, each monitored for tree crown condition (defoliation, discoloration and visible damage). Many Level I plots have been extended to undergo more extensive monitoring as part of the BioSoil program, which aims to develop a common baseline of European forest soils, as well as assessing forest biodiversity. A further subset of 800 strategically located plots (Level II) forms an intensive continual monitoring system. Here, additional measurements include tree growth and phenology, ground vegetation, atmospheric deposition, soil chemistry, soil solution chemistry, foliar nutrient levels, and water balances. Expert panels are in place to ensure the harmonisation of methods used in the field, and quality assurance and control in laboratories. These networks cover the major tree species in Europe, and involve the participation of 41 countries.

The ICP Forests network provides mycorrhizal researchers with a huge and largely un-tapped resource on which to conduct a large-scale assessment of distributions, measure responses to environmental gradients, and link community composition directly to forest ecosystem function. The intensively monitored Level II plots of the ICP Forests programme confer a number of advantages: (i) pre-established and maintained plots enable and facilitate project design, (ii) access to an existing, large, and harmonized forest monitoring dataset, (iii) access to information on site history, management practices and disturbances, allowing individual studies to control for, or take into account, specific inter-site variables, and (iv) a long-term perspective to detect, and validate predictions for, future change.

METHODOLOGICAL CONSIDERATIONS

Selection of sites across the ICP network can be randomized, or based on a statistically rigorous algorithm that stratifies sampling across the variables hypothesized to control fungal distribution at multiple scales. Ensuring adequate sampling across these variables can lay the foundation for rigorous modelling of species- and community-environment relationships to serve as the basis for predictions of current and future mycorrhizal communities across Europe.

Reliable across-site comparisons are difficult when sampling is not of sufficient intensity to capture species diversity (Peay et al., 2008; Taylor, 2002). Before launching into a large-scale study of ECM communities in forests plots, it would be ideal to first develop a standardised sampling design which maximises the chance of fully capturing ECM species diversity within plots, and this will likely require iterative fine-tuning as the project develops. Preliminary data on the number of samples required, and the scale at which samples should be taken to ensure independence, are currently available for pine, beech, oak, and spruce plots (F. Cox, unpublished data).

Identification of fungi colonising roots, versus fungi present in the soil as hyphae, can give contrasting views of the ECM fungal community (Koide et al., 2005), and the collection of both can provide a better overall picture of mycorrhizal diversity than one method in isolation of the other. High-throughput molecular ecology techniques would be essential for generating data with the speed and efficacy required for such a large study, and novel techniques such as pyrosequencing (e.g., Buee et al., 2009) may complement this approach, particularly if and when sample throughput increases so that bulking of roots and/or soil can be avoided. These technologies are in their infancy, and sampling methodologies still need to be validated to ensure accurate characterisation of fungal communities. For example, a recent study has shown that current methods of pooling samples can lead to inaccurate fungal community descriptions (Avis et al., 2009). Level II sites in which the ECM fungal community have previously been well characterised using Sanger sequencing of individual root tips, may offer a useful baseline upon which to validate new methodologies associated with these techniques.

Species delimitation of ECM fungi can be standardized initially by relying on nuclear ribosomal internal transcribed spacer DNA sequence similarity cut-offs to provide reproducible estimates of species boundaries (Nilsson et al., 2008), whilst multi-locus analyses, taxonomic collections and expertise will be useful when linking species names to DNA sequences (Brock et al., 2009; Hedh et al., 2008). The gradual

development of a publicly accessible online data-base of ECM fungal distributional data, and DNA sequence alignments of closely related taxa, would be an exceptionally useful outcome of this research for the scientific community.

IMMEDIATE RESEARCH OUTCOMES FROM A CONTINENTAL ASSESSMENT

A continental-scale survey of ECM fungi would produce immediate research outputs, as well as providing data key to the development of local and regional scale research that is ecologically relevant and hypothesis-driven. Carrying out a large-scale assessment of ECM fungi in plots throughout Europe would dramatically improve our understanding of the factors that drive function and biodiversity of mycorrhizal trees and fungi in a number of ways (Fig. 1, Phase 1).

The primary benefit of distribution data would be the ability to identify patterns in ECM community composition in forests across geographic regions, and link this to broad environmental variables recorded at each sampling location. With these data, it should be possible to predict mycorrhizal communities in un-sampled European forests as a result of site characteristics via GIS layers, enabling the mapping of current fungal distributions across the continent. Whilst predicting the distributions of infrequent species that constitute the major part of fungal diversity will prove challenging, realistic predictions for dominant taxa should be achievable, and they would represent a test of the power of predictive mapping. A baseline map, combined with information about dynamic responses to changing environmental conditions, can also allow the prediction of future distributions of dominant species as a result of changes in climate, atmospheric pollution and land use (Ellis et al., 2007).

Currently, mycorrhizal species ranges based on fruitbody collections have been described for some ECM fungal species at national scales (e.g., Courtecuisse et al., 2008). However, these data are lacking for the majority of species, do not take into account below-ground presence, and are poorly "harmonizable" over the continental scale due to differing morphospecies concepts. Through DNA sequence based distribution maps, we could infer the extent to which geographic dispersal constraints act on mycorrhizal fungal species distributions, and how these differ among species, higher taxonomic levels, and functional guilds.

Continental scale distribution data would also allow us to identify the most abundant and widespread mycorrhizal fungi across Europe, providing a prioritized list of fungal species to target as models for future research (e.g., genetics, ecology, physiology, genomics), increasing its applicability and relevance.

Linking environmental and mycorrhizal community data will allow the effects of variables such as temperature, rainfall, and soil type on the community assemblage of ECM fungi to be tested. These data would identify broad niches for individual species (host type, soil type, climatic constraints). In particular, we would be able to extend and test existing knowledge on mycorrhizal specialists and generalists (e.g., Molina et al. 1992) and edaphic endemics. In addition, we would test and identify fungi sensitive to particular agents of pollution, such as atmospheric N deposition and oxidants. It may also be possible for mycorrhizal community data to be linked directly to data on the composition of ground flora and environmental characteristics. Although such inferences would be limited by the power of regression and correlation analyses, they can still point the way forward for experiments designed to provide a mechanistic understanding of fungus-environment interactions.

Analysis of mycorrhizal diversity across ICP Forests plots would allow us to identify geographic areas with the greatest diversity, as well as areas of endemism. These mycorrhizal "hotspots" can then be the target for habitat conservation action to maintain soil biodiversity. In combination with environmental and geographic data, we should be able to model the factors that are likely contributors to hotspot generation, leading to predictions of hotspots elsewhere.

Although mycorrhizal fungi are less understood compared to other large perennial organisms, there is nevertheless evidence of a sharp decline in the reproductive output of some mycorrhizal fungi in polluted regions of Europe (Arnolds, 1991). Currently, declining, "Red list" and locally extinct mycorrhizal fungi are assessed and monitored only through their sporadic and ephemeral reproductive structure production. This information does, however, constitute a warning signal to uncover their distribution and source/sink dynamics belowground at the continental scale. Reliable large-scale distribution data would allow the conservation status of individual species to be assessed – species not previously considered rare may be identified as targets for conservation activities, and vice-versa. By sampling a large geographic area belowground, we will also have the opportunity to improve our imprecise estimates of the total number of ECM fungal species (Rinaldi et al. 2008), and discover what proportion have not been taxonomically described to date.

Lessons learnt from studies like these would undoubtedly inform work on communities of other groups of forest fungi which also play pivotal roles in forest vitality, and could provide basic knowledge to help design and carry out studies on general fungal biogeography.

SMALLER-SCALE RESEARCH FACILITATED BY DISTRIBUTIONAL DATA

A European survey of forest mycorrhizas is imperative to the development of further research and model systems that are focused, hypothesis-driven, and ecologically relevant. There are a number of key areas of ecosystem and mycorrhizal research that would be directly informed by a European survey in ICP Forests plots (Fig. 1, Phase 2).

The identification of dominant mycorrhizal species by indicator species analyses would be a first step for many potential future studies. The development of species-specific primers would permit the quantification of ECM in both roots and soils of plots known to contain the mycorrhizal species of interest. Dominant fungal taxa could also be targeted for the next sequencing programs of fungal genomes, which would provide

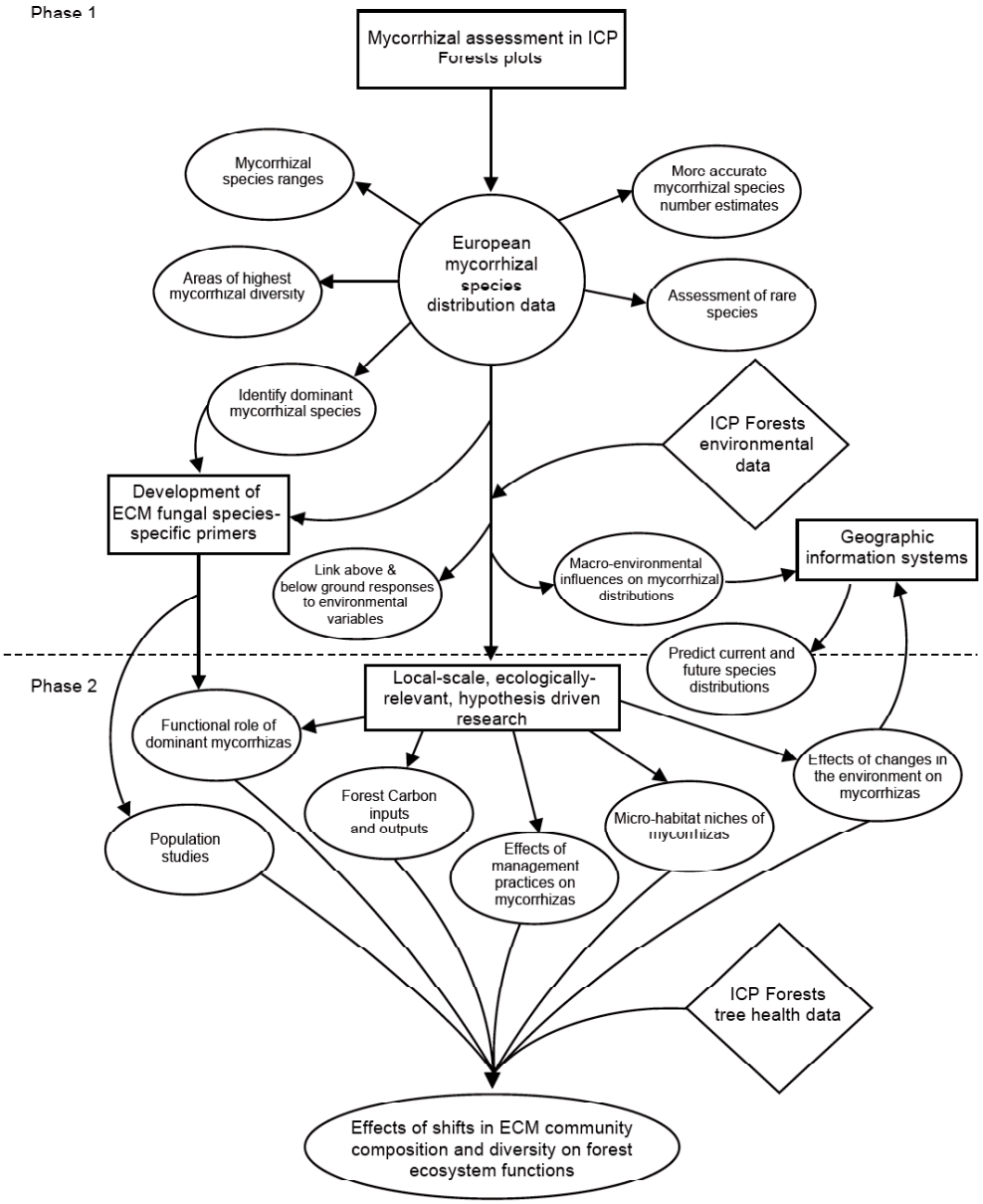


Figure 1. The potential research outputs of a European-wide survey of mycorrhizal fungi in biomonitoring plots. Rectangles represent a research effort, ovals indicate a research output and diamonds indicate an instance when data gathered from ICP Forest plots would enhance research efforts. Phase 1: Whilst the primary research achievement would be European-wide distributional data for mycorrhizal fungi, a number of immediate secondary research outputs would also be generated. Combining the mycorrhizal fungal survey data with environmental data would be critical to the development of Phase 2, which focuses on ecologically-relevant and hypothesis-driven research at smaller scales. Combining these data with tree health and environmental data can pinpoint key links between shifts in mycorrhizal fungal community composition and forest ecosystem health. ICP: The International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests.

genetics markers to gain some of the first insights into the population structure, recombination levels, genet size and overlap, as well as the extent and direction of gene-flow and dispersal between geographically separated populations (e.g., Kretzer et al., 2004).

The importance of mycorrhizal fungi as carbon sinks in forest ecosystems (Högberg et al., 2001; Smith and Read, 2008), and the potential for carbon demand to change due to elevated CO_2 and elevated nitrogen availability (Alberton and Kuyper, 2009; Bidartondo et al., 2001), makes it necessary to understand the role of mycorrhizal fungi in global carbon and nitrogen dynamics. A critical area for research will be whether evenness shifts, replacement of dominants, and allocation to root colonisation *vs.* soil colonisation as a result of environmental change will alter the carbon sink potential of forest ecosystems.

Pre-characterised mycorrhizal communities in ICP Forests plots would provide an unrivalled platform for experimental manipulations, such as nitrogen fertilisation. By selecting sites with similar initial mycorrhizal species composition, treating a subset of these sites, and then comparing the degree and direction of any changes in the mycorrhizal communities between treatment and control groups, the effects of elevated N availability could be ascertained at a larger scale, and in a more informed, replicated and controlled way, than has ever been possible.

Finally, assessing micro-scale habitats for a range of factors (e.g., soil pH, moisture, organic matter, coarse woody debris, nutrient availability), should allow correlations between the presence and/or abundance of mycorrhizal species and specific environmental conditions. When conducted at several sites, robust inferences about niches of individual fungi can be made, with implications for forest management practices (e.g., removal of brash and other deadwood) that alter the availability of micro-niche sites.

CONCLUSION

The functionally critical role of mycorrhizal fungi in forest ecosystems, and the imminent threat of climate change that may act to alter mycorrhizal functional biodiversity, means there is an urgent need for a regional to continental-scale assessment of mycorrhizal distributions. Until recently, it had not been possible to cost-effectively assess mycorrhizas precisely and accurately. A large-scale survey of ICP Forests plots would be only the first stage in answering many of the questions outlined above, but it is essential if future studies are going to address these questions with hypothesis-driven research in a cohesive manner, rather than remain independent for lack of a unified approach. The chance to utilise the vast network of biomonitoring plots at this time is a remarkable opportunity because it minimises the logistics and costs associated with achieving such an enormous effort and provides a rare stable – past and future – ground for forest ecosystem scientific investigation. In the face of rapid global change, we finally have an opportunity to accurately integrate mycorrhizal distribution data with long-term environmental monitoring, providing a basic understanding of functionally crucial organisms, and at the same time creating an invaluable resource for future research.

Acknowledgements: We would like to thank the NERC Centre for Population Biology and the Alice Holt Research Centre (Forest Research) for hosting the symposium from which this work stems; and the two anonymous reviewers for their helpful comments. This work was supported by a NERC Case Studentship (NER/S/A/2006/14012), and Forest Research's Chief Executive Fund.

REFERENCES

Alberton O. and Kuyper T.W., 2009. Ectomycorrhizal fungi associated with *Pinus sylvestris* seedlings respond differently to increased carbon and nitrogen availability: Implications for ecosystem responses to global change. Glob. Change Biol. 15: 166–175.

Arnolds E., 1991. Decline of ectomycorrhizal fungi in Europe. Agric. Ecosyst. Environ. 35: 209–244.

Avis P.G., Branco S., Tang Y., and Mueller G.M., 2009. Pooled samples bias fungal community descriptions. Mol. Ecol. Resour. Doi: 10.1111/j.1755-0998.2009.02743.x.

Baxter J.W. and Dighton J., 2005. Diversity-functioning relationships in ectomycorrhizal fungal communities. In: Dighton J., White J.F., and Oudemans P. (Eds.) The fungal community: Its organization and role in the ecosystem, Marcel Dekker, New York, pp. 383–398.

Bidartondo M.I., Ek H., Wallander H., and Söderström B., 2001. Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? New Phytol. 151: 543–550.

Børja I. and Nilsen P., 2009. Long term effect of liming and fertilization on ectomycorrhizal colonization and tree growth in old scots pine (*Pinus sylvestris* L.) stands. Plant Soil 314: 109–119.

Brock P.M., Döring H., and Bidartondo M.I., 2009. How to know unknown fungi: the role of a herbarium. New Phytol. 181: 719–724.

Buee M., Reich M., Murat C., Morin E., Nilsson R.H., Uroz S., and Martin F., 2009. 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol. 184: 438–448.

Chapela I.H., Osher L.J., Horton T.R., and Henn M.R., 2001. Ectomycorrhizal fungi introduced with exotic pine plantations induce soil carbon depletion. Soil Biol. Biochem. 33: 1733–1740.

Courtecuisse R., Moreau P.-A., and Daillant O., 2008. Suivi de la flore fongique : une énorme diversité difficile à mesurer – Partenariat avec les sociétés mycologiques de France. In: 15 Ans de suivi des écosystèmes forestiers, Hors Série no. 4, Rendez-Vous Techniques, Office National des Forêts, pp. 99–102.

Ellis C.J., Coppins B.J., Dawson T.P., and Seaward M.R.D., 2007. Response of British lichens to climate change scenarios: Trends and uncertainties in the projected impact for contrasting biogeographic groups. Biol. Conserv. 140: 217–235.

Fransson P.M.A., Anderson I.C., and Alexander I.J., 2007. Does carbon partitioning in ectomycorrhizal pine seedlings under elevated CO_2 vary with fungal species? Plant Soil 291: 323–333.

Gardes M. and Bruns T.D., 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: Above- and below-ground views. Can. J. Bot. 74: 1572–1583.

Gehring C.A., Theimer T.C., Whitham T.G., and Keim P., 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in environmental extremes. Ecology 79: 1562–1572.

Gorissen A. and Kuyper T.W., 2000. Fungal species-specific responses of ectomycorrhizal scots pine (*Pinus sylvestris*) to elevated [CO_2]. New Phytol. 146: 163–168.

Hedh J., Samson P., Erland S., and Tunlid A., 2008. Multiple gene genealogies and species recognition in the ectomycorrhizal fungus *Paxillus involutus*. Mycol. Res. 112: 965–975.

Hobbie J.E. and Hobbie E.A., 2006. N^{15} in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. Ecology 87: 816–822.

Högberg P., Nordgren A., Buchmann N., Taylor A.F.S., Ekblad A., Högberg M.N., Nyberg G., Ottosson-Löfvenius M., and Read D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411: 789–792.

Horton T.R. and Bruns T.D., 2001. The molecular revolution in ectomycorrhizal ecology: Peeking into the black-box. Mol. Ecol. 10: 1855–1871.

Johnson N.C., Graham J.H., and Smith F.A., 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol. 135: 575–586.

Kauserud H., Sige L.C., Vik J.O., Økland R.H., Høiland K., and Stenseth N.C., 2008. Mushroom fruiting and climate change. Proc. Natl. Acad. Sci. USA 105: 3811–3814.

Koide R.T., Xu B., and Sharda J., 2005. Contrasting below-ground views of an ectomycorrhizal fungal community. New Phytol. 166: 251–262.

Kretzer A., Dunham S., Molina R., and Spatafora J.W., 2004. Microsatellite markers reveal the below ground distribution of genets in two species of rhizopogon forming tuberculate ectomycorrhizas on Douglas fir. New Phytol. 161: 313–320.

Lilleskov E.A., Fahey T.J., Horton T.R., and Lovett G.M., 2002a. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83: 104–115.

Lilleskov E.A., Hobbie E.A., and Fahey T.J., 2002b. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytol. 154: 219–231.

Lilleskov E.A. and Parrent J.L., 2007. Can we develop general predictive models of mycorrhizal fungal community-environment relationships? New Phytol. 174: 250–256.

Molina R., Massicotte H., and Trappe J.M., 1992. Specificity phenomena in mycorrhizal symbioses: Community-ecological consequences and practical implications. In: Allen M.F. (Ed.), Mycorrhizal functioning: an integrative plant-fungal process, New York, Chapman and Hall, pp. 357–423.

Nilsson R.H., Kristiansson E., Ryberg M., Hallenberg N., and Larsson K.H., 2008. Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. Evol. Bioinform. Online 4: 193–201.

Peay K.G., Kennedy P.G., and Bruns T.D., 2008. Fungal community ecology: A hybrid beast with a molecular master. Bioscience 58: 799–810.

Rinaldi A.C., Comandini O., and Kuyper T.W., 2008. Ectomycorrhizal fungal diversity: Separating the wheat from the chaff. Fungal Divers. 33: 1–45.

Smith S.E. and Read D.J., 2008. Mycorrhizal symbiosis. Academic Press, London.

Taylor A.F.S., 2002. Fungal diversity in ectomycorrhizal communities: Sampling effort and species detection. Plant Soil 244: 19–28.

Wang B. and Qiu Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299–363.