Symbiosis, 37 (2004) 249–257
Balaban, Philadelphia/Rehovot

Review article.

Do Ectomycorrhizal Fungi have a Significant Role in Weathering of Minerals in Forest Soil?

HÅKAN WALLANDER* and DAVID HAGERBERG
Department of Microbial Ecology, Lund University, 223 62 Lund, Sweden,
Email. Hakan.Wallander@mbioekol.lu.se

Received October 19, 2003; Accepted January 14, 2004

Abstract

Ectomycorrhizal (EM) fungi are known to colonize minerals in forest soil, and in many laboratory experiments it has been confirmed that EM fungi stimulate dissolution of minerals such as apatite, biotite and feldspars. However, due to the low number of experiments performed in the field, and in forests with different soil fertilities, it is difficult to conclude whether this effect has any ecological significance for the overall cycling of nutrients in forest soils. A key question is to what extent EM-induced weathering can compensate for a developing nutrient deficiency situation by increasing dissolution of certain minerals in the soil. We have used ingrowth mesh bags amended with various minerals to study the interaction between EM fungi and minerals. Our results so far indicate that EM fungi were stimulated by the phosphorus (P) containing mineral apatite in a forest with low P status but not in a forest with adequate P. This could be either an effect of an increased allocation of carbon to external mycelium within each fungal taxa or a result of a changed EM community towards species that produce more external mycelium. Furthermore, the EM-induced dissolution of the apatite was more intense in the forests with low P status. We used rare earth elements as marker elements for quantifying transport from the mineral to the ectomycorrhizal roots. In contrast we found no indication that EM mycelia interacted with the potassium (K) containing mineral biotite whether in forests with deficient K or adequate K supply.

Presented at the 4th International Symbiosis Congress, August 17–23, 2003, Halifax, Canada

*The author to whom correspondence should be sent.

0334-5114/2004/$05.50

To confirm these results we suggest that future studies include a larger number of sites and investigate the influence of nutrient status of the trees on EM induced weathering. Such studies will increase our understanding of how forests will respond to a change from nitrogen (N) limitation to limitation by other nutrients such as P or K, a potential consequence of nutrient removal through intensified biomass harvesting and excessive N availability through anthropogenic deposition.

Keywords: Carbon allocation, mycorrhiza, nutrient deficiency, phosphorous, potassium, rare earth elements

1. Introduction

Ectomycorrhizal (EM) fungi form symbioses with forest trees. This relationship evolved when organic-matter began to accumulate in certain soils 200 million years ago. The tree host benefits through improved nutrient acquisition, since the fungus explores the soil efficiently for nutrients (especially N and P) in return for host carbon (C) (Smith and Read, 1997). EM mycelia can contribute to a substantial part of the soil microbial biomass (Högberg and Högberg, 2002; Wallander et al., 2001) and there are more than 6000 known EM species (Smith and Read, 1997) with a range of growth strategies in the soil. The density of ectomycorrhizal (EM) root tips is highest in the top organic layer of the soil (Stober et al., 2001) where the fungi are involved in utilizing nutrients from the organic material (Bending and Read, 1995).

Recent investigations with minirhizotrons and more thorough sampling approaches have however shown that many ectomycorrhizal root tips are also formed in the mineral soil layer (Majdi et al., 2001; Rosling et al., 2003). Different environmental conditions prevail at different soil depths, which may be one explanation of the broad diversity of EM fungi found in many boreal forest soils (Bruns, 1995). EM fungi have been suggested to play a role in weathering of minerals in forest soils and the mechanisms for these processes were recently reviewed by Landerweert et al. (2001). Numerous laboratory experiments have shown that EM fungi can have a stimulatory influence on weathering of minerals (Paris et al., 1995, 1996; Wallander and Wickman, 1999; Wallander, 2000a,b) but in order to evaluate if this effect is significant for the overall nutrient cycling of forest ecosystems, estimates of fungal induced weathering rates must also be performed in the field. Many forests in the northern hemisphere are normally N limited (Tamm, 1991), but due to the increased anthropogenic deposition of N together with the decrease in the storage of base cations due to acid rain, other mineral nutrients, e.g. K, P, magnesium (Mg) and calcium (Ca), may become limiting in some forest stands (reviewed by Thelin, 2000). Modelling of weathering rates and mass balance calculations in southern Sweden indicates that K will be an element in short supply in the future (Barkman and Sverdrup, 1996).

One question of fundamental importance is whether the influence of EM fungi on weathering of minerals is a constant contribution or if it increases when the trees are exposed to nutrient deficiency. EM fungi may increase weathering rates by increased production of organic acids or more intensively colonize deeper soil layers to obtain more nutrients from minerals (Landerweert et al., 2001). In the present review we summarize 1) results of the influence of host nutrient status on growth of EM fungi and 2) results obtained on EM induced weathering in field studies, with special focus on how this activity is related to nutrient status of the trees.

2. Influence of Nutrient Status of the Forest Trees on Growth of EM Fungi

EM fungi are adapted to N-limiting conditions and in forests with high N input, growth of EM fungi is usually inhibited. This condition is seen as reduced production of fruit bodies (Brandrud, 1995; Wiklund et al., 1995; Termorshuizen and Schaffers, 1991; Lilleskov et al., 2001) and a reduced diversity of EM fungi on the root tips in the soil (Wallenda and Kottke, 1998; Erland and Taylor, 2002; Kårén and Nylund, 1997; Taylor et al., 2000; Jonsson et al., 2000; Peter et al., 2001; Fransson et al., 2000; Lilleskov et al., 2002). The growth of the external mycelium from the root tips is usually reduced after N addition. This has been seen in laboratory experiments (Wallander and Nylund, 1992) with differences in species sensitivity among EM fungi (Arnebrant, 1994). Using ingrowth mesh bags to quantify EM mycelia in the field, Nilsson and Wallander (2003) demonstrated that growth of external mycelia was also reduced by ca 50% in field experiments with the addition of 100 kg N ha^{-1} yr^{-1} as $(NH_4)_2SO_4$ for 11 years in the Skogaby experimental forest in southern Sweden. Elevated N inputs to forests may thereby reduce the potential for EM fungi to influence weathering rates since lower amounts of C is allocated to the EM fungi under these conditions.

The nutrient status of trees has a strong influence on allocation between above-ground and below-ground parts. In general it could be assumed that nutrient deficiencies would result in larger allocation to roots to facilitate better nutrient uptake, but this is not always the case. Ericsson (1995) demonstrated in laboratory experiments that deficiencies of N and P resulted in the expected larger allocation of dry matter to the roots but K and Mg deficiencies on the other hand resulted in proportionally less dry matter allocated to roots. This was suggested to be a result of a reduction in carbon fixation by K and Mg deficiency, which led to shortage of carbohydrates, and a reduced allocation of C to below-ground parts. Similar results have been found for C allocation to EM fungi in laboratory systems. Wallander and Nylund (1992) and Ekblad et al. (1995) found increased allocation of dry matter to the EM symbionts under P deficiency whereas K deficiency resulted in reduced allocation of dry matter to EM symbionts. These findings suggest that the potential to counteract P deficiency by increasing weathering of P-containing minerals by EM fungi is much higher than the potential to counteract K deficiency by increasing weathering of K-containing minerals.

3. Weathering of Minerals in Forests Dominated by Ectomycorrhizal Trees

Tree species can have a strong influence on weathering of minerals. Augusto et al. (2000) demonstrated that weathering of feldspars added to forest soil was more intense in coniferous than in deciduous forests. Using strontium (Sr) isotopes and Ca/Sr ratios Blum et al. (2002) demonstrated that coniferous trees took up significantly more Ca from apatite sources in the B horizon of the soil compared to deciduous trees. Furthermore, trees colonized by arbuscular mycorrhizal fungi could not utilize apatite as a nutrient source. The higher weathering rates in coniferous compared to deciduous forests were probably an effect of higher acidity in the soil, but different communities of EM fungi in the two forest types may also have played a role. Bauhus and Messier (1999) suggested that conifers are more dependent on their EM symbionts than deciduous trees. This view was supported by data from Wallander et al. (2004), which indicated that EM mycelia in mixed stands obtained more carbon from spruce than from oak trees although the oaks produced more above-ground biomass than the spruce trees.

On the other hand, studies by Simard et al. (1997a,b) suggest that deciduous trees contribute with more C to EM mycelia than coniferous trees since the net C transfer via hyphal linkages between *Betula papyrifera* and *Pseuditsuga menziesii* was towards the coniferous tree both in field and in laboratory studies. The higher weathering rate in coniferous forests than in deciduous forests may be an effect of more intensive growth of EM mycelia in coniferous forests. Arocena et al. (1999, 2000) investigated soil solution and mineral composition in the ectomycorrhizosphere in subalpine fir in Canada and concluded that weathering rates of minerals were higher in the vicinity of root tips colonized by the EM fungus, *Piloderma* sp., than in surrounding soil. The capacity of *Piloderma* to extract K and Mg from different minerals was also confirmed in laboratory tests (Glowa et al., 2003). Hagerberg et al. (2003) estimated growth of external mycelia of EM fungi in Norway spruce forests in southern Sweden with different K status using ingrowth mesh bags. They found no influence of K status on growth of the EM mycelia. Furthermore they found no influence of adding the K-containing mineral, biotite, in the mesh bags on growth of the external mycelia in any of the forests.

In addition, the elemental composition of rhizomorphs growing in contact with biotite was not different from rhizomorphs growing in contact with pure quartz sand (Wallander et al., 2003) and no transport of ^{87}Sr, naturally enriched in the biotite, could be detected in EM root tips on the outside of the biotite amended mesh bags (Hargerberg, 2003). No sign of interaction between EM fungi and biotite was thus found in these field studies using ingrowth mesh bags buried in forest soil.

Apatite is an important P source in soil and several EM fungi have the potential to dissolve apatite to obtain P (Wallander, 2000a). Landeweert et al. (2003) and Rosling et al. (2003) demonstrated recently that the EM fungus *Suillus* sp. was detected in the B horizon but not in the upper soil horizons of a soil profile in northern Sweden. This finding is especially interesting since this fungus is known to produce oxalic acid and has the potential to solubilize apatite (Wallander, 2000a). Many soil-inhabiting fungi (including ectomycorrhizal fungi) are covered by calcium oxalate crystals (Cromack et al., 1979). Large amounts of Ca in EM hyphae growing in soil have been detected by scanning electron microscopy equipped with energy dispersive systems (SEM-EDS) (Arocena et al., 1999) and Particle Induced X-ray Emission (PIXE), especially when the fungus has been growing in contact with a Ca source such as apatite or wood ash (Wallander et al., 2003). Part of these calcium oxalate crystals could be residues after dissolving Ca rich minerals in the soil to obtain P.

In a field study Hagerberg et al. (2003) showed that growth of EM fungi in a forest with a low P status could be stimulated by addition of apatite. The amount of mycorrhizal root tips formed outside the surface of apatite amended mesh bags increased three-fold and the amount of mycelia inside the mesh bags increased by approximately 50%. This could either be an effect of an increased allocation of C to external mycelium within each fungal taxa or a result of a changed EM community towards species that produce more external mycelium. The strong stimulatory influence of apatite on formation of mycorrhizal root tips suggests that the tree responded to the P source in the soil by increasing the amount of C allocated to this site, which in turn resulted in increased formation of mycorrhizal root tips and external mycelia. It is likely that EM fungi adapted to P-poor soil would show higher responsiveness to local P sources in the soil and increase in abundance around the apatite amended mesh bags.

However it has also been shown that C allocation to single EM taxa can increase several-fold when it is exposed to P deficiency (Wallander and Nylund, 1992; Ekblad et al., 1995). A significant part of the C that was allocated to the EM fungi was probably used to produce oxalic acid to facilitate solubilization of the apatite although no data of oxalic acid production was provided in the study. The dissolution of the apatite was quantified by estimating the flux of rare earth elements from the apatite to the mycorrhizal roots. These elements are in high concentration in the apatite, and have been found to be transported by EM fungi, but are not transferred to the host tissue (Vesk et al., 2000). It was found that the EM fungi had a considerable influence on dissolution of the apatite and interestingly the EM induced dissolution increased by 40% in the P deficient soil compared to the P sufficient soil. This finding suggests that EM fungi can compensate for P deficiency in Norway spruce stands by increasing the dissolution of apatite. More studies including forests with a larger range of P status need to be performed to confirm these results. Hagerberg (2003) calculated the extent to which this process could compensate for nutrient losses induced by whole tree harvesting but found that EM induced weathering could compensate for at the most only half of the P lost from whole tree harvesting in Norway spruce forests in southern Sweden.

EM fungi may respond to P deficiency but not to K deficiency because of differences in C allocation within the plant, as discussed above (Ericsson, 1995). Another reason might be that P deficiency is common in many soils since phosphate is immobile and many soils have a large P-fixing capacity. It is therefore likely that certain EM fungi are adapted to these conditions and have evolved traits to utilize P sources efficiently. K on the other hand is much more mobile in the soil and K deficiency is probably a rare phenomenon in most ecosystems. Traits to utilize K sources specifically are therefore less likely to have evolved among EM fungi.

To conclude it seems likely that EM fungi have a significant role in the weathering of P minerals such as apatite in soil, especially in ecosystems that are limited by P availability. In contrast it seems much less likely that EM fungi have a significant role in weathering of K containing minerals such as biotite in natural ecosystems.

Acknowledgment

This work was supported by the Swedish National Energy Administration and the Royal Swedish Academy of Agriculture (grants to H. Wallander). We would like to thank Nancy Johnson for valuable comments on the manuscript.

REFERENCES

Arnebrant, K. 1994. Nitrogen amendments reduce the growth of extramatrical mycelium. *Mycorrhiza* **5**: 7–15.

Arocena, J.M. and Glowa, K.R. 2000. Mineral weathering in ectomycorrhizosphere of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) as revealed by soil solution composition. *Forest Ecology and Management* **133**: 61–70.

Arocena, J.M., Glowa, K.R., Massicotte, H.B., and Lavkulich, L. 1999 Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook) Nutt.) in the Ae horizon of a Luvisol. *Canadian Journal of Soil Science* **79**: 25–35.

Augusto, L., Turpault, M-P., and Ranger, J. 2000. Impact of forest tree species on feldspar weathering rates. *Geoderma* **96**: 215–237.

Barkman, A. and Sverdrup, H. 1996. Critical loads of acidity and nutrient imbalance for forest ecosystems in Skåne, Report 1. Department of Chemical technology II, Lund University, Lund, Sweden. 64 p.

Bauhus, J. and Messier, C. 1999. Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. *Canadian Journal of Forest Research* **29**: 260–273

Bending, G.D. and Read, D.J. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants V. Foraging behaviour and translocation of nutrients from exploited litter. *New Phytologist* **130**: 401–409.

Blum, J.D., Klaue, A., Nezat, C.A., Driscoll, C.T., Johnson, C.E., Siccama, T.G., Eagar, C., Fahey, T.J., and Liken, G.E. 2002. Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* **417**: 729–731.

Brandrud, T.E. 1995. The effects of experimental N addition on the ectomycorrhizal flora in an oligotrophic spruce forest at Gårdsjön, Sweden. *Forest Ecology and Management* **71**: 111–122.

Bruns, T.D. 1995. Thoughts on the processes that maintain local species diversity of Ectomycorrhizal fungi. *Plant and Soil* **170**: 63–73.

Cromack, K., Sollins, P., Graustein, W., Speidel, K., Todd, A., Spycher, H., Li, C., and Todd, R. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum. Soil Biology and Biochemistry* **11**: 463–468.

Ekblad, A., Wallander, H., Carlsson, R., and Huss-Danell, K. 1995. Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. *New Phytologist* **131**: 443–451.

Ericsson, T. 1995. Growth and shoot:root ratio of seedlings in relation to nutrient availability. *Plant and Soil* **168–169**: 205–214.

Erland, S. and Taylor, A.F.S. 2002. Diversity of ecto-mycorrhizal fungal communities in relation to the abiotic envirnment. In: *Mycorrhizal Ecology*, M.G.A van der Heijden, and I. Sanders, eds. Ecological Studies 157, pp. 163–224.

Fransson, P.M.A., Taylor, A.F.S., and Finlay, R.D. 2000. Effects of continous optimal fertlization upon a Norway spruce ectomycorrhizal community. *Tree Physiology* **20**: 599–606.

Glowa, K.R., Arocena, J.M., and Massicotte, H.B. 2003. Extraction of potassium and/or magnesium from selected minerals by *Piloderma*. *Geomicrobiology Journal* **20**: 99–111.

Hagerberg, D. 2003. The growth of external ectomycorrhizal mycelia in the field in relation to host nutrient status and local addition of mineral sources. Ph.D Thesis, Lund University, Lund, Sweden.

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

Högberg, M.N. and Högberg, P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–795.

Jonsson, L., Dahlberg, A., and Brandrud, T.-E. 2000. Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to nitrogen addition: above- and below-ground views. *Forest Ecology and Management* **132**: 143–156.

Kårén, O. and Nylund, J.-E. 1997. Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in southwestern Sweden. *Canadian Journal of Botany* **75**: 1628–1642.

Landeweert, R., Leeflang, P., Kuyper, T.W., Hoffland, E., Rosling, A., Wernars, K., and Smit, E. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. *Applied and Environmental Microbiology* **69**: 327–353.

Landeweert, R., Hoffland, E., Finlay, R., and van Breemen, N. 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution* **16**: 248–254.

Lilleskov, E., Fahey, T.J., and Lovett, G.M. 2001. Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. *Ecological Applications* **11**: 397–410

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

Majdi, H., Damm, E., and Nylund, J.-E. 2001. Longevity of mycorrhizal roots depend on branching order and nutrient availability. *New Phytologist* **150**: 195–202.

Nilsson, L.O. and Wallander, H. 2003. The production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* **158**: 409–416.

Paris, F., Botton, B., and Lapeyrie, F. 1996. *In vitro* weathering of phlogopite by ectomycorrhizal fungi II. Effect of K^+ and Mg^{2+} deficiency and N source on accumulation of oxalate and H^+. *Plant and Soil* **179**: 252–150.

Paris, F., Bonnaud, P., Ranger, J., Robert, M., and Lapeyrie, F. 1995. Weathering of ammonium or calcium saturated 2:1 phyllosilicates by ectomycorrhizal fungi *in vitro*. *Soil Biology and Biochemistry* **27**: 1237–1244.

Peter, M., Ayer, F., and Egli, S. 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytologist* **149**: 311–325.

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., and Finlay, R.D. 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol profile. *New Phytologist* **159**: 775–783.

Simard, S.W., Jones, M.D., Durall, D.M., Perry, D.A., Myrold, D.D., and Molina, R. 1997. Reciprocal transfer of carbon isotopes between ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*. *New Phytologist* **137**: 529–542.

Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., and Molina, R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**: 579–582.

Smith, S.E. and Read, D.J. 1997. *Mycorrhizal Symbiosis.* 2nd edn. Academic Press, San Diego.

Stober, C., George, E., and Persson, H. 2001. Root growth and response to nitrogen. In: *Carbon and Nitrogen Cycling in European Forest Ecosystems.* Schulze, E.-D., ed. Ecological studies vol. 142. Springer, Heidelberg, pp. 99–119.

Tamm, C.-O. 1991. *Nitrogen in Terrestrial Ecosystems. Questions of Productivity.* Springer-Verlag, Berlin, Germany.

Taylor, A.F.S., Martin, F., and Read, D.J. 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: *Carbon and Nitrogen Cycling in European Forest Ecosystems.* Schulze, E.-D., ed. Ecological studies vol. 142. Springer, Heidelberg, pp. 343–365.

Termorshuizen, A.J. and Schaffers, A. 1991. The decline of carpophores of ectomycorrhizal fungi in stands of *Pinus sylvestris* L. in The Netherlands: possible causes. *Nova Hedwigia* **53**: 267–289.

Thelin, G. 2000. Nutrient imbalance in Norway spruce. Ph.D. Thesis, Lund University, Lund, Sweden. 44 p.

Vesk, P.A., Ashford, A.E., Markovina, A.-L., and Allaway, A.G. 2000 Apoplasmic barriers and their significance in the exodermis and sheath of *Eucalyptus pilularis-Pisolithus tinctorius* ectomycorrhizas. *New Phytologist* **145**: 333–346.

Wallander, H. and Nylund, J.-E. 1992. Effects of excess nitrogen and phosphorous starvation on extramatrical mycelium in Scots pine seedlings. *New Phytologist* **120**: 495–503.

Wallander, H. and Wickman, T. 1999. Biotite and microcline as a K source in mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* **9**: 25–32.

Wallander, H. 2000a. Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. *Plant and Soil* **218**: 249–256.

Wallander, H. 2000b. Use of strontium isotopes and foliar K content to estimate weathering of biotite induced by pine seedlings colonised by ectomycorrhizal fungi from two different soils. *Plant and Soil* **222**: 215–229.

Wallander, H., Nilsson, L.O., Hagerberg, D., and Bååth, E. 2001. Estimation of the biomass and production of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* **151**: 751–760.

Wallander, H., Mahmood, S., Hagerberg, D., Johansson, L., and Pallon J. 2003. Elemental composition of ectomycorrhizal mycelia identified with PCR/RFLP and grown in contact with apatite or wood ash in forest soil. *FEMS Microbiology Ecology* **44**: 57–65.

Wallander, H., Göransson, H., and Rosengren, U. 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* **139**: 89–97.

Wallenda, T. and Kottke, I. 1998. Nitrogen deposition and ectomycorrhizas. *New Phytologist* **139**: 169–187.

Wiklund, K., Nilsson, L.O., and Jacobsson, S. 1995. Effect of irrigation, fertilization, and drought on basidioma production in a Norway spruce stand. *Canadian Journal of Botany* **73**: 200–208.