

Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles

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Abstract A decade ago, tunnels inside mineral grains were found that were likely formed by hyphae of ectomycorrhizal (EcM) fungi. This observation implied that EcM fungi can dissolve mineral grains. The observation raised several questions on the ecology of

these “rock-eating” fungi. This review addresses the roles of these rock-eating EcM associations in plant nutrition, biogeochemical cycles and pedogenesis. Research approaches ranged from molecular to ecosystem level scales. Nutrient deficiencies change EcM seedling exudation patterns of organic anions and thus their potential to mobilise base cations from minerals. This response was fungal species-specific. Some EcM fungi accelerated mineral weathering. While mineral weathering could also increase the concentrations of phytotoxic aluminium in the soil solution, some EcM fungi increase Al tolerance through an enhanced exudation of oxalate. Through their contribution to Al transport, EcM hyphae could be agents in pedogenesis, especially podzolisation. A modelling study indicated that mineral tunnelling is less important than surface weathering by EcM fungi. With both processes taken together, the contribution of EcM fungi to weathering may be significant. In the field vertical niche differentiation of EcM fungi was shown for EcM root tips and extraradical mycelium. In the field EcM fungi and tunnel densities were correlated. Our results support a role of rock-eating EcM fungi in plant nutrition and biogeochemical cycles. EcM fungal species-specific differences indicate the need for further research with regard to this variation in functional traits.

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Introduction

The term *rock-eating fungi* was coined by Jongmans et al. (1997), when they described microscopic tunnels contained within feldspar and hornblende grains in the E horizon of podzol soils. Podzols are characterised by four distinct soil horizons: a dark-coloured organic (O) horizon underlain by a white/ash-coloured eluvial (E) horizon, overlying a usually dark-coloured illuvial (B) horizon on top of the unaltered parent (C) material (Fig. 1). These tunnels had smooth and parallel-oriented walls with a constant diameter (3–10 μm) and rounded ends (Fig. 2), which distinguished them from (coalesced) etch pits and cracks caused by abiotic weathering processes known so far (Hoffland et al. 2002). Because (1) the size and shape of the tunnels perfectly fitted hyphae, (2) some tunnels were actually colonised by hyphae and (3) hyphae were seen to penetrate mineral grains (Fig. 3) a fungal role for rock weathering seemed likely. Jongmans et al. (1997) further hypothesised that such tunnels were formed through low molecular weight organic anions (LMWOA) exuded at hyphal tips. The

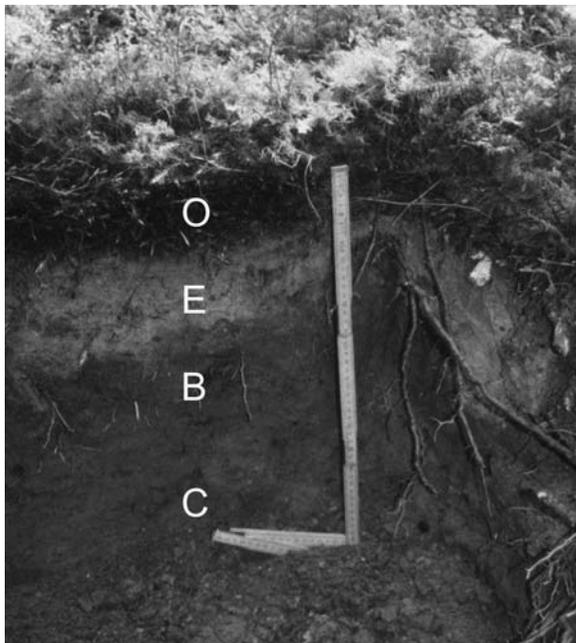


Fig. 1 A typical podzol profile in Nyänget, Mid Sweden, with horizons indicated

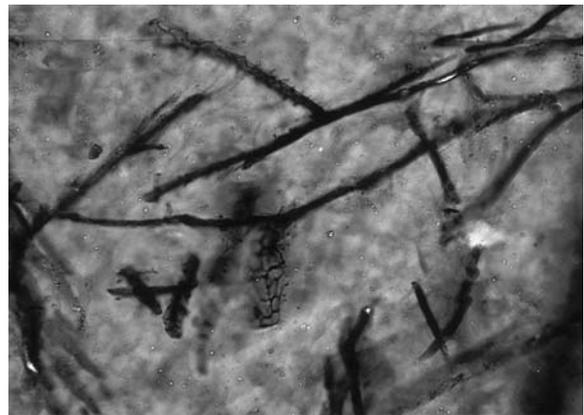


Fig. 2 Thin section micrograph of a feldspar grain from a podzol E horizon, criss-crossed by tunnels with a rounded end and a diameter of about 5 μm

term rock-eating fungi therefore refers to fungi that have the capacity to exude LMWOA to an extent that mineral weathering rates are significantly increased.

Ectomycorrhizal (EcM) fungi were considered the most likely candidates responsible for this tunnel formation. EcM fungi are known to exude LMWOA. EcM fungi associate with tree roots, where they exchange water and nutrients taken up by the hyphae for photosynthetically produced carbohydrates. Hence, EcM fungi have a potentially large source of carbohydrates available (Högberg et al. 2001). Lindahl et al. (2007) demonstrated vertical separation between

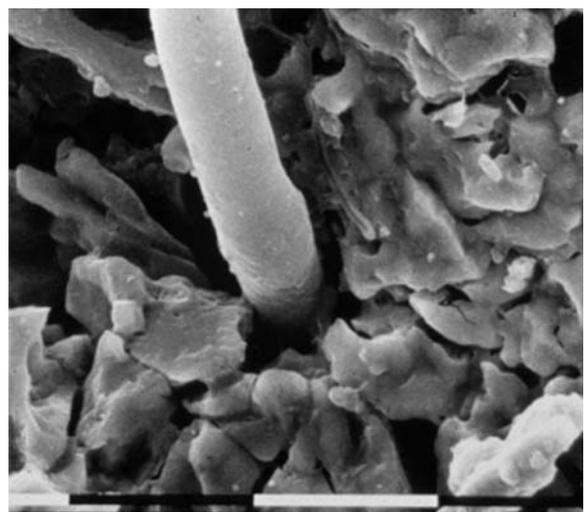


Fig. 3 Scanning electron micrograph showing a fungal hypha penetrating a mineral grain (bar size, 10 μm)

saprotrophic fungi that colonised relatively recently shed litter and EcM fungi that replaced saprotrophs deeper down the profile where the substrates became depleted in available energy. For saprotrophic fungi and bacteria the carbon source in the mineral E horizon, where organic matter content is low (usually <1%), is therefore very limited.

An extensive survey of 75 soils from Europe, Asia, North America and Australia revealed that tunnelled minerals occur almost exclusively in podzols in temperate and boreal zones, and also sometimes in acid brown forest soils (Hoffland et al. 2005). No tunnelled minerals were observed in podzol soils that had developed under the arbuscular mycorrhizal kauri (*Agathis australis*) in New Zealand. Northern temperate and boreal podzols typically develop under a vegetation of EcM coniferous trees, with an undergrowth of ericaceous shrubs, and develop in a time-scale of several hundred to thousand years (Lundström et al. 2000). These northern temperate and boreal podzols are therefore indicative for the long-term presence of EcM fungi.

The tunnelled minerals and the potential weathering capacity of EcM fungi raised novel questions regarding roles of EcM fungi in plant nutrition (especially uptake of base cations), biogeochemical processes and pedogenesis i.e. podzolisation (Van Breemen et al. 2000a; Van Breemen et al. 2000b; Landeweert et al. 2001). These questions have been addressed in the “Rock-eating Mycorrhiza: Where, Why, How?” research programme. This review discusses the results of that programme in the framework of the increased interest in EcM fungi as ecosystem engineers notably through interactions with soil minerals. We do not intend to review the field of mineral weathering by fungi, because several important reviews have appeared in the last few years (Hoffland et al. 2004; Wallander and Hagerberg 2004; Smits 2006; Wallander 2006; Gadd 2007; Gorbushina 2007). In this review we concentrate on the role of EcM fungal-mediated release of cations (K, Mg) and their role in alleviating aluminium toxicity and transport of Al in podzols. The role of EcM fungi in weathering of apatite and release of P has been reviewed by Wallander and Hagerberg (2004).

A major message from our review is the importance of EcM species diversity. In order to understand the ecosystem relevance of these processes, understanding of the spatial distribution of EcM fungi is imperative.

Role of rock-eating EcM fungi in plant nutrition

The hypothesis that EcM fungi contribute to uptake and subsequent transport to the tree of base cations through the enhanced weathering of soil mineral grains implied a new role of EcM in plant nutrition. The role of EcM fungi in the uptake of N and P is well established (Smith and Read 1997). EcM fungi occur in forests where low litter quality and low decomposition and mineralisation rates cause N and P limitation. EcM fungi have a suite of attributes that allow them to access organic N, also from recalcitrant sources. EcM fungi equally enhance uptake of P, both from recalcitrant inorganic and organic sources (Read et al. 2004). The role of EcM fungi in weathering and subsequent uptake of base cations is potentially increasingly important in situations where acid rain and intensive biomass harvesting increase losses and decrease storage of K, Mg and Ca. Under the present conditions, forest growth in some stands may become limited by K and/or Mg rather than N (Landman et al. 1997; Übel and Heinsdorf 1997; Thelin 2000).

In chronosequences, tunnelled minerals only emerged when easily weatherable base cation-containing minerals had disappeared (biotite) or were strongly weathered (hornblende), suggesting that EcM fungal weathering is driven by the (reduced) bioavailability of K, Ca and Mg (Hoffland et al. 2002; Smits et al. 2005). Microscopic tunnels occur exclusively in grains of the soil minerals feldspar and hornblende, which contain K, Ca and Mg, but which are scarce in N and P (Hoffland et al. 2002). EcM fungi have been found to respond to minerals containing K and Mg (Paris et al. 1996; Arocena et al. 2004; Glowka et al. 2003). EcM weathering of minerals containing base cations might therefore have a profound effect on plant uptake and ecosystem cycling of base cations.

An increased uptake of mineral-derived nutrients by tree seedlings when grown in symbiosis with EcM fungi has been found in several pot experiments (see review by Wallander 2006). The combined effect of EcM fungi on growth and nutrient uptake by the seedlings and on mineral weathering complicates interpretation of such studies. This problem can be overcome by making a complete mineral budget, with all pools of mobile elements quantified, an approach adopted lately in studies by Van Hees et al. (2006a) and by Van Schöll et al. (2006b). EcM colonisation by *Hebeloma longicaudum* increased the mineral

weathering of soil from a podzol E horizon compared to non-mycorrhizal seedlings, as measured from the fluxes of Al, Si and K (Van Hees et al. 2006a). Van Schöll et al. (2006b) determined the effect of EcM fungi on K and Mg availability through mineral weathering. Non-mycorrhizal and EcM tree seedlings were grown in pots with muscovite as the only K source or hornblende as the only Mg source. Weathering of muscovite was increased by tree seedlings by a factor 1.7, and was increased even further to a factor of 3.3 when tree seedlings were colonised by *Paxillus involutus* (Table 1). However, colonisation of the tree seedlings by the EcM fungi *Suillus bovinus* or *Piloderma croceum* did not have an additional effect. Weathering of hornblende was increased by tree seedlings by a factor of 1.5–2 compared to pots without tree seedlings, but was not affected by EcM fungi (Table 1).

The weathering of the K and Mg containing minerals by non-mycorrhizal and EcM tree seedlings corresponded well to their exudation of oxalate in response to omission of K and Mg. Non-mycorrhizal pine seedlings exuded mainly malonate (50–80% of all LMWOA; Van Schöll et al. 2006a). There were strong effects of EcM fungal colonisation on exudation of LMWOA, especially of oxalate (Table 1): Seedlings colonised by *P. involutus* significantly increased the exudation of oxalate, whereas colonisation by *H. longicaudum* decreased the exudation of total LMWOA and malonate compared to the non-mycorrhizal seedlings. Oxalate is probably one of the most potent weathering agents that is exuded by plant

roots and EcM fungal hyphae. The weathering potential of oxalate via complexation with Al, as indicated by the stability constant ($\log K_{Al}=6.5$), is higher than that of malonate ($\log K_{Al}=5.7$; Fox et al. 1990). In culture solution, Mg deficiency increased exudation of oxalate, but there were no significant differences between non-mycorrhizal or EcM seedlings (Van Schöll et al. 2006a). Only seedlings colonised by *P. involutus* significantly increased oxalate exudation under K deficiency. This K deficiency-induced oxalate exudation could explain the enhanced weathering of muscovite (Table 1).

The experiments showed that differences in response among EcM fungal species were as large as or larger than between nonmycorrhizal and EcM seedlings. These findings raise questions regarding diversity of EcM fungi and the spatial distribution of EcM fungi with high weathering potential through the soil profile (discussed below).

The role of EcM fungi in the uptake of K and Mg is further supported by studies showing an increased fungal exploitation of sites with higher cation availability, presenting a striking parallel to the intense hyphal proliferation in patches with organic materials such as litter (Bending and Read 1995) or when N or P are patchily supplied (Brandes et al. 1998; Jentschke et al. 2000). Wallander and Hagerberg (2004) hypothesised that EcM fungi are unlikely to have a significant role in weathering of K and Mg containing minerals, as K and Mg deficiency decreases below-ground carbon allocation to roots, whereas N and P deficiency increases allocation to roots and associated

Table 1 Ectomycorrhizal species-specific responses. Figures indicate differences between ectomycorrhiza-colonized *P. sylvestris* seedlings and their nonmycorrhizal control (=100; after Van Schöll et al. 2006a, b)

Fungal isolate	Weathering ^a (total K or Mg in plant tissue and soil solution)		Exudation			Transport
	Muscovite	Hornblende	Oxalate	Malonate	Total LMWOA ^b	Al
<i>Piloderma croceum</i>	101	100	161*	102	106	0
<i>Hebeloma longicaudum</i>	.	.	61	42*	49*	.
<i>Paxillus involutus</i> UK	180*	115	209*	27*	77	+
<i>Suillus bovinus</i>	97	97	.	.	.	0
<i>Rhizopogon roseolus</i>	+

For Al transport symbols indicate transport (+) or no transport (0) by fungi in pure culture (after Smits 2005)

^aMuscovite contains K; Hornblende contains Mg.

^bLow molecular weight organic anions

*significantly different from nonmycorrhizal control ($P<0.05$)

mycorrhizal fungi (Ericsson 1995). In contrast, increased carbon allocation by EcM tree seedlings and higher hyphal proliferation in patches with K-containing feldspar than in patches with quartz or in the surrounding soil (podzol E horizon) was shown by Rosling et al. (2004) in a microcosm experiment. Heinonsalo et al. (2004) showed that tree seedlings, growing on a reconstructed podzol in a microcosm, increased carbon allocation to mycorrhizas present in the mineral soil layers with high amounts of weatherable minerals. Increased hyphal foraging (exploitative growth) in response to either Mg or Mg+P addition in the hyphal compartment (not accessible to roots) of a two-compartment system was observed by Van Schöll (2006). Transport of Mg through EcM fungi, using a two-compartment pot system, has also been shown by Jentschke et al. (2001). They assumed this transport to be passive co-transport with P, driven by P deficiency. However, in the study by Van Schöll (2006) active hyphal transport of Mg, rather than passive co-transport of Mg with P was observed.

From these results it seems clear that EcM fungi and tree seedlings can adapt to base cation deficiencies by increased proliferation and by increased mineral weathering through enhanced exudation of LMWOAs. It remains however uncertain if this response is regulated by plant exudation or by fungal exudation when both organisms are grown in symbiosis. The exudation of oxalate and malonate by *P. sylvestris* seedlings and *P. involutus* in response to nutrient deficiencies differed strongly when grown either in pure culture or in symbiosis (Van Schöll et al. 2006a). Increased weathering was not *per se* reflected by increased uptake into the shoot, and with some EcM fungi the weathering products may have been retained partly in the mycelium. Such results emphasise that only experiments with both symbiotic partners can yield ecological realism. The underlying questions about the ‘who is in the driver’s seat’ of the mycorrhizal symbiosis (Zhu et al. 2001) and about the level of integration between fungal and plant processes remain unanswered to date.

Role of rock-eating EcM fungi in mineral weathering on ecosystem level

The results of Van Schöll et al. (2006a, b) provided further evidence that EcM fungi can enhance mineral

weathering through the exudation of organic anions in response to nutrient deficiencies, but their contribution to mineral weathering on ecosystem level remained unknown. Van Breemen et al. (2000a) estimated the contribution of EcM fungi to mineral weathering in a podzol soil via mineral tunnelling alone to be 50%. Sverdrup et al. (2002) estimated the contribution of organic ligands to total mineral weathering to be 15% and the direct contribution of all soil biota to be maximally 2%. This discrepancy made an improved estimate of EcM fungal weathering necessary. Mineral weathering is of fundamental importance in any ecosystem because it is responsible for a major influx of bioavailable elements and thereby contributes to soil fertility. It also serves as the main long-term buffer against soil acidification.

In the field, EcM fungal weathering cannot be distinguished from other weathering processes. The microscopic tunnels in mineral grains, which are visually distinct from other weathering phenomena, can however be used as a tool to quantify one aspect of EcM fungal weathering.

Even though a direct causal relationship between EcM fungi and mineral tunnelling cannot be established, field studies of productivity gradients have provided strong circumstantial evidence for the direct involvement of EcM fungi in mineral tunnelling. The productivity gradients consisted of a toposequence where the pH, N supply, mycorrhizal types and vegetation vary strongly over a short distance (<100 m), but where mineralogy is similar. EcM root tip density was positively correlated with tunnel frequency along two natural productivity gradients in Mid Sweden (Hoffland et al. 2003). No correlation was found between arbuscular mycorrhizal plants and tunnel frequency. Ericoid mycorrhizal fungi associate with *Vaccinium* spp., which grow in the understory in boreal forest, and therefore often co-occur with EcM fungi. These fungi can also produce high amounts of LMWOA (Martino et al. 2003), and may thereby contribute to tunnel formation. It seems unlikely, however, that ericoid mycorrhizal fungi are (solely) responsible for tunnel formation, as tunnels were found in sites with no ericoid mycorrhizal plants, and where they are unlikely to have grown considering the rapid establishment of the productivity gradient following deglaciation (Giesler et al. 1998).

A causal role for EcM fungi in tunnelling is further supported by the observation that tunnel length and

frequency increased with soil age and podzol formation. This relation was studied in a soil chronosequence in Mid Sweden that was formed through glacial rebound followed by gradual uplifting of land from sea ($9\text{--}50\text{ mm year}^{-1}$). Up to 25% of the feldspars from the uppermost 2 cm of the E horizon contained tunnels, and this percentage decreased rapidly with soil depth. Fungal hyphae were also observed inside the tunnels (Hoffland et al. 2002).

The contribution of EcM mineral tunnelling to total weathering was quantified via image analysis in soil thin sections (Smits et al. 2005) which were taken from a North Michigan dune chronosequence. Total weathering was quantified by comparing the mineralogy of the surface soil with the underlying parent material. Quantification revealed that mineral tunnelling accounted for less than 0.5% of total feldspar weathering in the upper 2 cm of the mineral soil. Tunnelling is however only one aspect of EcM weathering. Surface weathering by EcM fungi is likely to be quantitatively more important (Fig. 4),

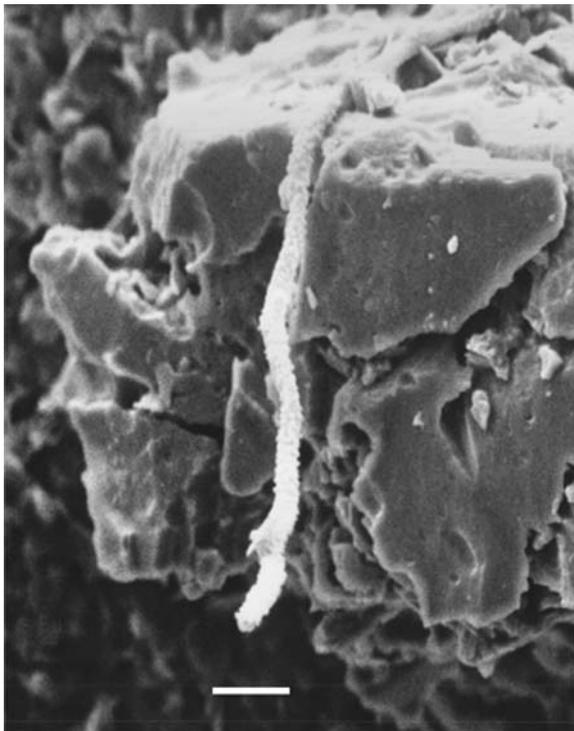


Fig. 4 Scanning electron micrograph of a hypha of *Piloderma croceum* on a mineral grain, indicating both surface weathering and tunnelling. The hypha is covered by characteristic wall incrustations of calcium oxalate (bar size, 10 μm)

as also proposed by Van Hees et al. (2004) based on a scanning electron microscope study.

Smits (2005) used a modelling approach to quantify surface weathering by EcM fungi. Feldspar weathering through oxalate exudation by EcM fungi hyphae was modelled by considering the kinetics of oxalate production, degradation and complexation. The production of oxalate by EcM fungi in the vicinity of feldspars was estimated from literature data on EcM biomass in podzol E horizons and *in vitro* oxalate production by EcM fungi, combined with data on fungal distribution over different minerals. Fungal distribution was assessed in stained soil thin sections from the dune chronosequence from which weathering by tunnelling was quantified. The relative abundance of hyphae in direct contact with the mineral surface was in the order Na/Ca-feldspar > K-feldspar > quartz.

Output from an improved version of the model (Smits et al., submitted for publication) indicated that surface weathering is an order of magnitude higher than weathering through tunnelling. Model calculations also indicated that EcM exudation of oxalate alone could account for 3% of microcline and 4% of plagioclase weathering in the upper 2 cm of the mineral horizon. Apart from oxalate, EcM fungi exude other LMWOA (citrate) and protons that also enhance weathering but are not accounted for in the model. Under deficiencies, exudation (Van Schöll et al. 2006a; Van Hees et al. 2006b) and hence weathering can be up-regulated, further enhancing EcM fungal weathering. Van Hees et al. (2006a) concluded that K weathering rates, induced by EcM conifers, were 3–10 times larger than historical weathering rates. These data imply that the contribution of EcM fungi alone to mineral weathering exceeds the 2% as estimated by Sverdrup et al. (2002) for the whole soil biota (including bacteria and saprotrophic fungi). On the basis of a modelling study Van Hees et al. (2005) pointed out that the relatively low-steady state concentrations of LMWOA as measured in the soil solution were caused by very high fluxes of both exudation and decomposition of these anions. Novel data on the balance between exudation and decomposition of LMWOA in the close vicinity of mineral surfaces, taking spatial variation in hyphal abundance, activity, and hence local soil solution chemistry into account, are especially required for model improvement and validation.

Role of rock-eating EcM fungi in aluminium toxicity and aluminium transport in podzols

Aluminium is a major component of most soil mineral grains. Consequently, mineral weathering will release not only base cations but also increase the concentration of Al in the soil solution of the mineral soil layers. In soils with a pH below 4, a situation typical for podzol soils, dissolved Al is present mainly in the form of the phytotoxic Al^{3+} (Kinraide 1991), and increased weathering will therefore contribute to Al toxicity. In the debate on the effect of acid deposition, Al toxicity is often seen as the most direct cause of forest deterioration. In the models that are used to quantify this effect and calculate acceptable critical loads of acid deposition, EcM associations are mostly overlooked.

Under Al toxicity, EcM seedlings maintained higher growth rates than nonmycorrhizal seedlings (Cumming and Weinstein 1990a; Cumming and Weinstein 1990b; Göransson and Eldhuset 2001; Hentschel et al. 1993; Schier and McQuattie 1995; Schier and McQuattie 1996). These higher growth rates may be the result of the fact that EcM fungi generally improve growth of their hosts through better nutrient supply. As shown by Schier and McQuattie (1996) increased nutrient supply alone can partly mitigate Al toxicity effects on tree seedlings.

The role of EcM fungi in the uptake of Ca and Mg under Al toxicity is especially of interest as Al^{3+} specifically inhibits the uptake of Ca and Mg by kinetic processes at the root cell wall, thereby inducing plant deficiencies of Ca and Mg. EcM fungi ensheath the root apices, which are the main sites for Al toxicity and uptake of Ca and Mg, and it seems likely that EcM fungi affect the uptake Ca and Mg at the root tips (Finlay 1995).

Under conditions of sufficient nutrient supply, which avoids differences in growth and biomass between non-mycorrhizal and EcM seedlings (Hobbie 2006), uptake of Ca and Mg under Al toxicity was not affected by EcM colonisation (Van Schöll et al. 2005). Mineral tunnelling by EcM hyphae through LMWOA exudation will give the tree direct access to base cations, while at the same time Al^{3+} is detoxified through chelation (Van Breemen et al. 2000a). By directly translocating the weathering products from these protected microsites, uptake is uncoupled from the toxic bulk solution.

EcM fungi might play an important role in the Al tolerance of their host trees by the exudation of LMWOA and subsequent complexation of Al and dissolution of Ca and Mg, a factor which is also not taken in consideration by most models. Aluminium toxicity can be – partly – mitigated by increasing the concentrations of dissolved Ca and Mg (Kinraide 2003) and by complexation of Al with LMWOA (Ryan et al. 2001).

Exudation of LMWOA seems a general response mechanism of Al tolerant plants (Barcelo and Poschenrieder 2002; Kochian et al. 2004; Ma et al. 2001; Ryan et al. 2001). Increased exudation of oxalate by tree seedlings under Al toxicity has been shown by Ahonen et al. (2000), and in several cases this was increased further by EcM fungi. Van Schöll (2006) demonstrated that oxalate exudation by EcM fungi was induced by Al, and exudation was further enhanced by simultaneous Al toxicity and Mg and P deficiency. The latter effect was stronger in non-mycorrhizal seedlings, which only exuded low amounts of oxalate under Al toxicity alone, and in seedlings colonised by *Rhizopogon roseolus*. Seedlings colonized by three other EcM fungi did not show this additive effect. The enhanced exudation in this experiment was apparently not an effect of Mg or P deficiency alone, because Mg or P deficiency without Al did not induce the exudation of oxalate. This outcome contrasts with results obtained before (Van Schöll et al. 2006a), where increased exudation of oxalate in response to Mg and P deficiency was observed. Possibly, this discrepancy is due to differences in experimental circumstances, which might also explain the substantially lower concentration of LMWOA found here. This difference emphasises that experimental conditions may, for unknown reasons, profoundly affect results.

The role of EcM fungi in mineral weathering and the exclusive occurrence of tunneled minerals in podzols have raised the question whether EcM fungi also play a direct role in Al transport and the podzolisation process. Cycling of Al from the mineral soil layers to the organic layer is an important but unexplained phenomenon in the podzolisation process. Existing theories on the podzolisation process focus on the formation of the mineral E and B horizon, but do not explain the high concentrations of Al observed in the organic O horizon. Budget studies on Al and Fe in podzols showed that much of the Fe

and Al that leached out of the E horizon into the B horizon originated from the O horizon (Giesler et al. 2000). Known input routes of Al and Fe into the O horizon, *i.e.* litter fall and atmospheric deposition, have been considered insufficient to explain the high concentrations of Al in the O horizon and output rates of Al from the O horizon to the E horizon. A possible explanation for this discrepancy is uptake and transport of Al and Fe from the E horizon towards the O horizon by tree roots and EcM hyphae that exploit the E horizon but colonise root tips in the O horizon (see below on occurrence of EcM root tips and hyphae). Data by Griffiths et al. (1994) for *Hysterangium crassum* and *Gautieria monticola* and by Van Hees et al. (2003) for *Paxillus involutus* also indicated enhanced dissolution and mobilization of Al and Fe by EcM fungi. Possibly, siderophores exuded by EcM hyphae could play a role here because siderophores are even more efficient complexing agents than LMWOA (Haselwandter and Winkelmann 2002; Holmström et al. 2004).

A prerequisite for an EcM fungal role in upward transport of Al is that these fungi are able to transport Al through their hyphae. An *in vitro* test of five different EcM fungi, growing in two-compartment Petri dishes, demonstrated that two of the five isolates tested were able to transport Al (Table 1; Smits 2005). In plants, transport of Al has been linked to complexes of Al with LMWOA, mostly citrate. For EcM fungi, complexation of Al to small soluble polyphosphate chains, very strong Al binders in biological systems (Martin et al. 1994), has also been suggested. An *in vitro* test did, however, not indicate any effect of P supply on the transport of Al through EcM hyphae (Smits 2005).

Transport of Al between soil layers cannot be studied directly because of the abundance of Al in podzols soils. Therefore Smits (2005) used gallium (Ga) as a tracer after *in vitro* tests showed the similarity of Ga and Al transport through EcM hyphae. Ga content in tree seedlings increased after Ga addition compared to controls without Ga addition. Ga content was not different between pots where tree roots were allowed or inhibited to grow into the mineral soil layer. These observations suggest a role for EcM fungi and roots in upward Ga (and by implication Al) transport. Whether or not hyphal and root transport of Al between the mineral and organic soil layers suffices to explain the surplus of Al in

podzol O horizons found in budget studies, requires further research.

Weathering by rock-eating EcM fungi – a further element of EcM diversity?

Diversity among EcM species in weathering potential (Table 1) and Al transport capacity (Smits 2005) is high. In order to study the role of EcM in plant nutrition and biogeochemical cycles it was necessary to relate this diversity to the spatial distribution of the EcM fungi over the soil horizons, and especially to establish which EcM species occur in the mineral E horizon in which the tunnels were found. While EcM species richness in podzols is high, little was known about their vertical distribution over the podzol soil profile. Studies on EcM assemblages and the functional roles of these species have often been confined to the organic horizon (see Read et al. 2004), but because chemical and mineralogical properties of soil horizons differ strongly, it seemed likely that different soil horizons provide different niches for EcM fungi.

Vertical niche differentiation of EcM root tips across soil horizons for a Swedish podzol was demonstrated by Rosling et al. (2003). Two thirds of the root tips were found in the mineral soil horizons. The percentage of root tips colonised ranged between 60 and 98%, but this was not related to soil horizon. A significant relationship between EcM fungal species composition and soil horizon was found, but species composition could not be related to specific chemical properties of the mineral E, B or C horizons. Most taxa typically occurred in one part of the soil profile only. *Tomentellopsis submollis*, three *Piloderma* species, *Cortinarius* subgen. *Dermocybe* spp. were found predominantly in the upper horizons while one other *Piloderma* sp., other *Cortinarius* spp., and *Suillus luteus* were associated with the lower mineral horizons. As much as half of the EcM fungal species were restricted to the mineral horizons.

Data on EcM fungi on root tips do however not necessarily reflect the simultaneous occurrence and activity of extraradical mycelium in the bulk soil of that soil horizon. Spatial differences in gene expression between EcM root tips and the extraradical mycelium have been found for the EcM fungus *Paxillus involutus* (Wright et al. 2005). Because EcM weathering is expected to take place at the

hyphal tips by exudation of LMWOA, it was necessary to identify and quantify hyphae of EcM fungi in bulk soil as well.

Landeweert et al. (2003a) successfully extracted basidiomycete DNA from the bulk soil, using soil samples from the same soil columns and soil horizons as in the accompanying root tip study by Rosling et al. (2003) described above. The amounts of DNA decreased with soil depth. There was strong vertical niche differentiation, with 16 out of 25 sequences occurring exclusively in the mineral layers of which 7 sequences occurred exclusively in the E horizon. At least 16 out of 25 fungal sequences belonged to EcM basidiomycetes, suggesting that they dominate over saprotrophic basidiomycetes in most soil layers. Comparison between the EcM root tip study and the soil mycelium study showed that both methods largely detected the same species. Of the 16 EcM fungal taxa identified in DNA extracts from root-free soil, all but one were also found on root tips. In several cases, however, species that were abundant on root tips were not detected in soil extracts, and others that were abundant in soil extracts were rarely detected on the roots. Discrepancies between EcM species composition and abundance based on root tip and hyphal analysis have been shown by several authors (Dickie et al. 2002; Kjølner 2006; Peintner et al. 2007) and indicate the need for a pluralistic approach to the analysis of EcM assemblages.

The molecular methods in these studies used only amplified basidiomycete DNA. The data do therefore not allow to rule out the possibility that ericoid mycorrhizal ascomycetes (associated with *Vaccinium* spp.) are also involved in mineral tunnelling. Quantitatively this is unlikely to be the case because Rosling et al. (2003) observed that over 95% of all root tips belonged to Basidiomycetes. Several EcM Ascomycetes, especially a group of dark septate endophytes that can both form EcM and ericoid mycorrhizas (Villarreal-Ruiz et al. 2004), are effective producers of LMWOA (Martino et al. 2003). Weathering experiments with that latter group are especially required.

EcM fungi showed vertical niche differentiation, however, the niche differentiation of EcM fungal hyphae did not occur between the organic versus mineral layers: in fact, the EcM assemblages in the organic horizon and the E horizon were more similar than the assemblages in the E horizon and the B or C

horizons. The co-occurrence of EcM fungi in the O and E horizon may seem surprising in view of the large differences in soil chemistry between both horizons, but may be a consequence of the proliferation in the E horizon of hyphae that are attached to root tips in the O horizon. Genney et al. (2006) also made the observation that the extraradical mycelium showed less clearly vertical niche differentiation than the ectomycorrhizas and exhibited a larger depth range. They argued that such patterns relate to the ability of the EcM fungus to utilise a wide range of substrates. The co-occurrence of fungal mycelium in O and E horizons therefore raises the question whether exudation of LMWOA could also confer benefits to EcM fungi in organic layers. While the highest tunnel density occurred in the upper part of the E horizon, EcM fungal species from the lower E or B horizon could also contribute to mineral weathering. The lower tunnel density may result from the lower hyphal density in the B horizon.

The molecular methods that were applied for identification did not truly allow quantification – even though clone numbers are often used as a surrogate for quantification. Molecular quantification methods require specific primers through which DNA of one fungal species can be amplified. While such methods can be successfully applied in microcosms where the researcher has control over EcM species composition (Landeweert et al. 2003b), they haven't yet been applied under field conditions with a suite of unknown species. Another research priority is therefore the development of a larger set of species-specific primers to quantitatively assess fungal occurrence. A further prospect of future research could be the development of a set of primers that allow amplification of genes that could be associated with weathering processes, e.g. transporter genes involved in the exudation of LMWOA or genes involved in siderophore synthesis.

For reasons of culturability the species choice for the experiments under controlled conditions described in this paper has been limited to the following species: *Paxillus involutus*, *Piloderma croceum*, *Hebeloma longicaudum*, *Suillus bovinus*, and *Rhizopogon luteolus*. *Paxillus involutus* produced several organic acids (Van Hees et al. 2003; Yamaji et al. 2005; Van Schöll et al. 2006a), enhanced the weathering of muscovite (Van Schöll et al. 2006b) and hyphae actively foraged for Mg (Van Schöll

2006). Some strains of this species (complex) showed high tolerance towards Al (Rudawska and Leski 1998) and one of two strains tested by Smits (2005) could transport Al (Table 1). *Paxillus involutus* was not observed on root tips and as mycelium in the Swedish podzol (Rosling et al. 2003; Landeweert et al. 2003a) but occurred in the middle part of a productivity gradient in Sweden (Toljander et al. 2006). In general, the species [or possibly species complex (Fries 1980)] occurs more frequently in organic horizons than in mineral horizons (Laiho 1970) and its ability to produce LMWOA could also be beneficial in the organic layers (see above).

Hyphae of *Piloderma croceum* with the characteristic wall incrustation of calcium oxalate were observed on mineral grains (Fig. 4). The species enhanced exudation of oxalate compared to non-mycorrhizal seedlings (Van Schöll et al. 2006a), released K and Mg (Arocena et al. 2004; Glowa et al. 2003), but did not show enhanced weathering of muscovite and hornblende in the study by Van Schöll et al. (2006b). The species was abundant in various horizons in the Swedish podzol (Rosling et al. 2003; Landeweert et al. 2003a). Both molecular data and the niche differentiation of the different molecular phylogenies suggest that *P. croceum* is a species complex, which might explain these different results.

Hyphae of *Hebeloma longicaudum* were found in the B horizon by Landeweert et al. (2003a), but EcM root tips were not mentioned by Rosling et al. (2003). The species showed strong foraging activity, subsequent exploitation, and oxalate exudation in mineral compartment (Rosling et al. 2004, Van Hees et al. 2006c – under the misapplied name *H. crustuliniforme*). A *Hebeloma* (or *Inocybe*-like) EcM morphotype increased release of K and Na in the mycorrhizosphere in a spruce forest (Glowa et al. 2003). However, pine seedlings colonised by this species down-regulated LMWOA production compared to non-mycorrhizal seedlings (Van Schöll et al. 2006a). *Hebeloma cylindrosporum* did not release oxalate (Arvieu et al. 2003; Casarin et al. 2003).

While *S. bovinus* was not observed in the Swedish podzol, two other *Suillus* species were observed either as root tips (*S. luteus* – Rosling et al. 2003) or as hyphae (*S. variegatus* – Landeweert et al. 2003a). Both *S. variegatus* (Ahonen et al. 2000; Wallander 2000) and the related *S. collinitus* (Arvieu et al. 2003)

produced large amounts of oxalic acid in soils with low pH and Al toxicity, and in soils with high pH and possibly associated P deficiency. However, *S. bovinus* did not enhance weathering of muscovite and hornblende in the study by Van Schöll et al. (2006b). *Rhizopogon roseolus* also produced large amounts of oxalic acid under a range of pH conditions (Ahonen et al. 2000; Arvieu et al. 2003; Casarin et al. 2003). It also up-regulated oxalate exudation, compared to non-mycorrhizal seedlings, under conditions of Al toxicity (Van Schöll 2006). Members of both *Suillus* and *Rhizopogon* are exclusively associated with conifers. Phylogenetically, *Suillus* and *Rhizopogon*, like *Paxillus*, are most closely related to brown-rot fungi such as *Serpula*, *Coniophora* and *Hygrophoropsis*, which also produce large amounts of oxalate (Fransson et al. 2004).

To clarify the relation between EcM functional traits and ecosystem variety, a further range of (different isolates of) EcM species, including EcM ascomycetes, obtained from different soil horizons, and from soils with low base cation versus high base cation supply should be used in future studies.

Concluding remarks

EcM fungi play an important role through weathering in plant base cation nutrition, and likely also in podzolisation and plant tolerance to Al. Generalisation of studies under controlled conditions to the natural situation is, however, limited by the species-specific and context-dependent responses of EcM fungi and still limited knowledge on EcM species distribution over soil horizons. With over 5,000 EcM fungal species known and only around 10 frequently used in research, assessing and understanding of EcM diversity and ecology is highly important. Our research on rock-eating EcM fungi (*where, why, how*) shows that, while we have made substantial progress in addressing the *how* question, we are only standing at the first steps of the route towards this understanding.

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