

Ectomycorrhizal weathering, a matter of scale?

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ABSTRACT

Boreal forest trees influence mineral weathering rates via exudation and uptake processes. Most trees in the boreal forest live in symbiosis with ectomycorrhizal (EcM) fungi that sheath most of the root tips and form the main interface between the tree and the soil. Current weathering models do not take into account the nature and scale of fungal-mineral interactions. Here we show for the first time grain-scale effects of EcM fungi in symbiosis with a host plant on mineral weathering under sterile conditions. EcM fungi actively direct their growth and energy flow towards mineral grains containing essential nutrient elements for the tree and fungus.

KEYWORDS: ectomycorrhiza, biological weathering, modelling, apatite, biotite, quartz, ¹⁴C.

Introduction

THE boreal forest is the world's largest vegetation system, covering a 1000–2000 km wide circum-polar belt around the northern hemisphere land masses (Read *et al.*, 2004). It provides economically important pine forests and is a major global store of carbon in biomass and soil. Boreal forest soils are young, with pedogenesis starting with the glacial retreats at the end of the Devensian ~10000 years ago, which left vast tracts of bare rock and new regolith on the surface. Soil development in this biome has been critically dependent on the weathering of raw mineral substrates to release essential nutrients for tree growth and to form clays and sesquioxides, which improve soil water and nutrient storage. Mineral weathering in the boreal region is also recognized as a key process buffering soils and lakes from anthropogenic acidification (Sverdrup, 1996).

The boreal forest not only depends on weathering for the supply of mineral nutrients, but it also positively influences weathering rates through several mechanisms: (1) acidification due to proton exchange with cationic nutrients; (2) CO₂ respiration; (3) exudation of organic

chelates; (4) uptake or complexation of dissolution inhibiting elements; and (5) mechanical forcing (Hoffland *et al.*, 2004). To understand the effects of a suite of interactions between boreal forest trees and mineral dissolution Sverdrup *et al.* (2002) developed the 'profile' model. Although the 'profile' model has significantly advanced understanding of tree-mineral weathering interactions, it still lacks some essential components, as it only takes into account the first three weathering mechanisms mentioned above, and partly addresses the fourth. Crucially, in modelling mineral dissolution the scale and nature of the interfaces between the tree and soil minerals needs to be understood and considered.

The tree-soil interface

In most boreal forest trees, the interface between the tree and the soil is not formed by the plants themselves, but by the symbiotic ectomycorrhizal (EcM) fungi that sheath over 90% of their root tips (Read *et al.*, 2004). The fungi extend from the roots forming an extensive differentiated mycelial network throughout the soil with thick multicellular transport structures, called rhizomorphs, and individual hyphae <10 µm wide, growing in intimate contact with mineral surfaces or organic

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particles. These fungi are the main conduits for nutrient and water uptake by plants and are a major pathway of energy flow to soil microbiota.

A matter of scale?

In developing the ‘profile’ model, both Sverdrup (1996) and Sverdrup *et al.* (2002) strongly criticized the view that EcM fungi play an important role in mineral weathering, and ascribe biotic weathering to the actions of the tree. Whilst the predictive power of ‘profile’ is very good, giving values that vary by <10% from field estimates of weathering (Sverdrup, 1996; though see Hodson *et al.*, 1997, and Hodson and Langan, 1999, for a different view), the underlying assumption that EcM are not important in weathering is unacceptable. In reality, the functioning of the trees and their mycorrhizal fungi are inseparably linked. All the major element fluxes between soil minerals and the trees are controlled by the actions of their EcM partners (exchange of protons for cations, and the exudation of organic chelates) and even respiration of CO₂ may be as great from mycorrhizal fungi as from roots (Högberg and Read, 2006). Consequently, the key chemical drivers of biotic weathering are dominated by the actions of EcM fungi. To model profile-scale weathering activity it is assumed that soil comprises homogeneous horizons with uniform concentrations of reactants (acids, chelators and CO₂) and minerals. This then develops the model outcome that large changes in bulk soil chemistry are required to alter weathering rates, and on this basis Sverdrup *et al.*

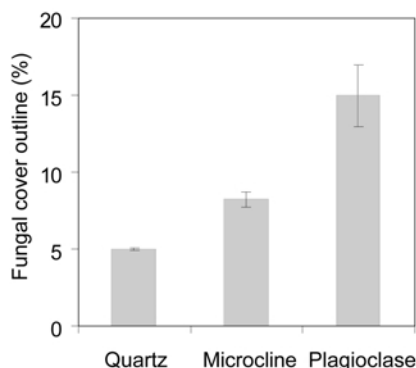


FIG. 1. Fungal cover of different mineral species in the upper 2 cm of the mineral soil in a North Michigan dune (data from Smits *et al.*, *in prep.*). Standard error of means are shown, $n = 30$.

(2002) concluded that there is no feedback between nutrient shortage in the tree and increased weathering of minerals containing these nutrients.

Since EcM fungi form the main absorptive and secretory interface between trees and soil, the interactions between individual 5–10 μm diameter hyphae and mineral crystals allows the fungi to penetrate, voids, etch pits and cleavage planes in crystals. Preferential colonization of plagioclase over other minerals (Smits *et al.*, *in prep.*) and increased fungal growth on individual apatite grains establishes grain-scale interactions of EcM hyphae (Figs 1, 2), (Leake *et al.*, 2008, this volume).

To investigate the spatial scale, mode of action, and role of EcM in mineral weathering, we have addressed four key questions: (1) Do the hyphae of EcM fungi show different amounts of growth on different minerals? (2) Do EcM fungi direct their weathering actions to specific mineral crystals containing essential plant or fungal elements? (3) What is the nature of the fungal-mineral interface? (4) Can locally enhanced activity of EcM in mineral weathering at the grain-scale account for a coupling between plant nutrient limitation and increased fungal weathering action?

We have developed novel microcosm reactors enabling plant-ectomycorrhizal fungal interactions with minerals to be studied with nano-

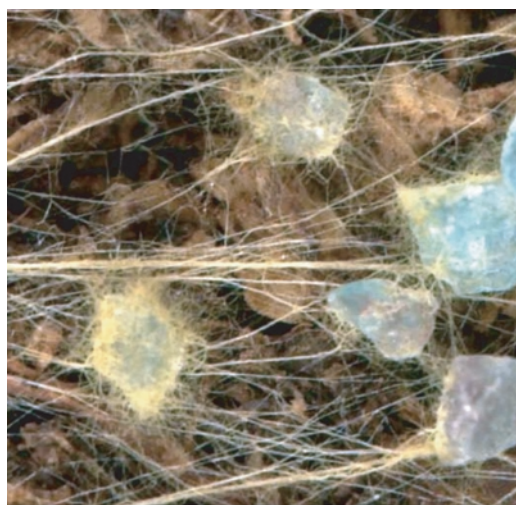


FIG. 2. Apatite grains (1 mm diameter) in a peat microcosm with Scots pine and the EcM fungus *Paxillus involutus*.

scale physical and chemical imaging technologies (see Leake *et al.*, 2008, this volume, for details).

Experiments

Pinus sylvestris trees mycorrhizal with *Paxillus involutus*, were grown under sterile conditions in 10 cm × 10 cm agar-based microcosms. Minerals were applied in weathering arenas which only the fungus can colonize (Leake *et al.*, 2008, this volume, for illustrations).

Apatite dissolution

Microcosms were established with two levels of P in nutrient agar (0 or 5 mg P per microcosm) and with or without apatite added in the weathering arenas (0.2 g total per microcosm, grain size 150–250 μm mixed with quartz grains, 5% apatite w/w). After three months of growth, P was measured in plant parts and external hyphae. In a parallel set of microcosms two pairs of weathering arenas were provided, one pair with quartz sand and the other with apatite mixed with quartz to test for preferential fungal colonization and carbon allocation to the apatite. After 3 months of growth the shoots were ¹⁴CO₂ labelled. After 48 h the systems were scanned under an autoradiographic imager and subse-

quently harvested. The ¹⁴C was measured in the different parts of the plant, fungus and weathering arenas.

In microcosms with apatite as the sole source of P, plants accumulate twice as much P compared to control systems without a P source (0.74 mg vs. 0.31 mg) (Fig. 3). P release from apatite in uncolonized wells is only 0.14 mg, indicating that fungal colonization increased weathering threefold. There was preferential allocation of ¹⁴C to the wells containing apatite, compared to the wells with only quartz sand (Fig. 4).

The nature of the fungal-mineral interface: biotite

Biotite flakes (0.5 cm × 0.5 cm, <1 mm thick) were cleaved, leaving an atomically flat surface. They were autoclave sterilized and added to microcosms containing *Pinus sylvestris* mycorrhizal with *Paxillus involutus*. After 3 months of colonization by the fungus, the mineral and fungal surface was analysed using atomic force microscopy (AFM) and a series of Force Ion Beam (FIB) sections were taken of the fungal mineral interface: close to the fungal tip (young) and subsequently further away (older) and studied using transmission electron microscopy (TEM) and scanning TEM (Bonneville *et al.*, 2008).

AFM analysis did not reveal any signs of surface change around the hyphae. Detailed analysis of the fungal cell wall showed hydrophobin-like structures and crystalline deposits. Force spectroscopic measurements confirmed the

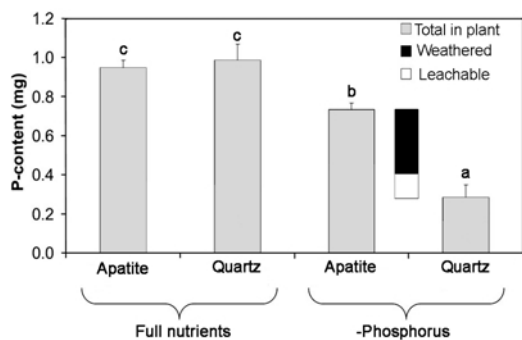


FIG. 3. The phosphorus content of *Pinus sylvestris* mycorrhizal with *Paxillus involutus* and grown with full mineral nutrients, or full nutrients except phosphorus, and provided with either quartz or apatite in weathering arenas. Significant differences between mean plant P content are indicated by bars not sharing the same letter (Tukey multiple-comparison test, $n = 3-4$). The white bar indicates the amount of water-soluble P that was leachable from apatite incubated in non-mycorrhizal control systems and black bar the additional P weathered from the apatite by mycorrhiza and transferred to the plant in the phosphorus treatment.

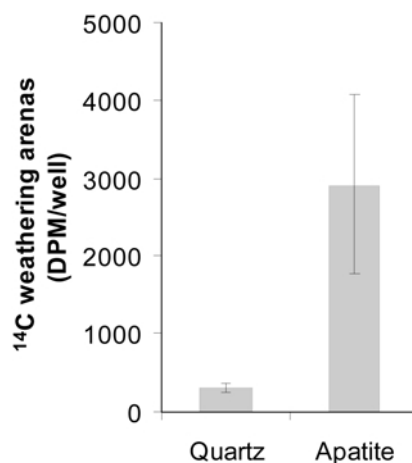


FIG. 4. ¹⁴C counts in the well without apatite ('Quartz') and wells with apatite mixed with quartz ('Apatite'). Standard errors of means are shown, $n = 3$.

hydrophobicity of the fungal cell walls (McMaster *et al.*, 2008). The analysis of the FIB sections suggests that the early stage of fungal colonization caused nanoscale cracks below the surface of the fungus, and at a later stage chemical alteration takes place (K-depletion).

Conclusions

We show for the first time grain-scale effects of EcM fungi in symbiosis with a host plant on mineral weathering under sterile conditions. The results confirm that EcM fungi are an active agent in weathering of minerals (apatite, biotite) and that photosynthate energy from the tree is actively transported to the fungus where it is preferentially allocated to areas with minerals containing weatherable supplies of essential nutrient elements. Detailed analyses of the fungal-mineral interface show that the actions of the fungus operate at the scale of individual hyphae and individual mineral grains. These factors need to be integrated into existing models of biotic weathering in the boreal forest, and in future experimental studies of plant-soil weathering interactions.

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