ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/fbr

Fungal Biology Reviews

Review

The fungal–mineral interface: challenges and considerations of micro-analytical developments

Mark M. SMITS[a,*], Anke M. HERRMANN[b], Michael DUANE[c], Owen W. DUCKWORTH[d], Steeve BONNEVILLE[e], Liane G. BENNING[e], Ulla LUNDSTRÖM[f]

[a]Microbial Ecology, Lund University, Ecology Building, SE-223 62 Lund, Sweden
[b]Department of Chemistry, Uppsala BioCenter, SLU, PO Box 7015, SE-750 07 Uppsala, Sweden
[c]Department of Earth and Environmental Sciences, Faculty of Science, PO Box 5969, Safat 13060, Kuwait
[d]Department of Soil Science, North Carolina State University, PO Box 7619, Raleigh, NC 27695-7619, USA
[e]Earth and Biosphere Institute, School of Earth and Environment, University of Leeds, Leeds LS2 9JT, United Kingdom
[f]Department of Natural Sciences, Technology and Mathematics, Mid Sweden University, SE-851 70 Sundsvall, Sweden

ARTICLE INFO

Article history:
Received 24 March 2009
Received in revised form
14 October 2009
Accepted 29 November 2009

Keywords:
FIB
Fungal weathering
SEM
Surface analysis
TEM
XRFS

ABSTRACT

Over recent years, the role of fungi, especially mycorrhizal fungi, in the weathering of rock-forming minerals has been increasingly recognised. Much of our understanding of the effects of fungi on mineral weathering is based on macroscopic studies. However, the ability of fungi to translocate materials, including organic acids and siderophores, to specific areas of a mineral surface leads to significant spatial heterogeneity in the weathering process. Thus, geomycologists are confronted with unique challenges of how to comprehend and quantify such a high degree of diversity and complicated arrays of interactions. Recent advances in experimental and analytical techniques have increased our ability to probe the fungal–mineral interface at the resolution necessary to decouple significant biogeochemical processes. Modern microscopy, spectroscopy, mass spectrometry, wet chemistry, and scattering techniques allow for the selective extraction of physical, chemical, and structural data at the micro- to nano-scale. These techniques offer exciting possibilities to study fungal–mineral interactions at the scale of individual hyphae. In this review, we give an overview of some of these techniques with their characteristics, advantages and limitations, and how they can be used to further our understanding of biotic mineral weathering.

1. Introduction

The transformation of rock into soils (i.e., weathering) is at the foundation of terrestrial ecosystem productivity, agriculture, and forest production. Initially, this process was primarily conceptualized in terms of abiotic reactions. However, growing evidence now suggests that soil microbiota associated with plant-roots largely contribute to, if not control, the weathering of rock-forming minerals (Hoffland *et al.*, 2004). Classical studies of biotic weathering have mainly focused

* Corresponding author. Tel.: +46 46 222 86 17; fax: +46 46 222 38 00.
E-mail address: mark.smits@mbioekol.lu.se (M. M. Smits).
1749-4613/$ – see front matter © 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.fbr.2009.11.001

on water–mineral interactions and the ability of plants and microorganisms to influence the soil solution chemical composition. Much of this research has been dedicated to organisms (mainly bacteria) cultivated in batch experiments in nutrient-rich liquid medium, and aims to evaluate their abilities to favour bulk dissolution reactions (see Hutchens *et al.*, 2006; Wu *et al.*, 2008). However, soils are typically under-saturated environments, in which most of the microorganisms are found firmly attached to the surfaces of minerals which are themselves only intermittently exposed to the soil solution. Therefore, most biotic weathering is restricted to the mineral–microorganism interface. As for any living organism, microbial metabolic function is dependent on energy supply. In this respect, filamentous fungi have an advantage over bacteria, namely, that they have the ability to concentrate energy and biomass towards specific weathering locations through carbon translocation *via* their mycelia (Rosling *et al.*, 2004; Leake *et al.*, 2008), avoiding the need to rely on diffusion through bulk soil solution. Moreover, many fungi that exist in soils and on rock surfaces are found in symbiosis with plant-roots (i.e., mycorrhizal) or algae (lichens), benefiting from a significant part of the carbon compounds produced by their phototrophic partners. This combination of both access to, and transport of energy from, a distant source, to specific locations makes fungi one of the prevalent microorganisms in the biotic weathering processes. Because of the microscopic nature of fungal filaments (hyphae), the biotic weathering operates and needs to be understood at a sub-micrometer level, a great challenge for scientists, even with the help of the latest techniques. Recent developments in conventional and synchrotron-based X-ray approaches, combined with advances in spatially resolved micro-analytical techniques [e.g., atomic force microscopy (AFM), scanning and transmission electron microscopy (SEM and TEM), and secondary ion mass spectrometry (SIMS)], open new avenues for such research. This suite of techniques, already widely used in environmental geochemistry (see Maurice and Hochella, 2009 and reference therein), gives mycologists unprecedented opportunities to advance the understanding of the nature of the fungal–mineral interface and to quantify the biogeochemical reactions associated with it. To further progress in the field of biotic weathering, the aim of this paper is therefore to (i) highlight the challenges in the study of fungal–mineral interactions, (ii) give an overview of the existing techniques with their specific applications and limitations in the field of fungal–mineral interactions, and (iii) provide an outlook on how micro-analytical methods can be integrated into a holistic approach.

2. Nature and challenges of the fungal–mineral interface

Fungi have the ability to degrade mineral substrates through biomechanical and biochemical processes (e.g., Burgstaller and Schinner, 1993; Banfield *et al.*, 1999; Burford *et al.*, 2003; Fomina *et al.*, 2005; Bonneville *et al.*, 2009). At the fungal–mineral interface, three categories of phenomenon levels are of importance in understanding biotic weathering: (i) mineral colonization; (ii) fungal exudation (iii) cell wall–mineral surface interactions. A schematic representation of these interactions is given in Fig 1.

Fungal hyphae grow in intimate contact with mineral grains (Van Breemen *et al.*, 2000) and they may exert mechanical forces by osmotically applied turgor pressure (Money, 2004), resulting in biomechanical weathering of the minerals (Bonneville *et al.*, 2009). Mineral penetration is supported by mucilaginous slime produced by fungi which may contain acidic and metal-chelating metabolites (Burford *et al.*, 2003). Fungal mineral colonization is highly heterogeneous and some minerals are preferentially colonized over others (Rosling *et al.*, 2004; Smits *et al.*, 2008), furthermore individual hyphae show a tendency to grow on areas with a high concentration of active sites, such as crystal planes, cleavage, cracks and etch pits (Banfield *et al.*, 1999; Burford *et al.*, 2003; Smits, 2006).

Exudation and uptake processes can dramatically alter the solution chemistry in the direct vicinity of the hyphae. Exudates can include a wide range of molecules, including protons, organic chelators, siderophores, and organic polymers (mucilage) (Hoffland *et al.*, 2004), which can all enhance dissolution reactions either by direct adsorption and destabilisation of the mineral surface or by inducing changes in the chemical parameters driving dissolution reactions (i.e., pH and redox conditions).

The fungus not only interacts with the mineral *via* the liquid interface. There is also, and in some cases (i.e., in under-saturated environments) more importantly, a direct contact between the fungal cell walls and the mineral surface. Recent studies that have focussed on the processes at the fungal–mineral interface indicate a close contact between the fungal hyphae and the mineral surface (Lee *et al.*, 2007; Bonneville *et al.*, 2009), and these studies show that weathering occurs *via* a combined biomechanical and biochemical process. In addition, reactive groups on the cell wall surface could directly interact with the mineral surface, influencing the weathering kinetics.

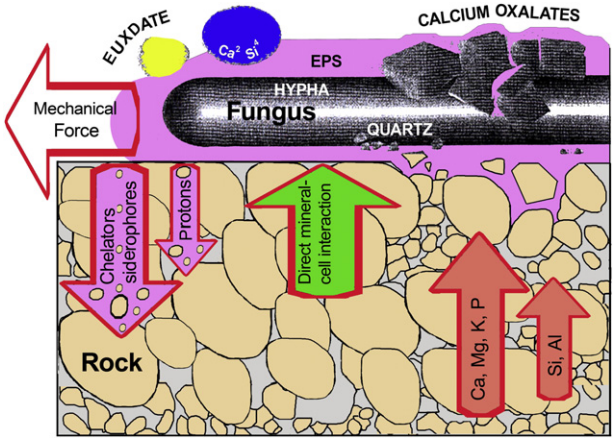

Fig. 1 – Schematic representation of the three-process-level interactions between mineral/rocks and the fungal hypha: (i) mechanical force, (ii) exudation of protons, organic chelators and siderophores, and (iii) direct cell wall–mineral surface interactions.

The three-process-level phenomena mentioned above (Fig 1) occur often concurrently, sometimes in a synergistic manner, and they can change simultaneously over various spatio-temporal scales. Geomycologists are therefore confronted with unique challenges of how to deal both conceptually and experimentally with such a high degree of diversitiy and complicated arrays of interactions. Firstly, the interface involves a crystalline mineral phase, a biotic fungal phase, and a liquid interface, with interactions that occur at molecular to microbial scales. Secondly, the interactions between fungi and minerals are highly dynamic, both in space and time. The fungal hypha is mostly active near the tip, and tip growth may be as fast as 10 μm/min (e.g., López-Franco *et al*., 1994). Finally, the fungal–mineral interface is far from being a closed system because fungal hyphae usually form extensive mycelial networks that enable the transport of water, nutrients, and other elements to and from the fungal–mineral interface and the host-plant. Many fungi live in symbiosis with a photosynthetic host organism (i.e., higher plants or algae), giving a major, distant source for carbon (photosynthates) and a sink for nutrients (uptake into the plant). The diversity and complexity of fungal–mineral interactions necessitate careful consideration of sampling strategies in order to understand and quantify biotic weathering at the fungal–mineral interface.

3. Recent developments in micro-analytical techniques

In the last 25 y, sensitivity and resolution of micro-analytical techniques has been improved significantly. In some cases, we now can analyse material at parts per million and nanometre resolution. A few examples are the development of the atomic force microscope (AFM) in 1986 (Binnig *et al*., 1986), improvement of the environmental scanning electron microscope (ESEM) in the late eighties (Danilatos, 1988), and the analytical developments in scanning transmission X-ray microscopy (STXM) (Ade *et al*., 1992). For instance, synchrotron-based X-ray microscopy allows for spatially resolved chemical characterization at the submicron level of the microorganism–mineral interface and, when combined with high-resolution microscopy [i.e., high resolution transmission electron microscopy (HR-TEM) and focused ion beam (FIB)], molecular level information can now be derived (Bonneville *et al*., 2009; Obst *et al*., 2009). In the following sections, we discuss the current state-of-the art of some techniques and the challenges faced in their application to the interdisciplinary field of geomycology. Table 1 provides an overview of information that can be obtained as well as sample size and limitations of each of these techniques.

Topography

Developed in 1986, AFM utilizes an optical level coupled with a microscopic tip to probe the microtopography of a surface (Binnig *et al*., 1986). This technique allows for sub-nanometre to micron scale spatial measurements under ambient conditions (*viz*. air or solution), and thus provides unique opportunities for studying fungal–surface interactions. Conventional imaging is accomplished by tapping or dragging (contact mode) a sharp tip mounted on the end of a cantilever across the mineral surface, and measuring the deflection of the tip with a laser. A more advanced AFM method to look at mineral surfaces is to probe the attractive or repulsive forces between the tip and surface without them touching (non-contact mode). In mineral weathering studies, AFM is typically used to monitor the development of surface topographic features during (*in situ*) or after reaction (*ex situ*). Abiotic mineral dissolution experiments have been conducted using biomolecules associated with fungal exudation, including oxalate (Sutheimer *et al*., 1999), small organic acids (Hong *et al*., 1997; Teng and Dove, 1997), siderophores (Buss *et al*., 2002) and extracellular polysaccharides (Buss *et al*., 2002; Perry *et al*., 2004). The use of an AFM flow-through cell allows for the measurement of the bulk solution chemistry, and thus gives the opportunity to determine and compare weathering rates derived from the AFM imaging capability (from 1×1 μm up to 100×100 μm in lateral resolution) and measured by the variation over time in bulk solution chemistry involving the total mineral surface area (Shiraki *et al*., 2000; Duckworth and Martin, 2004; Perry *et al*., 2004).

Although AFM imaging provides no direct chemical information about the surface, physical properties of the surface and its interactions with the tip can be probed by tapping the surface. The tip of the AFM not only gathers topographical information but also records variations in the phase lag between the tip and its driver, reflecting variation in physical surface properties. Similarly, lateral deflection of the cantilever during contact imaging provides information about the strength of the interaction between the tip and the surface. Although these ''phase'' and ''friction'' images do not give structural or chemical data on the mineral surface, it is a useful and sensitive method to map the spatial variations in surface properties at a nano- to micrometer scale. More detailed surface information can be gathered by using the AFM as a force spectrometer (FS) to measure repulsive or attractive interaction forces between surfaces and the tip. The tip can be ''functionalized'' with specific molecules (Dufrêne and van der Aa, 2002; Kendall and Hochella, 2003) or *via* the use of a voltage biased-tip (Kendall and Martin, 2007). FS has used to observe bacterial–mineral interactions (Lower *et al*., 2000, 2001a,b), and microfungi (Pouliot *et al*., 2005).

AFM does not require a high vacuum or specific sample preparation and can operate with or without fluids. It can even be applied to living fungi, observing *in situ* growth and cell wall development (Ma *et al*., 2005). The main limitations are the restricted field of view ($<100 \times 100$ μm) and the prerequisite of an extreme (atomically) flat surface. Although AFM has been used extensively to study bacterial interactions with surfaces during weathering (Grantham and Dove, 1996; Maurice *et al*., 1996; Grantham *et al*., 1997; Forsythe *et al*., 1998), it has been much less utilized less for fungal weathering studies. Recently, Balogh-Brunstad *et al*. (2008) used *ex situ* AFM, in conjunction with SEM and wet chemistry, to study the fungal attachment and weathering of biotite. Fungi were found to attach directly to the mineral surface and caused the formation of dissolution features with a concomitant

Table 1 – Overview of microscopy and spectroscopy techniques.

	Information	Spatial resolution	Detection limit	Limitations
FM (Atomic force microscopy) FS (Force spectroscopy)	3-D surface microtopography Interfacial forces	<10 nm		Max. sample size, require extreme flat surfaces
SEM (Scanning electron microscopy)	3-D spatial imaging	10–15 nm		No liquid phase (vacuum)
EDS (Energy dispersive spectroscopy)	Elemental compositional measurements	5 μm	~0.1 %	
TEM (Transmission electron microscopy) EELS (Electron energy loss spectroscopy)	2-D spatial imaging Elemental compositional measurements	0.1–0.2 nm 1 nm	~0.0001 %	Sample thickness, risk of artefacts with sample preparation using FIB (vacuum)
SAED (Selected area electron diffraction)	Crystal structure and long-range ordering	0.2–1 μm		
SIMS (Secondary ion mass spectroscopy)	Elemental and isotopic compositional measurements (dynamic mode), molecular information (static mode)	50 nm (NanoSIMS) 10–15 μm (SIMS)	~0.01 %	No liquid phase (high vacuum), destructive
XRD (X-ray diffraction)	Crystal structure and long-range ordering	5–10 μm (conv. XRD for single crystals) Sub-μm (synchrotron μXRD)		Spatial resolution
STXM (Scanning transmission X-ray microscopy)	Composition and structural chemistry (adsorption, binding)	5–30 nm		Sample thickness, risk of artefacts with sample preparation using FIB
NEXAFS (Near edge X-ray absorption fine structures)		5–30 nm	~0.001 %	
LC-ESI-MS/MS (Liquid chromatography electrospray ionization mass spectrometry)	Molecular identification and quantification	n.a.	50 pM–50 nM	Solution sampling limits spatial resolution
MALDI-MS (Matrix assisted laser desorption/ionization-mass spectrometry)	Molecular identification and quantification	10 μM	1 fmol $pixel^{-1}$	Variable ionization rate hinders quantification
Fluorescent confocal microscopy	3-D spatially resolved quantitative data of target molecules	0.5 μM	Variable	Lack of probes, sensitivity to variation in chemistry

increase in dissolution rates. Nevertheless, AFM does not provide information in relation to the interfacial contact zone between fungi and the mineral substrates where weathering processes are thought to be intense.

Elemental and isotopic analysis

SEM, including ESEM, has been used extensively for the surface visualisation of fungi in association with minerals (e.g., Fomina *et al.*, 2005; Gleeson *et al.*, 2005; Burford *et al.*, 2006; Rosling, 2003; Rosling *et al.*, 2007). SEM (and in particular Field Emission Gun – FEG-SEM) can provide spatial information at the micrometer-to sub-micrometer scales. Coupled with energy dispersive spectroscopy (EDS), this approach can reveal high-resolution elemental compositional measurements. Structural characterization, chemical analysis and imaging at the nanometre scale (*viz.* sub-cellular) can be achieved with TEM. Both SEM and TEM can provide elemental distribution maps when coupled with EDS, with Scanning Transmission Electron Microscopy (STEM-EDS) having a higher resolution. In addition, STEM can be used to measure and map the oxidation state of elements *via* electron energy loss spectroscopy (EELS). While EDS is particularly useful to determine chemical composition, especially heavier elements, EELS is optimal for lower weight elements (C–Zn). Additionally, structural analyses can be accomplished with TEM by using selected area electron diffraction (SAED) (see section below).

TEM analysis requires very thin samples (<100 nm) to allow the electron beam to pass through (transmission) the samples. Therefore, samples are commonly produced by microtoming or by the FIB approach. FIB is a spatially resolved ion milling technique that uses a focused ion beam of gallium ions to extract cross-sections from the mineral surface. Adequate sample preparation to avoid Ga incorporation into the cross-section is key for successful application of FIB coupled with TEM or other micro-analytical techniques (Lee *et al.*, 2007). FIB in combination with TEM has been used (Fig 2) to sample and study the surface of naturally weathered alkali feldspars (Benzerara *et al.*, 2005a,b; Lee *et al.*, 2007) and fungal–biotite interfaces (Bonneville *et al.*, 2009).

SIMS is an ion microprobe technology that provides spatially resolved, elemental and isotopic analysis of mineral surfaces at scales ranging from a few μm for conventional SIMS to 50 nm lateral resolution with the new SIMS generation (Cameca NanoSIMS 50®, referred to NanoSIMS in the following) (Herrmann *et al.*, 2007a). In the context of soil ecology, Cliff *et al.* (2002, 2007) used time-of-flight, secondary ion mass spectrometry (TOF-SIMS) to qualitatively describe the *in situ* assimilation of ^{15}N and ^{13}C into soil microorganisms. With NanoSIMS, the much improved lateral resolution allows for the analysis of isotopic signatures at a sub-cellular

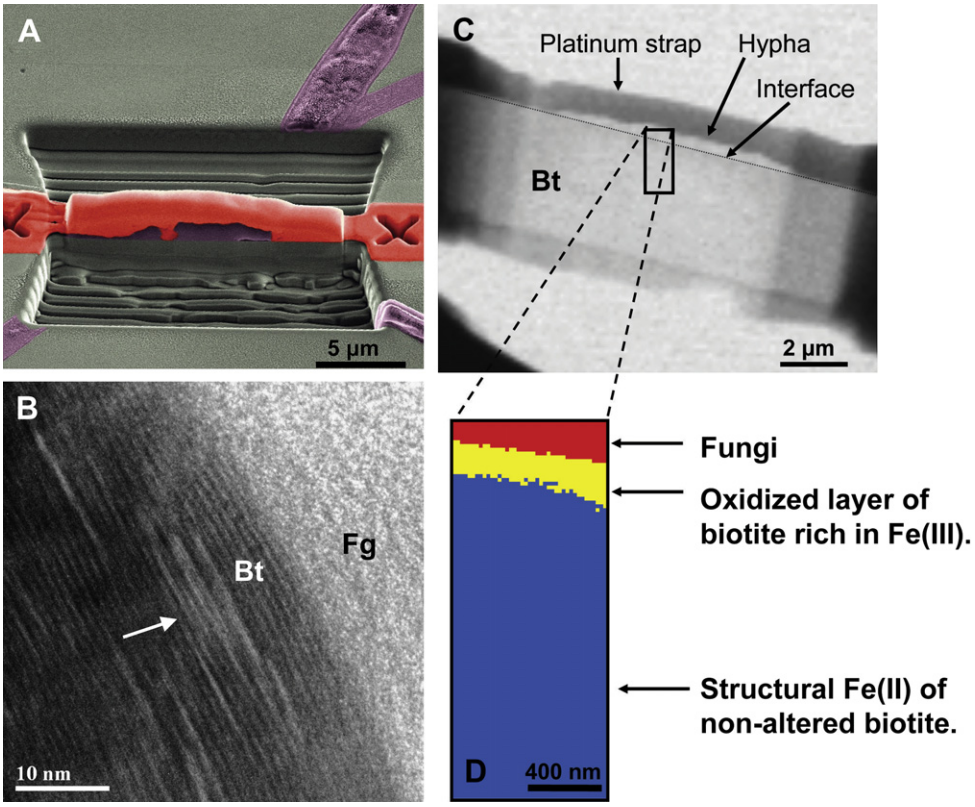

Fig. 2 – (A) SEM micrograph of ion milling process by FIB on a hypha at the surface of a biotite flake (red: protective platinum strap; purple: fungal hypha). (B) HR-TEM bright field micrograph of the hypha–biotite interface with stacking default within the biotite structure (white arrow), (C) NEXAFS image of a hypha–biotite FIB section at 700 eV. (D) Fe speciation map of the hypha–biotite interface derived from a principal component analysis of sequence of NEXAFS images at the Fe L2,3 edge. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

level which is particularly useful to the ecophysiology of microorganisms in complex environments and communities (Behrens *et al.*, 2008; Musat *et al.*, 2008). Herrmann *et al.* (2007a,b) showed that NanoSIMS can also be used to detect isotopically enriched bacteria in the soil matrix (Fig 3). Alternatively, by sputtering the mineral surface, SIMS can provide depth profiles of element abundances which could potentially prove to be useful in understanding the fungi–mineral interface.

Each of the above mentioned techniques has its own specific focus and sample restrictions. SIMS, specifically NanoSIMS, requires a stable, conductive and fairly flat surface (surface roughness < 5–$10\ \mu m$) (Herrmann *et al.*, 2007a). Charging effects (i.e., obscuring the boundaries between different mineral areas) may occur when depth profiling heterogeneous mineral surfaces as primary ion beam ablation may cause differences in variations in surface topography. Such effects, however, have not yet been checked but an electron flood gun can lessen emerging charging effects. SEM, except for ESEM, can accommodate non-flat and mineral grain-type samples however with conductive coating (carbon, platinum or gold) a few nanometers thick but the latter has an limited spatial resolution compared to SEM. TEM only requires ultra-thin samples but the high voltage of the TEM beam (80–300 kV) can induce significant beam damage to the sample and in some cases can hinder elemental analysis. Apart from ESEM which can operate in relatively humid and low vacuum conditions and without conductive coating, the common disadvantage of all the techniques listed above is the requirement of high vacuum which prevents the analysis of liquid phases around the fungi or possibly at the fungi–mineral interface (although some of the techniques can be applied with shock-frozen samples, see below ''Composition of the liquid phase of the interface'').

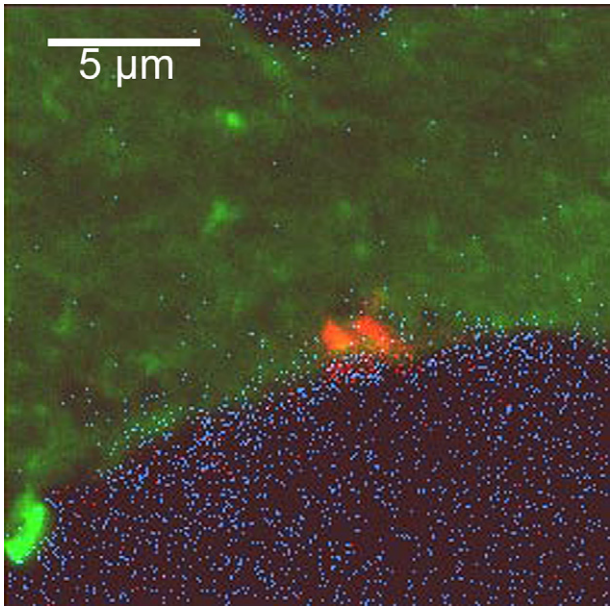

Fig. 3 – NanoSIMS image of a cross-section of ^{15}N enriched *Pseudomonas fluorescens* (red), attached to a mineral particle (Si in blue), surrounded by ^{14}N material (green) (see Herrmann *et al.*, 2007b for further information). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Structural analysis

TEM or SEM, in combination with EDS analysis and SIMS, can provide imaging, elemental and isotopic information at micro- to nanometre-scales. However, the analytical ideal for examining the fungal–mineral interface would be a method, or combination of methods, offering both structural, as well as elemental, composition data. One established technique to study mineral crystal structure is X-ray diffraction (XRD). The obtained diffractogram gives information on crystal structure and, when compared to a database, allows rapid identification of mineral species. XRD has been used extensively to identify biominerals at fungal–mineral interfaces (e.g., Wilson *et al.*, 1980; Krumbein and Jens, 1981; Adamo and Violante, 2000; Russ *et al.*, 1996). However conventional XRD usually analyses powders or single crystals and no capability of spatially resolved measurements is available.

Spatially resolved measurements of long- and short-range order can be achieved by modern diffraction and spectroscopy techniques. Selected area electron diffraction (SAED), using the electron beam of the TEM instead of X-rays to induce diffraction, has the great advantage of a high lateral resolution (i.e., from 0.2 to 1 μm) and also the possibility to spatially link structural data to element data within the same TEM sample. Compared to conventional XRD, synchrotron-based μ-X-ray techniques offer improved spatial resolution data and faster acquisition times than traditional XRD (Manceau *et al.*, 2002). X-ray diffraction can be measured down to the sub-μm level (Cloetens *et al.*, 1996). These advances make time-resolved and *in situ* measurements feasible.

Another family of synchrotron-based techniques, X-ray spectroscopy, provides information about the local electron and coordination structure of the targeted atom. Scanning transmission X-ray microscopy (STXM), coupled with near edge X-ray absorption fine structure (NEXAFS) or X-ray absorption near edge structure (XANES), can provide information on the structural organic/inorganic chemistry of soil/minerals at nanoscales. The technique provides both an image of soil particles as well as a spectrum for a given area on the respective image that can be used for functional group speciation, making it possible to evaluate relationships between organic matter chemistry and mineral surface composition. STXM coupled with NEXAFS has been used to study microbial–mineral interactions of filamentous microorganisms and biotic weathering products of Mg–Fe-orthopyroxene at nm-scales (Benzerara *et al.*, 2005a). Recently STXM–XANES was used to demonstrate local changes in the oxidation state of Fe in biotite near the fungal–mineral interface (Bonneville *et al.*, 2009), as shown in Fig 2. Samples for STXM can be small grains, but can also be prepared using the FIB approach described above (Benzerara *et al.*, 2005a; Bonneville *et al.*, 2009). The advantage of using FIB prepared samples is the ability to combine TEM and STXM techniques to a wide range of data on the same region of interest.

Composition of the liquid phase of the interface

Of all aspects of the fungi–mineral interactions, pH, redox status and chemical composition of fluids is probably the most challenging to study because of the extremely restricted volumes available and the highly temporally dynamic nature of organic solutes. However, recent developments in analytical tools enable measurements of organic exudates in the sub-pmol range. The combination of liquid chromatography with electrospray ionization mass spectroscopy (LC-ESI-MS/MS) allows measurement of low molecular weight organic acids and siderophores down to 50 pM in 100 μL samples (Bylund *et al.*, 2007; Moberg *et al.*, 2003, 2006) Currently, the micro-sampling at specific areas colonized by fungi actually limits the spatial resolution of studies of fluid chemistry. Techniques such as micro-suction (Dieffenbach and Matzner, 2000) and centrifugation (Geibe *et al.*, 2006) have been used but these are not suitable at the scale of individual hyphae. Nevertheless, these methods can help to link the local chemistry at this scale to the presence of fungal hyphae. Because of the small volume of sample needed for the determination of low molecular mass organic acids and siderophores, it would be possible to develop more precise sampling techniques.

A different approach utilizes shock-frozen samples combined with surface analytical techniques. Matrix assisted laser desorption/ionization coupled with a mass spectrometer (MALDI-MS) has been successfully used to study metabolite distribution at a cellular level in plant tissues (Burrell *et al.*, 2007). The ideal matrix appears to be water ice, which make this technique potentially valuable to obtain spatially resolved data of the fluid's chemical composition. To our knowledge, this technique has not yet been applied to extracellular aqueous chemistry. TEM has also been applied with frozen samples (Gustafsson *et al.*, 1995). With this technique the most critical step is handling of the ultra-thin cryo-TEM samples (MoberlyChan *et al.*, 2005). Up to now a combined cryo-FIB/SEM and TEM is hitherto not available in one instrument (pers. comm. Susan Stipp).

In the field of fungal and plant physiology, fluorescence imaging with confocal microscopy is a rapidly developing tool to study *in situ* molecular dynamics at the sub-cellular level (Emptage, 2001). The latest advances in confocal microscopy allow for the acquisition of spectral data at the sub-micrometer scale and in three dimensions. Also promising is the possibility to use fluorescent probes to target specific molecules and subsequently derive concentrations. This method was successfully applied in a study on bacterial–mineral interactions in which pH changes in biotite interlayers colonized by bacteria were measured (Barker *et al.*, 1998). A variety of fluorescent probes are now available to measure pH, the concentrations of a number of cations, and even hydrophobicity. The development of multiphoton microscopy improved fluorescence imaging considerably, by reducing the photobleaching and the out-of-focus light scattering. However, the lack of probes for most of the fungal exudates and the sensitivity currently limit the use of fluorescent probes to investigate fungi–mineral interactions and exudation products.

4. Outlook

Recent development of micro-analytical techniques offers exciting possibilities to study fungal–mineral interactions at the scale of individual hyphae. Most of these techniques are only applicable to solid phases (i.e., fungus and mineral components of the interface) as the requirements for sample preparation most often exclude the study the aqueous phase. The liquid–mineral phase interface remains probably the most challenging, but important, aspect to study in respect to fungal–mineral interactions.

Up to now, most studies have focussed on either the mineral, the fungus, or the liquid phase chemistry. A unified approach that focuses on all three aspects in the same study would provide unique insights into our understanding of fungal–mineral interactions (Banfield *et al.*, 1999). Advances in instrumentation have allowed for many of the above techniques to be combined into a single platform that can simultaneously probe different aspects of biotic mineral weathering. For instance, combined confocal and AF microscopy are capable of fluorescence imaging of microorganisms and solutes while monitoring changes in surface topography or surface chemistry. Other instrumentation pairs that once were mutually exclusive may now be used due to advances in sampling and sample preparation. For instance, ultra-thin samples, prepared for TEM and other transmission techniques, can also be analysed with NanoSIMS (Clode *et al.*, 2009), making it possible to spatially link structural visual (TEM), structural (SAED), elemental (EDS/EELS/NanoSIMS), chemical (STXM) and isotopic data (NanoSIMS). With further development of the cryo-instrumentation, the liquid phase could be included in most of these ultra-thin sample techniques.

The ability to study fungal–mineral interactions at high resolution raises a final key question: ''What are the implications of fungal–mineral interactions observed at the scale of the individual hyphae for biotic weathering at the ecosystem and global levels?'' Although it is critical to use these modern techniques to unravel the fundamental mechanisms of interactions at the fungal–mineral interface, a concerted effort with measurements needs to be placed in the larger context of biogeochemical cycling, environmental processes and ecosystem function. As fungal weathering is a spatially localized process (see e.g., Bonneville *et al.*, 2009), observations at the scale of the fungal–mineral interface are necessary to elucidate the mechanisms of fungal weathering. In order to extrapolate micron scale observation to soil or ecosystem scale, three aspects need to be considered: (i) spatial and (ii) temporal heterogeneity, and (iii) the rates of fungal–mineral interactions and processes. Experimental approaches at relevant spatial scales are required for accurate calibration of model parameters for modelling of fungal weathering at soil or ecosystem scales. Defining biogeochemical conditions in the fungal–mineral interface is likely to be the main challenge in developing accurate models (see Rosling *et al.*, 2010). However, recent developments of micro-analytical tools presented in this review will provide adequate sensitivity and resolution to obtain experimental data at the fungal–mineral

interface. The results of these studies, when combined with mathematical modelling approaches, can be reassembled to test and improve conceptual ideas in fungal–mineral interface research and they will further improve our understanding of biotic weathering.

Acknowledgements

MMS is supported by the Swedish National Energy Administration (STEM). AMH thanks the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS) for financial support. OWD thanks the North Carolina Agricultural Research Service for support. SB and LGB acknowledge funding from the UK Natural Environment Research Council ''Weathering Science Consortium'' grant no. NE/C004566/1 and also thank the staff of the PolLux beamline at the Swiss Light Source for their contribution during the STXM measurements as well as UK-Environsync2 for travel funds for the synchrotron work. The NanoSIMS image was obtained using the facilities at the Centre for Microscopy, Characterisation and Analysis (CMCA), The University of Western Australia. The authors acknowledge the facilities, scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the CMCA, The University of Western Australia, a facility funded by The University, State and Commonwealth Governments.

REFERENCES

Adamo, P., Violante, P., 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. Appl. Clay Sci. 16, 229–256.

Ade, H., Zhang, X., Cameron, S., Costello, C., Kirz, J., Williams, S., 1992. Chemical contrast in X-ray microscopy and spatially resolved XANES spectroscopy of organic specimens. Science 258, 972–975.

Balogh-Brunstad, Z., Keller, C.K., Dickinson, J.T., Stevens, F., Li, C.Y., Bormann, B.T., 2008. Biotite weathering and nutrient uptake by ectomycorrhizal fungus, *Suillus tomentosus*, in liquid culture experiments. Geochim. Cosmochim. Acta 72, 2601–2618.

Banfield, J.F., Barker, W.W., Welch, S.A., Taunton, A., 1999. Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. Proc. Natl. Acad. Sci. U.S.A. 96, 3404–3411.

Barker, W.W., Welch, S.A., Chu, S., Banfield, J.F., 1998. Experimental observations of the effects of bacteria on aluminosilicate weathering. Am. Mineral. 83, 1551–1563.

Behrens, S., Losekann, T., Pett-Ridge, J., Weber, P.K., Ng, W.O., Stevenson, B.S., Hutcheon, I.D., Relman, D.A., Spormann, A.M., 2008. Linking microbial phylogeny to metabolic activity at the single-cell level by using enhanced element labeling-catalyzed reporter deposition fluorescence *in situ* hybridization (EL-FISH) and NanoSIMS. Appl. Environ. Microbiol. 74, 3143–3150.

Benzerara, K., Yoon, T.H., Menguy, N., Tyliszczak, T., Brown, G.E., 2005a. Nanoscale environments associated with bioweathering of a Mg–Fe-pyroxene. Proc. Natl. Acad. Sci. U.S.A. 102, 979–982.

Benzerara, K., Menguy, N., Guyot, F., Vanni, C., Gillet, P., 2005b. TEM study of a silicate–carbonate–microbe interface prepared by focused ion beam milling. Geochim. Cosmochim. Acta 69, 1413–1422.

Binnig, G., Quate, C., Gerber, C., 1986. Atomic force microscope. Phys. Rev. Lett. 56, 930–933.

Bonneville, S., Smits, M.M., Brown, A., Harrington, J., Leake, J.R., Brydson, R., Benning, L.G., 2009. Plant-driven fungal weathering: early stages of mineral alteration at the nanometer scale. Geology 37, 615–618.

Burford, E.P., Fomina, M., Gadd, G.M., 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineral. Mag. 67, 1127–1155.

Burford, E.P., Hillier, S., Gadd, G.M., 2006. Biomineralization of fungal hyphae with calcite ($CaCO_3$) and calcium oxalate mono- and dihydrate in carboniferous limestone microcosms. Geomicrobiol. J. 23, 599–611.

Burgstaller, W., Schinner, F., 1993. Leaching metals with fungi. J. Biotechnol. 27, 91–116.

Burrell, M., Bendall, L., Earnshaw, C., Clench, M., Read, D., Leake, J., 2007. The cellular distribution of metabolites in mycorrhizal orchid roots measured by imaging MALDI. Comp. Biochem. Physiol. A 146, S222.

Buss, H.L., Luttge, A., Brantley, S.L., 2002. Etch pits and leached layers on iron-silicate surfaces during siderophore-promoted dissolution of iron silicates. Geochim. Cosmochim. Acta 66, A133.

Bylund, D., Norström, S.H., Essén, S.A., Lundström, U.S., 2007. Analysis of low molecular mass organic acids in natural waters by ion exclusion chromatography tandem mass spectroscopy. J. Chromatogr. A 1176, 89–93.

Cliff, J.B., Gaspar, D.J., Bottomley, P.J., Myrold, D.D., 2002. Exploration of inorganic C and N assimilation by soil microbes with time-of-flight secondary ion mass spectrometry. Appl. Environ. Microbiol. 68, 4067–4073.

Cliff, J.B., Bottomley, P.J., Gaspar, D.J., Myrold, D.D., 2007. Nitrogen mineralization and assimilation at millimetre scales. Soil Biol. Biochem. 39, 823–826.

Clode, P.L., Kilburn, M.R., Jones, D.L., Stockdale, E.A., Cliff, J.B., Herrmann, A.M., Murphy, D.V., 2009. In situ mapping of nutrient uptake in the rhizosphere using nano-scale secondary ion mass spectrometry. Plant Physiol., 151, 1751–1757; First published on October 7, doi:10.1104/pp.109.141499.

Cloetens, P., Barrett, R., Baruchel, J., Guigay, J.P., Schlenker, M., 1996. Phase objects in synchrotron radiation hard X-ray imaging. J. Phys. D Appl. Phys. 29, 133–146.

Danilatos, G.D., 1988. Foundations of environmental scanning electron microscopy. Adv. Electron. Electron Phys. 71, 109–250.

Dieffenbach, A., Matzner, E., 2000. *In situ* soil solution chemistry in the rhizosphere of mature Norway Spruce (*Picea abies* [L.] Karst.) trees. Plant Soil 222, 149–161.

Duckworth, O.W., Martin, S.T., 2004. Dissolution rates and pit morphologies of rhombohedral carbonate minerals. Am. Mineral. 89, 554–563.

Dufrêne, Y.F., van der Aa, B.C., 2002. *In situ* characterization of bacterial extracellular polymeric substances by AFM. Coll. Surf. B 23, 173–182.

Emptage, N.J., 2001. Fluorescent imaging in living systems. Curr. Opin. Pharmacol. 1, 521–525.

Fomina, M., Hillier, S., Charnock, J.M., Melville, K., Alexander, I.J., Gadd, G.M., 2005. Role of oxalic acid over-excretion in toxic metal mineral transformations by *Beauveria caledonica*. Appl. Environ. Microbiol. 71, 371–381.

Forsythe, J.H., Maurice, P.A., Hersman, L.E., 1998. Attachment of a *Pseudomonas sp*. to Fe(III)-(hydr)oxide surfaces. Geomicrobiol. J. 15, 293–308.

Geibe, C.E., Danielsson, R., van Hees, P.A.W., Lundström, U.S., 2006. Comparison of soil solution sampled by centrifugation, two types of suction lysimeters and zero-tension lysimeters. Appl. Geochem. 21, 2096–2111.

Gleeson, D.B., Clipson, N., Melville, K., Gadd, G.M., McDermott, F.P., 2005. Characterization of fungal community structure on a weathered pegmatitic granite. Microb. Ecol. 50, 360–368.

Grantham, M.C., Dove, P.M., 1996. Investigation of bacterial–mineral interactions using fluid tapping mode(TM) atomic force microscopy. Geochim. Cosmochim. Acta 60, 2473–2480.

Grantham, M.C., Dove, P.M., Dechristina, T.J., 1997. Microbially catalyzed dissolution of iron and aluminum oxyhydroxyide mineral surface coatings. Geochim. Cosmochim. Acta 61, 4467–4477.

Gustafsson, J., Arvidson, G., Karlsson, G., Almgren, M., 1995. Complexes between cationic liposomes and DNA visualized by cryo-TEM. Biochim. Biophys. Acta 1235, 305–312.

Herrmann, A.M., Ritz, K., Nunan, N., Clode, P.L., Pett-Ridge, J., Kilburn, M.R., Murphy, D.V., O'Donnell, A.G., Stockdale, E.A., 2007a. Nano-scale secondary ion mass spectrometry—a new analytical tool in biogeochemistry and soil ecology: a review article. Soil Biol. Biochem. 39, 1835–1850.

Herrmann, A.M., Clode, P.L., Fletcher, I.R., Nunan, N., Stockdale, E.A., O'Donnell, A.G., Murphy, D.V., 2007b. A novel method for the study of the biophysical interface in soils using nano-scale secondary ion mass spectroscopy. Rapid Commun. Mass Spectrom. 21, 29–34.

Hoffland, E., Kuyper, T.W., Wallander, H., Plassard, C., Gorbushina, A.A., Haselwandter, K., Holmström, S., Landeweert, R., Lundström, U.S., Rosling, A., Sen, R., Smits, M.M., van Hees, P.A.W., van Breemen, N., 2004. The role of fungi in weathering. Front. Ecol. Environ. 2, 258–264.

Hong, Q., Suarez, M.F., Coles, B.A., Compton, R.G., 1997. Mechanisms of solid/liquid interfacial reactions. The maleic acid driven dissolution of calcite: an atomic force microscope study under defined hydrodynamic conditions. J. Phys. Chem. B 101, 5557–5564.

Hutchens, E., Valsami-Jones, E., Harouiya, N., Chairat, C., Oelkers, E.H., McEldoney, S., 2006. An experimental investigation of the effect of bacillus megaterium on apatite dissolution. Geomicrobiol. J. 23, 177–182.

Kendall, T.A., Hochella, M.F., 2003. Measurement and interpretation of molecular-level forces of interaction between the siderophore azotobactin and mineral surfaces. Geochim. Cosmochim. Acta 67, 3537–3546.

Kendall, T.A., Martin, S.T., 2007. Water-induced reconstruction that affects mobile ions on the surface of calcite. J. Phys. Chem. A 111, 505–514.

Krumbein, W.E., Jens, K., 1981. Biogenic rock varnishes of the Negev Desert (Israel) an ecological study of Fe and Mn transformation by cyanobacteria and fungi. Oecologia 50, 25–38.

Leake, J.R., Duran, A.L., Hardy, K.E., Johnson, I., Beerling, D.J., Banwart, S.A., Smits, M.M., 2008. Biological weathering in soil: the role of symbiotic root-associated fungi biosensing minerals and directing photosynthate-energy into grain-scale mineral weathering. Mineral. Mag. 72, 85–89.

Lee, M.R., Brown, D.J., Smith, C.L., Hodson, M.E., MacKenzie, M., Hellmann, R., 2007. Characterization of mineral surfaces using FIB and TEM: a case study of naturally weathered alkali feldspars. Am. Mineral. 92, 1383–1394.

López-Franco, R., Bartnicki-Garcia, S., Bracker, C.E., 1994. Pulsed growth of fungal hyphal tips. Proc. Natl. Acad. Sci. U.S.A. 91, 12228–12232.

Lower, S.K., Tadanier, C.J., Hochella, M.F., 2000. Measuring interfacial and adhesion forces between bacteria and mineral surfaces with biological force microscopy. Geochim. Cosmochim. Acta 64, 3133–3139.

Lower, S.K., Hochella, M.F., Beveridge, T.J., 2001a. Bacterial recognition of mineral surfaces: nanoscale interactions between Shewanella and a-FeOOH. Science 292, 1360–1363.

Lower, S.K., Tadanier, C.J., Hochella, M.F., 2001b. Dynamics of the mineral–microbe interface: use of biological force microscopy in biogeochemistry and geomicrobiology. Geomicrobiol. J. 18, 63–76.

Ma, H., Snook, L.A., Kaminskyj, S.G.W., Dahms, T.E.S., 2005. Surface ultrastructure and elasticity in growing tips and mature regions of *Aspergillus* hyphae describe wall maturation. Microbiology 151, 3679–3688.

Manceau, A., Marcus, M.A., Tamura, N., 2002. Quantitative speciation of heavy metals in soils and sediments by synchrotron X-ray techniques. Rev. Mineral. Geochem. 49, 341–428.

Maurice, P.A., Hochella, M.F., 2009. Nanoscale particles and processes: A new dimension in soil science. Adv. Agronomy 100, 123–153.

Maurice, P.A., Forsythe, J., Hersman, L., Sposito, G., 1996. Application of atomic-force microscopy to studies of microbial interactions with hydrous Fe(III)-oxides. Chem. Geol. 132, 33–43.

Moberg, M., Bergquist, J., Bylund, D., 2006. A generic stepwise optimization strategy for liquid chromatography electrospray ionization tandem mass spectrometry methods. J. Mass Spectrom. 41, 1334–1345.

Moberg, M., Holmström, S.J.M., Lundström, U.S., Markides, K.E., 2003. Novel approach to the determination of structurally similar hydroxamate siderophores by column-switching capillary liquid chromatography coupled to mass spectrometry. J. Chromatogr. A 1020, 91–97.

MoberlyChan, W.J., Marko, M., Hsieh, C.E., 2005. Cryo-FIB for thinning cryo-TEM samples and evading ice during cryo-transfer. Microsc. Microanal. 11, 854–855.

Money, M.P., 2004. The fungal dining habit – a biomechanical perspective. Mycologist 18, 71–76.

Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F., Horreard, F., Amann, R., Jorgensen, B.B., Kuypers, M.M.M., 2008. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. Proc. Natl. Acad. Sci. U.S.A. 105, 17861–17866.

Obst, M., Dynes, J.J., Lawrence, J.R., Swerhone, G.D.W., Benzerara, K., Karunakaran, C., Kaznatcheev, K., Tyliszczak, T., Hitchcock, A.P., 2009. Precipitation of amorphous $CaCO_3$ (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. Geochim. Cosmochim. Acta 73, 4180–4198.

Perry, T.D., Duckworth, O.W., McNamara, C.J., Martin, S.T., Mitchell, R., 2004. The effects of the biologically produced polymer alginic acid on macroscopic and microscopic calcite dissolution rates. Environ. Sci. Technol. 38, 3040–3046.

Pouliot, J.M., Walton, I., Parkhouse, M.N., Abu-Lail, L.I., Camesano, T.A., 2005. Adhesion of *Aureobasidium pullulans* is controlled by uronic acid based polymers and pullulan. Biomacromolecules 6, 1122–1131.

Rosling, A., 2003. Responses of ectomycorrhizal fungi to mineral substrates. Doctoral Thesis, Acta Universitatis Agriculturae Sueciae, Silvestria, vol. 296, Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, ISSN:1404-6230, ISBN:91-576-6530-3.

Rosling, A., Lindahl, B.D., Finlay, R.D., 2004. Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. New Phytol. 162, 795–802.

Rosling, A., Roose, T., Herrmann, A.M., Davidson, F.A., Finlay, R.D., Gadd, G.M., 2010. Approaches to modelling mineral weathering by fungi. Fungal Biol. Rev., 23, 138–144.

Rosling, A., Suttle, K.B., Johansson, E., Van Hees, P.A.W., Banfield, J.F., 2007. Phosphorous availability influences the dissolution of apatite by soil fungi. Geobiology 5, 265–280.

Russ, J., Palma, R.L., Loyd, D.H., Boutton, T.W., Coy, M.A., 1996. Origin of whewellite-rich rock crust in the lower Pecos Region

of Southwest Texas and its significance to Palaeoclimate reconstructions. Quater. Res. 46, 27–36.
Shiraki, R., Rock, P.A., Casey, W.H., 2000. Dissolution kinetics of calcite in 0.1 M NaCl solution at room temperature: an atomic force microscope study. Aquat. Geochem. 6, 87–108.
Smits, M.M., 2006. Mineral tunnelling by fungi. In: Gadd, G.M. (ed), Fungi in Biogeochemical Cycles. Cambridge University Press, Cambridge, pp. 311–327.
Smits, M.M., Bonneville, S., Haward, S., Leake, J.R., 2008. Ectomycorrhizal weathering, a matter of scale? Mineral. Mag. 72, 135–138.
Sutheimer, S.H., Maurice, P.A., Zhou, Q.H., 1999. Dissolution of well and poorly crystallized kaolinites: Al speciation and effects of surface characteristics. Am. Mineral. 84, 620–628.
Teng, H.H., Dove, P.M., 1997. Surface site-specific interactions of aspartate with calcite during dissolution: implications for biomineralization. Am. Mineral. 82, 878–887.
Van Breemen, N., Finlay, R.D., Lundström, U.S., Jongmans, A.G., Giesler, R., Melkerud, P.A., 2000. Mycorrhizal weathering: a true case of mineral plant nutrition? Biogeochemistry 49, 53–67.
Wilson, M.J., Jones, D., Russell, J.D., 1980. Glushinskite, a naturally occurring magnesium oxalate. Mineral. Mag. 43, 837–840.
Wu, L., Jacobson, A.D., Hausner, M., 2008. Characterization of elemental release during microbe–granite interactions at T = 28 °C. Geochim. Cosmochim. Acta 72, 1076–1095.