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In vitro weathering of phlogopite by ectomycorrhizal fungi

I. Effect of K^+ and Mg^{2+} deficiency on phyllosilicate evolution

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Abstract

The ways in which ectomycorrhizal fungi benefit tree growth and nutrition have not been fully elucidated. Whilst it is most probably due to improved soil colonization, it is also likely that ectomycorrhizal fungi could be directly involved in nutrient cycling of soil reserves. This study assessed access by two species of ectomycorrhizal fungi to soil nonexchangeable K^+ reserves. The incubation of ectomycorrhizal fungi in bi-compartment Petri dishes with phlogopite led to cation exchange reactions and to crystal lattice weathering. *Paxillus involutus* COU led to irreversible phlogopite transformations, while *Pisolithus tinctorius* 441 led to reversible ones. Simultaneous depletion in K^+ and Mg^{2+} led to an enhanced weathering of phlogopite by *P. tinctorius* 441. The observation of phlogopite evolution shows that some specific Al^{3+} immobilization occurred under *P. tinctorius* 441. The data suggest that these bio-weathering mechanisms could be related to the release of fungal organic acids or other complex forming molecules.

Introduction

In ectomycorrhizas, the fungus plays a number of roles including improvement of water supply, regulation of nitrogen metabolism, provision of growth substances, protection of the root system against pathogens and improvement of nutrient uptake (Le Tacon et al., 1988; Marschner and Dell, 1994). Ectomycorrhizal root systems are well adapted, both in terms of total length and branching pattern, to exploit the soil in which they develop. Extramatrical hyphae and hyphal strands or rhizomorphs associated with ectomycorrhizas extend out into the soil beyond the root absorbing zone and form a complex branching mycorrhizal system (Bowen, 1973). The production and maintenance of ectomycorrhizas and the associated network of fungal hyphae is much less energetically expensive than the production of host plant roots (Marshall and Perry, 1987): roots need about 100 times more carbon than hyphae to exploit an equal surface area of soil (Harley, 1989). Ectomycorrhizal associations can greatly enhance the ability of a plant to take up elements from soil to such a point that rapidly growing trees inoculated with ectomycorrizal fungi could be used in decontamination programs for soils contaminated with biologically harmful radioelements: ectomycorrhizal *Pinus ponderosa* and *Pinus radiata* seedlings are able to remove in vitro 3-5 times more ^{90}Sr from contaminated soil than seedlings without ectomycorrhizas (Entry et al., 1994).

The mechanisms by which ectomycorrhizal roots efficiently take up elements have not been fully elucidated. Whilst it is most probably due to improved soil colonization, it is also likely that ectomycorrhizal fungi could be directly involved in nutrient cycling in forest

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ecosystems: they could enhance nutrient availability through biochemical solubilization and chelation of exchangeable and/or stable mineral reserves.

In this paper, we focussed on soil K^+ reserves. Many soils have large contents of total K^+ but relatively small amounts of available K^+ (Bhonsle et al., 1992). The release of K^+ by minerals to soluble and exchangeable forms and its adsorption from the soil solution by exchange sites are both forward and reverse (equilibrium) reactions (McLean and Watson, 1985). Nonexchangeable K^+ consists mainly of interlayer K^+ of nonexpanded clay mineral such as illites and of interlayer K^+ of micas as well as lattice K^+, the latter mainly present in feldspars (Mengel et al., 1993). Although it is defined as nonexchangeable, it may provide a significant part of the plant uptake of K^+ (Boyle and Voight 1973; Hinsinger et al., 1993; Niebes et al., 1993 ; Rahmatullah, 1992; Rosolem et al., 1993). However, most studies on plant access to K^+ reserves have dealt with non axenic culture systems where rhizospheric microorganisms could significantly contribute to mineral weathering processes. Leyval et al. (1991) demonstrated that dual inoculation of an acid-producing bacterium and an ectomycorrhizal fungus into the rhizosphere of *Pinus sylvestris* promoted the cation-exchange capacity of a mica (phlogopite).

The aim of this work was therefore to determine in vitro whether or not two ectomycorrhizal fungi have access to K^+ trapped inside phlogopite interlayer spaces and how nutritional conditions, i.e. depletions in K^+ and/or Mg^{2+}, influence their potential.

Materials and methods

Fungal strains

Experiments were conducted with *Paxillus involutus* (Batsch. ex Fr.) Fr. strain COU (isolated under *Eucalyptus* in France) and *Pisolithus tinctorius* (Pers.) Coker and Couch strain 441 (isolated under *Eucalyptus* in Brazil).

Phlogopite characteristics

The mineral used was a reference phlogopite collected in Madagascar. Its composition in percentage of oxides at 105°C is:

CaO	MgO	K_2O	Na_2O	FeO	Fe_2O_3
0.07	24.32	10.65	0.44	3.01	1.85
MnO	P_2O_5	Al_2O_3	BaO	SiO_2	TiO_2
0.14	0.01	17.2	0.63	39.76	1.01

It has the following structural formula (number of ions on the basis of 22 (O, OH) or 11 O equivalents), $(Si_{2.73}Al_{1.27})$ $(Fe^{3+}{}_{0.10}$ $Fe^{2+}{}_{0.17}Mg_{2.49}Mn_{0.02}Ti_{0.05})$ $(K_{0.93}Na_{0.06}Ca_{0.01})$ $O_{10}(OH)_2$; and a low cation exchange capacity (CEC), about 2 milliequivalents for 100 g of mineral (meq 100 g^{-1}).

Mineral preparation

Phlogopite was ground, sieved (50–100μm) and washed with distilled water. Quartz and ferrous oxides were removed by magnetic separation. Mineral purity was checked under a photonic microscope. The mineral was sterilized by Υ radiation (20 kGy). Mineral sterility was checked by plating on a Tryptic Soy Broth medium (0.3%, pH = 7).

Experimental device

To avoid any exchange of phlogopite interlayer cations with cations from the nutrient medium, fungi were grown in bi-compartment Petri dishes (100 mm diameter) (Paris et al., 1995). The first compartment, hereafter referred to as the "nutrient compartment", was filled with low glucose nutrient agar medium (for 1 liter: $NaH_2PO_4.2H_2O$, 0.869 g; KCl, 0.275 g; $MgSO_4.7H_2O$, 0.150 g; $CaCl_2.2H_2O$, 0.050 g; trace element stock solution (Kanieltra 6Fe, Hydro Azote Co, France: iron, 6.00 g L^{-1}; molybdenum, 0.27 g L^{-1}; boron, 8.45 g L^{-1}; manganese, 5.00 g L^1; copper, 0.625 g L^{-1}; zinc, 2.27 g L^{-1}), 0.1 mL; thiamine-HCl,100 μg; biotine, 0.4 μg; glucose D^+, 1.0 g; agar, 20 g; pH before autoclaving: 5.5). The nutrient compartment was inoculated with 6 fungal plugs (6 mm diameter) along the plate partition. Nitrogen was provided either as ammonium ($(NH_4)_2SO_4$, 1.250 g L^{-1}) or as nitrate ($NaNO_3$, 1.608 g L^{-1}). When specified, sources of K^+ and/or Mg^{2+} ions were omitted from the nutrient medium. The treatment with K^+ and Mg^{2+} provided in the nutrient compartment will be referred to as the "complete treatment", treatment without K^+ or Mg^{2+} as the "K-free treatment" or "Mg-free treat-

ment", and treatment without K^+ and Mg^{2+} sources as the "KMg-free treatment". The second compartment of the Petri dish, referred to as the "phlogopite compartment", was filled with high glucose water agar (for 1 litre: glucose, 5 g; agar, 20 g). It was overlaid with phlogopite (ca. 125 mg) gently sprinkled in between two cellophane films so that mineral samples could be easily recovered at the end of the experiment and chemically analysed without any hyphal remains in it. The plates were kept in dark incubators at 25°C up to 16 weeks for *P. involutus* and up to 20 weeks for *P. tinctorius*. Control plates were set up and treated exactly in the same way except that they were either not inoculated (fungal-free control) or prepared without mineral (phlogopite-free control). Subsamples of phlogopite were kept apart as original pre-experimental material for comparison with phlogopite from control plates. Fungal viability at the end of the experiments was confirmed by subculturing of fungal plugs on fresh nutrient media.

Phlogopite d spacing analysis: X-ray diffraction

Small samples (less than 10 mg) of phlogopite were collected with a scalpel from the two cellophane films. Phlogopite particles were settled onto glass plates (001) oriented sample slides). Phyllosilicate d spacing was assessed by X-ray diffraction (Siemens 5000, copper anticathode $K\alpha$= 1.541838 Å , 30 mA, 40 kV). The sample slide rotates from 1.5 to 20°2Θ at a speed of 0.2 s for each 0.01 °2Θ step (analysis range: 2 cm diameter). Relative humidity was maintained below 50%. For each treatment, between 5 and 19 phlogopite samples from 5 to 10 replicate plates were analysed. After X-ray diffraction analysis, some samples were individually resuspended in 2 mL of a 1 *M* KCl solution and shaken for 1 hour (performed twice). After washing with distilled water, a second X-ray diffraction analysis was performed on these samples. Samples, in which the d spacing did not completely collapse back to 10 Å after this KCl treatment, were then gradually heated at 110 °C (overnight), 220 °C (5 h) and 330 °C (5 h) till complete d spacing closure. This method, adapted from Rouiller et al.(1980), did not allow quantification of interlayer cations but gave access to their exchangeability for each individual sample.

Phlogopite cation exchange capacity analysis: I.C.P. spectrometry

Pooling phlogopite samples from two replicate plates was necessary for reliable analysis of exchangeable cation composition: 250 mg of dry phlogopite were shaken in 7.5 mL of a 1 *M* KCl solution for 1 hour (performed twice) and washed with distilled water (Rouiller et al., 1980). Ca, Mg, Mn, Na, Fe and Al cations in the 15 mL extraction solution were quantified by Inductively Coupled Plasma (ICP) spectrometry (Jobin JY 38 Plus). Protons in the same extraction solution were quantified by titration with NaOH 0.01 *N*. The sum of these cations was termed "apparent CEC " (aCEC) and expressed in milliequivalents for 100 g of mineral (meq 100 g^{-1}).

Composition of the agar medium in the "phlogopite compartment"

Agar medium under phlogopite from all treatments including controls was analysed. The agar medium of the "phlogopite compartment" was taken out of the Petri dishes, then cut into small cubes. A 48 μm pore size polyamide net (Scrynel NY 48HC) was invaginated into centrifugation tubes. The net was filled with the agar cubes while the edges were securely screwed together with the tube caps. The tubes were frozen (-20 °C), thawed at room temperature and centrifuged at 4000 rpm for 10 min. The liquid phase was collected at the bottom of the tube and filtered (0.20 μm). The concentrations (mg L^{-1} ± standard deviation) of Al^{3+} and Si^{4+} ions released from the mineral lattice were assessed in this liquid phase by ICP spectrometry. Mycelium development over phlogopite was recorded as well. As the Petri dishes were fully colonized by the end of the experiment, mycelium dry weight (mg) and contact surface (cm^2) with phlogopite were assessed so that the mass of mycelial mat per unit surface area over the phlogopite (mg cm^{-2}) could be calculated. As the mycelial development varied according to the nutrient media provided, the released cation concentration for each sample was related to fungal mat mass per unit surface area over the phlogopite (mg L^{-1} mg^{-1} cm^2 ± standard deviation).

Statistical analysis

Data were analysed with Abacus Concepts, StatView Sudent, (Abacus Concepts, Inc., Berkeley, CA 1991) software. Before running ANOVA and Fisher's Pro-

tected Least Significant Difference test, data were transformed to stabilize variance [$y = (x^{\lambda} - 1) / \lambda$; with $\lambda = 0.5$] (Chatfield,1988).

Results

Evidence of phlogopite evolution

At the end of the experiments (16 or 20 weeks), control phlogopite, from non-inoculated plates (Fig. lb), was characterized by the same 10.1 Å peak as the original non-incubated material (Fig. la). By this time, *P. involutus* COU (16 weeks incubation time) and *Pisolithus tinctorius* 441 (20 weeks incubation time) had fully colonized the "phlogopite compartment" of all inoculated Petri dishes. After colonization by ectomycorrhizal fungi, four types of phlogopite evolution, ranging from closed to open d spacings, could be characterized by four typical X-ray diffractograms: type I: one single 10 Å peak, type II: two peaks at 10 Å and 12 Å , type III: three peaks at 10 Å ,12 Å and 14 Å , type IV : two peaks at 10 Å and 14 Å (Fig.1). Each replicate sample was analyzed and classified into types I–IV according to their X-ray diffractograms.

After colonization of phlogopite by *P. tinctorius* 441, the frequencies of each alteration type among replicate plates appeared to be under the influence of the nutrient compartment (Fig. 2a, b). The general trend was that the most evolved phlogopite types (types III and IV) became prevalent when K^+ and Mg^{2+} were omitted from the nutrient compartment (KMg-free treatment). This trend was independent of the source of nitrogen (NH_4-N or NO_3-N) in the nutrient compartment. Sample evolution was easily reversible. Indeed after KCl treatment and the 110 °C heating treatment, the d spacing of the most evolved samples (Type IV) collapsed back to 10 Å (Figure not shown).

The aCEC (Ca, Mg, Mn, Na, Fe, Al, H) of the control phlogopite (fungal-free control) was 2.22 meq 100 g^{-1}($\pm$ 0.21, n=2), which was similar to the aCEC of the original pre-experimental material (2.07 meq 100 g^{-1}, $\pm$ 0.33, n=16) (Table 1). Following colonization by *P. tinctorius* 441, phlogopite aCEC reached 4.03 to 8.86 meq 100 g^{-1} depending on the nutrient medium feeding the mycelium (Table 1a,b). This increase was mainly related to interlayer K^+ substitution by Mg^{2+}. In KMg-free treatment, the aCEC was significantly higher than in any other treatment (6.37 meq 100 g^{-1} on NH_4-N ; 8.86 meq 100 g^{-1} on NO_3-N) (Table 1). This was associated with a significant increase of interlayer Mg^{2+} cations (Table 1) and was verified with either form of nitrogen (NH_4-N or NO_3-N) provided in the nutrient compartment.

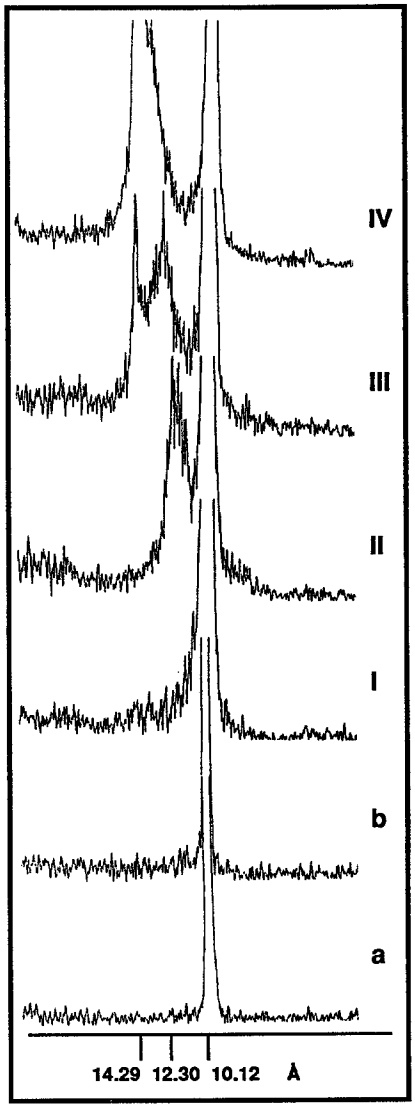

Fig. 1. X-ray diffractograms of the original non-incubated phlogopite (**a**), of phlogopite collected after a 16 week incubation time in the absence of a fungal colony (**b**) or under a *Paxillus involutus* COU colony (**I-IV**). Four different types of evolved phlogopite could be characterized, Type I, II, III and IV.

The evolution of the phlogopite followed different trends under the second ectomycorhizal fungus. Under *P. involutus* COU, grown on NH_4-N culture medium, 82 to 100% of phlogopite samples evolved toward types II, III or IV (data not shown) compared

Table 1. Exchangeable cation composition (milliequivalents per 100 g of phlogopite, mean ± standard deviation; n, number of replicates) of original non-incubated phlogopite (pre-experimental phlogopite), of phlogopite collected after a 20 week incubation time in the absence of fungal colony (control phlogopite) or under *Pisolithus tinctorius* 441 (*P.t.* 441) colonies maintained on various nutrient media. Nitrogen was provided either as NH_4 or NO_3 in the nutrient compartment of the Petri dishes (see Materials and methods section). K^+ and Mg^{2+} ions were either included (complete treatment), or omitted in the nutrient media (Mg-free, K-free and KMg-free treatments). Only the most striking differences recorded under mycelial mat were statistically tested. Within each column, values followed by the same letter are not significantly different (Fisher's Protected Least Significant Difference test, $p < 0.05$)

	Ca	Mg	Mn	Na	Fe	Al	H	aCEC
NH_4-N								
Pre-experimental phlogopite (n=2)	1.80 ± 0.25	0.26 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.07 ± 0.21
Control phlogopite (n=16)	1.55 ± 0.32	0.44 ±0.08	0.01 ± 0.00	0.20 ± 0.09	0.01 ± 0.01	0.01 ± 0.02	0.00 ± 0.00	2.22 ± 0.33
*P.t.*441 Complete treatment (n=4)	2.24 ± 0.16	1.06[a] ± 0.12	0.01 ± 0.00	0.62 ± 0.08	O.07 ± 0.02	0.02 ± 0.03	0.00 ± 0.00	4.03[a] ± 0.23
P.t. 441 Mg-free treatment (n=7)	2.30 ± 1.40	1.73[a] ± 0.74	0.01 ± 0.00	0.68 ± 0.15	O.04 ± 0.03	0.08 ± 0.14	0.00 ± 0.00	4.83[a] ± 1.13
P.t. 441 K -free treatment (n=4)	2.78 ± 0.52	1.12[a] ± 0.19	0.01 ± 0.00	0.54 ± 0.27	O.04 ± O.03	0.01 ± 0.02	0.00 ± 0.00	4.49[a] ± 0.82
P.t. 441 KMg-free treatment (n=5)	1.27 ± 0.71	4.59[b] ± 0.74	0.02 ± 0.01	0.32 ± 0.19	O.08 ± O.06	0.10 ± 0.19	0.00 ± 0.00	6.37[b] ± 1.50
NO_3-N								
Pre-experimental phlogopite (n=2)	1.80 ± 0.25	0.26 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.07 ± 0.21
Control phlogopite (n=16)	1.55 ± 0.32	0.44 ± 0.08	0.01 ± 0.00	0.20 ± 0.09	0.01 ± O.01	0.01 ± 0.02	0.00 ± 0.00	2.22 ± 0.33
P.t. 441 Complete treatment (n=5)	2.53 ± 0.16	1.27[a] ± 0.21	0.01 ± 0.00	0.43 ± 0.13	0.06 ± 0.06	0.02 ± 0.03	0.00 ± 0.00	4.30[ab] ± 0.29
P.t. 441 Mg-free treatment (n=4)	2.40 ± 0.43	0.87[a] ± O.18	0.01 ± 0.00	0.93 ± 0.65	0.01 ± O.01	0.02 ± 0.02	0.00 ± 0.00	4.24[a] ± 1.02
P.t. 441 K-free treatment (n=4)	3.05 ± 0.32	1.35[a] ± 0.22	0.01 ± 0.00	1.31 ± 0.42	0.04 ± O.05	0.02 ± 0.04	0.00 ± 0.00	5.78[b] ± 0.51
P.t. 441 KMg-free treatment	4.34 ± 0.99	3.32[b] ± 1.12	0.01 ± 0.01	1.06 ± 0.40	0.01 ± O.00	0.12 ± 0.08	0.00 ± 0.00	8.86[c] ± 2.42

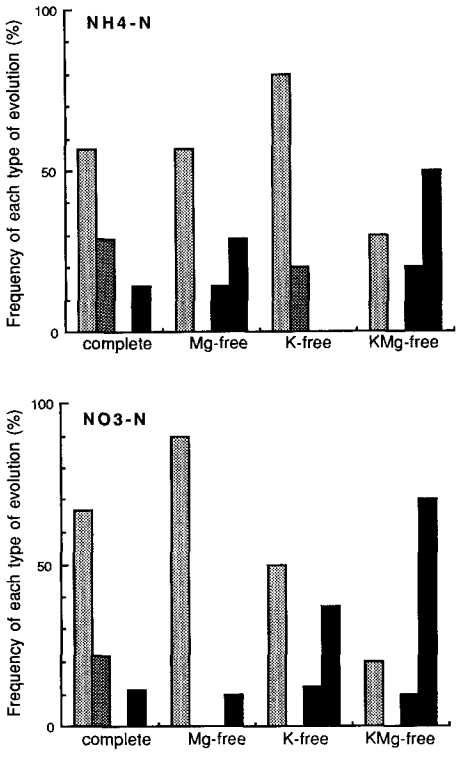

Fig. 2. Frequency of each type of phlogopite evolution (Type I ■, II ▒, III ▒, IV ■) among phlogopite samples colonized for 20 weeks by *Pisolithus tinctorius* 441 maintained on various nutrient media. Nitrogen was provided either as NH_4 (**a**), or NO_3 (**b**) in the nutrient compartment of the Petri dish. K^+ and Mg^{2+} were either included (complete medium), or omitted in the nutrient media (Mg-free, K-free and K and Mg-free treatments).

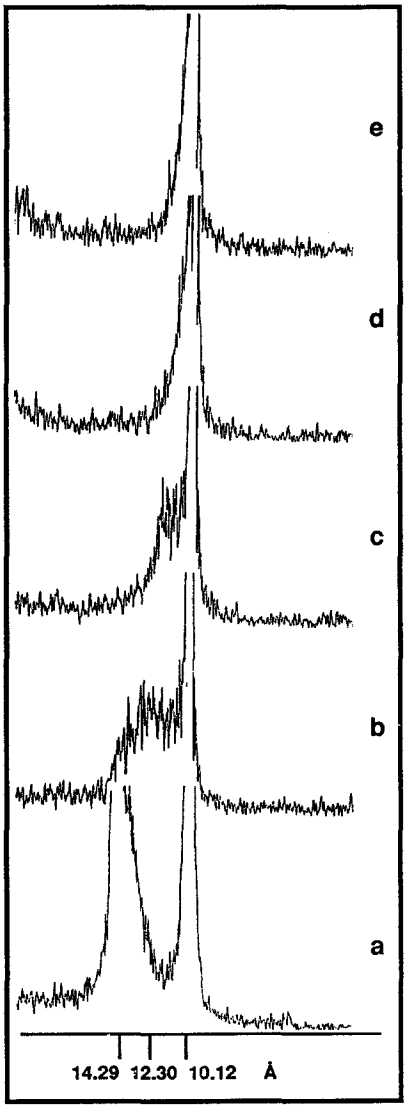

Fig. 3. X-ray diffractograms of the highly evolved (type IV) sample of phlogopite collected under *Paxillus involutus* COU (**a**), then treated with 1 *M* KCl to substitute interlayer cations with K^+ (**b**), and heated up to 110 °C overnight (**c**), 220 °C (**d**), and 330 °C (**e**) during 5 hours, until complete closure of the interlayer spaces.

to 20 to 70% under *P. tinctorius* 441 inoculated on various NH_4-N media (Fig. 2a). In contrast to *P. tinctorius* 441, some irreversible transformations could be observed under *P. involutus* COU (Fig. 3). Indeed, following KCl treatment (Fig. 3b) of type IV phlogopite samples (Fig. 3a), heating up to 330 °C (Fig. 3e) was necessary to obtain complete d spacing closure. Such irreversible transformations were independent of K^+ and/or Mg^{2+} presence in the nutrient compartment. Different trends were also recorded when comparing the aCEC evolution under *P. involutus* COU and under *P. tinctorius* 441 (Table 2). Phlogopite aCEC increased under both fungi but in contrast to the results obtained in experiments with *P tinctorius* 441, with *P. involutus* COU, Al^{3+} were detected as well as Mg^{2+} in phlogopite interlayer exchangeable locations and the aCEC in KMg-free treatment did not significantly differ from the aCEC in the other treatments (Table 2).

Cation release from phlogopite: Al^{3+}

Two lattice cations (Al^{3+} and Si^{4+}), released from the phlogopite mineral, were assessed in the agar medium under phlogopite colonized by *P. tinctorius* 441. Other lattice cations like K^+ and Mg^{2+}, were not assessed as they are major nutritional cations absorbed for fungal growth.

Table 2. Exchangeable cation compositions (milliequivalents per 100 g of phlogopite, mean ± standard deviation; n, number of replicates) of original non-incubated phlogopite (pre-experimental phlogopite), of phlogopite collected after a 16 week incubation time in the absence of fungal colony (control phlogopite) or under *Paxillus involutus* COU (*P.i.* COU) colonies maintained on various nutrient media as in Table 1. Nitrogen was provided as NH_4. Only the most striking differences recorded under mycelial mat were statistically tested. Within each column, values followed by the same letter are not significantly different (Fisher's Protected Least Significant Difference test, $p < 0.05$)

	Ca	Mg	Mn	Na	Fe	Al	H	aCEC
NH_4-N								
Pre-experimental phlogopite n=2)	1.80 ± 0.25	0.26 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.07 ± 0.21
Control phlogopite (n=4)	1.54 ± 0.21	0.29 ± 0.08	0.00 ± 0.01	0.08 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.90 ± 0.20
*P.i.*COU Complete treatment (n=2)	2.35 ± 0.35	0.91 ± 0.11	0.01 ± 0.01	0.14 ± 0.04	O.04 ± 0.00	0.55 ± 0.03	0.23 ± 0.03	4.22 [a] ± 0.21
P. i. COU Mg-free treatment (n=2)	2.22 ± 0.28	1.07 ± 0.0l	0.01 ± 0.0l	0.23 ± 0.21	O.03 ± 0.02	0.58 ± 0.08	0.00 ± 0.00	4.12 [a] ± 0.14
P. i. COU K-free treatment (n=2)	1.97 ± 0.17	0.81 ± 0.18	0.01 ± 0.01	0.49 ± 0.59	0.07 ± 0.07	0.81 ± 0.22	0.49 ± 0.24	4.63 [a] ± 1.12
P.i. COU KMg-free treatment (n=2)	2.51 ± 0.64	1.04 ± 0.29	0.01 ± 0.00	0.08 ± 0.03	0.02 ± 0.00	0.66 ± 0.01	0.00 ± 0.00	4.31 [a] ± 0.95

In the control, non-inoculated plates (fungal-free control), only 0.28 mg L^{-1}(± 0.15, n=25) of Al^{3+} could be detected in the agar medium after 20 weeks, this value was subtracted from all subsequent measurements. In inoculated plates without phlogopite (phlogopite-free control), the Al^{3+} concentration was 0.42 mg L^{-1}(± 0.40, n=26), resulting from hyphal release or transfer. These concentrations of Al^{3+}, low compared to those found under colonized phlogopite, were not taken into account.

When *P. tinctorius* was provided with NH_4-N (Table 3), the Al^{3+} concentration in the phlogopite compartment of the Petri dish was increased due to mycelial interaction with the mineral (from 2.84 mg L^{-1} to 18.26 mg L^{-1}). When K^+ and Mg^{2+} were absent (KMg-free treatment), the Al^{3+} concentration in the phlogopite compartment was significantly higher than in any other treatment (Table 3). Despite important variations, mycelium growth, expressed as mycelial mat mass per unit surface area over phlogopite (mg cm^{-2}), did not vary significantly between treatments (Table 3). When expressed as mg L^{-1} per mycelial mat mass per unit surface area (mg L^{-1} mg^{-1} cm^2) (Table 3), the concentration of Al^{3+} released from phlogopite was still significantly higher, two-fold, in the KMg-free treatment (78.16 mg L^{-1} mg^{-1} cm^2) than in the other treatments (from 29.41 to 40.83 mg L^{-1} mg^{-1} cm^2) (Fig. 4).

When *P. tinctorius* was provided with NO_3-N (Table 3), the Al^{3+} concentration (mg L^{-1}) in the phlogopite compartment was not significantly influenced by K^+ or Mg^{2+}. Despite important variations, mycelium growth over phlogopite did not significantly vary with treatment. When expressed as mg L^{-1}

Table 3. Accumulation of free Al and Si ions in water agar medium, under phlogopite colonized for 20 weeks by *Pisolithus tinctorius* 441 maintained on various nutrient media, as in Table 1. Fungal mat mass per surface unit over phlogopite (mg cm^{-2} ± standard deviation; n, number of replicates) was recorded. The released ions concentration (mg L^{-1} ± standard deviation), for each sample has been related to mat mass per surface unit (mg L^{-1} $mg^{-1}cm^2$ ± standard deviation). Within each column, values followed by the same letter are not significantly different (Fisher's Protected Least Significant Difference test, $p < 0.05$)

Nutrient compartment	Fungal mat mass per surface unit (mg cm^{-2})	Al		Si	
		(mg L^{-1})	(mg L^{-1} mg^{-1} cm^2)	(mg L^{-1})	(mg $L^{-1}mg^{-1}$ cm^2)
NH_4-N					
Complete treatment (n=2)	0.145 [a] ± 0.092	5.26 [a] ± 2.73	35.52 [a] ± 3.72	28.45 [a] ± 5.19	139.07 [a] ± 64.88
Mg-free treatment (n=4)	0.160 [a] ± 0.063	6.66 [a] ± 3.33	40.83 [a] ± 12.54	31.94 [a] ± 9.52	182.63 [a] ± 119.03
K-free treatment (n=3)	0.090 [a] ± 0.053	2.84 [a] ± 2.13	29.41 [a] ± 12.86	24.81 [a] ± 5.03	93.49 [a] ± 62.90
KMg-free treatment (n=4)	0.275 [a] ± 0.257	18.26 [b] ± 11.11	78.16 [b] ± 31.00	48.93 [b] ± 14.38	395.04 [b] ± 179.78
NO_3-N					
Complete treatment (n=7)	0.099 [a] ± 0.050	3.21 [a] ± 2.63	25.33 [a] ± 13.27	27.20 [a] ± 6.77	123.43 [a] ± 84.64
Mg-free treatment (n=9)	0.262 [a] ± 0.279	2.56 [a] ± 2.27	14.26 [b] ± 7.45	21.09 [b] ± 3.53	47.07 [b] ± 44.11
K-free treatment (n+8)	0.080 [a] ± 0.059	1.17[a] ± 0.36	14.10 [b] ± 5.74	18.38 [b] ± 1.69	13.14 [b] ± 21.24
KMg-free treatment (n=5)	.096 [a] ± 074	3.83 [a] ± 2.34	40.90 [a] ± 19.45	27.20 [a] ± 4.69	123.39 [a] ± 58.61

per mycelial mat mass per unit surface (Table 3), the release of Al^{3+} was significantly higher in the KMg-free treatment, than with K-free or Mg-free treatments but not significantly higher than with complete treatment (Fig. 4b).

The mycelial growth was not significantly affected by the form of nitrogen in the nutrient compartment

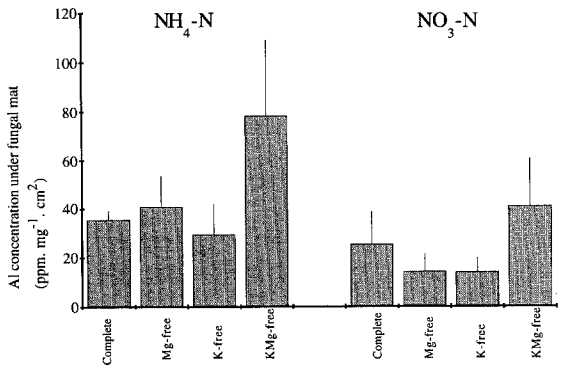

Fig. 4. Accumulation of free Al^{3+} in water agar medium, under phlogopite colonized for 20 weeks by *Pisolithus tinctorius* 441 maintained on various nutrient media, as in Table 1. Nitrogen was provided either as NH_4 (left), or NO_3 (right). The released Al^{3+} concentration, for each sample, was related to fungal mat density over phlogopite (mg L^{-1} $mg^{-1}cm^2$± standard deviation).

(Table 3). Only when K^+ or Mg^{2+} were not incorporated into the nutrient compartment (the K-free and the Mg-free treatments) the concentration of Al^{3+} released (mg L^{-1} mg^{-1} cm^2) was significantly higher on NH_4-N than on NO_3-N (Fisher's Protected Least Significant Difference test, $p < 0.05$) (Tables 3a, b).

Cation release from phlogopite: Si^{4+}

In the control non-inoculated plates (fungal-free control), 17.33 mg L^{-1} of Si^{4+}(± 1.91, n=25) could be detected in the agar medium after 20 weeks, this value was substracted to all subsequent measurements. In inoculated plates without phlogopite (phlogopite-free control), no release or transport of Si^{4+} by the hyphae was detected on either the NH_4-N or the NO_3-N media.

When *P. tinctorius* 441 was provided with NH_4-N (Table 3), Si^{4+} accumulated due to fungal interaction with phlogopite (24.81 48.93 mg L^{-1}). In the KMg-free treatment, the Si^{4+} concentration in the phlogopite compartment, just like the Al^{3+} concentration, was significantly higher than in any other treatment (Table 3), when expressed as mg L^{-1} or as mg L^{-1} per fungal mat mass per unit surface area.

When *P. tinctorius* 441 was provided with NO_3-N (Table 3), the Si^{4+} concentration in the phlogopite compartment followed the same trends as on NH_4-N except that the concentration of Si^{4+} released, in mg L^{-1} or in mg L^{-1} mg^{-1} cm^2, was significantly higher in the complete treatment as well as in the KMg-free treatment than in the Mg-free and K-free treatments (Table 3).

Except when K^+ and Mg^{2+} were incorporated into the system (complete treatment), the Si^{4+}'release (mg L^{-1} mg^{-1} cm^2) as for Al^{3+} release, was always significantly higher on NH_4-N than on NO_3-N (Fisher Protected Least Significant Difference test, $p < 0.05$) despite non significant variations of fungal growth (Table 3).

Discussion

The ectomycorrhizal strains investigated, *P. tinctorius* 441 and *P. involutus* COU, both had access to K^+ trapped in between the phlogopite 2:1 layers. This was established from d spacing openings from 10 Å to 14 Å and from interlayer K^+ substitutions. As phlogopite samples were separated from the mycelium by cellophane films, soluble fungal exudates are responsible for mica vermiculitization. Such a bio-induced evolution of primary phyllosilicates has been observed with saprophytic fungi (Boyle and Voigt, 1973) and in the rhizosphere of some higher plants, in the presence and in the absence of rhizospheric microorganisms (Hinsinger et al., 1993; Leyval et al., 1991). However, this is the first investigation concerning ectomycorrhizal fungi in pure culture.

The two species of ectomycorrhizal fungi did not lead to the same expanded mineral structure. Firstly, the opening of mica d spacing appeared more drastic under *P. involutus* COU: a higher proportion of analysed samples evolved toward a vermiculitic structure and to a higher degree. Secondly, in contrast to *P. tinctorius* 441, *P. involutus* COU not only leads to the substitution of nonexchangeable K^+ by exchangeable Mg^{2+}, but Al^{3+} also participates in K^+ substitution. Moreover, the samples collected under *P. involutus* COU did not collapse back to a 10 Å peak in spite of K-saturation and 110 °C heating, suggesting that non-exchangeable wedges prevent the collapse of the opened structure and render the transformation of the phyllosilicate irreversible. These particles might have evolved under *P. involutus* COU toward an hydroxy-aluminous vermiculite. Such crystallographic and crystallochemical changes necessarily involved an acid dissolution of part of the mica structure under *P. involutus* COU. As Mg^{2+} can originate in mycelial exudates, the phlogopite changes recorded under *P. tinctorius* 441 could be explained by exchange reactions.

However, significant amounts of Si^{4+} and Al^{3+} were recorded in the agar medium both under *P. involutus* COU and under *P. tinctorius* 441. As these cations necessarily originate from the phyllosilicate, it demonstrates that *P. tinctorius* 441 as well as *P. involutus* COU caused the dissolution of part of the phlogopite structure. As Al^{3+} freed by *P. tinctorius* from the crystal lattice are not found in the interlayer space, this suggests that other trapping mechanisms prevented their accumulation at this site. Considering that some ectomycorrhizal fungi are known to excrete large amounts of oxalate (Lapeyrie et al., 1987; Lapeyrie, 1988), a strong Al^{3+} complexing agent, or to accumulate Al^{3+} in vacuoles as Al-polyphosphates (Martin et al., 1994), such trapping mechanisms could be involved. In other respects, the contrasting Al^{3+} distribution could result from different kinetics of proton excretion during mycelial development, giving rise to various types of dissolution.

Based on the accumulation of Al^{3+} and Si^{4+} in the water agar compartment under weathered phlogopite, it seems that the weathering potential of *P. tinctorius* 441 is higher on NH_4-N than on NO_3-N. This is in agreement with frequent reports of abundant proton excretion associated with NH_4-N absorption (Marschner et al., 1986; Salsac et al., 1982).

P. tinctorius weathering potential appeared to be highly dependent on simultaneous K^+ and Mg^{2+} deficiency in the nutrient medium, which was observed on NH_4-N and on NO_3-N nutrient media. This suggests that some unidentified weathering mechanism is switched on by this double deficiency. It seems that when only one of these ions (e.g. K^+) is deficient the other ion (e.g. Mg^{2+}) can substitute for it to repress such mechanisms or their switch. These bio-weathering mechanisms could be related to fungal organic acids or other complexant molecules released. The accumulation of citric acid by *Aspergillus niger* is known to be inhibited by Mn^{2+} ions (Schreferl et al., 1986).

Here it could be noticed that the Si/Al ratio in water agar compartment under weathered phlogopite is in most cases significantly higher (up to a 8 fold on NO_3-N in absence of free K^+) than the expected ratio deduced from mineral structural formula (2.2). This again suggests specific Al^{3+} immobilization under the control of *P. tinctorius* 441.

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