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WEATHERING OF AMMONIUM- OR CALCIUM-SATURATED 2:1 PHYLLOSILICATES BY ECTOMYCORRHIZAL FUNGI *IN VITRO*

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Summary—The ways through which ectomycorrhizal fungi benefit tree growth and nutrition have not been totally elucidated. Our study was therefore aimed at assessing fungal access to soil exchangeable and stable mineral reserves. The growth of different ectomycorrhizal fungi in bi-compartment Petri dishes with NH_4^+ - or Ca-saturated vermiculite led to cation exchange reactions and to crystal lattice weathering. The presence or absence of soluble NH_4^+ or Ca did not seem to affect fungal mobilization potential. Oxalic acid appears to be involved in vermiculite weathering by *Paxillus involutus* and Ca ions could limit the acid dissolution of Ca-saturated vermiculite. The quantitative significance of such cation mobilization and mineral weathering still has to be assessed *in situ* and in association with ectomycorrhizal plants.

INTRODUCTION

Ectomycorrhizal fungi benefit tree growth and nutrition: plants inoculated with efficient ectomycorrhizal fungi grow faster than non-mycorrhizal plants, their shoot-to-root ratio is often larger and their nutrient concentration is sometimes increased compared to non-inoculated plants (Harley and Smith, 1983; Le Tacon *et al.*, 1988; Dixon and Hiol-Hiol, 1992). These common observations suggest that infected roots are more efficient per unit weight or length in extracting soil minerals than non-mycorrhizal ones. The mechanisms through which they develop such an efficiency have not been totally elucidated. It is most probably due to improved soil colonization. Infected root systems are well-adapted, both in terms of total length and branching pattern, to exploit the volume of soil in which they develop. Extramatricial hyphae, strands or rhizomorphs associated with ectomycorrhizas extend out into the soil beyond the root absorbing zone and form a complex mycorrhizal system of branches (Bowen, 1973). Yet, ectomycorrhizal fungi could also be directly involved in the nutrient cycling of forest ecosystems: they contribute to N translocation between trees connected by a common ectomycorrhizal mycelium (Arnebrant *et al.*, 1993), and could enhance nutrient availability through biochemical solubilization and chelation of exchangeable or stable mineral reserves.

Most of the work concerned with soil mineral-

microorganism interactions has focused so far on saprophytic fungi, bacteria or lichens (Eckhard, 1985; Jones and Wilson, 1985; Robert and Berthelin, 1986). One might assume that ectomycorrhizal fungi possess qualitatively the same properties as non-symbiotic rhizosphere soil microorganisms; however, as symbiotic fungi are directly connected to large sinks (i.e. the host plants), their contribution to plant nutrition could be quantitatively much more significant. Whatever hypothesis is prevalent *in situ*, symbiotic hyphae directly contribute to plant mineral nutrition; it is therefore essential to assess fungal access to the soil mineral compartment. The mineral nutrition of the host plant might depend more on hyphae-soil interface characteristics, than on root-soil interactions.

Ectomycorrhizal fungi are able to solubilize poorly-soluble phosphates *in vitro* (Bousquet *et al.*, 1985; Leyval and Berthelin, 1986; Lapeyrie *et al.*, 1991; Nguyen *et al.*, 1992), but there is still some controversy about whether or not they are able to give the plant access to new phosphate sources in soil (Thomas *et al.*, 1982). There is also some evidence that mycorrhizal fungi contribute to phlogopite alteration and to potassium mobilization in soil (Leyval and Berthelin, 1991; Hinsinger and Jaillard, 1993), but no information is available concerning mycorrhizal access to NH_4^+ or Ca^{2+} ions from clay minerals.

According to Paul and Clark (1989), the amount of fixed NH_4^+ in the top meter of soil increases from 5 to 13% of the total N in surface horizons to 50% of the total N in subsurface horizons. Stevenson *et al.* (1958) reported that in some subsoil samples as much as 70%

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of NH_4^+ could be part of clay complexes. In acidic soils, an exchangeable pool often represents the main source of available Ca for vegetation, this pool being maintained only by a very conservative biological cycle (low weathering rates and atmospheric inputs). Our aim was therefore to determine *in vitro* whether or not some ectomycorrhizal fungi have access to NH_4^+ or Ca trapped inside vermiculite interlayer space and how nutritional conditions influence their potential.

MATERIALS AND METHODS

Materials

Fungal strains. Experiments were conducted with *Paxillus involutus* strain COU (isolated under *Eucalyptus* in France). Six other ectomycorrhizal strains were used for comparison: *P. involutus* strain NAU, *Boletus edulis* strain BARL, *Cenococcum geophilum* strain SIV, *Hebeloma cylindrosporum* strain D15, *Pisolithus tinctorius* strain 441 and *Rhizopogon luteolus* strain KTP.

Vermiculite characteristics. The mineral used was a reference vermiculite collected at Santa Ollala (Spain) having the following structural formula:

It has a high cation exchange capacity (CEC): 160 mequiv 100 g⁻¹ mineral.

Mineral preparation. Vermiculite was ground, sieved (50–100 µm) and washed with distilled water. To avoid interactions, quartz and ferrous oxides were removed by magnetic separation. Vermiculite purity was checked under a photonic microscope. The mineral was sterilized by γ -radiations (20 kGy) before saturation under sterile conditions with NH_4^+ or Ca^{2+} cations. Vermiculite (10 g) was suspended (37°C, 180 rev min⁻¹ shaking) in 250 ml sterile 1 M NH_4Cl or CaCl_2 solutions. The incubation solutions were renewed every day for 8 days. Saturated vermiculite was finally rinsed in 4 × 250 ml of sterile distilled water and air-dried under a laminar flow beam.

Experimental device

To avoid any exchange of vermiculite interfoliar cations with cations from the nutrient medium, fungi were grown in bi-compartment Petri dishes (100 mm dia).

The first compartment was filled with basic low glucose nutrient agar medium [(NH_4)₂SO₄, 1.250 g l⁻¹; NaH₂PO₄·2H₂O, 869 mg l⁻¹; KCl, 275 mg l⁻¹; MgSO₄·7H₂O, 150 mg l⁻¹; CaCl₂·2H₂O, 50 mg l⁻¹; trace element stock solution (Kanieltra 6Fe, Hydro Azote Co, France: Fe, 6.00 g l⁻¹; Mo, 0.27 g l⁻¹; B₂O₃, 8.45 g l⁻¹; Mn, 5.00 g l⁻¹; Cu, 625 mg l⁻¹; Zn, 2.27 g l⁻¹), 0.1 ml l⁻¹; thiamine-HCl, 100 µg l⁻¹; biotine, 0.4 mg l⁻¹; glucose D⁺, 1.0 g l⁻¹; agar, 20 g l⁻¹; pH before autoclaving: 5.5]. Where specified, the sources of NH_4^+ or Ca^{2+} ions were omitted from the

nutrient medium. This first compartment was inoculated with 6 fungal plugs (6 mm dia) along the plate partition and will be referred to as the “nutrient compartment”.

The second compartment was filled with high glucose water agar (glucose, 5 g l⁻¹; agar, 20 g l⁻¹) and with vermiculite (ca. 125 mg) that was gently dispersed in between two cellophane films so that mineral material could be easily recovered at the end of the experiment and chemically analysed without any mycelium on it [Fig. 1(A)]. This second compartment will be referred to as the “vermiculite compartment”.

For scanning electron microscopy (SEM) observations, cellophane films were absent to allow direct colonization of vermiculite flakes by hyphae. The plates were kept in dark incubators at 25°C from 4 to 10 weeks for the mycelium to colonize the second compartment. Control plates were set up exactly in the same way except that they were not inoculated. Subsamples of saturated vermiculites were kept apart as original pre-experimental material for comparison with vermiculites from control plates.

Vermiculite analysis

X-ray diffraction. Small samples (<10 mg) of vermiculite were collected from between the two cellophane films with a scalpel. Vermiculite particles were settled onto glass plates [(001) oriented sample slides]. Phyllosilicate d-spacings were assessed by X-ray diffraction (Siemens 5000, copper anticathode $K_\alpha = 0.1541838$ nm, 30 mA, 40 kV). The sample slide rotates from 1.5 to 20°2 θ at a speed of 0.2 s for each 0.01°2 θ step (analysis range: 2 cm dia). Relative humidity was maintained <50%. For each treatment, between 2 and 14 vermiculite samples from 2 to 7 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 h (performed twice). After washing with distilled water, a second X-ray diffraction analysis was performed on these samples. Samples, of which d-spacing did not completely collapse to 1.0 nm after this KCl treatment, were then gradually heated at 110°C (overnight), 220°C (5 h), 330°C (5 h), 440°C (5 h) and 550°C (5 h) until complete d-spacing closure. This method, adapted from Rouiller *et al.* (1980), did not allow a quantification of interlayer cations but gave access to their exchangeability for each individual small sample.

Inductively coupled plasma (ICP) spectrometry. Pooling vermiculite samples from 2 replicate plates was necessary for reliable analysis of exchangeable cations composition: 250 mg of vermiculite were shaken in 7.5 ml of a 1 M KCl solution for 1 h (performed twice) and washed with distilled water (Rouiller *et al.*, 1980). Ca, Mg, Mn, Na, Fe, Al cations present in the 15 ml extraction solution were quantified by ICP spectrometry (Jobin JY 38 plus). The sum of these cations was named “apparent CEC” (aCEC), it was expressed in mequiv for 100 g of

mineral. As this would have required a specific analytical procedure and was not necessary for interpretation of the results, we did not check for NH_4^+ . Neither did we analyse K^+ , as a different extracting solution, such as NaCl, would have been necessary.

SEM. Vermiculite samples were air-dried, gold-coated and observed under SEM (Philips SM 525, 25.6 kV) equipped with a microprobe (Link Energy Dispersing System).

RESULTS

NH_4^+ -saturated vermiculite evolution

Between 50 and 65 days after inoculation, the vermiculite compartments of the Petri dishes were fully colonized by *P. involutus* COU and the experiment was stopped at that time. Control vermiculites, from non-inoculated plates, were characterized by the same 1.04 nm peak as the original non-incubated material [Fig. 2(A, B)]. Therefore, NH_4^+ ions should be considered as retrograded or "fixed".

When no soluble N was provided in the nutrient compartment. In this case the only source of N for mycelium nutrition was vermiculite interlayer NH_4^+ . Twenty-seven days after inoculation, the mycelium had colonized only half of the vermiculite compart-

ment [Fig. 1(A)]. Then, the active growth of hyphae over vermiculite, compared to restricted development over the nutrient medium (where only N was growth-limiting), suggested that retrograded NH_4^+ had become available to the fungus [Fig. 1(A)]. X-ray diffractograms of vermiculite sequentially sampled along the fungal colony radius exhibited various stages of phyllosilicate evolution (Fig. 3), from almost collapsed d-spacings for vermiculite flakes collected under the hyphal tips (major peak at 1.05 nm with a 1.21 nm raised peak) [Fig. 3(A)] to expanded d-spacings for vermiculite flakes collected under the oldest tissues along the plate partition (3 peaks at 1.05, 1.21 and 1.44 nm) [Fig. 3(F)]. Vermiculite interlayer expansion was, therefore, directly related to fungal progression.

Sixty-five days after inoculation, the X-ray diffractograms of all samples collected under fungal colonies differed from control vermiculite. Most of them (8 samples/14 samples analysed from 7 replicate Petri dishes) were characterized by 3 peaks, a major one at 1.44 nm with 2 minor peaks at 1.24 and 1.07 nm [Fig. 2(B), control vermiculite; Fig. 2(C), inoculated vermiculite]. The other remaining samples after incubation exhibited only 2 peaks: 2/14 samples were characterized by a major peak at 1.47 nm and a minor one at 1.07 nm, 4/14 samples by peaks at 1.19 and

Fig. 1. Vermiculite flakes, saturated by NH_4^+ (A, B) or Ca^{2+} ions (C, D) and colonized by the ectomycorrhizal fungus *P. involutus*. On the inoculated plate (A, right), the fungal colony spreading out from the inoculum plugs (i) was very dense (+) over NH_4^+ -saturated vermiculite (v) compared to other areas in the Petri dish; the control plates were incubated in the absence of fungus (A, left). NH_4^+ -saturated flakes were heavily colonized by hyphae (B). Following fungal colonization, Ca^{2+} -saturated flakes were embedded in a new crystalline material (C), including typical Ca oxalate crystals (D). Scale bars for SEM observations = 100 μm (B), 10 μm (C) and 10 μm (D).

Fig. 2. X-ray diffractograms of initially NH_4^+ -saturated vermiculites (A) collected after a 65-day incubation in the absence of fungal colony (B), or under a *P. involutus* colony (C). Vermiculite samples were treated with 1 M KCl to substitute interlayer cations with K^+ (D) and heated to 110°C overnight (E) and then to 220°C (F), 330°C (G), 440°C (H) and 550°C (I) for 5 h until complete closure of the interlayer spaces.

1.07 nm (diffractograms not shown). Although all *P. involutus*-inoculated vermiculite samples had been transformed by the end of the experiment, they formed a quite heterogenous population on the scale of a Petri dish.

Following X-ray diffraction analysis, 9 typically transformed samples [major peak at 1.44 nm, minor peaks at 1.24 and 1.07 nm; Fig. 2(C)] were individually

treated with a 1 M KCl solution; d-spacings were then measured again to test the exchangeability of interfoliar cations. For each sample, some d-spacings collapsed back to 1.05 nm, therefore, most vermiculite particles were closed again, but all samples still included some 1.38 nm d-spacings [Fig. 2(D)]. Gradual heating was necessary for complete interlayer collapsing. Most samples returned to 1.04 nm at 220°C but heating up to 550°C of the most evolved ones (2/9 samples) was necessary to allow complete interlayer space collapsing [Fig. 2(E–I)].

Minerals from 2 replicate plates were pooled together and treated with KCl to compare the exchangeable cation composition of control and evolved vermiculite (Table 1). In the NH_4^+ -saturated vermiculite (pre-experimental material) the sum of analysed exchangeable cations (Ca, Mg, Mn, Na, Fe, Al ions) was 3.44 ± 0.40 mequiv 100 g^{-1} , this fraction of the CEC was named aCEC. Therefore, the remaining fraction of the CEC is assumed to be occupied by NH_4^+ (about 156 mequiv 100 g^{-1}). The mean aCEC value of vermiculite from non-inoculated control plates was 7.37 ± 0.29 mequiv 100 g^{-1} , but after *P. involutus* COU colonization it reached

Fig. 3. X-ray diffractograms of initially NH_4^+ -saturated vermiculites collected at the edge of the *P. involutus* colony (A) and inward, towards the centre of the colony (B–F).

Table 1. Exchangeable cation composition (mequiv 100 g⁻¹ of vermiculite, mean $\pm$ SD; n = number of replicates) of initially NH₄⁺-saturated vermiculite after saturation (saturated vermiculite) and after a 65-day incubation without inoculation (control) or with inoculation by an ectomycorrhizal fungus: *P. involutus* COU (*P. i.*), *R. luteolus* (*R. l.*), *B. edulis* (*B. e.*) or *C. geophilum* (*C. g.*); soluble N was not provided in the nutrient medium

	Ca	Mg	Mn	Na	Fe	Al	aCEC
Saturated vermiculite ($n = 3$)	0.56 $\pm$ 0.13	0.42 $\pm$ 0.15	0.01 $\pm$ 0.00	2.42 $\pm$ 0.15	0.01 $\pm$ 0.01	0.03 $\pm$ 0.02	3.44 $\pm$ 0.40
Control ($n = 5$)	4.12 $\pm$ 0.34	1.70 $\pm$ 0.04	0.02 $\pm$ 0.00	1.42 $\pm$ 0.20	0.00 $\pm$ 0.00	0.11 $\pm$ 0.07	7.37 $\pm$ 0.29
<i>P. i.</i> ($n = 2$)	15.50 $\pm$ 1.02	13.01 $\pm$ 0.48	0.12 $\pm$ 0.01	9.13 $\pm$ 1.16	0.08 $\pm$ 0.02	1.81 $\pm$ 0.45	39.63 $\pm$ 2.18
<i>R. l.</i> ($n = 1$)	11.51	11.83	0.11	5.90	1.57	1.62	32.54
<i>B. e.</i> ($n = 1$)	16.65	6.58	0.07	6.26	0.05	1.79	31.40
<i>C. g.</i> ($n = 1$)	9.20	4.66	0.05	5.32	0.06	0.54	19.83

39.63 $\pm$ 2.18 mequiv 100 g⁻¹. Therefore approximately 32 mequiv 100 g⁻¹ of the initial NH₄⁺ cations have been substituted by Ca, Mg, Na and Al ions.

When soluble N was provided in the nutrient compartment. When abundant soluble N (NH₄SO₄) was added to the nutrient medium, *P. involutus* grew at the same rate in both compartments of the Petri dish, and was still able to induce vermiculite transformation. Fifty days after inoculation, all vermiculite samples analysed differed from control vermiculite (13 samples from 6 replicate Petri dishes). Three peaks were observed in most cases (11/13 samples) at 1.42, 1.25 and 1.05 nm, and 2 peaks at 1.42 and 1.05 nm for the other 2 samples (X-ray diffractograms not shown). All samples treated with 1 M KCl only partially returned to 1.06 nm, some interlayer spaces remained open at 1.33 nm (X-ray diffractograms not shown) and collapsed to 1.04 nm after heating at 220°C as previously described in the absence of soluble N.

Fungal strain comparisons. Five other ectomycorrhizal fungi grown without soluble N—*R. luteolus*, *B. edulis*, *C. geophilum*, *P. tinctorius*, *P. involutus* NAU—could also transform vermiculite. Such transformations were identified as expansion of interlayer spaces of NH₄⁺-saturated vermiculite (X-ray diffractograms not shown). aCEC (only determined following *R. luteolus*, *B. edulis* and *C. geophilum* colonization) increased in a way similar to that measured after *P. involutus* COU growth (Table 1): NH₄⁺ ions were mainly replaced by Na, Ca, Al and Mg ions.

Ca²⁺-saturated vermiculite evolution

Seventy days after inoculation, Petri dishes were fully colonized by *P. involutus* COU. The presence or absence of soluble Ca in the nutrient compartment of the Petri dish did not apparently affect fungal growth. At the end of the experiment, all X-ray diffractograms of vermiculite from non-inoculated control plates were characterized by a single peak at 1.46 nm as in the original pre-experimental material [Fig. 4(A, B)].

When no soluble Ca was provided in the nutrient compartment. Modified X-ray diffractograms related to fungal colonization were found in 6 out of 8 samples analysed from 5 replicate Petri dishes [Fig. 4(C)]. The major peak was at 1.45 nm, but a minor peak could be

observed at 1.27 nm. These too small samples were not individually checked for cation exchangeability in 1 M KCl solution.

Total vermiculite from 2 replicate plates was pooled together and treated with 1 M KCl to analyse the exchangeable cation composition (Table 2). We referred to this sum of cations aCEC (see Materials and Methods). The aCEC of the original pre-experimental material was 155.43 $\pm$ 4.47 mequiv 100 g⁻¹, 98.95% of it being occupied by Ca ions (153.80 $\pm$ 4.33 mequiv 100 g⁻¹). In control non-inoculated plates the sum of exchangeable cations remained stable (154.51 $\pm$ 1.53 mequiv 100 g⁻¹) but Ca saturation was then reduced to 87.53% (135.24 $\pm$ 1.441 mequiv 100 g⁻¹): this Ca release was compensated by Mg and Na ions and by traces of Al ions. Mg and Na ions could originate from agar or

Fig. 4. X-ray diffractograms of initially Ca²⁺-saturated vermiculites (A) collected after a 70-day incubation in the absence of fungal colony (B), or under a *P. involutus* colony (C).

Table 2. Exchangeable cation composition (mequiv 100 g⁻¹ of vermiculite, mean ± SD; n = number of replicates) of initially Ca²⁺-saturated vermiculite after saturation (saturated vermiculite) and after a 70-day incubation without inoculation (control) or with inoculation by the ectomycorrhizal fungus *P. involutus* (*P. i.*); soluble Ca was provided (+Ca) or omitted (–Ca) in the nutrient medium

	Ca	Mg	Mn	Na	Fe	Al	aCEC
Saturated vermiculite (n = 3)	153.80 ± 4.33	0.34 ± 0.12	0.02 ± 0.00	1.26 ± 0.02	0.00 ± 0.00	0.01 ± 0.02	155.43 ± 4.47
Control (n = 2)	135.24 ± 1.44	5.90 ± 0.86	0.08 ± 0.01	12.58 ± 0.54	0.00 ± 0.00	0.71 ± 0.24	154.51 ± 1.53
<i>P. i.</i> (–Ca) (n = 1)	111.09	12.66	0.12	8.03	0.82	2.68	135.40
<i>P. i.</i> (+Ca) (n = 1)	119.93	12.33	0.11	12.65	0.26	2.45	147.73

cellophane but, as our culture device was Al-free, Al ions must originate from the vermiculite crystal lattice. The aCEC of vermiculite collected under *P. involutus* mycelium was not drastically modified (135.40 mequiv 100 g⁻¹), Ca saturation was slightly reduced to 81.28% (111.09 mequiv 100 g⁻¹), a 2.1-fold increase in Mg²⁺ concentration and a 3.8-fold increase in Al³⁺ concentration were detected.

Following such KCl treatment of total vermiculite originating from 2 replicate plates, d-spacings of inoculated minerals collapsed to 1.09 nm just as with the control ones (diffractograms not shown). Therefore, there was no evidence of no non-exchangeable complexes remaining in the interlayer spaces. The difference in aCEC (154–135 mequiv) between control and inoculated vermiculites could, therefore, only be explained by non-measured exchangeable cations.

As the quantity of Na⁺ interlayer cations did not increase, we can infer that the 1.27 nm shoulder was due to some 1.0 nm d-spacings mixed with 1.4 nm d-spacings. K⁺ and NH₄⁺ cations are the only cations capable of such a closure; as these ions were not analysed in the KCl extraction solution, they could also explain the slight decrease in aCEC. The phyllosilicate might therefore have evolved towards a Ca-K-NH₄ interstratified vermiculite. A similar change was observed with a vermiculite treated with bi-ionic K⁺-Mg²⁺ solutions (Martin de Vidales *et al.*, 1991).

SEM observations showed that initially Ca²⁺-saturated vermiculite flakes directly colonized by mycelium were embedded within a Ca deposit [Fig. 1(C)] in which the typical Ca oxalate crystal structure could be recognized [Fig. 1(D)]. This new material was therefore assumed to be Ca oxalate; this hypothesis was confirmed by microanalysis as only Ca ions were detected in it. The only source of Ca being the vermiculite interlayer cations, this gave secondary evidence of fungal ability to mobilize interlayer Ca ions. Such neoformations were never observed on colonized NH₄⁺-saturated vermiculite [Fig. 1(B)].

When Ca²⁺-saturated vermiculite was isolated from mycelium by cellophane films, white deposits that showed the typical structure of Ca oxalate crystals under photonic microscopy were observed on the cellophane films, and on the vermiculite flakes. This raises the question of their eventual dissolution during the shaking of the mineral with 1 M KCl for interlayer cations extraction and aCEC analysis. As we do not

have a precise answer to this question, the number of mequiv 100 g⁻¹ of Ca ions thought to be originating from the interlayer spaces might therefore be overestimated. If so, fungal Ca mobilisation would be underestimated.

When soluble Ca was provided in the nutrient compartment. No modification of vermiculite d-spacings related to fungal colonization was detected (6 samples from 3 replicate Petri dishes analysed). The aCEC of the vermiculite collected under *P. involutus* mycelium was quite similar to that measured for the soluble Ca-free treatment, and the measured Ca desaturation was in a similar range (Table 2).

Fungal strain comparisons. Without soluble Ca in the nutrient medium, the vermiculite exposed to *P. tinctorius* for 100 days showed the same trends of d-spacing evolution as with *P. involutus* COU: 3/5 samples from 5 replicate Petri dishes were characterized by a shoulder at 1.27 nm rising up from a 1.46 nm peak.

DISCUSSION

Ectomycorrhizal fungi have access to NH₄⁺ and Ca²⁺ ions trapped in between the vermiculite 2:1 layers. As vermiculite samples were separated from the mycelium by cellophane films, soluble fungal exudates are responsible for phyllosilicate weathering. NH₄⁺ ions, usually considered as retrograded, were mobilized from the interlayer spaces and replaced by Mg²⁺, Al³⁺, Ca²⁺ and Na⁺ ions. Ca²⁺ ions were mainly replaced in interlayer spaces by Mg²⁺ and Al³⁺ ions but protons, K⁺ or NH₄⁺ ions could also have contributed to Ca²⁺ substitutions.

The access of microorganism to vermiculite N has only been investigated for nitrifying bacteria (Nömmik, 1957; Simon-Sylvestre *et al.*, 1991). Ca²⁺ release from Ca-saturated vermiculite by saprophytic fungi has been reported by Robert and Chenu (1992), but this is the first report concerning ectomycorrhizal fungi in pure culture.

Exchangeable Al³⁺ ions were detected in the interlayer spaces of NH₄⁺-saturated vermiculite after exposure to ectomycorrhizal fungi. It strongly suggests that the crystal lattice has been weathered, as it was the only Al³⁺ source in the experimental device. Some of the NH₄⁺-saturated vermiculite collected under the mycelium did not collapse to a peak at 1.0 nm in spite of K-saturation and 110°C heating. These non-exchangeable “wedges” prevent the collapse of the opened structure and render the transformation of the

phyllosilicate irreversible. Therefore, some NH_4^+ -saturated particles have probably evolved under mycelium towards an hydroxy-aluminous vermiculite. Such crystallographic and crystallochemical changes necessarily involved an acid dissolution of part of the mica structure. Our experimental approach does not give access to an eventual complete mineral dissolution phenomenon: however, this reaction can not be excluded.

A cation addition or depletion in the nutrient medium does not seem to affect fungal mobilization potential. Therefore such potential may be more constitutive than inducible.

When Ca^{2+} ions were mobilized from vermiculite, Ca oxalate crystal accumulation was easily detected. Oxalic acid is a strong complexing agent (Robert *et al.*, 1979) frequently associated with mineral weathering by lichens or fungi (Graustein *et al.*, 1977; Jones and Wilson, 1985; Robert and Berthelin, 1986). Oxalic acid excretion by ectomycorrhizal fungi including *P. involutus* has been reported many times (e.g. Lapeyrie *et al.*, 1987; Entry *et al.*, 1992; Griffiths *et al.*, 1994). Cromack *et al.* (1979) found higher oxalate concentrations and evidence for advanced clay weathering in *Hysterangium setchellii* mats of northwest U.S. soils. It is therefore not surprising that oxalate appears involved in the alteration of vermiculite by *P. involutus*. This should not exclude the possible involvement of other fungal organic-complexing molecules, siderophore-like, so far unidentified, as suggested by Ochs *et al.* (1993) when comparing $\gamma\text{-Al}_2\text{O}_3$ solubilization by mycorrhizal and non-mycorrhizal plant exudates. The ectomycorrhizal fungi *P. involutus* and *P. tinctorius* are also known to release a reducing substance (reduction of manganese oxide) (Cairney and Ashford, 1991). Robert (1971) experimentally verified the effect of oxido-reduction phenomenon in phyllosilicate transformation (vermiculitization).

Figure 1(C, D) shows initially Ca^{2+} -saturated vermiculite flakes in a dense network of hyphae covered by Ca oxalate crystals. Figure 1(B) shows bare hyphae in contact with initially NH_4^+ -saturated vermiculite flakes. We can infer that oxalate produced by these hyphae has reacted with Fe and Al ions originating from the vermiculite crystal lattice, thus accelerating weathering. This chemical reaction coupled with the large sink for N constituted by the mycelium could have led to the massive transformation of the NH_4^+ -saturated vermiculite. In contact with Ca^{2+} -saturated vermiculite, Ca oxalate formation is promoted by Ca^{2+} from vermiculite, impeding Fe and Al ions complexation. While oxalic acid could enhance the weathering of the NH_4^+ -saturated vermiculite, it might be ineffective on Ca^{2+} -saturated vermiculite.

In forest stands, tree species have an important action on soil functioning and vermiculite weathering (Ranger *et al.*, 1990). We established that some ectomycorrhizal fungi have access, *in vitro*, to various

ions from the phyllosilicate interlayers or crystal lattices. Endomycorrhizas compared to non-mycorrhizal roots are able to promote biotite weathering (Mojallali and Weed, 1978; Berthelin and Leyval, 1982). Here, the quantitative significance of such symbiotic mobilization and weathering by ectomycorrhizal fungi still has to be assessed *in situ* and in association with ectomycorrhizal plants. Indeed, not only mycorrhizal fungi and ectomycorrhizas but saprophytic microorganisms and non-mycorrhizal roots also interact with soil minerals. Interestingly, while oxalic acid may play an important role in mineral weathering by ectomycorrhizal fungi, widely different plant families have developed similar biogeochemical mechanisms, as non-mycorrhizal proteoid roots of *Banksia* release citric acid into their rhizospheres (Grierson, 1992).

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