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Biological mobilization of potassium from clay minerals by ectomycorrhizal fungi and eucalypt seedling roots

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Abstract

Growth, proton and oxalate efflux, K absorption and mineral depletion by one isolate of *Cenococcum geophilum* Fr., by a putative isolate of *Pisolithus microcarpus* and by two isolates of *Pisolithus* sp. were compared in pure liquid culture using either vermiculite or phlogopite as sole K source. An isolate from China (*Pisolithus* strain XC1) showed greatest biomass and K accumulation from both minerals. There were inverse correlations between reduction in HCl-extractable K and fungal proton efflux ($r = -0.912$, $P < 0.001$) and between the ratio (total K lost from the substrate: K accumulation in hyphae) and oxalate efflux ($r = -0.890$, $P < 0.001$). Protons effused from the fungi may have replaced interlayer K, and oxalate effused may have led to biological weathering of the minerals, especially under conditions of limited exchangeable K with phlogopite as K source. *Pisolithus* sp. and *Pisolithus* XC1 gave high depletion rates of $\text{CH}_3\text{COONH}_4^-$ and HCl-extractable K from the minerals and were therefore selected to study the growth and mobilization and utilization of K from the minerals by ectomycorrhizal eucalypt (*Eucalyptus globulus* Labill.) seedlings. Mycorrhizal seedlings extracted more K from the two minerals than did non-mycorrhizal controls, and those colonized by *Pisolithus* XC1 accumulated much more K than those inoculated with *Pisolithus* sp., a similar trend to fungal K accumulation *in vitro*. Non-mycorrhizal seedlings extracted very little HF-HClO₄-extractable K from the minerals following sequential extraction with H₂O, $\text{CH}_3\text{COONH}_4$ and HCl. In contrast, mycorrhizal seedlings significantly depleted K, although there was no difference between the two fungal strains. Patterns of depletion of sequentially extractable K fractions in the minerals by the fungi in pure culture *in vitro* differed from those exhibited by the fungus-root associations.

Introduction

The identification of nutrient uptake and mobilization mechanisms is a key issue in understanding the nutrition of ectomycorrhizal plants (Chalot et al., 2002). Ectomycorrhizal fungi cover roots and rootlets with a thick mantle of hyphae, effectively enlarging the surface areas of the roots in contact with clay minerals and soil pores that are not accessible to the root hairs (Schlecht, 1976). The finest lateral root-

lets, which can have a very short life and remain unbranched when not colonized, respond to mycorrhizal infection by growing for a longer period of time and by branching (Gerdemann, 1974), which is highly metabolism-dependent and partially related to the ion exchange between soil clay particles and roots and thus may enhance mineral nutrient uptake by plant roots. The extramatrical mycelium extending from the mantle may also play an important role in mobilization of nutrients (Smith and Read, 1997).

Numerous studies have shown that ectomycorrhizal plants extract mineral nutrients, including P,

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Ca, Mg and K, from soils and minerals (Blum et al., 2002; Jongmans et al., 1997; Wallander and Wickman, 1999). Potassium, an element required in slightly lower quantities than nitrogen, is one of the essential major nutrients for trees in forests. Forest trees deficient in K are commonly found in subtropical and tropical acid soils, and ectomycorrhizal inoculation might improve the growth and K nutrition of the trees in artificial plantations in those areas. Huang and Lapeyrie (1996) reported the utilization of HCl-extractable K by Douglas fir inoculated with *Laccaria bicolor* S238N and the concomitant acceleration of tree growth rate. Similarly, the growth of *Pinus sylvestris* seedlings was stimulated by *Paxillus involutus* when microcline was used as the K source (Wallander and Wickman, 1999). Moreover, simultaneous depletion of K and Mg in culture media leads to weathering of phlogopite by ectomycorrhizal fungi. This biological weathering, resulting in the release of otherwise unavailable K from minerals, seems to be related to the efflux of oxalate ions and protons by hyphae because Lapeyrie et al. (1987) have reported that the weathering of the minerals is promoted by oxalate, and the interlayer K in 2:1 clay minerals can be replaced by protons. Wallander and Wickman (1999) also found higher concentrations of citric and oxalic acids in solutions from media containing biotite that were used to grow pine seedlings colonized by *Suillus variegatus*. Thus, release of organic acids may be a mechanism by which ectomycorrhizas mobilize and then absorb unavailable K from soils. However, there is still no direct evidence for the exploitation by ectomycorrhizas of soil K pools that are unavailable to non-mycorrhizal plants.

Analysis of K concentrations in plant tissues has also failed to demonstrate unequivocally the involvement of ectomycorrhizas in soil K uptake and mobilization. Following ectomycorrhizal inoculation, promotion of K uptake by host trees from soils has been found, but in some cases K has been found to be at a lower concentrations in the tissues of plants inoculated with mycorrhizal fungi compared to uninoculated controls (Bowen, 1973; Smith and Read, 1997). This may be due to a dilution effect resulting from mycorrhizal stimulation of host plant growth and suggests that K uptake is a better indicator than K concentration of mycorrhizal effects on plant K nutrition. Fungal species have been shown to vary in their capacity to enhance the growth and nutrition of host plants (Bowen, 1973; Huang and Lapeyrie, 1996; Smith and Read, 1997). Among 16 ectomycorrhizal fungi studied by Burgess et al. (1993), only four (includ-

ing *Pisolithus* sp. in the present study and formerly *Pisolithus tinctorius* 2144) promoted the growth and P uptake of eucalypt seedlings. There are several possible explanations for these variable results, including the limited number of tree and fungal species (and especially fungus–root associations) that have been studied. The influence of host plant roots in symbiosis on fungal nutrient absorption and utilization should not be neglected. Furthermore, interference from confounding factors that influence nutrient mobilization and absorption by trees under field conditions such as soil type, soil available nutrient concentrations and age of trees, should be taken into consideration.

Biological weathering of phlogopite and vermiculite by ectomycorrhizal fungi was further studied *in vitro* by Paris et al. (1995a,b; 1996). Enhanced weathering of phlogopite by *P. tinctorius* 441 (now a putative strain of *P. microcarpus* according to Martin et al., 2002) under conditions of K^+ and Mg^{2+} depletion was found to be associated with specific Al^{3+} immobilization (Paris et al., 1995a), and the fungus accumulated oxalic acid when supplied with either NH_4^+-N or NO_3^--N , although *Paxillus involutus* accumulated oxalate only on NO_3^--N (Paris et al., 1996). Oxalic acid also appeared to be involved in vermiculite weathering by *Paxillus involutus* and the presence or absence of soluble NH_4^+ or Ca did not appear to affect fungal immobilization potential (Paris et al., 1995b).

Cation mobilization and mineral weathering has yet to be evaluated in mycorrhizal associations. It is thus important to study the utilization and mobilization of K from clay minerals by ectomycorrhizas formed by various fungal species with the host tree. The aim of the present study was to complement the work of Paris et al. (1995a,b; 1996) by evaluating further the capacity of several ectomycorrhizal fungal isolates to mobilize and then utilize K pools in 2:1 type clay minerals *in vitro* and to examine the fractions of K removed from the minerals using a sequential extraction procedure, and to extend the study to ectomycorrhizal plants by comparing the effects of two of the fungal strains on K mobilization and absorption by a eucalypt host species.

Materials and methods

Biological mobilization of K from clay minerals by ectomycorrhizal fungi in vitro

Fungal strains

Three of the ectomycorrhizal fungi used in the experiments, namely *Cenococcum geophilum* SIV, a putative isolate of *Pisolithus microcarpus* formerly referred to as *P. tinctorius* 441 (Martin et al., 2002) and *Pisolithus* sp. (previously *P. tinctorius* 2144) were provided by Dr Frédéric Lapeyrie of the Centre de Recherches Forestières de Nancy in France, and *Pisolithus* XC1 was obtained from the Microbiology Laboratory, Southwest Agricultural University, Chongqing, China. The putative isolate of *P. microcarpus* was originally isolated in Brazil, *Pisolithus* sp. in Australia and *Pisolithus* XC1 from Xichang, Sichuan province, China (all from mycorrhizal *Eucalyptus* spp.), and *C. geophilum* SIV was originally isolated from spruce mycorrhizas in Europe. Mycelium for inoculation was grown on Pachlewski agar medium for three weeks. The medium contained 0.5 g L⁻¹ tartrate, 1.0 g L⁻¹ KH₂PO₄, 0.5 g L⁻¹ MgSO₄, 20 g L⁻¹ glucose, 5.0 g L⁻¹ maltose, 0.1 g L⁻¹ vitamin B₁, 20 g L⁻¹ agar and 1 mL L⁻¹ microelement solution (1 L microelement solution contained 8.45 mg H₃BO₃, 5 mg MnSO₄, 6 mg FeSO₄, 0.625 mg CuSO₄, 2.27 mg ZnCl₂ and 0.27 mg (NH₄)₂MoO₄).

Preparation of the minerals

Phlogopite and vermiculite were collected from Jinchun Mine, Sichuan province, China (31°52' N, 103°55' E). They were air-dried and ground to pass a 0.1-mm sieve. Vermiculite was then saturated with 0.1 mol L⁻¹ KCl for one week. The KCl-saturated vermiculite was then washed with deionized water several times to remove water-soluble K. The sizes of various K pools in phlogopite and K-saturated vermiculite after sterilization are shown in Table 1.

Experimental procedure

Pachlewski liquid medium was prepared in which the KH₂PO₄ was replaced by NaH₂PO₄ with an equivalent amount of phosphorus. Aliquots (20 mL) of the liquid medium were transferred into 100-mL Erlenmeyer flasks with 0.500 g phlogopite or vermiculite added as K source. After steam-sterilization at 121 °C for 30 min each flask was inoculated with a plug of mycelium 5 mm in diameter. Blank control flasks were

set up in the same way but with no inoculation. The flasks were incubated for 3 weeks at 25 ± 1 °C in the dark. There were 12 replicates of each treatment.

Sampling and analysis

The fungi were harvested and washed with deionized water to remove culture medium from the surface of the mycelium. They were then oven-dried, weighed and digested with H₂SO₄-H₂O₂.

The culture solutions together with minerals were centrifuged at 4500 rpm for 10 min to separate the minerals. The minerals were then air-dried and sequentially extracted with H₂O and 1 mol L⁻¹ CH₃COONH₄ by shaking at 150 rpm under 30 °C for 30 min, and with 1 mol L⁻¹ HCl with boiling for 10 min (mineral:solution ratio = 1:10).

The culture solutions were analysed at harvest for pH using a pH meter and for oxalate by a KMnO₄ titration procedure following precipitation with saturated Ca(OH)₂ solution as CaC₂O₄ and dissolution with H₂SO₄ (Peng et al., 1985). The concentration of K in the digests, extracts and liquid media was determined by flame photometry.

Biological mobilization of K from clay minerals by ectomycorrhizal eucalypt seedlings

Preparation of ectomycorrhizal seedlings

On the basis of their capacities to deplete K pools in the two minerals in the experiment described above, *Pisolithus* sp. and *Pisolithus* XC1 were selected for inoculation of eucalypt seedlings for further study and were grown on Pachlewski agar medium. Eight mycelial plugs (each 5 mm in diameter) were inoculated in a row at the centre of one half of each 12-cm-diameter culture plate containing 20 mL Pachlewski agar medium from which all maltose and 95% of the glucose were omitted. Following cultivation at 25 °C for two weeks, 20–25 CaClO₃-sterilized seeds of *E. globulus* were sown in a line parallel with, and about 10 mm away from, the row of fungal colonies. The culture plates were then inclined with the seed row positioned horizontally above the mycelial row and cultured at 25 °C with a light intensity of 10000 lux for a further two weeks. Fungus-root associations were formed as the roots grew downwards and made contact with the fungal hyphae. Non-mycorrhizal seedlings were produced in the same way except that no fungal mycelia were grown in the plates.

Table 1. Analysis of potassium pools in the two minerals by sequential extraction

Clay or mica mineral	H ₂ O-K ⁺	CH ₃ COONH ₄ -K ⁺	HCl-K ⁺	HF-HClO ₄ -K ⁺
	(μg g ⁻¹)			(mg g ⁻¹)
Vermiculite	5.5	328	120	13.0
Phlogopite	2.5	125	400	34.5

Experimental design

Quartz sand (30 g, 1 mm in diameter) was mixed with 2 g of phlogopite or vermiculite and the mixture was placed in a plastic container 6.0 cm in diameter and 3.0 cm high. The two minerals were pre-treated in the same way as described above for the *in vitro* experiment (Table 1). Four mycorrhizal or non-mycorrhizal seedlings were transplanted into each container. Control pots were set up in the same way without seedlings so that changes in the K pools in the minerals attributable to the plants could be observed. There were five replicate pots of each treatment in a fully randomized design. Seedlings were grown in a glasshouse in the summer for 60 days. Potassium-free Hoagland nutrient solution was applied daily during the growth period.

Sampling and analysis

Harvested seedlings were washed and oven-dried and then weighed. The dried seedlings were digested with H₂SO₄-H₂O₂. The substrate in each experimental container was air-dried. The sand was then removed and the remaining minerals in the substrate were carefully collected. The K fractions in the minerals were determined by sequential extraction as described above for the *in vitro* experiment. The residual minerals were digested with HF-HClO₄. The K concentration in the digests and extracts was determined by flame photometry.

Statistical analysis

Data were tested by two-way analysis of variance with replication (Sokal and Rolff, 1995) using the SAS package (Statsoft, 1989–1996 by SAS Institute Inc., Cary, NC, USA). Mean values for reduction in mineral HCl-extractable K and fungal proton efflux, and for (total K lost from flask: K accumulation in hyphae) ratio and oxalate efflux, were compared by linear correlation with 8 degrees of freedom ($n = 10$).

Results

Biological mobilization of K from clay minerals by ectomycorrhizal fungi in vitro

Fungal growth and K absorption

As shown in Table 2, the four fungal isolates varied significantly in growth rate, with *Pisolithus* XC1 showing the highest mean biomass of about 48 mg, followed by *C. geophilum* SIV (34 mg), *Pisolithus* sp. (14 mg) and *P. microcarpus* with about 7 mg flask⁻¹. There was no significant difference between the two minerals in their influence on the growth of the fungi. A significant interaction between fungal isolate and clay mineral reflects the trend of slightly higher biomass of the three *Pisolithus* isolates grown with vermiculite compared with phlogopite and the opposite trend for *C. geophilum*. The three *Pisolithus* isolates had similar K concentrations irrespective of which clay mineral was the K source (range 13.2–15 g kg⁻¹) and this was about double the concentration in *C. geophilum* mycelium. Potassium accumulation by the fungi, calculated by multiplying biomass by K concentration, followed a similar trend to fungal biomass, with *Pisolithus* XC1 showing the highest K accumulation (669 μg flask⁻¹) and *P. microcarpus* showing the lowest (94 μg flask⁻¹). Despite the vermiculite having a larger proportion of more readily available P than phlogopite (Table 1), there was no difference between the minerals in terms of fungal growth, K concentration or K uptake from the medium (Table 2).

Efflux of protons and oxalate by fungi

Accumulation of protons in the culture solution also varied among fungal species (Figure 1), with the highest proton accumulation in the medium of *Pisolithus* XC1 (21.8 μg on average) and the lowest in the *P. microcarpus* solution (5.1 μg flask⁻¹). Proton efflux from *Pisolithus* isolates was unaffected by phlogopite versus vermiculite as K source but *C. geophilum* SIV released more protons into the medium with phlogopite compared to vermiculite (Figure 1).

Table 2. Mean biomass, K concentration and K accumulation in fungal hyphae grown *in vitro*

Fungal isolate	K source	Biomass (mg flask ⁻¹)	K concentration (g kg ⁻¹ DW)	K accumulation (μg flask ⁻¹)
<i>Pisolithus</i> XC1	Vermiculite	48.6	13.9	676
	Phlogopite	47.0	14.1	662
<i>Pisolithus</i> sp.	Vermiculite	14.1	15.0	211
	Phlogopite	13.0	15.3	199
<i>P. microcarpus</i>	Vermiculite	7.0	13.2	93
	Phlogopite	6.7	14.1	95
<i>C. geophilum</i> SIV	Vermiculite	32.4	7.3	238
	Phlogopite	34.8	6.5	227
Significance ^a due to:				
Fungus		***	***	***
K source		ns	ns	ns
Fungus × K source		**	ns	ns

^aBy analysis of variance; ***, $P < 0.001$; **, $P < 0.01$; ns, not significant

Differences among fungal isolates in accumulation of oxalate in the culture solution followed a similar trend to proton efflux (Figure 1) with *Pisolithus* XC1 culture solution containing 36.6 μg and *P. microcarpus* 18.3 μg flask⁻¹. Phlogopite promoted oxalate efflux by *Pisolithus* XC1 and *C. geophilum* SIV, but the K source had no effect on oxalate accumulation in the culture solutions of the other two *Pisolithus* isolates.

K pools in residual culture medium and clay minerals

There was more residual K in the culture liquid and in the H₂O- and NH₄Ac-extractable fractions of K in the clay minerals when vermiculite was the sole K source compared with phlogopite (Table 3), and all four fungal isolates had depleted the soluble K in the culture medium and the H₂O-extractable K in the vermiculite. Residual vermiculite contained more NH₄Ac-extractable K than HCl-extractable and phlogopite showed the opposite trend. Compared with control values, the fungi had depleted vermiculite NH₄Ac-extractable K in the sequence *Pisolithus* XC1 > *Pisolithus* sp. > *C. geophilum* SIV > *P. microcarpus*. *Pisolithus* XC1 had also removed more HCl-extractable K from vermiculite and phlogopite than the other isolates, and *Pisolithus* sp. had removed a relatively high proportion of this fraction. The significant interactions between fungal isolate and clay mineral indicate that NH₄-extractable K was more depleted by the fungi in vermiculite than in

phlogopite and that *Pisolithus* XC1 depleted HCl-extractable K from much more from both minerals than did the other fungi (Table 3).

Balance between accumulation of K in fungi and depletion of K in substrate

The amount of K lost from the substrates in the Erlenmeyer flasks, including K in the culture solution, and water-, CH₃COONH₄- and HCl-extractable fractions, was calculated from the initial amount minus that in every fraction in the flask at fungal harvest. The ratio between the summation of substrate K lost in each Erlenmeyer flask and K accumulated in fungal hyphae ranged from 0.29 to 0.78 (Figure 2) and was well below 1.00, suggesting that the fungi took up K from some more recalcitrant pools other than those extracted by our sequential protocol. Furthermore, *Pisolithus* XC1 showed a smaller ratio than the other fungi (which showed similar ratios), suggesting that *Pisolithus* XC1 utilized more of the recalcitrant K pools.

Biological mobilization of K from clay minerals by ectomycorrhizal eucalypt seedlings

Plant growth

Growth of the eucalypt seedlings was promoted by fungal colonization, and plant biomass was increased more by inoculation with *Pisolithus* XC1 than with *Pisolithus* sp. (Table 4). There was also a small but sig-

(a)

(b)

Figure 1. Mean (a) proton and (b) oxalate efflux per flask by four ectomycorrhizal fungi grown *in vitro* and supplied with two different minerals (vermiculite and phlogopite) as sole K source. Bars: $\pm$ one standard deviation (SD). P XC1, *Pisolithus* XC1; P sp, *Pisolithus* sp.; P mic, putative *Pisolithus* microcarpus; Cg SIV, *Cenococcum geophilum* SIV.

nificant decrease in root:shoot ratio with mycorrhizal colonization.

Seedling K concentration and uptake

There was little effect of fungal inoculation on K concentrations in seedling leaves, roots and stems compared with non-mycorrhizal controls except for a high concentration in the leaves of seedlings colonized by *Pisolithus* XC1 (Figure 3). However, the effects of fungal colonization on plant biomass were reflected in amount of K taken up by the eucalypt seedlings, with

three times as much taken up by seedlings in association with *Pisolithus* XC1 than by non-mycorrhizal controls (Table 4).

Changes in K pools in the minerals

Water-soluble K

Seedlings decreased H₂O-soluble K in the substrate minerals from 3.00 $\mu\text{g g}^{-1}$ (without seedlings) to 1.60 (*Pisolithus* XC1), 1.75 (*Pisolithus* sp.) and 1.50 $\mu\text{g g}^{-1}$ (non-mycorrhizal) on average (Table 5). No differences were found in H₂O-soluble K in the

Table 3. Mean concentration of K in residual culture solution and in sequentially extracted fractions of the clay minerals *in vitro* at fungal harvest

Fungal isolate	K pool			
	Culture liquid ($\mu\text{g ml}^{-1}$)	H ₂ O-extractable	NH ₄ Ac-extractable ($\mu\text{g g}^{-1}$)	HCl-extractable
Vermiculite				
Control	3.6	4.5	315.5	125.5
<i>Pisolithus</i> XC1	1.6	2.2	75.5	59.9
<i>Pisolithus</i> sp.	1.8	2.5	153.3	76.4
<i>P. microcarpus</i>	1.6	2.4	264.5	105.2
<i>C. geophilum</i> SIV	1.6	2.3	163.4	62.6
Phlogopite				
Control	1.6	2.0	105.0	412.0
<i>Pisolithus</i> XC1	1.5	1.8	48.1	77.6
<i>Pisolithus</i> sp.	1.5	1.7	45.4	225.2
<i>P. microcarpus</i>	1.3	1.8	48.5	364.3
<i>C. geophilum</i> SIV	1.4	1.6	53.0	210.5
Significance ^a of:				
Fungus	ns	ns	***	***
K source	***	***	***	***
Fungus $\times$ K source	ns	ns	***	***

^aBy analysis of variance; ***, $P < 0.001$; ns, not significant.

substrates following plant growth with or without fungal inoculation.

NH₄Ac-exchangeable K

As shown in Table 5, both non-mycorrhizal and mycorrhizal seedlings markedly lowered NH₄Ac-exchangeable K in the two minerals. Mycorrhizal plants decreased NH₄Ac-exchangeable K more than did non-mycorrhizal seedlings.

HCl-extractable K

Non-mycorrhizal seedlings depleted the HCl-extractable fraction of K in phlogopite to a much greater extent than in vermiculite, and mycorrhizal seedlings lowered HCl-extractable K in both minerals more than did non-mycorrhizal seedlings. Colonization with *Pisolithus* XC1 led to a greater depletion of HCl-extractable K in phlogopite by eucalypt seedlings than colonization with *Pisolithus* sp. but there was no such difference between isolates in the case of vermiculite.

HF-HClO₃-extractable K

After sequential extraction with H₂O, CH₃COONH₄ and HCl, there was little difference between the blank controls (without seedlings) and non-mycorrhizal

seedlings in residual K released by HF-HClO₃ digestion. Fungal colonization markedly lowered HF-HClO₃-digestible K in the minerals especially after inoculation with *Pisolithus* XC1 when vermiculite was the K source.

Discussion

Hyphal K uptake in pure culture was highest in the Chinese isolate *Pisolithus* XC1 and was also higher in eucalypt seedlings colonized by this isolate than those colonized by *Pisolithus* sp. The results indicate that this was due mainly to the effects of the fungus on plant growth, although K concentrations in the leaves of seedlings colonized by *Pisolithus* XC1 were also higher than those with the Australian isolate. Both fungal isolates were effective in promoting plant K uptake of mycorrhizal seedlings which was about twice or three times higher than in non-mycorrhizal controls. Our results are consistent with those of Burgess et al. (1993) who studied the effectiveness of 16 fungal isolates in forming ectomycorrhizas with *E. globulus* and *E. diversicolor* seedlings and found that only four of them (including *P. tinctorius* 2144, here referred to as *Pisolithus* sp.) promoted plant growth and P uptake.

Figure 2. Ratio between the total amount of K loss from the substrate in each flask (K loss, including K in the liquid medium and water-, $\text{CH}_3\text{COONH}_4$ - and HCl-extractable pools) and K accumulated in fungal tissues. P XC1, *Pisolithus* XC1; P sp, *Pisolithus* sp.; P mic, putative *Pisolithus microcarpus*; Cg SIV, *Cenococcum geophilum* SIV.

Table 4. Mean dry matter yield and K uptake of non-mycorrhizal and ectomycorrhizal eucalypt seedlings grown with vermiculite or phlogopite as sole K source

Fungal isolate	K source (mineral)	Biomass (g pot^{-1})	Root:shoot (ratio)	K uptake (mg pot^{-1})
Non-mycorrhizal	Vermiculite	2.44	0.83	8.4
	Phlogopite	1.92	0.82	6.7
<i>Pisolithus</i> sp.	Vermiculite	4.36	0.72	16.8
	Phlogopite	4.24	0.75	17.0
<i>Pisolithus</i> XC1	Vermiculite	5.60	0.80	23.7
	Phlogopite	6.36	0.75	27.6
Significance ^a of:				
Fungus		***	**	***
K source		ns	ns	ns
Fungus $\times$ K source		ns	ns	*

^aBy analysis of variance; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

They also report that *P. tinctorius* produced the largest plant growth increases and the isolates that developed more extensive mycorrhizas on roots produced the largest growth responses, and isolates that increased plant growth also increased plant P uptake. The finding that fungal isolates that are effective in enhancing host plant P uptake may also promote plant K uptake is also consistent with the belief (Smith and Read, 1997) that polyphosphate is stabilized by K^+ ions, and that both inorganic P and K may be transferred to the plant, thus maintaining charge balance in both symbionts.

Some tropical soils are rich in vermiculite, an expanded 2:1 type clay mineral (Mohr et al., 1972) and both P and K are commonly in short supply (Mohr et al., 1972). Under these conditions fungal inoculation may be important for the absorption of P and K.

Mica is an unexpanded 2:1 type clay mineral and is a primary K mineral with K^+ ions adsorbed between the clay layers. Some of the K^+ ions in the crystal lattice, often referred to as lattice-K, are encircled by six oxygen atoms and expansion between clay layers is difficult. However, vermiculite has lower binding forces between mineral layers than unexpanded clay minerals (Huang, 2000). Thus, vermiculite subjected to KCl-saturated treatment for one week contained three times as much exchangeable- K^+ as phlogopite, and HCl-extractable K in vermiculite was only up to 30% of that in phlogopite in our experiment. In other words, if the same weight of the minerals is supplied, vermiculite will provide more exchangeable K and less HCl-extractable K for fungal uptake than phlogopite.

Table 5. Changes in sequentially extracted K fractions in the minerals after harvesting of the seedlings

Treatment	K source (mineral)	H ₂ O-K ⁺	NH ₄ Ac-K ⁺ ($\mu\text{g g}^{-1}$)	HCl-K ⁺	HF-HClO ₄ -K ⁺ (mg g^{-1})
No seedlings (control)	Vermiculite	4.50	127.5	110.5	13.5
	Phlogopite	1.50	100.5	385.0	35.0
Seedlings non-mycorrhizal	Vermiculite	2.50	70.5	82.5	10.0
	Phlogopite	0.50	65.0	345.0	31.5
Seedlings with <i>Pisolithus</i> sp.	Vermiculite	3.00	47.5	40.5	6.0
	Phlogopite	0.50	40.0	295.5	26.0
Seedlings with <i>Pisolithus</i> XC1	Vermiculite	2.75	50.5	40.0	2.5
	Phlogopite	0.45	35.0	255.5	22.0
Significance ^a of:					
Treatment		***	***	***	***
K source		***	***	***	***
Treatment $\times$ K source		***	***	**	ns

^aBy analysis of variance; ***, $P < 0.001$; **, $P < 0.01$; ns, not significant.

Potassium extracted by 1 mol L⁻¹ CH₃COONH₄ from soils is mainly adsorbed on the surfaces of soil colloids and present in the interlayer of expanded 2:1 minerals and is exchangeable. It is thus available to plants and could be also available to ectomycorrhizal fungi. In contrast, HCl-extractable K consists mainly of interlayer K in unexpanded 2:1 type clay minerals and lattice K chiefly present in feldspars (Martin and Sparks, 1983, 1985; Mengel and Uhlenbecker, 1993), and is slowly released from soils and minerals. Numerous studies have indicated that HCl-extractable K in soils is often unavailable to plants in the short term (Brar et al., 1986; Grimme, 1974; Martin and Sparks, 1983, 1985; Mengel and Uhlenbecker, 1993). In a loess-derived soil (Luvisol), for example, HCl-extracted K was not even depressed by ryegrass, a plant which usually has well-developed root systems and a strong ability to absorb nutrients from soils (Mengel and Uhlenbecker, 1993). However, HCl-extractable K was markedly decreased by ectomycorrhizal fungi in our experiment. This may suggest that ectomycorrhizal fungi can utilize HCl-soluble K that is not available to plants and may explain why the HCl-extractable K fraction was substantially depressed by eucalypt seedlings inoculated with *Pisolithus* XC1 and *Pisolithus* sp. compared with non-mycorrhizal controls. A similar observation was made in Douglas fir inoculated with *Laccaria bicolor* S238N (Huang and Lapeyrie, 1996). It is noteworthy that the pattern of reduction in CH₃COONH₄- and

HCl-extractable K fractions in the two minerals was different in fungal pure culture and fungus-root associations, indicating some changes in fungal K utilization and mobilization in the presence of the host plants.

After sequential extraction with water, CH₃COONH₄ and HCl, the K remaining in the minerals may exist mainly in structural pools that are unavailable to plants unless the minerals are weathered. In the present work, however, 22 to 71% of the K accumulated in fungal hyphae was derived from structural pools (Figure 3) and this implies that weathering of the clay minerals and release of K from structural pools occurred in the fungal cultures. In eucalypt seedling culture, K obtained by HF-HClO₃ digestion after sequential extraction with water, CH₃COONH₄ and HCl was lowered very little (if at all) in the substrates of non-mycorrhizal seedlings compared to blank controls with no seedlings present. Fungal colonization significantly lowered HF-HClO₃-digestible K in the substrates. This indicates that that mineral weathering and K release may have occurred in the mycorrhizal seedling cultures and that ectomycorrhizal fungi may play a key role in mineral weathering and K release.

Both [Al (C₂O₄)₃]³⁻ and [Fe (C₂O₄)₃]³⁻ have very high chelation constants of 2.0×10^{16} and 3.9×10^{16} . Oxalate may therefore chelate Al³⁺ and Fe³⁺ in the crystal lattice of minerals containing K, resulting in weathering of these minerals and K release. Moreover, both protons and K are monovalent. The diameter of protons (0.32×10^{-10} m) is much smaller

Figure 3. Mean concentration of K in leaves, roots and stems of non-mycorrhizal eucalypt seedlings and seedlings inoculated with *Pisolithus* sp. and *Pisolithus* XC1. Bars: $\pm$ one standard deviation (SD). No ECM, no ectomycorrhiza (uninoculated control); P sp, *Pisolithus* sp.; P XC1, *Pisolithus* XC1.

ler than that of K (2.03×10^{-10} m). Protons may therefore have a strong capacity to move through the interlayer of layer minerals such as mica and phlogopite and replace the K^+ present. The weathering of minerals containing K caused by oxalate and the replacement of the interlayer K by protons have been demonstrated, albeit not unequivocally, by stimulation experiments (Lapeyrie et al., 1987). Simultaneous depletion of K and Mg^{2+} in culture medium *in vitro* leads to enhanced weathering of phlogopite and this biological weathering is related to the efflux of protons and oxalate by fungal hyphae (Lapeyrie et al., 1987; Paris et al., 1995a, b, 1996). Minerals collapse and K release may occur through the formation of chelates of oxalate with bivalent and trivalent cations in crystal lattices. In addition, protons produced by fungi may move to the interlayer of expanded and non-expanded 2:1 type clay minerals to replace interlayer K (Cromack et al., 1979). Efflux of protons and oxalate ions by ectomycorrhizal fungi colonizing root surfaces could therefore be very important in the mobilization of interlayer K and structural K from minerals. In the present study, significant negative correlations were found between the reduction in HCl-extractable K and fungal proton efflux ($r = -0.912$, $P < 0.001$) and between the ratio of total K lost in the flask to K accumulation in hyphae and oxalate efflux ($r = -0.890$, $P < 0.001$) under conditions of limited exchangeable K supply (phlogopite treatments). This suggests that protons and oxalate effused into the medium by ectomycorrhizal fungi under conditions of available K deficiency may replace interlayer K in clay minerals and lead to biological weathering of the min-

erals and supports the findings of Paris et al. (1995a, b, 1996).

Trees in forests and plantations may interact with hundreds of ectomycorrhizal fungal species (Dahlberg, 2001). Studies are therefore required on the possible biological weathering of K from minerals by ectomycorrhizal fungi under field conditions. Experiments in boreal forests in which mesh bags containing sand and various minerals have been buried in forest soils of varying K and P status and examined after 17 months have indicated that ectomycorrhizas had a low potential to ameliorate K limitation by mineral weathering (Hagerberg et al., 2003). However, under tropical and sub-tropical conditions biological weathering of K could be more important and therefore merits investigation under field conditions.

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