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Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings

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Abstract The aim of this study was to investigate the role of plants colonised by two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus variegatus*, in mobilising potassium (K) from biotite and microcline, two minerals common in acid to medium-acid bedrock. This was carried out in a 33-week pot study with seedlings of *Pinus sylvestris* growing in symbiosis with the fungi, where no K was added or where K was added in the form of biotite or microcline. The mineral additions were similar to those found in natural soils. All seedlings, including non-mycorrhizal, were able to access the K in biotite, leading to stimulated growth and K uptake relative to controls. Microcline addition induced growth depression in all seedlings except those colonised by *P. involutus*, which were stimulated. The soil solution from *S. variegatus*-colonised seedlings grown with biotite had higher concentrations of citric and oxalic acid. Citric acid concentration was positively correlated to the fungal biomass (ergosterol) in the soil, as well as to the foliar K in *S. variegatus*-colonised seedlings. Seedlings growing without K addition had low K concentrations in the shoot. Magnesium (Mg) concentrations were enhanced in seedlings with severe K shortage, indicating that Mg can substitute for K, while calcium concentrations did not vary significantly.

Key words Ectomycorrhiza · Feldspar · Mica · *Paxillus* · Pine · *Suillus* · Weathering

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Introduction

Roots with ectomycorrhizas have higher surface areas for nutrient uptake than non-mycorrhizal roots. In addition, hyphae of mycorrhizal fungi can affect nutrient uptake by altering soil chemistry. For example they exude organic acids (Cromack et al. 1979; Malajczuk and Cromack 1982; Lapeyrie 1988; Snetselaar and Whitney 1990; Jones et al. 1992; Rasanayagam and Jeffries 1992). Higher concentrations of organic acids, or a more complex suite of acids, have been found in the rhizospheric soil than in bulk soil, and in soils containing mats of ectomycorrhizal fungi than in non-mat soils (Grierson 1992; Griffiths et al. 1994). Apart from oxalic acid, low-molecular-weight organic acids identified in soils are often citric and formic acid (Fox and Comerford 1990). Using a mathematical model, Jones et al. (1996) predicted that 99% of the organic acids lost by the root remained within 1 mm of the root surface. Concentrations of organic acids may be high in the micro-environments around fine roots or fungal hyphae (Ochs et al. 1993; Drever and Vance 1994). Griffiths et al. (1994) found that mycelial mats formed by *Gautieria monticola* (high production of oxalic acid) weathered soil minerals more efficiently than mycelial mats formed by *Hysterangium setchellii* (low production of oxalic acid). Decomposition of organic matter, on the other hand, was more rapid in mats of *H. setchellii,* indicating different functions of different ectomycorrhizal fungi.

Weathering of silicate minerals by low-molecular-weight organic acids has been studied by Schenk et al. (1989) among others. Both citric acid and oxalic acid dissolve feldspars (Manley and Evans 1986). Boyle and Voigt (1973) also studied the weathering of microcline and biotite by non-mycorrhizal pine (*Pinus radiata*) seedlings and by fungi (*Aspergillus niger*, and mixtures of unknown forest fungi) in a culture medium, all without any other potassium (K) source. They attributed the weathering to several low-molecular-weight organic acids, of which they were able to identify citric and ox-

alic. Watteau and Berthelin (1994) studied weathering of biotite and the effect of the ectomycorrhizal fungus *Suillus granulatus* growing in a nutrient medium free of iron. Iron was released from the mineral in response to release of malic and citric acids by the fungus. Bio-accumulation of iron in the fungus was high (more than required). Thus, the dissolution could be attributed to the effects of acidity, complexation and modification of the equilibrium between the mineral and solution due to bio-accumulation.

Soil bacteria can also influence weathering. Bacteria can dissolve silicate minerals with the help of chelating compounds, for example organic acids (Vandevivere et al. 1994). On the other hand, Lundström and Öhman (1990) reported that certain soil bacteria consume organic acids present in the soil solution, which resulted in a reduced rate of feldspar dissolution.

Jongmans et al. (1997) recently found a network of numerous tubular pores in feldspars and hornblendes in podzol E horizons and granitic bedrocks at several locations in Europe. It was suggested that these pores were formed by ectomycorrhizal fungi and that the fungi could supply trees with basic cations such as K^+, Mg^{2+} and Ca^{2+} from protected sites inside the minerals. The aim of this present study was to elucidate the role of two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus variegatus,* in mobilising K from biotite and microcline when living in symbiosis with *Pinus sylvestris* seedlings. Biotite and microcline are common K-containing minerals in most soils from acid to medium-acid parent bedrock and the fungi are common associates of pine in these soils. A second aim was to examine a possible relationship between exuded low-molecular-weight acids and the efficiency of K uptake. The activity and biomass of bacteria in soils used in the present study were described by Olsson and Wallander (1998).

Material and methods

Plant and fungal material

Seeds of *Pinus sylvestris* (L.) were sown in vermiculite and irrigated with distilled water. After 4 weeks, the seedlings were placed on a 2-mm peat layer in observation boxes next to a *P. sylvestris* seedling already colonised by either *Paxillus involutus* (Fr.) Fr (isolate Pi 3, isolated in an 80-year-old *Picea abies* forest at lake Gårdsjön in southwest Sweden by A. Dahlberg, Swedish University of Agricultural Sciences) or *Suillus variegatus* (Fr.) O. Kuntze (isolate 2–10, isolated in a 100-year-old *P. sylvestris* forest at Månskogstjärn in northern Sweden by A. Dahlberg). The new seedlings (5 seedlings per treatment) were colonised within 2 weeks, and after 4 weeks the experimental treatments started. Non-mycorrhizal control seedlings were grown in similar observation boxes but without ectomycorrhizal inoculum (5 seedlings per treatment).

Potting mixture

The biotite (Table 1), from a pegmatite in Moen, Norway, was crushed in an electric coffee mill to particles of less than 250 μm (specific surface area 1.22 m^2g^{-1}). The microcline (Table 1), from a Rb and Cs naturally-enriched pegmatite in Varuträsk, Sweden, was crushed in jaw- and rollercrushers (Mogårdshammar) to particles less than 160 μm (specific surface area 0.26 m^2g^{-1}). After crushing, the minerals were washed in distilled water to eliminate the most readily released K and the very finest material. Acid-washed quartz sand (Table 1) was used (particle size 364–2000 μm, 99% SiO_2; Ahlsell, Sweden) as an inert mineral substratum. Peat (Table 1) was collected from a raised bog (Hanvedsmossen, south of Stockholm), partly dried and sieved through a 2.0-mm mesh.

Table 1 Chemical composition of the microcline, biotite, quartz sand, peat and nutrient solution used (n.m. denotes not measured). Element analyses were performed by atomic emission spectrometry of minerals after fusion with $LiBO_2$ and peat after HNO_3 digestion, with 1–3 replicates per sample (*nm* not measured)

Material	K (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})
Microcline	100	–	–
Biotite	78	72	0.79
Quartz sand	1.75	2.3	1.4
Peat	0.1	nm	nm
	K (mg l^{-1})	Mg (mg l^{-1})	Ca (mg l^{-1})
Nutrient solution	0.11	15	14.6

The basic potting mixture, which served as the control treatment (no added K), was made by mixing quartz sand with peat in a ratio of 3:1 (v/v). One litre of distilled water was added to the potting mixture before planting. In the two mineral treatments, biotite (3%) or microcline (25%) were added to the sand as K sources prior to mixing with the peat. These concentrations correspond to those normally found in forest soils in Sweden. The pH of the final substrate in all treatments was 4.8. It should be noted that the biotite and microcline particles were smaller than the quartz sand particles.

Experimental treatments

Seedlings were transplanted into 5 × 5 × 5 cm plastic pots. No attempt was made to separate the roots from added minerals. The pots were placed on a capillary mat on a tray, according to a method developed by Sen (1990). The capillary mat transported nutrient solution from a reservoir located 5 cm below the seedlings. The volume of the reservoir was adjusted to 6 l every 2 weeks. The solutions were unsterile but were replaced once every month during the last 4 months of the experiment to prevent excessive growth of algae and bacteria, which may immobilise nutrients in the solution. The nutrient solution was prepared according to Ingestad and Kähr (1985) but with K replaced by Na. Approximate concentrations of N and P were 50 mg l^{-1} and 7.5 mg l^{-1}, respectively (for details of other elements, see Nylund and Wallander 1989). The seedlings were cultivated in growth cabinets at approximately 300 μmole $m^{-2}s^{-1}$ PAR with an 18 h/6 h 18 °C/15 °C day/night cycle for 223 days.

Harvest

At harvest, roots were washed in distilled water to release rhizospheric soil particles. Excess water was removed by paper towels. Only shoots could be analysed for elements as it was not possible to remove all adhering mineral particles from the roots. Care was taken not to contaminate the shoots, and all shoots were washed in double-distilled water before freezing. Roots and shoots were freeze-dried and dry weights were recorded. A 120-g sample of the potting mixture was centrifuged in a Sorvall RC-5B centrifuge

for 30 min at 11 000 rpm. The solution was collected and the pH measured. The samples were stored at –20 °C prior to organic acid analysis.

Ergosterol

The roots and potting mixture were analysed for ergosterol as an estimate of fungal biomass (Wallander and Nylund 1992). Roots were milled in a ball mill to a fine powder and 20–30 mg extracted with 2 ml of 10% KOH in methanol and 0.5 ml cyclohexane. The soil was freeze dried and 5 g dry soil was extracted in 4 ml 10% KOH in methanol and 1 ml cyclohexane. Both root and soil samples were sonicated for 15 min, extracted overnight and then refluxed at 70 °C for 30 min. After cooling, 0.5 ml H_2O and 1.5 ml cyclohexane were added. The samples were mixed using a vortex apparatus for 20 s, centrifuged for 5 min at 3000 rpm and the hexane phase was transferred to another test tube. The methanol was extracted with a further 1.5 ml cyclohexane. The cyclohexane was evaporated under N_2 and the samples dissolved in methanol. Prior to quantification of ergosterol, the samples were filtered through a 0.5-μm teflon syringe filter (Millex LCR-4, Millipore, Milford, Mass., USA). The chromatographic system consisted of a HPLC (Pharamacia-LKB, Uppsala, Sweden, model 2248), UV detector (Pharmacia model 2141) and a C_{18} reverse-phase column (Nova-Pak 0.39 cm × 7.5 cm), preceded by a C_{18} reverse-phase guard column (Superguard Supelco, Milford, Mass., USA). Extracts were eluted with methanol at a flow rate of 1 ml min^{-1} and monitored at 282 nm. Ergosterol used as standard was obtained from Sigma. Bacterial activity in the soils was measured with the thymidine incorporation technique (Bååth 1992) as reported by Olsson and Wallander (1998).

pH and organic acid analysis of solutions

The pH of supernatants after centrifugation of soils was measured and the organic acid concentrations determined by ion chromatography, using a Varian 5000 HPLC to pump the eluent. The column system (Dionex) consisted of an anion trap column (ATC1, 4 mm, P/N 037151) to clean the eluent before sample injection, a guard column, AG11 (4 mm, P/N 044078), and an analytical column, AS11 (4 mm, P/N 044076), before the detector, which was a Conductomonitor III from LDC. A gradient of vacuum-degassed NaOH, 0.5 m*M* for 2.5 min, 0.5–5.0 m*M* for 3.5 min and 5.0–38.2 m*M* for 12 min, was used as the eluent with a flow rate of 2.0 ml min^{-1} (Ström et al. 1994). The analysis were performed at the Department of Plant Ecology, University of Lund.

Chemical analyses

Five ml concentrated HNO_3 was added to each shoot sample. A digestion block programme was: 12 h at 30 °C, increased temperature to 135 °C over 70 min, and 4 h boiling at 135 °C. The clear solutions were diluted with deionized water to 10 ml. Element analysis was performed by atomic emission spectrometry (Jobin Yvon, Stockholm, Inductively Coupled Plasma, JY24 sequential analyser).

Weathering budget

Since the element concentrations in roots could not be analysed (see above), data was used from another experiment to estimate root K concentration for the weathering budget. Pine seedlings (non-mycorrhizal or mycorrhizal with *S. variegatus*, isolate 2–10) were produced as described above and transplanted to nylon mesh bags (mesh size 100 μm, which is supposed to allow fungal but not root penetration), which were placed in plastic pots (7 × 7 × 7 cm). The cultures thus consisted of an inner compartment with both roots and fungal mycelium and an outer compartment with fungal mycelium alone. The volume of the mesh bags was the same as the volume of the pots used in the first experiment (125 cm^2). Biotite was added (2% w/w) to the outer compartment only. The seedlings were maintained for 153 days under the same mineral nutrient conditions as in the first experiment. At harvest, K concentrations of roots and shoots were analysed as described above.

A calculation for weathering using the K concentrations of plants and minerals was made as follows. It was assumed that the K in plants came from biotite or microcline weathering, K stored in the seedling before the start of the experiment, decomposition of peat, weathering of K-bearing compounds in the quartz sand and traces from the nutrient solution. To determine the amount of K derived from mineral weathering of biotite or microcline, the K content of seedlings (calculated from the K concentration in shoot, shoot weight, estimated root concentration and root weight) without K source was subtracted from the K content of seedlings with a mineral source, i.e.:

$$K_W = (K_M)_A - (K_M)_B \quad (1)$$

where K_W is the amount of K from weathering of the added mineral, K_M is the K content of seedlings with a specific mycorrhizal treatment, *A* denotes seedlings which have access to K from biotite or microcline and *B* denotes seedlings which had no added K. To calculate the amount of weathered mineral in each pot, K_w was divided by the percentage of K in the specific mineral (biotite or microcline).

Statistics

The data weathering, growth parameters and the concentrations of elements and organic acids were analysed by two-way analysis of variance (ANOVA). When ANOVAs indicated significant differences, least significant deviations (LSD) were used to evaluate differences between the treatments. All statistical analyses were performed with the program Systat 5.05 for Windows.

Results

Growth and K uptake from mineral weathering

According to the growth data (Table 2), K uptake and weathering budgets (Table 3), all seedlings were able to mobilise K from biotite. Seedlings colonised by *S. variegatus* tended to be more efficient than the other seedlings. There was a significant correlation between the amount of ergosterol in *S. variegatus*-colonised soil and foliar K uptake (Fig. 1). Addition of microcline resulted in stunted growth (Table 2) of non-mycorrhizal and *S. variegatus*-colonised seedlings, particularly affecting root production, as seen from the high shoot/root ratio. These seedlings showed low foliar uptake of K but high foliar K concentrations (Table 3). On the other hand, microcline addition improved growth of *P. involutus*-colonised seedlings, although foliar K concentrations were lower in these seedlings than others (Table 3) and no correlation was found between ergosterol in soil and foliar K uptake (data not shown).

Paxillus. involutus produced more biomass in roots (as measured by ergosterol) than *S. variegatus*-colonised seedlings. Biomass production by *S. variegatus* tended to be inhibited by microcline addition and very few mycorrhizal roots were visible at harvest in this treatment. The fungal biomass in the potting mixture was positively influenced by biotite addition.

Table 2 Growth parameters and ergosterol of mycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Different letters indicate significant differences ($P=0.05$) within each column

Mycorrhizal status	K source	Total dry weight (g)	Shoot/root	Ergosterol (root) (μg g^{-1} root dry wt.)	Ergosterol (soil) (μg g^{-1} sand dry wt.)
Non-mycorrhizal	No K	1.03 ± 0.04 bcd	0.57 ± 0.03 c	1 ± 1 d	0.26 ± 0.06 b
	Microcline	0.46 ± 0.11 d	1.14 ± 0.06 a	4 ± 2 d	0.24 ± 0.03 b
	Biotite	2.38 ± 0.39 a	0.55 ± 0.06 c	7 ± 2 d	0.24 ± 0.04 b
Mycorrhizal with *Suillus variegatus*	No K	0.89 ± 0.16 cd	1.04 ± 0.13 a	100 ± 4 cd	0.24 ± 0.04 b
	Microcline	0.57 ± 0.18 d	1.07 ± 0.15 a	40 ± 2 cd	0.25 ± 0.03 b
	Biotite	2.51 ± 0.14 a	0.65 ± 0.09 bc	160 ± 6 bcd	0.56 ± 0.09 ab
Mycorrhizal with *Paxillus involutus*	No K	1.07 ± 0.21 bcd	0.85 ± 0.05 b	230 ± 9 abc	0.32 ± 0.05 b
	Microcline	1.30 ± 0.28 bc	0.64 ± 0.04 c	350 ± 10 ab	0.63 ± 0.04 ab
	Biotite	1.64 ± 0.31 b	0.67 ± 0.06 bc	390 ± 12 a	1.03 ± 0.40 a
ANOVA (*P*-value)					
	Fungus	ns	0.003	0.000	0.022
	K-source	0.000	0.000	ns	ns
	Fungus*K source	0.001	ns	ns	ns

Table 3 Total foliar K content and foliar K and Mg concentrations in non-mycorrhizal *P. sylvestris* seedlings and seedlings colonised by *S. variegatus* or *P. involutus*. Significant *P*-values from ANOVA analyses are indicated for each K source. The amount of weathered mineral was calculated from the K data, based on the assumption that the concentration of K in roots does not differ from the concentration in shoots (see text). Different letters indicate significant differences within a column (*ns* not significant)

Mycorrhizal status	Potassium source	Foliar K content (mg)	Folia Mg concentration (mg g^{-1})	Foliar K concentration (mg g^{-1})	Weathered mineral (mg pot^{-1})
Non-mycorrhizal	Control	0.5 ± 0.1 b	2.2 ± 0.2	1.4 ± 0.2	–
+ *S. variegatus*	Control	0.7 ± 0.02 ab	1.8 ± 0.2	1.6 ± 0.2	–
+ *P. involutus*	Control	0.9 ± 0.1 a	2.0 ± 0.1	2.0 ± 0.2	–
ANOVA (*P*-value)		0.015	ns	ns	
Non-mycorrhizal	Microcline	1.2 ± 0.2	1.2 ± 0.2	5.1 ± 0.4	9.5 ± 3.6
+ *S. variegatus*	Microcline	1.6 ± 0.6	1.6 ± 0.6	5.4 ± 0.5	18.5 ± 10.4
+ *P. involutus*	Microcline	1.8 ± 0.1	1.8 ± 0.1	3.9 ± 0.5	26.1 ± 2.1
ANOVA (*P*-value)		ns	ns	ns	ns
Non-mycorrhizal	Biotite	3.1 ± 0.5	1.5 ± 0.07	3.9 ± 0.4	96.3 ± 16
+ *S. variegatus*	Biotite	4.5 ± 0.6	1.4 ± 0.06	4.7 ± 0.3	132.0 ± 7.2
+ *P. involutus*	Biotite	3.5 ± 1.0	1.6 ± 0.15	5.2 ± 0.5	85.4 ± 26
ANOVA (*P*-value)		ns	ns	ns	ns

Weathering budgets

In the experiment in which roots were grown in a mesh bag to separate roots from biotite particles, K uptake could not be compared between mycorrhizal and non-mycorrhizal seedlings since roots in some pots had penetrated the nylon mesh to various extents. Shoot and root K concentrations did not differ significantly between mycorrhizal and non-mycorrhizal seedlings and root concentrations were not significantly different from shoot concentrations (Table 4). It was, therefore, assumed that root and shoot concentrations were also the same in the first experiment when calculating the weathering budget. Approximately 9.5–26.1 mg microcline and 96–132 mg biotite was weathered during the experiment. This corresponds to 0.014–0.085% of the total added microcline and 1.1–2.7% of the total added biotite (Table 3). The effects of the different ectomycorrhizal fungi on the amount of weathered minerals were not statistically significant.

Table 4 Potassium concentrations in roots and shoots of *P. sylvestris* seedlings grown in nylon mesh bags separating added biotite from roots

Mycorrhizal status	K source	K (shoots) mg g^{-1}	K (roots) mg g^{-1}
Non-mycorrhizal	No K	2.1 ± 0.4	2.0 ± 0.6
	Biotite	3.8 ± 0.7	4.3 ± 0.3
+ *S. variegatus*	No K	2.5 ± 0.5	3.1 ± 0.8
	Biotite	4.9 ± 1.2	6.4 ± 2.3

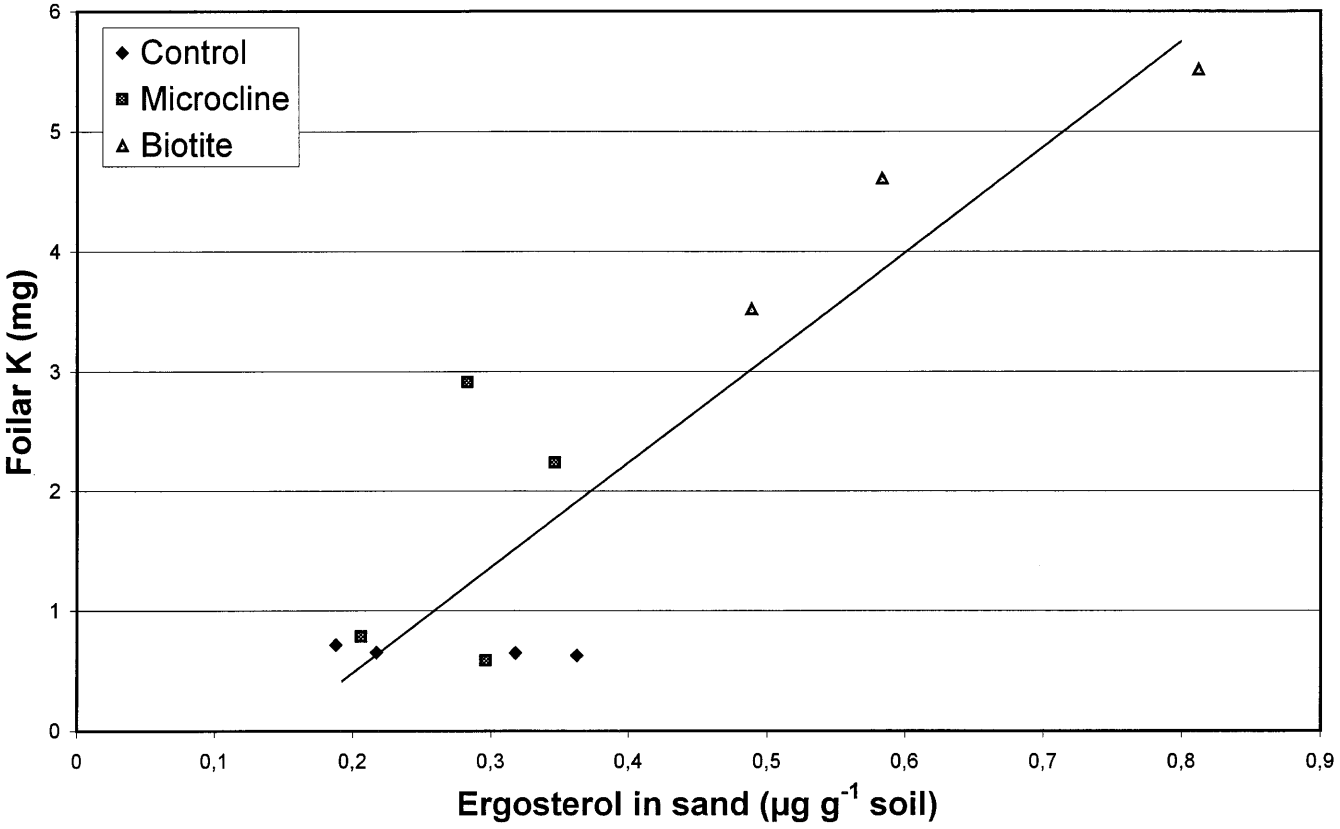

Fig. 1 Relationship between foliar K and soil ergosterol in *Suillus variegatus*-colonised seedlings. A linear trend line is fitted to the points ($Y = 8.5X–1.1$, $r^2 = 0.784$)

Magnesium and calcium in shoots

Seedlings without any K source had higher foliar concentrations of magnesium (Mg) (Table 3), and biotite addition resulted in higher foliar Mg concentrations than microcline addition (Table 3). Foliar calcium (Ca) concentrations were not significantly different between the treatments (mean values 3.4–5.1 mg g^{-1}, data not shown).

Organic acids

Malic, oxalic, citric and formic acids were identified in the soil solution at concentrations of up to 20 µM; variation between replicate samples was high. Biotite addition to *S. variegatus*-colonised soil was the only treatment producing statistically higher concentrations of all acids, except for formic acid (Table 5). This is interesting since the root biomasses of *S. variegatus* and non-mycorrhizal seedlings were similar when biotite was added. Mean pH values for different treatments were 4.8–5.2, with no significant variation amongst fungal or mineral treatments (data not shown).

Citric acid in the soil solution was positively correlated with ergosterol concentration in the *S. variegatus*-colonised soil (Fig 2). Furthermore, citric acid was positively correlated to the amount of K in the shoots of *S. variegatus*-colonised seedlings (Fig. 3). No significant correlations between organic acids, ergosterol and foliar K were found in the *P. involutus* treatment.

Discussion

The enhanced growth and high foliar K concentration in *P. sylvestris* seedlings when biotite was the K source indicate that K was supplied to the plants through weathering of the biotite. Seedlings colonised by *S. variegatus* tended to be more efficient in the uptake of K from biotite, and the significant positive correlation between ergosterol in *S. variegatus*-colonised soil and foliar K uptake may reflect an active role in K mobilisation by the fungus. However, data should be interpreted with care, since this correlation could also be explained by a more general growth stimulation, related or not to K uptake, induced by the fungus. The effect of the fungus may be more important in a natural forest, where the soil volume is not limited as in the present pot experiment. When microcline was added to non-mycorrhizal and *S. variegatus*-colonised seedlings, plant growth tended to be suppressed but foliar concentration of K was elevated. Thus, K did not limit growth in the microcline treatment. The relatively high concentration of microcline in the microcline treatment (25%) may have resulted in poorer aeration of the potting mixture than did other treatments. *P. involutus*-colonised seedlings

Table 5 Concentration of organic acids (μM) of centrifuged soil solutions from soil of ectomycorrhizal and non-mycorrhizal *P. sylvestris* seedlings. Different letters indicate significant differences ($P=0.05$) within each column

Mycorrhizal status	K source	Malic acid	Oxalic acid	Citric acid	Formic acid
Non-mycorrhizal	No K	0.2±0.1 b	1.8±0.8 b	0.7±0.7 b	4.6±0.2 b
	Microcline	0.17±0.1 b	2.2±0.9 b	1.5±0.6 b	6.2±3.0 b
	Biotite	1.9±0.4 b	2.4±0.8 b	1.6±1.3 b	6.2±2.6 b
+*S. variegatus*	No K	0.1±0.1 b	1.7±0.8 b	0.6±0.4 b	2.7±1.3 b
	Microcline	0.15±0.1 b	2.1±0.7 b	0.8±0.4 b	3.2±2.4 b
	Biotite	3.4±1.5 a	19.8±12.5 a	15.8±10.5 a	8.1±4.1 b
+*P. involutus*	No K	1.2±1.1 b	1.8±0.7 b	1.2±1.0 b	4.1±2.4 b
	Microcline	0.22±0.1 b	2.6±0.6 b	1.4±0.6 b	4.1±1.0 b
	Biotite	1.7±1.2 b	5.0±2.2 b	3.2±2.3 b	5.9±3.5 b
ANOVA *P*-value					
	Fungus	ns	ns	0.034	ns
	K source	0.03	0.006	0.017	ns
	Fungus*K source	ns	ns	0.040	ns

grew better than other seedlings in the microcline treatment. However, was not related to K uptake, since foliar K concentration was lower in these seedlings. Furthermore, no correlation was found between fungal biomass (ergosterol) and foliar K in *P. involutus*-colonised seedlings. This implies that the ectomycorrhizal colonisation of microcline particles was not important for mobilisation of K. The involvement of ectomycorrhizal fungi in the uptake of mineral nutrients from protected sites inside pores of feldspar particles, suggested by Jongmans et al. (1997), was thus not confirmed by the present study; it is unlikely that pores had formed during the limited experimental time. Boyle and Voigt (1973) studied weathering of microcline and biotite by non-mycorrhizal pine (*Pinus radiata*) seedlings and found the seedlings to be sensitive to low aeration in combination with biotite, resulting in suppressed growth. In that study, the seedlings received lower amounts of K from microcline than from biotite, but the uptake was inversely correlated with the size frac-

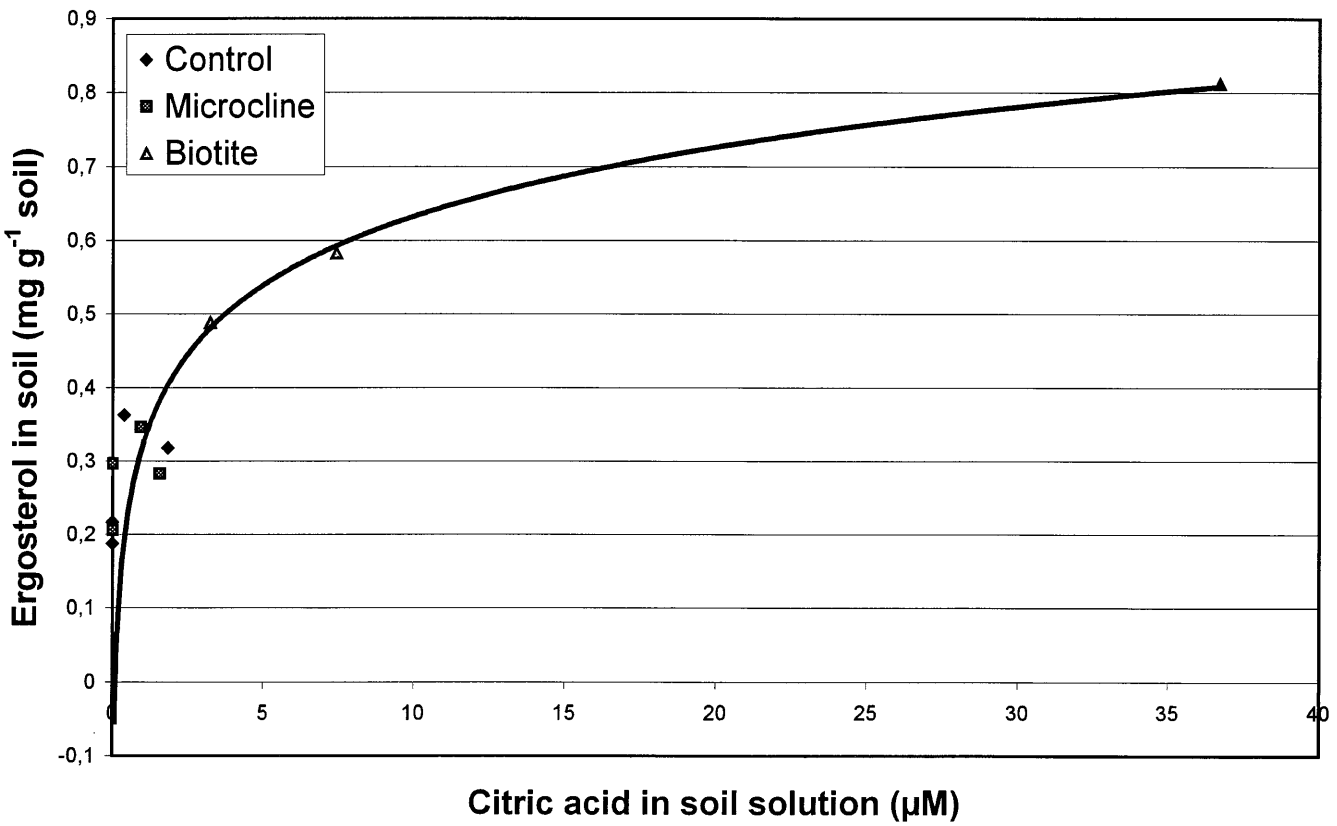

Fig. 2 Relationship between ergosterol in the soil and citric acid in the soil solution in *S. variegatus*-colonised soil. A logarithmic trend line is fitted to the points ($Y=0.13\,\mathrm{Ln}(X)+0.32$, $r^2=0.997$)

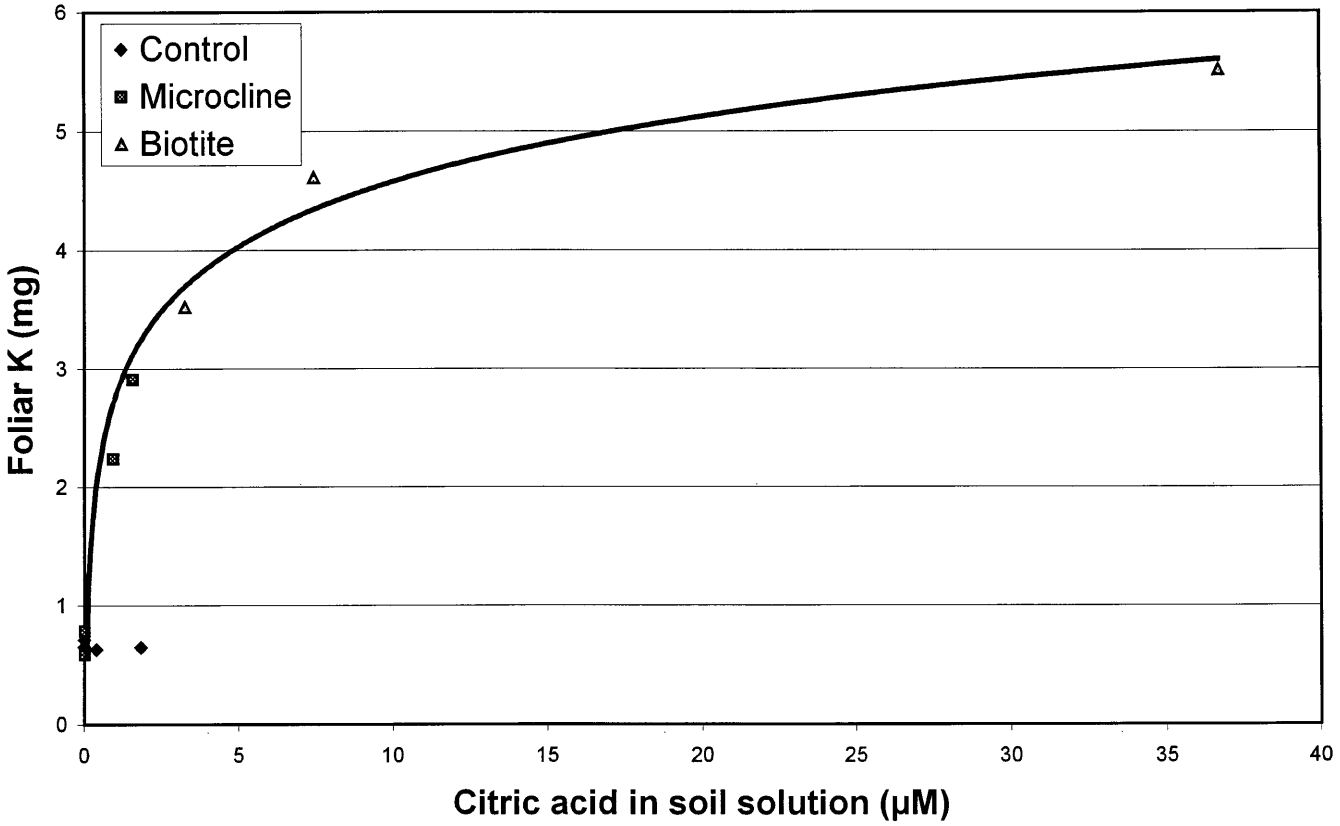

Fig. 3 Relationship between foliar K and citric acid in the soil solution of*S. variegatus*-colonised soil. A logarithmic trend line is fitted to the points ($Y = 0.79\ \mathrm{Ln(X)} + 2.8$, $r^2 = 0.945$)

tion. Thus, the authors proposed that availability was correlated with mineral surface area.

In the present study, the production of fungal biomass in *P. involutus*-colonised soil decreased when no K was added, compared with when biotite was added. The tendency was similar in *S. variegatus*- colonised soil, although it was only statistically significant when using the PLFA 18:2w6 as a marker for fungal biomass (Olsson and Wallander 1998). This suggests that less carbon was allocated to the ectomycorrhizal fungus under K deficiency. Ericsson (1995) found that shoot/root ratios increased in response to low K availability for Norway spruce seedlings and argued that less carbon is allocated below-ground at low K availability because of a decreased rate of photosynthesis.

The reason for the higher Mg concentrations found in plants suffering from K deficiency may be Mg substitution for K in many plant processes, such as stomatal regulation and osmoregulation (Marschner 1995). A probable reason for a higher Mg concentration in plants with biotite as a K source, compared with those with microcline, is that the biotite contains significant amounts of Mg which is released by weathering, whilst the microcline contains no Mg. It is possible that the observed growth stimulation with biotite was not solely an effect of increased K availability, as other nutrients are also released during weathering (Mg, Fe etc.), and the availability of some of these nutrients was higher in the biotite treatment than the balanced nutrient solution.

The concentration of organic acids in the soil solution does not necessarily reflect exudation by the plants because of the rapid turnover rate of these acids in the soil (Jones et al. 1996). However, in the biotite treatment, colonisation of *P. sylvestris* by *S. variegatus* resulted in much higher concentrations of oxalic and citric acid in the soil solution. The significant correlation between citric acid in the soil solution and ergosterol in *S. variegatus*- colonised soil suggests that, at least, citric acid was produced by the fungus. In addition, the significant correlation between citric acid and foliar uptake of K in *S. variegatus*-colonised seedlings may be an indication of citric acid involvement in K release from the biotite. The presence of biotite seemed to stimulate the production of organic acids in *S. variegatus*-colonised soil, since the amounts of citric and oxalic acid were 10 times higher, while the fungal biomass was only twice as high in the biotite than in the control treatment. Whether this increase in organic acid concentration in the soil solution is sufficient to induce the formation of pores in weatherable minerals remains to be investigated.

Wallander et al. (1997) found that *S. variegatus* ectomycorrhizas reduced soil pH more than non-mycorrhizal *P. sylvestris* seedlings or seedlings colonised by *P. involutus*. It was proposed that the pH decrease was caused by exudation of organic acids. Song and Huang

(1988) investigated the effect of citric and oxalic acid on K release from biotite and microcline, and reported K release to be 14–18 times faster from biotite than from microcline in both these organic acid solutions. The rates of release of structural cations from the minerals by organic acids followed the order Al, Fe, Mg>K>Si. Jones and Darrah (1994) studied different kind of soils and found citrate to be highly efficient in mobilising some di- and tri-valent cations into solution (Fe, Mg and Ca). By complexing Fe, weathering could be increased and thus more K made available. Jones and Darrah (1994) also found malate to be as effective as citrate for Ca and Mg mobilisation. In our study, malic acid production by *S. variegatus*-colonised *P. sylvestris* seedlings was significant higher when biotite was added.

The bacterial activity of the soils used in the present experiments was described by Olsson and Wallander (1998). It was found that bacterial activity was greatly stimulated by biotite addition in *S. variegatus*-colonised soil. It is thus possible that bacteria contributed to weathering, organic acid production and/or K uptake into the seedlings in the present study. It is also possible that bacterial activity is enhanced by exudation of organic acids from roots and ectomycorrhizal fungi, since these acids can serve as carbon sources for bacteria. It, therefore, still remains open whether roots, ectomycorrhizal fungi, saprophytic fungi or bacteria are the main contributors to organic acid production in soils and consequently to the release of K from minerals.

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