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REGULAR ARTICLE

Laccaria bicolor S238N improves Scots pine mineral nutrition by increasing root nutrient uptake from soil minerals but does not increase mineral weathering

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Abstract The role of ectomycorrhizal fungi on mineral nutrient mobilization and uptake is crucial for tree nutrition and growth in temperate forest ecosystems. By using a "mineral weathering budget" approach, this study aims to quantify the effect of the symbiosis with the ectomycorrhizal model strain *Laccaria bicolor* S238N on mineral weathering and tree nutrition, carrying out a column experiment with a quartz/biotite substrate. Each column was planted with one Scots pine (*Pinus sylvestris* L.) non-mycorrhizal or mycorrhizal with *L. bicolor*, with exception of the abiotic control treatment. The columns were continuously supplied with a nutrient-poor solution. A mineral weathering budget was calculated for K and Mg. The pine shoot growth was significantly increased (73%) when plants were mycorrhizal with *L. bicolor*. Whatever their mycorrhizal status, pines increased mineral weathering by factors 1.5 to 2.1. No difference between non-mycorrhizal and mycorrhizal pine treatments was revealed, however, mycorrhizal pines assimilated significantly more K and Mg. This suggests that in our experimental conditions, *L. bicolor* S238N improved shoot growth and K and Mg assimilation in Scots pine mainly by increasing the uptake of dissolved nutrients, linked to a better exploration and exploitation of the soil by the mycorrhizal roots.

Keywords Ectomycorrhiza · *Laccaria bicolor* S238N · Scots pine · Mineral weathering · Biotite · Nutrient uptake

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Introduction

For growth, trees need essential mineral macronutrients (N, K, Ca, Mg, P, S) and micronutrients (B, Cl, Cu, Fe, Mn, Mo, Ni, Zn). With the exception of N, soil minerals are the main source for all mineral nutrients required for tree growth in non-fertilized forest ecosystem (Marschner 1995). The major pool of these nutrients is, however, not directly available to

trees as the nutrients are entrapped in the mineral phase. Therefore mineral weathering, a process which promotes soil nutrient bioavailability, i.e. the degree to which a nutrient becomes assimilated by the plant roots, is crucial to satisfy plant nutritional needs (Barber 1995; Drever 2005). So far, a great variety of biological processes linked to plant root and micro-organism activities are known to affect soil mineral weathering and to increase the nutrient supply to the tree (Robert and Berthelin 1986; Hinsinger 1998; Van Breemen et al. 2000).

Notably, the ectomycorrhizae which represent the dominant symbiotic association between tree roots and fungi in temperate and boreal forest ecosystems, play an active role in the mobilization and acquisition of mineral nutrients from niches and sources not accessible to the plant roots (Smith and Read 1997; Chalot et al. 2002; Gadd 2007). For example, K and Mg derived from micro-sites only accessible to hyphae are expected to play a major role in acidic forest soils in which pools of readily available K and Mg are being depleted (Jongmans et al. 1997). Owing to their multiple functions, ECM fungi could thus represent a main pathway through which most trees obtain mineral nutrients and, as such, could play a critical role in terrestrial ecosystem functioning (Finlay 2004; Hoffland et al. 2004; Kernaghan 2005; Van Hees et al. 2006). However, the issue of mycorrhizal significance in improvement of K uptake is still a bit controversial and obviously calls for more research. For example, Hagerberg et al. (2003) showed that in a boreal forest the potential of ECM fungi to ameliorate K limitation through an increased exploitation of K-containing minerals appears to be low.

The beneficial effect of certain ECM fungi on tree growth is well known, especially in the case of *Laccaria bicolor* S238N which is commercially used in France for controlled mycorrhization of Douglas fir, because of its promoting effect on tree growth and nutrition in forest nurseries and plantations (Le Tacon et al. 2005). *Laccaria bicolor* S238N has been so well and widely studied that it has become a model organism in forest tree inoculation research in Europe (Heinonsalo et al. 2004). Until now, however, no study has addressed the question of the processes by which *L. bicolor* S238N improves tree nutrition from soil mineral stocks. The aim of this study is thus to quantify the respective contribution of the ECM fungal model *L. bicolor* S238N to the biotite weathering process and to the nutrient assimilation by pine roots, using an in vitro column experiment similar to that designed by Calvaruso et al. (2006).

Materials and methods

Laccaria bicolor S238N strain and inoculum preparation

Laccaria bicolor (Maire) P. D. Orton is a member of the ectomycorrhizal family *Tricholomataceae*. The original strain, S238O, was isolated in 1976 in Oregon by Trappe and Molina from a fruit body collected under *Tsuga mertensiana* (Bong.) Carr. The sub-culture transferred to the 4°C fungal collection (Institut National de la Recherche Agronomique, Nancy, France) in 1980 was named S238N (Di Battista et al. 1996). This strain is maintained on Pachlewski agar medium P5 (Pachlewski and Packlewska 1974). Fungal inoculum was prepared by growing the mycelium aseptically in a peat-vermiculite nutrient mixture (Duponnois and Garbaye 1991).

Pre-culture of non-mycorrhizal and mycorrhizal pine seedlings

One hundred Scots pine seeds (*Pinus sylvestris* L.; provenance: Haguenau forest, France) were planted either in a non-sterile peat-vermiculite substrate (1/1) or in a mixture of non-sterile peat-vermiculite substrate and *L. bicolor* S238N inoculum (9/1). Both non-inoculated and *L. bicolor* S238N-inoculated seedlings were grown in a greenhouse during 20 weeks under the following conditions: 60% humidity, a night temperature of 15°C and a day temperature of 22°C, a 16 h period of daylight, and an automatic mist watering of 2 min twice a day.

Mineral material, nutrient solution and experimental device

The column experiment was performed according to the protocol described by Calvaruso et al. (2006). Briefly, the columns (15 cm high and 4 cm in diameter) contained a K-, Mg-bearing phyllosilicate, i.e. biotite (2.8 g of 0.5 to 1 mm size particles) and quartz (10 g of 0.5 to 1 mm size particles and 35 g of

1 to 2 mm size particles), and were continuously supplied (1 ml per hour) with a nutrient-poor solution containing all macronutrients necessary to pine growth apart from those present in the biotite, i.e. 1.5 mg l^{-1} of sodium, 2 mg l^{-1} of phosphorus, 2.3 mg l^{-1} of calcium, 1.9 mg l^{-1} of sulphur, 11.2 mg l^{-1} of nitrogen. After the 20 weeks of pre-culture, one pine seedling was planted in each column, with exception of the abiotic control treatment simulating geochemical processes. The nutrient solution draining through the columns was collected individually in flasks placed below each column. The experiment included three treatments (with four replicates each): abiotic, non-mycorrhizal pine plants, and *L. bicolor* S238N mycorrhizal pine plants. The columns were placed in a growth chamber for 8 weeks with 60% humidity, 25–18°C (day–night) regime and under 17–7 h photoperiod.

Sampling

After 20 weeks of pre-culture, 15 pine seedlings mycorrhizal with *L. bicolor* S238N and 14 non-mycorrhizal pine seedlings were sampled randomly from the hundred pines cultivated, and harvested. Because the fresh weight and aerial part height of these harvested pines did not differ from those of the pines used in the column experiment (data not shown), the harvested pines were used as a measure of the initial mycorrhizal rate, initial root and shoot biomass and, initial K and Mg contents of the seedlings.

After 8 weeks of culture, the plants were removed from the columns. Their root systems were then slightly shaken by hand and were washed very carefully in sterile ultra-pure water to remove the mineral particles which remained adhering to the roots. These particles, which represent the rhizosphere soil fraction, were collected. All pine plants were then sampled to quantify their final mycorrhizal rate, final root and shoot biomass and, final K and Mg contents.

The drained solutions from the four replicates of each treatment were collected each week throughout the experiment.

Analyses

The volumes of all drained solutions were measured each week. The K and Mg concentrations of the solutions sampled were measured by ICP emission spectrometer (Plasma torch JY180 ULTRACE). The evolution of K and Mg amounts in the drained solutions through the experiment were thus obtained for each treatment. The total amounts of K and Mg present in the drained solutions were calculated for each treatment by adding together the drained solutions collected throughout the experiment.

The rhizosphere soil fraction was dried three days in a steam-air dryer at 30°C, the rhizosphere biotite particles were then separated from those of quartz and both portions were weighed. The percentage of rhizospheric biotite/quartz was calculated as the ratio of rhizospheric to total biotite/quartz dry weight. The surface and the mineral composition of biotite particles were analysed with a Hitachi S2500 Scanning Electronic Microscope (SEM) connected to a Thermonoran microanalysis system in order to observe dissolution figures on biotite surfaces.

The mycorrhizal rate of the pine roots was measured according to Frey-Klett et al. (1997). Briefly, after the 20 weeks of pre-culture, the proportion of short roots forming mycorrhizae with *L. bicolor* S238N was determined by examining a hundred short roots under a binocular microscope. The plants were dried at 65°C for five days and then the dry weight of the roots and shoots were measured. Then, 0.25 g of the oven-dried roots and shoots were ground and digested with H_2O_2 and $HCLO_4$ as described by Calvaruso et al. (2006). The elemental composition of the digests was determined by ICP emission spectrometer (Plasma torch JY180 ULTRACE). The amounts of K and Mg assimilated by pine seedlings during the 8-week experiment were calculated as the difference between final and initial K and Mg contents of the pines for each column.

Weathering budget

For each column, the weathering budget of the biotite W was calculated for K and Mg, two elements which were not re-precipitated in the columns as confirmed by SEM observations, as follows:

$$W = (D - N) + 1$$

D is the amount of the element in the drained solution,

N is the amount of the element in the nutrient solution (= 0 for K and Mg),

I is the amount of the element immobilized by the pine plant during the experiment which corresponds to the difference between final and initial element contents in the pine.

Statistical analyses

The effects of the plant and the fungal inoculation on the weathering budgets, on the evolution of the K and Mg amounts in the drained solutions, on the K and Mg assimilation by pines, on the rhizosphere biotite fraction and on the growth of the pine plants were determined using analyses of variance (ANOVA) at the threshold level of P=0.05, and the Bonferroni-Dunn test. The Superanova software (Abacus Concepts, Inc., Berkeley, CA) was used for all of these analyses.

Results

Evolution of K and Mg concentrations in the drained solutions

The K and Mg concentrations of the drained solutions remained stable in the abiotic control treatment (Fig. 1a and b). Whatever the plant treatment (mycorrhizal or non-mycorrhizal), the K and Mg concentrations of the drained solutions were significantly higher than those of the abiotic control treatment at the beginning of the experiment (P<0.001) and decreased progressively throughout the experiment (Fig. 1a and b). This decrease is faster when the pines were mycorrhizal with *L. bicolor* S238N. For this treatment, the amounts of K and Mg in the drained solutions were even significantly lower than those of the abiotic control treatment and the non-mycorrhizal pine treatments at the end of the experiment (P=0.0136 and 0.0089, respectively).

Weathering budget

According to the weathering budget for K and Mg, all pine plants were able to mobilize K and Mg from biotite (Table 1). Significantly more biotite was weathered by non-mycorrhizal and *L. bicolor* S238N mycorrhizal pine plants in comparison with the abiotic control treatment (Table 1). There was no significant difference between the non-mycorrhizal and *L. bicolor* S238N mycorrhizal pine plants as regards to the amounts of K and Mg mobilized from the biotite (Table 1).

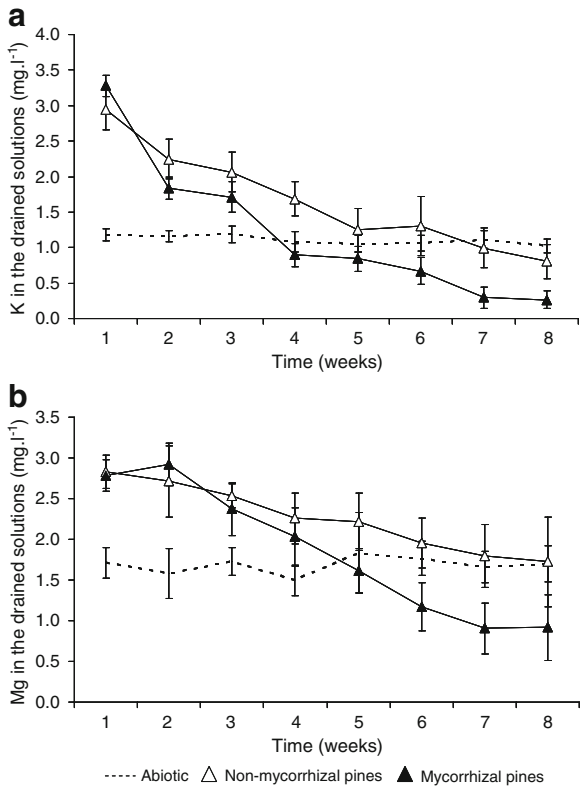

Fig. 1 Evolution of K (**a**) and Mg (**b**) concentrations in the drained solutions throughout the experiment. The *dotted line*, the *white triangle* and the *black triangle* represent the mean values of four replicates for the abiotic, the non-mycorrhizal pine and the *L. bicolor* S238N mycorrhizal pine treatments, respectively. *Bars* represent standard deviation

Weathering process

Plotting the total amount of K released from biotite (amount of K in the drained solution + amount of K assimilated by pine) against the total amount of Mg released from biotite revealed that biotite was dissolved in a stoichiometric way for the abiotic control treatment (Fig. 2). In contrast, biotite was dissolved in a non-stoichiometric way for the two plant treatments (non-mycorrhizal and mycorrhizal ones) (Fig. 2). There was a faster release of interlayer K for these latter. The SEM observations suggest that the surfaces of biotite particles collected at the end of the experiment in the columns containing non-mycorrhizal

Table 1 Total amounts of K and Mg mobilized from biotite (amounts released in the drained solutions + amounts assimilated by pines), amounts of K and Mg assimilated by pines, and ratio K and Mg assimilated by pines on total amount of K and Mg mobilized from biotite

Treatment	K			Mg		
	Mobilized from biotite (mg)	Assimilated by pines (mg)	Assimilated/ mobilized ratio	Mobilized from biotite (mg)	Assimilated by pines (mg)	Assimilated/ mobilized ratio
Abiotic	$1.15^{a}\pm0.21$	0	0	$1.63^{a}\pm0.32$	0	0
Non-mycorrhizal pine	$2.40^{b}\pm0.30$	$0.50^{a}\pm0.13$	$0.21^{a}\pm0.07$	$2.71^{b}\pm0.64$	$0.21^{a}\pm0.07$	$0.08^{a}\pm0.04$
Mycorrhizal pine	$2.13^{b}\pm0.36$	$0.87^{b}\pm0.16$	$0.41^{b}\pm0.10$	$2.38^{b}\pm0.30$	$0.39^{b}\pm0.06$	$0.16^{b}\pm0.03$
ANOVA *P* value	0.001	0.007	0.001	0.021	0.013	0.032

Each value is the mean value of four replicates ± standard deviation. For each variable, values followed by the same letter are not significantly different according to a one-factor (biological treatment) ANOVA (P=0.05), and the Bonferroni-Dunn test

or mycorrhizal pines were more weathered than those of the abiotic control treatment (Fig. 3).

Fraction of rhizosphere biotite and quartz

Significantly more biotite was collected in the rhizosphere of *L. bicolor* S238N mycorrhizal pine plants (108±13 mg) as compared with the non-mycorrhizal ones (67±16 mg) according to a one-factor (mycorrhizal status) ANOVA (P=0.028). That represents respectively about 3.9 and 2.4% of the total biotite amount contained in the column. Similarly, significantly more quartz was collected in the rhizosphere of *L. bicolor* S238N mycorrhizal pine plants (721±138 mg) as compared with the non-mycorrhizal ones (298±96 mg) according to a one-factor (mycorrhizal status) ANOVA (P=0.013). That represents respectively about 1.6 and 0.6% of the total quartz amount contained in the column.

Uptake of K and Mg from biotite weathering

At the beginning of the experiment, the amounts of K and Mg contained in the mycorrhizal plants were not different from those of the non-mycorrhizal ones. The initial K contents were 0.24 and 0.23 mg for the non-mycorrhizal and mycorrhizal plants, respectively. The initial Mg contents were 0.05 mg for both the non-mycorrhizal and mycorrhizal plants.

According to the weathering budget calculated for K and Mg, all pine plants were able to assimilate K and Mg from biotite throughout the experiment (Table 1). The proportions of K and Mg issued from biotite weathering and assimilated by the roots during the 8 weeks of the experiment for *L. bicolor* S238N mycorrhizal pine seedlings were approximately 1.9 times higher than those of the non-mycorrhizal plants (Table 1).

Growth of the pine seedlings

At the beginning of the experiment, the root, shoot, and total dry biomass of the mycorrhizal plants were not different from those of the non-mycorrhizal plants (Table 2). After 8 weeks of the experiment, the pine plants mycorrhizal with *L. bicolor* S238N had significantly higher shoot and total plant biomass than those of the non-mycorrhizal plants but root biomass was not significantly different between treatments (Table 2). The shoot and total plant biomass

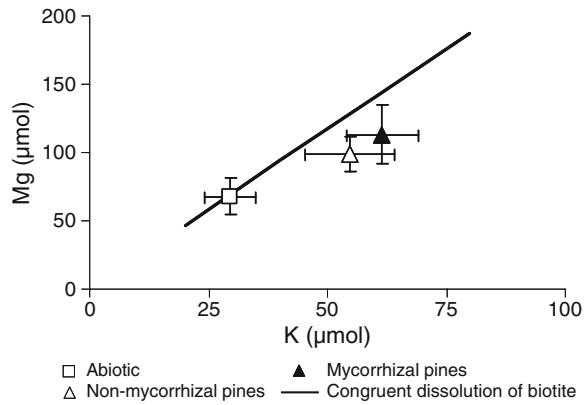

Fig. 2 Relationship between Mg and K amounts mobilized from the biotite during the experiment. The *square*, the *white triangle* and the *black triangle* represent the mean values of four replicates for the abiotic, the non-mycorrhizal pine and the *L. bicolor* S238N mycorrhizal pine treatments, respectively. *Bars* represent standard deviation. The *black curve* represents the Mg/K stoichiometry in the Bancroft biotite which refers to a congruent dissolution process

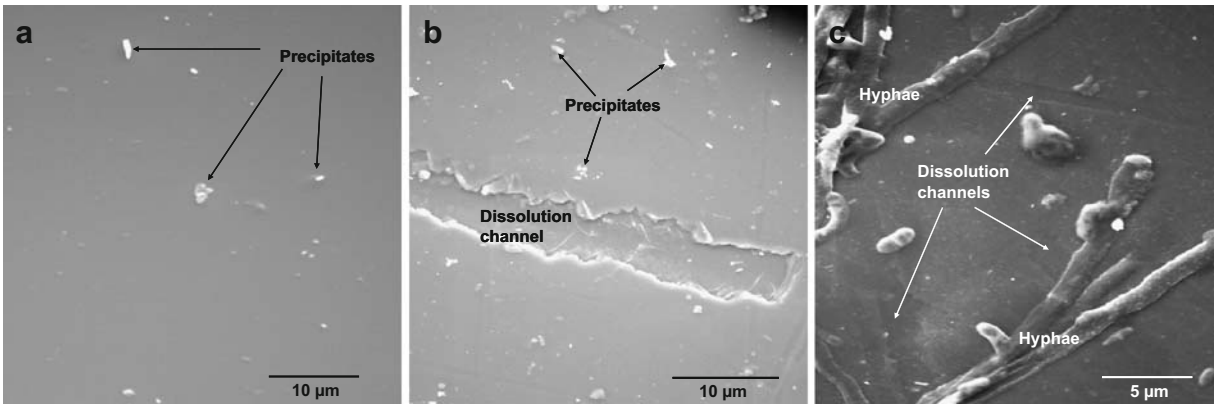

Fig. 3 Examples of SEM images of biotite surfaces. **a** Biotite surface in the abiotic treatment with secondary precipitates after 8 weeks of experiment. **b** and **c** Dissolution channels on biotite surfaces in the biotic treatment after 8 weeks of experiment

increase during the experiment was significantly higher when the pine plants were mycorrhizal with *L. bicolor* S238N (Fig. 4).

Discussion

The stability of K and Mg amounts released into the solutions during the experiment for the abiotic control treatment indicates that abiotic factors, notably mineral, were stable throughout the experiment. In consequence we can assess the effect of biological (plant and fungal) activities on the biotite weathering process. While the mycorrhizal rates of the pine seedlings decreased by 27% during the experiment, the fine roots mycorrhizal with *L. bicolor* S238N still represent more than to 37% of the total fine roots after eight weeks of experiment. This level of colonization is sufficient to assess the effect of the ECM fungi on mineral weathering, tree nutrition and growth.

The weathering budgets based on K and Mg demonstrated that whatever their mycorrhizal status, the pine roots increased biotite weathering by factors 1.5 to 2.1 in comparison with the abiotic control treatment. This result is in accordance with those obtained by Calvaruso et al. (2006) in a previous column experiment carried out under similar conditions, which showed that non-mycorrhizal Scots pine plants increased biotite weathering by factors 1.3 to 1.7 compared to abiotic treatment. Several physical and/or biochemical processes resulting from root and hyphal activities and involved in mineral weathering have been discussed in previous reviews (April and Keller 1990; Kelly et al. 1998; Landeweert et al. 2001; Gadd 2007). They include fragmentation and reorientation of mineral grains, nutrient uptake, and exudation of a great range of compounds such as CO_2, protons, anions, cations, mineral acid, and organic acids.

Our weathering budgets also revealed that K and Mg were not released in a stoichiometric way in the columns

Table 2 *L. bicolor S238N* mycorrhizal rate and root, shoot, and total plant dry biomass after pre-culture (initial) and at the end of the column experiment (final)

Mycorrhizal status	*L. bicolor S238N* mycorrhizal rate (% of short roots)		Root biomass (mg)		Shoot biomass (mg)		Total plant biomass (mg)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Non-mycorrhizal	0	0	39.8±9.8	79.8±13.0	70.8±17.4	198.6±35.6	110.6±27.2	278.5±25.2
Mycorrhizal	64.2*±8.4	37.3*±15.2	44.1±5.9	91.3±22.3	78.4±10.4	300.0*±39.7	122.5±16.3	391.3*±34.8
ANOVA *P* value	0.001	0.001	ns	ns	ns	0.009	ns	0.026

Each value is the mean value of four replicates ± standard deviation. For each variable, values followed by an asterisk are significantly different according to a one-factor (mycorrhizal status) ANOVA (P=0.05), and the Bonferroni-Dunn test

ns not significant

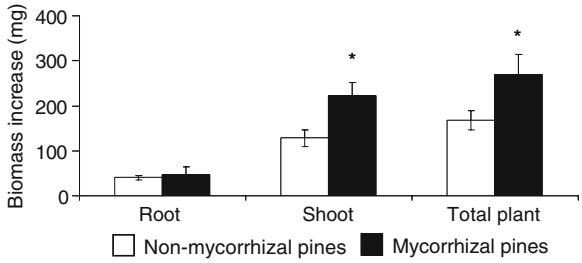

Fig. 4 Increase of root, shoot and total plant biomass during the experiment for non-mycorrhizal (*white*) and *L. bicolor* S238N mycorrhizal (*black*) pine plants. Each plot is the mean value of four replicates. *Bars* represent standard deviation. For each variable (root, shoot and total plant biomass), treatment associated with an asterisk is significantly different according to a one-factor (mycorrhizal status) ANOVA (P=0.05), and the Bonferroni-Dunn test

containing pine plants whatever their mycorrhizal status: the interlayer K was released faster than the Mg from the biotite structure. This means that biotite has mainly been dissolved by an incongruent phenomenon which corresponds to the partial transformation of biotite into vermiculite. Weathering of phyllosilicates into vermiculite is basically a cation-exchange process which can be accelerated by uptakes of K by plants (Spyridakis et al. 1967; Boyle and Voigt 1973; Hinsinger et al. 1992) and ECM fungi (Leyval and Berthelin 1991; Paris et al. 1995; Glowa et al. 2003). In fact, the uptake of K by roots and hyphae results in a depletion of K contained in the solution in the rhizosphere. The interaction between the liquid and the solid phases then requires the release of interlayer K from the biotite. In addition, protons produced by roots and fungi may move to the interlayer of expanded and non-expanded 2:1 type clay minerals to replace interlayer K (Cromack et al. 1979). Finally, organic acids exuded by roots and ECM fungi may chelate Al^{3+} and Fe^{3+} in the crystal lattice of minerals containing K, resulting in weathering of these minerals and release of interlayer K (Paris et al. 1995, 1996; Yuan et al. 2004).

After 8 weeks of culture in columns, pine plants mycorrhizal with *L. bicolor* S238N have produced 73% more shoot biomass than the non-mycorrhizal plants. We equally showed that the mycorrhizal pine plants assimilated significantly more K than the non-mycorrhizal plants during the experiment. The capacity of the ectomycorrhizal genus *Laccaria* to improve K nutrition of trees was previously reported by Leyval and Berthelin (1991) on Scots pine in a lysimeter experiment, by Quoreshi and Timmer (2000) on black spruce (*Picea mariana*) in a pot experiment, and by Baum et al. (2002) on Balsam poplar (*Populus Trichoporta*) in both a pot and a field experiment. Recent studies revealed that numerous ECM fungi can increase the supply of essential mineral nutrients such as K (Wallander and Wickman 1999; Wallander et al. 2002; Glowa et al. 2003; Yuan et al. 2004; Van Schöll et al. 2006), P (Brandes et al. 1998; Wallander 2000; Casarin et al. 2004; Torres Aquino and Plassard 2004), Ca (Blum et al. 2002; Wallander et al. 2002) and Mg (Jentschke et al. 2000; Glowa et al. 2003) to the host plant. This improvement of mineral nutrient acquisition is supposed to result from an increased element mobilization from the soil mineral reserve and/or from higher root uptake of element in solution (Smith and Read 1997; Chalot et al. 2002).

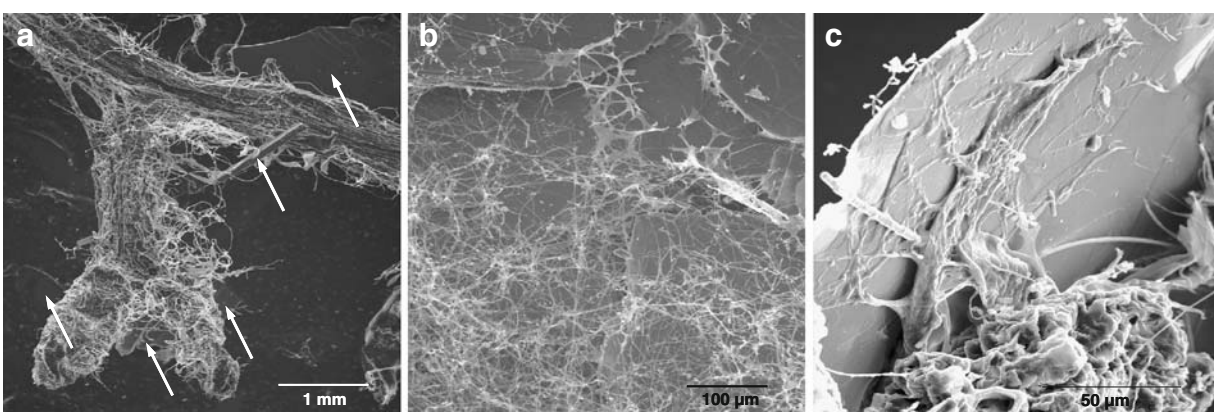

Fig. 5 Examples of SEM images of contact between biotite particles and hyphal network. **a** Biotite particles tangled up in the hyphal network in the mycorrhizal treatment after 8 weeks of experiment. The white arrows point to biotite particles. (**b**) and **c** Biotite surfaces covered with hyphal network in the mycorrhizal treatment after 8 weeks of experiment

The release of organic acids and siderophores by ECM fungi may be a mechanism by which ectomycorrhizas mobilize and then absorb unavailable nutrients (Cromack et al. 1979; Paris et al. 1996; Ahonen-Jonnarth et al. 2000; Arocena and Glowa 2000; Rosling et al. 2004; Balogh-Brunstad et al. 2008a). For example, Wallander and Wickman (1999) reported that the ECM fungus *Suillus variegatus* increased the release of K from biotite via oxalate and citrate production, resulting in enhanced *P. sylvestris* seedling growth and K foliar content. In our study, however, we did not observe an increase of biotite weathering in the presence of *L. bicolor* S238N mycorrhizal roots as compared with the non-mycorrhizal ones, suggesting that the production of weathering agents by the mycorrhizal roots and the related enhancement of nutrient bioavailability is not the main mechanism for the improvement of the pine nutrition.

In contrast, we demonstrated that the proportions of K and Mg mobilized from the biotite and assimilated by pine plants were twice as much for the mycorrhizal plants in comparison with those of the non-mycorrhizal plants. Secondly, we showed a significant depletion of K and Mg in the drained solutions when the pines were mycorrhizal with *L. bicolor* S238N in comparison with those of the non-mycorrhizal pine treatment. These observations tend to show that *L. bicolor* S238N improved pine nutrition through an increased uptake of the dissolved nutrients contained in the rhizosphere soil solution. This may be due to the fact that ECM hyphae that grow far from the ECM mantle into the surrounding soil are very efficient nutrient scavengers, due to their high surface area:mass ratio and their ability to penetrate microsites that are inaccessible to plant roots (Marschner and Dell 1994; Jongmans et al. 1997; Smith and Read 1997; Landeweert et al. 2001; Wallander et al. 2002). In addition, the extramatrical mycelium extends out from the mycorrhizal roots and increases the plant-mineral contact area of the root system (Harley 1989; Rousseau et al. 1994). This idea is confirmed by the quantification of the biotite adhering to the roots which revealed that about two times more biotite adhered to the mycorrhizal roots, and by the SEM investigation which showed that the hyphal network tangled up biotite particles (Fig. 5). Interestingly, Balogh-Brunstad et al. (2008b) demonstrated in a recent study, that the ectomycorrhizal hyphal network is able to transfer dissolved nutrients to the plant with minimum drainage losses. These authors suggest that hyphal network and associated bacterial biofilms could reduce transport of weathered elements out of the system in solution by isolating the root-hypha-mineral interface from the bulk soil solution. Such a mechanism could explain the significant K and Mg depletion we observed in the drained solutions when the pines were mycorrhizal with *L. bicolor* S238N. As suggested by Ahonen-Jonnarth et al. (2003), this ability of ECM fungi to acquire nutrients and restrict their loss through drainage may play an important role in tree nutrition in low-nutrient environments influenced by soil acidification.

To conclude, our results demonstrate that the ECM model strain *L. bicolor* S238N is not an efficient mineral weathering agent. However, it is very efficient for mineral nutrient assimilation from bio-available soil nutrients which can be released from the minerals by the tree roots themselves and/or by rhizosphere bacterial communities (Calvaruso et al. 2006). This is in accordance with the intrinsic properties of *L. bicolor* genome. Indeed, its recent sequencing revealed that the total number of predicted nutrient transporters is larger in *L. bicolor* as compared with other basidiomycetes (Martin et al. 2008). Based on the fact that *L. bicolor* S238N is the first ECM fungus whose genome was sequenced, it is now conceivable to decode the ECM physiological processes and genes involved in the improvement of plant nutrient acquisition from mineral weathering and thus to improve our understanding of one of the key mechanisms by which the ectomycorrhizal symbiosis contributes to forest sustainability.

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References

Ahonen-Jonnarth U, Van Hees PAW, Lundström US et al (2000) Production of organic acids by mycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings exposed to elevated concentrations of aluminium and heavy metals. New Phytol 146:557–567

Ahonen-Jonnarth U, Göransson A, Finlay RD (2003) Growth and nutrient uptake of ectomycorrhizal *Pinus sylvestris*

seedlings treated with elevated Al concentrations. Tree Physiol 23:157–167

April R, Keller D (1990) Mineralogy of the rhizosphere in forest soils of the eastern United States-Mineralogic studies of the rhizosphere. Biogeochemistry 9:1–18

Arocena JM, Glowa KR (2000) Mineral weathering in ectomycorrhizosphere of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) as revealed by soil solution composition. For Ecol Manag 133:61–70

Balogh-Brunstad Z, Keller CK, Dickinson J et al (2008a) Biotite weathering and nutrient uptake by ectomycorrhizal fungus, *Suillus tomentosus*, in liquid-culture experiments. Geochim Cosmochim Acta 72:2601–2618

Balogh-Brunstad Z, Keller CK, Gill RA et al (2008b) The effect of bacteria and fungi on chemical weathering and chemical denudation fluxes in pine growth experiments. Biogeochemistry 88:153–167

Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach. Wiley, New York, USA

Baum C, Stetter U, Makeschin F (2002) Growth response of *Populus trichocarpa* to inoculation by the ectomycorrhizal fungus *Laccaria laccata* in a pot and a field experiment. For Ecol Manag 163:1–8

Blum JD, Klaue A, Nezat CA et al (2002) Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. Nature 417:729–731

Boyle JR, Voigt GK (1973) Biological weathering of silicate materials. Implications for tree nutrition and soil genesis. Plant Soil 38:191–201

Brandes B, Golbold DL, Kuhn AJ et al (1998) Nitrogen and phosphorus acquisition by the mycelium of the ectomycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. New phytol 140:735–743

Calvaruso C, Turpault MP, Frey-Klett P (2006) Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. Appl Environ Microbiol 72:1258–1266

Casarin V, Plassard C, Hinsinger P et al (2004) Quantification of ectomycorrhizal fungal effects on the bioavailability and mobilization of soil P in the rhizosphere of *Pinus pinaster*. New Phytol 163:177–185

Chalot M, Javelle A, Blaudez D et al (2002) An update on nutrient processes in ectomycorrhizas. Plant Soil 244:165–175

Cromack K Jr, Sollins P, Grostein WC et al (1979) Calcium oxalate accumulations and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biol Biochem 11:463–468

Di Battista C, Selosse MA, Bouchard D et al (1996) Variations in symbiotic efficiency, phenotypic characters and ploidy level among different isolates of the ectomycorrhizal basidiomycete *Laccaria bicolor* strain S238. Mycol Res 100:1315–1324

Drever JI (2005) Surface and ground water, weathering, and soils. In: Holland HD, Turekian KK (eds) Treatise on geochemistry 5. Elsevier, Amsterdam

Duponnois R, Garbaye J (1991) Mycorrhization helper bacteria associated with the Douglas fir-*Laccaria laccata* symbiosis: effects in aseptic and in glasshouse conditions. Ann For Sci 48:239–251

Finlay RD (2004) Mycorrhizal fungi and their multifunctional roles. Mycologist 18:91–96

Frey-Klett P, Pierrat JC, Garbaye J (1997) Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas fir. Appl Environ Microbiol 63:139–144

Gadd GM (2007) Geomycology: biogeochemical transformation of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111:3–49

Glowa KR, Arocena JM, Massicotte HB (2003) Extraction of potassium and/or magnesium from selected soil minerals by Piloderma. Geomicrobiol J 20:99–111

Hagerberg D, Thelin G, Wallander H (2003) The production of ectomycorrhizal mycelium in forests: Relation between forest nutrient status and local mineral sources. Plant Soil 252:279–290

Harley JL (1989) The significance of mycorrhiza. Mycol Res 92:129–139

Heinonsalo J, Klett P, Pierrat JC et al (2004) Fate, tree growth effect and potential impact on soil microbial communities of mycorrhizal and bacterial inoculation in a forest plantation. Soil Biol Biochem 36:211–216

Hinsinger P (1998) How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Adv Agron 64:225–265

Hinsinger P, Jaillard B, Dufey JE (1992) Rapid weathering of a trioctahedral mica by roots of Ryegrass. Soil Sci Soc Am J 56:977–982

Hoffland E, Kuyper TW, Wallander H et al (2004) The role of fungi in weathering. Front Ecol Environ 5:258–264

Jentschke G, Brandes B, Kuhn AJ et al (2000) The mycorrhizal fungus *Paxillus involutus* transports magnesium to Norway spruce seedlings. Evidence from stable isotope labeling. Plant Soil 220:243–246

Jongmans AG, Van Breemen N, Lundstrom U et al (1997) Rock-eating fungi. Nature 389:682–683

Kelly E, Chadwick OA, Hilinski TE (1998) The effect of plants on mineral weathering. Biogeochemistry 42:21–53

Kernaghan G (2005) Mycorrhizal diversity: cause and effect? Pedobiologia 49:511–520

Landeweert R, Hoffland E, Finlay RD et al (2001) Linking plants to rock: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol Evol 16:248–253

Le Tacon F, Bouchard D, Churin JL et al (2005) Mycorhization contrôlée du Douglas et du chêne. For Entrep 164:33–37

Leyval C, Berthelin J (1991) Weathering of a mica by roots and rhizospheric micro-organisms of pine. Soil Sci Soc Am J 55:1009–1016

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Martin F, Aerts A, Ahrén D et al (2008) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452:88–92

Pachlewski R, Packlewska J (1974) Studies on symbiotic properties of mycorrhizal fungi of pine (*Pinus sylvestris*) with the aid of the method of mycorrhizal synthesis in pure culture on agar. Forest Research Institute, Warsaw

Paris F, Bonnaud P, Ranger J et al (1995) In vitro weathering of phlogopite by ectomycorrhizal fungi. 1. Effect of K+ and

Mg2+ deficiency on phyllosilicate evolution. Plant Soil 177:191–201

Paris F, Botton B, Lapeyrie F (1996) In vitro weathering of phlogopite by ectomycorrhizal fungi. 2. Effect of K+ and Mg2+ deficiency and N sources on accumulation of oxalate and H+. Plant Soil 179:141–150

Quoreshi AM, Timmer VR (2000) Early outplanting performance of nutrient-loaded containerized black spruce seedlings inoculated with *Laccaria bicolor*: a bioassay study. Can J For Res 30:744–752

Robert M, Berthelin J (1986) Role of biological and biochemical factors in soil mineral weathering. In: Huang PM (ed.) Interactions of soil minerals with natural organics and microbes. Soil Sci Soc Am, Madison, Wi, pp 453–495

Rosling A, Lindahl BD, Taylor AFS et al (2004) Mycelial growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. FEMS Microbiol Ecol 47:31–37

Rousseau JVD, Sylvia DM, Fox AJ (1994) Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. New Phytol 128:639–644

Smith SA, Read D (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Spyridakis DC, Chesters G, Wilde SA (1967) Kaolinisation of biotite as a result of coniferous and deciduous seedling growth. Soil Sci Soc Am Proc 31:203–210

Torres Aquino M, Plassard C (2004) Dynamics of ectomycorrhizal mycelial growth and P transfer to the host plant in response to low and high soil P availability. FEMS Microbiol Ecol 48:149–156

Van Breemen N, Finlay RF, Lundström U et al (2000) Mycorrhizal weathering: a true case of mineral plant nutrition. Biogeochemistry 49:53–67

Van Hees PAW, Rosling A, Lundström US et al (2006) The biogeochemical impact of ectomycorrhizal conifers on major soil elements (Al, Fe, K and Si). Geoderma 136:364–377

Van Schöll L, Smits MM, Hoffland E (2006) Ectomycorrhizal weathering of the soil minerals muscovite and hornblende. New Phytol 171:805–814

Wallander H (2000) Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. Plant Soil 218:249–256

Wallander H, Wickman T (1999) Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. Mycorrhiza 9:25–32

Wallander H, Johansson L, Pallon J (2002) PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil. FEMS Microbiol Ecol 39:147–156

Yuan L, Huang J, Li X et al (2004) Biological mobilization of potassium from clay minerals by ectomycorrhizal fungi and eucalypt seedling roots. Plant Soil 262:351–361