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Iron ore weathering potentials of ectomycorrhizal plants

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Abstract Plants in association with soil microorganisms play an important role in mineral weathering. Studies have shown that plants in symbiosis with ectomycorrhizal (ECM) fungi have the potential to increase the uptake of mineral-derived nutrients. However, it is usually difficult to study many of the different factors that influence ectomycorrhizal weathering in a single experiment. In the present study, we carried out a pot experiment where *Pinus patula* seedlings were grown with or without ECM fungi in the presence of iron ore minerals. The ECM fungi used included *Pisolithus tinctorius*, *Paxillus involutus*, *Laccaria bicolor* and *Suillus tomentosus*. After 24 weeks, harvesting of the plants was carried out. The concentration of organic acids released into the soil, as well as potassium and phosphorus released from the iron ore were measured. The results suggest that different roles of ectomycorrhizal fungi in mineral weathering such as nutrient absorption and transfer, improving the health of plants and ensuring nutrient circulation in the ecosystem, are species specific, and both mycorrhizal roots and non-mycorrhizal roots can participate in the weathering process of iron ore minerals.

Keywords Ectomycorrhizal fungi · Iron ore · Biohydrometallurgy · Organic acids · Particle size · Weathering

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Introduction

The soil biological system plays a major role in nutrient cycling by mediating mineral weathering processes (Arocena and Glowa 2000; Burford et al. 2003). In the process of establishing viable survival strategies, soil organisms notably plants, saprophytic and symbiotic microbes interact in different ways to produce different metabolites that are involved in mineral dissolution (Banfield et al. 1999; Schoenholtz et al. 2000). In the weathering of minerals where symbiotic organisms are involved, ectomycorrhizal (ECM) fungi are actively involved in the solubilisation of minerals (Smith and Read 2008). These fungi have the capabilities to absorb nutrients from hard mineral materials (Jongmans et al. 1997; Arocena and Glowa 2000; van Schöll et al. 2006; Smith and Read 2008).

Jongmans et al. (1997) have linked tunnels in mineral grains to the presence and establishment of ECM fungi. More recent evidence shows that ECM root density was proportional to both feldspar tunnelling and the release of nutrients from the minerals (Hoffland et al. 2003), while

several other studies (Burford et al. 2003; Jain and Sharma 2004; Balogh-Brunstad et al. 2008) have identified pH, nutrient limitation, mineral grain size, organic acid availability and mechanical penetration of the mineral as important factors that influence the dissolution of minerals.

Wallander et al. (1997) showed that the ECM fungus *Suillus variegatus* decreased the pH and increased the citric acid concentration of an apatite-amended soil, which resulted in an increase in the release of phosphorus (P) from the apatite. Olsson and Wallander (1998) demonstrated that although solubilisation of minerals by ECM fungi is substantially dependent on organic acid production, other factors such as bacterial community (biofilms), mineral type and other soil properties play an equally important role (Wallander and Wickman 1999; Burford et al. 2003; Jain and Sharma 2004). Oxalic, citric, acetic, lactic, tartaric, malic and malonic acids are examples of organic acids produced by ECM fungi that have been demonstrated to have mineral solubilising characteristics (Fox et al. 1990; Entry et al. 1992; Wallander et al. 1997). Rhizospheric soils containing dense mats of ECM mycelia usually have higher concentrations of organic acids than soils with lower concentrations of these constituents (Griffiths et al. 1994; Landeweert et al. 2001).

Actions associated with these acids comprise direct mineral attack by the metal complexing organic acid anions and protons (Gadd 1999). Because of the high chelation constants of $[Al\ (C_2O_4)_3]^{3-}$ (2.0×10^{16}) and $[Fe\ (C_2O_4)_3]^{3-}$ (3.9×10^{16}), mineral sites containing Al and Fe ions are easy targets for attack by organic acid anions. For instance, oxalate chelation of these cations can result in a structural imbalance that may lead to the dissolution and release of different elements and nutrients contained in minerals such as Si, K and P (Yuan et al. 2004; Delvasto et al. 2009). In addition, there is similarity (monovalent structures) between protons from organic acids and K from minerals like muscovite, but the size of these protons (0.32×10^{-10} m) is much smaller than K protons that are 2.03×10^{-10} m (Lapeyrie et al. 1987; Yuan et al. 2004). The size of the proton is therefore an advantage, as it enables the protons to replace interlayer K contained in layered minerals.

Previous studies on ECM weathering had focussed mostly on the use of different minerals separately or in combination (Wallander et al. 1997; Wallander and Wickman 1999; Calvaruso et al. 2006, 2010). In this study, we used iron ore consisting of different naturally bonded minerals. Generally, high content levels of K (>0.24%) and P (>0.03%) diminish the market value of iron ores and often render them economically non-viable (Parks et al. 1990; Yusfin et al. 1999; Williams and Cloete 2008; Delvasto et al. 2009; Adeleke et al. 2010). In view of this and other considerations, the goal of this study was to investigate potential mobilisation effects of ectomycorrhizal *Pinus patula* on the chemical composition of minerals contained in iron ore. For this purpose, non-exportable Sishen iron ores with high levels of K and P were used, and four ECM fungi were tested in order to enhance our understanding of the mechanisms and specifics of ectomycorhizal weathering. We did this by measuring the quantity of organic acids released in the soil as well as K, P and Fe released from iron ore in order to objectively assess the weathering potential of each fungus in symbiotic association with *Pinus patula*.

Materials and methods

Origin of ectomycorrhizal fungi and iron ore preparation

Origin of the ECM fungi (*Pisolithus tinctorius*, *Paxillus involutus*, and *S. tomentosus*) and the chemical composition of the iron ore mineral (KGT) that were used are described in Adeleke et al. (2010). In addition to the listed fungi in Adeleke et al. (2010), *Laccaria bicolor* UAMH 8232 was also obtained from the University of Alberta Microfungus Collection and Herbarium (http://tinyurl.com/d3t4fc4).

The iron ore sample (KGT conglomerates) was supplied by KUMBA Iron Ore Resources Ltd. The ore materials were milled and mesh-segregated into two particle sizes of 3.36–1.68 mm (particle size A) and 1.68–0.84 mm (particle size B). The iron ore materials were cleaned of fine dust through overnight soaking for 24 h in 0.1 M HCl (van Schöll et al. 2006) and thorough washing under distilled water. The pH was adjusted to 4 using 0.1 M HCl to reduce the grinding effect, which can lead to modification of the mineral when in contact with contaminants by exposing its surface (Pugh et al. 1995; van Schöll et al. 2006). This treatment was followed by the addition of distilled water to the ore mineral and shaking for 7 day at 100 rpm in order to remove residues of the acid

Preparation of seeds

Seeds of *Pinus patula* obtained from Komatiland Forest at SABIE, South Africa were surfaced-sterilised in 30% H_2O_2 for 15 min, washed continuously under distilled water for 3 min and soaked overnight in autoclaved distilled water with a drop of Tween 20. After 24 h, the seeds were re-sterilised in 10% sodium hypochlorite (NaOCl) for 60 s. This was followed by washing three to five times using distilled water before inoculation onto 15% water agar plates where they were pre-germinated for 4 weeks. Germinants were considered ready for mycorrhizal synthesis experiment when radicles were 1–2 cm in length.

Ectomycorrhizal synthesis experiment

Autoclavable Magenta boxes (Magenta. Corp., Chicago, IL, USA) were used for the experiment. Fifty millilitres aliquots

of modified Melin Norkrans (MMN) medium (Marx 1969) were used for the experiment. The medium contained malt extract (3 g/l), $(NH_4)_2HPO_4$ (0.25 g/l), $MgSO_4 \cdot 7H_2O$ (0.075 g/l), $CaCl_2 \cdot H_2O$ (0.067 g/l), NaCl (0.025 g/l), $FeCl_3$ (1%), thiamine (100 μg/l) and agar (10 g). Glucose was omitted from the medium composition in order to starve the fungi of carbon source, which they could get from their host plant by forming the mycorrhizal association. After the sterile medium was poured into the sterile Magenta boxes, they were covered with a layer of sterile cellophane paper to prevent the ectomycorrhizal root from penetrating the medium.

A 6-mm cork borer was used to cut plugs of the mycelial culture of the ECM fungi from the actively growing edge of a 7-day-old culture. Three plugs of ECM mycelia were inoculated on the cellophane paper at equal distances from one another and incubated at 28°C for 5 days. After 5 days, germinated seedlings of *Pinus patula* were introduced into the centre of the boxes containing growing mycelia of the fungi, and the boxes were incubated under sterile conditions for a period of 12 weeks at 23/16°C (days/night), 16 h photoperiod and 80% humidity. All the control plant seedlings (non-mycorrhizal) were also transferred to the Magenta boxes containing the same type of MMN medium but no ECM fungi. After 12 weeks, the root samples were selected at random and carefully examined for signs of ectomycorrhizal association. The root samples were observed using a dissecting microscope Leica S4E microscope (Leica Microsystems Imaging Solutions, Cambridge, UK) for hyphal sheath structural development as an indication of mantle formation. "Representative roots" (from extra replicates of each treatment) that could not be confirmed as colonised under dissecting microscopes were further examined using a light microscope after staining using the method described by Smith and Dickson (1997). Stained roots were not used in further experiments.

Soil treatments

The growth medium was natural sand obtained from Sable Marco inc., Pont-Rouge, QC, Canada. Sand was soaked in 0.1 M HCl overnight in order to remove fine dust and exchangeable bases. After 24 h, the sand was washed continuously for several hours using distilled water and later dried in the oven for 3 days. Sand were sieved with an electroformed sieve to particle size of 0.25–0.59 mm. This allowed an easy separation of sand from the iron ore minerals of particle sizes A and B at the end of the experiment. The sieved sand was sterilised in the autoclave at 121°C for 30 min and allowed to cool overnight before the sterilisation was repeated at the same temperature and time. The sand was kept sterile until the beginning of the weathering experiment.

Weathering experiment

Plastic pots (80×80×70 mm) were sterilised by soaking in 3% NaOCl overnight and washed sufficiently to remove the NaOCl using distilled water. These pots were then filled to the brim with the above described sterile/treated sand. Planting holes, big enough to hold seedlings, were created in the sand and partially filled with 4 g of the prepared iron ore samples. Healthy ectomycorrhizal colonised (EMC) and non-mycorrhizal (NMC) seedlings were then inserted in these holes and covered with sand. The growing conditions were at 23/16°C (days/night), 16 h photoperiod and 80% humidity. This experiment lasted for 24 weeks with all treatments being performed with four replicates.

Watering and nutrient supply

Watering of the seedlings was done every other day, and the nutrient supply was twice a week. The nutrient solution used in this experiment was a Hoagland solution adjusted to reduce the sources of K. In addition, sources of Mg were also halved to limit the alternative K source (Mg) available to the fungi during the weathering process (Van Schöll et al. 2006). The final solution of the Hoagland contained the following: 7 mL of 1 M $Ca(NO_3)_2$, 2 mL of 1 M $NH_4(PO_4)$ and 1 mL of 1 M $MgSO_4$; trace elements of 1 mL (2.8 g/l H_3BO_3, 1.8 g/l $MnCl_2 \cdot 4H_2O$, 0.2 g/l $ZnSO_4 \cdot 7H_2O$, 0.1 g/l $CuSO_4 \cdot 5H_2O$ and 0.025 g/l $Na_2MO_4 \cdot 2H_2O$) and 1 mL FeEDTA (15 g/l) and distilled water added to make up to 1 l.

Harvesting

All plants were harvested at the end of 24th week. The first step in harvesting included the careful separation of the seedling from the sand and iron ore samples through sieving. Thereafter, the roots were severed from the shoot system, washed free of the sand and transported to the laboratory for where they were thoroughly re-washed with distilled water to remove all traces of sand in preparation for light microscopy examination in order to determine the percentage root colonisation by counting of the percentage fine roots that were colonised. The plant shoots and roots were separately dried in the oven (65°C for 48 h) and weighed to obtain root dry mass (RDM) and shoot dry mass (SDM). The sieved sand samples free of the iron ore samples were split into two for nutrient analysis, dry mass calculation, pH determination and organic acid analysis.

Sand samples were dried in the oven at 70°C, ground in a Wiley mill and passed through a 2-mm sieve screen. A 0.5-g quantity of the each sample was then digested in a sulphuric acid and hydrogen peroxide mix (Parkinson and Allen, 1975). Determinations of P and K were made using the induction coupled plasma optical emission spectrometer

(ICP-OES) Optima 4300 DV (Perkin Elmer, Waltham, MA, USA).

Organic acid analyses and pH measurement

Four grams of the harvested sand was put into 25-ml centrifuge tubes, and 20 ml of 10 mM NaH_2PO_4 was added (Mimmo et al. 2008). The mixture was shaken for 4 h at room temperature and centrifuged at 9300 rph for 8 min at 20°C. Ten millilitres of the supernatant was collected and kept at −20°C for further analysis. A 1-ml subsample was collected and evaporated to dryness on a SAVANT Speed Vac Plus evaporator (SC210A) system (Fisher Scientific, Ontario, Canada) and then re-suspended in 200 μl of demineralised water, vortexed and left at room temperature for 15 min. The re-suspended samples were vortexed, transferred into 1.5-mL tubes and centrifuged at 13,000 rph. Fifty microlitres of each sample was analysed by high pressure liquid chomatography (Adeleke et al. 2010). The organic acid standards used included oxalic acid, citric acid, malonic acid and maleic acid, which were well separated under the described chromatographic conditions (Adeleke et al. 2010). The pH status of the sand (H_2O) was measured using a digital pH meter.

Elemental analyses

Iron ore samples collected during harvesting were repeatedly washed with 0.1 M HCl and later left in deionised water for 24 h. The samples were dried at 104°C before sending them for induction coupled plasma (ICP-OES Optima 4300 DV, Perkin Elmer, Waltham, MA, USA) analysis by UIS Analytical Services, Pretoria, South Africa. The analysis was able to detect the quantities of K, P and total Fe of the ore samples before and after the experiment. Percentage K or P loss was then calculated as stated in the formula below

$$\%K \text{ or } \%P \text{ loss} = \frac{\%K_{initial} \text{ or} \%P_{initial} - \%K_{final} \text{ or } \%P_{final}}{\%K_{initial} \text{ or} \%P_{initial}} \times 100$$

Dry root and shoot samples were also analysed for potassium and phosphorus content using the inductively coupled plasma–atomic emission spectrometer.

Experimental design and statistical analyses

The study involved different treatments with pine seedlings inoculated with four different types of ectomycorrhizal fungi (EMF) and those that were not inoculated. Both mycorrhizal and non-mycorrhizal seedlings were grown in the presence of iron ore particles. Two sizes of iron ore were used. At the end of the experiment, parameters measured include shoot and root P and K, loss of K and P from iron ore particles, P and K concentrations in the remaining sand as well as the concentrations of different organic acids in the sand.

A two-way ANOVA was used to compare the effect of EMF [five levels: *L. bicolor* (LBR), *Paxillus involutus* (PI), *Pisolithus tinctorius* (PT), *S. tomentosus* (ST), NMC] and particle size of iron ore (two levels: A and B) on each of the response variables. In addition, multiple comparisons using the protected least significant difference method were carried out for all significant sources of variation in the ANOVA table. The effect of the presence/absence of EMF was examined using a planned contrast, which compares the average response value of the four EMC (LB, PI, PT and ST) to the NMC (Table 1).

Results

The mycorrhizal synthesis experiment was successfully carried out with colonisation of between 40% and 100% recorded for all the four types of fungi used. More than half of the seedlings had >60% root colonisation rates, with the following mean±standard errors: *Pisolithus tinctorius* (64 ±2.48), *S. tomentosus* (72±4.57), *L. bicolor* (66±4.24) and *Paxillus involutus* (67±2.85).

Organic acids and pH

In general, citric acid concentration was the highest produced organic acid detected in the sand samples of all the treatments (Fig. 1 a–d). There was no consistent relationship in values of the concentration of citric acid in the sand and particle sizes of the iron ore samples. *S. tomentosus* (A) treatment was the only treatment with a significant difference in the concentration of citric acid compared to the non-mycorrhizal plants. *S. tomentosus* treatment was also the only treatment that recorded a significant difference in the concentration of citric acid secreted between particle sizes A and B (Fig. 1a). There was no significant effect of fungi and particle size on citric acid secretion (Table 1).

A similar result was obtained for oxalic acid, where the particle size A of *S. tomentosus* treatment and particle size B of *Pisolithus tinctorius* treatment showed significantly higher concentrations than the non-mycorrhizal plants (Fig. 1b). Only fungal type had a significant effect on oxalic acid secretions (Table 1).

For malonic acid, two (*Paxillus involutus* and *Pisolithus tinctorius*) out of five treatments showed a significant difference between particle sizes A and B. There were significant effects of fungal type and particle size on the malonic acid secretion (Table 1). A similar trend was obtained for maleic acid with four (*L. bicolor*, *Paxillus involutus*, *Pisolithus tinctorius* and NMC) out of five treatments showing significant differences between particle size A and B treatments. There

Table 1 ANOVA table showing *P* values for the fungal effect (4 *df*), the particle size effect (1 *df*) and the interaction of fungi and particle size (4 *df*) on the production of four different organic acids

	Oxalic			Citric		Malic		Malonic	
Source	*df*	*F*	*P* value	*F*	*P* value	*F*	*P* value	*F*	*P* value
Fungi	4	9.55	<0.0001	1.63	0.1974	2.00	0.1253	5.84	0.0018
Pres vs abs	1	1.81	0.1912	1.26	0.2732	0.01	0.9184	0.06	0.8159
Particle size	1	1.34	0.2583	1.86	0.1849	9.95	0.0042	8.46	0.0075
Fungi vs Particle size	4	2.52	0.0680	2.92	0.0414	5.90	0.0017	1.97	0.1299
(Pres vs Abs) vs Part.size	1	0.03	0.8741	0.04	0.8745	1.67	0.2083	0.05	0.8295

The interaction was significant at the 0.05 level for %K, shoot K, shoot DM and pH

Pres presence, *Abs* absence, *vs* versus

was a significant effect of particle size as well as interaction between fungal type and particle size on maleic acid secretion (Table 1). The average acid concentration with ectomycorrhizal treatment was not significantly different from the non-mycorrhizal treatment for all acids (Table 1).

The sand pH was significantly affected by fungal type, particle size as well as interaction between the fungal type and particle size (Table 2). A pH range of 4.41–5.19 was recorded in all treatments. The significant effects of particle size were only reflected in the results obtained for *Paxillus involutus* and *Pisolithus tinctorius*. Apart from *L. bicolor* (A and B) and *Paxillus involutus* (B) treatments, all the other sand mycorrhizal treatments were more acidic than non-mycorrhizal treatments (Table 3). *S. tomentosus* treatment had the highest concentration of sand citric acid as well as low pH values, indicating that there could be a relationship between organic acid secretion and sand pH (Table 3).

Mobilisation of K and P from iron ore minerals

Fungal type, particle size and the interaction between the two factors significantly affected the mobilisation of K from

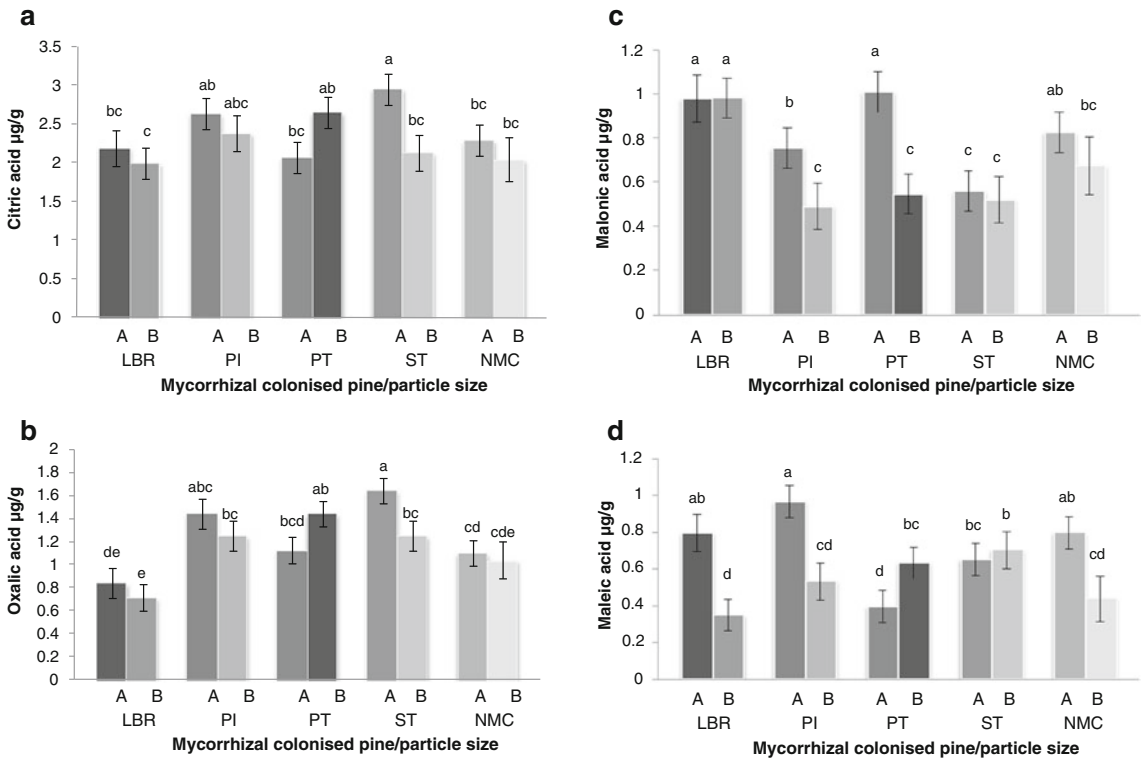

Fig. 1 Amount of citric acid (**a**), oxalic acid (**b**), malonic acid (**c**) and maleic acid (**d**) released into the soil by the four EMC (*Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius* and *Suillus tomentosus*) and the NMC roots grown in the presence of KGT iron ore (particle sizes A and B). Mean of four replicates. For each type of organic acids, *bars with the same letter* [standard errors (SE)] are not significantly different ($P<0.05$)

Table 2 Two-way analysis of variance (ANOVA) models with *F* and *P* values that showed the effects of fungal type, particle size and their interactions on K and P reduction from iron ore as well as the effects on K and P of the soil, shoot and root

Factors	*df*	Sources of variation			
		Fungi	Particle size	Fungi vs particle size	
		df	4	1	4
%K	26	*F*	19.08	37.51	9.11
		P value	<.0001	<.0001	<.0001
%P	26	*F*	2.16	12.48	1.29
		P value	0.1019	0.0016	0.3012
		df	4	1	8
Soil K	26	*F*	4.60	0.58	0.88
		P value	0.0061	0.4551	0.4894
Soil P	26	*F*	3.91	2.34	0.98
		P value	0.0129	0.1380	0.4378
		df	4	1	8
Shoot K	24	*F*	5.56	3.68	3.23
		P value	0.0026	0.0671	0.0298
Shoot P	23	*F*	1.31	0.00	0.81
		P value	0.2961	0.9447	0.5333
		df	4	1	8
Root K	25	*F*	10.52	0.12	1.14
		P value	<.0001	0.7268	0.3587
Root P	25	*F*	4.86	0.08	0.49
		P value	0.0049	0.7819	0.7460
		df	4	1	8
Shoot DM	26	*F*	11.54	0.02	5.74
		P value	<0.0001	0.8927	0.001
Root DM	26	*F*	31.46	0.13	2.33
		P value	<0.0001	0.7186	0.0829
		df	4	1	4
pH	26	*F*	12.71	28.47	3.91
		P value	<0.0001	<0.0001	<0.0129

The effects of these factors on pH, shoot and root DW (dry weight) were also shown. $P<0.05$ were considered significant

iron ore minerals, while only the particle size significantly affected the mobilisation of P from the iron ore minerals (Table 2). A higher concentration of % K and% P was removed from treatments with particle size A compared to particle size B (Figs. 2 and 3). Particle size therefore had a significant effect on % K and % P loss. The highest% K loss from the iron ore was from treatments containing fungal type *Pisolithus tinctorius* with particle sizes A and B (Fig. 2). In addition, the lowest % K reduction was from the NMC treatment (particle size B). The highest % P reduction from the iron ore was recorded from *Paxillus involutus* treatment of particle size A, while the lowest was from the NMC treatment with particle size B (Fig. 2).

Foliar, root and soil phosphorus/potassium

Except for foliar (shoot) P, fungal type had significant effects on foliar, root and sand P and K. There were also significant effects of particle size as well as the interaction between fungal type and particle size on foliar K (Table 2). The highest value of foliar K for the EMC treatment was recorded in *Pisolithus tinctorius* treatment with particle size A, while the lowest was from *L. bicolor* treatment with particle size B (Table 3). A similar trend was observed for foliar P where the treatment involving *Pisolithus tinctorius* (particle size A) had the highest shoot P, while the lowest was *S. tomentosus*, particle size A (Table 3). However, there was no significant difference between the foliar P in non mycorrhizal and the ectomycorrhizal treatments (Table 3).

The non-mycorrhizal treatments had significant higher root K values than ectomycorrhizal treatments of *Paxillus involutus* (A), *Pisolithus tinctorius* (A and B) and *S. tomentosus* (A and B) (Table 3). For the ectomycorrhizal treatments, highest root K was recorded in the *L. bicolor* treatment with particle size B, while the lowest was recorded from the *S. tomentosus* treatment with particle size B (Table 3). Except for *S. tomentosus* (A and B), there was no significant difference in the concentration of root P of both mycorrhizal treatments and the non-mycorrhizal treatments. *Paxillus involutus* treatments tended to retain more root P than all other EMC treatments, while the lowest P root retention was obtained in *S. tomentosus* with both particle sizes A and B (Table 3).

For sand nutrient status, there was no significant difference in the concentration of K in sand for all treatments except for particle size B of *L. bicolor* treatments whose values were significantly higher than treatment with particle size B of non-mycorrhizal treatments (Table 3). For both particle sizes, the lowest value of sand K recorded was from *Pisolithus tinctorius* treatment, particle size B (Table 3). Values for sand P in ectomycorrhizal and non-mycorrhizal treatments with particle sizes A and B were not significantly different except for *L. bicolor* (A) with significantly higher values than non mycorrhizal fungi (Table 3).

Shoot dry mass and root dry mass

There were significant effects of fungal type as well as the interaction between fungal type and particle size on SDM. For RDM; the only significant effect observed was for fungal type (Table 2). The RDM of *S. tomentosus* treatments were significantly higher than all other treatments, and a similar result was also obtained for SDM, with both particle sizes A and B of *S. tomentosus* treatments having highest SDM (Table 3).

Table 3 Amounts of measured foliar K (potassium) and P (phosphorus), root K and P, soil K and P as well as pH, and values of root and shoot DW for EMC and NMC

Fungi	Size	Root P (ppm)	Root K (ppm)	Foliar P (ppm)	Foliar K (ppm)	pH	Soil K (ppm)	soil P (ppm)	Root DM(g)	Shoot DM(g)
NMC	A	1339ab	1880a	2000ab	5788ab	4.91bc	3.21abcd	3.38bc	0.3081 cd	0.6035c
NMC	B	1194abc	1859a	2043ab	5526abc	4.95abc	2.22 cd	3.38bc	0.3188bcd	0.5899c
LBR	A	1152abc	1564abc	1983ab	5301bc	5.06ab	3.78ab	3.42bc	0.2074 d	0.491c
LBR	B	1208abc	1786a	1964ab	3879 d	5.19a	4.11a	4.45a	0.3515bcd	0.6001c
PI	A	1339ab	1406bcd	1789ab	4322 cd	4.42e	3.60abc	3.63ab	0.4289bc	0.7816b
PI	B	1465a	1604ab	1911ab	4960bcd	4.99abc	3.29abcd	3.56b	0.2903 cd	0.5256c
PT	A	1141bc	1326bcd	2269a	6952a	4.48e	2.62bcd	2.69c	0.4352bc	0.5768c
PT	B	1077bc	1165 d	1902ab	5050bcd	4.88bc	2.15 d	2.88bc	0.4849b	0.7965ab
ST	A	1000c	1254 cd	1649b	4208 cd	4.59de	2.59bcd	3.10bc	0.9131a	0.9498a
ST	B	930c	1151 d	1830ab	4457 cd	4.79 cd	3.01abcd	3.48bc	0.7806a	0.8659ab

Two groups that represented two particle sizes A and B were presented. Means with the same letters within a column are not significantly different ($P<0.05$)

Discussion

This study successfully combined different parameters that are rarely studied together in a single experiment to investigate the role of mycorrhizal plants in the weathering process by four different mycorrhizal plants. Results of this investigation indicate that although it is difficult to study all the factors that contribute to mineral weathering in a single experiment, it is still possible to gain informative insights by focussing on objectively selected parameters.

Pine roots, whether mycorrhizal or not, were able to mobilise between 9% and 29% K and more than 50% P from the iron ore samples, thereby confirming their participation in the weathering process. Higher values of P mobilisation indicate that roots were more efficient in mobilising P than K. This observation is in agreement with previous studies (Wallander and Wickman 1999; Calvaruso et al. 2006, 2010) that reported similar mobilisation capabilities by mycorrhizal and non-mycorrhizal roots and active participation in mineral weathering. These observations were further corroborated by Calvaruso et al. (2010) who reported the participation of both mycorrhizal and non-mycorrhizal pine root in biotite weathering, improvement of shoot growth by the former and K and Mg assimilation in Scots pine via increased uptake of dissolved nutrients.

Rates of K and P absorption were shown to vary among the different mycorrhizal plants used in this study. Nevertheless, on average, mycorrhizal plants significantly increased K mobilisation from iron ore compared to non-mycorrhizal plants, but there was no significant difference between non-mycorrhizal plant and mycorrhizal plant treatments for mobilisation of P. These divergent results make it difficult to directly correlate the mobilisation of nutrients

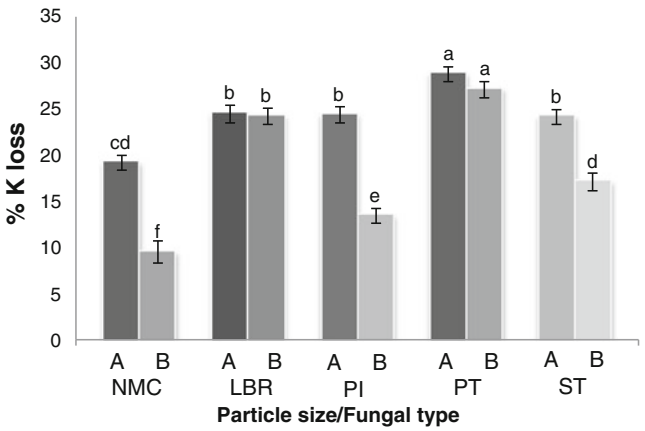

Fig. 2 Percentage of K reduction $[(\%K_{initial}-\%K_{final})/\%K_{initial}]\times100$ from iron ore mineral in response to the four different fungal treatments (*Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius* and *Suillus tomentosus*) and NMC using two particle sizes (A and B) of the iron ore materials. Mean of four replicates. *Bars with the same letter* [standard errors (SE)] are not significantly different ($P<0.05$)

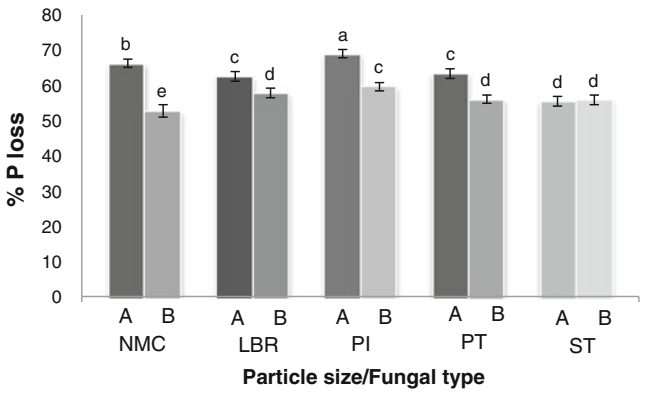

Fig. 3 Percentage of P reduction $[(\%P_{initial}-\%P_{final})/\%P_{initial}]\times100$ from iron ore mineral in response to the four different fungal treatments (*Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius* and *Suillus tomentosus*) and NMC using two particle sizes (A and B) of the iron ore materials. Mean of four replicates. *Bars with the same letter* [standard errors (SE)] are not significantly different ($P<0.05$)

from the iron ore to the presence of ECM fungi. It is possible to have similar efficiency in the mineral weathering process between non-mycorrhizal and mycorrhizal roots (Calvaruso et al. 2010).

Regarding the absorption of the dissolved mineral nutrients, mycorrhizal plants have been shown to be more efficient (Smith and Read 2008) due to the nature of ECM hyphal network, which facilitates the penetration of hard mineral surfaces and scavenging for nutrients by fungus that spread to cover large surface areas. Such effective absorption of dissolved mineral nutrients through ECM fungi has also been identified to facilitate mineral weathering processes. When weathering products are fast removed from the boundary layer surrounding the mineral surfaces through bio-uptake, there is little or no limitation to weathering rates because re-precipitation of weathering products on mineral surfaces is substantially reduced (Paris et al. 1995; Glowa et al. 2003; Sverdrup 2009).

Among the four organic acids analysed from the sand samples in this study, citric acid concentrations were the highest followed by oxalic acid. Several studies have highlighted the importance of these two organic acids in mineral weathering due to their high metal chelating capabilities (Wallander and Wickman 1999; Ahonen-Jonnarth et al. 2000; Smith and Read 2008; van Scholl et al. 2008). Wallander and Wickman (1999) observed large quantities of citric acid in a weathering experiment involving *Pinus sylvestris* seedlings inoculated with *S. variegatus* using biotite mineral as source of K. Similarly, Ahonen-Jonnarth et al. (2000) reported the increased production of oxalic acid by *S. variegatus* and *Rhizopogon roseolus* colonised plants when under elevated Al concentrations.

Neither the limitation of nutrient (K) nor the addition of iron ore minerals increased organic acid production by both mycorrhizal and non-mycorrhizal plants. With no statistically significant difference between the amount of citric acid detected in treatments with the highest (*Pisolithus tinctorius*) and lowest (NMC) K mobilisation, the quantity of organic acid produced in the present study can, therefore, not be directly correlated to mobilisation of either P or K from the iron ore minerals. This suggests that mineral weathering is induced by a combination of factors, not only in response to organic acid. This view was shared in the review by Banfield et al. (1999) where it was explained that other mechanisms such as scavenging by microbes can also lead to mineral weathering. This is contrary to the findings of Wallander and Wickman (1999), where weathering by *S. variegatus* was linked to the production of citric and oxalic acids. However, caution must be exercised in the interpretation of organic acid results because the response could vary with sand type, treatments, and storage as well as rhizospheric microbial effects (Bligh and Dyer 1959; Mimmo et al. 2008). For instance, Bligh and Dyer (1959) reported that organic acids produced by fungi may be wrongly calculated in weathering experiments because they sometimes serve as substrates for saprophytic growth of bacteria.

Particle size and mineral type are other factors that significantly affected the weathering process in this study. Generally, there was more nutrient release from larger particle size A than B. This is in contrast to the result obtained by Modak et al. (2001) that showed better mobilisation of calcium from finer mineral particles and allowing them to conclude that exposure of larger mineral particle surface area (finer particle size) to the leaching agents increases the possibility of solubilisation. The result also failed to corroborate our previous in vitro study where greater mobilisation of nutrients was obtained from the iron ore with the smallest particle size (Adeleke et al. 2010). There could be a number of reasons for this. For example, Sheng et al. (2008) showed in an experiment involving three silicate minerals that mineral type can determine the rate of nutrient release from minerals. This factor could have affected the result of our investigation as previous mineralogical characterisation of KGT iron ore identified minerals such as muscovite, apatite, woodhouseite, goyasite and gorceixite as P- and K-bearing minerals associated with this ore (Richards 1990–1992; Ogilvie 2002). In addition, the mineralogical heterogeneity of the iron ore samples further draws attention to the need for much more detailed chemical analysis of the variability in the iron ore's chemical contents. It also suggests a strong possibility that when the minerals were fractionated based on particle size, there could have been a co-fractionation based on mineral types, which could occur during the milling/grinding process. This could explain the variable results with regards to the greater percentage loss of P and K in the coarser iron ore.

Contrary to results from previous studies where pH of soil was shown to be lowered by mycorrhizal plants compared to non-mycorrhizal plants (Cromack et al. 1979; Berthelin 1983; Arocena and Glowa 2000), our investigation showed that both mycorrhizal and non-mycorrizal plants can lower the pH of the growth medium. This result suggests that non-mycorrhizal roots can also participate in weathering processes. Furthermore, statistical analyses of the pH values indicated that mycorrhizal plants could have significant effects on the pH but this depends on mycorrhizal type. Meanwhile, on average, mycorrhizal colonisation had little effects on pH, a situation that may be linked to significant effect of interaction between fungal type and particle size. Another reason may be the mediating effects of pH, which may lead to the oxidation of inorganics such as sulphur, the production of organic acid and, high rate of NH_4^+ uptake by ECM plant roots (Berthelin 1983; Marschner et al. 1987; Arocena and Glowa 2000). The lower pH in the presence of particle size A compared to B can be explained by

the positive effects of good aeration (Calvaruso et al. 2006) in sand with bigger iron ore particles that may lower the pH to levels favourable for mineral weathering by facilitating metabolic activities in the sand. The only exception was the *L. bicolor* where the presence of iron ore minerals raised sand pH. This is similar to the result of van Scholl et al. (2006) that reported how hornblende addition affected the pH and concluded that the effect of pH can be species specific.

It is difficult to confirm the effects of mycorrhizal inoculation on plant health. For shoot dry matter, only *Paxillus involutus* and *Suillus tomentosus* plants exposed to size A and only *Pisolithus tinctorius* and *S. tomentosus* exposed to size B were larger than NMC plants. Such variability could possibly be linked to other factors that were not measured in this study. On the other hand, effect of mycorrhizal inoculation in this study could also be mycorrhizal dependent as indicated in *S. tomentosus* treatments with higher RDM and SDM than other treatments.

Potassium and phosphorus released/absorbed from the iron ore did not seem to have much effect on the plant growth. *S. tomentosus* mycorrhizal treatments did not absorb much K and P compared to other mycorrhiza treatments but had higher SDM and RDM than *Pisolithus tinctorius* treatment with the highest K mobilisation. This may be due to the fact that both mycorrhizal and non-mycorrhizal plants need not only K and P for optimal growth but also additional nutrients such as N (Smith and Read 2008). In addition, the effect of other microorganisms cannot be excluded because the experiment was carried out under non-sterile conditions.

In a previous study, Wallander and Wickman (1999) observed that weathering and release of K from biotite by *S. variegatus* occurred because of the fungal production of oxalate and citrate, which promoted plant growth and increased foliar K. In contrast, Calvaruso et al. (2010) observed that colonisation by *L. bicolor* S238N did not cause any increase in biotite weathering when compared to non-mycorrhizal pine but significantly contributed to plant growth through absorption of weathered nutrients. These findings are in agreement with our observations that point to different rates of weathering and absorption by the four mycorrhizal fungi investigated. This indicated that the weathering role of ECM fungi is species specific. The results obtained in the present study provided additional information about the differences and relationships that could exist between weathering, absorption and transfer of nutrient to plants.

Our study has also shown that both mycorrhizal and non mycorrhizal pine roots were able to mobilise nutrients from iron ore minerals. However, depending on the species, mycorrhizal plants can be more effective in nutrient mobilisation compared to non-mycorrhizal plants. To the best of our knowledge, there has not been any study that directly investigated the effects of mycorrhizal plants on the mobilisation of nutrients from iron ore minerals. This study therefore provided an opportunity to look into nutrients release from an ore that consists of different minerals bonded together. Although it was inferred that both mycorrhizal and non-mycorrhizal plants can contribute to the release of nutrients from iron, the mineral absorption preferences of mycorrhizal plants was not investigated. Iron ore consists of different minerals, which serve as potential sources of nutrients for mycorrhizal plants. Despite the results obtained in this study, which showed the release of both P and K from the ore, it was not possible to indicate which mineral part of the ore contributed to the released elements. By this, it is still necessary to further investigate the fate of mycorrhizal plants when faced with a scenario of different mineral options. Are they going to absorb nutrients preferentially? If not, are they going to absorb nutrients from the minerals at equal rates? These are ideas that will eventually help shape our future understanding of nutrients absorption from minerals by mycorrhizal plants in complex environments.

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