CHAPTER TWO

IMPLICATION OF ECTOMYCORRHIZAL WEATHERING OF IRON ORE MINERALS: THE BIOLEACHING CONNECTION

Abstract

Ectomycorrhizal bioleaching as a stage in mineral weathering was investigated in order to establish the relationship between the effects of ectomycorrhizal (ECM) fungal and chemical constitution of minerals. Such relationships can have a great impact on mining as it can either improve or reduce the quality of the minerals. Low grade quality iron ore minerals from Sishen iron ore mine were utilised in this study in an experiment that involved both mycorrhizal and non-mycorrhizal *Pinus patula*. Four types of ECM fungi were used, namely *Pisolithus tinctorius* (PT), *Paxillus involutus* (PI), *Laccaria bicolor* (LB) and *Suillus tomentosus* (ST). 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 influence the chemical constitution of the iron ore minerals.

2.1 Introduction

Mineral weathering can be described as a combination of different physical, chemical and biological processes that can improve and boost the nutrients supply to plants (Smith and Read, 1908; van Breemen *et al*., 1900a; van Breemen *et al*., 1900b; Schoenholtz *et al*., 1900). Soil biological systems play a major role in mineral weathering (Arocena and Glowa, 2000). In the process of establishing some survival strategies, soil organisms such as plants, saprophytic and symbiotic microbes are able to interact in different ways to produce varieties of metabolites that are involved in weathering (Banfield *et al*., 1999; Schoenholtz *et al*., 1900). At the symbiotic level, ectomycorrhizal (ECM) fungi are actively involved in mineral weathering (Smith and Read, 2008). Ectomycorrhizal fungi are beneficial and like other types of mycorrhizal fungi, they form mutualistic associations with suitable plants (Smith and Read, 2008). These fungi colonise the roots of compatible plants by forming an association that is intercellular, just outside the root cells and the hyphae never penetrate the root cells (Smith and Read, 2008). They are able to absorb nutrients on behalf of their host plants, especially when nutrients are in short supply or unavailable to plants. Such nutrients are subsequently passed onto their host in return for carbon supply by the plants (Bolan, 1991; Smith and Read, 2008).

Ectomycorrhizal fungi have special capability to absorb nutrients from rocky and hard mineral materials (Smith and Read, 1908; Jongmans *et al*., 1997; Arocena and Glowa, 2000; van Scholl *et al*., 2006b). The link between weathering and ECM fungi was first noted by Jongmans *et al*., (1997) while investigating the tunnels found inside mineral grains obtained from podzolic soil. These authors suggested that the tunnels were created by ECM fungi over a long period of time. Their findings were further corroborated by another study that investigated feldspar tunneling. In that study, it was discovered that ECM root density was constantly proportional to the feldspar tunneling, which was also related to the release of nutrients such as K and Ca from the mineral (Hoffland *et al*., 2003). In another study by Leake *et al*., (2008), the ectomycorrhiza formed between *Paxillus involutus* and *Pinus sylvestris* was suggested to be actively involved in the

weathering of apatite, quartz and biotite, and also altered the potassium content of these minerals. Their study also confirmed that weathering rate is dependent on mineral type and proportional to the surface area-to-volume ratio of the mineral. Although other soil microorganisms contribute to mineral weathering (Banfield *et al*., 1999), ECM fungi play a major role through high production of low-molecular-weight HOC=O compounds known as organic acids (Paris *et al*., 1995; Jones, 1998; Smith and Read, 1908). Many studies had proposed that factors such as pH, nutrient limitation, grain size, organic acid production, and mechanical penetration of the mineral have different influences on mineral weathering (Burford *et al*., 1903; Jain and Sharma, 1904; Balogh-Brunstad *et al*., 1908). Of all these factors, production of organic acid is probably the most acknowledged (Burford *et al*., 1903; Jain and Sharma, 1904).

Investigation of mineral weathering in the mining industry has been mostly in two ways – bioleaching and bioremediation (bioremediation is outside the scope of present study) - both processes rely primarily on production of organic acids by microorganisms (Burford *et al*., 1903; Jain and Sharma, 1904). The word bioleaching is mostly used to describe the microbial solubilisation of metals for extraction purpose (Bosecker, 1997) and can also be used to estimate the weathering budget (Balogh-Brunstad *et al*., 1908; Calvaruso *et al*., 1909). Most biological weathering investigations that involved ECM fungi have focused on agricultural benefits (Balogh-Brunstad, *et al*., 1908; Calvaruso *et al*., 1909), leaving a gap about the knowledge of how this process can change the chemical constitution of minerals. It was therefore hypothesised in this study that when ECM fungi are present, their weathering capabilities can contribute to the quality of naturally occurring minerals through bioleaching. In this situation, non–exportable Sishen iron ore minerals, with high levels of K and P (Williams, 1909), were used. High levels of K (>0.24%) and P (>0.03%) contents diminish the market values of iron ore minerals and could render them non-exportable (Parks *et al*., 1990; Yusfin *et al*., 1999; Williams and Cloete, 1908; Delvasto *et al*., 1909). The purpose of this study was therefore to investigate potential mobilisation effects of ectomycorrhizal colonised plants on chemical constitution of minerals such as iron ore, through weathering.

2.2 Materials and Methods

2.2.1 Origin of ectomycorrhizal fungi

Ectomycorrhizal isolates used and their origins are *Pisolithus tinctorius* - # PT 7 (Plant Health Care Inc., Pittsburgh, USA), *Paxillus involutus* - NOF 2340 (Canada), *Laccaria bicolor* - LB (Canada) and *Suillus tomentosus* - UAMH 6252 (Canada).

2.2.2 Iron ore preparation

The iron ore sample used, KGT (conglomerates), was supplied by KUMBA iron ore resources Ltd. The KGT sample, originally contained an average of 0.995% of K_2O and 0.152% of P. KGT also contains SiO_2 (4.87%), Al_2O_3 (3.26%) and Fe_2O_3 (90.70%). Trace values of TiO_2, CaO, MgO, Na_2O, MnO, Cr_2O_3, NiO, V_2O_5 and ZrO_2 were present in the ore sample. The ore materials were milled and separated into two particle sizes of 3.36 mm - 1.68 mm and 1.68 mm - 0.84 mm with mesh of different sizes. Henceforth, these would be referred to as particle sizes A and B, respectively. The iron ore materials were treated with 0.1M HCl by soaking overnight to reduce the effects of high-energy surface sites created by grinding (van Scholl *et al*., 2006b). After 24 h, the samples were thoroughly washed under distilled water to remove fine particles and the pH adjusted to 4. This was followed by the addition of distilled water to the iron ore mineral and shaking for 7 d at 100 rpm. The iron ore materials were finally washed with distilled water and dried overnight in an oven at 60 °C.

2.2.3 Preparation of seeds

Seeds of *Pinus patula* obtained from Komatiland Forest (KLF), 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 3-5 times under 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 to 2cm long.

2.2.4 Mycorrhizal synthesis experiment

Autoclavable Magenta boxes (Magenta. Corp., Chicago, Ill., USA) were used for the experiment. A 50 ml aliquots of Modified Melin Norkrans (MMN) medium (Marx, 1969) containing Malt extract (3g/l), $(NH_4)_2HPO_4$ (0.25g/l), $MgSO_4.7H_2O$ (0.075g/l), $CaCl_2.H_2O$ (0.067g/l), NaCl (0.025 g/l), $FeCl_3$ (1%), thiamine (100µg/l) and Agar (10g) was used for the experiment. 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 autoclaved medium was poured into the boxes, they were covered with a layer of autoclaved cellophane paper, which was meant 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 7-d old cultures. Three plugs of ECM mycelia were inoculated on the cellophane paper at equal distances from one another. This was incubated at a temperature of 28 °C. After 5 d, germinated seedlings of *Pinus patula* were carefully introduced into the centre of the flasks containing growing mycelia of the fungi, and the boxes were incubated under sterile conditions at 23 °C/16 °C (d/night), 16 h photoperiod and 80% humidity for a period of 12 weeks. 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 mycorrhizal association. The root samples were observed under the dissecting microscope Leica S4E microscope (Leica Microsystems Imaging Solutions, Cambridge, UK) for hyphal sheath structural development as an indication of mantle formation. Representative roots that could not be confirmed as colonised under dissecting microscopes were further examined under a light microscope after staining using the method described by Smith and Dickson (1997) (Appendix I).

2.2.5 Soil Treatments

The growth medium was 100% sand soil obtained from Sable Marco Inc., Pont-Rouge, Québec Province, Canada. Soil was soaked in 0.1 M HCl overnight in order to remove fine dust and exchangeable bases. After 24 h, the soil was washed continuously for several hours under distilled water and later dried in the oven for 3 d. Soils were sieved with an electroformed sieve to particle sizes of 0.25 – 0.59 mm which allows an easy separation of soil from the iron ore minerals of particle sizes A and B (bigger) at the end of the experiment. The sieved soil 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 soil was kept sterile until the beginning of the weathering experiment.

2.2.6 Weathering experiment

Plastic pots of size (80 X 80 X 70 mm) were sterilised by soaking in 3% NaOCl overnight and washed several times under distilled water. These pots were then filled to the brim with the autoclaved/treated soil mentioned above. Planting holes, big enough to hold seedlings were created in the soil and were partially filled with 4 g of the prepared iron ore samples. In the same hole, ectomycorrhizal colonised (EMC) and non-mycorrhizal colonised root (NMC) sections - radicle of all the healthy seedlings - were introduced and covered with soil. The incubation was at 23 °C/16 °C (d/night), 16 h photoperiod and 80% humidity. This experiment lasted for 24 weeks. All treatments were performed with 4 replicates.

2.2.7 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

Scholl *et al*., 1906b). The final solution of the Hoagland contained the following – $Ca(NO_3)_2$ -7 ml, $NH_4(PO_4)$ – 2 ml, $MgSO_4$ – 1 ml, trace elements-1 ml (H_3BO_3 - 2.8 g/L, $MnCl_2.4H_2O$ - 1.8 g/L, $ZnSO_4.7H_2O$ - 0.2g/L, $CuSO_4.5H_2O$ - 0.1 g/L and $Na_2MO_4.2H_2O$ – 0.025 g/L) and 1 ml FeEDTA (15 g/L). For the controls that included 1.) no iron ore samples (CT) and 2.) no ECM fungi and no iron samples (CTR), the nutrient solution contained - $Ca(NO_3)_2$ - 7 ml, $KH_2(PO_4)$ – 2 ml, KNO_3 - 5 ml, $MgSO_4$ – 2 ml, Trace elements - 1 ml (H_3BO_3 - 2.8 g/L, $MnCl_2.4H_2O$ - 1.8 g/L, $ZnSO_4.7H_2O$ - 0.2 g/L, $CuSO_4.5H_2O$ - 0.1 g/L and $Na_2MO_4.2H_2O$ – 0.025 g/L) and 1 ml FeEDTA (15 g/L).

2.2.8 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 soil and iron ore samples through sieving. The roots were thereafter severed from the shoot system, washed free of the soil and transported to the laboratory for further processing. In the laboratory, the root samples were thoroughly washed again with distilled water to remove any trace of the soil and then prepared for light microscopy examination where percentage root colonisation was determined. The plant shoots and roots were separately dried in the oven and weighed to obtain root dry mass (RDM) and shoot dry mass (SDM). The sieved soil samples free of the iron ore samples were divided into two for dry mass calculation, pH determination and organic acid analysis.

2.2.9 Organic acid analyses

Four g of the harvested soil was put into 25 ml centrifuge tubes and 20 ml of 10 mM NaH_2PO_4 were added (Mimmo *et al*., 2008). The mixture was shaken for 4 hrs at room temperature and then centrifuged at a speed of 9300 rpm for 8 min at 20 °C. Ten ml of the supernatant were collected and kept at -20 °C until further analysis. A 1-ml subsample was collected and evaporated to dryness on a SAVANT Speed Vac Plus evaporator (SC210A) system (Fisher Scientific, ON, Canada) and then re-suspended in 200 µl of demineralised water, vortexed and left at room temperature for 15 min. The

resuspended samples were vortexed, transferred into 1.5 ml tubes and centrifuged at 13,000 rpm. Fifty µl of each sample were analysed by high pressure liquid chomatography (HPLC). The chromatographic conditions were a modification of the separation method described in Schneider *et al*. (1987). The HPLC analytic system was controlled by WATERS Empower software (WATERS, Milford , MA , USA) and was composed of Model 1525 pump, a Model 717 ^{plus} autosampler, and a Model 2487 dual absorbance detector. Organic acids were separated on a Bio-Rad HPX-87H column (Bio-Rad, Hercules, Ca, USA), eluted isocratically at 25 °C at a flow rate of 0.6 mL min^{-1} with 0.008 N sulfuric acid and detected on dual absorbance detector set at 210 nm. Peak identity and organic acid quantity were determined by comparison with standards. The organic acid standard included oxalic acid, citric acid, malonic acid and maleic acid that were well separated under the described chromatographic conditions.

2.2.10 Plant root and shoot analyses

Roots and shoots of the harvested plants were dried at 65 °C for 48 h. Dry weights were recorded and the root and shoot samples were further analysed for potassium and phosphorus content by inductively coupled plasma-atomic emission spectrometer (ICP-OES Optima 4300 DV, Perkin Elmer, Waltham, MA, USA).

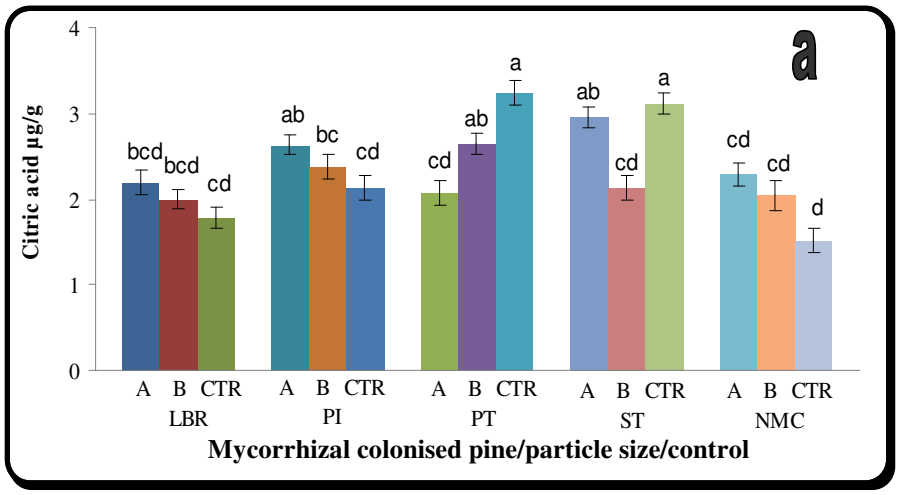
2.2.11 Statistical analyses

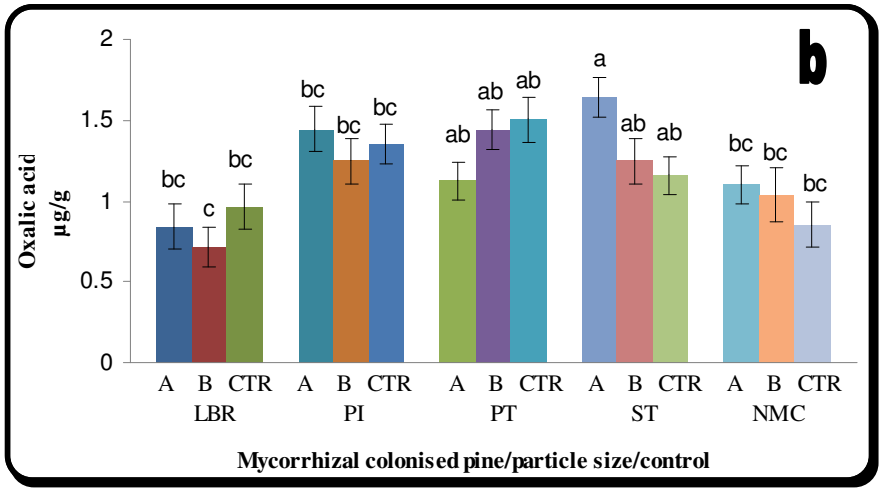
The weathering experiment was designed as a factorial involving the following treatments: 1.) Ectomycorrhizal-colonised plants with iron ore samples (EMC); 2.) Non-mycorrhizal plants (NMC) with iron ore samples; and 3.) Controls (CTR) for both EMC (EMC with no iron ore) and NMC (NMC with no iron ore). The statistical analyses for the weathering experiment therefore involved comparison of these treatments i.e. EMC, NMC and CTR. For organic acid analysis (oxalic, citric, maleic and malonic acids) and other dependant variables, two-way Anova model was used that included the plants colonised by the four fungal types for EMC and the NMC (i.e. PT, PI, ST, LBR and NMC), 3 levels of particle size (A, B and CTR). Similarly, for %K and %P loss, a two-

way Anova model was used with one group consisting of EMC and NMC (as described earlier) and the other consisting 2 levels of particle sizes (A and B). Following significant effects, multiple comparisons with the step down Bonferroni method were conducted to identify the differences between the treatments.

2.3 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 and more than half the entire population (EMC) having more than 60% colonisation rates. These levels of mycorrhization were enough to assess the effects of mycorrhization on the weathering process. The statistical analyses revealed different significant effects of both dependent and independent factors. For the organic acid analysis, there was a statistically significant interaction (P< 0.0001) between the fungal type, particle size and organic acids produced during the experiment.





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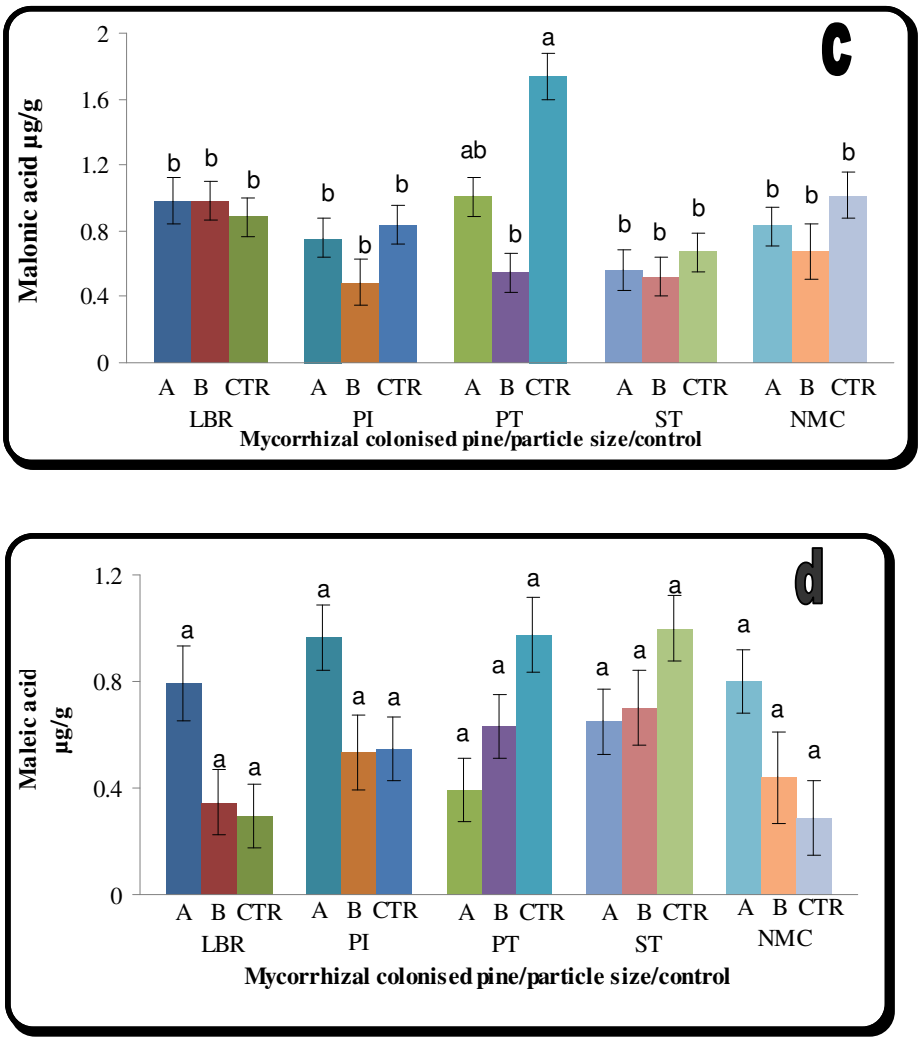


Figure 2.1: Amount of Citric acid (a), Oxalic acid (b), Malonic acid (c) and Maleic acid (d) released by the four EMC (LBR, PI, PT, ST) and the NMC roots grown in the presence of KGT iron ore (particle sizes A and B; CTR - control without iron ore). Bars with the same number are not significantly different (P<0.0001).

In general, citric acid was the highest produced organic acid detected in the soil samples of all the treatments (Fig. 2.1 a). Control and EMC (particle size A for ST and B for PT) treatments of both PT and ST produced the highest amount of citric acid. However, for

oxalic acid, there was no significant difference in the quantities produced among the EMC and CTR treatments, except for LBR and NMC/CTR treatments with statistically significant lower values (Fig. 2.1 b). There was no significant difference between the quantities of maleic and malonic acid detected from all the EMC and CTR treatments (Fig. 2.1 c and d).

Table 2.1: 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. The effects of these factors on shoot and root DM were also shown. P values <0.05 are considered significant

Factors	df		Sources of Variation		
			Fungi	Particle size	Fungi vs particle size
		df	4	1	4
%K	**134**	*F*	98.36	193.32	46.95
		P	<.0001	<.0001	<.0001
%P	**134**	*F*	11.13	64.34	6.63
		P	<.0001	<.0001	<.0001
		df	4	2	8
Soil K	**201**	*F*	24.09	1135.82	16.9
		P	<.0001	<.0001	<.0001
Soil P	**201**	*F*	35.63	14.31	5.35
		P	<.0001	<.0001	<.0001
		df	4	2	8
Shoot K	**189**	*F*	15.22	1868.44	8.74
		P	<.0001	<.0001	<.0001
Shoot P	**189**	*F*	11.81	35.43	5.39
		P	<.0001	<.0001	<.0001
		df	4	2	8
Root K	**197**	*F*	52.5	2030.39	35.82
		P	<.0001	<.0001	<.0001
Root P	**197**	*F*	29.44	3.44	13.98
		P	<.0001	0.034	<.0001
		df	4	2	8
Shoot DM	**197**	*F*	138.99	3.56	40.23
		P	<0.0001	0.0301	<0.0001
Root DM	**197**	*F*	265.09	1.84	37.41
		P	<0.0001	0.1616	<0.0001

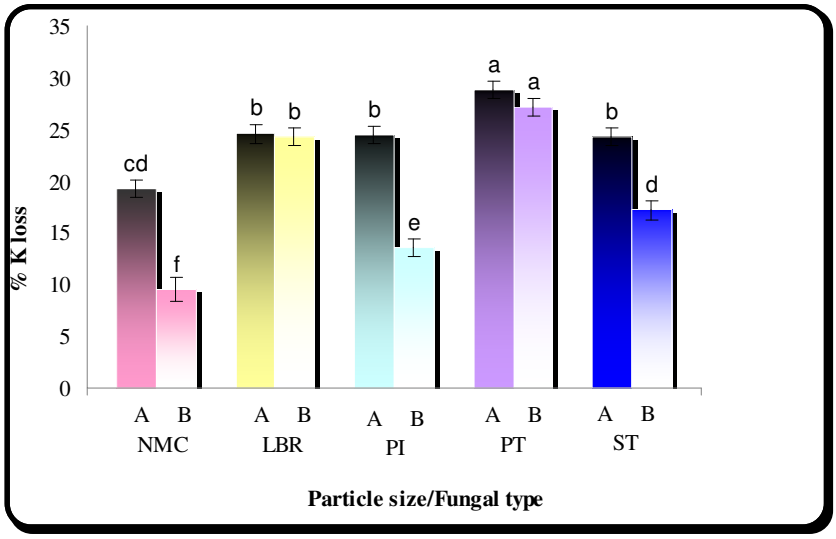


Figure 2.2: Percentage of K reduction from iron ore mineral in response to the four different fungal treatments (PFR, PI, PT and ST) and NMC using two particle sizes (A and B) of the iron ore materials. Bars represent standard errors (P<0.0001).

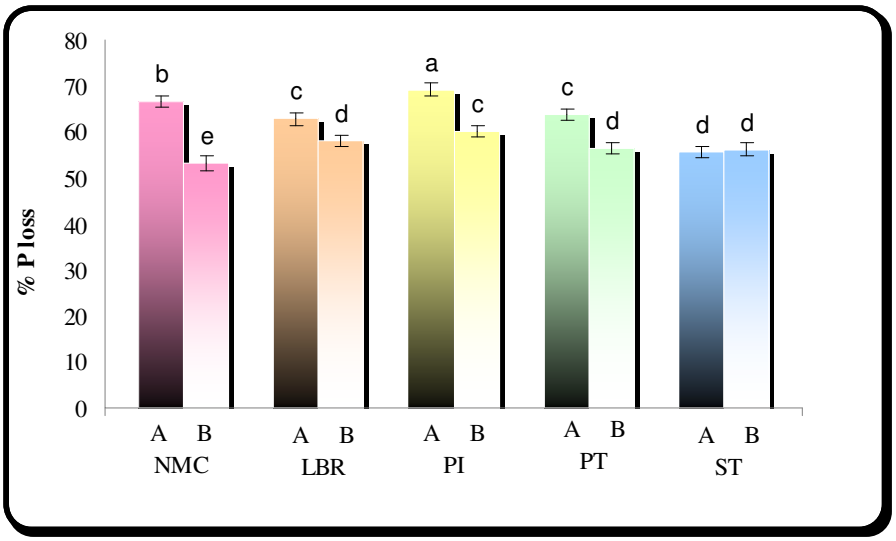
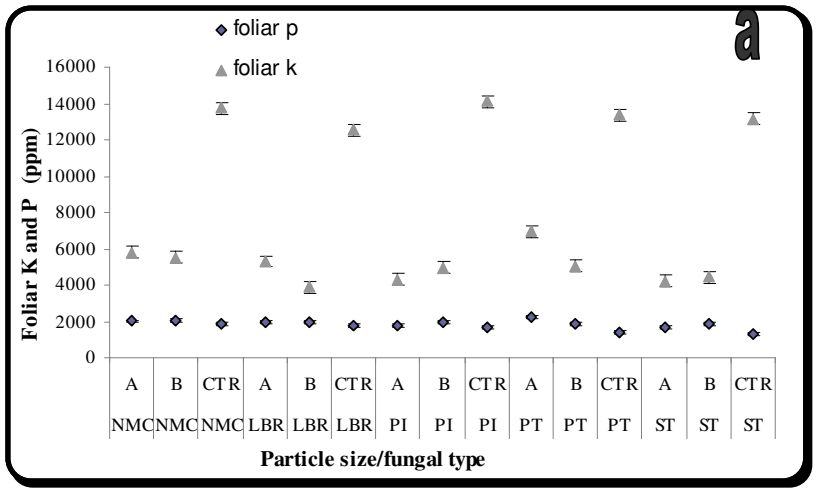


Figure 2.3: Percentage of P reduction from iron ore mineral in response to the four different fungal treatments (PFR, PI, PT and ST) and NMC using two particle sizes (A and B) of the iron ore materials. Bars represent standard errors (P<0.0001).

Fungal type, particle size and the interaction between the two factors significantly affected the mobilisation of K and P from iron ore minerals (Table 2.1). More K and P quantities were removed from treatments involving particle size A compared to B (Fig. 2.2 and 2.3). Highest amounts of % K loss (28.8%) from the iron ore was from treatments containing fungal type PT with particle size A (Fig. 2.2). In addition, the lowest K reduction was from the NMC treatment (particle size B). Similarly, more %P loss was recorded from treatments involving particle size A compared to B (Fig. 2.3). Meanwhile, the highest P reduction from the iron ore was recorded from PI treatment of particle size A (69%), while the lowest (53.16%) was from the CTR treatment with particle size B (Fig. 2.3). In addition, the soil pH was significantly affected by interaction between the fungal type and particle size (Table 2.1). All EMC (except LBR) and NMC plant treatments have a slightly lower pH compared to CTR treatments (Fig. 2.4 c). Therefore, mycorrhizal status seemed not to directly affect the pH status of the soil. Particle size was observed to have statistically significant effect on the soil pH. The pH was lower in presence of particle size A compared to particle size B.

All the interactions between the fungal type and particle size have effects on foliar, root and soil P and K (Table 2.1). Meanwhile, due to the full Hoagland nutrient solution used to fertilise CTR treatments, they had significantly higher values of foliar K compared to EMC and NMC treatments (Fig. 2.4 a). The highest value of foliar K for the EMC treatment was recorded in PT treatment with particle size A, while the lowest was from LBR treatment with particle size B (Fig. 2.4 a). Comparable trend was observed for foliar P where the treatment involving PT (particle size A) had the highest shoot P, while the lowest was ST, particle size A (Fig. 2.4 a). However, there were higher values of foliar P in both the EMC and NMC treatments compared to CTR treatments (Fig. 2.4 a). This is due to the presence of two different phosphate sources for these treatments during the experiment; one in the Hoagland solution and the other from iron ore minerals.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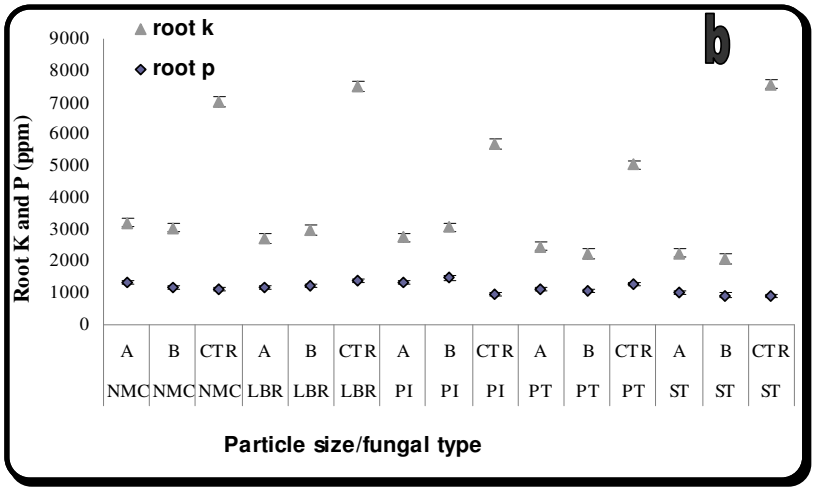
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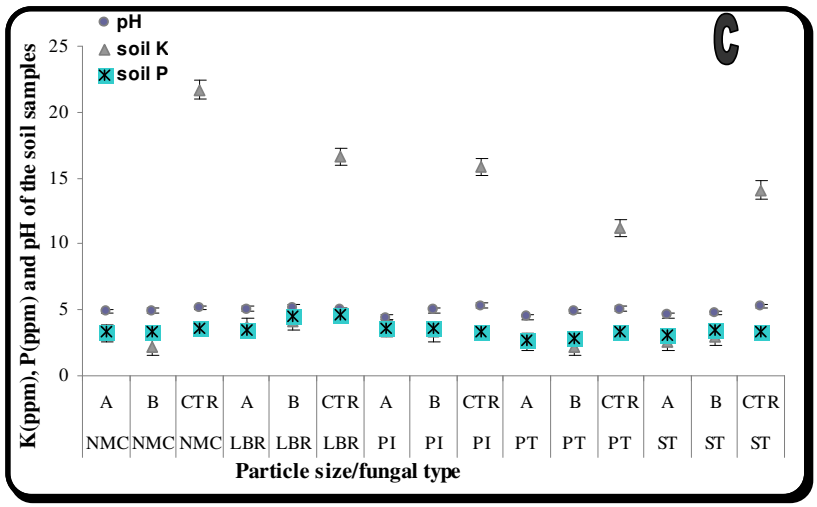
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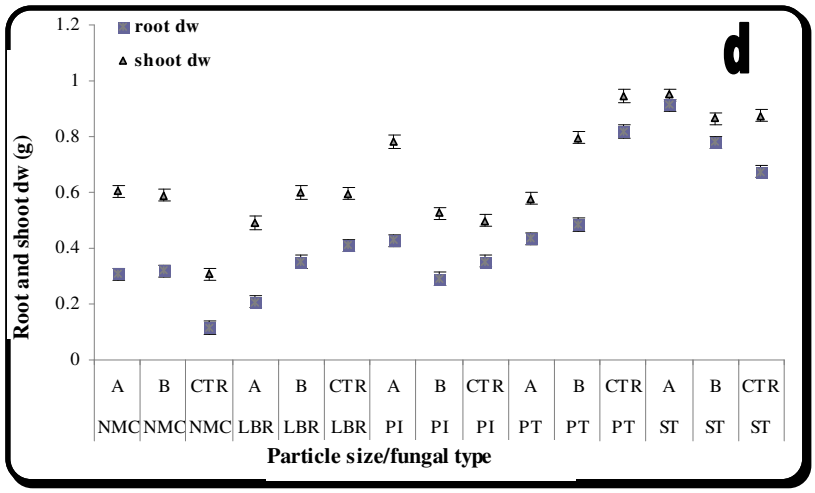

Figure 2.4: Amounts of measured (a) foliar K and P, (b) root K and P, (c) soil K and P, as well as pH, and (d) values of root and shoot DM for EMC (LBR, PI, PT and ST), NMC, as well as the controls (CTR). A and B are the two particle sizes. Bars represent standard errors (P<0.0001).

The NMC plant treatments with particle size A and B had statistically significantly higher root K values compared to EMC plant treatments (Fig. 2.4 b). For the EMC treatments, highest root K was recorded in LBR treatment with particle size B while the lowest was recorded from ST treatment with particle size B (Fig. 2.4 b). Plants with PI treatments retained more P than all other EMC treatments in the roots, while the lowest P root retention was obtained in ST with both particle size A and B (Fig. 2.4 b).

Statistical analyses of the soil nutrient status after the experiment revealed that there was no significant difference between the amount of K in soil with particle size A and B for all the mycorrhizal plant treatments (Fig. 2.4 c). However, as expected, CTR treatments (full hoagland nutrient solution supply), had higher amounts of soil K. Highest value of soil K recorded was from LBR treatment with particle size B, while the lowest was from PT treatment (Fig. 2.4 c). Similarly, values for soil P in both treatments with particle size A and B were not significantly different. The NMC treatments had slightly higher values of P than the EMC plants except, for PI where the values from the CTR treatment was slightly lower than those with iron ore of the two particle sizes (Fig. 2.4 c).

There were significant effects of fungal type, particle size and their interaction on both SDM and RDM (Table 2.1). The highest values of both shoot and root DM were recorded in ST treatments, while the lowest was in the CTR treatment of NMC (Fig. 2.4 d).

2.4 Discussion

Mineral weathering is a process that has been interpreted in different ways to enable its applicability in various industrial applications. Land rehabilitation and remediation, biohydrometallurgy, agroforestry, among others, are some of the technological advancements that have benefited from underlying mechanisms of mineral weathering (Ahonen *et al*, 2000; Vasan *et al*., 2001; Jain and Sharma, 2004; van Scholl *et al*., 2006b; van Scholl *et al*., 2008; Khosla and Reddy, 2008). Traditionally, mineral weathering is believed to largely depend on organic acid availability that affects the mineral dissolution through three major mechanisms. These mechanisms are complexation of cations from the mineral surface, lowering of the pH towards acidic range and selective binding of Fe and Al ions (Drever and Stillings, 1997). Both plant roots and their associated microorganisms have been implicated in organic acid production in the rhizosphere. Of the most important soil microbes that produce organic acids, ectomycorrhizal fungi have been well acknowledged (van Scholl *et al*., 2008). However, because of the complex microbial population mix in the rhizosphere, it is difficult to estimate the organic acid released into rhizosphere by ECM fungi (van Scholl *et al*., 2008). Nevertheless, investigators have managed to study this process under sterile and semi-sterile conditions, thereby reducing the risk of over- or under-estimation of the organic acids released by ECM fungi (van Scholl *et al*., 2006b; van Scholl *et al*., 2008).

This study has revealed that pine roots, whether mycorrhial or not, were able to mobilise between 9 and 29% K and more than 50% P from the iron ore sample, thereby confirming their participation in the weathering process. Higher values of P mobilisation indicate that roots are more efficient in P than in K mobilisation. This is in agreement with previous studies that identified both mycorrhizal roots and non-mycorrhizal roots as capable of participating in mineral weathering (Wallander and Wickman, 1999; Calvaruso *et al*., 2006; Calvaruso *et al*, 2009). For instance, Calvaruso *et al*. (2009) reported the participation of both mycorrhizal and non-mycorrhizal pine root in biotite weathering. Furthermore, this study shows that all the mycorrhizal plants had little

significant increase in K mobilisation from iron ore compared to non-mycorrhizal plants, while in contrast, non-mycorrhizal plant showed a better mobilisation of P than most mycorrhizal plant treatments. These contradictory results make it difficult to directly correlate the mobilisation of nutrients from the iron ore to the presence of ECM fungi. On the other hand, the present study clearly demonstrates the efficiency of PT to pass the absorbed K and P onto the shoot system, compared to the other mycorrhizal and non-mycorrhizal treatments. This suggests that PT participated in the absorption and transfer of dissolved mineral nutrients to the plant and the possibility of little drainage loss during the process. As explained by Calvaruso *et al*. (2009), it is possible to have similar efficiency in mineral weathering process between non-mycorrhizal roots and mycorrhizal roots. However, when it comes to the absorption of the dissolved mineral nutrients, mycorrhizal plants have been shown to be more effective (Smith and Read, 2008). This is due to the nature of ECM hyphal network, which usually spread to cover large surface area and to the ability of the fungus to penetrate hard mineral surfaces, while scavenging for nutrients. On the other hand, effective absorption of dissolved mineral nutrients through ECM fungi has also been identified to indirectly facilitate mineral weathering processes (Paris *et al*., 1995; Glowa *et al*., 2003). This can happen when there is a shortage of a particular nutrient in the growth medium. For instance, in the present study, the shortage of K in the nutrient solution available to mycorrhizal plants can favour scavenging as a feeding mechanism by the ectomycorrhizal hyphae (Banfield *et al*., 1999). The mineral constitution of the iron ore is probably another factor that affected the rate of K and P release from the iron ore. Sheng *et al*. (2008) showed in an experiment, involving three silicate minerals, that mineral type could determine the rate of nutrient release from minerals. The iron ore used in this study contained different types of minerals from which the K-bearing mineral was different from P-bearing mineral.

Among the four organic acids analysed from the soil samples in this study, citric acid was the highest produced, followed by oxalic acid. Several studies have highlighted the importance of these two organic acids in mineral weathering due to their high capability to chelate metals (Wallander and Wickman, 1999; Ahonen-Jonnarth, 2000; Smith and Read,

2008; van Scholl *et al*., 2008). Akin to the result obtained in this study, Wickman and Wallander (1999) reported large quantities of citric acid in weathering experiment involving *Pinus sylvestris* seedlings inoculated with *S. variegatus* using biotite mineral as source of K. Similarly, Ahonen-Jonnarth (2000) reported the increased oxalic acid production by *S. variegatus* and *Rhizopogon roseolus* colonised plants when Al concentrations were elevated.

Neither the nutrient (K) limitation nor the addition of iron ore minerals increased organic acid production by both mycorrhizal and non-mycorrhizal plants. For instance, the largest quantity of citric acid was produced by mycorrhizal (PT and ST) treatments that were fertilised with complete Hoagland's solution without iron ore minerals. This is similar to the result obtained by van Scholl *et al*. (2006a), where the P limitation did not increase the organic acid production by ECM plants compared to non-mycorrhizal plants. In that study, ECM colonisation did not increase total organic acid compared to non-mycorrhizal fungi, but affected the total organic acid production depending on the fungal species. With no statistically significant difference between the amount of citric acid detected in treatments with highest (PT) and lowest (NMC/CTR) 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 occurred as a result of 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 lead to mineral weathering. This is contrary to the findings of Wallander and Wickman (1999), where weathering by *Suillus variegatus* was linked to the production of citric and oxalic acid. However, caution must be exercised in the interpretation of organic acid result because the response could vary with soil type, treatments and storage (Mimmo *et al*., 2008).

Another factor that significantly affected the weathering process in this study was the particle size. Generally, there were more nutrients 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 particle size mineral and concluded that exposure of

larger surface area (finer particle size) of mineral particle to leaching agent increases the possibility of solubilisation. However, the opposite result obtained in the present study may be due to aeration of the growth medium. Larger particle size ores may allow more aeration of the growth medium, a factor which has been mentioned to affect mineral weathering by microorganisms (Calvaruso *et al*., 2006). Similar effects of aeration were also suggested by Kazantseva *et al*. (2009), especially for mycorrhizal plants. In addition, the importance of aeration to the functionality of mycorhizal and non-mycorrhizal roots was highlighted in the study by Wallander and Wickman (1999), where improper aeration was suggested as a factor affecting the weathering process.

In contrast to previous studies where pH of soil was shown to be lowered by mycorrhizal plants as compared to non-mycorrhizal plants (Cromack *et al*., 1979; Berthelin, 1983; Arocena and Glowa, 2009), the result in the present study showed that non-mycorrizal plants can also lower the pH of the growth medium. This suggests the capability of non-mycorrhizal roots to also participate in weathering processes. Furthermore, statistical analyses of the pH values indicated that mycorrhizal colonisation had little effects on K and P mobilisation, a situation which may be connected to significant effect of interaction between fungal type and particle size. Another reason could be the causal effects of pH, which may lead to the oxidation of inorganics such as sulfur, organic acid production and high rate of NH_4^+ uptake by ECM plant roots (Berthelin, 1983; Marschner *et al*., 1987; Arocena and Glowa, 2009). The lower pH in the presence of particle size A compared to B can be connected to positive effects of proper aeration (Calvaruso *et al*., 2006) on soils with bigger particle size of iron ore. This may allow better metabolic activities in the soil, thereby lowering the pH to favour mineral weathering. Statistical results of pH from non-mycorrhizal, PI, PT and ST treatments with iron ore minerals compared to their respective CT, suggest that the presence of iron ore minerals and probably the omission of K from the Hoagland solution, lowered the pH of the soil. The only exception was the LBR where the presence of iron ore minerals raised the pH of the soil. This is similar to the result of Scholl *et al*. (2006b) that reported how hornblende addition affected the pH, and concluded that the effect of pH can be species-specific.

The mycorrhizal inoculation seems to have positive effects on the health of the plants (especially for ST treatment) when RDM and SDM of both mycorrhizal and non-mycorrhizal plants were compared. This may be due to functionalities of the mycorrhizal hyphal network that penetrates and covers more area as compared to ordinary roots when in search of nutrients (Smith and Read, 2008). However, potassium and phosphorus released / absorbed from the iron ore do not seem to have much effect on the health of the plants, as indicated in the result. ST mycorrhizal treatments did not absorb much K and P compared to other mycorrhiza treatments, but had higher SDM and RDM than PT 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, effects of other microorganisms cannot be excluded because the experiment was carried out under non-sterile conditions.

In a previous study, Wallander and Wickman (1999) suggested that weathering and release of K from biotite by *Suillus variegatus* occurred because of the fungal production of oxalate and citrate, which promoted plant growth as well as an increase in foliar K. In contrast, Calvaruso *et al*. (2009), observed that colonisation by *Lacarria bicolor* S238N did not cause any increase in biotite weathering when compared to non-mycorrhizal pine, but significantly contributed to plant health through absorption of weathered nutrients. These two findings support the observation in the present study of different rates of weathering and absorption exhibited by the four mycorrhizal fungi investigated. This indicates that weathering roles of ECM fungi is species-specific. Therefore, the results obtained in the present study provided additional information about the differences that exist between weathering, absorption and transfer of nutrient to plants. There are many implications of weathering and other subsequent events that follow the process.

This study has shown that whether mycorrhizal or not, pine roots are 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. If ECM fungi are truly involved in mineral tunnelling then it could be suggested that they

are part of the biotic factors that determine the chemical constitution of minerals. Despite the positive results obtained in this study regarding this hypothesis, there are still more investigations that need to be carried out. This is very necessary in a natural environment where presence of ECM fungi could be directly linked to changes in chemical constitution of minerals. Long term studies are needed and these could be conducted by comparing minerals in ECM infested environments to those in non-ECM environments. Other factors such as biotic and abiotic factors should also be considered.

2.5 References

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