

METAL DISSOLUTION FROM RHIZOSPHERE AND NON-RHIZOSPHERE SOILS USING LOW MOLECULAR WEIGHT ORGANIC ACIDS.

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This research is part of an investigation of micro-biogeochemistry in the rhizosphere of co-occurring *Eucalyptus mannifera* and *Acacia falciformis* on the Southern Tablelands of New South Wales, Australia (Little *et al.* 2004, Little *et al.* 2005). While there is still considerable controversy in the literature regarding the role of low molecular weight organic acids in mineral weathering and soil processes (e.g., Drever & Stillings 1997, Jones *et al.* 2003), there is growing evidence suggesting these compounds, especially dicarboxylic acids, have dramatic effects on mineral weathering and metal mobility in the rhizosphere. Previous studies have examined the roles low molecular weight compounds like malic, oxalic and citric acid (exuded by roots of cropping plants) play in forming stable soluble and insoluble complexes that aid in nutrient up take. Or in the cases of Al and Cd, these may restrict their entry into the root (Curl & Trulove 1986, Gobran *et al.* 2001). In addition, there have been a number of controlled experiments undertaken using pure minerals (e.g., Welch & Ullman 2000, Welch *et al.* 2002) and with respect to soil organic matter. However, few attempts have been undertaken in the context of 'real' soil environments (e.g., Giesler *et al.* 2000), especially in Australia.

The purpose of this work is to determine the effect of three root exudates (citric, malic and oxalic acid) on metal mobilisation from near-natural dry sclerophyll forest soils and the implications for mineral weathering and soil formation processes. This work is the first attempt to understand the roles played by low molecular weight root and microbial exudates in mineral weathering and metal mobilisation processes in a dry sclerophyll forest soil anywhere along the eastern seaboard of Australia.

METHODS

Rhizosphere and non-rhizosphere bulk soil samples collected from the A and B horizons from adjacent *E. mannifera* and *A. falciformis* trees were subjected to four separate treatments in sets of 3 replicates: +oxalic acid; +malic acid; +citric acid; and, +NaCl control solution. These three acids were chosen because they are produced by roots and associated microbial communities, they form strong stable complexes with metals in solution, and because of their established associations with the mobilisation of such nutrient elements as phosphorus (P), iron (Fe), calcium (Ca). One gram of dried sterilized soil was added to 200 ml of a ~ 1 mM solution that had initial pH adjusted to ~ 4.5 to mimic bulk soil pH. Solution aliquots were collected at day 1, day 8 (or 9 for the rhizosphere experiments) and day 15, and filtered through a 0.45 µm filter. Solutions were analysed for pH using a pH meter and electrode calibrated with standard buffers, and acidified to ~ 0.1% HNO₃ for analysis of major and trace metals by ICP-AES and ICP-MS.

Soil geochemistry and mineralogy

X-ray Diffraction was used to determine the mineralogy of the bulk soil and the clay fraction. A nitric-perchloric acid digest was performed on subsamples of the rhizosphere and non-rhizosphere soils and the supernatant was analysed by ICP-AES to determine total Al, Ba, Ca, Cu, Fe, Mg, Mn, K, Na, Sr and Zn contents in the soils. Sample size was too small to examine the mineralogy of the rhizosphere soils which was assumed to be similar to the mineralogy of the corresponding non-rhizosphere sample, although the amorphous contents might be greater in abundance. The soil materials consisted mostly of quartz and X-ray amorphous phases (primarily Fe and Al oxyhydroxides), with minor amounts of K-feldspar, muscovite mica and Na-feldspar, weathering to interstratified illite-kaolinite and kaolinite, which is consistent with the weathering of the sedimentary parent rocks. Most variation in soil mineralogy was observed in the amorphous content, i.e., soil organic matter in the A₁ horizon and poorly refractive Fe-Al oxyhydroxides in the B₂ horizon.

RESULTS AND DISCUSSION

In general, the dissolution experiments show that citric acid was the most effective in promoting mineral dissolution compared to the inorganic control. In contrast, the effect of malic acid was usually minimal compared to the NaCl solution, however, the effects of individual organic acids depended on the element,

reacting phase and time. In all of the experiments there was a rapid release of metals to solution within the first day, followed by a much slower increase in concentration, and in some cases a decrease in concentration over the next two weeks. This change in dissolution rate reflects a change in pH and concentration of free available organic ligands as the solid phase reacts. The organic acids had little impact on the release of readily soluble elements such as the alkaline and alkaline earth metals (Na, K, Rb, Mg, Ca, Sr, Ba). However, for elements such as Al and Fe, whose solubility is strongly pH dependant and form very strong stable complexes with organic ligands, concentrations were increased by a factor of 2 to 50 compared to the inorganic control (Figures 1 and 2).

The major ion concentrations from the bulk soil dissolution experiments were similar for the A₁ and B₂ horizons associated with *E. mannifera* and *A. falciformis* trees. Results of the Fe concentration in solution for the four soils are plotted in Figure 1A to 1D. Dissolved Fe concentrations from reacting soils in citrate solutions were up to 20 times greater than for the NaCl control. Iron concentrations in oxalate were approximately 2-4 times greater than in the control. Iron concentrations were approximately 30% greater in the experiments with the B₂ horizon soils compared to the A₁ horizon soils. Similar effects were observed for dissolved Al from the bulk soils. The organic acids had little effect on Si release to solution, increasing Si concentrations by a factor of about 2 compared to the inorganic control. This suggests that the bulk of the solubilization is from reactions with the 'amorphous' Al and Fe oxyhydroxide minerals and soil organic matter, and not with primary silicate or clay phases.

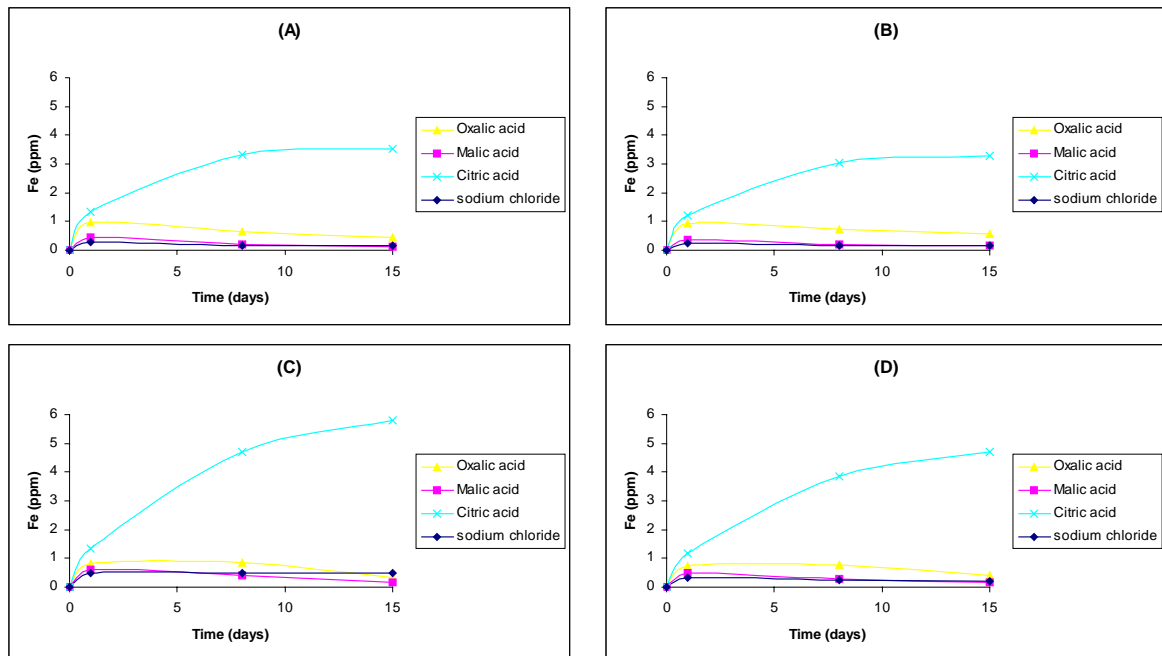


Figure 1: Dissolution trends (mg/L) over 15 days for Fe from 1 g rhizosphere soil samples using 1 mM organic acid solutions (A: eucalypt A₁ horizon; B: acacia A₁ horizon; C: eucalypt B₂ horizon; D: acacia B₂ horizon).

Results of the rhizosphere soils experiments show striking contrast between the A and B soil for metal solubilisation (Figure 2). For example, Fe release to solution in the A₁ horizon experiments increased rapidly in the first day of the experiments and then decreased slightly over the next two weeks. In the B₂ horizon experiments, Fe increased throughout, and total Fe concentrations were approximately 5 times higher than for the A₁ horizon rhizosphere soils. As in the bulk soil experiments, citrate increased Fe release approximately 10 times in the A₁ horizon compared to the NaCl controls, but up to 100 times higher concentrations were measured in the B₂ horizon experiments.

The differences in Fe leached from the rhizosphere versus bulk soils can be only partially explained by the Fe content in the solid phase. Fe concentration in the A₁ horizon for the bulk soils were 7.8 and 9.8 g/kg and in the B horizon were 13.8 and 13.2 g/kg for *A. falciformis* and *E. mannifera* trees respectively. This is consistent with the dissolution experiments that showed approximately 30-50% higher Fe concentrations in the B₂ versus A₁ soil horizons. The total amount of Fe solubilized in the experiments was at most 10% of the available mineral Fe. The Fe concentrations in the rhizosphere soils were slightly higher, 11.5 and 17.1 g/kg for A₁ horizon, and 11.3 g/kg and 24.7 g/kg for B₂ horizon for *A. falciformis* and *E. mannifera* trees

respectively. The leaching experiments for the A₁ horizon rhizosphere soils were very similar to the bulk soils, whereas, for the B₂ rhizosphere soils, leached Fe concentrations were up to 5 times greater, suggesting that the B₂ rhizosphere soil has a larger fraction of 'reactive iron'.

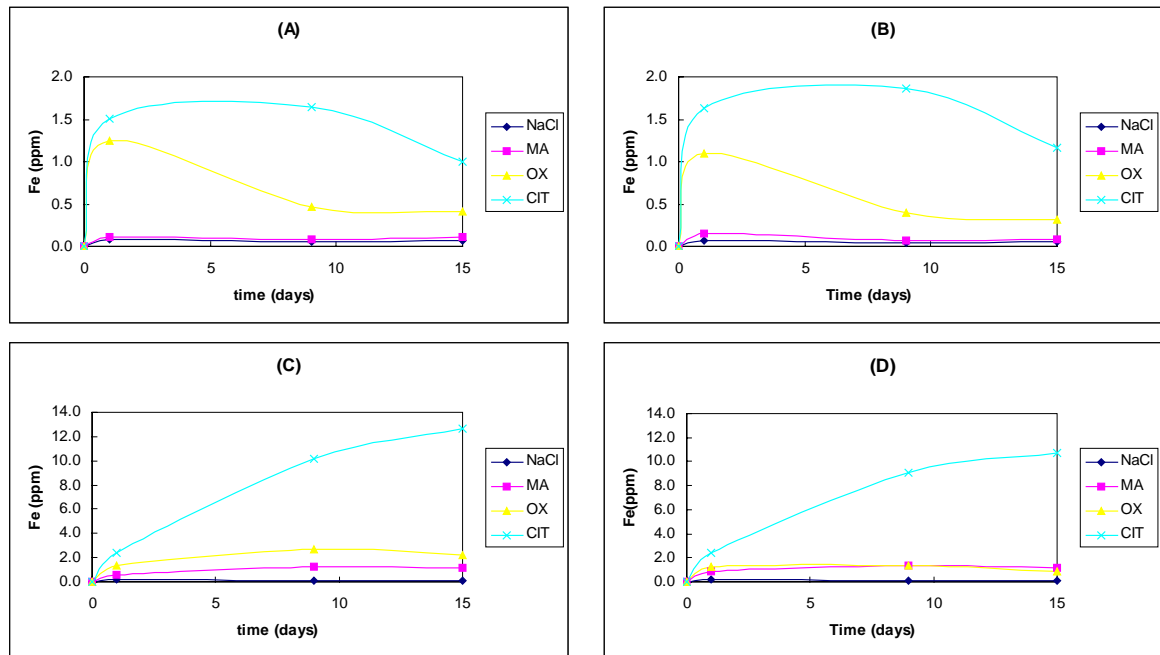


Figure 2: Dissolution trends ($\mu\text{g/L}$) over 15 days for Fe from 1 g rhizosphere soil samples using 1 mM organic acid solutions. (A: eucalypt A₁ horizon; B: acacia A₁ horizon; C: eucalypt B₂ horizon; D: acacia B₂ horizon).

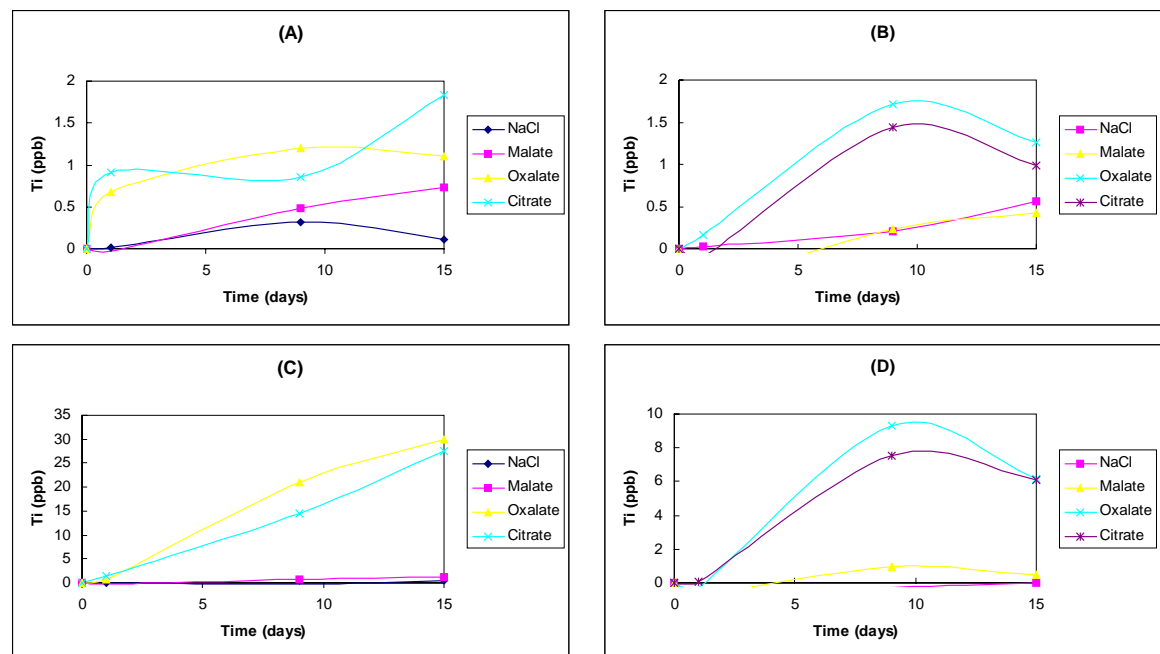


Figure 3: Dissolution trends ($\mu\text{g/L}$) over 15 days for Ti from 1 g rhizosphere soil samples using 1 mM organic acid solutions (A: eucalypt A₁ horizon; B: acacia A₁ horizon; C: eucalypt B₂ horizon; D: acacia B₂ horizon).

Trace metals leached from rhizosphere soils were much greater in the B₂ horizons than for the A₁ horizon or bulk soils. Trace elements, such as Pb, Ce, Ia, Ge, Ga, Cr, V leached to solution from the rhizosphere soil with concentrations 2 to 100 times higher in the organic acids solutions compared to the NaCl control. These results are consistent with similar experiments with pure mineral separates (Welch & McPhail 2003). In most

of the other experiments, trace metals were below detection. The behaviour of Ti and Zr in the rhizosphere soils (Figures 3 and 4) are of particular interest because they are generally considered to be immobile or conserved during mineral weathering and soil formation. However, in these experiments it has been shown that all of the organic acids, especially citric acid, have the ability to dissolve measurable quantities of these elements, thus allowing the possibility of their movement through the soil profile. This finding also supports work by Stiles *et al.* (2003) demonstrating the susceptibility of zircon and rutile to physical and chemical weathering processes. However, The dissolution of Ti and Zr follow that for Fe, and also Al (data not shown), suggesting that these elements may have been sorbed to Fe and Al oxyhydroxide phases.

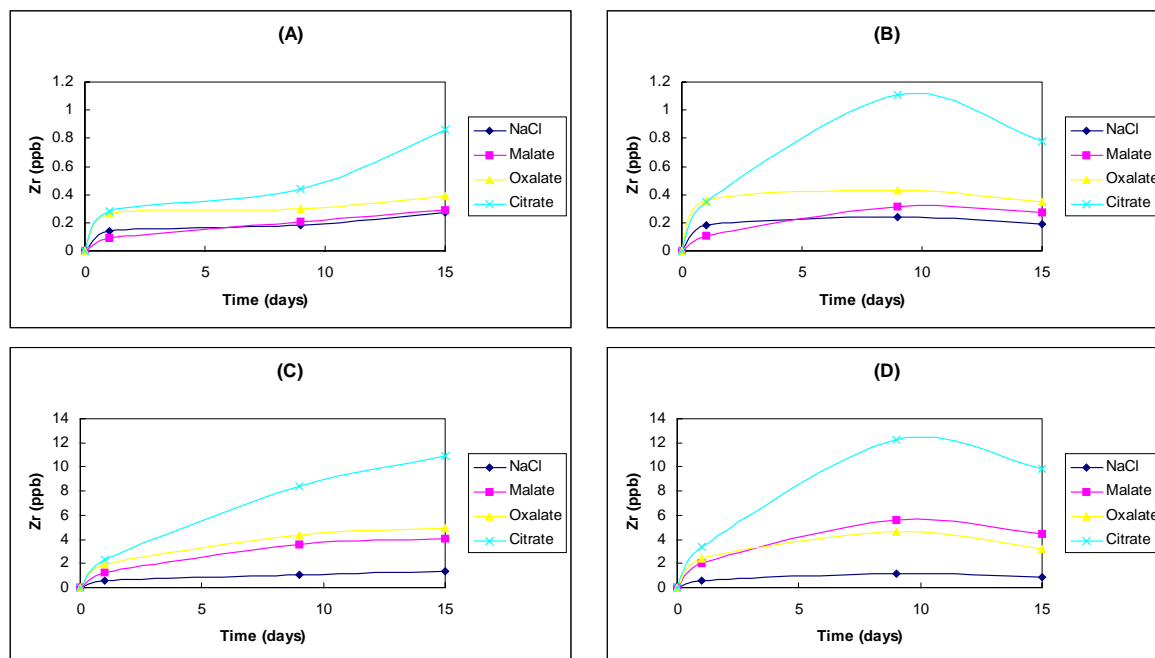


Figure 4: Dissolution trends ($\mu\text{g/L}$) over 15 days for Zr from 1 g rhizosphere soil samples using 1 mM organic acid solutions (A: eucalypt A₁ horizon; B: acacia A₁ horizon; C: eucalypt B₂ horizon; D: acacia B₂ horizon).

The results of these dissolution experiments with natural soils are consistent with other studies on pure mineral phases that show moderate concentrations of low molecular weight carboxylic acids enhance release of major ions, especially Al and Fe, from minerals and organic matter (Welch & Ullman 2000, Welch *et al.* 2002). Specifically, it has been shown that milli-molar concentrations of the organic acids under mildly acidic conditions greatly increased the release of major and trace elements to solution by up to 2 orders of magnitude compared to inorganic controls, and therefore have the potential to dissolve soil minerals and contribute to soil weathering. However, this work also shows that trace elements, many of which are micronutrients, can be preferentially mobilized by organic acids. This was particularly relevant to the rhizosphere soils, which showed high concentrations of trace metals mobilized by organic ligands compared to bulk soils. Further, these results also support findings of other studies demonstrating the beneficial effects of low molecular weight organic acids on metal uptake in nutrient limiting environments.

FUTURE WORK

Further investigation is required to:

1. Determine water soluble organic acid contents of the rhizosphere and non-rhizosphere;
2. Use acid mixtures similar to those observed in the water extracts to examine the combined influence of organic acids on mineral dissolution from these soils; and,
3. Correlate changes in geochemistry with microbial community dynamics

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