EFFECT OF MN DEFICIENCY AND LEGUME INOCULATION ON RHIZOSPHERE pH IN HIGHLY ALKALINE BAUXITE RESIDUE

P.M. Kopittke[A], N.W. Menzies[A], H.B. So[A] and I. Fulton[B]
[A] School of Land and Food Sciences, University of Queensland, Brisbane, Australia.
[B] Alcan Gove Pty Ltd, Nhulunbuy, Northern Territory, Australia.

Abstract
Although plant growth is often limited at high pH, little is known about root-induced changes in the rhizospheres of plants growing in alkaline soils. The effect of Mn deficiency in Rhodes grass (*Chloris gayana* cv. Pioneer) and of legume inoculation in lucerne (*Medicago sativa* L. cv. Hunter River), on the rhizosphere pH of plants grown in highly alkaline bauxite residue was investigated. Rhizosphere pH was measured quantitatively, with a micro pH electrode, and qualitatively, with an agar/pH indicator solution. Manganese deficiency in Rhodes grass increased root-induced acidification of the rhizosphere in a soil profile in which N was supplied entirely as NO_3^-. Rhizosphere pH in the Mn deficient plants was up to 1.22 pH units lower than that of the bulk soil, while only 0.90 to 0.62 pH units lower in plants supplied with adequate Mn. When soil N was supplied entirely as NO_3^-, rhizosphere acidification was more efficient in inoculated lucerne (1.75 pH unit decrease) than in non-inoculated lucerne (1.16 pH unit decrease). This difference in capacity to lower rhizosphere pH is attributable to the ability of the inoculated lucerne to fix atmospheric N_2 rather than relying on the soil N (NO_3^-) reserves as the non-inoculated plants.

Additional Keywords: legume, acidification, manganese deficiency, rhizosphere pH

Introduction
Approximately 30 MT of highly alkaline (pH 12) and sodic (ESP 100 %) residue are produced throughout the world annually from the refining of bauxite. Even following neutralisation with seawater and leaching with freshwater, pH (9.5) and sodicity (ESP 50 %) remain problematic. Revegetation of such areas often involves the use of salt tolerant groundcover species such as Rhodes grass (*Chloris gayana*) and legumes such as lucerne (*Medicago sativa*). Although the availability of many micronutrients (and in particular Mn) are low (Gherardi and Rengel 2001), plant growth in bauxite residue has been found to be better than anticipated. The mechanisms allowing growth in this hostile media were therefore investigated.

The solubility, concentration in the soil solution, ionic form, mobility and thus availability of soil nutrients often depends largely upon the soil pH. The availability of many nutrients including P, Mn, Fe, Al, Zn and Cu change rapidly with soil pH. Plant roots have been observed to substantially change the pH of their environment, thus affecting the nutrient dynamics. The rhizosphere pH has been observed to be up to 2 pH units higher or lower than the bulk soil (Marschner and Römheld 1983). Although much work has investigated these pH changes and corresponding effects on nutrient availability in acidic conditions, little work has focused on alkaline soils.

In this paper, we report the effect of plant nutritional status on the rhizosphere pH of Rhodes grass (*Chloris gayana* cv. Pioneer) growing in highly alkaline bauxite residue. Changes in rhizosphere pH of inoculated and non-inoculated lucerne (*Medicago sativa* L. cv. Hunter River) growing in alkaline residue were also compared.

Materials and Methods
All plant growth experiments were carried out in controlled conditions, with high pressure sodium lamps supplementing natural sunlight, providing 16 h of light per day. Temperature was maintained at 30 °C during the light period and 25 °C during the dark.

Validation of method
This experiment aimed to establish the contact time required for agar sheets placed on the soil surface to reach pH equilibrium, and also to determine the accuracy of the agar/micro-electrode method compared to the conventional pH measurement procedure. The agar contact method was validated as described by Pijnenborg et al. (1990). Hydrochloric acid (HCl) (0.00, 0.10, 0.25 and 0.50 mM H^+ g^{-1} soil) was incorporated into the seawater neutralised residue sand and allowed to equilibrate for 21 d. Agar solution, consisting of 15 g bacterial grade agar per litre of triple deionised (TDI) water, was adjusted to pH 8.0, 9.0 and 9.5 with 0.01 M NaOH. Glass sheets (420 x 150 mm) were covered in 3 mm agar solution, and the soil moistened to field capacity. The agar was brought into contact

with the soil and allowed to equilibrate. Agar pH was measured after 1, 2 and 3 h with a micro-electrode (MI-413 combination pH electrode, Microelectrodes Inc., 1.2 mm tip diameter). Each treatment was replicated four times in a completely randomised design (CRD).

Data were analysed as a CRD using a repeated measures analysis, GenStat 6 (GenStat 2002). Comparisons between means were made using Fisher's protected least significant difference (LSD) test. The variance ratios and LSDs for the time and interaction terms were adjusted for the degree of auto-correlation between times by the Greenhouse-Geisser epsilon (Greenhouse and Geisser 1959).

To allow comparison between the agar and standard pH methods, sub-samples were removed from the soil and air-dried for pH measurement using 1:5 soil:water suspensions (Rayment and Higginson 1992). Using GenStat 6 (GenStat 2002), a regression of agar pH against 1:5 suspension pH was performed to determine the relationship between the two variables.

Rhodes grass

An experiment was conducted to determine the effect of plant nutritional status on rhizosphere pH changes in alkaline soils. Fifteen wooden boxes (rhizotrons) (380 mm high x 150 mm wide x 150 mm deep) with a removable, 65° glass front panel were arranged in three treatments with five replicates; the first two consisting of a soil profile composed entirely of residue sand (350 mm deep), and the third a profile of residue sand (300 mm deep) capped with a layer of topsoil (50 mm deep).

A basal nutrient application was made to all three treatments, consisting of (mg 100 g^{-1} soil) 7.2 N-NO_3^- (150 kg N-NO_3^- ha^{-1}); 12.8 K (265 kg K ha^{-1}); 9.7 Ca (200 kg Ca ha^{-1}); 12.1 P (250 kg P ha^{-1}); 2.9 S (60 kg S ha^{-1}); and 2.9 Mg (60 kg Mg ha^{-1}) (assuming a 150 mm depth of incorporation and a bulk density of 1.38 g cm^{-3}). All nutrients (other than P) were thoroughly mixed through the soil. Due to the high P sorption capacity of residue sand, the P application was banded through the soil surface. An addition of 0.5 mg Mn 100 g^{-1} soil (10 kg Mn ha^{-1}) was also made to the second residue sand treatment.

All soil was moistened to field capacity with de-ionised (DI) water before addition to the rhizotrons, and the soil moisture content was held constant throughout the experiment by supplying DI water to the base of the boxes through capillary matting. Rhodes grass (*Chloris gayana* cv. Pioneer) seeds were planted and thinned to four plants per rhizotron after one week. The soil surface was covered with 10 mm polypropylene beads to limit evaporational losses. The plants were allowed to grow for a total of four weeks.

Agar solution was prepared as previously described, with 0.14 g thymol blue L^{-1} also incorporated into the solution (pKa = 8.9 (Covington 1972)). Using 0.01 M NaOH, the agar solution was adjusted to pH 9.5 and the glass sheets covered with a 3 mm layer. The front glass panel was removed from the rhizotron and the indicator agar sheet placed on the soil surface and allowed to equilibrate for 3 h. The indicator agar was then removed and digitally scanned. A second sheet of agar was prepared without pH indicator and placed on the soil surface to equilibrate for a further 3 h. The micro-electrode was then used to measure pH at distances of 1, 3, 5, 7 and 9 mm from the root tip, with bulk soil pH measured using both the agar method and standard method (1:5 soil:water suspension) (Rayment and Higginson 1992). Unusual growth characteristics were noted and shoot elemental concentrations determined by ICPAES after acid digestion as described by Martinie and Schilt (1976). Concentrations of diethylenetriaminepentaacetic acid (DTPA) extractable Cu, Zn, Mn and Fe were determined in both the neutralised residue sand and topsoil by ICPAES (Rayment and Higginson 1992).

Lucerne

An experiment was conducted to determine the effect of legume inoculation on rhizosphere pH changes in alkaline soils. Seawater neutralised residue sand (300 mm deep) and topsoil (50 mm deep) were added to ten rhizotrons. A basal nutrient application was made to both treatments, consisting of (mg 100 g^{-1} soil) 7.2 NO_3^- (150 kg NO_3^- ha^{-1}); 12.8 K (265 kg K ha^{-1}); 9.7 Ca (200 kg Ca ha^{-1}); 12.1 P (250 kg P ha^{-1}); 2.9 S (60 kg S ha^{-1}); and 2.9 Mg (60 kg Mg ha^{-1}), 0.5 Zn (10 kg Zn ha^{-1}), 0.5 Cu (10 kg Cu ha^{-1}), 0.5 Mn (10 kg Mn ha^{-1}), 0.2 B (5 kg B ha^{-1}), 0.1 Mo (2 kg Mo ha^{-1}) (assuming a 150 mm depth of incorporation and a bulk density of 1.38 g cm^{-3}).

Lucerne seeds (*Medicago sativa* L. cv. Hunter River) were inoculated with *Sinorhizobium meliloti* (*Rhizobium meliloti*) and planted into five of the rhizotrons, with non-inoculated seeds planted into the remaining five

rhizotrons. Plants were thinned to four per rhizotron after one week and then allowed to grow for a further five weeks. Rhizosphere pH was measured both with the agar/indicator mixture and with the micro-electrode as described above. Unusual growth characteristics were noted, and shoot tissue elemental concentrations measured as above. Tissue N concentrations were also measured as detailed by Rayment and Higginson (1992) using a LECO CNS 2000.

Results and Discussion

Validation of method

Results for the treatment combinations at each contact time are shown in Figure 1. The interaction between contact time and initial agar pH was significant ($p<0.001$, LSD (5 %) = 0.05), indicating different response patterns for the agar across time. The differences in initial agar pH significantly affected the measured values after 1 h equilibration; the lower the initial agar pH, the lower the measured pH after 1 h. However, no significant differences in pH were found between the three agar treatments after 3 h equilibration for any soil HCl treatment. Three hours was therefore sufficient to allow equilibration between soil and agar pH's (Figure 1). Working in the acidic range, Pijnenborg et al. (1990) also reported that 2 to 3 h contact were required to achieve equilibrium. The results obtained using the agar contact method were compared with those using a 1:5 soil:water suspension. A regression line was fitted and a strong relationship found between the two methods ($R^2 = 0.926$) (Figure 1). The correlation between the agar and standard methods was found to fit the linear relationship: $y = 0.64*x + 2.54$, crossing the theoretical line $y = x$ at pH 7.06. The agar method is therefore suitable for the direct measurement residue sand pH, with the correlation curve (Figure 1) allowing accurate conversion between the two methods as: soil-pH = 1.56 * agar-pH – 3.97.

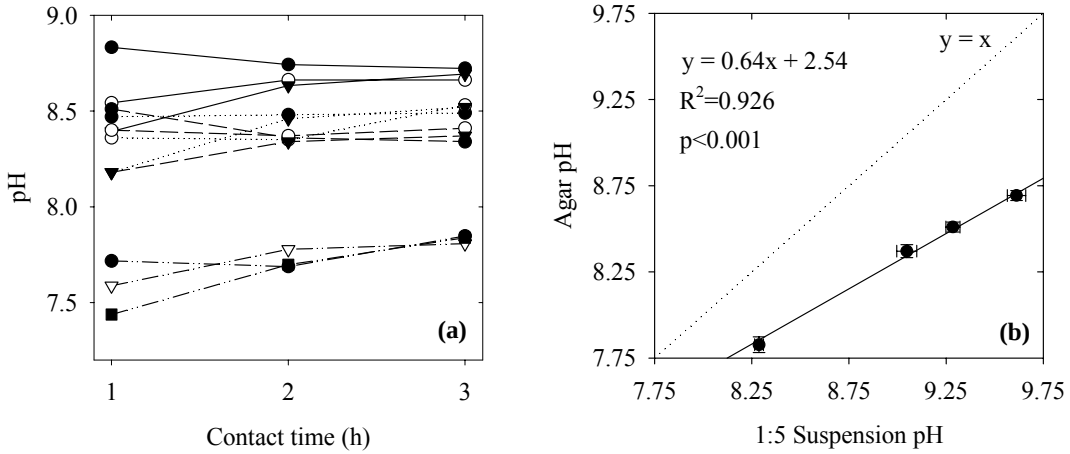

Figure 1. (a) pH values, measured with a microelectrode inserted in agar of differing initial pH (8.0 (■), 8.5 (▽) and 9.0 (●)) 1, 2 and 3 h after contact, of soils modified with the addition of 0.0 (−), 0.1 (···), 0.2 (−−), and 0.5 (−···−) mmol H^+ g^{-1} soil, and **(b)** regression between pH values obtained with the agar contact method (after 3 h equilibration) and the standard method (1:5 soil:water suspension) for residue sand.

Rhodes grass

Rhizosphere pH values were lower than the bulk soil pH in all three treatments, with soil pH tending to decrease with increasing proximity to the root (Figure 2). Root-induced pH change was greatest in the residue sand (-Mn) treatment where rhizosphere pH was 1.22 units below that of the bulk soil, compared to a 0.90 unit reduction in the residue sand (+Mn) treatment and a 0.62 unit reduction in the topsoil capped treatment.

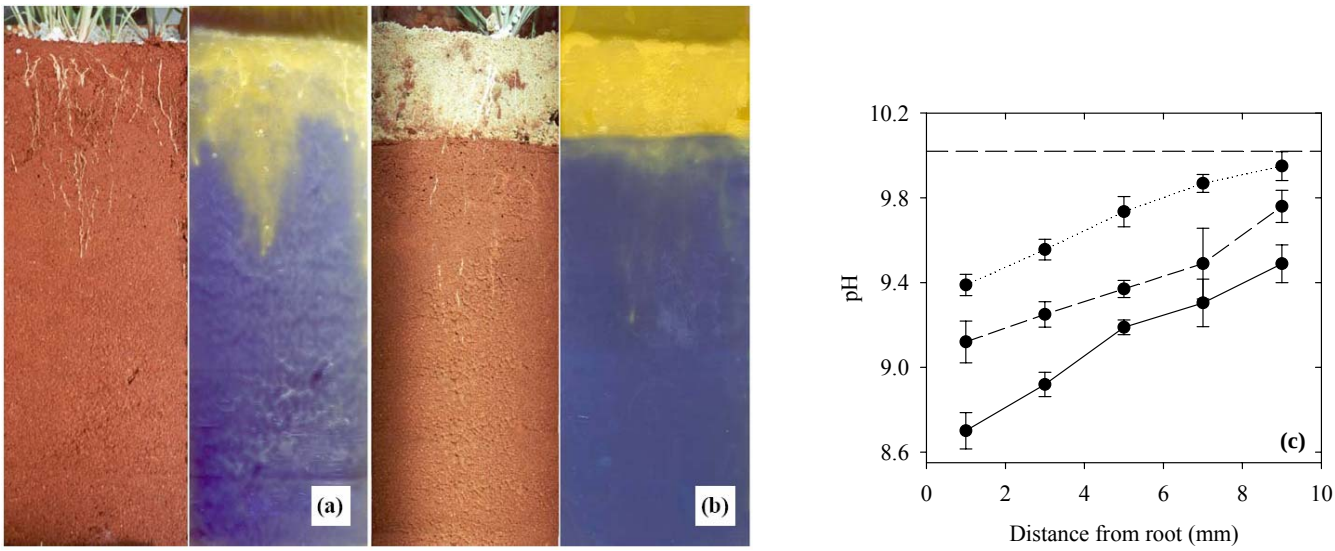

Figure 2. Rhizosphere pH of Rhodes grass (*Chloris gayana* cv. Pioneer) in **(a)** residue sand (-Mn) and **(b)** residue sand capped with topsoil in agar/pH indicator mixture after 3 h equilibration following four weeks growth, and **(c)** changes rhizosphere pH measured with a micro-electrode (converted to equivalent 1:5 suspension) after contact with agar for 3 h in residue sand (-Mn) (—), residue sand (+Mn) (– –), and residue sand capped with topsoil (···), (n = 5) (bulk soil pH = 10.02)

Symptoms of Mn deficiency were observed in the residue sand (-Mn) treatment after two weeks of growth. Leaves were observed to be a pale green, with the youngest emerging leaf failing to unroll (Smith 1973). These visual symptoms were confirmed by tissue analysis, with shoot Mn concentrations below the critical concentration for deficiency (Table 1). Shoot Mn concentrations of plants growing in the residue sand (+Mn) and topsoil capped residue sand treatments were not deficient. Concentrations of all DTPA extractable micronutrients were higher in the topsoil than in the residue sand, with concentrations of extractable Mn 15 times greater in the topsoil (Table 2).

Table 1. The average concentration of selected nutrients in Rhodes grass (*Chloris gayana* cv. Pioneer) plant tops following four weeks growth in seawater neutralised residue sand and topsoil capped seawater neutralised sand (n=5).

Treatment	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	S ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)	B ($\mu g\ g^{-1}$)
Residue sand	3.56	30.5	3.10	2.83	2.34	435	8.6	50.6	46.9
Residue sand (+Mn)	4.23	26.8	3.46	2.80	2.49	394	33.2	43.7	41.0
Topsoil capped	4.17	23.5	4.19	2.74	1.95	400	102	39.0	64.7
Critical Value*	2.0-3.0	15-20	2.0	2.5-5.0	1.3-2.0	50	25-50	20	5-10

*(Jones, Woolf *et al.* 1991)

Table 2. Concentrations of DTPA extractable Cu, Fe, Mn and Zn in seawater neutralised, freshwater leached residue sand, and in topsoil.

Soil	Cu	Fe	Mn	Zn
			($\mu g\ g^{-1}$)	
Residue sand	0.01	5.72	0.09	0.32
Topsoil	0.05	13.7	1.35	0.63

Although the form in which N is supplied is the most prominent factor influencing rhizosphere pH, pH changes may also be induced by mineral nutrient deficiencies. Decreases in rhizosphere pH have been observed in the rhizosphere of plants deficient in P (Li and Barker 1991; Liu, Shi *et al.* 1990), Zn (Chattopadhyay and Subrahmanyam 1993) and Fe (Chattopadhyay and Subrahmanyam 1993; Liu, Shi *et al.* 1990; Shi and Liu 1991), even if supplied with NO_3^-. This acidification is typically due to an increase in the net H^+ excretion resulting from an increase in the cation/anion uptake ratio (Marschner and Römheld 1996).

Rhizosphere acidification was greater in plants with Mn deficiency (1.22 pH units) than in those which had adequate Mn (0.90 pH units) (Figure 2 and Table 1). This increased acidification observed in the residue sand (-Mn) treatment was primarily due to a root-induced response to Mn deficiency. Residue sand has been observed to rapidly decrease the availability of added Mn, often transforming it to less-available forms within 24 h (Gherardi and Rengel 2001).

The addition of a topsoil capping, overlying the residue sand, provided a slightly acidic soil layer for the plant roots to exploit. Concentrations of DTPA extractable Mn in this layer were 15-fold greater than in the highly alkaline bauxite residue (Table 2). Rhizosphere acidification of the residue sand in this treatment was not as great as in either of the residue sand treatments (0.62 pH units below the bulk soil pH), with average shoot Mn concentrations ten fold higher than in the residue sand treatment and three fold higher than in the residue sand with added Mn (Table 1). Studying wheat (*Triticum aestivum*), Liu et al. (1999) also found that the acidification of the rhizosphere was greater in plants suffering Mn deficiency than in those with adequate Mn.

Lucerne

Rhizosphere pH was lower than that of the bulk soil in both the inoculated and non-inoculated treatments (Figure 3). Root-induced pH change was greatest in the inoculated treatment, where rhizosphere pH was 1.75 units below that of the bulk soil, compared to a reduction of 1.16 units in the non-inoculated treatment (Figure 3). Acidification also occurred to a distance greater than 9 mm in both treatments, with rhizosphere pH at 9 mm in the inoculated treatment 1.36 units lower than the bulk soil pH, and 0.70 units lower in the non-inoculated treatment. No symptoms of nutritional deficiencies were observed throughout the trial duration. This was confirmed with shoot concentrations of all nutrients higher than the critical concentration for deficiency in both treatments (Table 3).

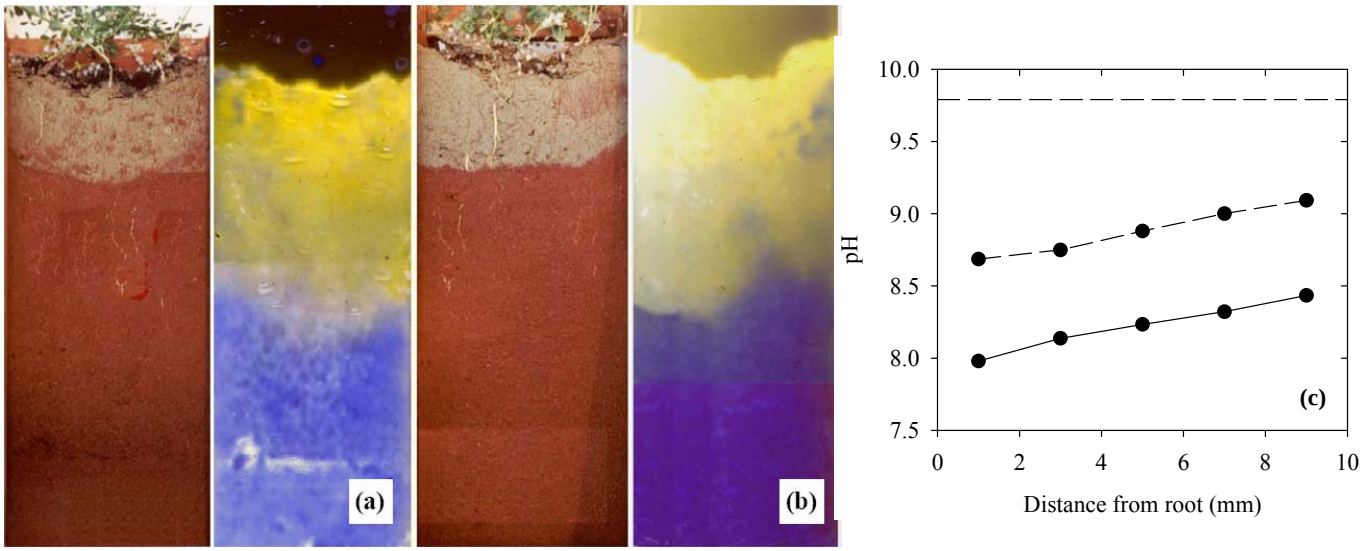

Figure 3. Rhizosphere pH of **(a)** inoculated lucerne (*Medicago sativa* L. cv. Hunter River), and **(b)** non-inoculated lucerne in agar/pH indicator mixture after three hours equilibration following six weeks growth, and **(c)** changes rhizosphere pH measured with a micro-electrode (converted to equivalent 1:5 suspension) after contact with agar for 3 h as effected by inoculation (inoculated (—), non-inoculated (···)) (n=5) (bulk soil pH = 9.79)

Table 3. The average concentration of selected nutrients in inoculated and non-inoculated lucerne (*Medicago sativa* L. cv. Hunter River) plant tops following six weeks growth in topsoil capped seawater neutralised sand

Treatment	N ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	S ($mg\ g^{-1}$)	Ca ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)	B ($\mu g\ g^{-1}$)
Inoculated	73.1	7.8	11.2	8.0	12.1	3.7	61.2	35.7	62.9	89.4
Non-inoculated	62.9	6.4	15.8	6.7	11.2	3.4	54.1	39.1	78.5	95.8
Critical Value	40-45	2.4	7.5	2.8	10	2-2.9	44	30	20.0	20

This difference in capacity to lower rhizosphere pH is attributable to the ability of the inoculated lucerne to fix atmospheric N_2, rather than relying on the soil N (NO_3^-) reserves as do the non-inoculated plants. Unable to fix atmospheric N_2 and reliant on the soil applied NO_3^-, the rhizosphere pH of the non-inoculated lucerne would be

expected to increase due to a decrease in H^+ excretion (Marschner and Römheld 1996). However, a decrease of 1.16 pH units was observed and is most likely due to an increase in the cation/anion uptake ratio resulting in an increased rate of H^+ excretion. Being able to fix atmospheric N_2, the inoculated lucerne was not reliant on this soil NO_3^-, and as a result, was able to increase the cation/anion uptake ratio further than the non-inoculated treatment, resulting in a greater rhizosphere pH decrease (Figure 3). Using soybean, Römheld (1984) found that legumes not infected with *Rhizobium* and supplied only NO_3^- slightly acidified areas in their apical zones but pH generally increased in the basal areas. However, those inoculated with *Rhizobium* were more efficient at acidifying their rhizosphere, with root-induced pH changes greater than in those without *Rhizobium*.

The acidification of the lucerne rhizosphere (up to 1.75 pH units) tended to be greater than that of the Rhodes grass rhizosphere (up to 1.22 pH units) (Figure 2 and Figure 3). Typically, the root CEC of dicots is higher than that of monocots (Keller and Deuel 1957), with increases in root cation exchange capacity (CEC) resulting in an increase in cation uptake (Smiley 1974). Therefore, the difference observed in rhizosphere acidification between the two species is possibly partially due to the higher cation uptake (and higher cation/anion uptake ratio) of the lucerne.

Conclusions

In conclusion, Mn deficiency in Rhodes grass has been shown to cause a root-induced acidification of the rhizosphere in highly alkaline soil in which N was supplied entirely as NO_3^-. The pH of the rhizosphere in these Mn deficient plants was up to 1.22 pH units lower than that of the bulk soil. Rhizosphere acidification was also observed to a smaller degree in plants which were not deficient in Mn (or any other mineral nutrient), reducing the bulk soil pH by 0.90 to 0.62 pH units. Rhizosphere acidification was also more efficient in inoculated lucerne (1.75 pH unit decrease) than in the non-inoculated lucerne (1.16 pH unit decrease) when supplied with only NO_3^-.

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References

Chattopadhyay A., Subrahmanyam K. (1993) Changes in yield, chemical composition and rhizosphere soil properties of citronella Java (*Cymbopogon winterianus* Jowitt) suffering from iron chlorosis. *Journal of the Indian Society of Soil Science* **41**, 166-167.

Covington A.K. (1972) Acid-base indicators. In 'Indicators'. (Ed. E Bishop) pp. 16-18 (Appendix 8). (Pergamon Press: New York)

GenStat (2002) GenStat for Windows. Release 6.1. Sixth Edition. In. (VSN International Ltd., Oxford.)

Gherardi M.J., Rengel Z. (2001) Bauxite residue sand has the capacity to rapidly decrease availability of added manganese. *Plant and Soil* **234**, 143-151.

Greenhouse S.W., Geisser S. (1959) On methods in the analysis of profile data. *Psychometrika* **24**, 95-112.

Jones J.B., Woolf B., Mills H.A. (1991) 'Plant analysis handbook: a practical sampling, preparation, analysis and interpretation guide.' (Micro-Macro Publishing: Athens, Georgia)

Keller P., Deuel H. (1957) Kationenaustauschkapazität und pektingehalt von pflanzenwurzeln. *Zeitschrift fur Pflanzenernahrung Dungung und Bodenkunde* **79**, 119-131.

Li Y., Barker S.A. (1991) Calculating changes of legume rhizosphere soil pH and soil solution phosphorus from phosphorus uptake. *Communications In Soil Science And Plant Analysis* **22**, 955-974.

Liu X.J., Lu S.H., Zhang F.S., Mao D.R. (1999) Effect of Mn fertilization on Mn nutrition and dynamics of available Mn in rhizosphere of two wheat genotypes. *Journal of China Agricultural University* **4**, 77-80.

Liu Z.Y., Shi W.M., Fan X.H. (1990) The rhizosphere effects of phosphorus and iron in soils. *Transactions of 14th International Congress of Soil Science* **2**, 147-152.

Marschner H., Römheld V. (1983) In vivo measurement of root-induced pH changes at the soil-root interface: effect of plant species and nitrogen source. *International Journal of Plant Physiology* **111**, 241-251.

Marschner H., Römheld V. (1996) Root-induced changes in the availability of micronutrients in the rhizosphere. In 'Plant Roots : The Hidden Half'. (Eds Y Waisel, A Eshel and U Kafkafi) pp. 557-579. (M. Dekker: New York)

Martinie G.D., Schilt A.A. (1976) Investigation of the wet oxidation efficiencies of perchloric acid mixtures. *Analytical Chemistry* **48**, 70-74.

Pijnenborg J.W.M., Lie T.A., Zehnder A.J.B. (1990) Simplified measurement of soil pH using an agar-contact technique. *Plant and Soil* **126**, 155-160.

Rayment G.E., Higginson F.R. (1992) 'Australian Laboratory Handbook of Soil and Water Chemical Methods.' (Inkata Press: Melbourne)

Römheld V. (1984) pH changes in the rhizosphere depending on nutrient supply. *Landwirtschaftliche Forschung* **40**, 226-230.

Shi W.M., Liu Z.Y. (1991) Secretion of phytosiderophore and its effects on soil Fe availability. *Pedosphere* **1**, 73-81.

Smiley R.W. (1974) Rhizosphere pH as influenced by plants, soils, and nitrogen fertilzers. *Soil Science Society of America Proceedings* **38**, 795-799.

Smith F.W. (1973) 'Foliar Symptoms of Nutrient Disorders in *Chloris gayana*.' Commonwealth Scientific and Industrial Research Organization, 13, Melbourne.