

H⁺/OH⁻ Excretion and Nutrient Uptake in Upper and Lower Parts of Lupin (*Lupinus angustifolius* L.) Root Systems

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The cultivation of narrow-leaved lupins (*Lupinus angustifolius* L.) increases rates of subsoil acidification, and this is thought to be partly related to their pattern of nutrient uptake and H⁺/OH⁻ excretion. The main hypothesis of this study was that H⁺ and OH⁻ excretion is not distributed evenly over the entire length of the root system but is limited to zones where excess cation or anion uptake occur. Seedlings of nodulated lupins were grown in solution culture using vertically split pots that allowed the upper and lower zones of the root system to be supplied with varying concentrations of K⁺ and NO₃⁻. Net H⁺/OH⁻ excretion was equated to the addition of NaOH/HCl required to maintain a constant pH in the nutrient solution during a 4-d treatment period and nutrient uptake was measured by depletion from solution in each zone of the split pots.

The excess of cation over anion uptake was positively correlated with H⁺ excretion in each rooting zone. In zones where K⁺ was supplied at 1200 μM, cation uptake was dominated by K⁺ and up to twice as much H⁺ was excreted than in zones where K⁺ was absent. In zones where NO₃⁻ was supplied at 750 μM, the anion/cation uptake was balanced, however H⁺ excretion continued to occur in the zone. When NO₃⁻ was supplied at 5000 μM, anion uptake exceeded cation uptake but there was no OH⁻ excretion. Organic acid anions may be excreted by lupins to maintain their internal electroneutrality when anion uptake exceeds cation uptake. Rhizosphere pH would not increase unless the pK_a of the excreted organic anions was greater than the external pH.

INTRODUCTION

Acidification is a slow natural process in most soils. In south-eastern Australia the use of pastures based on subterranean clover (*Trifolium subterraneum* L.) has increased the rate of soil acidification leading to low pH levels that seriously decrease crop and pasture production (Williams, 1980). Recently, it has been suggested that the growth of narrow-leaved lupins (*Lupinus angustifolius* L.) may also increase the rate of soil acidification, particularly at depth (Coventry and Slattery, 1991; Loss, Ritchie and Robson, 1993). Unlike acidity in the surface soil, subsoil acidity cannot be ameliorated economically with lime because of its slow downward movement in soils (Conyers and Scott, 1989).

The growth of many N₂-fixing legumes has been associated with increased soil acidification (Jarvis and Hatch, 1983; Lui, Lund and Page, 1989). In a grazed, grass-legume pasture growing in a mediterranean climate, Helyar and Porter (1989) estimated that 10–15% of soil acidification is caused by the excretion of H⁺ by roots and the subsequent removal of organic anions in plant products. When the charges of the cations and anions absorbed are not balanced, plants maintain their electroneutrality by excreting H⁺ or OH⁻ into their rhizosphere. Any charge imbalance within

the plant is corrected by the synthesis of organic acid anions, and when the plant material is not returned to the soil, the pH changes in the soil persist. For non-legumes NO₃⁻ and/or NH₄⁺ are the nutrients absorbed in the greatest quantities, and the relative uptake of each largely determines the cation-anion balance and pH changes in the rhizosphere. For legumes reliant on N₂ fixation, the uptake of cations is usually greater than the uptake of anions and acidification of the soil generally occurs (Israel and Jackson, 1978; Lui *et al.*, 1989).

The amount of H⁺ excreted by legumes reliant solely on N₂ fixation can be calculated from the chemical composition of the legume (Jarvis and Robson, 1983), or the ash alkalinity of the plant (Nyatsanga and Pierre, 1973) or by measuring the amount of OH⁻ required to maintain a constant pH in the growing medium (Jarvis and Hatch, 1983; Lui *et al.*, 1989). While it is relatively simple to measure the amount of acidity added to the soil by the growth and removal of legume material, it is more difficult to determine how this acidity is distributed in the soil profile.

Changes in soil pH with depth that are caused by plant growth will depend upon the distribution of roots and nutrients in the soil, the patterns of nutrient uptake and H⁺ excretion along roots, and the amount and distribution of organic matter returned to the soil. In the model of Helyar, Hochman and Brennan (1989), H⁺/OH⁻ excretion is equated from the excess of cation or anion uptake in various depth

intervals. Their assumption that the uptake of K, Ca, and Mg is proportional to the distribution of roots down the soil profile, could lead to considerable errors in their model predictions.

Several glasshouse studies have demonstrated that the pattern of H^+ excretion is uniform along the roots of young, rapidly growing plants with a constant and unlimited supply of nutrients (Römheld, Müller and Marschner, 1984; White and Robson, 1989), however this was not the case in split root experiments with rape (*Brassica napus* L.) (Moorby, Nye and White, 1985), and maize (*Zea mays* L.) (Römheld, 1986). Nye (1987) concluded from these results that H^+ and OH^- are excreted at the site of cation or anion uptake and that differences in nutrient concentrations between the surface soil and the subsoil will lead to different rates of acidification. This conclusion however, is based on results with non-legumes in split root experiments that divide root systems horizontally, whereas gradients of nutrient concentrations tend to occur vertically (i.e. with depth) in undisturbed soils.

The overall aim of our study was to determine whether lupins excrete H^+ or OH^- uniformly over the entire length of their root system or only in zones of high cation or anion uptake. This was achieved by designing a nutrient solution pot that splits root systems vertically, that is, into an upper and lower zone, and by varying the supply of K^+ and NO_3^- to the roots in each zone. Apart from NH_4^+ , plants take up K^+ in much larger quantities than other cations and the uptake of anions is dominated by NO_3^- , hence the balance of cation and anion uptake can be changed by varying the supply of these two nutrients.

MATERIALS AND METHODS

Experimental procedure

Seeds of *L. angustifolius* cv. Yandee were germinated on a stainless steel screen suspended on the surface of an aerated solution of 10^{-4} M $CaSO_4$ and 10^{-6} M H_3BO_3 . After 7 d, seedlings were transferred to an aerated, complete nutrient solution at a density of eight seedlings per 5.0 l pot. The complete nutrient solution contained the following nutrients (μ M): $CaSO_4$, 625; K_2SO_4 , 600; $NaNO_3$, 250; $MgSO_4$, 200; NaH_2PO_4 , 20; H_3BO_3 , 5; $FeNaEDTA$, 3; $MnSO_4$, 1.0; $ZnSO_4$, 0.75; $CuSO_4$, 0.2; $CoSO_4$, 0.2; Na_2MoO_4 , 0.03. These concentrations were chosen to provide an adequate but not excessive nutrient supply to the young seedlings. The pH of the nutrient solution was maintained between 5.0 and 6.5 daily with additions of 0.1 M KOH and the solutions were changed every second day.

Immediately after the transfer of seedlings to the complete nutrient solution, 5.0 ml of a suspension of commercial peat (10 g l^{-1}) containing *Bradyrhizobium* sp. (*Lupinus*) WU425 was added to each pot and again after the first solution change (a total inoculation time of 4 d). At 4 weeks of age, the cotyledons of the lupin plants turned yellow and dropped off, and nodules with pink interiors were clearly visible on a 6 cm portion of tap root, 8 cm from the hypocotyl. Sodium nitrate was excluded from the nutrient solution for the final week of seedling preparation to ensure the seedlings were relying solely on N_2 fixation.

After 5 weeks of pretreatment, two zones of nutrient supply were imposed to the root systems of the lupin seedlings by using vertical split-root pots described in detail by Tang, Robson and Dilworth (1991). This system splits the root system into an upper and a lower zone of nutrient supply. Nodules were only present in the upper zone.

During the 4 d treatment period (details below), pH was monitored and corrected to 6.0 with a known volume of 0.01 M NaOH or HCl solution four times a day in each zone. Water lost through transpiration and evaporation was replaced daily by adding de-ionized water so that the volume of nutrient solution was maintained constant, otherwise the solutions were left unchanged during the treatment periods. In each experiment, pots without plants were included as controls for comparisons of nutrient uptake and H^+ excretion.

Experimental designs

Experiment 1. The hypothesis for expt 1 was that nodulated lupin roots absorb a greater excess of cations and excrete more H^+ in zones supplied with high K^+ concentrations than those where K^+ was absent. We investigated the effects of two K^+ concentrations (0 and 1200 μ M) in two root zones (upper and lower) on nutrient uptake, cation-anion balance and H^+ excretion of nodulated lupin seedlings grown in nutrient solutions. The experiment was a factorial design with four replicates for each of the four treatments (Table 1), and was conducted in an air conditioned glasshouse in root cooling tanks maintained at 18–20 °C during October 1989.

Sixteen lupin seedlings were transferred to the vertical split pots and the treatment nutrient solutions were identical to the complete nutrient solution used in the pretreatment except for their NO_3^- and K^+ contents. $NaNO_3$ was absent in all treatments, hence the plants were reliant solely on N_2 fixation and K_2SO_4 was also replaced with Na_2SO_4 for the treatment where K^+ was absent, so that SO_4^{2-} concentrations between treatments were constant. The treatments are abbreviated as AA (K^+ absent in upper and lower zones), AP (K^+ present in lower zone only at 2500 μ M), PA (K^+ present in upper zone only at 2500 μ M) and PP (K^+ present in upper and lower zones at 2500 μ M).

Experiment 2. In expt 2 we tested the hypothesis that lupin roots absorb a greater excess of anions and excrete more OH^- in zones supplied with high concentrations of NO_3^- than in zones supplied with low NO_3^- concentrations, and that Ca^{2+} is absorbed more slowly than K^+ , hence supplying $Ca(NO_3)_2$ causes greater OH^- excretion than supplying KNO_3 . In the lower zone of the split pots, we studied the effects of two NO_3^- concentrations supplied as $Ca(NO_3)_2$ or KNO_3 and $NaNO_3$, on the nutrient uptake of H^+ or OH^- excretion from the roots of nodulated lupin seedlings. The experiment included six replicates per treatment and was conducted during April 1990, under conditions similar to expt 1.

Eighteen seedlings were transferred to the vertical split pots for expt 2. The nutrients in the upper root zones of all treatments were similar to the solution used in the

TABLE 1. The mean nutrient uptake, mean cation-anion balance (C-A) and the mean OH⁻ added to each zone over the 4 d treatments in expt 1

Treatment			Nutrient uptake [$\mu\text{mol (m root)}^{-1}$]						C-A	OH ⁻
Code	Root zone	K added (μM)	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻	H ₂ PO ₄ ⁻	[$\mu\text{eq (m root)}^{-1}$]	[$\mu\text{eq (m root)}^{-1}$]
AA	Upper	0	0.0	24.8	46.7	21.3	39.2	4.3	78.0 ± 9.1*	76.2 ± 8.7
	Lower	0	0.0	10.9	43.1	28.1	36.6	5.6	74.6 ± 9.2	72.7 ± 7.5
AP	Upper	0	0.0	26.3	25.6	17.1	29.4	2.6	50.3 ± 5.6	55.1 ± 7.7
	Lower	1200	83.2	0.0	47.1	27.0	63.6	4.3	99.7 ± 11.0	88.6 ± 9.5
PA	Upper	1200	56.6	0.0	31.8	12.8	31.7	2.4	80.0 ± 5.6	72.4 ± 3.3
	Lower	0	0.0	37.2	41.3	26.5	54.1	3.8	61.0 ± 13.7	61.4 ± 5.5
PP	Upper	1200	66.0	0.0	32.1	15.1	37.2	3.2	82.6 ± 8.5	87.4 ± 5.0
	Lower	1200	96.8	0.0	59.5	38.1	87.9	6.6	109.6 ± 12.0	108.8 ± 11.9

* Standard error, $n = 4$.

pretreatment except that NaNO₃, CaSO₄ and K₂SO₄ were replaced by a low concentration of Ca(NO₃)₂ and KNO₃ (each 250 μM). Sulphate was only supplied as MgSO₄ (200 μM). In the lower root zones, the supply of KNO₃ and Ca(NO₃)₂ was varied as follows: (a) as in the upper zone, i.e. 250 μM Ca(NO₃)₂ and 250 μM KNO₃, (coded in Table 2 as treatment Ca_uK_u); (b) 2500 μM Ca(NO₃)₂ and KNO₃ absent, (treatment Ca_lK_l); and (c) 2500 μM KNO₃, 2500 μM NaNO₃ and no Ca(NO₃)₂, (treatment Ca_lK_u).

Analyses and calculations

Plants were harvested after a 4 d treatment period in both experiments and root length in each zone was measured using a root length scanner (Comair®, Commonwealth Aircraft Corporation, Melbourne, Australia). A sample of the nutrient solution was taken from each zone and stored at 2 °C until analysis. Nutrient solutions were analysed for K, Na, Ca, and Mg using atomic absorption spectrophotometry and S and P concentrations using inductively coupled analysis. Nitrate concentrations were determined in expt 2 with an ion-selective electrode (Orion®, nitrate ion electrode 92-07).

When the seedlings were transferred to the vertical split pots an attempt was made to distribute the lengths of root evenly between the two zones but because of the lateral root development at the base of the tap root, this was not always possible. To account for any differences in root length between zones, all data were calculated per metre of root.

Nutrient uptake was measured in each zone from the difference in the concentration of nutrients between the control and the treatment pots. Cation-anion balance was determined by summing of the charges of the cations absorbed and subtracting the sum of the charges of the anions absorbed. The amount of H⁺/OH⁻ added was used to estimate the amount of OH⁻/H⁺ excreted by the roots in each zone.

RESULTS

The growth of the seedlings during the treatment phases of both experiments was satisfactory, with no visible symptoms of nutrient deficiency or pathogens. There was no effect of

the treatments on shoot or root growth ($P < 0.05$). In vertical split pots without plants, there was no change in the concentrations of nutrients nor the pH.

Experiment 1

Nutrient uptake. As was expected, K⁺ was absorbed in larger quantities [$66\text{--}97 \mu\text{mol (m root)}^{-1}$] than other cations when it was supplied over the 4-d experimental period (Table 1). In zones supplied with K⁺, Na⁺ was not absorbed despite pH correction with NaOH (up to 250 μM by the end of the treatment period), whereas in the zones where K⁺ was absent, up to 37 $\mu\text{mol (m root)}^{-1}$ of Na⁺ was absorbed. Where K⁺ was supplied, its rate of uptake was between 390 and 750 $\mu\text{mol (g root)}^{-1} \text{d}^{-1}$, similar to rates reported by Asher (1964). In all treatments SO₄²⁻ was the anion absorbed in the largest quantities [$29\text{--}88 \mu\text{mol (m root)}^{-1}$]. Less than 7 $\mu\text{mol (m root)}^{-1}$ of H₂PO₄⁻ was absorbed. The concentration of H₂PO₄⁻ was depleted by 85% over the 4-d treatment, while the depletion of the other nutrients was not greater than 70%.

The uptake of all nutrients per unit root length was greater in the lower than in the upper zone for all treatments (Table 1) with the exceptions of SO₄²⁻ and Ca²⁺ in the AA treatment (K⁺ absent in both zones). The uptake of all nutrients (except K⁺) from the upper zone was greater in the AA treatment than in the PP treatment, and the reverse was the case in the lower zone.

In general, the uptake of nutrients other than K⁺ was greater in the lower zone when K was present than when it was absent, regardless of whether K⁺ was present in the upper zone. In contrast, the uptake of nutrients other than K⁺ was decreased from the upper zone by the presence of K⁺ in either or both zones. The largest decrease was observed in the AP treatment except for Na⁺ which increased slightly.

Cation-anion balance. There was a trend for a more positive cation-anion balance (indicating that more cations than anions had been absorbed) where K⁺ was present than where K⁺ was absent, even when these treatments were imposed on different rooting zones of the same plant (Table 1). The greatest difference between the cation-anion balance

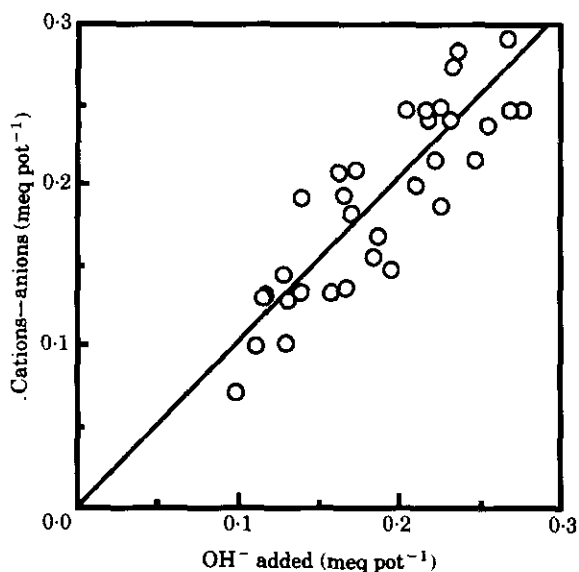


FIG. 1. The correlation between the cation-anion balance and the amount of OH^- added to each rooting zone. ($r^2 = 0.76$).

in rooting zones of the one plant was about $50 \mu\text{eq} (\text{m root})^{-1}$ in the AP treatment. For the PA treatment, the mean cation-anion balance was about $20 \mu\text{eq} (\text{m root})^{-1}$ greater in the lower zone than in the upper zone ($P < 0.1$). For the PP treatment, the mean cation-anion balance was greater in the lower zone than in the upper zone ($P < 0.1$).

NaOH addition. The total amount of OH^- required to maintain a constant pH of the solutions was linearly correlated with the cation-anion balance in both zones for individual replicates of each treatment ($r^2 = 0.76$; Fig. 1). The linear regression did not differ ($P < 0.05$) from a line with a slope of 1, and when curvilinear relationships were fitted to these data the variation accounted for did not increase.

Experiment 2

Nutrient uptake. In general, K^+ , Ca^{2+} and NO_3^- were absorbed in the greatest quantities. This was shown

particularly for Ca^{2+} and NO_3^- in the zone supplied with Ca_HK_A and for K^+ and NO_3^- in the zones supplied with Ca_AK_H (Table 2). Calcium uptake was least in the lower zone of the Ca_LK_L treatment, about ten times less than in expt 1. The uptake of Ca^{2+} from the Ca_HK_A treatment was more than 20 times the uptake in the lower zone of the control Ca_LK_L treatment, and between two and four times the Ca^{2+} uptake in expt 1. The uptake of K^+ in the Ca_AK_H treatment was more than seven times the uptake in the Ca_LK_L treatment, and up to three times the K^+ uptake in expt 1. The roots absorbed more equivalents of Ca^{2+} than K^+ over the 4-d experimental period except for the Ca_LK_L treatment. The uptake of SO_4^{2-} was up to nine times greater in expt 1 than in the expt 2, and it was very low in the Ca_HK_L treatment. Magnesium and H_2PO_4^- uptake were similar in both experiments.

Cation-anion balance. The cation-anion balance was not significantly different from zero ($P < 0.05$) in the upper zones of treatment Ca_LK_L . In the lower zones there was an excess of anion uptake of between $29 \mu\text{eq} (\text{m root})^{-1}$ in the Ca_LK_L treatment and $151 \mu\text{eq} (\text{m root})^{-1}$ in the Ca_AK_H treatment. The uptake of Ca^{2+} in the lower zone of the Ca_HK_A treatment was greater than the uptake of K^+ in the lower zone of the Ca_AK_H treatment, however because of the divalent charge of Ca^{2+} , there was a greater anion charge excess in the lower zone of the Ca_AK_H treatment.

NaOH addition. The pH of the nutrient solutions did not rise in any zone during the 4-d of treatments and hence, no addition of H^+ was required to maintain the pH of the solutions at 6.0. Significant quantities of OH^- were required in all upper zones, while the amounts added to the lower zones were not different from zero ($P < 0.05$). Unlike expt 1, the amounts of OH^- added to the zones were not related to cation-anion balance.

DISCUSSION

Proton excretion by lupin roots was not distributed evenly over the entire length of the root system but occurred in the zone of nutrient uptake. Hence, differences in nutrient uptake by lupin roots between the surface soil and the subsoil will lead to different rates of acidification. Lupins

TABLE 2. The mean nutrient uptake, mean cation-anion balance (C-A) and the mean OH^- added to each zone over the 4 d treatments in expt 2. High and low concentrations of Ca and K are indicated by $_H$ and $_L$ respectively, and $_A$ indicates absent. (See expt 2 treatments for actual concentrations)

Treatment		Nutrient uptake [$\mu\text{mol} (\text{m root})^{-1}$]							C-A	OH ⁻
Root zone	Ca/K added	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻	H ₂ PO ₄ ⁻	NO ₃ ⁻	[$\mu\text{eq} (\text{m root})^{-1}$]	[$\mu\text{eq} (\text{m root})^{-1}$]
Upper	Ca _L K _L	16.1	2.2	13.6	13.5	11.1	3.0	43.0	1.3 ± 2.7*	16.9 ± 1.3
Lower	Ca _L K _L	20.4	0.0	4.0	16.1	20.0	6.8	35.9	-28.7 ± 3.5	3.6 ± 2.3
Upper	Ca _L K _L	17.6	1.8	15.2	16.0	15.3	3.4	45.2	-0.9 ± 3.4	18.7 ± 0.6
Lower	Ca _H K _A	0.0	0.0	101.5	14.0	1.2	4.7	260.2	-41.1 ± 6.7	1.0 ± 0.5
Upper	Ca _L K _L	11.2	5.1	12.9	13.4	11.1	2.9	42.9	-2.1 ± 1.7	16.7 ± 1.2
Lower	Ca _A K _H	152.1	2.7	0.0	11.4	19.5	5.1	279.5	-151.0 ± 15.2	0.3 ± 0.2

* Standard error, $n = 6$.

did not excrete OH^- when they absorbed an excess of anions.

When nodulated lupins were supplied with an adequate concentration of nutrients in expt 1, they absorbed greater quantities of K^+ than other cations and up to twice as much H^+ excretion occurred in the zones where K^+ was supplied than in zones where K^+ was absent. This has implications for the patterns of soil acidification under lupin:wheat rotations. Rowland, Mason and Hamblin (1986) and Loss *et al.* (1993) presented data that suggest that the deep rooting patterns of lupins enable them to absorb K^+ from the subsoil and increase K^+ concentrations of the surface soil through organic matter cycling. In such situations, one would expect greater rates of acidification at depth than in the surface soil. Loss *et al.* (1993) measured greater rates of soil acidification under wheat:lupin than wheat:sub-clover based pasture rotations in the field, particularly below 20 cm.

The data from expt 1 were in agreement with the results of others that demonstrated a strong linear relationship between excess cation uptake and H^+ excretion by roots (Van Egmond and Aktas, 1977; Jarvis and Hatch, 1983; Lui *et al.*, 1989). However, the lack of correlation between excess anion uptake and OH^- excretion by lupins in expt 2, contrasts with previous observations with peas, *Pisum sativum* L. (Van Beusichem, 1981), sub-clover, *Trifolium subterraneum* L. (Jarvis and Robson, 1983) and castor oil plants, *Ricinus communis* L. (Van Beusichem, Kirkby and Baas, 1988). Proton excretion has been reported in plants supplied with NO_3^- , but only when cation uptake exceeded anion uptake; e.g. soybeans, *Glycine max* L. (Israel and Jackson, 1982), sunflower, *Helianthus annuus* L., (Bekele *et al.*, 1983) and buckwheat, *Fagopyrum esculentum* L. (Mitreva, 1989). Proton excretion has also been measured in chickpeas (*Cicer arietinum* L.) supplied with NO_3^- in response to Fe and P deficiency (Alloush and Sanders, 1990). The lupins in our experiments were supplied with adequate Fe and P, and did not show symptoms of deficiency.

It is possible that lupin roots excrete organic acid anions when anion uptake exceeds cation uptake (Fig. 2). The source of organic anions is a 'pH stat' mechanism that maintains a constant cellular pH when OH^- is produced from the excretion of H^+ and the oxidation of NO_3^- and SO_4^{2-} (Raven and Smith, 1973; Davies, 1973). Any rise in pH is prevented by the synthesis of organic acids from neutral carbon precursors. Malate and citrate are the most abundant organic anions in lupins and they have low pK_{a1} values (pK_{a1} values of 5.1 and 5.4 respectively), hence they would not affect the pH of the growing media at pH 6.0 to any great extent. The results of recent experiments concerning organic anion excretion by lupins will be published shortly in a following paper.

In a grass-legume pasture growing in a mediterranean climate, Helyar and Porter (1989) estimated that 40% of soil acidification is caused by the oxidation of organic N to NO_3^- which produces H^+ . This reaction is enhanced by the build up of soil N via N_2 fixation and the leaching of NO_3^- . More efficient use of soil NO_3^- is being explored as a method of decreasing the rate of soil acidification, because as much as 10 mM NO_3^- has been measured in the soil solution below

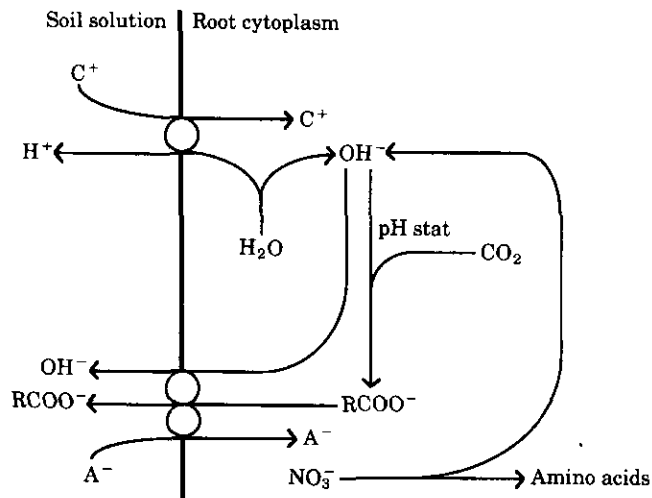


Fig. 2. The proposed uptake of anions in exchange for organic anions (RCOO^-) synthesised from a 'pH stat' mechanism.

15 cm in sandy soils (Carr, pers. comm.). Our work has shown that lupins do not excrete OH^- when they absorb an excess of anions. Even though lupins are deep rooted and therefore have a large potential to absorb leached NO_3^- and decrease soil acidification, any excess anion absorption would not increase the soil pH at depth unless the pH of the soil was below the pK_a of the excreted organic anions (Ritchie and Dolling, 1985). However, the organic anions may be broken down by micro-organisms in the rhizosphere, producing OH^- and CO_2 .

Our results indicate that the age of roots may be important in nutrient uptake and H^+ excretion. Differences in the proportion of lateral to main roots between the zones may also be important. The cation-anion balance and the amounts of NaOH required to maintain pH in the zones indicate that when K was supplied to both zones, more cations were absorbed and more H^+ was excreted from the lower than the upper zones of the vertical split. This was not because of the greater volume of nutrient solution in lower than in upper zones and hence there was a greater depletion of nutrients from the upper zone. Nutrients were depleted by similar amounts in both zones over the 4-d experimental periods and final concentrations were greater than 30% of their original concentrations. The lupin seedling may have a priority on the growth of the apical meristem of tap root causing the greater cation uptake and H^+ excretion in the lower zone.

The vertical split pot designed for use in this experiment had advantages over some horizontal split designs which have been used to examine a number of physiological hypotheses in tap rooted plants. Our method did not involve cutting the tap root to induce lateral root formation as performed by Singleton (1983) which may cause morphological changes affecting root physiology. Also the vertical split was more representative of nutrient gradients in the soil which occur vertically rather than horizontally. Errors in expt 1 were quite large because there was only one plant per pot and only four replicates, but errors were decreased in expt 2 with the use of six replicate pots. A

method of using more than one plant per pot may also reduce the variation in results.

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LITERATURE CITED

- Alloush GA, Sanders FE. 1990. Responses of chickpea (*Cicer arietinum*) to iron stress measured by using a computer-controlled continuous-flow hydroponic system. In: Van Beusichem ML, ed. *Plant nutrition—physiology and applications*. Dordrecht: Kluwer Academic Publishers, 197–206.
- Asher CJ. 1964. *Potassium uptake by pasture plants*. PhD (Agric) Thesis. University of Western Australia.
- Bekele T, Cino BJ, Ehlert PA, Van Der Maas AA, Van Diest A. 1983. An evaluation of plant-borne factors promoting the solubilization of alkaline rock phosphate. *Plant and Soil* 75: 362–378.
- Conyers MK, Scott BJ. 1989. The influence of surface incorporated lime on subsurface soil acidity. *Australian Journal of Experimental Agriculture* 29: 201–207.
- Coventry DR, Slattery WJ. 1991. Acidification of soil associated with lupins grown in a crop rotation in north-eastern Victoria. *Australian Journal of Agricultural Research* 42: 391–397.
- Davies DD. 1973. Control of and by pH. *Symposium of the Society of Experimental Biology* 27: 513.
- Helyar KR, Porter WM. 1989. Soil acidification, its management and the processes involved. In: Robson AD, ed. *Soil acidity and plant growth*. Sydney: Academic Press, 61–101.
- Helyar KR, Hochman Z, Brennan JP. 1989. The problem of acidity in temperate soil areas and its management. Canberra: *National Soils Conference*, 22–54.
- Israel DW, Jackson WA. 1978. The influence of nitrogen nutrition on ion uptake and translocation by leguminous plants. In: Andrew CS, Kamprath EJ, eds. *Mineral subtropical soils*. Melbourne: CSIRO, 113–129.
- Israel DW, Jackson WA. 1982. Ion balance, uptake and transport processes in N_2 fixing and nitrate and urea dependent soybean plants. *Plant Physiology* 69: 171–178.
- Jarvis SC, Hatch DJ. 1983. Rates of hydrogen ion efflux by nodulated legumes grown in flowing solution culture with continuous pH monitoring and adjustment. *Annals of Botany* 55: 41–51.
- Jarvis SC, Robson AD. 1983. The effect of nitrogen nutrition of plants on the development of acidity in West Australian soils. II. Effects of differences in cation–anion balance between plant species grown under non leaching conditions. *Australian Journal of Agricultural Research* 34: 355–365.
- Loss SPL, Ritchie GSP, Robson AD. 1993. The effect of lupins and pasture on soil acidification and fertility in Western Australia. *Australian Journal of Experimental Agriculture* 33: (in press).
- Lui WC, Lund LJ, Page AL. 1989. Acidity produced by leguminous plants through symbiotic dinitrogen fixation. *Journal of Environmental Quality* 18: 529–534.
- Mitreva NI. 1989. Sunflower and maize transorganal tissue pH gradients and problems related to ion transport mechanisms. *Plant and Soil* 113: 144–146.
- Moorby H, Nye PH, White RE. 1985. The influence of nitrate nutrition on the H^+ efflux by young rape plants (*Brassica napus* c.v. Emerald). *Plant and Soil* 84: 403–415.
- Nyatsanga T, Pierre WH. 1973. Effect of nitrogen fixation by legumes on soil acidity. *Agronomy Journal* 65: 936–940.
- Nye PH. 1987. Acid-base changes in the rhizosphere. *Advances in Plant Nutrition* 2: 129–153.
- Raven JA, Smith FA. 1973. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytologist* 76: 415–431.
- Ritchie GSP, Dolling PJ. 1985. The role of organic matter in soil acidification. *Australian Journal of Soil Research* 23: 569–576.
- Römheld V. 1986. pH changes in the rhizosphere of various crop plants, depending on nutrient supply. *Kali-Briefe* 18: 13–30.
- Römheld V, Müller C, Marschner H. 1984. Localisation and capacity of proton pumps in roots of intact sunflower plants. *Plant Physiology* 76: 602–606.
- Rowland IC, Mason MG, Hamblin J. 1986. Effects of lupins on soil fertility. *Proceedings of the 4th International Lupin Conference*, Geraldton. 96–111.
- Singleton PW. 1983. A split root system for evaluating the effect of salinity on components of soybean–*Rhizobium japonicum* symbiosis. *Crop Science* 23: 259–262.
- Tang C, Robson AD, Dilworth MJ. 1991. Which stage of nodule initiation in *Lupinus angustifolius* L. is sensitive to iron deficiency? *New Phytologist* 117: 243–250.
- Van Beusichem ML. 1981. Nutrient absorption by pea plants during dinitrogen fixation I. Comparison with nitrate nutrition. *Netherlands Journal of Agriculture* 29: 259–272.
- Van Beusichem ML, Kirkby EA, Baas R. 1988. Influence of nitrate and ammonium nutrition on the uptake, assimilation and distribution of nutrients in *Ricinus communis*. *Plant Physiology* 86: 914–921.
- Van Egmond F, Aktas M. 1977. Iron nutritional aspects of the ionic balance of plants. *Plant and Soil* 48: 685–703.
- White PF, Robson AD. 1989. Rhizosphere acidification and Fe^{3+} reduction in lupins and peas: Iron deficiency in lupins is not due to a poor ability to reduce Fe^{3+} . *Plant and Soil* 119: 163–175.
- Williams CH. 1980. Soil acidification under clover pasture. *Australian Journal of Experimental Agriculture and Animal Husbandry* 20: 561–567.