

Nitrate leaching stimulates subsurface root growth of wheat and increases rhizosphere alkalisation in a highly acidic soil

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Abstract Subsurface acidity is a major factor limiting crop yield in some agricultural soils. The surface application of lime has limited effect on the subsurface acidity due to the slow downward movement, while deep incorporation of lime is costly. This paper tested the concept of biologically ameliorating subsurface acidity in a highly acidic soil through the net uptake of anions by plant roots. Nitrogen was supplied to the top soil (0–10 cm) as $\text{Ca}(\text{NO}_3)_2$ at rates equivalent to 30–240 kg N ha^{-1} . Four water levels were imposed (40, 60, 80 and 100% of field capacity). Aluminium-tolerant wheat was grown for 58 days. The high N and high water treatments stimulated root growth below 15 cm, which in turn increased N capture, resulting in a greater excess anion uptake over cations and thus alkalisation of subsurface soil layers. This study suggests that it is feasible to exploit the process of nitrate uptake by an aluminium-tolerant wheat genotype to increase pH in acidic subsoil.

Keywords Cation-anion balance · Carbon allocation · Root-soil interactions · N and water interaction · Soil management · Soil acidity

Introduction

Subsurface acidity can be a major factor limiting crop yield in some agricultural soils (Farina and Channon 1988; McLay et al. 1994; Scott et al. 1997; Tang et al. 2001). Rooting depth and root growth at depth can be severely restricted under these conditions (Adams and Moore 1983; Adams 1984; Tang et al. 2003). The surface application or incorporation of lime has limited effect on subsurface acidity due to the slow downward movement of lime into the subsurface (Conyers and Scott 1989; Scott et al. 1997; Conyers et al. 2003). Furthermore, the deep incorporation of lime can be effective but is impractical due to the lack of suitable machinery, the high cost and the negative effect on soil structure (Simpson et al. 1979; Sumner et al. 1986; Scott et al. 1997). New approaches to combating subsurface acidity are required.

One such approach is to use plant roots to alleviate subsurface acidity by increasing net uptake of anions to alkalisate rhizosphere. When plant uptake of ions occurs where one type of charge exceeds the other, the plant maintains electroneutrality by excreting H^+ or OH^- , leading to acidification or alkalisation of the rhizosphere. The type of charge released by plant roots mainly depends on the form of nitrogen that is

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available to plants, while a large supply of NO_3^- -N resulting in excess anion uptake (Mengel et al. 2001) and rhizosphere alkalisation will occur in the same root zone where excess anion uptake took place (Moorby et al. 1985; Weligama et al. 2008). Therefore, in order to decrease acidity by releasing basic anions in the subsurface soil, a surplus supply of NO_3^- needs to occur in the subsurface.

There are a number of requirements for this approach to be feasible. The first is that there needs to be a means for delivering significant amounts of NO_3^- to the subsurface soil layers to ensure that there will be a surplus of anions in the subsurface. Thus, surface NO_3^- application and surface watering will be required. This approach has been successful in a soil column experiment where NO_3^- addition to the top 10 cm, followed by leaching together with plant growth resulted in a reduction in acidity throughout the 100-cm soil column (Tang et al. 2000).

The second requirement is that there should be adequate subsurface root growth to intercept and take up leaching nitrate in the subsurface layers. The presence of the leached NO_3^- in the subsurface layers can stimulate root growth in these layers. For example, Diggle and Bowden (1990) measured root mineral nitrogen and root length densities at 3 and 6 weeks after seeding of wheat in a soil column experiment. There was a remarkable similarity between root density profiles after 6 weeks and the mineral N profile 3 weeks after seeding. The root length densities had increased in soil layers where mineral N was high at the earlier time. As long as the rate of leaching is not excessive, then the expanding root system has the potential to capture leached NO_3^- . Thus, the leaching losses can be minimised and the capture of N from deep soil layers can be increased, if there is sufficient root growth in the subsurface (Poss et al. 1995; Smith et al. 1998; Thorup-Kristensen 2001; Dunbabin et al. 2003).

The final requirement for the above approach is that the plants must be tolerant to subsurface acidity, which is generally related to tolerance to Al toxicity, so that they can effectively grow roots in the hostile subsurface. Previous studies show that crops resistant to acidity or Al toxicity could be used as scavengers for nutrients deep in the profile (Romulo et al. 1997) or as ameliorators of subsurface acidity (Noble et al. 1987; Tang et al. 2000; Weligama et al. 2008) because of their ability to proliferate roots in deep acid layers.

This paper reports on a glasshouse soil-column experiment that aimed to test the concept of biologically ameliorating subsurface acidity in a highly acidic and Al-toxic soil through excess anion uptake by plant roots. The approach was to grow an Al-tolerant wheat genotype in 50-cm soil columns with varying amounts of NO_3^- in the surface and then impose different watering regimes to move the NO_3^- into the subsurface layers. We postulated that nitrate in subsurface layers can stimulate root proliferation in those subsurface layers, resulting in high nitrate capture and uptake and that the resulting surplus anion uptake over cations would lead to rhizosphere alkalisation.

Materials and methods

The treatments

A soil column experiment was carried out under naturally lit glasshouse conditions at the La Trobe University farm (37°42'S, 145°02'E). The experiment was factorially arranged with 4 nitrogen treatments and 4 water treatments and three replicates. The air temperature was kept at approximately 25°C for the day and 15°C for the night. Nitrogen was supplied to the surface (0–10 cm) as $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ at rates of 23.5, 47.0, 94.0 and 188.0 mg N column⁻¹. These amounts were equivalent to 30 (N30), 60 (N60), 120 (N120) and 240 (N240) kg N ha⁻¹ on a surface-area basis. The selection of the minimum rate was based on a previous experiment with the same type of soil (Weligama et al. 2008). Four water levels were maintained for each of nitrate treatments. Water was added up to 40% (FC40), 60% (FC60), 80% (FC80) and 100% (FC100) of field capacity (FC). The depth to the wetting front ranged from about 20 cm for the lowest and 50 cm for the highest water treatments (There were 16 treatments in the experiment).

Soil column construction

The PVC tube with 10-cm diameter and 60-cm height consisted of two equal halves that were taped together and a PVC cup was placed on the bottom. A 3-cm layer of high-density polyethylene (HDPE) beads was placed at the bottom of each column to facilitate any drainage. The soil column then received 4.8 kg of air-dried soil filling it to 15 cm from the top of each

column. The soil was compacted to a bulk density of 1.4 g cm^{-3} . Then an NO_3^- -containing layer of 1.2 kg of soil was compacted to the surface. The two soil layers were separated by a 2-cm layer of white HDPE beads so that they could be recognised at the end of the experiment. After wheat seeds were sown, the soil surface was covered with 2-cm layer of HDPE beads to minimize water evaporation. Two N treatments (N120 & N240) were also included as controls without plants. These columns were kept at 60FC and brought up to 100FC one week before the plant harvesting.

Soil and nutrient additions

A sandy soil with pH 3.66 (0.01 M CaCl_2) and low pH buffer capacity ($0.81 \text{ cmol kg}^{-1} \text{ pH}^{-1}$) was collected from virgin land in the Cranbourne area of Victoria. The site had no history of fertilizer application or any other agriculture practice in the past 50 years. The soil was air-dried, sieved through a 4-mm sieve and then mixed well. Some soil characteristics were presented in Weligama et al. (2008). The following basal nutrients (mg kg^{-1}) were added in solution to the soil; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ -17, KH_2PO_4 -175.8, K_2SO_4 -87.5, CaCl_2 -270, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ -10, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ -10, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -2, H_3BO_3 -0.67 and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ -0.17. The total amount of K supplied in all treatments was 89.8 mg kg^{-1} which ensured sufficient K supply to the plants (Weligama et al. 2008). The basal nutrients were added uniformly through the entire soil column for all treatments. The nutrient solutions were pipetted directly onto the soil. The soil was air-dried and then well mixed before filling the columns. Each layer was brought to 60% of FC (12% w/w) at the time of filling.

Planting and watering

Ten pre-germinated seeds of an aluminium-tolerant wheat (*Triticum aestivum*, L) genotype (ET8) were sown per column. The seedlings were thinned to five plants per column at 9 days after sowing (DAS). No water was added until the water content of all treatments reached 40% of FC. Water was then added as required every 7th day from 30 DAS until harvesting. The water status dropped up to 25–30% less than the target water level in the FC40 and FC60

treatments and up to 40–50% in FC80 and FC100% treatments during these 7 days. The columns were weighed every time before and after adding water. The amounts of water loss were recorded and used for calculation of water use by plants. No leaching was observed from the bottom of columns in any treatment during the experiment. The average water loss from columns without plants was deducted each time to calculate water loss by transpiration. Plant heights were measured seven times and the number of tillers was counted at 4 different stages of the plant growth. The plant shoots were harvested by removing them at the surface level on 58 DAS. The shoots were washed three times in de-ionised water and then oven dried at 70°C for 48 h before being weighed.

The soil was sampled by slicing the soil column into 0–10, 10–15, 15–25, 25–35 and 35–50 cm soil layers. The roots were separated from the soil with care. The rhizosphere soil was collected by shaking soil adhered to roots into a plastic container. The remaining soil was considered as bulk soil, which might be partly affected by root activities. The collected bulk and rhizosphere soils were air-dried and sieved through 2-mm sieve for chemical analysis. The rhizosphere soil mass was recorded and it accounted for <2% of the total soil mass in each layer. The separated roots were also washed. Root growth parameters were measured. The root samples were then dried at 70°C for 48 h. Root measurements in soil layers were presented on a soil volume basis (per cm^3).

Measurements

Root measurements including total root length and number of root tips were measured using an EPSON EU-35 scanner (Seiko Epson Corp., Japan) with the Win Mac Rhizo Pro version 2003b programme. Scanning was done at 400 dpi resolution. Greyscale images taken from the scan were analysed by the method of automatic thresholding. Oven-dried shoot and root samples were weighed for dry weights. The pH of rhizosphere and bulk soil were measured using a Thermo Orion 720 pH meter after extraction in 0.01 M CaCl_2 solution (1:5 w/v soil:solution ratio) by shaking for 17 h on an end-over-end shaker. Soil pH buffer capacity measured according to Xu et al. (2006). Briefly, knowing amounts of NaOH and HCl (cmol kg^{-1}) were added to extractions of soil (1:5 w/v soil: 0.01 M CaCl_2) over a range and

pH was plotted against added amounts of acid or alkali. Nitrate in soil was measured colorimetrically in 2M KCl extracts (Mulvaney 1996).

The shoot material was digested in 4:1 concentrated nitric: perchloric acids. Concentrations of Ca, Mg and P in digests were analysed by an inductively coupled plasma atomic emission spectrometry (ICPAES). The concentrations of K and Na were analysed in a Corning Clinical Flame Photometer 410C (Corning Ltd., England). Chloride was analysed using a Tosco 920 Chloride meter (TOSCO, Pty. Ltd., England) after extracting in water using the method described by Sargeant et al. (2006). The concentrations of N and S were determined using an Elementar Vario EL III (Elementar Analysensysteme GmbH, Germany). The excess anion concentration was calculated as the difference in the sum of the charge concentrations of cations ($\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^{+} + \text{Na}^{+}$) and anions ($\text{NO}_3^{-} + \text{H}_2\text{PO}_4^{-} + \text{SO}_4^{2-} + \text{Cl}^{-}$). In this instance, it was assumed that all N was taken up in nitrate form. The net (excess) anion uptake by plants was calculated by multiplying shoot yield by net excess anion concentration in the shoots. Total hydroxyl release per column of soil was calculated by adding hydroxyl released to each layer to increase rhizosphere and bulk soil pH. This was done by multiplying the change in pH (from the original soil pH at start of experiment) by pH buffering capacity of the soil (Xu et al. 2006).

The data were statistically analysed using Genstat Release 4.2, Lawes Agricultural Trust, Rothamsted Experimental Station. A two-way ANOVA was performed on shoot and root dry matter yields, shoot/root ratio, tiller numbers, water use, specific root length and root measurements below 15 cm. ANOVA was also performed to test the significance of main treatment effects and their interactions for pH, root measurements and residual NO_3^{-} concentrations in soil layers. The least significant difference (LSD) was used to compare any two individual means. Linear regression was conducted to correlate net anion uptake by shoots and total hydroxyl release to the soil columns.

Results

Shoot growth

Shoot DM increased significantly ($P < 0.001$) with increasing N rates and increasing soil water content in

the columns. However, significant N×Water interaction ($P < 0.01$) occurred for shoot DM yield (Table 1) as the shoot growth response to the highest N treatment depended on the water supply. When N addition was increased from N120 to N240, the only significant increase in shoot DM occurred where the water supply was maintained at FC100. Tiller numbers responded to N and water treatments in a similar manner to shoot yields with a regression coefficient of 0.93 between these parameters (data not presented). Plant height also responded to N and water treatments (data not presented) where only main effects of the N and water treatment were significant and the effect of N was evident from 15 DAS and the effect of water from 36 DAS. The total water use also highly correlated with shoot DM ($R = 0.90$) (data not presented).

Root growth

Total root DM responses to N and water treatments were different from those of the shoots, with only the main effects of N and water being significant ($P < 0.05$). Total root DM increased with increasing water supply (Table 1). However, maximum root DM occurred with the N60 and N120 treatments, with the main effect of N means decreasing when N addition was increased to N240. Total root length and number of root tips per column were also affected by N and water treatments with significant N×Water interactions occurring (data not presented) ($P < 0.05$), as the response in total root length and the number of root tips to N120 and N240 depended on soil water status. In this case, root length and number of root tips increased significantly where soil water supply increased from FC80 to FC100.

A significant N×Water interaction occurred in shoot/root ratio resulting from a greater increase in this ratio between N120 and N240 when soil was dry (FC40) relative to wet soil treatment (FC100) (Table 1). Little variation occurred in shoot/root ratios among various water treatments where N supply was low (N30/60).

Changes in root growth with depth

The analysis of root DM production in soil layers revealed a significant interaction of N×Water×Soil Layer ($P < 0.01$). The basis for this interaction was the different root DM responses to NO_3^{-} supply, occurring

Table 1 Effect of NO_3^- supply in 0–10 cm and soil water treatment on shoot and root growth, root/shoot ratios and proportion of root parameters below 15-cm layers of wheat plants grown in the acid soil columns for 58 days

Treatment		Shoot DM (g column ⁻¹)	Root DM (g column ⁻¹)	Shoot/ Root ratio	Root DM below 15 cm (% of total)	Root length below 15 cm (% of total)	No of root tips below 15 cm (% of total)
Water regime (% field capacity)	NO_3^- in surface (kg N ha ⁻¹)						
40	30	1.95	0.77	2.53	52	60	66
	60	2.54	1.13	2.28	61	69	71
	120	3.24	0.91	3.61	49	59	65
	240	2.81	0.57	4.96	36	50	65
60	30	2.64	1.14	2.35	69	79	80
	60	3.11	1.48	2.12	60	70	72
	120	3.86	1.57	2.48	53	65	68
	240	4.14	1.03	4.12	29	46	55
80	30	2.98	1.32	2.28	56	70	72
	60	3.71	1.69	2.21	54	68	65
	120	4.34	1.69	2.60	62	76	74
	240	4.70	1.10	4.28	49	65	68
100	30	3.18	1.11	2.87	50	67	63
	60	4.22	1.92	2.28	52	68	62
	120	4.74	1.81	2.68	60	76	71
	240	6.10	1.74	3.55	64	82	80
LSD ($P=0.05$) for any two means		0.58	0.36	0.71	14	11	9
Significance level							
Nitrate main effect (N)		***	***	***	**	**	n.s.
Water main effect (W)		***	***	**	n.s.	**	n.s.
N×W interaction		**	n.s.	*	***	***	***

Here n.s., *, ** and *** represent probability of >0.05, <0.05, <0.01 and <0.001, respectively

at different depths, as the water supply to the columns was increased (Fig. 1). Minimal responses to NO_3^- supply through the column occurred with the dry FC40 treatment, whereas a range of N responses in root growth occurred with the wet FC100 treatment. With this wet treatment, maximum root growth (significantly greater than the low N30 treatment) occurred in the 0–10 and 10–15 cm layers with N60. In the deeper 15–25 cm layer, maximum response occurred with the N120, while in 25–35 cm layer, the maximum response occurred with the N240, with the two high N treatments producing more root growth than the low N30 and N60 treatments.

Changes in root length with soil depth also indicated that a significant N×Water×Soil Layer interaction occurred ($P<0.001$) (Fig. 2). Again, the reason for this interaction was the increased root length generated in deep soil layers (20–40 cm) with

the highest rate of NO_3^- addition (N240) and the wettest soil treatment (FC100) (Fig. 2D). For example, root length per cm³ of soil in 25–35 cm soil layer nearly doubled from 2.4 to 4.6 cm with the high N treatment (N240) compared to the N30 treatment where the soil water status of the column was maintained at FC100. The opposite occurred with drier soil columns, where more root length occurred in of 25–35 cm layer with the supply of N30, N60 and N120, where soil water was maintained at FC40 or FC60. A similar increase in root length occurred with the second highest N treatment (N120) in the shallower 15–25 cm layer compared to other N treatments where the soil water status was maintained at FC80 or FC100 (Fig. 2C and D). There were no differences in root length in this layer between N treatments with the dry FC40 or FC60 treatments. The number of root tips produced in different soil layers

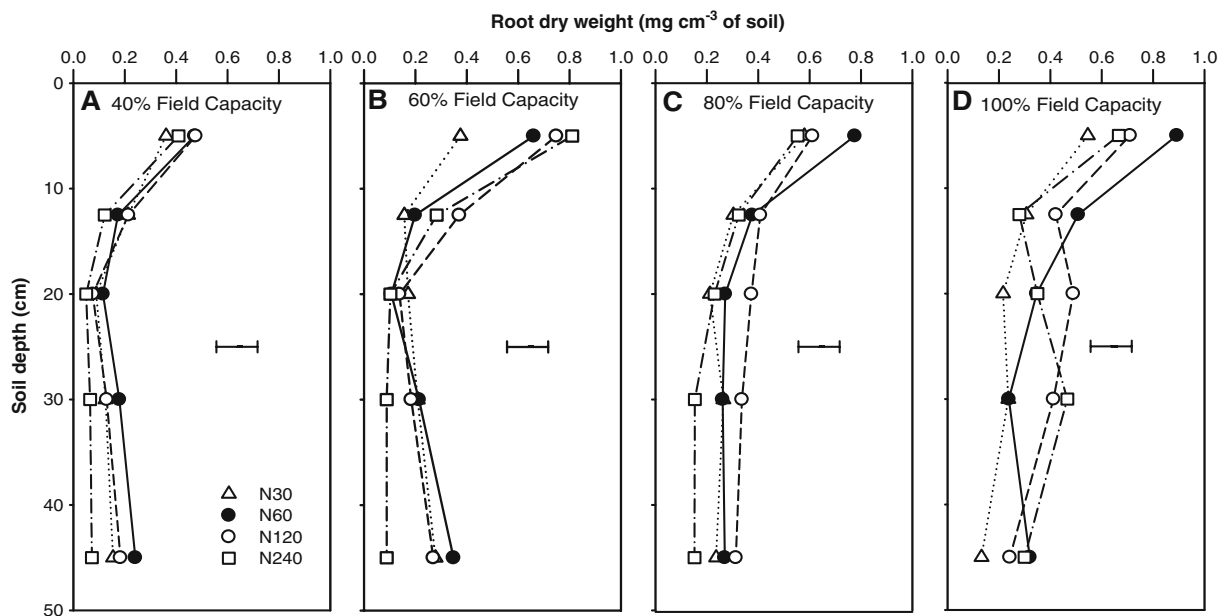


Fig. 1 Changes with soil depth in root dry matter of wheat plants grown for 58 days in soil columns receiving 30 (N30), 60 (N60), 120 (N120) and 240 (N240) kg N ha⁻¹ at various levels of water supply. Levels of significance for the main effects and interaction terms from ANOVA are Nitrogen (N) ($P < 0.001$),

Water (W) ($P < 0.001$), Soil Layer (SL) ($P < 0.001$), N \times W ($P < 0.05$), N \times SL ($P < 0.05$), W \times SL ($P < 0.01$) and N \times W \times SL ($P < 0.01$). Error bars represent the LSD ($P = 0.05$) for the N \times W \times SL interaction means

followed a similar pattern to the root length results (data not presented).

There was a significant interaction between N \times Water in subsurface root measurements (below 15 cm) in terms of root DM, length and number of tips (Table 1). Increasing N supply from N120 to N240 decreased subsurface root growth when water supply was kept at or below FC80. For example, the increase of N from N120 to N240 at FC80 resulted in almost 35% decrease of root dry mass below 15 cm. However, a similar N increase at FC100 did not decrease root dry mass below 15 cm. The greater subsurface root growth in N240-FC100 treatment contributed for a higher percentage of total root (mass, length or tips) located in soil below 15 cm.

Plant N uptake and cations and anions in shoots

Total N uptake by the shoots of wheat plants increased as both N and water supply were increased (Table 2). A significant N \times Water interaction occurred ($P < 0.05$), as a result of greatly increased N uptake where both N and water supply were increased together. For example, there was minimal increase in

plant N uptake in the dry soil treatment (FC40), when N addition was increased from N120 to N240. In contrast, there was a 6-fold greater increase in N uptake for these N treatments with the wettest FC100 water supply. Roots contributed $< 25\%$ of total N uptake by plants (data not presented).

The uptake and total concentration of anions (calculated as though unmetabolised) was substantially greater than the cation uptake and concentration in shoots, resulting in negative cation-anion balance (Fig. 3 and Table 2). The nominal concentration of anions in shoots increased with increasing N rates mainly because of the high nitrate uptake. This created significant main effects of N and Water on the cation-anion balance in shoots. Anion excess increased both with the increase in N application and the water regime.

Changes in soil pH

There was a clear pattern in the rhizosphere alkalisation occurring with increasing NO₃⁻ addition (Fig. 4A–D). Firstly, significant increases in rhizosphere pH only occurred with the highest rate of N

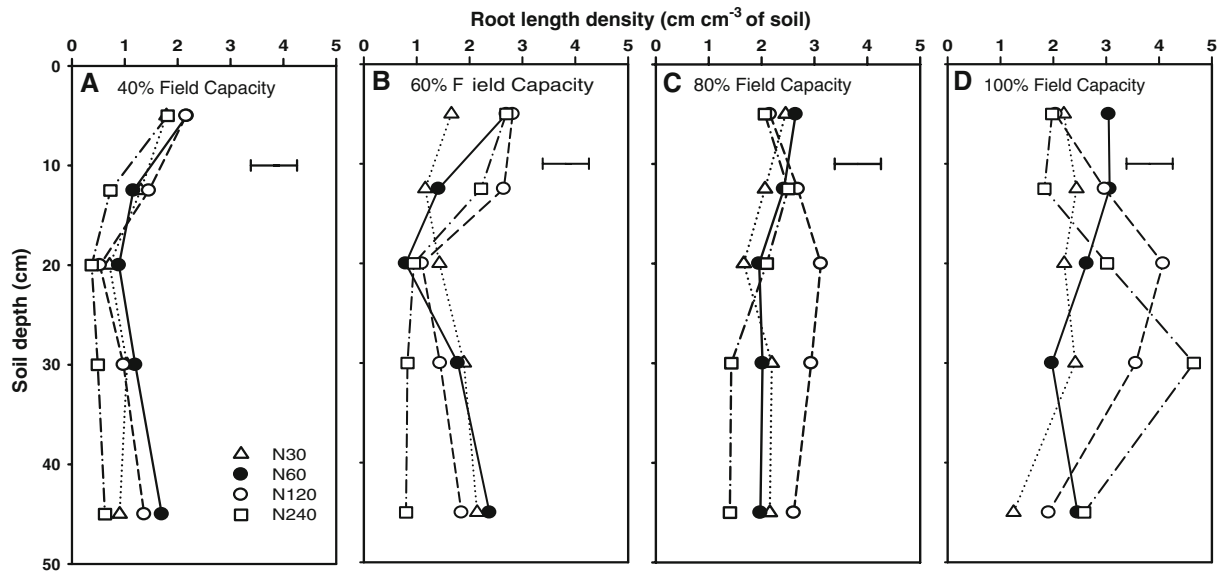


Fig. 2 Changes with soil depth in root length of wheat plants grown for 58 days in soil columns receiving 30 (N30), 60 (N60), 120 (N120) and 240 (N240) kg N ha⁻¹ at various levels of water supply. The main effects of nitrogen (N), water (W) and soil layer

(SL), and their interactions are all significant at $P < 0.001$ except for $N \times W$ ($P < 0.01$) and $N \times SL$ ($P < 0.05$). Error bars represent the LSD ($P = 0.05$) for the $N \times W \times SL$ interaction means

addition (N240) under the three drier water regimes (FC40, FC60 and FC80). Secondly, this alkalinisation only occurred in the top 25 cm of the soil column. However, there were exceptions to the above patterns with significant increase in rhizosphere pH occurring between the N120 and N30 treatments in the surface 10 cm layer in the FC40, FC60 and FC80 water treatments, and in the deeper 25–35 cm layer in the FC40 treatment with the highest N supply (N240).

The supply of N and water affected bulk soil pH in the soil columns (Fig. 4E–H). There was a similar pattern of pH increase to that which occurred in the rhizosphere, where bulk soil pH generally increased with high N treatment (N240) in the upper soil layers of the columns. However, there were some notable differences; one was that pH changes were generally smaller than that occurred in the rhizosphere. For example, rhizosphere pH was increased by 0.6 of a unit in the subsurface soil layer (10–15 cm) in the N240 treatment compared to the N30 in dry soil treatments (FC40). However, there was only 0.1 unit increase in bulk soil pH between these two treatments. Further, bulk soil pH increased more in the deeper soil layers in the wetter FC80 and FC100 treatments. The most notable difference was a significant increase in bulk pH with

N240 treatment in the wettest FC100 compared to the N30 treatment (Fig. 4H) in soil layers between 10 and 35 cm depths. Soil pH in the no-plant controls increased on average of 0.05 units uniformly in all soil layers compared to the pH of the original (data not presented).

NO₃⁻ content in soil layers

The concentration of NO₃⁻ in soil layers at harvest was another measure that depended on the N rate, water supply and soil layer (Fig. 5). The reason for this interaction was the very high NO₃⁻ concentration of around 23 mg NO₃⁻-N per kg of soil in the top 10 cm layer with the highest N240 treatment where soil water was maintained at FC40. This did not occur when water supply was increased to FC60, FC80 and FC100; there was no difference between treatments in NO₃⁻ concentration in the topsoil layer, where NO₃⁻ concentration was depleted to about 2–4 mg per kg of soil. Similarly, there was no difference in the NO₃⁻ concentration among N treatments at any depth for the wettest FC100 treatment (Fig. 5D) and for layers below 15 cm for the driest FC40 treatment. However, with the intermediate FC60 and FC80 treatments, there was increased NO₃⁻ concentration in the deeper

Table 2 Effect of NO_3^- -N supply in 0–10 cm and soil water treatment on the concentration and uptake of N in shoots, residual NO_3^- -N in soil columns, net anion uptake by shoots (calculated as though unmetabolised) and total OH^- release into the rhizosphere and bulk soil

Treatment		N concentration in shoots (%)	N uptake by shoots (mg N column ⁻¹)	Residual NO_3^- in column (mg N column ⁻¹)	Net anion uptake by shoots (mmole column ⁻¹)	Total OH^- release into soil (mmol column ⁻¹)
Water regime (% field capacity)	NO_3^- -N in surface (kg N ha ⁻¹)					
40	30	3.15	74.4	16.6	2.5	3.9
	60	2.93	92.8	17.4	2.7	2.9
	120	3.49	129.8	19.4	4.5	4.0
	240	4.61	141.6	46.3	5.2	5.7
60	30	2.96	95.4	15.3	3.1	3.1
	60	2.69	106.5	17.6	3.2	3.1
	120	2.89	134.0	16.7	4.2	5.8
	240	4.15	189.9	26.7	7.1	5.3
80	30	2.80	104.9	15.9	3.3	2.1
	60	2.58	119.2	18.3	3.4	3.6
	120	2.88	149.6	15.6	4.6	4.0
	240	4.16	216.7	21.8	8.1	6.1
100	30	2.91	110.0	16.6	3.7	3.1
	60	2.51	131.2	15.7	3.6	3.1
	120	2.91	165.4	17.0	5.3	6.4
	240	3.49	242.4	16.8	8.3	9.1
LSD ($P=0.05$) for any two means		0.32	20.5	8.0	1.0	2.2
Significance level						
Nitrate main effect (N)		***	***	***	***	***
Water main effect (W)		***	***	**	***	*
N×W interaction		*	**	**	*	n.s.

Here n.s. *, ** and *** represent probability of >0.05, <0.05, <0.01 and <0.001, respectively

soil layers, indicating less NO_3^- -N uptake by plants and some NO_3^- accumulation with the N60 and N240 treatments. A significant N×Water interaction also occurred for the amount of NO_3^- remaining in the soil columns at the end of the experiment. The basis for this interaction was the higher NO_3^- remaining in dry FC40 and FC60 treatment with the N240 (Table 2). The no-plant control with N240 had NO_3^- concentrations of 85 mg kg⁻¹ in 0–10 cm and around 14 mg kg⁻¹ below 15 cm (data not presented).

Discussion

The main objective of this study was to investigate the possibility of using plant roots to reduce the acidity in the subsurface layers of an acidic soil. An

aluminium-tolerant wheat genotype was grown in the relatively permeable acid soil with the supply of NO_3^- to the soil surface layer, together with sufficient water addition to leach the NO_3^- down into subsurface layers. It was postulated that the correct combination of NO_3^- -N supply and high water addition would stimulate root growth in the subsurface layer, which would in turn help to intercept and take up leached NO_3^- . The uptake of large quantities of leached NO_3^- would result in the excess uptake of anions over cations, leading to increases of rhizosphere and bulk soil pH. The results reported above confirm that these postulated outcomes did occur in this study.

The maximum alkalinisation of the bulk soil was observed in the soil column with the highest NO_3^- supply (N240), when soil was maintained at 100% of

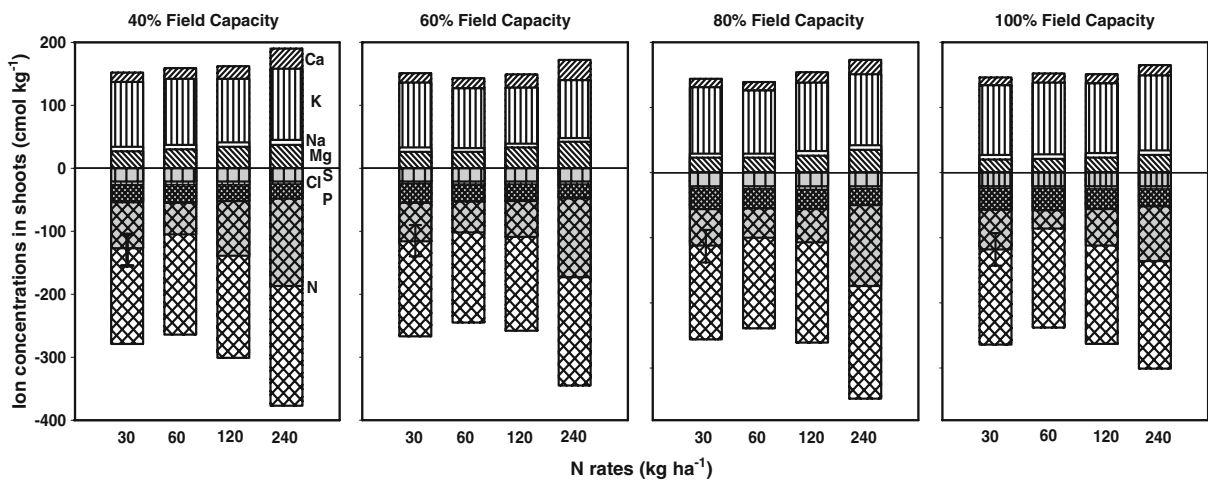


Fig. 3 Concentrations of cations (Ca^{2+} , K^+ , Na^+ and Mg^{2+}) and anions (NO_3^- , H_2PO_4^- , Cl^- and SO_4^{2-}), and the cation-anion balance in shoots (unmetabolised) indicated by the shaded area. Wheat plants were grown for 58 days in soil columns under various levels of nitrogen and water supply. It

was assumed that all N was taken up in NO_3^- form. Levels of significance for the main effects and interaction terms from ANOVA for cation-anion balance: Nitrogen (N) ($P < 0.001$), Water (W) ($P < 0.01$) and $\text{N} \times \text{W}$ ($P > 0.05$). Vertical bars represent LSD ($P = 0.05$) $\text{N} \times \text{W}$ interaction

FC. Slightly over 9 mmol of OH^- ions passed into the rhizosphere and bulk soil in this treatment (Table 2). The basis for this alkalisation in bulk soil was the surplus anions uptake over cations (Fig. 3), which occurred as a direct result of the very high NO_3^- uptake. This treatment had the highest N uptake (Table 2) and the N uptake would be in the NO_3^- form. This in turn resulted in the maximum total anion surplus (calculated as unmetabolised) in plants, as evidenced by the maximum net anion uptake by shoots (Table 2). The surplus anions uptake led to the maximum estimated OH^- ions released from wheat roots to the soil. The strong positive relationship between excess anion uptake over cation uptake in shoot and total estimated OH^- ions release to the soil ($R^2 = 0.70$), obtained from changes in rhizosphere and bulk soil pH and pH buffer capacity is evident in Fig. 6. The root might contribute an additional 20–25% of the estimated excess anion uptake based on the N content. Since the soil contained $12 \text{ mg-NH}_4\text{-N kg}^{-1}$, the uptake of soil NH_4^+ could be significant, and might partially counteract the alkalisation resulting from NO_3^- uptake. The inclusion of root's contribution to excess anion uptake would have shifted the regression line above the 1:1 line while inclusion of NH_4^+ uptake could have moved the regression line downward. Nonetheless, a striking feature of this study was the effect of soil water status. Increasing water supply from 40% to 100% of FC

resulted in increases in total N uptake, surplus anion uptake over cations by between 60 and 70% and OH^- release to the soil.

Another striking result from increasing soil water supply from 80 to 100% of FC together with the highest NO_3^- was the increase in the bulk soil pH relative to the rhizosphere pH (Fig. 4). For example, rhizosphere pH in 15–25 cm with the N240 treatment declined from 4.35 to 4.00 as the soil water status increased from 80% to 100% of FC (Fig. 4C & D), whereas the bulk soil pH increased from 3.80 to 3.95 (Fig. 4G & H). This indicates that the released basic anions ($\text{OH}^-/\text{HCO}_3^-$) were better able to move from the rhizosphere to the bulk soil with the increasing soil water content. Reasons for this are two-fold although the bulk soil might be partly affected by root activities. The first is that the movement of alkalinity occurs predominantly by diffusion (Nye 1986). The soil alkalinity diffusion coefficient (D_{HS}), a parameter which determines the alkalinity/acidity transfer rate, increases with the soil water fraction (Tinker and Nye 2000). This is because diffusion pathways become less tortuous with increasing soil water content. This means that $\text{OH}^-/\text{HCO}_3^-$ ions could diffuse faster in wet treatments than in dry soil treatments, resulting in lower pH difference between the root surface and bulk soil. A second reason is that the pH gradient between rhizosphere and bulk soil is likely to have been higher than in drier water treatments due to

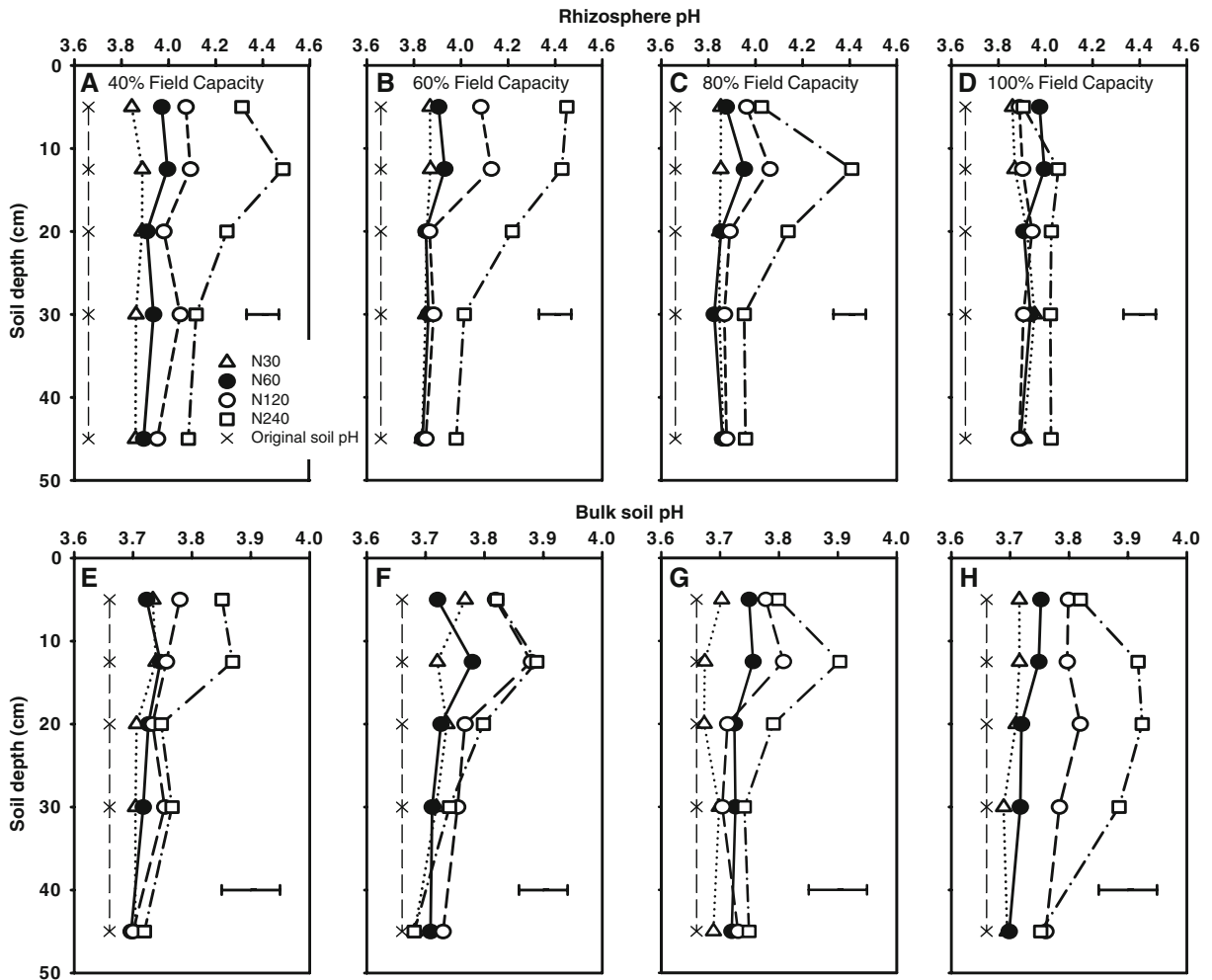


Fig. 4 Profiles of rhizosphere and bulk soil pH after growing wheat for 58 days in soil columns receiving 30 (N30), 60 (N60), 120 (N120) and 240 (N240) kg Nha^{-1} at various levels of water supply. The main effects of nitrogen (N) and water (W) are significant at $P < 0.001$ except the following. Rhizosphere pH: 40% FC–N \times W ($P < 0.05$), 60% FC–N \times W ($P < 0.01$), 80% FC–

N \times W ($P < 0.001$), 100% FC–W ($P > 0.05$), N \times W ($P < 0.05$). Bulk soil pH: 40% FC–N ($P < 0.01$), W ($P < 0.05$), N \times W ($P > 0.05$), 60% FC–N \times W ($P > 0.05$), 80% FC–N \times W ($P > 0.05$), 100% FC–N \times W ($P < 0.05$). Error bars represent the LSD ($P = 0.05$) for the N \times SL interaction means

greater uptake of nitrate resulting in a greater flux of $\text{OH}^-/\text{HCO}_3^-$ into the rhizosphere in wet soil treatments (Table 2).

A key contributing factor to the alkalinisation of the soil in the high N (N240) treatment maintained at 100% of FC was the large proliferated root system in the subsurface layers of the soil column (Table 1). The large root system was associated with the large shoot growth with this treatment, which would be able to supply sufficient photosynthate for root growth. The large shoot canopy transpired a large quantity of water that required more than 2 L of water to be added to the

surface in regular applications to replace transpiration losses and maintain the soil at 100% of FC (data not shown). This in turn would have leached a significant amount of NO_3^- into the subsurface layers, which then acted to stimulate root growth in the deeper soil layer. More than 60% of root mass and more than 80% of root lengths and root tips for this treatment were located below 15 cm of the soil column (Table 1). The stimulatory response of roots in soil volumes with high levels of localised N availability is well documented (Hackett 1972; Robinson 1994; Zhang et al. 1999; Forde and Lorenzo 2001; Linkohr et al. 2002). In

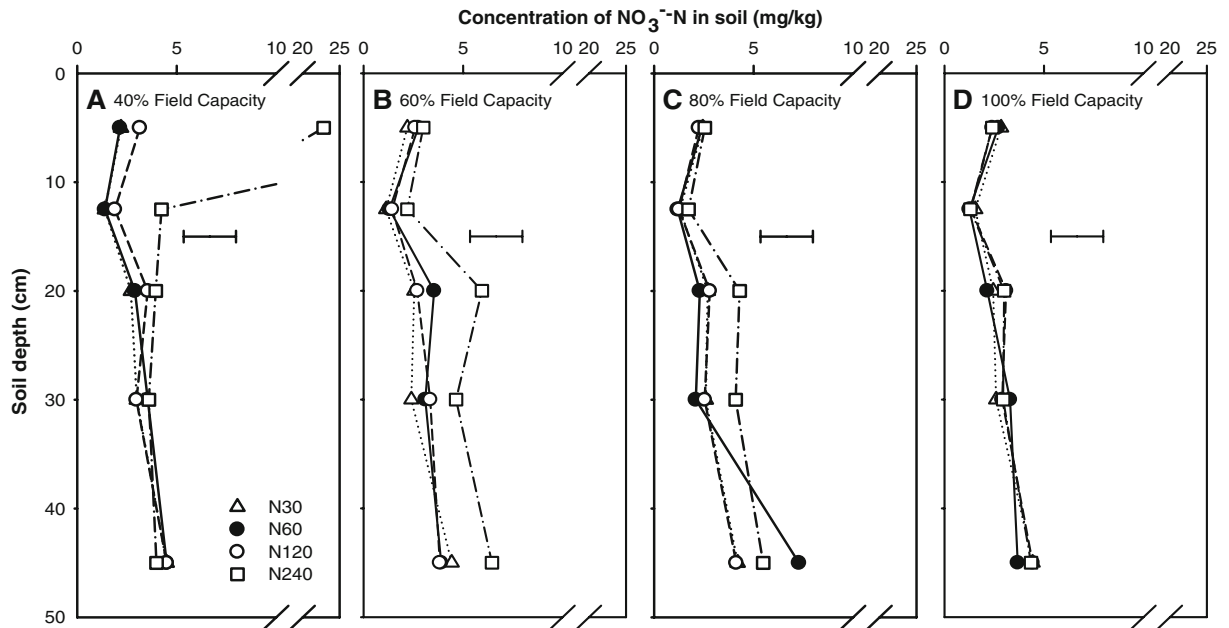


Fig. 5 Changes with soil depth in NO_3^- -N content in soil columns after growing wheat for 58 days under various levels of nitrogen and water supply. The main effects of nitrogen (N),

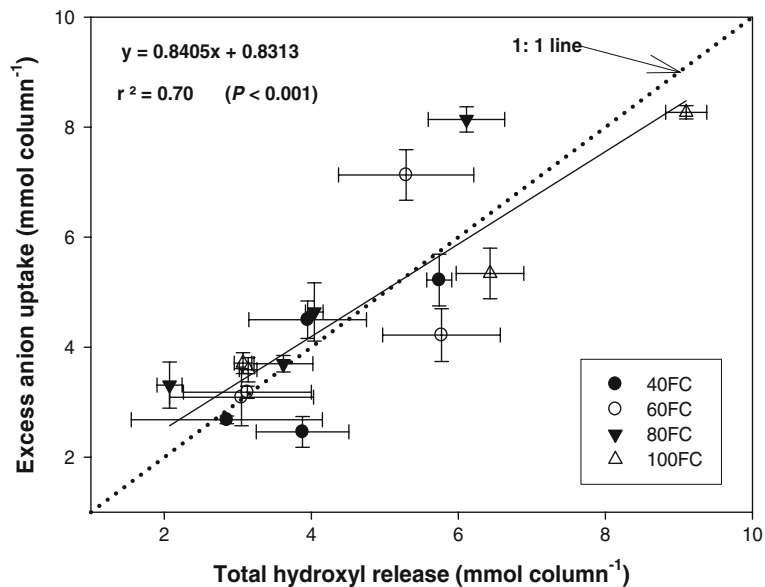
water (W) and soil layer (SL), and their interactions are all significant at $P < 0.001$. Error bars represent the LSD ($P = 0.05$) for the $N \times W \times SL$ interaction means

particular, the local availability of NO_3^- in the growing medium has been observed as having a stimulatory effect on lateral root elongation in *Arabidopsis* (Zhang and Forde 2000) and in maize (Bloom et al. 2003). Thus, stimulation of root growth in the subsurface layers in soil columns maintained at 100% of FC with high surface NO_3^-

supply can be attributed to high levels of N availability in subsurface soil layers, due to extensive nitrate leaching that occurred in the soil column. Similar results have been reported by Diggle and Bowden (1990, 1991) and Diggle et al. (1990).

The opposite effect on deep root growth occurred with high NO_3^- -N supply (N240) and drier soil

Fig. 6 The relationship between total hydroxyl release to soil and net excess anion uptake by plant shoots. Data were means of 3 replicates for four water treatments. Bidirectional bars indicate standard errors (40, 60, 80 and 100% of field capacity)



treatments, where soil water was maintained at 40% and 60% of FC. There appeared to be a suppression of deep root growth in these treatments (Table 1). For example, less than a quarter of root mass and root length that occurred below 15 cm at 100% of FC was observed for the drier soil treatments. This can be explained by the effect of the internal N status of plants on root growth. Shoot-root allocation of photosynthates is known to be governed by the internal nitrogen status of plants (Ågren and Ingestad 1987). The high N uptake could result in high internal N concentrations in shoots, which can alter carbon allocation in favour of shoots (Scheible et al. 1997). We did not measure NO_3^- in shoots; however, there is a strong linear relationship between shoot/root ratios (Table 1) and N concentrations in shoots with a regression coefficient of 0.96 in treatments of the highest N supply (N240) with four soil water status. The recent studies with *Arabidopsis* provide further explanations for the partitioning mechanism of biomass between shoot and roots (Zhang et al. 1999; Forde and Lorenzo 2001; Miller and Cramer 2004). The development of lateral roots can be suppressed by high internal N concentration of shoots, but high N concentrations did not occur with the relatively low N (N30 and N60) and dry soil water treatments (40% & 60% of FC). A greater root production was therefore observed in the deepest soil layer (below 35 cm) in these N treatments compared to the highest N treatments (N240) under the same soil water status (Figs. 1B & 2B). The increased root growth in the deeper layers resulted in decreased shoot/root ratios, which indicates a greater investment of photosynthate in roots (Table 1). The above two different responses in deep root growth highlight the plants plastic response to the available N in soil and the allocation of photosynthates to the root system to ensure that plants' demand for N are met.

The findings in this study highlight the importance of having a large proliferated root system to capture mobile nutrient ions such as nitrate that leach down from the soil profile. There was a minimal residual NO_3^- in the soil column with the highest N supply (N240) that was maintained at 100% of FC (Fig. 5). In contrast, when there were fewer roots in the deeper soil layers of the column, as seen in high N and dryer soil treatments maintained at 60% and 80% of FC (N240-FC60 & N240-FC80) where there was considerably more residual NO_3^- in the deeper layers of the soil column at the end of the experiment (Table 2). Similarly, the

modelling work of Dunbabin et al. (2003) shows that higher root densities as well as deeper rooting can increase nitrate capture in leaching conditions. This is further supported by field observations, which indicate that the capture of leached N reduced N stress in the latter part of the growing season in wheat (Halse et al. 1969). The high N uptake efficiency by the roots in subsurface soil can be attributed to the improvement of 'synchronisation' between NO_3^- availability and NO_3^- interception by roots before it moved beyond the rooting zone (Angus 2001; Liao et al. 2006).

A key finding from this study is that there needs to be a delicate balance between NO_3^- supply and water supply to the soil surface to ensure that there are sufficient roots in the subsurface layers to capture the leaching NO_3^- . For example, root growth in the deepest soil layer was insufficient to capture and take up all NO_3^- available in that layer where modest N (N60) was supplied at the surface and soil water was maintained at 80% of FC. Increasing N supply to N120 under the same water status increased root length density (Fig. 2C) and root branching (data not presented) in the deepest layer thereby capture of NO_3^- in that layer (Fig. 5). On the other hand, if excessive NO_3^- supply occurs in the surface with insufficient soil water supply, then deep root growth will be suppressed, and the successful capture of subsequent leached NO_3^- would be at risk. It will therefore be a challenging task to manipulate N leaching and N capture in rain-fed wheat crops where there is no control in the field over the amount of precipitation during crop development. Further research needs to be done under field conditions to examine the benefit of nitrate leaching and subsequent capture by plants from the subsurface as a measure of combating subsurface soil acidity.

Conclusion

This study shows that it is feasible to exploit the process of nitrate uptake to increase pH in acidic subsoil. There are several key requirements for this to happen; the crop should be tolerant of soil acidity, there needs to be a large amount of NO_3^- supplied to the surface soil and sufficient water to be added to leach the NO_3^- into subsurface soil layers. However, there is a delicate balance required between the NO_3^- supply and the water supply. Insufficient water supply

with high NO_3^- will restrict deep root growth and plants might not be able to capture subsequently leached NO_3^- . Similarly, insufficient NO_3^- supply and adequate water might also fail to produce sufficient deep roots to capture the leached NO_3^- . There also needs to be synchrony in timing, such that the root system has time to develop to capture NO_3^- from subsequent leaching events.

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