

Localised nitrate and phosphate application enhances root proliferation by wheat and maximises rhizosphere alkalinisation in acid subsoil

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Abstract The soil pH in the vicinity of the roots can be changed by an imbalance in supply of predominant anions or cations. A soil column experiment examined the effects of localised supply of nitrate and P on plant growth and pH change in a Podsol (pH 3.76 in 0.01 M CaCl₂ and pH buffering capacity 0.81 cmol kg⁻¹ pH⁻¹). Nitrate [(Ca(NO₃)₂)] and P [(NaH₂PO₄)] fertilizers were applied alone or in combination to either 0–5 cm or 10–15 cm layer of the soil column. Aluminium-tolerant (ET8) and sensitive (ES8) wheat (*Triticum aestivum*, L) were grown for 38 days. Plant height, water use and tiller number were measured during the growth period. Biomass production, root growth and soil pH were determined at the final harvest. On average, ET8 had a greater shoot biomass, root length and water use than ES8. The greatest shoot biomass and water use were achieved where N and P were applied together in the 0–5 cm layer, followed by

N and P together in the 10–15 cm layer and the lowest where N was applied in the 0–5 cm and P in the 10–15 cm layer. Root length density in the subsoil was greatest where N and P were applied together followed by N alone, and the lowest with the supply of P alone. The effect of localised supply was greater on rhizosphere pH than bulk soil pH. The application of N and P together in topsoil and subsoil layers increased rhizosphere pH by 0.4 and 0.5 units respectively, compared to the corresponding layers in the treatment where N and P were applied uniformly in the whole soil column. Changes in rhizosphere pH were similar under both genotypes, although ET8 produced more roots than ES8 in the soil profile. The results suggest that the combined application of nitrate and P is necessary to maximise root proliferation and root-induced alkalinisation in acid subsoil.

Keywords Biological amelioration · Cation-anion balance · Excess cation uptake · Localised supply · Nutrient placement · Subsoil acidity

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Introduction

Acidification of subsoils is becoming a major concern for agricultural production in Australia and around the world (Carr and Ritchie 1994; Scott et al. 1997; Tang et al. 2001). It is estimated that over 1 billion ha are affected globally by subsoil acidity (Mengel et al. 2001). Soil acidity in the surface soil can be easily

ameliorated by the conventional surface lime application. On the other hand, subsurface acidity is considered as “an irreversible problem” due to amelioration difficulties (Smith et al. 1994; Crawford et al. 1995). While surface application of lime is ineffective to ameliorate subsoil acidity due to the slow movement of lime in soil profile, deep incorporation of lime is not economically feasible, especially where rainfall is low and profit margins are small. Moreover, surface lime application can further increase the vertical differences in pH within the soil profile causing crop and pasture production problems (Helyar 1991; Coventry 1992; Tang et al. 2000; Conyers et al. 2003). New management strategies are needed to combat the deleterious effects of subsoil acidity on agricultural production.

Release of proton or hydroxyl ions by roots, resulting from differential ion uptake, is known to contribute to pH changes in the rhizosphere (Marschner 1995; Tang and Rengel 2003). The H^+ excretion due to predominant cation uptake is known to contribute to acidification in agricultural soils (Tang et al. 2000). In contrast, the plant's uptake of nitrate can create net alkalisation in soil layers (Noble et al. 1987; Poss et al. 1995; Tang et al. 1999). This mechanism may provide an opportunity for possible ways to biologically ameliorate soils with subsoil acidity.

Plants respond to the localised supply of nutrients by proliferating roots in these patches with high nutrient concentration (Hackett 1972; Drew 1975; Valizadeh et al. 2002; He et al. 2003; Trapeznikov et al. 2003; Hodge 2004). The banding of fertilizers at a certain depth in the soil is one way of creating conditions for the localised supply of nutrients available to plants. In practice, this can be achieved during tillage before sowing or by injection after the emergence (Robinson 1994). Banding fertilizer at sowing not only reduces cost for farmers but it also enhances fertilizer efficiency by placing the nutrients in positions where root contact is most likely (Moody et al. 1995). In addition, the localised application of P fertilizers in higher concentrations is a strategy to cope with a great capacity for P fixation, particularly in acid soils (Williams and Simpson 1965; Neumann and Römheld 2002).

Root systems in acid soils are normally shallow with few lateral roots, especially in subsurface layers, because of pH and its associated Al toxicity (Adams and Moore 1983; Pinkerton and Simpson 1986). However, some plant species and genotypes

within a species have deeper root systems than others, due to their ability to withstand high concentration of soluble Al (Ahlrichs et al. 1991; Romulo et al. 1997; Gilker et al. 2002; Tang et al. 2001, 2002;). This Al resistance in plants has been used in breeding programmes to develop crops with increased productivity in acid soils (Garvin and Carver 2003).

Root proliferation in acid soil in general and acid subsoil in particular is important in terms of productivity increase and possible biological amelioration. Localised application of N and P can play a vital role in the stimulation of root growth either in the surface or subsoil layer. Previous studies indicate that local concentrations of K had relatively little effect on root development where K was supplied (Drew 1975; Philipson and Coutts 1977). Hence the focus of this study was on P and N supply. Although it has been proven that localised supply of N and P enhances root proliferation, it is unknown how the localised effect of these two nutrients applied separately or together in soil layers will affect root growth and root-induced pH changes in acid soils. The present study aimed to examine the effects of localized supply of nitrate and P on root proliferation and the root-induced alkalization in acid subsoil and to provide basic information for possible biological amelioration of subsoil acidity through managing plant nutrient uptake. Two near-isogenic wheat genotypes differing in aluminium tolerance were compared for their effects on pH change.

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 5 nutrient placement treatments of two wheat genotypes in four replicates. Nitrate and P were applied in combination or separately to the surface (0–5 cm), subsurface (10–15 cm) or uniformly throughout the column (0–50 cm). The 5 placement treatments were: (1) NP applied throughout the 0–50 cm soil column as control (denoted as NP0–50); (2) NP applied in 0–5 cm soil layer (NP0–5); (3) N applied in 0–5 cm soil and P in 10–15 cm soil layer

(N0–5/P10–15); (4) P applied in 0–5 cm soil and N applied in 10–15 cm layer (P0–5/N10–15) and (5) NP applied in the 10–15 cm soil layer (NP10–15). The NP treatment without plants was not used as a control because our previous study showed that nitrate profiles were totally different during the experiment period between the treatments with and without plants (Tang et al. 2000). A preliminary experiment showed that the plants grew extremely poorly in this highly leached soil without N and P, and used much less water and nutrients than those grown in the above 5 placement treatments. Obviously, the treatments with and without supply of N & P were not comparable, and thus the treatment without NP was not included as another control.

The basal nutrients were added uniformly through the entire soil column for all treatments. The two wheat (*Triticum aestivum* L.) genotypes grown in the experiment were Al-tolerant ET8 and Al-sensitive ES8. These are near-isogenic lines (over 95%) differing in Al tolerance at Alt 1 locus (Delhaize et al. 1993). The air temperature was kept approximately 25 °C for the day and 15 °C for the night.

Soil column construction

The PVC tube with 10 cm diameter and 60 cm height consisted of two equal halves that were taped together. A 5-cm layer of high-density poly ethylene (HDPE) beads was placed at the bottom of each column to facilitate any excess water collection in the lower part of the column. Air-dried (4.2 kg) soil was filled up to 15 cm from the top of each column. The soil was compacted to a bulk density of 1.4 g cm⁻³. Then three soil layers were compacted to the surface. Each of these layers consisted of 600 g of treated soil. The soil layers were marked with white HDPE beads so that they could be recognised at the end of the experiment and also to disrupt capillary water movements between layers. Three micro-tubes with 5 mm internal diameter were vertically placed in each column, one for watering soil below 15 cm, one for 10–15 cm layer and the other for 5–10 cm layer. Water was added from the surface to the top (0–5 cm) layer. This was a precautionary measure to minimise nutrient leaching, especially nitrate from the surface (0–5 cm) and subsurface (10–15 cm) layers. After wheat seeds were sown, the soil surface was covered with 2-cm HDPE beads to minimize water evaporation.

Soil and nutrient additions

A sandy soil with pH (3.76 in 0.01 M CaCl₂) and pH buffer capacity (0.81 cmol kg⁻¹pH⁻¹) was collected from virgin land in the Cranbourne area, 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 before filling the columns. Some soil characteristics are presented in Table 1. The following types and amounts (mg kg⁻¹) of basal nutrients were added in solution to the soil: MgCl₂·6H₂O, 17; K₂SO₄, 200; CaCl₂, 270; MnSO₄·H₂O, 10; ZnSO₄·7H₂O, 10; CuSO₄·5H₂O, 2; H₃BO₃, 0.67; and Na₂MoO₄·2H₂O, 0.17. Nitrogen was applied as Ca(NO₃)₂·4H₂O at 397.2 mg column⁻¹. This N rate was equivalent to 60 kg N ha⁻¹. The P was applied as NaH₂PO₄·2H₂O at 118.6 mg column⁻¹, which resulted in an equivalent application of 30 kg P ha⁻¹ on a surface area basis. The solutions of nutrients were pipetted directly onto the soil. The soil was air-dried, then well mixed before filling the columns. Each layer was brought to field capacity (20% w/w) at the time of filling.

Planting and watering

Ten pre-germinated seeds were sown per column. The seedlings were thinned to 5 plants at 11 days after sowing (DAS). Water was added every 3 days during the first 22 days and then every 2 days till the end of the experiment. The water was added through the micro-tubes. The columns were weighed each time before adding water. The amounts of water added

Table 1 Soil characteristics at the commencement of the experiment

Characteristic	Value
pH (1:5 0.01 M CaCl ₂)	3.76
pHBC (cmol kg ⁻¹ pH ⁻¹)	0.81
Extractable Al (mg kg ⁻¹)	3.9
Nitrate (N mg kg ⁻¹)	1.0
Ammonium (N mg kg ⁻¹)	12.0
Phosphorous (mg kg ⁻¹)	2.0
Potassium (mg kg ⁻¹)	17.0
Iron (mg kg ⁻¹)	100.0
Organic carbon (mg g ⁻¹)	9.5
Conductivity (dS m ⁻¹) (1:5 H ₂ O)	0.108

were recorded and used for calculation of water use by plants. Evaporation loss was minimized by the beads layer on the surface. Plant heights were measured at 6 different stages of the study. The number of tillers was counted at 29 and 38 DAS. The plants shoots were harvested by removing them at soil level on 38 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 0–5, 5–10, 10–15, 15–20 and 20–50 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 container. The remaining soil was considered as the bulk soil which might be partly affected by root activities. The collected bulk and rhizosphere soil were air-dried and sieved through 2 mm sieve for chemical analysis. The separated roots were also washed. Root growth parameters were measured. The root samples were then dried at 70°C for 48 h.

Analyses

Root growth parameters including total root length, number of root tips and root volume from different soil layers were measured on a EPSON EU-35 scanner (Seiko Epson Corp., Japan) using Mac Rhizo Pro version 2003b Programme. Oven-dried shoot and root samples were weighed for dry weights. The pH of bulk and rhizosphere soil was measured using a Thermo Orion 720 pH meter after extraction in 0.01 M CaCl₂ solution (1:5 w/v soil: solution ratio) by shaking for 17 h on an end-over-end shaker. The following methods were used to analyse soil: extractable Al-pyrocatechol violet method (Conyers et al. 1991), organic carbon (Walkley and Black 1934), nitrate and ammonium nitrogen (Searle 1984), phosphorus (Colwell 1963) and Fe (Parfitt and Childs 1988).

The shoot material was digested in 4:1 concentrated nitric: perchloric acids. Concentrations of Ca, Mg and P in digestion were analysed by an inductively coupled plasma atomic emission spectrometry (ICPAES). The concentrations of Na and K 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). Total nitrogen and sulphur in shoots were determined using an Elementar Vario EL III (Elementar Analysensysteme GmbH, Germany). The non-nitrogenous excess cation concentration was calculated as the difference in the sum of the charge concentrations of cations (Ca²⁺+Mg²⁺+K⁺+Na⁺) and anions (H₂PO₄⁻ + SO₄²⁻ + Cl⁻). The cation–anion balance was calculated as the difference between non-nitrogenous excess cation and the concentration of N in shoot. In this instance, it was assumed that all N was taken up in nitrate form.

These data were statistically analysed using GenStat Release 4.2, Lawes Agricultural Trust, Rothamsted Experimental Station. A split-plot design of ANOVA was performed to test the significance of treatment effects and interactions between placement treatment, genotype and soil layer for root parameters and pHs, and the treatment effects and interactions between placement treatment, genotype and date (days after sowing) for shoot growth parameters. Least significant difference (LSD) was used to compare any two individual means. Linear regression was conducted to correlate pH change with root length densities.

Results

Shoot growth

Significant differences in the shoot growth of wheat were observed between the five NP treatments, and between the two genotypes. The application of N and P together in either the surface (0–5 cm) or the subsurface (10–15 cm) soil layers increased shoot growth compared to the treatments of N and P supplied in separate layers (Tables 2 and 3). The combined N and P treatments produced significantly ($P < 0.05$) greater shoot dry matter, more tiller numbers and taller plants after 38 days of growth. They generally out-yielded the treatments of N and P supplied separately by about 30% in terms of both dry matter and tiller number. Supply of N and P separately in both the surface layer and subsurface layer produced no significant differences in shoot growth. Supplying N and P together in the entire column resulted in intermediate growth in terms of shoot dry matter yield and tiller number, but not for plant heights (Table 3). Smaller differences in shoot

Table 2 Levels of significance for the main effects and interaction terms from the analysis of variance for various measurements made after 38 days of growth

Source of variation	Shoot dry matter (g column ⁻¹)	Plant height (cm)	Tiller numbers (no column ⁻¹)	Root dry matter (g per 5-cm soil layer)	Root length (cm per 5-cm soil layer)	Root tips (No per 5-cm soil layer)	pH	
							Bulk soil	Rhizosphere
Genotype (<i>G</i>)	**	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
Treatments (<i>T</i>)	***	***	***	***	***	***	***	***
<i>G</i> × <i>T</i>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Shoots								
Date (<i>D</i>)		***	*					
<i>D</i> × <i>T</i>		**	n.s.					
<i>D</i> × <i>G</i>		n.s.	n.s.					
Roots								
Soil layer (<i>L</i>)				***	***	***	***	***
<i>L</i> × <i>T</i>				***	***	***	***	***
<i>L</i> × <i>G</i>				n.s.	n.s.	n.s.	**	n.s.

Where n.s., *, ** and *** represent probability of >0.05, ≤0.05, ≤0.01 and ≤0.001, respectively

growth were observed between the two wheat cultivars, with ET8 having about 10% greater shoot dry matter yields and plant heights (Table 3) than ES8. Importantly, these NP effects occurred for both cultivars, as there were no interactions in shoot growth between genotype and the NP treatments (Table 2).

Effect of treatments on plant growth was evident at 17 DAS (Fig. 1). The mixed NP in the surface (0–5 cm) layer had the highest plants compared to all other treatments at this stage. However, the plant

height difference between the NP0–5 treatment and the NP10–15 treatment diminished after 25 DAS (Fig. 1b). The plants with N and P added separately and NP0–50 treatment had similar growth patterns during the whole period of the experiment (Fig. 1a). The final plant heights were similar between the NP0–5 and NP10–15 treatments (Table 3). However, there was a significant difference in heights between former two treatments and the treatments of P and N supplied separately.

Table 3 Dry weights of shoot and roots, tiller number, plant heights, total root length, number of root tips and total water use of Al-tolerant (ET8) and Al-sensitive (ES8) wheat grown with various combinations of nitrate and P placements in acid soil columns for 38 days

Main effect means	Shoot dry matter (g column ⁻¹)	Tiller number per column	Final plant height (cm)	Total root dry matter (g column ⁻¹)	Total root length (m column ⁻¹)	Total root tips (No column ⁻¹)	Total water use (ml column ⁻¹)
Genotypes							
Al sensitive ES8	1.85	11.5	45.5	0.97	55.6	2,445	992
Al tolerant ET8	2.05	12.4	46.5	1.06	62.3	2,557	1,116
<i>LSD</i> (<i>P</i> =0.05)	0.12	n.s.	1.0	n.s.	5.2	n.s.	61
Treatments							
NP 0–50 cm	1.95	12.0	44.0	0.94	54.1	2,053	1,034
NP 0–5 cm	2.29	13.5	48.3	1.40	75.9	3,024	1,255
N 0–5/P10–15 cm	1.66	10.3	44.3	0.86	48.9	2,352	875
P 0–5/N10–15 cm	1.74	9.9	45.9	0.83	54.6	2,436	984
NP 10–15 cm	2.11	14.1	47.6	1.03	61.3	2,638	1,123
<i>LSD</i> (<i>P</i> =0.05)	0.19	1.6	1.5	0.19	8.2	257	96

Each column had five wheat plants; values were means of four replicates

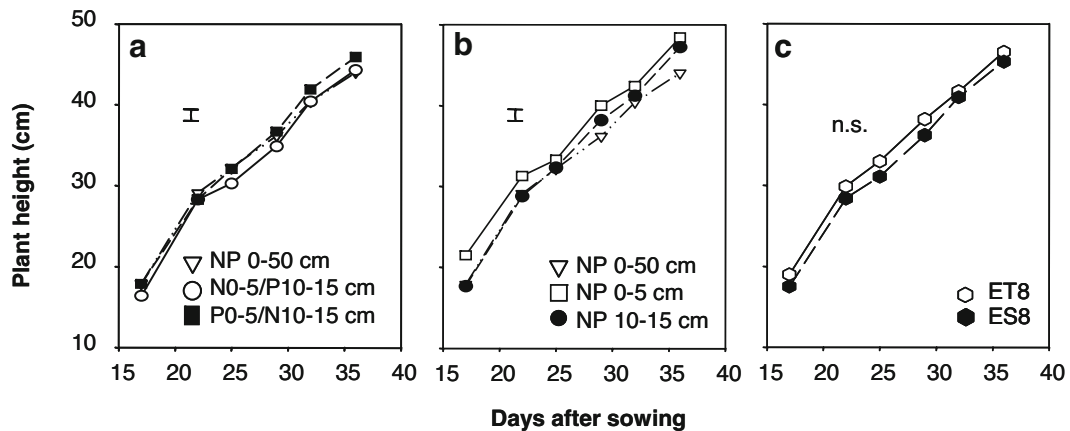


Fig. 1 Changes in plant heights at different stages of growth. The data were average of Al-tolerant (ET8) and Al-sensitive (ES8) isogenic wheat genotypes for various nutrient placement treatments (a and b) and average of various placement

treatments for ET8 and ES8 (c) because the interaction of genotype \times treatment was not significant. Vertical bars represent the LSD ($P=0.05$) for any two means. n.s. means not significant

Water use

Differences in water use followed similar patterns to shoot growth. ET8 transpired 12% more water than ES8. The mixed NP treatments used considerably more water over 38-day growth period, than the treatments of N and P supplied separately (Table 3). There was no genotype by NP treatment interaction (Table 2). However, the plants supplied with N and P together in the surface 0–5 cm used the greatest amount of water. The other mixed treatments, where N and P were supplied in either the 10–15 cm soil layer or throughout the 50-cm column, used lesser amounts of water, but significantly more than the treatments of N and P supplied separately (Table 3). Interestingly, the separated NP treatment where N was applied in the surface and P applied in the subsurface layers used the least amount of water. This treatment used only 2/3 of water as the treatment of N and P supplied together in the surface 0–5 cm layer.

Root growth

The surface NP treatment also produced the greatest total root growth. In this respect, the responses of total root growth in the soil columns to N and P supply differed from the responses of the shoots. All root growth parameters measured were significantly greater ($P<0.05$) in the treatment of N and P supplied together to the surface (NP 0–5) compared to the subsurface layer (Tables 2 and 3). Root dry weight,

root length and number of root tips in the NP 0–5 treatment were 36, 24 and 15% greater than the NP10–15 treatment, respectively. One notable result was that there were significantly fewer root tips in P0–50 than in all other treatments (Tables 2 and 3). ET8 had longer roots than ES8, but both produced similar root dry matter and number of root tips.

The NP treatments had a marked effect on the root mass in different soil layers. The basis for the highly significant ($P<0.001$) soil layer by treatment interaction (Table 2) was the large root mass in the surface 0–5 cm layer, where N and P were applied together (Fig. 2a and b). The root mass in NP0–5 was almost double that of NP10–15 and NP0–50 treatments (Fig. 2b). The other important finding from the root mass data was that when N was supplied alone to a soil layer, there was a significantly greater root mass ($P<0.05$) than when P was applied alone. For example, root mass in the surface layer was 62% more with the N0–5/P10–15 treatment than the same layer in the P0–5/N10–15 treatment. Similarly, root mass in the subsurface layer of the latter treatment exceeded that of the same layer of the former by 66%.

It should be noted that the wheat plants had greater root mass in the surface 0–5 cm layer, when there was neither N nor P supplied to the surface layer compared to the root mass in the 10–15 cm layer when N and P were together applied to this layer. Thus, the root mass in the surface (0–5 cm) layer was significantly greater than and almost double that in the subsurface (10–15 cm) layer for the NP10–15

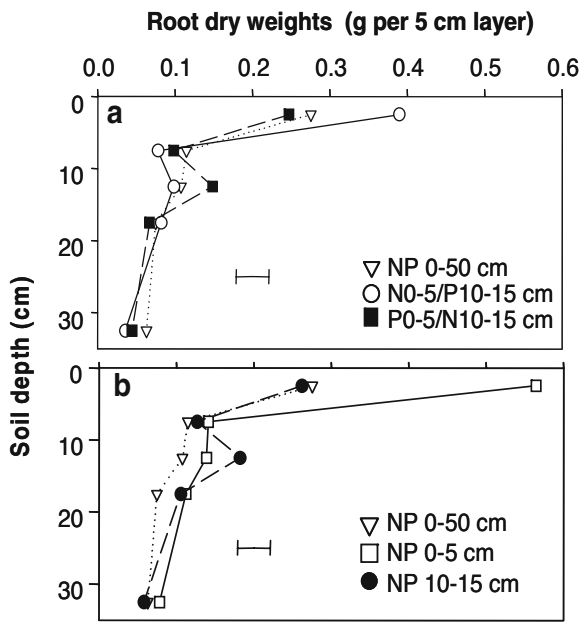


Fig. 2 Effect of the N and P treatments on changes in root dry weights with the depth. Values are means of Al-tolerant (ET8) and Al-sensitive (ES8) isogenic wheat genotypes grown for 38 days in columns with N and P separate (a) and with N and P together (b). Horizontal bars represent LSD ($P=0.05$) for comparing any two means. Genotype \times soil layer effect is not statistically significant

treatment (Fig. 2b) and similar to that for the NP0–50 treatment. In addition, the root mass in the surface layer with P added alone, was almost double that in the deeper 10–15 cm subsurface layer that received N alone in the P0–5/N10–15 treatment (Fig. 2a).

Total root length was also affected by NP treatments in the soil layers, as evidenced by the significance in treatment by soil layer interactions (Table 2 and Fig. 3). Again the basis for this interaction was the large total root length in the surface layer when the N and P were applied together to this layer. However, there were two key differences between the root length and the root mass data. The first was the very small total length of roots produced in the surface (0–5 cm) layer, when N and P were added together to the subsurface layer (10–15 cm; Fig. 3b). Root length in this surface layer was similar to that in the treatment with NP0–50. The second difference was the significantly longer roots produced in the subsurface (10–15 cm) layer, compared to the surface (0–5 cm) layer, when N and P were added together as the NP10–15 treatment or N was added alone in the P0–5/N10–15 treatment. Similar to the root mass data, N application alone

whether in the surface or the subsurface significantly increased total root length in the layer applied, compared to P alone applications to the same layer. Again, the NP10–15 treatment (neither N nor P was added to the surface 0–5 cm layer) resulted in similar total root length in the upper (0–5 and 5–10 cm) layers as for the mixed NP0–50 treatment.

The change in the production of root tips in different soil layers followed similar patterns to that

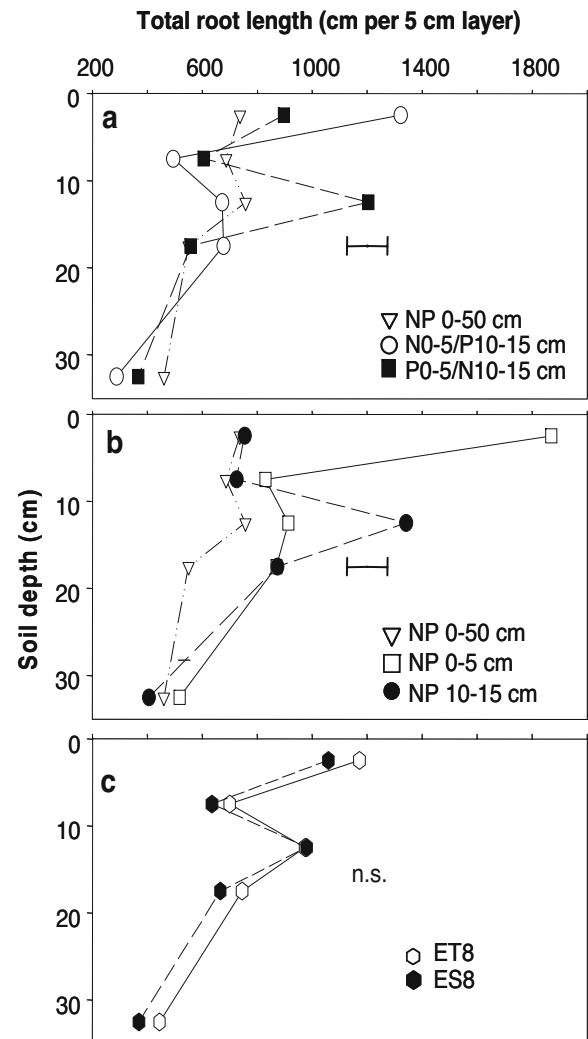


Fig. 3 Effect of N and P treatments on changes in total root length with depth. Values are means of Al-tolerant (ET8) and Al-sensitive (ES8) isogenic wheat genotypes for various nutrient placement treatments (a and b) and means of various placement treatments for ET8 and ES8 (c). The interaction of genotype \times treatments was not significant. Horizontal bars represent the LSD ($P=0.05$) for comparing any two treatments. *n.s.* means not significant

of total root length (Fig. 4). For example, the mixed NP applications either in the surface or the subsurface significantly increased the number of root tips in the respective layers, compared to other layers for all treatments. Similarly, the N application alone either in the surface or the subsurface significantly increased the number of root tips compared to the P application alone to the same layer. The changes in the root growth parameters in different soil layers were not significantly different for the Al-tolerant and Al-sensitive (Table 2) although ET8 tended to have greater root length in the soil column compared to ES8 (Fig. 3c).

Bulk soil pH

The NP treatments affected bulk soil pH values in different soil layers, resulting in a highly significant ($P < 0.001$) treatment by soil layer interaction (Table 2 and Fig. 5a and b). The basis for this interaction was similar to that for root growth, namely, the addition of N and P together in the surface (0–5 cm) increased

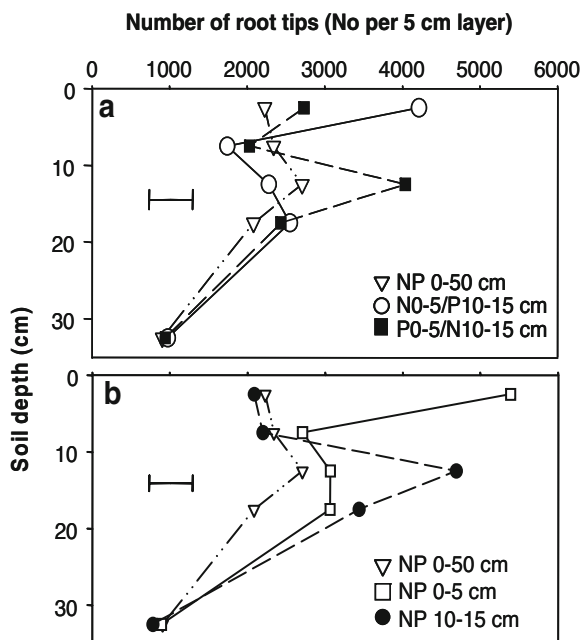


Fig. 4 Effect the N and P treatments on number of root tips in different soil layers. Values are means of two wheat genotypes grown for 38 days in columns with N and P separate (a) and with N and P together (b). The interaction of genotype \times treatments was not significant. Vertical bars represent the LSD ($P = 0.05$) for any two means

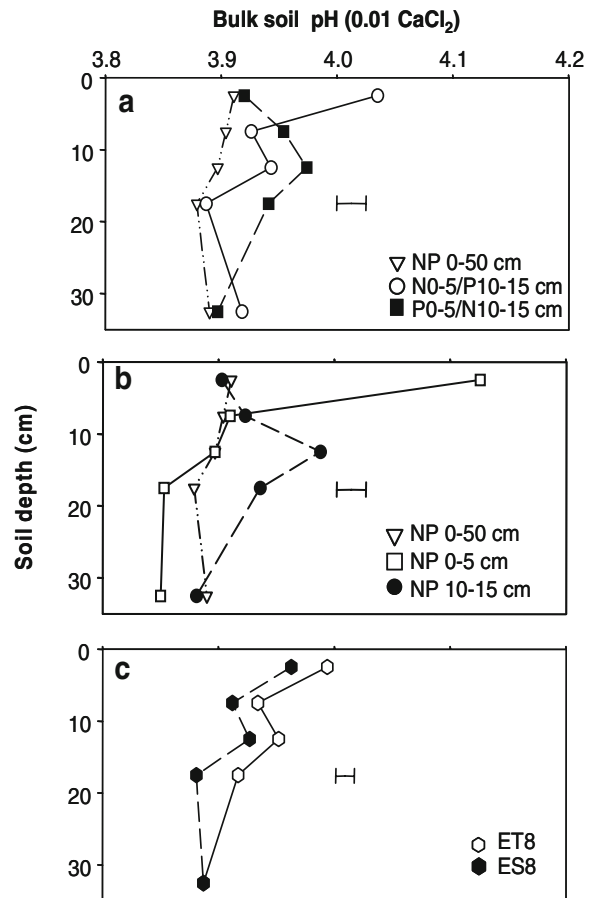


Fig. 5 Effect of the N and P treatments on changes in bulk soil pH with depth. The pH was measured in 1:5 soil: CaCl_2 0.01 M solution. The data were means of Al-tolerant (ET8) and Al-sensitive (ES8) isogenic wheat genotypes for various nutrient placement treatments (a and b) and means of various placement treatments for ET8 and ES8 (c) because the interaction of genotype \times treatment was not significant. Vertical bars represent the LSD ($P = 0.05$) for any two means

soil pH in that soil layer by 0.2 units (Fig. 5b). The second largest increase in bulk soil pH also occurred in the surface layer, where N alone was added in the N0–5/P10–15 treatment compared to deeper soil layers in this treatment. The increase in bulk soil pH in the surface layer of the N0–5/P10–15 treatment was twice that in the 10–15 cm layer for the NP10–15, where N and P were added together to subsurface layer (Fig. 5b).

There was also a significant genotype by soil layer interaction ($P < 0.01$) in bulk soil pH values (Table 2). The basis for this was the high bulk soil pH for the top 4 soil layers with the ET8 genotype, compared to

ES8 genotype (Fig. 5c), with no difference between the genotypes in the deepest soil layer.

Rhizosphere pH

The effect of NP treatments on rhizosphere pH was greater than the effect on bulk soil pH. Nitrate and P applied together to the surface (0–5 cm) and subsurface (10–15 cm) layers resulted in an increase of 0.4 pH units in rhizosphere pH compared to same layer of the NP0–50 treatment (Fig. 6a). Nitrate alone application

increased pH by 0.3 units in the surface (0–5 cm) and by 0.2 units in the subsurface (10–15 cm) layers compared to the NP0–50 treatment. In each case, the increase in rhizosphere pH in this N-enriched layer was 2–3 times greater than that for the bulk soil pH in that layer (Figs. 5a and 6a), and 3–4 times greater compared to the increase in the rhizosphere pH in the adjacent deeper soil layer (Fig. 6a). The addition of P alone to the soil layers, i.e. the P0–5/N10–15 or N0–5/P10–15 treatments, had minimal effect on rhizosphere pH in the soil layer enriched with P compared to the rhizosphere pH in that layer in the NP0–50 treatment (Fig. 6a). There was no pH change in the rhizosphere compared to the bulk soil when mixed NP was applied to the whole soil column (0–50 cm), and pHs remained fairly uniform over the soil depths (Fig. 6).

There were no significant differences in the bulk soil pH and rhizosphere pH of the whole profile between the two genotypes, and no genotype by soil layer interaction in rhizosphere pH values (Table 2 and Fig. 5). Irrespective of genotype and soil layer changes in rhizosphere pH as well as bulk soil pH correlated well with root length density (Fig. 7).

Nutrient uptake in plants

Concentrations of major nutrients in the shoots varied between the treatments (Table 4). Concentrations of N and K in treatments with mixed N and P in 0–5 and 10–15 treatments tended to be lower compared to the treatment with separate N and P applications and mixed NP0–50 treatment. Generally, mixing N and P throughout the column (0–50) resulted in lower Ca, Mg and P concentrations compared to other treatments. The concentration of P in shoots was higher when P was applied to the subsurface layer alone (N0–5/P10–15) and together with N (NP10–15) compared to surface application of P in the NP0–5 and P0–5/N10–15 and the NP0–50 treatments. The other treatment with subsurface P application (N0–5/P10–15) also had higher P concentration than that of NP0–50 and NP0–5 treatments. The concentrations of Mg were higher in mixed NP0–5 and NP10–15 treatments than in other treatments. Total nutrient uptake by shoots in the treatments with NP applied together was greater than the treatment with N and P in different layers due to greater biomass production (Tables 3 and 4). There was a negative correlation ($R^2=0.55$) between total

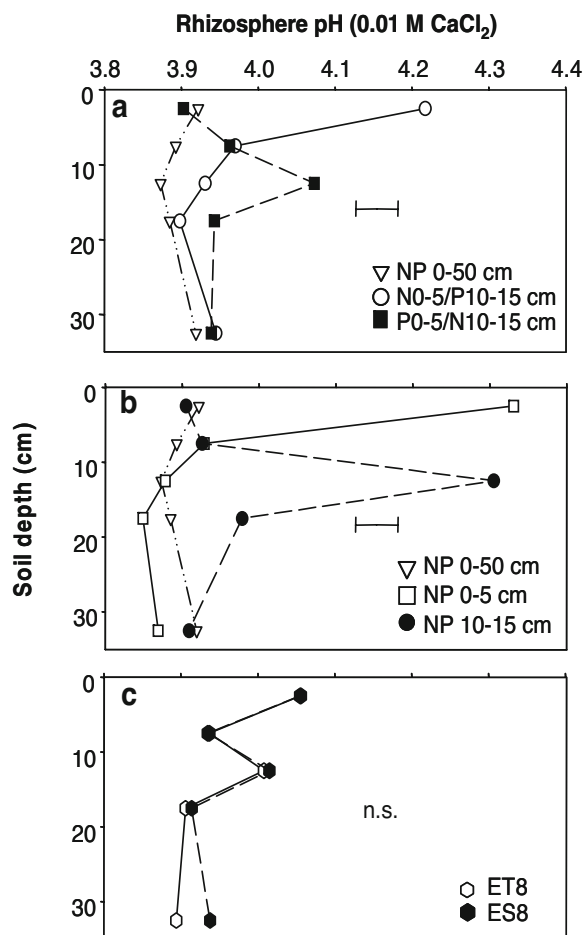


Fig. 6 Effect of the N and P treatments on changes in rhizosphere pH with depth. The pH was measured in 1:5 soil: CaCl_2 0.01 M solution. The data were means of Al-tolerant (ET8) and Al-sensitive (ES8) isogenic wheat genotypes for various nutrient placement treatments (a and b) and means of various placement treatments for ET8 and ES8 (c) because the interaction of genotype \times treatment was not significant. Vertical bars represent the LSD ($P=0.05$) for any two means

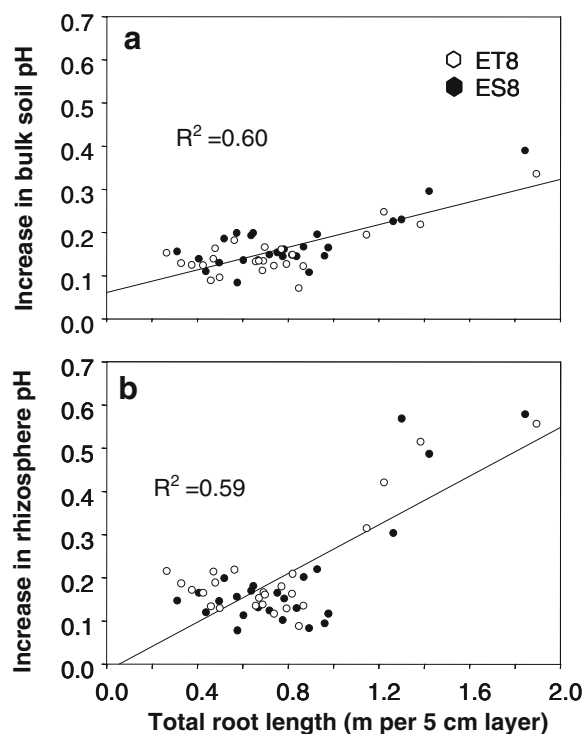


Fig. 7 The relationships between total root length (in 5 cm soil layer) and increases in bulk and rhizosphere soil pH compared to the original soil pH ($\Delta \text{pH} = \text{bulk/rhizosphere pH} - \text{original soil pH}$ at the commencement of the experiment)

root growth in treatments and total N concentrations in shoots (data not presented). In contrast, no correlation was found between total root length and P concentrations or Ca concentrations in shoots.

Table 4 Concentrations of N, Ca, K, Mg, P and S (g kg^{-1}), and non-N excess cation, cation–anion balance (cmol kg^{-1}) and total net anion uptake (mmol column^{-1}) in shoots of Al-tolerant

Main effect means	N	Ca	K	Mg	P	S	Excess cations	Cation–anion balance	Total net anion uptake
Genotypes									
Al sensitive (ES8)	48.0	3.26	53.0	2.58	4.17	5.32	138	–204	3.78
Al tolerant (ET8)	49.0	3.05	50.8	2.45	3.88	5.53	129	–228	4.69
LSD ($P=0.05$)	n.s.	0.10	2.0	n.s.	0.27	n.s.	6	17	0.17
Treatments									
1.NP 0–50 cm	50.2	1.99	53.8	2.05	3.40	5.26	132	–227	4.42
2.NP 0–5 cm	46.8	3.21	47.3	2.89	3.57	5.09	128	–207	4.74
3.N 0–5/P 10–15 cm	50.4	3.61	52.3	2.33	4.42	5.35	134	–226	3.75
4.P 0–5 /N10–15 cm	51.5	3.19	56.4	2.41	4.03	5.98	143	–226	3.92
5.NP 10–15 cm	46.0	3.79	49.8	2.90	4.71	5.45	132	–198	4.17
LSD ($P=0.05$)	4.1	0.15	3.2	0.32	0.43	0.59	9	27	0.27

Values are means of four replicates, and data between ES8 and ET8 were averaged in treatments because interaction of treatment by genotype effect was not significant.

Concentrations of Ca, K and P were higher in ES8 than in ET8 (Table 4). There was no significant variation in excess cation content between treatments except for the higher values for the P0–5/N10–15 treatment. However, ES8 had higher concentrations of excess cations compared to ET8.

Cation–anion balance also varied between the NP treatments (Table 4). The combined application of N and P resulted in less negative cation–anion balances. However, net anion uptake per column tended to be greater in all NP together treatments than in treatments where N and P applied separately (Table 4). Net anion uptake in ET8 was also greater than ES8.

Discussion

Impact of localised NP supply on rhizosphere alkalinisation

This study illustrated that localised supply of nitrate increased rhizosphere pH and to a lesser extent the bulk soil pH in acid soil layers. The maximum alkalinisation was observed where N and P were placed together. This alkalinisation was considerably greater than in the treatments where N and P were supplied separately. The maximum alkalinisation occurred in the soil layers with the NP applications and was correlated highly with total root length (Fig. 7) which in turn was related to the great root length density and number of

(ET8) and Al-sensitive (ES8) wheat genotypes grown for 38 days in soil columns with various combinations of nitrate and P placements

root tips in that layer (Figs. 3 and 4). Although N concentrations in the shoots tended to be lower when N and P were supplied together in the same soil layer than for other treatments, total N uptake by shoots per column was greater (Tables 3 and 4). Thus the alkalinisation observed in the NP-enriched layers can be attributed to the greater root growth and greater biomass production leading to great nitrate uptake from that layer.

It was apparent that the N supplied as calcium nitrate had the major effect on increasing soil pH in the rhizosphere. For example, a large increase in rhizosphere pH occurred when N was supplied alone, in the absence of P, as occurred in the surface layer with the N0–5/P10–15 treatment (Fig. 6). In contrast, there was no pH increase in the surface layer, when P was supplied alone in the P0–5/N10–15 treatment even though the original soil was deficient in P (Table 1). However, this latter treatment did increase pH in the deeper (10–15 cm) layer, where N was added alone. Given that plant N uptake can be responsible for 70% of total ion uptake by a plant (Van Beusichem et al. 1988) and that the N in this study was supplied as nitrate, then these N treatments would have resulted in a large excess supply of major anions to the plant. The increase in pH with nitrate addition can be attributed to the release of OH⁻ ions into the rhizosphere to maintain charge balance in the root tissue (Jarvis and Robson 1983; Marschner 1991; Neumann and Römheld 2002; Hinsinger et al. 2003).

The role of NO₃⁻ in root growth

Nitrogen placement in soil layers had a marked effect on root morphology in those soil layers. The changes in root morphology included increases in root mass, length and number of tips (Figs. 2, 3 and 4), compared to the roots in layers that did not receive N, or only received P. Nitrate increased root length and number of tips to a greater extent than the root mass, which was clearly seen in the layers with addition of N alone, especially in the subsurface 10–15 cm layer (Figs. 2, 3 and 4). The results are consistent with other studies (Hackett 1972; Robinson et al. 1994), where N placement resulted in greater root branching.

Advances in plant molecular biology provide new understanding of root proliferating mechanisms with localised nitrate supplies. Nitrate *per se* was identified

as a stimulator of meristematic activity in lateral root tips of *Arabidopsis* (Zhang et al. 1999; Zhang and Forde 2000). Zhang and Forde (2000) further revealed that the stimulatory effect of nitrate occurred as a result of the ability of lateral root tips to perceive signals coming from nitrate rather than other N sources. The pioneering studies with localised effect of nutrients show that the localised supply of nitrate stimulates lateral root initiation and elongation in cereal crops (Hackett 1972; Drew et al. 1973). On the other hand, in studies with *Arabidopsis*, the increased nitrate supply resulted only in an increase in lateral root elongation but not initiation (Zhang et al. 1999). In this study the greater total root length observed under nitrate localisation compared with that from P localisation was due to localised stimulatory effect of nitrate on lateral root initiation and elongation.

Combined effect of N and P on root growth

The localised application of P alone in this study had a minimal effect on root morphology and proliferation. This contradicts many previous studies with various plant species (Simpson and Lipsett 1973; Drew 1975; Drew and Saker 1978; Moody et al. 1995; Sun et al. 2002; Valizadeh et al. 2002; He et al. 2003). Those studies showed that locally available P in a soil or a root zone increased lateral root generation in that layer (Sun et al. 2002). In addition, when N and P were supplied locally, but in separate layers, total root growth was no better than the treatment where NP had been added uniformly through the entire column (Table 3). In some previous studies, locally supplied P or N stimulated root growth in other areas compared to treatments where there was uniform nutrient supply (Robinson et al. 1994; Tang et al. 2000; Trapeznikov et al. 2003). In those studies, where P was added locally, other nutrients were applied at adequate levels. In another study, however, root proliferation of *Arabidopsis* was not stimulated by localized supply of high P when inadequate N was applied in the high P layer (Linkohr et al. 2002). The lack of root responses to localized P supply alone in this present study could be related to (1) the inadequate N supply in the P-applied layer prevented the response of root growth to P, similar to that found with *Arabidopsis* (Linkohr et al. 2002), or (2) the negative impact of low soil pH on root growth (Yan et al. 1992) might counteract the stimulatory effect of localized P supply.

The maximum root proliferation was observed in the N plus P layers in this study. There are several possible explanations for the synergistic effect of nitrate and P on root growth in the acid soil. The first explanation could be due to the alkalisation occurring with nitrate uptake, increasing the P availability in the rhizosphere of the acid soil (Bagayoko et al. 2000). The second reason might be that the soil had a very small N content as stated above (Table 1). The layer supplied with P alone had little available N for plant uptake. Thus, the N content in the P alone layer might not be enough to achieve the potential root growth in that layer. The other possible reason for great root growth in NP mixed layers is the localised supply of Ca^{2+} ions with NO_3^- ions [nitrate was applied as $\text{Ca}(\text{NO}_3)_2$]. Although Ca was supplied as a basal nutrient, there was luxury Ca supply in the N added soil layer. Enhanced Ca status in acid subsoil layers is known to make better conditions for root proliferation by reducing the impact of exchangeable Al (Simpson et al. 1977; Sumner 1995) and Ca also act as an extrinsic ameliorant by electrostatically displacing Al^{3+} and H^+ from the surface of the plasma membrane (Kinraide 2003). Elevated Ca supply in N enriched layer might have played a role in reducing effects of Al^{3+} and H^+ toxicities. This needs to be tested with further research.

The root proliferation of plants is known to be regulated by the internal nutrient status of the plant (Robinson 1996; Scheible et al. 1997; Forde and Lorenzo 2001). New knowledge on plant's systematic response to nutrient supply gives some clue to understanding possible reasons for greater total root growth in treatments where N and P were applied together in the same soil layer. It has been shown that not only the nutrients themselves but their assimilates and phytohormones are also implicated in signalling in plant responses to external nutrients (Forde 2002). For example, a plant's response to a localised supply of nitrate is greater when the plant has low N status (Drew et al. 1973). The recent extensive studies with *Arabidopsis* show that lateral root development and elongation are governed by the plant's internal nitrate concentration (Zhang and Forde 1998; Zhang et al. 1999; Forde and Lorenzo 2001). High rates of nitrate supply delayed the lateral root development in *Arabidopsis*, which was closely connected to the high internal nitrate concentration of the plant. In this study, plants grown with N and P supplied locally and

together had a lower total N concentration than the other treatments (Table 4). In addition, there is a negative correlation between total N concentration ($R^2=0.55$) in shoots and total root length. It is expected that concentrations of total N in shoots was associated with nitrate content, and thus stimulated root development or elongation in treatments where N and P were applied together compared to those where N and P were supplied separately or supplied uniformly throughout the column.

Genotypic variation in response to N and P placement

The Al-tolerant wheat (ET8) in this study had significantly greater shoot mass and total root length than the Al sensitive (ES8) genotype. The former also used more water than the latter. The greater plant growth and water use by ET8 can be attributed to the greater root proliferation in the acid soil layers shown in this study (Table 2) and as reported in previous studies (Tang et al. 2001; 2002), and subsequent greater nitrate uptake. Release of carboxylates is known to be an important characteristic of plants resistant to Al toxicity in acid soils. For example, Al-tolerant maize and snapbeans release more citrate into the rhizosphere than Al-sensitive plants. Likewise, Al-tolerant wheat releases more malate and succinate than Al-sensitive wheat (Miyasaka et al. 1991; Delhazie and Ryan 1995; Pellet et al. 1996). Carboxylates chelate Al in the soil solution to create a less toxic environment for plant roots (Tesfaye et al. 2001).

It is interesting to note that the Al-tolerant wheat ET8 had a lower P concentration in shoot than the Al-sensitive ES8. This might be partly explained by a dilution effect on ET8 as ET8 produced more shoot biomass. However, the uptake of P per unit root mass, per unit length or surface area was very similar between the two genotypes (data not shown). Alternatively, the Al-tolerant genotype might have a greater P utilization efficiency than ES8. Further research is required to test whether this is the case.

Conclusion

This study demonstrated that localised supply of nitrate and P together maximised alkalisation in acid subsoil. This soil alkalisation had resulted from an increased uptake of nitrate and stimulated root

proliferation in the fertilizer-applied layer, which in turn enhanced shoot growth due to efficient exploration of resources in acid subsoil. While nitrate supply alone increased root growth, P placement alone did not stimulate root proliferation in the acid soil. The study suggests that P fertilizer should be applied together with nitrate-N to achieve maximal benefits in extra root growth and pH increase. This strategy can be applied as a part of an integrated approach to combat subsurface acidity and acidification in field conditions with other measures such as lime application and use of acid-tolerant cultivars.

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