Differential mobilization of P in the maize rhizosphere by citric acid and potassium citrate

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Abstract

The release of organic acid anions from plant roots into soil has been hypothesized to be a mechanism for enhancing phosphorus availability in the rhizosphere. Although these compounds are excreted from the cytoplasm as organic acid anions (e.g. citrate, malate), when the H⁺-ATPase is also upregulated there is evidence to suggest that they enter the soil as organic acids (e.g. citric acid, malic acid). The aim of this study was to evaluate the role of citric acid (H-citrate) and potassium citrate (K-citrate) in the mobilization and plant uptake of P from two acid soils contrasting in their P availability. Our results indicated that the mobilization of P from a KH₂PO₄ labelled patch of soil was soil type dependent, was controlled by its intrinsic P status, and that more P was made available by K-citrate than H-citrate. Similarly, the uptake of ³²P from the rhizosphere by Zea mays L. was greatest in the presence of K-citrate in comparison to H-citrate. However, a significant increase in shoot ³²P content was only observed in the more acidic soil with high P sorption potential (Haplic podzol) while no significant increase was observed in the less acidic soil with low P sorption potential (Eutric cambisol). We conclude that the chemical form of organic acid anion excretion may have a significant impact on its P mobilization capability. The contrasting results with the two acid soils indicate that organic acids may not provide a universal mechanism for enhancing P uptake from soil.

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1. Introduction

Phosphorus is often sparingly soluble in soils and consequently P deficiency in plants represents a major constraint to world agricultural production (Buresh et al., 1997). Consequently much research has concentrated on the potential to manipulate plants either through conventional breeding or genetic engineering to enhance their capability to mobilize P in soil (Kochian et al., 2004). Bhadoria et al. (2001) showed that only by increasing the soil solution P concentration by a factor of 5–6 could the P uptake by maize or groundnut be explained. Plants possess many potential mechanisms to increase P uptake from soil including an upregulation of P membrane transport systems, the increased growth of root hairs, enhanced mycorrhizal association, the release of phosphatases, changes in root architecture and the release of organic acids (Barber, 1995; Föhse et al., 1991; Marschner, 1995; Tinker and Nye, 2000). In addition, many indirect mechanisms for enhancing P availability in the rhizosphere have also been documented (Vessey, 2003; Zahir et al., 2004). The role of organic acids in the enhancement of P acquisition by plant roots remains controversial as genetic manipulation has yielded conflicting results and direct evidence for its role in soil in many plants is lacking (Jones, 1998; Jones et al., 2003). One exception is in Lupinus albus and members of the Proteaceae family where there is clear evidence that root exuded organic acids are capable of mobilizing P (Vance et al., 2003). In this situation, organic acid anions such as malate and citrate are released from specially adapted regions of roots (cluster roots) in very large concentrations resulting in a saturation of soil anion sorption sites and mineral
reduction both of which can result in P release into solution (Egle et al., 2003; Shen et al., 2003). Whilst the exudation of organic acids is enhanced from most plant roots under P deficiency, in many cases the rate of release is very low in comparison to L. albus and it is unclear whether this is a direct response to enhance soil P availability or an indirect response due to the increased membrane permeability experienced under P deficiency (Jones, 1998). When low concentrations of organic acids are released into soil their role in mineral weathering and mineral solubilization has been questioned (Drever and Stillings, 1997). When present at low concentrations organic acids are rapidly mineralized by the soil microbial community with a mean residence time in soil of between 0.5 and 2 h (Jones and Darrah, 1994; van Hees et al., 2003). In contrast, when high concentrations of organic acids are released into the soil it is hypothesized that saturation of microbial transport systems occurs at which point they can be expected to have a long residence time in soil and be involved in P mobilization (Jones and Darrah, 1994; van Hees et al., 2002). While the mechanism for organic anion release under Al toxicity has been well characterized at a physiological and molecular level (Ryan et al., 1995; Sasaki et al., 2004) comparatively less information exists on organic anion release from P deficient plants (Ryan et al., 2003; Kochian et al., 2004; Zhang et al., 2004). Organic acid anions such as malate and citrate are predicted to exist in the cytoplasm (pH 7.1–7.4) in a fully dissociated state (e.g. citrate$^3^-$, malate$^2^-$) rather than in the acid form (e.g. H$_3$-citrate$^0$, H$_2$-malate$^0$; Ryan et al., 2003). Current evidence suggests that upon exposure to rhizotoxic levels of Al, Triticum aestivum releases malate$^2^-$ with the release of two K$^+$ counter ions for charge balance (Ryan et al., 2003). Under Al toxicity an upregulation of the plasma membrane H$^+$-ATPase is not apparent presumably as this would reduce soil pH and exacerbate the toxic effect of Al$^{3+}$. However, under P deficiency there is strong evidence to suggest that the H$^+$-ATPase is upregulated and that H$^+$ release and organic acid anion release are both spatially and temporally co-ordinated (Yan et al., 2002; Hinsinger et al., 2003; Tang et al., 2004). In a calcareous soil, we have shown previously that the simultaneous presence of organic acids and H$^+$ enhances the availability of P (Jones and Darrah, 1994). However, the impact of these two factors on plant P uptake were not quantified and previous experiments indicated that while citrate could mobilize P in batch extracts no appreciable increase in plant P uptake was apparent when organic acids were added to the rhizosphere (Ström et al., 2002). The aim of this study was therefore to evaluate the effectiveness of organic acids in mobilizing P in two soils of contrasting P status and to determine the relative efficiency of organic acids (H-citrate) and organic acid anions (K-citrate) in plant P acquisition.

2. Materials and methods

2.1. Soil

Soil was obtained from two contrasting temperate oceanic agricultural grasslands located in Aberwyngregyn, Gwynedd, North Wales (53°14’ N, 4°01’ W). Soil A (Eutric cambisol) was collected from the surface Ah horizon (5–20 cm) of a lowland (15 m altitude) freely draining barley (Hordeum vulgare L.) field which receives regular fertilization (120 kg N, 60 kg K and 10 kg P yr$^{-1}$). Soil B (Haplic podzol) was collected from the surface Ah horizon (5–20 cm) of an upland (200 m altitude) freely draining, heavily leached, lightly sheep-grazed grassland which receives no fertilizer and supports a grassland sward consisting predominantly of sheep’s fescue (Festuca ovina L. var. Ovina) and common bentgrass (Agrostis capillaris L.). The mean annual soil surface temperature at 10 cm varies from 8 to 10°C and the annual rainfall at the lowland site is 1250 mm and at the upland site 1700 mm.

Soil was removed using a spade and stored in CO$_2$ permeable polypropylene bags for immediate transport back to the laboratory. In the laboratory, the soil was sieved (<10 mm) and then stored field-moist at 3°C in the same bags. Earthworms, above-ground vegetation and large masses of roots were removed by sieving.

Properties of the soils are listed in Table 1. Soil pH and electrical conductivity were determined in 1:1 (v/v) soil: H$_2$O extracts (Smith and Doran, 1996) and moisture by drying at 105°C for 24 h. Total C and total N were

| Table 1Chemical and physical characteristics of the two soils used in the study |
|---------------------------------|-----------------|-----------------|
|                                 | Eutric cambisol | Haplic podzol   |
| EC$_{1:1}$ (µS cm$^{-1}$)        | 80 ± 4          | 46 ± 7          |
| pH (H$_2$O)                     | 5.90 ± 0.03     | 4.33 ± 0.01     |
| CaCO$_3$ (g kg$^{-1}$)          | 0.11 ± 0.02     | <0.01           |
| Water holding capacity (g kg$^{-1}$) | 520 ± 20       | 690 ± 40        |
| Moisture content (g kg$^{-1}$)  | 160 ± 10        | 260 ± 2         |
| Organic C (g kg$^{-1}$)         | 21 ± 0.1        | 12 ± 0.1        |
| Total N (g kg$^{-1}$)           | 0.16 ± 0.01     | 0.8 ± 0.01      |
| C-to-N ratio                    | 13.3 ± 0.6      | 15.6 ± 1.3      |
| Soil solution NO$_3$ (mg N l$^{-1}$) | 13.7 ± 1.3     | 0.5 ± 0.106     |
| Soil solution NH$_4$ (mg N l$^{-1}$) | 1.40 ± 0.04    | 1.13 ± 0.09     |
| Exchangeable cations            |                 |                 |
| Na (mg kg$^{-1}$)               | 29 ± 3          | 37 ± 1          |
| K (mg kg$^{-1}$)                | 116 ± 18        | 77 ± 12         |
| Ca (mg kg$^{-1}$)               | 1595 ± 217      | 89 ± 8          |
| Mg (mg kg$^{-1}$)               | 89 ± 19         | 15 ± 2          |
| Al (mg kg$^{-1}$)               | 22 ± 2          | 323 ± 55        |
| Extractable P (mg kg$^{-1}$)    | 9.9 ± 0.3       | 0.22 ± 0.09     |
| Root biomass (g m$^{-2}$)       | 0.39 ± 0.01     | 0.17 ± 0.06     |
| Soil respiration (g CO$_2$ m$^{-2}$ h$^{-1}$) | 0.60 ± 0.02 | 0.25 ± 0.02     |

All values represent means ± SEM (n = 3).
determined with a CHN-2000 analyzer (Leco Corp., St Joseph, MI). Exchangeable cations were estimated by performing 1:10 (w/v) soil:0.5 M BaCl₂ extractions on a reciprocating shaker (60 min, 20 °C) followed by centrifugation at 10,000g (30 min, 4 °C) and storage of supernatant solutions at −20 °C. Exchangeable cations were determined by ICP-OES (JY138-Ultrtrace, Horiba-Jobin Yvon SAS, Longjumeau, France). CaCO₃ content was determined by the Van Slyke manometric method (Nelson, 1982).

Extractable P was measured by extraction with 0.5 M acetic acid (1 h, 200 rev min⁻¹) followed by centrifugation at 10,000g (30 min, 20 °C) and P analysis by the method of Murphy and Riley (1962). Soil solution was removed by the centrifugal-drainage method of Giesler and Lundström (1993) with NO₃⁻ and NH₄⁺ in solution determined with a Skalar San⁺ segmented flow autoanalyzer (Skalar UK Ltd, York). Root biomass was determined by wet sieving and drying the roots at 80 °C overnight. Soil respiration was determined with a SR1 automated soil respirometer (PP Systems Ltd, Hitchin, UK).

2.2. Plant growth and uptake of ³³P

Seeds of maize (Zea mays L. cv. ‘Pioneer 3377’) were soaked for 24 h in aerated, deionized water and then allowed to germinate on moistened filter paper for 48 h at 20 °C. After 3 days, each plant had one main root axis approximately 1.5 cm in length, at which point the seedlings were placed into individual soil microcosms. The microcosm was constructed from nylon tube, which composed a 250 mm long, 9 mm diameter main ‘rhizotube’ at 15 mm intervals down the length of the main rhizotube to ensure aeration. Before the addition of the germinated plant, the microcosms were filled with either the Eutric cambisol or Haplic podzol soil to a bulk density of 1.16 g cm⁻³. After plant addition, the microcosms were placed in a climate controlled growth room (Sanyo-Gallenkamp, Fi-totron PG660/C/RO/HQI, Loughborough, UK) with day/night rhythm of 18/22 °C, 70% relative humidity, photoperiod of 16 h and light intensity of 500 mol photons m⁻² s⁻¹ PAR at canopy height. Carbon dioxide concentrations within the growth cabinets were maintained at 350 ppm by regular changes with external air. Microcosms were kept moist by a wick placed in the bottom of the rhizotube connected to a soil reservoir held close to field capacity and through addition of distilled water to the soil surface (1 ml day⁻¹ equivalent to ca. 3 mm day⁻¹).

When the roots had filled the entire microcosm (plants 14 days old), 250 μl of a KH₂PO₄ solution (92 kBq ml⁻¹; 100 TBq mmol⁻¹; Amersham Pharmacia Biotech Ltd, Little Chalfont, Bucks) was injected through a hole halfway down the rhizotube into the soil. KH₂PO₄ was added to the soil at two contrasting concentrations (10 μM and 5 mM) to give a low (0.005 μmol P g⁻¹) and high (2.5 μmol P g⁻¹) rate of soil P addition. Consequently, the final available P concentration in the Eutric cambisol at the low P addition rate was 0.32 μmol P g⁻¹ and at the high P addition rate it was 2.82 μmol P g⁻¹ (Table 1). Similarly, the available P concentration in the Haplic podzol at the low P addition rate was 0.012 μmol P g⁻¹ and at the high P addition rate it was 2.51 μmol P g⁻¹ (Table 1). Lateral roots and root hairs (2 mm long emerging at 90° to the main root axis) filled the microcosm ensuring that the entire microcosm contained rhizosphere soil. After ³³P addition, organic acid solutions (500 μl) were injected into the microcosms at the same location 1 and 16 h after ³³P injection. The injection solutions contained either citric acid (H-citrate; pH 2.7) or tri-potassium citrate (K-citrate; pH 7.9) at concentrations of 5 mM. This resulted in approximately 2.5 μmol citrate g⁻¹ soil. Distilled water or KCl (5 mM) injections (500 μl) were used as controls. After 24 h, the shoots were harvested, dried (60 °C, 24 h), ashed (450 °C, 6 h) and the ³³P content determined by liquid scintillation counting (Wallac 1409 Liquid Scintillation Counter and Optiphase 3 scintillation fluid; EG&G Ltd, Milton Keynes, UK). At least five replicates of each experimental treatment were performed.

2.3. Mineralization studies

To determine the rate of microbial citrate mineralization in each of the soils, 500 μl of a 5 mM, ¹⁴C-labelled H-citrate or K-citrate solution (1.04 kBq ml⁻¹; 1.5,¹⁴C-citrate; ICN Pharmaceuticals Inc., Irvine, CA; 3.7 GBq mmol⁻¹) was added to 5.0 g of field-moist rhizosphere soil contained in a 60 ml polypropylene tube (Jones et al., 1996a). Rhizosphere soil was obtained from maize microcosms, from which all discernable roots were removed. Following organic anion addition, the soils were incubated at 20 °C. The degree of citrate mineralization was determined 1, 4, 21, 30, and 48 h after the addition of the organic anion to soil by catching evolved ¹⁴CO₂ in 1 M NaOH traps and measurement of radioactivity in the traps by liquid scintillation counting. After 48 h, the tubes were shaken with 0.5 M KH₂PO₄ for 20 min (200 rev min⁻¹) to recover citrate remaining in the soil, centrifuged at 16,000g for 5 min and the supernatant recovered for ¹⁴C analysis as determined above. Three replicates of each treatment were performed.

In an additional mineralization experiment, a uniformly ¹³C-labelled glucose (Sigma-Aldrich Ltd; 50 mM; 9.25 MBq mol⁻¹) or an amino acid mixture (ICN Pharmaceuticals Inc., Irvine, CA; 10 mM; Jones et al., 2004) was added to the two soils, and ¹⁴CO₂ evolution monitored as described above.

Substrate half-life in the soil (t½) was determined from the ¹⁴CO₂ evolution curves by linear interpolation using a least squares iteration routine in SigmaPlot 8.0 (SPSS Inc., Chicago, IL).
2.4. \( ^{31}P \) and \( ^{33}P \) availability in soil

To determine the availability of the added \( ^{33}P \) and the intrinsic \( ^{31}P \) in the soil, 1000 \( \mu L \) of a 5 mM \( ^{33}P \) solution (1.5 kBq ml\(^{-1} \)) was added to 2.5 g of microcosm rhizosphere soil and left for 48 h at 18 °C. The soil was then shaken with 5 ml of citric acid (1 or 5) or distilled water for 30 min on an orbital shaker (300 rev min\(^{-1} \)). The soil was then centrifuged (15,000 g, 15 min) and the \( ^{33}P \) in the supernatant solution determined by liquid scintillation counting and the native \( ^{31}P \) by the method of Murphy and Riley (1962). The ratio of \( ^{31}P\)-to-\( ^{33}P \) in the equilibrium solution was then determined. Three replicates of each treatment were performed.

Additionally, 15 g of the Eutric cambisol or Haplic podzol were shaken with 15 ml of either H-citrate, K-citrate or a 50:50 (v/v) mixture of H-citrate and K-citrate (5 mM) in polypropylene tubes on a reciprocating shaker (300 rev min\(^{-1} \)) for time periods of up to 24 h. At known times (0.08, 0.25, 0.5, 1, 3, 6, and 24 h), the soil suspension was centrifuged (15,000 g, 15 min) and the supernatant pH and \( ^{31}P \) in the supernatant solution determined as described above. Soils were also shaken with distilled water as a control. Three replicates of each treatment were performed.

2.5. Citrate and \( P \) sorption

Sorption of H-citrate and K-citrate to the Eutric cambisol and Haplic podzol were determined according to Jones and Brassington (1998). A \( ^{14}C \)-labelled organic acid solution (2.5 ml, 5 mM; specific activity 0.07 kBq ml\(^{-1} \)) was added to 0.5 g of soil contained in 6 ml polypropylene vials [soil-to-solution ratio 1:5 (w/v)]. Following addition, the samples were shaken for 10 min on a reciprocating shaker (320 rev min\(^{-1} \)), the samples centrifuged (16,000 g, 5 min) and the supernatant solution recovered. The equilibrium solution citrate concentration was determined by liquid scintillation counting. The soil’s solid-to-solution partition coefficient for citrate (\( b \); also known as the buffer power; Barber, 1995) was calculated using the following equation

\[
b = C_{\text{tot}}/C = (C \times \Theta) + (A \times \gamma)
\]

where \( A \) is the amount of anion adsorbed (\( \mu mol \, g^{-1} \)), \( C_{\text{tot}} \) is the total amount of anion in the soil (\( \mu mol \, cm^{-3} \)), \( C \) is the soil solution concentration (\( \mu mol \, cm^{-3} \)), \( \Theta \) is the volumetric water content (\( cm^{3} \, cm^{-3} \)) and \( \gamma \) is the soil bulk density (\( g \, cm^{-3} \)). The value of \( \Theta \) used was 0.3 cm\(^3\) cm\(^{-3}\) and for \( \gamma \) it was 1.16 g cm\(^{-3}\). Three replicates of each treatment were performed. Citrate sorption performed on autoclaved soil or in the presence of 10 mM Hg to minimize microbial activity yielded almost identical results to those using fresh soil (data not presented).

\( P \) sorption isotherms were determined as described in van Hees et al. (2003). The buffer power of \( P \) was calculated for the added \( P \) only, i.e. in Eq. (1) instead of \( C_{\text{tot}} \) and \( C, \Delta C_{\text{tot}} \) and \( \Delta C \) caused by the \( P \) addition were used.

2.6. Statistical analysis

Statistical analysis (\( t \)-tests and ANOVA followed by the Bonferroni method for significance level adjustments due to multiple comparisons) was performed with the computer programs Excel 12.0 (Microsoft Corp.) and Minitab 14.0 (Minitab Inc., State College, PA).

3. Results

3.1. Soil characteristics

Two contrasting agricultural soils were used in this study (Table 1). One soil was a fertile Eutric cambisol which exhibited a high base saturation, available \( P \) content and relatively high soil respiration. In addition, the Eutric cambisol dissolved inorganic N pool was dominated by NO\(_3^-\) reflecting the high nitrification potential in this soil (Jones et al., 2004). In comparison, the Haplic podzol was relatively acid and possessed a lower base saturation, available \( P \) and soil biological activity. The rate of nitrification in this soil was also low (Jones et al., 2004) which is reflected by the dominance of NH\(_4^+\) in soil solution. The above-ground annual grass yield is also much greater in the Eutric cambisol in comparison to the Haplic podzol.

3.2. Effect of citrate on plant \( ^{33}P \) uptake

At the end of the experimental period there was no significant differences in shoot height or biomass between any of the citrate treatments (\( P > 0.05 \); data not presented), however, significant differences were observed in shoot \( ^{33}P \) accumulation. The effect of K-citrate and H-citrate on the uptake of \( ^{33}P \) from the soil by maize plants is shown in Fig. 1. At both added soil \( P \) levels the shoot uptake of \( ^{33}P \) was always greatest from the Eutric cambisol in comparison to the Haplic podzol (\( P < 0.001 \)). On average, at harvest, the shoots contained 1.39 ± 0.08% of the \( ^{33}P \) initially added to the soil in the Eutric cambisol while in the Haplic podzol the shoots contained only 0.12 ± 0.05% of the \( ^{33}P \) initially added to the soil. At the low soil \( P \) concentration (0.005 \( \mu mol \, ^{33}P \, g^{-1} \)) neither K-citrate or H-citrate resulted in a significant increase in shoot \( P \) concentration relative to the control plants in the Eutric cambisol (\( P > 0.05 \)). In contrast, in the Haplic podzol, K-citrate caused a 2-fold increase in shoot \( ^{33}P \) uptake relative to the control plants (\( P < 0.05 \) whilst H-citrate had no significant effect (\( P > 0.05 \)). In the soils to which a high level of \( P \) was added (2.5 \( \mu mol \, ^{33}P \, g^{-1} \)) no significant effect of either K-citrate or H-citrate was seen in the Eutric cambisol (\( P > 0.05 \)). In contrast, the addition of both K-citrate and to a lesser extent H-citrate caused a significant increase in shoot...
33P accumulation in the Haplic podzol relative to the plants in which distilled water was added (P < 0.05 for H-citrate and P < 0.001 for K-citrate). Similar trends were observed in the Eutric cambisol and Haplic podzol when H-citrate concentrations were varied from 1 to 20 mM (data not presented).

3.3. Citrate mineralization

The time-dependent mineralization of 14C-labelled K-citrate and H-citrate to 14CO2 in the Eutric cambisol and Haplic podzol is shown in Fig. 2. Generally, the initial rate of organic acid mineralization was 4-fold greater in the Eutric cambisol in comparison to the Haplic podzol (P < 0.05). At the end of the experimental incubation period (48 h), a significant reduction in the mineralization rate was apparent which is consistent with an exhaustion of substrate from the solid phase (Jones et al., 2004). This is supported by extraction of the soil with 0.5 M KH2PO4 at the end of the experiment at which point only 5 ± 1% of the citrate in the Eutric cambisol and 6 ± 1% of the citrate in the Haplic podzol could still be recovered. No significant difference in the amount of citrate recovered by 0.5 M KH2PO4 from the individual treatments was apparent after 48 h (P > 0.05). The mean half-life of the organic acids in the Eutric cambisol was 29 ± 1 h whilst in the Haplic podzol it was significantly higher at 47 ± 1 h (P < 0.05). These half-life values are similar to those reported for citrate in coniferous forest ecosystems in the UK and Sweden (van Hees et al., 2002). Overall, the patterns of K-citrate and H-citrate mineralization were very similar and not statistically significantly different from each other in both soil types (P < 0.05).

3.4. Glucose and amino acid mineralization

The mineralization of 14C-labelled glucose and a mixture of free amino acids in the Haplic podzol and Eutric cambisol are shown in Fig. 3. Generally, the pattern of mineralization
was similar to that of citrate in the two soils. The rate of substrate mineralization was significantly greater in the Eutric cambisol in comparison to the Haplic podzol for both glucose and amino acids \((P < 0.05)\). The difference in mineralization rate between the two soils was similar to that of citrate (Table 2).

### 3.5. Effect of citrate on P release from soil

The effect of adding citrate to the soil on the equilibrium solution P concentration is shown in Fig. 4. In the Eutric cambisol, the addition of citrate enhanced the equilibrium solution P concentration significantly in comparison to soil in which distilled water was added \((P < 0.05)\). Generally, H-citrate initially released significantly more P than K-citrate in the Eutric cambisol \((P < 0.05)\). The elevated levels of P persisted in solution for up to 24 h (data not presented). The addition of KCl alone (5 mM) did not yield any significant increase in solution P concentration relative to the distilled water controls \((P > 0.05\); data not presented). The equilibrium solution P concentration in the Haplic podzol was approximately two orders of magnitude lower than in the Eutric cambisol \((P < 0.05)\). In contrast to the Eutric cambisol, the addition of H-citrate to the Haplic podzol did not yield a significant increase in solution P concentration relative to the distilled water controls \((P > 0.05\); data not presented). However, K-citrate caused a significant 10-fold increase in solution P concentration in comparison to the distilled water controls which persisted for 24 h \((P < 0.05)\). Generally, in both soils, the addition of a 1:1 mixture of H-citrate and K-citrate (H-K-citrate) resulted in an intermediate equilibrium solution P concentration between those observed with H-citrate and K-citrate. The relative increase of P by K-citrate in the Haplic podzol was significantly greater than the stimulation of P release by H-citrate in the Eutric cambisol.

Extraction experiments similar to those performed above with different concentrations of citrate (1–5 mM) and a \(^{33}\)P labelled soil indicated that P release from the soils increased with increasing citrate concentration (data not presented) in agreement with previous reports (Jones and Darrah, 1994).

The ratio of \(^{33}\)P-to-\(^{31}\)P released by distilled water and the different concentrations of citrate in the Eutric cambisol remained constant \((4.7 \pm 0.2 \text{ kBq} \ \text{mol}^{31}\text{P}^{-1})\). Unfortunately, the low concentrations of \(^{31}\)P in the Haplic podzol did not permit a similar calculation to be made.

### 3.6. Effect of citrate on soil pH

The effect of citrate addition on soil pH is shown in Fig. 5. As expected, the addition of H-citrate to soil resulted in a significant decrease in the pH of both the Eutric cambisol and Haplic podzol the effect of which persisted for up to 24 h \((P < 0.05)\). This drop in solution pH equated to an increase in solution H\(^+\) concentration of approximately 30-fold in the
Eutric cambisol (Δ[H\(^+\)] = 0.03 mM) and 20-fold in the Haplic podzol (Δ[H\(^+\)] = 0.92 mM). The addition of K-citrate caused a significant increase in soil pH in the Haplic podzol (P < 0.05), however, no significant change was observed in the Eutric cambisol (P > 0.05). In the Haplic podzol, K-citrate caused an 8-fold drop in solution H\(^+\) concentration (Δ[H\(^+\)] = 0.03 mM). In both soils, the addition of a 1:1 mix of H-citrate and K-citrate (H-K-citrate) resulted in an intermediate equilibrium solution pH between those observed with H-citrate and K-citrate.

### 3.7. Citrate and P sorption

The amount of citrate sorption to the soil’s solid phase is shown in Table 3. Generally, at the high concentration of citrate employed here (5 mM) the amount of citrate sorbed to both soils was similar but relatively low in comparison to previous studies using lower concentrations (10–100 μM; van Hees et al., 2003). This low rate of sorption is reflected in the low values for the solid-solution partition coefficient (b, buffer power) which on average was 1.3 ± 0.1. The rate of H-citrate sorption was slightly greater than that of K-citrate in the Eutric cambisol (P < 0.05) whilst in the Haplic podzol the reverse pattern was observed.

Table 4 shows the P sorption characteristics of the two soils. The Eutric cambisol even with no P addition had a solution concentration of 4.7 mM while in the Haplic podzol no P could be detected (<0.1 mM). The adsorption intensity of the Haplic podzol was much higher than that of the Eutric cambisol as can be recognized by the lower increase of the solution concentration after adding P and by the approximately 100-fold higher buffer power value. The large SEM of the buffer power values for the Haplic podzol at low P addition is due to the low accuracy when measuring P in solution at concentrations below 1 μM.

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**Table 3**

| Sorption characteristics for citric acid (H-citrate) and potassium citrate (K-citrate) after the addition of a 5 mM solution to two soils (Eutric cambisol and Haplic podzol) |
|---------------------------------|------------------|-----------------|
|                                  | Amount sorbed    | Final solution  | Buffer power |
|                                  | (mmol kg\(^{-1}\))| conc. (mM)       |                |
| **Eutric cambisol**              |                  |                 |                |
| H-citrate                        | 2.97±0.03        | 2.51±0.03       | 1.40±0.02     |
| K-citrate                        | 2.35±0.06        | 3.02±0.05       | 1.01±0.03     |
| **Haplic podzol**                |                  |                 |                |
| H-citrate                        | 3.23±0.06        | 2.28±0.05       | 1.61±0.05     |
| K-citrate                        | 2.78±0.05        | 2.66±0.04       | 1.26±0.03     |

Values represent means±SEM (n = 4). Sorption is expressed on a dry weight basis. The solid-to-solution partition coefficient or buffer power is described in Section 2 (Eq. (1)).

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**Table 4**

| Amount of P sorbed to the solid phase or present in solution after the addition of P to two soils (Eutric cambisol or Haplic podzol) |
|---------------------------------------------------------------|------------------|-----------------|
| Soil and P added                                               | Amount of added P sorbed | Final solution conc. (μM) | Buffer power |
| (mmol kg\(^{-1}\))                                             |                  |                 |                |
| **Eutric cambisol**                                            |                  |                 |                |
| 0                                                             | 4.7±0.5          |                 |                |
| 0.33                                                          | 0.32a            | 16.3±0.3        | 33±1          |
| 2.67                                                          | 2.38a            | 425.9±3.4       | 7±1           |
| **Haplic podzol**                                              |                  |                 |                |
| 0                                                             | <0.5             |                 |                |
| 0.33                                                          | 0.33a            | 0.27±0.1        | 2281±1222     |
| 2.67                                                          | 2.66a            | 3.96±0.3        | 788±52        |

Sorption is expressed on a dry weight basis. The solid-to-solution partition coefficient or buffer power is described in Section 2 (Eq. (1)).

a SEM of the adsorbed P was very small because most of the P added was sorbed.
4. Discussion

4.1. Citrate release into the rhizosphere

The release of low molecular weight organic acid anions from plant roots has been hypothesized to be involved in many mechanisms for enhancing nutrient acquisition from the rhizosphere (Ryan et al., 2003). Most of this evidence has come from the growth of plants in hydroponic culture where under nutrient deficiency roots release greater quantities of organic acid anions into the external medium (Jones, 1998). Despite the evidence from hydroponic cultures, there is less direct evidence to support their role in mobilizing nutrients in soil environments. In the experiments described here we have directly manipulated citrate concentrations in the soil to reflect a high rate of exudation normally seen in plants with cluster roots (e.g. Proteaceae, lupins; Dinkelaker et al., 1989; Shen et al., 2003). In these plants, current evidence suggests that organic acid anion release appears to be a spatially and temporally co-ordinated event triggered by P deficiency (Vance et al., 2003). In comparison, monocotyledonous crop plants such as wheat and maize typically release relatively low amounts of organic acid anions under nutrients stress (Ryan et al., 2003). Based upon the diffusion coefficients of organic acid anions in agricultural soils, we have previously predicted that organic acid concentrations at the root surface of wheat plants grown in sterile soil may be in the region of 100–500 μM declining rapidly away from the root and falling to background concentrations (1–50 μM) within 100 μm of the root surface (Jones et al., 1996b). At present, no experimental techniques have been developed to enable sampling with sufficient spatial resolution to validate these mathematical model predictions. However, studies in forest and grassland soils have shown that organic acids are rapidly degraded in the rhizosphere often having a half-life of only a few hours. Further, rapid sorption to the soil’s solid phase may also significantly lower soil solution concentrations (Ryan et al., 2003).

4.2. Enhancing P uptake of crop plants

Although the measured rates of organic acid exudation are low in monocotyledonous crop plants such as wheat and maize it has been suggested that plants could be engineered either through conventional breeding or genetic manipulation (GM) to overproduce and excrete large amounts of organic acids into the soil (De la Fuente et al., 1997; Ryan et al., 2003). Previous studies have indicated that a GM strategy may enhance the uptake of P and the detoxification of toxic metals, although the results remain controversial (Deltcheva et al., 2001, 2003). The results presented here in two acid soils and elsewhere suggest that the effectiveness of this strategy can be expected to be highly soil type dependent. In addition, our results indicate that the form that the organic acids are released in (anionic or acid form) may significantly affect the efficiency of the mechanism.

Although time consuming the traditional approach of selecting and breeding P efficient cultivars on broad soil types (e.g. oxisols, rendzinas) remains an effective mechanism for developing plants adapted to P deficient soils. Early GM approaches to manipulate P efficiency where only single genes responsible for organic acid metabolism were targeted met limited success (e.g. citrate synthase; Delhaize et al., 2003). This is particularly relevant to soils where P deficiency is synonymous with other constraints to growth and where alteration of multiple traits is required (e.g. Al and Mn toxicity in acid soils and micronutrient deficiency in calcareous soils). However, with the continued advancement in genomics techniques and the whole genome sequencing of crop plants it is likely that it will soon be possible to genetically manipulate suites of proteins involved in the P deficiency response. Indeed, the enormous potential of this approach has already been realized in successfully moving genes regulating malate efflux and conferring Al tolerance from wheat to barley (Delhaize et al., 2004). We would, however, also like to insert a note of caution. From an environmental perspective it may not be advantageous in the long term to excessively mine P from the soil using highly P use efficient crops, particularly if P is not replenished by fertilizers. Therefore, a balance needs to be achieved in preserving food production while maintaining soil quality and sustainability.

4.3. Citrate release and soil acidification and root P uptake

Most studies to date have shown that the release of organic acids occurs concomitantly with an acidification of the surrounding medium (Dinkelaker et al., 1989). Previously it was postulated that the root was excreting organic acids directly into the soil [e.g. (H3-citrate)0]. However, based upon the pH of the cytoplasm (pH 7.1–7.4) where citrate is fully dissociated [(citrate)3−] and the transporters responsible for their release, excretion as citric acid is unlikely (Ryan et al., 2003). It has been shown in wheat that to maintain electroneutrality in the plasma membrane the release of malate through anion channels upon exposure to Al is accompanied by a concomitant release of K+ (Ryan et al., 1995). As this response is only triggered in acid soils the release of citrate3− or malate2− anions into an acid environment will induce the formation of (H3-citrate)0 and (Al-citrate)0 raising the pH, lowering Al3+ and consequently reducing toxicity. In the case of P deficiency, however, the counter-ion released during malate and citrate release remains unknown, however, it is clear that the simultaneous upregulation of the H+·ATPase often occurs. Assuming that the H+·ATPase and organic anion channels are co-located (Hoffland et al., 1989; Hoffland, 1992) we presume that the excreted H+ and organic acid anions will equilibrate in the cell wall or apoplast producing
organic acids which then diffuse into the soil. This is supported by most reports showing that the release of malate and citrate is synonymous with an acidification and not an alkalization of the rhizosphere. Our results suggest, however, that alkalization of the rhizosphere by the release of K-citrate rather than H-citrate enhances P mobilization in the Haplic podzol and enhances the recovery of P from soil. This P mobilization was particularly evident when the concentrations of P in the Haplic podzol were relatively high. However, at lower concentrations the impact of both citric acid and K-citrate was much reduced. This would indicate that citrate is able to solubilize P that is not to strongly adsorbed. Our study clearly showed a difference in P response between the acid soils with citrate only being effective at mobilizing P in the Haplic podzol. We hypothesize that this occurs due to the soil having a low intrinsic P status and a slower rate of citrate mineralization (Table 4). Field trials have indicated that this soil is P limiting in addition to other nutrients such as N, whereas the Eutric cambisol responds little to added P due to the long term annual fertilization of the site. We hypothesize that in the Eutric cambisol the plants are not P limited and while the addition of citrate is capable of mobilizing P in the Haplic podzol and enhances the recovery of P from soil. At the lower added33P concentration the P uptake above that required by the plant. In contrast, in the Haplic podzol where plant available P is limited, the roots readily responded to citrate addition and actively took up the mobilized P. At the lower added33P concentration the P mobilization efficiency of citrate was very low and consequently only small amounts were made available to the plants. In contrast, at the higher added P concentration (2.5 nmol g−1) the binding strength of P to the soil was much lower due to a greater saturation of the sorption sites. Consequently, the capacity for citrate to replace P on sorption sites was much greater making it proportionally more available for plant uptake.

In conclusion, our results suggest that the efficiency of organic acids and their anions in mobilizing P from the rhizosphere remains critically dependent upon a range of soil factors and in particular the soils intrinsic P status and its organic acid and P sorption characteristics. Other factors such as microbial activity, water content, pH and the availability of complexing cations (e.g. Al, Ca) are also probably of importance. From a plant perspective, our results indicate that the amount of organic acid released and its ionic form significantly influence its ability to mobilize P from the soil. In addition, the plant’s intrinsic P status is also likely to be a key determinant in the potential for P capture after organic release. Further work is required to characterize the nature of the transport processes occurring under P deficiency (e.g. ionic form of organic acid release and spatial coordination with H++ATPase activity) and to similarly establish the coordination of these events with those of P transporter activities and other P related metabolic events.

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References


