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Organic acids in the rhizosphere – a critical review

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Abstract

Organic acids, such as malate, citrate and oxalate, have been proposed to be involved in many processes operating in the rhizosphere, including nutrient acquisition and metal detoxification, alleviation of anaerobic stress in roots, mineral weathering and pathogen attraction. A full assessment of their role in these processes, however, cannot be determined unless the exact mechanisms of plant organic acid release and the fate of these compounds in the soil are more fully understood. This review therefore includes information on organic acid levels in plants (concentrations, compartmentalisation, spatial aspects, synthesis), plant efflux (passive versus active transport, theoretical versus experimental considerations), soil reactions (soil solution concentrations, sorption) and microbial considerations (mineralization). In summary, the release of organic acids from roots can operate by multiple mechanisms in response to a number of well-defined environmental stresses (e.g., Al, P and Fe stress, anoxia): These responses, however, are highly stress- and plant-species specific. In addition, this review indicates that the sorption of organic acids to the mineral phase and mineralisation by the soil's microbial biomass are critical to determining the effectiveness of organic acids in most rhizosphere processes.

Introduction

Organic acids are low-molecular weight CHO containing compounds which are found in all organisms and which are characterised by the possession of one or more carboxyl groups. Depending on the dissociation properties and number of these carboxylic groups, organic acids can carry varying negative charge, thereby allowing the complexation of metal cations in solution and the displacement of anions from the soil matrix. For this reason, they have been implicated in many soil processes including the mobilisation and uptake of nutrients by plants and microorganisms (e.g., P and Fe), the detoxification of metals by plants (e.g., Al), microbial proliferation in the rhizosphere, and the dissolution of soil minerals leading to pedogenesis (e.g., podzolisation) (Marschner, 1995). Until recently, however, there has been little direct *in vivo* evidence to support any of these hypotheses. Indeed, the reactions of organic acids in soil are extremely complex, a fact which is often ignored by researchers.

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A schematic diagram which shows the major organic acid fluxes and pools in soil and which will form the basis for this review is shown in Figure 1. Without a knowledge of the size and rates of flux between these pools it has been impossible to determine the importance of organic acids in a complex soil environment. The aim of this review is therefore to summarise past and present research on the behaviour of organic acids in plants and soil and to assess the functional significance of organic acids with particular reference to the rhizosphere.

Organic acids in plants

Root content

Typically roots contain many organic acids varying in chain length with lactate, acetate, oxalate, succinate, fumarate, malate, citrate, isocitrate and aconitate being the primary anion components. Certainly for crop plants, we have a good understanding of the biosynthetic pathways involved in the production of

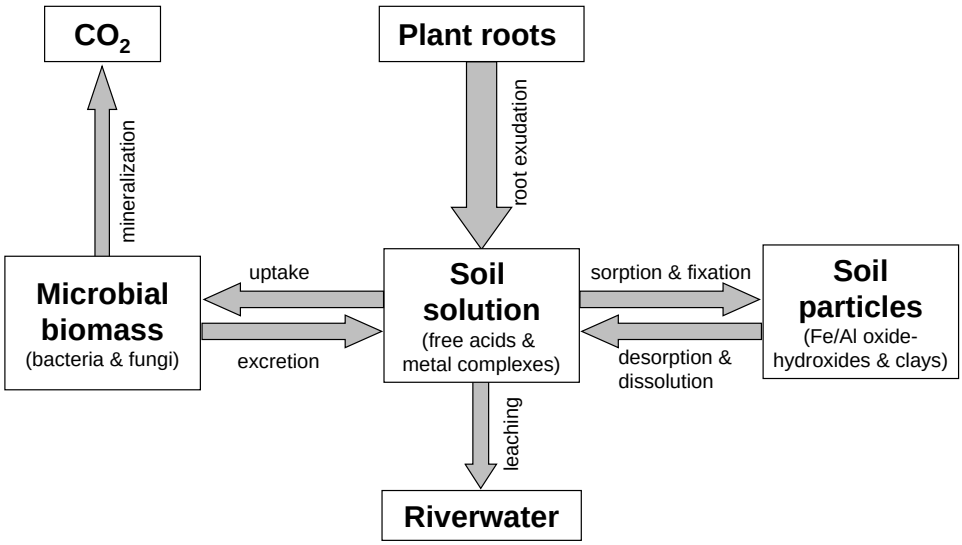

Figure 1. Schematic diagram showing the major organic acid fluxes and pools in soil.

these organic acids of which further details can be found in Dennis et al. (1997) and Marschner (1995). While some of these organic acids are involved in energy production as intermediates in the tricarboxylic (TCA) cycle (e.g., citrate, malate), others are primarily present in cells for cation charge balancing or for maintaining osmotic potential (e.g., malate, malonate, oxalate). The organic acid content of plants is governed primarily by their type of C fixation (e.g., CAM, C_3 or C_4), their nutritional status and age. A summary of the typical organic acid concentrations found in plant roots under varying conditions is provided in Table 1. Typically the total concentration of organic acids in roots is around 10–20 m*M* (1–4% of total dry weight) which can be compared, at least for maize, with the other main organic solutes present in root cells, namely amino acids (10–20 m*M*) and sugars (90 m*M*) (Table 1; Jones and Darrah, 1994a, 1996). The typical spatial distribution of these solutes in maize roots is shown in Figure 2 and illustrates the spatial heterogeneity of solutes within a single root.

One of the primary factors determining organic acid levels in roots is their degree of cation–anion imbalance. In situations where roots take up an excess of cations (particularly K^+), the negative charge required to balance this is often provided by organic acids, such as malate, malonate, citrate and aconitate (Chang and Roberts, 1991; Osmond, 1976). Roots grown on NH_4^+ have lower organic acid concentrations than those grown on NO_3^-, for reasons discussed in Marschner (1995).

One of the problems with the interpretation of root organic acid concentrations and its resultant effect on organic acid efflux is that rarely has the cellular compartmentation of the organic acids in the root been investigated. The vacuole can occupy a significant proportion of root cells, varying from 15% at the root cap to 90% of the cellular volume 1 cm away from the apex (Patel et al., 1990). Although the vacuolar pool may be important as a storage pool for organic acids, it is the concentration gradient between the cytosol and soil solution which ultimately determines the rate of efflux. It can be expected that the concentration of organic acids in the cytosol must be closely controlled in order to conform to the kinetic and inhibitory requirements of the enzymes involved in cellular metabolism. Therefore, when an accumulation of organic acids is observed in roots, this may simply reflect an increase in vacuolar concentration or vacuolar volume. This is supported by flux analysis and ^{13}C-NMR spectroscopy, which have shown that typically the concentration of metabolic intermediates like malate and citrate in the cytosol rarely exceeds 5 m*M*, whereas concentrations in the vacuole can be 1–10 fold higher (Chang and Roberts, 1989, 1991; Gout et al., 1993; Osmond, 1976; Osmond and Laties, 1969).

Extreme caution must also be shown when comparing whole root system organic acid concentrations with efflux data, especially since metabolite concentration can change significantly along the length of a root (Figure 2; Chang and Roberts, 1989; Jones and

Table 1. Organic acid content of plant roots as a function of nutritional status. Organic acid concentrations are given in the same order as the treatments. +P or +Fe or +K and −P or −Fe or −K represents either P/Fe/K sufficient or P/Fe/K deficient conditions respectively. −Al and +Al indicates plants exposed to zero or rhizotoxic levels of Al (20–200 μM), respectively. +nut indicates plants grown at a high nutrient supply, while −nut indicates plants grown without P and micronutrients. The units nmol g^{-1} FW root are approximately equivalent to the root concentration in μM. SEM indicates standard error. – indicates not determined.

Plant species	Treatment	Location[a] in root	Plant content (nmol g^{-1} FW root)		Reference
			Malate	Citrate	
Brassica napus	+P, −P	tip	3350–21 700	1100–8100	Hoffland (1992)
Brassica napus	+P, −P	base	3150–10 400	2700–5450	Hoffland (1992)
Sisymbrium officinale	+P, −P	tip	9700–4700	8500–6700	Hoffland et al. (1992)
Sisymbrium officinale	+P, −P	base	2500–3600	1000–2500	Hoffland et al. (1992)
Sorghum bicolor	+P, −P	wr	930–1910	1260–1860	Schwab et al. (1983)
Sorghum bicolor	−Al, +Al	wr	7200–18 000	1500–4830	Cambraia et al. (1983)
Triticum aestivum	−Al, +Al	tip	493–525	–	Delhaize et al. (1993)
Hordeum vulgare	−Al, +Al	wr	2686–8358	1888–1666	Foy et al. (1987)
Zea mays	−Al, +Al	wr	145–1043	15–24	Pellet et al. (1995)
Zea mays	+nut, −nut	wr	1430–2240	1090–1560	Jones and Darrah (1995)
Zea mays	−K,+K	wr	23 000–33 000	12 000–19 700	Kraffczyk et al. (1984)
Phaseolus vulgaris	+Fe, −Fe	wr	18 000–68 000	800–5100	Schwab et al. (1983)
Range			150–58 000	15–19 700	
Mean ± SEM			10 250 ± 3000	4060 ± 1000	

[a] wr indicates whole root system data.

Darrah, 1995; Stumpf and Burris, 1981). Many researchers have also tried to link increases in organic acid efflux with rises in the levels of organic acid synthesising enzymes. In some cases this has shown good correlations, while in others no correlation has been observed (Hoffland et al, 1992; Johnson et al., 1996a; Ryan et al., 1995a). Where new enzymes are required for the production of a novel compound, protein synthesis is obviously a prerequisite. However, for simple intermediates like citrate and malate where the enzymatic machinery is already present in the cell, all that is required is an increased flux through enzymes such as PEP carboxylase and citrate synthase with a continual removal of product. Further, enzyme assays performed *in vitro* with whole tissue extracts under optimal conditions (e.g., substrate, H^+ and co-factor concentrations) should be extrapolated with caution to *in vivo* situations where cellular conditions may be very different. Future research must therefore be directed towards a more *in vivo* approach whereby gene expression reporters (e.g., β-glucuronidase; GUS), protein reporters (green fluorescent proteins; GFPs) or alternatively more informative *in vivo* enzyme assays are used as visual measures of enzyme levels (Ismail et al., 1997). Further, more detailed cytosolic-vacuolar organic acid compartmentation analyses need to be performed with techniques such as ^{13}C-NMR where adequate consideration is also given to spatial differences in concentrations along the root length.

Root release of organic acids

The release of organic compounds from plant roots is well documented (Curl and Trueglove, 1986). From early investigations using semi-quantitative techniques, it soon became evident that the composition of root exudates is highly variable and dependent on plant species, plant age and physiochemical environment (for a review see Curl and Trueglove, 1986). Despite this early pioneering work, however, the mechanisms controlling organic acid release from roots have only recently started to be elucidated.

Theoretical considerations

From a purely theoretical standpoint it is known that release of organic acids into the rhizosphere must always be occurring. This is based upon the fact that the concentration of organic acids in the cytosol (ca. 0.5–10 mM; Table 1) is about 1000-fold greater than that present in the soil solution (0.5–10 μM; Table 2). In addition, a substantial electric charge gradient exists across the plasma membrane (ca. −180 mV) due to the operation of ATP-driven proton pumps

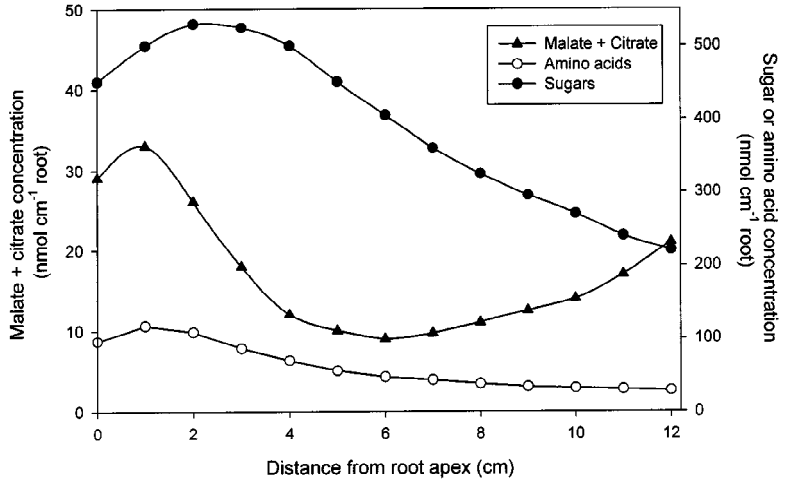

Figure 2. The spatial distribution of the major low molecular weight organic solutes in maize roots. For reference to the later discussion, only malate and citrate are shown although other organic acids are also present in appreciable quantities (e.g., aconitate). The data is taken from Jones and Darrah (1994a, 1995, 1996).

Table 2. Soil solution organic acid concentrations reported in the literature. – indicates not determined, bdl indicates below detection limits

Matrix type	Organic acid concentration (μM)					Reference
	Acetate	Formate	Citrate	Malate	Oxalate	
Lupinus albus proteoid root mat[a]	–	–	4700	–	–	Dinkelaker et al. (1989)
Banksia rhizosphere soil	–	–	70	35	bdl	Grierson (1992)
Bulk soil from near *Banksia*	–	–	0.8	0.7	bdl	Grierson (1992)
Forest floor	10	0.7	<0.001	–	3.3	Krzysowska et al. (1996)
Beech forest soil[b]	14	5	0.8	1.5	2.0	Shen et al. (1996)
Eutric cambisol	8.5	5.0	0.8	0.7	2.4	Ström (1997)
Rendzic leptosols (calcareous)	10.0	5.1	4.1	2.5	7.1	Ström (1997)
Forest soil	–	bdl-90	bdl-12	118	10	Hue et al. (1986)
Cultivated soil	–	bdl-8	bdl	4	5	Hue et al. (1986)
Ultic hapludalf cultivated soil[c]	2737	9	61	–	–	Elkhatib (1990)
Typic hapludult cultivated soil[d]	786	579	122	–	–	Elkhatib (1990)
Trifolium rhizosphere soil[a]	1430	bdl	bdl	1472	bdl	Bolan (1994)
Elytrigia rhizosphere soil[a,e]	630	563	bdl-22	bdl-4	bdl-198	Baziramakenga et al. (1995)
Soil containing *Elytrigia* residues[a,e]	3151	2277	bdl-110	bdl-26	bdl-417	Baziramakenga et al. (1995)
Oak leaf litter leachate	–	–	–	bdl	bdl	Pohlman and McColl (1988)
Douglas fir litter leachates	–	–	–	bdl-68	bdl-190	Pohlman and McColl (1988)
Decomposing yellow pine wood	–	–	–	–	550	Micales (1997)
Picea abies podzolic topsoil	bdl-1829	bdl-117	160-370	60-165	–	Van Hees et al. (1996)
Picea abies podzolic subsoil	bdl	bdl	bdl-40	bdl-25	–	Van Hees et al. (1996)

[a] Conversion factor of 4.3 used to convert μmol g^{-1} soil to μmol cm^{-3} soil solution.
[b] Mean of six soils under varying vegetation types growing on a Dystric cambisol.
[c] Grey brown podzolic soil.
[d] Red-yellow podzolic soil.
[e] Humo-ferric podzolic soil.

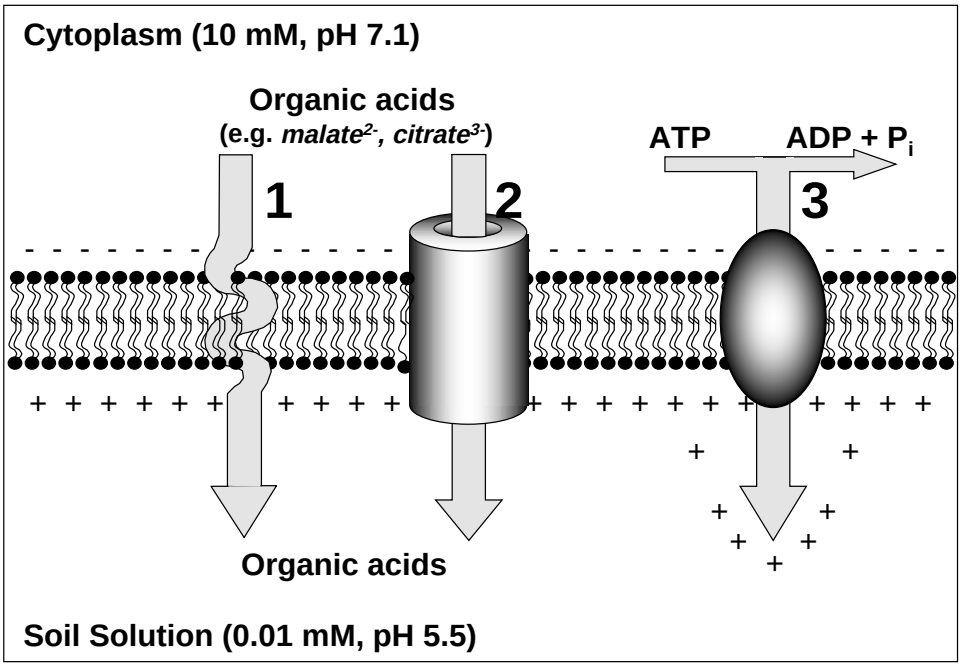

Figure 3. Schematic representation of the two main routes of organic acid efflux from the cytoplasm of root cells into the soil solution. The first (1) represents the slow passive diffusion across the lipid bilayer, whereas the second (2) represents efflux through a plasma membrane channel protein. The direction of transport by both mechanisms is controlled by the electrochemical potential gradient (charge and concentration) across the membrane which is partly generated by the H^+-ATPase (3).

(H^+-ATPases) and the large cytosolic K^+ diffusion potential (Samuels et al., 1992). While the H^+ expelled into the apoplast by these H^+-ATPases creates a charge gradient to facilitate the uptake of cations from the soil, it also tends to draw anions (e.g., citrate^{3-}, malate^{2-}) out of the cells and into the external soil solution. Organic acids typically flow across the lipid bilayer at a slow rate in response to this electrochemical gradient (baseline exudation); however, efflux can be increased greatly by the opening of channels embedded in the lipid bilayer (Dennis et al., 1997). A schematic representation of the two main routes of efflux from living root cells is shown in Figure 3.

From a knowledge of membrane biophysics and the cellular concentrations of organic acids, the net (baseline) exudation of solutes can be predicted using the net flux density equation (Nobel, 1991) where the net flux (F) can be derived from

$$F = F_{in} - F_{out} = A\left(\frac{PzE_{\mathrm{m}}F}{RT}\right)\left(\frac{1}{e^{zE_{\mathrm{m}}F/RT}-1}\right)\left(C_{out} - C_{in}e^{zE_{\mathrm{m}}F/RT}\right)$$

where F_{in} is the inward flux, F_{out} is the outward flux, A is the root surface area, P is the membrane permeability coefficient, z is the charge of the ion, E_{m} is the membrane potential, F is the Faraday constant, R is the gas constant, T is temperature, C_{out} is the concentration in the soil solution and C_{in} is the concentration in the cytosol. For example, to calculate the net baseline flux of malate from a wheat root tip, the following parameters are used; a root area of 5.7×10^{-6} m^2 mg^{-1} FW root, P of 1.2×10^{-9} m s^{-1}, E_{m} of -0.120 V, F of 9.649×10^4 J mol^{-1} V^{-1}, R of 8.3143 J mol^{-1} K^{-1}, T of 293 K, C_{out} of 10 μM, C_{in} of 500 μM and assuming a cytosolic pH of 7.2, net charge of malate (z) to be -2 (Delhaize et al., 1993; Lüttge and Smith, 1984; Nobel, 1991; Papernik and Kochian, 1997). From this equation, the theoretical net efflux of malate from a wheat root tip into the soil solution can be calculated at 3.3×10^{-2} pmol mg^{-1} root FW s^{-1}. This is in close agreement with experimentally derived malate efflux rates for wheat root tips which range from 1.4×10^{-2} pmol mg^{-1} root FW s^{-1} under normal conditions rising up to 3.3×10^{-1} pmol mg^{-1} root FW s^{-1} in the presence of toxic levels of Al and when transport is probably enhanced through the opening of channel proteins (Table 3; Ryan et al., 1995a).

Table 3. Summary of root organic acid exudation rates

Plant	Exudate location[a]	Treatment[b]	Organic acid exudation rate			Reference
			Malate	Citrate	Oxalate	
			(pmol g^{-1} root FW s^{-1})			
Rape	tip	+P, −P	10–59	4–18	–	Hoffland (1982)
Rape	base	+P, −P	2–14	0.5–9	–	Hoffland (1982)
Lupin	proteoid	+P, −P	8–141	6–158	–	Johnson et al. (1996a)
Alfalfa	wr	+P, −P	0.8–1.7	0.2–0.8	–	Lipton et al. (1987)
Sorghum	wr	+P, −P	bdl.	0.5–5.8	–	Schwab et al. (1983)
Maize	tip	−Al, +Al	1–8	0.1–36	–	Pellet et al. (1995)
Wheat	tip*/wr**	−Al, +Al	14–338*	1.3–3.1**	–	Delhaize et al. (1993)
Maize	wr	ns, nd	5–165	3.3–38	–	Jones et al. (1995)
Barley	wr	+Fe, −Fe	1–40	–	–	Fan et al. (1997)
Maize	wr	+K, −K	0.25–0.42	0.02–0.04	–	Kraffczyk et al. (1984)
Agropyron cristatum	wr	ns	0.25	–	–	Klein et al. (1988)
Agropyron smithii	wr	ns	0.17	–	–	Klein et al. (1988)
Bouteloua gracilis	wr	ns	<0.1	–	–	Klein et al. (1988)
Calcifuge plants	wr	9 species (ns)	0.08	0.06	0.44	Ström et al. (1994)
Calcicole plants	wr	9 species (ns)	0.08	0.38	1.22	Ström et al. (1994)

[a] tip indicates exudation measured at the root apex, base indicates measurement at root base, wr indicates measurement from a whole root system, proteoid represents measurement of proteoid roots only.
[b] + indicates in the presence of, and – indicates deficient conditions, ns indicates nutrient sufficient while nd indicates nutrient deficient.

In addition to the net organic acid flux, the concentration at which malate can passively re-enter the root can be calculated using the Ussing–Teorell equation (Nobel, 1991) where

$$\frac{F_{in}}{F_{out}} = \frac{C_{out}}{C_{in} e^{zE_{\mathrm{m}}F/RT}} = 1$$

For a root with total malate concentration of 500 μM and cytosolic pH of 7.2, the concentration of each malate species in the cytosol can be predicted at 497 μM for malate^{2-}, 1.8 μM for malate^{1-} and 0.4 nM for malate^{0} assuming no metal interactions (Parker et al., 1995). Therefore the soil solution concentration required to start driving malate back into the root can be calculated at 6673 mm for malate^{2-} 205 μM for malate^{1-} and 0.4 nM for malate^{0}. At least for malate^{2-}, this concentration is six orders of magnitude greater than typically present in the soil solution (Table 2). Therefore purely based on theoretical calculations it can be hypothesised that malate (and citrate) release from the root will be predominantly a unidirectional passive transport process using the cell's electrochemical potential gradient. In contrast, root influx of organic anions such as malate, citrate and oxalate at soil pHs >5.5 (i.e. when the anions are negatively charged) must be an active (energy-requiring) transport process, conclusive evidence for which has yet to be presented (Jones and Darrah, 1995).

Experimental considerations

Most root organic acid exudation studies have been performed in solution culture which facilitates the collection and analysis of exudate components (e.g., Jones and Darrah, 1995). However, this technique suffers from a series of severe drawbacks. The most serious criticism is that roots grown under these conditions may be morphologically and physiologically very different from those growing in a real soil (e.g., no root hairs, no cortical degeneration, different branching patterns, no mechanical impedance or water stress). In addition, the aeration, microbial and nutrient status of these hydroponic cultures is often very different from those in a typical soil environment. Certainly for roots placed in distilled water, the lack of Ca^{2+} can severely and irreversibly damage cell membranes, which in some cases can lead to the bursting of root cells and the loss of cell contents into the external medium (Jones et al., 1995). In other previous efflux experiments, roots have been removed from soil and then washed, inevitably leading to the release of organic acids from damaged cells. In addition, with non-sterile studies, no account is ever made for the use

of exudates by the microorganisms which are known to rapidly build up on the rhizoplane (e.g., Matsumoto et al., 1979). However, despite all these methodological limitations, comparative studies of this nature strongly suggest that organic acids play an important functional role in many plant responses to nutrient stress. While many roles of organic acids have been postulated, direct evidence for a functional role only appears to be available for P, Al and Fe and it is upon these elements that this review will concentrate.

It must also be stressed that results can also be highly biased by the method used to calculate organic acid release rates. For example, in some species organic acid release appears to be highly localised to the tip region (see later). If exudate release rate is calculated on a root tip basis, and, even better, if exudate release can be spatially quantified, the results will be accurate. However, if results are expressed for total root mass, then exudate release rates are greatly underestimated and differences in tip:weight ratio between treatments can severely bias results and in some circumstances make the conclusions invalid. For this reason it is often difficult to interpret many studies in which exudation is integrated over the whole root system and over extended time periods ($>$24h) and where no account of root growth is made (e.g., Kraffczyk et al., 1984; Schwab et al., 1983). Future studies should therefore measure exudate release over short time periods ($\leq$12 h) and, if possible, spatially isolate root regions so that exudation rates can be accurately quantified (e.g., Delhaize et al., 1993; Hoffland et al., 1992).

Organic acid release by calcicole versus calcifuge plants

Most previous studies have focused on the role of nutrient deficiencies in triggering organic acid release. However, a role for root exudates in general plant ecology and the ability of different plants to establish on either calcareous or silicate soils is now being investigated. For plants to establish and successfully colonise calcareous soils requires a mechanism for solubilising Fe and P in the rhizosphere which are the primary limitations to growth in this environment. It appears from preliminary studies that calcicole plants (i.e. those that can establish on calcareous soils) have a greater capacity to release organic acids than calcifuge plants, which mainly establish on silicate soils (Table 3; Ström, 1997; Ström et al., 1994; Tyler and Ström, 1995). Further work is certainly needed to confirm the functional significance of these promising findings.

Organic acid release under Fe deficiency

Plant Fe deficiency normally occurs on calcareous soils (pH $>$ 7.0) due to the insolubility of Fe at high pH (Marschner, 1995). Organic acids such as citrate and malate are potent complexers of Fe in soil and induce the dissolution of previously unavailable insoluble ferric oxyhydroxides (Gerke, 1992; Jones et al., 1996a). Recently is has been proposed that citrate may play an important role in supplying Fe to dicotyledonous plants (Jones et al., 1996a). Fe deficiency induces a substantial accumulation ($\approx$5 fold increase) of organic acids in root tissues and also induces a large (5-10 fold) increase in H^+ and organic acid excretion (De Vos et al., 1986; Guerinot and Yi, 1994; Landsburg, 1981; Ohwaki and Sugahara, 1997). Under Fe deficiency, root H^+-ATPases are highly up-regulated and are therefore likely to be the predominant source of the excreted H^+ (Rabotti and Zocchi, 1994; M L Guerinot, pers. comm.). In calcareous soils, the combination of the acidification power of the H^+-ATPase alongside the complexing ability of citrate (which increases with decreasing pH) could provide a viable means of mobilising Fe in the rhizosphere. Once Fe^{3+}-citrate complexes form in the soil solution, dicotyledonous roots can access the Fe using a plasma-membrane ferric reductase which reduces Fe^{3+} to Fe^{2+} releasing the citrate back into solution. Fe^{2+} can then be transported into the root using the root's electrochemical potential gradient, probably in combination with a divalent cation channel (Fox et al., 1996; Figure 4). The mechanisms for Fe^{3+} reduction and Fe^{2+} transport have been shown to be highly up-regulated upon Fe deficiency with the enzymatic machinery present along most of the root length but surprisingly absent from the root apex (Welch et al., 1993). The widespread nature of organic acid release and the exact location of efflux in dicotyledonous roots under Fe deficiency remains unknown. However, based on mathematical calculations it has been predicted that the amount of organic acid release from *Brassica napus* roots, even under non-Fe stressed conditions, may be sufficient to supply a substantial amount of the plant's Fe demand (Jones et al., 1996a). Further experimental work, however, is required to confirm the existence and operation of this mechanism in soil.

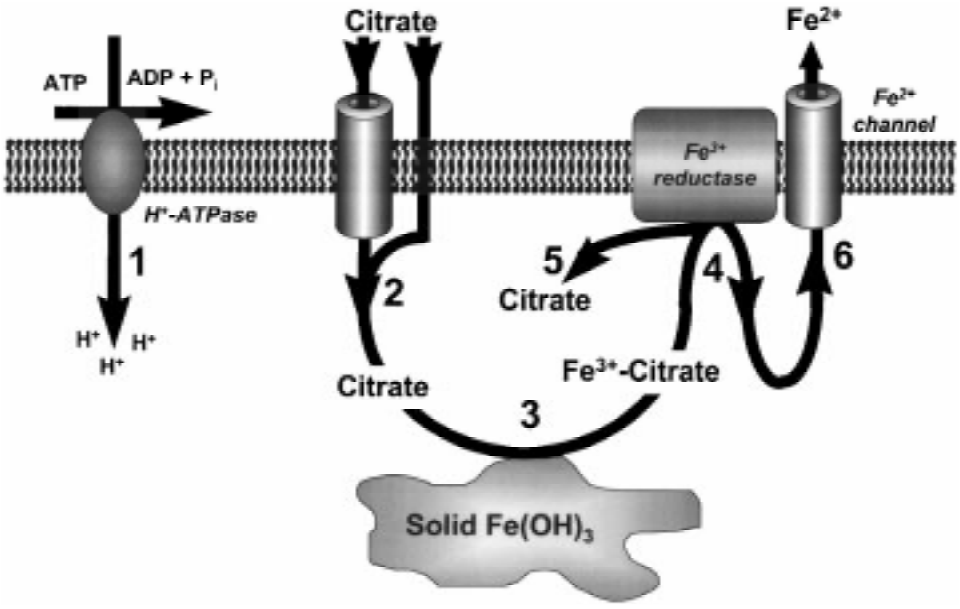

Figure 4. Schematic representation of the role of organic acids in Fe uptake by dicotyledonous plant roots. The major steps are: (1) acidification of the rhizosphere by H^+-ATPases; (2) the release of citrate from the cytoplasm into the soil (see Figure 1); (3) the reaction of citrate with $Fe(OH)_3$ in the soil forming Fe^{3+}-citrate complexes; (4) the reduction of the Fe^{3+}-citrate complex at the plasma membrane surface by the Fe^{3+} reductase enzyme; (5) the release of the citrate chelator back into the soil solution; and (6) the uptake into the root of the reduced Fe^{2+} by a divalent cation channel.

Organic acid release under phosphorus deficiency

Due to its insolubility and high sorption capacity in soil, phosphate supply can be one of the major constraints to plant growth (Marschner, 1995). However, there is now overwhelming evidence to suggest that some plants can directly modify the rhizosphere in order to gain access to previously unavailable soil P reserves. This can include the manipulation of root hair length/density, the extra provision of C for mycorrhizal exploitation of non-rhizosphere soil, the release of phosphatases to release organically bound soil P, and the release of organic acids and H^+ to solubilise inorganic P. It has now been demonstrated that some dicotyledonous plant roots, and especially non-mycorrhizal plants such as *Lupinus albus* (but not *L. angustifolia*) and *Brassica napus*, are capable of releasing large amounts of organic acids into the rhizosphere in response to P deficiency, while other dicotyledonous (e.g., *Sisymbrium officinale*) and graminaceous (wheat, maize) plants do not appear to express the trait (Table 3; Gerke, 1994; Hoffland et al., 1992; Imas et al., 1997a; Johnson et al., 1996a, b; Laheurte and Berthelin, 1988; Lipton et al., 1987; Schwab et al., 1983). However, it is also well established that P deficiency significantly increases the leakiness of the root plasma membrane to solutes indicting that for some exudation studies the observed increases in organic acid release may be an indirect root response of minimal importance (Ratnayake et al., 1978). This is especially true where only small increases ($\leq$2 fold) in organic acid release and C diversion into root exudation (0.3%) are observed upon long-term P deficiency (Lipton et al., 1987), and where the calculation of results may cause significant biasing (see above section on Experimental considerations).

Malate and citrate appear to be the primary components released by roots under P deficiency. In *Brassica napus* the 4-fold increase in organic acid exudation is largely associated with the root apex, while smaller amounts are also released from mature root regions (Hoffland et al., 1989, 1992). In contrast, except under extremely high P levels, lupin and other species with cluster roots (e.g., *Banksia*) induce the development of short branched, tertiary lateral roots (proteoid or 'cluster' roots) (Dinkelaker et al., 1995; Keerthisinghe et al., 1998). These roots are directly responsible for the 13–40-fold increase in the citrate and malate excretion which constitutes >90% of the total root exudate under P deficiency and which commences 3 days after proteoid root development (Dinkelaker et al., 1989; Gardner et al., 1983; Grierson, 1992; John-

son et al., 1996a, b). This organic acid exudation under P deficiency constitutes a drain of 5–25% of the plant's photosynthetically fixed C, however, this does not appear to significantly affect dry matter production (Dinkelaker et al., 1989; Gardner et al., 1983; Johnson et al., 1996a, b; Keerthisinghe et al., 1998). To sustain this level of root exudation obviously requires a sustained production of organic acids as exudation under P stress can deplete the entire root organic acid reserves within hours (Johnson et al., 1996a). In lupins, it appears that C is mainly supplied in the form of phloem-translocated sugars (70%) whilst some is also supplied in the form of root-fixed inorganic C (30%) (Johnson et al., 1996a, b). The phloem-translocated sugars are subsequently converted to organic acids via the enzymes PEP carboxylase, malate dehydrogenase and citrate synthase at the site of release (Hoffland et al., 1992; Johnson et al., 1994, 1996a, b). The transport mechanisms controlling organic acid release and the number and regulation of genes determining this P deficiency trait, however, have still to be identified.

Role of organic acids in aluminium detoxification

Aluminium (Al) rhizotoxicity is one of the biggest limitations to crop production in the world and is characterised by the inhibition of root cell elongation and to a lesser extent cell division (Kochian, 1995; Kochian and Jones, 1997). However, some plants appear to be able to resist concentrations of Al which are typically toxic to most plants ($>5\ \mu M$). Of the two resistance mechanisms identified to date, both appear to involve the detoxification of Al either outside (apoplast) or inside (vacuole) the root through a complexation reaction with organic acids (Kochian, 1995; Ma et al., 1997). Only the apoplastic (rhizosphere) mechanism will be addressed here.

Excretion of organic acids by Al-resistant crop plants in response to Al exposure is now well established (Delhaize et al., 1993; Miyasaka et al., 1991; Pellet et al., 1995). Roots specifically release either malate (wheat) or citrate (snapbean and maize) into the apoplast and external solution upon exposure to high levels of Al, with the amount released directly proportional to the external Al concentration (0–200 μM). Above Al concentrations of 200 μM, malate release becomes saturated (Ryan et al., 1995a). Once these organic acids have left the root, they rapidly form Al-organic acid complexes in the apoplast and soil solution, rendering Al non-toxic and making the roots 5–20 times more resistant to Al (Delhaize et al., 1993). In wheat, malate is specifically released upon exposure to the monomeric Al^{3+} species with other forms of Al (e.g., Al_{13}, $Al(OH)_3$), heavy metals, or changes in pH unable to trigger the malate release (Ryan et al., 1995a). In addition, once the Al is removed from the external medium, malate exudation rates quickly drop to baseline (non-stressed) levels, indicating a very sensitive Al sensing and controllable organic acid excretion mechanism (Ryan et al., 1995a). It has been hypothesised from genetic analysis of the Al tolerance trait in hexaploid wheat that the inducible organic acid release appears to be predominantly controlled by a single gene with fine control provided by a series of other minor regulatory genes (Ryan et al., 1995b). From kinetic analysis of organic acid release in root tips of wheat and maize, malate and citrate release appears to be almost instantaneous and thus cannot involve *de novo* synthesis of proteins. From these results, some researchers have speculated that the genes controlling malate release (e.g., *Alt1*) encode not the organic acid transport proteins but rather signalling elements necessary to trigger the release of malate and citrate. The nature of this signalling protein, however, remains unknown but may constitute an Al^{3+}receptor located in the plasma membrane. In addition, the release of root exudates in response to Al only occurs at the root apex (0–3 mm) which correlates well with this being the primary site of Al toxicity (Kochian, 1995).

From a knowledge of the temporal, spatial and Al-specific nature of organic acid release from cereal roots, it has been hypothesised that the release of organic acids is a unidirectional flux involving a plasma-membrane anion channel (Jones and Darrah, 1995; Ryan et al., 1995a). Other evidence for this comes from work with biochemical anion-channel antagonists (e.g., niflumic acid), electrochemical potential measurements, and the fact that only specific organic acids are released. Work is now underway around the world to confirm the existence of root plasma membrane efflux channels using both molecular and biophysical techniques. Current evidence suggests that the gating of the anion-efflux channel may involve a complex signal transduction cascade (Papernik and Kochian, 1997; Ryan et al., 1997).

Excised wheat root tips can release more than three times more malate as present at the start of the experimental period (Ryan et al., 1995a). Sustained exudation, therefore, requires the continued synthesis of new malate within the root apex. As the concentration of the key malate-synthesising enzymes (malate dehydrogenase, PEP carboxylase) are not present in

higher quantities in Al-resistant roots either in the presence or absence of Al, and as the time required for protein synthesis is not consistent with the time course of Al release, an increased flux of intermediates through these enzymes must control organic acid synthesis upon exposure to elevated Al (Ryan et al., 1995a).

Organic acid release by roots under other nutrient stresses

Increases in organic acid efflux have also been observed under a variety of other stresses, including K^+ deficiency (Kraffczyk et al., 1984) and a general nutrient deficiency (Jones and Darrah, 1995), and also in response to the type of N nutrition (Imas et al., 1997b). It is likely that Ca^{2+} and Zn^{2+} deficiency, which increases membrane permeability, will also cause increases in organic acid efflux (Cakmak and Marschner, 1988). However, experiments in which malate, citrate or oxalate were continually added to soil or hydroponic solutions have showed little effect on Zn accumulation by wheat (Chairidchai and Ritchie, 1993a, b; Evans, 1991). A role for organic acids in mobilising Zn and other micronutrients (e.g., Cu) in the rhizosphere, however, cannot be fully discounted at this time.

Organic acid release by roots under oxygen stress

Plants differ widely in their ability to cope with anaerobic stress. Plants have developed two main strategies to tolerate hypoxic (or under extreme circumstances anoxic) conditions. The first involves the slow hormone-induced development of aerenchyma within the root cortex which stimulates air flow (Marschner, 1995). The second involves rapid changes in carbon metabolism and organic acid transport (Marschner, 1995). Under hypoxia, roots have a restricted aerobic metabolism and significant fermentative metabolism, which produces potentially phytotoxic metabolic end-products (e.g., lactic acid and alanine; Ricard et al., 1994). In some plants it appears that the rhizosphere can be used as a 'dumping' zone for lactic acid thereby preventing its build-up in the cellular metabolic pool (Rivoal and Hanson, 1993; Xia and Roberts, 1994). The rates of lactic acid efflux from maize root tips under anaerobic conditions has been estimated at between 0.55–1.58 pmol mg^{-1} root FW s^{-1}, greater than most reported rates of citrate and malate release (Table 3). This, however, probably reflects the dominance of aerobic respiration in root metabolism (Xia and Saglio, 1992). Further, work with protein synthesis inhibitors indicates that the transport of lactate across the plasma membrane may involve specific transport proteins (Xia and Saglio, 1992). Whether this lactic acid excretion strategy is present in species other than maize and *Limonium* remains to be identified. In addition, its impact on rhizosphere colonisation and pathogen infection also needs to be addressed in light of evidence linking anaerobic stress to plant pathogen attack (Smucker and Erickson, 1987).

Do organic acids cause rhizosphere acidification?

Despite early work suggesting that organic acids can acidify the rhizosphere (Marschner, 1995), it now appears that H^+ release and organic acid release are probably two biochemically separate but spatially coordinated transport events. It is well documented that the cytosolic pH of roots ranges from 7.1–7.4 (Marschner, 1995). At this pH, it can be predicted from chemical equilibria models that the primary organic acid species in the cytosol will be the fully dissociated forms of malate and citrate, namely mal^{2-} and cit^{3-}. However, during passage of the organic acids across the membrane into the apoplast, a counter ion is also required to maintain electrical neutrality. Under Al rhizotoxic conditions, no stimulation of H^+ excretion and no change in rhizosphere pH is observed when malate is being released, indicating that H^+ is not the counter ion (Kochian, 1995). This is functional as a lowering of the external pH would increase Al^{3+} solubility in the rhizosphere and increase Al rhizotoxicity. In the case of malate release from wheat roots, it appears that K^+ is the counter cation which accompanies malate across the membrane with a K:malate stoichiometry of 2:1 (Ryan et al., 1995a). For other organic acid release situations the counter ion has yet to be identified. If indeed a H^+ counter ion is involved, some way will be needed to confirm this by separating out direct H^+-organic acid coupled transport events from uncoupled transport events.

Organic acids in soil

Soil solution concentrations

There is now overwhelming evidence showing that significant and sustained quantities of organic acids can be found in soil solutions. This information is

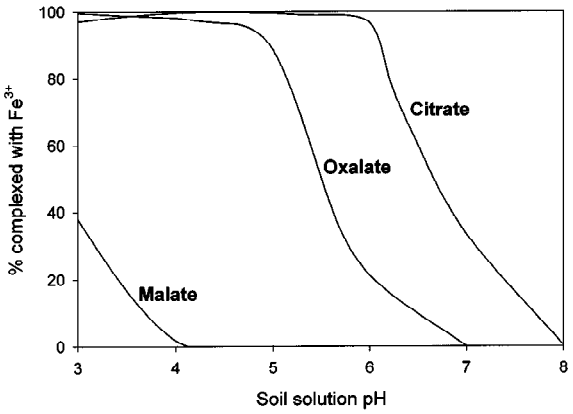

Figure 5. Ability of three organic acids to form stable complexes with Fe^{3+} as a function of pH as predicted with the chemical speciation program Geochem-PC (Parker et al., 1995). The Fe^{3+} and organic acid concentration was fixed at 100 μM.

partly due to recent advances in organic acid analysis which allows detection and quantification at concentrations in the nM range (Fan et al., 1997; Krzyszowska et al., 1996; Shen et al., 1996). While some of the organic acids present in the soil solution will be added in atmospheric dry and wet deposition (rainwater from urban areas typically contains 10–500 μM acetate and formate; Millet et al., 1997), the predominant input will be from root exudates, dead plant material and microbial decomposition products. Organic manures can also contain large amounts of organic acids (Bolan et al., 1994). All of the major plant originating organic acids have now been detected in the soil solution in appreciable quantities (0.1–100 μM) and constitute about 2% of the total dissolved organic C in podzolic soils (Table 2; Krzyszowska et al., 1996; Pohlman and McColl, 1988; Shen et al., 1996). Generally higher concentrations are found in rhizosphere soil compared to those present in the bulk soil. Care must be taken, however, in the interpretation of results depending on the methodology employed to extract the soil solution. While centrifugation-based soil solution extraction techniques do not release significant amounts of organic acids from live roots and microbes (Zabowski, 1989), extraction techniques whereby soil is shaken with distilled water for extended periods (>10 min) inevitably lead to contamination from damaged microbial cells and plant residues.

Metal complexation reactions in the soil solution

Organic acids have the capacity to complex metals in solution. The degree of complexation, however, depends on the particular organic acid involved (number and proximity of carboxyl groups), the concentration and type of metal and the pH of the soil solution. Organic acids with only one carboxyl group (lactate, formate and acetate) have very little metal-complexing ability. From the stability constants shown in Table 4 it can be seen that malate, citrate and oxalate all have a high affinity for trivalent metals such as Al^{3+} and Fe^{3+} and it is indeed these metals which are mobilised (and immobilised) most readily by organic acids in most soils (Jones and Kochian, 1996; Pohlman and McColl, 1986). However, this does not always imply efficient complexation at all soil solution pHs (Mench and Martin, 1991). For example, using the chemical equilibria speciation program Geochem-PC (Parker et al., 1995), it can be predicted that the complexation of

Table 4. Stability constants of organic acid-metal complexes (Martell and Smith, 1976–1989). Where possible stability constants represent conditions at 25 °C and zero ionic strength. M:H:L ratio indicates the stoichiometry of the complex where m indicates metal, L indicates ligand and H indicates protons

Metal	M:L:H ratio	Malate	Citrate	oxalate
H^+	1:1	5.10	6.40	4.27
	2:1	3.46	4.76	1.25
	3:1	–	3.13	–
K^+	1:1	0.40	0.56	0.90
Mg^{2+}	1:1	1.55	4.84	3.43
	1:2	–	–	4.24
	1:1:1	0.77	2.59	–
Ca^{2+}	1:1	2.72	4.85	3.19
	1:1.$H_2O_{(s)}$	–	–	−8.80
	1:2	–	–	2.69
	1:1:1	1.39	2.93	1.38
Mn^{2+}	1:1	2.24	3.70	3.95
	1:2	–	–	4.40
Zn^{2+}	1:1	3.32	4.70	4.87
	1:2	5.90	7.65	
	1:1:1	2.00	2.96	1.72
Cu^{2+}	1:1	3.33	5.90	6.23
	1:2	8.40	–	4.00
	1:1:1	1.96	3.70	
	2:2	8.0	13.2	
Fe^{3+}	1:1	7.1	11.50	7.74
	1:2	–	–	13.64
	1:3	–	–	18.49
	2:2	12.85	–	–
Al^{3+}	1:1	6.00	7.87	6.1
	1:2	–	11.7	11.1
	1:3	–	–	15.12

Fe by malate, citrate and oxalate is highly dependent upon soil solution pH with little or no complexation at high soil pHs (Figure 5). Therefore, to establish a link between organic acid release and nutrient uptake by plant roots requires theoretical considerations like those undertaken by Cline et al. (1982). In addition, unlike citrate and malate, oxalate also has the tendency to precipitate in the presence of Ca^{2+}. While this will reduce its potential complexing ability with some nutrients it may be of importance in the release of P from Ca^{2+} containing minerals such as apatite.

P, Mn and Fe mobilisation

Phosphate dissolution rates can be greatly accelerated in soil in the presence of organic acids such as malate, citrate and oxalate leading to 10–1000-fold higher soil solution P concentrations depending on soil type and speciation and concentration of organic acid (Earl et al., 1979; Fox et al., 1990a,b, 1992; Gerke, 1994; Jones and Darrah, 1994b). Generally speaking the extraction efficiency of inorganic P by the organic acids appears to follow the series citrate > oxalate > malate > acetate, with P release dependent on the ability of the anion to complex Al (i.e. a log K_{al} greater than 3.5; Furrer and Stumm, 1986; Lan et al., 1995). However, organic acid-induced P release depends on many factors, including pH and soil mineralogy (Bolan et al., 1994; Fox et al., 1990a, b; Jones and Darrah, 1994b; Lan et al., 1995). There are at least two mechanisms by which P release can occur. The first involves direct ligand exchange, whereby citrate directly replaces P on ligand exchange surfaces (e.g., on crystalline $Al(OH)_3$ or $Fe(OH)_3$). The second could involve the complexation of metal ions in the solid which constitute the exchange matrix holding the P (e.g., Ca^{2+} in rock phosphate or Fe^{3+} in $Fe(OH)_3$). As metal ions are often released from soil concomitantly to P release it implies that the second pathway may be dominant although in reality both are probably operating simultaneously. However, the desorption/release of P is extremely soil dependent with generally high concentrations of organic acids (> 100 μM for citrate, >1 mM for oxalate, malate and tartrate) required to mobilise significant quantities of P into the soil solution (Earl et al., 1979; Jones and Darrah, 1994b; Lan et al., 1995; Lopez-Hernandez et al., 1986). In some soils, no P appears to be mobilised upon the addition of organic acids (Lan et al., 1995; Jones and Darrah, 1994b). Indeed, many studies of this kind have used unnaturally high concentrations of organic acids (> 1 mM) which is 100-fold higher that of a typical soil solution (10 μM; Table 2). While these experiments are significant in situations where soil solution concentrations of organic acids are high (e.g., in the proteoid rhizosphere of lupin) their importance in most other situations must remain speculative. In general, when the organic acid-mediated dissolution of P-containing minerals occurs, P release is also accelerated by a decrease in soil solution pH, a phenomenon that is often associated with roots experiencing P deficiency (Dinkelaker et al., 1989).

In addition to mobilising inorganic P, organic acids also appear to be able to efficiently mobilise P held in humic-metal complexes. The mechanisms involved in P release from humics, however, appear to be different from those associated with inorganic P (Fox et al., 1990a,b; Gerke, 1992, 1993, 1994; Lan et al., 1995). Gerke (1992) has shown that the P mobilised by citrate may persist in the soil solution for long periods (>10 weeks) although the extent to which this P is still plant available remains unknown.

Organic acids have also been implicated in the release of Mn in the rhizosphere (Godo and Reisenauer, 1980). Although the effect of Mn deficiency on root organic acid release has not been quantified, it is clear that organic acids such as malate and citrate can release Mn from synthetic MnO_2 through a combination of oxidation and complexation (Jauregui and Reisenauer, 1982). Mn dissolution is highly pH dependent with very little release above pH 5.0 and where the amount of Mn released is much smaller (if any) than the amounts of Fe, Al and other ions mobilised by organic acids in soil (Jones and Darrah, 1994b; Mench and Martin, 1991). More work is required with naturally occurring Mn-containing minerals to confirm the existence and importance of such a Mn-mobilising mechanism in soil.

Organic acids can also rapidly release Fe held in goethite and ferrihydrite, while mobilisation from Fe_2O_3 and Fe_3O_4 is extremely slow (Jones et al., 1996a). As occurs for Mn, dissolution has been shown to be extremely pH dependent with the Fe mobilisation potential reducing with increasing pH (Jones et al., 1996a). Dissolution also appears to depend on the amount of P associated with the ferric hydroxide surface and on the composition of the other ions in solution (Jones et al., 1996b). In general, however, soil extracts have indicated that between 1 and 40% of any citrate present in the soil solution of podzolic soils may be present as an Fe-citrate or Fe-malate complex (Jones et al., 1996a, b).

Organic acid sorption in soils

Due to the negative charge associated with their carboxyl groups, organic acids can become rapidly and readily sorbed to the soil's solid phase, a fact which is often ignored. Sorption appears to be largely dependent on soil type (as for P) but the general sorption trend is phosphate > oxalate > citrate > malate > sulfate > acetate (Earl et al., 1979; Jones and Brassington, 1998; Jones and Darrah, 1994b; Jones et al., 1996b). It is also probable that all these anions share similar sorption sites, as citrate, malate and oxalate are all capable of inducing P desorption or preventing the sorption of newly added P (Bolan et al., 1994; Earl et al., 1979; Jones and Darrah, 1994b; Nagarajah et al., 1970).

Studies with synthetic ferric and aluminium hydroxides have shown that organic acid sorption is highly pH dependent with increasing sorption with decreasing solution pH (Jones and Brassington, 1998; Karltun, 1998). Although the sorption of organic acids appears to be almost instantaneous, the reversibility (desorption) of these reactions remains unknown. The degree of sorption also appears to be controlled by the presence of cations in the soil solution and the anions associated with the soil's exchange surface (Jones and Brassington, 1998).

Concentration gradients of organic acids in the rhizosphere

Of critical importance to understanding rhizosphere processes is a knowledge of the organic acid concentrations in the rhizosphere. From computer simulation modelling and *in vitro* studies, it has been predicted that a steep gradient of di- and tricarboxylic acids exists in the rhizosphere with an effective sphere of influence in the rhizoplane of between 0.2 and 1.0 mm, depending on soil type, organic acid type and time (Darrah, 1991a; Jones et al., 1996a). The distance for non-sorbing compounds such as glucose and monocarboxylic acids such as acetate can, however, be much greater (>5 mm; Darrah, 1991b). Using mathematical models and anion adsorption isotherms, it can be predicted that most of the organic acids (>60%) will be rapidly adsorbed to the soil's exchange phase while the resultant concentration in the soil solution will be in the range of 1–50 μM depending on Al and P stress level (Jones et al., 1996a). This is similar to experimentally measured concentrations of organic acids extracted from rhizosphere soil (1–100 μM; Table 2). Calculation of organic acid concentrations at the surface of Al-resistant wheat root tips grown in hydroponic culture (i.e. concentration at the root surface in the unstirred layer) has indicated that malate concentrations are in the region of 10–100 μM (Pellet et al., 1997). Future soil metal mobilisation studies should reflect these low organic acid concentrations rather than high ones often favoured ($\geq$1 mM) in many experiments.

Organic acids in mineral dissolution and pedogenic processes

It is clear from the above discussion that di- and tricarboxylic acids can be potent metal complexers bringing about the dissolution of soil minerals and modulating the size of humic substances (Albuzio and Ferrari, 1989; Huang and Keller, 1972; Jones and Kochian, 1996; Pohlman and McColl, 1986; Ritchie, 1994). In general, organic acids appear to be able to induce a 2–4 fold increase in mineral dissolution rate in comparison with rainwater alone; however, this is highly dependent on mineral type, pH, Al content of the mineral and organic acid type (Jones and Kochian, 1996; Lundström and Öhman, 1990; Pohlman and McColl, 1986). Certainly the dissolution of Fe and Al oxyhydroxides can be greatly increased in the presence of organic acids (Jones and Kochian, 1996). With respect to silicate mineral weathering (e.g., feldspars), a more detailed review can be found in Drever and Stillings (1997). In this review they conclude that the impact of organic acids on weathering rate is of little significance as the typical concentrations of organic acids found in soil solutions are too low to have any appreciable affect.

In a leaching environment organic acid-metal complexes have the potential to migrate downwards through the soil leading to the redistribution of metals (e.g., Fe) within the soil profile (Lundström, 1993). Indeed, this may be one of the principal mechanisms (but not the only one) by which Fe and Al are redistributed in podzols (Anderson et al., 1982; Lundström, 1993). It has also been clearly demonstrated that microbes reduce the effectiveness of this metal dissolution and transportation, presumably through the mineralisation of the organic complexing ligands en route down the profile (Lundström, 1993, 1994; Lundström et al., 1995).

Table 5. Transport kinetics of organic acids by microorganisms. DW indicates dry weight

Species	Soil or culture	Organic acid	K_m (μM)	V_{max}[a]	Reference
Mixed population	soil	malate	1400	14 nmol kg^{-1} soil s^{-1}	Jones et al. (1996c)
Mixed population	soil	malate	1100	17 nmol kg^{-1} soil s^{-1}	Jones et al. (1996c)
Mixed bacteria	culture	malate	935	25 nmol g^{-1} protein s^{-1}	Jones et al. (1996c)
Mixed bacteria	culture	malate	955	4638 nmol g^{-1} protein s^{-1}	Jones et al. (1996c)
Rhizobium I110	culture	malate	6.7	208 nmol g^{-1} protein s^{-1}	SanFrancisco et al. (1985)
Rhizobium 3278	culture	malate	6.1	127 nmol g^{-1} protein s^{-1}	SanFrancisco et al. (1985)
Rhizobium 3278	culture	succinate	10.0	383 nmol g^{-1} protein s^{-1}	SanFrancisco et al. (1985)
Rhizobium I110	culture	succinate	7.5	375 nmol g^{-1} protein s^{-1}	SanFrancisco et al. (1985)
Rhizobium I110	culture	succinate	3.8	444 nmol g^{-1} protein s^{-1}	McAllister and Lepo (1983)
Rhizobium I217	culture	succinate	1.8	916 nmol g^{-1} protein s^{-1}	McAllister and Lepo (1983)
Lactobacillus plantarum	culture	malate	540	4166 nmol g^{-1} protein s^{-1}	Olsen et al. (1991)
Saccharomyces pombe	culture	malate	3700	666 nmol g^{-1} protein s^{-1}	Finan et al. (1981)
Rhodobacter capsulatus	culture	malate	2.9	722 nmol g^{-1} protein s^{-1}	Shaw and Kelly (1991)
Halobacterium distributum	culture	citrate	30	72 nmol g^{-1} protein s^{-1}	Tarasov et al. (1991)
Klebsiella pneumoniae	culture	citrate	7.2	136 nmol g^{-1} vesicles s^{-1}	Van der Rest et al. (1992)
Klebsiella pneumoniae	culture	citrate	12	83 nmol g^{-1} DW cells s^{-1}	Van der Rest et al. (1991)
Candida utilis	culture	malate	56	380 nmol g^{-1} DW cells s^{-1}	Cassio and Leao (1991)
Hansenula anomala	culture	malate	38	200 nmol g^{-1} DW cells s^{-1}	Côrte-Real and Leao (1990)
Hansenula anomala	culture	succinate	76	670 nmol g^{-1} DW cells s^{-1}	Côrte-Real and Leao (1990)
Candida sphaerica	culture	malate	100	439 nmol g^{-1} DW cells s^{-1}	Côrte-Real et al. (1989)
Esterichia coli	culture	succinate	14	333 nmol g^{-1} DW cells s^{-1}	Lo et al. (1972)
Bacillis subtilis	culture	citrate	2300	2416 nmol g^{-1} DW cells s^{-1}	Willecke and Pardee (1971)
Bacillis subtilis	culture	citrate	450	2416 nmol g^{-1} DW cells s^{-1}	Willecke et al. (1972)

[a] 1.7×10^9 cells = 0.4 mg protein.

Impact of the soil microbial biomass on organic acids

The impact of the soil microbial biomass on rhizosphere processes involving root exudates has largely been ignored. However, considering the vast number of microbial cells and the enormous activity and C utilisation capacity in the rhizosphere this view must not persist. This is especially true in view of recent work on the impact of microbes on phytometallophore efficiency (Crowley et al., 1992; Von Wiren et al., 1995). Many researchers have used the argument that organic acid release is often restricted to the root apical region which corresponds to a zone of reduced microbial activity. However, studies in which rhizosphere bacteria have been tracked using fluorochromes suggest that microbes rapidly colonise the root apex (Bowers et al., 1996; Mawdsley and Burns, 1994; Wiehe et al., 1994). Studies on the mineralisation of exudates have shown that organic acids such as citrate and malate added at realistic rhizosphere (10–100 μM) concentrations are rapidly degraded in non-rhizosphere (bulk) soil with an average half life of 2–3 h depending on soil type (Jones and Darrah, 1994; Jones et al., 1996c). Generally speaking, decomposition is much faster in organic surface horizons than in low organic content subsoils, while decomposition rates in rhizosphere soil are 2–3 fold faster than in bulk soil (DL Jones, data not presented). Microbial decomposition of organic acids in both soil and solution culture appears to conform to Michaelis–Menten kinetics with typically 60% of the organic acid mineralised to CO_2 and 40% incorporated into new cell biomass (Table 5; Jones and Darrah, 1994b; Jones et al., 1996c). In addition, mineralisation in soil does not appear to be affected by the presence of other root exudate components such as simple sugars and amino acids (Jones et al., 1996c). In bacterial cultures, it has been demonstrated that soil bacteria are capable of regulating the amount and type of transporters required for the uptake of organic acids into the cell based on their available C supply (Jones et al., 1996c). Generally culture studies have shown an up-regulation of transport activity in response to additions of malate. However, the extent to which

this could occur in soil remains unknown (Jones et al., 1996c; McAllister and Lepo, 1983). Kinetic and molecular studies of bacterial and yeast organic acid utilisation have shown that uptake occurs via metabolically driven transporter proteins which tend to be specific for either dicarboxylic or tricarboxylic acids (Bergsma and Konings, 1983; Finan et al., 1981; Jones et al., 1996c; SanFrancisco and Jacobson, 1985).

Many microorganisms appear to have the capacity to use organic acid metal complexes as well as 'free' non-complexed organic acids (Bergsma and Konings, 1983). In particular, Fe transport systems involving citrate are present in both prokaryotic and eukaryotic microorganisms (Fedorovich et al., 1989). This can involve either a direct uptake of Fe-citrate into the bacterial cells or a splitting of the complex at the cell surface using a reductase mechanism similar to that described for dicotyledonous plants above (Ecker and Emery, 1983; Fedorovich et al., 1989; Meyer, 1992; Figure 4). In some cases, Fe-citrate complexes can provide a more readily available source of Fe than some microbial siderophores (Francis and Dodge, 1993; Francis et al., 1992; Meyer, 1992). However, in other cases the formation of organic acid metal complexes appears to either inhibit the transport of organic acids into microbial cells or their subsequent metabolism once inside (Brynhildsen and Allard, 1994; Brynhildsen and Rosswall, 1989; Madsen and Alexander, 1985). Most of these studies, however, have been performed in culture media in which bacteria have become adapted to using non-complexed C sources. All the evidence available suggests that in soil, where microbes may be adapted to using organometallic complexes, this inhibition does not occur (Brynhildsen and Rosswall, 1997). This is also supported by experiments showing rapid organic acid mineralisation in soils where metal complexes are known to predominate (Jones and Darrah, 1994b; Jones et al., 1996c). Unlike other non-charged exudate components (e.g., glucose), the biodegradation of organic acids appears to be highly dependent on the amount and type of sorption to soil particles, with Al and Fe hydroxides providing the greatest protective effect (Boudot, 1992; Boudot et al., 1986, 1989; Jones and Edwards, 1998; Jones et al., 1996c). Indeed, almost no biodegradation of citrate occurs when citrate is held on the surface of Fe and Al hydroxides (Boudot, 1992; Jones and Edwards, 1998). Whether the inhibition of biodegradation by Fe/Al hydroxides represents chemical or physical protection of the substrate remains unknown.

The release of large amounts of organic acids into the rhizosphere can be expected to not only induce a growth of pre-existing rhizosphere bacteria, but also to act as chemoattractants inducing the movement (chemotaxis) of motile microbes such as flagellate bacteria and fungal hyphae towards the roots. Experiments with pure cultures of rhizosphere bacteria have shown concentrations of 10 μM are sufficient to induce a chemotactic response, similar to concentrations found in rhizosphere soil solutions (Barbour et al., 1991; Shen et al., 1996; Zheng and Sinclair, 1996; Table 2).

While microbes can consume root exudates, they are also responsible for the production of a wide range of organic acids especially in situations where nutrients may be limiting (Rózycki, 1985; Rózycki and Strzelczyk, 1986). The release of large amounts of oxalate and the presence of Ca^{2+}-oxalate crystals on the surface of fungal hyphae both in wood and soil is now well documented (Dutton and Evans, 1996; Takao, 1965). It has been speculated that oxalate is involved in a number of processes, including the acquisition of nutrients (e.g., P, Fe), free-radical formation, extracellular pH modification, calcium precipitation and subsequent pectin hydrolysis, and acid catalysis of hemicellulose/cellulose (Green and Highley, 1997; Micales, 1997). The microbial product, ketogluconic acid, has also been identified in the rhizosphere of wheat; it is capable of mobilising small amounts of soil P, albeit only at very high concentrations (10 mM; Moghimi et al., 1978).

Conclusions

Organic acids potentially perform a diverse range of functions in the rhizosphere. At present, most of these hypothesised processes appear to be beneficial to plants. However, future research needs to be directed towards gaining a more holistic understanding of the behaviour of organic acids in a contrasting range of plants and soil types. The reaction of organic acids can be expected to vary both spatially and temporally in the rhizosphere with the operation of many fluxes simultaneously. With respect to future research needs, firstly the physiological and molecular mechanism by which organic acids are released from plants needs to be understood, and secondly, the reactions of these organic acids in the soil need to be more fully investigated (i.e. sorption, complexation and mineralisation). In the past, most research has focused on organic

acid release from hydroponically grown plants out of which far-reaching conclusions have been drawn. We are now at the stage, however, where the pieces of the jigsaw puzzle are presented before us. We should now be aiming to place those pieces into some semblance of order through which the importance of these hypothesised organic acid-mediated processes can be determined and their ecological significance ascertained.

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References

Albuzio A and Ferrari G 1989 Modulation of the molecular-size of humic substances by organic acids of the root exudates. Plant Soil 113, 237–241.

Anderson H A, Berrow M L, Farmer V C, Hepburn A, Russell J D and Walker A D 1982 A reassessment of podzol formation processes. J. Soil Sci. 33, 125–136.

Baziramakenga R, Simard R R and Leroux G D 1995 Determination of organic acids in soil extracts by ion chromatography. Soil Biol. Biochem. 27, 349–356.

Barbour W M, Hattermann D R and Stacey G 1991 Chemotaxis of *Bradyrhizobium japonicum* to soybean exudates. Appl. Environ. Microbiol. 57, 2635–2639.

Bergsma J and Konings W N 1983 The properties of citrate transport in membrane-vesicles from *Bacillus subtilis*. Eur. J. Biochem. 134, 151–156.

Bolan N S, Naidu R, Mahimairaja S and Baskaran S 1994 Influence of low-molecular-weight organic-acids on the solubilization of phosphates. Biol. Fert. Soils 18, 311–319.

Boudot J P 1992 Relative efficiency of complexed aluminum, non-crystalline Al hydroxide, allophane and imogolite in retarding the biodegradation of citric acid. Geoderma 52, 29–39.

Boudot J P, Belhadj B A and Chone T 1986 Carbon mineralization in andosols and aluminum-rich highland soils. Soil Biol. Biochem. 18, 457–461.

Boudot J P, Brahim A B H, Steiman R and Seiglemurandi F 1989 Biodegradation of synthetic organo-metallic complexes of iron and aluminum with selected metal to carbon ratios. Soil Biol. Biochem. 21, 961–966.

Bowers J H, Nameth S T, Riedel R M and Rowe R C 1996 Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. Phytopathol. 86, 614–621.

Brynhildsen L and Rosswall T 1997 Effects of metals on the microbial mineralization of organic acids. Water Air Soil Pollut. 94, 45–57.

Brynhildsen L and Allard B 1994 Influence of metal complexation on the metabolism of citrate by *Klebsiella-oxytoca*. Biometals 7, 163–169.

Brynhildsen L and Rosswall T 1989 Effects of cadmium, copper, magnesium, and zinc on the decomposition of citrate by a *Klebsiella* sp. Appl. Environ. Microbiol. 55, 1375–1379.

Cakmak I and Marschner H 1988 Increase in membrane-permeability and exudation in roots of zinc deficient plants. J. Plant Physiol. 132, 356–361.

Cambraia J, Galvani F R, Estevao MM, Santanna R 1983 Effects of aluminum on organic-acid, sugar and amino-acid-composition of the root-system of sorghum (*Sorghum bicolor* L *moench*). J. Plant Nutr. 6, 313–322.

Cassio F and Leao C 1991 Low-affinity and high-affinity transport-systems for citric acid in the yeast *Candida utilis*. Appl. Environ. Microbiol. 57, 3623–3628.

Chairidchai P and Ritchie G S P 1993a The effect of citrate and pH on zinc uptake by wheat. Agron. J. 85, 322–328.

Chairidchai P and Ritchie G S P 1993b Zinc adsorption by sterilized and nonsterilized soil in the presence of citrate and catechol. Comm. Soil Sci. Plant Anal. 24, 261–275.

Chang K J and Roberts J K M 1989 Observation of cytoplasmic and vacuolar malate in maize root-tips by ^{13}C-NMR spectroscopy. Plant Physiol. 89, 97–203.

Chang K J and Roberts J K M 1991 Cytoplasmic malate levels in maize root-tips during K^+ ion uptake determined by ^{13}C-NMR spectroscopy. Biochim. Biophys. Acta 1092, 29–34.

Cline G R, Powell P E, Szaniszlo P J and Reid C P P 1982 Comparison of the abilities of hydroxamic, synthetic, and other natural organic acids to chelate iron and other ions in nutrient solution. Soil Sci. Soc. Am. J. 46, 1158–1164.

Côrte-Real M and Leao C 1990 Transport of malic-acid and other dicarboxylic-acids in the yeast *Hansenula anomala*. Appl. Environ. Microbiol. 56, 1109–1113.

Côrte-Real M, Leao C and VanUden N 1989 Transport of L-malic acid and other dicarboxylic-acids in the yeast *Candida sphaerica*. Appl. Microbiol. Biotechnol. 31, 551–555.

Crowley D E, Römheld V, Marschner and Szaniszlo P J 1992 Root-microbial effects on plant iron uptake from siderophores and phytosiderophores. Plant Soil 142, 1–7.

Curl E A and Trueglove B 1986 The Rhizosphere. Springer-Verlag, Berlin.

Darrah P R 1991a Measuring the diffusion-coefficient of rhizosphere exudates in 2. The diffusion of sorbing compounds. J. Soil Sci. 42, 421–434.

Darrah P R 1991b Measuring the diffusion-coefficient of rhizosphere exudates in soil. 1. The diffusion of non-sorbing compounds. J. Soil. Sci. 42, 413–420.

Delhaize E, Ryan P R and Randall P J 1993 Aluminum tolerance in wheat (*Triticum aestivum* L.) 2. Aluminum stimulated excretion of malic acid from root apices. Plant Physiol. 103, 695–702.

Dennis D T, Turpin D H, Lefebvre D D and Layzell D B 1997 Plant Metabolism. Longman Group, Essex.

De Vos CR, Lubberding HJ and Bienfait HF 1986 Rhizosphere acidification as a response to iron-deficiency in bean plants. Plant Physiol. 81, 842–846.

Dinkelaker B, Hengeler C and Marschner H 1995 Distribution and function of proteoid roots and other root clusters. Bot. Acta 108, 183–200.

Dinkelaker B, Römheld V and Marschner H 1989 Citric-acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ. 12, 285–292.

Drever J I and Stillings L L 1997 The role of organic acids in mineral weathering. Colloids Surfaces A: Physicochem. Eng. Aspects 120, 167–181.

Dutton M V and Evans C S 1996 Oxalate production by fungi – its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol. 42, 881–895.

Earl K D, Syers J K and McLaughlin J R 1979 Origin of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. Soil Sci. Soc. Am. J. 43, 674–678.

Ecker D J and Emery T 1983 Iron uptake from ferrichrome A and iron citrate in *Ustilago sphaerogena*. J. Bacteriol. 155, 616–622.

Elkhatib E A 1990 Simultaneous determination of low molecular-weight organic-acids in soil solution by ion chromatography. Z. Pflanzenernähr. Bodenk. 153, 201–205.

Evans A 1991 Influence of low-molecular-weight organic-acids on zinc distribution within micronutrient pools and zinc uptake by wheat. J. Plant Nutr. 14, 1307–1318.

Fan T W M, Lane A N, Pedler J, Crowley D and Higashi R M 1997 Comprehensive analysis of organic ligands in whole root exudates using nuclear magnetic resonance and gas chromatography mass spectrometry. Anal. Biochem. 251, 57–68.

Fedorovich D V, Shavlovskii G M, Dedyshin E E 1989 System of iron transport with participation of citrate in *Pichia guilliermondii*. Microbiology 58, 289–294.

Finan T M, Wood J M and Jordan D C 1981 Succinate transport in *Rhizobium leguminosarum*. J. Bacteriol. 148, 193–202.

Fox T R and Comerford N B 1990 Low-molecular-weight organic-acids in selected forest soils of the Southeastern USA. Soil Sci Soc. Am. J. 54, 1139–1144.

Fox T R and Comerford N B 1992 Influence of oxalate loading on phosphorus and aluminum solubility in spodosols. Soil Sci. Soc. Am. J. 56, 290–294.

Fox T R, Comerford N B and McFee W W 1990a Phosphorus and aluminum release from a spodic horizon mediated by organic acids. Soil Sci. Soc. Am. J. 54, 1763–1767.

Fox T R, Comerford NB and McFee W W 1990b Kinetics of phosphorus release from spodosols – effects of oxalate and formate. Soil Sci. Soc. Am. J. 54, 1441–1447.

Fox T C Shaff J E, Grusak M A, Norvell W A, Chen Y, Chaney R L and Kochian L V 1996 Direct measurement of Fe^{59}-labeled Fe^{2+} influx in roots of pea using a chelator buffer system to control free Fe^{2+} in solution. Plant Physiol. 111, 93–100.

Foy C D, Lee E H and Wilding S B 1897 Differential aluminum tolerances of two barley cultivars related to organic acids in their roots. J. Plant Nutr. 10, 1089–1101.

Francis A J and Dodge C J 1993 Influence of complex structure on the biodegradation of iron-citrate complexes. Appl. Environ. Microbiol. 59, 109–113.

Francis A J, Dodge C J and Gillow J B 1992 Biodegradation of metal citrate complexes and implications for toxic-metal mobility. Nature 356, 140–142.

Furrer G and Stumm W 1986 The coordination chemistry of weathering. 1. Dissolution kinetics of ∂Al_2O_3 and BeO. Geochim. Cosmochim. Acta 50, 1847–1860.

Gardner W K, Barber D A and Parberry D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant Soil 70, 107–114.

Gerke J 1992 Phosphate, aluminum and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Z. Pflanzenernähr. Bodenk. 155, 339–343.

Gerke J 1993 Solubilization of Fe(III) from humic-Fe complexes, humic Fe oxide mixtures and from poorly ordered Fe-oxide by organic acids: consequences for P-adsorption. Z. Pflanzenernähr. Bodenk. 156, 253–257.

Gerke J 1994 Kinetics of soil phosphate desorption as affected by citric acid. Z. Pflanzenernähr. Bodenk. 157, 17–22.

Godo G H and Reisenauer H M 1980 Plant effects on soil manganese availability. Soil Sci. Soc. Am. J. 44, 993–995.

Gout E, Bligny R, Pascal N and Douce R 1993 ^{13}C nuclear-magnetic-resonance studies of malate and citrate synthesis and compartmentation in higher-plant cells. J. Biol. Chem. 268, 3986–3992.

Green F and Highley T L 1997 Mechanism of brown-rot decay: Paradigm or paradox. Int. Biodeterioration Biodeg. 39, 113–124.

Grierson P F 1992 Organic-acids in the rhizosphere of *Banksia integrifolia* L. Plant Soil 144, 259–265.

Guerinot M L and Yi Y 1994 Iron: nutritious, noxious, and not readily available. Plant Physiol. 104, 815–820.

Hoffland E 1992 Quantitative evaluation of the role of organic-acid exudation in the mobilization of rock phosphate by rape. Plant Soil 140, 279–289.

Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. 2. Local root exudation of organic acids as a response to P starvation. Plant Soil 113, 161–165.

Hoffland E, Van den Boogaard R, Nelemans J and Findenegg G 1992 Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. New Phytol. 122, 675–680.

Huang W H and Keller W D 1972 Organic acids as agents of chemical weathering of silicate minerals. Nature 239, 149–151.

Hue N V, Craddock G R and Adams F 1986 Effect of organic-acids on aluminum toxicity in subsoils. Soil Sci. Soc. Am. J. 50, 28–34.

Imas P, BarYosef B, Kafkafi U and Ganmore-Neumann R 1997a Phosphate induced carboxylate and proton release by tomato roots. Plant Soil 191, 35–39.

Imas P, BarYosef B, Kafkafi U and Ganmore-Neumann R 1997b Release of carboxylic anions and protons by tomato roots in response to ammonium nitrate ratio and pH in nutrient solution. Plant Soil 191, 27–34.

Ismail I, DeBellis L, Alpi A and Smith S M 1997 Expression of glyoxylate cycle genes in cucumber roots responds to sugar supply and can be activated by shading or defoliation of the shoot. Plant Mol. Biol. 35, 633–640.

Jauregui M A and Reisenauer H M 1982 Dissolution of oxides of manganese and iron by root exudate components. Soil Sci. Soc. Am. J. 46, 314–317.

Johnson J F, Allan D L and Vance C P 1994 Phosphorus stress induced proteoid roots show altered metabolism in *Lupinus albus*. Plant Physiol. 104, 657–665.

Johnson J F, Allan D L, Vance C P and Weiblen G 1996a Root carbon dioxide fixation by phosphorus-deficient *Lupinus albus*: Contribution to organic-acid exudation by proteoid roots. Plant Physiol. 112, 19–30.

Johnson J F, Vance C P and Allan D L 1996b Phosphorus deficiency in *Lupinus albus*: altered lateral root development and enhanced expression of phosphoenolpyruvate carboxylase. Plant Physiol. 112, 31–41.

Jones D L and Brassington D S 1998 Sorption of organic acids in acid soils and its implications in the rhizosphere. Eur. J. Soil Sci. 49, 447–455.

Jones D L and Darrah P R 1994a Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. Plant Soil 163, 1–12.

Jones D L and Darrah P R 1994b Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. Plant Soil 166, 247–257.

Jones D L and Darrah P R 1995 Influx and efflux of organic-acids across the soil-root interface of *Zea mays* L. and its implications in rhizosphere C flow. Plant Soil 173, 103–109.

Jones D L and Darrah P R 1996 Re-sorption of organic-compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. 3. Characteristics of sugar influx and efflux. Plant Soil 178, 153–160.

Jones D L and Edwards A C 1998 Influence of sorption on the biological utilization of two simple carbon substrates. Soil Biol. Biochem. (*In press*).

Jones D L and Kochian L V 1996 Aluminum-organic acid interactions in acid soils. 1. Effect of root-derived organic-acids on the kinetics of Al dissolution. Plant Soil 182, 221–228.

Jones D L, Shaff J E and Kochian L V 1995 Role of calcium and other ions in directing root hair tip growth in *Limnobium stoloniferum*. 1. Inhibition of tip growth by aluminum. Planta 197, 672–680.

Jones D L, Darrah P R and Kochian L V 1996a Critical-evaluation of organic-acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant Soil 180, 57–66.

Jones D L, Prabowo A M and Kochian L V 1996b Aluminum-organic acid interactions in acid soils. 2. Influence of solid-phase sorption on organic acid-Al complexation and Al rhizotoxicity. Plant Soil 182, 229–237.

Jones D L, Prabowo A M and Kochian L V 1996c Kinetics of malate transport and decomposition in acid soils and isolated bacterial-populations – the effect of microorganisms on root exudation of malate under Al stress. Plant Soil 182, 239–247.

Karltun E 1998 Modelling SO_4^{2-} surface complexation on variable charge minerals. II. Competition between SO_4^{2-}, oxalate and fulvate. Eur. J. Soil Sci. 49, 113–120.

Keerthisinghe G, Hocking PJ, Ryan P R and Delhaize E 1998 Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (*Lupinus albus* L.) Plant Cell Environ. 21. (*In press*).

Klein D A, Frederick B A, Biondini M and Trlica M J 1988 Rhizosphere microorganism effects on soluble amino acids, sugars and organic-acids in the root zone of *Agropyron cristatum, Agropyron smithii* and *Bouteloua gracilis*. Plant Soil 110, 19–25.

Kochian L V 1995 Cellular mechanisms of aluminum toxicity and resistance in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46, 237–260.

Kochian L V and Jones D L 1997 Aluminum toxicity and resistance in plants. *In* Research Issues in Aluminum Toxicity. Eds. R A Yokel and M S Golub. Taylor and Francis Publishers, Bristol, PA.

Kraffczyk I, Trolldenier G and Beringer H 1984 Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. Soil Biol. Biochem. 16, 315–322.

Krzyszowska A J, Blaylock M J, Vance G F and David M B 1996 Ion-chromatographic analysis of low molecular weight organic acids in spodosol forest floor solutions. Soil Sci. Soc. Am. J. 60, 1565–1571.

Laheurte F and Berthelin J 1988 Effect of a phosphate solubilising bacteria on maize growth and root exudation over four levels of labile phosphorus. Plant Soil 105, 11–17.

Lan M, Comerford N B and Fox T R 1995 Organic anions effect on phosphorus release from spodic horizons. Soil Sci. Soc. Am. J. 59, 1745–1749.

Landsberg E C 1981 Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3, 579–591.

Lipton D S, Blanchar R W and Blevins D G 1987 Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315–317.

Lo T C Y, Rayman M K and Sanwal B D 1972 Transport of succinate in *E. coli*. I. Biochemical and genetic studies of transport in whole cells. J. Biol. Chem. 247, 6323–6331.

Lopez-Hernandez D, Siegert G and Rodriguez J V 1986 Competitive adsorption of phosphate with malate and oxalate by tropical soils. Soil Sci. Soc. Am. J. 50, 1460–1462.

Lundström U S 1993 The role of organic-acids in the soil solution chemistry of a podzolized soil. J. Soil Sci. 44, 121–133.

Lundström U S 1994 Significance of organic-acids for weathering and the podzolization process. Environ. Int. 20, 21–30.

Lundström U S and Öhman L O 1990 Dissolution of feldspars in the presence of natural, organic solutes. J. Soil Sci. 41, 359–369.

Lundström U S, Van Breemen N and Jongmans A G 1995 Evidence for microbial decomposition of organic acids during podzolization. Eur. J. Soil Sci. 46, 489–496.

Lüttge U and Smith J A C 1984 Mechanism of passive malic-acid efflux from vacuoles of the CAM plant *Kalanchoe daigremontiana.* J. Memb. Biol. 81, 149–158.

Ma J F, Hiradate S, Nomoto K, Iwashita T and Matsumoto H 1997 Internal detoxification mechanism of Al in *Hydrangea* – Identification of Al form in the leaves. Plant Physiol. 113, 1033–1039.

Madsen E L and Alexander M 1985 Effects of chemical speciation on the mineralization of organic compounds by microorganisms. Appl. Environ. Microbiol. 50, 342–349.

Marschner H 1995 Mineral Nutrition of Higher Plants. Academic Press, London.

Martell A E and Smith R M 1976–1989. Critical Stability Constants, 6 vol. Plenum Press, New York.

Matsumoto H, Okada K and Takahashi E 1979 Excretion products of maize roots from seedling to seed development stage. Plant Soil 53, 17–26.

Mawdsley J L, Burns R G 1994 Root colonization by a *Flavobacterium* species and the influence of percolating water. Soil Biol. Biochem. 26, 861–870.

McAllister C F and Lepo J E 1983 Succinate transport by free-living forms of *Rhizobium japonicum*. J. Bacteriol. 153, 1155–1162.

Mench M and Martin E 1991 Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L, *Nicotiana tabacum* L and *Nicotiana rustica* L. Plant Soil 132, 187–196.

Meyer J M 1992 Exogenous siderophore-mediated iron uptake in *Pseudomonas aeruginosa:* possible involvement of porin OprF in iron translocation. J. Gen. Microbiol. 138, 951–958.

Micales J A 1997 Localization and induction of oxalate decarboxylase in the brown-rot wood decay fungus *Postia placenta*. Int. Biodeterioration Biodegrad. 39, 125–132.

Millet M, Wortham H, Sanusi A and Mirabel P 1997 Low molecular weight organic acids in fogwater in an urban area: Strasbourg (France). Sci. Tot. Environ. 206, 57–65.

Miyasaka S C, Buta J G, Howell R K and Foy C D 1991 Mechanism of aluminum tolerance in snapbeans: root exudation of citric acid. Plant Physiol. 96, 737–743.

Moghimi A, Tate M E and Oades J M 1978 Characterization of rhizosphere products especially 2-ketogluconic acid. Soil Biol. Biochem. 10, 283–287.

Nagarajah S, Posner A M and Quirk J P 1970 Competitive adsorptions of phosphate with polygalacturonate and other anions on kaolinite and oxide surfaces. Nature 228, 83–84.

Nobel P S 1991 Physiochemical and Environmental Plant Physiology. Academic Press, London.

Ohwaki Y and Sugahara K 1997 Active extrusion of protons and exudation of carboxylic acids in response to iron deficiency by roots of chickpea (*Cicer arietinum* L.).Plant Soil 189, 49–55.

Olsen E B, Russell J B and Henick-Kling T 1991 Electrogenic L-malate transport by *Lactobacillus plantarum:* a basis for energy derivation from malolactic fermentation. J. Bacteriol. 173, 6199–6206.

Olsson S, Bååth E and Söderström B 1987 Growth of *Verticillium dahliae* Kleb. hyphae and of bacteria along the roots of rape (*Brassica-napus* L.) seedlings. Can. J. Microbiol. 33, 916–919.

Osmond C B 1976 Ion absorption and carbon metabolism in cells of higher plants. *In* Encyclopedia of Plant Physiology, New Series, Vol. 2, Part A. Eds. U Lüttge and M G Pitman. pp 347–372. Springer-Verlag, Berlin.

Osmond C B and Laties G G 1969 Compartmentation of malate in relation to ion absorption in beet. Plant Physiol. 44, 7–14.

Osothsilp C and Subden R E 1986 Malate transport in S*chizosaccharomyces pombe*. J. Bacteriol.168, 1439–1443.

Papernik L A and Kochian L V 1997 Possible involvement of Al-induced electrical signals in Al tolerance in wheat. Plant Physiol. 115, 657–667.

Parker D R, Chaney R L and Norvell W A 1995 Chemical equilibria models: Applications to plant research. *In* Chemical Equilibria and Reaction Models, Special Publication 42. Eds. R H Loeppert, A P Schwab and S Goldberg. pp 163–200. SSSA-ASA, Madison, WI.

Patel D D, Barlow P W and Lee R B 1990 Development of vacuolar volume in the root-tips of pea. Ann. Bot. 65, 159–169.

Pellet D M, Grunes D L and Kochian L V 1995 Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). Planta 196, 788–795.

Pellet D M, Papernik L A, Jones D L, Darrah P R, Grunes D L and Kochian LV 1997 Involvement of multiple aluminium exclusion mechanisms in aluminium tolerance in wheat. Plant Soil 192, 63–68.

Pohlman A A and McColl J G 1986 Kinetics of metal dissolution from forest soils by soluble organic acids. J. Environ. Qual. 15, 86–92.

Pohlman A A and McColl J G 1988 Soluble organics from forest litter and their role in metal dissolution. Soil Sci. Soc. Am. J. 52, 265–271.

Rabotti G and Zocchi G 1994 Plasma membrane-bound H^+-ATPase and reductase activities in Fe-deficient cucumber roots. Physiol. Plant. 90, 779–785.

Ratnayake M, Leonard R T and Menge J A 1978 Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal infection. New Phytol. 81, 543–552.

Ricard B, Couee I, Raymond P, Saglio P H, Saintges V and Pradet A 1994 Plant-metabolism under hypoxia and anoxia. Plant Physiol. Biochem. 32, 1–10

Ritchie G S P 1994 Role of dissolution and precipitation of minerals in controlling soluble aluminum in acidic soils. Adv. Agron. 53, 47–83.

Rivoal J and Hanson A D 1993 Evidence for a large and sustained glycolytic flux to lactate in anoxic roots of some members of the halophytic genus *Limonium*. Plant Physiol. 101, 553–560.

Rózycki H 1985 Production of organic acids by bacteria isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Acta Microbiol. Polon. 34, 301–308.

Rózycki H and Strzelczyk E 1986 Organic acids production by *Streptomyces* spp isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant Soil 96, 337–345.

Ryan P R, Delhaize E and Randall P J 1995a Malate efflux from root apices and tolerance to aluminum are highly correlated in wheat. Aust. J. Plant Physiol. 122, 531–536.

Ryan P R, Delhaize E and Randall P J 1995b Characterization of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots. Planta 196, 103–110.

Ryan P R, Skerrett M, Findlay G P, Delhaize E and Tyerman S D 1997 Aluminum activates an anion channel in the apical cells of wheat roots. Proc. Natl. Acad. Sci. Am. 94, 6547–6552.

Samuels A L, Fernando M and Glass A D M 1992 Immunofluorescent localization of plasma membrane H^+-ATPase in barley roots and effects of K nutrition. Plant Physiol. 99, 1509–1514.

SanFrancisco M J D and Jacobson G R 1985 Uptake of succinate and malate in cultured-cells and bacteroids of two slow-growing species of *Rhizobium*. J. Gen. Microbiol. 131, 765–773.

Schwab S M, Menge J A and Leonard R T 1983 Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. Plant Physiol. 73, 761–765.

Shaw J G and Kelly D J 1991 Binding protein dependent transport of C4 dicarboxylates in *Rhodobacter capsulatus*. Arch. Microbiol. 155, 466–472.

Shen Y, Ström L, Jonsson J A and Tyler G 1996 Low-molecular organic-acids in the rhizosphere soil solution of beech forest (*Fagus sylvatica* L.) cambisols determined by ion chromatography using supported liquid membrane enrichment technique. Soil Biol. Biochem. 28, 1163–1169.

Smucker A J M and Erickson A E 1987 Anaerobic stimulation of root exudates and disease of peas. Plant Soil 99, 423–433.

Ström L 1997 Root exudation of organic acids: importance to nutrient availability and the calcifuge and calcicole behaviour of plants. Oikos 80, 459–466.

Ström L, Olsson T and Tyler G 1994 Differences between calcifuge and acidifuge plants in root exudation of low molecular organic-acids. Plant Soil 167, 239–245.

Stumpf D K and Burris R H 1981 Organic-acid contents of soybean: Age and source of nitrogen. Plant Physiol. 68, 989–991.

Takao S 1965 Organic acid production by basidiomycetes I. Screening of organic acid producing strains. Appl. Microbiol. 13, 732–737.

Tarasov A L, Zvyagintseva I S and Plakunov V K 1991 Citrate transport in *Halobacterium distributum* cells. Microbiology 60, 19–23.

Tourdot-Marechal R, Cavin J F, Dricicachon Z and Divies C 1993 Transport of malic-acid in *Leuconostoc oenos* strains defective in malolactic fermentation: A model to evaluate the kinetic-parameters. Appl. Microbiol. Biotech. 39, 499–505.

Tyler G and Ström L 1995 Differing organic-acid exudation pattern explains calcifuge and acidifuge behavior of plants. Ann. Bot. 75, 75–78.

Van der Rest M E, Abee T, Molenaar D and Konings W N 1991 Mechanism and energetics of a citrate-transport system of K*lebsiella pneumoniae*. Eur. J. Biochem. 195, 71–77.

Van der Rest M E, Molenaar D and Konings W N 1992 Mechanism of Na^+-dependent citrate transport in *Klebsiella pneumoniae*. J. Bacteriol. 174, 4893–4898.

Van Hees P A W, Andersson A M T and Lundström U S 1996 Separation of organic low-molecular-weight aluminum complexes in soil solution by liquid-chromatography. Chemosphere 33, 1951-1966.

Von Wiren N, Romheld V, Shioiri T and Marschner H 1995 Competition between microorganisms and roots of barley and sorghum for iron accumulated in the root apoplasm. New Phytol. 130, 511–521.

Welch R M, Norvell W A, Schaefer S C, Shaff J E and Kochian L V 1993 Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status – does the root-cell

plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake ? Planta 190, 555–561.

Wiehe W, Hechtbuchholz C and Hoflich G 1994 Electron-microscopic investigations on root colonization of *Lupinus albus* and *Pisum sativum* with two associative plant-growth promoting rhizobacteria, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* bv *trifolii*. Symbiosis 17, 15–31.

Willecke K and Pardee A B 1971 Inducible citrate transport in the gram positive bacterium *Bacillus subtilis*. J. Biol. Chem. 246, 1032–1040.

Willecke K, Fries E M and Oehr P 1972 Coupled transport of citrate and Mg in *Bacillus subtilis*. J. Biol. Chem. 248, 807–811.

Xia J H and Roberts J K M 1994 Improved cytoplasmic pH regulation, increased lactate efflux, and reduced cytoplasmic lactate levels are biochemical traits expressed in root-tips of whole maize seedlings acclimated to a low-oxygen environment. Plant Physiol. 105, 651–657.

Xia J H and Saglio P H 1992 Lactic acid efflux as a mechanism of hypoxic acclimation of maize root tips to anoxia. Plant Physiol. 100, 40–46.

Zabowski D 1989 Limited release of soluble organics from roots during the centrifugal extraction of soil solutions. Soil Sci. Soc. Am. J. 53, 977–979.

Zheng X Y and Sinclair J B 1996 Chemotactic response of *Bacillus megaterium* strain B153-2-2 to soybean root and seed exudates. Physiol. Mol. Plant Path. 48, 21–35.

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