Interactions between rhizosphere microorganisms and plants governing iron and phosphorus availability

Petra Marschner[A], David Crowley[B] and Zed Rengel[C]

[A]Soils, School of Agriculture, Food and Wine, Faculty of Sciences, University of Adelaide, Adelaide, Australia, Email petra.marschner@adelaide.edu.au
[B]Department of Environmental Sciences, University of California, Riverside, USA
[C]Soil Science and Plant Nutrition, School of Earth and Geographical Science, The University of Western Australia, Australia

Abstract
Because Fe availability is low in most aerobic soil, microorganisms and plants release low molecular-weight compounds (chelators) which increase Fe availability. Microorganisms appear to be far more competitive than plants: they can utilise Fe bound to plant-derived chelators and decompose them, whereas microbial chelators are poor Fe sources for plants. However, some plants, such as grasses, grow well in Fe-deficient soils, which may be explained by the spatially and temporarily concentrated release of phytosiderophores. Plants and microorganisms have developed a number of strategies to increase soil P availability. Microorganisms can increase plant P uptake by mobilising more P than they require and by stimulating root growth and mycorrhizal colonisation. However, microorganisms may also decrease P availability by (i) net P immobilisation in their biomass, (ii) decomposition of P-mobilising root exudates and (iii) decreasing root growth or mycorrhizal colonisation.
Depending on the availability of carbon, the microbial biomass can influence Fe and P availability to plants by acting as either a source or a sink. We propose the following hypothesis: at high availability of carbon such as in the zone immediately behind the root tip, Fe and P immobilisation dominates, whereas in the mature root zones with decreased C availability, mineralisation is dominant. While net Fe and P immobilisation behind the root tip is likely to directly decrease plant uptake, net mineralisation along the mature root zones that have a low capacity for nutrient uptake may have a relatively small effect on plant uptake.

Key Words
Carbon availability, competition, microbial biomass, mobilisation, phytosiderophores, siderophores

Interactions between microorganisms and plants for iron
The total Fe content in soil is relatively high, but its availability to organisms is low in aerated soils because the prevalent form (Fe^{3+}) is poorly soluble. Plants and microorganisms have developed mechanisms to increase Fe uptake (Marschner 1995). In plants, there are two different strategies in response to Fe deficiency. Strategy I plants (dicots and non-graminaceous monocots) release organic acid anions which chelate Fe. Iron solubility is also increased by decreasing the rhizosphere pH, and Fe uptake is enhanced by an increased reducing capacity of the roots ($Fe^{3+} \rightarrow Fe^{2+}$). Strategy II plants (*Poaceae*) release phytosiderophores that chelate Fe^{3+}. Iron is taken up in the chelated form as Fe-phytosiderophore (Roemheld 1991; Von Wiren *et al.*, 1993). Phytosiderophores are released only for a few hours per day at the root tip (Roemheld, 1991).
Under Fe deficiency stress, microorganisms release organic acid anions or siderophores that chelate Fe^{3+}. After movement of the ferrated chelate to the cell surface, Fe^{3+} is reduced either outside or within the cell (Neilands, 1984). Microorganisms produce a range of siderophores, e.g. ferrichromes by fungi, and enterobactin, pyoverdine and ferrioxamines by bacteria.
Bacterial siderophores are usually poor Fe sources for both monocot and dicot plants (Bar-Ness *et al.*, 1992; Crowley *et al.*, 1992; Walter *et al.*, 1994). However, in some cases microbial siderophores have alleviated Fe deficiency-induced chlorosis in dicots (Jurkevitch *et al.*, 1988; Sharma *et al.*, 2003; Wang *et al.*, 1993; Yehuda *et al.*, 2000). On the other hand, plant-derived Fe-phytosiderophore complexes appear to be a good Fe source for bacteria (Jurkevitch *et al.*, 1993; Marschner and Crowley, 1998).
The interactions between different Fe chelators depend on the affinity of the chelators towards Fe and their relative concentrations. Compared to phytosiderophores, bacterial siderophores such as pyoverdine have a higher affinity towards Fe (Yehuda *et al.*, 1996). If siderophores and phytosiderophores are present at similar concentrations, Fe is preferentially bound to the siderophores, which may even remove Fe from the Fe-phytosiderophore complex. In contrast to many bacterial siderophores, rhizoferrin from the fungus *Rhizopus arrhizus* has only a slightly higher affinity towards Fe compared to phytosiderophores. Rhizoferrin is a good

Fe source for barley, probably because of exchange of Fe from rhizoferrin to the phytosiderophore (Yehuda *et al*., 1996).

Thus, microorganisms appear to be highly competitive for Fe compared to plant roots. However, not only the affinity of the chelators towards Fe, but also their relative concentration is important (Yehuda *et al*., 1996). The diurnal rhythm of phytosiderophore release by grasses results in a high concentration of phytosiderophore at the root tips at certain times of the day (Crowley and Gries, 1994). Under these conditions, phytosiderophores may be efficient Fe chelators that could even remove Fe from bacterial siderophores, particularly because the density of microorganisms at the root tip is low. Even if a proportion of the phytosiderophores is decomposed by microorganisms, the concentration remaining is likely to be sufficient to mobilise adequate amounts of Fe.

Interactions between microorganisms and plants for phosphorus

Although the total amount of P in the soil may be high, it is mainly present in forms that are unavailable to plants and microorganisms. Under P deficiency, plants may increase the soil volume exploited by increasing root growth and root hair length, or decreasing root diameter (Föhse and Jungk, 1983). Plants and microorganisms can increase the solubility of inorganic P by releasing protons, OH^- or CO_2, and organic acid anions such as citrate, malate and oxalate; and they can mineralise organic P by release of various phosphatase enzymes. The effectiveness of these mechanisms may depend on soil type and/or P forms present in the soil. For example, a given citric acid concentration mobilized more P and had a more persistent effect in an oxisol than in a luvisol (Gerke, 1992).

Rhizosphere microorganisms can increase or decrease the availability of P to plants (Marschner, 2009). Rhizosphere microorganisms increase P uptake by solubilising or mineralising more P than they require and by stimulating root growth. They can also indirectly enhance plant P uptake by releasing plant growth regulators that stimulate root or root hair growth or mycorrhizal colonisation.

A large number of microorganisms with high P solublisation *in vitro* have been isolated (Banik and Dey, 1983; Whitelaw *et al*., 1999). Inoculation with such P-solubilising microorganisms can either lead to increased P uptake and plant growth (Gerretsen, 1948; Kumar and Narula, 1999; Kundu and Gaur, 1980) or be ineffective (Azcon-Aguilar *et al*., 1986; Badr el-Din *et al*., 1986). Mycorrhizal fungi can also increase plant P uptake, but they will not be discussed in this overview.

Up to 80% of soil P can be in organic form (Richardson 2001). Phytate, which is considered to be the dominant form of organic P in soils (Turner *et al*., 2003), is a poor P source for some plants grown under sterile conditions (Hayes *et al*., 2000; Richardson *et al*., 2001). Microorganisms, on the other hand, excrete phytase which breaks down phytate (George *et al*., 2007; Richardson and Hadobas, 1997); thus, inoculation with soil microorganisms strongly increased plant P uptake from phytate (Richardson *et al*., 2001).

Rhizosphere microorganisms can reduce plant P availability by immobilisation of P in the microbial biomass, decomposition of P-mobilising root exudates and by inhibition of root growth or mycorrhizal colonisation. Organic acid anions released by plant roots could potentially mobilise P, but are rapidly decomposed by soil microorganisms (Van Hees *et al*., 2002). However, roots may also release compounds such as phenolics or cell-wall degrading enzymes that inhibit microbial growth and hence decomposition of organic acid anions (Weisskopf *et al*., 2006).

In the presence of a readily available carbon source such as root exudates, P is rapidly immobilised in the microbial biomass. However, when C is depleted, microbial growth rates decrease and a proportion of the microbial biomass may die off, releasing P. Hence, an active microbial biomass with a high turnover rate can rapidly take up P, but may also represent a slow source of available P through release from dead microbial cells (Oberson *et al*., 2001; Seeling and Zasoski, 1993). The importance of the microbial biomass for plant P uptake was shown to differ among plant families. Microbial biomass P in the rhizosphere was positively correlated with P uptake by three *Poaceae*, but not with P uptake by three Brassicas (Marschner *et al*., 2007), although the concentration of microbial biomass P in the rhizosphere of the species from two plant families was similar.

Hypothesis for the ratio of net nutrient immobilization and mobilization by the microbial biomass along the root axis

We hypothesise that the ratio of nutrient mobilisation to immobilisation and hence, plant Fe and P availability, varies along the root axis. At the root tip, where microbial density in the rhizosphere is relatively low, root exudates will be able to mobilise nutrients without strong competition by microorganisms. The high rate of root exudation at the root tip will stimulate growth of rhizosphere microorganisms immediately behind the root tip, accompanied by strong net Fe and P mobilisation. However, most of the mobilised Fe

and P will be taken up by the microorganisms; they may also take up Fe and P mobilised by root exudates; resulting in net immobilisation. A few centimeters behind the root tip, where root exudation is lower, Fe and P mobilisation will equal immobilisation and some of the P mobilised by root exudates can be taken up by the plant. In the older root zones, the lack of easily decomposable C sources results in lower microbial growth rates and hence Fe and P demand as well as death of microorganisms. Hence, microbial biomass Fe and P are likely to become available to the plant. However, plant uptake of the nutrients released from the microbial biomass in the older root zones may be low because of inherently low nutrient uptake rates in these root zones (Häussling *et al*., 1988; Colmer and Bloom, 1998; Fang *et al*., 2007).

References
Azcon-Aguilar C, Gianinazzi-Pearson V, Fardeau JC, Gianinazzi S (1986) Effect of vesicular-arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria on growth and nutrition of soybean in a neutral-calcareous soil amended with 32P-45Ca-tricalcium phosphate. *Plant and Soil* **96**, 3-15.

Badr el-Din SMS, Khalafallah MA, Moawad H (1986) Response of soybean to dual inoculation with Rhizobium japonicum and phosphate dissolving bacteria. *Zeitschrift fuer Pflanzenernaehrung und Bodenkunde* **149**, 130-135.

Banik S, Dey BK (1983) Phosphate-solubilizing potentiality of the microorganisms capable of utilizing al phosphate as a sole phosphorus source. *Zentralblatt fuer Mikrobiologie* **138**, 17-23.

Bar-Ness E, Hadar Y, Chen Y, Roemheld V, Marschner H (1992) Short-term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. *Plant Physiology* **100**, 451-456.

Colmer TD, Bloom AJ (1998) A comparison of NH_4^+ and NO_3^- net fluxes along roots of rice and maize. *Plant Cell and Environment* **21**, 240-246.

Crowley DE, Gries D (1994) Modeling of iron availability in the plant rhizosphere. In 'Biochemistry of metal micronutrients in the rhizosphere'. Eds. JA Manthey, DE Crowley, DG Luster, pp. 199-224 (Lewis Publishers, Boca Raton).

Crowley DE, Roemheld V, Marschner H, Szaniszlo P J (1992) Root-microbial effects on plant iron uptake from siderophores and phytosiderophores. *Plant and Soil* **142**, 1-7.

Fang Y, Babourina O, Rengel Z, Yang XE, Pu P (2007) Spatial distribution of ammonium and nitrate fluxes along roots of wetland plants. *Plant Science* **173**, 240-246.

Föhse D, Jungk A (1983) Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant and Soil* **74**, 359-368.

George TS, Simpson R J, Gregory P J, Richardson A E (2007) Differential interaction of *Aspergillus niger* and *Peniophora lycii* phytases with soil particles affects the hydrolysis of inositol phosphates. *Soil Biology & Biochemistry* **39**, 793-803.

Gerke J (1992) Phosphate, aluminium and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. *Zeitschrift für Pflanzenernährung und Bodenkunde* **155**, 339-343

Gerretsen FC (1948) The influence of microorganisms on the phosphate intake by the plant. *Plant and Soil* **1**, 51-81.

Häussling M, Jorns C A, Lehmbecker G, Hecht-Buchholz C, Marschner H (1988) Ion and water uptake in relation to root development in Norway spruce (*Picea abies* (L.) Karst.). *Journal of Plant Physiology* **133**, 486-491.

Hayes JE, Simpson RJ, Richardson AE (2000) The growth and phosphorus utilisation of plants in sterile media when supplied with inositol hexaphosphate, glucose-1-phosphate of inorganic phosphate. *Plant and Soil* **220**, 165-174.

Jurkevitch E, Hadar Y, Chen Y (1988) Involvement of bacterial siderophores in the remedy of lime- induced chlorosis in peanut. *Soil Science Society of America Journal* **52**, 1032-1037.

Jurkevitch E, Hadar Y, Chen Y, Chino M, Mori S (1993) Indirect utilization of the phytosiderophore mugineic acid as an iron source to rhizosphere fluorescent Pseudomonas. *Biometals* **6**, 119-123.

Kumar V, Narula N (1999) Solubilization of inorganic phosphates and growth emergence of wheat as affected by Azotobacter chroococcum mutants. *Biology and Fertility of Soils* **28**, 301-305.

Kundu BS, Gaur AC (1980) Establishment of nitrogen-fixing and phosphate-solubilizing bacteria in the rhizosphere and their effect on yield and nurtrient uptake of wheat crop. *Plant and Soil* **57**, 223-230.

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

Marschner P, Crowley DE (1998) Phytosiderophore decrease iron stress and pyoverdine production of *Pseudomonas fluorescens* Pf-5 (pvd-inaZ). *Soil Biology and Biochemistry* **30**, 1275-1280.

Marschner P (2009) The role of rhizosphere microorganisms in relation to P uptake by plants. In 'The ecophysiology of plant-phosphorus interactions' Eds PJ White, JP Hammond, pp 165-176, Plant Ecophysiology Series, Springer, Heidelberg

Neilands JB 1984 Siderophores of bacteria and fungi. *Microbiological Sciences* **1**, 9-14.

Oberson A, Friesen DK, Rao IM, Buehler S, Frossard E (2001) Phosphorus transformations in an oxisol under contrasting land-use systems: the role of the soil microbial biomass. *Plant and Soil* **237**, 197-201.

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology* **28**, 897-906.

Richardson AE, Hadobas PA (1997) Soil isolates of *Pseudomonas* spp. that utilize inositol phosphates. *Canadian Journal of Microbiology* **43**, 509-516.

Richardson AE, Hadobas PA, Hayes JE, O'Hara CP, Simpson RJ (2001) Utilisation of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhance by the presence of soil micro-organisms. *Plant and Soil* **229**, 47-56.

Roemheld V (1991) The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species : an ecological approach. *Plant and Soil* **130**, 127-134.

Seeling B, Zasoski RJ (1993) Microbial effects in maintaining organic and inorganic solution phosphorus concentrations in a grassland topsoil. *Plant and Soil* **148**, 277-284.

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium *Pseudomonas* sp strain GPR(3) influences iron acquisition in mungbean (*Vigna radiata* L. Wilzeck). *Soil Biology & Biochemistry* **35**, 887-894.

Shen J, Rengel Z, Tang C, Zhang F (2003) Role of phosphorus nutrition in development of cluster roots and release of carboxylates in soil-grown *Lupinus albus*. *Plant and Soil* **248**, 199-206.

Turner BL, Mahieu N, Condron LM (2003) The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. *Organic Geochemistry* **34**, 1199-1210.

Van Hees PAW, Jones DL, Godbold DL (2002) Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology & Biochemistry* **34**, 1261-1272.

Von Wiren N, Roemheld V, Morel JL, Guckert A, Marschner H (1993) Influence of microorganisms on iron acquisition in maize. *Soil Biology & Biochemistry* **25**, 371-376.

Walter A, Roemheld V, Marschner H, Crowley DE (1994) Iron nutrition of cucumber and maize: effect of *Pseudomonas putida* YC3 and its siderophore. *Soil Biology & Biochemistry* **26**, 1023-1031.

Wang Y, Brown HN, Crowley DE, Szaniszlo PJ (1993) Evidence for direct utilization of a siderophore, ferrioxamine B, in axenically grown cucumber. *Plant, Cell and Environment* **16**, 579-585.

Weisskopf L, Abou-Mansour E, Fromin N, Tomasi N, Santelia D, Edelkott I, Neumann G, Aragno M, Tabacchi R, Martinoia E (2006) White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. *Plant Cell and Environment* **29**, 919-927.

Whitelaw MA, Harden TJ, Helyar KR (1999) Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum*. *Soil Biology & Biochemistry* **31**, 655-665.

Yehuda Z, Shenker M, Hadar Y, Chen Y (2000) Remedy of chlorosis induced by iron deficiency in plants with the fungal siderophore rhizoferrin. *Journal of Plant Nutrition* **23**, 1991-2006.

Yehuda Z, Shenker M, Roemheld V, Marschner H, Hadar Y, Chen Y (1996) The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants. *Plant Physiology* **112**, 1273-1280.