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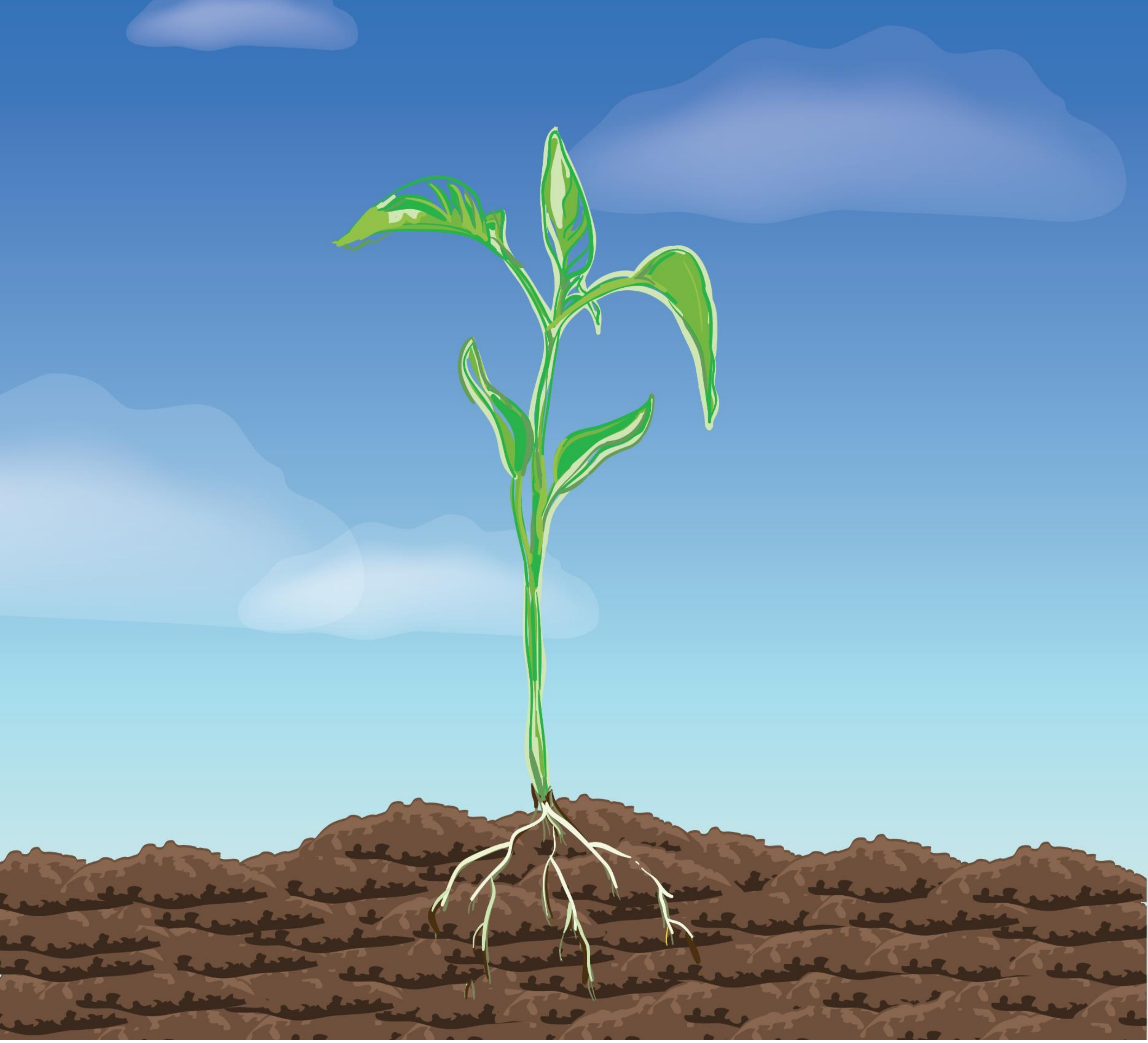

D.H. Keuskamp, R. Kimber, P. Bindraban, C. Dimkpa, and W.D.C. Schenkeveld

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Plant exudates for nutrient uptake

D.H. Keuskamp,[1,2*] R. Kimber,[3*] P. Bindraban,[4] C. Dimkpa[4] and W.D.C. Schenkeveld[3*]

[1] Soil Quality, Wageningen University, The Netherlands
[2] Nature Conservation and Plant Ecology, Wageningen University, The Netherlands
[3] Department of Environmental Geosciences, University of Vienna, Austria
[4] Virtual Fertilizer Research Center

* Authors contributed equally.

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Washington, D.C., USA

Executive Director
Virtual Fertilizer Research Center
Suite 800
1901 Pennsylvania Avenue NW
Washington, D.C. 20006
USA
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Contents

List of acronyms and abbreviations

AMF	Arbuscular Mycorrhizal Fungi
B	Boron
Ca	Calcium
Cl	Chlorine
Cu	Copper
Fe	Iron
HS	Humic Substances
K	Potassium
LMWOA	Low Molecular Weight Organic Acids
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Ni	Nickel
OA	Organic Acids
P	Phosphorus
PS	Phytosiderophore
S	Sulfur
Zn	Zinc

Abstract

Plants require nutrients for unimpaired growth. Many plant strategies for acquiring nutrients from the soil involve root exudates that facilitate the detachment from the soil solid phase and the transport to the plant root. In this report, root exudation related to acquisition of nutrients other than nitrogen (N) and phosphate (P) has been considered.

In this context, three important classes of root exudates can be identified: low molecular weight organic acids (LMWOA), phytosiderophores (PS) and reductants. The mechanisms by which these exudates can enhance bioavailability include ligand exchange, ligand-promoted dissolution, mineral dissolution by lowering solution saturation state through complexation, co-exudation of protons and chemical reduction. These mechanisms are not specific to a certain class of exudates, and a single class of exudates can be involved in multiple mechanisms.

The efficiency of exudates in mobilizing nutrients from soil depends on the chemical affinity of the exudate ligand for the targeted nutrient element (the denticity of the exudate ligand plays an important role in this respect), the characteristics of the soil, and the susceptibility of the exudate to microbial degradation, adsorption and binding of non-targeted elements.

A meta-analysis of available literature data on the response of root exudation levels by different crop species to the availability of specific nutrients was carried out. The relative change in root exudation level as a result of a decrease in the availability of specific nutrients was investigated. The responsiveness and the magnitude of these responses seem to be strongly plant species, cultivar and nutrient specific.

Available data on exudation proved biased towards certain nutrients, specifically iron (Fe) and zinc (Zn), and comparisons between studies were often complicated due to differences in experimental approach. Furthermore, at present there are very few published data on exudation under actual rhizosphere conditions. Despite the shortage of data, the potential for utilizing root exudates for making better use of soil nutrient reserves and improving nutrient acquisition, e.g., in intercropping systems, looks promising and needs to be further explored.

1 Introduction

Plants need nutrients for unimpaired growth and reproduction. Upon depletion of the nutrient reserves from the seed lobes after germination, the primary source of nutrients is the soil. In the course of evolution, plants have developed diverse strategies for acquiring nutrients from the soil. Many of these strategies involve so-called root exudates (Marschner, 2012). Upon release into the rhizosphere, exudates can affect the physical, chemical and biological conditions in the direct vicinity of the root, and hence increase the bioavailability of nutrients (Hinsinger et al., 2009). Different classes of exudates can be distinguished, which, due to their distinct chemical nature, will affect rhizosphere conditions in different ways and may facilitate nutrient uptake through a range of mechanisms (e.g., Jones 1998; Dakora et al., 2002; Oburger et al., 2014).

The essential nutrients that plants need can be divided into classes based on the required amounts: macronutrients (N, P, K), secondary nutrients (Ca, Mg, S) and micronutrients (Fe, Mn, Zn, Cu, B, Mo, Cl, Ni). The strategies employed by plants to acquire nutrients are diverse and plant-specific and, as a consequence, so are the composition and exudation rates of root exudates (e.g., Jones, 1998; Gransee et al., 2000). Also, the developmental stage (e.g., Boeuf-Tremblay et al., 1995; Chaparro et al., 2013) and even cultivar (e.g., Aulakh et al., 2001; Li et al., 2013) can have a large effect on root exudation. Furthermore, the amount and composition of root exudates strongly depend on the nutrient status of the plant (e.g., Dakora et al., 2002; Carvalhais et al., 2011). Exudation can be strongly up-regulated, particularly under conditions of nutrient deficiency. The degree to which plant species or even cultivars of a single species are resistant to conditions of limited availability of a specific nutrient relates both to the type and amount of exudates that are released. As a result, certain types of plant species are highly adapted to conditions of low availability of specific nutrients.

In agricultural practice, nutrient deficiency leads to reduced yields and reduced crop quality (Schenkeveld et al., 2008, 2010). Selection of species and cultivars that can efficiently utilize available nutrient resources, a.o., through release of exudates, can lower fertilizer requirements as well as losses through leaching and reduce the risk of crop failure. An improved understanding of plant-specific root exudation related to nutrient acquisition will aid a successful selection of crop species and cultivars and may be useful in crossbreeding programs. Furthermore, knowledge of differences among species will generate insight in the potential for intercropping – i.e., a cropping system in which different plant species are grown together and, ideally, mutually enhance nutrient availability, resulting in an increased yield of both species (Xiong et al., 2013).

The aim of the present report is to review the scientific literature on the role of root exudates in nutrient acquisition (except for nitrogen and phosphorus). We identify and describe the principal classes of root exudates involved in nutrient acquisition (Chapter 2), as well as the nutrient acquisition mechanisms in which these exudates are involved (Chapter 3). Subsequently, the effectiveness of the exudates in mobilizing nutrients from soil is discussed (Chapter 4). A meta-analysis of available literature data on root exudation in relation to plant species and cultivars and nutrient availability has been carried out. The results of the meta-analysis are presented in Chapter 5. Root exudates not primarily exuded for the purpose of nutrient acquisition but which might have secondary effects are also briefly discussed (Chapter 6). Chapter 7 concludes the report with a summary and discussion and recommendations based on the presented findings.

2 Characteristics of exuded compounds

A wide range of compounds are exuded by plants (Table 1), however, only a few of the proposed effects of these compounds have been established so far, with many remaining speculative and unproven (Uren, 2007). In this report, we will focus on classes of root exudates which have been demonstrated to increase nutrient availability in the rhizosphere and potentially increase nutrient uptake by plants. We have identified three main groups of exudates, classified by their chemical properties and mechanism of nutrient mobilization: low molecular weight organic acids (LMWOA), phytosiderophores (PS) and reductants. Although enzymes also form a well-defined class of compounds exuded in relation to plant nutrition, to our knowledge their effect is limited to phosphorous mobilization, and so they are not discussed in the context of this report. Details of the physicochemical properties which govern these classes are provided in this chapter.

Table 1. *Root exudates detected from higher plants*

(adapted from Badri and Vivanco [2009], Neumann and Römheld [2007] and Uren [2007])

Low molecular weight organic acids	Formic, acetic, butyric, popionic, malic, citric, isocitric, oxalic, fumaric, malonic, succinic, maleic, tartaric, oxaloacetic, pyruvic, oxoglutaric, glycolic, shikimic, cis-aconitic, trans-aconitic, valeric, gluconic
Phytosiderophores	*Mugineic acid, deoxymugineic acid, hydroxymugineic acid, epihydroxymugineic acid, avenic acid, hydroxyavenic acid, epihydroxy-deoxymugineic acid, hydroxy-deoxymugineic acid, distichonic acid*
Reductants	Flavonols, flavones, flavanones, isoflavonoids, coumarins, ascorbic acid, caffeic acid
Enzymes	Amylase, deoxyribonuclease, ribonuclease, invertase, peroxidase, phenolase, acid phosphatase, phytase, pyrophosphatase apyrase, polygalacturonase, protease
Aromatic acids	p-Hydroxybenzoic, p-coumaric, ferulic, gallic, gentisic, protocatechuic, salicylic, sinapic, syringic
Amino acids (other than phytosiderophores)	All 20 proteinogenic amino acids, aminobutyric acid, homoserine, cystathionine
Sugars and Polysaccharides	Arabinose, glucose, fructose, galactose, maltose, deoxyribose, mannose, mucilages, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose
Fatty Acids	Linoleic, linolenic, oleic, palmitic, stearic
Sterols	Campesterol, cholesterol, sitosterol, stigmasterol
Miscellaneous	Vitamins, ethanol, H^+, metal cations (e.g., K^+), nitrate, phosphate, HCO_3^-

2.1 Low molecular weight organic acids

This class of compounds consists of low molecular weight (<250 MW), water-soluble, aliphatic, carboxyl containing compounds and includes the carboxylic acids: citric acid, oxalic acid, fumaric acid and malonic acid. Some of these (citric acid, malic acid, fumaric acid) are produced in the Krebs cycle, which is used by all aerobic organisms to generate energy through ATP production. At the near-neutral pH of the cytosol (typically 7.3-7.6) in most plant cells, these LMWOA largely exist as fully deprotonated anions (Fig. 1) and hence are expected to be released in their conjugate base form (Hinsinger, 2001; Hinsinger et al., 2009; Kirkby, 2012). The electrical potential difference across the plasma membrane of plant cells ensures that the electrical gradient favors the passive transport of these anions out of the cell (Ryan et al., 2001). However, large bursts of organic anions can also be released into the rhizosphere and are thought to be related to environmental stimuli such as metal toxicity (particularly Al^{3+}), nutrient deficiency (particularly P) and anoxia (Carvalhais et al., 2011; Egle et al., 2003; Gherardi and Rengel, 2004; Hoffland et al., 2006; Jones, 1998; Neumann and Römheld, 1999; Ohwaki and Sugahara, 1997; Ryan et al., 2001). Increased accumulation of LMWOA in cluster roots under nutrient deficient conditions is thought to occur from enhanced biosynthesis and reduced turnover of the acids, resulting in increased exudation/secretion (Neumann et al., 2000). However, it must be noted that, next to root exudation, LMWOA may also be present in the soil due to microbial excretion and the decomposition of soil organic matter (Jones, 1998; Jones et al., 2003).

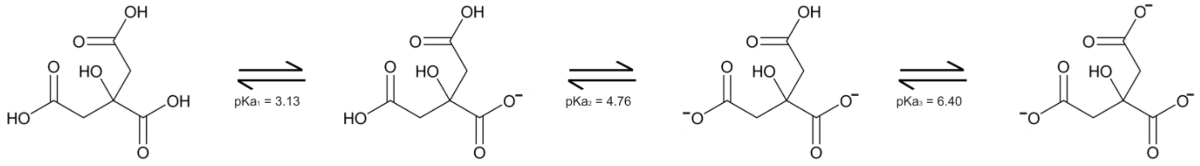

Figure 1. *Structure and pKa values of a LMWOA (citrate). The pKa values of LMWOA are typically lower than the cytosol pH (7.3-7.6) and hence are expected to be exuded as fully deprotonated anions.*

The release of negatively charged organic compounds from the plant must be counterbalanced by either an equivalent efflux of cations or influx of anions in order to maintain the charge balance. The release of H^+ as the counter ion can lead to acidification of the rhizosphere and a resulting drop in rhizosphere pH. However, if the counterion released is a cation other than H^+, such as K^+, the rhizosphere pH will be unaffected. Furthermore, if the released LMWOA are deprotonated upon entering the rhizosphere, for example if citrate^{3-} is released into the rhizosphere with a pH close to or below the pKa for citrate^{2-}/citrate^{3-} (6.40), and the counterion released is a cation other than H^+, for example K^+, then an increase in the rhizosphere pH may occur due to protonation of the acid anion. Therefore, the difference in pH between the cytosol and rhizosphere could be an important factor in controlling exudate-induced pH changes.

Denticity, i.e., the number of donor groups in a single ligand able to bind to a metal, affects the stability of the metal-ligand complex. For LMWOA, the number and proximity of the carboxyl groups of the organic acids influences their affinity for forming metal complexes. Monodentate carboxylic acids containing only one carboxyl group, such as lactate, formate and acetate, have very little affinity for metals. In contrast, dicarboxylates (malate, oxalate, malonate) and tricarboxylates (citrate) have much stronger affinity for metal binding. As a rule of thumb, the stability of metal complexes with LMWOA (which are hard Lewis bases) also increases with increasing charge to ionic radius ratio (Kraemer et al., 2014). So for metals with similar ionic radius, the stability of complexes with LMWOA will increase from monovalent (e.g., Cu(I)), to divalent (e.g., Zn(II)) to trivalent (e.g., Fe(III)).

2.2 Phytosiderophores

Phytosiderophores (PS), or mugineic acids (MAs), are hexadentate chelating ligands secreted by graminaceous plants (grasses) for Fe acquisition, although they are also reported to bond to other nutrients including Zn, Mn, Cu and Ni (Schenkeveld et al., 2014). PS are non-proteinaceous amino acids synthesized from the precursor compound, nicotianamine (NA), through hydroxylation of the amine group. Although this parent compound is found throughout the plant kingdom, it is only graminaceous plants (so-called Strategy II plants) which are found to secrete MAs (Reichman and Parker, 2005). Nicotianamine is itself an intermediate compound in the synthesis of MAs, which begins with the amino acid methionine (Ma et al., 1995). Since the identification of the first phytosiderophore, mugineic acid (Takemoto et al., 1978), nine analogous compounds have been identified (Ma, 2005). The structures of some of the important members of the PS group are presented in Figure 2. The molecular weight of PS ranges from 294-336, and hence, PS are lighter than siderophores secreted from microorganisms, which have molecular weights ranging from 500 to 1000 (Ma, 2005). Biosynthesis of PS occurs during the day where they accumulate within the root cells and are then secreted the next morning (Ma and Nomoto, 1996). Secretion occurs with a diurnal cycle, generally beginning around three hours after sunrise and lasting for approximately three hours. Secretion timing was found to be controlled by the temperature around the root environment (Ma et al., 2003). High temperatures result in early secretion of PS, whereas low temperatures delay their release. The secretion of PS varies along the root but is most pronounced in the apical root zone (Marschner et al., 1987). Biosynthesis of all PS follows the same pathway from methionine via nicotianamine to the PS 2'-deoxymugineic acid, with subsequent steps depending on plant species and cultivar. In order to maintain high levels of MAs synthesis, it is suggested that the biosynthesis of 2'-deoxymugineic acid is associated with the recycling pathway of methionine (Ma et al., 1995).

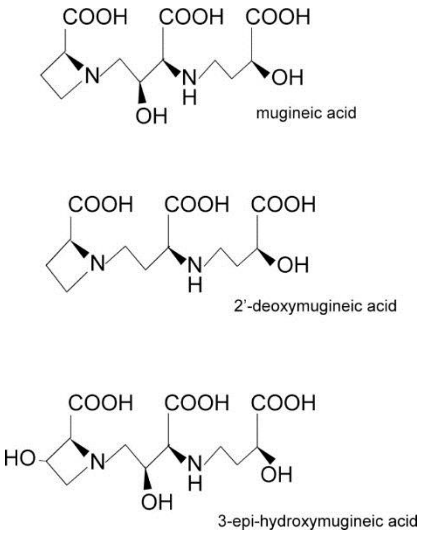

Figure 2. *Structure of some of the important phytosiderophores found in root exudates*

The binding of iron requires deprotonation of the functional groups of the PS. The resulting complex has a pH-dependent net negative over most typical soil pH ranges (von Wiren et al., 2000). The pK_a values of the carboxylate groups of MA, DMA and epi-HMA range from 2.3 to 3.4, whereas their secondary and tertiary amino groups have pK_a values between 7.1 and 10.0 (Kraemer et al., 2006). The terminal hydroxyl group has a pK_a of approximately 17.1 (Murakami et al., 1989).

2.3 Reductants

Reductants are elements or compounds which can donate one or more electrons to another chemical species in a redox reaction (e.g., to nutrients in their oxidized form such as Fe(III), Mn(III/IV), Cu(II), N(V) and SO_4(VI)). Thus, the species receiving the electron becomes reduced whilst the species donating the electron, the reductant, becomes itself oxidized. A wide variety of both inorganic and organic compounds can act as reductants.

Phenolic compounds are a common type of reductant found in root exudates and are characterized by a hydroxyl group attached to a carbon atom of an aromatic ring. They include polyphenol compounds such as coumarin and flavonoids. Some phenolics, such as flavonoids and isoflavonoids, also display antifungal properties and hence have been suggested to play a role in preventing organic acid degradation by microorganisms in order to increase their ability to mobilize nutrients (Weisskopf et al., 2006) (see also Chapter 6).

Flavonoids are polyphenolic, secondary metabolites synthesized via the phenylpropanoid or acetate-malonate pathways and contain a basic skeleton with 15 carbon atoms consisting of two phenyl rings and one heterocyclic ring. They can be divided into subclasses depending on their structure. These subclasses include flavones, flavanones, isoflavones, chalcones and flavanols (Fig. 3). Flavonoids can bind to cationic mineral sites and have a moderate water-solubility, resulting in sorption to organic polymers (Neumann, 2006). Increased biosynthesis of flavonoids and their subsequent release has been linked to upregulation of phenylpropanoid associated with various stress factors, including nutrient deficiencies, plant damage, pathogen infection, oxidative stress and UV-irradiation (Cesco et al., 2010; Weston and Mathesius, 2013). Plant species capable of forming cluster roots (Lupines) mainly release flavonoids during distinct development stages of formation of such roots (Dinkelaker et al., 1997; Neumann et al., 2000; Tomasi et al., 2008; Weisskopf et al., 2006). Recent work, including specific inhibitor studies, has suggested that ABC transporters may play a role in the root exudation of flavonoids (Loyola-Vargas et al., 2007; Sugiyama et al., 2007; Sugiyama et al., 2008). MATE transporters have also been suggested to be involved in flavonoid exudation; however, although their involvement in flavonoid uptake has been shown (Marinova et al., 2007), their potential role in exudation requires further study (Cesco et al., 2010).

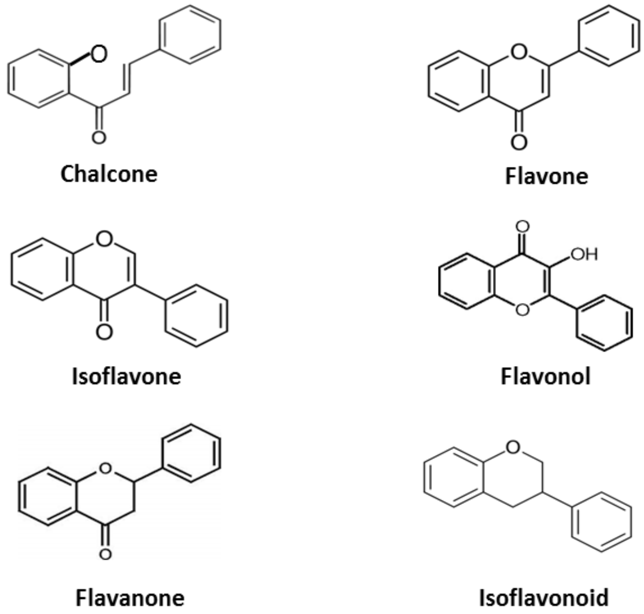

Figure 3. *Chemical structure of the subclasses of flavonoid compounds (adapted from Cesco et al. [2012])*

Another class of phenolic reductants released by plants are coumarins, which are ketone derivatives of benzopyran, a polycyclic organic compound consisting of a fused benzene and heterocyclic pyran ring. Coumarin derivatives, scopoletin and esculetin, have recently been observed in root exudates (Fourcroy et al., 2014; Schmid et al., 2014).

Caffeic acid, a key intermediate in the biosynthesis of lignin, is an aromatic acid found in root exudates which can act as a reductant. It contains both a phenolic and acrylic functional group. The formation of caffeic acid appears to require p-coumaric acid as a precursor, which is then hydrolyzed by p-coumarate hydroxylase detected in roots of tomato plants (Olsen et al., 1981).

3 Mechanisms of nutrient mobilization by plant exudates

Nutrients can be mobilized through a number of mechanisms, and while some of these are specific to a certain class of exudates, many are not unique or restricted to a particular class of compounds. For example, both LMWOA and PS act as ligands and so share a number of mechanisms by which they mobilize nutrients such as ligand-promoted dissolution and ligand-exchange. Although a basic mechanism may be shared among different classes of compounds, differences exist in the exact process, which takes place due to the chemical properties of the exudates. Although PS and LMWOA can both complex metals, the higher denticity of PS means it forms stronger complexes than LMWOA.

3.1 Low molecular weight organic acids

Organic acids can increase nutrient availability through a variety of mechanisms, including ligand exchange, ligand-promoted dissolution and metal complexation. These processes are influenced by numerous factors, including soil properties and pH, mineralogy, organic acid structure and microbial degradation.

3.1.1 Ligand exchange at mineral surfaces

Ligand exchange, or ligand substitution, involves the exchange of one ligand in a complex for another. LMWOA usually exist in their deprotonated state in the rhizosphere (see Chapter 2) and hence act as anionic ligands. Nutrients which also exist as anionic ligands (for example, inorganic phosphate, molybdate and sulfate) are often found sorbed to soil mineral surfaces, particularly to positively charged Fe-oxide surfaces, thus limiting their availability in certain soils (Tejedor-Tejedor and Anderson, 1990). LMWOA can therefore increase the availability of these negatively charged nutrients by exchanging with the nutrient ligand sorbed to the mineral surface (Eq. 1).

$$Fe\ oxide - P + L \rightarrow Fe\ oxide - L + P_{aq} \qquad [1]$$

where *L* = organic acid Fe-complexing agent (ligand) and *P* = inorganic phosphate

3.1.2 Ligand exchange with soil organic matter

LMWOA can also increase the availability of nutrients which are complexed with humic substances (HS). These HS are present in soils as compounds with variable molecular mass and solubility. As well as complexing metals such as Fe, HS may act as sorbents for other nutrients such as P. LMWOA can increase Fe nutrient availability via ligand exchange with the larger, less-soluble humic substances (Eq. 2). Earlier work by Gerke (1992, 1993) indicated that OA, particularly citrate, are effective in mobilizing Fe from Fe-humic complexes. This author postulated that by complexing the metal, citrate was able to break Fe bridges between humic molecules, which resulted in the lowering of the molecular size of the humic complex, thus enhancing its solubility. Furthermore, citrate was also found to mobilize P bound to the Fe-humic complexes more effectively than P bound to metal oxides.

$$HS - Fe + L \rightarrow HS - L + Fe_{aq} \qquad [2]$$

where HS = Humic substances and L = Fe-complexing agent (ligand)

3.1.3 Ligand-promoted dissolution

LMWOA can also liberate nutrients from soil minerals through ligand-promoted dissolution of the particle surface. This process is proposed to occur via a three step procedure (Furrer and Stumm, 1986). It begins with ligand sorption to the surface via ligand exchange and formation of an inner-sphere complex. This polarizes the bonds between the metal and oxygen of the crystal lattice, thus promoting the detachment of the metal complex into solution as the second step. The final step involves transport of the metal complex into the bulk solution and fast regeneration of the mineral surface. The rate of dissolution is proportional to a rate coefficient k_L and the amount of ligand adsorbed $[L]_{ads}$. The rate limiting step of most dissolution processes is controlled by detachment of the surface complex.

As ligand-promoted dissolution results in the slow dissolution of the mineral surface, it can mobilize nutrients that are sorbed to the mineral surface as well as those which are part of the mineral structure (Eq. 3). This can also contribute to weathering of minerals, in which nutrients contained within the mineral structure are released as the mineral is dissolved.

$$Fe\ oxide - P + L \rightarrow Fe\ oxide < \begin{matrix} P \\ L \end{matrix} \rightarrow Fe\ oxide + Fe^{3+} - L + P_{aq} \qquad [3]$$

where L = organic acid Fe-complexing agent (ligand) and P = inorganic phosphate

3.1.4 Reducing the solution saturation state of metals

Complexation of metal ions in solution with LMWOA reduces the metal's solution saturation state and thus can result in further desorption or dissolution of the metal from soil phases in order to maintain equilibrium (Lindsay and Norvell, 1978). However, it has been estimated that citrate does not have sufficient affinity for iron to increase the solubility of poorly crystalline ferrihydrite at pH ≥7 to levels which could support plant growth (Jones et al., 1996).

3.1.5 Influence of protons

In a model system consisting of Fe(hydr)oxide with adsorbed phosphate studied by Johnson and Loeppert (2006), both phosphate and Fe release in the presence of LMWOA was found to increase with decreasing pH. Decreasing pH increases the protonation of oxide OH surface groups, thus weakening the structural Fe-O bonds and enhancing the rate of Fe detachment. Inner-sphere adsorption of ligands is favored at low pH due to the relative ease of exchanging H_2O compared with OH- from the positively charged surface. Ligand adsorption is also enhanced at lower pH values due to the greater positive surface charge of the oxide increasing the affinity of the anionic ligand to form outer-sphere complexes. However, as ligands become more protonated with decreasing pH, there is likely to be a pH at which dissolution is at a maximum and below which begins to decrease (Johnson and Loeppert, 2006).

3.1.6 Influence of organic acid structure

The structure of LMWOA influences the stability of the ligand-surface complexes and the subsequent dissolved ligand-metal complexes and thus is a factor in determining dissolution rates and , hence, potential nutrient mobilization. The lability of surface metal ions complexed with inner-sphere coordinating ligands increases with increasing denticity of the surface complex formed (Margerum et al., 1978), and hence bi- and tridentate ligands are more efficient in the dissolution of oxides than monodentate ligands (Ludwig et al., 1995). Furthermore, the stability of the chelate ring formed by bidentate ligands also influences dissolution. Dissolution rates of Al oxide were found to decrease when bidentate ligands formed chelate rings with more members (increasing from five to seven) (Stumm et al., 1985). Among bidentate ligands, maximum dissolution of Fe from both ferrihydrite and goethite occurred with oxalate, which formed a five-membered ring, then decreased with malaonate, which forms a six-membered ring, and further with seven-member ring forming succinate, malate and tartrate (Johnson and Loeppert, 2006). Dissolution of appreciable Fe from the oxide surface will naturally result in concomitant release of surface bound nutrients, such as phosphate. However, the increasing rates of dissolution do not necessarily result in increasing nutrient mobility. For example, Johnson and Loeppert (2006) found that although oxalate dissolved more Fe than the other LMWOA studied, little corresponding phosphate P release was observed. Therefore, it was suggested that oxalate resulted in relatively rapid phosphate release but was not as effective as other OA, such as citrate, in competing with P for fresh surface sites and hence was not able to prevent significant P readsorption.

3.2 Phytosiderophores

The process by which PS increase nutrient availability and enhance nutrient uptake can typically be divided into four steps: (1) biosynthesis of the PS in the roots; (2) secretion of the PS into the rhizosphere; (3) chelation of the insoluble metal, particularly iron, and subsequent solubilization; and (4) uptake of the PS-metal complex by the roots. Although PS differ in chemical structure among plant species and even cultivars, all coordinate Fe(III) via their 2 amine, 3 carboxylate and 1 hydroxyl groups, forming an octahedral complex (Kraemer et al., 2006; Ma et al., 2003). Graminaceous plants are capable of acquiring Fe via uptake of the Fe(III)-PS complex as an undissociated molecule across the plasma membrane of root cortex cells (Römheld and Marschner, 1986) where strict recognition of the specific stereostructure occurs (Ma and Nomoto, 1996).

3.2.1 Ligand-promoted dissolution model studies

In a model study investigating goethite (α-FeOOH) dissolution in the presence of PS, Reichard et al. (2005) found a linear relationship between the steady-state dissolution of the oxide and adsorbed DMA, consistent with a surface controlled, ligand-promoted dissolution mechanism (as described in Section 3.1.3). Maximum dissolution rates occur at near neutral pH, which corresponds to the typical rhizosphere pH range in calcareous soils (Kraemer et al., 2006; Reichard et al., 2005). At low concentrations of PS, the apparent steady-state dissolution of iron(hydr)oxide minerals can be increased by the presence of a LMWOA (particularly oxalate), suggesting that the co-exudation of LMWOA and PS may have a synergistic effect on nutrient mobilization (Reichard et al., 2005). Diurnal release of PS results in fast, non-steady-state dissolution of the Fe oxide. This dissolution rate is enhanced when low concentrations of OA are already present, further indicating a synergistic role between OA and PS in Fe mobilization. However, readsorption of the Fe-PS complex onto the iron oxide or other soil components can lead to lower Fe availability than predicted by net dissolution rates and hence must also be considered (Reichard et al., 2005).

3.2.2 *Ligand exchange*

PS can also enhance Fe availability to plants via ligand exchange (as described in Sections 3.1.1 and 3.1.2). Iron complexed with other organic ligands in complexes with sufficiently low thermodynamic stability can undergo ligand exchange with PS substituting for the organic ligand. For example, Cesco et al. (2000) demonstrated that iron can be mobilized from an insoluble iron-hydroxide by water-extractable humic substances, which can then undergo ligand exchange with a PS and thus create an Fe-PS complex which can be directly taken up by graminaceous plants.

3.2.3 *Solution saturation reduction*

As is also the case for OA, PS can increase Fe solubility without direct interaction with the mineral surface by reducing the solution saturation state of Fe with respect to the oxide (see Section 3.1.4). The high affinity of PS for Fe ($\log\beta_{110} \approx 18\text{-}19$; I = 0.1 M Murakami et al. [1989]) can reduce the solution saturation state even at low free ion concentrations (Kraemer et al., 2006).

3.3 Reductants

Reductive dissolution of soil minerals can mobilize nutrients which are part of the mineral structure as well as those which are sorbed to the mineral surface. In this process, a reductant typically forms an inner sphere complex with the oxide, enabling the transfer of electrons between the reductant and the oxide mineral which acts as the oxidant. This results in the formation of a reduced ion species on the surface of the oxide which, being much more soluble than the oxidized species; results in depolymerization and subsequent release of the reduced species into solution. Reductive dissolution often occurs at a higher rate than proton-promoted or ligand-promoted dissolution due to weakening of the bonds between the reduced species and oxygen of the mineral lattice, relative to the bonds formed by the oxidized species, thus making release of the reduced species more thermodynamically favorable (Suter et al., 1991). However, like proton- and ligand-promoted dissolution, reductive dissolution is also a surface controlled process, and thus the dissolution rate is dependent on the surface concentration of the reductant species. The lability of reduced metals bound to organic matter is also increased relative to the oxidized metal, and hence reductants can increase the availability of organically bound nutrients.

For example, an exuded reducing ligand, such as caffeic acid, can form a surface complex with an iron oxide mineral, such as hematite (Eq. [3]). Electron transfer between the reductant (caffeic acid) and the Fe(III) results in a formation of surficial Fe(II), which is subsequently released into solution (Eq [4]). However, in the presence of oxygen, the reduced species can be rapidly re-oxidized and hence re-immobilized.

$$Fe\,(III) \;+\; R \;=\; Fe(II) \;+\; R^{+} \qquad [4]$$

where R = a reductant which transfers an electron to the oxidized Fe(III)

In a model study, the surface concentration of ascorbate was found to decrease with decreasing pH; however, the dissolution rate and rate constants increased (Suter et al., 1991). With decreasing pH, surface protonation increases the rate of dissolution, and thus exudation of reductants by roots can lead to nutrient mobilization via reductive dissolution, with co-exudation of protons accelerating this process.

Metals such as Fe can co-precipitate with other plant nutrients, including P (forming ferric phosphate). Reductants can therefore indirectly increase the availability of co-precipitated nutrients, such as P, through reductive dissolution of the sparingly soluble phosphate minerals (Tomasi et al., 2008). In addition, reductants such as polyphenolic flavonoids can compete with P ions for sorption sites and participate in ligand exchange reactions (as described in Section 3.1.1) resulting in P desorption from mineral surfaces (Cesco et al., 2012). Fe and P solubility may also be increased by the chelating properties of phenolics, which can form relatively stable chelates with Fe and Al present in Fe- and Al-phosphates (Cesco et al., 2010; Dakora and Phillips, 2002; Schmid et al., 2014).

4 Effectiveness of root exudates in increasing nutrient availability

As discussed in Chapter 3, the mechanisms by which nutrients are mobilized are not necessarily specific to one class of exudates. Moreover, chemical differences between compounds either of the same or of different classes mean that certain compounds are observed to perform better than others in nutrient mobilization. For example, the number of functional groups of LMWOA or PS influences the denticity of the metal-ligand complex, which in turn affects the stability and lability of the complex. The picture is complicated even further as soil properties such as pH, mineralogy, organic content and texture can all influence the mobility of nutrients and the effectiveness of exudates. Other rhizosphere processes, such as microbial respiration and adsorption to mineral surfaces, can inhibit the effectiveness of certain exudates by reducing their persistence in the rhizosphere.

4.1 Low molecular weight organic acids

4.1.1 Effect of different LMWOA

As a tridentate ligand, citrate forms metal complexes which are more stable than those formed by bi- and monodentate ligands (e.g., oxalate and acetic acid, respectively). As such, it would be expected that citrate exudation would result in a larger mobilization of nutrients relative to oxalate or other bidentate LMWOA. This has indeed been observed in a number of studies, but is, however, dependent upon the nutrient involved and the soil type. Khademi et al. (2009) observed Mn mobilization was a factor of >5 higher for citrate compared to oxalate. At 100 mM LMWOA concentrations, Fe mobilization by citrate was also greater than that by oxalate. However, for both Zn and Cu, little mobilization was observed for either citrate or oxalate (Fig. 4a). The concentration of LMWOA is also clearly important in nutrient mobilization. At concentrations of 10 and 100 mmol kg^{-1} soil, both the acid and K-salt forms of citrate and oxalate were able to mobilize Fe (Fig. 4b). However, whereas both citric acid and K-citrate were able to mobilize Fe at 1 mmol kg^{-1} soil, the same concentration of oxalic acid did not mobilize more Fe than was mobilized by water alone (25 µmol kg^{-1} soil) (Fig. 4b). However, it must be noted that even a concentration of 1 mmol per kg soil is considered high relative to concentrations of LMWOA detected in the rhizosphere (Table 2) of maize grown on soil from the A horizon of a Eutric cambisol, which were in the low micromolar range. Oxalate was the most abundant among the LMWAO examined. The data in Table 2 do not indicate the existence of a concentration gradient of LMWOA as a function of distance from the root. This would suggest that the majority of LMWOA detected were not of plant origin. The study does not mention any indication that the plants would suffer from nutrient deficiency stress; nutrient requirements could possibly be met without (elevated) LMWOA exudation. Alternatively, potentially higher microbial activity near the roots could result in faster turnover of LMWOA.

Table 2. *Approximate concentrations (µM) of LMWOA in rhizosphere soil solution at defined distances from a root layer of maize grown in rhizoboxes (Oburger et al., 2013)*

	Distance from Root Layer			
	1 mm	**2 mm**	**3 mm**	**Bulk Soil**
Citrate	0.4	0.4	1.4	0.9
Malate	0.8	1.0	1.0	2.1
Malonate	0.6	0.6	0.1	1.6
Oxalate	5.1	3.8	4.6	8.2

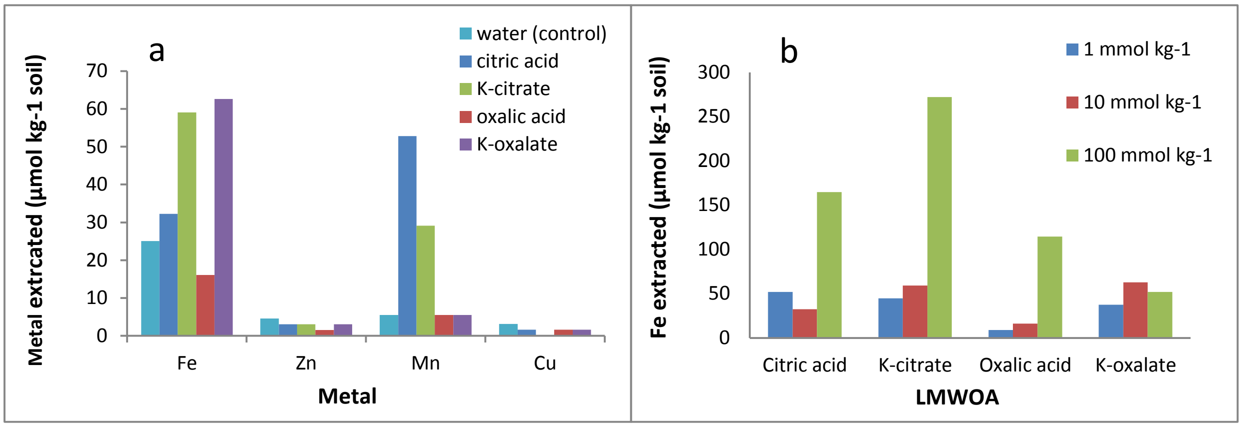

Figure 4. *Extraction of various nutrients as affected by (a) the type and (b) the concentration of LMWOA applied (Khademi et al., 2009). The concentration of LMWOA used in graph (a) was 10 mmol kg^{-1} soil.*

Citrate was also found to be the most effective LMWOA (compared with malate, oxalate and malonate) in Fe and P mobilization in a study by Oburger et al. (2011). Although the efficiency of citrate in mobilizing Fe was strongly affected by different soils, it was almost always more effective than the other LMWOA studied (Fig. 5). The exception being in an andosol soil (an unfertilized Ahp horizon), in which little mobilization of Fe was observed by any of the LMWOA. Mobilization of P (data not shown) was also lowest in this soil, and the authors postulated that the presence of a high amount of anionic binding sites in this soil may have limited nutrient mobilization, possibly through rapid readsorption of mobilized Fe and P. In the same study, LMWOA were observed to have little effect on the mobilization of Ca.

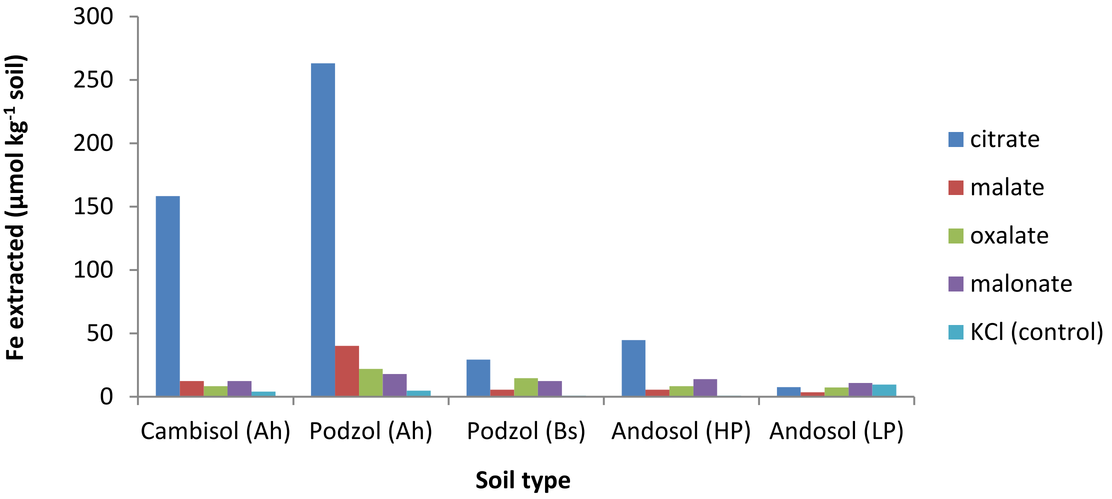

Figure 5. Comparison of Fe mobilization by various LMWOA in different soil types

Soil pH was 5.8, Cambisol; 4.7 - 4.9, Podzols; 5.8, Andosols. DTPA-extractable Fe (µmol kg^{-1} soil) was 21.5, Cambisol; 10.7-14.3, Podzols; 10.7-23.3, Andosols. 2.5 mmol LMWOA extractant was used (Oburger et al., 2011). The soil horizon is indicated in parentheses. HP refers to a fertilized Ahp horizon, and LP refers to an unfertilized Ahp horizon. Detailed soil characteristics are found in (Oburger et al., 2011).

4.1.2 Effect of soil properties

As has already been discussed in the previous section, soil type clearly influences the effectiveness of LMWOA in mobilizing nutrients. Due to the wide range of variables in soil properties, it can be difficult to identify specific conditions under which LMWOA (and other exudates) operate most efficiently. Soils are often classified by texture, as determined by their sand, silt and clay content, which influences their ability to retain water and nutrients. An investigation into Mn mobilization by citric and oxalic acid on 10 different soils comprising of four different textures (Maqsood et al., 2011) found little correlation between the amount of Mn mobilized and the soil texture (Fig. 6). However, there was a correlation between the amount of Zn mobilized and the amount of Zn bound to the Fe/Mn oxide fraction (Fig. 6), potentially due to partial ligand-promoted dissolution of the oxides by the LMWOA. Interestingly, bidentate maleic acid mobilized more Zn than the tridentate citric acid (Maqsood et al., 2011). A possible explanation is that as citric acid mineralization has been observed to occur at a faster rate than for most other LMWOA (Oburger et al., 2009; Ström et al., 2001; Ström et al., 2002), microbial degradation could limit its role in nutrient mobilization in non-sterile conditions. LMWOA are also prone to sorption and complexation in the rhizosphere. Although this can increase nutrient availability when sorption or complexation to a target nutrient occurs, when the LMWOA sorbs or complexes with a non-target nutrient, the LMWOA is lost with no beneficial effect on nutrient mobilization.

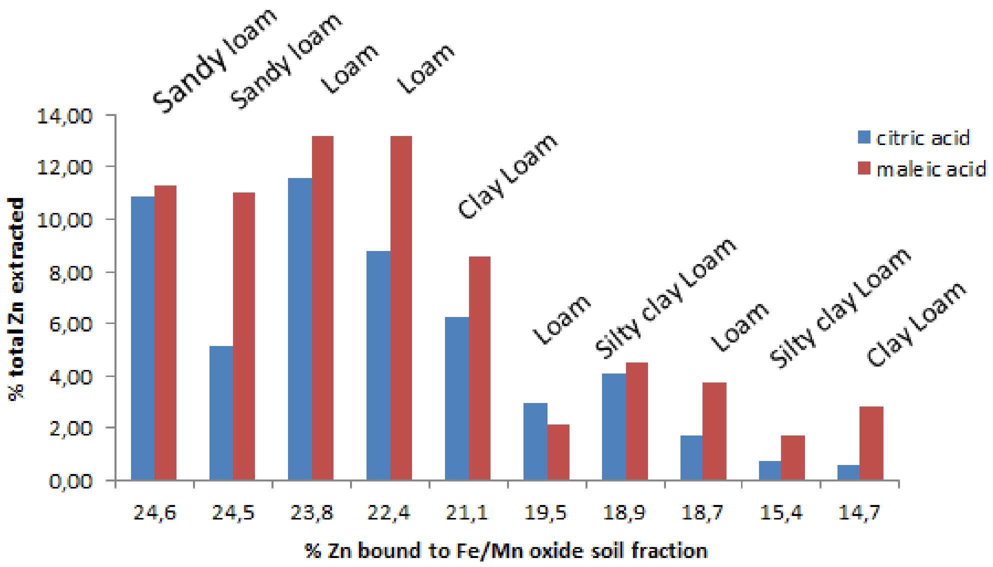

Figure 6. *Zn mobilization by citric and maleic acid as a function of the percentage of total Zn bound to the Fe/Mn oxide soil fraction*

The texture of each soil is given above the relevant bar (Maqsood et al., 2011).

Soil acidity may also influence nutrient mobilization by LMWOA. Mn extraction was significantly higher in a Eutric cambisol (acidic) than in two calcareous Rendzic leptosols (Fig. 7), although Fe mobilization was not affected so strongly. Again, the type of LMWOA used influenced the amount of Mn and Fe mobilized. However, it is difficult to draw many conclusions from this study as different concentrations of LMWOA were used, thus making comparisons between their mobilization efficiency difficult (Ström, 1997). However, it would appear that monodentate acetic acid is relatively ineffective in Mn and Fe mobilization despite being used at a very high concentration (300 mmol kg^{-1}). Citric and oxalic acid were effective in mobilizing Mn from the acidic soil but not from the calcareous soils. Mobilization of Fe was also usually more effective in the acidic soil. However, as with many other studies, the concentrations of LMWOA used were very high (100-300 mmol kg^{-1} soil) and so do not accurately reflect concentrations expected in the rhizosphere. (Oburger et al., 2013).

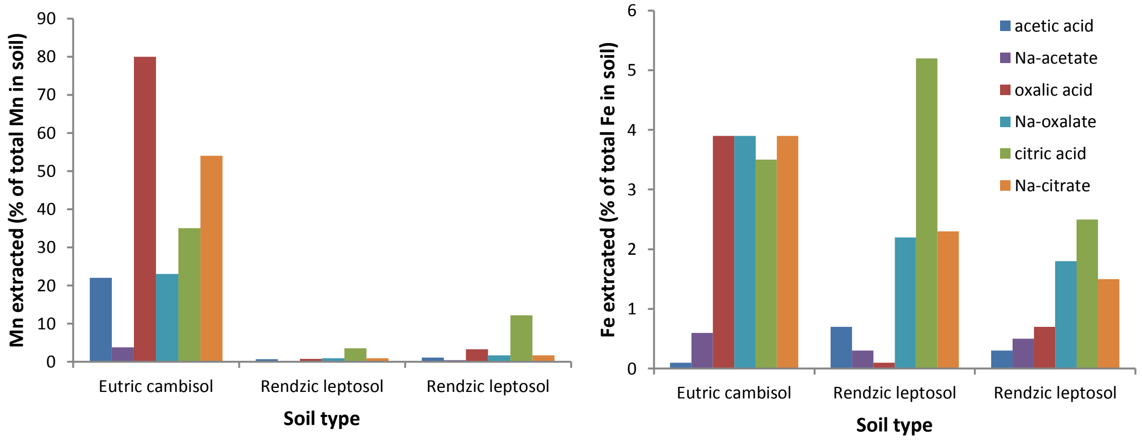

Figure 7. *Mn and Fe extracted by various LMWOA in an acidic (Eutric cambisol) and calcareous soil(s) (Rendzic leptosols) (Ström, 1997)*

4.2 Phytosiderophores

4.2.1 Loss of PS from solution

Once exuded, PS participate in rhizosphere processes such as adsorption, degradation and metal mobilization. Sorption of both the PS ligand and PS-metal complexes to soil solid phases can occur, which can inhibit the efficiency of the PS ligand in mobilizing metals as well as reduce the availability of nutrients complexed with the PS. In a 1:1 (w/v) soil solution experiment, sorption was reported to be responsible for the loss from solution of DMA of between 25% and 62% (Schenkeveld et al., 2014). Biodegradation is also a major cause of PS loss from the rhizosphere (Reichman et al., 2011). Yields of PS can be up to 10 times higher in solution culture studies which are sterile compared to non-sterile conditions (Römheld, 1991). However, it has been suggested that the diurnal release of PS from apical root zones with a low density of rhizosphere microorganisms is a mechanism to limit degradation of the PS (Von Wirén et al., 1993).

4.2.2 Mobilization of nutrients

PS have been shown to mobilize a number of nutrients including Fe, Zn, Mn, Cu and Ni (Awad and Römheld, 2000; Schenkeveld et al., 2014; Takagi et al., 1988; Treeby et al., 1989; Zhang, 1993; Zhang et al., 1991). Awad and Römheld (2000) observed that PS from exudates of Fe-deficient wheat[1] were able to mobilize significant Fe and Zn in calcareous soils. PS-extracted Fe was 3.8 to five times higher than DTPA-extracted Fe, whereas PS-extracted Zn was between 4.8 and 8.2 times higher than DTPA-extracted Zn. However, the concentration of PS used was extremely high (10,000 µmol kg soil^{-1}) and not reflective of concentrations expected in the rhizosphere, which were found to be in the lower to sub-micromolar range for wheat (cv Tamaro) grown in several calcareous soils (Oburger et al., 2014). In a calcareous sandy loam, Treeby et al. (1989) found that PS collected from exudates (500 µmol kg-1 soil) of Fe-deficient barley extracted similar amounts of Fe and Cu as DTPA (51.7 and 61 mol kg^{-1} soil for Fe and 10.5 and 15.7 µmol kg^{-1} soil for Cu, respectively). Zn extracted by the PS (70 µmol kg^{-1} soil) was 35 times higher than that extracted by DTPA (2.4 µmol kg^{-1} soil). However, PS-extracted Mn (58.3 µmol kg^{-1} soil) was 3.1 times

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[1] DMA is the only PS known to be exuded by wheat.

lower than that extracted by DTPA (182 µmol kg^{-1} soil). Schenkeveld et al. (2014a,b) recently investigated nutrient mobilization by the PS, DMA, using concentrations (down to 3 µmol kg^{-1} soil) more relevant to those expected in the rhizosphere. Mobilization of Fe, Zn, Mn and Cu was observed (Fig. 8), though at much lower levels than has been reported in previous studies using higher amounts of PS per kg of soil (Awad and Römheld, 2000; Takagi et al., 1988; Zhang, 1993; Zhang et al., 1991). Fe extraction was between six and 60 times higher in two of the sandy soils compared with clay soils. However, in a third sandy soil studied, the level of DMA-extracted Fe was lower than any of the clay soils. In this soil, available Mn and Ni concentrations were much higher than in the other soils, suggesting that competition with other metals can inhibit Fe mobilization. The correlation between DMA- and DTPA-extracted nutrients was weakest for Fe (R^2=0.79) but stronger for Cu (R^2=0.81) and Zn (R^2=0.85), further suggesting that Fe mobilization by DMA is strongly affected by competition with other cations (Fig. 9). Cu and Zn mobilization was greatest in the clay soils, whereas relatively little Mn was mobilized in any soil type. Therefore, the latter study from Schenkeveld et al. (2014) highlights the importance of investigating nutrient mobilization at concentrations of PS relevant to the rhizosphere, as previous studies may have overestimated the efficiency of PS induced nutrient mobilization.

In two studies comparing the mobilization of Fe by different PS (including DMA, MA, HMA and AA), little difference was observed between the efficiency of each PS in Fe mobilization (Takagi et al., 1988; Zhang, 1993).

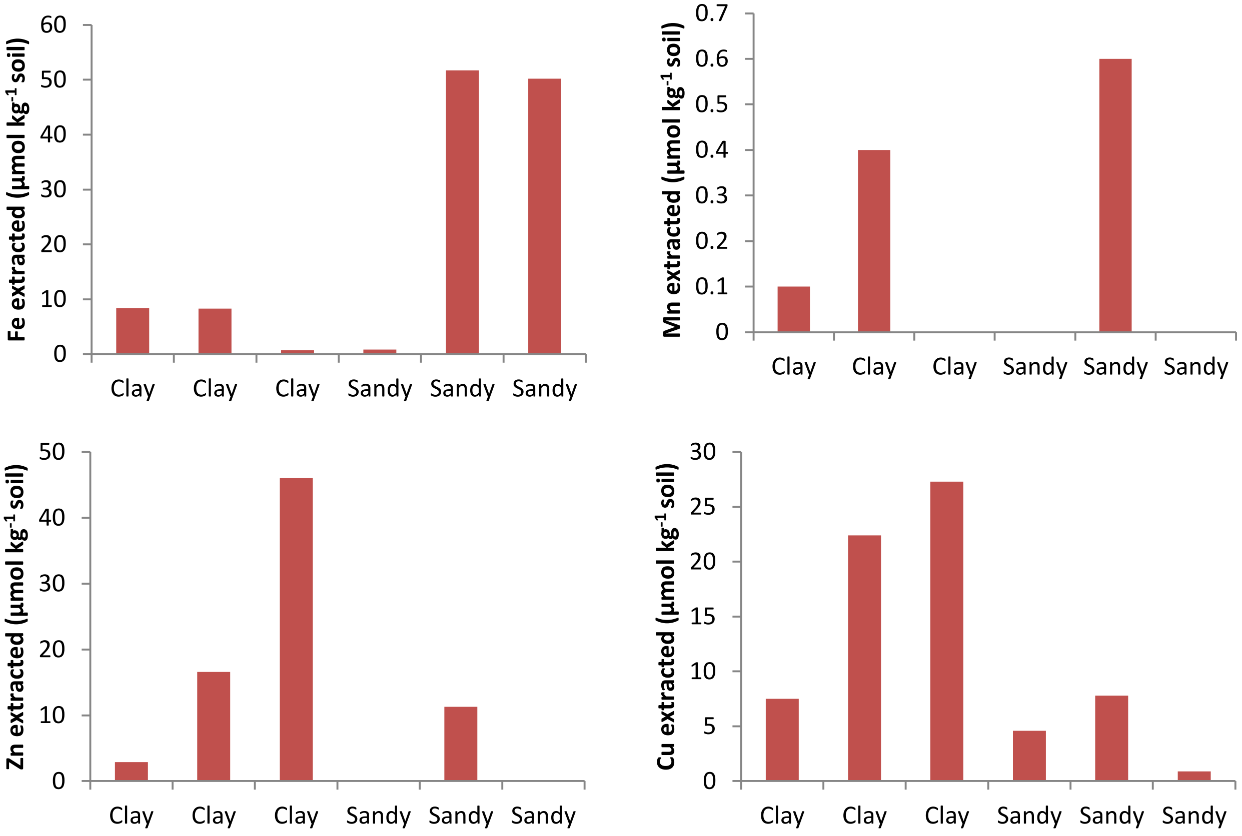

Figure 8. *DMA extracted nutrients in clay and sandy soils after four hours by 100 µM DMA (SSR = 1) (Schenkeveld et al., 2014a)*

Note the different scales used on the vertical axis for each nutrient.

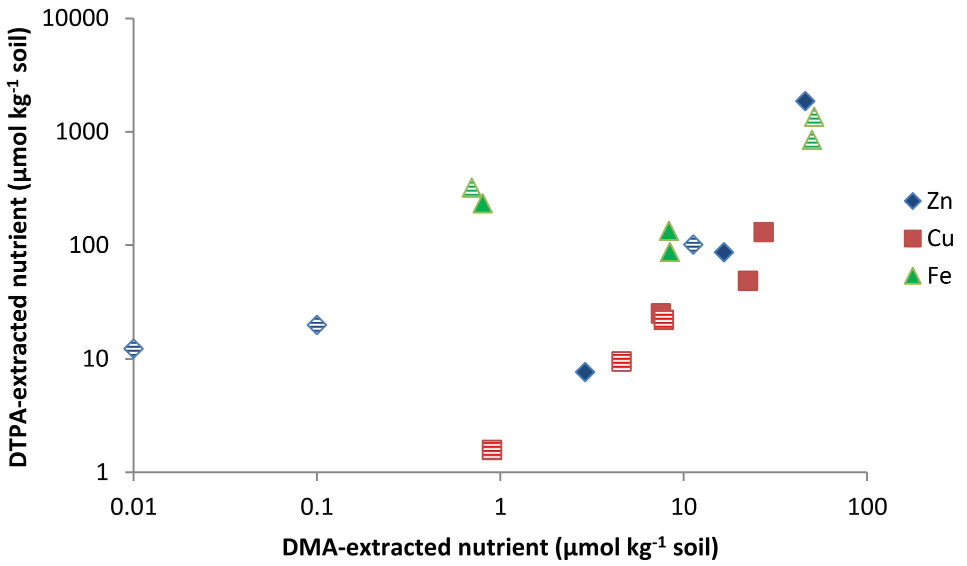

Figure 9. *Correlation between DMA- and DTPA-extracted nutrients*

The filled symbols represent the nutrient extracted in clay soils. The dashed symbols represent the nutrient extracted in sandy soils (Schenkeveld et al., 2014).

4.2.3 Uptake of PS-metal complex

In graminaceous plants, increased uptake of Fe in the presence of PS occurs via direct uptake of the PS-Fe complex (Römheld and Marschner, 1986). The uptake of PS-Fe complexes has been shown to be affected by sulfur supply in Fe-deficient barley plants (Astolfi et al., 2006). Only extreme sulfur starvation resulted in a decrease in PS exudation, however, even at moderate (60 µmol L^{-1}) sulfur supply, uptake of PS-Fe complexes was reduced by up to 40% relative to optimal (1200 µmol L^{-1}) sulfur supply. As with other grass species, rice is also capable of direct uptake of Fe(III)-PS complexes. However, it has been reported that some rice species are capable of direct uptake of Fe^{2+} in a unique uptake system different from other graminaceous and non-graminaceous species (Ishimaru et al., 2006). This may provide an additional mechanism for rice plants to survive in submerged calcareous soils, where under the anoxic conditions high concentrations of soluble Fe^{2+} can occur. Increased PS exudation has also been reported to occur under Zn-deficient conditions and have been observed to increase Zn uptake (Ishimaru et al., 2011a; Suzuki et al., 2008). Uptake of both the free Zn cation and the undissociated Zn-PS complex has been reported (Suzuki et al., 2008; von Wirén et al., 1996).

4.3 Reductants

4.3.1 Remobilization of Fe

The highly negative cell walls of the root apoplast can serve as a sink for cationic nutrients. Up to 75% of Fe in roots has been reported to be contained in the apoplast, and therefore it may act as an important pool of Fe, which can be remobilized under Fe-deficient conditions (Bienfait et al., 1985). Jin et al. (2007) reported that phenolics exuded by Red Clover roots may play an important role in remobilization of apoplastic Fe. In the presence of phenolics, a significant decrease in the apoplastic content of Fe was observed, along with regreening of the plant leaves. When the phenolics were removed, reutilization of the apoplastic Fe was almost completely inhibited. Phenolic removal also resulted in an increase in root ferric chelate reductase activity, a process normally induced by Fe deficiency in strategy I plants such as clover. However, in rice plants, exudation of phenolics also appears to play a role in remobilization of precipitated apoplastic Fe, and rice strains that possess the phenolic efflux transporter were able to grow better in calcareous soils than mutants without the phenolic transporter (Ishimaru et al., 2011b). Rodriguez-Celma et al. (2013) also found that exudation of phenolic compounds (by *Arabidopsis thaliana* and *Medicago truncatula*), such as flavins, increases Fe uptake during low Fe bioavailability.

4.3.2 Reduction and dissolution of nutrient containing minerals

Phenolic compounds have also been shown to enhance nutrient availability through mineral dissolution. Under Fe-deficient conditions, alfalfa plants release an isoflavonoid which displayed a capacity to dissolve poorly available ferric phosphate 62 times faster than exudates produced under Fe-sufficient conditions, thus increasing the availability of both P and Fe (Masaoka et al., 1993). Caffeic acid released by Fe-deficient tomato plants can also solubilize Fe from insoluble sources (Olsen et al., 1981). Furthermore, the oxidized products of caffeic acid have been shown to form both soluble and insoluble complexes with Fe(III) and Fe(II), which may further enhance Fe availability (Deiana et al., 2003). The ability of phenolics to dissolve Fe- and Al-phosphates could make them an important factor in influencing P supply in acidic soils containing high amounts of Fe and Al minerals (Northup et al., 1998).

Flavonoid addition to vivianite (a hydrated iron phosphate mineral) suspensions resulted in release of both Fe and P, although not as efficiently as citrate (Tomasi et al., 2008). However, the flavonoids also decreased microbial respiration and citrate mineralization. Therefore, in addition to directly increasing nutrient mobilization, flavonoids may also indirectly increase nutrient mobility through a synergistic effect with co-exuded LMWOA by reducing the mineralization of the LMWOA and hence increasing their persistence in the rhizosphere (Tomasi et al., 2008; Weisskopf et al., 2006). Other studies have suggested that exuded reductants do not play a significant part in plant-induced Fe reduction, contributing less than 7%, with ferric chelate reductases being much more significant (Schmidt, 1999; Wei et al., 1997; Zheng et al., 2003). Therefore, reductants (particularly phenolics) may primarily be exuded as part of a synergistic mechanism for nutrient acquisition with LMWOA acting as the primary nutrient mobilizers and reductants (phenolics) acting to lessen LMWOA mineralization.

4.3.3 Coumarins

Recently, it has been shown that the exudation of coumarins under Fe deficiency could improve Fe uptake and limit chlorosis (Fourcroy et al., 2014). Coumarins may improve Fe availability through chelation of Fe(III) and subsequent transport to the root ferric chelate reductase or through reduction of Fe(III) to the more soluble Fe(II) (Fourcroy et al., 2014; Mladěnka et al., 2010). It has been postulated that coumarins, upon release, may undergo modifications in the root apoplast to derivatives which are effective in Fe(III) chelation and reduction, such as esculetin (Schmid et al., 2014).

4.4 Flooding

Flooding may occur as part of normal agricultural practice of a farming system, such as with rice production, or as an adverse effect of soil compaction and high precipitation. Flooding has a significant impact on plant production.

In the 1970s, 12% of the world's soils were estimated to be waterlogged at some point in time, while flooding events are expected to increase as a result of climate change, especially in Asia and Europe (Arnell et al., 2014). Waterlogging of soils exerts a large influence on plant nutrient uptake, either directly, via inhibition of uptake, or indirectly, via changes in the bioavailability of nutrients. Waterlogging changes a host of soil parameters, including redox potential (Eh), pH and oxygen availability.

Through the filling up of pore spaces with water, the diffusion of O_2 is inhibited. As the roots and microorganisms in the soil consume oxygen through their metabolism, oxygen is depleted, and the redox potential decreases. The reduction of soil compounds consumes H^+-ions so that pH increases, most notably in poorly buffered soils. While nutrients having just a single oxidation state (e.g., K, Na) are influenced only by pH, nutrients having multiple oxidation states (e.g., N, Fe, Mn, S) are strongly influenced by redox status (Husson, 2012).

Upon lowering of Eh, these nutrients are reduced in a well-established redox cascade either chemically or through the metabolic activity of anaerobic microorganisms (Laanbroek, 1990). Solubility generally increases upon reduction, but this is dependent on pH. For example, upon waterlogging, Mn availability reaches toxic levels in acidic soils, while a shortage is induced in alkaline soils (Husson, 2012). Analogously, ferrous iron toxicity is known to be a serious problem in rice farming, but only on acidic soils (Jackson, n.d.). SO_4^- is reduced to the gaseous compound H_2S. This is extremely toxic to plants, but as H_2S is only formed on very low Eh, this mostly only occurs after prolonged periods of flooding. The combined effects of pH and Eh on the bioavailability of a number of nutrients is depicted in Figure 10 (adapted from Husson [2012]). Waterlogging increases diffusion rates of soluble nutrients, even further increasing their availability on the short term. On the long term, however, availability of soluble nutrients may decrease as a result of leaching.

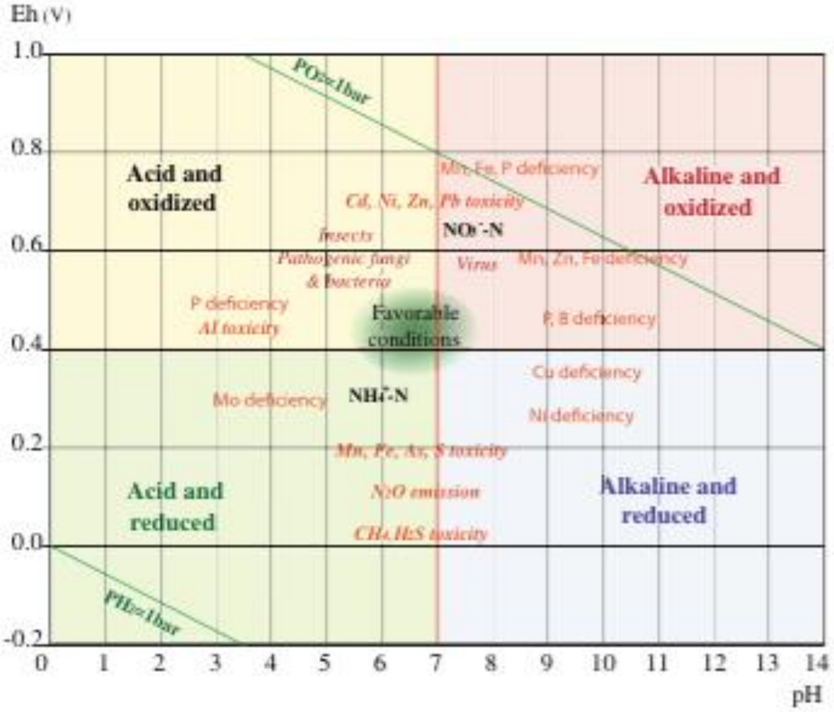

Figure 10. *Synthesis of agronomic constrains and estimation of optimum conditions of plant growth depending on soil Eh and pH (adapted from Husson [2012])*

While waterlogging generally increases nutrient availability, the capacity to acquire these nutrients dramatically decreases if the rhizosphere becomes anoxic. As a result of oxygen shortage and associated metabolic changes, ion transport systems are down-regulated and the plasma membrane is depolarized (Elzenga and van Veen, 2010). This depresses nutrient uptake while increasing leakage of ions, especially K+ (Elzenga and van Veen, 2010). In barley and wheat, waterlogging results in a severe shortage of K+, and N, P, Mg, Cu, Zn and Mn concentrations decrease (Hocking et al., 1987; Steffens et al., 2005). These shortages may be even more important in inhibiting growth than formation of toxic compounds in waterlogged soils (Steffens et al., 2005). Nutrient shortage may, however, be avoided by foliar nutrient application, as has been shown for Barley (Pang et al., 2007).

Most plant species that are not adapted to waterlogging exhibit symptoms such as wilting or leaf senescence. Plant species adapted to flooding or waterlogging, such as rice, outgrow the water and form specialized oxygen-conductive tissue (aerenchyma), which enables them to oxygenate their rhizosphere. As a result, rice is able to diminish most of the negative effects of waterlogging. In addition to higher root uptake, reduced compounds such as H_2S are oxidized upon entering the rhizosphere, thus neutralizing their toxic effects (Nishiuchi et al., 2012). The group of plants that can form aerenchyma also includes other important crop species such as sugarcane and maize.

5 Effect of nutrient deficiency on exudates

The adaptive reactions of plants in response to nutrient deficiency involve both morphological and metabolic changes. Examples of responses that increase the uptake capacity are an increase in the root/shoot-ratio, increased numbers and length of root hairs and the increased capacity of plasma membrane transporters. Another important factor is the water content of the soil as nutrient uptake depends on its amount in the soil solution, its transport to the root surface by mass flow and diffusion, followed by its uptake into the root.

The changes mentioned above primarily serve the goal of increasing the potential uptake capacity, but plants are also able to increase the actual bioavailability of many nutrients with their root exudates. This chapter will discuss the outcome of the meta-analysis that was done on all available literature data, on root exudation in relation to plant species and cultivars and nutrient availability that has been carried out. To collect the articles, both the search engines “Scopus” and “Web of Science” were used, where all relevant data were extracted from these articles. The data will be discussed per nutrient and is mainly focussed on OA and PS, and there will be special attention for differences between species and among cultivars of the same species.

5.1 Iron deficiency and root exudates

Iron is a trace element essential for the growth of almost all forms of life, including plants, due to its necessity in photosynthesis and its role as a co-factor in enzyme activity. Even though Fe is one of the most abundant elements on earth, its bioavailability is limited because of its low solubility in the environmentally relevant pH range. Therefore, Fe deficiency is common in most plants, particularly on calcareous soils. Crops grown on such soils show distinct Fe deficiency symptoms such as chlorosis (Marschner, 1995). As a result of this common stress, plants have evolved different mechanisms to solubilize and absorb iron from soil by their root exudates (Lemanceau et al., 2009). Several studies have shown that Fe deficiency increased the level of specific OA in the roots of pea, bean, apple tree and cucumber plants among others (Landsberg et al., 1981; de Vos et al., 1986; Sun et al., 1987; Rabotti et al., 1995), suggesting also that the levels of OA in the root exudates are upregulated upon Fe deficiency. Strategy II plants are able to increase the bioavailability of Fe through the excretion of phytosiderophores (PS) (Lemanceau et al., 2009). The excretion of phenolic compounds is known to be increased in response to iron deficiency, although Treeby et al. (1992) show that not all plant species respond this way. In this study, we mainly focused on the relation between the bioavailability of Fe and its influence on the OA and PS levels in root exudates.

5.1.1 Iron deficiency and Phytosiderophores

Figure 11a-c shows the relative change in PS exudation of several plant species (each with their own color) and their cultivars, grown at different Fe (offered as Fe-EDTA) concentrations. The change in exudation level at the Fe deficiency treatment was made relative to the exudation level at the non-deficiency treatment and presented on a log2-scale. This means that Figure 11a shows the average change in the PS exudation level at 0 µM Fe of each plant species/cultivar relative to the LMWOA exudation level at 100 µM Fe of that same plant species/cultivar (of the same study). The data is presented on a log2-scale because of the large differences between the plant species/cultivar in the magnitude of their response but also to give a better insight in the possible down regulation of in this case PS exudation.

The data was extracted from multiple studies (n=12), in which the growth conditions and the way PS exudation was expressed differed per study. For this reason, the PS exudation in the Fe deficiency treatment was made relative to the PS exudation under control conditions of that same study. For example, Figure 11a shows the exudation data of plants grown at 0 µM Fe relative to 100 µM on a $\log_2$ scale (Chaignon et al., 2002; Römheld et al., 1990; Zhang et al., 1998; Yousfi et al., 2009; Suzuki et al., 2008; Ma et al., 1996; Wulandri et al., 2014). This figure shows that the exudation response can differ significantly between plant species and even between cultivars from the same species. The different barley (brown) cultivars had a large PS exudation response under Fe deficiency, whereas the relative increase in PS exudation was much lower in the Sorghum cultivars (grey). The response to Fe deficiency is much less clear for the different wheat cultivars. Where “songlen” exuded less PS under 0 µM Fe in comparison to 100 µM Fe, the “nourin 61” cultivar showed the largest relative increase. From the data in Figure 11a, we can conclude that most species exude more PS when grown under Fe deficiency, but that the relative increase differs per species and that even the response of cultivars can differ strongly for a single species.

Figure 11b shows an interesting difference between wheat and barley: the barley cultivars consistently have a larger relative increase in PS exudation in comparison to the wheat cultivars (Kudo et al., 2013; Alam et al., 2008; Pedler et al., 2000; Erenoglu et al., 1996). The most interesting difference between species is shown in Figure 11c (Brown et al., 1991). These data show that oat had a clear response to Fe deficiency, but that corn did not show any changes in PS exudation, and moreover corn had an extremely low PS exudation (maximum was 0.02 mg/L). These findings are in contrast to data in Figure 11a showing that certain other corn cultivars are able to exude PS, since the “garbo” cultivar showed a clear response (with a maximum of 2 µmol PS gram root dry weight^{-1} day^{-1}).

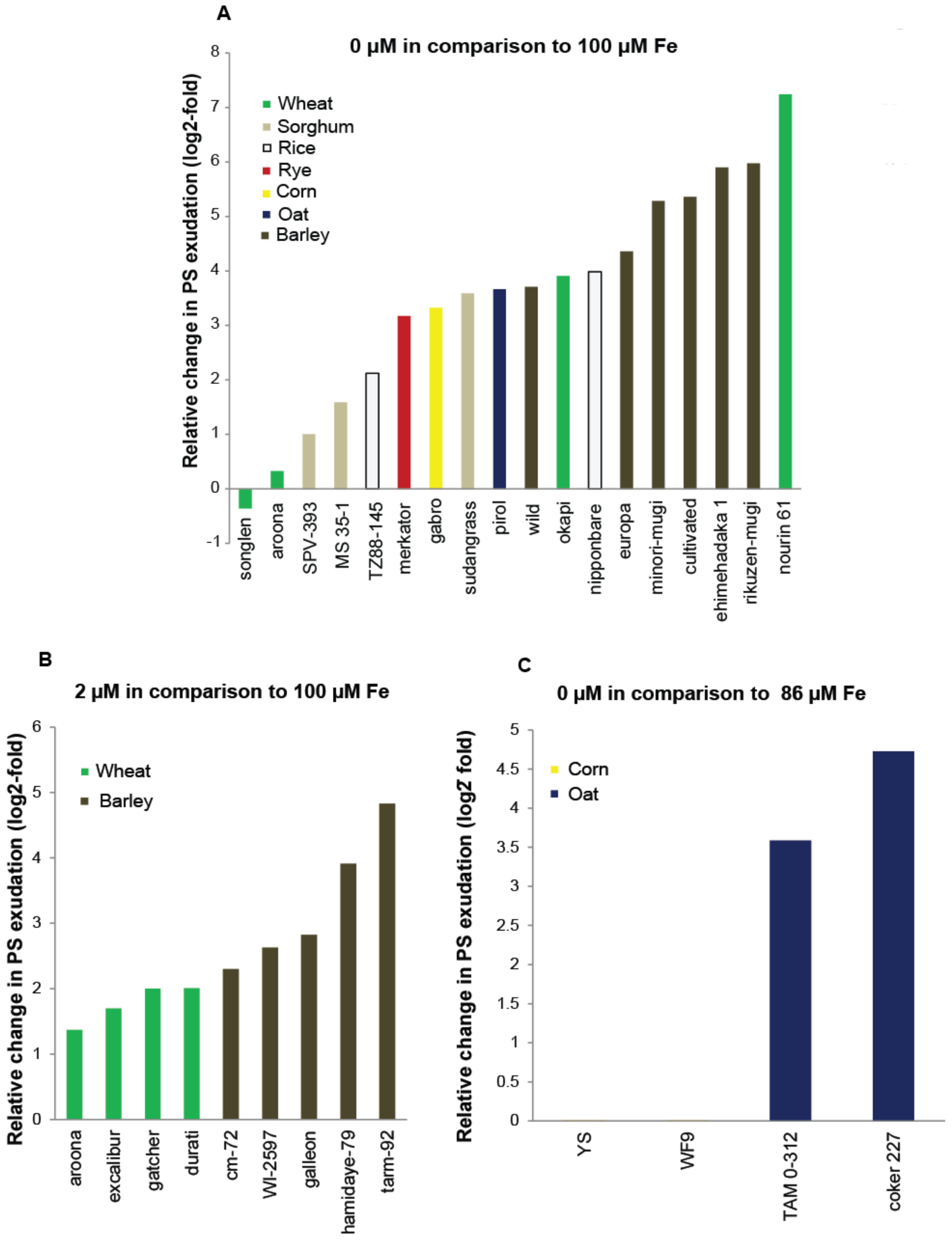

Figure 11. *The relative change in PS exudation for several plant species and their cultivars*

(Chaignon et al., 2002; Römheld et al., 1990; Zhang et al., 1998; Yousfi et al., 2009; Suzuki et al., 2008; Ma et al., 1996; Wulandri et al., 2014; Kudo et al., 2013; Alam et al., 2008; Pedler et al., 2000; Erenoglu at al., 2008; Brown et al., 1991).

Figure 12 shows the relationship between the relative change in Fe concentration in the plant and the relative change in PS exudation (Erenoglu et al., 1996; Yousfi et al., 2009; Kudo et al., 2013; Suzuki et al., 2006; Pedler et al., 2000; Brown et al., 1991; Chaignon et al., 2002). Here, the data were also expressed relatively because the studies presented their results differently and were performed under different conditions. Based on these data, there is not a strong linear correlation between the relative change in Fe concentration of the plant and the relative change in PS exudation (R^2=0.272). Even within the species, there was no clear correlation. However, there were some striking conclusions that could be made from this figure: (i) barley seems to have, on average, a large relative PS response, already at lower changes in the plant Fe, especially the cultivars “minorimugi” and “Ehimehadaka no. 1”; (ii) the oat cultivars surprisingly showed a very diverse pattern since all data were from only two different cultivars (Coker 227 and TAM 0-312); and (ii) corn did not show any response independent of the plant Fe concentration.

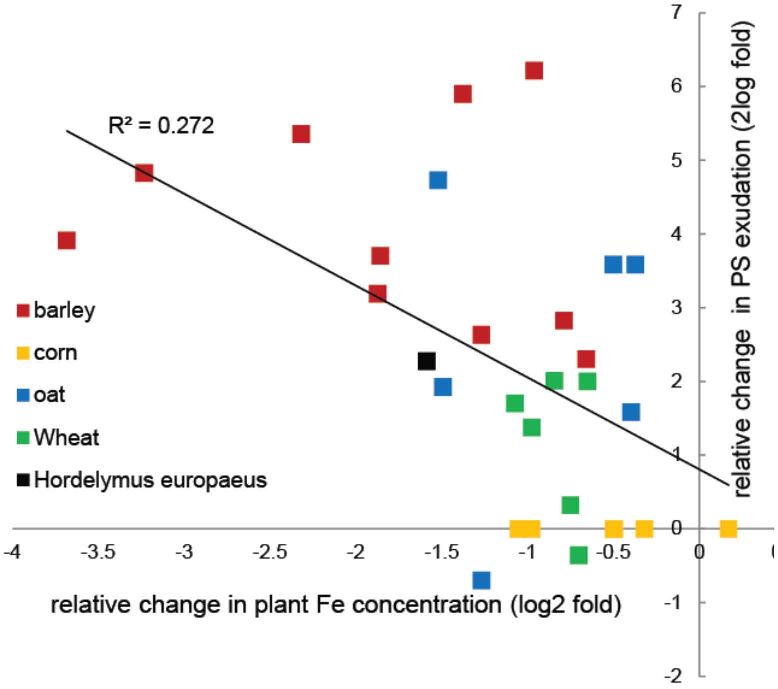

Figure 12. *The relative change in the Fe concentration of the plant against the relative change in PS exudation for several plant species and their cuftivars*

(Erenoglu et al., 1996; Yousfi et al., 2009; Kudo et al., 2013; Suzuki et al., 2006; Pedler et al., 2000; Brown et al., 1991; Chaignon et al., 2002)

Figure 13 shows the absolute data from Rengel et al. (2000) on the PS exudation of different wheat cultivars when grown under no Fe conditions in relation to the total dry weight. The figure shows a clear divide: the exudation rate of one-half of the cultivars is around 100-150 nmol PS $3h^{-1}$ $plant^{-1}$ and that of the other half around 250-300 nmol PS $3h^{-1}$ $plant^{-1}$. These differences in the PS exudation rate did not result in a change in shoot dry weight. When Fe is the growth limiting factor in the presence of an Fe source (e.g., soil Fe or FeEDTA), differences in dry weight could be expected because PS can enhance the bioavailability of Fe. However, in absence of an Fe source (i.c., no addition of FeEDTA), no positive effect from higher PS levels is expected because, in this case, Fe availability remains unaffected by PS exudation. The exudation rate is merely a measure for the stress response under extreme Fe deficiency stress. Therefore, no correlation could be made between de-exudation pattern and shoot biomass.

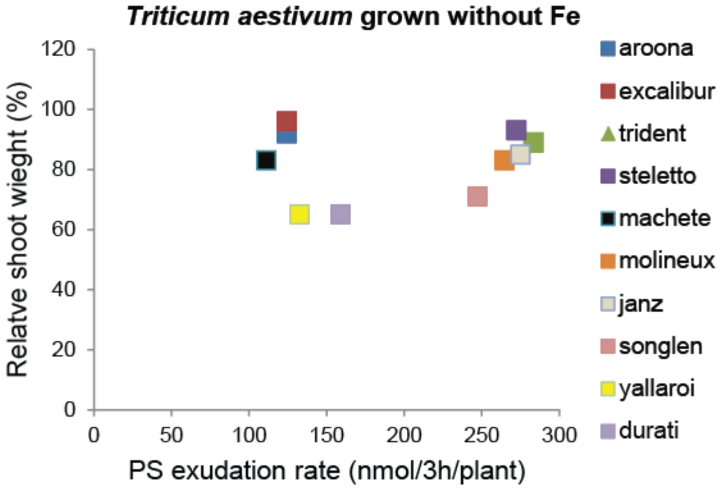

Figure 13. *The PS exudation rate of different cultivars of Triticum aestivum against the relative change in shoot biomass (Rengel et al., 2000)*

5.1.2 Iron deficiency and Low Molecular Weight Organic Acids

Figure 14 shows the relative change in the exudation of LMWOA upon Fe deficiency (El-Baz et al., 2004; Carvalhais et al., 2011; Martinez-Cuenca et al., 2013; Zocchi et al., 2007). These data show that corn, orange tree, soybean and cucumber can increase the exudation of OA under Fe deficiency. Interestingly, the two cucumber cultivars show a big difference. Whereas the “beit alpha” cultivar showed a negative response to LMWOA exudation, the “dotto” cultivar had a largely positive response. Although the amount of data is limited, these data are in line with earlier studies demonstrating that organic acid levels not only increase within the roots due to Fe deficiency, but also in the rhizosphere of the root.

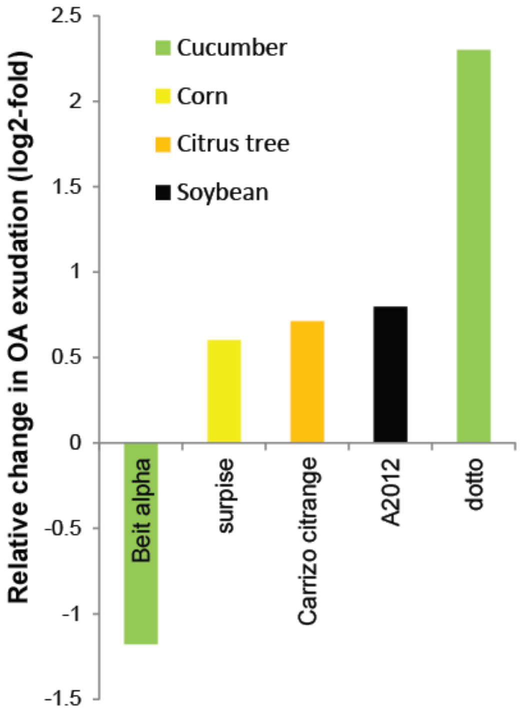

Figure 14. *The relative change in LMWOA exudation of different plant species as a result of Fe deficiency*

(El-Baz et al., 2004; Carvalhais et al., 2011; Martinez-Cuenca et al., 2013; Zocchi et al., 2007)

5.1.3 Iron deficiency and reductants

Another group of exudates that have not been discussed in this chapter up to this point are the reductants such as phenolic acids. Several studies have shown that cucumber, soybean and red clover induce the exudation of phenolic acids and increase the reductant activity upon Fe deficiency (Zheng et al., 2005; Zocchi et al., 2007; El-Baz et al., 2004). Table 3 shows that different citrus trees increase their phenolic exudation rate and their reductase activity upon Zn deficiency, but also that the magnitude of the response differs per tree species. The big exception is the *Citrus sinensis* and *Poncirus trifoliata* hybrid, which did not show increase in phenolic exudation but did have an increased reductase activity upon Zn deficiency.

Table 3. *The phenolic exudation rate and reductase activity of different Citrus trees at different Fe levels*

(Treeby et al., 1992)

Species	Cultivar	Fe (µM)	Phenolics (nmoles/g root DW/h)	Reductase Activity (nmoles/g root DW/h)
Citrus jambhiri	Lush	10	0.53	30.56
Citrus jambhiri	Lush	0	2.8	110.74
Citrus reticulata	Blanca	10	1.25	20.36
Citrus reticulata	Blanca	0	2.2	52.43
Citrus aurantium		10	1.62	22.55
Citrus aurantium		0	2.3	123.86
Citrus sinensis	Osbeck	10	0.87	51.7
Citrus sinensis	Osbeck	0	1.53	85.23
C. sinensis x P. trifoliata hybrid		10	1.31	84.5
C. sinensis x P. trifoliata hybrid		0	1.22	150.1
Poncirus trifoliata	Cooper	10	0.63	58.99
Poncirus trifoliata	Cooper	0	1.01	150.83

Based on these data, it can be concluded that most of the (tested) species showed a clear increase in the exudation of PS, phenolics or OA in response to Fe deficiency stress. Based on the functions of these compounds, it can be expected that these differences would lead to variations in biomass or plant Fe concentrations between plant species and/or cultivars as well. However, the data did not show a clear pattern between exudation and Fe status of the plant (Fig. 12). Also, there was no clear correlation between the Fe status of the plant and the relative change in biomass (Fig. 13 & data not shown). One explanation could be that other factors are as important such as root hair length or the amount of Fe transporters, which were not included in this study, or the different experimental conditions under which the studies were conducted.

5.2 Zinc deficiency and root exudates

Zinc is an important component of many essential plant molecules such as certain proteins, enzymes and membrane lipids. Zn is the most common micronutrient deficiency in crops, especially in alkaline and calcareous soils (Alloway, 2008). Zn is taken up from the rhizosphere by the plant root system as Zn^{2+} or attached to organic ligands (Grusak, 2001).The bioavailability of Zn in soil is determined by the soil solution concentration of Zn as well as by the size of the adsorbed Zn-pool that can replenish the Zn concentration when it is taken up. Soil parameters that influence the bioavailability of Zn include: total zinc content, pH, organic matter content, clay content, calcium carbonate content, phosphate content and redox conditions. The low bioavailability of Zn in alkaline soils is, thus, the result of low concentrations of free Zn^{2+}. Due to its crucial role in important cellular molecules, shortage of Zn causes various symptoms such as chlorosis, small thin stems, reduced leaf size, decreased growth, root apex necrosis, internode shortening and bronze leaf coloring (Hacisalihoglu et al., 2003).

Plants have several mechanisms to cope with or relieve Zn deficiency, such as changes in root morphology, mycorrhizal associations, differences in the uptake and translocation of Zn or simply a more efficient use of Zn by the plant (Cakmak et al., 1996). Rye, pea and carrot species tend to be Zn-efficient (more effectively function under low soil Zn conditions), while Zn-inefficient species include wheat, maize, bean, rice and tomato species

(Hacisalihoglu et al., 2003). There are also variations in efficiency between different cultivars of the same species, which is probably based on a difference in both physiological and molecular mechanisms of Zn acquisition (Hacisalihoglu et al., 2003; Cakmak, 1997). Reid et al. (1996) suggest that Zn uptake at concentrations above 0.5 µM (nutrient-sufficient conditions) is dominated by a low-affinity system, whereas plants growing under nutrient-limiting conditions (e.g., natural soils) would depend on a high-affinity system. The exact mechanism of this regulation is largely unknown; however, an abundance of Zn (500 µM) repressed the expression of a high-affinity divalent metal ion transporter in *Arabidopsis thaliana* (Connolly et al., 2003), supporting the notion of non-high affinity transport of Zn under non-limited conditions.

Similarly to Fe, root exudates play a crucial role in regulating the bioavailability of Zn, which is not only increased by a decrease in pH, but also potentially by the exudation of LMWOA and/or PS. This study will focus on the relation between Zn availability in the soil and the exudation patterns of several species. Figure 11a shows the change in PS exudation when wheat and barley plants are grown at 0 Zn level relative to 1 µM Zn (Cakmak et al., 1996; Chaignon et al., 2006; Erenoglu et al., 1996). These data show that the response in PS exudation differs greatly among wheat cultivars. Where “kunduru 1149” and “songlen” show a negative response, “kirag-66” shows a large increase of PS exudation upon Zn deficiency (an 11.5-fold increase). The results presented in Figure 15a do not show a clear distinction between wheat and barley cultivars. In Figure 15b, which presents data from a study in which different wheat and barley cultivars were grown at both 8 µM and 0.2 µM Zn (Pedler et al., 2000), a clear difference between wheat and barley can be seen. These data show that the PS exudation response was larger for all of the barley cultivars when compared to the wheat cultivars, although the differences are small. Since all the data was from one single study, a comparison can be made between the absolute data. These data show that, although the response of the wheat cultivar was lower, the PS exudation was higher in comparison to the barley cultivars (3.75 µmol/g root dry weight and 9.33 µmol/g root dry weight, respectively). When corrected for the root dry weight, the average PS exudation was 0.69 µmol/plant for barley and 1.41 µmol/plant in wheat. So, although barley had a larger relative response to Zn deficiency, the absolute exudation levels, which are of bigger biological relevance, were higher for wheat. These absolute values can, however, not be compared between studies.

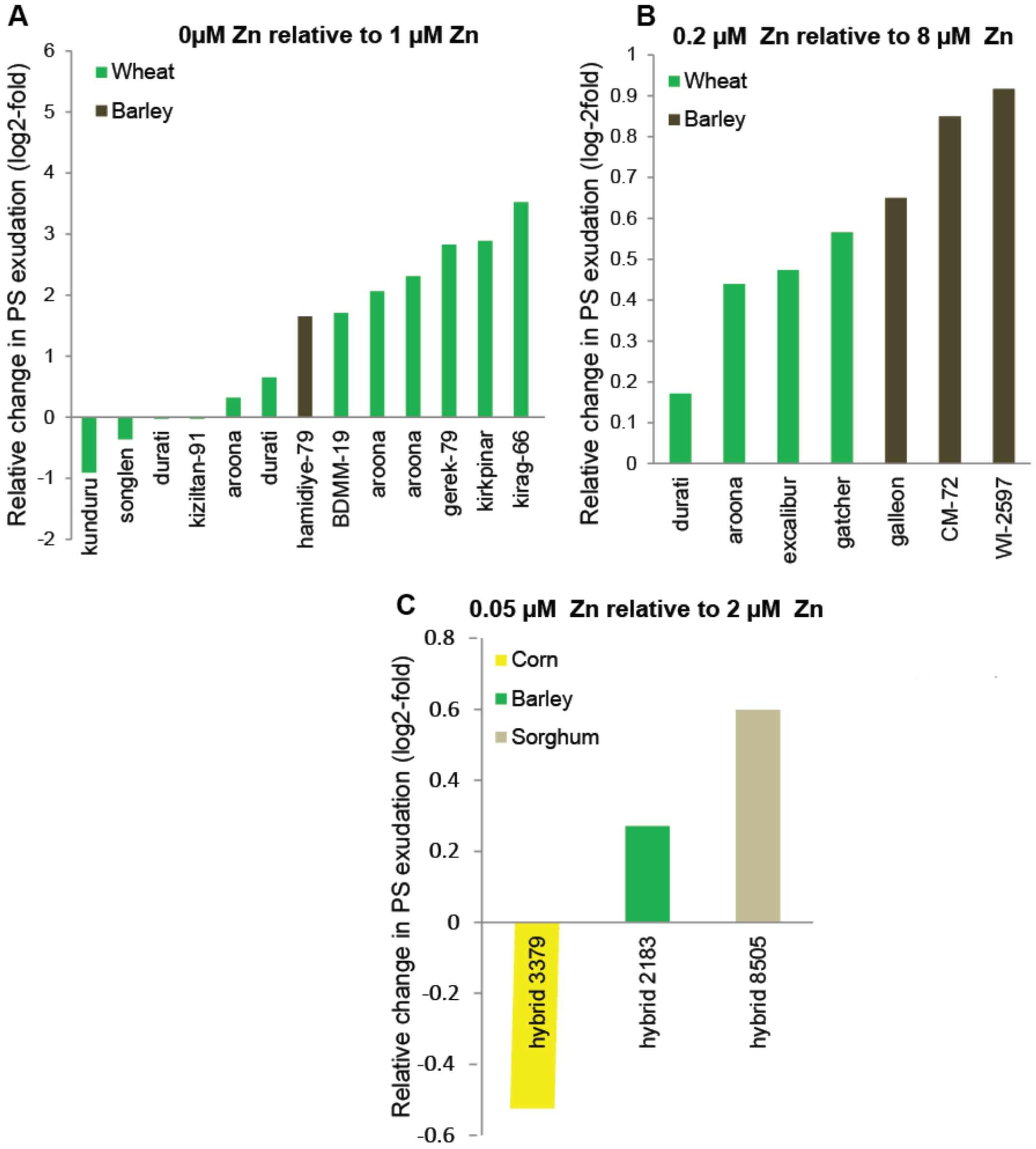

Figure 15. *The relative change in PS exudation of several plant species and their cultivars in response to Zn*

(Cakmak et al.,1996; Chaignon et 81.,2006; Erenoglu et 81.,2008; Pedler et al., 2000; Hopkins et al., 1998)

Figure 15c shows the data from Hopkins et al. (1998) and indicates that the response differs more strongly between corn, which had a negative response in terms of PS exudation rates in comparison to the positive response of wheat and sorghum. The response here was also quite limited when compared to Figure 15a. The main reason for these differences could be the fact that the treatment of Figure 15a compares 0 μM Zn, which is less Zn than the other studies, and thereby induces a stronger response.

Work by Broadley et al. (2009) showed that rice exudes PS, but there is almost no exudation response to Zn deficiency. In contrast, as shown in Figure 16a, there is a response in the exudation of LMWOA, in this case malate, by different rice cultivars (Gao et al., 2009). The exudation rate of most cultivars is around 35 nmol/g root dry weight/s, except for "hongke", which had a higher exudation rate of 53 nmol/g root dry weight/s in the 0 Zn treatment. Unfortunately, the root dry weight data was not available, so the absolute PS levels per plant could still differ between the rice cultivars. Figure 16b shows data from a field study (Hoffland et al., 2006) in which citrate and oxalate were measured. This study also shows that there are differences among rice cultivars in their response to Zn deficiency. The "507"cultivar showed the largest relative response, mainly caused by a large increase in oxalate.

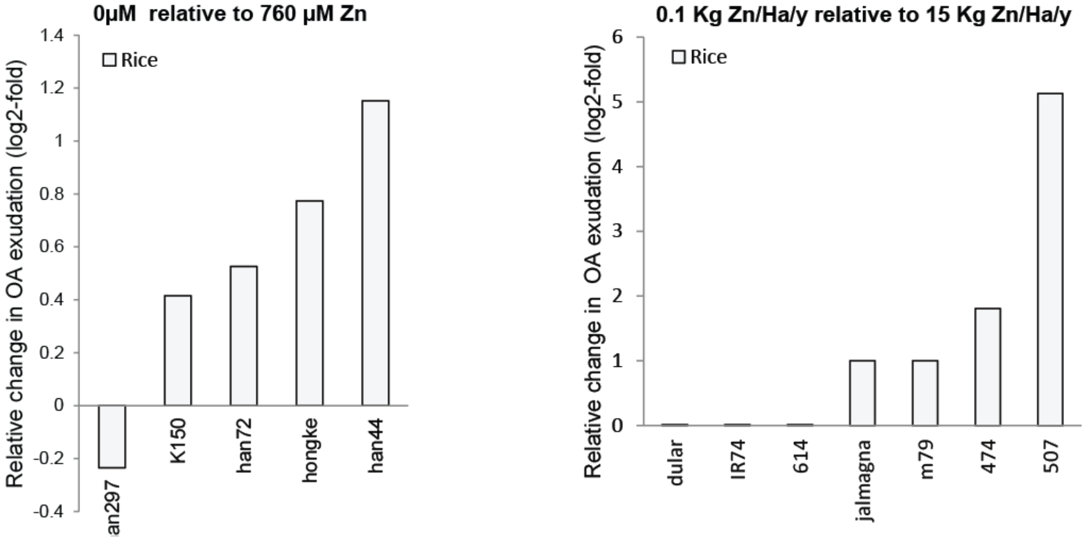

Figure 16. *The relative changes in OA exudation of different rice cultivars as a result of Zn deficiency (Gao et al., 2009; Hoffland et al., 2006)*

The overall data demonstrate differences between species and among cultivars in their relative LMWOA and PS exudation responses upon Zn deficiency. The next step would be to obtain more insight into the relationship between Zn status in the plant and the exudation rate. Figure 17 shows the correlation between the relative change in the plant's Zn concentration and the relative change in OA or PS exudation (Pedler et al., 2000; Erenoglu et al., 1996; Hopkins, 1998; Gries et al., 1998; Chaignon et al., 1996; Broadley et al., 2009). Interestingly, there seems to be a linear correlation between these two factors (independent of the species), except for two outliers: corn and the barley cultivar "tarm-92" (R^2=0.6343 without these two outliers).

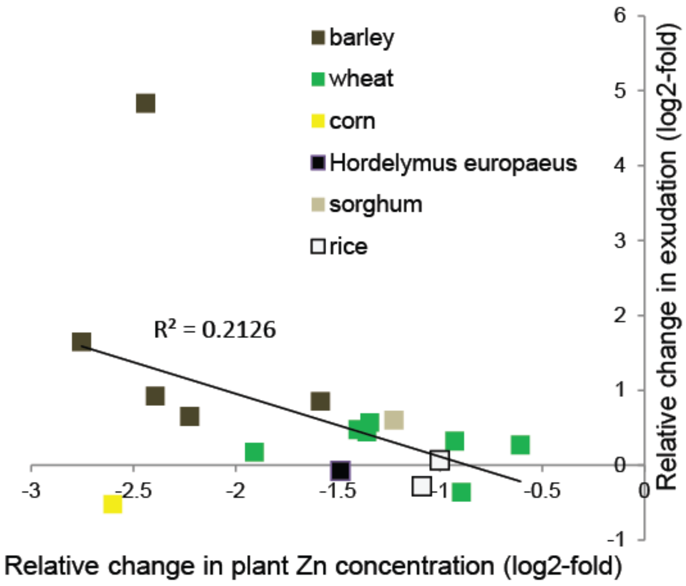

Figure 17. *The relative change in the Zn status of different plant species and their cultivars against the relative change in PS exudation*

(Pedler et al., 2000; Erenoglu et al., 1996; Hopkins, 1998; Gries et al., 1998; Chaignon et al., 1996; Broadley et al., 2009)

Another very interesting linear correlation can be found in Figure 18, which shows the PS exudation rate against the relative shoot dry weight of different wheat cultivars (Rengel et al., 2000). These data could suggest that in wheat, an increase in the PS exudation rate possibly leads to a higher biomass under Zn deficiency conditions (0 µM), or that the more Zn efficient cultivars exude more PS because of their larger biomass. This is in contrast to Fe deficiency of the same study where there was no correlation between PS exudation and shoot biomass (Fig. 13). No linear correlation was found for LMWOA exudation and plant biomass (data not shown), possibly due to the limited amount of data and/or the fact that not all LMWOA were measured in these experiments.

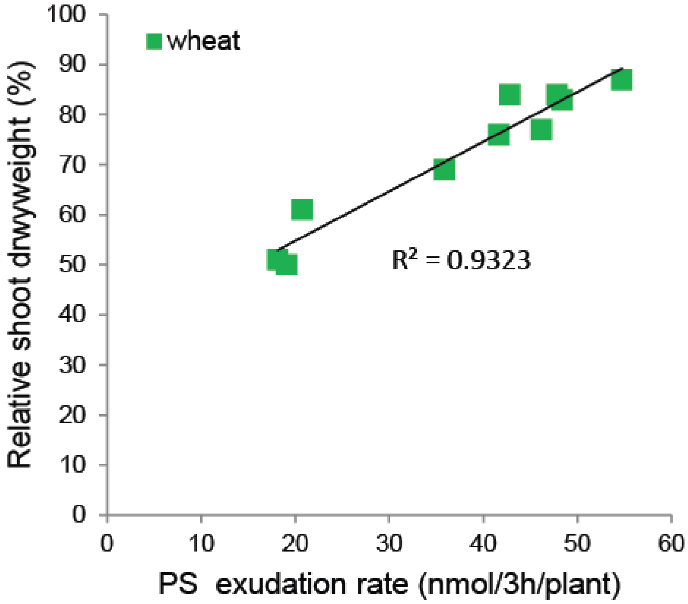

Figure 18. *The PS exudation rate of different wheat cultivars against the relative change in shoot dry weight (Rengel et al., 2000)*

Hence, also for Zn there are differences between and within plant species in their exudation response to Zn deficiency, where wheat, barley and rice show increase, while corn shows decrease, in their PS exudation. Based on Figure 18 showing a positive correlation between PS exudation rate and plant biomass under Zn deficiency, it can be assumed that corn would be intolerant to Zn deficiency. It should, however, be added that there are mechanisms other than PS exudation by which plants can cope with Zn deficiency. Although it was not included in one of the graphs, Zheng et al. (2005) showed that red clover had lower root reductase activity at low Zn levels.

5.3 Manganese deficiency and root exudates

Manganese is needed in plants for chloroplast formation and photosynthesis, nitrogen metabolism and synthesis of various enzymes. Manganese in soils is present in three oxidation states: Mn^{2+}, Mn^{3+} and Mn^{4+}, of which Mn^{2+} is the primary form of absorption by plants. The rapid uptake of Mn is reversible and non-metabolic, with Mn^{2+} and Ca^{2+} or other cations being freely exchanged in the rhizosphere. The other way is not as fast, with Mn^{2+} being less readily exchanged. The uptake is dependent on plant metabolism, although the exact mechanisms are not clear. Manganese availability is optimal at soil pH of 5 to 6.5. At lower pH, Mn may become too available to plants, leading to accumulation and toxicity. Mn deficiency symptoms (such as interveinal chlorosis) are first seen in the young leaves, because Mn is highly immobile once inside the plant (Millaleo et al., 2010). Mn deficiency increases the root length and the root/shoot-ratio as well (Gherardi and Rengel, 2004).

Occurrence of genotypical differences in tolerance to Mn deficiency is well-known. Tolerant plant species have the ability to extract greater amounts of Mn compared to intolerant ones. This differential tolerance to Mn deficiency has only been found in soils, but not in solution culture. This suggests a role of specific rhizosphere processes and dynamics of microbial rhizosphere populations in the bioavailability of Mn (Gherardi and Rengel, 2004).

The data on the effect of Mn deficiency on root exudates are limited. Table 4 shows the results of two studies by Gherardi and Rengel (2004) and Rosas et al. (2007) where they measured LMWOA in root exudates under conditions differing in Mn concentrations. These data shows that alfalfa, ryegrass and white clover exude more LMWOA when the available Mn is lower and that these LMWOA levels differ between species but also between the two Alfalfa cultivars. Interestingly, the differences in LMWOA exudation between the two cultivars at 0 Mn might explain the differences in the plant dry weight. Cultivar “Salado” exuded more LMWOA and had a higher biomass dry weight at 0 Mn in comparison to cultivar “sirosal,” whereas their dry weights were similar at 500 nM Mn. A possible explanation could be that Mn deficiency is the limiting factor for their growth and that the difference in Mn uptake as a consequence of these differences in LMWOA exudation resulted in the differences in dry weight. Strangely enough, “sirosal” did have a higher Mn plant concentration in comparison to “salado,” although the total amount of Mn taken up was not different (approximately 11 µg). These results imply that there are other (possibly LMWOA related) factors involved, resulting in the difference in dry weight between these two Alfalfa cultivars at 0 Mn. Although it was not included in one of the graphs, Zheng et al. (2005) showed that red clover had lower root reductase activity at 0 Mn levels.

Table 4. *LMWOA exudation rates, dry weight (DW) and Mn concentration in alfalfa, rye grass and white clover plants under different Mn conditions*

(Gherardi and Rengel 2004; Rosas et al., 2007); ND= not detected.

Species	Cultivar	Mn Treatment (nM)	Total LMWOA (µmol/g root DW/h)	Biomass (g DW/plant)	Mn in Plant (µg/g DW)
Alfalfa	Salado	0	647.78	1.09	9.8
Alfalfa	Salado	5	446.06	1.76	29
Alfalfa	Salado	500	336.97	2.12	252
Alfalfa	Sirosal	0	406.52	0.77	14.2
Alfalfa	Sirosal	5	272.49	1.3	32
Alfalfa	Sirosal	500	249.64	2.12	334
Ryegrass	Nui	0	1.14	0.09	90
Ryegrass	Nui	2,400	0.5	0.15	187
White clover	Huia	0	2.14	0.09	136
White clover	Huia	2,400	0.8	0.12	ND

Table 5 shows the results of two studies by Gries et al. (1995, 1998), where exudation of PS by barley under different Mn conditions was measured. The “CM72” cultivar showed a clear increase in PS exudation when grown at no Mn addition, in comparison to 0.6 µM Mn, whereas “Harz” had no increase in PS exudation. Interestingly, the “Harz” cultivar showed a larger decrease in biomass due to Mn deficiency than the “CM 72” cultivar (40% against 70%). This implies that, besides OA differences in PS, exudation rate can also influence the tolerance for Mn stress.

Table 5. *The PS exudation rates, dry weight (DW) and Mn concentration in Barley cultivars under different Mn conditions (Gries et al., 1995, 1998)*

Mn concentration was not determined in the CM 72 cultivar.

Species	Cultivar	Mn treatment (µM)	PS (µmol/g root dw/2h)	DW	Mn (µmol/g root dw/2h)
Barley	Harz	0.6	2.22	0.46 (g DW/plant)	1.16
Barley	Harz	0	2.4	0.14 (g DW/plant)	0.19
Barley	CM 72	0.6	1.33	100 (%)	-
Barley	CM 72	0	2.43	60.53 (%)	-

5.4 Copper deficiency and root exudates

Copper is essential for its role in photosynthesis, respiration, ethylene perception, reactive oxygen metabolism and cell wall remodeling. However, Cu can also be very toxic at relatively low levels. Redox cycling between Cu^{I+} and Cu^{2+} can also catalyze the production of highly toxic hydroxyl radicals and can cause displacement of other essential metals in proteins. Typically, symptoms of deficiency start when Cu decreases below 5 pg/g biomass DW, while toxicity levels are observed above 20 pg/g biomass DW (Burkhead et al., 2009). This threshold of Cu concentrations associated with healthy plant development is much lower than for other nutrients, such as Fe or Zn (e.g., Wong and Bradshaw, 1982). Symptoms of Cu deficiency include decreased growth rate, chlorosis in young leaves, a decrease in fruit formation and lower seed viability. Excess Cu concentrations in the soil tend to decrease root growth before shoot growth because of preferential Cu accumulation in the roots and the induction of chlorosis in vegetative tissue. Copper toxicity can also reduce Fe uptake, even to the point of deficiency, depending on the form of Fe available in the soil (Marschner, 1995).

Soils with a high pH or soils with a high level of soil organic matter have lower levels of bioavailable Cu. Cu enters the cytosol of root cells trough specific transporters in the cell membrane, which can be negatively regulated by Cu. These proteins transport Cu in a reduced form (Cu(I)), whereas most copper in soils is present as Cu(II), so the reduction of Cu will likely increase its uptake. *Arabidopsis* and other dicots use root cell, surface ferric chelate reductases (FCR) to regulate root reductase activity, which is increased upon Cu deficiency (Zheng et al., 2005). Table 6 shows the PS exudation rate of two different barley genotypes grown at two different Cu levels (in combination with HEDTA) (Gries et al., 1995, 1998). These data suggest that at least the “Harz” cultivar was able to increase the PS level in its root exudates.

Table 6. *PS exudation rate, biomass and Cu concentration of two Barley cultivars at two different Cu levels (Gries et al., 1995 and 1998)*

Species	**cultivar**	**Cu** (µM)	**PS** (µmol/g root DW/2h)	**Biomass** **(%)**	**Cu** (µmol/g DW)

Barley	Harz	0	5.07	43.50	0.06
Barley	Harz	2	2.22	100	0.2
Barley	CM 72	0	0.78	33.50	–
Barley	CM 72	2	1.33	100	–

The LMWOA levels were measured by Meier et al. (2012) at various Cu levels (Table 7) in different plant species. All four species showed lower LMWOA exudation at 0.2 µM in comparison to 2.2 µM, and in the case of *O. picensis* and *I. condensata,* PS exudation increased at even higher Cu levels. These data highlight the opposite LMWOA exudation response from, e.g., Fe or Zn deficiency and could imply Cu toxicity. The metal tolerance mechanisms developed by plants is the exudation of different compounds which can modify bioavailability of metals, including Cu. Exudation of OA by roots is important in the tolerance for high metal concentrations because these compounds can also reduce the bioavailability of toxic metals in the rhizosphere through chelation. The changes in biomass suggests that at 0.2 µM Cu is a deficient level limiting plant growth (except for *H. annuus*) and does not seem to be toxic at 2,2 µM. Although not included in the table, all four species showed an increase in the exudation rate of phenolic compounds at 0.2 µM Cu in comparison to 2.2 µM and also Zheng et al. (2005) showed that red clover had higher root reductase activity at 0 Cu levels.

Table 7. *The LMWOA exudation, biomass and Cu concentrations in four plant species at two different Cu conditions (Meier et al., 2012).*

Species	**Cu** (µM)	**LMWOA** **(**µmol/g DW/h)	**Dry Weight** (mg/plant)	**Cu in Plant** (µg/g DW)
Oenothera picensis	0.2 µ	313.98	96.7	150.25
Oenothera picensis	2.2	431.57	103.5	851.74
Iimperata condensata	0.2	164.93	443.6	75.62
Iimperata condensata	2.2	178.815	577	135.32
Lupinus albus	0.2	34.98	376	75.62
Lupinus albus	2.2	48.219	462	150.25
Heliantus annuus	0.2	45.745	526.2	90.55
Heliantus annuus	2.2	48.529	412.1	180.1

5.5 Calcium deficiency and root exudates

Calcium is a crucial regulator of growth and development in plants. It plays a role in cell wall and membrane structure and in membrane permeability, which is an important factor in the uptake of other nutrients. The concentration of Ca may be very high in soil, but Ca-deficiency disorders often occur in plants. Ca is immobile in the phloem, and its transport through the plant is thereby restricted to the xylem and thus fully dependent on transpiration. Ca deficiency can cause fruit and blossom rot among other responses. Ca is a ubiquitous intracellular secondary messenger in plants in a diverse array of signals and responses, many related to plant defense against environmental cues. This means that Ca deficiency could potentially affect these responses as well (Kitano et al., 1999; Yang et al., 2011).

Considering the scant available data, there does not seem to be a role for root exudates in Ca acquisition. This is suggested from the study of Liu et al. (2011). Table 8 shows the effect of Ca availability on the exudation of different LMWOA in two rice cultivars. Although there are clear differences between these cultivars, there are no clear differences between the treatments, except for oxalic acid, which seems to increase with decreasing Ca levels. Collectively, these findings indicate that Ca deficiency does not affect the LMWOA exudation pattern of rice and therefore is not likely to be relevant in inducing root exudation of OA. Moreover, the Ca levels in the plant did not change (data not shown).

Table 8. *The level of different LMWOA (µ/g DW) of two different rice cultivars at different calcium levels (Liu et al., 2011)*

Species	Cultivar	Ca (µM)	Oxalic (µ/g DW)	Citric (µ/g DW)	tartaric (µ/g DW)	Malic (µ/g DW)	Acetic (µ/g DW)	Maleic (µ/g DW)	Succinic (µ/g DW)	Total (µ/g DW)
Rice	Yangdao 6	470	101.4	261.6	834.5	505.4	922.1	11.9	128.5	2,765.4
Rice	Yangdao 6	235	77.3	243.5	837	510.4	942.1	12.8	132.5	2,755.6
Rice	Yangdao 6	0 Ca	70.9	243.5	815.8	518.5	938.9	12.6	124.6	2,724.8
Rice	Yangjing 9538	470	555.1	132.5	813.6	501.8	232.2	18.7	82.5	2,336.4
Rice	Yangjing 9538	235	456.9	127.5	829.9	516.2	217.8	18.5	86.7	2,253.5
Rice	Yangjing 9538	0 Ca	372.4	122.5	811.4	528.8	225.6	17.7	84.5	2,162.9

5.6 Sulfur deficiency and root exudates

Sulfur occurs in the environment in a variety of oxidation states that range from sulfide (S(-II)) to sulfate (S(VI)). Under atmospheric conditions, inorganic S occurs predominantly in the form of sulfate. Sulfur dioxide (SO_2) and hydrogen sulfide (H_2S) are emitted into the atmosphere as a result of volcanic activity, decomposition of biological tissues and anthropogenic activities. Plants utilize S as sulfate (SO_4^{2-}); insufficient S nutrition reduces plant growth, crop yield, chlorophyll content and the resistance to abiotic and biotic stresses (Astolfi, 2006; Lewandowska and Sirko, 2008). On the other hand, sulfur fertilization increases pathogen resistance of plants. In the last decades, S availability in soils has become a major limiting factor for plant production due to significant reduction of anthropogenic S emission and the large-scale use of S-free fertilizers. The acquisition of S by plants has therefore become increasingly important. Studies have shown that the transcription of several genes involved in the uptake and assimilation of S are up-regulated under S deficiency. A decrease in S availability increases S uptake capacity of tobacco and maize plants by an increase in the abundance of high affinity S transporters (Quaggiotti et al., 2002). A deficit of S causes a cascade of changes in gene expression (reviewed in Hirai and Sato, 2004) and extensive changes in the metabolic profile of different plant species (reviewed in Nikiforova et al., 2005).

Not much is known about the effect of S deficiency on root exudates. Table 9 illustrates that no change in PS was found at different S levels (Astolfi et al., 2006). Interestingly, this study also showed that when barley plants were deprived of Fe, S deficiency induced the PS levels in the root exudates threefold greater than under Fe deficiency alone. These findings imply that the internal level of S and its possible effects on root exudation pattern is part of a complex interaction with other nutrient stresses. Other studies have suggested that there is a complex interaction between S and nitrogen, since nitrogen deficiency inhibits S uptake (Quaggiotti et al., 2002). This interaction is often not observed due to the default application of S containing fertilizers (e.g., ammonium sulfate and super phosphate) (Yamaguchi, 1999) in many cropping systems.

Table 9. *Effect of S-availability on the exudation of PS in barley (Astolfi et al., 2006)*

Species	Cultivar	S application (mol/L)	PS
barley (*Hordeum vulgare*)	Europe	60	Not detected
barley (*Hordeum vulgare*)	Europe	0	Not detected

5.7 Potassium deficiency and root exudates

Potassium plays an important role in a number of crop physiological processes related to growth, yield, quality and stress resistance. Soil K reserves are generally large, however, large agricultural areas of the world are deficient in K, partly due to low bioavailability. Soils inherently low in K are often sandy, waterlogged, saline, or acidic. Also, plants grown on intensively used agricultural soils encounter K deficiency, in particular coarse-textured or organic soils. Water-soluble K is directly available to plants and potentially subjected to leaching. Exchangeable K is bound to clay minerals and humic substances. Both these exchangeable K-pools are often considered to be easily available to crops, but the size of both pools is very small (1-2% of the total soil-K). The utilization of non-exchangeable K sources is an important factor for the K tolerance of crops and is known to differ between species and cultivars. For example, ryegrass and sugar beet are more efficient in mobilizing K than wheat and barley (reviewed in Zörb et al., 2014). Interestingly, organic acids are known to facilitate the weathering of soil minerals through the formation of metal-organic complexes and by enhancing the exchange of H^+ for K^+ (reviewed in Zörb et al. [2014]). Song et al. (1988) showed that both oxalic and citric acid increased available K of several rock types.

Table 10. *The LMWOA levels in the root exudates of different corn and rice cultivars under different potassium levels*

(Kraffczyk et al., 1984; Liu et al., 2011)

Species	**Cultivar**	**K Level** (mM)	**OA in root exudates** (mg/g root DW)
Corn	Prior	0.5	4.29
Corn	Prior	3	3.97
Corn	Prior	7.75	4.62
Corn	Garbo	0.5	15.87
Corn	Garbo	1	3.42
Corn	Garbo	4	4.72
			OA in root exudates (µmol/g DW)
Rice	Yangdao 6	0.25	2,765.4
Rice	Yangdao 6	0.125	2,715.6
Rice	Yangdao 6	0	2,625
Rice	Yangjing 9538	0.25	2,336.4
Rice	Yangjing 9538	0.125	2,305.3
Rice	Yangjing 9538	0	2,282.7

Table 10 shows the level of total organic acid exudation of corn and rice under different K conditions (Kraffczyk et al., 1984; Liu et al., 2011). These data revealed a clear increase in LMWOA exudation by the corn cultivar “garbo” under low K concentrations, whereas cultivar “prior” did not. The increased exudation of OA by ‘‘garbo’’ under low K could be attributed to the enhanced production of fumaric acid (please give the exact reference for this). With rice, the two studied cultivars did not show any difference in their exudation of OA under low K. Unfortunately, there is, to our knowledge, no available data to correlate these differences in OA exudation to possible differences K content of the plant.

Besides the solubilization of mineral K by root exudates, plants have other systems to cope with limited K availability. Plants can alter their root morphology including root foraging, increasing root length, biomass and root hairs and their K uptake capacity (Wang et al., 2011). The K uptake system in plants is considered to consist of low- and high-affinity components; channels and transporters respectively (Ashley et al., 2006).

5.8 Conclusion

Chapter 5 discussed the response of root exudation on the deficiency of different nutrients, but not for all nutrients. To our knowledge, there are no available data for boron, chloride and molybdenum, possibly because these nutrients are often not limiting for plant growth or that they do not affect the root exudation pattern (like for example Ca), although unpublished data suggest that the boron concentration affects the total amount of dissolved organic carbon in root exudates.

An important difference between the studies in this report is the fact that some were conducted under sterile conditions, whereas others were not. There is a complex interaction between roots and soil micro-organisms, whereby micro-organisms influence root exudation, root exudates attract and/or suppress specific micro-organisms and micro-organisms exude chemical compounds themselves, which in turn all have an effect on plant health and nutrient bioavailability (reviewed in Badri and Vivanco (2009) and Berendsen et al. (2012)). These and other functions of root exudates that are not directly related to nutrient mobilization and uptake will be discussed in Chapter 6.

6 Other functions of root exudates

6.1 Root-root communication

Plants can recognize the presence of neighboring plants by their root exudates and are even able to adjust their exudation pattern in response to other plant species and/or genotypes (e.g., Badri et al., 2012; Semchenko et al., 2014). Phytotoxic secondary metabolites released by living plants have the potential to reduce growth and development of neighboring plants species. This process, known as allelopathy, has the potential to be exploited, e.g., weed control purposes (Bhadoria, 2011). Rice has been well studied in regards to selecting allelopathic strains and identifying potential allelopathic exudates (Kato-Noguchi, 2004; Seal et al., 2004). Although phenolics are often detected in allelopathic rice strains, they are not exuded in significantly greater quantities than from non-allelopathic strains and never reach phytotoxic levels (Kato-Noguchi, 2011; Kato-Noguchi et al., 2010). Recent work suggests that one of such allelochemicals, momilactones, may play a significant role in the interaction of rice with other species (Kato-Noguchi, 2011; Kato-Noguchi and Peters, 2013). Plants can, however, also benefit from allelopathic interactions, e.g., in intercropping, through the biocontrol of pathogens or directly by the root exudates of neighboring plant species (Zuo and Zhang, 2008).

6.2 Root-microbe interactions

Pathogens can have a severe impact on plant health. However, plants are able to regulate the composition of the root-microbe community by secretion of compounds that specifically stimulate or repress members of the microbial community. Secondary metabolites with antimicrobial properties are exuded by plants as a defense against pathogenic soil microbes. For example, fungal resistant cultivars of *Gladiolus* exude increased amounts of phenolic compounds relative to fungal susceptible *Gladiolus* cultivars, and thus these phenolics may be responsible for inhibiting fungal germination (Perry et al., 2007). Plant roots can also secrete secondary metabolites that inhibit growth of specific microbes in the rhizosphere For example, benzoxazinoids, which are phytochemicals found in large amounts in the root exudates of wheat and rye, are known to repress specific rhizospheric microbes (Berendsen et al., 2012). Similarly, increased secretion of phenolics under Fe deficiency in clover influences the rhizosphere microbial community structure and population (Jin et al., 2006, 2010).

Rhizosphere colonization by beneficial soil microorganisms can increase the plant's acquisition of nutrients and defense against pathogens in exchange for sugars, proteins and secondary metabolites exuded by the plant. Initially, microbes respond to chemical signals released by the plant and move towards the root through chemotaxic attraction (Perry et al., 2007). Flavonoids, for example, are chemo-attractants for arbuscular mycorrhizal fungi (AMF) and *Rhizobium*. These microbes can, among others, increase nutrient availability through mechanisms including: N-fixation, P solubilization and Fe mobilization through siderophore production (Dimkpa et al., 2009; Vessey, 2003; Zafar et al., 2012). Strigolactones, which are involved in the interaction with AMF, also serve as a chemo-attractor for striga (witchweed), a group of parasitic plant species. Hence, the same compound can have both a negative and a positive effect on plant performance (Smith, 2014).

6.3 Toxicity relief

In acidic soils, aluminum (Al) toxicity is a major stress factor for plants and can strongly limit root growth. Al forms stable complexes with a number of OA including citrate, oxalate, tartrate and, to a lesser extent, malate (Neumann and Römheld, 2007). These complexes are less toxic than free Al ions, and increasing evidence suggests that increased exudation of LMWOA during elevated external Al concentrations is a mechanism for alleviating the toxic effects of Al. The Al resistance of plant species and cultivars appears to be related to both the amount of exuded LMWOA and the plant's ability to maintain exudation (Neumann and Römheld, 2007). Similar responses are reported for Cd or Cu toxicity (Pinto et al., 2008; Meier et al., 2012).

7 Discussion and conclusion

7.1 Summary

In this report, we have shown that plants exude a wide range of exudates. Different classes of root exudates that play a role in nutrient acquisition have been identified, including LMWOA, PS, reductants and enzymes. The latter are mainly of importance in the context of P acquisition and have not been considered in detail in this report. Scientific literature reports that the mechanisms by which exudates increase the bioavailability of nutrients are not specific to a certain class of exudates, and a single class of exudates can be involved in multiple mechanisms.

Mechanisms by which LMWOA increase the bioavailability of nutrients include ligand exchange (either with a ligand adsorbed to a mineral surface or with a ligand binding a nutrient ion), ligand-promoted dissolution, mineral dissolution by lowering solution saturation state through complexation and co-exudation of protons. For PS, ligand exchange, ligand-facilitated dissolution and lowering solution saturation state are considered the principal mechanisms. Reductants can chemically reduce nutrient elements to species that are more bioavailable.

The extent to which exudates can be efficient in availing plants of nutrients depends on their properties affecting the binding and interaction with the nutrient (e.g., denticity, binding strength, stoichiometry of the complex), the manner in which the exudate participates in soil processes (e.g., their tendency to adsorb to reactive soil constituents, to be (bio)mineralized and to interact with non-target elements), as well as on soil properties (e.g., pH, redox conditions and abundance of reactive soil constituents).

Several LMWOA have been demonstrated to mobilize nutrients from soils. Denticity seems to play a clear role in the effectiveness of this role: monodentate acids (e.g., acetic acid) are less effective than bidentate (e.g., oxalate) or tridentate (e.g., citrate) acids. For mobilization of Fe and Mn, citrate is most efficient in most soil types. There is no simple relation between soil texture and effectiveness of LMWOA in mobilizing nutrients. For instance, for Zn mobilization, the effectiveness of the LMWOA correlated with the mineral-oxide bound Zn instead. Although citrate and oxalate are relatively efficient in mobilizing metals, they are also biodegraded relatively rapidly.

PS are believed to be primarily exuded for Fe acquisition, but can mobilize a range of metals from soil, including Fe, Cu, Zn, Ni, Co, Mn and Cd. Similar to LMWOA, the effectiveness of PS in mobilizing metals can be compromised by biodegradation and adsorption. Plants exude PS in a diurnal pulse release, which has been suggested to be related to overcoming depletion of soil solution concentration by biodegradation. Fe mobilization by PS can be severely hampered by mobilization of competing cations. Differences among PS in mobilizing metals are small. Mobilization of metals by PS correlates with the soil nutrient availability parameter ‘DTPA-extractable metal content.’

Reductants have so far mainly been associated with nutrient (particularly Fe) remobilization and uptake from the apoplast. Their potential role in the soil environment in the context of nutrient acquisition needs to be further explored.

In Section 5.1, it is shown that Fe deficiency induces several responses including a relative increase in exudation of PS, LMWOA and phenolics. Unfortunately, there was little overlap in the species which were used to measure these different exudation responses. Most citrus tree species showed a phenolic response (Table 3) and a diverse group of species showed a LMWOA exudation response (Fig. 14). Barley cultivars had a relatively larger PS exudation response than the wheat cultivars (Fig. 11), and barley cultivars had a larger relative PS response at lower Fe-concentrations in the plant (Fig. 12). This might indicate that certain barley cultivars could be more suitable for intercropping with other crops or could grow under lower nutrient conditions than wheat cultivars, especially since the relative PS response of barley seems to be larger than that of wheat under Zn deficiency as well (Fig. 15). Although the correlation between PS exudation rate and relative biomass could not be found for Fe deficiency, there was a clear correlation for wheat cultivars under Zn deficiency. The mechanisms underlying this correlation may not be clear, but the correlation suggests that higher PS exudation rates may lead to a higher relative biomass.

Also for Cu and Mn responses in LMWOA, PS and reductant exudation were found (paragraphs 5.3 and 5.4), but unfortunately there was little overlap in the species used in comparison to the species used in other nutrient deficiency treatments. On the other hand, it showed that a diverse group of plants, such as citrus trees, legumes, grasses and gourds are all able to respond in their exudation to metal deficiency.

Although the amount of data was limited, deficiency of other nutrients such as calcium, sulfur and potassium also appears to induce the exudation of LMWOA (paragraphs 5.5-7). On other exudates, no data were found. Even for the LMWOA response, it was very species- and/or cultivar-specific, making it hard to arrive at general conclusions.

7.2 Discussion

While exploring and comparing the available literature data on root exudates in relation to nutrient acquisition, we came to notice the following issues:

- The amount of available data on root exudates is strongly dependent on the nutrient. There is much data available in relation to Fe, P (not treated in this report) and Zn; little on Cu, Mn, K, Ca and S; and, to our knowledge, none on B, Mo, Cl and Mg. This may reflect the incidence of deficiencies in these nutrients, along with a potential role of root exudates in alleviating these deficiencies. However, a more complete analysis of root exudates in relation to overall nutrient deficiencies is required.
- Although hydroponic studies are very useful for identifying root exudates as well as comparative trends among species and cultivars, absolute exudation levels may differ strongly from those under rhizosphere conditions, particularly when plants are completely deprived of specific nutrients. This may lead to an overestimation of the potential effects of LMWOA and PS.
- There is very little data available reflecting exudate concentration levels under rhizosphere conditions. In nutrient mobilization experiments, generally very high amounts LMWOA and PS per amount of soil are used. Such high concentrations make it more likely that an effect will be observed, but competition effects become less pronounced at such high concentrations. As a result, the distribution in mobilized metals will presumably be very different under actual rhizosphere conditions. To address this issue, there is a need to measure exudate concentrations under rhizosphere conditions and to investigate the behaviour of the exudates in soil systems at comparable concentrations levels. Speciation modelling of the root exudates may serve as a useful predictive tool in this context when sampling and analytical tools fall short to quantify rhizosphere concentrations.
- Often, it is difficult to make sound comparisons between studies because of differences in the experimental setup: different parameters were considered or reported in different ways, the experiments were carried out under different conditions, characterization of the experimental system (e.g., soil) was conducted only to limited extents, etc. It would, therefore, be useful to grow a specific set of species/cultivars under the same conditions except for nutrient levels and measure the same parameters. This could help to obtain conclusive outcomes with respect to differences between cultivars/species and to determine possible relations between the different nutrient deficiencies.
- There seems to be a knowledge gap in the effect of flooding on the root exudates for specific crops such as rice. Flooding has clear effects on the bioavailability of different nutrients and on the plant functioning. It is expected that root exudation will change depending on the duration of the flooding (including the after-effects of flooding) and whether the plant is able to reach the water surface and form aerenchyma. Under anoxic conditions, plant metabolism is shut down and, with that, any form of active uptake transport or exudation processes. Aerenchyma can release this stress, but the oxygen leakage from the root has large effects on the (toxic) nutrient conditions in the rhizosphere. Differences in these responses could have great implications not only on the root exudation pattern, but also on plant yield. Hence, flooding and its effect on root exudates could be of great significance.

7.3 Recommendations

Nutrient acquisition by plants is a complex and challenging interdisciplinary field of research. Nutrient availability is controlled by a range of soil parameters that are highly soil dependent, nutrients differ strongly in their behavior and bioavailability in the soil, and plants employ a range of nutrient acquisition strategies that differ in specifics and efficiency even at the level of cultivars. Because of these numerous variables in the rhizosphere, there is no one-size-fits-all solution for utilizing the potential of root exudates in nutrient acquisition in agricultural practice.

Moreover, not only the effectiveness of the individual exudate should be considered, but also the complex interplay between exudates. Many of these interactions are still poorly understood, and further elucidating them is challenging. Nonetheless, the examples of synergistic interactions that have been reported, e.g., between LMWOA and PS, between flavonoids and LMWOA, may hold promise for utilization in agricultural practice. Creating or enhancing synergistic effects between exudates can be considered for a single species or between species. In the context of intercropping, it has already been demonstrated that plants can benefit from each other's exudates for nutrient acquisition; e.g., Strategy I plants can benefit from PS exudation by neighboring graminaceous species for Fe acquisition (Zuo and Zhang, 2008). The results of this study show that (based on the root exudation responses) barley would have a large potential to be beneficial for intercropping under conditions of Fe and Zn deficiency. Barley cultivars showed the largest responses overall in PS exudation upon the deficiencies of the essential metals (e.g., Fig. 11), but also showed the largest PS exudation responses at relative small changes in the Fe level of the shoot (Fig. 12). Although further research is needed to be able to compare actual exudation levels for different plant species and nutrient conditions, barley seems the most promising for intercropping with other (non-graminaceous) plant species based on its responsiveness and the magnitude of these responses.

For obtaining meaningful synergisms between plants, accurate characterization and understanding of the specifics of the field site under considerations as well as tailored crop selection are vital. Root exudates are clearly not the only consideration in this context; pest resistance, compatibility of the species, competition for water, etc. should all be considered. Nevertheless, intercropping plant species that complement each other in nutrient acquisition may be able to facilitate a more efficient usage of nutrient reserves in the soil.

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