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„Beneficial effects on plant iron acquistion in calcareous soils“

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Abbreviations

Al	aluminium
a.o	and others
asc.	ascorbate
Co	cobalt
Cu	copper
DMA	2'-deoxymugineic acid
DOC	dissolved organic carbon
dw	dry weight [g]
Fe	iron
FePS	iron-phytosiderophore complex
fw	fresh weight [g]
MA	mugineic acid
MAs	mugineic acids
MeDMA	metal-DMA complex
MePS	metal-phytosiderophore complex
Mn	manganese
Ni	nickel
PS	phytosiderophore
SOM	soil organic matter
SSR	soil to solution ratio [kg L^{-1}]
TETA	triethylenetetramine dihydrochloride
Zn	zinc

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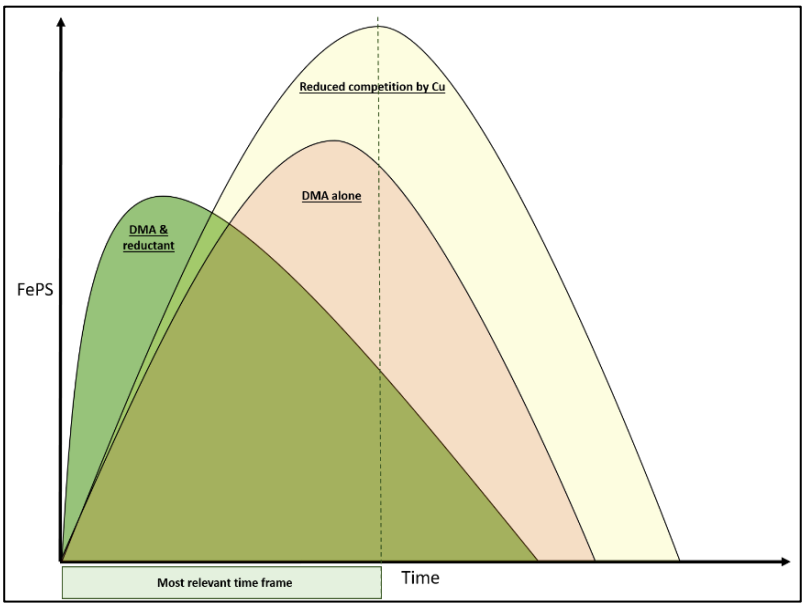
Abstract

This thesis sheds light on beneficial effects on iron acquisition in calcareous soil. The mobilization of the metal ions iron, copper, nickel cobalt and manganese by the phytosiderophore DMA was examined in batch experiments. Solution concentrations of FeDMA decreased over time after an initial increase. This was caused by competition of other metal ions on Fe complexation, due to decreasing concentrations of uncomplexed DMA and competitive displacement of FeDMA. Initial mobilization during the first hours was in the same range for three different concentrations of DMA, for all metals mobilized by the phytosiderophore, but differences increased over time until the ratio between DMA supplied and MeDMA in solution was well matched after 168 hours.



Conceptual model of this thesis

To strengthen findings obtained, a method to relate MeDMA concentrations obtained under different soil to solution ratios (SSR) was applied. Results were in good agreement between the two SSRs tested, but not with previously published values.

A previously reported synergistic effect on iron mobilization by the combined application of DMA and the reductant ascorbate was examined over multiple concentrations of DMA and ascorbate. The synergistic effect found, might substantially benefit plant iron acquisition in the environmentally most relevant time frame, but maximum solution concentrations were lower and earlier in time than under no ascorbate conditions. It was shown, that increased competition affected the synergistic effect on iron mobilization negatively, as in particular cobalt and nickel showed synergistically elevated solution concentrations, outcompeting iron for free and complexed DMA. A ratio dependency of ligand and reductant was concluded, in which the positive effects of the combined application of DMA and ascorbate dominate.

To shed more light on the mechanism underlying the synergistic effect on iron acquisition the combined application of DMA and ascorbate was investigated under absence of oxygen. Results showed in general similar mobilization under anoxic conditions as under oxic, indicating no strong effect of oxygen present in soil solution. Reductive dissolution of Mn-oxides was found to be the most prominent result of ascorbate addition.

The compounds coumarin, esculetin, fraxetin, scopoletin and caffeic acid were tested for their potential synergistic effect on iron mobilization. Fraxetin was the only compound tested showing a

clear synergistic effect in combined application with DMA in the concentration range tested. Indications for a potential synergistic effect on Iron mobilization by caffeic acid, scopoletin and esculetin with DMA were found and discussed. Scopoletin and fraxetin alone mobilized Fe from a calcareous soil, a process that has not been observed previously.

As copper has been identified to be the major competitor for the complexation by DMA an additional beneficial effect onto iron acquisition by *Triticum aestivum sp. Tamaro* was investigated in a pot trial. By reducing the availability of copper for complexation by phytosiderophores, iron availability may become relatively elevated without providing additional Fe to the soil. Copper availability in Santomera soil was reduced by adding the chelating agent triethylenetetramine dihydrochloride (TETA). Plants grown in TETA containing soil showed no negative effects on plant health, but increased biomass and elevated leaf chlorophyll content over the time period of the experiment. Plant tissue metal concentrations showed indications for reduced phytosiderophore efflux, while Fe tissue concentrations remained in similar concentrations as in the control group.

1 Introduction

1.1 Iron as a micronutrient

Iron (Fe) is after aluminium (Al) the second most abundant metal in the earth crust, with concentrations of 4.6% (Scheffer et al., 2010; Broadley et al., 2012). It is an essential micronutrient for plants and animals (Scheffer et al., 2010). In plants it is needed to form Heme-Proteins like Cytochrome, which is an essential part in the redox system of chloroplasts, mitochondria and nitrate-reductase (Broadley et al., 2012). It is also used in Fe-Sulphur proteins like ferredoxin, necessary for metabolic, and a variety of other redox and enzymatic processes (Broadley et al., 2012).

Both iron deficiency and toxicity can occur under natural conditions and may have severe impact on crop production, with the former being especially problematic on calcareous soils, and the latter attributed in particular to water logged paddy soils (Mengel et al., 2001; Broadley et al., 2012). Plants experiencing Fe deficiency have in general lower levels of chlorophyll and ferredoxin and therefore a reduced photosynthetic activity, which can cause reduced sugar and starch concentrations in the plant (Broadley et al., 2012). Chlorosis is a visible symptom of iron deficiency in plants, expressed as pale green to yellow leaves, often associated with darker coloured veins (Schenkeveld, 2010; Broadley et al., 2012).

1.2 Iron in soils

In soils the iron content ranges from 0.2 to 5 % of the soil solid constituents. It occurs in the form of primary minerals (olivine, augite, biotite a.o.) and most prominently as secondary minerals (Scheffer et al., 2010) with Fe(III) (hydr)oxides like goethite, haematite and ferrihydrite being the most abundant of those secondary minerals (Mengel et al., 2001). Additionally, soluble and exchangeable Fe might be present in some soils, in dependence of pH and Eh. Fractions of Fe can also be bound to organic matter in soluble or insoluble forms (Colombo et al., 2014).

The bioavailability of iron is determined by chemical reactions in the soil (Lindsay, 1995). Due to the low solubility of Fe(III) (hydr)oxides, the concentrations in soil solution in presence of oxygen are generally very low, ranging from 0.02 to 0.3 mg l^{-1} (Scheffer et al., 2010). The solubility of Fe oxides in soils is often described using the hypothetical mineral $Fe(OH)_{3\ soil}$ with a log K° of 2.70. The dissolution of that model mineral is given by equation 1 (Lindsay & Schwab, 1982).

$$Fe(OH)_{3\ soil} + 3H^{+} = Fe^{3+} + 3H_2O \quad (1)$$

As becomes apparent by equation 1 dissolution of $Fe(OH)_{3\ soil}$ is highly pH dependent (compare also Figure 1). It decreases with increasing pH, having its minimum in the circumneutral range. In calcareous soils, where pH values of 7 and higher can be expected (Scheffer et al., 2010; Colombo et al., 2014), this results in soluble inorganic Fe concentrations in the order of $10^{-10.4}$ M (Lindsay, 1995). As the concentrations required by plants to maintain health are approximately 4 orders of magnitude higher, they need a mechanism to manipulate Fe availability in order to survive in pH ranges above 5 (Lindsay, 1995).

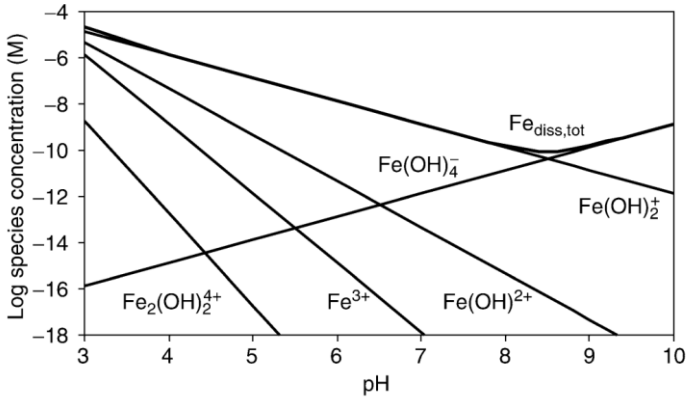


Figure 1: Iron solubility in equilibrium conditions for ‘‘soil iron oxide’’; (Kraemer et al., 2006)

1.3 Iron uptake in the rhizosphere

The rhizosphere can be defined as “the field of action or influence of a root” (Lynch, 1994). It is the zone of interactions between plant roots, microorganisms and soil. Making up only a small volume, it plays a central role to all life on earth (A.A.M. De Leij et al., 2007; Hinsinger et al., 2009). Conditions in the rhizosphere are of great spatial and temporal heterogeneity in terms of their ecological, physical and chemical properties (Hinsinger et al., 2009). Boundaries depend on the question of interest and may vary significantly, when observing for example nutrient availability or pH gradients (Neumann & Römheld, 2012). Iron uptake takes place in the rhizosphere and processes described in the following thesis need to be examined under consideration of the complex environment – the rhizosphere - in which they take place.

As has been showed so far Fe is abundant in most soils, but due to its low activity, bioavailability is often limited. So far two distinct mechanisms of Fe acquisition have been described, by which plants can manipulate the rhizosphere of soils with low Fe availability. These are called Strategy I and Strategy II (Römheld & Marschner, 1986).

1.3.1 Iron uptake by plants - Strategy I

Strategy I is utilized by all dicotyledonous and monocotyledonous plants, with the exception of graminaceous species (Marschner et al., 1986; White, 2012). In general Strategy I plants take up iron in in the form of Fe^{2+} (Grusak et al., 1999). A plasma membrane bound reductase reduces Fe^{3+} to Fe^{2+}. The Fe^{2+} is then transported into the cytoplasm, where it is complexed by nicotianamine and further distributed in the plant (Marschner et al., 1986; Mengel et al., 2001; White, 2012).

Under iron deficient conditions, the plant responses consist of root morphological changes and alteration of the rhizosphere chemistry. Morphological changes include inhibition of root elongation, larger apical root zones and increased root hair formation (Broadley et al., 2012). An increase in high surface root transfer cells is accompanied with Fe deficiency in some species (Schmidt, 2003).

Changes in rhizosphere chemistry are achieved by increasing the Fe(III) reduction capacity, rhizosphere acidification by net increase in proton exudation via H^+-ATPase and excretion of organic acids and phenolic compounds, which can serve as chelating agents and/or reductants in the case of phenolics (Römheld & Marschner, 1986; Grusak et al., 1999; Kraemer et al., 2006; Broadley et al., 2012; White, 2012). Those processes can increase the Fe solubility by lowering the pH (compare Figure 1) and by chelation of Fe, leading to increased dissolution (Grusak et al., 1999). Increasing reduction capacity may increase Fe availability by reducing ferric to the more mobile ferrous iron (Lindsay, 1995) and facilitate Fe^{2+} uptake (Grusak et al., 1999).

Enhanced Fe solubility by rhizosphere acidification is compromised in soils with a high pH buffer capacity. Due to high pH and bicarbonate levels in calcareous soils, Strategy I plants may still experience Fe deficiency here (Marschner et al., 1986).

1.3.2 Iron uptake by plants - Strategy II

Strategy II is only carried out by graminaceous plant species, including cereal crops of high agricultural significance like wheat, oat, barley etc. (Marschner & Römheld, 1995; Kobayashi & Nishizawa, 2012). Strategy II plants exude specific chelating agents called *''phytosiderophores''*. Phytosiderophores (PS) are chelating agents from the mugeneic acid family (MA), with a high affinity for Fe(III) (Schmidt, 2003; Kraemer et al., 2006; Kobayashi & Nishizawa, 2012). Dissolution of otherwise insoluble Fe sources is promoted by maintaining disequilibria and ligand promoted dissolution (Kraemer et al., 2006; Crowley & Kraemer, 2007).

Release and uptake of PS is thought to be highest in the apical zone behind the root tip (Marschner & Römheld, 1995; Reichman et al., 2005). The release of phytosiderophores follows a diurnal exudation pattern (Marschner & Römheld, 1995). Shortly after the onset of light, PS get exuded over a time frame of around 4 to 6 hours (Römheld, 1991; Oburger et al., 2014). Under Fe deficient conditions PS release is significantly increased (Römheld & Marschner, 1986). The amount and type of PS released by plants, varies with species and cultivar. Marschner et al. (1986) showed that species with increasing chlorosis resistance exude higher amounts of phytosiderophores in the sequence barley > oat > maize> sorghum > rice. Rye and wheat, the latter has been used for a pot experiment in this thesis, are considered to be in the range of barley in terms of PS exudation (Grusak et al., 1999; von Wirén et al., 2000).

The iron-phytosiderophore (FePS) complexes are mobile in soil solution and are taken up as a whole by a specific membrane bound transporter (Schmidt, 2003). Reduction of Fe(III) therefore does not take place at the root surface. The further transport in the plants is not yet completely resolved. It is however believed, that the FePS complex is split up and the PS are released again into the rhizosphere to continue chelation. Fe taken up is reduced and chelated by nicotianamine or transported by a MA in ferric form (Grusak et al., 1999; Kobayashi & Nishizawa, 2012).

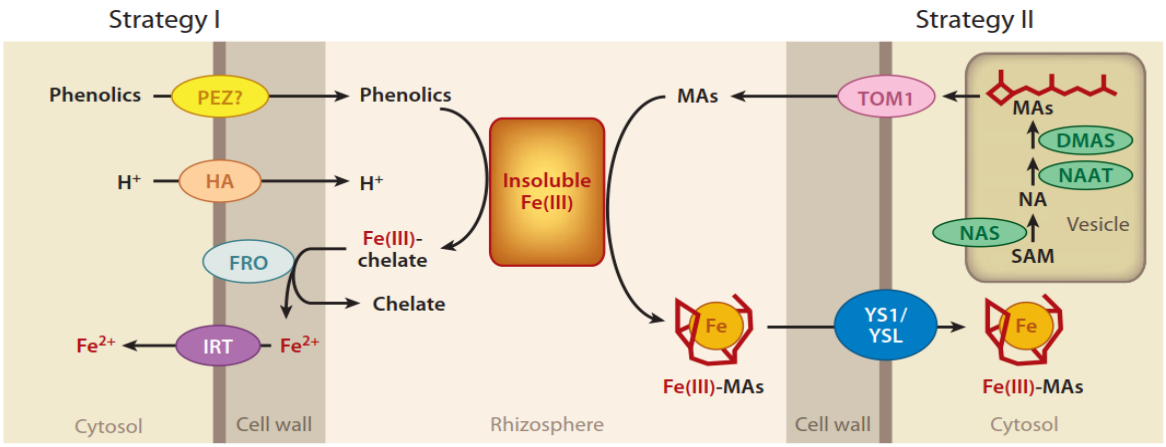


Figure 2: Mechanisms of iron uptake in higher plants. Oval shapes represent transporters and under Fe deficiency induced enzymes. Abbreviations DMAS, deoxymugineic acid synthase; FRO, ferric-chelate reductase oxidase; HA, H+ -ATPase; IRT, iron-regulated transporter; MAs, mugineic acid family phytosiderophores; NA, nicotianamine; NAAT, nicotianamine aminotransferase; NAS, nicotianamine synthase; PEZ, PHENOLICS EFFLUX ZERO; SAM, S-adenosyl-L-methionine; TOM1, transporter of mugineic acid family phytosiderophores 1; YS1/YSL, YELLOW STRIPE 1/YELLOW STRIPE 1–like. (Kobayashi & Nishizawa, 2012)

It has been demonstrated in models and experiments, that PS do not exclusively complex Fe, but also chelate other metals (Murakami et al., 1989; Crowley et al., 1991; von Wirén et al., 2000; Reichman et

al., 2005; Schenkeveld et al., 2014a). If those MePS complexes are used to overcome deficiencies in other micronutrients like Zn and Cu is still not completely resolved, but questioned due to lower affinities for uptake of those complexes in comparison with FePS (Grusak et al., 1999; White, 2012).

1.3.2.1 Chemical Properties of phytosiderophores

Up to now 9 types of mugeneic acids have been identified, that are synthesized in the same pathway deriving from S-adenosyl-L-methionine (Kobayashi & Nishizawa, 2012).

Commonly 3 types are described which are displayed in Figure 3. Those are called mugineic acid (MA), 3-epihydroxymugineic acid (epi-HMA) and 2'-deoxymugineic acid (DMA) (Murakami et al., 1989).

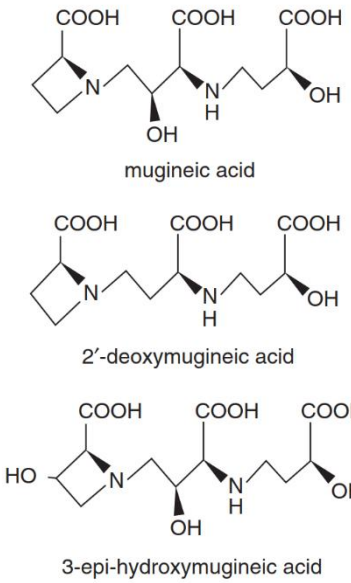


Figure 3: : Mugineic acid and its derivates (Reichman et al., 2005)

For convenience they are often called mugineic acids or MAs (Kraemer et al., 2006). MAs are derived by hydroxylation of nicotianamine, which is also found in Strategy I plants (Kraemer et al., 2006). MAs have three carboxyl and two amino functional groups in common with variating hydroxyl groups (Dell'mour et al., 2010). Under environmentally common conditions MAs are negatively charged (von Wirén et al., 2000). They can complex bi- and trivalent metals, by forming hexadentate 1:1 metal complexes with an octahedral shape (Dell'mour et al., 2010). Only when binding a trivalent metal ion like Fe(III) or Co(III) the terminal hydroxy group deprotonates (Kraemer et al., 2006). Metals potentially mobilized by PS, in addition to both Fe(II) and Fe(III), are essential micronutrients or beneficial elements like Mn, Zn, Co, Ni and Cu, but also Cd which is of concern, due to its high toxicity to humans and animals (Reichman et al., 2005; Neumann & Römheld, 2007; Scheffer et al., 2010).

Murakami et al. (1989) have published stability constants for MAs. They showed, that the Irving-Williams order, with bindings strengths in the sequence Mn(II) < Fe(II) < Ni(II) < Cu(II) > Zn(II) (Irving & Williams, 1953), holds true for MAs. Reichman et al. (2005) introduced the need for conditional stability constants depending on pH. Further they resolved that at a pH of 7 copper forms more stable complexes with DMA than iron(III) and at a pH of 8 the same holds true for nickel. They concluded that Fe(III) complexation in alkaline soil might be affected significantly by competition from Cu and Ni, lowering the overall ability to mobilize Fe. Schenkeveld et al. (2014a) included soil specific multi-surface modelling to predict the dominant metals mobilized under equilibrium conditions by DMA. They showed that depending on the metal activity in the respective soil either Fe, Ni or Cu was the dominant species in solution under equilibrium conditions.

1.3.2.2 Rhizosphere Concentrations

Phytosiderophores are occurring in a distance up to 1mm of the root surface (Reichman et al., 2005). They are subject to microbial degradation and adsorption to the soil solid phase (Römheld & Marschner, 1986; Reichman et al., 2005; Oburger et al., 2014). Rhizosphere concentrations of PS are still subject of investigation. Reichman et al. (2005) stated concentrations between a few and 100µM based on previous research. Just recently the DMA exudation of wheat (*Triticum aestivum*) grown in soil could be determined quantitatively, resulting in 156µM and 31µM considering a hotspot release

via root tips, or maximum rhizosphere concentrations averaged over the entire root system of 22µM and 3µM for two respective sampling dates (Oburger et al., 2014).

1.3.2.3 Window of Fe Acquisition

The mobilization of Fe by DMA is not only driven by thermodynamics, but also complexation kinetics and the reactive pool of available iron (Schenkeveld et al., 2014a). Recently Schenkeveld et al. (2014b) introduced the conceptual "*Window of Fe acquisition".* This model considers biogeochemical processes affecting rhizosphere concentrations of FePS complexes over time. The main processes are microbial degradation and adsorption of free PS or MePS complex. Additionally, competition by other metals was identified to affect FePS concentrations significantly. The authors identified two principal mechanism of competition: 1) the chelation of metals other than Fe leading to a faster decline in free ligand and therefore less relative Fe mobilization and 2) already complexed Fe being displaced by a metal that forms a thermodynamically more stable complex, under the specific conditions of the soil, and consequential becoming depleted from solution. Interestingly this competitive displacement seemed to strongly influence the solution speciation, increasing solution concentrations of especially Cu, Ni and to some extent Co at the expense of Fe and Zn (Schenkeveld et al., 2014b). Therefore it was assumed, that there is a certain time frame in which sufficient Fe can become complexed and made plant available, whereas over time degradation and competition will reduce the amount of complexed Fe and therefore close the window of Fe acquisition (Schenkeveld et al., 2014b).

1.3.3 Microbial and fungal iron uptake

In this thesis the main focus is on the iron acquisition by plants. However, microbes and fungi are known to exude iron chelating agents, which in fact make up the vast majority of the so far discovered siderophores (Kraemer et al., 2006; Robin et al., 2008; Hider & Kong, 2010; Harrington et al., 2015). Reported values for stability constants of microbial siderophores are in the range of 10^{23} to 10^{52} (Colombo et al., 2014). Interactions of fungal and microbial siderophores may alter the rhizosphere chemistry and significantly enhance mineral weathering (Colombo et al., 2014). Additionally contribution, and negative effects to plant iron uptake by microbial and fungal siderophores have been discussed (Kraemer et al., 2006; Crowley & Kraemer, 2007). Furthermore synergistic effects on the dissolution rate of goethite in presence of the microbial siderophore and low molecular weight organic acids have been reported (Reichard et al., 2007; Wang et al., 2015), showing the dynamic interactions potentially present in the rhizosphere. Mycorrhizal symbiosis in the context of iron has been discussed (Marschner & Dell, 1994; Caris et al., 1998). Microbial degradation of phytosiderophores is known to alter solution concentrations significantly and therefore affects the conceptual window of iron acquisition in plants (Marschner & Römheld, 1995; Schenkeveld et al., 2014b).

1.4 Reductants and redox conditions in the rhizosphere

1.4.1 General

Root exudates known to possess reducing properties are flavonols, flavones, flavanones, isoflavonoids, coumarins, ascorbic acid and caffeic acid (Dehner et al., 2010; Keuskamp et al., 2015). Ascorbate has recently been reported to be increasingly exuded by Fe deficient grapevine (López-Rayo et al., 2015). Microbial mitigated redox reactions are omnipresent in soils (Scheffer et al., 2010). Microbial and plant root respiration may also significantly affect the oxygen content and subsequently overall redox conditions in the rhizosphere (Zausig et al., 1993; Uteau et al., 2015). Mucilage has been discussed as a mean of contact reduction onto the soil solid phase by plant roots (Uren, 1993). Additionally water saturation especially in soil pores will fluctuate over time, changing the redox conditions on those sites significantly (Crowley & Kraemer, 2007; Sposito, 2008). All those processes and compounds contribute

to the spatial and temporal heterogeneity of redox potentials in soil and in particular the rhizosphere. Scheffer et al., 2010 state a range from Eh = +0.8V in well aerated highly acidic soils to Eh = -0.35V under anoxic neutral to alkaline soils, displaying the vast range of possible conditions present in nature.

1.4.2 Coumarins

Coumarins are polyphenolic secondary metabolites deriving from the cinnamic acid family (Bresinsky et al., 2013). Many hundred coumarins have been found so far in a wide variety of dicots (Clemens & Weber, 2016). Some coumarins are known allelochemicals and have been investigated closely from that perspective (Gelsomino et al., 2015; Niro et al., 2016).

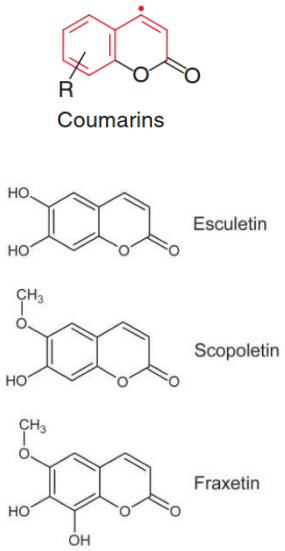


Figure 4: Basic Structure of coumarins and structure of esculetin, scopoletin and fraxetin; (adapted from Bresinsky et al., 2013; Clemens and Weber, 2016)

Phenolic compounds like coumarins and flavonoids, have been discussed as possible chelators and reductants in the context of Fe acquisition in Strategy I plants for a long time (Römheld & Marschner, 1983; Marschner & Römheld, 1995). However, until recently they received less attention than other parts of the Strategy I mechanism. Coumarins are now known to be increasingly exuded under Fe deficient conditions (Schmidt et al., 2014). Several studies have shown coumarins to be crucial for plants under alkaline conditions (Fourcroy et al., 2014; Schmid et al., 2014; Schmidt et al., 2014). Especially scopoletin and its glycone scopolin, fraxetin and its conjugated form fraxin as well as esculetin could be identified in root exudates of *Arabidopsis thaliana* (Clemens & Weber, 2016). It was even demonstrated, that mutant plants without the ability to synthesize coumarins themselves, can be saved from Fe deficiency by external supply of those compounds in presence of an iron source (Schmid et al., 2014). The exact mechanism by which coumarins help to overcome Fe deficiency is not yet understood. The possible role as a chelator and/or reductant has been discussed (Römheld & Marschner, 1983).

So far it seems as if only esculetin and closely related compounds can chelate Fe(III) due to its two hydroxyl groups in *ortho* position, forming 3:1 complexes. Fe mobilization from a highly calcareous soil has been reported for esculetin by Schenkeveld et al. (2016). Scopoletin has been reported to not be able to chelate Fe(III) due to the inaccessibility of the 6'-hydroxy group, which is striking as scopoletin has been identified to be much more abundant in root extracts than esculetin (Schmid et al., 2014). Schmidt et al. (2014) showed that esculetin, fraxetin and scopoletin have the ability to both reduce Fe(III) and chelate Fe(II).

1.5 Aim of this thesis

In this thesis two main pathways have been followed to display beneficial effects on plant iron acquisition in calcareous soil.

One pathway will try to shed light on synergistic effects between plant borne compounds with reducing properties and the phytosiderophore DMA. Here it will be shown, that by increasing the absolute availability of Fe, in a soil poor in readily plant available iron, significantly more Fe can become mobilized. Hypotheses 1-4 will focus on this. The reader may see evidence for significant interactions between plant exudates found in the rhizosphere, that have in the past been overlooked. It will become apparent, that the interplay of a reductant and the phytosiderophore DMA can significantly increase the mobilization of iron in the environmentally most relevant time frame. Those so called synergistic effects will be shown to be possible by several plant borne compounds and a phytosiderophore, showing the potential relevance under natural conditions, as well as the limitations of using single compound batch experiments to describe a complex environment like the rhizosphere. It will however also be shown, that the synergistic effect, does not come without a cost. As competitive effects for the complexing agent are increased as well, iron mobilization becomes compromised, eventually counteracting the benefits of a combination of the reductant ascorbate and the phytosiderophore DMA.

Competition is then the center of a second approach in exploring possible beneficial effects on iron acquisition by a wheat plant grown in iron poor, highly calcareous soil. By reducing the availability of copper, the main competitor on iron mobilization by DMA, the relative availability of iron to the plant may become increased. This potentially opens up interesting possibilities in terms of in example fertilizer development, as it could help the plant to satisfy its nutrional needs, without increasing the actual amount of available Fe in the soil.

1.5.1 Hypothesis 1

So far it has been demonstrated, that DMA mobilizes iron and other metals (Cu, Zn, Ni, Co and in very high DMA additions, Mn) from Santomera soil (Schenkeveld et al., 2014a). Competition for DMA by other metals in particular Cu and Ni has been reported to be the main influence onto the conceptual 'window of iron acquisition', due to competitive displacement (Schenkeveld et al., 2014b). Recently cobalt has also been identified to be a potential competitor for iron complexation by DMA (Schenkeveld et al., 2016).

A synergistic effect on metal mobilization in soil is observed, when the sum of metal in solution mobilized by the combined treatment of two compounds like ascorbate and DMA, is larger than the metal in solution mobilized by the individual application of those treatments (Schenkeveld et al., 2016). This data is only comparable at the same time points, due to the systems kinetics.

When combining the application of DMA and ascorbate, iron solution concentrations were synergistically elevated in Santomera soil initially. This effect was only temporary, and antagonistic effects of the combined treatment reduced Fe solution concentrations over time, as has been demonstrated by Schenkeveld et al. (2016b). Nickel and cobalt were reported in the same study to experience an even stronger synergistic effect than iron, without showing signs for antagonistic effects over time. Copper appeared to be hardly affected by the combined treatment, therefore remaining a strong potential competitor for chelating agent (Schenkeveld et al., 2016). For those reasons an increase in competition for free ligand is expected in a combined application of ascorbate and DMA. Schenkeveld et al. (2014b) showed that decreasing amounts of free DMA in solution were correlating with decreasing concentrations of FeDMA solution concentrations.

It is therefore hypothesized (**Hypothesis 1**):

A combined application of DMA and ascorbate will result in an initial synergistic effect on iron mobilization. With increasing ascorbate concentrations, the positive correlation between ascorbate added and iron mobilized will decrease eventually. The timewise extent of the synergistic effect on iron mobilization will additionally become compromised by increasing ascorbate addition, due to increased competition for DMA.

Experiment 1 is therefore evaluating the mobilization of iron and other metals (Cu, Ni, Co, Zn and Mn) by three concentrations of DMA and three concentrations of ascorbate over time. The findings are related to the mobilization by treatments including only ascorbate and only DMA. In order to see the effects of increasing DMA additions to the soil solution chapter 3 first addresses the metal mobilization by DMA alone. The underlying research question is the following:

How big is the gain in iron mobilization from increasing the concentrations of DMA in an environmentally relevant time frame?

In order to address influences of the soil to solution ratio (SSR) a recently published method on relating experimental data of metal mobilization by organic ligands (Schenkeveld et al., 2017) is presented in this paragraph relating data from experiments 1 and 2.

Chapter 4 then shows the interplay of ascorbate and DMA. Hypothesis 1 is explored based on the research question how the overall rate of all metals mobilized by DMA is affected by the addition of different concentrations of ascorbate.

Individual metal mobilization by the combined treatments is discussed in the following. By focussing on the research question how the solution concentrations of iron and other metals are affected by variating the concentration of ascorbate and DMA.

1.5.2 Hypothesis 2

Parts of experiment 1 were repeated under the absence of oxygen in experiment 3, to further elaborate the mechanisms responsible for the synergistic effect on iron acquisition. It has been reported, that ascorbate addition leads to reductive dissolution of Fe(hydr)oxides and Mn-oxides, while ascorbate gets oxidized in the process (Martin, 2005). Schenkeveld et al. (2016b) observed increasing solution concentrations of Mn, but not of Fe when adding only ascorbate to Santomera soil under oxic conditions, indicating reductive dissolution of Mn-oxides present in the soil. The presence of oxygen, may compromise the effectiveness of ascorbate by oxidation reactions, before reaching iron- or manganese-oxide surfaces (Wang et al., 2015). This and possible reoxidation and precipitation reactions are the reason why reductive dissolution reaction rates are generally obtained under absence of oxygen (Stone & Morgan, 1984; Dos Santos Afonso et al., 1990; Suter et al., 1991).

Based on that it is hypothesized (**Hypothesis 2**):

The effects of ascorbate alone and in combination with DMA, when not compromised by the presence of oxygen will be more pronounced under anoxic conditions. Namely the reductive dissolution of manganese oxides, the increased complexation availability of metals associated with those minerals and therefore increased competition for 'free' DMA, are expected to be more pronounced under anoxic than under oxic conditions.

Experiment 3 therefore evaluates the interplay of two DMA and two ascorbate concentrations added to Santomera soil over time under the absence of oxygen. So far no research has been conducted elaborating the metal mobilization by DMA alone or in combination with a reductant in soil under

anoxic conditions. Therefore, in paragraph 4.2 individual metal mobilization by DMA and ascorbate alone and in combined treatments is evaluated and compared to that under conditions with oxygen present. In the following the effects of the combined application of ascorbate and DMA are discussed and evaluated in terms of their synergistic effect and compared to their counterparts under presence of oxygen.

1.5.3 Hypothesis 3

Santomera soil has a pH ($CaCl_2$) of 7.8 and the prevalent form of Fe in Santomera soil is crystalline Fe(III) (hydr)oxides (Schenkeveld et al., 2014b). This makes Fe(III) the only prevalent oxidation state in soil solution under presence of oxygen. Ligand facilitated dissolution is known to work on the one hand by decreasing the solutions saturation state and on the other hand by attacking the mineral surface in a ligand controlled dissolution mechanism (Kraemer et al., 2006). As has been discussed in section 1.4.2, Schmid et al. (2014) found esculetin, resembling a catechol -type siderophore most closely, to mobilize Fe(III) from a not further specified Fe hydroxide suspension, but not scopoletin. They attributed this to its chemical structure, in particular the inaccessibility of the 6'-hydroxy group. No other compound tested in experiment 4 has an accessible catechol group as esculetin does.

Based on that **Hypothesis 3** was formulated the following:

It is not expected, that scopoletin, fraxetin, coumarin and caffeic acid can mobilize significant amounts of Fe from Santomera soil by chelation of Fe.

Experiment 4 explores this hypothesis by interaction of Santomera soil and esculetin, scopoletin, fraxetin, coumarin and caffeic acid in two time points.

1.5.4 Hypothesis 4

Under environmental conditions plants suffering from iron deficiency release a wide variety of different compounds into the rhizosphere (Fan et al., 2001; Broadley et al., 2012; Colombo et al., 2014). Compounds belonging to the family of coumarins like esculetin, scopoletin and fraxetin have just recently been discovered to be increasingly exuded under Fe deficiency by *Arabidopsis* and *Brassica* species (Schmid et al., 2014; Schmidt et al., 2014). Those compounds mentioned as well as the compounds namesake coumarin and the closely related caffeic acid are polyphenolic compounds, which are considered to have reducing capacities (Keuskamp et al., 2015). It is therefore expected, that a synergistic effect onto the mobilization of Fe, but also of potential competitors such as cobalt and nickel can be observed in combined application of those compounds with DMA.

Hypothesis 4 therefore states the following:

Plant borne compounds, other than ascorbate, with known reducing properties are expected to synergistically elevate iron, cobalt and nickel solution concentrations in combined treatments with DMA.

Experiment 4 therefore combined a fixed amount of plant borne compounds with a fixed amount of DMA and examined the mobilization of Al, Fe, Mn, Zn, Cu, Co and Ni on two time points. A relative index has been developed to quickly assess potential synergistic effects per element.

1.5.5 Hypothesis 5

It has been reported, that Fe mobilization by DMA is affected by competition for free DMA in solution by other metal ions. The conceptual model of a "*Window of Fe acquisition"* was introduced based largely due to the potential negative effects of competition onto Fe acquisition by phytosiderophores. Especially copper has been identified as the main competitor in Santomera soil (Schenkeveld et al.,

2014a). Previous batch experiments in our group (Gasser, 2015) (unpublished), have shown that the combined application of DMA and the compound TETA – a strong copper chelating agent - could increase solution concentrations of Fe significantly compared to the same amount of DMA alone, while solution concentrations of Cu were significantly reduced.

Based on those findings Hypothesis 5 states that:

By reducing the availability of copper in Santomera soil, wheat plants can experience positive effects in their nutritional status reflected by increased chlorophyll content, plant biomass and tissue metal concentrations.

Experiment 5 consists of a pot trial in which *Triticum aestivum cv. Tamaro* was grown in Santomera soil. Two concentration of TETA have been amended to the soil and the plants status was evaluated against an untreated control group., during the growing period and after harvest.

2 Materials and Methods

2.1 Soil parameters

The soil used in all experiments presented is an alkaline calcareous topsoil (0-20cm) from the Murcia region in Spain. Table 1 shows the properties as reported by Schenkeveld et al. (2014b).

Table 1:Properties of Santomera soil as reported by Schenkeveld et al. (2014b)

General properties		Extraction	Element	Concentration
Origin (name)	Santomera	CBD	Fe (g kg^{-1})	10.2
Region	Murcia	AmOx	Fe (g kg^{-1})	0.5
Country	Spain		Fe (mg kg^{-1})	4.9
USDA classification	Entisol		Cu (mg kg^{-1})	1.6
pH ($CaCl_2$)	7.8		Ni (mg kg^{-1})	0.3
EC (mS cm^{-1})	0.11	DTPA	Zn (mg kg^{-1})	0.5
SOC (g kg^{-1})	7.3		Co (mg kg^{-1})	0.0
Clay (g kg^{-1})	300		Mn (mg kg^{-1})	3.1
$CaCO_3$ (g kg^{-1})	500			

The soil has been used in a variety of studies due to its high pH and low Fe availability (Oburger et al., 2014; Schenkeveld et al., 2014a; b, 2016; Walter et al., 2016) and even showed to cause chlorosis in wheat plants grown on it (Oburger et al., 2014).

2.2 Experiment 1: Mobilization by 2'-deoxymugineic acid only and in combination with ascorbate

1.25 g of Santomera soil was weighed into 50mL polypropylene centrifugation tubes (Greiner Bio-One GmbH; Germany).

$CaCl_2$ (analytical grade; Merck KGaA, Germany) was added as a background electrolyte in a final concentration of 10mM. In order to prewet the soil samples 90% of the final volume was pipetted into each sample in a concentration of 11.11mM and put into an end over end shaker (15 rpm) in the dark 2 days before the start of the experiment.

Bronopol (2-Bromo-2nitro-1,3-propanediol) (98% pure; Acros Organics) in a concentration of 0.222 g/L was added together with $CaCl_2$, resulting in a final concentration of 0.2 g/L.

On the day of the experiment a previously prepared in house stock of 2'-deoxymugineic acid (DMA) was thawed and allowed to equilibrate to room temperature. When not used, DMA was stored cool and dark.

Ascorbic acid (Reagent grade, Fisher Scientific; USA) was prepared fresh multiple times and was not used if older than 30 minutes. The pH was adjusted to be in the range of 7 by carefully adding NaOH solution.

All solutions were prepared using ultrapure water (UPW) and acid washed glassware.

All batch experiments have been carried out under temperature controlled conditions at 21 ± 1°C.

Final solution concentrations of DMA and ascorbate were:

DMA: 3.75µM; 6.25µM and 12.5µM; Ascorbate: 37.5µM, 62.5µM and 125µM

The respective concentrations of DMA and/or ascorbate were added and UPW was used to make to final volume of 10ml (SSR 1:8). 10 interaction times (time inside the shaker) were tested: 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h, 48h, 96h, 168h. All treatments were carried out in duplicates. Blank treatments and treatments containing only ascorbate were included for the 1h, 8h, 24h, 168h time points. Note that for the series including 6.25µM and 3.75µM DMA the 0.5h time point was adapted to an interaction of 25 minutes. This is accounted for, in all data presented.

After interaction, samples were allowed to settle for 2 minutes and the supernatant was pipetted off and filtered over 0.45µm cellulose acetate filters (Sartorius, Austria) into 12ml round bottom polystyrene tubes (Greiner Bio-One GmbH; Germany). Samples were transferred into 12ml polystyrene tubes (Greiner Bio-One GmbH; Germany) and acidified with HNO_3 (trace metal grade; Fisher Scientific) and stored in the fridge. The pH value was recorded before acidification.

Metal concentrations were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Optima 5300 DV, PerkinElmer). Metal-DMA concentrations were determined by substracting the mean concentration of treatments containing solely the background electrolyte and bronopol (Blank corrected). Data analysis was carried out in Microsoft Excel.

2.3 Experiment 2: Mobilization by DMA only in SSR 1:4

Experiment 2 was carried out in the same way as experiment 1, without the pipetting of the supernatant. The SSR of 1:4 was reached by interaction of 1.5g of Santomera soil with 6ml final solution volume. No adjustment had to be carried out for any time point resulting in a time series of 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h, 48h, 96h, 168h.

Final solution concentrations of DMA and ascorbate were:

DMA: 7.5µM; 12.5µM and 25µM; Ascorbate: 0.5 mM, 0.75 mM, 1.25mM, 2.5 mM

NOTE: Treatments including ascorbate were not included in this thesis as data interpretation suggested depletion of oxygen in the tubes head space.

2.4 Experiment 3: Mobilization by DMA and ascorbate under anoxic conditions

1.25 g of Santomera soil was weighed into 50mL polypropylene centrifugation tubes (Greiner Bio-One GmbH; Germany).

All samples and materials were introduced into the anaerobic chamber(Coy Lab Products, USA) on the day previous to the start of the experiment. A N_2-H_2 atmosphere was maintained over the whole period of the experiment. Oxygen detectors and two palladium catalysts ensured anoxic conditions throughout the experiment.

$CaCl_2$ and Bronopol (2-Bromo-2nitro-1,3-propanediol) were used as a background electrolyte as already described for experiment 1.

The in house stock of DMA was thawed, Bronopol was added in a concentration of 0.2 g/L to prevent microbial degradation of the compound and stripped of oxygen by bubbling N_2 through the solution

for more than 45 minutes, and introduced into the anaerobic chamber on the day before the experiment. Water losses due to evaporation were marginal, but adjusted for once introduced into the chamber.

Ascorbic acid (Reagent grade, Fisher Scientific; USA) was prepared fresh at the day of the experiment. The pH was adjusted to be in the range of 7 by carefully adding NaOH solution.

All solutions were prepared using ultrapure water stripped of oxygen (UPW) and acid washed glassware.

Final solution concentrations tested were:

DMA: 3.75µM and 6.25µM; ascorbate: 37.5µM and 62.5µM

The respective concentrations of DMA and or ascorbate were added and UPW was used to make to final volume of 10ml (SSR1:8). 10 interaction times (time inside the shaker) were tested: 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h, 48h, 96h, 168h. All treatments were carried out in duplicates. Blank treatments were included for 0.25h, 1h, 8h, 24h, 168h.

After interaction samples were allowed to settle for 2 minutes and the supernatant was pipetted off and filtered over 0.45µm cellulose acetate filters (Sartorius, Austria) into 12ml round bottom polystyrene tubes (Greiner Bio-One GmbH; Germany). Samples were transferred into 12ml polystyrene tubes (Greiner Bio-One GmbH; Germany) and acidified with HNO_3 (trace metal grade) and stored in the fridge as soon as the experiment was terminated. The pH value was recorded before acidification. Due to the limitations of the anaerobic chamber, samples were split to start interaction on two consecutive days.

Analysis was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7700 Series).

2.5 Experiment 4: Scanning experiment Coumarins & DMA

1.5 g of Santomera soil were weighed into 50mL polypropylene centrifugation tubes (Greiner Bio-One GmbH; Germany).

$CaCl_2$ (analytical grade; Merck KGaA, Germany) was added as a background electrolyte to obtain a final concentration of 10mM. In order to prewet the soil samples 90% of the final volume was pipetted into each sample in a concentration of 11.11mM and put into an end over end shaker (15 rpm) in the dark 2 days before the start of the experiment.

On the day of the experiment a previously prepared in house stock of DMA was thawed and allowed to equilibrate to room temperature. When not used DMA was stored cool and dark.

Stock solution (5mM) were prepared not later than 30 minutes before addition to soil solution.

Table 2 displays the properties of the respective stock solution prepared and states if ultrasonifiaction and or pH adjustment was needed to dissolve compound. Note that not all compounds tested are discussed in this thesis.

Table 2: Overview on preparation of compound stocks

Compound	Ultrasonification	pH final	Adjusted by adding NaOH solution?
coumarin	10 minutes	n.r	No
scopoletin	No	7.5	Yes
fraxetin	15 minutes	7.6	Yes
caffeic acid	15 minutes	> 5	Yes
Riboflavin	No	9.8	Yes
$MnCl_2$	No	7.9	Yes
$FeCl_2$	No	5.4	Yes
AQS	5 minutes	7.7	Yes
AQDS	5 minutes	n.r	No

All solutions have been prepared using ultrapure water (UPW) and acid washed glassware.

All batch experiments have been carried out under temperature controlled conditions at 21 ± 1°C.

25 µM of DMA and or 250µM of compound were added and UPW was used to make to final volume of 6ml (SSR1:4). Interaction times tested were 15 minutes and 4 hours. All treatments were carried out in duplicates. Blank treatments were included at both time points.

After interaction time samples were centrifuged for 3 minutes at 4500 rpm and filtered over 0.45µm cellulose acetate filters (Sartorius, Austria) into 12ml round bottom polystyrene tubes (Greiner Bio-One GmbH; Germany). Samples were transferred into 12ml polystyrene tubes (Greiner Bio-One GmbH; Germany) and acidified with HNO_3 (trace metal grade) and stored in the fridge. The pH value was recorded before acidification.

Analysis was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7700 Series). Metal-DMA and Metal-Ligand concentrations were determined by substracting the mean concentration of treatments containing solely the background electrolyte and bronopol (Blank corrected). Data analysis was carried out in Microsoft Excel.

2.6 Experiment 5: Pot trial: Effects of Triethylenetetramine dihydrochloride addition to a calcareous soil on iron acquisition by wheat

16 one liter pots (polypropylene) were thoroughly cleaned and rinsed with deionized water. Santomera soil (4mm sieved) was prewetted with deionized water (DIW) (SSR 5:1) and fertilized according to (Schenkeveld et al., 2008).

Triethylenetetramine dihydrochloride (TETA) was given in two treatments in the concentrations 500µM (in the following referred to as Low TETA) and 2000µM (in the following referred to as High TETA) relative to the pore water content. Those treatments proved to be effective in previous batch interaction studies in a SSR of 5 (Gasser, 2015). To ensure homogeneity TETA and the fertilizers were stepwise mixed with the air dried Santomera soil.

Special care was taken to not cause precipitation of the fertilizer solutions and to avoid aggregate formation of the clay rich soil, by stepwise addition of individual nutrient and TETA solutions followed by a thoroughly mixing period by hand. Appr. 1 kg soil was added to the pots and the final weight recorded. A quartz sand column was included in the centre of each pot to facilitate watering throughout the experiment. Effects of the sand column on soil pH and introduction of micronutrients were considered to be neglectable.

Initial weight of each pot was recorded in order to maintain constant moisture conditions throughout the experiment.

Each treatment and the control treatment was carried out in quadruplicates. Additional 2 pots of the Control treatment and the High TETA treatment were equipped with Rhizons (ceramic membrane, pore size: 0.12 – 0.18 μm, Rhizosphere research products, Netherlands) to sample pore water extracts during the growth phase.

The cultivar chosen was wheat (*Triticum aestivum cv Tamaro*). Previous studies showed iron deficiency for this cultivar in Santomera soil (Oburger et al., 2014). Per pot 8 seeds *Triticum aestivum cv Tamaro* were planted. After germination was ensured, shoots were removed from pots and 4 plants per pot were grown from there on.

Germination was conducted under temperature controlled conditions (±20°C) in the dark. After germination pots were transferred into a greenhouse. Temperature was controlled in the greenhouse at 25 ± 5°C. The experiment was conducted between April and June. Primary source of light was sunlight assisted by artificial lights on cloudy days.

Water addition was carried out carefully to avoid potential soil solution losses with deionized water. The SSR was kept at around 5. Duration of the growing phase was 45 days.

A solution of 0.2 mM Cu, 0.82 mM Mn,0.27 mM Zn and 0.04mM Mo was prepared containing 1ml/L solution of the surfactant Agronetz (BASF, Austria) and added after 28 and 40 days into the experiment by foliar fertilization.

The chlorophyll content was measured 2-4 times a week with a SPAD meter (Konica Minolta, Japan) to monitor the leaf chlorophyll content of the youngest and second youngest leaf. Per pot 8 youngest and 8 second youngest leaves were measured each session.

Rhizon sampling was conducted regularly (Note that the amount of sample was often not sufficient to provide a substantial data contribution).

At harvest, all above ground plant material was cut with a metal free knife, fresh weight (fw) was recorded and the plant material was thoroughly rinsed with DIW. Cuttings were carefully placed into bags and dried at 70°C until no weight loss could be recorded anymore (3 days). The dry weight was recorded immediately after.

The soils were carefully cleaned from sand and root material and prepared for pore water extraction on the day of harvest. The pore water extraction was carried out in two compartment centrifuge tubes for 10 minutes at 7000rpm according to Schenkeveld et al. (2008).

The pore water was collected and split into subsamples. One half was prepared for metal analysis as is described for experiments 1-4 and measured by ICP-MS. The other half was frozen for later analysis of DOC. DOC analysis was carried out by acidification followed by combustion using a total organic carbon analyzer (TOC-L, Shimadzu, Japan). Method blanks were included during the preparation process.

The plant material was ground to fine powder using a granite mortar and liquid nitrogen, until all sample was homogenized.

The plant material was weighed into PTFE vials (Savillex, USA) and wet digested on a heating block by HNO_3 & H_2O_2 according to (Wheal et al., 2011). The reference material NCS DC73349 (“bush branches”) was used to assess the metal recovery, a blank was included during the digestion. Metal solution concentrations was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7700 Series). Data analysis was carried out using MS Excel.

3 Iron and other metal mobilization by DMA

In this thesis metal mobilization refers to the amount of respective metal brought into solution by interaction of the soil solution with the phytosiderophore 2'-deoxymugineic acid (DMA). Metal mobilization is determined by correcting the solution concentrations of the respective metal with DMA for the mean solution concentrations in treatments without DMA (Blanks).

In this chapter the mobilization of 3 different concentrations of DMA applied in a SSR of 1:8 are discussed in detail by assessing the concentration of the total metals mobilized (paragraph 3.1) and the individual metal mobilization (paragraph 3.2) over time. This will then be compared to the mobilization obtained under a SSR of 1:4 in paragraph 3.3. Paragraph 3.4 will discuss the key conclusions drawn.

3.1 Total metal mobilization by different DMA additions

Figure 5 displays the sum of all metal concentrations in solution for three different concentrations of DMA. The metals summed up (Fe, Cu, Co, Ni, Zn) are known to form complexes with DMA (Reichman et al., 2005). Mn and Cd have also been reported to form DMA complexes, but their concentration in treatments with DMA did not differ from the treatments without DMA in this experiment. This is in agreement with other studies related to metal mobilization by DMA in Santomera soil, where substantial amounts of MnDMA was found only in much higher DMA additions (1mM DMA – SSR 1) (Schenkeveld et al., 2014a; b, 2016; Walter et al., 2016).

Figure 5 shows a decreasing rate of total metal mobilization over time, with variations depending on the amount of DMA supplied. The treatment with 3.75µM DMA (in the following referred to as low DMA treatment) mobilized around 0.75 µM total metals after 15 minutes and concentration increased to 2.9 ± 0.3µM in solution after 8 hours. From there on only little changes in total metals in solution were observed with solution concentrations of 3.0 µM after 168 hours. The intermediate treatment of 6.25µM DMA mobilized 0.9µM total metals initially and solution concentration increased thereafter. Between the 24 hour and 168 hour time point the rate of total metal mobilization became significantly reduced with solution concentration increasing from 4.4µM to 4.8 µM. Given the fact that mobilization by the background electrolyte only was deducted (SI Table 1), all metals displayed here can be considered being complexed by DMA. This is in agreement with (Schenkeveld et al., 2014b). Based on that in the low DMA treatment 75% of the ligand supplied was complexing and mobilizing a metal after 8h and 81% after 168h. For the intermediate DMA scenario 73% ligand was complexing and mobilizing a metal after 24h and 79% of it after 168h. The treatment with the highest (12.5µM) DMA addition showed increasing solution concentration the longest, starting at solution concentrations of 1µM after 15 minutes. However, the increase between the 96h time point and the 168h time point appeared to be less strong than in previous time points with 66% and 68% of the ligand supplied, mobilizing a metal.

Even though the sum of total metals in solution did not change drastically in the low and intermediate DMA treatments for large parts of the later time series, the systems did not reach equilibrium conditions in that time frame. This became obvious by the solution concentration changes of the individual metals (compare paragraph 3.2).

This serves as an indication that the amount of available free DMA in solution was decreasing over time by ongoing complexation and partial adsorption to, but also desorption of the MeDMA complexes from, the soil solid phase. This slowed down the rate of total mobilization. Schenkeveld et al., 2014b found an approximately exponential decline of metal DMA solution concentration increase over time. This finding is in very good agreement with the data obtained here.

Initial solution concentrations did not differ largely. The lowest treatment (3.75µM DMA) mobilized around 75% of the amount the highest treatment (12.5µM DMA) mobilized after 15 minutes. This was striking as DMA concentrations differed by a factor 3 between low and high DMA treatment. Ligand promoted dissolution is triggered by adsorption of DMA to the soil solid phase (Kraemer et al., 2006). Adsorption of free DMA supplied to a soil system is fast (Schenkeveld et al., 2014a), but complexation and desorption kinetics have to be taken into considerations here, which are element dependant and can be rather slow (Schenkeveld et al., 2014a). The discrepancy between the treatments did grow over time, until at the end of the experiment the ratio between metals in solution and ligand supplied to the system was almost matched exactly for low and intermediate DMA treatment and approaching that ratio for the highest DMA treatment.

However considering in particular the first 8 hours the difference between treatments were not yet that distinct with 2.9 ± 0.3µM total metals mobilized by the lowest DMA treatment and 4.1 µM mobilized in the highest treatment. Under environmental conditions PS are subject to microbial degradation (Marschner & Römheld, 1995), with most of the ligand being depleted from solution concentration during the first 24 hours (Schenkeveld et al., 2014b). The initial mobilization of total metals displayed showed, that an increase in DMA supply by more than a factor 3, in the concentration range and soil to solution ratio examined, did not impact total metal mobilization immediately.

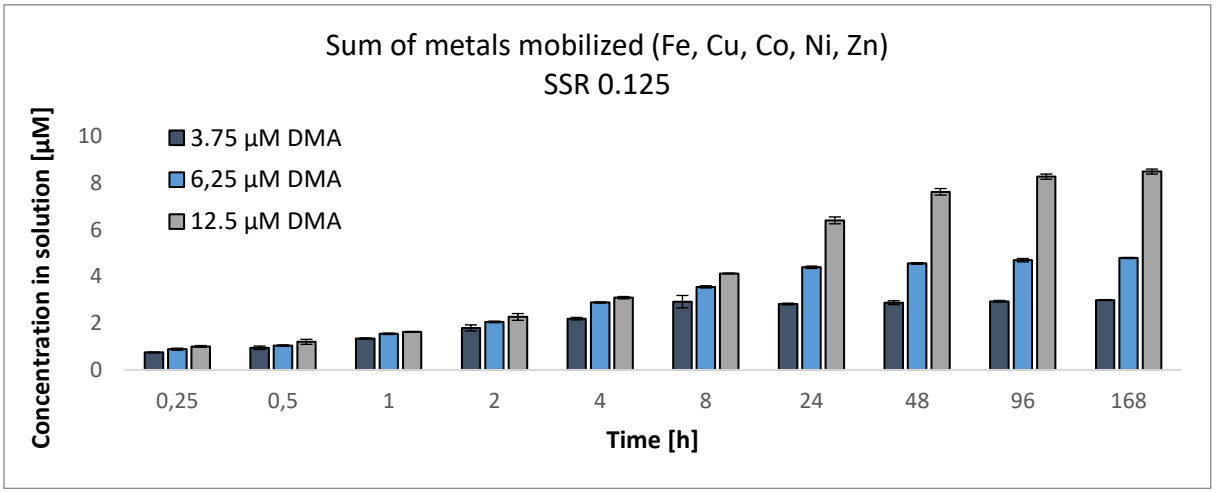


Figure 5: Sum of metals mobilized (Fe, Cu, Co, Ni, Zn) in Santomera soil; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant); error bars indicate standard deviation (n=2);

It has been demonstrated that a reduced supply of DMA to the system narrows the "Window of Fe acquisition" (Schenkeveld et al., 2014b). However, the rate of total metals mobilized here might serve as one indicator, as to the point in time when most of the ligand in solution is complexing a metal.

Under ligand limited conditions, competitive displacement is influencing the MeDMA speciation in solution, moving towards the thermodynamically most favourable species to complex (Schenkeveld et al., 2014b). Modelling has shown Cu and Ni to be the dominant species mobilized by DMA in Santomera soil when equilibrium conditions are reached (Schenkeveld et al., 2014a). Iron on the other hand is, depending on the soil pH and its availability, less likely to become mobilized by DMA than those two elements in this soil (Reichman et al., 2005; Schenkeveld et al., 2014a). Indications of the system approaching conditions of limited free ligand are displayed in Figure 5 and its consequences are presented in paragraph 3.2. Cu, Ni and Co, which was not included in the equilibrium model by Schenkeveld et al. (2014) due to lack of CoDMA stability constants, showed an increasing solution concentration trend over time. Iron and zinc on the other hand were decreasing under those conditions.

3.2 Individual metals mobilized by DMA

Figure 6 gives an overview on the individual metal-DMA (MeDMA) species in solution over time. A general pattern visible is, that in the beginning of the time series all DMA treatments mobilized the respective element in a similar concentration range. Over time differences in solution concentrations became stronger, with the treatment with the highest DMA addition mobilizing the most metals, while the treatment with the lowest DMA addition mobilized least. This has also been observed in a higher soil to solution ratio of 1:4 (compare paragraph 3.3) and is in agreement with the total metal mobilization displayed in Figure 5.

3.2.1 Iron

Iron (Figure 6a) did not reach its maximum concentration after 168h in any of the scenarios tested. The largest relative differences in peak concentrations by varying ligand concentration can be observed for this element. All treatments mobilized around 0.3µm Fe initially. Concentrations increased thereafter with a relatively small maximum delta between the three treatments of 0.1µM onto the 1 hour time point. Here the low DMA treatment could not mobilize as much as the other treatments and deviated, reaching a maximum solution concentration of 1.2µM after 8 hours. The delta between the intermediate and high DMA treatments increased in the following time points, until the intermediate DMA treatment reached its maximum of 1.9µM after 24 hours and decreased to concentrations of around 0.6µM after 168 hours thereafter. The high DMA treatment reached its maximum of 4.1µM after 48 hours. In the following solution concentrations decreased again to 3.3µM at termination of the experiment.

When paying especially close attention to the first 8 hours of the experiment, differences between highest and lowest DMA treatment started to have an impact after the 1 hour time point and began to influence the solution concentrations between the intermediate and high treatment from 4 hours onward. After 8 hours the concentrations of the low (3.75µM) DMA and high (12.5µM) DMA treatment differed by a factor of approximately 2, and between the intermediate (6.25µM) and high DMA treatment by a factor of 1.3.

When comparing the peaks of Fe solution concentration (Figure 6a) with the total metals mobilized by the respective treatment (Figure 5) one might see a relationship. The maximum concentration of the low and intermediate DMA treatment matched well with the point in time, where the rate of total metal mobilization slows down significantly, approaching equilibrium like total metal concentrations, but not solution speciation. This dependency was not that obvious for the high DMA treatment, but here as well the total metals in solution increase was smaller between the 48h time point and the 96h time point, compared to earlier in the experiment.

It has already been shown, that FeDMA is not the thermodynamically most favourable MeDMA complex in Santomera soil. The patterns of Fe mobilization displayed here are in line with previously reported data on FeDMA complexation in Santomera soil (Schenkeveld et al., 2014a, 2016), with differences in time points of maximum mobilization, which might be caused by variation of the soil to solution ratio (SSR) and resulting deviations in ligand - solid interaction. Additionally prewetting of the soil has not been carried out in aforementioned studies, but has recently been shown to significantly alter the metal mobilization by DMA from soil (Schenkeveld et al., 2017).

The availability of other metals in Santomera soil is determining the fate of FeDMA presence in solution. Therefore, the mobilization of those potential competitors for free or already complexed ligand needs to be evaluated.

3.2.2 Zinc

Zinc (Figure 6b) did also not reach its maximum concentration after 168 hours in any of the treatments tested. In all treatments initial solution concentration after 15minutes were around 0.3µM. From there on a simultaneous increase in solution concentration was observed.

Concentrations in the 3.75µM DMA treatment reached plateau like conditions after 1 hour and remained in a concentration range below 0.4µM. After the 8 hour time point concentrations steadily decreased until almost all Zn was depleted from solution after 168 hours. The intermediate and high DMA treatments caused Zn concentrations to increase simultaneously, until the maximum concentrations for the intermediate DMA treatment of 0.45µM was reached after 4 hours and concentrations decreased to below 0.2µM after 168 hours. In the high DMA treatment ZnDMA increased further until it reached a maximum of 0.6µM after 48 hours. From here the concentration decreased slightly, but stayed in the range of 0.6µM after 168h.

Alike Fe, Zn experienced depletion over time. Zinc has a lower stability constant with DMA than Fe (Murakami et al., 1989; von Wirén et al., 2000) and equilibrium modelling shows it to be of even lower concentration than iron in Santomera soil (Schenkeveld et al., 2014a SI). However, solution concentrations decreased less strongly over time after maximum concentrations are reached, compared to Fe. This indicates a lower sensitivity of Zn to become displaced from the DMA complex by Co, Ni or Cu than Fe (Schenkeveld et al., 2017).

In general, observed solution concentrations of Zn stayed below 10% of ligand supplied, indicating a rather limited potential to impact Fe acquisition by competition. Additionally, Zn is an essential micronutrient and increased PS efflux has been discussed as a potential mean to overcome Zn deficiency (Reichman et al., 2005; Broadley et al., 2012). For a Strategy II plant a mild competition on iron mobilization by PS mediated Zn mobilization might therefore be not entirely negative.

3.2.3 Copper

Copper concentrations increased over the entire time series of the experiment (168h). Initially all treatments mobilized around 0.3µM CuDMA. In the following very similar mobilization for the first 8 hours was observed in all treatments. After that the concentrations for the 3.75µM DMA treatment deviated and reached a maximum of 2.0 µM in solution after 168h, accounting for approximately 2/3 of all MeDMA in solution. The concentrations in the intermediate and high DMA treatment remained in similar concentration range up until the 48 hour time point. Here the 6.25µM DMA treatment deviated, to reach a maximum of 2.5µM (more than 50% of total MeDMA) in solution after 168h. The highest DMA treatment (12.5µM) reached its maximum of 2.8µM (around 1/3 of all MeDMA) after 168 hours.

Copper is considered to be a possible competitor for Fe complexation by DMA (Murakami et al., 1989; von Wirén et al., 2000). In this soil Cu is thermodynamically more favourable to be complexed by DMA than Fe (Schenkeveld et al., 2014a). The data obtained provides evidence for that. Relatively independent of the amount of ligand, Cu solution concentrations continued to increase over time. Even though Ni has been shown to be the dominant species at equilibrium by Schenkeveld et al. (2014a), Cu can be considered to be the main competitor to iron acquisition in Santomera soil, due to the slow mobilization kinetics of Ni (Schenkeveld et al., 2014b). Its impact onto Fe acquisition was well visualized in the low DMA scenario (3.75µM). After 8h both Cu and Fe were in approximately the same solution concentration range of 1.1µM ±0.1µM. Thereafter Cu concentration continued to increase steadily, whereas Fe concentration steadily decreased and was hardly present in solution after 168h. As previously pointed out the sum of all metal concentrations did not change largely during the 8 to

168 hours time span (Figure 5). The gain of Cu from 8 hours on matched surprisingly well with the loss of Fe in that time.

Even though also Cu is an important micronutrient for plants (Broadley et al., 2012), under Fe deficient conditions the preferential mobilization of Cu might significantly hinder the uptake of desperately needed iron. In fact in copper contaminated soils of former vineyards decreased Fe concentrations in durum wheat have been reported (Michaud et al., 2007).

3.2.4 Nickel

Nickel (Figure 6d) reached a maximum concentration in all treatments after 168 hours. It was mobilized to the same extent for the first 8h, with concentrations for the intermediate and high DMA treatment being in very good agreement with each other and slightly reduced for the low DMA treatment. After 8 hours NiDMA concentrations in the low DMA treatment did not increase similarly as the other treatments, reaching a maximum concentration of 0.7 μM. The intermediate and high DMA addition deviated only slightly resulting in maximum solution concentration of 1.1μM and 1.2μM respectively after 168 hours.

Equilibrium modelling suggested that NiDMA will become the dominant species in Santomera soil eventually. Due to its slow complexation kinetics this was not yet the case in the time span of the experiment, which is in agreement with findings by Schenkeveld et al. (2014a). In an environmental system biodegradation is constantly depleting concentrations of organic compounds such as DMA (Römheld, 1991). That most likely limits the unfavourable impact of Ni onto Fe mobilization under more natural conditions significantly, as can be inferred by the results presented by (Schenkeveld et al., 2014b). Here biodegradation of MeDMA complexes was analysed and only little remained in solution after 24 hours. However, under conditions where Ni is more available, Ni might become a significant competitor for Fe acquisition.

3.2.5 Cobalt

Cobalt (Figure 6e) mobilization was below 0.1μM in the first 8 hours for all treatments. Thereafter in the low DMA treatment Co solution concentrations increased only slightly to remain at below 0.2μM. The intermediate and high treatments showed similar concentrations for the first 24 hours. Both treatments continued to increase until termination of the experiment, with different rates. The CoDMA concentration in the intermediate DMA treatment reached a maximum of 0.4μM, whereas the high DMA treatment mobilized a maximum of 0.6μM after 168 hours incubation time.

The lack of stability constants and certainty about the redox state of CoDMA - Co(II) and Co(III) exist in soils (Scheffer et al., 2010), make it difficult to predict its behaviour with DMA in soils. The fact that in the data obtained here Co solution concentrations could still increase while Fe and Zn were decreasing, did indicate a thermodynamically more favourable complexation. Under the conditions displayed here, cobalt mobilization by DMA, like nickel, appeared not to be of highest concern considering its impact onto Fe mobilization, due to its late increase in solution concentration.

3.2.6 Manganese

Schenkeveld et al. (2014b) showed that by increasing the amount of DMA significantly (1mM in a SSR of 1) Mn can get mobilized substantially from Santomera soil over time. Mn concentrations measured here did not show mobilization by DMA alone. However, over time Mn was mobilized in treatments with and without DMA to the same extent with the highest concentrations of 0.7μM $\pm$ 0.1 μM reached after 168h (compare SI Table 1). This was likely to be related to Mn oxide dissolution over time. Elements associated with Mn oxides like Ni and Co (Scheffer et al., 2010), may have experienced increased availability due to that.

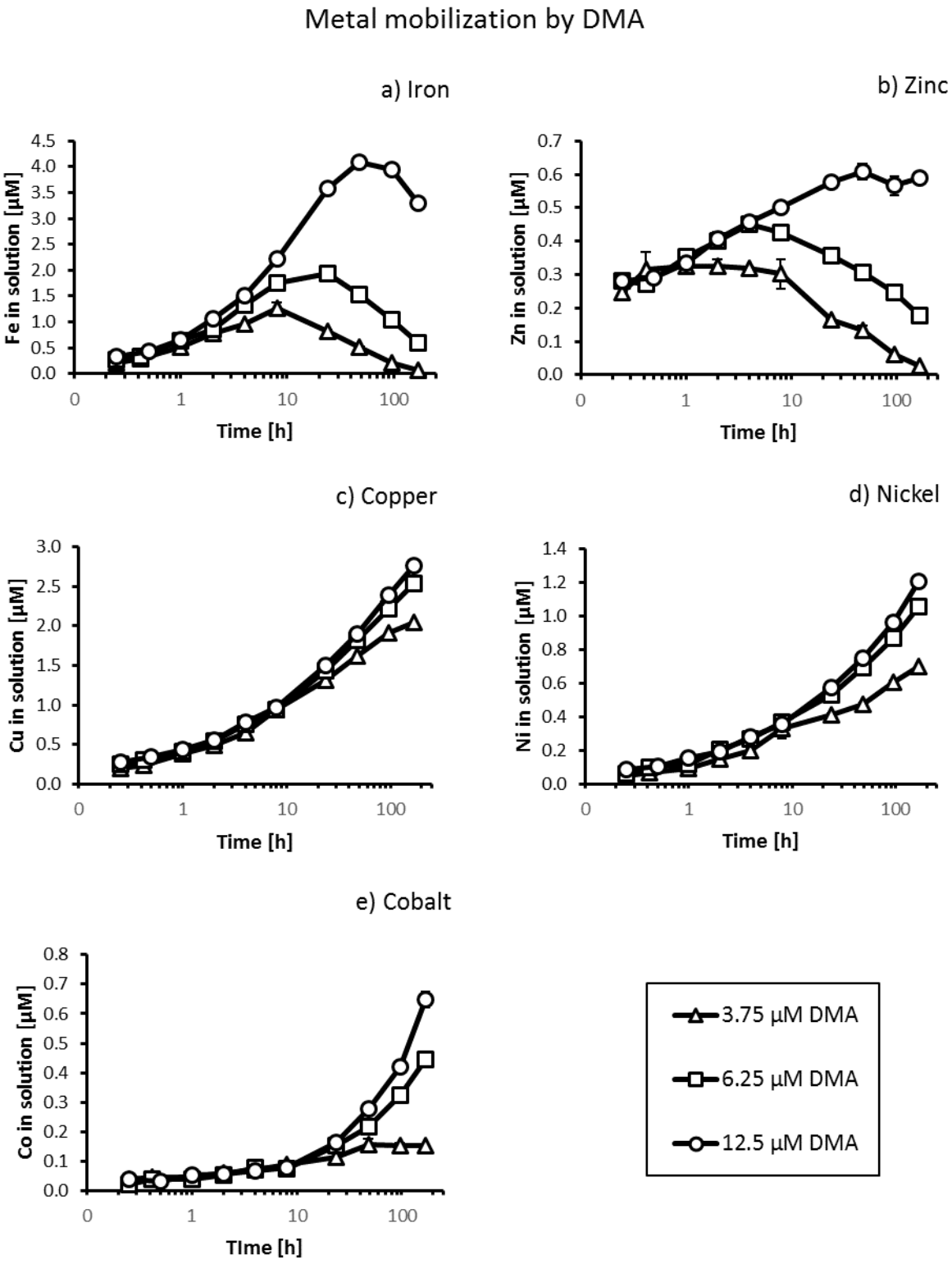


Figure 6: Metal mobilization by 3.75µM, 6.25µM and 12.5µM DMA from Santomera soil over time; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant); error bars indicate standard deviation (n=2)

3.3 Comparison metal mobilization SSR 1:8 and SSR 1:4

SI Figure 1 & SI Figure 2 show the total and individual metal mobilization by DMA alone in a soil to solution ratio (SSR) of 1:4. The amount of DMA supplied to the system was increased by a factor of 2 in comparison to the treatments tested in a SSR of 1:8. The general trends of mobilization described in detail in section 3.2 were well in agreement, , with those obtained in a soil to solution ratio of 1:4, both for the individual and subsequently the total metal mobilization. In the following a method of translating the results obtained in a lower SSR to a higher SSR shall be applied.

3.3.1 Approach

Batch experiments are carried out in vast range of soil to solution ratios. The majority of studies conducted in our group, are however carried out in a SSR of 1 (Schenkeveld et al., 2014a; a, 2016; Walter et al., 2016). Sometimes it may be more convenient to work in a lower soil to solution ratio. In example when the compound under investigation is scarce or hardly soluble, a lower soil to solution ratio may be preferred. Many processes involving ligands, like ligand promoted dissolution, are influenced by reactive surface sites in soil. A change in the ratio of soil to solution with a certain concentration of ligand therefore alters the number of surface sites the ligand interacts with. Therefore an adaption of the amounts applied to the system must be undertaken, to avoid possible misinterpretation of the data obtained. As solution concentrations are influenced by adsorption of the complex to the soil solid phase, adsorption isotherms have to be included when evaluating changes in solution concentration. The adsorption isotherm is highly soil dependant. For adsorption of Metal DMA (MeDMA) complexes to Santomera soil those have been derived in a previous study (Walter et al., 2016).

Walter et al. (2016) proposed a simple determination of total species concentration. The proposed mechanism combines measured solution concentrations and the adsorbed fraction calculated by a soil specific adsorption isotherm.

The adsorbed content [mol kg^{-1}] can be derived by multiplying the adsorption coefficient with the solution concentration measure (Equation 1).

$$Q = \alpha * C_L \quad (1)$$

With Q = adsorbed content [mol kg^{-1}], α = adsorption coefficient [l kg^{-1}], C_L = solution concentration [M]

As the adsorbed concentration depends on the number of reactive surface sites, the soil to solution ratio needs to be considered (Equation 2).

$$C_s = Q * SSR \quad (2)$$

With C_S = adsorbed concentration [M], SSR soil to solution ratio [kg l^{-1}]

By combining the concentrations in solution and the concentration in solution one can deterimine the total concentration (Equation 3)

$$C_{T_n} = C_{S_n} + C_{L_n} = C_{L_n} * (1 + \alpha SSR_n) \quad (3)$$

With C_T = total concentration [M] and n being the respective treatment (e.g. field or batch experiment)

The determined adsorption coefficients are stated in Table 3.

Table 3: Adsorption coefficient for MeDMA complexes determined by Walter et al. (2016)

Species	**Adsorption coefficient for Santomera soil [l kg-1],**	**R^2**
FeDMA	0.71	0.999
CuDMA	0.61	0.998
NiDMA	1.35	0.998
ZnDMA	1.17	0.998
DMA	0.88	0.997

As this approach considers linear adsorption of the respective DMA complex a much more accurate translation of experimental data can be achieved. Here it shall be investigated how well the data derived from an experiment carried out in a SSR of 0.125 is able to predict the concentrations measured in a SSR of 0.25.

The amounts of ligand added are related in following fashion:

$$L_{SSR_2} = L_{SSR_1} * (\frac{SSR_2}{SSR_1}) \quad (4)$$

With L_{SSR_n} = the amount of ligand added [M]; in experiment with respective soil to solution ratio (SSR) [kg l^{-1}].

By comparing the total concentration of species the different adsorbed fraction of MeDMA complexes by the soil solid fraction are taken into account. In order to compare the results obtained another simple transformation can normalize the total concentrations.

$$C_{T_{SSR_2}} = C_{T_{SSR_1}} * (\frac{SSR_2}{SSR_1}) \quad (5)$$

Or

$$(\frac{C_{T_{SSR_1}}}{C_{T_{SSR_2}}}) = (\frac{SSR_1}{SSR_2}) \quad (6)$$

With $C_{T_{SSR_1}}$ being the total concentration in the SSR normalized to.

The amounts of solution concentrations can also be directly related into each other by combining equation 3 and 5 resulting in equation 7 (Schenkeveld et al., 2017)

$$C_{L_1} = \frac{C_{L_2} * (1 + \alpha * SSR_2)}{(1 + \alpha * SSR_1)} * \frac{SSR_1}{SSR_2} \quad (7)$$

3.3.2 Results

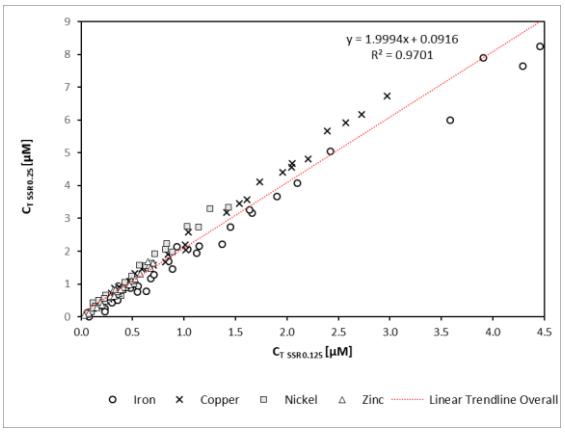


Figure 7: Total concentrations (C_T) of Fe, Co, Ni, Zn calculated according to Equation 3 for SSR 0.25 over SSR 0.125; standard deviation not considered in display

When displaying the total concentrations calculated by equation 3 for a soil to solution ratio of 0.25 over the total concentration derived from Experiment 1 in a SSR of 0.125 (Figure 7), it becomes apparent that the data obtained in both experiment is overall in good agreement with each other. The slope of the overall trendline derived from all respective total concentrations over their respective counterpart in a lower SSR is shown in Table 3. Ideally that slope should be 0.25/0.125 = 2 according to equation 6. The fact that the overall slope is so close to that ideal value highlights the overall ability to relate the total concentrations with each other.

For the individual elements iron was the only metal with a $C_{T\ SSR\ 0.25}$ / $C_{T\ SSR\ 0.125}$ value below 2, indicating relatively higher total concentrations in the lower SSR. This was in particular driven by the relatively elevated peak concentrations in the lower SSR as becomes apparent in Figure 8. Those might be related to an incomplete desorption of the FeDMA complexes from the soil solid phase as has been reported by (Walter et al., 2016). Apart from that solution concentrations of Fe are very well represented. Especially while Fe solution and subsequently total concentrations were still increasing Figure 8 shows good agreement between estimated and measured values. As has been elaborated earlier in this thesis and is reported in literature(Schenkeveld et al., 2014a, 2017), it is likely that still free DMA is present in the system while Fe solution concentrations increase and Fe becomes subject to increased competition by competitive displacement when the free ligand becomes scarce.

With incomplete desorption, which is especially pronounced for FeDMA complexes (Walter et al., 2016), more FeDMA remains at the solid surface and is potentially subject to catalysed competitive displacement, as has been demonstrated for the ligand EDDHA (Schenkeveld et al., 2012). This effect might become more pronounced according to the amount of reactive surface sites. Indications for that can actually be seen in the mass balance of the total concentrations for experiment 1 displayed in SI Table 2. In particular in the low DMA treatment the total MeDMA calculated, accounted for 112% of the ligand supplied after 168 hours. This indicated competitive displacement of FeDMA at least partly happening at the soil surface, when neglecting the possibility of experimental problems. The fact that this was observed most prominently in the lowest DMA treatment – with the most reactive surface sites per ligand to interact with, might be plausible under those circumstances.

Table 4 Slopes of linear trendlines

	Slopes $C_{T\ SSR\ 0.25}$ / $C_{T\ SSR\ 0.125}$	R^2
Zn	2.39	0.97
Ni	2.53	0.98
Cu	2.27	1.00
Fe	1.89	0.99
Overall	1.999	0.97

The values for all other metals displayed $C_{T\ SSR\ 0.25}$ / $C_{T\ SSR\ 0.125}$ trendlines are above 2, indicating relative larger total concentrations in the higher SSR experiment. The measured values in solution for the SSR 0.25 experiment (Figure 8) are subsequently consistently higher than estimated based on the SSR 0.125. The differences become more pronounced over time. Which especially for Cu and Ni may be related to the observed lower Fe solution concentrations.

Figure 8 demonstrates that in general solution concentrations mobilized by DMA can be effectively translated between different SSR.

Schenkeveld et al. (2017) showed clearly that the soil to solution ratio needs to be considered and amount of ligand needs to be scaled accordingly, in order to effectively model the potential metal mobilization by phytosiderophores under field conditions. It has been shown in this thesis as well as in other studies (Schenkeveld et al., 2014a; b, 2017), that the amount of free ligand in solution is an important driver especially on Fe mobilization or depletion from solution. When applying higher amounts of ligand in relation to the amount of soil surface sites reactive pools may become depleted, competitive displacement underestimated, and other effects like manganese mobilization substantially overestimated (Schenkeveld et al., 2017). By simply considering adsorption of the respective MeDMA complex to the soil solid phase and adjusting the ligand to the SSR, misinterpretations of the overall impact of, in this case, DMA onto Fe and other metal mobilization can be avoided.

Counterintuitively comparing the mobilization patterns presented here and the mobilization patterns presented in studies carried out in a SSR of 1 with DMA on the same soil by Schenkeveld et al. (2016) it becomes obvious, that the solution concentrations are not in agreement with each other (the reader may be referred to SI Figure 3). It is obvious that the peaks in mobilization for Fe and Zn shift towards earlier time points, which can be related to the fact, that lower quantities of free DMA remain in solution in increased SSR. What causes the consistently elevated higher solution concentrations (around a factor 1.5 for Cu, Ni and Fe) especially in the first time points remains unclear. Soil age may play a role in that respect as it has just recently been demonstrated, that metal ions related to SOM are affected by air drying and subsequent rewetting (Schenkeveld et al., 2017).

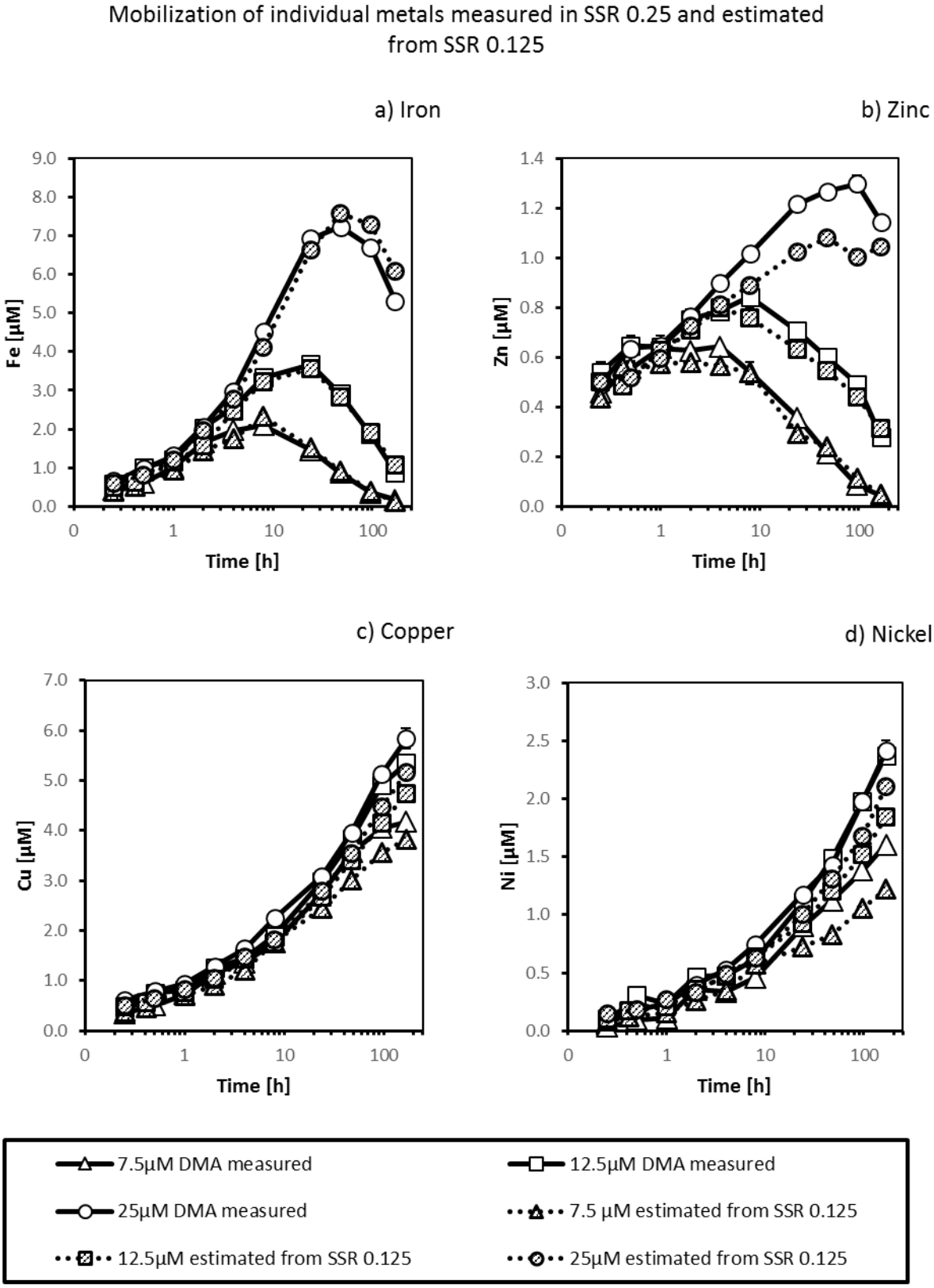


Figure 8: Measured solution concentrations in SSR 0.25 and estimated solution concentrations based on measured solution concentration in SSR 0.125 calculated by Equation 7; Error bars indicate measured standard deviation of respective experiment. 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

3.4 Discussion

It was demonstrated, that adjusting soil to solution ratio and amount of ligand supplied to a soil system led to similar trends in MeDMA solution concentrations. It was shown directly by (Schenkeveld et al., 2014a) and indirectly in this thesis via evaluation of the MeDMA mobilization, that the amount of free DMA present in the soil solution resulted in altered individual metal mobilization. Therefore it is necessary to scale the amount of ligand supplied with the soil to solution ratio, if comparison between results obtained under different soil to solution ratios is wanted (Schenkeveld et al., 2017).

Estimating MeDMA solution concentrations by experimental data obtained under SSR 0.125 to a SSR of 0.25 worked well for all elements, where adsorption isotherms of the MeDMA complex on Santomera soil were available. The trends in solution metal concentrations found in a SSR of 1:8 held true in a SSR of 1:4, strengthening several key findings for the metal mobilization by the ligand DMA with Santomera soil.

In all cases did the high (12.5µM in SSR 0.125; or 25µM in SSR 0.25) DMA treatment mobilize most, the intermediate (6.25µM or 12.5µM) DMA treatment second most and the low (3.75µM or 7.5µM) DMA treatment least of the respective element approaching equilibrium conditions. Changes in solution speciation however indicated that equilibrium conditions were not yet reached.

In all treatments and for all elements, there was an initial increase in solution concentration observable. The duration was depending on the relative element and concentration of ligand. This increase was also reflected by the increase in total metals in solution. That indicates incomplete ligand complexation in the beginning of the time series and the existence of free DMA in solution or adsorbed to the solid phase. This finding is in agreement with (Schenkeveld et al., 2014a) where it has been demonstrated, that free DMA was even the dominant species in solution after 4 hours in Santomera soil solution (100µM DMA; SSR 1).

Zinc and iron reached their maximum solution concentrations during the investigated time series and decreased thereafter. All other elements had their maximum at 168 hours. Given the fact that microbial degradation has been repressed, and the total metals in solution changed only little after certain, treatment specific points in time, competitive displacement was the most likely reason for changes in solution concentrations over time (Schenkeveld et al., 2014a). Which metal was complexed approaching equilibrium was most likely related to their respective stability constants with DMA and available metal pools driven by the free metal activity in this soil (Schenkeveld et al., 2014a).

On the other hand Zn and Fe were most strongly affected by increasing DMA concentrations. Interestingly the concentration ranges investigated did not mobilize significantly higher initial solution concentrations. Increasing DMA additions rather allowed the solution concentrations to increase for a longer time period and accordingly to reach higher peak concentrations. Thermodynamical modelling suggested that Fe and Zn will be hardly present in solution under equilibrium conditions in Santomera soil (Schenkeveld et al., 2014a). The trends observed here were in agreement with that.

Schenkeveld et al. (2014b) defined the concept of a “Window of Fe acquisition”. Here we see support for that concept. Increasing DMA concentrations clearly led to later and higher maximum FeDMA concentrations. Conceptually this widens the window of iron acquisition significantly. For the lowest treatments observed in both soil to solution ratios the window of iron acquisition was already closed and for the intermediate treatment it was rapidly closing approaching the 168 hours time point. However, considering an environmental system, where biodegradation is present the window closes earlier by default. In fact experimental results indicated that most FeDMA is degraded after 24 hours (Schenkeveld et al., 2014a). Schenkeveld et al. (2017) showed that pre-equilibration with the soil

solution had an effect on the available metal pools, but not on the lag phase of microbial degradation. This degradation might be even faster than reported, when considering an already active and substrate adapted microbial community in the rhizosphere of graminaceous plants (Schenkeveld et al., 2014b, 2017).

Keeping that in mind the initial mobilization can be considered particular significant for Fe acquisition. In the concentration range observed only small differences was seen in the first hours of the experiment. Experimental results obtained under a SSR of 1:4 strengthened this finding, as the trends held also in a higher SSR. This was somewhat striking, as the concentrations of DMA added, differed by more than a factor 3 from the lowest to the highest treatment. In terms of energy investment for a plant suffering from Fe deficiency, this might be an additional increase in costs without a significant gain in Fe uptakte during the initial hours after exudation.

Under environmental conditions phytosiderophore release by Strategy II plants cannot be considered as a pulse or point in time release, but rather as a time period of significantly increased efflux into the rhizosphere (Marschner et al., 1986). How this fact will alter the mobilization pattern of Fe and other metals cannot be resolved with the data presented here. However, the data did suggest that in the first hours of the experiment in all observed treatments there is still free DMA present in soil solution. This might indicate that environmental conditions were resembled more closely in those first hours than when most ligand was already complexing a metal (compare Figure 5), but more research is needed to investigate this thoroughly.

It has been demonstrated that when the available pool of Fe is larger (i.e. in soils richer in amorphous Fe), initial mobilization increases significantly (Schenkeveld et al., 2014a). Recently Schenkeveld et al. (2016) showed that there is a synergistic effect on Fe and other metal mobilization in treatments where the reductant ascorbate and DMA are present together. The synergistic effect reported was especially pronounced in the first hours of the time series investigated. Chapter 4 investigates this process more closely and relates it to DMA concentrations used in this paragraph.

4 Synergistic effect on iron and other metal mobilization by DMA and ascorbate

In this paragraph the terms synergistic and antagonistic effects are going to be used in the following manner:

A synergistic effect is established when the combined application of two compounds (in this case DMA and ascorbate) mobilized more of a respective metal, than the sum of the individual treatments.

Therefore: $AB > A + B$ (8)

An antagonistic effect is the opposite of that, with the sum of the individual treatments mobilizing more.

Therefore: $AB < A + B$ (9)

4.1 Synergistic effect on iron and other metal mobilization by DMA and ascorbate in presence of oxygen

In this section selected results from Experiment 1 (paragraph 2.2) shall be presented. SI Table 1 provides all data obtained.

4.1.1 Total Metal mobilization

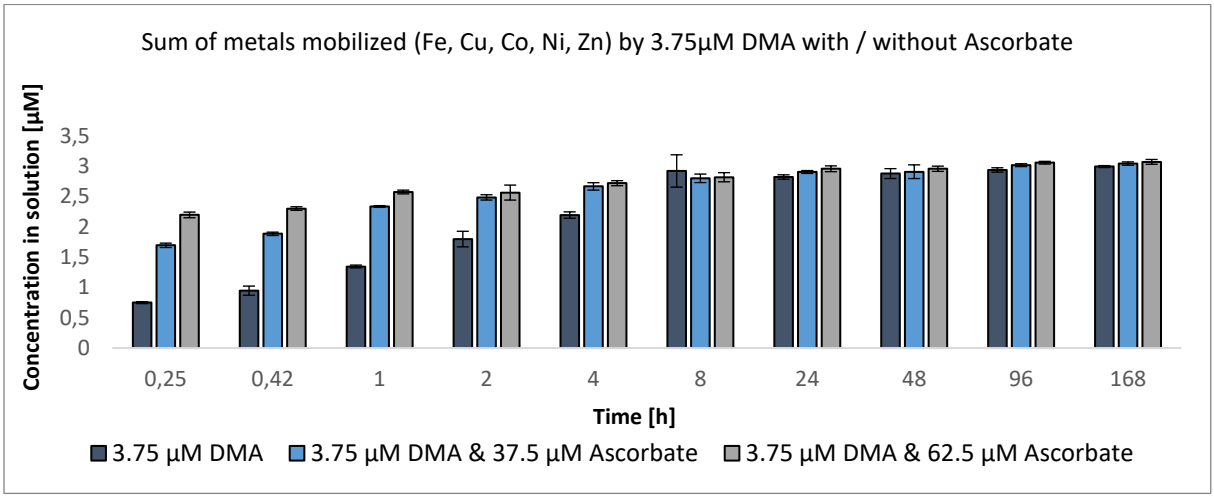


Figure 9: Sum of metals excluding Mn mobilized by 3.75µM DMA under oxic conditions and no, 37.5µM and 62.5µM ascorbate in Santomera soil; Error bars indicate sum of standard deviation of individual elements; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Figure 9 shows the sum of metals brought into solution by DMA (Fe, Cu, Co, Ni & Zn) after addition of 3.75µM DMA alone, in combination with 37.5µM or with 62.5µM ascorbate. After 168 hours the sum of metals in solution was almost the same for each treatment observed here. The treatments including ascorbate mobilized significantly more metals in the first time points of the experiment. The data indicated an increasing rate of total metal mobilization, with increasing reductant concentration. Differences in total metals in solution were especially pronounced in the first 4 hours between ligand only and combined treatments of ligand and reductant. Between the combined treatments with higher and lower ascorbate levels those differences were especially visible in the first hour of the experiment.

Figure 12 and Figure 13 show the metal DMA complex as a function of time. It becomes apparent that the metals included in Figure 9 were not at all or in the case of Ni only to a neglectable extent mobilized by ascorbate addition alone. Similar as in Chapter 3.1 it can be concluded, that the elements displayed here were actually chelated by the ligand DMA. The combined treatments did not mobilize more

metals in absolute amounts, as all treatments reached very similar solution concentrations over time. The addition of DMA and ascorbate combined therefore increased the rate by which DMA complexed those metals in Santomera soil. The amount of reductant was correlated positively with that rate, resulting in the high ascorbate treatment (3,75µM DMA & 62.5µM Ascorbate) in almost 60% of the total ligand added, complexing some metal and bringing it into solution after 15 minutes.

The total mobilization by the higher ligand concentration of 6.25µM DMA is displayed in Figure 10. Again, the increase in mobilization rate by increased ascorbate addition prevailed and solution concentrations levelled out over the time of the experiment. However, clear concentration differences between the ligand only and combined treatments lasted longer (up until the 24 hour time point) and clear differences between the combined treatments were visible for the first 4 hours of the experiment.

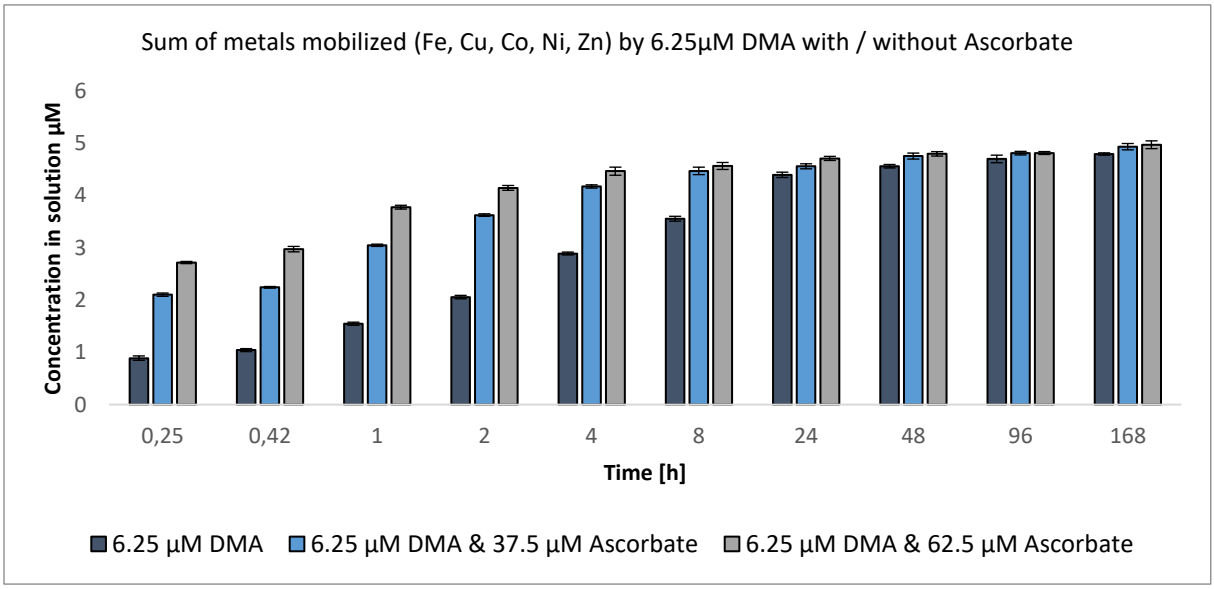


Figure 10: Sum of metals excluding Mn mobilized by 6.25µM DMA under oxic conditions and no, 37.5µM and 62.5µM ascorbate in Santomera soil; Error bars indicate sum of standard deviation of individual elements; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

The combination of ascorbate and DMA appeared to enhance the speed of metal complexation. The amount of reductant was clearly determining the rate of initial mobilization, and the amount of ligand drove the timewise extent of those differences. This dependency is logical, as there was more ligand available for complexation, and therefore extending the time period in which total metal complexation could be affected by the reductant. Even if ascorbate reacted quickly with the soil constituents, the data suggested that its addition had a longer lasting effect on the reactive metal pool in the soil, which got mobilized by DMA.

In Paragraph 3.1 it has been shown, that decreasing amount of free DMA in solution enhanced competition for the remaining free ligand and triggered competitive displacement to become a driving force of solution speciation. Iron has been shown to not be the most favourable metal in this soil system to be complexed by DMA (Schenkeveld et al., 2014a; b). Recently Schenkeveld et al. (2016b) have shown, that there is an initial synergistic effect between ascorbate and DMA on Fe mobilization. This may appear counterintuitive based on the total metals mobilized. However, as will be shown and discussed in Paragraph 4.1.2 the enhanced competition did affect Fe mobilization over time, but an initial synergistic effect was observed .

4.1.2 Mobilization by the combined treatments Results and Discussion

Figure 12 and Figure 13 show that the only metal responding strongly to the addition of ascorbate alone is manganese (Figure 12 f & Figure 13f). For all metals except Mn the highest ascorbate addition tested (125µM) is displayed (Compare also SI Table 1). Nickel concentrations were affected slightly by ascorbate alone, but remained in a concentration range below 0.1µM at all time points (Figure 12 d & Figure 13 d). Experimental data obtained in experiment 3 and reported in literature (Schenkeveld et al., 2016) provides clear evidence for having no different mobilization by ascorbate only after 15 minutes than after 1 hour for any element tested other than Mn.

4.1.2.1 Iron

Iron solution concentrations are displayed in Figure 12 a) and Figure 13 a). Fe mobilization was initially affected by a synergistic effect, that was followed by an antagonistic effect over time, leading to a faster depletion of solution concentrations.

In the treatments with a low supply of DMA (Figure 12 a) – 3.75µM DMA) both combined treatments mobilized around 0.3µM Fe after 15 minutes. For the 62.5µM ascorbate treatment the synergistic gain turned into an antagonistic effect after 0.5h, and solution concentrations decreased from 4 hours onward. The lower ascorbate treatment mobilized more Fe than the treatment with DMA alone, until after 1h into the experiment. Thereafter an antagonistic effect was observed, resulting in an earlier (after 4h vs. after 8h) and reduced (0.7µM vs 1.3 ±0.1 µM) maximum solution concentration compared to the no ascorbate treatment.

In the combined treatments with a higher supply of DMA (Figure 13 a – 6.25µM DMA), the synergistic gain became more pronounced. Both combined treatments mobilized similar concentrations of Fe initially, and were significantly enhanced compared to the DMA alone treatment. The net gain was in the range of a factor 2 after 15 minutes. Thereafter both combined treatments increased in a similar manner, with lower concentrations recorded for the high ascorbate treatment after 2 hours. From there on the 62.5µM ascorbate treatment resulted in an antagonistic effect, but mobilized Fe still increased to a maximum in solution of around 1.2µM after 4 hours, with decreasing solution concentrations thereafter. The lower ascorbate treatment (37.5µM) showed elevated concentrations compared to the DMA only scenario until after the 4h time point. From there on the concentration peak was reached with 1.4µM Fe in solution. An antagonistic effect was observed in the following and FeDMA was almost completely depleted from solution after 168 hours.

The treatment with 37.5µM ascorbate and 6.25µM DMA was in terms of synergistic effect on Fe mobilization the more effective treatment. Here an initial net gain of a factor 1.9 was reached compared to the sum of the DMA and ascorbate alone treatments, and concentrations remained elevated for the first 4 hours of the experiment. However, the antagonistic effect resulting in earlier and lower peak concentrations (1.4µM vs. 1.9µM) shall not be considered neglectable. The fact that in terms of iron acquisition more (reductant) is not always better, becomes obvious in this treatment. There appeared to be a delicate ratio dependency between enhancing the availability of Fe and increasing the competition so much, that antagonistic effects on Fe mobilization dominated immediately. On the other hand, with decreasing amount of reductant the mobilization patterns resembled those of the ligand only treatments more closely, and the initial synergistic effect may become less strong.

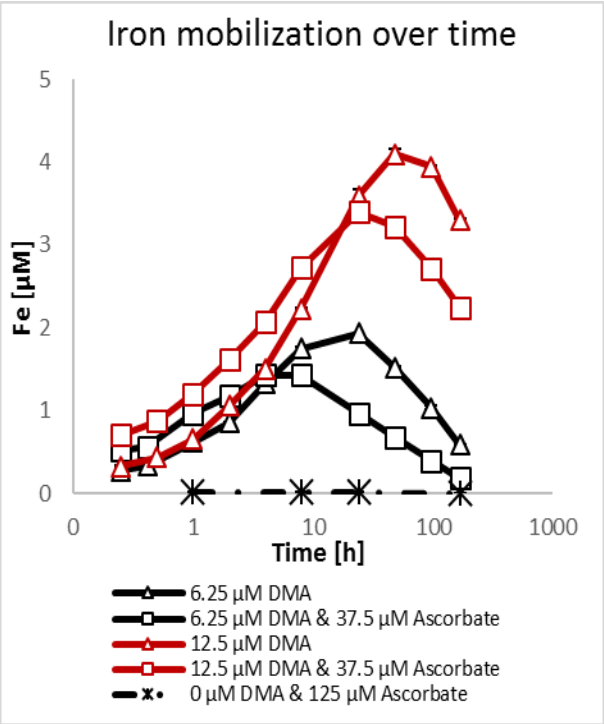


Figure 11:: Fe mobilization by selected amounts of ligand and reductant in Santomera soil; Error bars indicate Standard deviation; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Figure 11 shows the Fe mobilization of 6.25µM and 12.5 µM DMA with and without 37.5µM ascorbate added. Both combined treatments showed a very effective initial mobilization with around a factor 2 elevated concentrations compared to the treatment without ascorbate. Over time the synergistic effect wore off and antagonistic effects dominated. The time window in which the synergistic effect was present was extended for the combined treatment with 12.5µM DMA, yielding a net gain for the first 8 hours and only slightly reduced concentrations after 24 hours. A striking finding here is the higher Fe mobilization by 6.25µM DMA and 37.5µM ascorbate combined, compared to 12.5µM DMA alone in the first 4 hours. In an environmental context, where the first hours can be considered of highest importance in terms of Fe acquisition this interplay has the ability of significantly reduce the amount of ligand needed by Strategy II plants.

The ratio dependency of synergistic mobilization is driven by the competition by other metals. This is due to the fact, that the rate of free ligand depletion was very likely correlated with increasing ascorbate additions. And other metals, in this soil in particular Co, Ni and Cu outcompeted Fe for the complexing agent DMA. Therefore, the effects of the combined treatments onto potential competitors for DMA must be taken into consideration. Without their effect there would be no antagonistic effect observable. This has been shown by (Schenkeveld et al., 2016)) with the microbial siderophore Desferrioxamine B (DFOB), where solution concentration became synergistically elevated and continued to increase over the entire time series tested, as Fe(III) is thermodynamically most favourable complexed by DFOB and initial competition by Aluminium was reduced over time, likely due to competitive effects of elevated Fe availability onto Aluminium complexation.

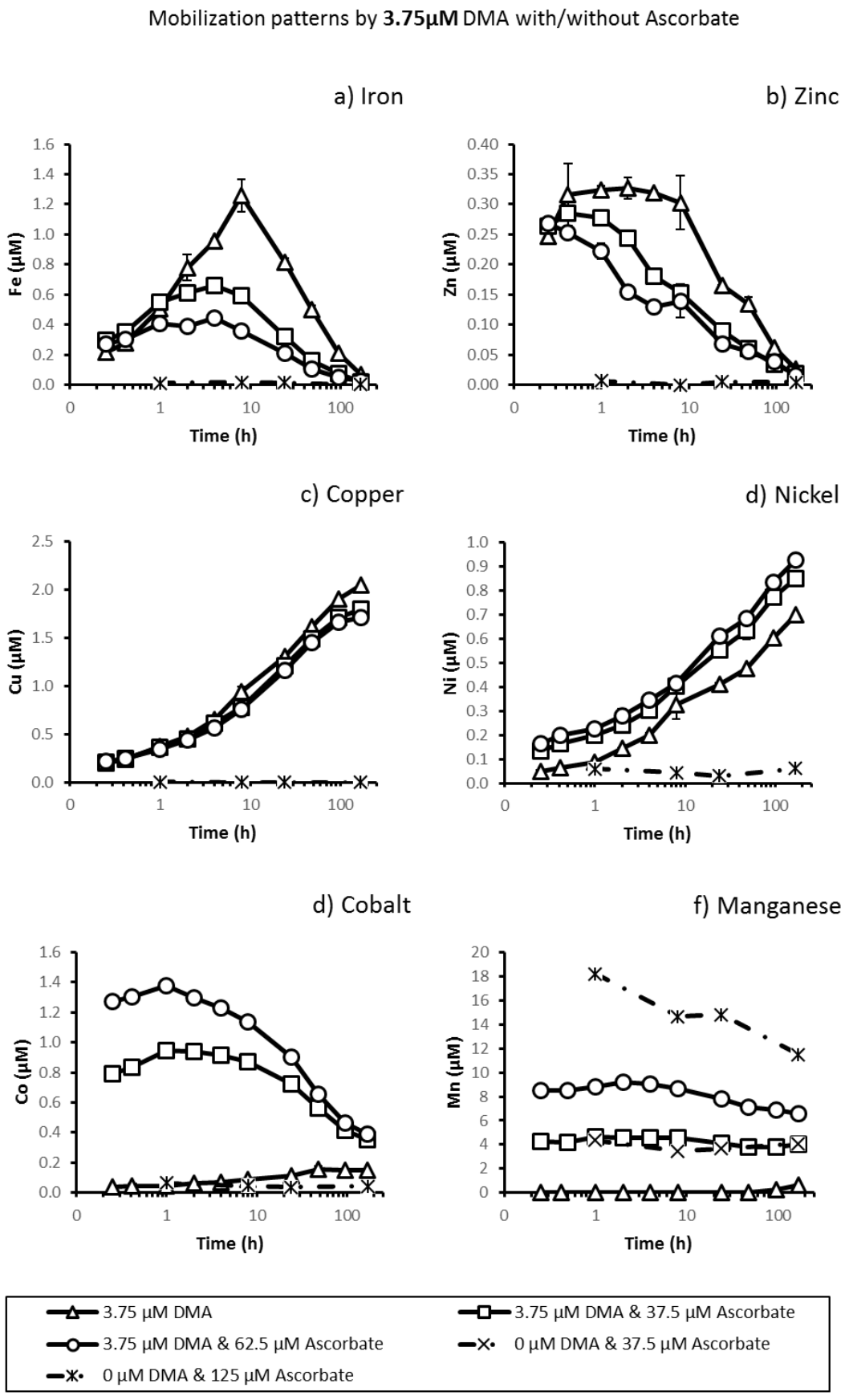


Figure 12: Mobilization patterns of individual elements by 3.75µM DMA with/without ascorbate addition and ascorbate alone in Santomera soil (Note: a)-e) display highest Asc addition tested; a) iron, b) zinc, c) copper, d) nickel, e) cobalt, f) manganese, Error bars indicate standard deviation; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

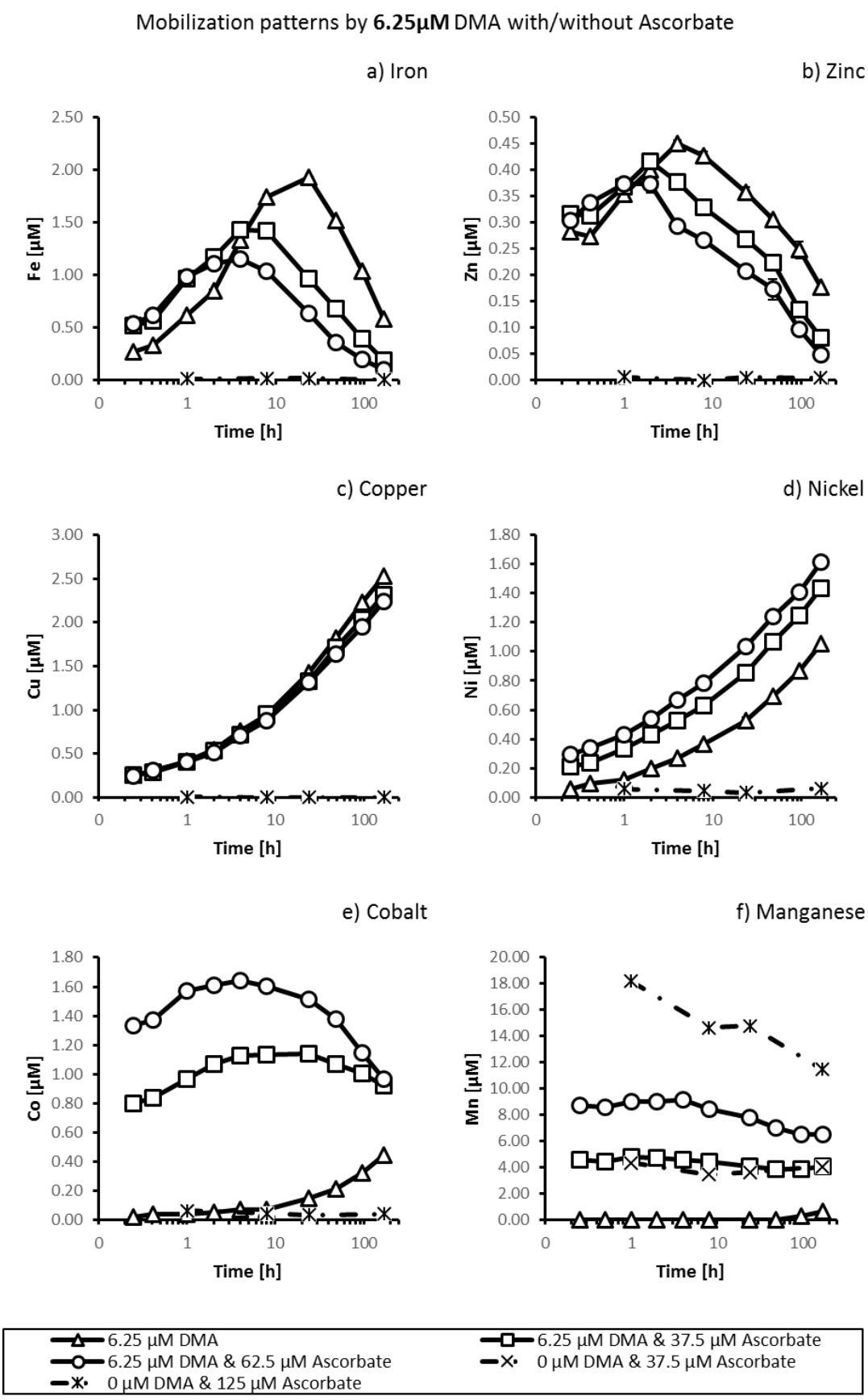


Figure 13: Mobilization patterns of individual elements by 6.25µM DMA with/without ascorbate addition and ascorbate alone in Santomera soil (Note: a)-e) display highest Asc addition tested); a) iron, b) zinc, c) copper, d) nickel, e) cobalt, f) manganese, Error bars indicate standard deviation; SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

4.1.2.2 Zinc

The overall behaviour of zinc in solution is similar in both DMA concentrations displayed (Figure 12b & Figure 13 b). As Zn did not get mobilized by the addition of ascorbate only, the slightly elevated solution concentrations in the first time point for the 3.75µM DMA treatment (Figure 12b) and in the first hour for the 6.25µM DMA treatment Figure 13 b) can be attributed to a moderate synergistic effect of reductant and ligand combined. However, this small synergistic effect quickly turned into an antagonistic effect over time, with clearly reduced solution concentration over the longest period of the experiment. The antagonsitic effect was stronger, with increased reductant concentrations, with earlier and lower concentration peaks. As Zn(II) has a rather low complexation constant with DMA compared to copper, nickel and iron (Reichman et al., 2005), this was most likely driven by preferential complexation of other metals becoming more available by the increase in reductant concentration.

4.1.2.3 Copper

Copper (Figure 12 c & Figure 13 c) followed the same trends independent of the ligand concentration. The solution concentrations were hardly affected by the amount of reductant added to the system. No synergistic, but a slight antagonistic effect was observed over time, where the treatments with the highest ascorbate additions showed the lowest Cu mobilization at termination of the experiment. This is very likely due to the fact, that solution concentrations of Co and especially Ni (Schenkeveld et al., 2014a), were elevated in those treatments and copper therefore experienced a slight antagonistic effect over time. Cu is mainly associated with the organic fraction in Santomera soil (Schenkeveld et al., 2014a). Schenkeveld et al., (2016) reported a synergistic effect of DMA and ascorbate on metals associated with the oxy(hydro)oxide soil fraction, but not the ones mainly associated with SOM. The findings here, indicating no synergistic Cu mobilization in the combined treatments, agreed with this.

However, copper hardly reacted to the increase in competition for free DMA indicated by the total metal mobilization displayed in Figure 9 & Figure 10. Especially in the first 8 hours of the experiment this enhanced competition made virtually no difference in rate and extent of its mobilization. It has already been discussed, that copper is the main competitor for iron to become mobilized by DMA alone from Santomer soil, in an environmentally significant time frame (Schenkeveld et al., 2014b; Walter et al., 2016). In the data obtained here, it became apparent how stable the complexation of Cu is. Being the thermodynamically more favourable metal to be complexed by DMA than Fe in this soil (Reichman et al., 2005; Schenkeveld et al., 2014a), an increase in competition hardly affected the overall mobilization rate. This was significantly affecting Fe solution concentrations in DMA alone treatments and continued to do so in all combined treatments tested. Apart from increased additional competition by other metals such as cobalt and nickel, copper solution concentrations continued to increase over time, remaining a major competitor for free and already complexed DMA.

4.1.2.4 Nickel

Nickel concentrations (Figure 12 d and Figure 13 d) were elevated in the combined treatments with the higher ascorbate concentration yielding the highest solution concentrations consistently. The sum of Ni concentrations mobilized by the ascorbate only and DMA only treatment was, at all time points observed, smaller than the concentrations in the combined treatments. Therefore a strong and lasting synergistic effect on Ni mobilization was concluded.

The extent of that effect is ligand driven, with higher solution concentrations in the combined treatments with 6.25µM DMA (compare Figure 13 d). Interestingly, the synergistic effect was so strong, that the combined treatments with 3.75µM DMA both mobilized more Ni than 6.25µM DMA alone for

first 48 hours, and even more than by 12.5µM DMA alone for the first 24 hours (SI Table 1). This was likely driven by the slow complexation kinetics of Ni, as has been discussed in paragraph 3.2. The addition of ascorbate therefore clearly increased the availability for complexation of Ni in this soil. As a consequence, the competition by Ni onto Fe acquisition by DMA increased in the combined treatments with ascorbate than in those with DMA or ascorbate individually.

4.1.2.5 Cobalt

Cobalt (Figure 12 e and Figure 13 e) reacted the strongest of all metals investigated to the combined treatments initially and experienced a very strong synergistic effect. Solution concentrations showed initially to be strongly correlated to the amount of reductant added. Co solution concentrations were extraordinarily similar for the combined treatments with 37.5µM ascorbate in the first hour and very similar for the combined treatments with 62.5µM ascorbate in the first two time points, regardless of a higher or lower supply of ligand.This effect was also seen for the treatment of 12.5µM DMA with 37.5µM ascorbate (not displayed, compare SI Table 1).

Interestingly, over time the amount of ligand supplied determined the CoDMA solution concentrations. Both combined treatments with 3.75µM DMA (Figure 12 e) reached their respective maximum concentrations after 1 hour and decreased from there on reaching solution concentrations in the same range of around 0.4µM after 168h. A similar pattern was seen for the combined treatments with 6.25µM DMA (Figure 13 e), where both, after an initial increase and large differences in initial mobilization, approached solution concentrations of around 1µM and 0.9µM respectively after 168h. This was likely related to the thermodynamic equilibrium of the system, indicating a lower complex stability for CoDMA than for Ni, and Cu complexes, as CoDMA concentrations decreased, while NiDMA and CuDMA were still increasing. DMA can potentially chelate Co^{2+} and Co^{3+} with different stability constants (Walter et al., 2016). Which oxidation state to which extent is chelated here may be subject of future research, but curiosity is triggered by the fact, that both low and high ascorbate combined treatments reached similar solution concentrations over time.

The obtained results indicated synergistic mobilization by a combined application of DMA and ascorbate for nickel and cobalt over the whole period of the experiment in different ligand to reductant ratios. Cobalt is redox sensitive whereas nickel is not (Schenkeveld et al., 2016). Both elements are known to be associated with metal-oxy(hydro)oxide in soils, in particular Mn-oxides (Scheffer et al., 2010). As ascorbate appeared to mobilize Mn mainly by reductive dissolution of Mn-oxides, the heavy metals associated with them became more available. Interestingly, there appears to be a ligand needed to scavenge the metals, which otherwise were not seen in significant amount in solution concentration. This is in agreement with Schenkeveld et al. (2016). DMA alone mobilized only little Co over time. Whether its increase in solution was linked to increasing Mn concentrations, both in blank and DMA only treatments over time, cannot be resolved here, but would be plausible in the context of increasing availability with Mn-oxide dissolution.

Iron mobilization was negatively affected by increased competition of Co. This became obvious by the fact, that Co solution concentrations could benefit strongly from increasing ascorbate additions initially, whereas Fe remained in a similar concentration for combined treatments with higher and lower reductant concentrations and experienced earlier antagonistic effects in the higher (62.5µM) ascorbate combined treatments. Co on the other hand clearly profited from increased levels of ascorbate and solution concentrations did not converge where Fe already decreased, highlighting its increased role as a competitor for Fe mobilization in the combined treatments.

4.1.2.6 Manganese

Figure 12 f) and Figure 13 f) show the concentrations of manganese over time. Mn concentrations were strongly driven by the amount of ascorbate added to the system. DMA alone did not mobilize Mn to above the blank levels and concentrations did not differ significantly between the combined treatments with varying DMA concentrations (SI Table 1). There were no clear indications for a synergistic effect in mobilization of Mn in the combined treatments, compared to the ascorbate only treatment. In all treatments observed, the concentrations decreased over time, which may be due to slow oxidation of Mn^{2+} (Schenkeveld et al., 2016). It has to be pointed out, that the solution concentrations remained at a plateau and even increased for both combined treatments with 62.5µM ascorbate (Figure 12 f and Figure 13 f) in the first 8 hours of the experiment. Whether this is caused by a ligand controlled process cannot be resolved with the data obtained here, but it is unlikely given the fact that total metals in solution including the delta between combined treatments and ascorbate only would exceed the amount of ligand applied in some treatments.

The main driver of Mn concentration however, did appear to be reductive dissolution of the soils manganese oxides. Manganese oxides are hardly soluble and exist as Mn(III/IV)oxides in soil with Mn(IV) being the more prevalent form (Scheffer et al., 2010). Ascorbate can reduce Mn(III/IV)oxides by forming inner sphere surface complexes and transfering electrons. A Mn(II) ion is formed inside the oxides lattice surface structure. Resulting Mn(II)oxides are much more soluble and are subsequently released into solution (Stone & Morgan, 1984; Martin, 2005). Reoxidation kinetics of Mn(II) are rather slow in a pH range < 8 (Martin, 2005; Scheffer et al., 2010), resulting in the slow decrease in solution concentration observed.

Mn(III/IV) oxide reductive dissolution takes place by the following half reactions (Martin, 2005):

$$MnO_2(s) + 2\,e^- + 4\,H^+ \rightarrow Mn^{2+} + 2\,H2O, \qquad pE^0 = 20.8,\ E^0_{1/2} = +1.23\ V \qquad (10)$$

$$MnOOH(s) + e^- + 3\,H^+\ Mn^{2+} + 2\,H2O, \qquad pE^0 = 25.4,\ E^0_{1/2} = +1.50\ V \qquad (11)$$

An Adsorption Isotherm for Mn^{2+} added to Santomera soil is presented in SI Figure 4. Based on that the µmoles of total Mn^{2+} (sorbed and in solution) exceeds the µmoles of ascorbate added by a factor of 1.5 to 1.8 (Compare SI Table 4). Equation 10 shows that, to dissolve Mn(IV)oxides 2 electrons are needed. Ascorbate gives off 2 electrons to form dehydroascorbate (Dos Santos Afonso et al., 1990). In soils mixtures of Mn(III/IV)oxides are common (Scheffer et al., 2010). Mn(III)oxides dissolve by equation 11. However, dehydroascorbate has been shown to dissolve hematite in batch suspensions, indicating the possibility of further oxidation (Dos Santos Afonso et al., 1990). Reductive dissolution of Mn-oxides is energetically more favourable than that of Fe(hydr)-oxides (Martin, 2005). Based on the observed ratios between total Mn (in solution and sorbed) and ascorbate, it can be concluded that ascorbate was mainly affecting Mn-oxides. Previous experiments with Santomera soil (SI Table 2) proved evidence for the possibility to increase total concentrations of Mn to around a factor 3 higher than recorded in the highest combined treatment presented here, before substantial amounts of Fe^{2+} were recorded in solution. However, reoxidation happens fast for Fe^{2+} and the possibility of electron transfer to the Mn-oxides via temporarily reduced surface Fe shall not be excluded.

Schenkeveld et al. (2016) found synergistic mobilization of Mn by ascorbate and DMA in Santomera soil, however the data presented here was obtained in a different soil to solution ratio and is therefore not completely comparable. The ascorbate only treatment has its first data point after 1 hour, where an initial synergistic effect might have already wore off. However, in the combined treatments tested, the amount of DMA supplied did not influence Mn solution concentrations, while the amount of

reductant supplied strongly did so. This provides evidence that there is, if any, at least no strong synergistic effect on manganese mobilization under DMA concentrations supplied here.

4.1.3 Synthesis

The data presented indicates that ascorbate mainly affects the system due to reductive dissolution of Mn-oxides. The elements associated with those Mn-oxides, cobalt and nickel (Scheffer et al., 2010), showed the strongest reaction to the combined treatments tested. Iron did nevertheless initially profit from a synergistic effect, particularly in the environmentally most relevant first hours of interaction. Initial synergistic Fe mobilization was impeded by the apparently preferential complexation of cobalt. Over time synergistically elevated concentrations of nickel, and increasing concentrations of copper caused enhanced competition. This led to antagonistic effects of reduced and earlier maximum FeDMA concentrations, compared to treatments without ascorbate. A ratio dependency of reductant and ligand became obvious considering this delicate interplay.

The mechanism driving the observed synergistic effect on synergistic Fe mobilization remains still unresolved. Schenkeveld et al. (2016) state bond labilization by electron input into Fe (hydr)-oxide minerals and subsequently facilitation of ligand promoted dissolution as one possible mechanism. Additionally reductive dissolution of Fe (hydr)oxide minerals and rapid chelation of the short lived Fe^{2+} or chelation of reoxidized transient Fe(III), with an increased availability should be considered as possible ways Fe can get mobilized synergistically (Schenkeveld et al., 2016).

Additionally, cations like Li, Na, K, Ca, Ba, Al and Fe should also be incorporated in the mineral structure of Mn-oxides (Scheffer et al., 2010). The amount of incorporated iron in Mn-oxides is in the range of 10^{-3} parts per mass oxide (Boenigk, 2004). Reductive dissolution of those Mn-oxides by ascorbate may therefore possibly increase the Fe availability further. A simple mass balance based on the total Mn concentrations calculated by the adsorption isotherm of Mn^{2+} to Santomera soil, showed that this might contribute, but is unlikely to be the only mechanism behind the synergistic effect observed here, as potentially associated Fe was rather little (SI Table 4).

Paragaph 4.2 describes the synergistic effect under anoxic conditions in order to gain more insights into the mechanism underlying synergistic effects, like the formation of Fe^{2+} in solution.

4.2 Synergistic effect on iron and other metal mobilization by DMA and ascorbate in absence of oxygen

In natural soils heavy rainfalls and fluctuating groundwater levels are omnipresent and can result in (micro)sites in which microbial and root respiration causes sub- and anoxic conditions (Sposito, 2008). Those sites contribute to the heterogeneity in soils by drastic changes in the redox potential and subsequent leaching and precipitation processes. All microbial processes have been supressed in the experimental data presented and the rhizosphere is an environment of great spatial and chemical heterogeneity. The results shall in no means be understood as a general mobilization of metals by phytosiderophores under anoxic conditions, but rather help to shed light on the main processes of the synergistic effect on iron acquisition.
For materials and methods please be referred to paragraph 2.4. The mobilization under anoxic conditions, grouped by the amount of DMA supplied is displayed in SI Figure 5 and SI Figure 6.

In general there was a surprisingly small effect on the overall mobilization of metals by the absence of oxygen. The general patterns of the synergistic effect described for conditions including oxygen are maintained under anoxic conditions. In the following paragraphs the mobilization of the individual elements in comparison to the mobilization under presence of oxygen is discussed in detail.

4.2.1 Iron

Figure 14 shows iron solution concentrations obtained under anoxic conditions and relates it to the concentrations obtained under presence of oxygen.

Figure 14 a) shows the mobilization both under presence (dotted lines) and absence (full lines) of oxygen by 3.75µM and 6.25µM DMA without ascorbate. DMA alone mobilized Fe over time in both concentrations tested. In the scenario with 3.75µM DMA supplied, 0.2µM Fe were mobilized initially, whereas in the treatment with 6.25µM DMA 0.3 µM Fe were brought to solution. Thereafter solution concentrations of both treatments increased in a similar fashion. The 3.75µM DMA only treatment had its recorded peak concentration after 4h at around 1µM, while the 6.25µM DMA only treatment reached its maximum Fe in solution of 1.6µM after 8 hours.

Initially there was no significant difference, with mobilization rates being in a similar range for both treatments, between the oxic and anoxic system. Over time Fe was mobilized less under anoxic conditions, leading to lower and earlier maximum solution concentrations. This 'shift in peak concentrations' has been observed in previous treatments, where it was attributed to an increase in competition for a scarcer amount of complexing agent by other metals.

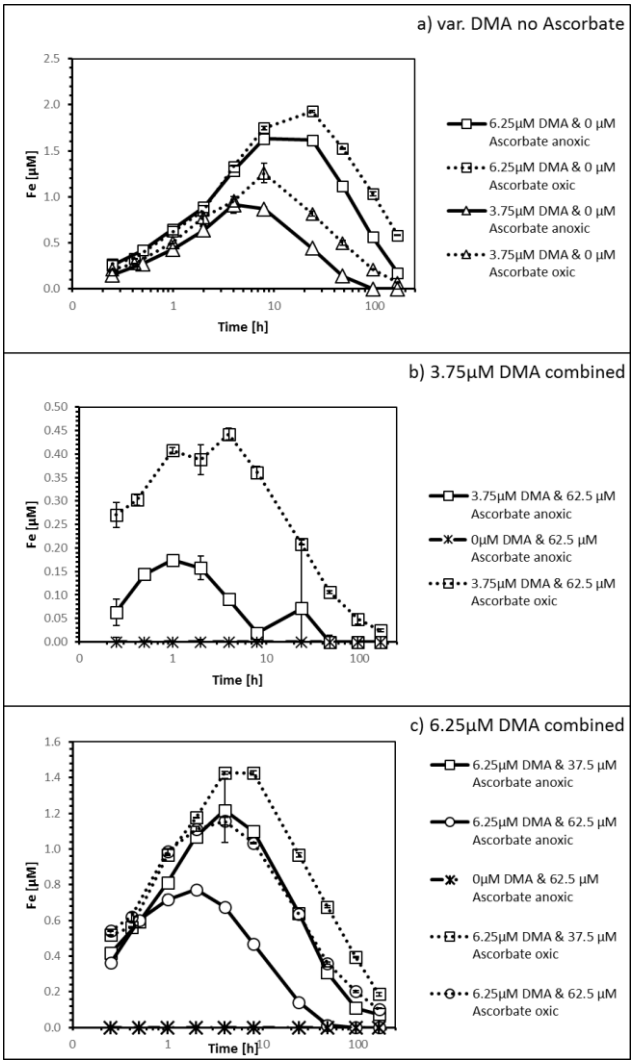


Figure 14: Iron mobilization by a) 6.25µM and 3.75µM DMA, b) 3.75µM DMA and 62.5µM Ascorbate and c) 6.25µM DMA in combination with 37.5µM and 62.5µM Ascorbate; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Figure 14 b) & c) showed that, similar to the addition of ascorbate under presence of oxygen, no Fe was mobilized by ascorbate alone in the concentration ranges tested. This indicated, that reductive dissolution of soil Fe(hydr)oxides is, if at all, only a minor effect of ascorbate addition, as no substantial amount of the more soluble form of Fe^{2+} is measured in solution. Temporary reduction to ferrous iron and rapid reoxidation, with e.g Mn-oxides serving as an electron acceptor, cannot be excluded by the data obtained.

Especially in the treatment with 3.75µM DMA and 62.5µM ascorbate Fe solution concentrations were compromised under anoxic conditions (compare Figure 14 b). Here solution concentrations never exceeded 0.2µM Fe, with a maximum mobilization after 1 hour and depletion from solution after 8 hours.

When supplied with 6.25µM DMA (Figure 14 c) a small synergistic effect was observed in both combined treatments with ascorbate, here both initially mobilized Fe in a similar range of 0.4µM. The combined treatments with 6.25µM DMA increased initially in a similar fashion, before the combined

treatment with 62.5µM ascorbate mobilized less Fe after 1 hour and an antagonistic effect was observed after 2 hours. Here maximum solution concentrations of 0.85µM were reached and solution concentration decreased from there on, until no iron was mobilized after 48 hours. The combined treatment with 37.5µM ascorbate increased for a longer period. The recorded maximum solution concentration of 1.2µM Fe was reached after 4 hours. Thereafter solution concentrations decreased, with Fe being mobilized in the range of 0.1µM after 96 hours. A synergistic effect on Fe mobilization occurred for the first hour in case of the high ascorbate combined treatment and for the first 2-4 hours in case of the low ascorbate treatment.

When comparing the mobilization over time of both 6.25µM DMA combined treatments under absence and presence of oxygen it becomes obvious, that iron was consistently mobilized to a smaller extent under anoxic conditions. A 'shift in peak' in time and concentration was observed for both high and low ascorbate treatment. The delta between maximum concentrations in the 6.25µM DMA and 37.5µM ascorbate treatment and the 6.25µM DMA and 62.5µM ascorbate case was larger under anoxic than under oxic conditions, indicating a stronger negative correlation between ascorbate addition and maximum Fe mobilization under absence of oxygen.

This relation was confirmed when evaluating the total metal mobilization (Fe, Zn, Cu, Ni & Co) under absence and presence of oxygen (SI Figure 7 and SI Figure 8). General trends described in paragraph 4.1, such as an increase in total metals in solution with increasing ascorbate addition in the earlier time points of the experiment and similar solution concentrations of all treatments depending on the amount of DMA supplied, were observed under absence of oxygen as well.

In addition, evidence for an increased rate of total metal mobilization by DMA with and without ascorbate under anoxic conditions was found. The increased rate of total metal mobilization was positively correlated to the amount of reductant supplied (SI Figure 8). An increase in competition for the complexing agent was therefore found to be a likely consequence, with iron mobilization being compromised by the preferential complexation of other metals discussed in the following paragraphs.

The pattern of earlier peaks with reduced solution concentrations was therefore attributed to increased competition for complexing agent by other metals. In particular the elevated concentrations of cobalt and nickel under anoxic conditions are likely to play the major role here. Especially in the 3.75µM DMA and 62.5µM ascorbate treatment already 71% of the ligand supplied is mobilizing a metal and bringing it into solution after 0.25 hours. Cobalt is by far the biggest fraction here, with 66% of the total metals in solution.

The ratio dependency of ligand and reductant observed under oxic conditions was therefore found to also apply to the synergistic mobilization of Fe under anoxic conditions. Furthermore the effects of the reductant applied appeared to be stronger under anoxic conditions, which is plausible considering the lack of oxygen potentially oxidizing ascorbate, before it was able to react with the soil constituents.

4.2.2 Zinc

Figure 15 displays the mobilization of Zn over time under anoxic conditions in comparison to the mobilization under presence of oxygen. In general Zn solution concentrations were elevated under anoxic conditions throughout, in comparison to their counterparts under oxic conditions.

Maximum concentrations were higher and were reached later in treatments including only DMA (Figure 15a) in comparison to treatments under oxic conditions. Zn has been shown in a previous study to be mainly associated with SOM in Santomera soil (Schenkeveld et al., 2014a). Whether anoxic conditions in general facilitate SOM-bound Zn complexation by DMA, remains subject of future research, with metal retention and mobilization from the anaerobic rhizosphere being still under investigation (Jacob & Otte, 2003).

Ascorbate alone had no effect on Zn mobilization (Figure 15 b, c). If any, only a very small synergistic effect, followed by a strongly pronounced antagonistic effect from 0.5h on, was observed in the 3.75µM DMA – 62.5µM ascorbate treatment. Interestingly, solution concentrations appeared to be less affected by increased competition, than those of iron (compare Figure 14 b).

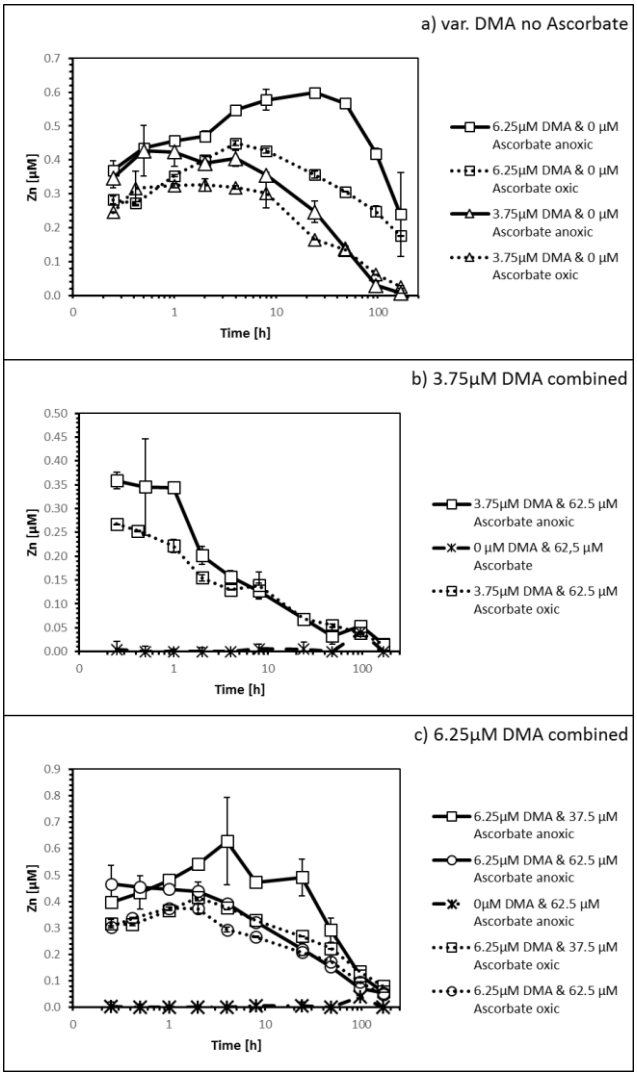


Figure 15: Zinc mobilization by a) 6.25µM and 3.75µM DMA, b) 3.75µM DMA and 62.5µM Ascorbate and c) 6.25µM DMA in combination with 37.5µM and 62.5µM Ascorbate; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

In the combined treatments with 6.25µM DMA no clear synergistic effect was seen. Solution concentrations were in a similar range until antagonstic effects decreased solution concentrations. More ascorbate led to a stronger antagonistic effect, with concentrations reaching an earlier lower maximum concentration.

Maximum solution concentrations of Zn were in the range of 10% of respective ligand supplied and Zn is not the strongest competitor for FeDMA complexation from a thermodynamically perspective. In terms of impact onto iron acquisition Zn complexation can therefore be considered as being of smaller importance, than Cu, Ni and Co.

4.2.3 Copper

Figure 16 displays the mobilization of Cu over time under anoxic conditions in comparison to the mobilization under presence of oxygen. In general Cu solution concentrations were in good agreement with each other independent of presence or absence of oxygen.

Cu was reacting to the addition of DMA alone in a similar fashion as has already been described in paragraph 4.1.2.3. Both DMA only treatments mobilized around 0.3µM and increased at the same rate thereafter, with the 6.25µM DMA treatment mobilizing more Cu from 4 hours on (Figure 16a). The decrease of Cu in solution for the 3.75µM DMA only scenario after 96 hours has not been observed in previous experiments. Competitive displacement by Ni might play a role here, but more research, is needed to resolve that interplay over a longer period of time. Copper mobilization followed the same trend under aerobic and anaerobic conditions. Over time solution concentrations differed between the combined treatments according to the amount of ligand and amount of reductant applied.

Figure 16 b) and c) display the mobilization of Cu in the presence of ascorbate with or without DMA. Ascorbate alone had no mobilizing effect on copper. As under oxic conditions (paragraph 4.1.2.3), a small antagonisitic effect was observed in treatments combining DMA and ascorbate. This was more pronounced under anoxic conditions, with a delta of 0.5µM after 168h interaction between the 6.25µM DMA only and the combined treatment with 6.25µM DMA and 62.5µM ascorbate, compared to a delta of 0.3µM under oxic conditions.

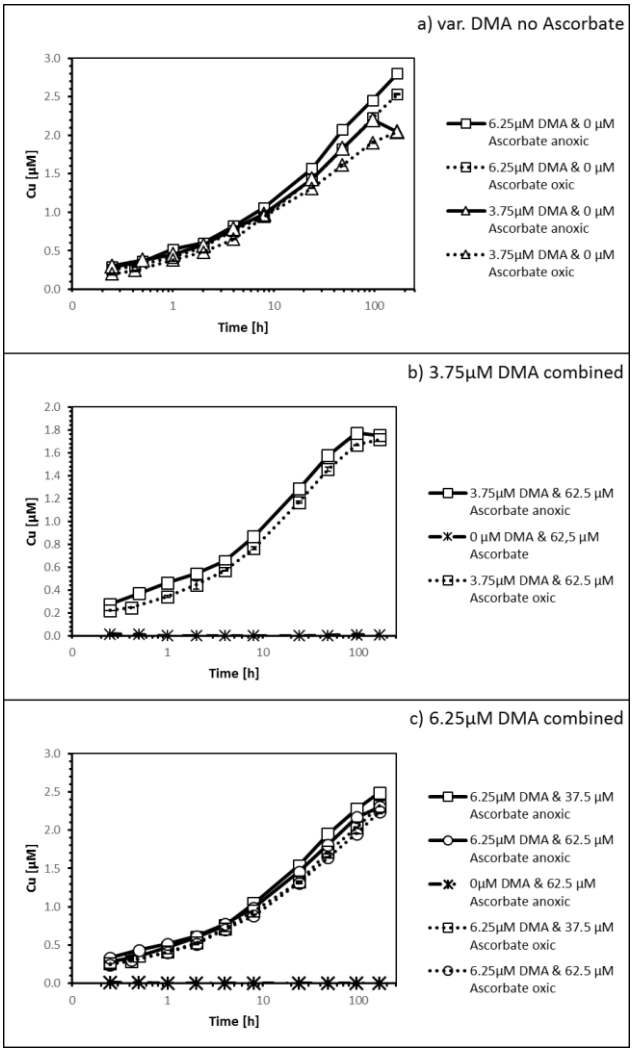


Figure 16: Copper mobilization by a) 6.25µM and 3.75µM DMA, b) 3.75µM DMA and 62.5µM Ascorbate and c) 6.25µM DMA in combination with 37.5µM and 62.5µM Ascorbate; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Overall copper concentrations were elevated under anoxic conditions in comparison to the oxic system. The differences remained however in the range of +0.2µM. As Cu like Zn is associated with SOM in Santomera soil (Schenkeveld et al., 2014a), this might further strengthen the thought that the anoxic conditions facilitate complexation of those species mainly associated with SOM.

For Fe the impact of Cu as a major competitor remained present, as anoxic conditions apperared rather beneficial for Cu complexation by DMA than impeding. Copper therefore remained to be a major competitor for free and already complexed DMA. This is proven by the fact that CuDMA was the dominant fraction of MeDMA approaching equilibrium conditions in all treatments tested.

4.2.4 Manganese

Figure 17 displays the mobilization of Mn over time under anoxic conditions and compares it to the mobilization in the presence of oxygen. Similar to the mobilization under oxic conditions Mn solution concentrations were strongly driven by the amount of reductant supplied.

Figure 17 a) shows that only minor differences between the combined treatments of DMA and ascorbate and the treatments containing only ascorbate occurred. Comparing the combined treatments with 6.25µM DMA and 3.75µM DMA respectively, no significant differences were observed. A clear synergistic effect on Mn mobilization by combined application of DMA and ascorbate under anoxic conditoins can therefore not be concluded in the concentration range tested.

DMA alone did not mobilize Mn to above blank levels throughout the time series and no differences between 3.75µM and 6.25 µM DMA were recorded (Figure 17 b). When comparing the mobilization under oxic and anoxic conditions, Mn solution concentrations were elevated significantly after 96 hours under anoxic conditions. This increase in solution concentration agreed with the recorded Mn solution concentrations in the blank treatments. Interestingly the mean blank concentrations between oxic and anoxic conditions differed only slightly (0.66µM oxic vs 0.75µM anoxic), emphasizing the kinetic effect on Mn mobilization in blank treatments. Whether cation exchange processes, or increased dissolution over time, due to changes in the redox potential play a role here, may be subject of future research.

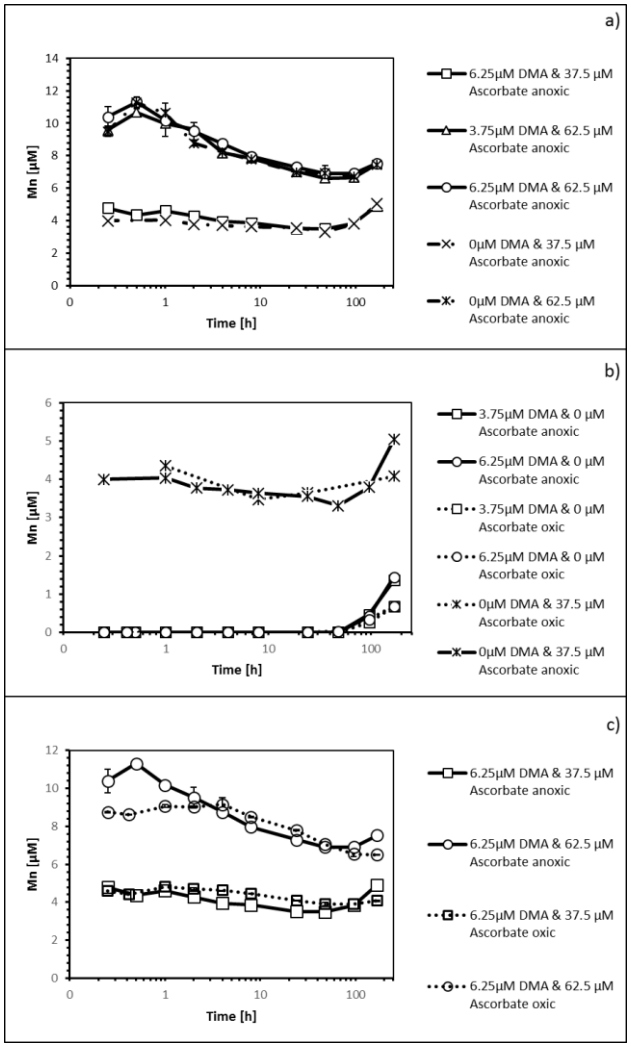


Figure 17: Manganese mobilization a) under anoxic conditions by Ascorbate with and without DMA, b) under presence and absence of oxygen with Ascorbate only and DMA only, c) under presence and absence of oxygen with selected combined treatments; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Figure 17 c) displays the mobilization of Mn by 6.25µM DMA with 37.5µM and 62.5µM ascorbate under presence and absence of oxygen. For the lower ascorbate treatment, initial concentrations were in a similar range of 4.6µM Mn in solution. Thereafter solution concentrations decreased with the oxic treatment mobilizing more Mn between the 2 hour and 48 hour time point.

The higher ascorbate treatment mobilized significantly higher amounts of ascorbate during the first 2 hours of the experiment under anoxic conditions. This indicates an increased dissolution of Mn-oxides under anoxic conditions in the higher ascorbate treatment. A synergistic Mn mobilization is less likely, based on the small differences between the combined and ascorbate only treatments displayed in Figure 17 a) and the virtually inexistent differences between the combined treatments with varying

DMA concentration discussed. However, a direct comparison of the treatments including ascorbate alone as in Figure 17 b) was not possible for the 62.5µM ascorbate treatment, due to the experimental setup, so uncertainty does remain.

4.2.5 Nickel

Figure 18 displays the mobilization of Ni over time under anoxic conditions in comparison to the mobilization under presence of oxygen. In general Ni solution concentrations were elevated under anoxic conditions compared to their counterparts obtained in the presence of oxygen.

Figure 18 a) shows that Ni solution concentrations were mobilized in a similar concentration range during the first 8 hours of the experiment independent of the higher or lower DMA addition and presence or absence of oxygen, while no ascorbate was added. The slow complexation kinetics of NiDMA have already been discussed in this thesis and are the most likely explanation for this. Thereafter Ni was mobilized to a larger extent under anoxic conditions than under the oxic scenario, resulting in a delta of +0.2µM (3.75µM DMA treatment) and +0.3µM (6.25µM DMA treatment) respectively after 168 hours. This difference might be related to the generally increased Mn-oxide dissolution under anoxic conditions, as has been pointed out in the previous paragraph.

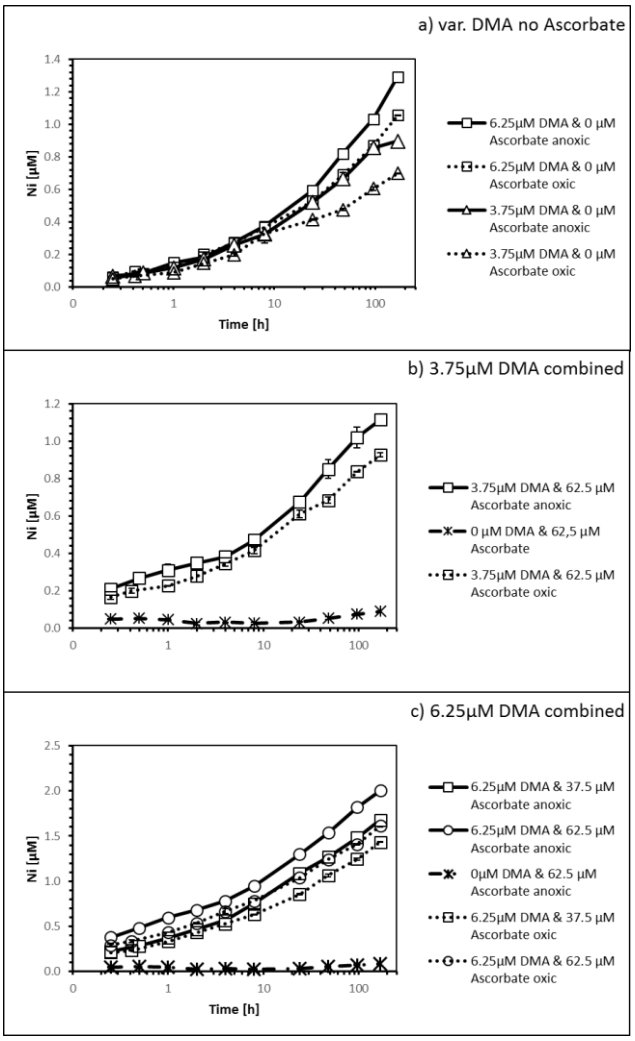


Figure 18: Nickel mobilization by a) 6.25µM and 3.75µM DMA, b) 3.75µM DMA and 62.5µM Ascorbate and c) 6.25µM DMA in combination with 37.5µM and 62.5µM Ascorbate; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Nickel did not substantially get mobilized by ascorbate alone under anoxic conditions, with solution concentrations staying below 0.1µM at all times during the experiment (Figure 18 b).

A synergistic effect on Ni mobilization was observed in all combined treatments under anoxic conditions. Interestingly patterns, such as the 3.75µM DMA & 62.5µM ascorbate treatment being effective enough, to yield higher solution concentrations than the 6.25µM DMA only treatment in the first 48 hours, were found under anoxic conditions as well as under oxic conditions. Synergistic mobilization was initially only significantly elevated in the combined treatment with highest DMA and ascorbate addition (6.25µM DMA & 62.5µM ascorbate), compared to its oxic counterpart. Here, an initial delta of +0.1µM increased over time to a difference of +0.4µM (anoxic-oxic) (Figure 18 c). All other treatments tested, mobilized similar concentrations initially, but deviated in favour of the anoxic treatment over time. The reasoning for this might be a combination of slow complexation kinetics and increased Mn-oxide dissolution under anoxic conditions. As is displayed in Figure 17 c) Mn-oxide dissolution appeared to be increased in the 62.5µM ascorbate treatments for the first 4

hours. With more ligand being available for complexation Ni appeared to become mobilized more effectively in the 6.25µM DMA – 62.5µM ascorbate combined treatment.

Nickel showed to profit over time from both the lack of oxygen and the synergistic effect of DMA and ascorbate. For iron mobilization, this increased the competition further. Nickel has been determined to be the dominant species in Santomera soil under equilibrium conditions (Schenkeveld et al., 2014a). In the 3.75µM DMA combined treatment, we see first evidence for a potential competitive effect on Cu by Ni. While Ni concentrations still increased until the168 hour time point, copper showed a drop in solution concentration. At this time point all Fe, Zn and most of Co had been depleted from solution concentration and SI Figure 7 indicates, that all ligand is complexing a metal. Iron is thermodynamically not such a strong competitor for DMA complexation in this system as Cu. Increased Ni mobilization therefore heavily affects Fe mobilization over time. In the first hours of the experiment however, the slow complexation kinetics of Ni reduced this effect substantially.

4.2.6 Cobalt

Figure 19 a) shows the mobilization of Co by DMA alone under presence and absence of oxygen. Trends of mobilization over time were similar, both under oxic and anoxic conditions, for the first 24 hours of the experiment. From there on, the treatments under absence of oxygen mobilized more Co, with differences increasing with increased supply of DMA. This might be related to the increased dissolution of Mn-oxides under anoxic conditions. Whether this is the only reason for the increased Co concentrations over time, cannot be completely resolved with the data obtained here, as cobalt is redox sensitive and might experience different bonding environments, depending on its oxidation state. It does however coincide with the increased solution concentrations of Ni displayed in Figure 18 a), emphasizing the correlation between Mn, Ni and Co in solution, and therefore strengthening the argument that increased dissolution of Mn-oxides is increasing the availability of Ni and Co for complexation by DMA.

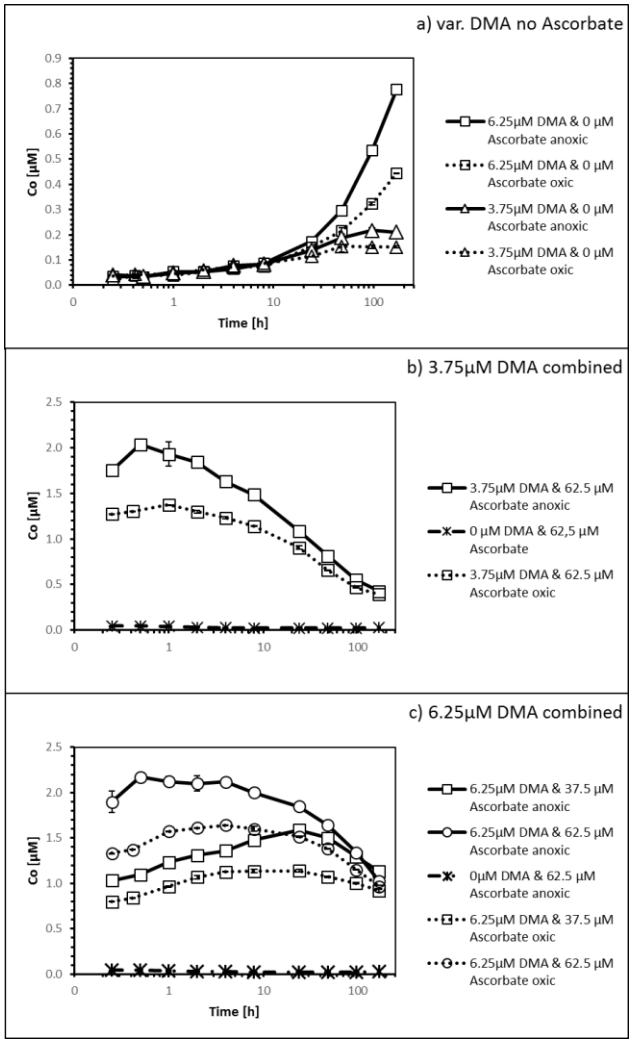


Figure 19: Cobalt mobilization by a) 6.25µM and 3.75µM DMA, b) 3.75µM DMA and 62.5µM Ascorbate and c) 6.25µM DMA in combination with 37.5µM and 62.5µM Ascorbate; dotted lines indicate results in presence of oxygen. Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

Figure 19 b) shows that ascorbate alone had no effect on Co mobilization under anoxic conditions, agreeing with the findings of paragraph 4.1.2.5 and Schenkeveld et al. (2016), A ligand is obviously needed to bring Co into solution. Synergistic mobilization was even more pronounced under anoxic conditions than under oxic conditions in all scenarios tested (compare also Figure 19 c). Intial mobilization was, as under oxic conditions,

strongly driven by the amount of ascorbate added. In both 62.5µM ascorbate combined treatments similar amounts of Co were mobilized in the first hour of the time series, with maximum concentrations after 0.5h with 2.0 and 2.2µM in solution respectively (low & high DMA treatment). Thereafter solution concentrations decreased steadily. Solution concentrations of Co in the combined treatment with 37.5µM ascorbate increased from 1.0µM up until 1.5µM Co in solution after 24 hours and decreased from that point in time.

Cobalt has in Chapter 4.1.2 already been identified as the most sensitive element to the synergistic effect between ascorbate and DMA. It has been shown, that the amount of ascorbate determined the Co solution concentration initially, whereas the amount of DMA supplied determined the solution concentration over time. Those trends were seen under anoxic conditions as well, with solution concentrations reaching similar levels after 168 hours, according to the amount of DMA supplied.

In addition, the final concentration appeared to be hardly affected by the presence or absence of oxygen in the system. This finding demonstrates, that the effect of ascorbate onto the system is temporary and eventually solution concentrations will be driven by thermodynamic properties of the CoDMA complex.

Throughout the experiment the combined treatments showed higher solution concentrations of Co under anoxic conditions, with differences decreasing over time. This might be related partly to an increase in Mn oxide dissolution and therefore increased availability, which is shown in the data obtained for the higher ascorbate concentrations tested. On the other hand, cobalt is redox sensitive and its availability for chelation, when incorporated into Fe- or Mn-oxides may be affected by its oxidation state (Frierdich & Catalano, 2012). This could explain the initially increased Co concentrations in the combined treatment with 37.5µM ascorbate (Figure 19 c), where only minor differences in Mn solution concentration were seen (Figure 17 a).

There is still more research needed to better understand the behaviour of CoDMA complexes over time. However, it is striking, that solution concentrations both under oxic and anoxic conditions appeared to ‘level out’ at the 168h time point, despite the fact that DMA is able to chelate both Co(II) and Co(III) (Murakami et al., 1989; Schenkeveld et al., 2016; Walter et al., 2016). Further investigation into the behaviour of Co(II)DMA and Co(III)DMA complexes in soils, in particular adsorption and desorption behaviour and competitive displacement is needed. Whichever Co species might have been present here, appeared to form less stable complexes with DMA as Ni and Cu, which both still increased over most time points and concentrations tested, while Co solution concentrations decreased.

Iron mobilization is strongly affected by the initially increased solution concentrations of Co. The initial strong response of Co to the combined treatments appeared to be the reason for Fe being mobilized less under anoxic than under oxic conditions. As the initial mobilization might be the most important in order to overcome Fe deficiency, strongly elevated Co mobilization might significantly impede the success of an iron starving plant in some soils. The ratio dependency of ligand and reductant mentioned earlier, appeared to be different for a system lacking oxygen. While the 6.25µM DMA and 37.5µM ascorbate treatment showed to be very successful in terms of a synergistic effect on Fe mobilization for the first 4 hours in an oxic system, the effect was diminished by increasing solution concentrations of Co under anoxic conditions.

4.3 Discussion synergistic effect on iron mobilization

The following main conclusions can be drawn from both experiments addressing the synergistic effect of ascorbate and DMA in combined application.

The overall mobilization was only mildly affected by the presence or lack of oxygen. The main characteristics of metal and in particular iron mobilization by DMA alone, and the synergistic effect on metal mobilization identified in the presence of oxygen, hold true under absence of oxygen.

Manganese concentrations were strongly driven by the amount of reductant applied and were initially elevated under anoxic conditions in the higher reductant treatments (62.5µM ascorbate) applied. This indicates increased dissolution of Mn-oxides. However, Mn solution concentrations were still in the same range as under oxic conditions, indicating that the effects of ascorbate alone were not substantially altered by oxygen present in the soil solution.

Adsorption isotherms of manganese in Santomera soil have been generated (SI Figure 4). The data suggests, that potentially most ascorbate supplied is used for the reductive dissolution of Mn-oxides. The presence or absence of oxygen in the system is apparently no driver of significant changes in solution, and subsequently total, concentrations of Mn^{2+}.

A synergistic effect on Fe mobilization was visible in most treatments tested, both under presence and absence of oxygen. The effect on Fe was affected initially by the mobilization of cobalt, which was profiting from synergistic mobilization the most in all scenarios tested. Over time the competition by copper and nickel became the main driver of Fe solution concentrations. Therefore, a strong ratio dependency of ligand and reductant to mobilize iron was concluded. It became apparent, that both in presence and absence of oxygen Fe can profit more strongly in a system with less reductant (37.5µM ascorbate) than with more (62.5µM ascorbate) in combined treatments of the same amount of ligand (6.25µM DMA).

The mechanism behind the synergistic mobilization of Fe is still to be resolved. Under anoxic conditions ascorbate alone did not mobilize substantial amounts of Fe into solution. A reductive dissolution of Fe(hydr)oxides therefore appears to be less likely the main driver of the synergistic effect on Fe mobilization. Without an adsorption isotherm of Fe^{2+} to Santomera soil this can however not be resolved completely. This is due to the fact, that ferric iron reduction, followed by near instantaneous adsorption of ferrous iron onto the soil solid phase might happen, resulting in not measurable solution concentrations in the time points recorded in this experiments. In Experiment 4 (paragraph 2.5) Fe^{2+} was supplied to Santomera soil. The data showed no iron present in solution after 15 minutes, but elevated Mn solution concentrations (compare SI Table 6). This demonstrates the potential of Fe^{2+} to serve as an electron donor to Mn(III/IV) oxides present in Santomera soil. A mechanism involving reduction of Fe (hydr)oxides to ferrous iron, instantaneous adsorption and reoxidation by reduction of Mn oxides appears not unplausible based on those observations. Santomera soils Fe is mostly in the crystalline fraction (Schenkeveld et al., 2014a). A reoxidized Fe(hydr)oxide species might very well be less crystalline and easier to complex by DMA. Fe availability would be increased by that, and therefore significantly contribute to the synergistic effect observed. As discussed previously, incorporation of Fe into the structure of Mn oxides has been reported (Boenigk, 2004), reductive dissolution of Mn oxides might therefore additionally contribute to the increased availability of Fe when, ascorbate is added to the soil system.

Under conditions resembling the rhizosphere more closely with increased DMA input over a period of time, the Fe mobilization may look differently. The synergistic effects between reductant and ligand may be of even greater importance here, as it can be considered to be a system with rather continuous

resupply of ligand. That may reduce the impact of competitive displacement and mobilize more Fe than observed in the experimental setup here. Future research should try to move closer towards those conditions in order to get a better understanding of the environmental significance of the synergist effect on iron mobilization described here.

What the data obtained clearly indicates is that metal and in particular iron mobilization is heavily influenced by the presence of a reducing agent in the soil solution. A vast amount of root exudates, omnipresent in the rhizosphere, are known to possess reductive properties. Those include low molecular weight organic acids like phenols (Keuskamp et al., 2015). Additionally, microbial processes like respiration, heavily impact the reduction potential in soils (Sposito, 2008). Therefore, exudates without immediate reductive properties can serve as a carbon source and indirectly influence the soil redox potential (Neumann & Römheld, 2007). Furthermore, plant root respiration depletes oxygen in the proximity of the plant roots and increases spatial, temporal and chemical heterogeneity in the rhizosphere.

Under Fe deficiency a variety of exudates is released into the rhizosphere of graminaceous plants (Fan et al., 1997, 2001). Therefore, the data obtained here, can serve as a clear indication, that it is insufficient to investigate the effects of phytosiderophores, without considering their interplay with other rhizosphere compounds over time.

5 Scanning experiment interplay plant borne compounds and DMA

5.1 Results

In the following selected results of a scanning experiment are presented, investigating the interplay of DMA and other plant borne compounds, in particular coumarins and closely related compounds. For Materials and Methods, the reader may be referred to paragraph 2.5 and for the complete data obtained to SI Tables 6-8.

5.1.1 Compound only

Figure 20 shows the metal mobilization (Al, Fe, Co, Ni, Cu and Zn) by the respective compounds alone after 15 minutes (a), 4 hours (b) and the Mn mobilization (c). Scopoletin, esculetin, fraxetin and coumarin belong to the polyphenolic group of compounds commonly referred to as coumarins. Some coumarins are known to have both reducing and chelating properties, so Mn mobilization could be derived from reductive and/or ligand promoted dissolution processes. If partly chelation of Mn was causing the Mn mobilization observed cannot be excluded. Considering the Irving Williams series of metal complex stabilities in the order of Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) (Irving & Williams, 1953), one might assume, that at least partly mobilization of Cu and Zn could be observed, if Mn was brought into solution by chelation. Figure 20 a&b shows that no significant mobilization of those ions was observed, while Mn solution concentrations were still strongly elevated in solution. The Mn mobilization therefore might serve as an indication on the reducing capacity of the respective compound. However, many properties of this system are unknown, like the redox state of Mn or compound specific complex stability constants, making it uncertain if the Irving-Williams series is valid here. Chelation of Mn by the respective compound displayed in Figure 20 a&b can therefore not be excluded.

Evaluating the performance of the individual compounds alone several interesting findings could be made.

The compound coumarin itself did not mobilize any metal significantly to above blank levels, neither after 15 minutes, nor after 4 hours. This indicated that coumarin, lacking a catechol group was not able to chelate iron and other metals from Santomera soil (Figure 20 a&b). Additionally, no indications for a reduction of the soil constituents were found, as the Mn solution concentrations did not increase on addition of coumarin alone to above blank levels (Figure 20c).

Caffeic acid did not mobilize more than 0.25µM of any metal displayed in Figure 20 a&b ,neither after 15 minutes, nor after 4 hours. On the other hand, it was the compound with the strongest Mn mobilization after 15 minutes with 24.7 (± 0.6) µM and solution concentrations increased to 27.0 (± 0.5) µM after 4 hours (Figure 20 c). Caffeic acid is a compound with a strong reduction potential (Magnani et al., 2014), which might help to explain the fast mobilization of Mn, that remained in a similar concentration range, due to rather slow oxidation kinetics of Mn^{2+} in solution.

Esculetin is known to be a chelating agent for Al(III), Cu(II), Pb(II) and Fe(III) forming 2:1 or 3:1 complexes with depending on the metal ion (Schmid et al., 2014; Le Person et al., 2014). Additionally it has already been reported to mobilize Fe from Santomera soil (Schenkeveld et al., 2016). Fe was mobilized to levels of around 0.3µM after 15 minutes with solution concentrations decreasing thereafter to around 0.1µM after 4 hours. The mobilization of Fe from Santomera soil by esculetin was in agreement with previous findings (Schenkeveld et al., 2016). Aluminium was the dominant species mobilized by esculetin in Figure 20 a&b, reaching solution concentrations of 0.9 (± 0.1) after 15 minutes, but decreasing to around 0.2µM after 4 hours. Mn mobilization by esculetin was in the range

of 10 μM, with increased concentrations after 4 hours. Schenkeveld et al. (2016) observed Mn mobilization by esculetin from Santomera soil as well. The total amounts mobilized here and in their study were well in agreement with each other, considering the adsorption isotherm of $Mn^{2+}$to Santomera soil (compare SI Figure 4). They also observed a small synergistic effect on Mn mobilization in a combined treatment of esculetin and ascorbate, complicating the distinction between reductive and ligand promoted dissolution of Mn oxides here. However, from all compounds, tested here, which had an observable effect on Mn solution concentrations, esculetin mobilized the least, indicating a lower reduction capacity than scopoletin, fraxetin and caffeic acid.

Fraxetin showed only little mobilization of any metal other than Mn after 15 minutes. Interestingly Mn solution concentrations increased significantly between the tested 15 minutes and 4 hour time point from 14.8 (± 0.1) μM to 28.0 (± 0.3) μM, indicating a not immediate reduction of the soils Mn oxides. Fe solution concentrations also increased during that time period significantly from 0.2 μM after 15 minutes to 0.5μM after 4 hours, making it the compound mobilizing the most iron in that time point.

Scopoletin was the compound mobilizing most iron after 15 minutes (1.1 ± 0.1 μM Fe) and significant amounts of aluminium (0.8 ± 0.1 μM Al). Mobilization of Co, Ni, Cu and Zn was not significantly elevated at solution concentrations of around 0.1μM (Figure 20 a). After 4 hours solution concentrations of Al and Fe decreased (0.1μM Al and 0.3μM Fe in solution), while the other metals tested remained at low concentration levels (Figure 20 b). Scopoletin mobilized around 14.8 (± 0.2) μM Mn after 15 minutes and 16.9 (± 0.2)μM after 4 hours. This may serves as an indication for reductive dissolution of the soil Mn oxides. The elevated concentrations after 4 hours compared to those after 15 minutes could indicate slower redox kinetics of the compound or possible reaction of former complexed Fe(II) to with the soils Mn oxides.

Under Fe deficient conditions fraxetin, scopoletin and esculetin have just recently been shown to be increasingly exuded by *Arabidopsis thaliana* (Schmid et al., 2014). An included interaction study with a not further specified Fe-hydroxide suspension showed that only esculetin mobilized Fe, but not scopoletin (Schmid et al., 2014). Here we see indications, that fraxetin and scopoletin can mobilize significant amounts of Fe. To the authors knowledge, this is the first time Fe mobilization from soil by those two compounds has been shown. The mechanism behind this remains still to be subject of future research. A follow up kinetic experiment including fraxetin and scopoletin with Santomera soil conducted by the author, (not included in this thesis, unpublished) validated the finding that scopoletin mobilized significant amounts of iron, but yielded contradicting results for the interaction of fraxetin with Santomera soil. Based on the results presented here, it has to be concluded that Hypothesis 3 did not hold true. Scopoletin, even though not having a catechol group with two hydroxy groups accessible, did mobilize Fe, in particular in short term interaction. It is unlikely that the Fe in solution measured derived from the consumption of oxygen and the production of not chelated Fe^{2+} in solution. Would this have been the case, it should have also been observed for the strong reductant caffeic acid, which mobilized more Mn, but could not significantly increase Fe solution concentrations.

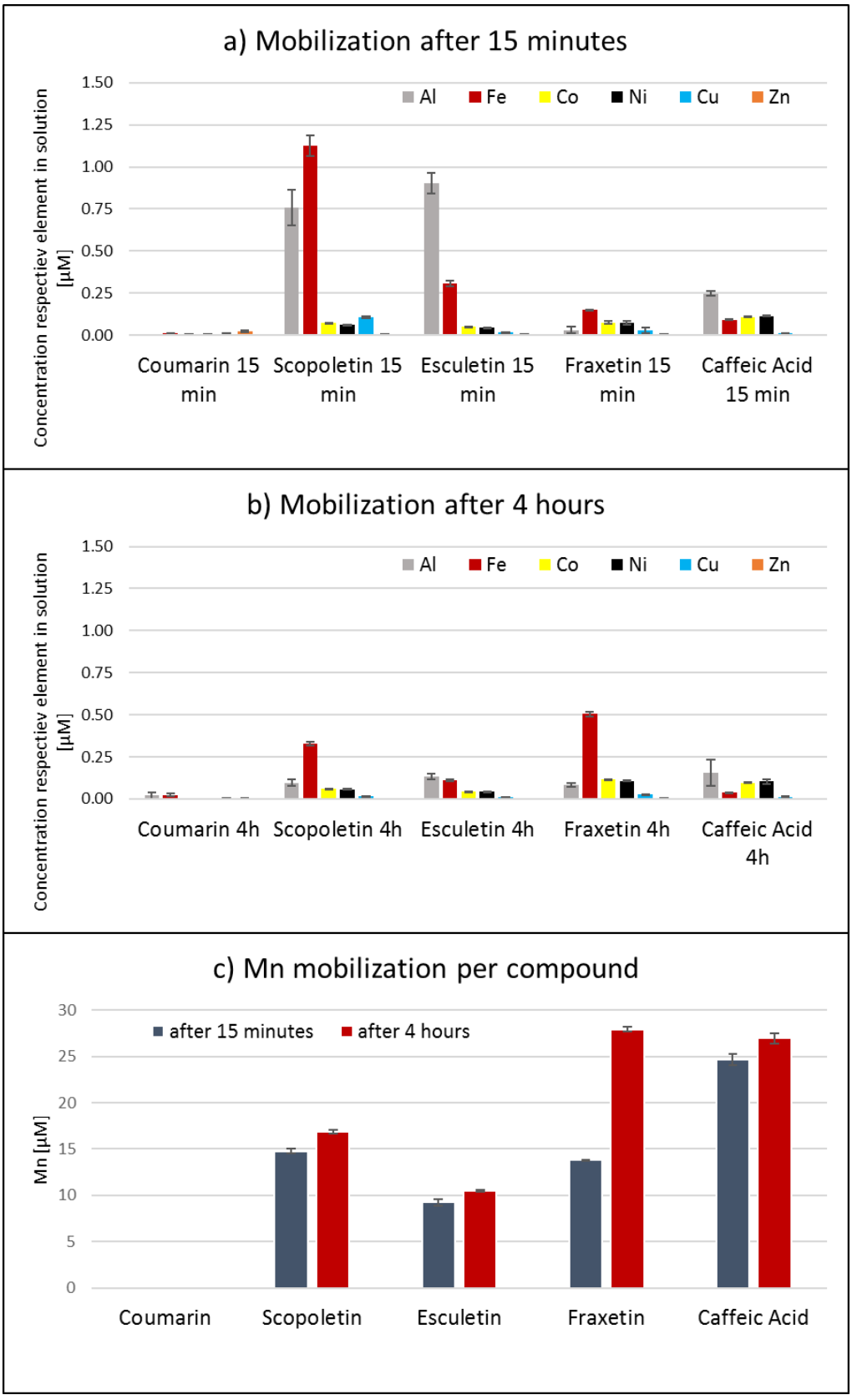


Figure 20: Mobilization of Fe, Co, Ni, Cu and Zn by selected plant bourne compounds alone a) after 15 minutes and b) after 4 hours; c) Mobilization of manganese, by selected plant bourne compounds alone after 15 minutes and after 4 hours; Error bars indicate Standard deviation (n=2); SSR 0.25; amount of compound supplied: 250µM; 10mM $CaCl_2$ as background electrolyte

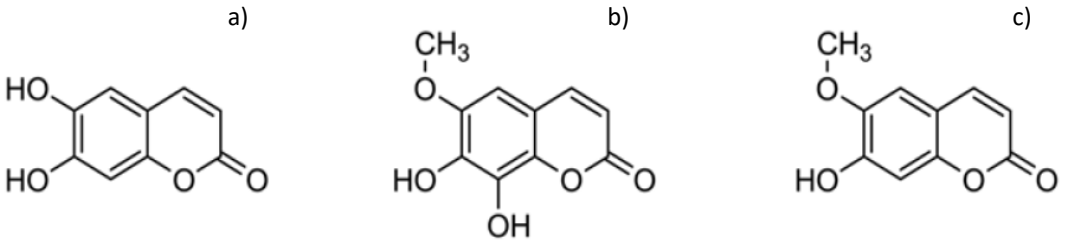


Figure 21: Structure of a) esculetin; b)fraxetin; c) scopoletin; (Schmid et al., 2014 adapted)

The structure of the respective compounds might help understanding processes leading towards the mobilization observed. Esculetin Figure 21 a) has been reported to have reducing and chelating capacities. An oxidation of the compound results in structural changes, including the possible formation of esculetin oligomers (Deiana et al., 2000). The mobilization of Mn shown by both fraxetin and scopoletin is very likely to its largest part driven by reductive dissolution of Mn oxides. This reaction with the soil solid phase of scopoletin or fraxetin might result in structural changes of the molecule, resulting in a demethylation and transformation into a hydroxy group. This process has been reported for the oxidation of Scopoletin by Miller et al. (1975), where esculetin (*''compound II''* (Miller et al., 1975)) was reported to be one possible oxidation product. A sequential reduction of the soil constituents and following chelation process might be carried out by scopoletin (and fraxetin) and their oxidation products. In the context of this work, where an interplay of a reductant and a ligand was investigated in the previous chapter and found to substantially increase Fe mobilization, a compound potentially using both, reduction and chelation in sequence appears fascinating. More research is needed in order to evaluate the results and understand the biogeochemical processes more thoroughly. As there is already evidence, that under Fe deficient conditions increasing amounts of the three compounds displayed in Figure 21 are exuded (Schmid et al., 2014; Schmidt et al., 2014), an investigation into their interplay might yield fascinating results and broaden the understanding of plant responses to Fe deficiency significantly.

5.1.2 Compound and DMA – synergistic effect on metal mobilization

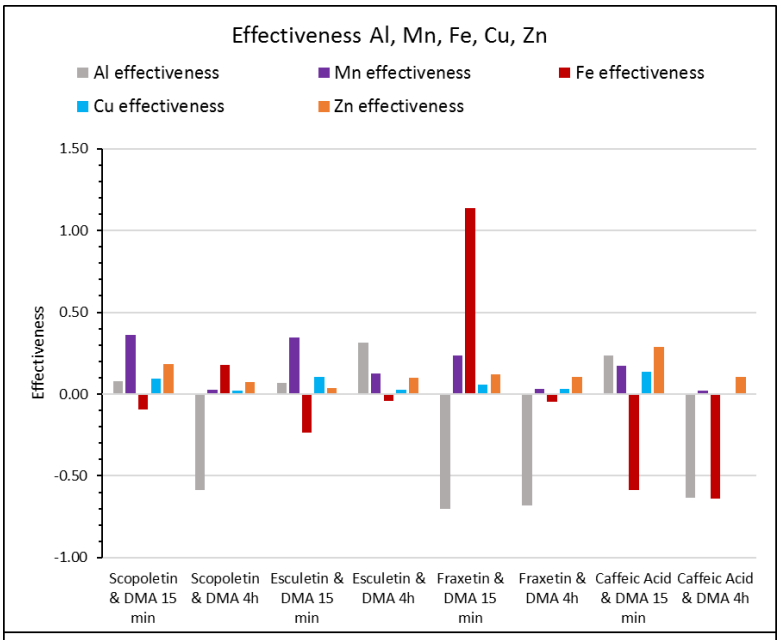
The combination of respective compound and DMA can serve as a first overview on the potential for synergistic effects on iron mobilization. In this section the main findings are presented and potential fields of future research elaborated. Individual mobilization after 15 minutes and after 4 hours interaction time are displayed in SI Figure 9.

In order to get a comprehensive overview on the potential synergistic mobilization one can apply the following equation:

$$Effectiveness = \frac{T_{combined} - Stdv_{combined}}{T_{reductant} + Stdv_{reductant} + T_{DMA\ only} + Stdv_{DMA\ only}} - 1 \quad (12)$$

With T_n being the respective treatment [µM] and $Stdv_n$ being the treatments standard deviation [µM].

Note that in order to get the most conservative assessment the respective standard deviation is substracted from the combined and added to the individual treatments. All positive values signify a net gain (synergistic effect) in the combined over the individual treatments, all negative values a net loss (antagonistic effect). Effectiveness values close to 0 can be considered as unaffected by synergistic or antagonistic effects.



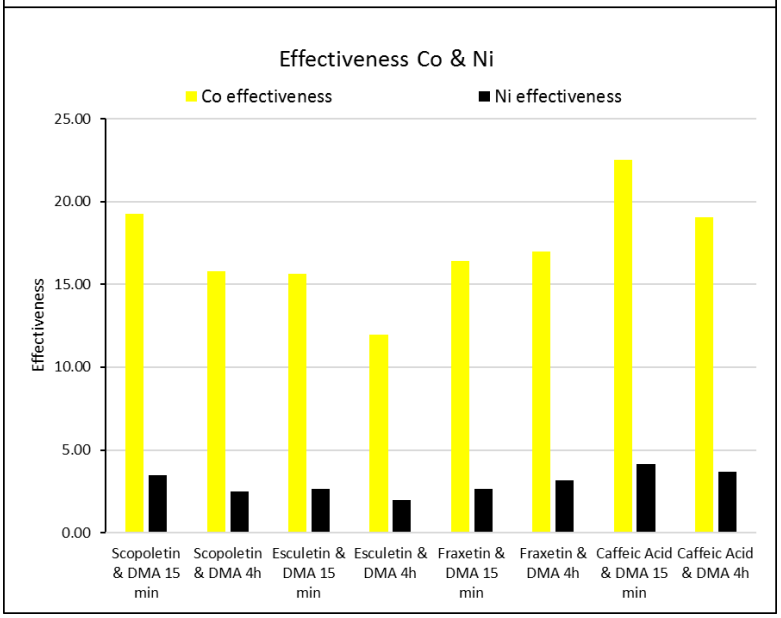


Figure 22: Synergistic or antagonistic effects of selected combined treatments. Effectiveness calculated by equation 12; SSR 0.25; amount of compound supplied: 250µM; amount of DMA supplied: 25µM; 10mM $CaCl_2$ as background electrolyte

Figure 22 shows the effectiveness of the respective combined treatments. It becomes apparent that in particular cobalt and nickel were able to profit the most by the combination of DMA and respective compound. This has been observed previously in paragraph 4, where the ratio of ligand and reductant, could not benefit Fe mobilization but rather Co and Ni.

Caffeic acid and scopoletin mobilized the most Co and Ni after 15 minutes. This is in accordance with the total Mn mobilization displayed in SI Figure 9. Over time all treatments except for fraxetin lost in effectiveness for Co and Ni. Again, Mn mobilization might explain this behaviour as fraxetin appeared to react more slowly, yielding Mn solution concentrations of around a factor 2 higher after 4 hours than after 15 minutes.

Manganese itself was elevated in all combined treatments in comparison to the sum of the individual counterparts, indicating a small synergistic effect on Mn, with scopoletin and esculetin after 15 minutes mobilizing most (effectiveness around 0.35).

Iron is of particular interest here. Caffeic acid is the only treatment, where a clear antagonistic effect onto iron mobilization was observed, with effectiveness values around – 0.6 in both time points. The fact that Co and Ni mobilization was relatively most elevated in this treatment, matches the observations in paragraph 4.1.2, indicating an antagonistic effect on iron mobilization by strongly elevated Co and Ni complexation. Both treatments shown to mobilize substantial amounts of Fe on their own after 15 minutes – scopoletin and esculetin (compare Figure 20) – experienced a slight antagonistic effect in the first 15 minutes. After 4 hours however, there was no clear antagonistic effect on esculetin and a synergistic effect (effectiveness 0.2) on scopoletin visible. Fraxetin was the compound yielding the highest relative effect on Fe mobilization after 15 minutes with an effectiveness of around 1. This means that iron solution concentrations are doubled in comparison to the sum of the

individuals. This effect wore off over time, but did not turn into an antagonistic effect in the time frame observed.

5.2 Discussion

The results of this short time scanning experiment indicate some interesting findings. All compounds displayed, except for coumarin, which appeared inert in terms of metal mobilization, had an effect on manganese mobilization. This can serve as an indication for a reductive dissolution of the soils Mn-oxides and therefore the reducing capacities of the compound. Potential Mn chelation has to be considered however.

Fraxetin and scopoletin alone mobilized substantial amounts of iron from Santomera soil. Schmidt et al. (2014) showed the ability of scopoletin and fraxetin to chelate Fe(II). They proposed a Fe(III) reduction and a Fe(II) chelation as the underlying mechanism of increased compound exudation under Fe deficiency. The results presented in Chapter 4 might contradict this process as being responsible for the observed mobilization. However, it is possible that reduction and complexation carried out by the same compound and not by two separated compounds will result in Fe(II) chelation, even under presence of oxygen.

Future research should be conducted to understand the mechanism lying behind this more profoundly. Structural changes of the respective compound due to reductive dissolution of the soils Mn-oxides might be a valuable explanation. Schenkeveld et al. (2016) showed an increased initial mobilization of Fe in Santomera soil, by a combination of ascorbate and esculetin (1mM esculetin & 1mM ascorbate in SSR 1) and no substantial mobilization by the individual treatments. Esculetin did however affect Mn mobilization in a concentration range that agrees with the data presented here. In a suspension with not further specified Fe hydroxides esculetin showed to mobilize Fe (Schmid et al., 2014). Whether the structural changes resulting in oxidation of fraxetin and scopoletin facilitated chelation of Fe(III) by an oxidation product resembling the structure of esculetin more closely appears to be a valid hypothesis to be checked by future studies. Hypothesis 3 however has to be declared as invalid. Scopoletin has clearly been shown to mobilize Fe from Santomera soil, in which oxidation state Fe is brought to solution still remains unclear based on the data presented here. A follow up study conducted by the author (not included in this thesis) showed that the majority of mobilized Fe by Scopoletin is in fact Fe(II). As the species, for which increased exudation of coumarins under Fe deficiency has been reported, are Strategy I plants, it would be plausible, from an evolutionary point of view, for the plant to chelate the form of Iron it can immediately take up.

In terms of synergistic metal mobilization esculetin, fraxetin, scopoletin and caffeic acid mobilized significantly higher amounts of Co and Ni in the combined treatments with DMA, than in comparison with the respective treatments alone. Therefore, a synergistic effect on mobilization of those elements can be concluded. The findings obtained in paragraph 4, show a clear ratio dependence of the amount of ligand and the amount of reductant to benefit iron acquisition. In case of a ratio with “too high” reductant concentrations, metals like cobalt and nickel get chelated preferentially and iron mobilization experiences antagonistic effects in Santomera soil. This might very well have been the case here. Further studies should be conducted in order to gain more insights into ratios being beneficial for iron acquisition of Strategy II plants in relation to those plant borne reductants.

Of particular interest for future research would be the interplay of different compounds exuded by the same plant species. Scopoletin and fraxetin are the compounds that appear most promising for a synergistic effect on iron mobilization, but also the interplay with a stronger reductant like caffeic acid and those compounds respectively or even combined in a mixed application of three exudates could yield fascinating new insights into plant responses under Fe deficiency.

6 Effects of TETA onto Iron acquisition of *Triticum aestivum sp. Tamaro*

In paragraph 3 and paragraph 4 of this thesis, copper has been shown to be consistently outcompeting Fe in terms of complexation by DMA over time. Triethylenetetramine dihydrochloride (TETA) is a chelating agent with an especially high affinity for copper. It is used as a therapeutic compound to treat Wilsons disease in humans, where it chelates and removes excessive copper from the body (Nurchi et al., 2013). Previous batch experiments conducted in our group, showed that TETA has the ability to reduce the amounts of copper in Santomera soil solution as well. Additionally, when combined with DMA, more iron could be mobilized from the soil, due to reduced competition by Cu (Gasser, 2015). Those findings led to the hypothesis, that TETA may have beneficial effects on the overall iron acquisition of graminaceous plants, leading to a beneficial effect of the overall growth.

The experimental design is displayed in paragraph 2.6. Two treatments of TETA have been chosen, based on previous batch experiments. Treatments of 4000µM TETA (High TETA treatment) and 500µM TETA (Low TETA treatment) in a SSR of 5:1 were evaluated against an untreated control group.

6.1 Description of Growth phase

All treatments showed 4 or more shoots after 4 days into the experiment. Thereafter all plants grew in similar fashion without visible differences (SI Figure 11 - SI Figure 16). In no treatment, clearly visible signs of chlorosis could be observed throughout the experiment. During the 45 days of the experiment no obvious indications for plant biomass differences became apparent. The rate of new leaf development and tillers was not obviously different in any group. Main shoots developed 8 or more leaves with most plants developing between 2 and 4 tillers. Stems of the main shoot started to elongate leading to a growth stage classification of Z30 (Zadoks et al., 1974). At termination of the experiment the pots were densely covered by plant roots. The soil can therefore be considered rhizosphere soil (Oburger et al., 2014). No negative effects on plant health have been observed in treatments including high or low concentration of TETA in comparison to the control group.

6.2 Pore water metal content

Table 5: Pore water metal concentrations; ICP-MS; standard deviation indicated by uncertainty n=2

Treatment	**Fe** µM	**Co** µM	**Ni** µM	**Cu** µM	**Zn** µM
Control	0.95 ± 0.05	0.04 ± 0.00	0.23 ± 0.02	0.43 ± 0.06	2.21 ± 0.46
Low TETA	1.15 ± 0.12	0.07 ± 0.01	0.26 ± 0.04	0.41 ± 0.09	2.70 ± 1.31
High TETA	0.99 ± 0.03	0.08 ± 0.01	0.20 ± 0.02	0.34 ± 0.05	2.07 ± 0.41

The pore water extractions after harvest showed only minor differences between respective treatments. The iron concentrations in the pore water were in a similar range. Cobalt concentrations were low for all treatments, with significantly lower concentrations in the Control group. Nickel concentrations were in a similar range for all treatments. Zinc showed to be the most prominent element in the pore water extracts with the largest standard deviations. The values obtained were not different between treatments, when considering the standard deviations. Copper showed indications of being affected by the addition of TETA, with the mean values being negatively correlated to the

amount of TETA added. Differences between the High TETA and Control group were however not significant (P=0.051).

The pore water concentrations did not differ sufficiently to clearly resolve the impact of TETA onto the system. However copper solution concentrations might hint towards a small still lasting effect, binding Cu more strongly to the solid phase. TETA is subject to biodegradation as previous experiments have shown. Gasser (2015) demonstrated, that the performance of TETA on Cu removal from solution after two weeks exposure to unsterilized soil was reduced, but still in a similar range as when freshly added.

The soil in this experiment has not been sterilized. Microbial communities in real rhizosphere conditions can additionally be expected to be more divers and active than under non-fed batch conditions (A.A.M. De Leij et al., 2007). Therefore, it can be expected that the influence of TETA decreases over time due to biodegradation. Being not able to see clear trends at the end of the 45 days growth period might therefore not mean, that TETA addition did not affect the soils overall metal activity throughout.

Furthermore solubility of Cu from Santomera soil is generally low, as has been observed multiple times during data analysis of the batch experiments. Pore water extracts obviously do not represent all bioavailable metals in a soil (McLaughlin et al., 2000), so copper might have still been more available to the control group than to the TETA treated groups.

6.3 SPAD measurements

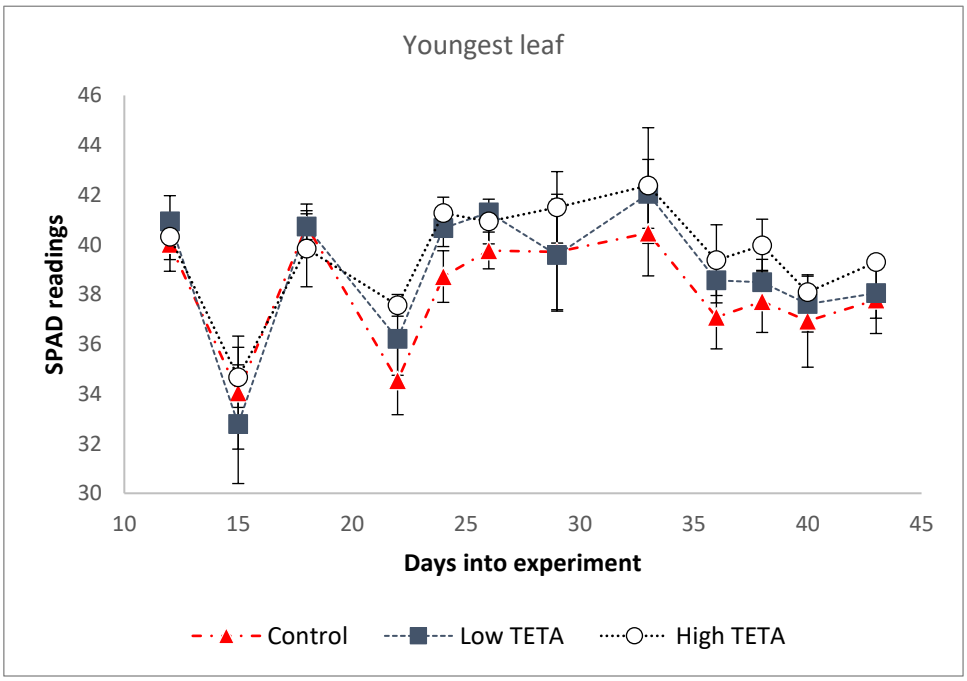


Figure 23:Mean SPAD readings of the youngest leaf per treatment; Measurements per pot = 8, pots per treatment = 4; Error bars indicate Standard deviation of means per pot

Figure 23 shows the mean SPAD readings of the youngest leaves throughout the growing phase per treatment. In order to yield reliable data, each mean consists of 8 measurements per pot and 4 pots per treatment. Special care was taken to consistently measure in the same distance from the leaf tip, to avoid influences deriving from leaf structure.

Initial fluctuations observed, were attributed to the shedding of new leaves, which were significant lighter especially in the early stages of the growing phase. For the first 18 days into the experiment no significant differences could be detected between the treatments. From the fourth measurement on

(22 days into experiment), differences became statistically significant (P<0.05). Over the entire time period following, the High TETA treatment yielded significantly higher SPAD values than the Control treatment (exception 40 days measurement). The overall time series of the High TETA treatment was significantly different than the Control treatment (P<0.05). This indicates a small beneficial effect on the chlorophyll content of the High TETA treatment in comparison to the Control group.

The Low TETA treatment showed an intermediate trend between Control and High Treatment, matching the expected results. However, differences were not significantly different to the control group over the whole series and only partly for individual measuring dates.

6.4 Dry weight

Table 6: Mean dry weight per treatment; with a) Control and b) Low TETA; letters indicate significant difference (P < 0.05)

Treatment	Mean dry weight [g]	Standard deviation
Control	4.11	0.09
Low TETA	4.10	0.15
High TETA	4.36 [a,b]	0.17

Table 6 shows the mean dry weight of the respective treatments at harvest. There was no significant difference between Control group and Low TETA treatment. But the mean dry weight concentration of the High TETA treatment were elevated. This results in a significant increase in biomass of +6% in comparison to the Control group.

6.5 Plant tissue metal content

Table 7: Metal concentration in above ground tissue determined by HNO_3-H_2O_2 digestion; n=4; Recovery relative to NIST DC73349 (''Bush branches'');Critical value of deficiency according to (Broadley et al., 2012)

Sample	Mn		Fe		Ni		Cu		Zn	
	mg/kg dw	Stdv	mg/kg dw	Stdv	mg/kg dw	Stdv	mg/kg dw	Stdv	mg/kg dw	Stdv
Control	101.31	5.98	65.55	7.39	0.47	0.06	8.22	0.56	22.66	4.23
Low TETA	94.58	2.87	60.82	6.00	0.39	0.05	7.82	0.18	16.53	0.96
High TETA	100.03	5.50	71.76	17.97	0.30	0.03	7.50	0.53	16.34	2.01
Recovery %	109		81		82		98		97	
Critical value* mg/kg dw	20		50-150		n.a / toxicity > 10		1-5		15-20	

Table 7 gives the metal content in above ground plant tissue as determined by the HNO_3-H_2O_2 wet digestion (Wheal et al., 2011). The recovery of Fe and Ni is poor with 81% and 82% and those results should be interpreted with care. Copper and zinc show very good recoveries and manganese was within ± 10%.

Manganese and copper concentrations were in a similar concentration range and no significant differences between the treatments were observed. Iron tissue concentration was not significantly different for the low TETA treatment and the control group. Elevated Iron concentrations in the High TETA treatment were compromised by the high standard deviation in that treatment. The concentrations of Zn and Ni were significantly elevated in the Control treatment, compared to the High TETA treatment. The concentrations of tissue iron were generally low in all treatments. Broadley et al. (2012) state the critical value of iron deficiency to be in the range of 50-150 mg/kg dry weight. The levels measured here indicated, that iron might indeed have been limited to the plants, but the recovery relative to the standard material limited the interpretation. No deficiency symptoms, like chlorotic leaves, could be determined during the growth phase. The tissue iron levels were not in agreement with Oburger et al. (2014), where the same cultivar on the same soil was reported to show chlorotic symptoms, but tissue Fe concentrations were more than a factor 4 higher than found here. Zinc tissue concentrations were on the other hand in very good agreement with the values presented by Oburger et al. (2014) for the Control group.

The relative lower concentrations of Ni for the High TETA treatment were in agreement with results obtained from batch experiments with TETA and DMA (Gasser, 2015). Here less Cu, Ni and Co but more Fe and Zn were mobilized by a combination of DMA and TETA. The lower values for Ni in treatments with TETA might therefore be a direct effect of the TETA addition. Copper and Zn have been applied to the plants by foliar fertilization. The small differences in Cu uptake might have been related to the foliar application of that micronutrient, levelling out effects otherwise visible.

Table 8: Mean uptake of respective element (normalized to biomass); HNO3 H2O2 digestion; n=4

Sample	Mn		Fe		Ni		Cu		Zn	
	mg	Stdv	mg	Stdv	mg	Stdv	mg	Stdv	mg	Stdv
Control	416.1	21.0	269.9	34.5	1.9	0.2	33.8	2.0	92.8	15.5
Low TETA	387.7	11.4	249.9	30.7	1.6	0.2	32.1	1.1	67.7	2.0
High TETA	436.0	22.3	313.9	83.2	1.3	0.1	32.6	1.0	71.6	11.5

The elevated zinc concentrations in the control group are particularly interesting. Table 8 suggests that elevated concentration in plant tissue were not compromised by increased biomass in the High TETA treatment, but total zinc uptake was elevated in the Control throughout. Several studies have found enhanced phytosiderophore exudation under Zn deficiency (Zhang et al., 1989; Cakmak et al., 1994; Erenoglu et al., 2000). Gries et al. (1995) showed a clearly enhanced PS exudation under Fe deficiency, enhanced PS exudation to Zn deficiency when symptoms were already severe, but none to Cu and Mn deficiency in barley. A specific uptake mechanism for copper, manganese and zinc complexed by phytosiderophores is however heavily questioned and arguments have been brought forward, that symptoms of Zn deficiency compromise Fe translocation from roots to shoots and therefore result in enhanced PS exudation (Grusak et al., 1999).

The data obtained here can be interpreted as an enhanced exudation of phytosiderophores in the control group relative to the groups treated with TETA. When considering the increased tissue concentrations of Zn in the Control group, being the result of increased mobilization of Zn by phytosiderophores in the rhizosphere. The lower relative availability of Fe in the control treatment might have triggered an enhanced exudation of PS, resulting in similar Fe tissue concentrations, similar concentrations of copper, which was applied in a foliar fertilization, and elevated concentrations of Zn. Ni tissue concentrations further strengthen this thought, as it has not been supplied by foliar fertilization and the tissue levels decrease with increasing soil TETA concentration. In other words, the plants in the control treatment might have had to exude more PS, to mobilize and take up similar amounts of Fe.

6.6 Discussion

No clear visible evidence for chlorosis in any treatment was found during the experiment. The cultivar *Triticum aestivum sp. Tamaro* is known to produce high amounts of phytosiderophores (Reichard et al., 2005). Being a cultivar with a high efficiency in iron uptake it is therefore not be completely unexpected to not see chlorotic plants in any treatment, including the control group. However a previous study with the same cultivar and soil led to visible chlorosis (Oburger et al., 2014). Other environmental factors like light regime, soil temperature and moisture content play an important role in Fe deficiency (Marschner et al., 1986). Rooting density, seed and soil age additionally alter conditions and may yield in different results than expected (Lock & Janssen, 2003; Broadley et al., 2012).

However, several indications of a beneficial effect by the addition of TETA were found. The SPAD measurements showed elevated relative chlorophyll content in the High TETA group, in comparison to the control, suggesting beneficial effects on photosynthetic performance. The elevated SPAD readings did however not correlate with an elevated iron content in the total plant tissue and the differences in SPAD readings levelled out with leaf age.

Tissue metal concentrations were showing several interesting indications. While uptake of Fe, Cu, and Mn was in similar ranges, concentrations of nickel and Zn were elevated in the Control group. This might be related to the fact that an enhanced phytosiderophore exudation in the control group was needed to reach similar concentrations of Fe as in the treatments containing TETA.

The fact that the mean dry weight of the High TETA group was significantly larger than that of the Control group indicates an beneficial overall effect of TETA onto the plants growth. A possible lower Fe stress in the High TETA treatment might have led to increased biomass production in comparison to the Control group (Chapin, 1991), where increased energy investment might have been needed by PS production for Fe acquisition.

6.7 Future research and possible applications for TETA

The data presented here suggests beneficial effects of TETA onto iron acquisition in *Triticum aestivum*. However, future research is needed in order to clearly provide undisputable evidence for this effect. Several possible changes to the study conducted shall be discussed, in order to guide future studies.

The major drawback compromising the findings presented here, was that no visible chlorosis was present in the control groups. Santomera soil is a very challenging soil in terms of Fe availability (see paragraph 2.1). Wheat (*Triticum aestivum*) however, is a very Fe efficient species and the cultivar *Tamaro* has been used in past studies (Reichard et al., 2005) in order to "harvest" phytosiderophores for further research, due to its high exudation rates. Marschner et al. (1986) proposed the hierarchy of barley, (wheat, rye) > oats > maize > sorghum in terms of Fe efficient Strategy II plants. Selecting a

less Fe efficient cultivar might therefore result in more clearly distinguishable effects. A pre-screening with several cultivars and species might give a better feeling about the overall performance of the respective plant in Santomera soil and help to select a suitable candidate. Agricultural practise should not be neglected in this selecting a cultivar that is commonly grown under high calcareous, low Fe availability soils like oats (Eurostat, 2012).

Santomera is a soil of relatively low bio available iron and increased availability of copper. However the soil cannot be considered to be contaminated by Cu (Tóth et al., 2016). There has been evidence brought forward, showing decreasing Fe shoot concentrations with increasing copper concentrations in former vineyard soils (Chaignon et al., 2002; Michaud et al., 2007). In vineyards Cu-containing fungicides are used regularly, leading to possibly Cu contaminated, or significantly enriched soils (Michaud et al., 2007). Working with such a high Cu soil, might be an elegant solution to provide clear evidence for both, the impact of Cu on Fe acquisition by phytosiderophores and the effectiveness of TETA to decrease that competition.

To closer investigate the possibility of Cu deficiency caused by TETA application and to not ‘‘override’’ differences in plant metal uptake, the possibility of excluding foliar Cu fertilization should be considered.

The recovery of the plant digest was only around 80% for iron, making interpretation and reliability of the results obtained complicated. A vast amount of digestion methods can be found in literature. A digestion including HF eliminates the possibility of retention of any metals due to incorporation into silicates (Temminghoff & Houba, 2004). Therefore, this technique, even though associated with risks due to HF handling might be more desirable in future studies.

Special care has been taken to keep the soil water content constant and steady for all treatments. However, a watering system by irrigation wicks can rule out any water stress and reduces the operational effort significantly (Wenzel et al., 2001; Kuntz, 2013),

Agricultural practise on calcareous soils with low Fe plant availability is typically fertilization with Fe chelates like FeEDDHA (Schenkeveld, 2010). It has been demonstrated that copper is able to replace Fe from those chelate complexes (Schenkeveld et al., 2012). A combined application of TETA and FeEDDHA might therefore significantly increase the effectiveness of the iron fertilization, especially on high Cu calcareous soils (Chaignon et al., 2002). Additionally, comparison experiments with TETA and FeEDDHA in combination and alone might help to link an increase in growth and possible reduction of fertilizer application. A 6% increase in Biomass between the high TETA and Control group has been observed in this study. A longer experiment over the whole growing cycle might yield additional information about the possible benefits onto grain yield and help to estimate the economic benefits deriving from the application of TETA.

Rhizosphere research is an interdisciplinary field of study in which soil scientists, biogeochemists, microbiologists and plant physiologists are working. In order to evaluate the impact of TETA onto rhizosphere and plant an interdisciplinary approach might result in more in depth findings, additional research questions and a more holistic analysis as can be carried out by individual researchers. Rhizosphere research is in that respect a great example on future challenges, but also opportunities to develop and use a research network consisting of specialists from all fields of environmental sciences, contributing to find answers to challenging problems arising, as soil contamination and demands for nutritious food increase.

7 Conclusions

In calcareous soils, beneficial effects on plant iron acquisition are a story of competition. I tried to shed light on multiple aspects of Fe acquisition by plants, with special focus on graminaceous plants and phytosiderophores. It becomes apparent that iron acquisition by phytosiderophores is strongly driven by the kinetic nature of iron complexation and can be increased temporarily or on the long term to prove beneficial.

In chapter 3 it was shown that the interaction between the ligand DMA and Santomera soil mobilized the metals, iron, zinc, copper, nickel and cobalt. The mobilization of those metals was driven by kinetic and thermodynamic properties of the system. However, the amount of ligand supplied to the system did not immediately result in increased solution concentrations of individual and total metals. Findings were strengthened by the investigation of the metal mobilization in a higher soil to solution ratio, which yielded similar trends throughout. Competitive displacement was found to be the main driver of solution concentrations, under conditions where free ligand becomes scarce and copper was identified to be the main competitor on Fe acquisition by DMA from Santomera soil.

Competition on iron acquisition was also the repeating feature in chapter 4. Here the combined application of the reductant ascorbate and the phytosiderophore DMA showed to result in an initial synergistic effect on Fe mobilization. This effect may be of great environmental importance, as it elevated solution concentrations of Fe in the environmentally most relevant time frame – the first hours of interaction. In fact, it was even shown, that in this time frame a 'good' ratio of ascorbate and DMA was able to mobilize more Fe, than twice as much DMA applied alone. As exudation of phytosiderophores is associated with metabolic costs, this synergistic effect of reductant and phytosiderophore on Iron mobilization may be of great importance to a deficient plant.

However, competition affected Fe mobilization even stronger in combination with ascorbate, as mobilization of Ni and Co was synergistically elevated as well. The increased availability of those elements for complexation by DMA was attributed to the reductive dissolution of the soils Mn oxides. An adsorption isotherm for Mn^{2+} was used to conclude, that most ascorbate supplied, resulted in the reductive dissolution of Mn oxides. Copper showed to remain unaffected by the combination of ascorbate and DMA. Iron on the other hand was strongly affected by the increased competition of copper, cobalt and nickel for DMA, resulting in an antagonistic effect on solution concentrations over time. A repetitive feature identified throughout the thesis was reduced and earlier maximum solution concentrations of Fe as a result of increased competition for scarcer amounts of free ligand (DMA).

It was concluded that there is a delicate ratio dependency between the amount of reductant and the amount of DMA added, in order to profit Fe mobilization in Santomera soil most. As under Fe deficient conditions a whole variety of compounds, including potential reductants, are exuded (Fan et al., 2001), synergistic effects between compounds may be of great importance to iron acquisition and have until recently (Schenkeveld et al., 2016) not been investigated.

Under anoxic conditions similar trends as under oxic conditions were found, with Fe peak concentrations being compromised under absence of oxygen by increased competition. Again, this was shown by reduced and earlier peaks in Fe solution concentrations. The ratio between ligand and reductant found suitable under oxic condition, was not as effective under anoxic conditions, due to a strong synergistic effect on cobalt mobilization. Nevertheless, there was still an initial synergistic effect on Fe mobilization observed in most treatments (6.25µM DMA & var. ascorbate) under anoxic conditions.

Interestingly there were no significant differences on Mn mobilization apparent under anoxic conditions and no Fe was measured in solution in treatments containing only Ascorbate, indicating that most of the ascorbate supplied, affected the Mn oxides under both scenarios (oxic and anoxic) efficiently. A mechanism likely to cause the synergistic mobilization of Fe in this soil is the temporary reduction of the soil Fe(hydr)oxides and the reoxidation of the produced Fe^{2+} by reductive dissolution of Mn oxides, resulting in transient, poorly crystalline Fe(III)oxides, being more available for complexation, as well as the increased availability of Fe by release of in Mn oxides incorporated Fe.

More research is needed to understand the synergistic effect on Fe mobilization throughout, but this thesis contributed substantially by identifying a ratio dependency between ligand and reductant and displaying the kinetic nature of competition, affecting iron mobilization in the 'window of iron acquisition' of this soil. Additionally, it has been shown, that Fe and other metal mobilization by DMA is relatively unaffected by the presence or absence of oxygen in soil. This helps to understand natural conditions better, where anoxic (micro)sites in soil are frequent (Sposito, 2008).

In a scanning experiment plant borne compounds belonging to the cinammic acid family have been investigated for their potential to mobilize Fe alone and synergistically in combination with DMA. Again, competition was encountered when looking into synergistic effects of the respective compounds and DMA. The compounds scopoletin, esculetin, caffeic acid and fraxetin showed to synergistically elevate solution concentrations of Ni and in particular Co in both time points investigated. Only fraxetin showed a synergistic effect on Fe mobilization in one time point. Mn mobilization was, as before, found to be correlated with the mobilization of Ni and Co. Fraxetin mobilized significantly less Mn in the time point, where the synergistic effect on Fe could be determined, indicating slower reduction kinetics on Mn oxides, than the other compounds tested. This led to the conclusion, that the chosen ratio between ligand (DMA) and reductant (cinammic acid compound) was not ideal and a synergistic effect on Fe mobilization between the compounds tested and DMA is very likely to occur, when reducing the amount of cinammic acid compound. As it appears now, possible synergistic effects on Fe mobilization were outcompeted by Ni and Co in most treatments.

Furthermore, scopoletin and fraxetin alone mobilized iron from Santomera soil. This is to the authors knowledge the first time those compounds have been shown to mobilize Fe from soil. A possible mechanism causing this was hypothesized to be the oxidation and consecutive demethylation leading to an oxidation product with two accessible hydroxy groups and subsequent chelation of Fe.

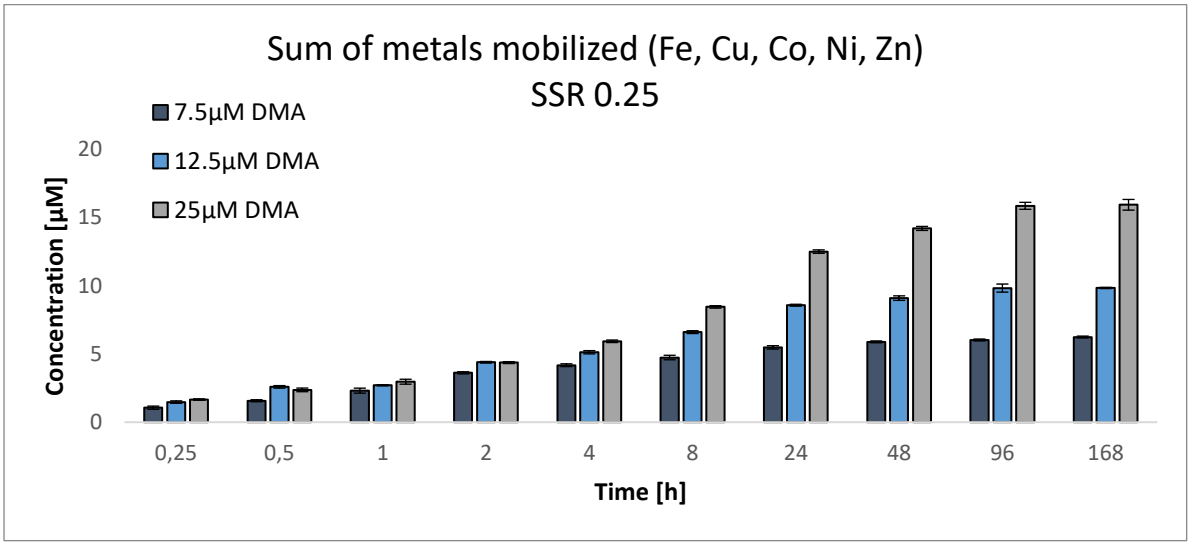
Up until chapter 6 beneficial effects have been investigated in batch experiments by increasing the amount of DMA supplied and by investigating synergistic effects between DMA and reducing agents. Another approach was tested in a pot trial where the availability of the main competitor for iron complexation by DMA – copper - was reduced. Indications for a beneficial effect of the copper specific chelating agent TETA on the cultivar *Triticum aestivum sp. Tamaro* were observed. Plants grown on Santomera soil treated with TETA showed to have increased biomass, and SPAD readings indicated higher chlorophyll contents in the youngest leaves. Tissue metal concentrations provided some evidence for decreased phytosiderophore exudation in the TETA treatment group. No negative effects of the compound TETA on plant health were observed. Possible implications of the findings presented here may be of particular relevance for fertilizer development and provide a new, more holistic approach on tackling plant nutrient demands by taking the rhizosphere biogeochemistry more into account. More research is however needed to clearly resolve the potential extent of the beneficial effect. Ideas for future research have been proposed in this work.

8 Supporting Information

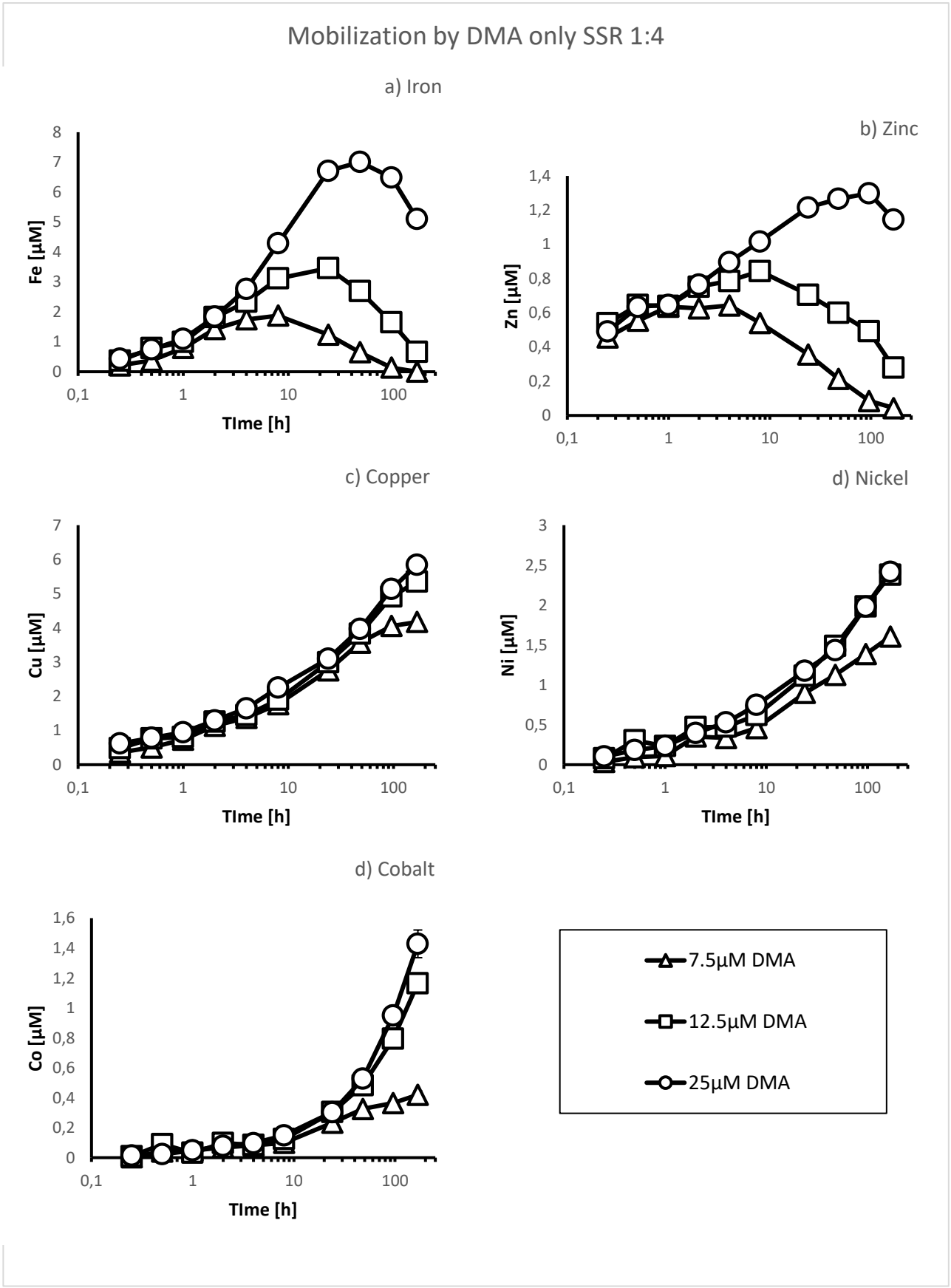
8.1 Supporting Figures and Tables

Table of SI Figures

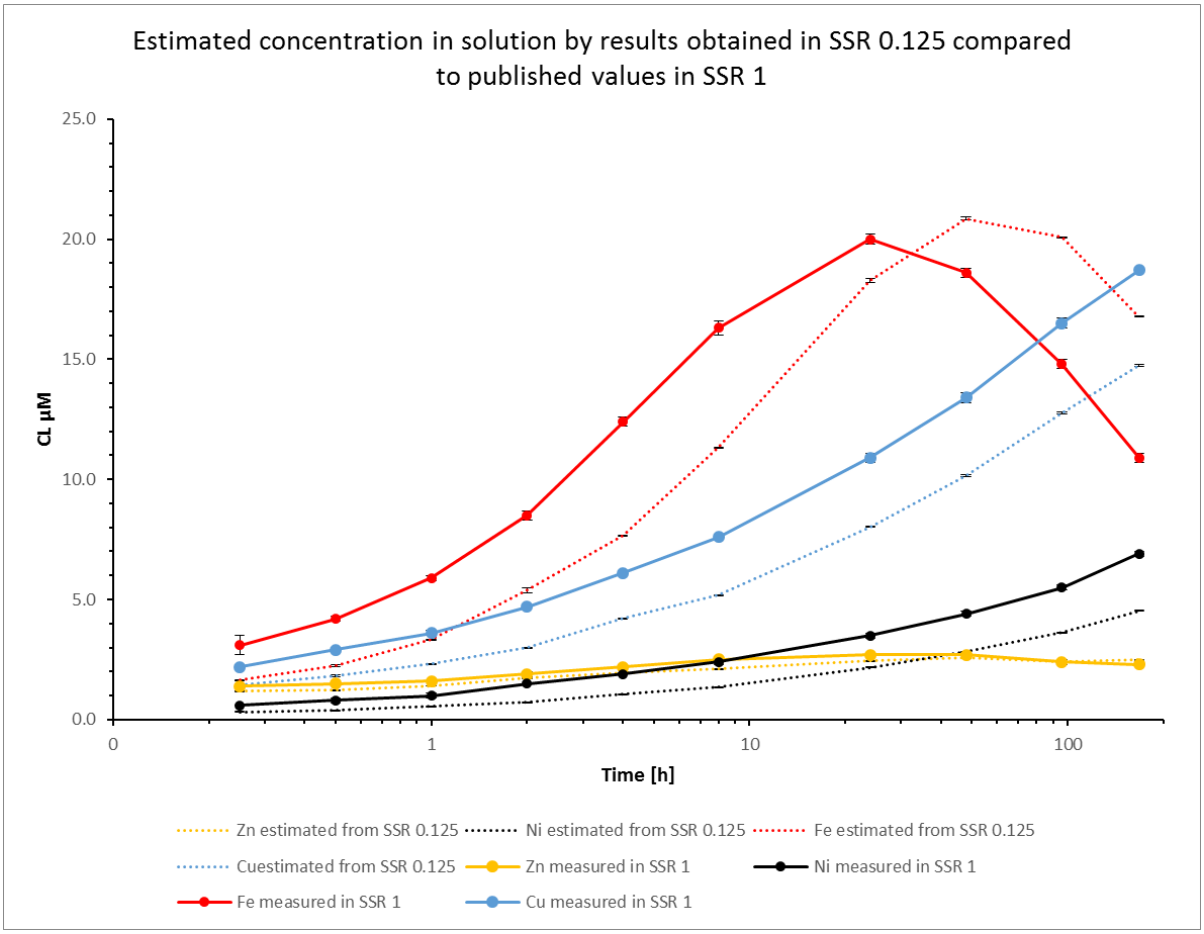
Table of SI Tables



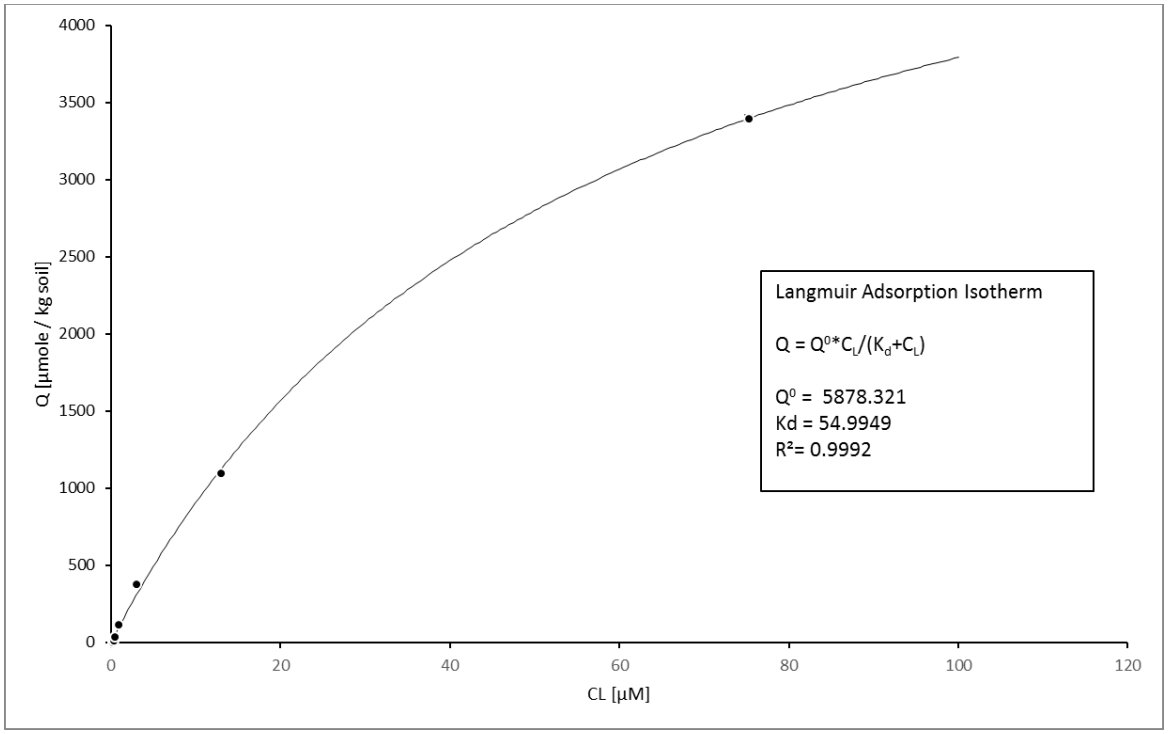
SI Figure 1: Total metals mobilized by DMA in a soil to solution ratio of 1:4;Error bars indicate standard deviation (n=2) Error bars indicate sum of Standard deviation; SSR 0.25; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)



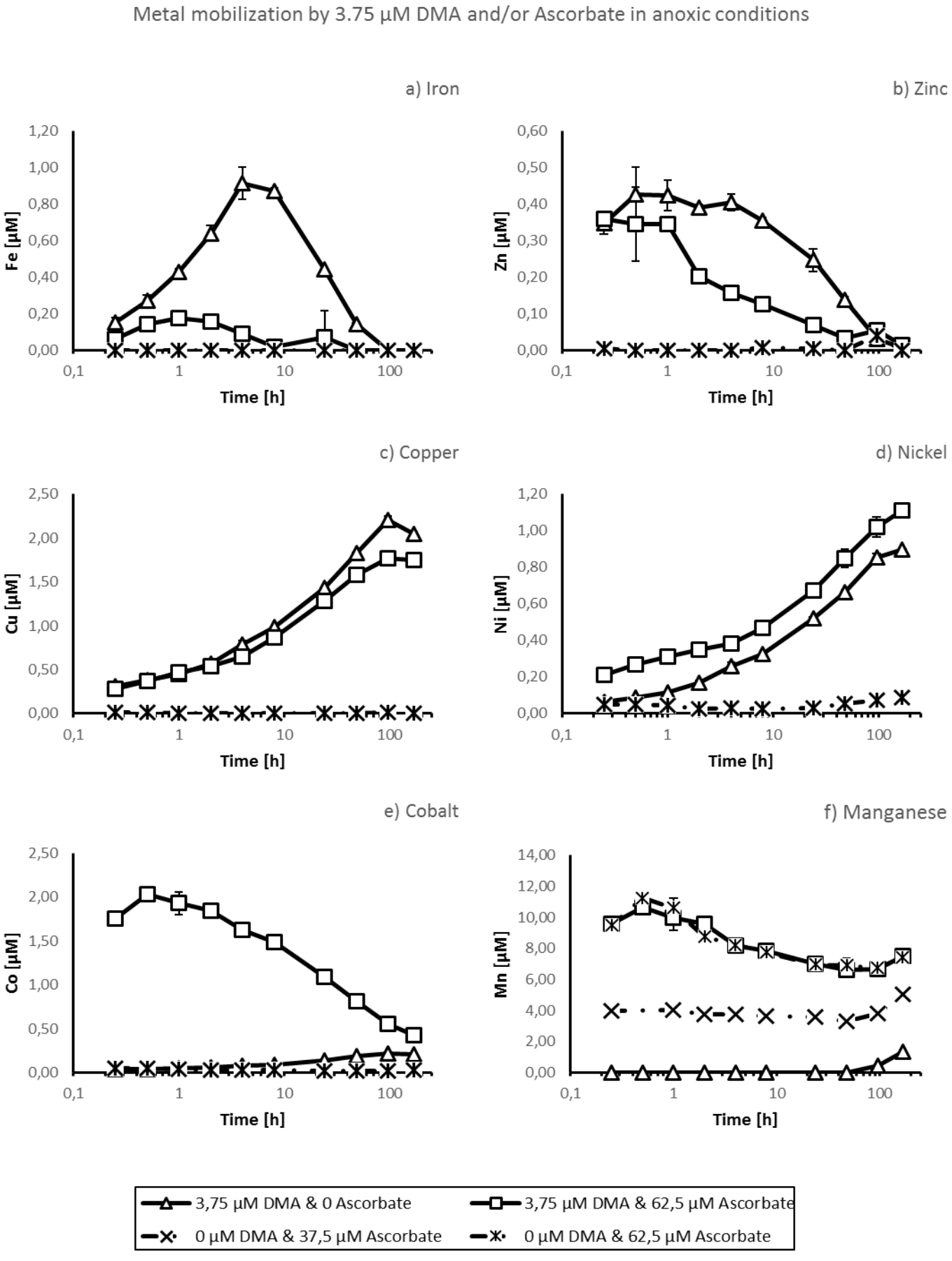
SI Figure 2: Individual metals mobilized SSR 1:4; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)



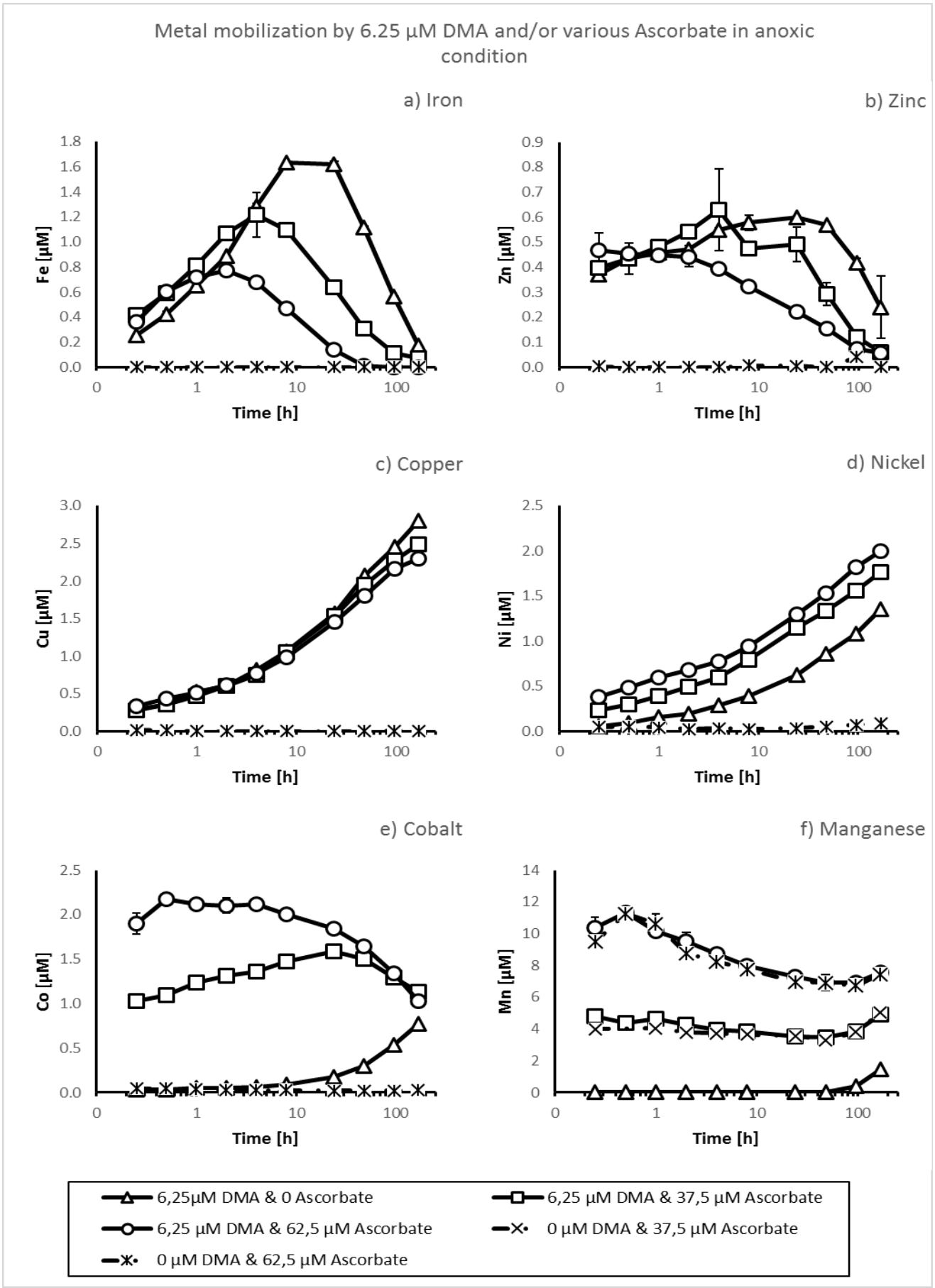
SI Figure 3: Comparison of CL concentrations mobilized by DMA based on experimental data presented in paragraph 3.1 and values obtained by Schenkeveld et al. (2016b) in a SSR of 1; SSR experimental data = 0.125; error bars indicate measured standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant) for both treatments



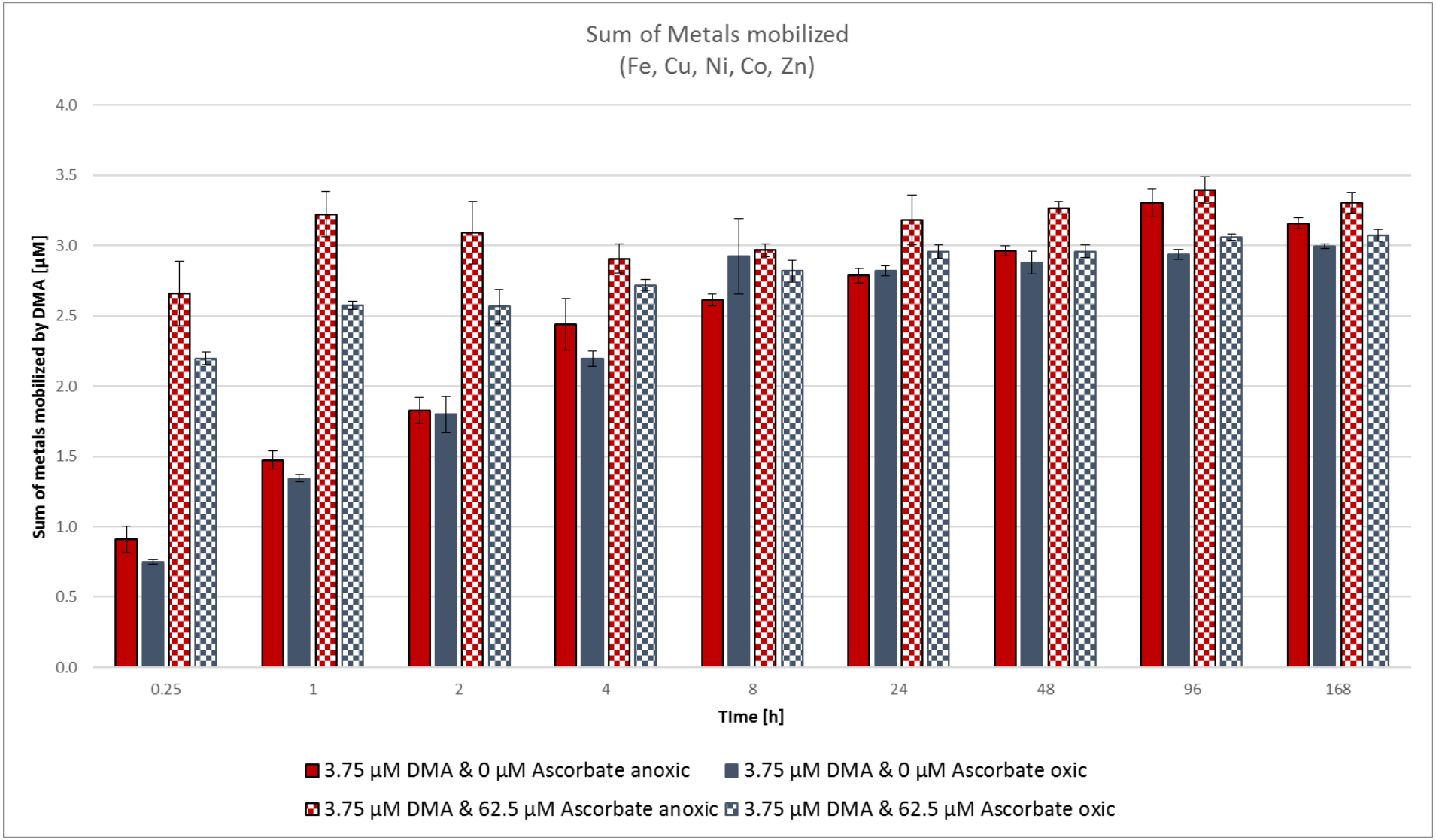
SI Figure 4: Adsorption Isotherm of Mn^{2+} to Santomera soil; error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; t of equilibration: 4 hours; conducted under anoxic conditions



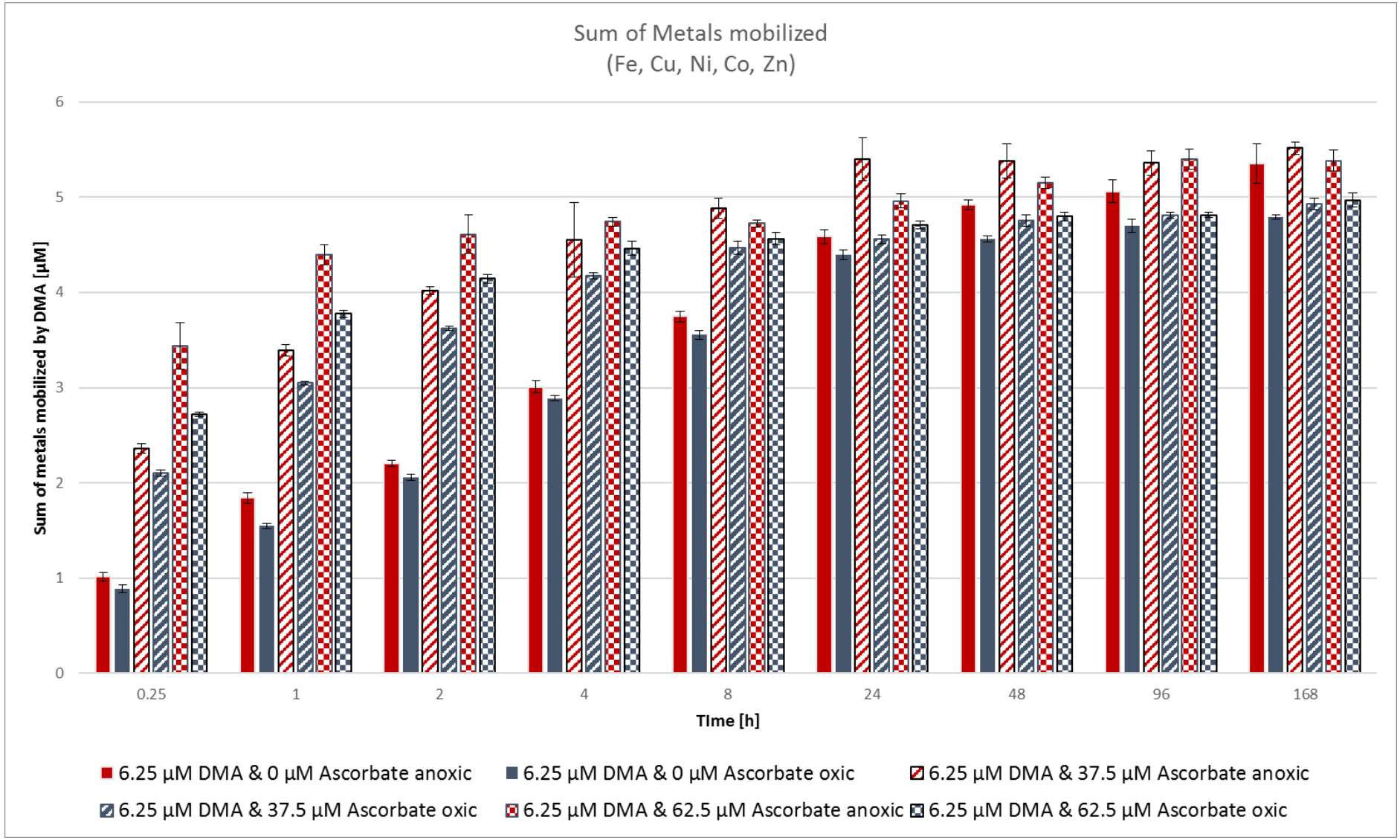
SI Figure 5 Metal mobilization by 3.75µM DMA and/or ascorbate under anoxic conditions in Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)



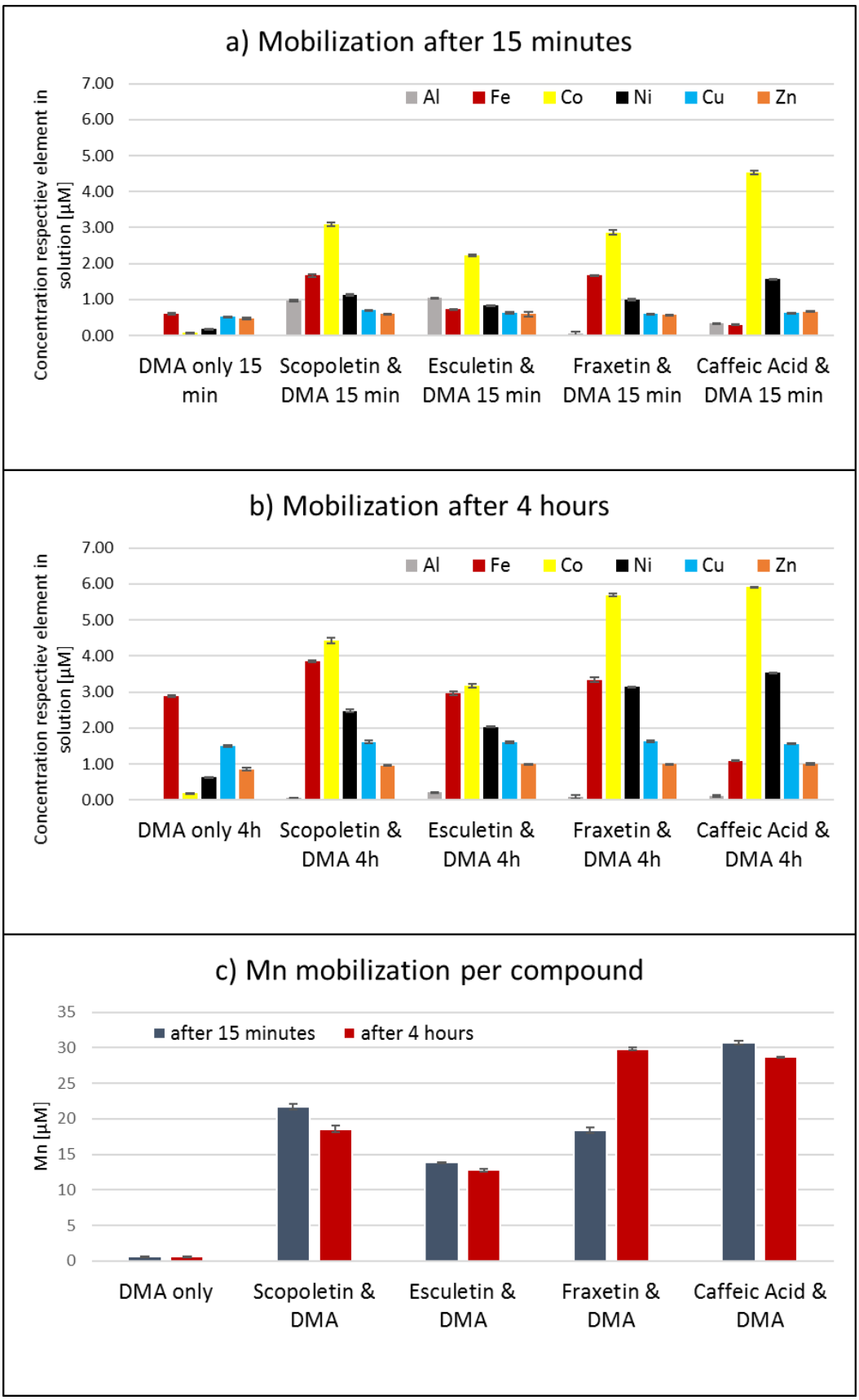
SI Figure 6: Metal mobilization by 6.25µM DMA and/or ascorbate under anoxic conditions in Santomera soil; SSR = 0.125; Error bars indicate standard deviation (n=2); 10mM CaCl2 as background electrolyte; 0.2g/L Bronopol (steriliant)



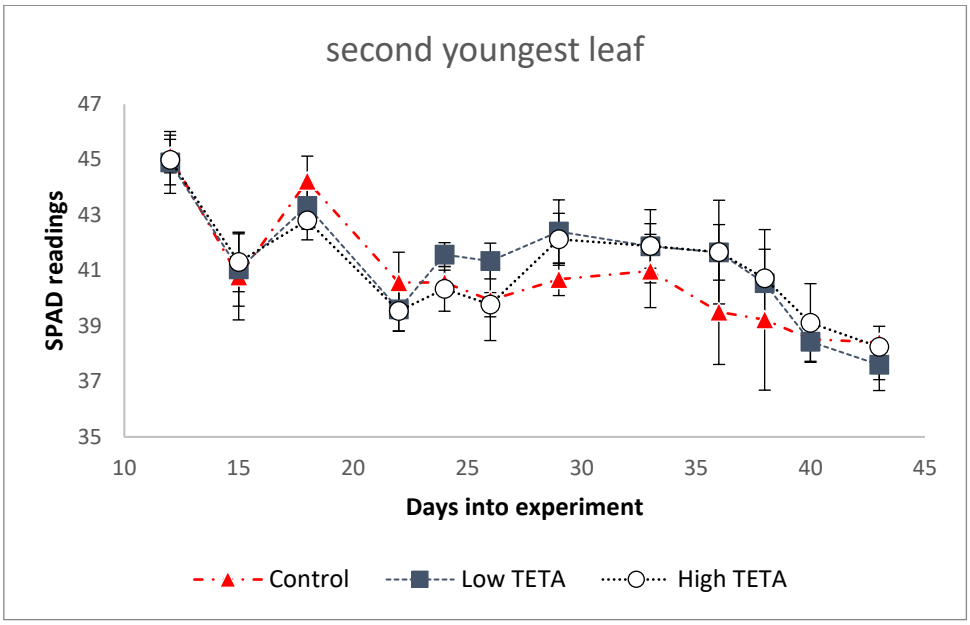
SI Figure 7:Sum of metals mobilized by 3.75µM DMA with and without ascorbate in the presence and absence of oxygen; Error bars indicate sum of standard deviation respective metals (n=2); SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)



SI Figure 8: Sum of metals mobilized by 6.25µM DMA with and without ascorbate in the presence and absence of oxygen; Error bars indicate sum of standard deviation respective metals (n=2) SSR = 0.125; 10mM $CaCl_2$ as background electrolyte; 0.2g/L Bronopol (steriliant)

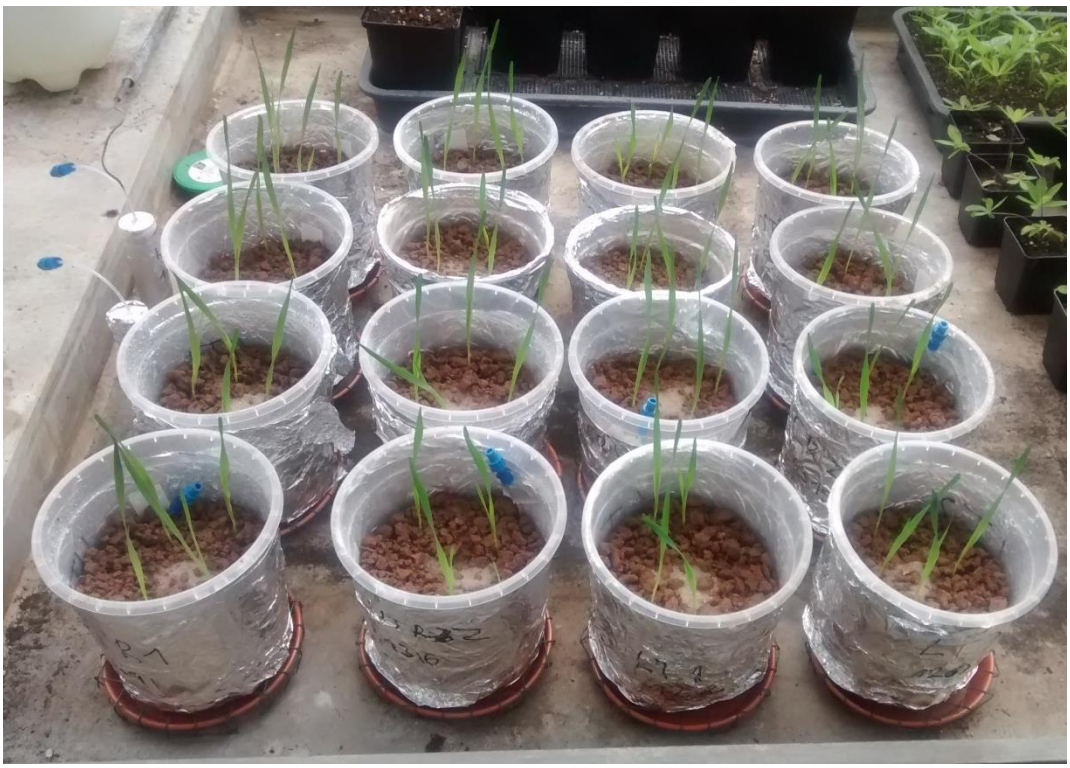


SI Figure 9: Mobilization of Fe, Co, Ni, Cu and Zn by DMA alone and in combined treatments after 15 minutes interaction time (a); after 4 hours interaction time (b) and Mn mobilization after 15 minutes and 4 hours (c); error bars indicate standard deviations (n=2); SSR 0.25; amount of compound supplied: 250µM; amount of DMA supplied: 25µM; 10mM $CaCl_2$ as background electrolyte

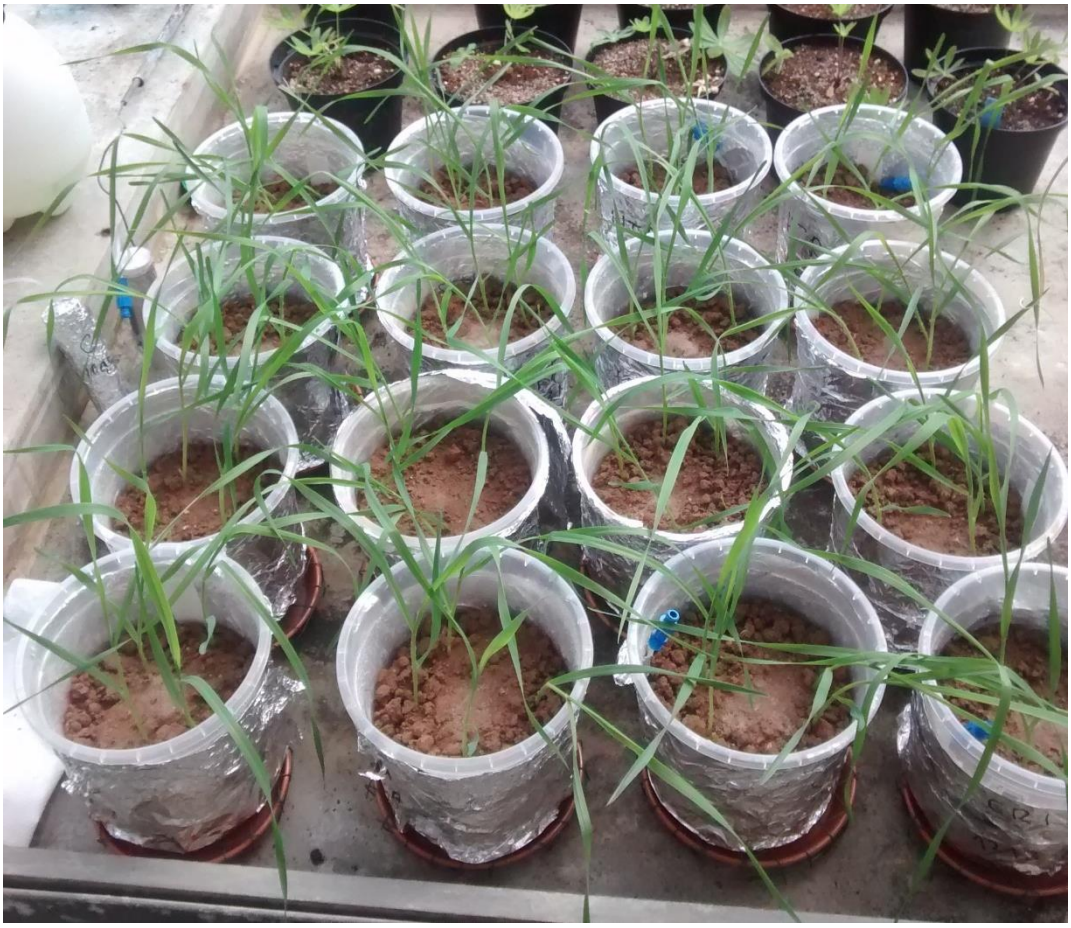


SI Figure 10: Mean SPAD readings of the second youngest leaf per treatment; Measurements per pot = 16;; pots per treatment = 4; Error bars indicate Standard deviation of means per pot

SI Figure 11: Pot trial; 4 days into experiment

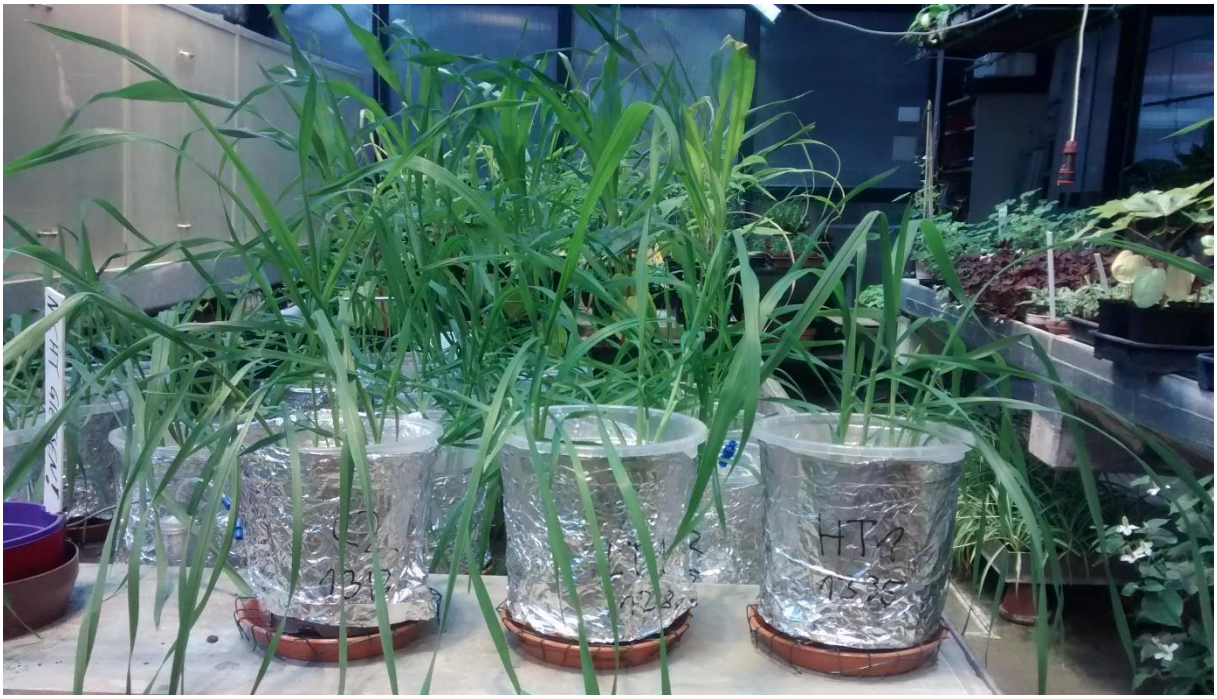


SI Figure 12: Pot trial; 7 days into experiment

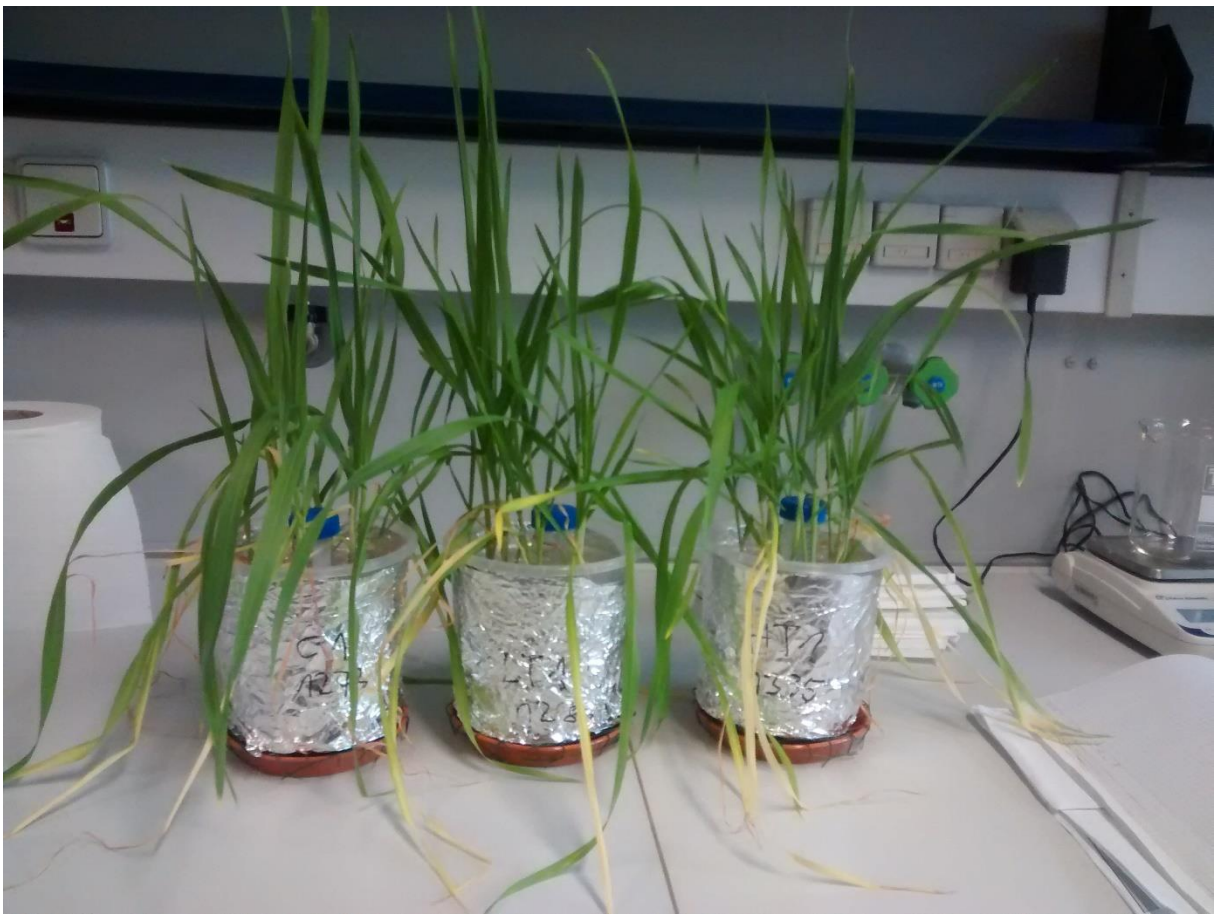


SI Figure 13: Pot trial; 15 days into experiment

SI Figure 14: Pot trial; 22 days into experiment



SI Figure 15: Selected pots after 29 days into experiment; From left to right: Control, Low TETA, High TETA



SI Figure 16: Selected pots at harvest after 40 days into experiment; From left to right: Control, Low TETA, High TETA

SI Table 1: Results Experiment 1 ''presence of oxygen''; ICP OES; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2

DMA [µM]	Ascorbate [mM]	Time [h]	Zn [µM]	Ni [µM]	Fe [µM]	Mn [µM]	Co [µM]	Cu [µM]	Zn STDV	Ni STDV	Fe STDV	Mn STDV	Co STDV	Cu STDV
0	0	1	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.05	0.01	0.00
0	0	8	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01
0	0	24	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02
0	0	168	0.00	0.02	0.00	0.78	0.01	0.00	0.00	0.00	0.01	0.04	0.01	0.01
0	0.0375	1	0.00	0.02	0.00	4.35	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.01
0	0.0375	8	0.00	0.00	0.01	3.48	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.00
0	0.0375	24	0.00	0.01	0.00	3.65	0.01	0.00	0.01	0.01	0.00	0.06	0.00	0.01
0	0.0375	168	0.00	0.04	0.00	4.08	0.02	0.01	0.00	0.00	0.00	0.07	0.00	0.01
0	0.125	1	0.01	0.06	0.01	18.20	0.06	0.01	0.01	0.00	0.01	0.13	0.00	0.00
0	0.125	8	0.00	0.05	0.02	14.66	0.05	0.00	0.01	0.01	0.01	0.22	0.00	0.00
0	0.125	24	0.01	0.03	0.02	14.81	0.04	0.00	0.00	0.01	0.00	0.09	0.00	0.02
0	0.125	168	0.00	0.06	0.00	11.48	0.04	0.00	0.00	0.00	0.00	0.17	0.01	0.00
12.5	0	0.25	0.28	0.09	0.33	0.00	0.04	0.27	0.00	0.01	0.01	0.00	0.00	0.01
12.5	0	0.5	0.29	0.10	0.44	0.00	0.03	0.34	0.02	0.01	0.04	0.13	0.00	0.04
12.5	0	1	0.34	0.15	0.65	0.00	0.05	0.44	0.00	0.00	0.00	0.00	0.01	0.00
12.5	0	2	0.41	0.19	1.06	0.00	0.06	0.56	0.02	0.01	0.11	0.01	0.00	0.01
12.5	0	4	0.46	0.28	1.50	0.00	0.06	0.79	0.01	0.00	0.01	0.01	0.01	0.03
12.5	0	8	0.50	0.36	2.23	0.00	0.08	0.97	0.00	0.00	0.01	0.01	0.01	0.00
12.5	0	24	0.58	0.58	3.59	0.00	0.16	1.50	0.01	0.02	0.08	0.03	0.01	0.02
12.5	0	48	0.61	0.75	4.09	0.09	0.28	1.90	0.02	0.01	0.06	0.04	0.01	0.03
12.5	0	96	0.57	0.96	3.94	0.35	0.42	2.39	0.03	0.00	0.02	0.01	0.02	0.04
12.5	0	168	0.59	1.20	3.29	0.67	0.65	2.76	0.00	0.02	0.02	0.03	0.03	0.04
12.5	0.0375	0.25	0.30	0.29	0.70	5.04	0.81	0.30	0.01	0.00	0.00	0.02	0.00	0.01
12.5	0.0375	0.5	0.31	0.30	0.87	4.30	0.77	0.36	0.01	0.01	0.01	0.03	0.02	0.02
12.5	0.0375	1	0.36	0.40	1.20	4.50	0.88	0.44	0.01	0.01	0.01	0.02	0.00	0.01
12.5	0.0375	2	0.40	0.47	1.62	4.18	0.94	0.57	0.00	0.01	0.02	0.03	0.00	0.00
12.5	0.0375	4	0.45	0.59	2.07	4.00	1.03	0.75	0.01	0.01	0.00	0.09	0.01	0.01
12.5	0.0375	8	0.49	0.72	2.72	3.67	1.08	0.95	0.01	0.01	0.02	0.06	0.00	0.01
12.5	0.0375	24	0.59	1.06	3.40	3.65	1.32	1.50	0.01	0.00	0.00	0.06	0.01	0.01
12.5	0.0375	48	0.60	1.26	3.22	3.63	1.42	1.88	0.02	0.01	0.04	0.03	0.01	0.05
12.5	0.0375	96	0.55	1.51	2.72	3.44	1.57	2.28	0.01	0.01	0.03	0.09	0.02	0.02
12.5	0.0375	168	0.51	1.77	2.24	3.94	1.77	2.70	0.01	0.02	0.01	0.16	0.01	0.01
12.5	0.125	0.25	0.37	0.68	0.90	19.60	2.31	0.32	0.00	0.01	0.00	0.26	0.04	0.01
12.5	0.125	0.5	0.38	0.74	1.25	16.98	2.16	0.38	0.00	0.01	0.03	0.30	0.02	0.00
12.5	0.125	1	0.42	0.91	1.64	17.00	2.31	0.44	0.00	0.01	0.03	0.12	0.01	0.00
12.5	0.125	2	0.43	0.98	1.78	16.32	2.40	0.58	0.01	0.00	0.04	0.29	0.02	0.00

12.5	0.125	4	0.43	1.17	1.99	16.31	2.50	0.75	0.01	0.00	0.00	0.03	0.00	0.00
12.5	0.125	8	0.42	1.34	1.81	14.96	2.48	0.92	0.01	0.01	0.03	0.17	0.01	0.02
12.5	0.125	24	0.52	1.97	1.87	14.50	2.77	1.42	0.01	0.01	0.01	0.06	0.00	0.00
12.5	0.125	48	0.48	2.21	1.58	14.02	2.73	1.78	0.03	0.03	0.01	0.24	0.02	0.01
12.5	0.125	96	0.43	2.53	1.12	12.32	2.73	2.27	0.00	0.01	0.01	0.00	0.00	0.01
12.5	0.125	168	0.37	2.82	0.86	11.84	2.75	2.67	0.01	0.08	0.02	0.58	0.03	0.03
0	0	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.01	0.00
0	0	8	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.00	0.02
0	0	24	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00
0	0	168	0.00	0.00	0.00	0.69	0.01	0.01	0.00	0.01	0.01	0.02	0.01	0.00
3.75	0	0.25	0.25	0.05	0.21	0.00	0.04	0.20	0.00	0.01	0.01	0.01	0.00	0.00
3.75	0	0.42	0.32	0.07	0.28	0.00	0.04	0.24	0.05	0.00	0.00	0.01	0.02	0.00
3.75	0	1	0.32	0.09	0.51	0.00	0.05	0.38	0.01	0.00	0.01	0.01	0.00	0.00
3.75	0	2	0.33	0.15	0.78	0.00	0.06	0.49	0.02	0.00	0.09	0.02	0.01	0.01
3.75	0	4	0.32	0.20	0.95	0.00	0.07	0.65	0.01	0.01	0.02	0.00	0.01	0.01
3.75	0	8	0.30	0.33	1.26	0.00	0.09	0.95	0.04	0.06	0.11	0.08	0.01	0.05
3.75	0	24	0.17	0.41	0.82	0.00	0.11	1.31	0.00	0.00	0.03	0.00	0.00	0.00
3.75	0	48	0.13	0.48	0.50	0.00	0.15	1.61	0.01	0.01	0.03	0.02	0.02	0.01
3.75	0	96	0.06	0.60	0.21	0.26	0.15	1.91	0.00	0.01	0.00	0.01	0.01	0.01
3.75	0	168	0.03	0.70	0.07	0.68	0.15	2.05	0.00	0.00	0.00	0.06	0.00	0.00
3.75	0.0375	0.25	0.26	0.13	0.30	4.29	0.79	0.21	0.01	0.01	0.00	0.05	0.02	0.00
3.75	0.0375	0.42	0.29	0.17	0.35	4.17	0.83	0.25	0.00	0.00	0.01	0.02	0.02	0.00
3.75	0.0375	1	0.28	0.20	0.55	4.65	0.95	0.36	0.00	0.00	0.00	0.02	0.00	0.00
3.75	0.0375	2	0.24	0.24	0.61	4.53	0.94	0.45	0.01	0.00	0.01	0.00	0.01	0.01
3.75	0.0375	4	0.18	0.30	0.66	4.58	0.91	0.61	0.01	0.01	0.02	0.08	0.02	0.01
3.75	0.0375	8	0.15	0.41	0.59	4.56	0.87	0.78	0.00	0.01	0.01	0.00	0.02	0.04
3.75	0.0375	24	0.09	0.55	0.32	4.11	0.73	1.21	0.00	0.00	0.00	0.02	0.01	0.01
3.75	0.0375	48	0.06	0.64	0.16	3.81	0.56	1.49	0.00	0.04	0.00	0.02	0.05	0.02
3.75	0.0375	96	0.03	0.77	0.07	3.84	0.42	1.72	0.00	0.00	0.00	0.06	0.01	0.00
3.75	0.0375	168	0.02	0.85	0.02	4.01	0.35	1.80	0.00	0.01	0.00	0.03	0.01	0.01
3.75	0.0625	0.25	0.27	0.17	0.27	8.51	1.27	0.22	0.00	0.01	0.03	0.07	0.01	0.01
3.75	0.0625	0.42	0.25	0.20	0.30	8.53	1.30	0.25	0.00	0.01	0.01	0.01	0.00	0.00
3.75	0.0625	1	0.22	0.23	0.41	8.82	1.38	0.34	0.01	0.00	0.01	0.22	0.00	0.01
3.75	0.0625	2	0.15	0.28	0.39	9.23	1.30	0.45	0.01	0.03	0.03	0.20	0.02	0.04
3.75	0.0625	4	0.13	0.35	0.44	9.02	1.23	0.57	0.00	0.01	0.01	0.14	0.01	0.00
3.75	0.0625	8	0.14	0.42	0.36	8.63	1.14	0.76	0.03	0.02	0.01	0.03	0.01	0.01
3.75	0.0625	24	0.07	0.61	0.21	7.84	0.90	1.17	0.00	0.02	0.00	0.07	0.02	0.01
3.75	0.0625	48	0.05	0.68	0.11	7.09	0.66	1.46	0.00	0.02	0.00	0.05	0.00	0.02
3.75	0.0625	96	0.04	0.84	0.05	6.86	0.47	1.67	0.01	0.00	0.01	0.02	0.00	0.00

3.75	0.0625	168	0.01	0.93	0.02	6.56	0.39	1.71	0.00	0.01	0.00	0.12	0.01	0.01
6.25	0	0.25	0.28	0.06	0.27	0.00	0.02	0.25	0.01	0.01	0.01	0.01	0.00	0.01
6.25	0	0.42	0.27	0.10	0.33	0.00	0.04	0.30	0.00	0.00	0.00	0.02	0.01	0.02
6.25	0	1	0.35	0.12	0.62	0.00	0.04	0.41	0.00	0.00	0.02	0.00	0.00	0.00
6.25	0	2	0.40	0.20	0.85	0.00	0.05	0.55	0.01	0.00	0.01	0.00	0.00	0.01
6.25	0	4	0.45	0.27	1.33	0.00	0.08	0.76	0.01	0.00	0.01	0.01	0.01	0.00
6.25	0	8	0.43	0.37	1.75	0.00	0.07	0.94	0.01	0.01	0.02	0.00	0.00	0.00
6.25	0	24	0.36	0.53	1.93	0.00	0.15	1.43	0.01	0.02	0.01	0.01	0.01	0.00
6.25	0	48	0.31	0.69	1.52	0.03	0.22	1.82	0.00	0.01	0.00	0.03	0.01	0.00
6.25	0	96	0.25	0.87	1.03	0.33	0.32	2.22	0.02	0.01	0.03	0.04	0.01	0.02
6.25	0	168	0.18	1.06	0.58	0.68	0.44	2.53	0.00	0.00	0.01	0.01	0.00	0.01
6.25	0.0375	0.25	0.32	0.21	0.52	4.61	0.80	0.26	0.02	0.00	0.00	0.08	0.00	0.01
6.25	0.0375	0.42	0.31	0.24	0.56	4.43	0.84	0.29	0.01	0.00	0.00	0.00	0.00	0.00
6.25	0.0375	1	0.37	0.34	0.97	4.80	0.97	0.41	0.00	0.01	0.00	0.10	0.01	0.00
6.25	0.0375	2	0.42	0.43	1.18	4.71	1.07	0.53	0.00	0.00	0.00	0.05	0.02	0.00
6.25	0.0375	4	0.38	0.53	1.43	4.62	1.13	0.71	0.01	0.01	0.01	0.01	0.00	0.00
6.25	0.0375	8	0.33	0.63	1.43	4.46	1.13	0.95	0.01	0.01	0.01	0.01	0.02	0.03
6.25	0.0375	24	0.27	0.86	0.97	4.09	1.14	1.33	0.00	0.01	0.01	0.01	0.02	0.01
6.25	0.0375	48	0.22	1.06	0.68	3.90	1.07	1.72	0.00	0.02	0.01	0.10	0.01	0.03
6.25	0.0375	96	0.13	1.24	0.39	3.90	1.00	2.03	0.00	0.01	0.00	0.02	0.00	0.01
6.25	0.0375	168	0.08	1.43	0.19	4.08	0.92	2.31	0.00	0.00	0.01	0.03	0.02	0.03
6.25	0.0625	0.25	0.30	0.29	0.54	8.75	1.33	0.25	0.00	0.01	0.00	0.03	0.00	0.00
6.25	0.0625	0.42	0.34	0.34	0.62	8.62	1.37	0.30	0.00	0.02	0.02	0.02	0.00	0.01
6.25	0.0625	1	0.37	0.43	0.99	9.06	1.57	0.41	0.01	0.00	0.02	0.08	0.01	0.01
6.25	0.0625	2	0.37	0.54	1.11	9.03	1.61	0.51	0.02	0.00	0.02	0.05	0.00	0.01
6.25	0.0625	4	0.29	0.67	1.16	9.16	1.64	0.71	0.01	0.03	0.02	0.35	0.00	0.02
6.25	0.0625	8	0.27	0.78	1.03	8.49	1.60	0.88	0.00	0.02	0.01	0.04	0.03	0.01
6.25	0.0625	24	0.21	1.04	0.63	7.79	1.52	1.31	0.00	0.01	0.02	0.02	0.00	0.01
6.25	0.0625	48	0.17	1.24	0.36	7.06	1.38	1.64	0.02	0.01	0.01	0.01	0.00	0.01
6.25	0.0625	96	0.10	1.41	0.20	6.55	1.15	1.95	0.00	0.01	0.01	0.13	0.01	0.00
6.25	0.0625	168	0.05	1.61	0.10	6.50	0.97	2.24	0.00	0.01	0.01	0.01	0.02	0.03

*SI Table 2: **Mass balance** metal mobilization by DMA with and without Asc. (Exp. 1); CT calculated according to Walter et al. (2016); Adsorption isotherm of CoDMA assumed to be 0.2* CL; Incomplete desorption of FeDMA complex from Santomera soil accounted for in ''Fe corr.'' (0.7 * CT max – CT); Values presented are based on blank corrected values presented in SI Table 1*

Time [h]	DMA [µM]	Asc. [mM]	Zn [µM] CT	Ni [µM] CT	Fe [µM] CT	Fe corr.	Co [µM] CT	Cu [µM] CT	TOTAL	% of LIGAND
0.25	12.5	0	0.3	0.1	0.4	-	0.0	0.3	1.1	9%
0.5	12.5	0	0.3	0.1	0.5	-	0.0	0.4	1.3	11%
1	12.5	0	0.4	0.2	0.7	-	0.1	0.5	1.8	14%
2	12.5	0	0.5	0.2	1.2	-	0.1	0.6	2.5	20%
4	12.5	0	0.5	0.3	1.6	-	0.1	0.8	3.4	27%
8	12.5	0	0.6	0.4	2.4	-	0.1	1.0	4.5	36%
24	12.5	0	0.7	0.7	3.9	-	0.2	1.6	7.0	56%
48	12.5	0	0.7	0.9	4.5	-	0.3	2.0	8.4	67%
96	12.5	0	0.6	1.1	4.3	0.1	0.4	2.6	9.2	73%
168	12.5	0	0.7	1.4	3.6	0.6	0.7	3.0	9.9	79%
0.25	12.5	0.0375	0.3	0.3	0.8	-	0.8	0.3	2.6	21%
0.5	12.5	0.0375	0.4	0.4	0.9	-	0.8	0.4	2.8	23%
1	12.5	0.0375	0.4	0.5	1.3	-	0.9	0.5	3.6	28%
2	12.5	0.0375	0.5	0.6	1.8	-	1.0	0.6	4.3	35%
4	12.5	0.0375	0.5	0.7	2.3	-	1.1	0.8	5.3	43%
8	12.5	0.0375	0.6	0.8	3.0	-	1.1	1.0	6.5	52%
24	12.5	0.0375	0.7	1.2	3.7	-	1.4	1.6	8.6	69%
48	12.5	0.0375	0.7	1.5	3.5	0.1	1.5	2.0	9.3	74%
96	12.5	0.0375	0.6	1.8	3.0	0.5	1.6	2.5	9.9	79%
168	12.5	0.0375	0.6	2.1	2.4	0.9	1.8	2.9	10.7	86%
0.25	12.5	0.125	0.4	0.8	1.0	-	2.4	0.3	4.9	39%
0.5	12.5	0.125	0.4	0.9	1.4	-	2.2	0.4	5.3	42%
1	12.5	0.125	0.5	1.1	1.8	-	2.4	0.5	6.2	49%
2	12.5	0.125	0.5	1.1	1.9	-	2.5	0.6	6.7	53%
4	12.5	0.125	0.5	1.4	2.2	-	2.6	0.8	7.4	59%
8	12.5	0.125	0.5	1.6	2.0	0.1	2.5	1.0	7.7	61%
24	12.5	0.125	0.6	2.3	2.0	0.1	2.8	1.5	9.4	75%
48	12.5	0.125	0.5	2.6	1.7	0.3	2.8	1.9	9.9	79%
96	12.5	0.125	0.5	3.0	1.2	0.7	2.8	2.4	10.6	85%
168	12.5	0.125	0.4	3.3	0.9	0.9	2.8	2.9	11.2	90%
0.25	3.75	0	0.3	0.1	0.2	-	0.0	0.2	0.8	22%
0.42	3.75	0	0.4	0.1	0.3	-	0.0	0.3	1.0	28%
1	3.75	0	0.4	0.1	0.6	-	0.0	0.4	1.5	40%
2	3.75	0	0.4	0.2	0.8	-	0.1	0.5	2.0	53%
4	3.75	0	0.4	0.2	1.0	-	0.1	0.7	2.4	64%
8	3.75	0	0.3	0.4	1.4	-	0.1	1.0	3.2	86%
24	3.75	0	0.2	0.5	0.9	0.3	0.1	1.4	3.4	91%
48	3.75	0	0.2	0.6	0.5	0.6	0.2	1.7	3.7	99%
96	3.75	0	0.1	0.7	0.2	0.8	0.2	2.1	4.0	107%
168	3.75	0	0.0	0.8	0.1	0.9	0.2	2.2	4.2	112%
0.25	3.75	0.0375	0.3	0.2	0.3	-	0.8	0.2	1.8	48%
0.42	3.75	0.0375	0.3	0.2	0.4	-	0.9	0.3	2.0	54%
1	3.75	0.0375	0.3	0.2	0.6	-	1.0	0.4	2.5	67%
2	3.75	0.0375	0.3	0.3	0.7	-	1.0	0.5	2.7	71%

4	3.75	0.0375	0.2	0.4	0.7	-	0.9	0.7	2.9	77%
8	3.75	0.0375	0.2	0.5	0.6	0.1	0.9	0.8	3.1	82%
24	3.75	0.0375	0.1	0.6	0.4	0.3	0.7	1.3	3.4	91%
48	3.75	0.0375	0.1	0.7	0.2	0.4	0.6	1.6	3.5	95%
96	3.75	0.0375	0.0	0.9	0.1	0.4	0.4	1.8	3.7	100%
168	3.75	0.0375	0.0	1.0	0.0	0.5	0.4	1.9	3.8	102%
0.25	3.75	0.0625	0.3	0.2	0.3	-	1.3	0.2	2.3	62%
0.42	3.75	0.0625	0.3	0.2	0.3	-	1.3	0.3	2.5	65%
1	3.75	0.0625	0.3	0.3	0.4	-	1.4	0.4	2.7	73%
2	3.75	0.0625	0.2	0.3	0.4	-	1.3	0.5	2.7	73%
4	3.75	0.0625	0.1	0.4	0.5	-	1.3	0.6	2.9	78%
8	3.75	0.0625	0.2	0.5	0.4	0.1	1.2	0.8	3.1	82%
24	3.75	0.0625	0.1	0.7	0.2	0.2	0.9	1.3	3.4	90%
48	3.75	0.0625	0.1	0.8	0.1	0.3	0.7	1.6	3.5	93%
96	3.75	0.0625	0.0	1.0	0.1	0.3	0.5	1.8	3.6	97%
168	3.75	0.0625	0.0	1.1	0.0	0.3	0.4	1.8	3.7	98%
0.25	6.25	0	0.3	0.1	0.3	-	0.0	0.3	1.0	16%
0.42	6.25	0	0.3	0.1	0.4	-	0.0	0.3	1.2	18%
1	6.25	0	0.4	0.1	0.7	-	0.0	0.4	1.7	27%
2	6.25	0	0.5	0.2	0.9	-	0.1	0.6	2.3	36%
4	6.25	0	0.5	0.3	1.4	-	0.1	0.8	3.2	51%
8	6.25	0	0.5	0.4	1.9	-	0.1	1.0	3.9	63%
24	6.25	0	0.4	0.6	2.1	-	0.2	1.5	4.8	77%
48	6.25	0	0.4	0.8	1.7	0.3	0.2	2.0	5.3	85%
96	6.25	0	0.3	1.0	1.1	0.7	0.3	2.4	5.8	93%
168	6.25	0	0.2	1.2	0.6	1.0	0.5	2.7	6.3	100%
0.25	6.25	0.0375	0.4	0.2	0.6	-	0.8	0.3	2.3	36%
0.42	6.25	0.0375	0.4	0.3	0.6	-	0.9	0.3	2.4	39%
1	6.25	0.0375	0.4	0.4	1.1	-	1.0	0.4	3.3	53%
2	6.25	0.0375	0.5	0.5	1.3	-	1.1	0.6	3.9	63%
4	6.25	0.0375	0.4	0.6	1.6	-	1.2	0.8	4.5	72%
8	6.25	0.0375	0.4	0.7	1.6	-	1.2	1.0	4.9	78%
24	6.25	0.0375	0.3	1.0	1.1	0.3	1.2	1.4	5.3	85%
48	6.25	0.0375	0.3	1.2	0.7	0.6	1.1	1.8	5.8	92%
96	6.25	0.0375	0.2	1.5	0.4	0.8	1.0	2.2	6.0	97%
168	6.25	0.0375	0.1	1.7	0.2	0.9	0.9	2.5	6.3	102%
0.25	6.25	0.0625	0.3	0.3	0.6	-	1.4	0.3	2.9	47%
0.42	6.25	0.0625	0.4	0.4	0.7	-	1.4	0.3	3.2	51%
1	6.25	0.0625	0.4	0.5	1.1	-	1.6	0.4	4.1	65%
2	6.25	0.0625	0.4	0.6	1.2	-	1.7	0.6	4.5	71%
4	6.25	0.0625	0.3	0.8	1.3	-	1.7	0.8	4.8	77%
8	6.25	0.0625	0.3	0.9	1.1	0.1	1.6	0.9	5.0	80%
24	6.25	0.0625	0.2	1.2	0.7	0.4	1.6	1.4	5.5	88%
48	6.25	0.0625	0.2	1.4	0.4	0.6	1.4	1.8	5.8	93%
96	6.25	0.0625	0.1	1.6	0.2	0.7	1.2	2.1	6.0	96%
168	6.25	0.0625	0.1	1.9	0.1	0.8	1.0	2.4	6.3	100%

*SI Table 3: Results Experiment 2 ''higher soil to solution ratio'' SSR=0.25; ICP OES; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; NOTE row indicated by * not used for blank correction due to apparent contamination*

Time	DMA [µM]	Asc. [mM]	Fe [µM]	Cu [µM]	Ni [µM]	Co [µM]	Zn [µM]	Mn [µM]	Fe STDV	Cu STDV	Ni STDV	Co STDV	Zn STDV	Mn STDV
1	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8*	0	0	5.3	0.0	0.2	0.3	0.0	49.1	1.9	0.0	0.0	0.1	0.0	6.9*
8	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0	0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
168	0	0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.1
168	0	0	0.0	0.1	0.1	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.1
1	0	0.5	0.4	0.0	0.2	0.1	0.0	43.0	0.0	0.0	0.0	0.0	0.0	0.2
8	0	0.5	0.9	0.0	0.1	0.1	0.0	38.2	0.0	0.0	0.0	0.0	0.0	0.0
24	0	0.5	1.0	0.1	0.2	0.2	0.0	35.8	0.0	0.0	0.0	0.0	0.0	0.3
168	0	0.5	0.4	0.1	0.2	0.1	0.0	25.6	0.0	0.0	0.0	0.0	0.0	0.2
1	0	0.75	1.1	0.0	0.1	0.2	0.0	49.3	0.0	0.0	0.0	0.0	0.0	0.2
8	0	0.75	2.8	0.9	0.5	1.3	0.1	23.9	2.8	0.9	0.5	1.3	0.1	24.3
24	0	0.75	4.5	0.0	0.1	0.4	0.0	38.9	0.0	0.0	0.0	0.0	0.0	0.2
168	0	0.75	3.0	0.0	0.1	0.2	0.0	26.1	0.0	0.0	0.0	0.0	0.0	0.3
1	0	1.25	2.0	0.0	0.2	0.2	0.0	58.8	0.0	0.0	0.0	0.0	0.0	0.4
8	0	1.25	5.5	0.0	0.2	0.3	0.0	51.6	0.0	0.0	0.0	0.0	0.0	0.1
24	0	1.25	9.8	0.1	0.3	0.7	0.0	49.4	0.1	0.0	0.0	0.0	0.0	0.7
168	0	1.25	11.1	0.0	0.3	0.7	0.0	35.6	0.2	0.0	0.0	0.0	0.0	0.1
1	0	2.5	4.1	0.0	0.2	0.3	0.0	61.1	0.0	0.0	0.0	0.0	0.0	0.3
8	0	2.5	7.2	2.3	3.1	3.8	0.2	55.4	0.1	0.4	1.9	1.9	0.2	0.4
24	0	2.5	13.9	0.0	0.2	0.9	0.1	50.8	0.2	0.0	0.0	0.0	0.0	0.5
168	0	2.5	29.2	0.1	0.3	1.5	0.2	41.9	5.7	0.0	0.1	0.3	0.1	5.6
0.25	7.5	0	0.43	0.36	0.03	0.00	0.46	0.00	0.03	0.01	0.02	0.03	0.03	0.02
0.5	7.5	0	0.60	0.50	0.09	0.04	0.55	0.00	0.01	0.02	0.02	0.00	0.03	0.04
1	7.5	0	1.01	0.73	0.11	0.05	0.64	0.00	0.06	0.06	0.04	0.01	0.02	0.03
2	7.5	0	1.65	1.14	0.35	0.07	0.63	0.00	0.03	0.00	0.01	0.03	0.01	0.01
4	7.5	0	1.97	1.37	0.33	0.08	0.64	0.00	0.06	0.03	0.00	0.00	0.00	0.02
8	7.5	0	2.10	1.77	0.46	0.10	0.54	0.00	0.07	0.04	0.01	0.03	0.01	0.00
24	7.5	0	1.46	2.76	0.90	0.23	0.36	0.00	0.06	0.00	0.04	0.00	0.01	0.05
48	7.5	0	0.87	3.57	1.13	0.33	0.21	0.00	0.02	0.00	0.03	0.01	0.01	0.01
96	7.5	0	0.36	4.05	1.38	0.37	0.08	0.30	0.02	0.02	0.01	0.01	0.01	0.05
168	7.5	0	0.17	4.17	1.60	0.42	0.04	0.88	0.00	0.02	0.01	0.03	0.00	0.26
0.25	12.5	0	0.59	0.48	0.08	0.01	0.54	0.00	0.00	0.01	0.01	0.02	0.04	0.00
0.5	12.5	0	1.01	0.76	0.31	0.09	0.65	0.00	0.01	0.01	0.02	0.03	0.02	0.05
1	12.5	0	1.21	0.81	0.23	0.03	0.64	0.00	0.00	0.00	0.01	0.02	0.00	0.04
2	12.5	0	2.04	1.26	0.47	0.10	0.75	0.00	0.00	0.00	0.02	0.01	0.02	0.03
4	12.5	0	2.55	1.45	0.48	0.08	0.79	0.00	0.05	0.02	0.02	0.00	0.03	0.01
8	12.5	0	3.34	1.90	0.62	0.12	0.84	0.00	0.00	0.01	0.02	0.02	0.04	0.02
24	12.5	0	3.68	2.99	1.11	0.30	0.71	0.00	0.01	0.01	0.02	0.02	0.01	0.02
48	12.5	0	2.91	3.83	1.49	0.48	0.60	0.00	0.09	0.02	0.01	0.03	0.01	0.00
96	12.5	0	1.87	4.91	1.98	0.79	0.49	0.36	0.02	0.14	0.07	0.05	0.01	0.06

168	12.5	0	0.88	5.35	2.38	1.16	0.28	1.05	0.03	0.00	0.00	0.00	0.01	0.04
0.25	25	0	0.65	0.62	0.10	0.01	0.49	0.01	0.01	0.01	0.00	0.02	0.02	0.02
0.5	25	0	0.97	0.78	0.18	0.02	0.64	0.11	0.06	0.00	0.00	0.02	0.05	0.02
1	25	0	1.32	0.94	0.23	0.05	0.65	0.02	0.07	0.05	0.00	0.01	0.04	0.07
2	25	0	2.06	1.29	0.40	0.08	0.77	0.08	0.01	0.01	0.00	0.02	0.03	0.00
4	25	0	2.98	1.64	0.53	0.10	0.90	0.00	0.00	0.02	0.01	0.04	0.03	0.01
8	25	0	4.51	2.25	0.75	0.15	1.02	0.00	0.04	0.02	0.01	0.01	0.01	0.00
24	25	0	6.93	3.09	1.18	0.30	1.22	0.00	0.09	0.00	0.01	0.02	0.00	0.02
48	25	0	7.23	3.96	1.43	0.53	1.27	0.09	0.03	0.03	0.05	0.03	0.01	0.02
96	25	0	6.71	5.14	1.98	0.95	1.30	0.51	0.09	0.07	0.00	0.07	0.03	0.07
168	25	0	5.32	5.84	2.41	1.43	1.15	0.88	0.00	0.19	0.09	0.09	0.01	0.06

*SI Table 4 Solution Mn (C_L) and total Mn concentration in experiment 1 based on adsorption isotherm of Mn2+ to Santomera soil. Mn sorbed derived by Q * SSR with $Q = Q^0*CL/(K_d+C_L)$; SSR = 0.125; 10mM CaCl2 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2*

Time [h]	DMA [µM]	Ascorbate [mM]	Mn [µM]	Mn STDV	Mn sorbed [µmole / kg soil] Q*SSR	Mn total [µM]	Mn to Ascorbate ratio	µM Fe potentially mobilized (assuming fraction of 0.5%)
1	0	0	0.0	0.1	0.0	0.0		
8	0	0	0.0	0.0	0.0	0.0		
24	0	0	0.0	0.0	0.0	0.0		
168	0	0	0.8	0.0	10.2	11.0		0.0
1	0	0.0375	4.4	0.0	53.9	58.2	1.6	0.1
8	0	0.0375	3.5	0.0	43.7	47.1	1.3	0.1
24	0	0.0375	3.6	0.1	45.7	49.4	1.3	0.1
168	0	0.0375	4.1	0.1	50.8	54.8	1.5	0.1
1	0	0.125	18.2	0.1	182.7	200.9	1.6	0.4
8	0	0.125	14.7	0.2	154.6	169.3	1.4	0.3
24	0	0.125	14.8	0.1	155.9	170.7	1.4	0.3
168	0	0.125	11.5	0.2	126.9	138.4	1.1	0.3
0.25	12.5	0	0.0	0.0	0.0	0.0		0.0
0.5	12.5	0	0.0	0.1	0.0	0.0		0.0
1	12.5	0	0.0	0.0	0.0	0.0		0.0
2	12.5	0	0.0	0.0	0.0	0.0		0.0
4	12.5	0	0.0	0.0	0.0	0.0		0.0
8	12.5	0	0.0	0.0	0.0	0.0		0.0
24	12.5	0	0.0	0.0	0.0	0.0		0.0
48	12.5	0	0.1	0.0	1.2	1.3		0.0
96	12.5	0	0.3	0.0	4.6	5.0		0.0
168	12.5	0	0.7	0.0	8.9	9.5		0.0
0.25	12.5	0.0375	5.0	0.0	61.7	66.7	1.8	0.1
0.5	12.5	0.0375	4.3	0.0	53.3	57.6	1.5	0.1
1	12.5	0.0375	4.5	0.0	55.6	60.1	1.6	0.1
2	12.5	0.0375	4.2	0.0	51.9	56.1	1.5	0.1
4	12.5	0.0375	4.0	0.1	49.8	53.8	1.4	0.1
8	12.5	0.0375	3.7	0.1	46.0	49.6	1.3	0.1
24	12.5	0.0375	3.6	0.1	45.7	49.4	1.3	0.1
48	12.5	0.0375	3.6	0.0	45.5	49.1	1.3	0.1
96	12.5	0.0375	3.4	0.1	43.2	46.6	1.2	0.1
168	12.5	0.0375	3.9	0.2	49.1	53.1	1.4	0.1
0.25	12.5	0.125	19.6	0.3	193.1	212.7	1.7	0.4
0.5	12.5	0.125	17.0	0.3	173.3	190.3	1.5	0.4
1	12.5	0.125	17.0	0.1	173.5	190.5	1.5	0.4
2	12.5	0.125	16.3	0.3	168.2	184.5	1.5	0.4
4	12.5	0.125	16.3	0.0	168.1	184.4	1.5	0.4
8	12.5	0.125	15.0	0.2	157.1	172.1	1.4	0.3
24	12.5	0.125	14.5	0.1	153.3	167.8	1.3	0.3
48	12.5	0.125	14.0	0.2	149.3	163.3	1.3	0.3
96	12.5	0.125	12.3	0.0	134.5	146.8	1.2	0.3
168	12.5	0.125	11.8	0.6	130.2	142.0	1.1	0.3

0.25	3.75	0	0.0	0.0	0.0	0.0		0
0.42	3.75	0	0.0	0.0	0.0	0.0		0
1	3.75	0	0.0	0.0	0.0	0.0		0
2	3.75	0	0.0	0.0	0.0	0.0		0
4	3.75	0	0.0	0.0	0.0	0.0		0
8	3.75	0	0.0	0.1	0.0	0.0		0
24	3.75	0	0.0	0.0	0.0	0.0		0
48	3.75	0	0.0	0.0	0.0	0.0		0
96	3.75	0	0.3	0.0	3.4	3.7		0.0
168	3.75	0	0.7	0.1	8.9	9.6		0.0
0.25	3.75	0.0375	4.3	0.0	53.2	57.5	1.5	0.1
0.42	3.75	0.0375	4.2	0.0	51.8	55.9	1.5	0.1
1	3.75	0.0375	4.7	0.0	57.3	62.0	1.7	0.1
2	3.75	0.0375	4.5	0.0	56.0	60.5	1.6	0.1
4	3.75	0.0375	4.6	0.1	56.5	61.1	1.6	0.1
8	3.75	0.0375	4.6	0.0	56.2	60.8	1.6	0.1
24	3.75	0.0375	4.1	0.0	51.0	55.2	1.5	0.1
48	3.75	0.0375	3.8	0.0	47.6	51.4	1.4	0.1
96	3.75	0.0375	3.8	0.1	47.9	51.7	1.4	0.1
168	3.75	0.0375	4.0	0.0	49.9	53.9	1.4	0.1
0.25	3.75	0.0625	8.5	0.1	98.5	107.0	1.7	0.2
0.42	3.75	0.0625	8.5	0.0	98.7	107.2	1.7	0.2
1	3.75	0.0625	8.8	0.2	101.5	110.4	1.8	0.2
2	3.75	0.0625	9.2	0.2	105.6	114.8	1.8	0.2
4	3.75	0.0625	9.0	0.1	103.5	112.5	1.8	0.2
8	3.75	0.0625	8.6	0.0	99.6	108.3	1.7	0.2
24	3.75	0.0625	7.8	0.1	91.7	99.5	1.6	0.2
48	3.75	0.0625	7.1	0.1	83.9	91.0	1.5	0.2
96	3.75	0.0625	6.9	0.0	81.5	88.4	1.4	0.2
168	3.75	0.0625	6.6	0.1	78.3	84.9	1.4	0.2
0.25	6.25	0	0.0	0.0	0.0	0.0		0.0
0.42	6.25	0	0.0	0.0	0.0	0.0		0.0
1	6.25	0	0.0	0.0	0.0	0.0		0.0
2	6.25	0	0.0	0.0	0.0	0.0		0.0
4	6.25	0	0.0	0.0	0.0	0.0		0.0
8	6.25	0	0.0	0.0	0.0	0.0		0.0
24	6.25	0	0.0	0.0	0.0	0.0		0.0
48	6.25	0	0.0	0.0	0.4	0.4		0.0
96	6.25	0	0.3	0.0	4.4	4.8		0.0
168	6.25	0	0.7	0.0	8.9	9.6		0.0
0.25	6.25	0.0375	4.6	0.1	56.8	61.5	1.6	0.1
0.42	6.25	0.0375	4.4	0.0	54.8	59.2	1.6	0.1
1	6.25	0.0375	4.8	0.1	59.0	63.8	1.7	0.1
2	6.25	0.0375	4.7	0.1	58.0	62.7	1.7	0.1
4	6.25	0.0375	4.6	0.0	56.9	61.5	1.6	0.1
8	6.25	0.0375	4.5	0.0	55.1	59.6	1.6	0.1
24	6.25	0.0375	4.1	0.0	50.8	54.9	1.5	0.1
48	6.25	0.0375	3.9	0.1	48.6	52.5	1.4	0.1
96	6.25	0.0375	3.9	0.0	48.7	52.6	1.4	0.1

168	6.25	0.0375	4.1	0.0	50.8	54.9	1.5	0.1
0.25	6.25	0.0625	8.8	0.0	100.9	109.6	1.8	0.2
0.42	6.25	0.0625	8.6	0.0	99.6	108.2	1.7	0.2
1	6.25	0.0625	9.1	0.1	103.9	113.0	1.8	0.2
2	6.25	0.0625	9.0	0.0	103.6	112.6	1.8	0.2
4	6.25	0.0625	9.2	0.4	104.9	114.1	1.8	0.2
8	6.25	0.0625	8.5	0.0	98.3	106.8	1.7	0.2
24	6.25	0.0625	7.8	0.0	91.2	99.0	1.6	0.2
48	6.25	0.0625	7.1	0.0	83.6	90.7	1.5	0.2
96	6.25	0.0625	6.6	0.1	78.2	84.8	1.4	0.2
168	6.25	0.0625	6.5	0.0	77.7	84.2	1.3	0.2

SI Table 5: anoxic Results Experiment 3; ‘‘absence of oxygen’’ ICP MS; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2

DMA [µM]	Asc, [mM]	Time [h]	Mn [µM]	Mn STDV	Fe [µM]	Fe STDV	Co [µM]	Co STDV	Ni [µM]	Ni STDV	Cu [µM]	Cu STDV	Zn [µM]	Zn STDV
0	0	0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	0	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	0	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	0	24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	0	168	1.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	0.25	4.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	1	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	2	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	4	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	8	3.6	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	24	3.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	48	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	96	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	37.5	168	5.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
0	62.5	0.25	9.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	0.5	11.3	0.4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
0	62.5	1	10.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	2	8.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	4	8.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	8	7.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	24	7.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	62.5	48	6.9	0.5	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
0	62.5	96	6.7	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
0	62.5	168	7.4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
3.75	0	0.25	0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.0	0.3	0.0	0.3	0.0
3.75	0	0.5	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.0	0.4	0.0	0.4	0.1
3.75	0	1	0.0	0.0	0.4	0.0	0.0	0.0	0.1	0.0	0.5	0.0	0.4	0.0
3.75	0	2	0.0	0.0	0.6	0.0	0.1	0.0	0.2	0.0	0.6	0.0	0.4	0.0
3.75	0	4	0.0	0.0	0.9	0.1	0.1	0.0	0.3	0.0	0.8	0.0	0.4	0.0
3.75	0	8	0.0	0.0	0.9	0.0	0.1	0.0	0.3	0.0	1.0	0.0	0.4	0.0
3.75	0	24	0.0	0.0	0.4	0.0	0.1	0.0	0.5	0.0	1.4	0.0	0.2	0.0
3.75	0	48	0.0	0.0	0.1	0.0	0.2	0.0	0.7	0.0	1.8	0.0	0.1	0.0
3.75	0	96	0.5	0.1	0.0	0.0	0.2	0.0	0.9	0.0	2.2	0.0	0.0	0.0
3.75	0	168	1.4	0.0	0.0	0.0	0.2	0.0	0.9	0.0	2.0	0.0	0.0	0.0

3.75	62.5	0.25	9.6	1.1	0.1	0.0	1.8	0.1	0.2	0.0	0.3	0.0	0.4	0.0
3.75	62.5	0.5	10.7	0.2	0.1	0.0	2.0	0.1	0.3	0.0	0.4	0.0	0.3	0.0
3.75	62.5	1	10.0	0.3	0.2	0.0	1.9	0.0	0.3	0.0	0.5	0.0	0.3	0.1
3.75	62.5	2	9.6	0.8	0.2	0.0	1.8	0.1	0.3	0.0	0.5	0.0	0.2	0.0
3.75	62.5	4	8.2	0.1	0.1	0.0	1.6	0.1	0.4	0.0	0.7	0.0	0.2	0.0
3.75	62.5	8	7.9	0.1	0.0	0.0	1.5	0.0	0.5	0.0	0.9	0.0	0.1	0.0
3.75	62.5	24	7.0	0.1	0.1	0.1	1.1	0.0	0.7	0.0	1.3	0.0	0.1	0.0
3.75	62.5	48	6.6	0.2	0.0	0.0	0.8	0.0	0.9	0.0	1.6	0.0	0.0	0.0
3.75	62.5	96	6.7	0.2	0.0	0.0	0.6	0.0	1.0	0.0	1.8	0.0	0.1	0.0
3.75	62.5	168	7.5	0.0	0.0	0.0	0.4	0.0	1.1	0.1	1.8	0.0	0.0	0.0
6.25	0	0.25	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.0	0.3	0.0	0.4	0.0
6.25	0	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.1	0.0	0.4	0.0	0.4	0.0
6.25	0	1	0.0	0.0	0.7	0.0	0.1	0.0	0.1	0.0	0.5	0.0	0.5	0.0
6.25	0	2	0.0	0.0	0.9	0.0	0.1	0.0	0.2	0.0	0.6	0.0	0.5	0.0
6.25	0	4	0.0	0.0	1.3	0.0	0.1	0.0	0.3	0.0	0.8	0.0	0.5	0.0
6.25	0	8	0.0	0.0	1.6	0.0	0.1	0.0	0.4	0.0	1.1	0.0	0.6	0.0
6.25	0	24	0.0	0.0	1.6	0.0	0.2	0.0	0.6	0.0	1.6	0.0	0.6	0.0
6.25	0	48	0.0	0.0	1.1	0.0	0.3	0.0	0.8	0.0	2.1	0.0	0.6	0.0
6.25	0	96	0.4	0.0	0.6	0.0	0.5	0.0	1.0	0.0	2.5	0.1	0.4	0.0
6.25	0	168	1.4	0.1	0.2	0.0	0.8	0.0	1.3	0.0	2.8	0.1	0.2	0.1
6.25	37.5	0.25	4.8	0.0	0.4	0.0	1.0	0.0	0.2	0.0	0.3	0.0	0.4	0.0
6.25	37.5	0.5	4.4	0.0	0.6	0.0	1.1	0.0	0.3	0.0	0.4	0.0	0.4	0.1
6.25	37.5	1	4.6	0.1	0.8	0.0	1.2	0.0	0.4	0.0	0.5	0.0	0.5	0.0
6.25	37.5	2	4.3	0.0	1.1	0.0	1.3	0.0	0.5	0.0	0.6	0.0	0.5	0.0
6.25	37.5	4	3.9	0.0	1.2	0.2	1.4	0.0	0.6	0.0	0.8	0.0	0.6	0.2
6.25	37.5	8	3.9	0.1	1.1	0.0	1.5	0.0	0.8	0.0	1.0	0.0	0.5	0.0
6.25	37.5	24	3.5	0.0	0.6	0.0	1.6	0.1	1.1	0.1	1.5	0.0	0.5	0.1
6.25	37.5	48	3.5	0.1	0.3	0.0	1.5	0.0	1.3	0.0	1.9	0.1	0.3	0.0
6.25	37.5	96	3.8	0.0	0.1	0.0	1.3	0.0	1.5	0.0	2.3	0.0	0.1	0.0
6.25	37.5	168	4.9	0.1	0.1	0.0	1.1	0.0	1.7	0.0	2.5	0.0	0.1	0.0
6.25	62.5	0.25	10.4	0.6	0.4	0.0	1.9	0.1	0.4	0.0	0.3	0.0	0.5	0.1
6.25	62.5	0.5	11.3	0.1	0.6	0.0	2.2	0.0	0.5	0.0	0.4	0.0	0.5	0.0
6.25	62.5	1	10.2	0.2	0.7	0.0	2.1	0.1	0.6	0.0	0.5	0.0	0.4	0.0
6.25	62.5	2	9.5	0.5	0.8	0.0	2.1	0.1	0.7	0.0	0.6	0.0	0.4	0.0
6.25	62.5	4	8.7	0.2	0.7	0.0	2.1	0.0	0.8	0.0	0.8	0.0	0.4	0.0
6.25	62.5	8	8.0	0.2	0.5	0.0	2.0	0.0	0.9	0.0	1.0	0.0	0.3	0.0
6.25	62.5	24	7.3	0.2	0.1	0.0	1.8	0.0	1.3	0.0	1.5	0.0	0.2	0.0
6.25	62.5	48	6.9	0.1	0.0	0.0	1.6	0.0	1.5	0.0	1.8	0.0	0.2	0.0
6.25	62.5	96	6.9	0.1	0.0	0.0	1.3	0.0	1.8	0.0	2.2	0.1	0.1	0.0

6.25	62.5	168	7.5	0.1	0.0	0.0	1.0	0.0	2.0	0.0	2.3	0.0	0.1	0.0

SI Table 6: Metal mobilization by selected plant borne reductants/chelators; SSR 0.125;10mM $CaCl_2$; Values corrected for mean blank concentrations; n=2

Treatment	Al [µM]	Mn [µM]	Fe [µM]	Co [µM]	Ni [µM]	Cu [µM]	Zn [µM]
Blank 15 min	0.02	0.00	0.00	0.00	0.00	0.00	0.00
DMA only 15 min	**0.00**	**0.64**	**0.60**	**0.08**	**0.18**	**0.53**	**0.48**
Blank 4h	0.00	0.02	0.00	0.00	0.00	0.00	0.00
DMA only 4h	**0.00**	**0.58**	**2.89**	**0.19**	**0.63**	**1.50**	**0.85**
Coumarin 15 min	0.00	0.00	0.01	0.00	0.00	0.01	0.02
Coumarin & DMA 15 min	**0.01**	**0.52**	**0.60**	**0.07**	**0.18**	**0.53**	**0.52**
Coumarin 4h	0.02	0.00	0.02	0.00	0.00	0.01	0.00
Coumarin & DMA 4h	**0.00**	**0.45**	**3.01**	**0.16**	**0.63**	**1.55**	**0.80**
Scopoletin 15 min	0.76	14.78	1.12	0.07	0.06	0.11	0.00
Scopoletin & DMA 15 min	**0.96**	**21.71**	**1.67**	**3.10**	**1.13**	**0.71**	**0.60**
Scopoletin 4h	0.10	16.85	0.33	0.06	0.06	0.01	0.00
Scopoletin & DMA 4h	**0.05**	**18.58**	**3.86**	**4.42**	**2.48**	**1.61**	**0.96**
Esculetin 15 min	0.90	9.23	0.31	0.05	0.04	0.02	0.00
Esculetin & DMA 15 min	**1.04**	**13.84**	**0.73**	**2.23**	**0.83**	**0.63**	**0.60**
Esculetin 4h	0.14	10.47	0.11	0.04	0.04	0.01	0.00
Esculetin & DMA 4h	**0.21**	**12.80**	**2.96**	**3.16**	**2.03**	**1.60**	**0.99**
Fraxetin 15 min	0.03	13.79	0.15	0.08	0.07	0.03	0.00
Fraxetin & DMA 15 min	**0.06**	**18.39**	**1.67**	**2.87**	**1.00**	**0.61**	**0.58**
Fraxetin 4h	0.08	27.95	0.51	0.12	0.11	0.03	0.00
Fraxetin & DMA 4h	**0.08**	**29.85**	**3.34**	**5.69**	**3.13**	**1.63**	**1.00**
Caffeic Acid 15 min	0.25	24.66	0.09	0.11	0.11	0.01	0.00
Caffeic Acid & DMA 15 min	**0.33**	**30.69**	**0.30**	**4.54**	**1.57**	**0.63**	**0.66**
Caffeic Acid 4h	0.16	26.95	0.04	0.10	0.10	0.01	0.00
Caffeic Acid & DMA 4h	**0.11**	**28.73**	**1.09**	**5.92**	**3.53**	**1.56**	**1.00**
Riboflavin 15 min	0.01	0.05	0.01	0.00	0.01	0.02	0.00
Riboflavin & DMA 15 min	**0.00**	**0.77**	**0.66**	**0.08**	**0.18**	**0.57**	**0.48**
Riboflavin 4h	0.00	0.06	0.00	0.00	0.00	0.02	0.00
Riboflavin & DMA 4h	**0.00**	**0.61**	**3.27**	**0.19**	**0.64**	**1.66**	**0.87**
Mn(II) 15 min	0.03	21.93	0.00	0.00	0.00	0.01	0.00
Mn(II) & DMA 15 min	**0.01**	**29.04**	**0.62**	**0.09**	**0.16**	**0.51**	**0.50**
Mn(II) 4h	0.01	14.47	0.00	0.00	0.01	0.01	0.00
Mn(II) & DMA 4h	**0.05**	**18.38**	**3.29**	**0.32**	**0.71**	**1.59**	**0.89**
Fe(II) 15 min	0.00	4.59	0.01	0.02	0.02	0.00	0.00
Fe(II) & DMA 15 min	**0.00**	**4.57**	**20.56**	**0.55**	**0.07**	**0.25**	**0.12**
Fe(II) 4h	0.13	1.90	0.09	0.05	0.04	0.02	0.02
Fe(II) & DMA 4h	**0.01**	**3.64**	**18.88**	**0.74**	**0.22**	**1.04**	**0.26**
AQS 15 min	0.00	0.02	0.02	0.00	0.00	0.01	0.00
AQS & DMA 15 min	**0.01**	**0.71**	**0.64**	**0.08**	**0.19**	**0.58**	**0.49**
AQS 4h	0.00	0.03	0.00	0.00	0.00	0.01	0.00
AQS & DMA 4h	**0.00**	**0.51**	**3.12**	**0.18**	**0.65**	**1.64**	**0.85**
AQDS 15 min	0.00	0.01	0.00	0.00	0.00	0.01	0.00
AQDS & DMA 15 min	**0.01**	**0.63**	**0.66**	**0.08**	**0.18**	**0.55**	**0.51**
AQDS 4h	0.00	0.04	0.01	0.00	0.00	0.01	0.00
AQDS & DMA 4h	**0.00**	**0.55**	**3.24**	**0.20**	**0.66**	**1.61**	**0.91**

SI Table 7: STDV determined in experiment 4; SSR 0.125;10mM $CaCl_2$; Values corrected for mean blank concentrations; n=2

Treatment Name	Al Stdv	Mn Stdv	Fe Stdv	Co Stdv	Ni Stdv	Cu Stdv	Zn Stdv
Blank 15 min	0.02	0.00	0.00	0.00	0.00	0.00	0.00
DMA only 15 min	**0.00**	**0.02**	**0.02**	**0.00**	**0.00**	**0.00**	**0.02**
Blank 4h	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DMA only 4h	**0.00**	**0.03**	**0.03**	**0.01**	**0.01**	**0.03**	**0.04**
Coumarin 15 min	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Coumarin & DMA 15 min	**0.01**	**0.02**	**0.01**	**0.00**	**0.00**	**0.01**	**0.05**
Coumarin 4h	0.02	0.00	0.01	0.00	0.00	0.00	0.00
Coumarin & DMA 4h	**0.00**	**0.01**	**0.01**	**0.00**	**0.00**	**0.01**	**0.01**
Scopoletin 15 min	0.11	0.23	0.06	0.00	0.00	0.00	0.00
Scopoletin & DMA 15 min	**0.03**	**0.40**	**0.03**	**0.05**	**0.03**	**0.00**	**0.00**
Scopoletin 4h	0.02	0.17	0.01	0.00	0.00	0.00	0.00
Scopoletin & DMA 4h	**0.00**	**0.51**	**0.02**	**0.08**	**0.05**	**0.03**	**0.01**
Esculetin 15 min	0.06	0.34	0.02	0.00	0.00	0.00	0.00
Esculetin & DMA 15 min	**0.01**	**0.05**	**0.01**	**0.01**	**0.00**	**0.02**	**0.07**
Esculetin 4h	0.02	0.09	0.00	0.00	0.00	0.00	0.00
Esculetin & DMA 4h	**0.01**	**0.22**	**0.05**	**0.05**	**0.01**	**0.02**	**0.02**
Fraxetin 15 min	0.02	0.06	0.00	0.01	0.01	0.02	0.00
Fraxetin & DMA 15 min	**0.04**	**0.44**	**0.02**	**0.06**	**0.03**	**0.00**	**0.01**
Fraxetin 4h	0.01	0.25	0.01	0.00	0.00	0.00	0.00
Fraxetin & DMA 4h	**0.05**	**0.17**	**0.06**	**0.04**	**0.02**	**0.02**	**0.01**
Caffeic Acid 15 min	0.01	0.61	0.00	0.00	0.00	0.00	0.00
Caffeic Acid & DMA 15 min	**0.01**	**0.29**	**0.01**	**0.05**	**0.01**	**0.01**	**0.02**
Caffeic Acid 4h	0.08	0.54	0.00	0.00	0.02	0.01	0.00
Caffeic Acid & DMA 4h	**0.03**	**0.06**	**0.02**	**0.00**	**0.01**	**0.01**	**0.03**
Riboflavin 15 min	0.00	0.02	0.01	0.00	0.01	0.01	0.00
Riboflavin & DMA 15 min	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.01**	**0.01**
Riboflavin 4h	0.00	0.01	0.00	0.00	0.00	0.01	0.00
Riboflavin & DMA 4h	**0.00**	**0.02**	**0.02**	**0.01**	**0.01**	**0.00**	**0.00**
Mn(II) 15 min	0.00	0.16	0.00	0.00	0.00	0.00	0.00
Mn(II) & DMA 15 min	**0.00**	**0.13**	**0.02**	**0.00**	**0.00**	**0.01**	**0.01**
Mn(II) 4h	0.01	0.40	0.00	0.00	0.00	0.00	0.00
Mn(II) & DMA 4h	**0.00**	**0.33**	**0.06**	**0.05**	**0.04**	**0.07**	**0.03**
Fe(II) 15 min	0.00	0.17	0.00	0.00	0.00	0.00	0.00
Fe(II) & DMA 15 min	**0.00**	**0.09**	**0.15**	**0.01**	**0.00**	**0.01**	**0.01**
Fe(II) 4h	0.13	1.90	0.05	0.04	0.03	0.02	0.02
Fe(II) & DMA 4h	**0.01**	**0.29**	**0.09**	**0.03**	**0.00**	**0.02**	**0.01**
AQS 15 min	0.00	0.01	0.02	0.00	0.00	0.00	0.00
AQS & DMA 15 min	**0.01**	**0.04**	**0.01**	**0.00**	**0.00**	**0.01**	**0.00**
AQS 4h	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AQS & DMA 4h	**0.00**	**0.08**	**0.06**	**0.03**	**0.02**	**0.02**	**0.02**
AQDS 15 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AQDS & DMA 15 min	**0.01**	**0.03**	**0.01**	**0.00**	**0.00**	**0.00**	**0.03**
AQDS 4h	0.00	0.01	0.00	0.00	0.00	0.00	0.00
AQDS & DMA 4h	**0.00**	**0.05**	**0.06**	**0.01**	**0.02**	**0.04**	**0.03**

SI Table 8: Effectiveness of all treatments scanned in experiment 4; SSR 0.125;10mM $CaCl_2$; Values corrected for mean blank concentrations; n=2

Treatment Name	Al effectiveness	Mn effectiveness	Fe effectiveness	Co effectiveness	Ni effectiveness	Cu effectiveness	Zn effectiveness
Coumarin & DMA 15 min	-	-0.24	-0.07	-0.24	-0.09	-0.05	-0.11
Coumarin & DMA 4h	-1.00	-0.29	0.02	-0.21	-0.02	0.01	-0.11
Scopoletin & DMA 15 min	0.08	0.36	-0.09	19.25	3.50	0.10	0.18
Scopoletin & DMA 4h	-0.58	0.02	0.18	15.81	2.48	0.02	0.07
Esculetin & DMA 15 min	0.07	0.35	-0.23	15.62	2.63	0.10	0.04
Esculetin & DMA 4h	0.31	0.13	-0.04	11.99	1.98	0.03	0.10
Fraxetin & DMA 15 min	-0.70	0.24	1.14	16.39	2.65	0.06	0.12
Fraxetin & DMA 4h	-0.68	0.03	-0.05	17.00	3.17	0.03	0.11
Caffeic Acid & DMA 15 min	0.24	0.17	-0.58	22.53	4.16	0.14	0.29
Caffeic Acid & DMA 4h	-0.64	0.02	-0.64	19.03	3.67	0.00	0.10
Riboflavin & DMA 15 min	-1.00	0.04	0.02	0.02	-0.10	0.01	-0.07
Riboflavin & DMA 4h	-	-0.13	0.11	-0.06	-0.01	0.07	-0.02
Mn(II) & DMA 15 min	-0.59	0.27	-0.05	0.13	-0.13	-0.08	-0.03
Mn(II) & DMA 4h	0.80	0.17	0.10	0.37	0.03	-0.01	-0.04
Fe(II) & DMA 15 min	-	-0.17	31.08	4.57	-0.66	-0.54	-0.78
Fe(II) & DMA 4h	-1.00	-0.24	5.15	1.51	-0.69	-0.35	-0.73
AQS & DMA 15 min	-	-0.03	-0.03	0.01	0.00	0.06	-0.02
AQS & DMA 4h	-	-0.34	0.05	-0.20	-0.02	0.06	-0.06
AQDS & DMA 15 min	-	-0.11	0.03	-0.08	-0.05	0.02	-0.04
AQDS & DMA 4h	-	-0.25	0.09	-0.06	0.00	0.02	-0.01

SI Table 9: SPAD readings of pot trial; measurement per leaf (youngest & 2. youngest) per pot = 8; pots per treatment = 4

Days	Treatment	Mean youngest leaf	Stdv youngest	Mean second youngest	Stdv second youngest
12	Control	40.0	1.1	45.1	0.6
15	Control	34.1	2.3	40.8	1.6
18	Control	40.8	0.9	44.2	0.9
22	Control	34.5	1.4	40.6	1.1
24	Control	38.7	1.0	40.6	0.5
26	Control	39.8	0.7	39.9	1.5
29	Control	39.7	2.3	40.7	0.6
33	Control	40.5	1.7	41.0	1.3
36	Control	37.1	1.3	39.5	1.9
38	Control	37.7	1.2	39.2	2.5
40	Control	36.9	1.9	38.5	0.8
43	Control	37.8	1.4	38.4	0.6
12	High TETA	40.3	0.9	45.0	0.9
15	High TETA	34.7	1.2	41.3	1.1
18	High TETA	39.8	1.5	42.8	0.7
22	High TETA	37.6	0.4	39.5	0.7
24	High TETA	41.3	0.6	40.3	0.8
26	High TETA	40.9	0.9	39.8	0.4
29	High TETA	41.5	1.4	42.1	0.9
33	High TETA	42.4	2.3	41.9	0.8
36	High TETA	39.4	1.4	41.7	1.9
38	High TETA	40.0	1.1	40.7	1.8
40	High TETA	38.1	0.1	39.1	1.4
43	High TETA	39.3	0.9	38.2	1.6
12	Low TETA	40.9	1.0	44.9	1.1
15	Low TETA	32.8	2.4	41.0	1.3
18	Low TETA	40.7	0.5	43.3	0.8
22	Low TETA	36.2	1.5	39.6	0.8
24	Low TETA	40.7	0.7	41.6	0.4
26	Low TETA	41.3	0.3	41.3	0.6
29	Low TETA	39.6	2.3	42.4	1.1
33	Low TETA	42.0	1.4	41.9	1.3
36	Low TETA	38.6	0.9	41.7	1.0
38	Low TETA	38.5	0.9	40.5	0.2
40	Low TETA	37.6	1.1	38.4	0.3
43	Low TETA	38.0	1.0	37.6	0.5

8.2 Further Experiments carried out (not discussed in thesis)

8.2.1 Displacement Experiment of Al-DFOB and Fe-DFOB

8.2.1.1 Materials and Methods

Preparation of the Fe and Al-DFOB complexes was conducted the following:

A stock solution (0.1M) of $AlCl_3$ and $FeCl_3$ was made and pH was adjusted to below 2. Desferal (Novartis) was dissolved and $AlCl_3$ resp. $FeCl_3$ was added in an excess of 2%. The solutions were made to pH of around 8 by carefully adding NaOH. Solutions were covered and left overnight to ensure precipitation of excess Al or Fe. The next day Al and Fe-DFOB stock colutions were filtered over 0.1µM cellulose acetate filters (Sartorius, Austria) and made straight to final volume. Final stock concentrations were 2mM. All solutions were prepared using acid cleaned glassware and UPW.

The Soil to Solution ratio used was 1:1 resulting in 5 g of Xeraco R soil and 5 ml total solution. Concentrations of $CaCl_2$ and Bronopol were applied and preequilibrated with the soil as discussed in experiment 1.

Concentrations of Al-DFOB and Fe-DFOB added was: 50, 100 and 200µM respectively

Time points sampled were: 0.25 h, 1h, 4h, 8h, 24h, 96h, 168h

Blank samples were included in the 4h, 24h and 168h treatments.

All treatments were carried out in duplicates.

Samples were centrifuged at 4500 rpm for 3 minutes filtered over 0.1µM cellulose acetate filters (Sartorius, Austria) and thereafter treated as described for previous experiments. Measurement was carried out by ICP-OES (Optima 5300 DV, PerkinElmer).

8.2.1.2 Results

SI Table 10: Results Displacement Experiment of Al-DFOB and Fe-DFOB 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1; Xeraco soil

Al DFOB supplied µM	Time h	Al µM	Fe µM	Al StDv	FeStDv
50	0.25	6.105	0.116	0.231	0.028
50	1.00	6.088	0.072	0.033	0.001
50	4.00	5.826	0.257	0.201	0.083
50	8.00	5.352	0.217	0.050	0.030
50	24.00	5.769	0.346	0.142	0.042
50	96.00	4.989	0.785	0.091	0.003
50	168.00	4.647	1.168	0.085	0.015
100	0.25	11.939	0.125	0.015	0.005
100	1.00	11.679	0.113	0.176	0.013
100	4.00	11.433	0.163	0.289	0.024
100	8.00	11.063	0.258	0.076	0.015
100	24.00	10.821	0.502	0.079	0.006
100	96.00	9.634	1.403	0.077	0.042
100	168.00	8.323	2.062	0.349	0.011
200	0.25	29.773	0.131	4.417	0.007
200	1.00	23.789	0.141	0.126	0.006
200	4.00	23.399	0.263	0.179	0.028
200	8.00	22.582	0.417	0.123	0.018

200	24.00	22.359	0.878	0.210	0.011
200	96.00	19.741	2.726	0.173	0.055
200	168.00	17.717	4.348	0.192	0.057

SI Table 11: Results Displacement Experiment of Al-DFOB and Fe-DFOB 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1; Xeraco soil

Fe DFOB supplied µM	Time h	Al µM	Fe µM	Al StDv	FeStDv
50	0.25	0.14	8.45	0.08	0.14
50	1.00	0.05	8.19	0.11	0.17
50	4.00	0.19	7.88	0.05	0.06
50	8.00	0.07	7.69	0.03	0.03
50	24.00	0.88	7.59	0.12	0.00
50	96.00	0.87	7.11	0.07	0.02
50	168.00	0.28	7.04	0.11	0.03
100	0.25	0.44	16.55	0.46	0.04
100	1.00	0.25	16.34	0.25	0.03
100	4.00	0.00	15.88	0.24	0.19
100	8.00	0.00	15.64	0.07	0.22
100	24.00	0.70	15.12	0.26	0.06
100	96.00	0.62	13.82	0.23	0.05
100	168.00	0.93	13.60	0.04	0.09
200	0.25	0.00	35.08	0.22	0.21
200	1.00	0.43	33.90	0.01	0.28
200	4.00	0.26	32.12	0.00	0.21
200	8.00	0.00	31.55	0.18	0.31
200	24.00	0.39	30.47	0.07	0.44
200	96.00	0.85	28.34	0.00	0.53
200	168.00	0.85	28.23	0.10	0.28

8.2.2 Interaction of 0 – 100µM DFOB over two time points

8.2.2.1 Materials and Methods

0.6 g of Xeraco R soil was weighed into 50mL polypropylene centrifugation tubes (Greiner Bio-One GmbH; Germany) and $CaCl_2$ and Bronopol was added as previously described for preequilibration. On the day of the experiment a 1mM DFOB stock solution was prepared by dissolving Desferal (Novartis) and added in the concentrations 0,1, 2.5, 5, 10,25,50 and 100µM, UPW was added if needed, resulting in a total solution of 3 ml (SSR 1:5). Interaction times chosen were 8 hours and 24 hours. All treatments were carried out in duplicates. After interaction samples were treated as described in paragraph 8.2.1.1.

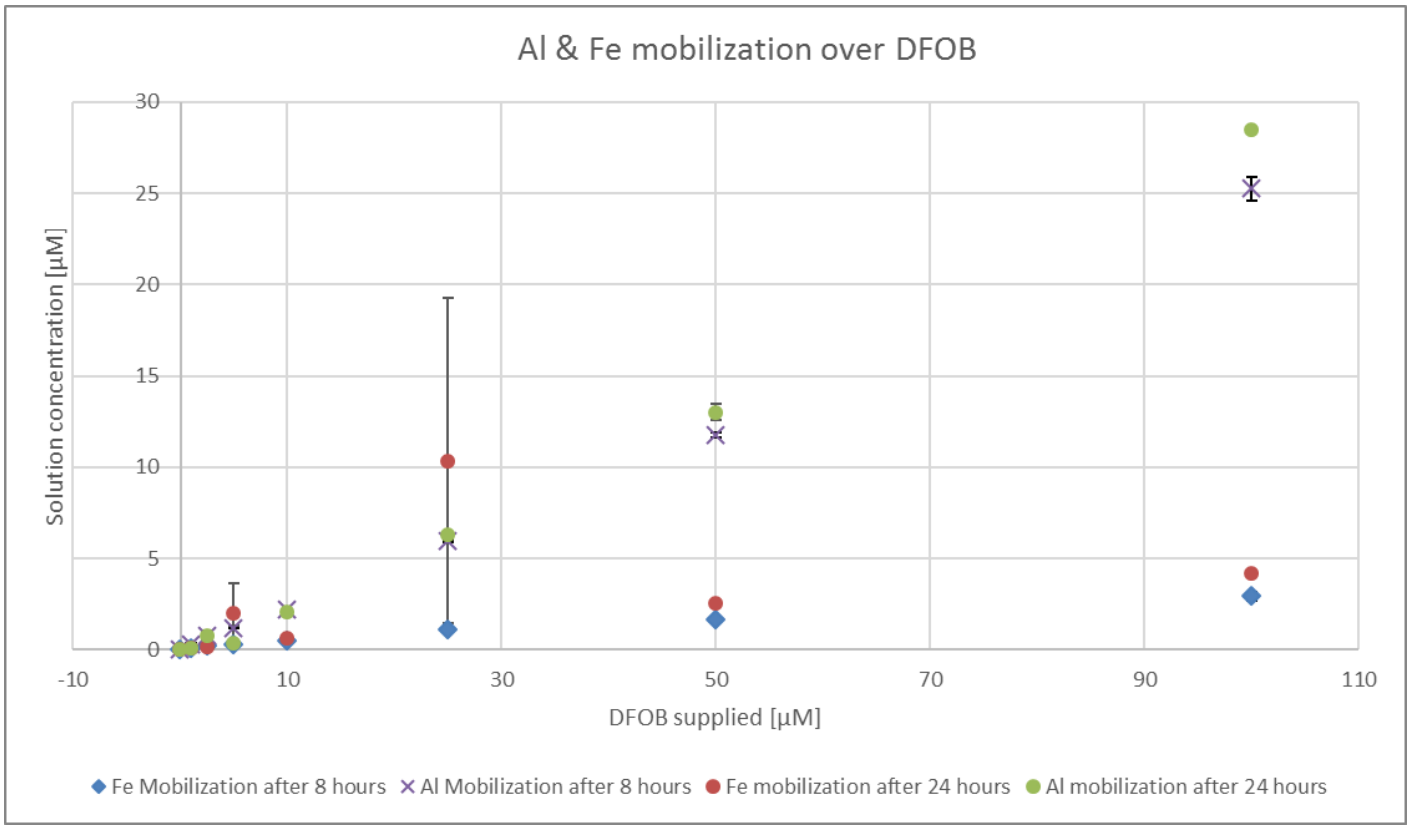
8.2.2.2 Results

SI Table 12 Results Interaction of 0-100 µM DFOB 8h; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil

Time [h]	DFOB [µM]	Al [µM]	Fe [µM]	Al StDv	Fe StDv
8	0	0.00	0.00	0.07	0.00
8	1	0.29	0.06	0.09	0.00
8	2.5	0.75	0.24	0.07	0.06
8	5	1.20	0.28	0.01	0.04
8	10	2.18	0.47	0.05	0.02
8	25	5.96	1.11	0.09	0.01
8	50	11.74	1.67	0.14	0.02
8	100	25.25	2.93	0.62	0.22

SI Table 13: Results Interaction of 0-100 µM DFOB 24h; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil

Time [h]	DFOB [µM]	Al[µM]	Fe[µM]	Al StDv	Fe StDv
24	0	0.03	0.00	0.16	0.01
24	1	0.07	0.08	0.11	0.01
24	2.5	0.77	0.17	0.25	0.01
24	5	0.33	2.00	0.99	1.66
24	10	2.08	0.60	0.10	0.02
24	25	6.30	10.35	0.02	8.91
24	50	13.02	2.57	0.46	0.09
24	100	28.51	4.16	0.00	0.07



SI Figure 17 Results Interaction of 0-100 µM DFOB 8h & 24h; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil

8.2.3 Kinetic experiment with DFOB, DFOD, DFOE

8.2.3.1 Materials and Methods

The compounds DFOB (Desferal – Novartis), DFOD (in house stock) and DFOE (in house stock) were interacted in a kinetic experiment with Xeraco soil. Concentrations of the compounds applied were 10µM. Preparation of the compound DFOB has already been described in paragraph 8.2.2. Preparation of the compound DFOE was conducted by weighing the compound into a 50mL polypropylene centrifugation tube (Greiner Bio-One GmbH; Germany) and slowly dissolving it in UPW. After adjusting the pH to around 7 and mixing the solution for around 20 minutes in an ultrasonic bath, the compound dissolved completely. The final stock solution of 300µM was frozen after preparation and thawed on the day of the experiment.

DFOD dissolution proved difficult. The compound was dissolved in a glass beaker after multiple additions of NaOH and vortexing the solution. The final solution pH was 11.3. A stock solution of 150µM was obtained, transferred into 50mL polypropylene centrifugation tubes (Greiner Bio-One GmbH; Germany) and frozen. The effect of high pH of the solution was investigated, by adding the compound, quickly mixing the soil solution with it and measuring the soil solution pH (initial value 7.5 – gradually decreasing) and found neglectable.

10µM of the respective compound were added after preequilibration of 0.6g Xeraco soil with $CaCl_2$ and Bronopol as described previously. UPW was added if needed, resulting in a total solution of 3 ml (SSR 1:5).

Further treatment of samples and Data was conducted as described in paragraph 8.2.1.1 and previous.

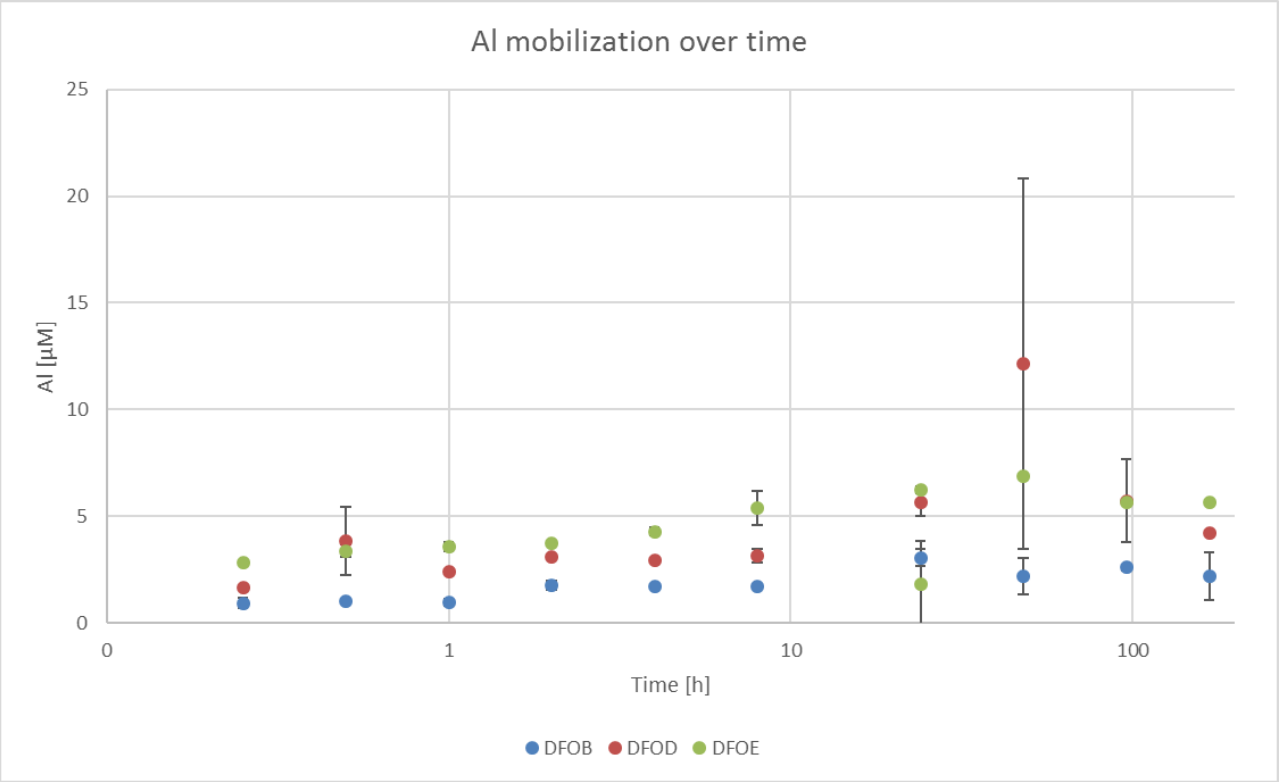
8.2.3.2 Results

SI Table 14: Results Kinetic experiment with DFOB; DFOD; DFOE; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil

Time	Treatment	Al	Fe	Al StDv	Fe StDv
1	Blank	0.00	0.01	0.10	0.00
1	Blank	0.00	0.00	0.01	0.01
8	Blank	0.00	0.00	0.13	0.00
8	Blank	0.30	0.00	0.00	0.00
24	Blank	0.00	0.00	0.09	0.00
168	Blank	0.21	0.02	0.21	0.00
168	Blank	0.00	0.00	0.25	0.00
0.25	DFOB	0.93	0.32	0.07	0.00
0.5	DFOB	1.04	0.31	0.15	0.01
1	DFOB	0.96	0.39	0.21	0.03
2	DFOB	1.77	0.45	0.07	0.05
4	DFOB	1.74	0.49	0.02	0.06
8	DFOB	1.72	0.53	0.39	0.01
24	DFOB	3.07	0.71	0.84	0.03
48	DFOB	2.21	0.81	0.14	0.04
96	DFOB	2.62	0.93	1.13	0.05
168	DFOB	2.19	1.03	0.05	0.02
0.25	DFOD	1.69	0.59	0.05	0.00
0.5	DFOD	3.87	1.07	1.60	0.42
1	DFOD	2.41	0.66	0.06	0.00
2	DFOD	3.10	0.85	0.01	0.08
4	DFOD	2.96	0.91	0.02	0.00
8	DFOD	3.15	1.02	0.32	0.02
24	DFOD	5.66	1.64	0.63	0.10

48	DFOD	12.13	3.14	8.67	1.84
96	DFOD	5.73	1.94	1.95	0.62
168	DFOD	4.24	1.59	0.07	0.00
0.25	DFOE	2.84	0.78	0.05	0.01
0.5	DFOE	3.38	0.99	0.29	0.06
1	DFOE	3.57	1.00	0.22	0.03
2	DFOE	3.72	1.16	0.04	0.05
4	DFOE	4.28	1.17	0.19	0.10
8	DFOE	5.39	1.25	0.78	0.04
24	DFOE	6.24	1.51	0.16	0.02
24	DFOE	1.84	0.61	2.01	0.61
48	DFOE	6.86	1.70	0.05	0.04
96	DFOE	5.67	1.74	0.13	0.03
168	DFOE	5.65	1.64	0.00	0.14

SI Figure 18: Fe mobilization by DFOB; DFOD; DFOE over time; 10mM $CaCl_2$ 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil



SI Figure 19: Al mobilization by DFOB; DFOD; DFOE over time; 10mM CaCl2 0.2g/L Bronopol; Values presented are corrected for the mean blank concentrations; n=2; SSR 1:5; Xeraco soil

8.3 Abstract in german - Zusammenfassung

Diese Masterarbeit behandelt verschiedene positive Einflüsse auf die Eisenaufnahme von Pflanzen in kalkreichen Böden. Die Mobilisierung der Metalle Eisen, Kupfer, Nickel Cobalt und Mangan durch den Phytosiderophor DMA wurde unter Laborbedingungen untersucht. Lösungskonzentrationen von FeDMA – Komplexen nahm, nach einer Zunahme zu Beginn der Zeitserie, ab. Dies wurde der Konkurrenz zugeordnet, die andere Metallionen, durch Reduzierung der Lösungskonzentration nicht komplexierter DMA und kompetitiver Verdrängung von bereits komplexierter FeDMA, auf die Eisenkomplexierung ausübten. Die Mobilisierung durch DMA Zugaben in drei verschiedenen Konzentrationen lag während der ersten Stunden des Experiments in einem ähnlichen Bereich. Die Unterschiede wuchsen jedoch im Verlauf an, bis sich nach 168 Stunden das Verhältnis zwischen zugegebenem DMA und MeDMA - Komplexen eingestellt hatte.

Um die Ergebnisse zu validieren, wurde eine Methode angewandt um MeDMA Konzentrationen unterschiedlicher Boden zu Lösungsverhältnisse miteinander zu vergleichen. Die Resultate zeigten gute Vergleichbarkeiten zwischen zwei durchgeführten Experimenten, jedoch nicht mit publizierten Werten.

Die kombinierte Zugabe des Reduktanten Ascorbinsäure und DMA, für die zuvor synergistische Effekte bei der Eisenmobilisierung berichtet wurden, wurde in verschiedenen Konzentrationen von DMA und Ascorbinsäure untersucht. Die gefundenen synergistischen Effekte, könnten die Eisenaufnahme von Pflanzen, in dem unter natürlichen Bedingungen relevantesten Zeitraum, zu erheblichem Maße positiv beeinflussen. Allerdings waren die Maximalkonzentrationen von Eisen früher und in niedrigerem Maße erreicht, als in den Testreihen ohne Reduktant. Es zeigte sich, dass die erhöhte Konkurrenz durch andere Metallionen den synergistischen Effekt für die Eisenmobilisierung negativ beeinflusste. Insbesondere Nickel und Cobalt Konzentrationen in Lösung waren, auf Kosten von Eisen, ebenfalls synergistisch erhöht, wodurch die Komplexierung von freiem DMA zunahm und bereits komplexiertes FeDMA verdrängt wurde. Ein Verhältnis zwischen Reduktant und Ligand wurde festgestellt, in dem die positiven Effekte der synergistischen Eismobilisierung dominierten.

Um die den synergistischen Effekten zugrunde liegenden Mechanismen besser zu verstehen , wurde die Kombination aus DMA und Ascorbinsäure zusätzlich unter anoxischen Bedingungen untersucht. Im Allgemeinen zeigte die Metalmobilisierung ähnliche Resultate, was keinen großen Einfluss von Sauerstoff in der Bodenlösung anzeigte. Die reduktive Auflösung von Manganoxiden wurde als dominantes Ergebnis der Ascorbinsäurezugabe identifiziert.

Kumarin, Esculetin, Fraxetin, Scopoletin und Kaffeesäure wurden in Kombination mit DMA als weitere, potentiell synergistische Eisenmobilisierung fördernde, Stoffe untersucht. Fraxetin war hierbei der einzige Stoff, im getesteten Verabreichungsbereich, der einen eindeutigen synergistischen Effekt auf die Eisenmobilisierung hatte. Es wurden jedoch Hinweise für einen möglichen synergistischen Effek für die Stoffe Esculetin, Scopoletin und Kaffeesäure gefunden. Scopoletin und Fraxetin zeigten von sich aus signifikante Mobilisierung von Eisen aus dem kalkreichen Boden, ein Prozess der für diese Stoffe bislang nicht bekannt war.

Nachdem Kupfer bekanntermaßen der Hauptkonkurrent der Eisenkomplexierung durch DMA ist, widmete sich ein Pflanzenexperiment einem weiteren möglichen positiven Einfluss auf die Eisenaufnahme des Weizens *Triticum aestivum sp. Tamaro. Durch V*erringerung der Verfügbarkeit von Kupfer für die Komplexierung durch Phytosiderophore, könnte die Eisenverfügbarkeit relativ zunehmen, ohne eine zusätzliche Eisenquelle im Boden zur Verfügung zu stellen. Die Kupferverfügbarkeit wurde duch Zugabe des chelatierenden Stoffes triethylenetetramin dihydrochlorid (TETA) vermindert. Die Pflanzen die in TETA-haltigem Boden wuchsen, zeigten keine negativen Effekte durch den Stoff. Allerdings waren die Biomasse und die Blattchlorophyllgehalte erhöht. Die Metallkonzentrationen in den Pflanzen zeigten Anzeichen für reduzierte Ausschüttung von Phytosiderophore, während die Eisenkonzentrationen in gleicher Menge wie in der Kontrollgruppe blieben.

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