

Geochemical Processes Constraining Iron Uptake in Strategy II Fe Acquisition

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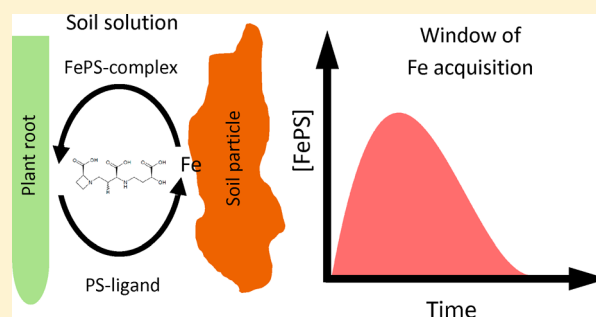
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S Supporting Information

ABSTRACT: Phytosiderophores (PS) are natural chelating agents, exuded by graminaceous plants (grasses) for the purpose of Fe acquisition (Strategy II). They can form soluble Fe complexes with soil-Fe that can be readily taken up. PS are exuded in a diurnal pulse release, and with the start of PS release a “window of iron uptake” opens. In the present study we examined how this window is constrained in time and concentration by biogeochemical processes. For this purpose, a series of interaction experiments was done with a calcareous clay soil and the phytosiderophore 2'-deoxymugineic acid (DMA), in which metal and DMA speciation were examined as a function of time and DMA concentration. Various kinetically and thermodynamically controlled processes affected the size of the window of Fe uptake. Adsorption lowered, but did not prevent Fe mobilization by DMA. Microbial activity depleted DMA from solution, but not on time scales jeopardizing Strategy II Fe acquisition. Complexation of competing metals played an important role in constraining the window of Fe uptake, particularly at environmentally relevant PS concentrations. Our study provides a conceptual model that takes into account the chemical kinetics involved with PS-mediated Fe acquisition. The model can help to explain how success or failure of PS-mediated Fe acquisition depends on environmental conditions.



INTRODUCTION

Iron is a micronutrient essential to plants for its role in chlorophyll synthesis and several other enzymatic processes.¹ Despite its abundance in soils, the bioavailability of iron is limited as a result of the low solubility of iron(hydr)oxide minerals, in particular in soils with a neutral to alkaline pH.² Plants have developed strategies to cope with this limited iron availability. Strategy II Fe acquisition, which is employed by graminaceous plants (grasses, including staple crops such as wheat, corn, and rice), is characterized by the root exudation of chelating ligands called phytosiderophores (PS).^{3,4} PS can bind and mobilize iron into soil solution, and hence facilitate Fe transport to and uptake at the root surface.⁵ The type and amount of PS released depend on plant species and cultivar.^{6,7} The chemical structures of the different types of PS are closely related.^{5,8}

PS are exuded in the apical root zone and exudation is enhanced under Fe deficiency.^{9,10} The exudation follows a diurnal pulse release; it starts shortly after the onset of light and lasts for approximately 4–6 h.^{4,10,11} Once exuded, PS participate in rhizosphere processes including adsorption, degradation, and mobilization of iron and other metals.

Adsorption onto the soil solid phase accounts for a significant loss of PS from soil solution in the rhizosphere at relevant pH values (7–8.5).¹² Both the ligand and the Fe complex of the PS mugineic acid (MA) were shown to adsorb to Fe(hydr)oxide minerals. Adsorption of the ligand decreases with increasing pH and increases with specific surface area of the Fe(hydr)oxide mineral.¹³ Adsorption of anions such as sulfate and phosphate onto the Fe(hydr)oxide surface inhibits the adsorption of PS.¹⁴ The degree of adsorption is soil dependent, accounting for 25–62% removal from solution of the PS 2'-deoxymugineic acid (DMA) in a 1:1 (w/v) soil interaction experiment with 100 μ M DMA.²⁷

Biodegradation is a major cause of PS loss from the rhizosphere.¹² Microorganisms have been reported to degrade PS in soil,^{15,16} and Takagi et al.¹⁶ have isolated bacteria from barley roots that utilize PS as their sole carbon source. It has been suggested that the pulse release of PS, mainly from the

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apical root zones, along with a low population density of rhizosphere microorganisms in these zones may allow a temporal PS accumulation in the presence of degrading microorganisms.^{17,18}

PS have been shown to mobilize Fe from soils (e.g., refs 16 and 19) and iron(hydr)oxide minerals commonly occurring in soils.²⁰ For the interaction of DMA with goethite, a surface-controlled ligand-promoted dissolution mechanism was found, and organic acids commonly occurring in soils can have a synergistic effect on the Fe dissolution rate.^{20–23} Also, it was shown that siderophore-promoted Fe dissolution can be influenced by other biogenic compounds including surfactants,²⁴ and by fulvic acid.²⁵

Besides Fe, PS can also mobilize other metals from soils including Cu, Zn, Ni, Co, Cd, and Mn.^{16,26,27} Competition of these metals for complexation by the PS ligand reduces Fe mobilization, and hence the effectiveness of PS-mediated Fe acquisition, potentially to the point where Fe requirements are no longer met and plants become Fe deficient. Both in chemical equilibrium modeling and experimental work it was shown that, in particular, mobilization of Cu, Zn, and Ni may compromise Fe mobilization by PS.^{12,27–29}

In a recent study it was shown that multisurface equilibrium modeling failed to provide an adequate prediction of metal mobilization by PS in uncontaminated calcareous soils.²⁷ In contrast to model predictions, PS-mediated Fe mobilization from such soils was found in experiments. This discrepancy was explained from the fact that chemical equilibrium was not reached within the 7-day interaction experiments. Considering the diurnal PS release, the time to reach equilibrium is much longer than the time between two PS release events. For this reason we hypothesize that Strategy II Fe acquisition can only be understood when both the thermodynamic and kinetic factors involved are appreciated.

With the start of PS release, a “window of iron uptake” opens for grasses for acquiring Fe. The aim of the present study was to develop a conceptual model to help constrain this window of iron uptake in terms of processes occurring in the rhizosphere. For this purpose a series of soil interaction experiments was done in which metal and PS speciation (i.e., the chemical forms and concentrations in which metals and PS are present) were examined as a function of time and PS concentration.

MATERIALS AND METHODS

Materials. An uncontaminated calcareous (i.e., calcium carbonate containing) clay soil was collected from the top layer (0–20 cm) at a site in Santomera, Spain. The soil was air-dried and sieved over 2 mm. Selected soil parameters are presented in Table 1. The soil has a high pH (7.8) and a high CaCO₃ content (50%). The soil organic carbon (SOC) content is low (0.73%), and so are the Fe availability parameters DTPA-extractable Fe (4.9 mg kg^{−1} Fe) and oxalate extractable Fe (0.5 g kg^{−1} Fe). Fe deficiency chlorosis has been reported in both Strategy I and Strategy II plants grown on this soil.^{11,30}

Mono ammonium DMA salt (Supporting Information (SI) Figure 1) was synthesized in-house, in accordance with Namba et al.³¹ The compound was characterized by LC-ESI-TOF-MS (Agilent time-of-flight LC/MS 6220 system) and had a purity of >95%, as determined with ¹H NMR. The compound readily dissolves in water. DMA is structurally very similar to other PS, and therefore a suitable representative of this class of compounds. Analytical-grade chemicals and ultrapure water were used for preparing experimental solutions.

Table 1. Soil Characteristics

origin/name	Santomera	
region	Murcia	
country	Spain	
soil classification	entisol	
pH CaCl ₂	7.8	
EC (mS cm ⁻¹)	0.11	
SOC (g kg ⁻¹)	7.3	
clay (g kg ⁻¹)	300	
CaCO ₃ (g kg ⁻¹)	500	
Extraction		
CDB	Fe (g kg ⁻¹)	10.2
AmOx	Fe (g kg ⁻¹)	0.5
DTPA	Fe (mg kg ⁻¹)	4.9
	Cu (mg kg ⁻¹)	1.6
	Ni (mg kg ⁻¹)	0.3
	Zn (mg kg ⁻¹)	0.5
	Co (mg kg ⁻¹)	0.0
	Mn (mg kg ⁻¹)	3.1

Experiment. DMA speciation was examined as a function of time in a series of soil interaction experiments. DMA solutions of different concentration (3, 30, 100, and 1000 μM) interacted with soil in a soil to solution ratio (SSR) of 1 (w/v); these concentrations are in the range of those estimated and measured in soil and rhizosphere.^{11,12,32} The influence of microbial degradation of the DMA ligand on metal mobilization was considered by including treatments with and without sterilant. Treatments without DMA, both with and without sterilant, were included as blanks. CaCl₂ (10 mM) was used as background electrolyte, and 2 g L^{−1} NaN₃ was applied as sterilant.²⁷ Experiments were carried out in duplicate. Samples were taken after 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 h. For *t* = 1, 8, and 24 h, additional treatments with 0.1, 0.3, 1, 10, and 300 μM DMA with sterilant were included to obtain a more detailed understanding of metal mobilization as a function of DMA concentration. Experiments were carried out in 50-mL polypropylene centrifuge tubes (VWR Eur. Cat. no 525-0224), which were put in an end-over-end shaker rotating at 18 rpm in the dark at 20 °C. The samples were centrifuged for 5 min at 4500 rpm (4.0 × 10³g) and the supernatant was filtered through 0.45-μm cellulose acetate filter (Whatman Aqua 30/0,45 CA). The pH of the filtrates was measured, and the filtrates were further analyzed for metal and DMA ligand concentrations.

Analytics. Fe concentrations were analyzed by ICP-OES (Optima 5300 DV, PerkinElmer); Co, Cu, Mn, Ni, and Zn concentrations were analyzed by ICP-MS (PerkinElmer, ELAN 6100); for further details, see SI Table 1. Samples were acidified with nitric acid prior to analysis. The concentrations of metal–DMA complexes were calculated as the difference between the metal concentrations in the treatments with and without DMA. Throughout the text, FeDMA concentration refers to the sum of all FeDMA complex species concentrations; at circumneutral pH, FeOHDMA is the dominant species (SI Figure 2). The total DMA ligand concentration was analyzed by HPLC (Agilent 1200SL) combined with an ESI-MS/MS mass selective detector (Agilent 6410 triple quadrupole system), as described in Schindlegger et al.³³

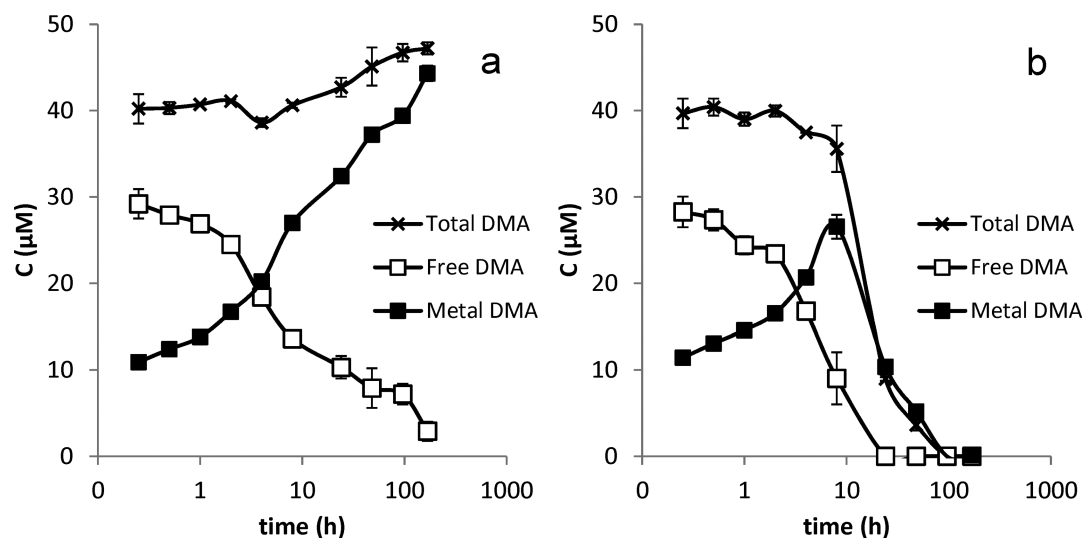


Figure 1. DMA speciation upon interaction of a 100 μM DMA solution with Santomera soil as a function of time (on a log scale) (a) with and (b) without the addition of 2 g L^{-1} NaN_3 (SSR = 1; 0.01 M CaCl_2). Error bars indicate the standard deviation.

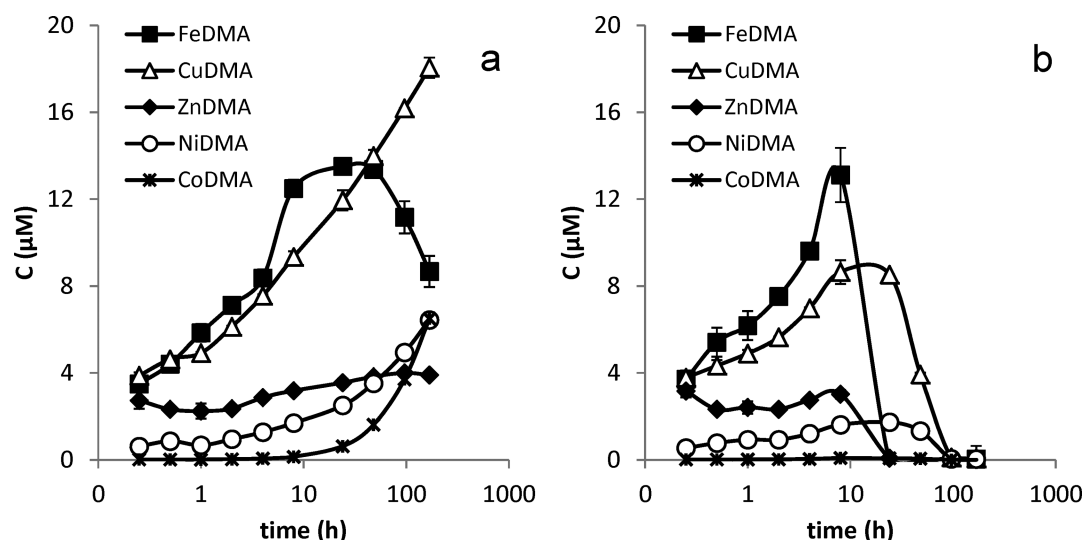


Figure 2. Metal mobilization from Santomera soil by a 100 μM DMA solution as a function of time (on a log scale) (a) with and (b) without the addition of 2 g L^{-1} NaN_3 (SSR = 1; 0.01 M CaCl_2). Error bars indicate the standard deviation.

RESULTS AND DISCUSSION

Adsorption. Upon interaction of a 100 μM DMA ligand solution containing azide with Santomera soil in a SSR of 1, approximately 60% of the DMA was removed from solution within 0.25 h (Figure 1a; Total DMA), indicating that adsorption kinetics of the free DMA ligand are fast. After the near instantaneous concentration drop, the total DMA concentration in solution mildly increased from 39 μM after 4 h to 47 μM after 168 h. From the lack of decline in total DMA concentration in solution after initial adsorption, it was concluded that azide was an adequate sterilant for the duration of the experiment. During the experiment, the metal–DMA concentration (i.e., the sum of concentrations of all metal DMA complexes) gradually increased from 11 μM after 0.25 h to 43 μM after 168 h at the expense of the free DMA concentration which decreased from 29 μM after 0.25 h to 3 μM after 168 h. Initial metal mobilization was fast; approximately 25% of the total metal mobilization occurred within 0.25 h after applying the treatment. The rate by which the metal–DMA concen-

tration increased declined approximately exponentially, presumably both as a result of the declining free DMA ligand concentration and a declining availability of metals bound to soil reactive compounds. The mild increase in total DMA concentration in solution over time suggests that the free DMA ligand (which throughout the experiment becomes gradually depleted in favor of metal DMA–complexes) is adsorbed to a larger extent than the metal–DMA complexes.

Microbial Activity. When no sterilant was applied with the DMA treatment (Figure 1b), the total DMA, metal–DMA, and free DMA concentrations initially (up to 8 h) evolved in the same way as when azide was included with the treatment (Figure 1a). This indicates that the 2 g L^{-1} sodium azide does not substantially affect the adsorption and metal mobilization behavior of the DMA ligand. After 8 h, corresponding concentrations of treatments with and without sterilant started to deviate. In the absence of sterilant, metal mobilization reached a maximum after 8 h and the rate of decline in free DMA concentration was enhanced, resulting in concentrations

below the limit of quantification (LOQ) after 24 h, presumably due to microbial degradation.

After 96 h also the total metal–DMA concentration (and hence the total DMA concentration) declined below the LOQ. However, given the fact that PS are exuded in a diurnal rhythm, providing new input of PS ligands on a daily basis,⁴ in the current experimental setup, microbial activity would not inhibit Fe acquisition by depleting the PS ligand from solution. It was found that rhizosphere bacteria can utilize PS as carbon source.¹⁶ Therefore, biodegradation seems a likely explanation for the observed decrease in DMA concentration. However, based on our experimental data, uptake of the DMA up as a whole by microorganisms cannot be excluded.

The lag phase between application of the DMA and its presumed microbial degradation most likely represents a lag phase of microbial growth and metabolism typically observed after emergence of a microbial community from a stationary phase, allowing adaptation to new environmental conditions and use of new substrates. These new conditions include an increase in moisture content from addition of the experimental solutions to the dried soils,³⁴ the use of DMA as substrate, and the release of easily degradable carbon compounds after rewetting that may serve as cosubstrates.³⁴ In rhizosphere soil of grass species, microbes are already active and possibly used to utilizing PS; the implications of this for the time of the lag phase and the rate at which microbial activity depletes PS from solution need to be further examined. However, the lag phase seemed to have lasted up to a maximum of 8 h, while the DMA ligand only became depleted after 48 h. Hence, in a regime of diurnal DMA release, DMA concentrations would not be depleted even if no lag phase occurred.

Fe Mobilization. The addition of DMA solution to Santomera soil led to a mobilization of Fe, in both the treatment with and without sterilant (Figure 2). Like the metal–DMA concentration, the Fe mobilization started to deviate between the treatments after 8 h. In both treatments, the FeDMA concentration reached a maximum: in the treatment with sterilant, the FeDMA concentration gradually increased until 24 h, after which it gradually declined (Figure 2a), whereas in the treatment without sterilant, the FeDMA concentration gradually increased until 8 h (Figure 2b), after which it dropped below the LOQ between 8 and 24 h after DMA application. The maximum FeDMA concentrations did not differ between the treatments ($13.5 \pm 0.3 \mu\text{M}$ with sterilant and $13.1 \pm 1.2 \mu\text{M}$ without sterilant). The rate by which Fe was mobilized until the maximum concentration was reached decreased over time. After 0.25 h, the FeDMA concentration was around $3.6 \mu\text{M}$, which already accounted for 25–30% of the maximum FeDMA concentrations. This observed trend corresponds with findings from previous soil interaction studies.^{16,19,26} The time frame over which FeDMA was depleted from solution in the treatment without sterilant corresponds to the time frame of depletion of the free DMA ligand; not to the time frame of depletion of the total DMA.

Mobilization of Other Metals. Upon addition of the 100 μM DMA treatments to Santomera soil, Cu, Ni, Zn, and Co also were mobilized to a substantial degree (Figure 2). To a lesser extent, Mn (submicromolar concentrations) and Cd (nanomolar concentrations) were mobilized by DMA as well (data not shown). In the treatment with sterilant, Cu was mobilized approximately to the same extent as Fe, until the FeDMA concentration started to decline (Figure 2a). After 168 h, the CuDMA concentration ($18 \mu\text{M}$) accounted for

approximately 40% of the total DMA ligand in solution. Ni ($6.4 \mu\text{M}$), Co ($6.5 \mu\text{M}$), and Zn ($3.9 \mu\text{M}$) were mobilized to a lesser extent. CuDMA, NiDMA, and CoDMA concentrations increased throughout the experiment. In the case of CuDMA and NiDMA, the rate gradually decreased, while for CoDMA, the rate increased from 1 to 48 h, after which it remained constant. The ZnDMA concentration remained approximately constant throughout the experiment ($3\text{--}4 \mu\text{M}$).

When no sterilant was added (Figure 2b), the trends in metal mobilization were identical up to and including 8 h. Similarly to FeDMA, the ZnDMA concentrations dropped below the limit of detection after 24 h; CuDMA and NiDMA concentrations dropped below the limit of detection after 96 h. Co mobilization was significant, but remained small because of its relatively low initial rate and the effects of microbial activity already set in after 8 h. The fact that FeDMA and ZnDMA were removed from soil solution before NiDMA and CuDMA can have both thermodynamic and kinetic grounds: under Santomera soil conditions NiDMA and CuDMA are more stable (i.e., have the highest product of complexation constant and activity) as predicted with equilibrium modeling.²⁷ The rate by which microbial activity affects the NiDMA and CuDMA concentrations may also be slower.

Competition. In the 100 μM DMA treatments, the cumulative amount of the DTPA-extractable metals in Santomera soil (Table 1) exceeds the amount of DMA added. Therefore, metals will compete for complexation by the DMA ligand. Conceptually, two competition effects can be distinguished in view of Fe acquisition: (1) complexation of other metals leads to a faster decline in free DMA ligand concentration, which results in a slower Fe mobilization rate compared to a situation in which no competing metals would be present; and (2) displacement of Fe from the FeDMA complex by competitive complexation of other metals leads to an actual decline in FeDMA concentration.

The second competition effect is well illustrated from the decline in FeDMA concentration after 24 h in the treatment with sterilant, while the concentrations of other metal complexes (Figure 2a) and the total DMA concentration (Figure 1a) increase. The FeDMA concentration started to decline before the free DMA ligand became depleted from solution. This can be explained from the mass law equation derived from the complexation reaction of Fe by DMA (eq 1), assuming that the FeDMA concentration only increases until the complexation equilibrium is reached. When the pH and Fe activity are considered imposed by the soil and constant, the ratio between free DMA ligand and Fe complex remains fixed once equilibrium is reached (K_C^* ; eq 1). Preservation of this equilibrium requires complex dissociation of the FeDMA complex, when the free DMA ligand concentration declines further as a result of complexation of other metals. For the time point at which the FeDMA concentration was highest ($t = 24$ h), the Fe activity in Santomera soil, and hence the solubility of the soil-Fe(hydr)oxide phase can be estimated from the ratio of the FeDMA and the free DMA ligand concentration. The pFe^{3+} (negative logarithm of the free Fe^{3+} activity) and $\text{p}K_{\text{sol}}(\text{Fe}(\text{hydr})\text{oxide})$ (negative logarithm of the Fe(hydr)oxide solubility product) were estimated from eq 1 (for K_C) and the Fe(hydr)oxide solubility mass law equation at 20.9 and 40.4, respectively. The calculated solubility is somewhat lower than the average value reported for soils ($\text{p}K_{\text{sol}} = 39.3^2$). This can be explained by the high ratio of crystalline to amorphous Fe(hydr)oxides in this soil.

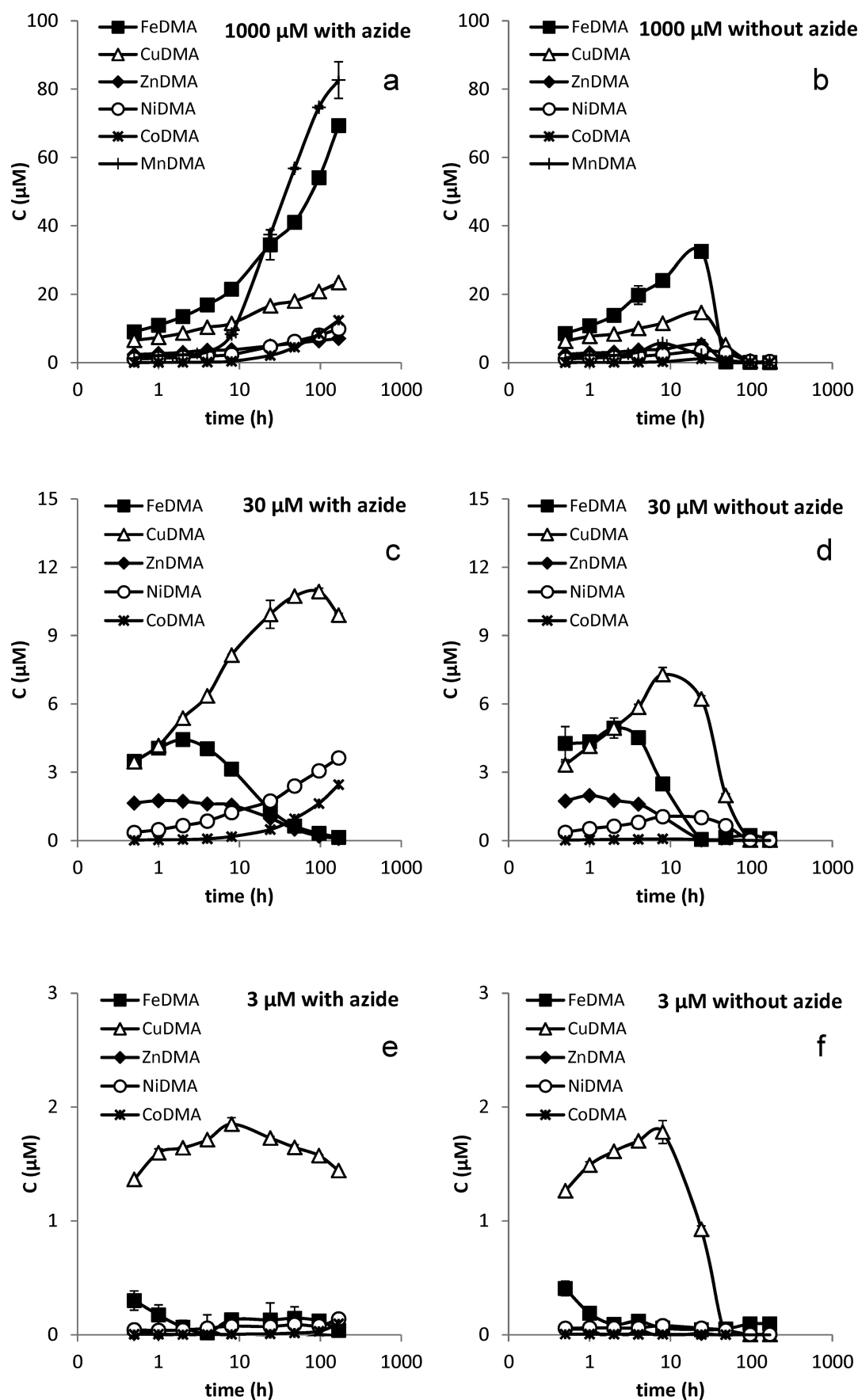


Figure 3. Metal mobilization from Santomera soil by a DMA solution of (a–b) 1000 μM , (c–d) 30 μM , and (e–f) 3 μM as a function of time (on a log scale), with and without the addition of 2 g L^{-1} NaN_3 (SSR = 1; 0.01 M CaCl_2). Error bars indicate the standard deviation.

$$K_C = \frac{(\text{FeOHDMA})(\text{H}^+)}{(\text{Fe}^{3+})(\text{DMA})}; \quad K_C^* = \frac{(\text{FeOHDMA})}{(\text{DMA})} \quad (1)$$

On the basis of the mobilized concentrations, Cu is the principal competing metal for complexation by DMA in Santomera soil (Figure 2). A strong competitive effect from Cu complexation on Fe mobilization from this soil had been previously found for the synthetic chelating agent ethylene diamine-*N,N'*-bis(hydroxy phenyl acetic acid) (EDDHA).³⁵ For this ligand the displacement of Fe from the FeEDDHA complex is surface catalyzed.³⁶

Effect of DMA Concentrations: 1000 μM DMA. To decrease the effect of competition between metals for complexation by DMA, we performed experiments with 1000 μM DMA additions. This concentration is in excess of the amount of available metals according to the DTPA-extraction (Table 1). When sterilant was added with the treatment, the concentrations of all metal DMA complexes continuously increased throughout the experiment, reaching a maximum at the final time point (168 h; Figure 3a). The increase in maximum concentration in comparison to the 100 μM treatment strongly depended on the metal; the maximum increased for CuDMA from 18.0 to 23.4 μM (30%), for NiDMA from 6.4 to 9.8 μM (53%), for ZnDMA from 4.0 to 7.1 μM (78%), for CoDMA from 6.5 to 12.4 (91%), for FeDMA from 13.5 to 69.3 μM (413%), and for MnDMA from 0.7 to 82.6 μM (11 700%). The increase in maximum concentrations was smallest for the strongest competing metals (Cu and Ni). The increase in Fe mobilization relative to the 100 μM treatment is high compared to most other metals, suggesting that high DMA concentrations in the rhizosphere are favorable for FeDMA mobilization, because competition effects are less strong. Contrary to dissolution experiments with the crystalline Fe(hydr)oxide mineral goethite and DMA,²⁰ no steady state net Fe mobilization was observed when the free DMA ligand was in large excess of the amount of metals mobilized. The ammonium oxalate extractable Fe content of Santomera soil (a measure for the noncrystalline Fe; Table 1), exceeded the amount of Fe mobilized by DMA, suggesting Fe mobilization will mainly take place from kinetically more labile phases such as organically bound Fe and amorphous Fe(hydr)oxide minerals rather than from crystalline minerals such as goethite. Possibly, heterogeneity in kinetic lability among the noncrystalline Fe-bearing phases present in the soil was so large that no steady state was reached.

When sterilant was omitted, the maximum FeDMA concentration (32.5 μM) was reached after 24 h (Figure 3b), which was later than in the corresponding 100 μM treatment (13.1 μM , after 8 h). The later drop in FeDMA concentration in the 1000 μM treatment compared to the 100 μM treatment seems to suggest that microbes are interested in the DMA as carbon sources rather than in FeDMA as Fe source. After 96 hours, removal of metal DMA complexes from solution was complete. This corresponds with the 100 μM treatment.

Effect of DMA Concentrations: 30 μM DMA. In the 30 μM DMA treatments, both with and without addition of sterilant, the maximum FeDMA concentration was reached after 2 h (4.4 μM (Figure 3c) and 4.9 μM (Figure 3d) respectively), which was earlier than in the 100 μM and 1000 μM treatments. In both treatments, FeDMA and ZnDMA were eventually entirely removed from solution. In the treatment with sterilant this was after 168 h, solely as a result of competition; in the treatment without sterilant it was already after 24 h, also as a result of

microbial activity. In the treatment with sterilant, the CuDMA concentration reached a maximum after 96 h (Figure 3c), and afterward decreased in favor of the NiDMA and CoDMA concentrations. The fraction of DMA in solution binding Cu increased in comparison to the 100 μM DMA treatments; in the treatment with sterilant, CuDMA accounted for 62% of the DMA in solution after 168 h, compared to about 40% in the 100 μM treatment.

Effect of DMA Concentrations: 3 μM DMA - Naturally Occurring Concentrations. The concentration of 3 μM DMA is in the same order of magnitude as the DMA concentrations measured in soil solution during a pot trial with wheat grown on Santomera soil.¹¹ Both in the treatment with and without sterilant, the maximum FeDMA concentration was observed after 0.5 h (0.3 μM (Figure 3e) and 0.4 μM (Figure 3f), respectively). In both treatments the Fe concentration decreased to background levels within 2 h after addition of the DMA. CuDMA was the dominant DMA species with a maximum concentration of 1.8 μM after 8 h, making Cu the principal competing cation within the relevant time frame and concentration range. In the treatment with sterilant, NiDMA (0.1 μM) and CoDMA (0.1 μM) concentrations increased toward the end of the experiment at the expense of CuDMA. In the treatment without sterilant, DMA was removed from solution after 48 h, and besides Cu and Fe no other metals were mobilized.

In Figure 4a, metal mobilization from Santomera soil 1 h after DMA addition is presented as a function of the DMA

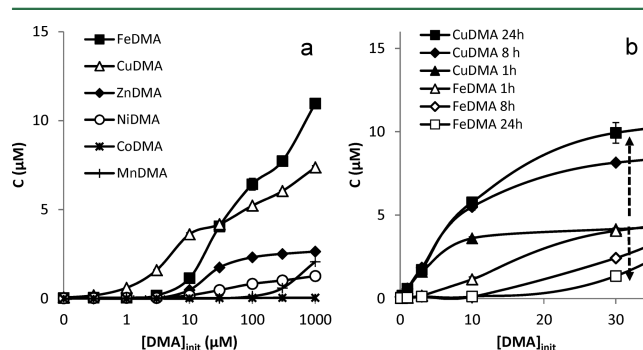


Figure 4. (a) Metal mobilization from Santomera soil as a function of DMA concentration added after 1 h; (b) CuDMA and FeDMA concentration as a function of the DMA concentration added to Santomera soil after 1, 8, and 24 h. The treatment contained 2 g L^{-1} NaN_3 and 0.01 M CaCl_2 ; SSR = 1. Error bars indicate the standard deviation.

concentration added. At low DMA concentrations, only Cu was mobilized; at DMA concentrations of 3 μM or higher Fe also was mobilized, and at 10 μM or higher Ni and Zn also were mobilized. Mn started to become mobilized only at 100 μM or higher and there was no substantial Co mobilization after 1 h. This confirms that a minimum initial DMA concentration is required to mobilize Fe for a timespan that plants can actually make use of it. Metal mobilization after 8 and 24 h is presented in SI Figure 3. As a result of further metal mobilization and metal displacement from DMA complexes, the concentration trends changed over time. For the dominant DMA species in the lower concentration range, CuDMA and FeDMA, this is illustrated in Figure 4b for initial DMA concentrations up to 30 μM . For the 10 and 30 μM DMA treatments it shows that CuDMA concentration increased at the expense of the FeDMA

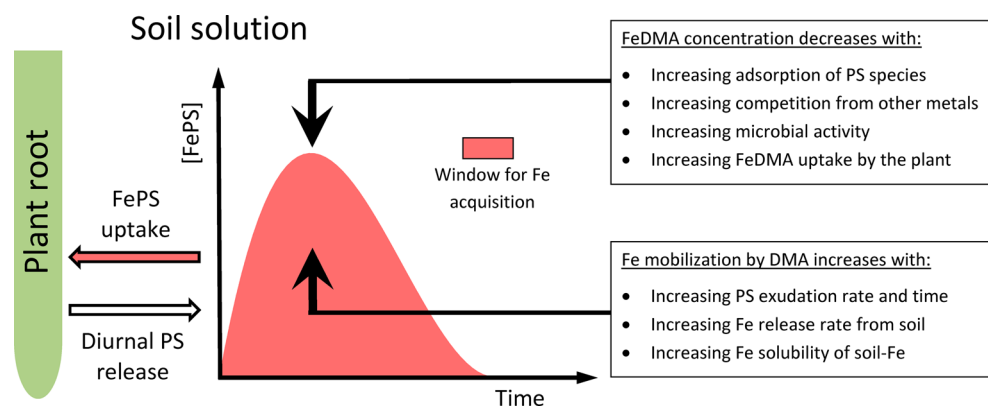


Figure 5. Window of Fe uptake in strategy II Fe acquisition. The window is defined as the integral of the FePS concentration over time. The size of this window, both in terms of time and soluble iron concentrations, is critical for iron uptake by graminaceous plants. Processes and factors increasing Fe mobilization enlarge the window, but processes and factors limiting Fe mobilization and removing FePS from solution reduce the size of the window.

concentration; for the 10 μM DMA treatment, FeDMA in solution was depleted after 8 h. This indicates that lower DMA release not only implies a lower Fe mobilization, but also a shorter timespan for which Fe is mobilized and available for plant uptake.

Time and Concentration Window of Iron Uptake in Strategy II Fe Acquisition. The diurnal exudation of PS by strategy II plants leads to the opening of a time window in which Fe concentrations increase and efficient Fe uptake by the plant can occur (Figure 5). The size of this window, both in terms of time and soluble Fe concentrations, is critical for plant iron uptake. It is constrained by various kinetically and thermodynamically controlled processes, which have either a beneficial or an adverse effect on the size of the window (Figure 5). In this context, adsorption, microbial activity, Fe mobilization, and competition from other metals for binding to the PS ligand have been considered, in relation to the PS concentration applied to the soil. The FeDMA concentration (Figure 5) increases as long as the rate of Fe mobilization by DMA is higher than the cumulative rate of processes removing FeDMA from solution; it reaches a maximum when the two rates are equal, and decreases when the rate of FeDMA removing process has become larger.

Although the adsorbed fraction of the ligand was substantial, adsorption was limiting but not preventing the mobilization of Fe by DMA; time-wise adsorption did not constrain the window of iron uptake for plants (Figure 1). Microbial activity clearly led to a depletion of DMA from solution. However, the experiments presented here do not indicate that the effects of microbial activity are rapid enough to deplete PS from solution on the time scales of plant PS exudation and FePS uptake, even if no lag phase occurs (Figure 1b). And indeed, PS at micromolar concentrations have been observed in the rhizosphere of nonsterile plants by our group, previously.¹¹

It was shown that next to microbial activity, competition from other metals may play an important role in constraining the window of Fe uptake (Figures 2 and 3), in particular at PS concentrations in the low micromolar range (Figure 3e and f), which appear to be most environmentally relevant.¹¹ With decreasing DMA concentrations the window of Fe uptake decreased both in terms of duration and in concentration (Figure 3). The maximum FeDMA concentration was both lower and reached earlier (Figure 3). This is related to the fact that with application of a lower DMA concentration, the free

DMA ligand becomes depleted earlier. In the 3 μM treatments, the window of iron uptake was solely constrained by competition from metals other than Fe for the DMA ligand (Figure 3e and f); microbial activity affected neither the concentration nor the duration FeDMA remained in solution, because free DMA was depleted before microbial activity started to affect concentrations. Clearly, this concentration level and the reduction of the window of iron uptake by competition with other metals seems relevant to plant systems: wheat plants (*Triticum* spp, cv Tamaro) grown on Santomera soil became chlorotic,¹¹ suggesting that Cu competing for complexation by DMA narrowed the window of iron uptake for Fe acquisition beyond the level of sufficiency.

The “window of Fe uptake of Strategy II Fe acquisition” is a useful conceptual framework that takes into account both the chemical kinetics (e.g., metal mobilization rates) and thermodynamics (e.g., solubility equilibria) involved in PS-mediated Fe acquisition. It is able to explain Fe mobilization by DMA, and potential Fe acquisition by Strategy II plants, from soils for which equilibrium modeling suggests this would not be possible.²⁷ Because of its conceptual nature, the model’s applicability is not limited to Santomera soil. Metal mobilization data for a second uncontaminated calcareous soil (Xeraco L) have been included in SI Figure 4 to illustrate this. In principal the model applies to any soil, although the relative importance of the individual processes may (strongly) vary, potentially leading to large variation in the size of the window. The model can help to explain how success or failure of PS-mediated Fe acquisition depends on environmental conditions, such as SSR, temperature, ionic strength, and adaptation of microbes to utilizing PS. How the relative importance of the processes drawing up the window alters when shifting from batch to natural systems including plant roots, a daily pulse release of PS, and rhizosphere conditions needs to be addressed in follow-up studies.

■ ASSOCIATED CONTENT

📄 Supporting Information

DMA structure (Figure 1), the pH-dependent speciation of Fe complexed by DMA (Figure 2), data on metal mobilization from Santomera soil after 8 and 24 h of interaction, as a function of DMA concentration (Figure 3), data on metal mobilization from Xeraco L soil by 100 μM DMA as a function of time (Figure 4), details concerning ICP-OES/MS analysis

(Table 1), and soil properties of Xeraco L soil (Table 2). This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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Notes

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