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Phytosiderophore-induced mobilization and uptake of Cd, Cu, Fe, Ni, Pb and Zn by wheat plants grown on metal-enriched soils

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ABSTRACT

We investigated to which extent phytosiderophores (PS), released by grasses for the acquisition of iron, solubilize other metals in contaminated soils, and how this affects metal mobilization and uptake in wheat plants. A plant-based bioassay ('RHIZOtest') and batch extraction scheme were carried out for assessing metal mobilisation in soil, PS exudation and metal accumulation in wheat. Increased PS exudation was observed in Fe-deficient wheat, leading to enhanced Zn, Cu, Mn and Ni concentrations in wheat shoots on some soils. In contrast, plant Cd and Pb concentrations were not affected. Likewise, in the batch experiment, strongly increased extractable Cu, Ni and Zn concentrations were observed, in particular when 100 or 1000 μM PS were added. Our results suggest that Fe deficiency can enhance the accumulation of some metals in shoots of grass species. Although our results indicate that the risk of enhanced accumulation of Cd and Pb in Fe deficient wheat shoots is rather small, further experiments conducted on soil for the complete vegetation period would be needed to confirm this observation.

1. Introduction

Phytosiderophores (PS) are root exudates released by members of the Poaceae family for the acquisition of Fe. PS mediated Fe uptake is referred to as strategy II iron acquisition (Marschner et al., 1986). Due to the high affinity of PS for other metals, they can also solubilize micronutrients like Cu, Zn and Ni (Murakami et al., 1989; Zhang et al., 1991; Awad and Römheld, 2000; Schenkeveld et al., 2014a,b). The solubilisation efficiency depends on the quantity of released PS, biogeochemical soil characteristics and the activity of microbes (Schenkeveld et al., 2014a). In the vast majority of previous studies, PS exudation rates were determined in hydroponic culture with zero Fe supply (see Oburger et al., 2014, and references therein) and only one study so far has shown that PS release rates in a soil of low Fe availability was ~50 times lower than in zero-Fe nutrient solution (Oburger et al., 2014).

Due to the PS-induced solubilisation of different metals, the bioavailability of micronutrients other than Fe can be enhanced (Cakmak et al., 1996a). On the other hand, competing metals may substantially limit the Fe solubilisation efficiency (Schenkeveld et al., 2014a). Schenkeveld et al. (2014b) investigated metal mobilization by PS from two soils that were also tested in this study (REDL, ARN A; Table 1) and found that in particular Cu and Ni, but in one soil also Zn, were quantitatively the most important elements competing with Fe for complexation by the PS 2′-deoxymugineic acid (DMA). Under conditions of metal contamination in soil, PS release of Fe-deficient plants may enhance the bioavailability, uptake and accumulation of pollutants in plants. Already some early studies in the 1990s reported on increased Cd, Ni and Zn accumulation in Fe-efficient species and cultivars with presumably higher PS release rates vs. Fe-inefficient ones (Awad and Römheld, 2000; Mench and Fargues, 1994; Römheld and Awad, 2000). Also Kudo et al. (2007) found that PS might enhance the accumulation of Cd in Fe-deficient barley. Chaignon et al.

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Table 1
Selected soil properties of the four experimental soils.

	Units	Soils			
		ARN A	ARN D	SK	REDL
MWHC[a]	$g\,kg^{-1}$	453	617	612	525
pH ($CaCl_2$)	-	7.3	6.2	5.3	6.6
$CaCO_3$	$g\,kg^{-1}$	323	1.5	4.6	0.0
Soil organic carbon	$g\,kg^{-1}$	33	25	16	14
CEC	$mmol_c\,kg^{-1}$	568	261	258	319
Fe oxides	$g\,kg^{-1}$	3.7	4.9	3.5	3.3
Sand	$g\,kg^{-1}$	506	662	743	468
Silt	$g\,kg^{-1}$	356	243	184	359
Clay	$g\,kg^{-1}$	137	95	74	173
DTPA-extractable	$mg\,kg^{-1}$				
Fe		18	45	19	20
Cu		8.3	13	16	1.0
Zn		122	627	222	0.65
Ni		0.7	1.1	0.0	30
Cd		11	11	4.1	0.0
Pb		1770	404	115	0.2
Total content[b]	$mg\,kg^{-1}$				
Cu		57	106	85	37
Zn		1040	1880	1070	75.7
Ni		18	47	21	1220
Cd		18	14	7.1	0.2
Pb		3810	1450	341	12

[a] Maximum water holding capacity.
[b] Extracted in *aqua regia*.

(2002) observed that under iron starvation the release of PS and the accumulation of Cu by wheat grown on a Cu-contaminated soil was enhanced. The release of phytosiderophores can in contrast also play a protective role: Sterckeman et al. (2005) found that in spite of strongly mobilized Cd this element was not taken up by ryegrass. Excess Cu, Ni and Zn in the growth substrate was also found to suppress the uptake of Fe (Ma et al., 1993; McBride, 2001) To further investigate the accumulation of heavy metals in Fe-deficient grasses, we conducted an extraction experiment and a plant test for addressing the following questions: 1) What is the effect of increasing DMA concentrations on metal extractability on contaminated smelter and serpentine-derived soils? 2) How does PS exudation change the mobilization and uptake of metal by wheat plants? 3) Is there a risk of increased pollutant uptake in Fe-deficient wheat when grown on such metal-rich soils?

2. Materials and methods

2.1. Soil and plant characteristics

Four soils were used in the experiments described below. Two soils originated from Arnoldstein (ARN A and ARN D), Austria, and one from Banská Štiavnica (SK), Slovakia. These soils were classified as Cambisols and the A horizons (0–20 cm) were sampled. They show high content of Zn, Cd, Pb and moderately elevated concentrations of Cu due to atmospheric deposition from metal smelter activities over several hundred years. The fourth soil was sampled from the A horizon of a serpentine soil near Redlschlag (REDL), Austria, which is classified as Eutric Leptosol (Wenzel and Jockwer, 1999). The soils were characterised according to Blum et al. (1996) and selected properties (partly taken from Puschenreiter et al., 2013) are shown in Table 1. All soils were air-dried, passed through a 2-mm sieve and stored under dry and dark conditions until further use.

Common wheat (*Triticum aestivum* cv. Tamaro) was chosen as experimental plant. The seeds originated from Nyon, Switzerland (Département fédéral de l'économie DFE Station de recherche Agroscope; Changins-Wädenswil ACW Département de recherche en Amélioration des plantes et ressources génétiques).

2.2. Mobilization of trace elements by DMA

Solutions containing different concentrations of in-house synthesized DMA (Division of Organic Chemistry, University of Natural Resources and Life Sciences Vienna) were added to the four experimental soils in increasing concentration: 0, 0.1, 1, 10, 100 and 1000 μM DMA. For all extractions, 10 g of air-dried soils were weighted into 50 mL polyethylene centrifuge vials and 10 mL of extraction solution was added. The extraction solution included 10 mM $CaCl_2$ serving as background electrolyte as well as 2 $g\,L^{-1}$ of NaN_3 to avoid microbial degradation during extraction period of 4 h. Samples were placed in end-over-end shaker rotating in the dark at 18 rpm. The batch experiments were carried out in duplicates. For all treatments the suspension was centrifuged at 4500 rpm (4.0 10^3 *g*) for 10 min, filtered through 0.45 μm syringe filters (Aquatron, Whatmann) and split into aliquots for analysis. Samples were acidified with nitric acid prior to analysis. Fe, and for certain samples Zn concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300 DV, Perkin Elmer, Waltham, US), whereas Ni, Cu, Cd, Pb and partly Zn concentrations were analysed by inductively coupled plasma mass spectrometry (ICP-MS, 7700x, Agilent Technologies, Waldbronn, Germany).

2.3. RHIZOtest

The RHIZOtest is a standardized approach according to Chaignon and Hinsinger (2003) for assessing rhizosphere characteristics and element fluxes into plants. This bioassay is also available as ISO norm (ISO, 2015). In this approach, the plants were grown in specifically designed units, in which the plants developed a planar mat of roots on the surface of a polyamide mesh. Initially, the plants were grown in nutrient solution and later transferred onto a ~3 mm thick soil layer, that was separated from the root mat by the mesh and connected to a reservoir of nutrient solution via a filter paper wick. A detailed description can be found in Chaignon and Hinsinger (2003).

All used materials were washed in 10% (v/v) HNO_3 acid solution and finally rinsed with laboratory water type 1 (0.055 $\mu S\,cm^{-1}$, TKAGenPure, Thermo Electron LED GmbH, Niederelbert, Germany) before use. The RHIZOtest was conducted under controlled environmental conditions in the greenhouse with an average day/night temperature of 27/20 °C and a 16 h photoperiod at 500 $\mu mol\,m^{-2}\,s^{-1}$ (photosynthetically active radiation).

During the first stage, wheat seedlings were grown under hydroponic conditions. The seeds were surface sterilized with 6% (v/v) H_2O_2 for 10 min before they were placed into cylindrical containers (inner diameter: 34 mm), which were closed at the bottom with a nylon mesh (mesh size 30 μm, SEFAR 03-30/18) that allowed the development of a dense and planar root mat. The containers were floating in 6-L buckets filled with continuously aerated nutrient solution. During the first week, seeds germinated in a nutrient solution containing 600 μM $CaCl_2$ and 2 μM H_3BO_3. For the first three days the buckets were covered with aluminium foil to avoid inhibited growth caused by intense radiation. After root development, acid washed PE-LD microgranulars (Lyondell Bassell PELD, Lupolen, Ehnus Mainzer GmbH, Germany) were placed in the plant containers to protect roots from light radiation.

After seven days, plants were subjected to different nutritional growth conditions. Part of the wheat plants were grown in a complete nutrient solution at pH 5.5 (sufficient Fe supply; +Fe) consisting of 500 μM KH_2PO_4, 2000 μM KNO_3, 10 μM H_3BO_3, 1 μM $ZnSO_4$, 1 μM $CuCl_2$, 2 μM $MnCl_2$, 0.05 μM Na_2MoO_4, 1000 μM $MgSO_4$, 2000 μM $Ca(NO_3)_2$ and 100 μM NaFe(III)EDTA. The other half was grown in the same solution but without 100 μM NaFe(III) EDTA (deficient Fe supply; −Fe). All reagents were analytical grade

and the experimental solutions as well as dilutions of samples for analytical measurements were conducted with high-purity water (Elix3® Millipore). The nutrient solutions were renewed every third day. After two weeks of plant growth under hydroponic conditions, five replicates of each treatment were collected and analysed (see below) to determine exudation rates and plant nutritional status prior to soil contact as a reference point.

For the soil stage of the RHIZOtest, the plant containers were brought in contact with soils by transferring them onto soil discs (3–4 mm thickness, 40.5 mm diameter) filled with 4.5 g soil (dw). During the soil stage, the following nutrient solution (i.e. soil contact solution) was provided to the soil via a filter paper wick: 50 μM KH_2PO4, 2000 μM KNO_3, 2000 μM $Ca(NO_3)_2$ and 1000 μM $MgSO_4$. The soil contact solution was exchanged every second day. Five unplanted replicates for each soil and treatment served as control (=bulk soil). Before starting plant exposure, soils were incubated for 24 h in darkness at 20 °C with the soil contact solution for equilibration at 70% of MWHC. After ten days plants and soils were separated for further analysis.

2.4. Collection and quantification of phytosiderophores

After the hydroponic and the soil stage and prior to plant harvest DMA release rates were determined. Therefore, two hours after the onset of the light period roots were washed for 1 min with deionized water and transferred into 100 mL vials containing 50 mL deionized water with 0.01 g L^{-1} of Micropur (Katadyn Products Inc, Switzerland) for preventing biodegradation of DMA during the sampling period. After a 4-h collection period plants were harvested and the hydroponic solution was filtered through 0.45 μm syringe filters (Rotilabo®, Carl Roth GmbH). For DMA analysis, an aliquot (15 mL) of the collected hydroponic solution was mixed with 0.5 mL of an internal standard solution containing 10 μM 13-C DMA (Walter et al., 2014) and 3% formic acid. Samples were freeze dried (Christ Alpha 1-2 LO; −50 °C; 0.06 mbar), then re-dissolved in 1.5 mL LC–MS CHROMASOLV® water (Fluka, Sigma-Aldrich GmbH, Germany) to gain a final concentration of 3.33 μM 13-C DMA concentration. The DMA measurement was conducted by liquid chromatography electrospray ionization tandem mass spectrometer (LC-ESI–MS/MS; Agilent 6410 Triple Quad LC/MS) according to Schindlegger et al. (2014). The remaining hydroponic solution was stored in the freezer (−20 °C) before dissolved organic carbon (DOC) was measured using a TOC analyser (Elementar Vario TOC cube).

2.5. Plant and soil analyses

After collection of DMA, roots and shoots were rinsed separately with deionized water. Shoots and roots were oven dried at 60 °C until constant weight was reached. Subsequently, root and shoot biomass were determined by weighing. After grinding and homogenization in a stainless steel mill (MF 10, IKA® Werke, Staufen, Germany), subsamples of 0.03–0.2 g were digested in 4 mL HNO_3 (puriss. p.a., Sigma-Aldrich Handels GmbH, Vienna, Austria) and 1 mL H_2O_2 (puriss. p.a., Sigma-Aldrich Handels GmbH, Vienna, Austria) at 225 °C using a microwave (Multiwave 3000, Rotor 64 MG 5, Anton Paar) and then analysed for metal concentrations by ICP-MS (Elan 9000 DRCe, Perkin Elmer). Metal acquisition by plants during the soil stage was calculated by multiplying metal concentrations with the plant biomass (dw) at the end of the experiment, diminished by the amount of metals already present at the start of the soil stage.

Planted (rhizosphere) and unplanted soils were collected at harvest and the water content was determined subsequently by drying a subsample at 105 °C for 24 h. Soil (4.5 g) was weighed in 50 mL polyethylene centrifuge vials and 10 mL of the soil contact solution was added. The soil suspension was then placed on an end-over-end shaker (20 rpm) for 2 h. Afterwards, samples were centrifuged at 3000 × g for 15 min and the supernatant was filtered through 0.45 μm syringe filters (Rotilabo®, Carl Roth GmbH). For DMA analysis, samples were split into aliquots and 0.25 mL internal standard (^{13}C DMA) was added as described above. DMA and metal concentrations were analysed as described above. In the remaining soil extracts pH (Thermo Scientific Orion3Star pH Benchtrap) was determined.

2.6. Statistical analyses

Statistical analyses of the extraction assay were done using the statistical software package R (R Core Team, 2015). First, all extractable metal concentrations were log-transformed on the basis of e. Then, a weighted analysis of covariance (ANCOVA) was performed individually for each metal with a factor 'Soil' consisting of four levels (ARN A, ARN D, SK, and REDL) and 'DMA concentration' as covariate. Additionally, interactions were incorporated in the ANCOVA model to allow for individual, soil-specific slopes of the DMA concentration. The ANCOVA was performed using the R package 'car' (Fox and Weisberg, 2011). To compensate for the heteroscedasticity of the data the within group variances at each DMA concentration were taken as weights. All post-hoc tests were done by multiple comparisons using the R package 'multcomp' (Hothorn et al., 2008). The simultaneous inference procedures used therein adjust for multiplicity and thus control the overall type I error rate. Statistical analyses on the RHIZOtest results were performed with IBM SPSS Statistics 21. The significance of differences between the means of the treatments was evaluated by the student's *t*-Test ($p < 0.05$) Here, two levels of significance were applied: $0.05 \leq p < 0.1$ (indicated by +) and $p < 0.05$ (indicated by *).

3. Results

3.1. Rhizotest experiment

Release rates of total C and DMA were determined in the hydroponic stage and after the soil contact period (Fig. 1). Generally, the DMA exudation rates clearly exceeded previously reported values (see Oburger et al. (2014) and references therein). The largest exudation rate was found for −Fe plants on soil ARN D (2.37 nM DMA g root dw^{-1} s^{-1}). The DMA release contributed considerably to the total exuded carbon (max. 38.0%, −Fe on ARN D). In the Fe deficient treatment, total C exudation rates were significantly ($p < 0.1$) higher in ARN D and SK than in Fe sufficient treatments, whereas the DMA release was enhanced in ARN D ($p < 0.05$) and REDL ($p < 0.1$). For the other soils and the hydroponic stage no significant differences in C and DMA exudation were found between Fe-deficient and Fe-sufficient wheat plants. In spite of some significant increase in DMA and total C exudation by Fe-deficient plants the extractable DOC concentrations in the rhizosphere soils were not different between Fe treatments (data not shown).

Visual Fe deficiency symptoms (leaf chlorosis) were observed for the −Fe plants already after two weeks growth under Fe deficiency during the hydroponic stage. At the end of the experiment, shoot biomass was significantly lower in the −Fe treatment for ARN D, SK ($p < 0.05$) and REDL ($p < 0.1$), whereas for roots only on soil ARND smaller biomass ($p < 0.1$) was observed on Fe-deficient plants (Table 2). Metal concentrations in shoots and roots are shown in Fig. 2. Shoot Fe concentrations were higher in plants grown on Fe-sufficient solution before soil exposure, but only remained significantly higher in the shoots exposed to ARN A and ARN D, and in the roots growing on ARN A. In contrast, the

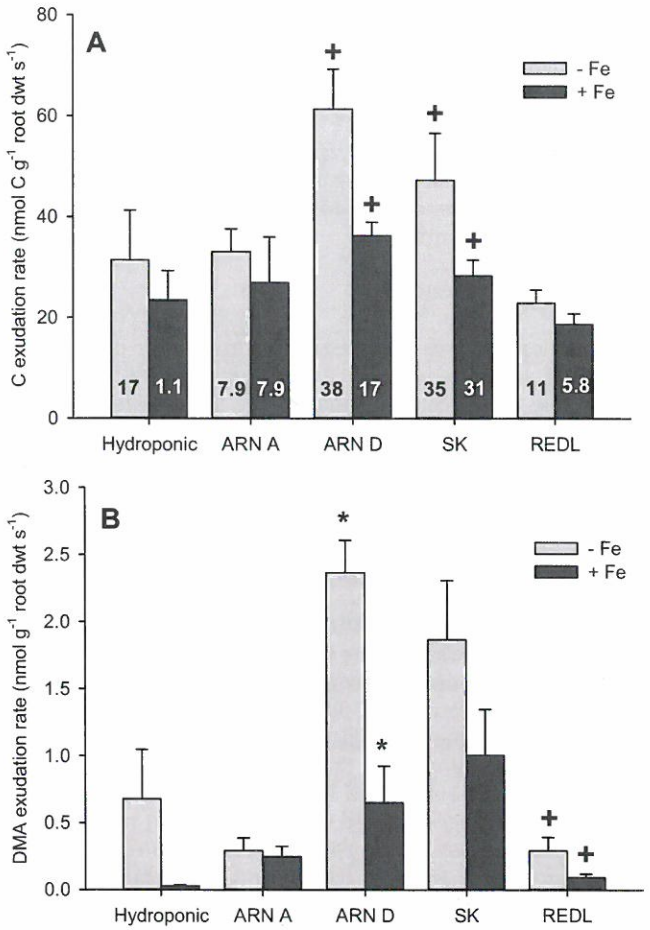

Fig. 1. Total root exudation, expressed as the amount of carbon released by roots, (A) and DMA exudation (B) of wheat plants during the hydroponic stage and during the soil contact period. The relative amounts of DMA in the total released exudate are given as percentages. Error bars indicate the standard error of the mean (n = 5). Significant differences between the −Fe and the +Fe treatment are indicated by the following symbols: +$p < 0.1$, * $p < 0.05$.

concentrations of shoot Zn were enhanced in Fe-deficient plants grown on ARN A ($p < 0.05$) and REDL ($p < 0.1$) and in the plants of the hydroponic stage ($p < 0.05$), while Cu shoot concentrations were only increased in Fe-deficient plants on ARN D ($p < 0.1$). For the other soils only a non-significant tendency of enhanced concentrations was observed. Ni concentrations in the shoots of −Fe plants were increased during the hydroponic stage. After the soil stage, only Ni concentration in roots was significantly ($p < 0.1$) higher in the −Fe treatment on the REDL soil. Another micronutrient affected by Fe deficiency was manganese: In all soils except SK Mn concentration in wheat shoots was enhanced under iron-limited conditions (data not shown). For Cd and Pb no changes in tissue concentrations were detected before and after soil exposure (Fig. 2).

The acquisition of metals during the soil contact period is shown in Fig. 3. Although on certain soils (ARND and REDL) DMA exudation by Fe-deficient plants was enhanced, the Fe acquisition was only marginally significantly only for ARN D ($p < 0.1$) larger in plants provided with sufficient Fe. The mobilisation and uptake of metals on soil REDL was reflected by a significantly ($p < 0.05$) larger Cu acquisition by Fe-deficient plants, whereas Ni acquisition on the same soil followed the opposite pattern ($p < 0.1$). For the other metals no clear trend was observed.

Differences in metal uptake were not reflected in the extractable metal fractions in the corresponding rhizosphere soils, except for Cu and Ni in REDL, for which the concentrations were higher in soils exposed to +Fe plants compared to the unplanted controls (data not shown). Differences in soil pH between rhizosphere and bulk soil were not found for any soil or treatment (data not shown). DMA concentrations in soil extracts were below the LOQ.

Table 2
Root and shoot biomass (g) of the harvested plants after the hydroponic and after the soil contact stage (mean ± standard deviation; n = 5). Significant differences between the −Fe and the +Fe treatments are indicated by the following symbols: +$p < 0.1$, * $p < 0.05$.

	−Fe	+Fe	−Fe	+Fe
	shoots		roots	
Hydroponic	0.12 ± 0.04	0.12 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
ARN A	0.43 ± 0.10	0.50 ± 0.12	0.17 ± 0.06	0.19 ± 0.11
ARN D	0.28 ± 0.04	0.48 ± 0.10*	0.08 ± 0.04	0.20 ± 0.08+
SK	0.30 ± 0.10	0.37 ± 0.08*	0.09 ± 0.06	0.13 ± 0.01
REDL	0.30 ± 0.06	0.43 ± 0.08+	0.10 ± 0.05	0.11 ± 0.03

3.2. Extraction experiment

Extractable metal concentrations solubilized by different DMA concentrations are shown in supplementary Table S1 and Fig. 4. For all metals except Cd a significant influence of the DMA addition on metal extractability was found (Table 3). Furthermore, the ANCOVA confirmed that – except for Cd and Cu – the effect of DMA addition was significantly different for the tested soils (Table 3). In general, DMA concentrations of 0.1–10 μM had no or very little effect. Increased extractability was observed upon addition of 100 μM and, to the largest extent, 1000 μM. In the 1000 μM DMA treatment, Zn, Cu and Fe were substantially mobilized, with the smallest effects on Cu and Zn in REDL and on Zn in SK. A strong mobilization of Ni was found for all soils, but particularly in the serpentine-derived REDL soil when adding 100 or 1000 μM DMA. Mobilization of Cd and Pb was not observed or marginal, even in the 1000 μM treatment. Fig. 5 shows the relative metal distribution relative to the sum of the six measured elements as affected by the DMA concentration in the extraction solution. In the mildly acidic soils ARN D and SK, Zn was the dominating metal (>95%) in treatments without DMA addition, but its relative abundance decreased with increasing DMA concentration, mainly due to the increased solubility of Cu and to a lesser extent also of Fe. In the alkaline soil ARN A, Zn and Cu were the most abundant and Fe and Pb the second most abundant metals at low DMA concentrations. The relative proportion of Zn increased with increasing DMA addition to 84%. At up to 10 μM DMA also Cu was enhanced, but when DMA concentrations further increased its proportion also decreased to 11%. In the serpentine REDL soil, Ni was by far the dominating metal and only after 1000 μM DMA the relative proportion of Fe increased. Like for ARN A, the relative amount of extractable Cu was largest after the addition of 10 μM DMA.

4. Discussion

PS are mainly released for the acquisition of Fe, but they also solubilize Cu, Zn, Ni and to a lesser extent other metals (Murakami et al., 1989; Zhang et al., 1991; Awad and Römheld, 2000; Schenkeveld et al., 2014a,b). In this way, PS can also contribute to improved uptake of Zn and Cu under conditions of Zn and Cu deficiency (Cakmak et al., 1996a,b; Gries et al., 1998). Fe acquisition by Strategy II plants relies on a time and PS concentration-dependent 'window of Fe acquisition' during which PS may increase the Fe concentration in soil solution and facilitate Fe uptake. (Schenkeveld et al., 2014a). This window is constrained by

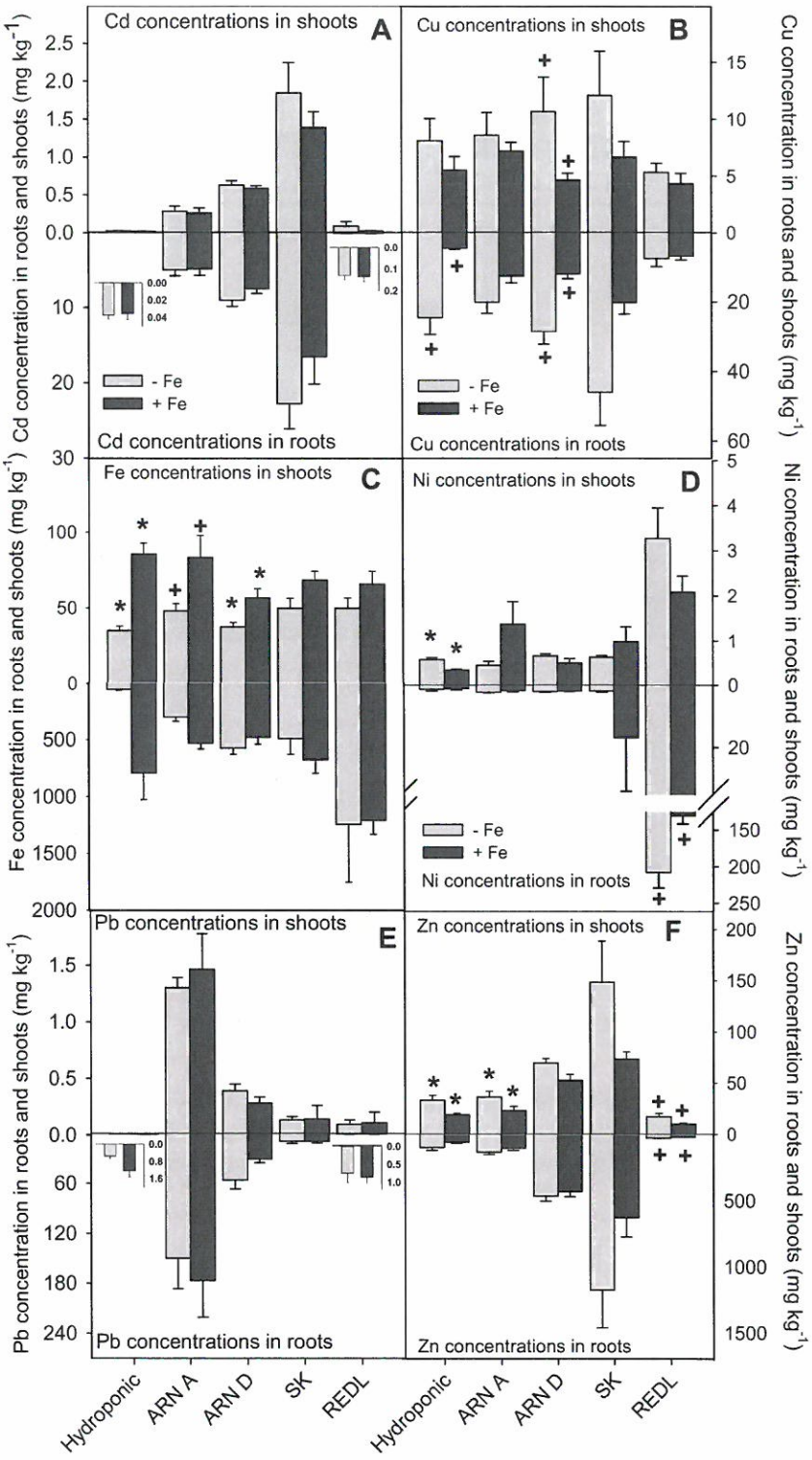

Fig. 2. Metal concentrations in wheat shoots and roots. Error bars indicate the standard error of the mean (n = 5). Significant differences between the −Fe and the +Fe treatment are indicated by the following symbols: +$p < 0.1$, * $p < 0.05$.

the PS exudation rate (Oburger et al., 2014), adsorption of PS and metal-PS complexes (Walter et al., 2016), microbial degradation of PS (Oburger et al., 2016) and the availability of metals that compete for complexation by PS (Schenkeveld et al., 2014a,b). Consequently, in contaminated soils, where specific metals are present in much larger concentrations compared to unpolluted soils, the competition effects may further limit the Fe solubilisation efficiency, potentially to the extent that Strategy II Fe acquisition fails (Schenkeveld et al., 2014b). Furthermore, the accumulation of metal pollutants might be enhanced in crops of the Poaceae family due to PS-facilitated mobilization. The experimental soils tested in this study were contaminated with Cd, Pb and Zn or naturally enriched with Ni. Indeed, we observed a (partly) significantly enhanced accumulation of Zn in Fe-deficient wheat. Also for non-significant differences the tendency was likewise. Cd and Pb were, however, not affected. Increased root Ni concentrations ($p < 0.1$)

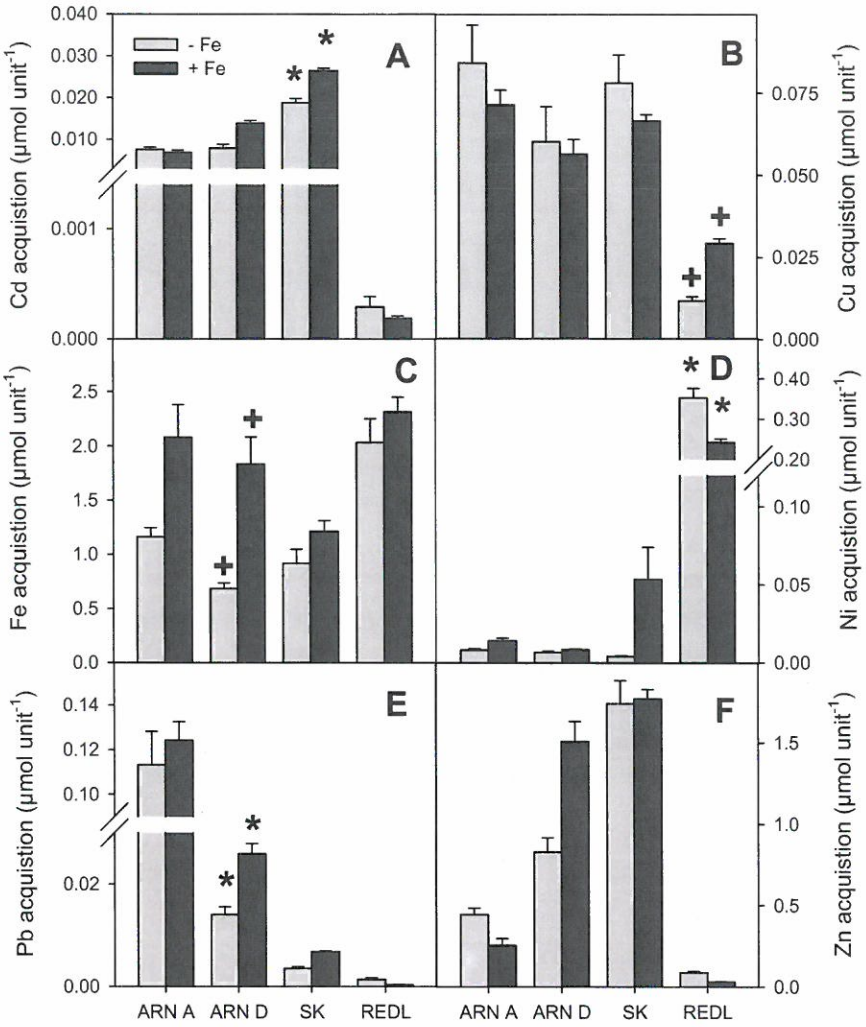

Fig. 3. Metal acquisition by wheat plants during the soil contact period. Error bars indicate the standard error of the mean (n = 5). Significant differences between the −Fe and the +Fe treatment are indicated by the following symbols: +$p < 0.1$, * $p < 0.05$.

and a non-significant tendency for enhanced Ni shoot concentrations were observed for the REDL soil.

DMA concentrations in soil extracts were too low and could therefore not directly be measured. However, the comparison of the metal concentration differences in wheat shoots between the −Fe and the +Fe treatments and the increases of metal extractability with increasing DMA concentrations suggests that the DMA concentrations in the RHIZOtest soils were presumably in the range of 10 µM. However, it is also possible that even higher (i.e. close to 100 µM) PS concentrations might have occurred (e.g. near root tips), but consequent higher metal extractability was not leading to likewise increased shoot metal concentrations. This might be related to differences in the increase of metal solubility and the uptake of the different metal-PS complexes that varies between different cell membrane transporter types (Araki et al., 2011).

DMA exudation rates in absolute amounts and also as the relative fraction among total exuded carbon were much higher compared to the reported values from soil grown wheat in Oburger et al. (2014), which was probably caused by metal toxicity. Previously, Kudo et al. (2013) reported an increase of PS release by barley when exposed to higher Cu concentrations in the growth medium; likewise, Meda et al. (2007) found a 7-fold increase of DMA release by hydroponically grown maize upon exposure to cadmium. In line with our findings the authors reported no protective chelation effect by DMA due to the weak nature of the Cd-DMA complex. However, they could show that Cd had a negative effect on Fe-DMA uptake rates that in turn triggered Fe deficiency responses. This suggests that, based on the soil extractions with planted and unplanted experimental soils (data not shown), the higher Cd solubility in the soils ARN D (50.0 µg kg^{-1}) and SK (143 µg kg^{-1}) compared to the other soils (<15 µg kg^{-1}) may have been at least partly responsible for high DMA exudation rates observed in these soils. Despite about 3 times higher soluble Cd concentrations in SK, the relative share of Cd of the total soluble metal pool was smaller in SK compared to ARN D (Fig. 5). This suggests that the presence of high concentrations of Zn might alleviate some of the inhibitory effect of Cd on DMA-Fe uptake.

Other factors like salt stress have also been suggested to increase the DMA release, as reported by Oburger et al. (2014) and Daneshbakhsh et al. (2013). Furthermore, PS exudation rates have been found to decrease with increasing plant age, which further has to be considered when comparing results from different studies. The exudate sampling approach itself may additionally have contributed to the high exudation rates, as previously demonstrated by Oburger et al. (2014). The higher exudation rates would support the assumed DMA concentrations (~10 µM) in the RHIZOtest soils, which were probably higher than previously reported. Oburger et al. (2014) found DMA concentrations in

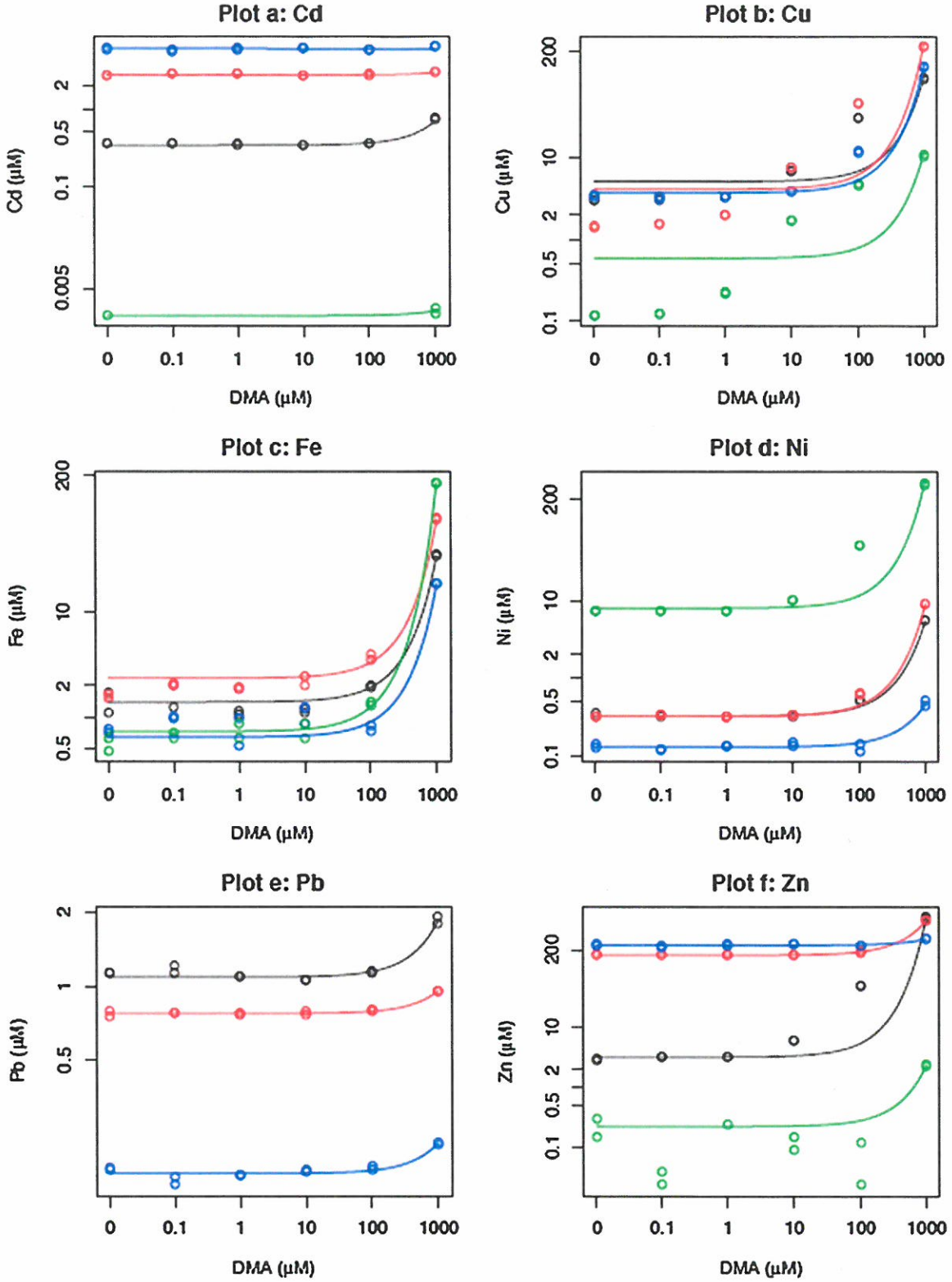

Fig. 4. Effect of increasing DMA concentration on metal extractability. Measured concentrations are shown as dots, whereas the estimated parameters of the weighted ANCOVA model were used to draw soil-specific regression lines. The results of the post-hoc tests are shown in Table 3. The soil-specific data are shown in black for ARN A, red for ARN D, blue for SK and green for REDL. Pb concentrations in REDL soil were below the limit of quantification (0.002 µM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

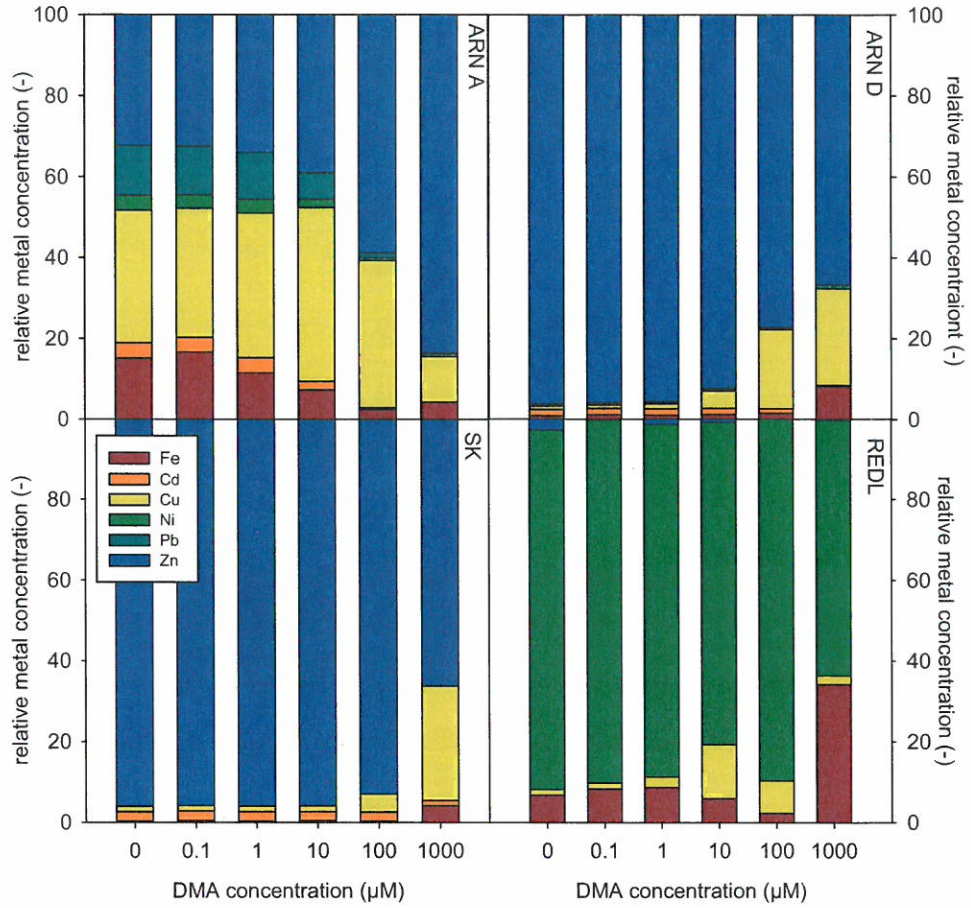

Fig. 5. Effect of different DMA concentrations on the relative amount (in µM) of extractable metal concentrations. For the soils ARN A and REDL relative metal distribution data were already previously published in Schenkeveld et al. (2014b).

rhizosphere soil solution of wheat grown in an uncontaminated calcareous soil in the lower µM range.

In spite of the high DMA release rates, the Fe concentrations in shoots were within or even below the critical range (50–150 mg kg^{-1}) of Fe tissue concentrations. This suggests that the plants were struggling to acquire sufficient amounts of Fe when exposed to the metal-enriched soils. As shown before, competing metals may strongly limit the Fe mobilization efficiency of PS (Schenkeveld et al., 2014a). Schenkeveld et al. (2014b) showed that in the ARN A soil most of the DMA was complexed with Zn and Cu and only a very little fraction could form the Fe-DMA complex. Likewise, most of the DMA added to the REDL soil was complexing Ni and Cu and again very little Fe. These observations were confirmed by the results of the extraction assay. With no or little DMA concentration Zn, Zn and Cu, or Ni (depending on the soil) were the dominating mobilized metals and only at higher DMA concentrations the Fe mobilization was substantial (Fig. 5). This corroborates the hypothesis presented by Schenkeveld et al., 2014b; who showed that Fe mobilization efficiency is limited by competing metals, but would increase with higher DMA concentrations/exudation rates. Our results clearly showed that in metal-enriched soils this competition effect is strongly reducing the Fe solubilisation efficiency, which can partly be compensated by increasing DMA release.

The acquisition of metals during the soil stage was, with a few exceptions, not different between −Fe and +Fe plants. The significantly higher Fe acquisition in +Fe plants exposed to ARN D despite higher PS release rates in the −Fe plants may indicate that Fe deficient plants are more sensitive to the inhibitory effect of Cd on DMA-Fe uptake. Due to the larger root mass and surface area, the plants could take up more Fe per RHIZOtest unit and thus compensate the likely lower Fe mobilisation, as indicated by the smaller DMA exudation rates of +Fe plants. The higher Cu concentrations in +Fe plants exposed to REDL soil might be related to the observed relative increase of Cu mobilization in the 10 and 100 µM DMA treatments (Fig. 5). The increase of Cd concentrations in +Fe plants grown on SK soil might have been caused by the tendentiously higher root biomass. In this case, other exudates than DMA might have mobilized more Cu and Cd in the +Fe treatment compared to the −Fe plants. Ni acquisition in plants exposed to REDL soil was significantly higher in Fe-deficient plants, which is corresponding well to the higher DMA exudation rates of −Fe wheat.

In spite of the (partly) enhanced trace element concentrations observed in Fe-deficient wheat shoots, the elements that might primarily cause health effects for humans, i.e. Cd and Pb, were not affected. The shoot Pb concentration on all soils was within the background range for unpolluted soils, whereas shoot Cd on soils ARN A, ARN D and in particular on SK were clearly above background concentrations (Kabata-Pendias, 2011). Nevertheless our results demonstrate that an additional increase of the risk of enhanced transfer of Cd and Pb into the human food chain due to plant Fe deficiency is unlikely, unless the excluder mechanisms are affected by severe metal toxicity (Baker, 1981). The elements that were mainly affected by Fe deficiency, i.e. Fe, Zn, Mn and Cu, are important micronutrients for animals and man, thus conditions of (slight) Fe deficiency might even lead to the biofortification of these elements.

Table 3
Results of the post-hoc test performed after the ANCOVA analysis of the data from the extraction experiment. Homogeneous subgroups separated by the factor 'Soil' are indicated by small letters, whereas homogeneous subgroups separated by the interaction of soil and DMA concentration are indicated by capital letters.

Metal/Soil	Factor Soil	Factor DMA*Soil
Cd		
ARN A	a	A
ARN D	b	B
SK	c	B
REDL	d	B
Cu		
ARN A	a	A
ARN D	a	A
SK	a	A
REDL	b	A
Fe		
ARN A	a	A
ARN D	b	B
SK	c	AB
REDL	c	C
Ni		
ARN A	a	A
ARN D	a	AC
SK	b	B
REDL	c	C
Pb		
ARN A	a	A
ARN D	b	B
SK	c	B
Zn		
ARN A	a	A
ARN D	b	B
SK	c	C
REDL	d	D

5. Conclusions

Our results obtained from the extraction assay confirmed that PS can mobilize a substantial amount of metals other than Fe when present in excess in contaminated soils. Depending on the soil characteristics and the metals present, the main competing metals were Zn, Ni and Cu. Substantial amounts of Fe were only mobilized at higher (>100 μM) DMA concentrations. The plant experiment, performed using the RHIZOtest approach, also showed that the mobilization of other metals than Fe may increase the uptake of Zn, Cu, Mn and Ni in Fe-deficient wheat plants when exposed to metal-rich soils, but the relative increase rates remained rather low. Other contaminants, i.e. Cd and Pb, were hardly affected and no concentration increase in wheat shoots was found, suggesting that increased transfer of these potentially toxic elements to the food chain is apparently not enhanced in iron-deficient wheat. However, long-term experiments on soil would be required for confirming this observation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2017.03.011.

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