Interaction of Root Exudates & Humic Ligands with Heavy Metal Uptake and Sequestration by Plants

Teresa W.-M. Fan, Fabienne Baraud, and Richard M. Higashi,
University of California, Davis

INTRODUCTION

Intracellular production and root exudation of metal ion ligands (MIL) are keys to metal ion mobilization and uptake by vascular plants, or conversely how they might help sequester metals in soil. This is well-exemplified by the exudation of phytosiderophores such as mugineic acid and derivatives in the acquisition of Fe (III) and Zn (II) by graminaceous plants. However, the genetic and biochemical mechanism(s) for the mobilization and sequestration of most pollutant metal ions in soils remain unknown, due largely to a lack of knowledge in MIL involved in the process. This information is critically needed (see Linkage to Site Needs) for developing and engineering plant-mediated remediation of metal contamination at DOE, DOD, and other industrial facilities.

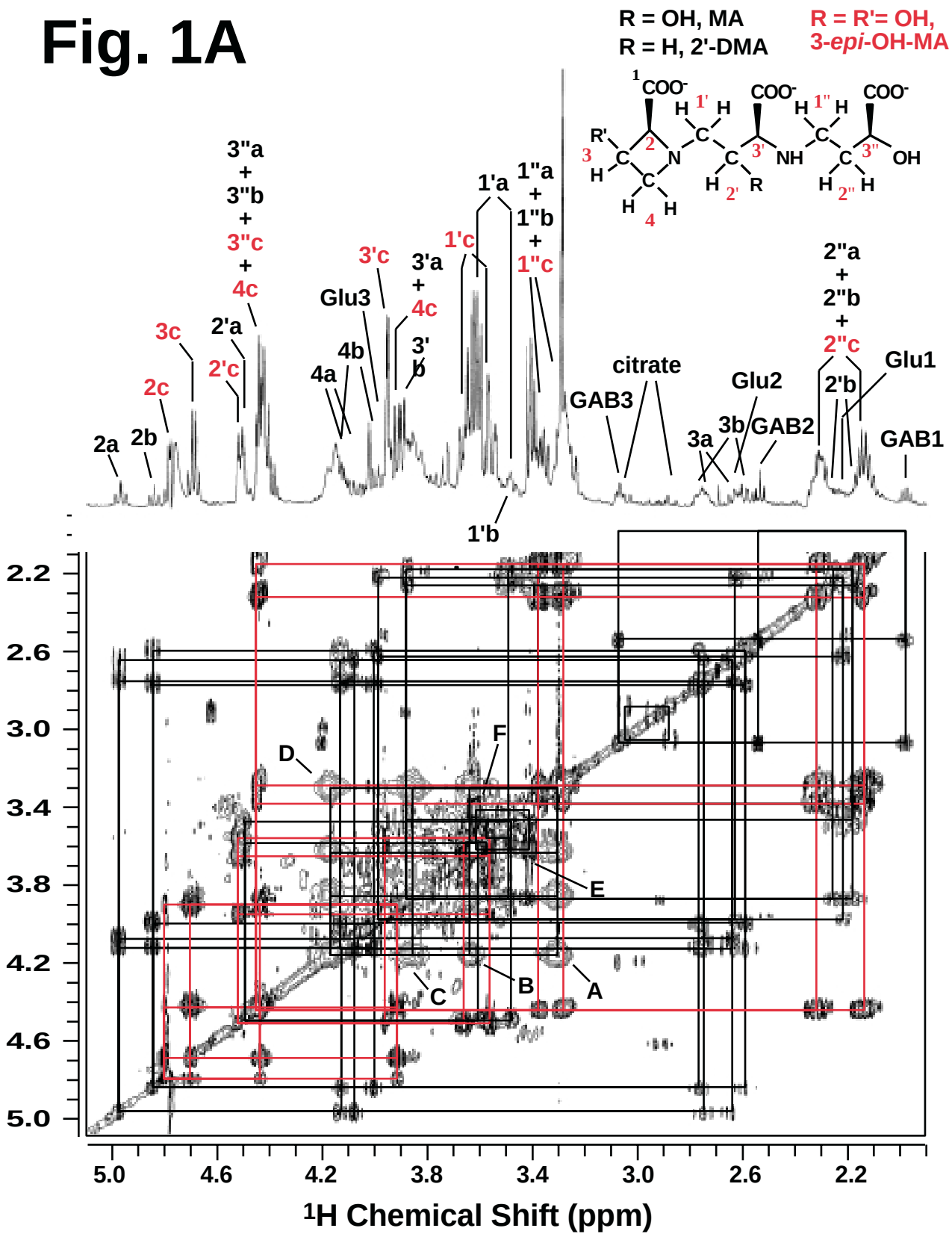
OBJECTIVES

(1) Determine major MIL involved in heavy metal uptake and sequestration by plants;
(2) Characterize genotypic variations in metal sequestration and MIL profiles to acquire clues to their genetic basis;
(3) Examine interaction of plant types, soil ligands, and microbes in effecting plant-mediated sequestration in soils.

In order to define major organic ligand composition in plant root exudates, tissues, and soils without *apriori* knowledge, we employed multi-nuclear/multi-dimensional NMR, pyrolysis GC-MS, and peptide gel electrophoresis. The comprehensive ligand information from NMR can be used to "calibrate" the more sensitive GC-MS for quantifying biogenic ligands directly in small quantities of rhizospheric soils.

We have utilized these techniques to probe the role(s) of phytosiderophores and other MIL, including soil humates, in the acquisition of Cd and transition metal ions by barley, various genotypes of wheat, and rice plants.

Fig. 1A


R = OH, MA
R = H, 2'-DMA
R = R'= OH,
3-*epi*-OH-MA
2a
2b
2c
3c
2'a
2'c
3"a
+
3"b
+
3"c
+
4c
4a
4b
Glu3
3'c
3'a
+
4c
3'
b
1'c
1'a
1'b
1"a
+
1"b
+
1"c
GAB3
citrate
3a
3b
Glu2
GAB2
2"a
+
2"b
+
2"c
2'b
Glu1
GAB1
A
B
C
D
E
F
2.2
2.6
3.0
3.4
3.8
4.2
4.6
5.0
^{1}H Chemical Shift (ppm)

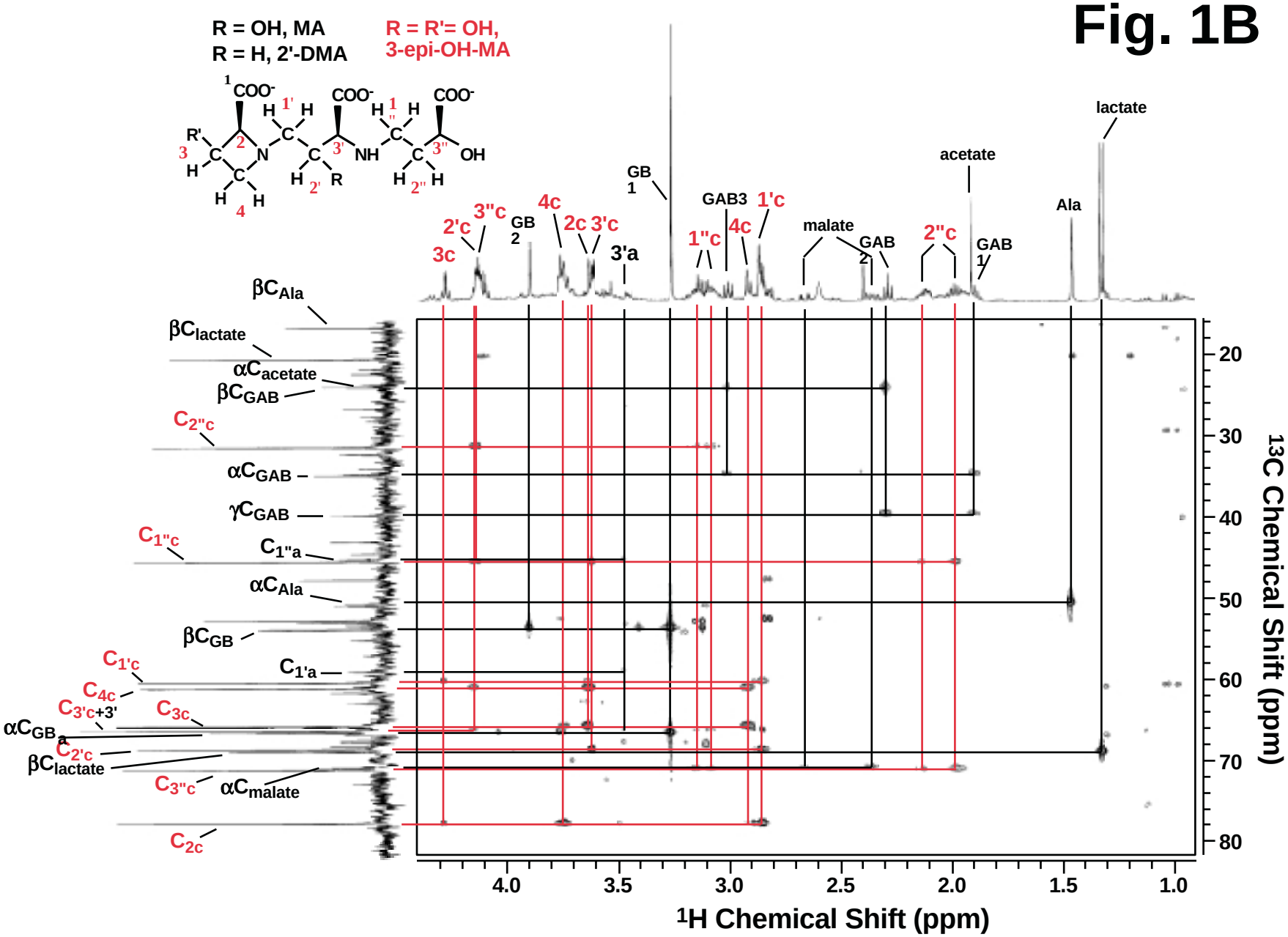


Fig. 1B
R = OH, MA
R = H, 2'-DMA
R = R'= OH,
3-epi-OH-MA
COO-
OH
NH
R'
R
GB 1
GB 2
GAB3
GAB 2
GAB 1
lactate
acetate
Ala
malate
3c
2'c
3"c
4c
2c
3'c
3'a
1"c
4c
1'c
2"c
βC Ala
βC lactate
αC acetate
βC GAB
C 2"c
αC GAB
γC GAB
C 1"c
C 1"a
αC Ala
βC GB
C 1'c
C 1'a
C 4c
C 3'c+3'
C 3c
αC GB a
C 2'c
βC lactate
C 3"c
αC malate
C 2c
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1H Chemical Shift (ppm)
20
30
40
50
60
70
80
13C Chemical Shift (ppm)

Fig. 1 Multinuclear 2-D NMR identifies ligands in whole barley root exudate. 2-D ^{1}H total correlation (TOCSY) (A) and ^{1}H-^{13}C multiple bond correlation (HMBC) (B) spectra were acquired from barley root exudates collected under Fe deficiency. The 2-D spectra are displayed as contour plots while the corresponding 1-D ^{1}H and ^{13}C spectra are presented along the 2-D axes. Also shown are the structures of the mugineic acid (MA) phytosiderophores which are critical to Fe acquisition by grasses. The boxes in (A) and lines and (B) trace the ^{1}H-^{1}H and ^{1}H-^{13}C coupling, respectively, of exudate components. Those in red trace the pattern for 3-*epi*hydroxy MA [c]. These and other 2-D spectra identified the structures of three MA's (2'-deoxy MA [a], MA [b], and 3-*epi*hydroxy MA) directly from whole exudates without fractionation. Also determined are ligands including Glu: glutamate; GAB: γ-aminobutyrate; A-F: morpholinoethanesulfonate (MES); citrate.

Fig. 2

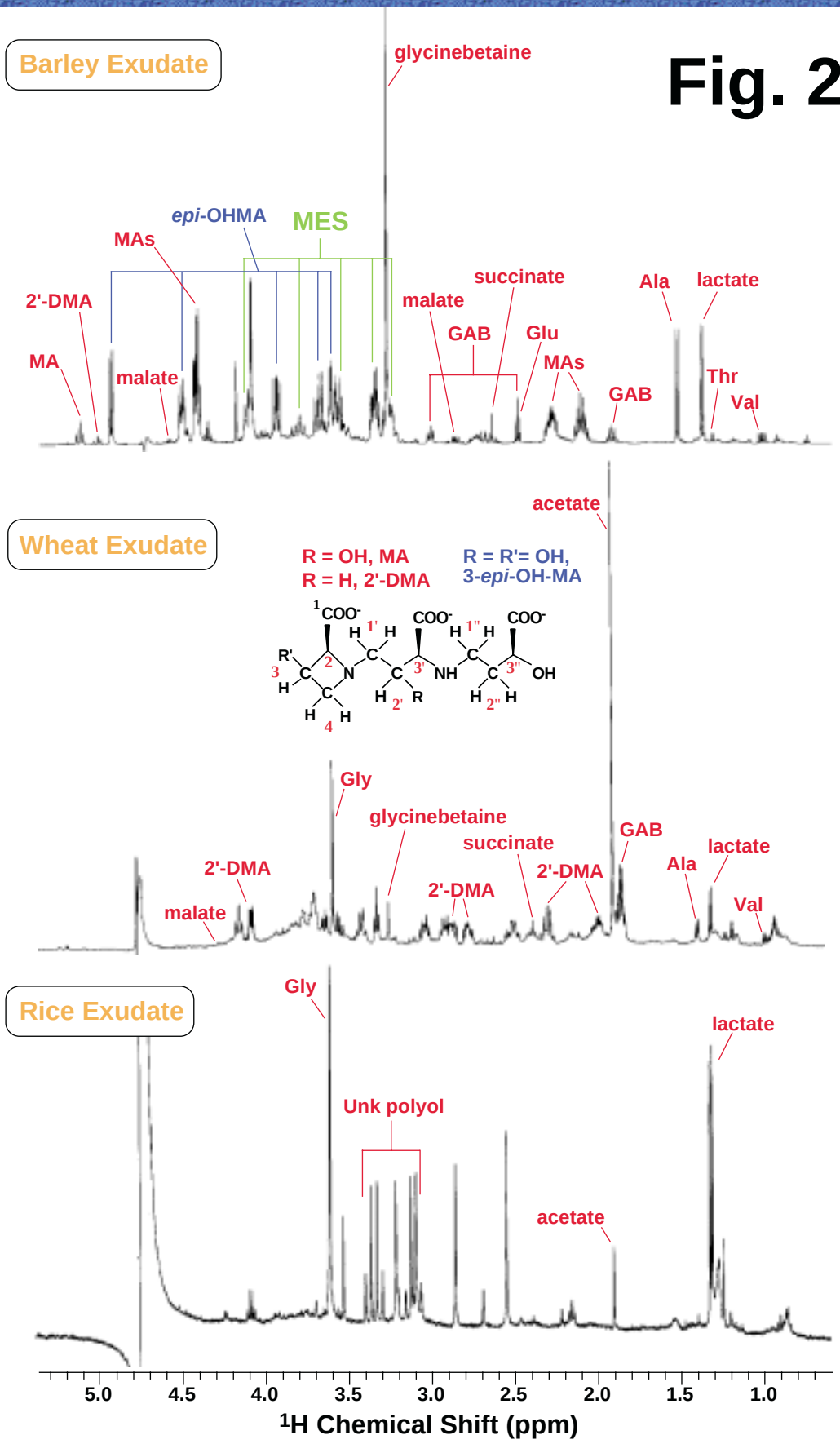

Barley Exudate
glycinebetaine
epi-OHMA
MES
MAs
2'-DMA
MA
malate
malate
succinate
GAB
Glu
MAs
GAB
Ala
lactate
Thr
Val
Wheat Exudate
acetate
R = OH, MA
R = H, 2'-DMA
R = R'= OH,
3-epi-OH-MA
Gly
glycinebetaine
succinate
2'-DMA
2'-DMA
2'-DMA
malate
GAB
Ala
lactate
Val
Rice Exudate
Gly
lactate
Unk polyol
acetate
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1H Chemical Shift (ppm)

Fig. 2 Exudate profiles differ among barley, wheat, and rice plants as revealed by ^{1}H NMR analysis. All root exudates were collected under a combined Fe deficient and Cd treatment. GAB, γ-aminobutyrate; MES, morpholinoethanesulfonate; *epi*-OHMA, 3-*epi*-hydroxy mugineic acid; 2'-DMA, 2'-deoxy-mugineic acid; Unk polyol, unknown polyol. The barley and wheat exudates were relatively more abundant in mugineic acid phytosiderophores and amino acids than the rice exudate. These differences may be related to the higher Fe acquisition capacity of barley and wheat than rice.

Fig. 3

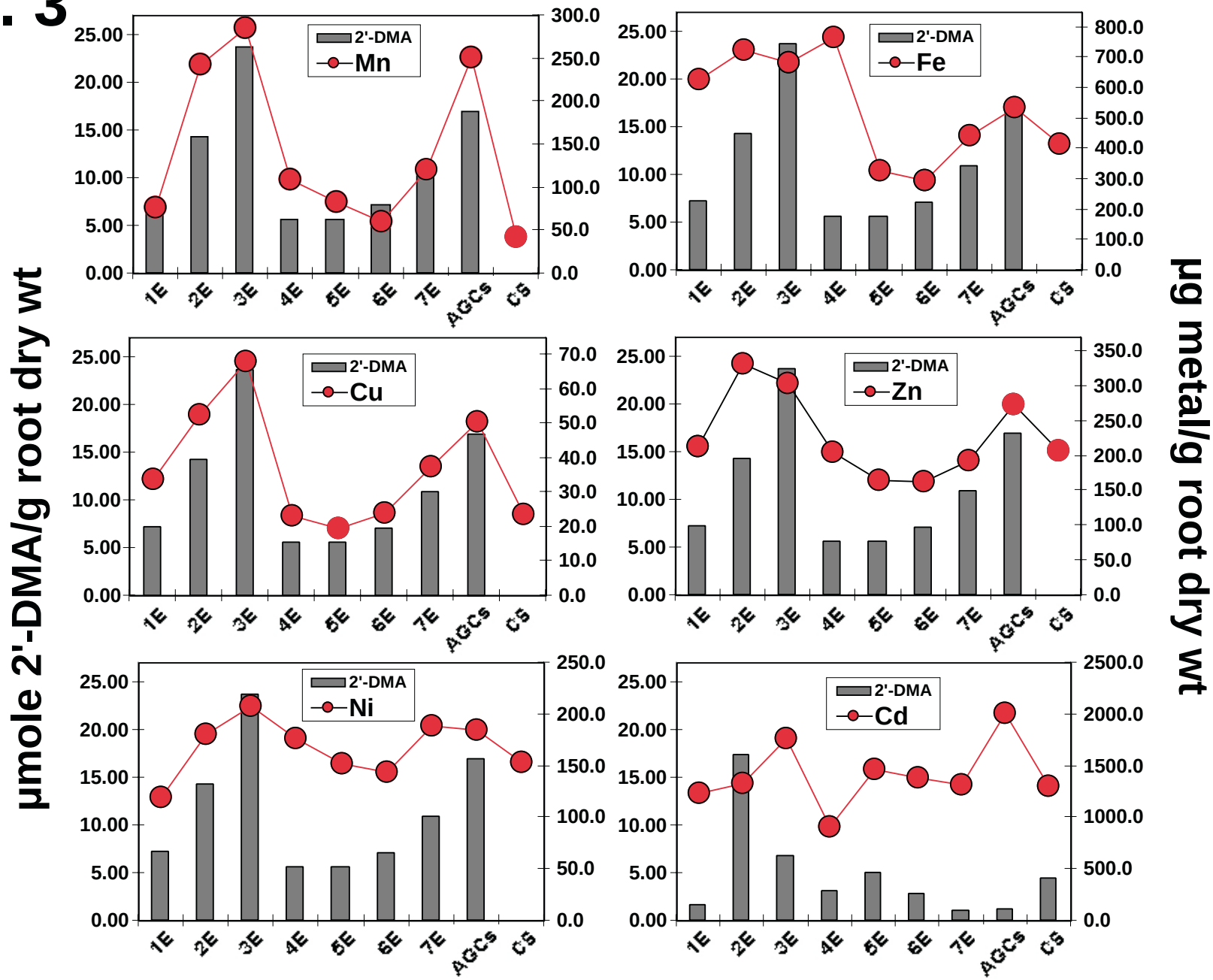
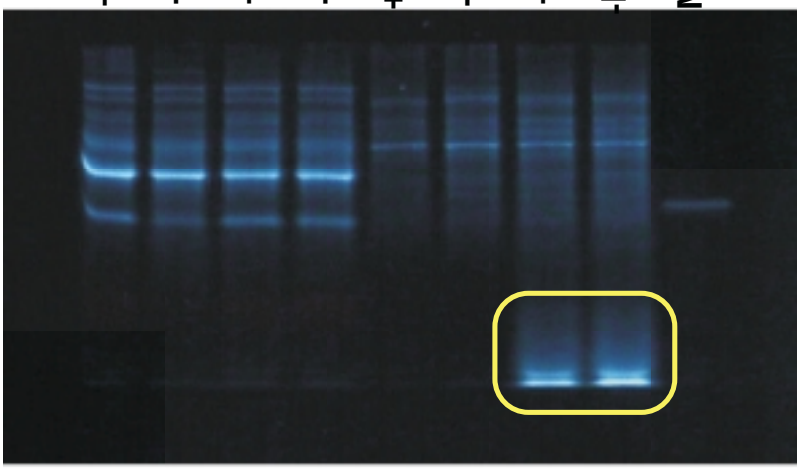

µmole 2'-DMA/g root dry wt
µg metal/g root dry wt
2'-DMA
Mn
Fe
Cu
Zn
Ni
Cd
1E
2E
3E
4E
5E
6E
7E
AGCs
C5

Fig. 3 2'-DMA exudation covaries with transition metals but not Cd in roots of different wheat genotypes. Exudate and root metal profiles were compared for seven wheat genotypes 1E, 2E, 3E, 4E, 5E, 6E, 7E (each with disomic addition of one wheatgrass chromosome) plus AgCS (an amphiploid with a full complement of wheatgrass chromosomes) and the Chinese Spring wheat parent (CS). Exudation of 2'-DMA exhibited genotypic covariation with Mn, Zn, Cu, and to some extent with Fe and Ni but not with Cd content in root tissues.

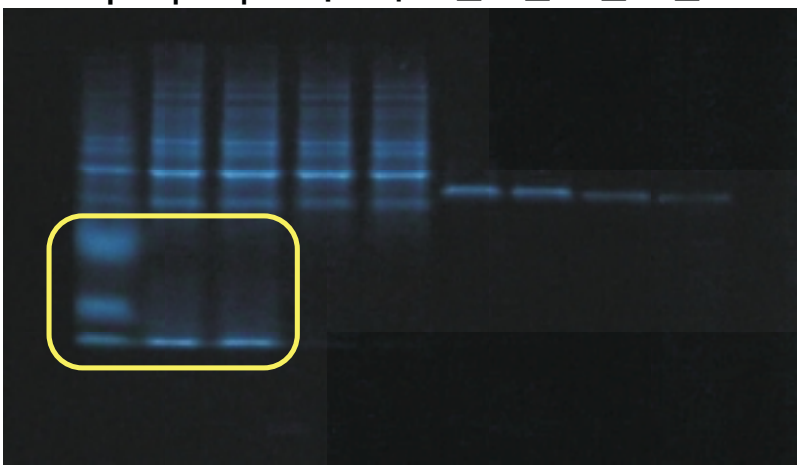
Fig. 4

A



PC (µg/g)	nd	nd	2.2	nd	nd	nd	909	1367
Cd (mM)	nd	nd	0.2	0.6	nd	0.2	17.5	14.8

B



PC (µg/g)	263	241	nd	nd
Cd (mM)	6.0	2.1	nd	nd

nd = not detected

Fig. 4A-B SH-rich peptides are induced in Cd-treated wheat and rice plants. A convenient polyacrylamide gel electrophoretic (PAGE) method was developed for assaying SH-rich peptides (e.g. phytochelatins, PC) that may be involved in intracellular sequestration of Cd(II). Peptidic SH groups were derivatized by bromobimane, then separated on PAGE followed by fluorescence image analysis. Panels A-B show the analysis for wheat and rice plants, respectively after Fe/Cd treatments. The yellow boxes highlight the regions where SH-rich peptides are induced by Cd acquisition. Also illustrated is the analysis of a metallothionein (MT) standard, from which a calibration curve was used to determine tissue "PC" content (µg MT equivalent/g dry wt). The Cd content was obtained from X–ray fluorescence (XRF) analysis.

Fig. 5

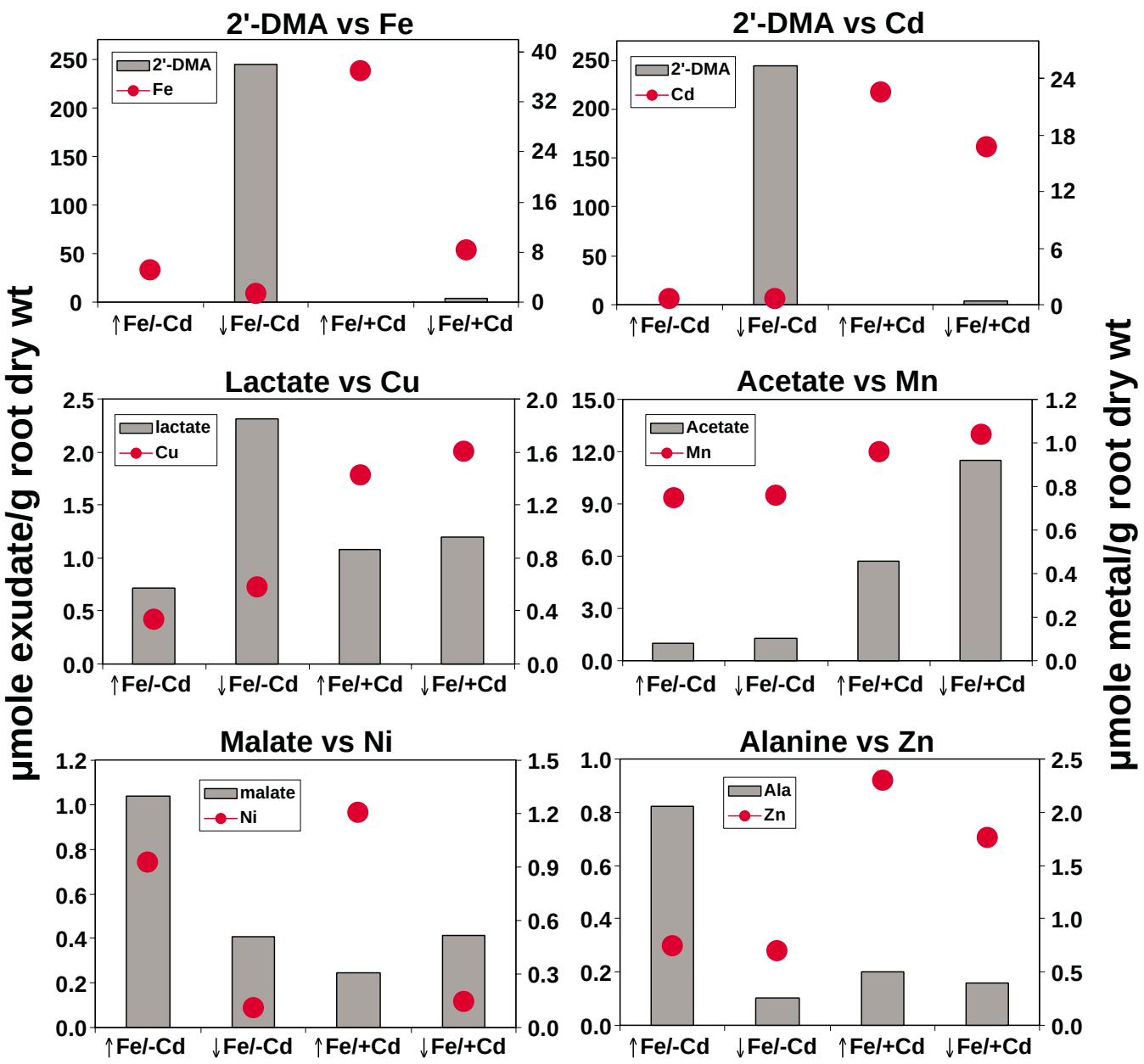

2'-DMA vs Fe
2'-DMA
Fe
2'-DMA vs Cd
2'-DMA
Cd
Lactate vs Cu
lactate
Cu
Acetate vs Mn
Acetate
Mn
Malate vs Ni
malate
Ni
Alanine vs Zn
Ala
Zn
↑Fe/-Cd
↓Fe/-Cd
↑Fe/+Cd
↓Fe/+Cd
µmole exudate/g root dry wt
µmole metal/g root dry wt

Fig. 5 Sequestration of Cd and transition metals interacts in wheat roots. Root metal content was compared with individual exudate profiles for Chinese Spring wheat under a combination of Fe sufficient without (↑Fe/-Cd) or with 1 ppm Cd (↑Fe/+Cd) and Fe deficient without (↓Fe/-Cd) or with (↓Fe/+Cd) 1 ppm Cd treatments. The presence of Cd elicited a substantial increase in the sequestration of Fe, Cu, Zn, and Mn but a large decrease in the exudation of 2'-DMA (most abundant), regardless of the Fe status. In contrast, the exudation of acetate (2nd in abundance) was enhanced by the Cd treatments, which correlated with the elevated Cu and Mn, and Zn content. These results show that the mechanism(s) of target metal ion sequestration in roots can be altered by co-contaminant metals such as Cd.

Fig. 6

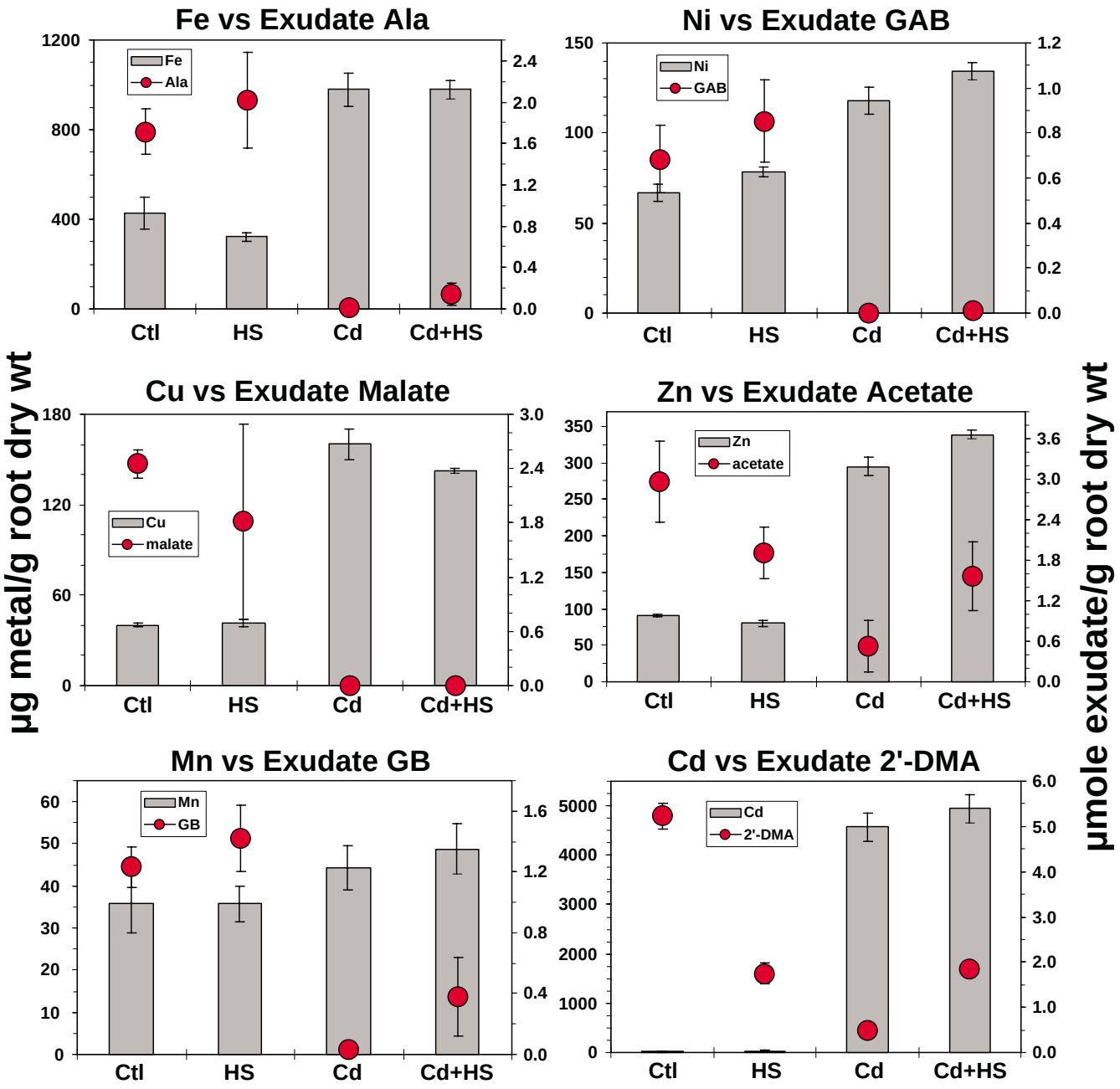

Fe vs Exudate Ala
Fe
Ala
Ni vs Exudate GAB
Ni
GAB
Cu vs Exudate Malate
Cu
malate
Zn vs Exudate Acetate
Zn
acetate
Mn vs Exudate GB
Mn
GB
Cd vs Exudate 2'-DMA
Cd
2'-DMA
Ctl
HS
Cd
Cd+HS
µg metal/g root dry wt
µmole exudate/g root dry wt

Fig. 6 Soil humate further enhances heavy metal sequestration into wheat roots. Root metal content was compared with individual exudate profiles for Chinese Spring wheat under a combination of soil humate (5 ppm) and Cd (5 ppm) treatments. At a higher Cd concentration (than in Fig. 5) a large increase in the sequestration of transition metals in roots was also observed while the exudation of all organic ligands was drastically reduced. Adding humate to the Cd treatment led to a further increase in the root content of Cd, Zn, Ni, and Mn while the exudation of 2'-DMA and a few other ligands was partially restored. These results indicate that humate (a dominant extant soil ligand) may directly and/or indirectly (via exudation) mediate heavy metal sequestration.

Fig. 7

A

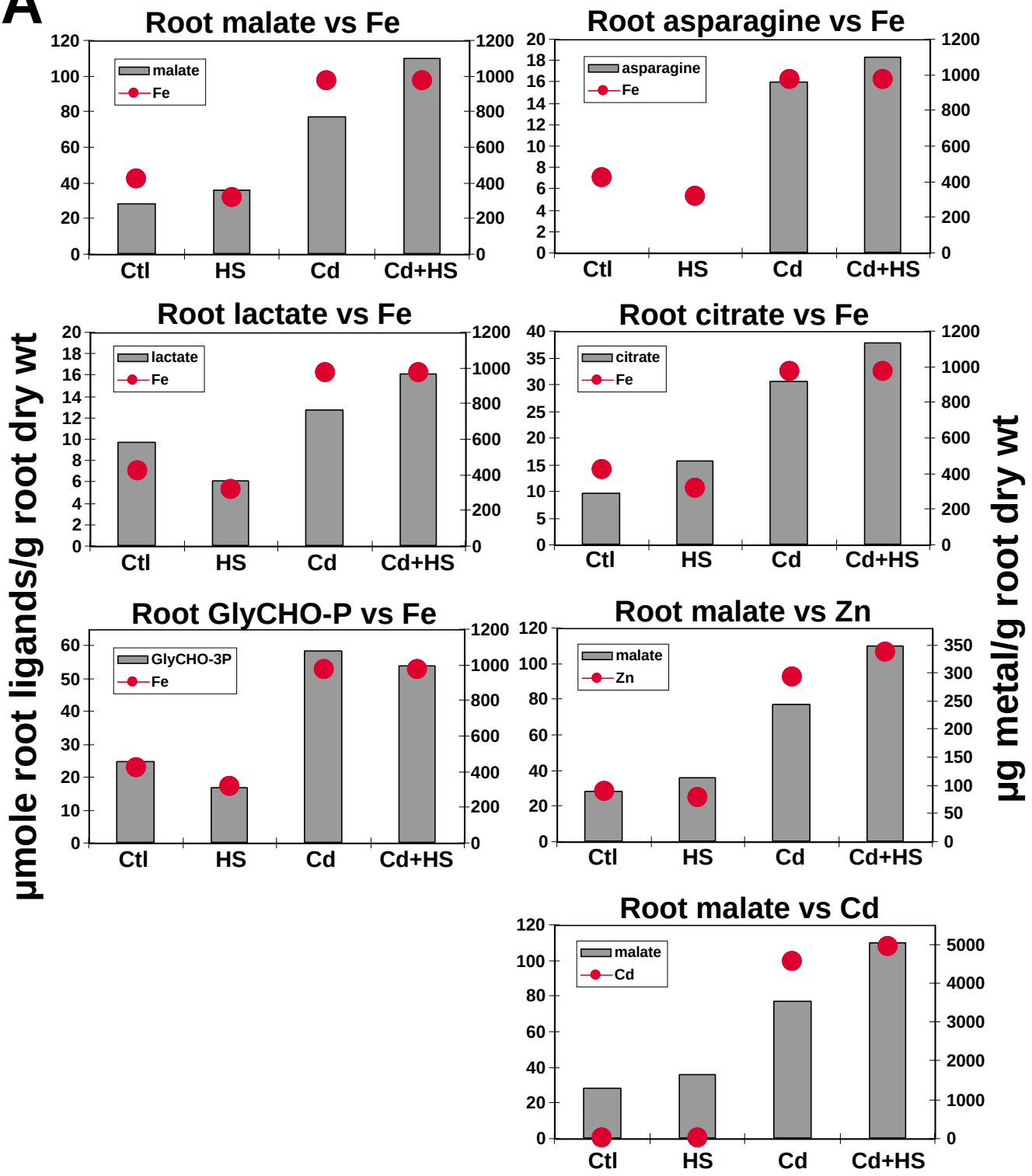
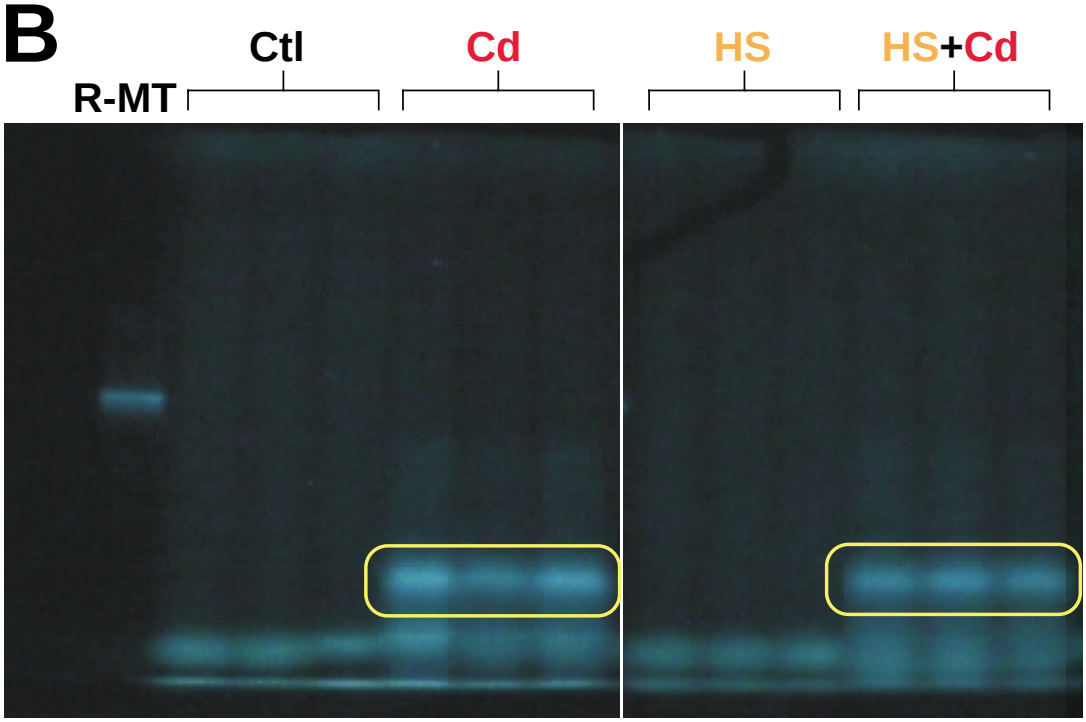

Root malate vs Fe
malate
Fe
Root asparagine vs Fe
asparagine
Fe
Root lactate vs Fe
lactate
Fe
Root citrate vs Fe
citrate
Fe
Root GlyCHO-P vs Fe
GlyCHO-3P
Fe
Root malate vs Zn
malate
Zn
Root malate vs Cd
malate
Cd
Ctl
HS
Cd
Cd+HS
µmole root ligands/g root dry wt
µg metal/g root dry wt

Fig. 7

B


Ctl
Cd
HS
HS+Cd
R-MT

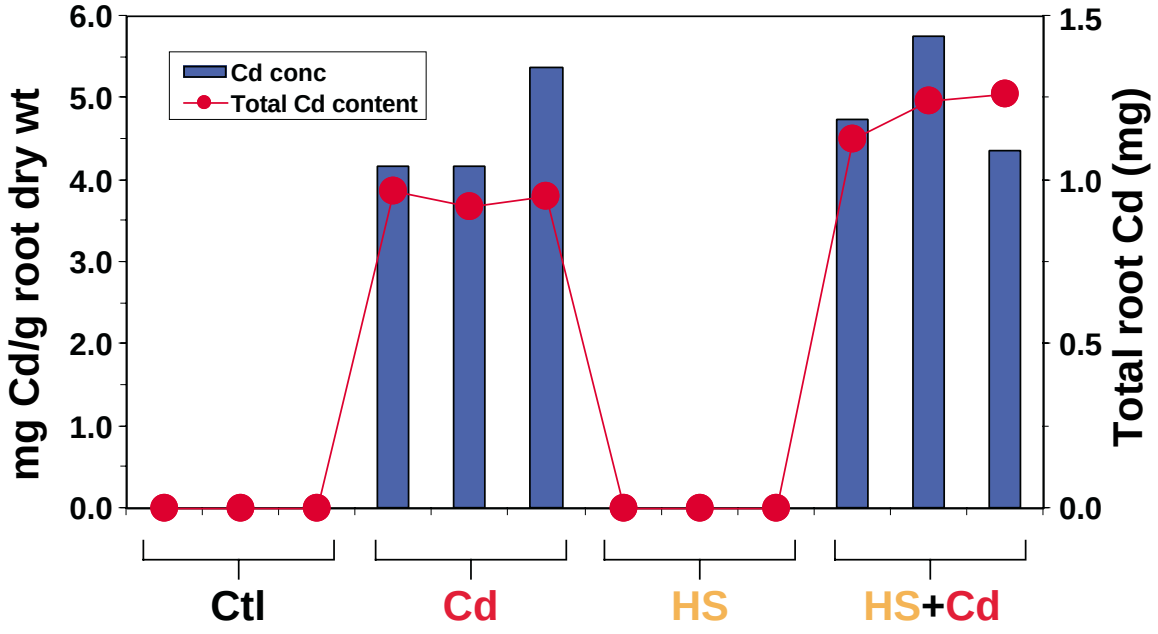

Cd conc
Total Cd content
mg Cd/g root dry wt
Total root Cd (mg)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.5
1.0
0.5
0.0
Ctl
Cd
HS
HS+Cd

Fig. 7 Intracellular ligands participate in heavy metal sequestration in wheat root. Root metal content was compared with tissue ligand profiles for Chinese Spring wheat under a combination of soil humate (5 ppm) and Cd (5 ppm) treatments. Accumulation of organic acids, amino acids, and phosphate metabolites (A) correlated with that of transition metals and Cd. In addition, induction of thiol-rich peptides (e.g. 1.4 kDa band in yellow box, B) strongly correlated with Cd content in roots. Soil humate appeared to enhance accumulation of carboxylic ligands (A), but attenuate the production of thiol-rich peptides induced by Cd (B). These results suggest that organic ligands in wheat roots participate in metal ion sequestration and that soil humate may also contribute to this process. Understanding the subsequent fate of these root-sequestered metals is important to the design of plant-mediated sequestration in soils.

Linkage to Site Needs

- Understanding natural ligand-mediated metal mobilization and/or sequestration in plant-soil systems facilitates the development of low-cost *in situ* stabilization strategies, a "Priority 1" need at INEEL (Need ID: ID-6.1.32) and phytoremediation strategies at Savannah River Site, including "Monitored Natural Attenuation" (Need ID: SR00-3026).

- Comprehensive natural ligand characterization helps predict metal speciation and biogeochemical reactions in complex real systems, and therefore the outcome of natural attenuation, in vegetated soils and vadose zone below (e.g. Need ID: SR00-3026).

- Key limitations that are ranked "Priority 1" (INEEL), such as the unknown presence of chelators, unknown Kd values for these, and presence, type, and sizes of organic colloids in facilitated transport, are being addressed through the broad-screen natural ligand characterization and identification (Need ID: S.1.10).

R&D Transition Strategy

- Examine aging effects of plant-sequestered metal ions in soils, uncover reliable aging markers, and investigate how plant and microbial consortia affect this process.
- Test the efficacy of low-cost organic amendments, including animal yard ("brown") and crop waste ("green") manures, in facilitating plant-mediated metal sequestration and aging of sequestered metals.
- Conduct pilot-scale test in contaminated field sites to validate laboratory results.

Acknowledgements

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