soil systems

Supporting Information

From plant to paddy – How rice root iron plaque can affect the paddy field iron cycling

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Plant growth containers.

Plant growth containers were made of transparent plexiglass (25 cm × 25 cm × 3 cm i.d.). Under sterile conditions and constant anoxic gas flow (100% N_2) 1.75 cm^3 anoxic Hoagland solution (100%, 35 °C, pH 6.8) amended with 500 μM $Fe(II)_{aq}$ (from $FeCl_2$) and 0.3% Gelrite (Carl Roth, Karlsruhe, Germany) were filled into the containers. When cooling down to room temperature, the solution formed a transparent solid soil matrix. Approximately 100 mL of 20% Hoagland solution was constantly kept on top of the growth gel to prevent desiccation. The growth containers were wrapped in aluminum foil to protect the soil matrix from illumination. The leaf biomass, however, was illuminated by light. These setups were kept in a specifically-designed greenhouse to maintain constant light, temperature and humidity conditions. Penetration of O_2 from the atmosphere into the gel was monitored by microelectrodes and found to be relevant only in the upper 0.7 cm of the growth gel and therefore considered to be neglectable for the investigation of the rhizosphere.

Mössbauer spectroscopy.

Within an anoxic glovebox (100% N_2), sampled root biomass was dried at constant 30°C. Dried sample material was mortared, and subsequently loaded into plexiglas holders (area 1 cm^2), forming a thin disc. Prior to analysis, samples were stored anoxically at -20°C to suppress recrystallization processes or microbial activity. Samples were transported to the instrument within airtight bottles which were only opened immediately prior to loading into a closed-cycle exchange gas cryostat (Janis cryogenics) to minimize exposure to air. Spectra were collected at 77 K using a constant acceleration drive system (WissEL) in transmission mode with a $^{57}Co/Rh$ source. All spectra were calibrated against a 7 μm thick α-^{57}Fe foil that was measured at room temperature. Analysis was carried out using Recoil (University of Ottawa) and the Voigt Based Fitting (VBF) routine [77]. The half width at half maximum (HWHM) was constrained to 0.127 mm s^{-1} during fitting.

Isolation of Fe(III)-reducing bacteria from a paddy field rhizosphere.

Wet soil was collected from a paddy field located in Vercelli, Italy and transported to the laboratory under cool conditions. In order to enrich Fe(III)-reducing bacteria, soil material was homogenized and aliquots of 1 g were added to 100 mL anoxic and sterile mineral medium [38] amended with 20 mM acetate and 10 mM ferrihydrite. From these Fe(III)-reducing enrichment incubations, dilution series were performed following Muehe et al., (2013) [38]. Positive tubes were easily identified by a change in color from red to black which indicated the reduction of ferric iron to ferrous iron minerals. A sample from the highest positive dilution was tested for purity by fluorescence microscopy and by 16S rRNA gene cloning and sequencing. This culture was constantly transferred (10 %; v/v) into fresh culture tubes.

Root surface and iron plaque surface area.

Root surface area increased over time during plant growth over 45 days (Figure S1a). A simple linear regression was applied to determine the increase in root surface area over time and resulted in a trending increase of approx. 4.3 cm² root surface area per day ($R^2 = 0.97$). Root iron plaque development during plant growth over 45 days was identified by image analysis and shown in one experimental replicate as shown in Figure S1 B-G.

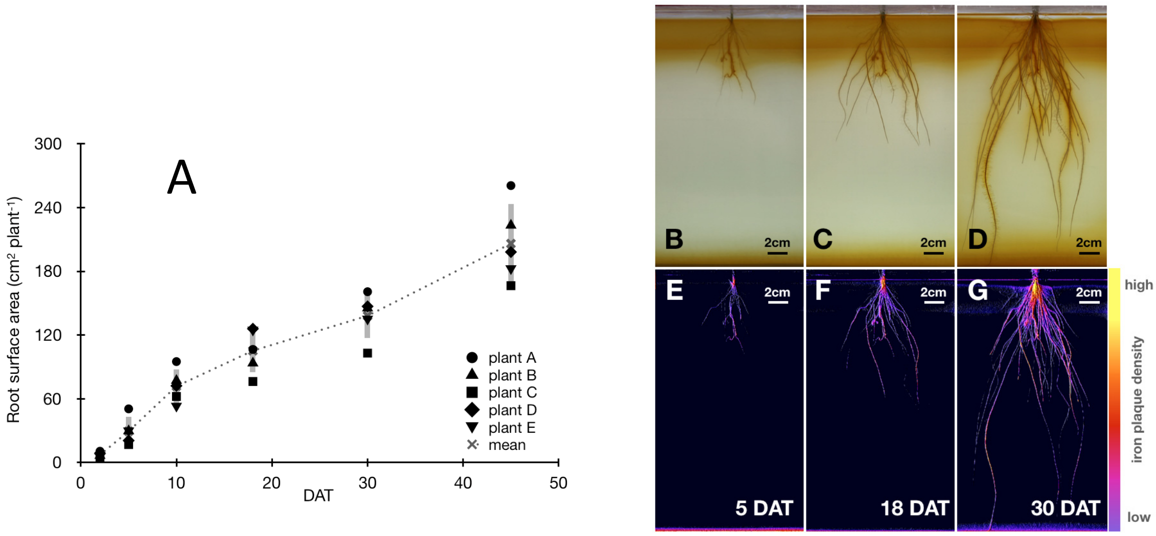

Figure S1. Root surface and iron plaque area. A: Root surface area (cm² per plant) in rhizotrons during plant growth (DAT = days after transfer) of five replicate plants (filled symbols), mean root surface area (cross & punctuated line) and standard deviation (grey bars). E-G: Iron plaque identification on roots by imaging and pixel analysis 5, 18 and 30 DAT and non-dimensional estimated iron plaque density (legend bar).

Mössbauer spectroscopy on aged root iron plaque minerals.

Iron plaque minerals were collected at the end of the growth experiment from (i) the basal root zone (approx. 40 days old), (ii) the middle part of the root (approx. 20 days old), and (iii) from young root tips (approx. 2 days old). Transmission spectra of root iron plaque collected from the basal root zone (Figure S2 A) was dominated by a narrow doublet with a low quadrupole splitting (ΔE_Q). The best-fit model suggested two individual components to be fitted as doublet 1 (Db1) and doublet 2 (Db2) (Table S1). The low ΔE_Q for both doublets suggests the presence of two Fe(III) species. The hyperfine field parameters of Db1 can be attributed to the presence of ferrihydrite as most abundant Fe(III) species, while Db2 likely represents the presence of approx. 8 % lepidocrocite. The presence of a sextet in the spectrum of iron plaque from the basal root zone suggests the presence of an iron species undergoing magnetically ordering at 77 K. Hyperfine field parameters of the best-model fit suggest the presence of goethite in this sample with a relative abundance of approx. 17%.

The fitted components in spectrum of iron plaque collected from 20-days old roots (Figure S2B) showed similar characteristics as iron plaque from basal roots. However, the relative abundance of lepidocrocite was estimated to be 3-fold higher compared to iron plaque from basal root zone while ferrihydrite remained at the same level with approx. 70 % relative abundance (Table S1). In the background of the spectrum a poorly-developed sextet was visible. The best-fit parameters for the sextet feature suggest a higher-crystalline magnetically ordered mineral phase with a relative abundance of approx. 5 % while hyperfine field parameters are similar to goethite as being detected previously.

The spectrum for freshly-precipitated iron plaque collected from root tips was clearly dominated by a narrow doublet (Figure S2 C). Here, the best-fit model suggests the presence of only one component (Db1). Hyperfine field parameters for Db1 can be attributed to the presence of ferrihydrite as the dominant (>95 %) iron mineral phase in this sample (Table S1). Additionally, a poorly developed wide doublet with a high quadrupole splitting suggests the presence of a high-spin Fe(II) phase. However, the hyperfine field parameters do not allow a clear identification as iron mineral but suggest the presence of potentially adsorbed/complexed Fe(II) in this sample.

Table S1. Mössbauer spectra hyperfine parameters for root iron plaque minerals.

Sample	**Temp. (K)**	**Phase**	**CS (mm/s)**	**ΔE_Q (mm/s)**	**ε (mm/s)**	**B_{hf} (T)**	**Pop (%)**	**±**	**χ^2**	**Mineral phase**
basal roots (45 days old)	77	Db1	0.47	0.75			74.5	0.5	0.60	Fh
		Db2	0.46	0.60			8.5			Lep
		Sxt1	0.45		-0.14	49.7	17			Gt
middle roots (20 days old)	77	Db1	0.46	0.82			72.0	0.6	0.58	Fh
		Db2	0.49	0.64			23.0			Lep
		Sxt1	0.47		-0.16	47.4	5.0			Gt
Root tips (2 days old)	77	Db1	0.47	0.71			96.5	0.3	0.66	Fh
		Db2	1.32	2.10			3.5			Fe(II)

CS – Center shift, ΔE_Q – Quadrupole splitting, ε – Quadrupole shift, B_{hf} – Hyperfine field, Pop. – relative abundance, χ2 – goodness of fit, identified mineral phase (Fh – ferrihydrite, Fe(II) – ferrous iron species, Lep – Lepidocrocite, Gt – Goethite).

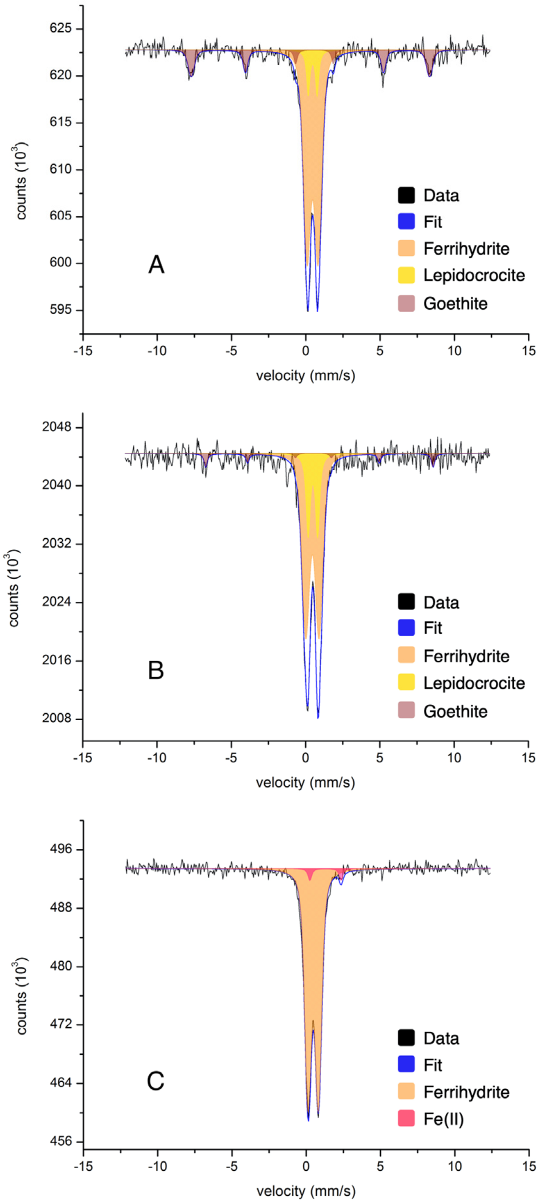

Figure 2. – Mössbauer transmission spectra of root iron plaque minerals collected at 77 K. A: Root iron plaque from basal root zone (45 days old) dominated by ferrihydrite (orange) and lepidocrocite (yellow) as fitting components for the narrow doublet (Db) and goethite (brown) as higher-crystalline iron mineral species represented by the magnetically-ordered sextet (Sxt). B: Root iron plaque from middle part of roots (20 days old) composing of ferrihydrite and lepidocrocite as fitting components for the narrow doublet (Db) and a small proportion goethite as higher-crystalline iron mineral species represented by the magnetically-ordered sextet (Sxt). C: Root iron plaque on root tips (2 days old) dominated by ferrihydrite and potentially sorbed Fe(II) (red) detected as wide doublet.

Identification of reduced iron plaque minerals by Mössbauer spectroscopy

Samples collected from experimental setups with root covered in iron plaque minerals which were exposed to an Fe(III)-reducing culture were identified by Mössbauer spectroscopy. The transmission spectrum of abiotic control incubation with inhibited cells showed the presence of a well-defined dominant doublet structure (Figure S3 A). The best-fit model using the VBF fitting routine suggested two individual components to fit the measured data. These components were assigned to a doublet feature Db1 and Db2, respectively. Both, Db1 and Db2 were characterized by a narrow ΔE_Q <0.8 which suggests the presence of a low-spin Fe(III) phase for both doublets. The individual hyperfine field parameters for Db1 likely can be attributed to the presence of ferrihydrite by >80 %, while Db2 suggests lepidocrocite to be abundant by approx. 20 % (Table S2).

The transmission spectrum for the active incubation was dominated by a wide doublet feature (Figure S3 B) with a relatively high ΔE_Q which indicates the presence of a high-spin Fe(II) phase being present in this sample (Db1; Table S2) The other hyperfine field parameters are close to reference parameters of siderite as ferrous iron carbonate being present by approx. 60 % relative abundance. Additionally, a narrow doublet (Db2) with a low quadrupole splitting was overlapping with Db1. The hyperfine field parameters are similar to ferrihydrite, while the fitting model suggests an approx. 20 % relative abundance. Moreover, a poorly defined sextet was observed in the spectrum of this sample (Figure S3 B). The collapsed and low hyperfine field of 27 T only cannot be referenced to any commonly known reference iron phase. However, collapsed feature and the beginning of a magnetic ordering at 77 K suggests the presence of a short-range ordered iron (oxyhydr)oxide. Although this observation does not allow a clear iron phase identification, the corresponding iron mineral extraction data suggests the presence of an Fe(II) compound. Potentially associated with sulfur species which caused the distortions of iron atoms, the resulting sextet feature in the recorded spectrum might represent some sort of Fe(II) sulfur compound as it was observed for other iron sulfur species [78, 79]. We therefore assigned the relative abundance of the observed sextet by approx. 10 % to the presence of a yet unknown Fe(II)–S species (Table S2).

Table S2. Mössbauer spectra hyperfine parameters for non/reduced root iron plaque minerals.

Sample	Temp.	Phase	CS	ΔE_Q	ε	B_{hf}	Pop	±	χ^2	Mineral phase
	(K)		(mm/s)	(mm/s)	(mm/s)	(T)	(%)			
non-reduced root iron plaque	77	Db1	0.49	0.78			72.6	0.9	0.71	Fh
		Db2	0.42	0.57			27.4			Lep
reduced root iron plaque	77	Db1	1.35	2.89			63.6	0.4	0.83	Sid
		Db2	0.41	0.83			19.5			Fh
		Sxt1	0.39		-0.01	27.4	16.9			Fe(II)–S

CS – Center shift, ΔE_Q – Quadrupole splitting, ε – Quadrupole shift, B_{hf} – Hyperfine field, Pop. – relative abundance, $\chi 2$ – goodness of fit, identified mineral phase (Fh – ferrihydrite, Lep – Lepidocrocite, Sid – Siderite, Fe(II)–S – ferrous iron sulfur species).

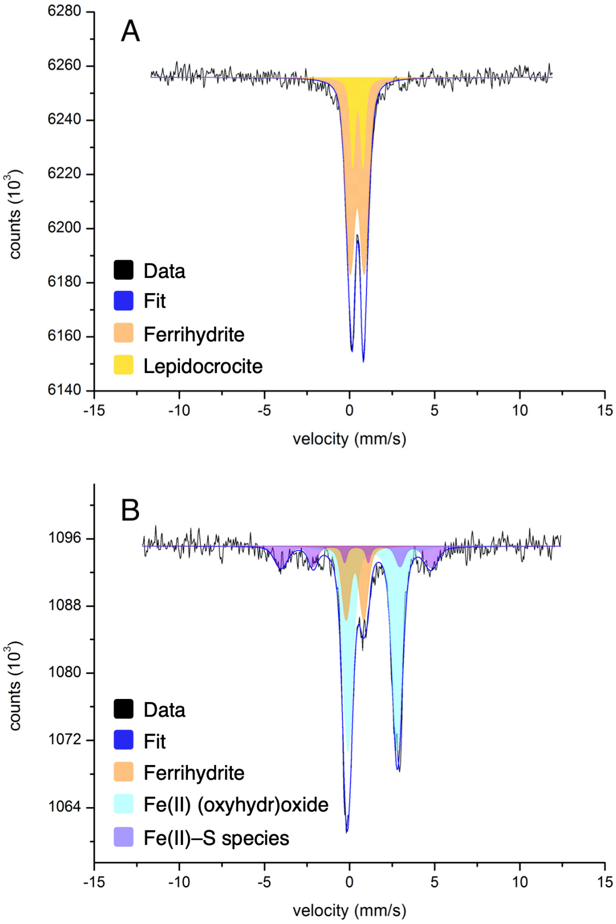

Figure S3. Mössbauer spectra of root iron plaque reduction experiments measured at 77K. A: Transmission spectrum of non-reduced root iron plaque minerals collected from inhibited control incubations. Mainly ferrihydrite (brown doublet) and lepidocrocite (yellow) were the two main iron phases. B: Transmission spectrum of reduced root iron plaque minerals collected from roots exposed to an Fe(III)-reducing culture. An Fe(II) mineral phase, most likely siderite (light blue), some resilient ferrihydrite (brown) and a collapsing sextet which is potentially representing a short-range ordered yet unknown Fe(II)–S species (purple). .

Hyperfine field parameters and Mössbauer spectrum of reduced iron plaque in rhizotron.

Table S3. Mössbauer spectra hyperfine parameters for non/reduced root iron plaque minerals.

Sample	Temp.	Phase	CS	ΔE_Q	ε	B_{hf}	Pop	±	χ^2	Mineral phase
	(K)		(mm/s)	(mm/s)	(mm/s)	(T)	(%)			
reduced root iron plaque	77	Db1	1.29	3.19			34.6	0.9	0.71	Viv (A)
		Db2	1.32	2.63			19.1			Viv (B)
		Db3	0.46	0.71			22.8			Fh
		Sxt	0.41		-0.02	28.3	23.5			Fe(II)–S

CS – Center shift, ΔE_Q – Quadrupole splitting, ε – Quadrupole shift, B_{hf} – Hyperfine field, Pop. – relative abundance, χ2 – goodness of fit, identified mineral phase (Viv – Vivianite coordination A/B, Fh – ferrihydrite, Fe(II)–S – ferrous iron sulfur species).

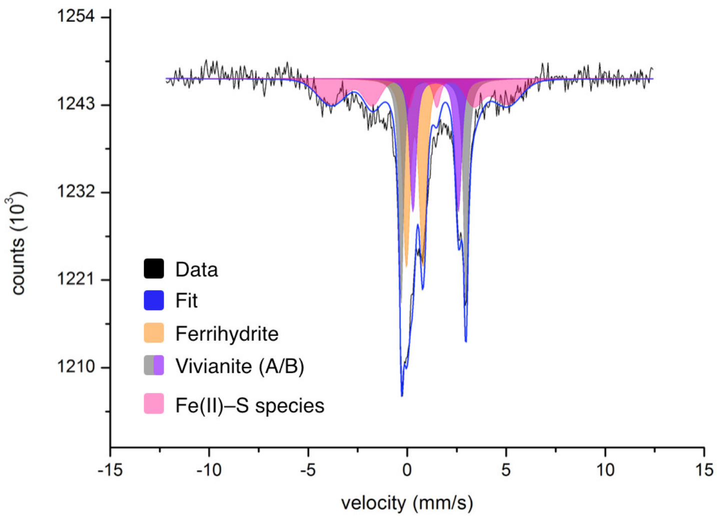

Figure S4. Mössbauer spectra of root iron plaque reduction experiments in rhizotron measured at 77K. Transmission spectrum of reduced root iron plaque minerals collected from rhizotron exposed to an Fe(III)-reducing culture. An Fe(II) mineral phase, most likely vivianite (grey/purple, represent two iron coordination states in vivianite), some resilient ferrihydrite (orange) and a collapsing sextet which is potentially representing a short-range ordered yet unknown Fe(II)–S species (pink).