SUPPORTING INFORMATION

Iron Lung

– How Rice Roots induce Iron Redox Changes in the Rhizosphere and create Niches for Microaerophilic Fe(II)-oxidizing Bacteria

*Markus Maisch[1], Ulf Lueder[1], Andreas Kappler[1,2], Caroline Schmidt[1]**

[1] Geomicrobiology, Center for Applied Geoscience, University of Tuebingen, 72076 Tuebingen, Germany

[2] Center for Geomicrobiology, Department of Bioscience, University of Aarhus, 8000 Aarhus, Denmark

* corresponding author:

Dr. Caroline Schmidt

Eberhard-Karls-University Tuebingen, Center for Applied Geoscience, Geomicrobiology

Sigwartstrasse 10, 72076 Tuebingen, Germany

caroline.schmidt@uni-tuebingen.de

Experimental setup: transparent rhizotrons:

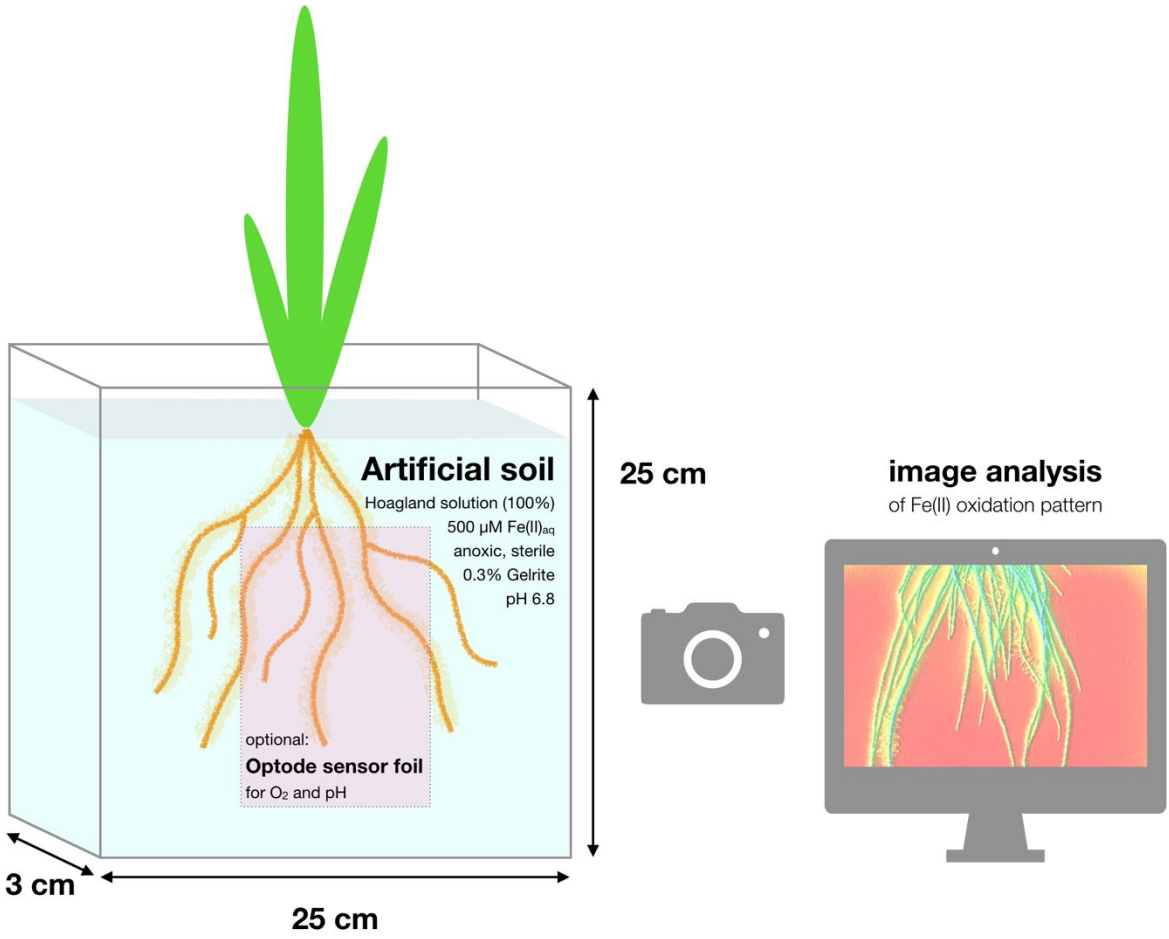

Figure S1. Representation of the experimental setup. Rhizotron dimensions, artificial soil composition, position of optode sensor foils inside the rhizotron for non-invasive mapping of O_2 and pH, and the schematic outline for image analysis of Fe(II) oxidation patterns.

Voltammetric measurements for $Fe(II)_{aq}$

Dissolved ferrous iron ($Fe(II)_{aq}$) concentrations were determined by voltammetric measurements using a three-electrode system hooked onto a DLK-70 web-potentiostat (Analytical Instrument Systems, Flemington, NJ). The working electrode was a lab-

constructed glass-encased 100 µm gold amalgam (Au/Hg) electrode.[1] A silver (Ag) wire, coated with AgCl and a platinum (Pt) wire were used as reference and counter electrode, respectively. Calibration for $Fe(II)_{aq}$ was performed applying the pilot ion method[1, 2] with $Mn(II)_{aq}$ standards. Cyclic voltammograms for $Fe(II)_{aq}$ and $Mn(II)_{aq}$ were collected by conditioning the electrode at -0.05 V for 2 s and subsequent scanning from -0.05 V to -2 V and reverse with a scan rate of 1000 mV s^{-1}. Prior to each scan, a potential of -0.9 V was applied for 5 s to precondition the electrode surface. Ten scans were run at every measuring position and the final three voltammograms were analyzed using the VOLTINT add-on for Matlab.[3] Measured $Fe(II)_{aq}$ concentrations were measured 5 mm in the artificial soil matrix. For high-resolution in-situ Fe(II) measurements around individual roots, the spatial resolution between measuring points was 2 mm. Microsensor adjustment was controlled with a 3-D micromanipulator. Abiotic Fe(II) oxidation kinetics and Fe(II) half-life times within the rhizotron were calculated according to Maisch et al., (2019).[4]

Calculation of abiotic Fe(II) oxidation kinetics and Fe(II) half-life times

Abiotic Fe(II) oxidation rates at a specific time point (considering only homogeneous oxidation) and half-life times for Fe(II) were calculated following Maisch et al., 2019[4]. Spatial Fe(II) oxidation rates were determined considering the respective spatial Fe(II) concentration and the corresponding mean O_2 concentration within 2 mm of the measuring point. Measuring conditions (25°C, and corresponding pH conditions at the measuring point) were considered for calculation of Fe(II) oxidation rates and Fe(II) half-life times, respectively.

Non-invasive oxygen and pH mapping using planar optode sensors

For non-invasive O_2 quantification in the rhizotrons, planar optode sensor foils (SF-RPSu4 & SF-HP5R, PreSens, Regensburg, Germany) were used. The monitoring is based on fluorescence ratiometric imaging (FRIM). The sensor foils contain two different fluorophores, an indicator and a reference dye. The ratiometric approach compensates for inhomogeneities in the sensor foil as well as in the excitation light field. Both fluorophores are excited in the blue range of the spectrum, one is emitting in the green, and one in the red range of the spectrum. The reference fluorophores emission is constant whereas the indicator emission is depending on the oxygen concentration. The indicator is a dye composed of transition metal complexes. These show an effect called dynamic luminescence quenching, i.e. their fluorescence intensity as well as the fluorescence lifetime is quenched in the presence of oxygen. The quenching follows the Stern-Volmer relationship: $I0/I = 1 + KSV * [O_2]$. KSV is a known parameter and specific for the respective indicator dye used in the calibration algorithm suggested by the manufacturer. The correlation of the ratiometric imaging is linearly correlated to O_2 concentrations. A two-point calibration allows a reliable non-invasive quantification of O_2 concentrations within a detection uncertainty range of ±0.02 µM O_2 for the setup used in the current study.

The spatial resolution of the VisiSens TD detection unit (12bit) is depending on the field of view (FOV). When using the Big Area Imaging Kit (as we did in the current study) and a FOV of 30 x 18 cm^2, the pixel resolution is about 200 µm. The O_2 sensor foils not only contain molecular components but particles. Hence, the spatial resolution is also depending on the sensor foil. The particles in the sensor foil for O_2 are small enough that the spatial resolution of the detection unit is the limiting factor. The particles in the pH sensor foil are

larger and the lower limit for the spatial resolution on the foil is 100 µm. Using the Big Area Imaging Kit the spatial resolution is limited to 200 µm in the experimental setup.

Image analysis and Fe(II) quantification

Protocols and scripts were calibrated with in-situ Fe(II) microsensor measurements in a blank container with abiotic oxidation patterns and known $Fe(II)_{aq}$/(III) concentrations. Image analysis correlated proportionally to Fe(II) concentrations in a range of 50 – 500 µM Fe(II). Step size for Fe(II) quantification via image analysis was set to 50 µM Fe(II) (and a total range from 50 – 500 µM Fe(II)). Areas outside the measuring range or within the step sizes remained as blank in plots. A rhizotron setup with a plant was sacrificed for a destructive quantification of Fe(III) minerals on roots (see below).

Iron plaque quantification

Root material covered in iron plaque precipitates was collected at the end of the growth experiment and the root cut into sections of 1 cm. Iron minerals were extracted in 2M HCl for 2 hours and dissolved total iron quantified by ferrozine assay[5] as described in Materials and Methods. Collected root sections were referenced to positions in rhizotrons and quantified iron plaque referred to pixel identity of roots in rhizotron.

Oxygen concentration patterns during plant growth

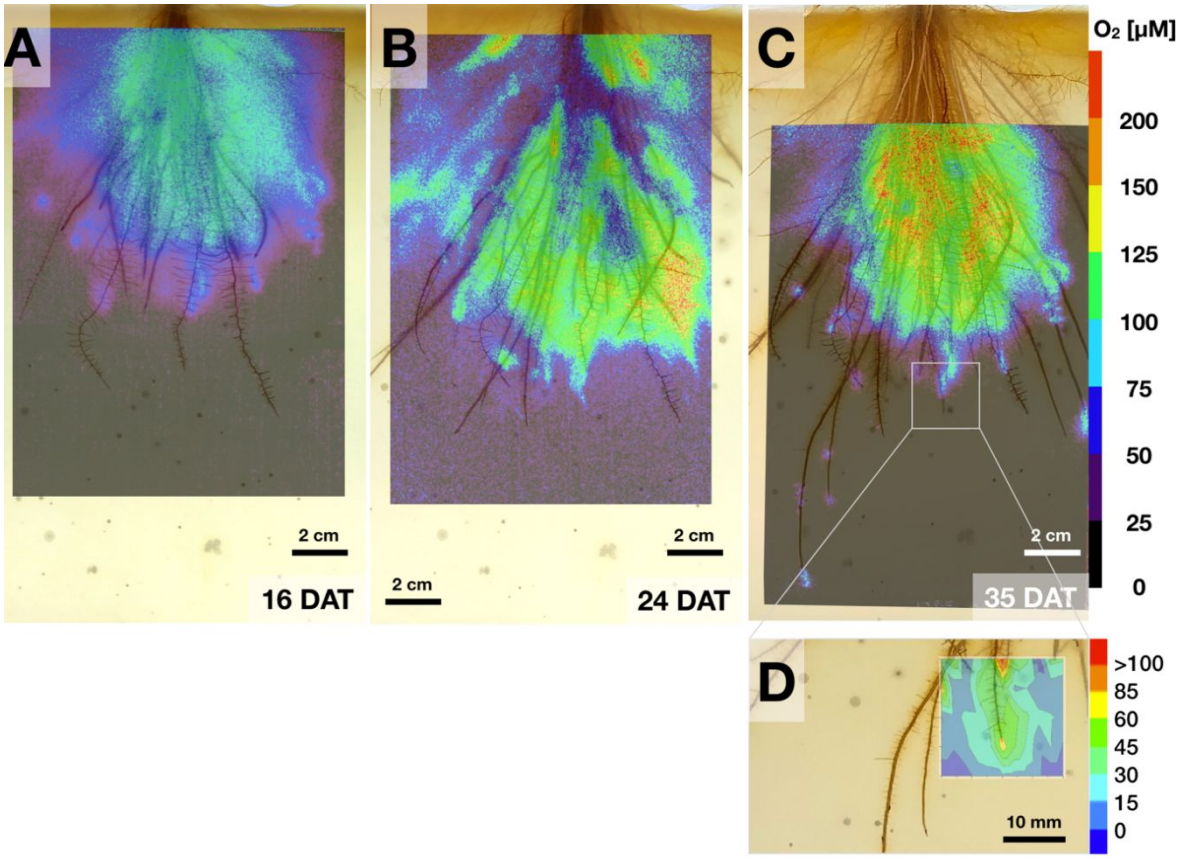

Figure S2. Oxygen concentrations in the rhizosphere. Non-invasive mapping of O_2 concentrations at A) 16 DAT, B) 24 DAT and C) 35 DAT (DAT = Days after transfer). D: High-resolution O_2 mapping around a single root using O_2 microsensors.

Abiotic Fe(II) oxidation kinetics and Fe(II) half-life times along rhizosphere transect

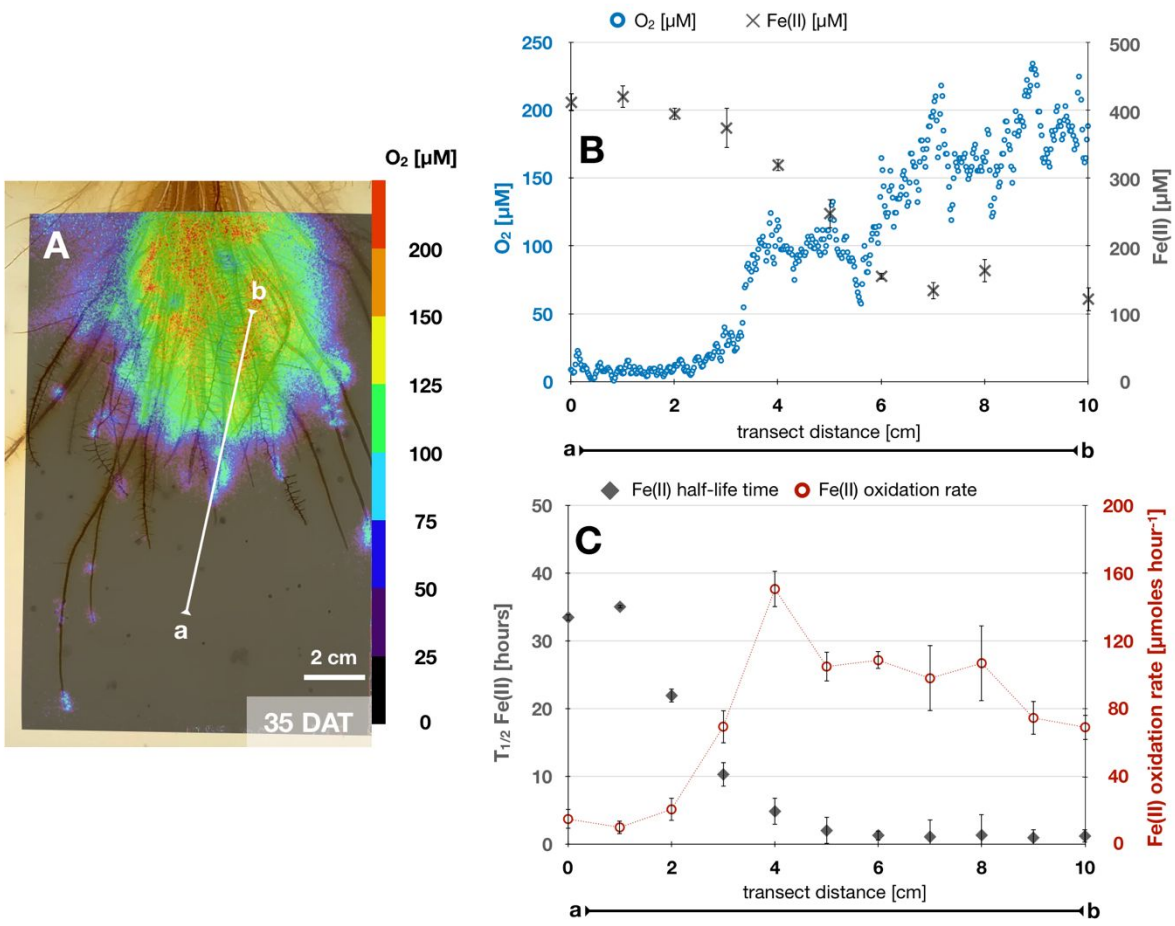

Figure S3. A: Oxygen concentrations within the rhizosphere of a rice plant 35 DAT (= days after transfer) and transect for spatial calculation of Fe(II) oxidation kinetics and Fe(II) half-life times; B: Spatial O_2 and Fe(II) concentrations along transect a–b and C: calculated Fe(II) half-life times and abiotic Fe(II) oxidation rates at specific positions along transect.

Habitable zones for microaerophilic Fe(II)-oxidizing bacteria during plant growth

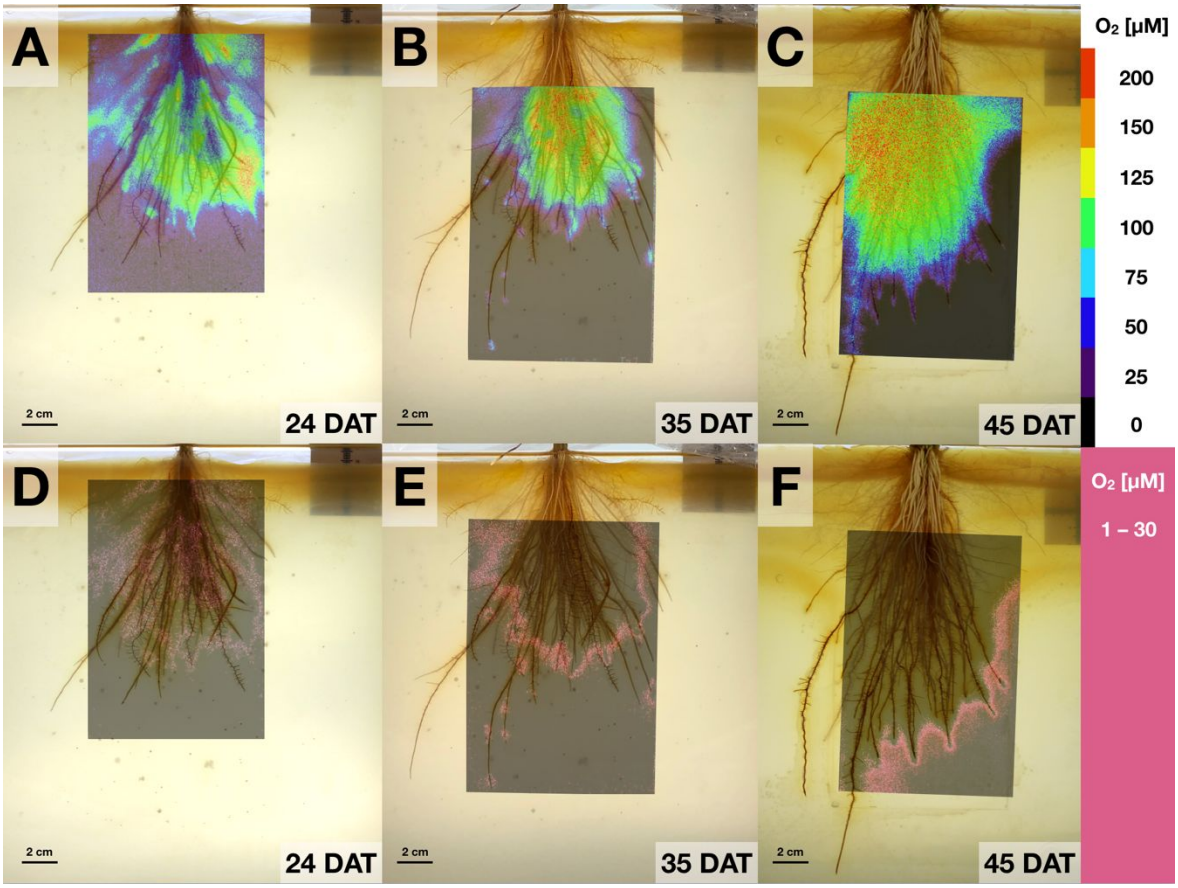

Figure S4. Habitable zones for microaerophilic Fe(II)-oxidizing bacteria. Non-invasively measured O_2 concentrations in the rhizosphere during plant growth (A-C) and identified zones with microoxic conditions (1-30 µM O_2) that provide ideal O_2 concentrations for microFeOx (D-F) over time (DAT = Days after transfer). The relative expansion of this zone increases during root and plant growth from 24 DAT to 45 DAT by more than 30%.

Mössbauer spectroscopy of iron plaque minerals.

Sample preparation. Within an anoxic glovebox (100% N_2), root biomass was collected from rhizotrons and 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.[6] The half width at half maximum (HWHM) was constrained to 0.127 mm/s during fitting.

Results. The spectra collected at 77K showed a dominant narrow doublet (Db) characterized by a low center shift (CS) ranging from CS = 0.47 mm/s and a quadrupole splitting (ΔE_Q) of ΔE_Q = 0.78 mm/s. These hyperfine parameters can be attributed to ferrihydrite as iron(III) mineral phase precipitated on and around the roots.

Table S1. Iron plaque Mössbauer parameters. Hyperfine field parameters of Mössbauer spectrum collected at 77 K.

Sample	Temp (K)	Phase	CS (mm/s)	ΔE_Q (mm/s)	Pop (%)	±	χ^2	Mineral phase
Fe plaque	77	Db	0.47	0.78	100	0.3	0.99	Fh

CS – Center shift, ΔE_Q – Quadrupole splitting, Pop. – relative abundance, χ^2 – goodness of fit, identified mineral phase (Fh – ferrihydrite).

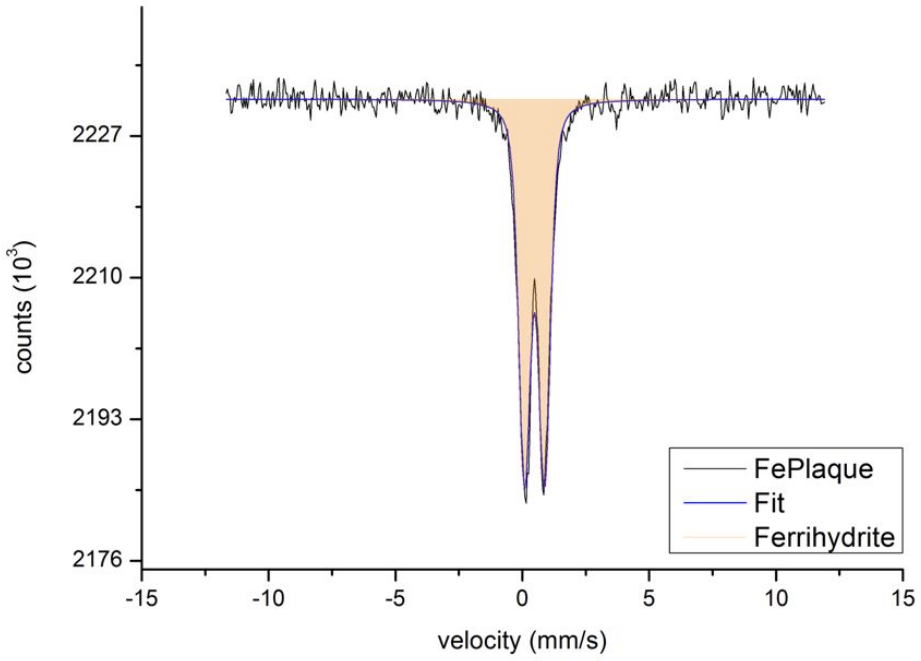

Figure S5. Mössbauer spectrum of iron plaque minerals. Iron plaque minerals were identified by Mössbauer spectroscopy at 77 K. The dominant doublet feature in the spectrum can be attributed to the presence of ferrihydrite as the only identified Fe(III) mineral phase in the sample.

Radial oxygen loss (ROL) and iron plaque formation

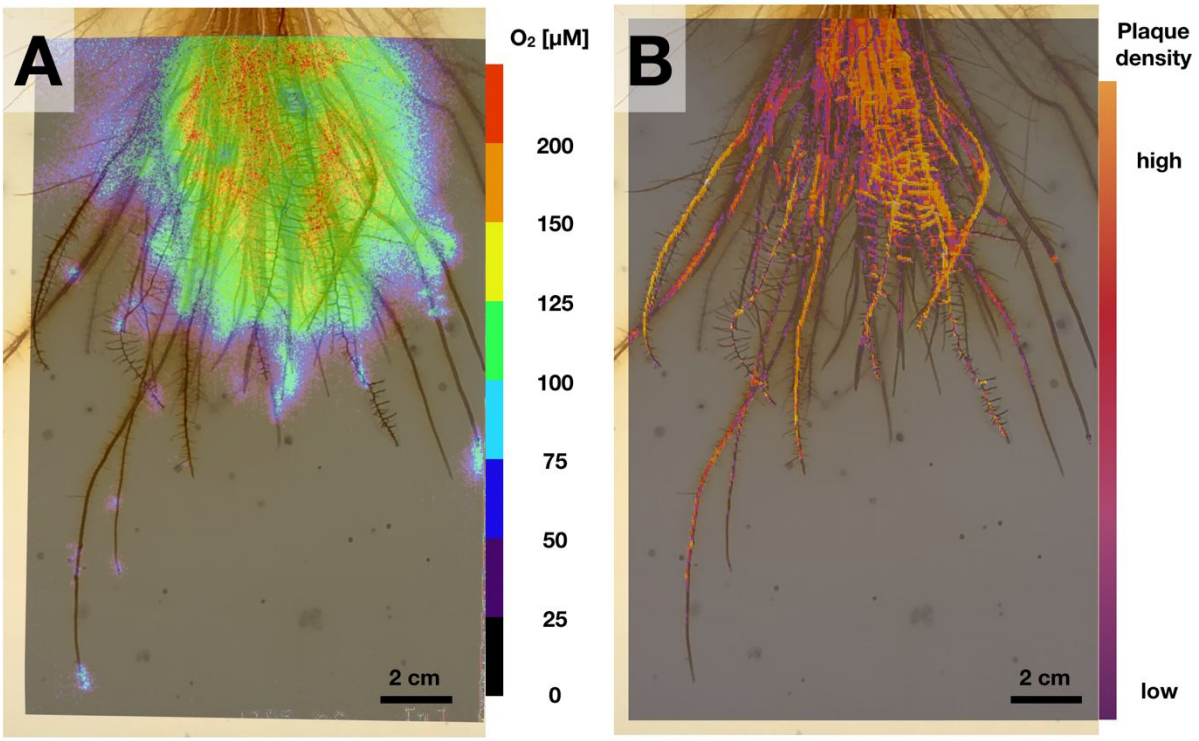

Figure S6. O_2 concentration patterns and identified iron plaque mineral precipitation. A) O_2 concentration measured with optodes. B) Iron plaque precipitation (orange areas) was detected non-invasively via image analysis by pixel thresholding along the roots and correlated with increases in local O_2 concentrations in the rhizosphere before 16 days after transfer.

Quantification of oxidized area in the rhizosphere

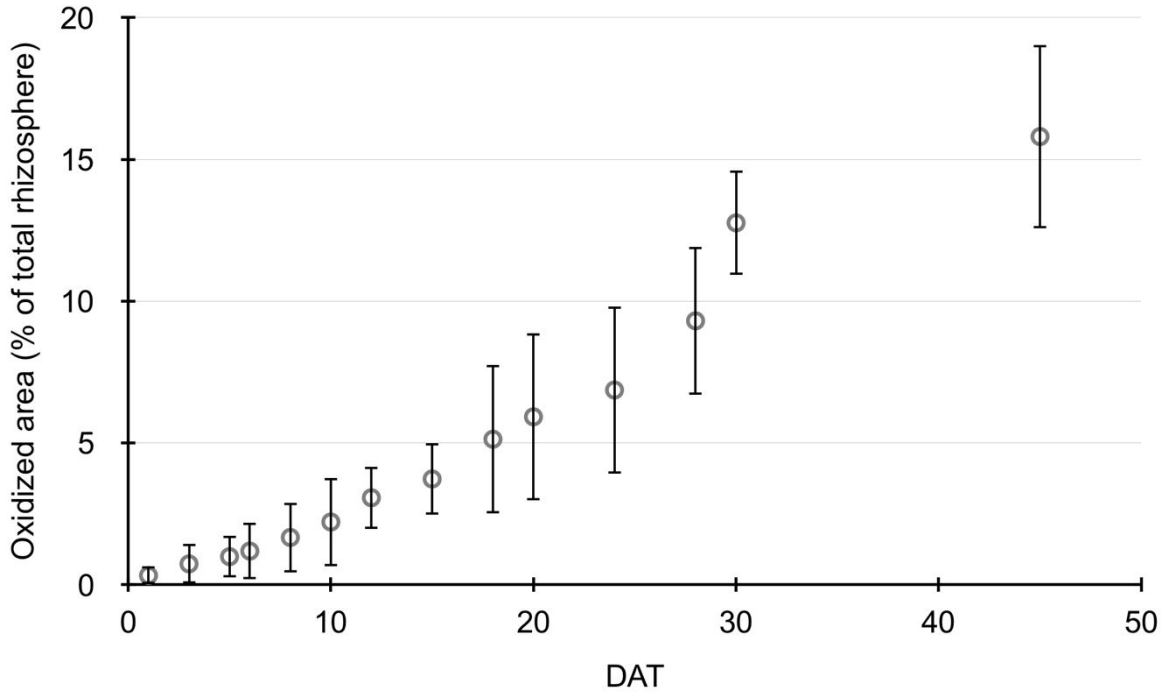

Figure S7. Quantification of oxidized rhizosphere over time. Oxidized zones in the rhizosphere were identified by image analysis and quantified non-invasively. The extension of relative area showing Fe(II) oxidation increased exponentially in all measured replicate plants (n=7) during plant growth. DAT = days after transfer.

Iron plaque formation and pH changes

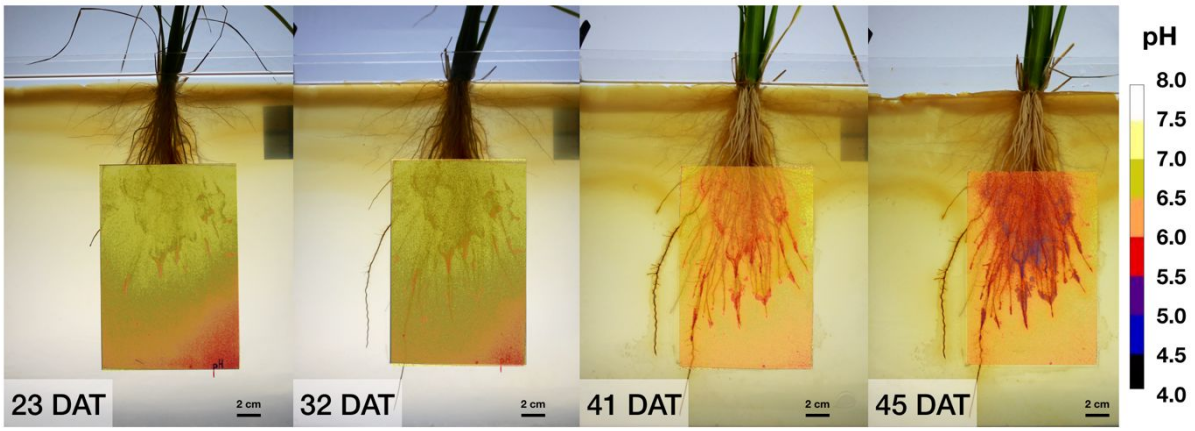

Figure S8. Local pH changes in the rhizosphere during iron plaque formation. Non-invasive mapping of the pH demonstrated that the pH decreased during iron plaque formation: A) 23 DAT, B) 32 DAT, C) 41 DAT, D) 45 DAT. (DAT = days after transfer).

References

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