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Iron Lung: How Rice Roots Induce Iron Redox Changes in the Rhizosphere and Create Niches for Microaerophilic Fe(II)-Oxidizing Bacteria

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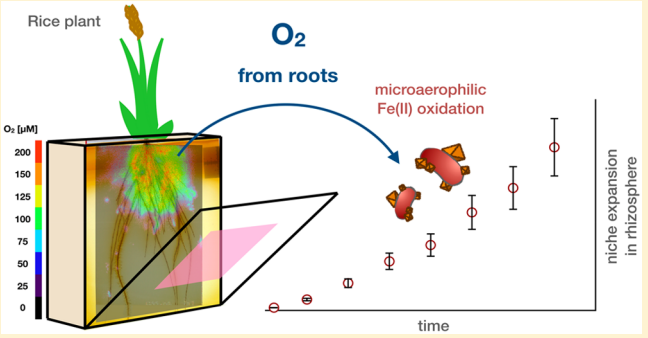
Supporting Information

ABSTRACT: Although water-logged rice paddies are characterized by anoxic conditions, radial oxygen loss (ROL) from rice roots temporarily oxygenates the soil rhizosphere. ROL not only triggers the abiotic oxidation of ferrous iron (Fe(II)) but also provides the electron acceptor for microaerophilic Fe(II)-oxidizing bacteria (microFeOx). Both processes contribute to the formation of ferric (Fe(III)) iron plaque on root surfaces. Redox interactions at single roots have been studied intensively. However, temporally resolved spatial changes of ROL in the entire rhizosphere and the impact on redoximorphic biogeochemistry are currently poorly understood. Here, we show how ROL spatiotemporally evolves and correlates with Fe-redox transformations. Applying noninvasive measurements in a transparent artificial soil, we were able to visualize opposing O_2 and Fe(II) gradients that extend from the root surface 10−25 mm into the rhizosphere. The microoxic zone expanded exponentially in size throughout the entire rhizosphere creating niches for microFeOx. Following iron mineral formation and pH, we show that root-related ROL induces iron redox transformations on and around roots and correlates with rhizosphere acidification. These findings highlight the dynamic nature of roots in the rice plant rhizosphere, and our approach spatiotemporally resolved their impact on iron redox chemistry and microbial niche formation in the rice plant rhizosphere.



INTRODUCTION

Paddy soils represent more than 6% of the world's cultivated and arable land surface. Traditionally cultivated under water-logged conditions, rice plants serve as an important conductor for gas−water exchange across the soil−atmosphere interface, including CH_4, N_2O, CO_2, and in particular O_2.[1] Oxygen from the atmosphere gets passively transported through the aerenchymatous tissue of rice plants and is released from roots as radial oxygen loss (ROL).[2] This plant-mediated oxygenation of anoxic soils strongly impacts local biogeochemistry, including metal speciation,[3,4] soil properties such as porosity and pH,[5,6] and microbial community structures,[7] as well as nutrient availability[8,9] and contaminant mobility.[10] In particular, ROL triggers the oxidation of Fe(II) and the formation of Fe(III) (oxyhydr)oxides that precipitate on the roots as ferric iron plaque.[11,12] There is a general agreement that ROL is the dominant driver for the formation of iron plaque.[13] However, it has been speculated that the formation of iron plaque can, at least, partly be attributed to microaerophilic Fe(II) oxidation (microFeOx). Such bacteria were found on the roots of many wetland plants and successfully isolated from rice paddy fields and numerous wetland environments.[14,15] In laboratory-controlled setups, that mimicked environmental parameters where microFeOx isolated from[14,16] these bacteria have been shown to actively contribute to Fe(III) mineral precipitation by up to 40% under microoxic conditions (5−30 μM O_2)[17] and even up to 60% on the roots of wetland plants in hydroponic culture.[15]

Although, geochemical conditions and redox processes at the root−soil interface of individual rice roots have been studied as a function of varying O_2, pH, and Fe(II)/(III) conditions, a systematic spatiotemporal quantification of biogeochemical gradients in the entire rhizosphere is still lacking. Especially, the remarkably high root density of rice plants with more than 20 cm root length per cm^3 surface soil (0−30 cm)[18] underline the impact rice plant roots can have on the atmospheric O_2 input into the rhizosphere of anoxic paddy soils. So far, little is known about the spatiotemporal ROL variation and its impact on the entire biogeochemical iron redox cycle. In the current study, we followed ROL during plant growth spatially and concluded on the extent of potential

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niches for microaerophilic Fe(II)-oxidizing bacteria. Different to previous studies that rely on single root measurements,[1,2,11] we hypothesize that ROL not only impacts ferric iron mineral formation at the root surface but also in the entire soil matrix where it creates suitable microoxic niches and expands potential living space for microFeOx throughout large areas of the rhizosphere. We expect that during plant and root growth, the oxygenation of soil-borne Fe(II) and the extension of zones that provide optimum O_2 (O_2 1−30 μM) and Fe(II) concentrations for microFeOx dynamically expand into the otherwise anoxic rice plant rhizosphere.

In order to exclude complex interactions between numerous environmental parameters, we established a transparent plexiglass rhizotron setup that allows a two-dimensional spatiotemporal quantification and visualization of distinct geochemical parameters in a less complex anoxic artificial Fe(II)-rich soil matrix. We conducted time-resolved high-resolution mapping of O_2, pH, and dissolved Fe(II) ($Fe(II)_{aq}$) and developed a noninvasive method to spatially quantify Fe(II) and ferric iron precipitates ($Fe(III)_{ppt}$) in the entire rhizosphere. Our findings show how (i) ROL drives iron biogeochemistry in rice paddy soils, (ii) triggers iron mineral deposition on and around the root surface, and (iii) reflects the importance of the entire rice plant rhizosphere as potential living space for microFeOx.

MATERIALS AND METHODS

Plant Growth Containers. Eight rhizotrons (growth containers made of transparent plexiglass; 25 cm × 25 cm × 3 cm i.d., Figure S1) were filled under sterile and anoxic conditions with 1.73 cm^3 anoxic Hoagland solution[19] (100%, 35 °C, pH 6.8) that was amended with 500 μM $Fe(II)_{aq}$ (from $FeCl_2$). Similar $Fe(II)_{aq}$ concentrations have been reported for many wetland environments where microFeOx has been observed and were documented as common Fe(II) concentrations for water-logged paddy fields.[20−24] The liquid matrix was stabilized by adding 0.3% Gelrite (Carl Roth, Karlsruhe, Germany) as a gelling agent forming a transparent solid artificial soil matrix at room temperature.[25] Approximately 100 mL of 20% Hoagland solution was constantly kept on top of the growth gel to prevent desiccation. The advantage of working with a transparent artificial soil matrix over real soil is to apply a suite of visual analyzing techniques that allow high spatial resolution of temporal geochemical patterns in the rice plant rhizosphere. The simplification of the natural system lacks numerous geochemical interactions substantially impacting (iron redox) geochemistry. However, the transparent setup allows us to prevent interference of such processes and provides the opportunity to visually follow only specific parameters (Fe(II)/(III), O_2, pH) and to quantify the maximum impact of ROL on the iron (bio)geochemistry.

Rice seeds (*Oryza sativa* Nipponbare) were sterilized (0.1% H_2O_2 rinse, sterile ultrapure water) and pregrown on sterile Hoagland (50%) solution at pH 6.8. Then, the seedlings were transferred into rhizotrons at the three-leaf stage. Plants were grown under temperature-controlled conditions (25−27 °C), at constant relative humidity (70%) and illumination conditions (high pressure sodium lamp, 10,000−12,000 lx) at day (14 h)−night (10 h) cycles. Rhizotrons were wrapped in opaque fabric to prevent any illumination of the roots. The front panel of the rhizotron was removable for in situ microsensor measurements.

Geochemical Measurements. Voltammetric microsensor measurements were applied to quantify $Fe(II)_{aq}$ in situ in a reference rhizotron container for color referencing (see below). Cu−Au sensors with a Hg−Au alloy surface (100 μm) were constructed in the lab[26] and hooked to a potentiostat (DLK-70, Analytical Instrument Systems, Flemington, NJ, USA). In order to perform in situ microsensor measurements inside the rhizosphere, the rhizotron was positioned horizontally, and the front cover was removed (lifted) under anoxic conditions. Measurements were performed as described in the Supporting Information.

Oxygen and pH measurements were performed using noninvasive planar optode sensor foils (SF-RPSu4 and SF-HP5R, PreSens, Regensburg, Germany) (O_2 and pH: 15 cm× 9 cm) that were glued onto the inside of a plexiglass front window, a fluorescing light source (Big Area Imaging Kit, PreSens, Regensburg, Germany), and a camera detector (VisiSens TD, PreSens, Regensburg, Germany). Prior to measurements, sensor foils were calibrated using the recommended procedures given by the provider at constant temperature (25 °C) in a Hoagland solution (100%) matrix. Images were recorded at constant temperature conditions in a dark room without any light sources, except the fluorescing illumination system required for optode records. Further information on two-dimensional O_2 and pH detection is given in the Supporting Information. Digital images of the root zone were taken after the simulated daylight period (14 h) within a maximum of 1 h using a camera (EOS 1200D, Canon) with constant camera and illumination settings. Potential habitable zones for microFeOx were assigned accounting for O_2 threshold concentrations of 1−30 μM O_2 (data obtained from O_2 optode sensor images using the software VisiSens, ScientifiCal (Presens, Regensburg, Germany)). The two-dimensional extension of assigned zones was calculated using imageJ.[27] The two-dimensional relative area (in %) for designated zones was calculated related to the total area of the artificial soil (575 cm^2).

Iron Mineral analyses. Iron plaque and iron mineral formation was followed using image analysis. For image-based iron mineral identification, the rhizotron was carefully positioned horizontally, and the plexiglass front window (with planar optode foil) was exchanged to a fully transparent one under anoxic and sterile conditions. Formation of orange Fe(III) (oxyhydr)oxide minerals in the gel matrix and on the root surface was followed based on color-coded pixel analysis.[28,29] Image analysis was performed on the basis of color thresholding and pixel color code identity using the software Fiji (Ver.1.0, ImageJ) and a color correlation function calibrated for in situ Fe(II) combined with identity of pixel matrices using Matlab (Supporting Information).

RESULTS AND DISCUSSION

Rice Roots as a Pipeline for O_2—Creating a Niche for Microaerophilic Fe(II) Oxidation. Rice root growth and the accompanied changes in geochemistry were monitored in transparent rhizotron cultivation chambers in order to quantify ROL and to detect niches for microFeOx on a spatiotemporal scale. Atmospheric O_2 that passively diffused through the aerenchymatous tissues was released as ROL and showed a maximum of 10−20 μM O_2 after 6−14 days after transfer (DAT) of plants in patchy patterns restricted to a narrow zone of 1−3 mm around single roots. After 15 DAT, O_2 concentrations increased, forming a constantly oxic zone

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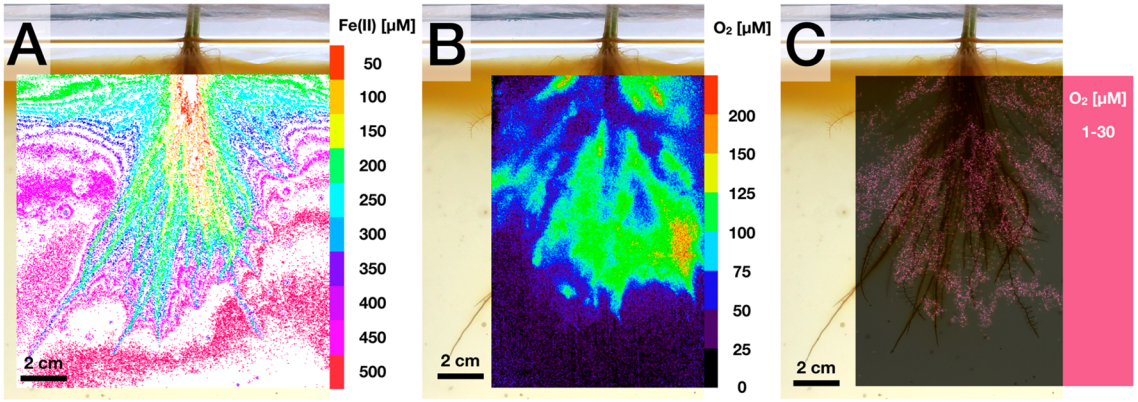

Figure 1. Rhizosphere of rice plant 28 DAT. (A) Noninvasive mapping of $Fe(II)_{aq}$ in the artificial soil matrix. In the unrooted zone, $Fe(II)_{aq}$ concentrations remained high at initial $Fe(II)_{aq}$ concentrations of 500 μM. In the rhizosphere, $Fe(II)_{aq}$ concentrations decrease gradually around roots. (B) Noninvasive mapping of O_2 in the rhizosphere. Oxygen concentrations increase in the immediate vicinity of roots and show steep gradient from high (>100 μM) to low (<25 μM) O_2 concentrations from roots to rhizosphere. (C) Microoxic zone with O_2 concentrations (1−30 μM) providing suitable O_2 conditions for microFeOx.

(with a relative soil area of approximately 32 ± 10.2 cm^2) with O_2 > 50 μM in the upper zone where basal roots formed a dense root zone that occupied more than 5% of the total rhizosphere (Figure S2). After greater than 35 DAT, the basal root zone was dominated by high O_2 concentrations (O_2 > 100 μM) and a constantly oxygenated zone with a relative area of approximately 56.4 ± 21.3 cm^2, which reflected approximately 10% of the total rhizosphere. Using microsensors, we were able to resolve O_2 gradients around individual roots on a μm resolution. On the surfaces of lateral roots, O_2 concentrations of more than 100 μM O_2 were detected, depleting gradually to <5 μM O_2 at a 2 mm distance away from the root into the rhizosphere (Figure S2D). The O_2 from ROL that diffuses toward soil-borne $Fe(II)_{aq}$ would rapidly oxidize $Fe(II)_{aq}$ chemically and form ferric (oxyhydr)oxides.[30] However, chemical Fe(II) oxidation negatively correlates with O_2 and slows with decreasing O_2 concentrations[31] which are found at a distance of 10−20 mm away from the roots along the O_2 diffusion gradient (Figure S3). From 5 DAT until the end of plant growth (45 DAT), we measured O_2 concentrations less than 30 μM O_2, in a distance of approximately 5−25 mm away from root surfaces. Under these conditions, Fe(II) half-life times were calculated to be in the range of more than 10 h (Figure S3), which would prolong the persistence of Fe(II) and increase the bioavailability for microaerophilic Fe(II)-oxidizing bacteria.[15,16,32−34]

Previous studies suggested that these microaerophilic Fe(II)-oxidizing bacteria have their physiological niche in a microoxic environment with O_2 concentrations ranging from approximately 1 to less than 50 μM.[16,32] Recent observations showed that microaerophilic bacteria from a paddy field can contribute efficiently up to 40% to the formation of Fe(III) minerals and under optimum O_2 conditions between 1 and 30 μM O_2.[17] Such bacteria were even shown to enhance iron plaque formation on roots of wetland plants.[4] We detected that areas with optimum $Fe(II)_{aq}$ and O_2 conditions for microFeOx (1−30 μM O_2) occur spontaneously, especially on young rice roots (Figure 1A and B), while the redoximorphic transition zone from a constantly oxic bulk root zone to the anoxic rhizosphere provided a rather stable opposing gradient of greater than 200 μM $Fe(II)_{aq}$ and less than 30 μM O_2. Geochemical conditions in these zones shifted drastically within 7−10 days with a significant increase in ROL creating oxygenated zones in the bulk root zone with O_2 concentrations grater than >30 μM. The increase in O_2 concentrations was shown to enhance abiotic Fe(II) oxidation reactions by up to 30%, favoring chemically induced Fe(III) mineral formation (Figure S3). Thus, potential niches for microFeOx around rice roots are only short term (<7 days) and turn into oxygenated zones with rapid abiotic Fe(II) oxidation which would kinetically outcompete microaerophilic Fe(II) oxidation.[32] However, studies have demonstrated that bacteria (<2 μm, which was reported as size for microaerophilic Fe(II)-oxidizing representatives[35]) show a relatively high passive mobility (more than 10% of total cells) within the soil porewater.[36] Additionally, the active motility of microFeOx[37] could enhance the mobility of microFeOx within the soil matrix and allow it to continue occupying the habitable niche within the rice plant rhizosphere.

Following O_2 and $Fe(II)_{aq}$ distribution patterns, we observed that during plant growth optimal O_2 conditions for microaerophilic Fe(II)-oxidizing bacteria expanded along the O_2 diffusion gradient, away from the root surface into the rhizosphere (Figure 1C). On root surfaces, along basal roots and in the dense bulk root zone, significantly high O_2 (>100 μM O_2) and considerably low $Fe(II)_{aq}$ concentrations (<100 μM $Fe(II)_{aq}$) were detected (Figure 1A). In these zones, dominated by high O_2 concentrations, more than 60% of the $Fe(II)_{aq}$ that was initially present was oxidized and precipitated as Fe(III) minerals. In these zones, Fe(II) half-life times were calculated to be considerably low (<5 h) which decreases the Fe(II) availability for microFeOx and thus increases the competition pressure for microbes with chemical Fe(II) oxidation (Figure S3). Microaerophilic Fe(II)-oxidizing bacteria would find suitable conditions only along the opposing gradients of $Fe(II)_{aq}$ and O_2 either (i) in the redoximorphic zone between the root surface (10−25 mm) and anoxic rhizosphere or (ii) in dynamic geochemical hotspots along the roots with periodic ROL, creating temporal microoxic zones (Figure 1C).

The first hypothesis is strongly supported by observations made by Weiss et al.[7] who observed significantly more (3.7×10^6 g^{-1} soil) microaerophilic Fe(II)-oxidizing bacteria in the soil matrix than directly with the roots of various wetland plants (5.9×10^5 g^{-1} root). This relative two-dimensional area in the rhizosphere, that provided suitable conditions for

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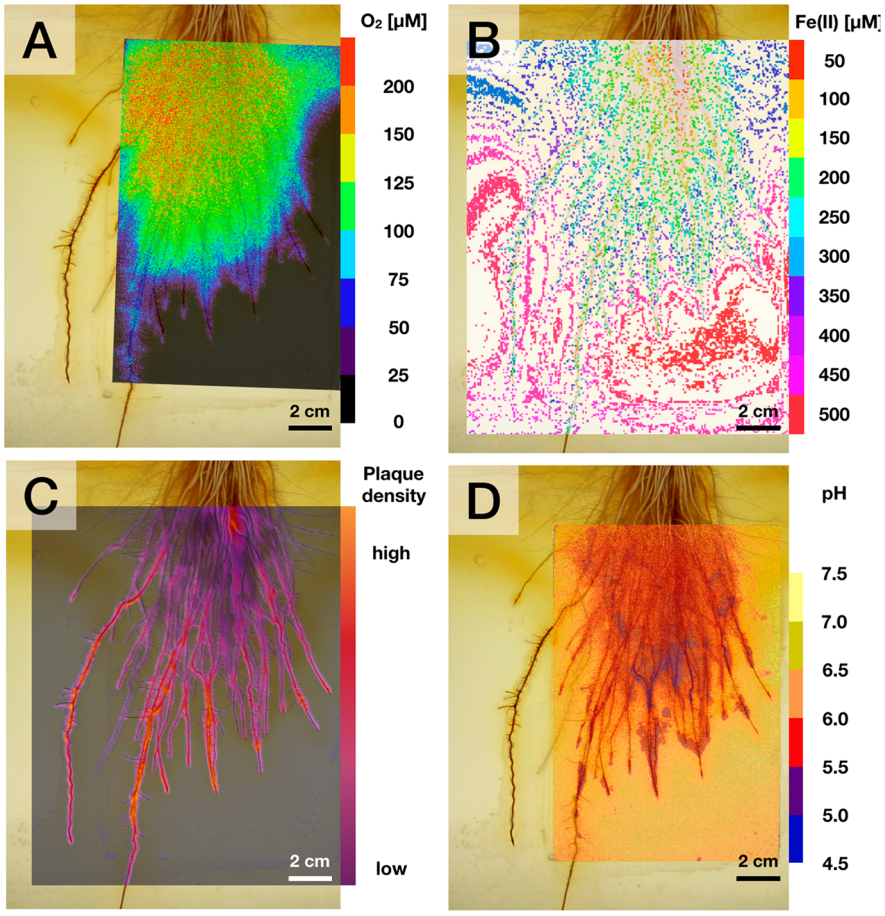

Figure 2. Rhizosphere of rice plant 45 DAT: oxygen, $Fe(II)_{aq}$, iron plaque precipitation, and pH patterns. (A) Oxygen was mainly detected in the basal root zone, forming a steep gradient around from O_2 > 200 μM in the bulk root zone to O_2 < 25 μM in the unrooted soil matrix. (B) $Fe(II)_{aq}$ concentrations decrease in the bulk root zone to $Fe(II)_{aq}$ < 200 μM and increase gradually toward the soil matrix to 500 μM $Fe(II)_{aq}$. (C) Iron plaque formed dense Fe(III) (oxyhydr)oxide plaque on young roots, while basal roots showed less iron plaque. (D) Soil matrix pH decreased around root tips, potentially due to iron plaque formation on/around root surface.

microFeOx, was found in the current study to expand exponentially during plant growth and increased from initially 4.8 ± 1.3 cm^2 (<1% of total rhizosphere) at 11 DAT to more than 32 ±11.4 cm^2 (>5% of rhizosphere) 45 DAT (Figure S4). Such spatiotemporal fluctuations in Fe(II) and O_2 availability that were found throughout the entire rhizosphere not only highlight the dynamic character of the rice plant rhizosphere but also might explain why microFeOx has been shown to metabolically respond very effectively to geochemical changes in highly dynamic environments like rhizosphere hot spots.[35] The substantial relative abundance of microFeOx in wetland plant rhizospheres (1%−6% of total microbial community[9,15]) such as paddy soils suggests that these bacteria can widely impact the iron cycle not only around the roots of rice plants in paddy fields but also around the roots of any wetland plant.[7] In addition to that, the current findings quantitatively demonstrate that ROL from roots expands the habitable niche of microFeOx throughout the entire rhizosphere which strongly increases the impact of microaerophilic Fe(II) oxidation during plant growth.

Rusty Roots—Iron Mineral Precipitation Driven by Radial Oxygen Loss. In order to spatiodynamically correlate ROL from plant roots to iron redox chemistry, the formation of Fe(III) minerals and changes in pH were monitored noninvasively during plant growth. Within 14 DAT, fresh orange/reddish iron plaque formed on young root surfaces (shown to be ferrihydrite; Table S1 and Figure S5) in heterogeneous patterns that considerably correlated with measured O_2 concentrations (Figure S6). After 16 DAT, Fe(III) mineral formation was not only limited to the surface of the roots, where highest O_2 concentrations were measured, but was also detected a distance of up to 15 mm away from the roots where O_2 concentrations reached 30−100 μM. Based on image analysis, we quantified that the relative area that was affected by Fe(II) oxidation expanded exponentially (R^2 = 0.95, n = 7) from less than 10 cm^2 (3 DAT) to a maximum of 95 ± 12.8 cm^2 (approximately 16.5% of total rhizosphere) on average by 45 DAT (Figure S7).

Fe(II) oxidation and consequent iron plaque formation induced a decrease in pH, which was monitored across the rhizosphere (Figure 2). After 23 DAT, pH dropped from initially 6.8 to 6.0−6.5 at the redoximorphic root−soil matrix interface (Figure S8), potentially caused by protons generated during the precipitation of Fe(III) (oxyhydr)oxides and acidification by the release of plant exudates, chelating agents, or plant-derived organic acid molecules[38] (found to decrease soil pH by up to 2 pH units).[39,40] The acidification of the unbuffered artificial rhizosphere proceeded until the end of the growth experiment after 45 DAT (pH 5.0−5.5; Figure S8) and significantly correlated to areas with the highest extent in

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Fe(III) mineral formation (Figure 2C and D). The observed pH decrease induced by ROL in our setup has been observed in a similar manner in various wetland soils and was hypothesized to be attributed to ROL-driven Fe(II) oxidation.[40−43] These observed changes in soil pH will not only positively affect Fe(III) solubility but also increase Fe(II) half-life times[28] and bioavailability for microbial Fe(II) turnover. However, in natural rice paddy soils, the soil pH buffer capacity would potentially decrease extensive changes in soil pH related to Fe(II) oxidation.

Transferability to Soil Rhizosphere Systems. The artificial soil matrix represents a simplified rhizosphere system compared to natural settings. However, this approach not only allows us to decipher the impact of ROL on iron speciation within the entire rhizosphere by visualizing geochemical gradient via imaging techniques but also facilitates the correlation of pH changes with ROL and visible Fe(III) (oxyhydr)oxide formation on and around the rice roots. Optimum application of imaging techniques and the quantification of a maximum impact of ROL on rhizosphere geochemistry enable us to construct and predict niches for microaerophilic bacteria on an extremely high spatial and temporal resolution. Diffusion coefficients for O_2 and other gases in water-saturated rhizosphere soils ($3.2−8.0 \times 10^{-10}$ m^2 s^{-2} [44,45]) are slightly smaller compared to O_2 diffusivity in the growth gel matrix ($1.6−2.1 \times 10^{-9}$ m^2 s^{-2} [46]). Thus, O_2 diffusion properties for the artificial soil are in a comparable range to real soil matrices. However, for the empirical modeling of spatiodynamic fluxes (e.g., root-derived O_2, mobility in soil, and Fe(II) oxidation) using the presented approach, differences in diffusion coefficients between the artificial soil system to real paddy soil need to be considered. For a qualitative extrapolation of habitable niches for microFeOx from the artificial growth matrix to real paddy soils, differences in O_2 diffusivity are minor and within the natural variation of a soil matrix.[47−49]

Nevertheless, in a natural rhizosphere, not only the physical heterogeneity[50] but also a variety of aerobic and microaerophilic soil microorganisms (e.g., heterotrophs and methanotrophs) will affect O_2 mobility away from the root and compete for O_2, consequently decreasing the availability for microFeOx and potentially narrow down their relative niche expansion.[51] Other reducing agents in a natural rhizosphere, such as redox-active exudates, chelating complexes such as small organic acids, or reducing metal sulfides, might additionally scavenge O_2 from ROL and considerably decrease the habitable niche for microFeOx in environmental settings. Additionally, these (plant- or microbially derived) organic molecules may trigger heterotrophic processes and or Fe(III)-reducing processes that shift redox conditions in either direction.[52] Nevertheless, the gathered data in the artificial soil replacement clearly proves the power of ROL to favor the formation of habitable niches for microFeOx in an otherwise anoxic environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.9b00403.

One table and eight figures. (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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