Geomicrobiology Journal, 24:65–73, 2007
Copyright © Taylor & Francis Group, LLC
ISSN: 0149-0451 print / 1521-0529 online
DOI: 10.1080/01490450601134309

Taylor & Francis
Taylor & Francis Group

Returning to Their Roots: Iron-Oxidizing Bacteria Enhance Short-Term Plaque Formation in the Wetland-Plant Rhizosphere

Scott C. Neubauer and Gloried E. Toledo-Durán
Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

David Emerson
American Type Culture Collection, Manassas, VA 20110, USA

J. Patrick Megonigal
Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

In the wetland rhizosphere, high densities of lithotrophic Fe(II)-oxidizing bacteria (FeOB) and a favorable environment (i.e., high Fe(II) availability and microaerobic conditions) suggest that these organisms are actively contributing to the formation of Fe plaque on plant roots. We manipulated the presence/absence of an Fe(II)-oxidizing bacterium (*Sideroxydans paludicola*, strain BrT) in axenic hydroponic microcosms containing the roots of intact *Juncus effusus* (soft rush) plants to determine if FeOB affected total rates of rhizosphere Fe(II) oxidation and Fe plaque accumulation. Our experimental data highlight the importance of both FeOB and plants in influencing short-term rates of rhizosphere Fe oxidation. Over time scales ca. 1 wk, the FeOB increased Fe(II) oxidation rates by 1.3 to 1.7 times relative to FeOB-free microcosms. Across multiple experimental trials, Fe(II) oxidation rates were significantly correlated with root biomass, reflecting the importance of radial O_2 loss in supporting rhizosphere Fe(II) oxidation. Rates of root Fe(III) plaque accumulation (time scales: 3 to 6 wk) were ∼70 to 83% lower than expected based on the short-term Fe(II) oxidation rates and were unaffected by the presence/absence of FeOB. Decreasing rates of Fe(II) oxidation and Fe(III) plaque accumulation with increasing time scales indicate changes in rates of Fe(II) diffusion and radial O_2 loss, shifts in the location of Fe oxide accumulation, or temporal changes in the microbial community within the microcosms. The microcosms used herein replicated many of the environmental characteristics of wetland systems and allowed us to demonstrate that FeOB can stimulate rates of Fe(II) oxidation in the wetland rhizosphere, a finding that has implications for the biogeochemical cycling of carbon, metals, and nutrients in wetland ecosystems.

Keywords Fe plaque, iron oxidation, iron-oxidizing bacteria (FeOB), *Juncus effusus*, Rhizosphere processes, Wetland biogeochemistry

Received 5 June 2006; accepted 30 October 2006.
Neubauer's Current address: Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442, USA.
Current address for Toledo-Durán, University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico 00681.
We thank Kim Givler and Amy Wolfe for laboratory assistance. We also thank two anonymous reviewers for constructive comments that improved the manuscript. This work was supported by NSF grant DEB-9986981 to J.P.M and D.E.
Address correspondence to Scott C. Neubauer, Baruch Marine Field Laboratory, University of South Carolina, PO Box 1630, Georgetown, SC 29442, USA. E-mail: scott@belle.baruch.sc.edu

INTRODUCTION

Microbially mediated reactions and chemical processes are responsible for the formation of iron oxyhydroxide coatings (i.e., Fe plaque) on the surface of many wetland plant roots. This plaque is a visual indication that subsurface oxidative processes are occurring in otherwise anoxic wetland soils and sediments. Oxygen that leaks out of plant roots (a processes known as radial O_2 loss; Armstrong 1964) can react with ferrous iron [i.e., Fe(II)] to form iron oxides that are deposited on or near plant roots. Iron oxides may also form in the absence of molecular O_2 if NO_3^- or perchlorate are used as oxidants (Straub et al. 1996; Lack et al. 2002), although the significance of anaerobic Fe(II) oxidation in the rhizosphere is currently unknown. Root Fe deposits are biogeochemically significant and can sequester significant amounts of PO_4^{3-} and metals including As, Zn, Cu, and Pb (Taylor and Crowder 1983; Peverly et al. 1995; Weis and Weis 2004; Chen et al. 2005). Subsequent reduction of iron plaque can suppress methanogenesis if Fe(III)-reducing bacteria outcompete methanogens for electron donors (Roden and Wetzel 1996; Neubauer et al. 2005). Because abiotic iron oxidation can be rapid under circumneutral conditions (Stumm and Morgan 1981), it has been largely assumed that plaque formation is primarily a chemically-driven process (Mendelssohn et al. 1995). Recent evidence, however, suggests that iron-oxidizing bacteria (FeOB) may play a key role in mediating rhizosphere Fe(II) oxidation and plaque formation.

The wetland rhizosphere is an interface between aerobic and anaerobic environments and contains a diverse community of both aerobic and anaerobic microbes. Many microbial cells are imbedded in Fe plaque (e.g. Trolldenier 1988; St-Cyr et al. 1993), but it was not until the late 1990s that the presence of lithotrophic FeOB in the rhizosphere was conclusively demonstrated (Emerson et al. 1999; Weiss et al. 2003). To date, at least 4 strains of FeOB have been enriched from the rhizosphere of wetland plants (Emerson et al. 1999; Weiss et al. in review). Despite some phylogenetic diversity among these FeOB, all are lithotrophic and acquire energy via the oxidation of Fe(II) under microaerobic conditions, an environment where rates of chemical Fe(II) oxidation are depressed. A survey of mid-Atlantic (USA) wetlands and aquatic habitats revealed that these FeOB are widespread and may comprise up to 5% of the total microbial community in the wetland rhizosphere (Weiss et al. 2003). The favorable environment in the rhizosphere (i.e., high Fe(II) availability and microaerobic conditions) and high FeOB densities are circumstantial evidence that these organisms are actively contributing to the formation of Fe plaque.

Methodological difficulties associated with working in complex microbial-plant-soil systems have limited our ability to quantify how lithotrophic FeOB affect rates of rhizosphere Fe(II) oxidation and plaque formation. In a series of batch culture laboratory experiments, Neubauer et al. (2002) studied the Fe(II) oxidation kinetics of a rhizosphere-isolated lithotrophic FeOB and determined that this bacterium mediated up to 62% of the total (biotic + abiotic) Fe(II) oxidation. In addition to successfully competing with chemical reactions for Fe(II) and O_2, the FeOB were able to accelerate total Fe(II) oxidation rates by up to 18%. There was also evidence that the FeOB inhibited rates of chemical Fe(II) oxidation, perhaps by temporarily binding Fe(II) within a matrix of bacterially-produced exopolymers (Neubauer et al. 2002). In the present study, we used a series of hydroponic microcosms containing the roots of intact *Juncus effusus* (soft rush) plants to determine if FeOB have an effect on total rhizosphere Fe(II) oxidation rates. Based on our previous work (Neubauer et al. 2002), we hypothesized that microcosms inoculated with FeOB would have higher rates of Fe(II) oxidation and Fe(III) plaque accumulation than microcosms without FeOB. The microcosms were designed to eliminate the microbial complexity of a natural microbial-plant-soil system so the role of FeOB could be specifically studied, while also having the FeOB exposed to conditions representative of the wetland rhizosphere.

METHODS

Description of Iron-Oxidizing Bacteria. Strain BrT is a neutrophilic Fe(II)-oxidizing bacteria (FeOB) isolated from the rhizosphere of *Typha latifolia* (broad-lead cattail) growing in a created marsh in Maryland. This lithoautotrophic FeOB requires Fe(II) and microaerobic conditions for growth. It cannot grow using heterotrophic media, formate, Mn(II), H_2, or reduced sulfur compounds as electron donors, nor can it use NO_3^- as an electron acceptor. Genotypically, BrT is a strain of *Sideroxydans paludicola* and lies within the ß-proteobacteria (Weiss et al. in review). Additional details about strain BrT and other similar rhizosphere FeOB can be found elsewhere (Emerson et al. 1999; Neubauer et al. 2002; Weiss et al. 2003; Weiss et al. in review). All experiments described herein were conducted with BrT cells maintained in gradient tubes with opposing gradients of O_2 and Fe(II).

Microcosm Setup. Approximately 3 months before the April and July experiments, *Juncus effusus* seeds were planted in a 50:50 mixture of moistened peat moss and potting soil. Following seed germination, the soil mix was consistently saturated with water or flooded during plant growth because the O_2 demand of the substrate can influence aerenchyma development and rates of radial O_2 loss (Sorrell and Armstrong 1994; Sorrell 1999). For the microcosm experiments, the roots of *J. effusus* plants were gently washed to remove any adhering soil particles, carefully inserted through a hole in the lid of each microcosm, and the space surrounding the base of the plant stem was filled with wax to support the plant stems and seal the opening (Figure 1). The plant roots and the underside of the microcosm lids were surface sterilized by agitation in a 0.2% sodium dodecyl sulfate (SDS) solution for 5 minutes, followed by an overnight soaking in an antibiotic solution containing streptomycin (0.1 g L^{-1}), nalidixic acid (0.1 g L^{-1}), ampicillin (0.05 g L^{-1}), and amphoterecin B (0.005 g L^{-1}) (Calhoun and King 1997). Microscopic examination of roots treated with SDS and antibiotics, and subsequently stained with a BacLight Live/Dead viability kit (Molecular Probes, Eugene, Oregon), showed that few live cells remained on the root surface (data not shown). Our goal was not to completely sterilize the microcosms for the entire 3- to 6-week

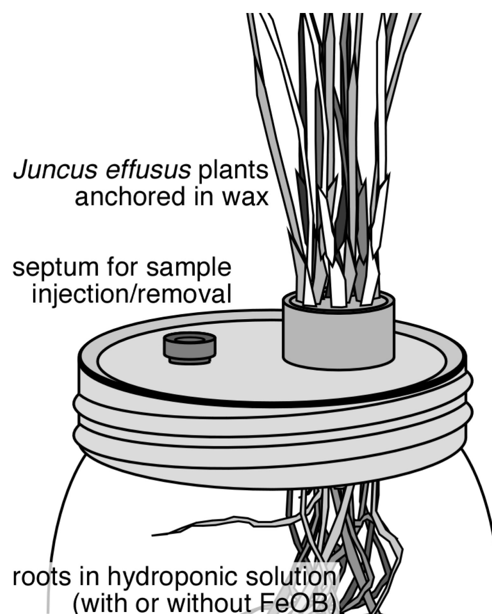

FIG. 1. Schematic of microcosms used for Fe(II) oxidation experiments.

duration of each experiment but rather to allow us to manipulate the presence/absence of FeOB at the start of each experiment. Following surface sterilization, the roots were rinsed 3 times in sterile DI water and placed into a 0.94 L glass jar containing 800 ml of sterile 0.25X Hoagland's solution, buffered with 5 or 10 mmol L^{-1} 2-(N-Morpholino)ethanesulfonic acid (MES). After the microcosm lid was sealed to the jar, the hydroponic solution inside each microcosm was bubbled overnight with 0.2 μm filter-sterilized N_2 to remove O_2. Following bubbling, a filter-sterilized and deoxygenated $NaHCO_3$ solution containing trace vitamins and minerals was added to adjust the pH to ∼6 (final $NaHCO_3$ concentration = 5.6 mmol L^{-1}).

Three microcosm types were established for each experiment: (i) plants inoculated with live cells of the Fe(II) oxidizer BrT ("+FeOB" microcosms; n = 10 or 20), (ii) plants inoculated with killed (autoclaved) BrT cells ("–FeOB;" n = 10 or 20), and (iii) experimental blanks containing no plants or Fe(II) oxidizers ("blanks;" n = 5). Collectively, the +FeOB and –FeOB microcosms are called the treatment microcosms. The experimental blanks were used to determine rates of O_2 leakage and Fe(II) oxidation in the absence of plants or microbes. Following inoculation, sterile deoxygenated $FeCl_2{\cdot}4H_2O$ was added to each microcosm to give a final Fe(II) concentration of ∼1000 μmol L^{-1}. Additional $FeCl_2{\cdot}4H_2O$ was added as needed whenever Fe(II) concentrations fell below ∼150 μmol L^{-1}. To minimize O_2 leakage through the sealed lids, each microcosm was submerged until the top of the wax-filled cylinder holding the plant was under water. Thus, the only source of O_2 to support Fe(II) oxidation was radial O_2 loss from the plant roots. All microcosms were wrapped in foil to keep light out of the hydroponic solution and were incubated in an environmental growth chamber at 28°C under a light regime of 14 h light:10 h dark.

Sampling the Microcosms. At 1- to 2-day intervals, aliquots of the hydroponic solution were removed from each microcosm using sterile needles and syringes. The concentration of soluble Fe(II) (i.e. Fe^{2+}) was determined using 0.1% ferrozine in 50 mmol L^{-1} HEPES buffer; samples were read on a spectrophotometer at 562 nm within minutes of sample collection. At the beginning of each experiment and after ∼3 weeks (April: 21 d; July: 18 d) and 6 weeks (41 d, April only), a subset of the microcosms were destructively sampled so root Fe plaque concentrations could be measured. After the microcosms were opened, the pH of the hydroponic solution was measured.

The roots were removed from the solution and rinsed at least 5X in sterile DI water to remove any cells and Fe that were not bound to the root surface (i.e., were not part of the Fe plaque). This operational approach will provide a conservative estimate of rhizosphere Fe(II) oxidation if the influence of the roots extends beyond the Fe plaque boundary. However, this protocol provides an easy and reproducible means of defining and distinguishing root plaque from other Fe pools and is consistent with our previous studies that have examined root-influenced Fe cycling (e.g., Weiss et al. 2003, 2005). Approximately half of the roots were extracted in 0.5 mol L^{-1} HCl for 30 minutes. An aliquot of the HCl extract was then added to ferrozine and analyzed for Fe(II) (as above) and total Fe (following reduction of Fe(III) with 0.25 mol L^{-1} hydroxylamine hydrochloride in 0.25 mol L^{-1} HCl). The green (aboveground) biomass, roots extracted for Fe plaque, and all remaining roots were separately dried at 80°C and weighed.

Data Analysis and Calculations. For the treatment microcosms, an exponential decay equation was used to describe temporal changes in soluble [Fe(II)] during the experiments (e.g., Figure 2A).

$$[\mathrm{Fe(II)}] = a \times e^{(-b\times \mathrm{time})} + c \quad [1]$$

where [Fe(II)] is in μmol L^{-1}, time is h since a pulse of Fe(II) was added to the microcosm, and a, b, and c are empirically determined coefficients . At any given point along the curve, the rate of Fe(II) disappearance is the first derivative of equation 1 with respect to time, or

$$\mathrm{d[Fe(II)]/dt} = -a \times e^{(-b\times \mathrm{time})} \times (-b) \quad [2]$$

By convention, the rate of Fe(II) oxidation is calculated as – d[Fe(II)]/dt. In other words, when Fe(II) concentrations

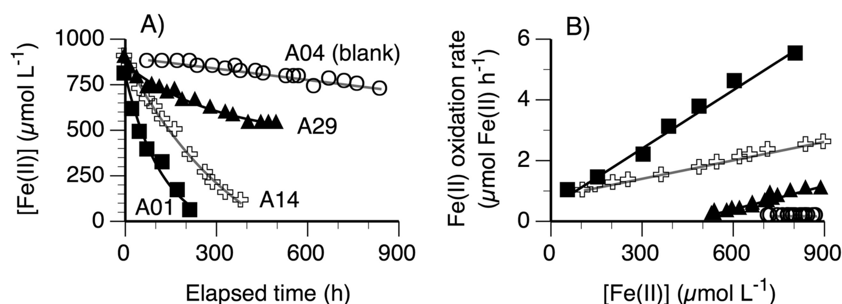

FIG. 2. Examples of data used to calculate Fe(II) oxidation rates. The plotted data were selected to illustrate some of the variability in Fe(II) oxidation rates between microcosms. (A) Fe(II) vs. time curves for treatment microcosms (e.g., A01, A14, A29) were well-described by exponential curves of the form Fe(II) = $a\times e^{-b\times \mathrm{time}} + c$; Fe(II) concentrations decreased linearly in blank microcosms (e.g. A04). (B) The rate of Fe(II) oxidation varied linearly as a function of [Fe(II)] in the treatment microcosms. For between-microcosm comparisons, Fe(II) oxidation rates were calculated at 750 μM Fe(II).

decrease in the microcosm (i.e., $d[Fe(II)]/dt < 0$), there is a positive rate of Fe(II) oxidation. Because equation 1 is non-linear, the rate of Fe(II) oxidation varies as a function of the ambient Fe(II) concentration (which is, itself, a function of time) (Figure 2B). To allow meaningful comparisons between microcosms and among treatments, we calculated the Fe(II) oxidation rate at a standard Fe(II) concentration of 750 μmol L^{-1}, a value that was within the range of Fe(II) values in the microcosms across both experimental runs and is typical of Fe(II) concentrations in wetland soils. Thus, we can solve for t_{750} (that is, the time when the target Fe(II) concentration, $Fe(II)_{target}$, equals 750 μmol L^{-1}) as

$$t_{750} = \ln[(Fe(II)_{target} - c)/a]/(-b) \quad [3]$$

Replacing the variable "time" in equation 2 with the output from equation 3 allows us to calculate the Fe(II) oxidation rate for all microcosms at a standardized Fe(II) concentration of 750 μmol L^{-1}. Multiplying the results of equation 2 by the volume of solution in the microcosms (0.8 L) gives the Fe(II) oxidation rate in μmol Fe(II) oxidized h^{-1}.

For each experiment, the maximum rate of Fe(II) oxidation (determined by linear regression) across all replicate blank microcosms was subtracted from Fe(II) oxidation in the treatment microcosms to correct for any Fe(II) oxidation that was occurring due to O_2 leakage through the microcosm seals. Microcosms that had Fe(II) oxidation rates lower than those in the blank microcosms were excluded from all subsequent analyses (see below for additional discussion). In the treatment microcosms, rates of blank-corrected Fe(II) oxidation were expressed on a per-microcosm basis and were also normalized to dry green (i.e., aboveground) biomass and dry root biomass.

Statistics. Exponential or linear regressions were fit to the Fe(II) versus time data, as described previously. Differences in Fe(II) oxidation rates, root plaque concentrations, plant biomass, and pH as a function of treatment (+FeOB vs. −FeOB), month (April vs. July), and time of sampling (0, 3, and 6 weeks) were assessed using t-tests and standard least squares models, as appropriate. Unless otherwise noted, statistical significance was set at $p \leq 0.10$ since preliminary experiments showed a high level of variability between microcosms but it was logistically unfeasible to increase the number of replicate microcosms in each experiment. Thus, we are more likely incorrectly to reject the null hypothesis of no difference between treatments (type I error) than if a more conservative alpha level of 0.05 had been used, but less likely to incorrectly accept the null hypothesis (type II error). All curve fitting and statistical analyses were conducted using JMP v.5.0 (SAS Institute, Cary, NC).

RESULTS

Temporal Patterns of Microcosm [Fe(II)] and Oxidation Rates. For the microcosm experiment initiated in April, the decrease in [Fe(II)] with time was well-described by an exponential equation (equation 1 and Figure 2A), with r^2 values for treatment microcosms ranging from 0.96 to 1.0. In the blank microcosms, Fe(II) concentrations decreased linearly with time, with r^2 values of 0.87 to 0.98. Fe(II) oxidation rates calculated at a standard [Fe(II)] of 750 μmol L^{-1} ranged from 0.8 to 5.3 μmol Fe(II) oxidized h^{-1} for the treatment microcosms ($n = 20$); rates were significantly lower in the blank microcosms (0.17 to 0.31 μmol h^{-1}; $n = 4$). For microcosms where multiple pulses of Fe(II) were added, reported Fe(II) oxidation rates are only for the initial Fe(II) addition. There were no consistent trends in Fe(II) oxidation rates across multiple Fe(II) pulses within a single microcosm (data not shown).

In July, equation 1 was a good fit to the Fe(II) versus time data for approximately half of the treatment microcosms ($n = 24$ of 40 total, average $r^2 = 0.90$). For the remaining treatment microcosms ($n = 16$) and the blanks ($n = 5$), linear regressions were fit to the data ($r^2 = 0.69$ to 0.96). At a Fe(II) concentration of 750 μmol L^{-1}, the July Fe(II) oxidation rates in the treatment microcosms (0.17 to 2.06 μmol h^{-1}) were significantly lower than in April, whereas rates in the blank microcosms (0.46 to 0.81 μmol h^{-1}) were higher than in April. As a result, some of the treatment microcosms from July were excluded from further analysis because calculated oxidation rates were lower than in the blank microcosms, presumably due to lower rates of radial O_2 loss as well as experimental difficulties in limiting O_2 leakage in the blank microcosms.

Effect of FeOB on Fe(II) Oxidation Rates. After correcting for Fe(II) oxidation in the blank microcosms, Fe(II) oxidation rates in the treatment microcosms were expressed as whole microcosm rates (μmol Fe(II) oxidized h^{-1}) and as normalized to dry green biomass and root biomass (μmol g^{-1} h^{-1}). In April, average Fe(II) oxidation rates tended to be greater in the +FeOB relative to the −FeOB microcosms when expressed as whole microcosm rates (2.78 ± 0.43 vs. 2.13 ± 0.43 μmol h^{-1}; averages $\pm$ 1 standard error), and when normalized to green biomass (6.95 ± 0.91 vs. 5.33 ± 0.90 μmol g^{-1} h^{-1}) or root biomass (6.05 ± 0.81 vs. 5.14 ± 0.83 μmol g^{-1} h^{-1}) but these differences were not statistically significant ($p > 0.10$; Figure 3). In July, whole microcosm oxidation rates were 1.6 times greater in +FeOB microcosms (0.45 ± 0.10 vs. 0.28 ± 0.06 μmol h^{-1}; $p = 0.08$). As in April, there was a trend for greater biomass-normalized oxidation rates in the +FeOB microcosms (green-biomass normalized: 1.45 ± 0.24 vs. 1.06 ± 0.29 μmol g^{-1} h^{-1}; root-biomass normalized: 8.31 ± 3.42 vs. 3.63 ± 0.91 μmol g^{-1} h^{-1}).

The Fe(II) oxidation rates were also expressed relative to average –FeOB oxidation rates within that month (e.g., right axes on Figure 3). When the data were pooled in this manner, we found that Fe(II) oxidation rates in the + FeOB microcosms were greater than in the –FeOB microcosms, regardless of whether the data were calculated as whole microcosm rates (1.5x greater, $p = 0.04$) or normalized to green biomass (1.3x greater, $p = 0.06$) or root biomass (1.7x greater, $p = 0.08$). The raw rate data could not be similarly pooled because Fe(II) oxidation rates differed substantially between experiments (note scales on April and July y-axes in Figure 3).

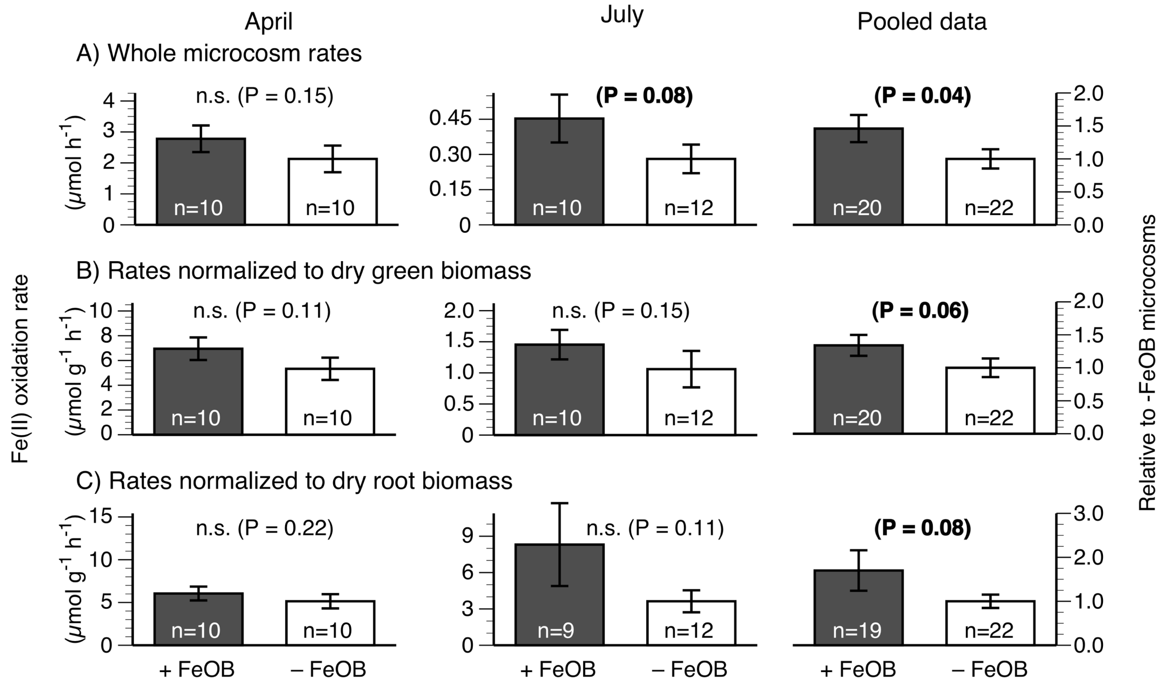

FIG. 3. Fe(II) oxidation rates expressed as: (A) whole microcosm rates, and as normalized to (B) dry green plant biomass and (C) dry root biomass. All rates have been corrected for Fe(II) oxidation in blank microcosms. Fe(II) oxidation is expressed both as a raw rate (left axes) and relative to the average oxidation rate in the –FeOB microcosms (values provided on right axis). Note that the right axis applies for all graphs within a particular row, but the scale of the left axes within a row varies between graphs. One-way t-tests were used to test the hypothesis that Fe(II) oxidation rates were greater in microcosms with FeOB than in −FeOB microcosms. n.s. = not significant (i.e., $p > 0.10$). Error bars are ± 1 standard error.

Fe Plaque Accumulation. In April, total root plaque Fe concentrations [Fe(III) + Fe(II)] increased significantly from 11.7 μmol g^{-1} at the beginning of the experiment to ~950 μmol g^{-1} after 3 to 6 weeks (Table 1). There was no difference in total Fe plaque accumulation between the 3 and 6 week sampling points. In July, there was an order of magnitude increase in root Fe plaque between 0 and 3 weeks, from 81.9 to 850.7 μmol g^{-1}. Across the sampling dates, Fe(III) accounted for 82 to 91% of the total plaque Fe, with the exception of the initial sampling in July (~70% of plaque Fe was Fe(III); Table 1). Long-term Fe(III) accumulation rates averaged ~40 μmol g^{-1} d^{-1} for the samples analyzed after 3 weeks, and 22.6 μmol g^{-1} d^{-1} for the 6 week microcosms sampled in April (Table 1). For each month and sampling date, there were no differences in any root plaque parameter (concentrations, %Fe(III), or long-term Fe(III) accumulation rate) between the +FeOB and −FeOB treatments (data not shown).

General Microcosm Parameters. The biomass of above-ground ("green") vegetation averaged ~0.4 g in each month (Table 2) and did not vary between months or as a function of time of sampling (i.e., initial microcosms vs. those sampled at end of experiments). Similarly, root biomass did not differ

TABLE 1
Root Fe plaque concentrations and accumulation rates

Month	Time sampled (d since start)	n	Plaque Fe(II) + Fe(III) (μmol g^{-1})	% Fe(III) (% of total)	Fe(III) accumulation rate (μmol g^{-1} d^{-1})
April	0	10	11.7 ± 0.9^a	84.4 ± 3.4^a	—
	21	10	943.8 ± 135.6^c	88.4 ± 2.4^a	41.0 ± 6.5^a
	41	10	955.1 ± 92.2^c	91.4 ± 1.5^a	22.6 ± 2.3^b
July	0	10	81.9 ± 11.0^b	67.6 ± 5.1^b	—
	18	21*	850.7 ± 98.7^c	81.6 ± 1.9^a	39.8 ± 5.0^a

Values are means ± standard error. Within a column, values with the same superscript were statistically similar (standard least-squares model with Tukey's hsd, $p < 0.05$).

*n = 22 for % Fe(III).

TABLE 2
Plant biomass

	Time sampled (d since start)	n	Green biomass (g dry weight)	Root biomass (g dry weight)
April	0	10	0.397 ± 0.038^a	0.478 ± 0.038^a
	21	10	0.424 ± 0.035^a	0.434 ± 0.043^a
	41	10	0.385 ± 0.044^a	0.442 ± 0.042^a
July	0	9	0.406 ± 0.098^a	0.086 ± 0.029^b
	18	21	0.458 ± 0.062^a	0.106 ± 0.013^b

Values are means ± standard error. Within a column, values with the same superscript were statistically similar (standard least-squares model with Tukey's hsd, $p < 0.05$).

between the beginning of each experiment and in destructively sampled microcosms after 3 or 6 weeks. However, average root biomass was about 4 times greater in April than in July (Table 2). Within each month, there were no differences in biomass between +FeOB and −FeOB microcosms (data not shown). The pH of the hydroponic solution ranged from 5.8 to 6.0 and did not vary between months, treatments, or time of sampling (data not shown).

DISCUSSION

To date, all measurements of the activity of circumneutral, lithotrophic, Fe(II)-oxidizing bacteria (FeOB) have been conducted in experimental systems (e.g., diffusion gradient tubes and bioreactors, Emerson and Revsbech 1994; Sobolev and Roden 2001; Neubauer et al. 2002) that eliminate much of the complexity of a natural wetland plant-microbe-soil system. It is methodologically difficult to study these FeOB *in situ* because there are no known specific inhibitors for microbial Fe(II) oxidation, and both stable and radioisotope techniques have unresolved issues (Roden and Lovley 1993; Emerson 2000; Bullen et al. 2001; Croal et al. 2004). In the FeOB-*Juncus effusus* microcosms discussed herein, we have replicated one element of the rhizosphere environment (namely, O_2 provided only via radial O_2 loss from roots) within a system where it is (relatively) easy to manipulate the presence or absence of FeOB and measure rates of Fe(II) oxidation and Fe plaque accumulation.

Factors Affecting Rates of Fe(II) Oxidation. Our experiments showed that FeOB increased Fe(II) oxidation rates by 1.3 to 1.7 times relative to –FeOB (i.e., FeOB-free) microcosms. The differences between +FeOB and –FeOB microcosms persisted when Fe(II) oxidation rates were expressed on a whole microcosm basis, or normalized to the aboveground or belowground biomass of the plants. Our finding that FeOB accelerated rates of Fe(II) oxidation is consistent with that of Neubauer et al. (2002) who found that FeOB strain BrT (the same FeOB used herein) could accelerate total Fe(II) oxidation rates by up to 18% relative to cell-free treatments. Similarly, Fe(II) oxidation rates were higher in FeOB-containing microbial mat and groundwater samples than in killed controls (Emerson and Revsbech 1994; James and Ferris 2004). However, other studies have reported that Fe(III) accumulation rates in diffusion-limited gradient cultures did not differ between +FeOB cultures and abiotic controls (Emerson and Moyer 1997; Sobolev and Roden 2004). Regardless of the net effect of FeOB on Fe(II) oxidation rates, these lithotrophic organisms are utilizing energy released by Fe(II) oxidation to support cellular metabolism and growth. Our data did not allow us to determine if the observed increases in Fe(II) oxidation in the +FeOB microcosms were due solely to microbially-mediated oxidation or if rates of abiotic oxidation also increased.

For FeOB to accelerate Fe(II) oxidation rates, the microbes must increase the availability of Fe(II) or O_2, or affect the reaction kinetics. If chemical Fe(II) oxidation is limited by Fe(II) or O_2 availability, FeOB could increase total rates by oxidizing Fe(II) pools that cannot be accessed chemically, by oxidizing Fe(II) at lower substrate concentrations than chemical oxidation, or by increasing rates of Fe(II) and/or O_2 diffusion to the rhizosphere. If Fe(II) oxidation rates are not limited by substrate availability, rates could increase if FeOB increase the kinetics of the reaction. This could occur enzymatically or if the surfaces of FeOB cells significantly increase chemical oxidation. Rates of Fe(II) oxidation could have been higher in +FeOB microcosms if FeOB were able to utilize Fe(II) that was otherwise bound to organic matter (e.g., as hypothesized by Emerson and Moyer 1997; Neubauer et al. 2002) and was unavailable for chemical oxidation in the –FeOB microcosms. Previous work has documented that Fe(II)-organic matter interactions are complex and can either inhibit, accelerate, or have no effect on rates of Fe(II) oxidation, depending on the concentrations and nature of organic matter, as well as concentrations of O_2 and Fe(II) (Theis and Singer 1974; Johnson-Green and Crowder 1991; Stone 1997; Roth et al. 2000). Regardless of the mechanism, enhanced consumption of both Fe(II) and O_2 in the rhizosphere of the +FeOB microcosms due to Fe(II) oxidation would lead to steeper concentration gradients that would further drive the delivery of both Fe(II) and O_2 to the root surface. Indeed, the presence of FeOB in the rhizosphere does indicate that these microbes are successfully competing for (and consuming) both Fe(II) and O_2. However, in a natural microbial-plant-soil system, however, it is unlikely that FeOB would dramatically affect O_2 concentration

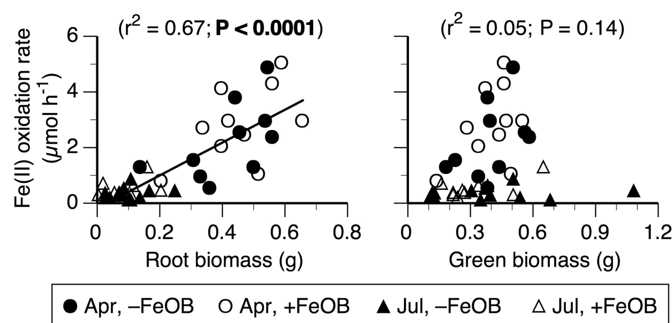

FIG. 4. Whole microcosm Fe(II) oxidation rates (μmol h^{-1}) were not related to dry green biomass. In contrast, dry root biomass was a significant predictor of the Fe(II) oxidation rate across both experiments: Rate = 5.91 × root biomass − 0.18. For each panel, statistics are for all data points, regardless of month or treatment.

gradients due to the presence of other aerobic microbes and O_2-consuming chemical reactions in the rhizosphere.

There were significant differences in rates of Fe(II) oxidation between the April and July experiments, regardless of treatment, that suggest that the presence/absence of FeOB is not the only factor that affects Fe(II) oxidation rates. When data from the two trials were combined, there was a strong correlation between whole microcosm Fe(II) oxidation rates and root biomass (Figure 4), indicating that between-month differences in root biomass (Table 2) explained a large part of the between-month differences in Fe(II) oxidation rates. In contrast, there was no relationship between green biomass and Fe(II) oxidation rates (Figure 4). Roots can affect Fe(II) oxidation by controlling rates of radial O_2 loss to the rhizosphere (i.e., more root mass = more radial O_2 loss), providing surface area for FeOB to colonize, or affecting rates of autocatalytic Fe(II) oxidation onto existing Fe(III) oxides on the root surface.

There is considerable between-plant variability in rates of radial O_2 loss and the mechanisms that drive O_2 transport through aerenchyma (e.g., passive diffusion vs. through-flow of gases) (Colmer 2003), but several field-based studies support a connection between plant biomass and rates of Fe(II) oxidation. For example, Sundby et al. (2003) found that seasonal cycles of root growth and decay in a salt marsh affected the degree of rhizosphere oxidation and porewater Fe^{2+} availability. Similarly, Weiss et al. (2005) suggested that temporal variations in porewater Fe^{2+} concentrations in a *J. effusus* wetland were driven by plant-microbial-environmental interactions that affect rates of rhizosphere Fe(II) oxidation and Fe(III) reduction. In a tidal freshwater marsh, high rates of radial O_2 loss and Fe(II) oxidation coincident with peak aboveground biomass were hypothesized to account for the high contribution of Fe(III) reduction to total anaerobic metabolism (Neubauer et al. 2005). As in these field studies, our microcosm experiments suggest that plant biomass plays a key role in driving rates of radial O_2 loss and rhizosphere Fe(II) oxidation.

In addition to plant activity (as proposed above), Fe(II) oxidation rates can be affected by pH, temperature, the Fe(II) supply rate, and concentrations of O_2, Fe(II), and Fe(III) (Singer and Stumm 1970; Theis and Singer 1974; Sung and Morgan 1980; Stumm and Morgan 1981; Liang et al. 1993). However, these factors were probably not major contributors to the between-month differences in Fe(II) oxidation rates in this study. The same experimental protocol was followed in the April and July experiments, so the pH, temperature, composition of the hydroponic solution, and concentrations of Fe(II) were comparable between months. Furthermore, the Fe(II) oxidation rates shown in Figure 3 were all calculated at a Fe(II) concentration of 750 μmol L^{-1}, eliminating another possible difference between months.

Fe(II) Oxidation versus Fe(III) Accumulation. Measuring rates of Fe(II) disappearance provides a relatively short-term measure of Fe(II) oxidation rates, whereas the accumulation of plaque onto plant roots represents a longer, more-integrated indicator of Fe(II) oxidation. In contrast to the above-described Fe(II) oxidation rate data, there were no long-term differences in Fe plaque accumulation between +FeOB and –FeOB microcosms. Furthermore, short term Fe(II) oxidation rates were 3 to 6 times higher than Fe(III) accumulation rates (e.g., compare Figure 3 with Table 1) which, in part, is because Fe(II) oxidation rates were calculated at 750 μmol Fe(II) L^{-1} whereas Fe(III) plaque accumulation rates were integrated over the entire range of Fe(II) concentrations (~100 to 1000 μmol L^{-1}) throughout each incubation.

Differences between Fe(II) oxidation and Fe(III) accumulation rates over time could result from temporal changes in the availability of Fe(II) and or O_2 in the rhizosphere, or the composition of the microbial community. As roots become coated with Fe plaque (typical thickness of 10s of μm, but up to 0.4 cm; e.g., Taylor et al. 1984; Vale et al. 1990) and the site of oxidation moves farther from the root surface, increased resistance to diffusion will theoretically decrease the rate at which Fe(II) or O_2 are made available for oxidation. In each experiment, we observed visible Fe oxide accumulation on the plant roots and in the hydroponic solution. If the surface chemistry of the roots and Fe plaque changed sufficiently during the course of the incubations, the focal site of Fe(III) accumulation could have shifted from the root surface early in the incubations to the bulk hydroponic solution later during the experiments. This is highlighted in the April experiment where the ratio of accumulated root Fe plaque (μmol microcosm^{-1}) to total Fe(II) oxidized (μmol microcosm^{-1}; based on Fe(II) disappearance across all Fe pulses) averaged 0.49 ± 0.04 after 3 weeks and 0.31 ± 0.03 after 6 weeks (n = 10 microcosms per time point), demonstrating that the fraction of oxidized iron associated with the roots decreased throughout the duration of the experiment. In part, the accumulation of Fe oxides in the hydroponic solution is an artifact of using a hydroponic system. In a root/soil system, the soil matrix itself would help keep oxides in close association with the roots. Either of the above mechanisms would cause measured root Fe(III) accumulation rates to decline over time.

However, the lack of consistent patterns in Fe(II) oxidation rates over the 3 to 6 weeks of each experiment indicates that a simple unidirectional change in rates of radial O_2 loss or diffusion rates is unlikely to explain differences between Fe(II) oxidation and Fe(III) accumulation rates.

In a study that measured rates of Fe(III) plaque accumulation and reduction on the roots of *Juncus effusus* in the field, Weiss et al. (2005) reported that rates of plaque accumulation averaged 0.08 to 0.24 μmol Fe(III) g^{-1} h^{-1} (averaged over 5 months; Fe(III) was ~79% of total Fe plaque). Average rates of Fe(III) reduction were considerably higher and illustrate that Fe(III) plaque accumulation in the field is the net result of the competing processes of Fe(II) oxidation and Fe(III) reduction. While our sterilization techniques substantially reduced the numbers of cells on root surfaces (as verified by microscopy), it is unlikely the sterilization was 100% effective. Thus, some microbes probably survived, either by antibiotic resistance or by "hiding" inside the roots, and subsequently recolonized the root surface during the 3 to 6 week experiments. If Fe(III) reducers were present in the community of surviving microbes, longer-term Fe(III) accumulation rates may represent net Fe(II) oxidation whereas the short-term Fe(II) oxidation rates may be closer to gross oxidation rates. Population increases of other microaerobes within the microcosms would also have reduced the amount of O_2 available to support Fe(II) oxidation.

The change in *Juncus effusus* Fe oxidation and accumulation rates over time was well-described by an exponential equation that is similar in form to equation 1 (Figure 5), even though the data set includes a combination of both laboratory (this study; Snowden and Wheeler 1995) and field data (Weiss et al. 2005). Together, these data indicate that changes in Fe oxidation and accumulation rates over time are not simply a function of artifacts associated with our experimental set-up but instead reflect fundamental changes in the underlying mechanisms responsible for plaque formation (e.g., rates of Fe(II) diffusion and radial O_2 loss) or temporal changes in the microbial community.

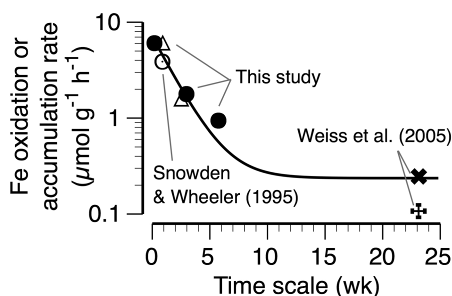

FIG. 5. Fe(II) oxidation rates on *Juncus effusus* roots decrease over time. Data include the short-term Fe(II) oxidation rates shown in Figure 3, Fe(III) accumulation rates reported in Table 1, laboratory Fe accumulation rates from Snowden and Wheeler (1995), and Fe(III) accumulation rates calculated from Weiss et al. (2005) based on field data. The regression line fit to the data is in the form of eq. 1, where $a = 6.87$, $b = 0.53$, and $c = 0.24$; regression $r^2 = 0.94$. Symbols are: ● this study, April; △ this study, July; ⊙ Snowden and Wheeler; × Weiss et al. transplants; and 5 Weiss et al. root in-growth data.

Environmental Relevance. Circumneutral FeOB have been found on the roots of *Juncus* spp. or in the soils of *Juncus*-dominated wetlands in Virginia, West Virginia, and Alabama (Emerson et al. 1999; Sobolev and Roden 2001; Weiss et al. 2003). The presence of FeOB on the roots of *J. effusus* may be common since this plant can have moderate to high rates of radial O_2 loss (Sorrell 1999; Wießner et al. 2002) and a high tolerance to dissolved Fe(II) (Snowden and Wheeler 1995). The environmental conditions used in our microcosm experiments approximate conditions in the rhizosphere and near-surface soils and sediments. The pH used in the microcosms (5.8 to 6.0) is within the range of porewater pH values reported in a study that found circumneutral FeOB in 13 diverse wetland and aquatic habitats (Weiss et al. 2003). Porewater Fe(II) concentrations are highly variable between wetlands and vary spatially (e.g., with depth or distance from a root) and temporally.

By periodically replenishing Fe(II) within the microcosms, we were able to prevent Fe(II) limitation of microbial Fe oxidation and maintain Fe(II) concentrations between ~100 to 1000 μmol L^{-1}, values that are typical of many wetland environments where FeOB have been observed or dynamic Fe cycles have been proposed (e.g., Roden and Wetzel 1996; Weiss et al. 2003; Neubauer et al. 2005; Weiss et al. 2005).

Unlike other studies that have examined the effects of FeOB in laboratory settings, this study mimicked the rhizosphere in that radial O_2 loss from plant roots was the only source of O_2 to support Fe(II) oxidation. To determine how FeOB affect Fe(II) oxidation rates, it was necessary to simplify the microcosms to exclude other microbes. Clearly, the rhizosphere in a natural setting will have a diverse and complex mixture of both aerobic and anaerobic microbes that are competing for resources including O_2, Fe(II), and carbon. Determining rates of microbial Fe(II) oxidation within a larger wetland microbial community remains a daunting challenge that is not likely to be overcome until the in situ activities of these organisms can be identified or it becomes possible to selectively inhibit microbially-mediated Fe(II) oxidation.

FeOB appear to be ubiquitous in circumneutral wetlands (Weiss et al. 2003), but little is known about the factors that control their distribution, abundance, activity, and ecological importance. Is FeOB activity affected by plant biology (e.g., variations in root O_2 loss rate), microbial biology (e.g., competition with other aerobic microbes), or physiochemical variables (e.g., soil mineral content or pH)? Do the factors that increase FeOB activity also positively affect Fe(III) reducing bacteria, leading to an active rhizosphere Fe cycle, or are there conditions under which Fe plaque can accumulate and increasingly sequester metals and nutrients? Are there geochemical differences between Fe oxides produced via microbial or abiotic processes that affect ecosystem-scale metal sequestration or rates of Fe(III) reduction?

Given the importance of root Fe plaque in the biogeochemical cycling of carbon, nutrients, and metals, our finding that FeOB increase rates of Fe(II) oxidation in the rhizosphere raises

important questions about the largely un-quantified role that these organisms play in complex microbial-plant-soil-environmental interactions.

REFERENCES

Armstrong W. 1964. Oxygen diffusion from the roots of some British bog plants. Nature 204:801–802.

Bullen TD, White AF, Childs CW, Vivit DV, Schultz MS. 2001. Demonstration of a significant iron isotope fractionation in nature. Geology 29:699–702.

Calhoun A, King GM. 1997. Regulation of root-associated methanotrophy by oxygen availability in the rhizosphere of two aquatic macrophytes. Appl Environ Microbiol 63:3051–3058.

Chen Z, Zhu Y-G, Liu W-J, Meharg AA. 2005. Direct evidence showing the effect of root surface iron plaque on arsenite and aresnate uptake in rice (*Oryza sativa*) roots. New Phytol 165:91–97.

Colmer TD. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ 26:17–36.

Croal LR, Johnson CM, Beard BL, Newman DK. 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. Geochim Cosmochim Acta 68:1227–1242.

Emerson D. 2000. Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH. In: Lovley DR, editor. Environmental Microbe-Metal Interactions. Washington, DC: American Society for Microbiology. p 31–52.

Emerson D, Moyer C. 1997. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. Applied and Environmental Microbiology 63:4784-4792.

Emerson D, Revsbech NP. 1994. Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: Laboratory studies. Appl Environ Microbiol 60:4032–4038.

Emerson D, Weiss JV, Megonigal JP. 1999. Iron-oxidizing bacteria are associated with ferric hydroxide precipitates (Fe-plaque) on the roots of wetland plants. Appl Environ Microbiol 65:2758–2761.

James RE, Ferris FG. 2004. Evidence for microbial-mediated iron oxidation at a neutrophilic groundwater spring. Chem Geol 212:301–311.

Johnson-Green PC, Crowder AA. 1991. Iron oxide deposition on axenic and non-axenic roots of rice seedlings (*Oryza sativa* L.). J Plant Nutr 14:375–386.

Lack JG, Chaudhuri SK, Chakraborty R, Achenbach LA, Coates JD. 2002. Anaerobic biooxodation of Fe(II) by *Dechlorosoma suillum*. Microb Ecol 43:424–431.

Liang L, McNabb JA, Paulk JM, Gu B, McCarthy JF. 1993. Kinetics of Fe(II) oxygenation at low partial pressure of oxygen in the presence of natural organic matter. Environ Sci Technol 27:1864–1870.

Mendelssohn IA, Kleiss BA, Wakely JS. 1995. Factors controlling the formation of oxidized root channels: a review. Wetlands 15:37–46.

Neubauer SC, Emerson D, Megonigal JP. 2002. Life at the energetic edge: Kinetics of circumneutral iron oxidation by lithotrophic iron-oxidizing bacteria isolated from the wetland-plant rhizosphere. Appl Environ Microbiol 68:3988–3995.

Neubauer SC, Givler K, Valentine S, Megonigal JP. 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334–3344.

Peverly JH, Surface JM, Wang T. 1995. Growth and trace metal absorption by *Phragmites australis* in wetlands constructed for landfill leachate treatment. Ecol Eng 5:21–35.

Roden EE, Lovley DR. 1993. Evaluation of ^{55}Fe as a tracer of Fe(III) reduction in aquatic sediments. Geomicrobiol J 11:49–56.

Roden EE, Wetzel RG. 1996. Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnol Oceanogr 41:1733–1748.

Roth RI, Panter SS, Zegna AI, Levin J. 2000. Bacterial endotoxin (lipopolysaccharide) stimulates the rate of iron oxidation. J Endotoxin Res 6:313–319.

Singer PC, Stumm W. 1970. Acidic mine drainage: The rate-determining step. Science 167:1121–1123.

Snowden RED, Wheeler BD. 1995. Chemical changes in selected wetland plant species with increasing Fe supply, with specific reference to root precipitates and Fe tolerance. New Phytol 131:503–520.

Sobolev D, Roden EE. 2001. Suboxic deposition of ferric iron by bacteria in opposing gradients of Fe(II) and oxygen at circumneutral pH. Appl Environ Microbiol 67:1328–1334.

Sobolev D, Roden EE. 2004. Characterization of a neutrophilic, chemolithoautotrophic, Fe(II)-oxidizing ß-protobacterium from freshwater wetland sediments. Geomicrobiol J 21:1–10.

Sorrell BK. 1999. Effect of external oxygen demand on radial oxygen loss by *Juncus* roots in titanium citrate solutions. Plant, Cell Environ 22:1587–1593.

Sorrell BK, Armstrong W. 1994. On the difficulties of measuring oxygen release by root systems of wetland plants. J Ecol 82:177–183.

St-Cyr L, Fortin D, Campbell PGC. 1993. Microscopic observations of the iron plaque of a submerged aquatic plant (*Vallisneria americana* Michx). Aquat Bot 46:155–167.

Stone AT. 1997. Reactions of extracellular organic ligands with dissolved metal ions and mineral surfaces. In: Banfield J, Nealson KH, editors. Geomicrobiology: Interaction between Microbes and Minerals. Washington, DC: Mineralogical Society of America. p 309–344.

Straub KL, Benz M, Schink B, Widdel F. 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl Environ Microbiol 62:1458–1460.

Stumm W, Morgan JJ. 1981. Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters. New York: Wiley-Interscience. 780 p.

Sundby B, Vale C, Caetano M, Luther III GW. 2003. Redox chemistry in the root zone of a salt marsh sediment in the Tagus Estuary, Portugal. Aquat Geochem 9:257–271.

Sung W, Morgan JJ. 1980. Kinetics and product of ferrous iron oxygenation in aqueous systems. Environ Sci Technol 14:561–568.

Taylor GJ, Crowder AA. 1983. Uptake and accumulation of heavy metals by *Typha latifolia* L. in wetlands of the Sudbury, Ontario region. Can J Bot 61:63–73.

Taylor GJ, Crowder AA, Rodden R. 1984. Formation and morphology of an iron plaque on the roots of *Typha latifolia* L. grown in solution culture. American Journal of Botany 71:666-675.

Theis TL, Singer PC. 1974. Complexation of iron(II) by organic matter and its effect on iron(II) oxygenation. Environ Sci Technol 8:569–573.

Trolldenier G. 1988. Visualization of oxidizing power of rice roots and of possible participation of bacteria in iron deposition. Zeitschrift Fur Pflanzenernährung Und Bodenkunde 151:117–121.

Vale C, Caterino F, Cortesão C, Caçador I. 1990. Presence of metal-rich rhizoconcretions on the roots of *Spartina maritima* from the salt marshes of the Tagus Estuary, Portugal. Sci Total Environ 97/98:617–626.

Weis JS, Weis P. 2004. Metal uptake, transport and release by wetland plants: Implications for phytoremediation and restoration. Environ Inter 30:685–700.

Weiss JV, Emerson D, Backer SM, Megonigal JP. 2003. Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the root zone of wetland plants: Implications for a rhizosphere iron cycle. Biogeochemistry 64:77–96.

Weiss JV, Emerson D, Megonigal JP. 2005. Rhizosphere iron(III) deposition and reduction in a *Juncus effusus* L.-dominated wetland. Soil Sci Soc Amer J 69:1861–1870.

Weiss JV, Rentz JA, Plaia T, Neubauer SC, Floyd MM, Lilburn T, Bradburne C, Megonigal JP, Emerson D. in review. Phenotypic and molecular characterization of neutrophilic Fe(II)-oxidizing bacteria isolated from the wetland plant rhizosphere and description of *Ferritrophicum radicicola* gen. nov. sp. nov, and *Sideroxydans paludicola* sp. nov. Geomicrobiol J.

Wießner A, Kuschk P, Kästner M, Stottmeister U. 2002. Abilities of helophyte species to release oxygen into rhizospheres with varying redox conditions in laboratory-scale hydroponic systems. Inter J Phytoremed 4:1–15.