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Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the root zone of wetland plants: Implications for a rhizosphere iron cycle

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Abstract. Iron plaque occurs on the roots of most wetland and submersed aquatic plant species and is a large pool of oxidized Fe(III) in some environments. Because plaque formation in wetlands with circumneutral pH has been largely assumed to be an abiotic process, no systematic effort has been made to describe plaque-associated microbial communities or their role in plaque deposition. We hypothesized that Fe(II)-oxidizing bacteria (FeOB) and Fe(III)-reducing bacteria (FeRB) are abundant in the rhizosphere of wetland plants across a wide range of biogeochemical environments. In a survey of 13 wetland and aquatic habitats in the Mid-Atlantic region, FeOB were present in the rhizosphere of 92% of the plant specimens collected (n = 37), representing 25 plant species. In a subsequent study at six of these sites, bacterial abundances were determined in the rhizosphere and bulk soil using the most probable number technique. The soil had significantly more total bacteria than the roots on a dry mass basis (1.4×10^9 cells/g soil vs. 8.6×10^7 cells/g root; $p < 0.05$). The absolute abundance of aerobic, lithotrophic FeOB was higher in the soil than in the rhizosphere (3.7×10^6 /g soil vs. 5.9×10^5 /g root; $p < 0.05$), but there was no statistical difference between these habitats in terms of relative abundance ($\sim$1% of the total cell number). In the rhizosphere, FeRB accounted for an average of 12% of all bacterial cells while in the soil they accounted for < 1% of the total bacteria. We concluded that FeOB are ubiquitous and abundant in wetland ecosystems, and that FeRB are dominant members of the rhizosphere microbial community. These observations provide a strong rationale for quantifying the contribution of FeOB to rhizosphere Fe(II) oxidation rates, and investigating the combined role of FeOB and FeRB in a rhizosphere iron cycle.

Abbreviations: DCB – dithionite-citrate-bicarbonate, FeOB – Fe(II)-oxidizing bacteria, FeRB – Fe(III)-reducing bacteria, ROL – radial O_2 loss, MPN – most probable number, MWMM – modified Wolfe's mineral media, PVC – polyvinyl chloride, SAV – submerged aquatic vegetation, SERC – Smithsonian Environmental Research Center

Introduction

Wetland ecosystems are sites of rapid biogeochemical cycling due to interactions between the aerobic soil surface and deeper anaerobic soils. The oxic-anoxic interface is further extended by the presence of wetland vegetation that releases oxygen from their root system in a process known as radial oxygen loss (ROL) (Armstrong 1964). Molecular oxygen (O_2) and Fe(II) react to form a rust-colored precipitate on the root surface referred to as iron plaque. Iron plaque is widely observed on the roots of wetland and submersed aquatic plant species where it can account for a large pool of Fe(III) (Chen et al. 1980; Mendelssohn et al. 1995) and influence the mobility of trace metals and phosphorus (Greipsson and Crowder 1992; Christensen and Sand-Jensen 1998; Hansel et al. 2001).

Iron plaque formation is known to be influenced by Fe(II) availability, ROL rates, pH, soil texture, and redox potential (Mendelssohn et al. 1995). Although bacteria have been found in iron plaque (Trolldenier 1988; St-Cyr et al. 1993), their role in its formation is unclear. Our discovery of lithotrophic Fe(II)-oxidizing bacteria (FeOB) on the roots of wetland plants (Emerson et al. 1999) raises the possibility that plaque-associated microbes may directly influence plaque formation. In laboratory studies, FeOB mediate between 45 and 90% of Fe(II) oxidation (Emerson and Revsbech 1994; Sobolev and Roden 2001; Neubauer et al. 2002), suggesting FeOB could play an important role in the formation of iron plaque.

O_2 availability in the rhizosphere varies both spatially along a root system and temporally (e.g. diurnal and seasonal), creating a mosaic of aerobic and anaerobic microsites. Observations of both FeOB (Emerson et al. 1999) and Fe(III)-reducing bacteria (FeRB) on the roots of wetland plants (King and Garey 1999) suggest that Fe(II) oxidation and Fe(III) reduction are coupled within the rhizosphere, promoting a localized iron cycle. A cycle in which poorly-crystalline Fe(III) is continually regenerated through the action of FeOB has important biogeochemical implications, such as a suppression of methane production (Roden and Wetzel 1996; van der Nat and Middelburg 1998).

Although the existence of an iron cycle promoted by the presence of wetland vegetation has been proposed (Giblin and Howarth 1984; Roden and Wetzel 1996), the possibility that FeOB and FeRB may be involved in such a cycle has not been investigated. In this study, we hypothesized that FeOB and FeRB are important components of the wetland plant rhizosphere microbial community. Specifically, our goals were to: (i) determine if FeOB are found in the rhizosphere of aquatic plants across a variety of biogeochemically-distinct environments, (ii) compare the abundance of FeOB, FeRB, and total bacteria in the rhizosphere and surrounding soil, and (iii) scale the abundances of FeOB and FeRB to the ecosystem level. All microbial investigations were limited to the study of aerobic, lithotrophic FeOB and acetate-utilizing FeRB. Furthermore, we define the ″rhizosphere″ as a root's zone of influence on Fe(II) oxidation and use the presence of iron plaque to delineate this zone.

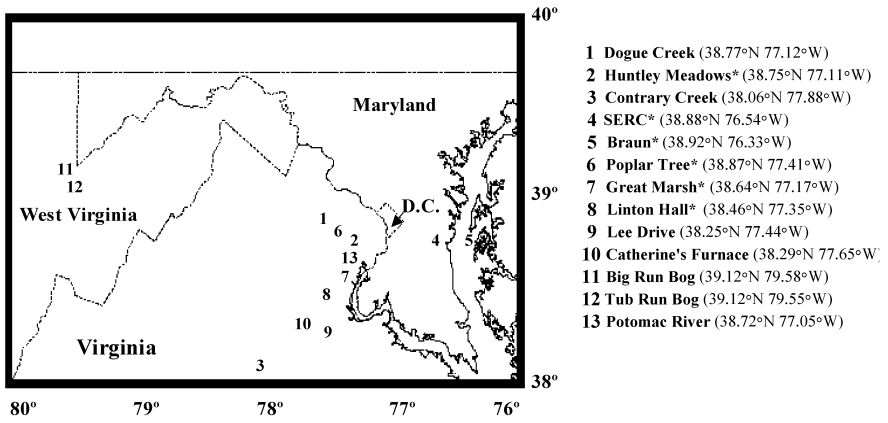

Figure 1. Location of sites sampled for FeOB associated with the roots of wetland and aquatic plants. A sub-set of the sites (*) were also sampled to obtain abundances of FeOB and FeRB associated with wetland plant roots and soil.

Material and methods

Survey of Fe(II)-oxidizing bacteria

Thirteen wetland and aquatic habitats in Virginia, Maryland, and West Virginia were sampled for rhizosphere FeOB between October of 1997 and November 1999 (Figure 1). The sites included tidal and non-tidal freshwater marshes, a salt marsh, riparian forests, a bottomland hardwood forest, bogs, and submersed aquatic vegetation (SAV) (Table 1). At each site, two to three individuals of the dominant plant species were removed in an intact soil block (approximately 15 × 15 × 25 cm) that contained the plant and most of its root system. Water samples were collected from the closest surface water at submerged sites, or from porewater that filled the soil block cavity. Subsamples collected for measuring Fe(II) concentration were added immediately to ferrozine, then subsequently read at 562 nm on a spectrophotometer within 6 h of collection (Stookey 1970). The pH of the porewater and the soil (1:1 slurry with DI water) were measured *in situ* with a portable Cole Parmer pH/con 10 series meter.

The intact blocks were transported to the lab, stored at 4 °C, then dissected within 24 hours. The oxidized layer of the soil surface was discarded, then roots and soil from the reduced zone were separated using sterile techniques. Roots were washed in sterile deionized water and subsampled to determine the presence or absence of FeOB and quantify iron plaque concentrations. Soil samples were taken from the root zone and dried at 110 °C for two days for physical and chemical analyses.

Table 1. Physical and chemical characteristics of wetlands and aquatic habitats sampled for Fe(II)-oxidizing and Fe(III)-reducing bacteria. All measurements are the average of three soil blocks ± 1 standard deviation

Site	Habitat Type	pH		Soil Organic	C (%)	N (%)	Soil Texture (%)		Texture classification
		Soil	Water	Content (%)			Sand	Clay	
Dogue Creek	Riparian forest	5.4 (0.3)	6.4 (0.3)	5 (1)	2.3 (0.6)	0.17 (0.04)	81 (3)	9 (5)	Loamy sand
Huntley Meadows	Freshwater marsh	6.5 (0.1)	6.5 (0.1)	16 (0)	8.7 (0.2)	0.50 (0.04)	48 (2)	29 (0)	Sandy clay loam
Contrary Creek	Mine-impacted riparian strip	4.2 (0.5)	3.9 (0.2)	4 (1)	1.5 (0.5)	0.10 (0.03)	79 (3)	11 (3)	Sandy loam
SERC	Salt marsh	6.1 (0.5)	6.5 (0.2)	69 (5)	38.1 (1.6)	2.22 (0.04)	84 (10)	7 (3)	Loamy sand
Braun	Constructed herbaceous wetland	6.6 (0.5)	5.9 (0.1)	2 (0)	0.9 (0.1)	0.10 (0.01)	37 (11)	45 (11)	Clay
Poplar Tree	Freshwater marsh	6.8 (0.3)	6.6 (0.2)	8 (3)	2.5 (1.3)	0.20 (0.11)	39 (6)	43 (10)	Clay
Great Marsh	Freshwater tidal marsh	5.9 (0.2)	6.3 (0.1)	38 (15)	21.6 (5.3)	1.36 (0.41)	7 (5)	47 (14)	Silty clay
Linton Hall	Freshwater marsh	6.6 (0.1)	7.2 (0.1)	7 (2)	3.0 (1.3)	0.29 (0.11)	47 (4)	32 (2)	Sandy clay loam
Lee Drive	Bottomland hardwood	4.2 (0.5)	5.2 (0.1)	13 (3)	7.8 (1.4)	0.46 (0.18)	80 (8)	13 (9)	Sandy loam
Catherine's Furnace	Riparian forest	5.0 (0.2)	5.8 (0.3)	4 (1)	1.5 (0.7)	0.15 (0.07)	61 (3)	18 (4)	Sandy loam
Big Run Bog	High elevation bog	3.7 (0.1)	4.4 (0.1)	54 (30)	29.4 (15)	1.42 (0.61)	69 (5)	14 (2)	Sandy loam
Tub Run Bog	High elevation mine-impacted bog	3.9 (0.1)	4.0 (0.1)	24 (4)	13.6 (0.9)	0.77 (0.06)	52 (15)	24 (7)	Sandy clay loam
Potomac River	Submersed aquatic vegetation	6.0 (0.1)	6.1 (0.0)	5 (0)	2.7 (0.3)	0.18 (0.01)	54 (8)	28 (6)	Sandy loam

Physical and chemical analyses

For the initial survey of 13 sites, we used dithionite-citrate-bicarbonate (DCB) to extract iron on the roots (Taylor and Crowder 1983) and the soil (Darke and Walbridge 1994), followed by analysis on an atomic absorption spectrometer (Perkin-Elmer model 5100). The two methods differ slightly in the amount of sodium dithionite that is recommended; we used 1 g for all samples taken for enumeration of iron bacteria. Because we determined that DCB removes 99% of the iron plaque (Weiss et al., in preparation), iron removed with DCB will be referred to as total plaque iron. Due to a larger pool of non-DCB-extractable iron in the soil, the term ″DCB-extractable soil iron″ will be used throughout. Soil organic matter content was calculated by loss on ignition at 430 °C for 16 hours (Nelson and Sommers 1982). Total soil C and N were determined using a CHN analyzer (Perkin Elmer Series II 2400), and soil texture was measured with the hydrometer method (Day 1965).

Enrichment for Fe(II)-oxidizing bacteria

The technique used to enrich for aerobic, lithotrophic rhizosphere FeOB has been previously described (Emerson and Moyer 1997; Emerson et al. 1999). Briefly, a minimum of four 1-cm subsections of root from each plant specimen were individually inoculated into gradient tubes containing an Fe(II)-sulfide plug overlaid with a bicarbonate-buffered mineral salt solution containing 0.15% low-melting agarose. All gradient tubes that were used for enrichment and enumeration of FeOB had a pH of 6.5. However, each of our four rhizosphere FeOB isolates can tolerate a wide range of pHs, from 4 to 7 or 8 (Weiss et al. in preparation).

Because air was present in the headspace, these tubes provided opposing diffusion gradients of O_2 and Fe(II). The tubes were considered positive if a discrete band of Fe(III) oxides formed around the root surface (Emerson et al. 1999). The presence of organisms such as sulfur-oxidizers in the initial enrichments does not appear to affect the growth of FeOB. No differences in Fe(III) oxide band formation were observed between enrichments and pure cultures or when FeOB were grown on another Fe(II) substrate such as ferrous carbonate. Subsets of samples were examined microscopically to confirm the presence of bacteria within the Fe(III) oxides.

Enumeration of total bacteria

Six of the initial 13 sites were sampled in the summer of 2000 to enumerate total bacteria, FeOB and FeRB in the rhizosphere and surrounding soil. The sites were chosen because of the presence of *Typha* spp., a common freshwater emergent macrophyte with high rates of radial oxygen loss (Michaud and Richardson 1989). *Typha latifolia* was collected at all sites with the exception of the Smithsonian Environmental Research center (SERC) where *Typha angustifolia* occurs. At each site, three *Typha* specimens were collected, stored, and dissected as described above.

Soil samples were collected solely from within the root zone of the plant, and generally within 5 cm of the root surface. In order to collect samples representative of the full range in iron plaque concentrations, roots varying in color from white to dark red were collected from many positions along the length of the root system. Roots were washed by repeatedly vortexing with sterile anaerobic water until the water became clear. No rhizomes were sampled. Aliquots of either washed root or soil were preserved in 2% gluteraldehyde and stored at 4 °C for up to 3 months until enumeration of total bacteria.

Rhizosphere bacteria were enumerated by total direct counts after the extraction and filtration of the iron plaque as described by Emerson et al. (1999). Acid ammonium oxalate at pH 3 (Darke and Walbridge 1994) was substituted for 0.5 M hydroxylamine/0.5 M HCl because it yielded higher total cell numbers while extracting all of the visible iron from the root surface (data not shown). Sterile oxalate solution (1 mL/cm root) was added to excised, washed roots and shaken in the dark at 125 rpm until the roots appeared white (1–3 hrs). Ten μL/mL of 0.1% acridine orange was added to a portion of the extractant and allowed to sit four minutes. The stained cells were filtered under low pressure onto 0.2 μM black polycarbonate membrane filters then counted using epifluorescence microscopy at 1000 × (Olympus BX 60 microscope). A minimum of 15 fields was counted for each extracted root sample. A subsample of the extracted roots was stained and examined directly under the microscope to ensure that no cells were still associated with the root surface. In order to further investigate the relationship between iron plaque and rhizosphere bacteria (Emerson et al. 1999), oxalate-extractable iron (equivalent to total iron in the root samples) was determined in each of the root extracts. A portion of the oxalate extract was added to 0.5 M hydroxylamine/0.5 M HCl to reduce all of the extracted Fe(III) to Fe(II) (Phillips and Lovley 1987). After shaking the sample solution for 1 hour, Fe(II) was determined by the addition of a subsample to ferrozine and measurement at 562 nm.

The total number of soil bacteria was determined by diluting 3 aliquots of homogenized, preserved samples by a factor of 200. After vortexing the diluted sample for 1 minute, two 10-μL spreads were made on agar-coated (1%), circle-imprinted microscope slides and allowed to dry (Gold Seal Flourescent Antibody Microslide). Ten μL of 250 μM SYTO (Molecular Probes, Eugene, OR), a fluorescent DNA-binding dye (Mason et al. 1998), was added to each circle. SYTO reduced the background fluorescence observed with acridine orange and proved extremely effective at estimating bacterial cells associated with particles. After staining, the soil samples were enumerated in the same manner as the root samples.

Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria

Aerobic, lithotrophic FeOB and acetate-utilizing FeRB were enumerated using the most probable number (MPN) method (Koch 1996). Root slurries were made by adding 0.1 g of root to 1 mL of sterile water and gently grinding with a mortar and pestle. Because this extract was enriched in solubilized carbon released from the root, it was washed by vortexing for 30 seconds, then centrifuged at 16,000 × g for

5 minutes to pellet the bacteria and remaining root tissue. The supernatant was discarded and replaced by sterile anaerobic water at the beginning of each of three successive washes. The root pellet was then resuspended in sterile water. Soil samples were homogenized through vortexing and kept under anaerobic conditions until dilution into MPN tubes.

The root slurry and homogenized soil were diluted in modified Wolfe's mineral media (MWMM) and inoculated into gradient tubes for the enumeration of FeOB. This dilution series was used as a source to inoculate Fe-reducing media consisting of sulfate-free MWMM, 10 mM sodium acetate, 50 mM sodium bicarbonate, 0.01% yeast extract (w/v), 1 μL/mL Wolfe's trace minerals, and 10% poorly crystalline Fe(III) adjusted to pH 7.0 (final concentration about 10 mmol/L). Sulfate-free media was used to reduce the possibility of chemical reduction of iron by sulfides (Jacobson 1994). A slightly modified enrichment was used for the salt marsh site in which the MWMM in both FeOB and FeRB media was replaced with water from the site. In these samples, 10 mM sodium molybdate was added to inhibit sulfate-reducing bacteria from producing sulfides that are capable of chemically reducing Fe(III) (Lovley et al. 1993).

A three-tube MPN was performed with dilution levels ranging from 10^{-3} to 10^{-8}. The development of a discrete band at the oxic-anoxic interface within the gradient tubes indicated the presence of FeOB. Positive Fe(III)-reducing tubes were visually assessed for consumption of the Fe(III) oxides and measured for the presence of Fe(II) by color development with ferrozine. When questionable, the ferrozine samples were read on a spectrophotometer. A subset of tubes positive for FeOB and FeRB was examined microscopically to confirm cell growth. Abundances of FeOB and FeRB were calculated using MPN tables (Eaton and Franson 1995)

Calculation of root and soil bulk density

In order to express our data on a volume basis, intact soil cores were collected from six *Typha*-dominated wetlands in February and March of 2001. At each site, six cores centered on a single *Typha* shoot were taken to a depth of 20 cm using a sharpened PVC pipe (15 cm diameter). The cores were taken as pairs; one core was oven-dried at 90 °C to determine bulk density and the other was washed to determine root biomass. The cores for root biomass were washed through a 2 mm sieve, then sorted into living and dead roots, rhizomes, and litter. All samples were dried at 90 °C until no weight change was observed. The sum of live and dead root biomass was assumed to approximate live root biomass at the peak of the growing season when samples for iron bacteria were taken. Rhizome biomass was not included in the total because no rhizomes were evaluated for iron or bacterial densities in this study. Soil density was calculated as the difference between bulk density and the sum of roots, rhizomes, and litter mass.

Statistical analyses

All statistics were performed in Microsoft Excel using the add-in statistical package XLSTAT version 5 (Kovach Computing Services, Wales, UK). Because much of the data was not normally distributed, correlations were performed using spearman rank correlation analyses. Relative and absolute bacterial abundances were compared with the Wilcoxon signed-rank tests. All standard errors were calculated according to Sokal and Rohlf (Sokal and Rohlf 1995). Unless otherwise noted, relationships with p-values < 0.05 were considered to be statistically significant.

Results

Soil characteristics and relationship to iron plaque

Our first objective was to conduct a survey for rhizosphere FeOB across a biogeochemically-diverse sample of wetland and aquatic habitats. The pH of the sites ranged from ~ 4 at Contrary Creek and Tub Run, both mine-impacted sites, to ~ 7 (Table 1). Percent organic matter ranged from 2.5% at Braun, a constructed wetland, to 69% at the salt marsh. Sand generally dominated the mineral fraction of the soil with notable exceptions at Braun and Poplar Tree where the clay fraction was significant and Great Marsh where silt was abundant.

The habitats sampled for FeOB also varied with respect to iron in the porewater, soil, and on the roots (Table 2). Porewater Fe(II) concentrations ranged from below detection at the Potomac River site to > 12 mg L^{-1} at Big Run Bog, Tub Run Bog, and Braun constructed wetland. DCB-extractable soil iron concentrations ranged from < 10 mg Fe/g dry weight (gdw^{-1}) of soil at Lee Drive and Big Run Bog to $>$ 100 mg Fe gdw^{-1} in some samples collected at Contrary Creek and Great Marsh. Iron plaque concentrations ranged from < 1 to > 225 mg Fe gdw^{-1} root.

No statistically significant relationships were found between iron plaque concentrations and porewater Fe(II), DCB-extractable soil Fe, pH, or soil texture. As was observed previously for *Typha latifolia* (Macfie and Crowder 1987), we found a weak negative correlation between iron plaque concentrations and soil organic content ($r^2 = 0.40$, $n = 34$, $p = 0.01$). Organic matter may bind iron, making it less available for iron cycling (Perret et al. 2000). Correlations were better when Fe plaque was expressed on a mass basis than a surface-area basis (Table 2).

Presence and abundance of FeOB and FeRB

A total of 37 plant specimens were sampled for rhizosphere FeOB. Aerobic, lithotrophic FeOB were enriched from the rhizosphere of 92% of the plant specimens, representing 23 of the 25 plant species collected (Table 2). The three plant specimens lacking FeOB had moderate to high levels of iron plaque.

Table 2. The plant species collected and examined for Fe(II)-oxidizing bacteria and associated root, soil, and water Fe(II) concentrations. Water samples are site averages because they could not be associated with individual plant samples (n = 3). Soil DCB-extractable iron concentrations are reported as ± 1 standard error (n = 2). The presence of Fe(II)-oxidizing bacteria (FeOB) is denoted by "+" and the absence is denoted by a "–"

Site	Plant Species	Pore Water Fe(II) (mg/L)	DCB-extractable soil Fe (mg/g)	DCB-extractable root Fe (mg/g)	DCB-extractable root Fe (mg/cm^2)[1]	Presence of root FeOB
Dogue Creek	*Microstegium vimineum*	4.1	14.6 (3.0)	3.3	0.03	+
	Lindera benzoin		26.0 (4.7)	125.7	1.04	+
Huntley Meadows	*Typha latifolia*	3.8 (0.2)	61.6 (1.7)	76.7	0.40	+
	Scirpus cyperinus			14.0	0.08	+
	Saururus cernuus			90.6	0.69	+
Contrary Creek	*Juncus effusus*	4.3 (2.2)	49.5 (8.2)	68.7	0.57	+
	unknown sedge		49.1 (9.5)	224.9	0.71	+
	Leersia oryzoides		130.5 (6.5)	43.1	0.23	+
SERC	*Spartina patens*	0.8 (0.4)	4.2 (1.2)	0.3	0.00	+
	Scirpus americanus		8.8 (0.5)	0.6	0.00	+
	Typha angustifolia		15.3 (4.5)	9.5	0.04	+
Braun	*Typha latifolia*	14.2 (3.3)	14.4 (1.1)	10.7	0.05	+
	Scirpus americanus		18.2 (0.8)	21.7	0.06	+
	Echinochloa colona		9.2 (1.5)	83.4	0.22	+
Poplar Tree	*Typha latifolia*	6.1 (2.5)	69.9 (26.4)	24.0	0.06	+
	Scirpus cyperinus		57.7 (10.4)	47.8	0.34	–
	Penthorum sedoides		27.6 (2.1)	46.1	0.16	+
Great Marsh	*Typha latifolia*	10.4 (3.0)	79.1 (3.0)	7.8	0.02	+
	Zizania aquatica		102.0 (75.9)	30.4	0.12	+
	Rosa palustris		86.0 (9.6)	1.6	0.01	+
Linton Hall	*Typha latifolia*	0.5 (0.4)	26.4 (8.3)	13.1	0.08	+
	Scirpus cyperinus		46.7 (1.3)	5.6	0.03	+
	Scirpus validus		9.6 (3.5)	30.4	0.14	+
Lee Drive	*Magnolia virginiana*	3.4 (0.8)	4.0 (1.4)	25.3	0.26	+
	Liquidambar styraciflua		5.8 (2.4)	0.5	0.01	+
	Osmunda cinnamomea		6.4 (1.8)	3.5	0.04	–
Catherine's Furnace	*Cinna arundinacea*	0.4 (0.2)	23.2 (4.2)	4.9	0.04	+
	Scirpus sp.		23.0 (2.3)	8.5	0.08	+
	Viburnum dentatum		21.0 (5.7)	35.3	0.47	+
Big Run Bog	*Juncus acuminatus*	12.6 (0.4)	5.2 (0.1)	42.4	0.33	+
	Rubus sp.		4.0 (0.4)	8.2	0.13	–
	unknown sedge		4.5 (0.6)	5.4	0.05	+
Tub Run Bog	*Juncus effusus*	15.9 (0.2)	6.1 (0.1)	63.6	0.85	+
	Hypericum densiflorum		13.6 (1.7)	13.5	0.02	+
	Dulichium arundinaceum		6.6 (1.7)	6.9	0.10	+
Potomac River	*Vallisneria americana*	< 0.1	15.7 (5.7)	91.0	0.72	+
	Hydrilla verticillata		12.6 (2.1)	97.1	1.29	+

[1]Root surface area was calculated from measurements of root length and diameter for each root in the sample.

At the sites where *Typha* was abundant, total rhizosphere bacteria averaged 8.6 $\times 10^7$ cells gdw^{-1} (Figure 2A). The highest FeOB abundances, 1.2×10^6 FeOB gdw^{-1} root, were observed at Linton Hall, a freshwater marsh with moderately high iron plaque concentrations. The lowest abundance of rhizosphere FeOB was 3.4×10^2 FeOB gdw^{-1} in the salt marsh (SERC), which also had the lowest levels of iron on the roots and 80% soil organic matter content. Abundances at the other sites ranged from 10^4 to 10^5 cells gdw^{-1}. Excluding the SERC site, the average FeOB abundance increased from 5.9×10^5 FeOB gdw^{-1} to 6.7×10^5 FeOB gdw^{-1}.

Rhizosphere FeRB density was one to two orders of magnitude higher than FeOB density at every site except Linton Hall, averaging 9.2×10^6 FeRB gdw^{-1}. At SERC, there were almost 4,000-times more FeRB gdw^{-1} than FeOB. Microbial Fe(III) reduction at this site was confirmed by transferring positive Fe(III)-reducing tubes to media containing sodium molybdate to inhibit sulfate reduction. Nonetheless, some of the organisms enumerated at the SERC site may have been sulfate-reducers capable of Fe(III) reduction that were not inhibited by sodium molybdate and/or false positives due to chemical reduction of Fe(III) by sulfides (Lovley et al. 1993). A significant relationship was found between the amount of iron plaque and the density of FeRB ($r^2 = 0.31$, $p = 0.01$). There was evidence of a relationship between FeOB densities and iron plaque concentrations. FeRB and FeOB abundances were not significantly related to each other.

In soil, total microbial abundance averaged 1.4×10^9 cells gdw^{-1} soil and was significantly higher than on roots ($p < 0.001$, Figure 2B). FeOB abundance was also significantly higher in the soil than on roots, but FeRB were more abundant on roots than in the soil ($p < 0.001$). On average, FeOB were slightly more abundant that FeRB in the soil. As was observed in the rhizosphere iron bacteria, soil FeOB and FeRB abundances were not significantly correlated with each other. No significant relationships were observed between DCB-extractable soil Fe and the abundance of FeOB or FeRB. DCB-extractable Fe concentrations were consistently lower in the soil than on the roots on a dry weight basis.

Proportions of FeOB and FeRB on roots and in soil

The contributions of FeOB and FeRB to total microbial abundances varied widely among sites and between the rhizosphere and soil (Table 3). The percentage of FeOB in the rhizosphere ranged from 0.001% at SERC to 6.3% at Linton Hall. On average, about 1.4% of the rhizosphere microbial community was aerobic, lithotrophic FeOB. In contrast, FeRB averaged 12.5% of the rhizosphere microbial community with sites such as Great Marsh and Braun yielding 20 to 30%. The percentage of iron bacteria in the soil microbial community was much lower, averaging 0.5% for FeOB and 0.2% for FeRB. The percentage of soil FeRB and FeOB were weakly correlated ($r = 0.51$, $p = 0.02$). The difference between root and soil percentages of FeRB was highly significant ($p < 0.001$).

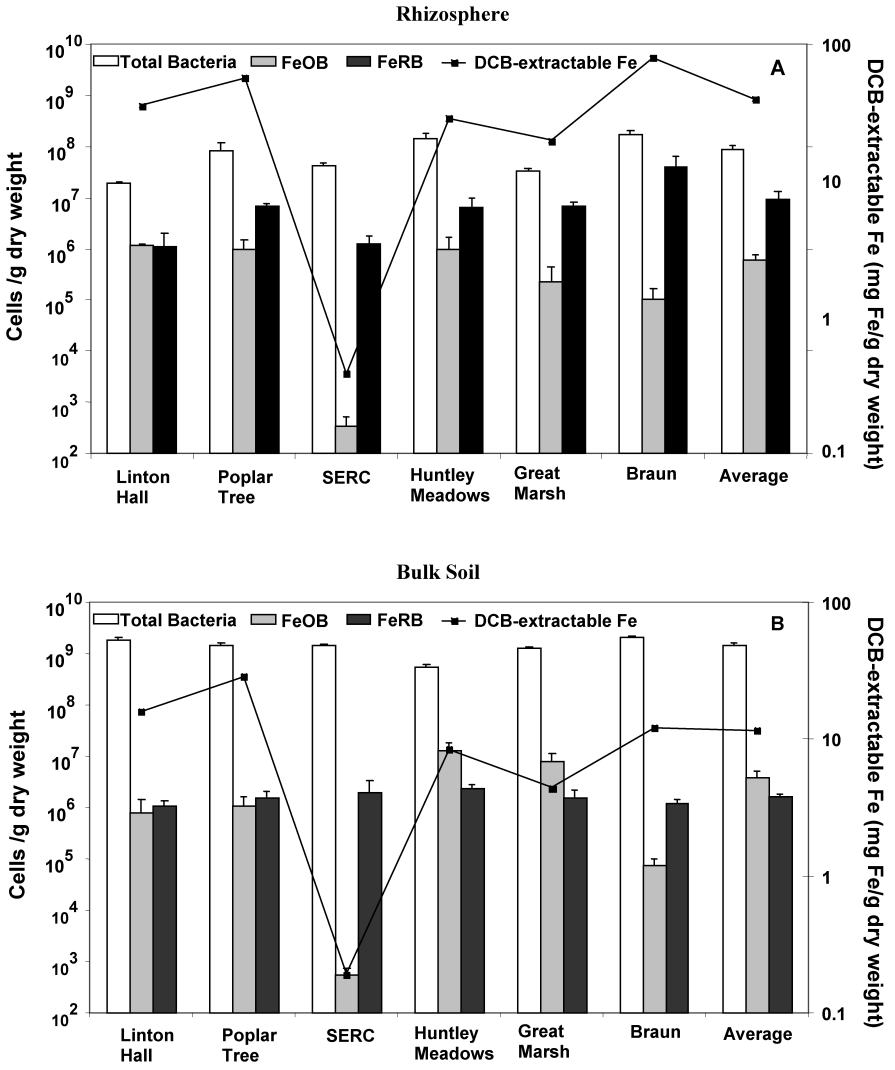

Figure 2. A comparison of total bacteria, lithotrophic FeOB, acetate-utilizing FeRB and DCB-extractable iron in the rhizosphere of *Typha* spp. (A) and in the soil (B). With the exception of root bacterial abundances at Linton Hall and Braun n = 2, all site data is a composite of three plant or soil samples. Mean bacterial abundances are shown with ± 1 standard error.

Root and soil density

On average, the minerals and soil organic matter of these wetland soils constituted > 95% of the total mass while roots constituted 1.5% (Table 4). Root iron concentrations ranged from < 0.001 mg cm^{-3} at SERC to 0.09 mg cm^{-3} at Braun. In contrast, soil iron concentrations ranged from 0.002 mg cm^{-3} at SERC to 14 mg cm^{-3}

Table 3. The relative proportions (%) of FeOB and FeRB in the rhizosphere of *Typha* spp. and in bulk soil. All values are presented as the average of three plant specimens or three soil samples ± 1 standard error. Percentages were calculated by dividing the abundance of each group by the total bacterial counts. The overall average is based on all the sampled plants or soils (n = 18). The asterisks (*) denote a significant difference ($p < 0.001$) between the rhizosphere and bulk soil in the relative abundance of FeRB; there was no significant difference between the rhizosphere and soil with respect to FeOB.

Site	Rhizosphere		Bulk Soil	
	FeOB	FeRB	FeOB	FeRB
Linton Hall	6.3 (0.6)	5.4 (3.6)	0.04 (0.03)	0.06 (0.01)
Poplar Tree	1.8 (1.1)	12.2 (4.2)	0.08 (0.04)	0.10 (0.04)
SERC	< 0.01	3.5 (1.6)	< 0.01	0.14 (0.10)
Huntley Meadows	0.6 (0.3)	5.8 (4.0)	2.10 (0.86)	0.42 (0.08)
Great Marsh	0.8 (0.7)	20.1 (3.4)	0.60 (0.25)	0.12 (0.06)
Braun	0.1 (0.1)	32.1 (22.5)	< 0.01	0.06 (0.01)
Average	1.4 (0.6)	12.5 (9.0)*	0.47 (0.22)	0.15 (0.04)*

at Poplar Tree. On a volume basis, the abundance of rhizosphere FeOB and FeRB was two to three orders of magnitude lower than in the soil. On sites with low to moderate levels of organic matter, < 50% of the cells and iron minerals were directly associated with the root surface.

Discussion

The role of microbes in plaque deposition

We recently showed that putatively lithotrophic FeOB exist in the rhizosphere of plants growing in a mining-impacted riparian wetland with low porewater pH and high levels of Fe(II) (Emerson et al. 1999). The present study greatly extends those results by demonstrating that FeOB are ubiquitous in wetlands, occurring in the rhizosphere of 25 plant species growing in 13 biogeochemically-diverse habitats. In a more detailed analysis of the roots of *Typha* spp. at a subset of these sites, recoverable FeOB had an average density of 10^5 FeOB gdw^{-1} root, and accounted for 1% of the total microbial population. Although no significant correlation was found between FeOB abundance and iron plaque concentration, the lowest numbers of FeOB were observed at the site with the lowest amount of iron plaque (i.e. the SERC salt marsh). Furthermore, we found that FeRB were 12.5% of the rhizosphere microbial community of the same plants. Although the MPN method does not assess the activity of these organisms, high abundances suggest that Fe(II)-oxidizing and Fe(III)-reducing bacteria may have a strong influence on the iron biogeochemistry of the rhizosphere.

The prevailing wisdom is that rhizosphere Fe(II) oxidation at circumneutral pH is largely an abiotic process (Howeler and Bouldin 1971; Faulkner 1994; Kirby et

Table 4. Root and soil bulk densities, iron, and iron bacteria. Volumetric Fe concentrations (mg Fe/cm^3) and abundances of iron bacteria (cells/cm^3) were calculated by multiplying the root and soil density by mass-based iron concentrations and abundances (Figure 2). Averages represent the composite of 3 different cores (density) or samples (iron and bacteria). Averages are ± 1 standard error

Site	ROOT				SOIL			
	Density (mg/cm^3)	DCB-extractable Fe (mg/cm^3)	FeRB (cells/cm^3)	FeOB (cells/cm^3)	Density (mg/cm^3)	DCB-extractable Fe(mg/cm^3)	FeRB (cells/cm^3)	FeOB (cells/cm^3)
Linton Hall	2.29 (0.56)	0.07 (0.02)	2.5×10^3 (6.1×10^2)	2.7×10^3 (6.8×10^2)	808 (23)	12.8 (0.5)	8.6×10^5 (2.4×10^4)	6.3×10^5 (1.8×10^4)
Poplar Tree	0.90 (0.06)	0.04 (0.01)	6.2×10^3 (4.1×10^2)	9.0×10^2 (6.0×10^1)	500 (14)	14.2 (0.7)	7.5×10^5 (2.1×10^4)	5.3×10^5 (1.5×10^4)
SERC	6.06 (0.85)	0.00 (0.00)	8.9×10^3 (1.7×10^2)	2.4 (0.05)	94 (1)	0.0 (0.0)	1.8×10^5 (2.7×10^3)	5.1×10^1 (0.1)
Huntley Meadows	1.17 (0.28)	0.02 (0.01)	6.6×10^3 (2.2×10^3)	1.0×10^3 (3.3×10^2)	321 (12)	2.7 (0.2)	7.5×10^5 (2.6×10^4)	4.1×10^6 (1.4×10^5)
Great Marsh	3.94 (0.79)	0.05 (0.02)	3.0×10^4 (6.5×10^3)	1.0×10^3 (2.2×10^2)	112 (16)	0.5 (0.1)	1.7×10^5 (3.2×10^4)	8.7×10^5 (1.7×10^5)
Braun	1.22 (0.25)	0.09 (0.03)	5.0×10^4 (1.0×10^4)	1.3×10^2 (2.6×10^1)	995 (43)	12.1 (0.9)	1.2×10^6 (5.2×10^4)	7.4×10^4 (3.2×10^3)

al. 1999; van Bodegom et al. 2001). In one of the only studies to experimentally examine the role of bacteria in iron plaque formation, microbial respiration in the rhizosphere was hypothesized to limit the amount of O_2 available for Fe(II) oxidation, resulting in a negative correlation between microbial abundance and plaque formation (Johnson-Green and Crowder 1991). However, because no precautions were taken to mimic the microaerophilic environment of an *in situ* rhizosphere, it is possible that relatively high O_2 concentrations in the experimental microcosms favored abiotic oxidation of Fe(II). In another set of studies using rice as a model system, van Bodegom et al. (2001) determined that iron was the primary O_2 sink in the rhizosphere and that Fe(II) oxidation was entirely abiotic. The description of their experimental set-up, which included periods of vigorous agitation, again suggested conditions that would artificially favor abiotic Fe(II) oxidation (Neubauer et al. 2002). Because iron plaque can potentially form rapidly due to autocatalytic oxidation, elucidating the specific contribution of FeOB in plaque formation will require experimental manipulations of FeOB abundance or the development of direct techniques for distinguishing biotic Fe(II) oxidation using isotopic tracers or specific inhibitors.

There are a number of reasons why we may have underestimated FeOB abundance. Because cultivation-based methods commonly recover < 1% of the *in situ* microbial population diversity, there may have been organisms capable of oxidizing Fe(II) that would not grow in the culture medium we used. For example, densities of nitrate-reducing FeOB comparable to the O_2-reducing FeOB values reported in the current study have been found in sediments from the top 3–4 mm of a rice paddy soil with substantial nitrate concentrations (Ratering and Schnell 2001) and in the profundal sediments of a deep lake (Hauck et al. 2001). In contrast, a study of European aquatic sediments found that nitrate-reducing FeOB comprised $\leqslant$ 0.04% of the total bacterial community (Straub and Buchholz-Cleven 1998). Generally, the use of NO_3^- as a terminal electron acceptor in wetland soils will be limited due to assimilation of NO_3^- by plants and microorganisms (Bodelier et al. 1998). We also did not quantify the abundance of aerobic heterotrophic FeOB such as members of the *Siderocapsaceae*, a group of organisms found in a wide range of aquatic environments (Hanert 1992). Although these organisms have been hypothesized to contribute to Fe(II) oxidation (Lunsdorf et al. 1997), there is little known about the exact mechanism of heterotrophic Fe(II) oxidation (Emerson 2000) and, to our knowledge, no studies have examined their natural abundances or potential rates of iron oxidation. Further studies are needed to determine the contribution of nitrate-reducing and aerobic heterotrophic FeOB to Fe(II) oxidation in wetlands. Our observation that FeOB are a substantial proportion of the wetland rhizosphere microbial community (1% average, 6% maximum) can be considered conservative.

The root as a site of Fe(III) reduction

Large numbers of acetate-utilizing FeRB were also associated with the roots of *Typha* spp., contributing an average of 12% of the rhizosphere microbial community compared to just 0.02% in the soil. One factor favoring the relatively high

densities of FeRB in the rhizosphere may be the high abundance of poorly-crystalline Fe(III) minerals that are superior substrates for FeRB (Thamdrup 2000). Poorly-crystalline Fe(III) oxides have been observed on plants growing in natural and mine-impacted wetlands (Taylor et al. 1984; Fisher and Stone 1991; Batty et al. 2000; Hansel et al. 2001). Conversely, much of the Fe(III) in aquatic sediments is in a crystalline form not readily reduced by FeRB (Phillips et al. 1993; Thamdrup 2000). In a separate study, we found a significantly higher percentage of oxalate-extractable iron on the roots (66%) versus the soil (23%) ($p < 0.05$), and a significant positive correlation between the percentage of oxalate-extractable iron and the percentage of FeRB ($r^2 = 0.55$, $n = 5$ sites, $p = 0.01$, Weiss et al. in preparation). Because oxalate extracts primarily poorly-crystalline Fe(III), these results imply that relatively large amounts of poorly-crystalline Fe(III) in the rhizosphere promote the high abundances of FeRB that were observed on the roots of wetland plants.

Another factor contributing to the large difference in the percentage of FeRB in the rhizosphere versus the soil may be the higher levels of labile carbon associated with roots. High levels of acetate have been reported in the vicinity of the roots of aquatic plants (Hines et al. 1994; Dannenberg and Conrad 1999), and concentrations can increase dramatically under anaerobic conditions (Conrad and Klose 1999). Due to its abundance in the rhizosphere and common use as a carbon source by FeRB such as *Geobacter* spp. (Lovley 2000), acetate was used in this study to enrich for FeRB. Other carbon compounds associated with the rhizosphere that can be used by *Geobacter* spp. include ethanol, propionate, and butyrate (Mendelssohn et al. 1981; Conrad and Klose 1999). It is possible that non-acetate-utilizing FeRB such as *Shewanella* spp. are also present in the rhizosphere, but a number of studies have indicated that members of the Geobacteracae dominate subsurface environments (Lovley 2000). Humic-acids derived from roots may also be used as electron shuttles by FeRB, further promoting high abundances of FeRB in the rhizosphere (King and Garey 1999).

Because the observation of Fe(III) reduction in the rhizosphere is a novel one, few studies have considered the role of this process in plaque formation and dissolution. King and Garey (1999) found that excised roots could reduce up to 28 mg Fe(III) gdw^{-1} day^{-1}, illustrating that Fe(III) may be reduced from the root surface quickly under anaerobic conditions. Our finding that high numbers of FeRB are associated with roots and the dominance of poorly-crystalline iron in the rhizosphere helps explain the high potential rates reported in that study. More studies are needed to determine how quickly Fe(III) reduction occurs *in situ* where variability in Fe(III) and O_2 concentrations might limit Fe(III) reduction potential. The presence of iron plaque dominated by Fe(III) (Weiss, in preparation) under many different environmental conditions indicates that rates of Fe(II) oxidation in the rhizosphere are generally faster than Fe(III) reduction.

The lack of significant relationships between environmental parameters and iron plaque concentrations was not surprising due to the dual control of plaque accumulation by iron deposition and solubilization. Because both of these processes are influenced by physical, chemical, and biological factors varying across spatial and

temporal scales, the relationship between iron plaque and any combination of factors is unlikely to be linear.

A rhizosphere iron cycle

Previous salt marsh studies have discussed the possibility of a geochemical iron cycle promoted by the presence of wetland vegetation (Giblin and Howarth 1984; Jacobson 1994), but did not specifically consider the role of FeOB and FeRB. Our observation of high abundances of FeOB and FeRB on the same 1-cm subsection of root suggests that oxidation and reduction are occurring simultaneously and both processes are mediated, at least in part, by bacteria. In this cycle, Fe(II) is oxidized by both autocatalytic and biotic mechanisms using O_2 from roots as a terminal electron acceptor. Under anaerobic conditions, FeRB use root-derived poorly crystalline Fe(III) and labile carbon for Fe(III) reduction. On a microscale, these two processes are likely to be separated spatially and temporally. The edge of the plaque may switch from microaerobic to anaerobic with diurnal variations in oxygenic photosynthesis (e.g. Flessa (1994)). Older parts of the roots have been shown to release less O_2 than the root tip (Armstrong (1971, 1979); Brix and Schierup 1991; Flessa 1994) and should support significant zones of Fe(III) reduction. Rhizosphere Fe(II) oxidation may dominate during the summer when increases in plant biomass stimulate radial O_2 loss (Chen et al. 1980), while Fe(III) reduction dominates at other times (Crowder and Macfie 1986).

The roots clearly have a smaller pool of iron bacteria and Fe(III) than the soil on a volume basis (Table 4). However, we did not determine the microbial activity of the FeOB and FeRB and several lines of evidence suggest the rhizosphere iron pool is more dynamic than the larger soil pool. Over 12% of the root-associated microbial community are FeRB as compared with less than 1% in the soil; differences in the availability of labile carbon and reducible Fe(III) may contribute to this difference. The roots are also the primary source of O_2 available to aerobic FeOB living in otherwise anaerobic subsurface soils. In addition, the roots can have a much larger aerobic surface area than the surface soil. A rough estimate of the root surface area (calculated by converting gdw^{-1} to cm^{-2} using Table 2) is 16-times higher than the surface area of the aerobic-anaerobic soil interface in our cores. Higher availability of O_2, labile carbon, and poorly crystalline Fe(III) may all contribute to a more active iron cycle in the rhizosphere than in the bulk soil in wetlands. More studies are needed to quantify to ecosystem-level rates of microbially-mediated iron cycling in the rhizosphere and the precise role that high abundances of iron bacteria have on such a cycle.

Conclusions

Aerobic, lithotrophic FeOB were found on 25 different species sampled from a wide range of biogeochemical conditions. In *Typha*-dominated wetlands, FeOB ac-

counted for an average of 1.4% of all rhizosphere bacteria. On the same 1-cm sections of root, FeRB accounted for an average of 12% of the rhizosphere microbial community. In contrast, FeOB and FeRB abundance in non-rhizosphere soil averaged 0.5% and 0.2% respectively. Relatively high proportions of FeOB, FeRB, poorly-crystalline Fe(III), O_2, and labile C suggest that the rhizosphere is a 'hotspot' of microbially-mediated iron cycling in wetlands. These observations provide a strong rationale for quantifying the contribution of FeOB to rhizosphere Fe(II) oxidation rates, and investigating the combined role of FeOB and FeRB in iron cycling. Such a cycle has important biogeochemical implications including the suppression of methane production.

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