Published online September 29, 2005

Rhizosphere Iron (III) Deposition and Reduction in a *Juncus effusus* L.-Dominated Wetland

Johanna V. Weiss,* David Emerson, and J. Patrick Megonigal

ABSTRACT

Iron (III) plaque forms on the roots of wetland plants from the reaction of Fe(II) with O_2 released by roots. Recent laboratory studies have shown that Fe plaque is more rapidly reduced than non-rhizosphere Fe(III) oxides. The goals of the current study were to determine in situ rates of: (i) Fe(III) reduction of root plaque and soil Fe(III) oxides, (ii) root Fe(III) deposition, and (iii) root and soil organic matter decomposition. Iron (III) reduction was investigated using a novel buried-bag technique in which roots and soil were buried in heat-sealed membrane bags (Versapor 200 membrane, pore size = 0.2 μm) in late fall following plant senescence. Bags were retrieved at monthly intervals for 1 yr to assess changes in total C and Fe mass, Fe mineralogy, Fe(II)/Fe(III) ratio, and the abundances of Fe(II)-oxidizing bacteria (FeOB) and Fe(III)-reducing bacteria (FeRB). The soil C and Fe pools did not change significantly throughout the year, but root C and total root Fe mass decreased by 40 and 70%, respectively. When total Fe losses were adjusted for changes in the ratio of Fe(II)/Fe(III), over 95% of the Fe(III) in the plaque was reduced during the 12-mo study, at a peak rate of 0.6 mg Fe(III) g dry weight^{-1} d^{-1} (gdw^{-1} d^{-1}). These estimates exceed the crude estimate of Fe(III) accumulation [0.3 mg Fe(III) g dry weight^{-1} d^{-1}] on bare-root plants that were transplanted into the wetland for a growing season. We concluded that root plaque has the potential to be reduced as rapidly as it is deposited under field conditions.

Iron-oxide deposits known as Fe plaque are commonly observed on the roots of wetland and aquatic plants (Mendelssohn et al., 1995). Iron plaque forms when O_2 leaking from plant roots reacts with Fe(II) produced in surrounding anaerobic soils (Armstrong, 1964). As a result, the presence of vegetation dramatically alters subsurface biogeochemistry by concentrating large amounts of solid-phase Fe(III) in the rhizosphere (Kostka et al., 2002; Ratering and Schnell, 2000). This Fe(III) pool is dominated by amorphous Fe(III) oxides (Taylor et al., 1984; Batty et al., 2000; Weiss et al., 2004), formed either by abiotic oxidation or FeOB (Weiss et al., 2003). In laboratory studies, FeOB isolated from the wetland rhizosphere were found to mediate between 18 and 90% of Fe(II) oxidation (Neubauer et al., 2002; Sobolev and Roden, 2001), suggesting that they could play an important role in the formation of Fe plaque.

The rhizosphere can vary between oxic and anoxic depending on microbial or chemical O_2 demand in the soil and rates of radial oxygen loss. Radial O_2 loss is in turn influenced by plant activity (Bedford et al., 1991; Kuehn and Suberkropp, 1998) and morphological characteristics such as suberized and lignified roots (Armstrong et al., 2000). Under anaerobic conditions, the amorphous Fe(III) oxides in root plaque serve as an excellent substrate for FeRB that mediate Fe(III) reduction in freshwater environments (Lovley, 2000). Iron(III) reducing bacteria have been successfully enriched from the rhizosphere (King and Garey, 1999) where they can account for up to 12% of all bacterial cells (Weiss et al., 2003).

The juxtaposition of oxic and anoxic conditions in the rhizosphere, separated either temporally or spatially, results in a rhizosphere Fe cycle in which Fe plaque is alternately deposited and then reduced. Previous studies have reported a higher Fe(III) reduction potential in the rhizosphere Fe(III) pool than the non-rhizosphere soil pool. For example, we recently reported that >75% of the Fe plaque is reduced in 10 d vs. <40% of the soil Fe(III) oxide pool (Weiss et al., 2004). Results from another short-term (7 d) experiment examining rates of Fe(III) reduction in salt marshes support the idea of more rapid Fe(III) reduction in rhizosphere than non-rhizophere soils (Gribsholt et al., 2003). However, both of these studies presented relatively short-term rates determined in a laboratory environment. To our knowledge, the kinetics of Fe(III) reduction have not been previously investigated in situ over periods of time >1 wk. Furthermore, although researchers have reported observations suggesting rapid rates of Fe(II) oxidation in the rhizosphere (Roden and Wetzel, 1996), no data set exists for in situ rates of Fe plaque accumulation.

The goals of the current study were to quantify: (i) Fe(III) reduction rates in the rhizosphere and non-rhizosphere soil following fall senescence, (ii) net Fe plaque deposition on selected wetland plants during a growing season, and (iii) decomposition rates of roots and soil organic matter. Based on our previous findings, we hypothesized that the rhizosphere Fe pool would be more dynamic than the non-rhizosphere Fe pool, resulting in significant amounts of plaque deposition and reduction over an annual cycle.

MATERIALS AND METHODS

For the purposes of this study the term "rhizosphere" is defined as the root surface and its associated Fe plaque. This operational definition is conservative because it is likely that radial O_2 loss and Fe(II) oxidation penetrate to some extent beyond the boundary of the Fe plaque. The advantage of this

J.V. Weiss, George Mason Univ., Fairfax, VA 22030; Present address: U.S. Geological Survey, Reston, VA 20142; D. Emerson, American Type Culture Collection, Manassas, VA 20110; J.P. Megonigal, Smithsonian Environmental Research Center, Edgewater, MD 21037-0028.
Received 3 Jan. 2005. *Corresponding author (jweiss@usgs.gov).

Published in Soil Sci. Soc. Am. J. 69:1861–1870 (2005).
Wetland Soils
doi:10.2136/sssaj2005.0002

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: BPDS, bathophenanthroline disulfonate; diH_2O, deionized water; DO, dissolved oxygen; FeOB, Fe(II)-oxidizing bacteria; FeRB, Fe(III)-reducing bacteria; MPN, most probable number.

definition is that Fe plaque is easily delineated and separated from the bulk soil, providing an objective and reproducible means of sampling the rhizosphere Fe pool. The non-rhizosphere soil in this study was >0.5 cm from the root surface.

All experiments were performed in a *Juncus effusus*-dominated marsh located near Contrary Creek in Mineral, VA, approximately 60 miles southwest of Washington, DC. The water level in the marsh varied seasonally, but there was continuous standing water in excess of 0.5 m. For the purposes of consistency and adequate comparison, all measurements were made and reported on a gram dry weight (gdw^{-1}) basis.

Net Iron Plaque Deposition

Two methods were used to estimate net rhizosphere Fe deposition over a growing season. Rhizosphere Fe accumulation was estimated by transplanting bare-root specimens of *Juncus effusus* (n = 8) and *Pontedaria cordata* L. (n = 11) into the marsh on 19 May 1999. The roots of two to three additional specimens of each species were immediately harvested to determine initial Fe plaque concentrations (i.e., $[Fe]_0$). The remaining transplants were harvested on 29 Oct. 1999 and washed with deionized water (diH_2O) to remove the soil and determine $[Fe]_{5\ mo}$. Despite the possibility that small amounts of soil may have adhered to the root after washing (Azcue, 1996), roots visually appeared to contain insignificant residual soil. Total rhizosphere Fe was extracted with a dithionate-citrate-bicarbonate solution and measured on an atomic absorption spectrometer as described in Weiss et al. (2003). The roots were then dried at 70°C until no weight change was observed.

In-growth cores were also employed during the same time period to determine root production and associated rhizosphere Fe accumulation. Eight 30-cm deep soil cores were extracted from a monotypic stand of *Juncus effusus* with a serrated PVC pipe (diam. = 7 cm). The cores were sieved (mesh size = 2 mm) to remove all roots. The resulting root-free soil was placed in its original location, its location marked by two flags on the edge of the core separated by 180°, and removed 5 mo later. Roots that grew into these soil cores over a 5-mo period were collected by washing the core through a 2-mm sieve and subsequently divided into two size classes: coarse roots (>1-mm diam.) and attached fine roots (<1-mm diam.). Much of the fine root mass was recovered because they were attached to the larger course roots. However, unattached fine roots <2-mm diam. may have been lost from analysis. The samples were extracted for total Fe and dried as described above.

In Situ Iron Reduction Rates

The rate of in situ rhizosphere Fe(III) reduction was quantified with a modified "litter bag" technique. Specimens of native *J. effusus* and adjacent bulk soil were collected in November 1999 to simulate the plant senescence that occurs in the fall. Roots were washed in diH_2O, and dead roots were discarded. The bulk soil was sieved (mesh size = 2 mm) and homogenized in a blender. Because root and soil samples were processed under aerobic conditions, the initial pools of poorly crystalline Fe(III) oxides may have been larger than those in situ (see results).

Approximately 5 g of roots and 25 g of soil (both wet weight) were placed into a decomposition bag made from Versapor 200 membrane (nominal pore size = 0.2 μm) that was heat sealed on all sides. This bag was placed into a protective nylon mesh bag (pore size = 47 μm) and kept at 4°C until transport within 48 h to the wetland. Sixty bags were buried vertically within the rooting zone of the wetland soil; the tops of the bags were 10 cm below the soil surface and extended to 25 cm. The bags were placed along three 12-m transects in a densely vegetated portion of the wetland. To confirm the persistence of anaerobic conditions, sanded steel rods that accumulated rust under aerobic conditions were buried to 0.3 m in the soil at the ends and middle of each transect.

The pore size of the bags (0.2 μm) was small enough to prevent exchange of solid phases Fe minerals but allow equilibration of pore-water solutes. The bags also prevented exchange of microorganisms with the surrounding soil, but we assumed that this was not an important issue because each bag initially contained roots, soil organic matter, and the full diversity of their associated microbial communities. More problematic was that we harvested the *Juncus* roots before they died. This allowed us to begin the experiment with freshly dead, plaque-encrusted roots, but it may have introduced more labile C than expected for roots that undergo natural senescence. If so, we may have overestimated in situ Fe(III) reduction rates.

Five buried bags were randomly sampled monthly for 1 yr, ending in November 2000. The time zero collection occurred immediately after the full set of bags was buried. Upon collection, the bags were immediately placed in an anaerobic container, transported to the laboratory where they were kept at 4°C, and processed in an anaerobic glove bag within 24 h of collection. Soil recovered from the bags was placed in 50-mL centrifuge tubes. To minimize O_2 exposure, the tubes were initially flushed with N_2 gas, and tightly capped. Roots recovered from the same bags were cut into small pieces (<1 cm), then washed and homogenized by continual vortexing in anaerobic diH_2O until the water remained clear. Although it is possible that some loosely attached microbes were lost during washing, previous microscopy work indicated that the Fe plaque and imbedded microbial community remained intact after the washing process (Emerson et al., 1999). All washings were collected in beakers and dried (70°C) to account for soil attached to the roots. The roots and soil recovered from each bag were weighed wet, then subdivided for the following analyses: wet-dry weight conversion factor, FeOB and FeRB abundance, sequential extraction of the Fe pool, and Fe(II) and Fe(III) proportion. The root and soil wet weights were converted to dry weights using the conversion factor for each particular bag. Mass loss rates were fit to an exponential decay function (Wieder and Lang, 1982). All mass data are reported on a dry weight basis.

Microbial Analyses of Buried Bags

Aerobic, lithotrophic FeOB, and acetate-utilizing FeRB in the buried bags were enumerated using the most probable number (MPN) method described in Weiss et al. (2003). Briefly, root slurries were made by adding 0.1 g of root to 1 mL of sterile water and gently ground with a mortar and pestle followed by three washes with ddH_2O. Soil samples were homogenized by 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, then dilutions ranging from 10^{-3} to 10^{-8} were used to inoculate tubes of both FeOB- and FeRB-media (Weiss et al., 2003). The presence of FeOB was indicated by the development of a discrete band of Fe oxides at the oxic-anoxic interface in the gradient-type MPN FeOB tubes (Emerson et al., 1999). Most probable number tubes for putative FeRB were visually assessed for consumption of the Fe(III) oxides and measured for Fe(II) by addition and color development of ferrozine (Stookey, 1970). Abundances of FeOB and FeRB were calculated using MPN tables (Eaton and Franson, 1995).

Geochemical Analyses

Changes in the forms of Fe on the roots and in the soil were monitored by a sequential extraction procedure modified from Wieder and Lang (1986) as detailed in Weiss et al. (2004). The only deviation was that all extraction steps were performed anaerobically on ≈1 g of fresh weight root or soil. Because anaerobic oxalate extractions can remove crystalline Fe in the presence of high amounts of Fe(II) (Phillips et al., 1993), a sequential extraction series in which 0.5 *M* HCl was substituted for oxalate was also run in the last 2 mo of the experiment. Use of 0.5 *M* HCl has been found to be suitable for extracting poorly crystalline Fe under both aerobic and anaerobic conditions (Lovley and Phillips, 1986). Quantities of total Fe(II) and Fe(III) were determined at each sampling interval with a dual-chelation technique using 1 m*M* EDTA or bathophenanthroline disulfonate (BPDS) to chelate the Fe(III) and Fe(II), respectively (Wang and Peverly, 1999; Weiss et al., 2004).

Solution Chemistry

The solution chemistry was monitored at the water surface and the soil–water interface of the study transects. Because some parameters were not measured in the initial months of the experiment, measurements were continued after the final bag collection to obtain a full year of seasonal data. Floodwater dissolved oxygen (DO) concentration was measured in situ with a DO meter (Yellow Springs Instruments 95) and pH with a Cole Parmer pH/con 10 series meter. Water samples for Fe(II) analysis were collected at the water surface and water–soil interface using a syringe followed by filtering (0.2 μm) into an acid-washed vial. Pore water Fe(II) was estimated by burying 10-mL polypropylene bottles containing anaerobic diH_2O to a depth of 15 cm and capped with a securely fitted membrane (Versapor 200, pore size = 0.2 μm). These pore water equilibration bottles were deployed monthly for 30-d periods. Iron (II) concentrations in all water samples were determined by immediate addition to ferrozine, then subsequent measurement at 562 nm on a spectrophotometer within 6 h of collection (Stookey, 1970).

The vertical distribution of pore water Fe(II) was characterized with pore water equilibrators or "peepers" (Hesslein, 1976). One peeper was buried to a depth of approximately 75 cm from the water surface for consecutive 1-mo intervals between August and October 2000. On the last sample date, two peepers were deployed. Because there was >30 cm of standing water, peepers were buried to a maximum soil depth of 20 cm, which encompassed most of the rooting zone. The peepers contained 26 1-cm wide wells overlaid with Versapor 200 membrane (pore size = 0.2 μm). Samples were collected by piercing the membrane of each well with a pipette tip and measured for Fe(II) concentrations using a ferrozine assay as described above.

Statistical Analyses

All statistics were performed in SAS, using the JMP 5.1 for Windows (SAS Institute, 2002). Pearson correlations were run between environmental parameters and decomposition rates, Fe(III) reduction rates, and abundances of FeOB and FeRB. One-way ANOVA followed by the Tukey-Kramer method of means separation was used to evaluate differences between roots and soils over the course of the field incubations with respect to Fe(II) and Fe(III) proportions, Fe mineral fraction concentrations, FeOB and FeRB abundances, and biomass. A paired *t* test was used to test for differences in the sequential extraction using 0.5 *M* HCl versus anaerobic oxalate. All significant differences are reported at the $P < 0.05$ level.

RESULTS

Solution Chemistry

The pH at the water–soil interface varied between 5.8 and 6.1 and decreased steadily from a high in March to a low in November (Fig. 1A). The pH of the surface floodwater, although generally higher, showed the same trend.

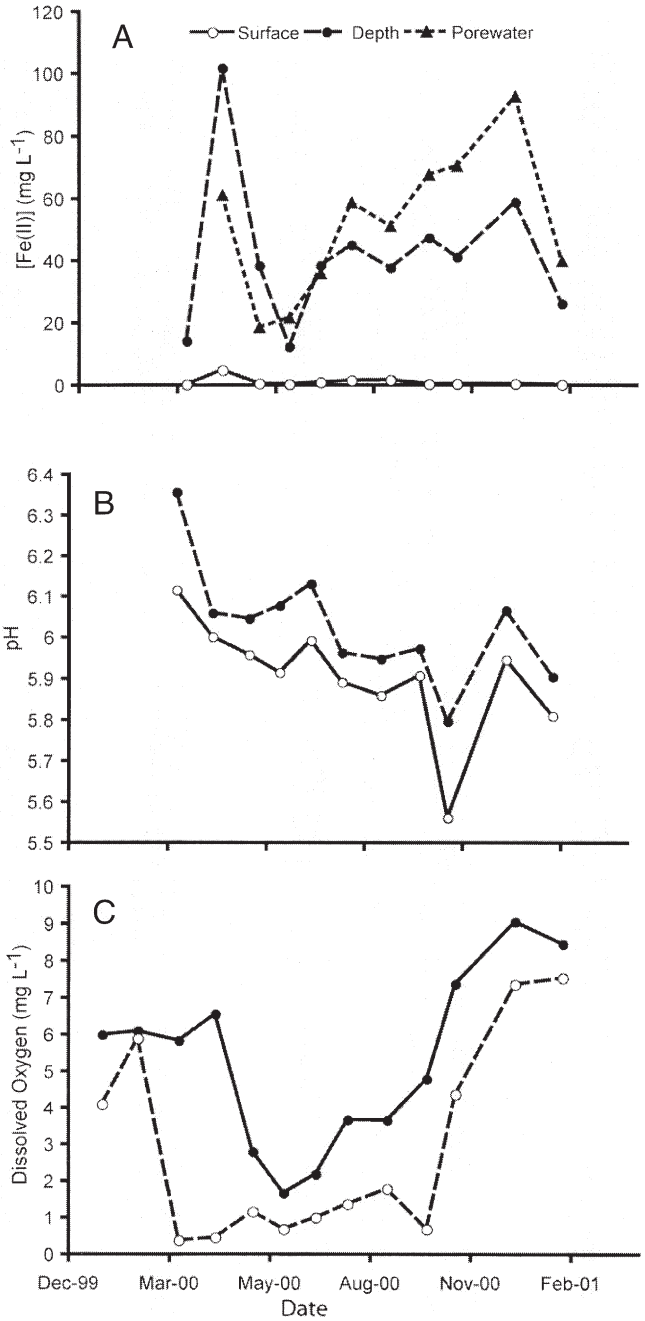

Fig. 1. Physical and chemical characteristics of a *Juncus*-dominated wetland. Each data point in Panels A through C is the average of six duplicate readings along the transect, two at each end and in the middle. Measurements were taken at the standing water surface (SURFACE), at the water–soil interface (DEPTH), and in the pore water (where applicable).

Table 1. Net rates of Fe plaque accumulation on the bare-roots of *Juncus effusus* and *Pontedaria cordata* over 5 mo.

Plant species	$[Fe]_0$†	$[Fe]_{5\,mo}$	Rate of Fe accumulation
	mg gdw^{-1}	mg g^{-1} root	mg g^{-1} root d^{-1}
Juncus effusus	0.34 ± 0.06 (n = 3)	65.80 ± 3.26 (n = 5)	0.40 ± 0.00
Pontedaria cordata	0.60 ± 0.13 (n = 3)	54.39 ± 10.24 (n = 4)	0.33 ± 0.07

† ±1 standard error.

Dissolved ferrous iron concentrations [i.e., $Fe(II)_{aq}$] ranged up to 100 mg L^{-1} (Fig. 1B). Surface water $Fe(II)_{aq}$ concentrations were generally <2 mg L^{-1} (Fig. 1A). $Fe(II)_{aq}$ concentrations increased 1 to 2 orders of magnitude at the water–soil interface and peaked at depths that overlapped with the buried reduction bag. Using pore water equilibrators buried at three 1-mo periods between August-November 2000, $[Fe(II)_{aq}]$ in the surface waters were confirmed to be very low, generally <0.5 mg Fe(II) L^{-1} (data not shown). $[Fe(II)_{aq}]$ increased to 250 mg $Fe(II)_{aq}$ L^{-1} at the soil surface with a maximum concentration of ≈275 mg $Fe(II)_{aq}$ L^{-1} 10 cm below the soil surface, corresponding to the upper rooting zone. We observed much higher pore water $[Fe(II)_{aq}]$ using the peeper than the buried pore water exchange bottle [200 vs. 60–80 mg $Fe(II)_{aq}$ L^{-1}], likely due to a relatively higher exchange surface area to water volume ratio in the peeper than the bottle.

The lowest $Fe(II)_{aq}$ concentrations were observed in May and June 2000 followed by general increases through the late summer and fall months. $[Fe(II)_{aq}]_{pore\ water}$ was inversely related to surface temperature (data not shown, $r = -0.64$, $n = 12$). No relationship was observed between $[Fe(II)_{aq}]$ and [DO] (Fig. 1C), with [DO] at the water–soil interface consistently <1–2 mg L^{-1} from March to October. The buried iron rods had no rust accumulation at depths below the water–soil interface, indicating that there was no significant flux of O_2 during the experiment at the depth the reduction bags were buried.

Net Iron Plaque Deposition

Iron plaque concentrations of transplanted bare-root specimens of *J. effusus* and *P. cordata* plants increased from an initial value of <1 to 66 mg Fe g^{-1} and 54 mg Fe g^{-1} at 5 mo, respectively (Table 1). Assuming that Fe(III) accumulated at a steady rate during the experiment (see discussion), the average rate of Fe accumulation was 0.37 mg Fe g root d^{-1}.

Table 2. Root production and Fe plaque accumulation in in-growth cores during the growing season.

Sample	Root production†	Root Fe accumulation	
	g root m^{-2} soil	mg Fe g dry weight^{-1} root	g plaque Fe m^{-2} soil‡
Coarse	106 ± 30§	30.99 ± 3.83	2.84 ± 0.88
Fine	136 ± 23	16.01 ± 1.78	2.25 ± 0.37
Total	243 ± 45	21.02 ± 2.48	5.09 ± 1.07

† Although cores were taken in a nearly monotypic *Juncus effusus* stands, we cannot rule out the presence of other unidentified plant species.
‡ Cores were taken to a depth of 30 cm.
§ ±1 standard error.

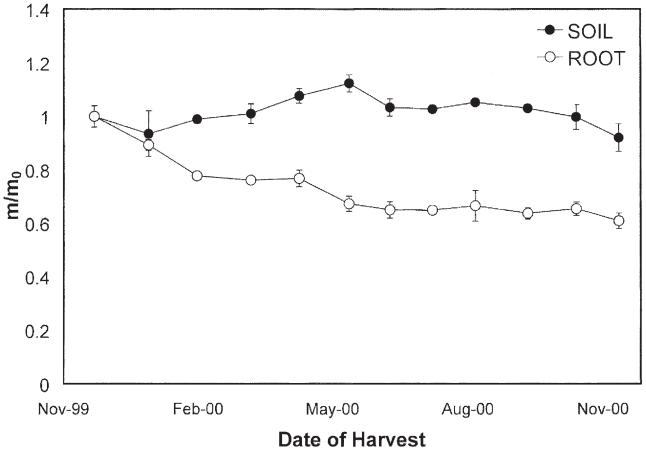

Fig. 2. Changes in root and soil biomass during anaerobic decomposition. All data points represent the average of five harvested bags ± 1 standard error. The m_0 is the initial biomass and the m is biomass at each time point.

Total root production in the in-growth cores from May-October was 243 g root m^{-2} of soil with Fe concentrations averaging 21 mg Fe g^{-1} root (Table 2). Using root production and Fe accumulation to scale Fe deposition to a soil-area basis yielded an estimate of >5 g Fe m^{-2} that was root-associated, to a depth of 30 cm. Fine roots accounted for a substantial portion of both root production and Fe plaque accumulation, as expected from use of the in-growth core method. The overall rate of plaque accumulation, calculated by dividing the average amount of Fe accumulation by the length of the incubation period, was 0.13 mg Fe g^{-1} root d^{-1}.

Root and Soil Decomposition

We observed no significant loss in soil organic matter over the 12-mo period in contrast to a 40% decrease in the root biomass (Fig. 2). The largest losses in root biomass were observed in the first 2 mo (Dec–Jan, $P < 0.0001$), with small, albeit nonsignificant, decreases in biomass after this period. The roots decayed at a rate of 0.12% d^{-1} ($r^2 = 0.84$) as determined by an exponential decay function.

In Situ Fe(III) Reduction Rates

Total Fe plaque concentrations, as determined by the sum of the fractions recovered by sequential extraction, decreased significantly from an initial value of 78 mg Fe g^{-1} root to 29 mg Fe g^{-1} at 2 mo (Fig. 3, $P < 0.0001$). With the exception of a brief increase in March, Fe plaque concentrations remained at 25–30 mg Fe g^{-1} for the final 7 mo of the experiment and were not statistically different. Soil Fe concentrations were initially 20 mg Fe g^{-1} soil and did not decrease below 14 to 17 mg Fe g^{-1}. No significant differences were observed in total soil Fe concentrations throughout the experiment.

With the exception of the residual fraction in the soil, the proportions of all the Fe fractions in the total Fe pool changed significantly during the 12-mo incubation period (Fig. 3, $P < 0.0001$). The exchangeable fraction, comprised primarily of adsorbed Fe(II) and Fe(III), in-

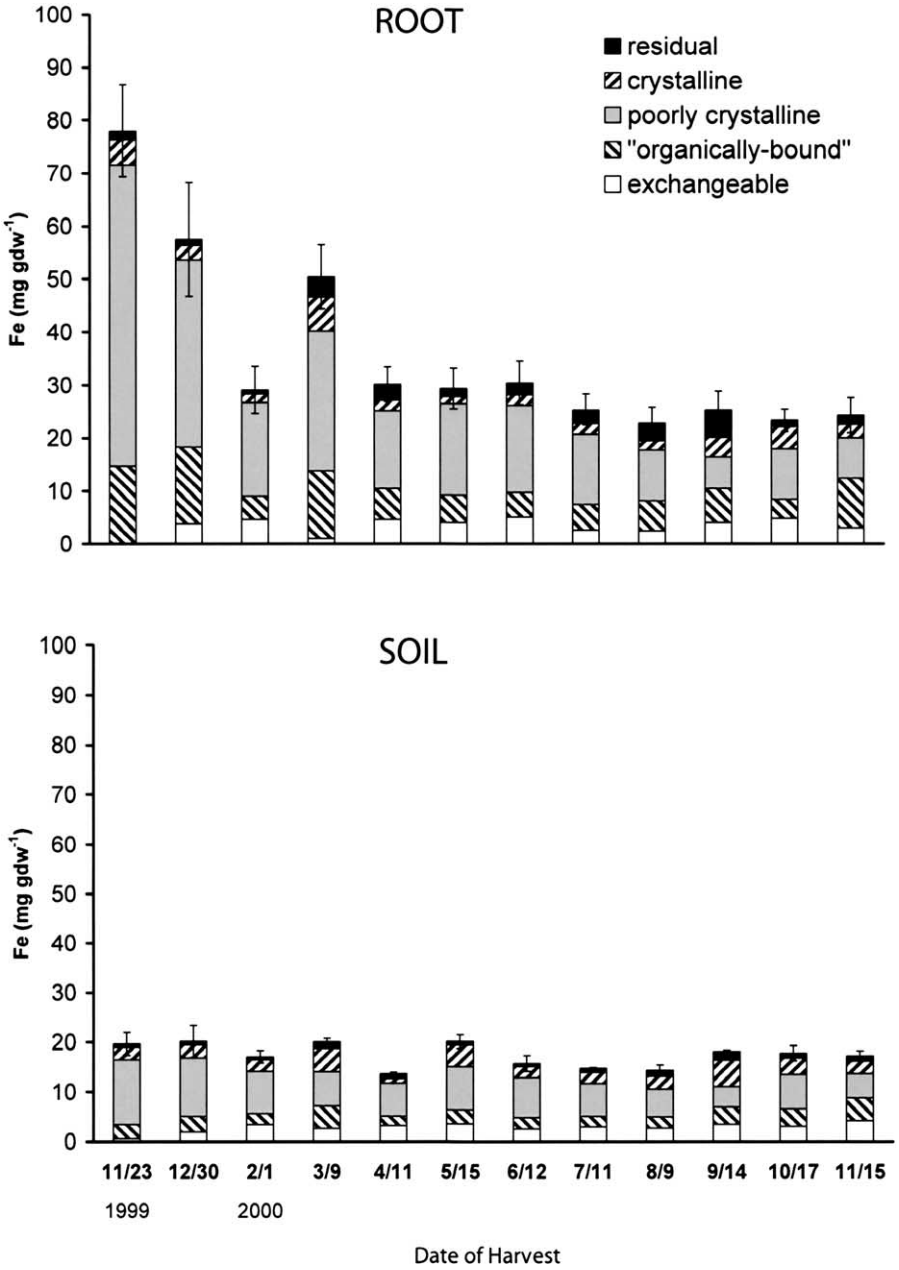

Fig. 3. Changes in Fe fractions during anaerobic decomposition. All columns represent the average total Fe of five buried bags collected at each time interval ±1 standard error.

creased from <0.1 to ≈20% in the roots and from 3 to >20% in the soils. The pyrophosphate ("organically bound") fraction increased 10 to 15% in the roots and soil, though this is largely due to overall decreases in the total Fe with the bulk amount of pyrophosphate generally decreasing in the first 4 mo of the experiment. Despite some differences in the crystalline fractions during the course of the experiment, no consistent trends were observed with months 0 and 11 being statistically the same. A significant increase in the residual fraction (from 2–10%, $P < 0.0001$) was observed in the roots.

Perhaps the most notable change was in the oxalate-extractable fraction, which approximates the poorly crystalline (amorphous or non-crystalline) Fe. In the root plaque, the poorly crystalline fraction significantly decreased from 73% of the total Fe pool to 50% by March (Month 3) and then to ≈30% by the end of the experiment (Fig. 3, $P < 0.0001$). The oxalate-extractable fraction in the soil also significantly decreased from 66% initially to 30–40% in the last months of the experiment ($P < 0.0001$). No significant differences were found between the anaerobic oxalate and HCl extraction methods for any of the Fe fractions in the root samples ($P > 0.20$), indicating that anaerobic oxalate extractions accurately estimated poorly crystalline Fe under conditions prevailing at the end of the experiment. However, in the soils there were significant decreases in the proportion of total Fe extracted with HCl versus oxalate ($P < 0.01$), accompanied by an increase in the crystalline Fe fraction. These results indicate that the use of oxalate

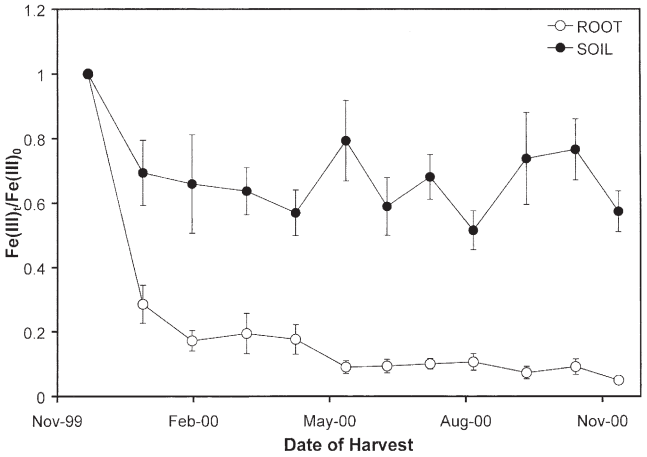

Fig. 4. Fe(III) reduction of root and soil Fe pools during anaerobic incubation. The Fe measured at each data point was converted to mg Fe and then multiplied by the percentage of Fe(III) at each harvest time to obtain the ratio of $Fe(III)_t$ to $Fe(III)_0$. Error bars represent ±1 standard error.

may have overestimated the amount of poorly crystalline Fe in the soils but not on the roots, largely due to the dominance of poorly crystalline in the plaque and crystalline Fe in the soil (Weiss et al., 2004). Because the sum of all Fe fractions was not significantly different using oxalate or HCl, the issue did not affect our estimates of Fe(III) reduction rates.

The percentage of Fe(II) in the Fe plaque changed significantly during the experiment, increasing from 18% in the initial samples to ≥40% after Month 6 ($P < 0.0001$, data not shown). However, the percentage of Fe(II) in the soil did not change and was consistently 20–30% of the total Fe. The total amount of Fe extracted with 1m*M* EDTA and BPDS was not significantly different from the total Fe recovered during the sequential extraction in the roots, but it was significantly lower by ≈25% in the soils. The trends in EDTA/BPDS-total Fe agreed well with the two other methods for quantifying total Fe; all methods showed that total Fe plaque decreased dramatically in the first 6 mo compared with much smaller losses in the soil Fe pool.

By multiplying the total amount of Fe in the root plaque and non-rhizosphere soil by the proportion of Fe(III) at each time point, the net reduction of the Fe(III) pool through the 12-mo study was calculated (Fig. 4):

$$Fe(III)_t = Fe(total)_t \times \%\ Fe(III)_t$$

where Fe(total) was determined with a DCB extraction, and %Fe(III) was determined with the dual chelation technique (see methods). Root plaque Fe(III) decreased 71% after 1 mo 83% after 2 mo, and 95% at the end of the 12-mo study. The soil Fe(III) pool decreased to 69% during Month 1 but changed little afterward. The peak rate of Fe(III) reduction of the plaque (i.e., during Month 1) was 0.6 mg Fe(III) g^{-1} root d^{-1} compared with ≤0.1 mg Fe(III) g^{-1} soil d^{-1}. The rates of Fe(III) reduction of the plaque and bulk soil Fe pool did not fit an exponential decay model because of very rapid Fe(III) reduction during Month 1, followed by much slower rates during the remainder of the study.

Microbial Analyses

Densities of FeOB and FeRB were highly variable over time, but there were some discernible patterns in the data. Rhizosphere FeOB density significantly decreased from an initial average of 3.5×10^6 FeOB g^{-1} root to 1.5×10^4 FeOB g^{-1} root by February and then to 2.7×10^2 FeOB g^{-1} root by August ($P = 0.05$) (Fig. 5). Iron(II)-oxidizing bacteria densities then appeared to begin to increase through November. Initial rhizosphere FeRB abundances (4.5×10^3 FeRB g^{-1} root) were significantly lower than FeOB and showed a moderate increase to 3.8×10^4 FeRB g^{-1} root after 1 mo. Abundances of FeRB generally remained at 10^3 and 10^4 for the duration of the experiment and only dramatically outnumbered the FeOB during August and September.

Similar to the trend observed in the rhizosphere samples, numbers of soil FeOB densities significantly decreased from the initial levels of 3.8×10^6 FeOB g^{-1} soil ($P < 0.0001$); however, there was considerable variability in subsequent months with densities vacillating between 10^4 and 10^5 FeOB g^{-1} soil. As with the roots, the lowest densities occurred in the summer months. Initial soil FeRB density was 6.2×10^4 FeRB g^{-1} soil and density generally remained between 10^3 and 10^4 FeRB g^{-1} soil, with no significant differences.

DISCUSSION

Previous studies have noted the presence and size of the rhizosphere Fe plaque pool in wetland soils, but very little is known about the dynamics of this pool. To our knowledge, the current study is the first to investigate in situ rates of iron deposition and reduction in the wetland rhizosphere. We observed substantial amounts of Fe deposition on the roots during the growing season, presumably driven by the release of O_2 into the root zone. Under anaerobic conditions > 70% of the Fe(III) in the plaque was reduced in the first month compared with <30% reduction of the soil Fe(III) pool. These results verify that previously reported differences in microbial Fe(III) reduction potential between rhizosphere and non-rhizosphere Fe(III) pools observed in laboratory studies (Weiss et al., 2004; Gribsholt et al., 2003) are realized in situ.

Net Iron Accumulation in the Rhizosphere

Rhizosphere Fe(II) oxidation is potentially rapid at circumneutral pH (Emerson and Weiss, 2004). A process-level understanding of the role of rhizosphere plaque as a sink for Fe and coprecipitated elements requires estimates of net Fe(III) accumulation rates. We estimated net total Fe accumulation rates of 0.13 and 0.37 Fe g^{-1} root d^{-1} in the root production and bare-root transplant studies, respectively. When adjusted for the average proportion of Fe(III) to total Fe in plaque (79%), the rate of Fe(III) accumulation for *J. effusus* was 0.3 mg Fe(III) g^{-1} root d^{-1}. This value is a minimum estimate of Fe(III) plaque deposition rates due to the possibility that plaque deposition was initially rapid then slower over time (as opposed to the constant rate of

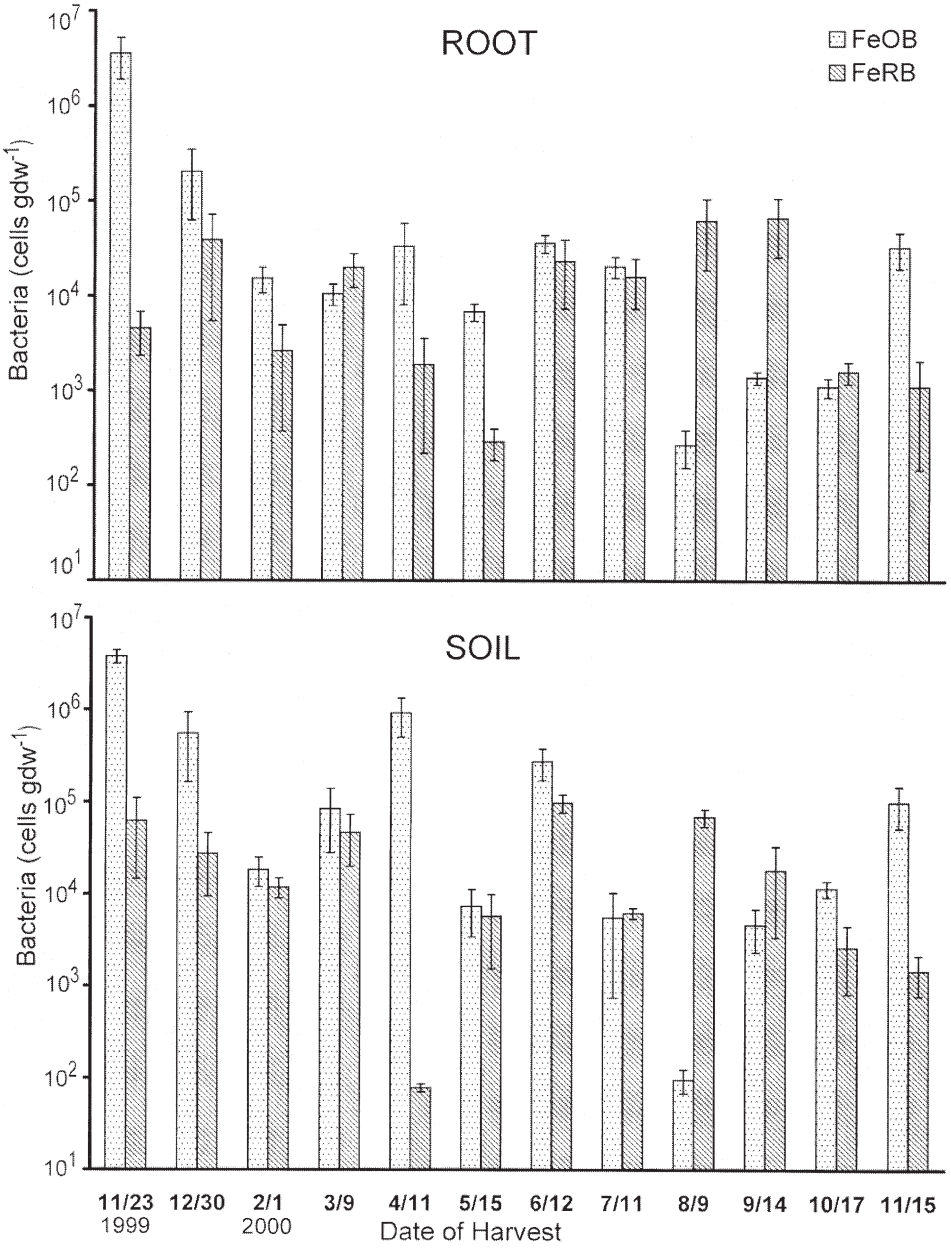

Fig. 5. Changes in Fe bacteria during anaerobic decomposition. All columns represent the average number of FeOB or FeRB of five bags collected at each harvest ±1 standard error.

deposition assumed in the calculations) as well as the potential for simultaneous Fe(III) reduction in the transplant and in-growth cores. Although we observed no significant difference in plaque accumulation rates between *J. effusus* and *P. cordata*, differences in radial oxygen loss between plant species are likely to influence plaque accumulation and reduction (see below). The fact that Fe(III) plaque accumulates in the rhizosphere of many wetland plant species (Weiss et al., 2003) is evidence that oxidation rates can outpace reduction rates (Begg et al., 1994).

Rhizosphere Fe(III) Reduction Rates

The peak Fe(III) reduction rate in the in situ experiments, 0.6 mg Fe(III) g^{-1} root d^{-1}, was slower than potential rates found in laboratory incubation studies. For example, a reduction rate of 3.4 mg Fe(III) g^{-1} root d^{-1} was reported in anaerobic laboratory incubations of unamended *Typha latifolia* roots (Weiss et al., 2004), and a rate of 8 to 30 mg Fe(III) g^{-1} root d^{-1} was reported for several freshwater macrophytes amended with exogenous Fe(III) (King and Garey, 1999). Judging from the shape of the decomposition curve, it is likely that peak rate of Fe(III) reduction in the present study was higher than the rate estimated by waiting 1 mo to retrieve the first bags. The initial burst of Fe(III) reduction may have occurred in a few hours, days, or weeks, despite low soil temperatures when the bags were deployed (<10°C). Low temperature may explain slower Fe(III) reduction rates observed in the present study than in laboratory

incubations (30°C). Future in situ studies should quantify initial Fe(III) reduction rates over intervals shorter than 30 d.

The higher reduction rates of the root plaque versus the soil Fe pool were likely due to some combination of enhanced availability of poorly crystalline, high surface area Fe(III) minerals, and labile organic C (Roden and Wetzel, 2002). Studies employing a wide range of techniques have reported that root plaque is dominated by amorphous Fe minerals, primarily ferrihydrite (Taylor et al., 1984; Batty et al., 2000; Hansel et al., 2001; Weiss et al., 2004). In contrast to the strongly crystalline Fe oxides that dominate bulk soils, amorphous Fe(III) oxides are subject to rapid and nearly complete dissolution (Thamdrup, 2000; Lovley, 2000). In a companion laboratory study using incubations, we reported the dissolution of >75% of the rhizosphere Fe(III) pool compared with <40% of the non-rhizosphere soil Fe(III) pool over 10 d, primarily due to dissolution of poorly crystalline Fe minerals (Weiss et al., 2004).

Because the field results shown here closely mirror our laboratory results (Weiss et al., 2004), we suspect that the difference in in situ Fe(III) reduction rates between root and soil Fe pools was also largely controlled by differences in iron mineral composition. The soil and root Fe pools decreased by nearly 30 and 70%, respectively, primarily in the first month of the study. This conclusion seems to conflict with the initial amounts of poorly crystalline Fe, which were similar in the soil and the roots (about 70% in both cases). We suspect the amount of poorly crystalline Fe in the soil pool was overestimated by the anaerobic oxalate procedure because it also extracted a portion of the strongly crystalline Fe pool (see the results section). This effect would not have occurred in extractions of the root Fe pool. This interpretation is also consistent with the results of a survey of several wetlands soils in which the average poorly crystalline Fe content was 23% (Weiss et al., 2004). If initial content of poorly crystalline soil Fe was indeed 68%, then the reduction of just 30% of the pool in 1 yr suggests the process was limited by a factor other than poorly crystalline Fe(III) availability.

The availability of labile C compounds is another key controller of in situ Fe(III) reduction rates (Roden and Wetzel, 2002; Thamdrup, 2000). The rapid decomposition of roots under anaerobic conditions in the initial months of the experiment indicated that a large pool of labile carbon was leached or consumed by indigenous microorganisms, leaving behind a more refractory C pool. These two distinct phases of organic matter decomposition are well known (Wieder and Lang, 1982) and have been observed in previous studies of wetland root decomposition (Day et al., 1989; Tupacz and Day, 1990). The heterogeneity in carbon degradability is mirrored by the Fe(III) oxide pool, with an initial phase of rapid dissolution of amorphous or 'labile' Fe(III) oxides followed by a slower reduction phase of more recalcitrant compounds. The simultaneous introduction of both labile organic carbon and Fe(III) oxides on root death undoubtedly contributed to higher Fe(III) reduction rate on the roots than the soil. The exhaustion of the root-derived labile C pool in the latter months of the experiment may have contributed to the persistence of poorly crystalline Fe in the root samples (about 10% of the initial HCl-extractable pool).

Rhizosphere FeRB and FeOB Abundance

Iron(II)-oxidizing bacteria and FeRB were previously shown to be abundant on the root surface of *Typha* spp. and associated no-rhizosphere soil (Weiss et al., 2003). Iron(II)-oxidizing bacteria and FeRB abundances were monitored in the buried-bag portion of the present study to assess whether the onset of anaerobic conditions and Fe(III) reduction coincided with changes in microbial community composition. Despite an overall decrease in FeOB abundances from 10^6 g^{-1} to 10^3 to 10^5 g^{-1} during the incubation period, there was no consistent trend in FeOB abundance after the first 3 mo of the study. This may be due to the fact that the MPN technique provides only a rough estimate of the number of organisms present and does not indicate their level of in situ activity. Although bulk redox information (obtained with the buried iron rods) indicate the bags remained anaerobic during the study, it is possible that the bags were exposed to short periods of oxygen through radial oxygen loss by nearby intact *Juncus* plants during the course of the experiment. However, it is likely that the remaining FeOB were able to tolerate anoxic conditions. The ability to survive without O_2 has been observed for FeOB in a mining environment (Wielinga et al., 1999) and for other groups of aerobes including methane-oxidizing bacteria (Gilbert and Frenzel, 1998) and ammonia-oxidizers (Bodelier et al., 1996). This adaptation may be a critical component of a rhizosphere Fe cycle, allowing FeOB to recover quickly when O_2 becomes available for consumption. Under these conditions, FeOB could accelerate the deposition of Fe in the rhizosphere (Neubauer et al., 2002), particularly when they occur at high densities (Weiss et al., 2003). In laboratory experiments, FeOB have been found to accelerate Fe(II) oxidation by about 20 to 90% (Neubauer et al., 2002; Sobolev and Roden, 2001).

The FeRB abundance of 10^3 to 10^4 cells g^{-1} were similar to those reported by Weiss et al. (2003) and Sobolev and Roden (2002). It is possible that initial FeRB abundance was affected by the aerobic processing of the samples. However, contrary to what we anticipated, a large increase in FeRB abundance was not observed during the experiment. Perhaps monthly samples were too infrequent to detect changes in root-associated FeRB, particularly those during the days following the start of the experiment when populations should have been the largest. Because the rhizosphere Fe(III) supply had already fallen by 70% after 1 mo when the first sample was taken, FeRB population growth may have already become limited by poorly crystalline Fe. However, concentrations of poorly crystalline Fe stabilized at 8 to 10 mg Fe g^{-1} at the end of the experiment, indicating that organic carbon may also be limiting Fe(III) reduction rates. Finally, it is possible that FeRB activity rather

FeRB population size responds to changes in environmental conditions.

Dynamics of in situ Iron Cycling

Although this study considered Fe(III) reduction on dead roots and net plaque deposition on living roots, the two processes may occur simultaneously on intact roots in situ. Seasonal variations in the pore water $Fe(II)_{aq}$ concentrations can provide insight on the relationship of Fe(II) oxidation and Fe(III) reduction with changes in plant activity during the growing season. The sharp decrease in pore water DO concentrations in February-March was followed by a large peak in $Fe(II)_{aq}$ in April (Fig. 1) and was probably due to an increase in plant and microbial O_2 demand associated with rising temperature (Megonigal et al., 1996; Sundby et al., 2003). The subsequent decline in $Fe(II)_{aq}$ coincided with development of the plant canopy and presumably increasing rates of radial O_2 loss, Fe(II) oxidation, and root plaque accumulation (Sundby et al., 2003). Decreasing rates of radial O_2 loss after peak biomass in June would have favored low rates of Fe(II) oxidation, increasingly reduced conditions, and a steady increase in $Fe(II)_{aq}$ concentrations. Although speculative and clearly influenced by the ecosystem type and dominant plant species, we interpret seasonal changes in pore water $Fe(II)_{aq}$ concentrations as an interplay between plants, microorganisms and environmental factors, some aspects of which were demonstrated in the present Fe(II) oxidation and Fe(III) reduction studies. There is a need for manipulative process-level studies that elucidate these interactions more clearly.

SUMMARY AND CONCLUSIONS

Using a novel buried-bag technique, this study is the first to demonstrate that Fe(III) plaque is rapidly reduced and solubilized under field conditions in the absence of radial O_2 loss. Reduction of the non-rhizosphere soil Fe(III) pool followed a similar pattern, but 30% of the soil Fe(III) pool was reduced in 1 mo versus about 70% of the rhizosphere pool. This difference between rhizosphere and non-rhizosphere Fe pools is consistent with the relative abundance of poorly crystalline Fe minerals, which are known to be more labile for FeRB than strongly crystalline minerals. Because losses of Fe(III) from the roots were accompanied by decay losses of root mass, it is likely that higher amounts of labile C in the roots versus the soil also contributed to the different rates of Fe(III) reduction. In addition to the rapid Fe(III) reduction rates of root plaque during this simulation of plant senescence, high rates of net Fe accumulation on roots were observed on intact plants during the growing season. Pore water $Fe(II)_{aq}$ data suggest that the balance between Fe(II) oxidation and Fe(III) reduction varies during the growing season, perhaps due to changes in plant activity.

ACKNOWLEDGMENTS

This work was funded by grants from the National Science Foundation (MCB-9723459 to DE and DEB-9985922 to PM and DE), the Jeffress Memorial Trust, and a NSF Dissertation Improvement Grant and American Association of University Women American Fellowship to JW. We gratefully acknowledge field assistance from Chris Bradburne, Scott Neubauer, Stephanie Backer, and Alex Karlsen.

REFERENCES

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

Armstrong, W., D. Cousins, J. Armstrong, D.W. Turner, and P.M. Beckett. 2000. Oxygen distribution in wetland plant roots and permeability barriers to gas-exchange with the rhizosphere: A microelectrode and modeling study with *Phragmites australis.* Ann. Bot. 86:687–703.

Azcue, J.M. 1996. Comparison of different cleaning procedures of root material for analysis of trace metals. Int. J. Environ. Anal. Chem. 62:137–147.

Batty, L.C., A.J.M. Baker, B.D. Wheeler, and C.D. Curtis. 2000. The effect of pH and plaque on the uptake of Cu and Mn in *Phragmites australis.* Ann. Bot. 86:647–653.

Bedford, B.L., D.R. Bouldin, and B.D. Beliveau. 1991. Net oxygen and carbon-dioxide balances in solutions bathing roots of wetland plants. J. Ecol. 79:943–959.

Begg, C.B.M., G.J.D. Kirk, A.F. Mackenzie, and H.U. Neue. 1994. Root-induced iron oxidation and pH changes in the lowland rice rhizosphere. New Phytol. 128:469–477.

Bodelier, P.L.E., J.A. Libochant, C.W.P.M. Blom, and H.J. Laanbroek. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Appl. Environ. Microbiol. 62:4100–4107.

Day, F.P., J.P. Megonigal, and L.C. Lee. 1989. Cypress root decomposition in experimental wetland mesocosms. Wetlands 9:263–282.

Eaton, A.D., and M.A.H. Franson. (ed.) 1995. Multiple-Tube Fermentation Technique for Members of the Coliform Group. p. 9–45–9–51. *In* Standard methods for the examination of Water and Wastewater American Public Health Association, Washington, DC.

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

Emerson, D., and J.V. Weiss. 2004. Bacterial iron oxidation in circumneutral freshwater habitats: Findings from the field and the laboratory. Geomicrobiol. J. 21:405–414.

Gilbert, B., and P. Frenzel. 1998. Rice roots and CH_4 oxidation: The activity of bacteria, their distribution and the microenvironment. Soil Biol. Biochem. 30:1903–1916.

Gribsholt, B., J.E. Kostka, and E. Kristensen. 2003. Impact of fiddler crabs and plant roots on sediment biogeochemistry in a Georgia salt marsh. Mar. Ecol. Prog. Ser. 259:237–251.

Hansel, C.M., S. Fendorf, S. Sutton, and M. Newville. 2001. Characterization of Fe plaque and associated metals on the roots of mine-waste impacted aquatic plants. Environ. Sci. Technol. 35:3863–3868.

Hesslein, R.H. 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21:912–914.

King, G.M., and M.A. Garey. 1999. Ferric iron reduction by bacteria associated with the roots of freshwater and marine macrophytes. Appl. Environ. Microbiol. 65:4393–4398.

Kostka, J.E., B. Gribsholt, E. Petrie, D. Dalton, H. Skelton, and E. Kristensen. 2002. The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments. Limnol. Oceanogr. 47:230–240.

Kuehn, K.A., and K. Suberkropp. 1998. Decomposition of standing litter of the freshwater emergent macrophyte *Juncus effusus.* Freshwater Biol. 40:717–727.

Lovley, D.R. 2000. Fe(III) and Mn(IV) reduction. p. 3–30. *In* D.R Lovley (ed.) Environmental microbe-metal interactions. ASM Press, Washington, DC.

Lovley, D.R., and E.J.P. Phillips. 1986. Organic-matter mineralization with reduction of ferric iron in anaerobic sediments. Appl. Environ. Microbiol. 51:683–689.

Megonigal, J.P., S.P. Faulkner, and W.H. Patrick. 1996. The microbial activity season in southeastern hydric soils. Soil Sci. Soc. Am. J. 60:1263–1266.

Mendelssohn, I.A., B.A. Kleiss, and J.S. Wakeley. 1995. Factors controlling the formation of oxidized root channels—A review. Wetlands 15:37–46.
Neubauer, S.C., D. Emerson, and J.P. Megonigal. 2002. Life at the energetic edge: Kinetics of circumneutral Fe oxidation by lithotrophic iron oxidizing bacteria isolated from the wetland plant rhizosphere. Appl. Environ. Microbiol. 68:3988–3995.
Phillips, E.J.P., D.R. Lovley, and E.E. Roden. 1993. Composition of non-microbially reducible Fe(III) in aquatic sediments. Appl. Environ. Microbiol. 59:2727–2729.
Ratering, S., and S. Schnell. 2000. Localization of iron-reducing activity in paddy soil by profile studies. Biogeochemistry 48:341–365.
Roden, E.E., and R.G. Wetzel. 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.
Roden, E.E., and R.G. Wetzel. 2002. Kinetics of microbial Fe(III) oxide reduction in freshwater wetland sediments. Limnol. Oceanogr. 47:198–211.
SAS Institute. 2002. JMP(R) Introductory Guide, Version 5. The SAS Institute Inc. Cary, NC.
Sobolev, D., and E.E. Roden. 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., and E.E. Roden. 2002. Evidence for rapid microscale bacterial redox cycling of iron in circumneutral environments. Anton. van Leeuw. 81:587–597.
Stookey, L.L. 1970. Ferrozine: A new spectrophotometric reagent for iron. Anal. Chem. 42:779–781.
Sundby, B., C. Vale, M. Caetano, and G.W. Luther. 2003. Redox chemistry in the root zone of a salt marsh sediment in the Tagus Estuary, Portugal. Aquat. Geochem. 9:257–271.
Taylor, G.J., A.A. Crowder, and R. Rodden. 1984. Formation and morphology of an iron plaque on the roots of *Typha latifolia* grown in solution culture. Am. J. Bot. 71:666–675.
Thamdrup, B. 2000. Bacterial manganese and iron reduction in aquatic sediments. Adv. Microb. Ecology 16:41–84.
Tupacz, E.G., and F.P. Day. 1990. Decomposition of roots in a seasonally flooded swamp ecosystem. Aquat. Bot. 37:199–214.
Wang, T.G., and J.H. Peverly. 1999. Iron oxidation states on root surfaces of a wetland plant (*Phragmites australis*). Soil Sci. Soc. Am. J. 63:247–252.
Weiss, J.V., J.P. Megonigal, D. Emerson, and S.M. Backer. 2003. Enumeration of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the root-zone of wetland plants: Implications for a rhizosphere Fe cycle. Biogeochemistry 64:77–96.
Weiss, J.V., D. Emerson, and J.P. Megonigal. 2004. Geochemical control of microbial Fe(III) reduction potential in wetlands: Comparison of the rhizosphere to non-rhizosphere soil. FEMS Microbiol. Ecol. 48:89–100.
Wieder, R.K., and G.E. Lang. 1982. A critique of the analytical methods used in examining decomposition data from litter bags. Ecology 63:1636–1642.
Wieder, R.K., and G.E. Lang. 1986. Fe, Al, Mn, and S chemistry of *Sphagnum* peat in 4 peatlands with different metal and sulfur input. Water Air Soil Pollut. 29:309–320.
Wielinga, B., J.K. Lucy, J.N. Moore, O.F. Seastone, and J.E. Gannon. 1999. Microbiological and geochemical characterization of fluvially deposited sulfidic mine tailings. Appl. Environ. Microbiol. 65:1548–1555.