The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments

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Abstract

   This study was carried out to quantify the effects of higher organisms, invertebrate macrofauna, and macrophyte plants on the rates and pathways of microbial respiration coupled to organic matter oxidation in saltmarsh sediments. Sediment geochemistry, rates of microbial metabolism, and the abundance of anaerobic respiratory bacteria were determined at sites differing in the abundance of fiddler crab (*Uca pugnax*) burrows and vegetation (*Spartina alterniflora*) coverage. Solid-phase Fe(III) concentrations were 50 to 100 times higher, and solid sulfide concentrations were eight times lower in bioturbated, vegetated sediments (BVL) as compared to nonbioturbated, unvegetated (NUC) sediments. Integrated sulfate reduction rates were 10 times lower in BVL (2 mmol m^{-2} d^{-2}) as compared to NUC sediments (20 mmol m^{-2} d^{-1}). Directly measured Fe(III) reduction rates were high at the BVL site, whereas no Fe(III) reduction was detected at NUC or in killed sediment treatments. Molybdate, a specific inhibitor of sulfate reduction, inhibited 70% of carbon oxidation when added to NUC sediment but showed no effect on Fe(III) reduction or C oxidation in BVL sediments. Counts of Fe(III)-reducing bacteria (FeRB) were two orders of magnitude higher in BVL sediments (10^7 cells g^{-1}) in comparison to NUC sediments (10^5 cells g^{-1}). Fe(III) respiration comprised up to 100% of carbon oxidation in BVL sediments, whereas sulfate reduction was the dominant respiration process ($\geq$70% of C oxidation) at NUC. We provide strong evidence to show that macroorganisms stimulate FeRB to outcompete sulfate-reducing bacteria in saltmarsh sediments by supplying an abundance of reactive Fe(III) through reoxidation processes.

Salt marshes are among the most productive ecosystems on earth (Alongi 1998). These wetlands are vital components of coastal marine ecosystems, because they provide nursery grounds for commercially important fish and shellfish species, limit nutrient exchange at the land–sea boundary, and protect coastal municipalities from catastrophic storms (Pomeroy and Wiegert 1981).

Along the east coast of the U.S., primary production in salt marshes is dominated by the macrophyte smooth cordgrass, or *Spartina alterniflora* (Pomeroy and Wiegert 1981). Twice the production of this grass occurs belowground as roots and rhizomes as is produced aboveground as shoots

and leaves (Schubauer and Hopkinson 1984). The roots and shoots of *Spartina* are an important conduit for chemical exchange between the sediments and tidal waters or the atmosphere (Dacey and Howes 1984; Morris and Whiting 1985). For example, studies have shown that the roots of *Spartina* may be a primary source of organic matter (Schubauer and Hopkinson 1984) to the sediment. In addition, *Spartina* roots may inject O$_2$ into subsurface sediment via evapotranspiration (Dacey and Howes 1984; Morris and Whiting 1985) or passive diffusion.

Fiddler crabs (*Uca* spp.) are the most abundant macroinvertebrates observed in the majority of salt marshes along the east coast of the U.S., and studies have linked the activities of *Uca* to nutrient cycling and primary productivity in salt marshes (Bertness 1985). However, previous studies have focused on the ecology of *Uca,* and few if any researchers have investigated the quantitative effects of macrofauna on microbial respiration reactions, which mediate nutrient remineralization and release in saltmarsh sediments. Fiddler crab burrows generally extend from 5 to 25 cm deep and are commonly observed at densities of 224–480 burrows m^{-2} in saltmarsh sediments (Bertness 1985). Fiddler crabs are burrowing deposit-feeders that have been shown to affect sediment biogeochemistry in two general ways. First, the excavation of semipermanent burrows leads to in-

creased sediment surface area and drainage, a higher sediment redox potential, or both (Bertness 1985). Second, through their deposit-feeding activities, fiddler crabs continually rework the top 2–3 cm of marsh sediment (Bertness 1985).

Saltmarsh sediments are rich in organic matter and relatively impermeable clay minerals. Therefore, oxygen is utilized rapidly in surface sediments (in the top few millimeters; King 1988), and most organic matter remineralization occurs under anoxic conditions (Howarth 1993; Alongi 1998). Because of the abundance of sulfate in seawater, microbial respiration coupled to the terminal decomposition of organic matter in saltmarsh sediments is thought to be dominated by sulfate reduction (Howarth 1993; Alongi 1998). Microbial respiration reactions represent a transfer of carbon and nutrients between the organic and inorganic reservoirs (Jahnke and Craven 1995), and an understanding of this transfer is vital to the modeling of biogeochemical dynamics in estuaries.

Sulfate reduction rates have been measured in salt marshes on the east coast of the U.S. since the late 1970s (Howarth and Giblin 1983; King 1988; Hines et al. 1989; *see* review by Howarth 1993). Few studies, however, have explored spatial variability such as that caused by vegetation coverage within the marsh (King 1988; Hines et al. 1989; Kostka et al. 2001), and to our knowledge, no studies have incorporated an extensive comparison of adjacent bioturbated versus nonbioturbated sites within the marsh. Salt marshes are exposed to a large number of physical, biological, and chemical forcings, resulting in complex biogeochemical cycles. This complexity has clouded the interpretation of mechanisms by which microorganisms interact with surrounding higher organisms (plants, macrofauna) to control predominating diagenetic reactions.

Several studies have now quantified the contribution of microbial Fe(III) reduction to C oxidation in a range of subtidal marine sediments (*see* reviews by Thamdrup 2000; Thamdrup and Canfield 2000). Previous biogeochemical studies have suggested that microbial Fe(III) reduction may also contribute to carbon cycling in intertidal saltmarsh sediments, and Fe(III)-reducing bacteria have been detected in abundance in these environments (Jacobson 1994; Kostka and Luther 1995; Lowe et al. 2000). However, rates of Fe(III) reduction have not been measured in saltmarsh sediments; therefore, the importance of Fe(III) reduction to carbon oxidation in saltmarsh sediments continues to be questioned (Howarth 1993; Kostka and Luther 1995; Alongi 1998).

This study was undertaken to constrain the effects of higher organisms on the rates and pathways of microbial respiration coupled to organic matter oxidation in saltmarsh sediments. Because sulfate and Fe(III) minerals are the most abundant electron acceptors available for microbial respiration in saltmarsh sediments, we focused on sulfate reduction and Fe(III) reduction processes. We hypothesized that bioturbation by macrofauna (*Uca*) and processes mediated by the roots of *Spartina* should be important factors limiting microbial respiration in saltmarsh sediments. Hence, a sampling scheme was designed to include contrasting sites in the marsh that vary according to abundance of crab burrows and the density of *Spartina.*

Materials and methods

Sampling area—This study was conducted at the Saltmarsh Ecosystem Research Facility (SERF) adjacent to the Skidaway Institute of Oceanography on Skidaway Island in Savannah, Georgia, during August 2000. At SERF, the marsh is dominated by a nearly monospecific stand of smooth cordgrass *Spartina alterniflora,* and the mud fiddler crab *Uca pugnax* was observed in abundance throughout the marsh. We will henceforth refer to burrow excavation and deposit-feeding collectively as ''bioturbation,'' which we loosely define as sediment mixing by fiddler crabs. Though we recognize that other members of the infauna/epifauna may be involved in bioturbation, our study centered on the effects of *Uca.*

Sediments were sampled at three sites, which differed in the abundance of fiddler crab (*Uca*) burrows and vegetation (*Spartina*) coverage. These sites will henceforth be referred to as the nonbioturbated, unvegetated creek bank (NUC); the bioturbated, unvegetated creek bank (BUC); and the bioturbated, vegetated levee (BVL). BVL and NUC sites were located within 100 m of each other in the same salt marsh and were sampled at similar elevations above mean low water. The BUC site, which was bioturbated by *Uca* but contained no visibly higher plants, was sampled within a few meters of NUC on the same creek bank. *Uca* burrows exhibited nearly equal abundances of 330.7 ± 95.8 m^{-2} and 273.7 ± 70.5 m^{-2} at BVL and BUC sites, respectively. At BVL, the sediments were further inhabited by the tall form of *Spartina* (mean height $= 138 \pm 17$ cm) to average densities of 84.9 ± 33.7 shoots m^{-2}.

Ecological measurements—*Uca* burrows were counted inside randomly placed quadrats of 25 by 25 cm (Bertness 1985). Using the same quadrats, the density and height of tall *Spartina* was measured in random portions of the marsh. The height of *Spartina* was determined as the distance from the sediment surface to the tip of each shoot.

Sediment handling and pore-water extraction—For geochemical analyses, sediments were sampled by driving a polycarbonate core liner (6 cm i.d.) into the salt marsh, and cores were immediately sealed with butyl rubber stoppers. Within 1 h of sampling, cores were transferred to a nitrogen-filled glove bag, where the sediment was sectioned into 1- to 6-cm depth intervals. Triplicate cores were sampled at each site, and cores were only accepted when the surface appeared to be unaffected by disruption during coring. The sediment was loaded into polypropylene centrifuge tubes in a N$_2$-filled glove bag. The tubes were tightly-capped and centrifuged for 10–20 min. at $5,000 \times g$. After reintroduction into the glove bag, pore waters were sampled and filtered through 0.2-μm cellulose acetate syringe filters. Sediments for solid-phase analysis were frozen under nitrogen for later use.

Rate measurements—For determination of carbon mineralization rates and pathways, sediment from 0 to 6 cm deep was sampled using a trowel into a polypropylene bucket that was immediately sealed with no headspace under aseptic conditions. Within 1 h of sampling, sediment was homogenized, treated where appropriate, and loaded into 50-ml centrifuge tubes in a N_2-filled glove bag. The tubes were placed within larger N_2-filled bags to further maintain anoxic conditions and were incubated at in situ temperature (30°C) in the dark. The tubes were then sampled at regular intervals, and the pore waters were extracted by centrifugation/filtration as described above. Buffered formaldehyde (pH 7) and sodium molybdate were added to selected treatments to final concentrations of 3% (v/v) and 20 mM, respectively. For sterile treatments, sediment was sealed anoxically into bottles and autoclaved on three consecutive days for 2 h at 121°C prior to incubation.

Sulfate reduction rates were determined in triplicate on intact 10-cm-long cores (2 cm i.d.) with $^{35}SO_4^{2-}$ (Jørgensen 1978) at in situ temperature. At termination, the sediment was fixed in 20% Zn acetate and frozen. The reduced ^{35}S was recovered by distillation with boiling acidic Cr^{2+} solution according to Fossing and Jørgensen (1989).

Pore-water and solid-phase geochemistry—Pore water for the determination of ΣCO_2 and NH_4^+ analyses was filtered into 1.8-ml glass vials that were capped with Teflon-coated butyl rubber septa, leaving no gas phase and maintaining anoxia. The samples were stored at 0°C and analyzed within a few days of sampling by flow injection with conductivity detection (Hall and Aller 1992; SD = 2% for both ΣCO_2 and NH_4^+), as modified for hydrogen sulfide interference according to Lustwerk and Burdige (1995).

Dissolved Fe^{2+} was determined immediately after filtration by colorimetry with a ferrozine solution (detection limit = 1 μM; SD = 2%; Stookey 1970). Dissolved sulfide was determined in pore waters fixed in Zn using the methylene blue method (detection limit = 1 μM, SD = 5%; Cline 1969). Sulfate and chloride concentrations were measured in acidified pore water using ion chromatography. Sediment pH was determined with a glass electrode, calibrated with NBS standards, that was inserted directly into the sediment or in filtered pore water.

Wet chemical extractions were used to determine the poorly crystalline Fe oxide minerals (Kostka and Luther 1994). Iron was extracted in 0.5 M HCl for 1 h (Kostka and Luther 1994). The oxidation state of Fe in the extract was further determined by analysis in (1) ferrozine buffer (50 mM HEPES, 0.1% ferrozine, pH 7) and (2) ferrozine buffer + 1% (wt/v) hydroxylamine hydrochloride (pH 7). Iron determined in the HCl extract with hydroxylamine addition is operationally defined as the total HCl-extractable fraction (Fe(II) + Fe(III)), whereas Fe in the HCl extract without hydroxylamine addition is defined as the HCl-extractable Fe(II). Solid Fe(III) was determined by difference between these two fractions. Calibration experiments with pure Fe phases have confirmed the selectivity of this extraction toward poorly crystalline Fe phases (Kostka and Luther 1994).

Total reduced sulfur (TRS), which included both acid-volatile sulfide (AVS = FeS + H_2S) and chromium-reducible sulfur (CRS = S^0 + FeS_2), was determined after single-step distillation with cold 2 M HCl and boiling 0.5 M Cr^{2+} solution (Fossing and Jørgensen 1989). Sediment fixed with Zn acetate from the SO_4^{2-} reduction measurements was used for sulfur determinations.

Abundance of respiratory bacteria—Iron(III)-reducing bacteria (FeRB) and sulfate-reducing bacteria (SRB) populations were enumerated by the three-tube most probable number (MPN) assay using 10-fold serial dilutions of sediment in growth medium. A carbonate-buffered, minimal medium that resembles seawater was prepared and dispensed into Hungate tubes per Widdel and Bak (1992). For the SRB, sulfate was added as the sole electron acceptor, and sulfide was added as a reductant to the medium (Widdel and Bak 1992). For the FeRB, Fe(III) oxyhydroxide or ferrihydrite ($Fe(OH)_3$; Lovley and Phillips 1988) was added as the electron acceptor, and $FeCl_2$ was added as a mild reductant. Carbon substrates (lactate + acetate) were added anoxically from sterile stocks to 10 mM final concentration. Prior to sealing with a butyl rubber stopper, MPN tubes were gassed with a 90% N_2/10% CO_2 gas mixture. The tubes were incubated at in situ temperature (30°C) for 3 months in the dark and analyzed at least two times for reduction activity. Reduction activity was scored by measuring the respiration products, dissolved sulfide (Cline 1969), or Fe(II) (Lovley and Phillips 1988) in comparison to autoclaved controls. The MPN index was determined from statistical tables published by the American Public Health Association (1969).

Results

Pore water chemistry—At the NUC site, sulfate concentrations decreased substantially (by 10 mM) and dissolved sulfide accumulated to millimolar levels below 5 cm depth (Fig. 1A,B). In contrast, no sulfate depletion or sulfide accumulation was observed at the BVL site. A large subsurface maximum in pore-water Fe was observed at the BVL below 4 cm depth, indicative of Fe reduction, whereas a much smaller maximum occurred in the top 2 cm at NUC (Fig. 1C). Pore-water pH was substantially lower (by $\geq$ 1 pH unit) at the BVL as compared to NUC and showed a middepth maximum at both sites between 4 and 6 cm (Fig. 1D). The distribution of mineralization products, ΣCO_2 and NH_4^+, was drastically different between sites. At NUC, mineralization products exhibited parallel distributions and the curvature of the profiles was indicative of accumulation from rapid organic matter decomposition (Fig. 1E,F). In comparison, little or no ΣCO_2 and NH_4^+ accumulated, and no depth gradient was observed in the pore waters at the heavily bioturbated BVL (Fig. 1E,F). Chloride concentrations ranged from 0.5 to 0.6 M, were not significantly different between sites, and showed no change with sediment depth (data not shown). The molar ratio of sulfate : chloride (calculated from values in Fig. 1A) was similar to that of seawater (0.047 to 0.057) and did not change with sediment depth at the BVL. At NUC, the molar ratio was similar to that at the BVL, with the exception that the ratio decreased to $\leq$0.04 below 5 cm depth, indicative of sulfate depletion from sulfate reduction.

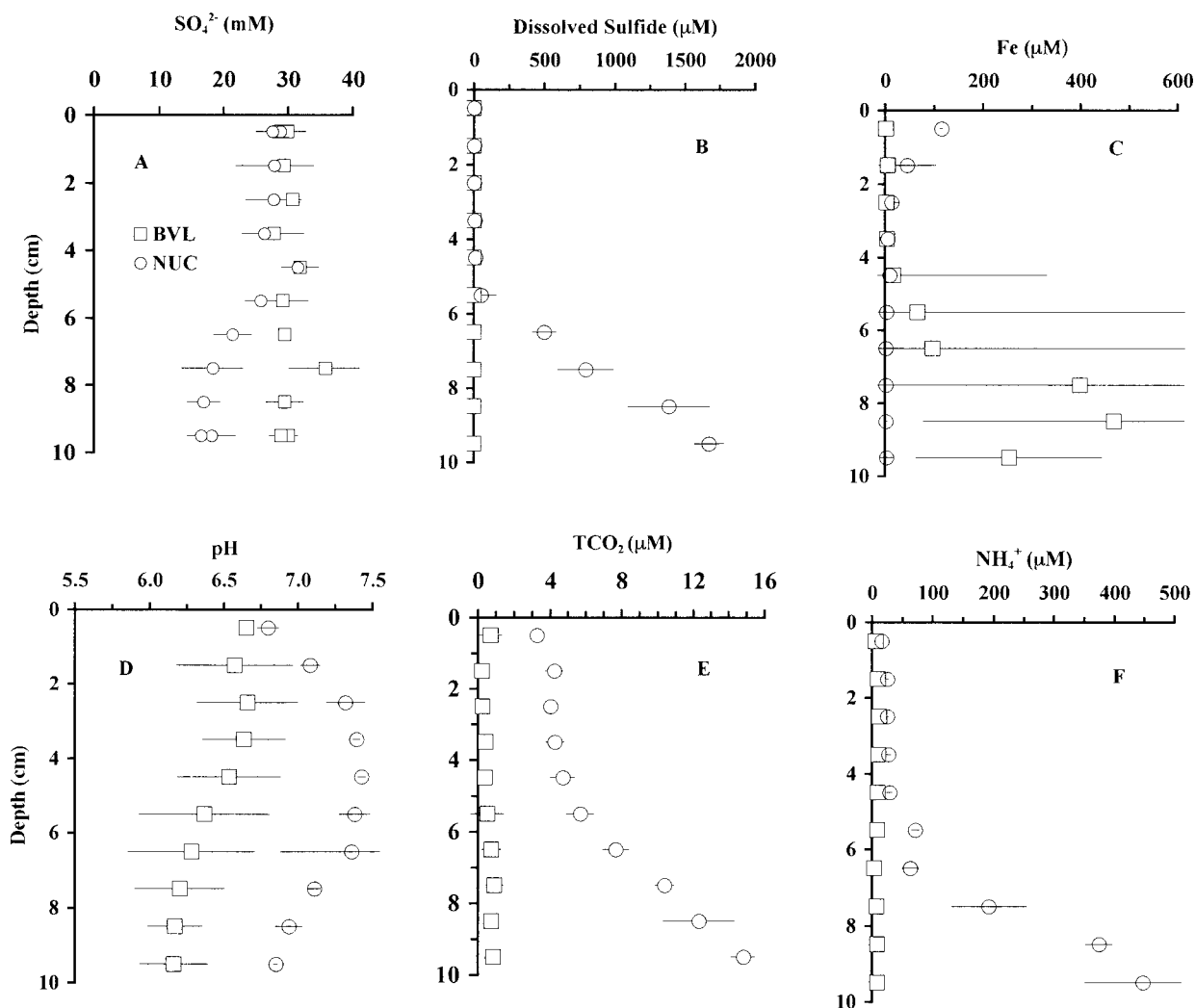A summary comparison of the sediment geochemistry ob-

Fig. 1. Vertical profiles of pore-water constituents at the nonbioturbated, unvegetated creek bank (NUC) and bioturbated, vegetated levee (BVL) sites. Symbols and error bars represent the mean $\pm$ 1 SD from triplicate cores, respectively.

Table 1. Summary of pore-water and solid-phase geochemistry data collected from all sites sampled.* Values are averages for the 0–6-cm depth interval.

	NUC	BUC	BVL
Pore water			
SO_4^{2-} (mM)	30.3	30.9	33.2
H_2S (μM)	14.7	—	0.8
Fe (μM)	12.4	—	12.5
pH	7.2	7.0	6.5
ΣCO_2 (mM)	4.7	1.7	0.5
NH_4^+ (μM)	32.6	—	8.6
Solid phase			
Reactive Fe(III) (μmol cm^{-3})	ND†	16.1	123.6
TRS (umol cm^{-3})	148.8	116.1	13.9

* BUC, bioturbated, unvegetated creek bank; BVL, bioturbated, vegetated levee; NUC, nonbioturbated, unvegetated creek bank; TRS, total reduced sulfur.
† ND, none detected.

served at the three sites sampled is provided in Table 1. This table reveals that the BUC showed a pore-water and solid-phase chemistry that was intermediate between that of the NUC and BVL sites. The geochemistry of the BUC site more closely resembled that of the NUC than that of the BVL. For example, as at NUC, pore-water pH was close to neutral, and the average solid sulfide concentration was high (> 100 μmol cm^{-3}) at BUC.

Solid phase distributions—Sediments sampled in the present study were clay-rich silts. Sediment porosity and wet density ranged from 0.8 to 0.9 and from 1.1 to 1.2 g cm^{-3}, respectively. Organic carbon concentrations (as loss on ignition) averaged 15 ± 3% at all sites.

Wet chemical extractions were used to determine the poorly crystalline Fe(III) oxide pool because these compounds are thought to be available for bacterial respiration (Kostka and Nealson 1998; Lovley and Phillips 1988). The total amount of poorly crystalline Fe oxide (hereafter referred to as solid Fe(III)) extracted at the BVL was on the high end
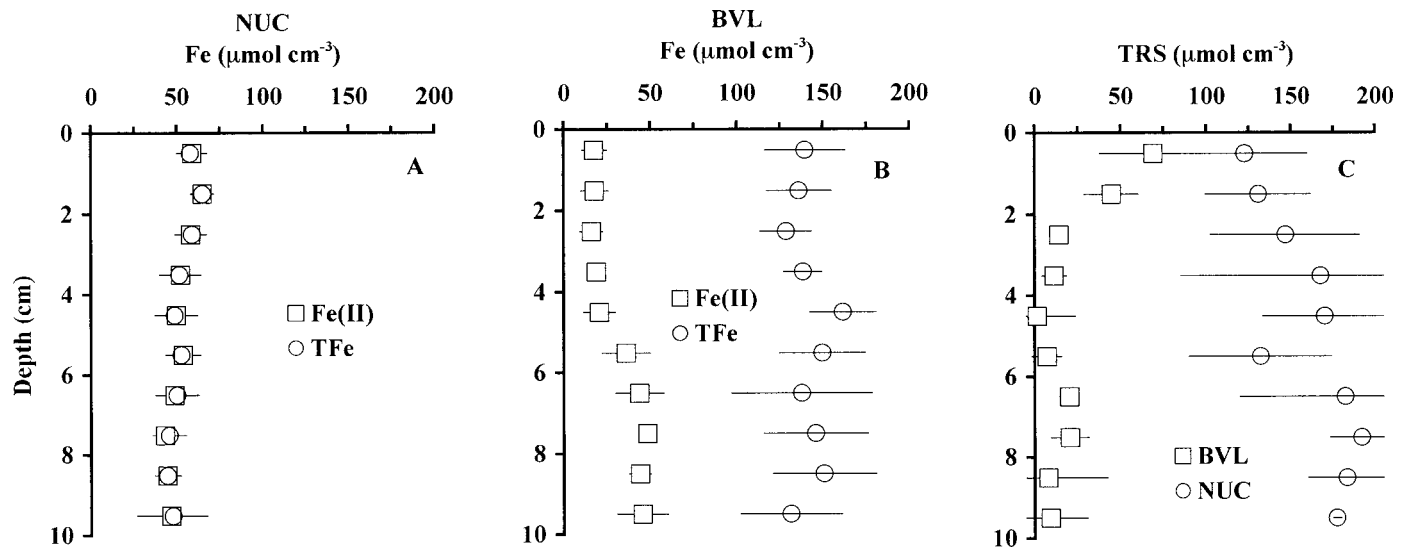
Fig. 2.   Distribution of solid-phase Fe and S. (A) Speciation of solid Fe at NUC. (B) Speciation of solid Fe at BVL. (C) Total reduced sulfur (TRS). Symbols and error bars represent the mean ± 1 SD from triplicate cores, respectively.

of the range observed in temperate marine sediments (*see* review by Thamdrup 2000) indicating that a large amount of reactive Fe(III) was available for respiration. At the BVL, total Fe concentrations averaged 125 to 150 μmol cm^{-3}, and the majority of this (100–125 μmol cm^{-3}) was found to be oxidized (Fig. 2B). In contrast, total Fe averaged 50–60 μmol cm^{-3} at NUC where no extractable Fe(III) was detected (Fig. 2A). A small gradient in solid Fe(II), consistent with the corresponding vertical pore-water Fe gradient (Fig. 1C) indicative of Fe(III) reduction, was observed at the BVL from 4 to 6 cm depth (Fig. 2B).

Profiles of total reduced sulfur (TRS = H$_2$S + S^0 + FeS + FeS$_2$) showed the mirror image of each other at the two sites sampled. TRS concentration decreased with sediment depth at the BVL and increased with depth at NUC (Fig. 2C). Upon integration of TRS concentration to 10 cm depth, we calculate an inventory that is nearly eight times higher at NUC (16.1 mol m^{-2}) relative to the BVL (2.0 mol m^{-2}).

Rate measurements—Intact cores revealed dramatic differences in sulfate reduction rates (SRR) between sites. Depth-integrated rates were 10 times higher at NUC (20.05 mmol m^{-2} d^{-1}) as compared to the BVL (2.25 mmol m^{-2} d^{-1}; Fig. 3). Rate profiles further matched with the distributions of TRS with sediment depth, showing that more sulfide accumulated where sulfate reduction rates were higher.

In order to further constrain the pathways of C oxidation, we measured the accumulation of ΣCO$_2$, sulfate depletion, and Fe(III) reduction rates in a variety of treatments of whole saltmarsh sediment collected from the 0–6-cm depth interval at each site and incubated for 1 week. In general, rates in sediment incubations were highly linear showing two distinct phases with time. At each site, rates were two to six times higher in the first 35 h of incubation in comparison to rates determined from 35 to 163 h of incubation (Figs. 4–6; Table 2).

Rates of organic carbon oxidation determined from the accumulation of ΣCO$_2$ were similar at the two sites. However, upon addition of molybdate, a specific metabolic inhibitor of sulfate reduction, C oxidation rates decreased by 75% in incubations of NUC sediments, whereas no change was observed in incubations of BVL sediments (Fig. 4A,C). Microorganisms appeared to be killed in autoclaved sediments because little or no ΣCO$_2$ accumulated over 5 d (Fig. 4B, D). Although the addition of formalin appeared to alter the carbonate chemistry of sediment pore waters, microbial activity also appeared to cease in formalin treatments because no substantial increase in ΣCO$_2$ was observed over 7 d of incubation (Fig. 4B,D).
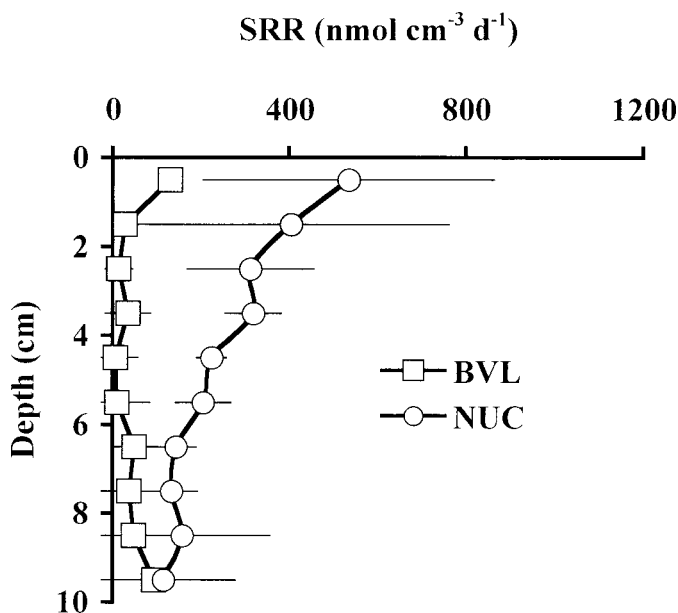

Fig. 3.   Depth profiles of sulfate reduction rates (SRRs) measured in triplicate intact cores. Symbols and error bars represent the mean ± 1 SD from triplicate cores, respectively.

Table 2. Summary comparison of rate measurements made in whole sediment incubations. Rates were integrated over the 0–6-cm depth interval (mmol m^{-2} d^{-1}). In order to minimize the influence of homogenization, the 36–163-h interval was used to calculate rates.

	NUC		BVL	
	Rate	Stoichiometry of respiration†	Rate	Stoichiometry of respiration
Unamended				
SRR, intact	20.0	0.41		
SRR, incubated	33.4	0.68	1.3	0.02
C oxidation	48.9		79.7	
Fe(III) reduction	ND‡		346.3	4.3
+Molybdate				
SRR	ND		ND	
C oxidation	22.3		115.9	
Fe(III) reduction	ND		522.3	4.5

* BVL, bioturbated, vegetated levee; NUC, nonbioturbated, unvegetated creek bank; SRR, sulfate reduction rates.
† Stoichiometry of electron acceptor respired divided by the C oxidized.
‡ ND, no respiration activity detected.

Fe(III) reduction rates were directly measured as the increase or accumulation of extractable Fe(II) with time in sediment incubations. No Fe(III) reduction was detected in incubations of NUC sediments because solid Fe(II) and total Fe concentration did not change with time in any of the treatments (Fig. 5A–D). In contrasting sediments from the heavily bioturbated BVL site, Fe(III) reduction rates were rapid and biphasic (Fig. 6A,B), showing similar trends to those of ΣCO_2 accumulation. Fe(III) reduction rates were similar or slightly stimulated in treatments where sulfate reduction was inhibited by molybdate addition (Fig. 6B). In sediments where microorganisms were killed by formalin addition or autoclaving, no Fe(III) reduction was observed, indicating that this process was biologically mediated in BVL sediments (Fig. 6C,D).

As expected, no change in pore-water sulfate concentration was observed with time in molybdate treatments or in killed sediments (data not shown).

Abundance of respiratory bacteria—MPN determinations of Fe(III)-reducing bacteria (FeRB) in the 0–6-cm depth interval were two orders of magnitude higher at the BVL (10^7 cells cm^{-3} wet sediment) than at the NUC site (10^5 cells cm^{-3}) (Table 3). In contrast, MPN counts of SRB were similar at each site (10^5 cells cm^{-3}; Table 3).

Discussion

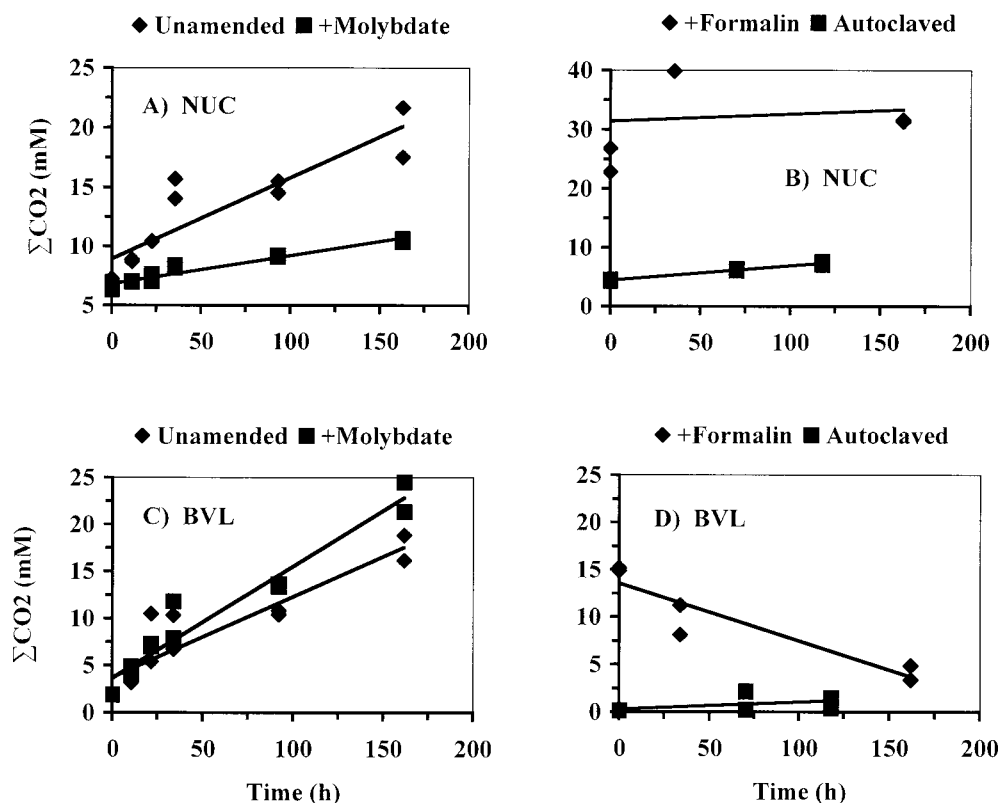Using a comprehensive approach, we provide strong evidence to show that Fe(III) reduction is the dominant micro-

Fig. 4. Carbon oxidation rates measured as the accumulation of ΣCO_2 over time in sediment incubations. Symbols represent duplicate incubations.
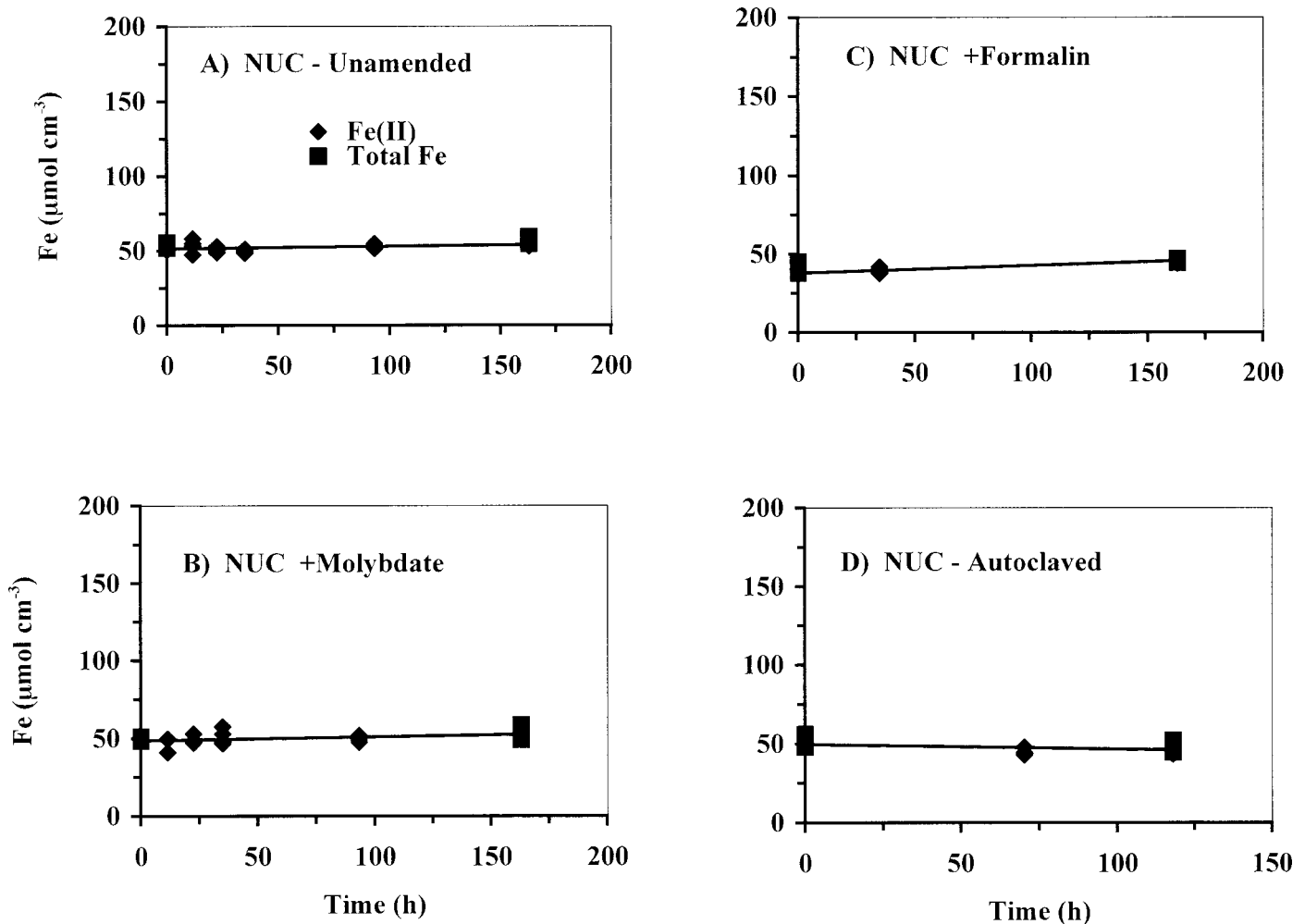
Fig. 5.   Fe(III) reduction rates measured as the accumulation of extractable Fe(II) in incubations of NUC sediments. Symbols represent duplicate extractions from duplicate incubations. Sediments are the same as those represented in Fig. 4A,B.

bial respiration process coupled to C oxidation in bioturbated/vegetated saltmarsh sediments, whereas sulfate reduction predominates in sediments not affected by macrofauna or macrophytes. All geochemical parameters, rate measurements, and bacterial count data together support these conclusions.

In this study, we reported densities of *Uca* burrows and *Spartina* plants at the BVL site that match the middle of the range reported above for salt marshes, indicating that the SERF marsh environment should be substantially but not inordinately influenced by macroorganismal activities. Even at these average densities of higher organisms, we have observed drastic effects on sediment biogeochemistry in comparison to adjacent environments less affected by such higher organisms. Our studies have focused on biogeochemistry driven by microbial respiration reactions coupled to organic matter mineralization.

Effect of macroorganisms on carbon oxidation pathways—Many previous studies have shown that macrofauna and macrophytes substantially affect the geochemical cycles of Fe and S in marine sediments (Aller 1980; Hines 1991;

Kostka and Luther 1995). However, previous research of marine sediments in general, and the salt marsh in particular, has not coupled a study of geochemical effects with a complete partitioning of the predominant carbon oxidation pathways, microbial Fe reduction and sulfate reduction that are largely influenced by macroorganisms. Sulfate reduction has been previously viewed as the predominant terminal electron-accepting process coupled to organic matter oxidation in saltmarsh sediments (*see* reviews by Howarth 1993; Alongi 1998). Although a few past studies have suggested that microbial Fe(III) reduction may be coupled to carbon oxidation in saltmarsh sediments (Jacobson 1994; Kostka and Luther 1995), most Fe(III) reduction was believed to be catalyzed abiotically through reaction with dissolved sulfides produced by sulfate reduction.

Through a combination of intact core and whole sediment incubations, our results show that Fe(III) reduction is biological and it comprises the majority of carbon oxidation in vegetated, bioturbated saltmarsh sediments. Although no Fe(III) reduction was observed in killed or unvegetated/non-bioturbated sediments, some of the highest Fe(III) reduction rates ever observed in marine sediments were recorded for
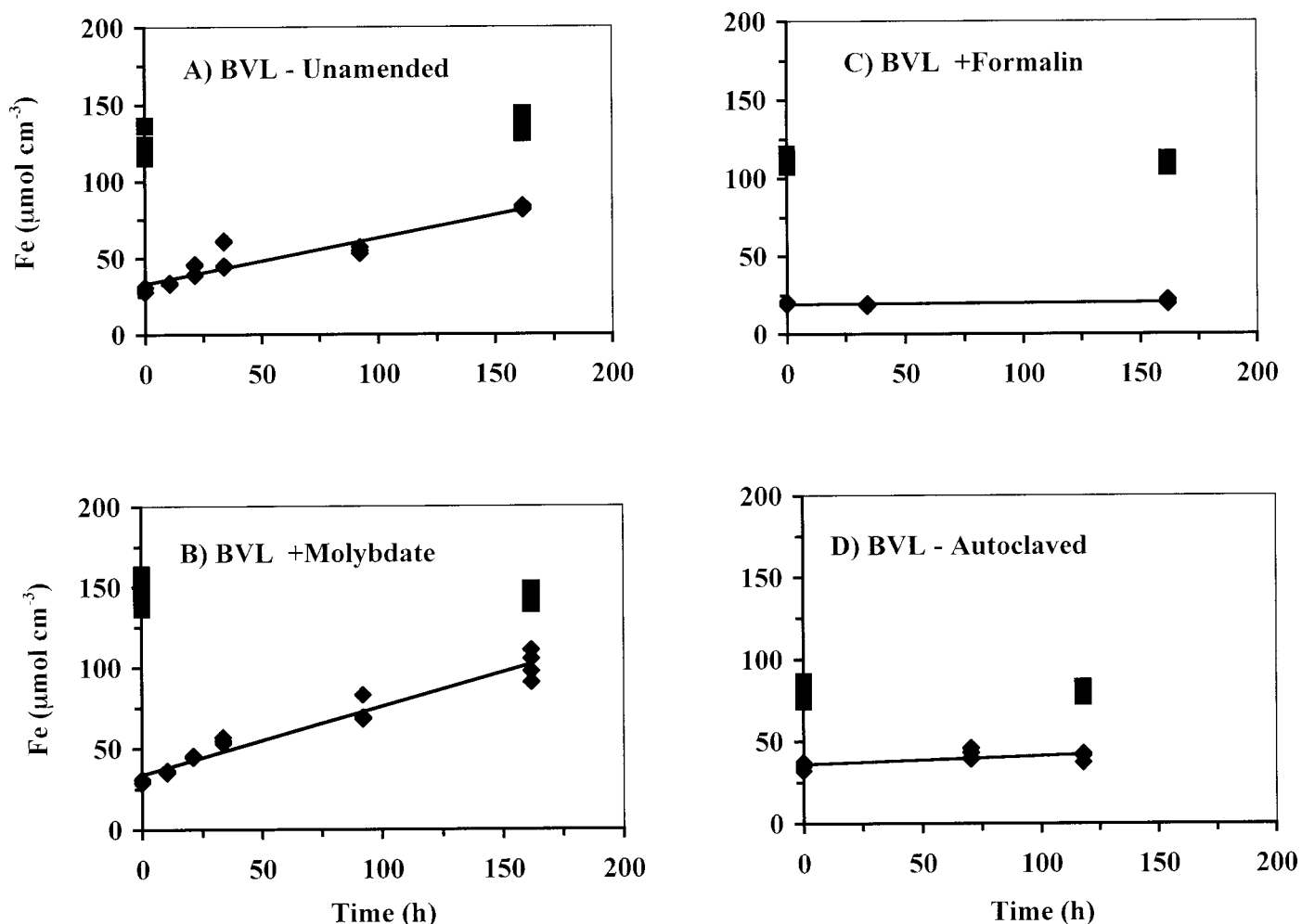
Fig. 6. Fe(III) reduction rates measured as the accumulation of extractable Fe(II) in incubations of BVL sediments. Symbols represent duplicate extractions from duplicate incubations. Sediments are the same as those represented in Fig. 4C,D.

Table 3. Most probable number (MPN) counts of Fe(III)-reducing and sulfate-reducing bacteria in Georgia saltmarsh sediments.* The 95% confidence interval was within one order of magnitude for each count.

	MPN (cells cm^{-3})	Cell-specific respiration rate (fmol $cell^{-1}$ d^{-1})
FeRB		
BUC	4.5×10^6	
BVL	2.8×10^7	207
NUC	2.7×10^5	ND†
SRB		
BUC	7.4×10^6	
BVL	1.8×10^6	20.8
NUC	5.1×10^5	655

* FeRB, Fe(III)-reducing bacteria; SRB, sulfate-reducing bacteria.
† ND = no respiration activity detected.

vegetated/bioturbated levee sediments in this study (Figs. 5, 6). Addition of sodium molybdate, a specific metabolic inhibitor of sulfate reduction, had no effect on Fe(III) reduction rates in vegetated, bioturbated sediment. The close coupling between Fe(III) reduction and carbon oxidation was further supported by the similar trends observed for the accumulation of ΣCO_2 and by the stoichiometry of microbial respiration pathways observed in various sediment incubation treatments (*see* Table 2). Fe(III) reduction rates closely paralleled those of ΣCO_2 accumulation in the same incubations of BVL sediment (Figs. 4, 6).

Previous work has suggested that acetate is the most abundant microbial fermentation product driving microbial respiration reactions in marine sediments, including the salt marsh (Jørgensen 2000). Therefore, the stoichiometry of sulfate reduction and microbial Fe(III) reduction have been considered according to the following equations (Jørgensen 2000; Thamdrup 2000).

$$SO_4^{2-} + CH_3COO^- + 2H^+$$

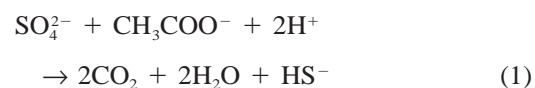$$\rightarrow 2CO_2 + 2H_2O + HS^- \qquad (1)$$

Table 4. Summary of the partitioning of C oxidation pathways. Values are calculated as a percentage of C oxidation rates using the stoichiometry presented in Eqs. 1, 2.*

		NUC	BVL
SRR	Intact cores	82%	6%
	Sediment incubations	137%	3%
Fe(III) reduction	Unamended	ND†	109%
	+Molybdate	ND	113%

* BVL, bioturbated, vegetated levee; NUC, nonbioturbated, unvegetated creek bank; SRR, sulfate reduction rate.
† ND = No Fe(III) reduction was detected in these sediments.

$$8FeOOH + CH_3COO^- + 17H^+$$
$$\rightarrow 2CO_2 + 14H_2O + 8Fe^{2+} \quad (2)$$
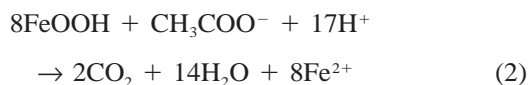
Following this accepted stoichiometry, our results indicate that microbial Fe(III) reduction is coupled to nearly 100% of C oxidation in BVL sediment, whereas the majority of C oxidation is driven by sulfate reduction in NUC sediment (Table 4). The 1:2 stoichiometry of sulfate reduction coupled to C oxidation that we observed (Table 2) has been previously confirmed in marine sediments using a combination of radiotracer assays and measurements in sediment incubations (Jørgensen 2000). However to our knowledge, no previous published work in marine sediments has confirmed the 4:1 stoichiometry of Fe(III) reduction coupled to C oxidation using direct rate measurements of each process. Roden and Wetzel (1996) observed a similar stoichiometry of Fe(III) reduction coupled to carbon oxidation in freshwater wetland sediments.

The predomination of microbial Fe(III) reduction in vegetated, bioturbated sediment suggests that higher organisms (macrofauna and plants) stimulate Fe(III)-reducing bacteria (FeRB) to outcompete the sulfate-reducing bacteria (SRB) for the utilization of carbon substrates such as acetate. This argument was supported by the bacterial counts carried out in the same sediments for which rate measurements were reported. Counts of FeRB were two orders of magnitude higher in the BVL sediment relative to the NUC (Table 3). Furthermore, unvegetated creek bank sediment (BUC), in which similar numbers of *Uca* burrows were observed, contained numbers of FeRB which were intermediate between the BVL (vegetated + bioturbated) and the NUC (unvegetated + nonbioturbated). Regression of the FeRB counts presented in Table 3 with directly measured solid Fe(III) concentrations (Fig. 2) over all sites studied revealed a high correlation ($r^2 = 0.92$). Although the number of SRB was not significantly correlated with solid Fe(III) ($r^2 = 0.01$), SRB counts were roughly two times higher than FeRB counts at NUC where sulfate reduction was the dominant respiration pathway.

To our knowledge, these are the first counts of FeRB that have been carried out using a MPN approach in saltmarsh sediments. We calculate cell-specific Fe(III) reduction rates (207 fmol cell^{-1} d^{-1}) for marsh sediments that are well within the range of cellular reduction rates measured for pure cultures of FeRB. Using the average cell number and the amount of Fe(II) produced upon completion in pure cultures

of dissimilatory FeRB (*Geobacter metallireducens, Shewanella putrefaciens*), we calculate a range of cellular Fe(III) reduction rates from 30 to 600 fmol cell^{-1} d^{-1} (Lovley and Phillips 1988; Kostka et al. 1999). Cellular sulfate reduction rates (21–655 fmol cell^{-1} d^{-1}) were consistent with those measured previously in marine sediments and in pure cultures (Hines et al. 1999; Sahm et al. 1999).

Supply of solid Fe supporting microbial Fe(III) respiration—As observed in previous studies (Dacey and Howes 1984; Hines 1991), pore-water and solid-phase geochemistry clearly indicate that vegetated sediment, bioturbated sediment, or both sediments are more oxidized (Table 1; Figs. 1, 2), further suggesting that macroorganisms control the partitioning of microbial respiration pathways by mediating sediment mixing or chemical exchange in the SERF marsh. Solid-phase Fe(III) concentrations, Fe(III) reduction rates, and populations of FeRB were all high in vegetated/bioturbated sediments and lower to nonexistent in nonbioturbated sediments with no grass roots. In his recent review of Fe(III) reduction in aquatic sediments, Thamdrup (2000) observed that poorly crystalline Fe(III) mineral concentration is a master parameter controlling the relative importance of Fe(III) and sulfate reduction coupled to C oxidation. The input or supply of reactive Fe(III) through sediment accumulation alone has been observed to be negligible in comparison to organic matter mineralization rates. Thus, the supply of reactive Fe(III) is thought to be controlled by enhanced advection–diffusion or sediment reworking (Thamdrup 2000). In the saltmarsh sediments of our study, reactive Fe(III) is likely supplied by an infusion of oxygen through the roots of *Spartina* or by macrofaunal bioturbation, which leads to the reoxidation of Fe(II). Although no quantitative studies of Fe reduction have been carried out previously in saline marshes, Roden and Wetzel (1996) found that Fe reduction comprised 40–65% of C oxidation in a freshwater marsh. They further hypothesized that reactive Fe(III) was supplied by the reoxidation of Fe(II) mediated by roots of the dominant marsh grass, *Juncus.* Kristensen et al. (2000) also observed Fe reduction to account for the majority of carbon oxidation in rooted mangrove forest sediment.

Macrophytes have been shown to increase the intrusion of oxidants into marine sediments, thereby enhancing the recycling of redox-sensitive elements like Fe and S (Hines et al. 1989; Hines 1991; Kostka and Luther 1995). In addition, several previous studies have implicated macrofaunal bioturbation as the primary mechanism by which reactive Fe(III) is supplied in marine subtidal sediments where microbial Fe(III) reduction has been observed to successfully compete with sulfate reduction (*see* recent reviews by Thamdrup 2000; Thamdrup and Canfield 2000). Both of the above supply mechanisms are likely active in supporting Fe reduction in the Georgia salt marsh. The activities and abundance of *Uca*, the dominant bioturbating organisms in the *Spartina* marsh, are well documented and the roots of *Spartina* have been shown to inject O$_2$ into subsurface sediment (*see* introduction). Further studies are required to separate the individual effects of macrophytes and macrofauna on the supply of solid Fe(III). However, we have shown definitively that higher organisms stimulate FeRB to outcompete the SRB in

saltmarsh sediments by supplying an abundance of reactive Fe(III) through reoxidation processes.

An important consideration about the accuracy of our sediment incubation method concerns the effect of sediment homogenization on respiration and C oxidation rates. The effects of homogenization on rate measurements are not yet clear according to a recent review by Hansen et al. (2000). At NUC, where sulfate reduction was the dominant respiration pathway, measured C oxidation rates from sediment incubations (270 mmol m^{-2} d^{-1}; 0–35 h; Fig. 4) were an average of at least six times higher than C oxidation rates calculated from intact core measurements (40.0 mmol m^{-2} d^{-1}). Our results show an initial stimulation by a factor of two to six that may be attributed to homogenization in saltmarsh sediments, but the effect appeared to diminish after the first 2 d of incubation (Figs. 4, 6). The stoichiometry of respiration processes remained the same throughout; therefore, we recommend that the incubation period of 2 to 4 d should be used in these saltmarsh sediments to more accurately estimate rates of in situ processes.

Sediments exposed to vegetation and bioturbation (at organismal densities similar to our study) are the norm rather than the exception in *Spartina* marshes along the east coast of the U.S. At the SERF marsh, fiddler crab burrows were observed in equal abundance on creek banks and on levees that were inhabited by the tall form of *Spartina.* As has been shown previously (*see* above discussion), we have observed that sediments of the bioturbated creek bank and tall *Spartina* zones are clearly more oxidized, exhibiting less accumulation of sulfides and ammonium relative to the midmarsh/short *Spartina* zone (Figs. 1, 2; Kostka et al. 2001). We estimate from aerial photos that creek bank and tall *Spartina* zones in the SERF marsh occupy approximately 10 and 25%, respectively, of total marsh area (data not shown). Therefore, roughly a third of surface saltmarsh sediments may be affected by higher organisms, as shown in this study. Ammonium is the most abundant form of inorganic nitrogen available for organisms in marsh sediments, and it is thought to be primarily taken up by macrophytes and recycled in the marsh (Alongi 1998). Dissolved sulfide has been shown to affect nitrogen cycling in marine sediments substantially by inhibiting coupled nitrification–denitrification (Joye and Hollibaugh 1995). Although the root zone of *Spartina* has not been studied in detail, about 25% of applied ammonium was shown to be lost through coupled nitrification–denitrification in the root zone of other macrophytes (Reddy et al. 1989). Clearly, by mediating chemical exchange (i.e., sulfide removal) in surface sediments, higher organisms could potentially stimulate a large removal of nitrogen through coupled nitrification–denitrification in the salt marsh. Therefore, in order to completely understand the fluxes of nutrients limiting primary production on a marshwide scale, further studies are required to assess the quantitative effects of macroorganisms on saltmarsh sediment biogeochemistry.

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