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Seasonal oscillation of microbial iron and sulfate reduction in saltmarsh sediments (Sapelo Island, GA, USA)

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Abstract. Seasonal variations in anaerobic respiration pathways were investigated at three saltmarsh sites using chemical data, sulfate reduction rate measurements, enumerations of culturable populations of anaerobic iron-reducing bacteria (FeRB), and quantification of *in situ* 16S rRNA hybridization signals targeted for sulfate-reducing bacteria (SRB). Bacterial sulfate reduction in the sediments followed seasonal changes in temperature and primary production of the saltmarsh, with activity levels lowest in winter and highest in summer. In contrast, a dramatic decrease in the FeRB population size was observed during summer at all sites. The collapse of FeRB populations during summer was ascribed to high rates of sulfide production by SRB, resulting in abiotic reduction of bioavailable Fe(III) (hydr)oxides. To test this hypothesis, sediment slurry incubations at 10, 20 and 30 °C were carried out. Increases in temperature and labile organic carbon availability (acetate or lactate additions) increased rates of sulfate reduction while decreasing the abundance of culturable anaerobic FeRB. These trends were not reversed by the addition of amorphous Fe(III) (hydr)oxides to the slurries. However, when sulfate reduction was inhibited by molybdate, no decline in FeRB growth was observed with increasing temperature. Addition of dissolved sulfide adversely impacted propagation of FeRB whether molybdate was added or not. Both field and laboratory data therefore support a sulfide-mediated limitation of microbial iron respiration by SRB. When total sediment respiration rates reach their highest levels during summer, SRB force a decline in the FeRB populations. As sulfate reduction activity slows down after the summer, the FeRB are able to recover.

Introduction

Stratified waters and sediments typically exhibit a vertical succession of oxic, suboxic and anoxic zones. According to the classical paradigm, this chemical redox zonation corresponds spatially to organic matter oxidation pathways that utilize a sequence of terminal electron acceptors in order of decreasing free energy yield

(Froelich et al. 1979). However, a simple one-to-one correlation between vertical redox zones and microbial respiratory pathways breaks down in systems characterized by steep spatial and temporal chemical gradients, particularly organic-rich, bioturbated sediments in nearshore environments (e.g., Aller (2001)). In such sediments, several carbon oxidation pathways may coexist within the same horizontal sediment layer (Sørensen 1982; Jørgensen and Bak 1991; Canfield and Des Marais 1991; Canfield 1993; Canfield et al. 1993; Jacobson 1994; Brandes and Devol 1995; Thamdrup and Canfield 2000). Physical proximity forces intense competition among microorganisms for available energy substrates and terminal electron acceptors (e.g., Lovley and Klug (1986) and Lovley and Phillips (1987), Aller and Rude (1988), Hoehler et al. (1998)). Furthermore, chemical (abiotic) redox pathways may compete for the same chemical substrates as microbial (enzymatic) pathways.

Saltmarshes are highly productive ecosystems (Pomeroy et al. 1981; Schubauer and Hopkinson 1984) and therefore exhibit high rates of sediment respiration. Previous studies have suggested that organic matter oxidation in the upper 10–50 centimeters of saltmarsh sediments is dominated by microbial sulfate reduction (Howarth and Teal 1979; Howarth and Hobbie 1982; Howarth and Giblin 1983; Howes et al. 1984; Howarth and Merkel 1984; King 1988; Hines et al. 1989; Kostka et al. 2002b). Saltmarsh sediments often contain significant quantities of reactive Fe(III) (hydr)oxides and exhibit high concentrations of reduced pore water Fe(II), indicative of active Fe(III) reduction. Nevertheless, microbial iron reduction is generally not considered a significant pathway of organic matter breakdown in saltmarsh sediments (Jacobson 1994; Alongi 1997), because sulfide produced by SRB is a powerful reductant of Fe(III) (hydr)oxides (Pyzik and Sommer 1981; Elsgaard and Jørgensen 1992; Peiffer et al. 1992; dos Santos Afonso and Stumm 1992; Yao and Millero 1996). However, Lowe et al. (2000) recently found evidence that active populations of iron-reducing bacteria (FeRB) inhabit the topmost centimeters of sediments at a variety of locations in saltmarshes of Sapelo Island, Georgia. Kostka et al. (2002a) further proposed that microbial iron respiration dominates organic matter oxidation in some bioturbated, vegetated saltmarsh sediments, in agreement with the mounting evidence that microbial iron reduction accounts for a significant fraction of organic matter oxidation in coastal marine environments (see reviews by Burdige (1993), Thamdrup (2000) and Thamdrup and Canfield (2000)).

In order to gain more insight into the competitive interactions and environmental conditions controlling microbial iron reduction, we conducted a seasonal study of FeRB populations in sediments sampled along a transect through a saltmarsh on Sapelo Island. Work by Kostka et al. (2002b) at the same sites demonstrates strong seasonal fluctuation of rates of total organic carbon oxidation and sulfate reduction. These rates correlate closely with changes in surface temperature and primary production of the marsh; similar observations have been made in other saltmarsh sediments (Hines 1991; Hines et al. 1989, 1999). The main objective of the present study is to determine the response of FeRB population to the seasonal cycles of total sediment respiration and sulfate reduction.

Previous analysis of total sediment-extracted rRNA pools indicate that FeRB species *Shewanella putrefaciens* and FeRB family *Geobacteraceae* are not domi-

nant members of the microbial population in Sapelo Island saltmarsh sediments (Lowe et al. 2000). Furthermore, in contrast to SRB, it is currently not possible to screen for FeRB using 16S rRNA probes. Hence, enumerations of culturable FeRB were used to infer relative changes of the *in situ* population sizes of the FeRB. Cultivation techniques only provide a partial view of the resident microbial community because many groups of microorganisms resist cultivation (e.g., Stephen et al. (1996) and Amann et al. (1997)). Furthermore, microbial community size does not necessarily correlate with activity (e.g., Ward et al. (1998) and Hines et al. (1999), Luna et al. (2002)). The culture data were therefore complemented with information on seasonal chemical changes of sediments and pore waters, as well as sulfate reduction rate measurements. In addition, sediment slurry experiments were performed to determine the responses of the FeRB population density and activity to variations in environmental conditions.

Methods

Field

Three sampling sites were chosen along a transect through a saltmarsh on Sapelo Island, a barrier island located off the coast of Georgia, southeastern USA (Figure 1). The sites include an exposed, unvegetated creek bank immediately adjacent to a large tidal creek, a densely vegetated levee site with *Spartina alterniflora* reaching heights of up to 1.5 m, and a sparsely vegetated ponded marsh site with *S. alterniflora* reaching approximately 0.5 m (Figure 2). All three sites are intensely bioturbated by fiddler crabs and are influenced by tides of 2–3 m. Surface air temperature ranges from approximately 10 °C in winter to approximately 30 °C in summer (Figure 3). Indices of primary productivity of the marsh, e.g. the aboveground plant biomass, show seasonal patterns similar to that of temperature (see for example: Dame and Kenny (1986) and Dai and Wiegert (1996)).

Vertical profiles of pore water pH, alkalinity, dissolved Fe(II)/(III), ΣNH_3, ΣH_2S, Mn(II), ΣPO_4^{-3}, and SO_4^{-2} were measured in May and August 1997, January, June and November 1998, and January 1999, using dialysis samplers, or 'peepers' (Hesslein 1976), with twenty 0.5 × 1.5 × 14 cm chambers at 1 cm intervals, followed by fifteen 1 × 1.5 × 14 cm chambers at 2 cm intervals. Peepers were acid-cleaned in dilute HCl or HNO_3, rinsed thoroughly in deionized water and assembled in deionized water prior to deployment. Between assembly and deployment in the field, peepers were kept in a Plexiglas box filled with deionized water and bubbled continuously with N_2 for a minimum of 3 days. Peepers were transported directly to the field site with portable N_2 tanks attached to the Plexiglas box by tygon tubing, and were left to equilibrate with the surrounding pore water for approximately four weeks before removal. Upon retrieval, the peepers were immediately placed in a portable vinyl globe bag flushed with N_2, and transported directly to the field laboratory. Pore waters were extracted using 10 mL latex-free, polypropylene sy-

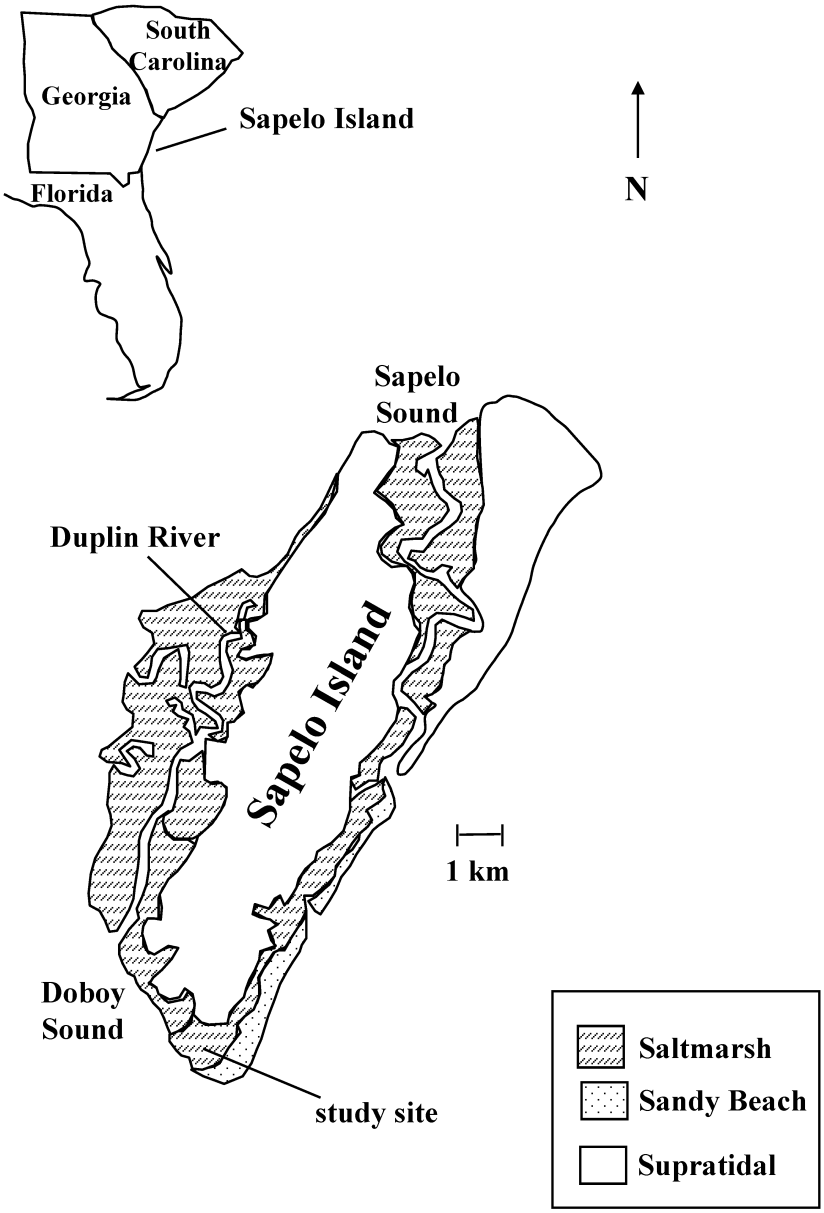

Figure 1. Map of Sapelo Island, GA. Sites described in this study were located in a saltmarsh at the southern end of Sapelo Island in close proximity to the University of Georgia Marine Institute.

ringes with stainless steel needles by puncturing the nylon glove bag and the porous nylon membrane covering the peeper chambers and directly withdrawing the fluids while N_2 was flushed into the bag to maintain overpressure. The extracted

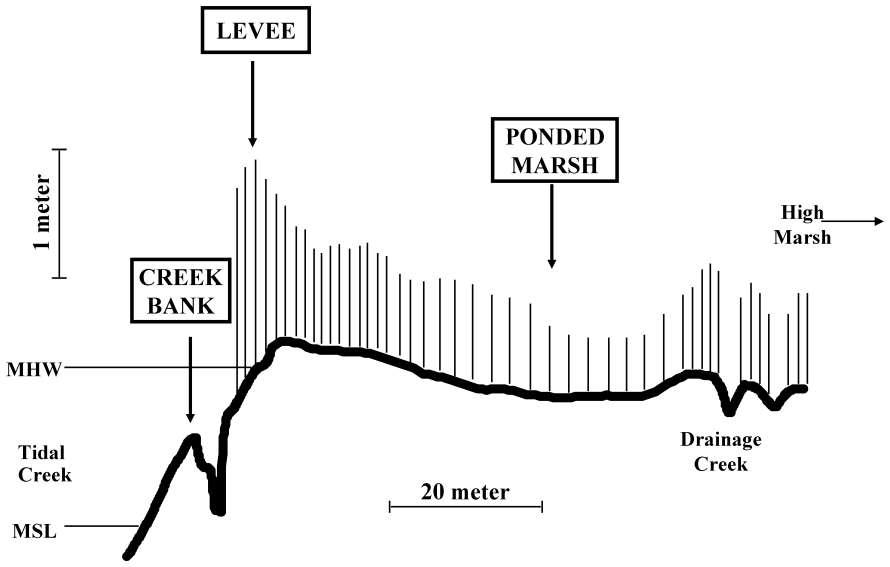

Figure 2. Topography of the saltmarsh transect. Sediments were sampled next to a large tidal creek (creek bank site), at the adjacent levee and at a ponded marsh site, approximately 30 m from the levee. Note that horizontal and vertical scales are not equal. Heights and density of *Spartina alterniflora* are represented schematically by the vertical lines and are based on measurements carried out in January 1999.

pore waters were filtered through polypropylene 0.2 μm pore size syringe filters into a 15 mL polypropylene centrifuge tube, and the pH was measured. Immediately following the pH measurement, the sample was pipetted into a series of 4 mL polypropylene vials with polyethylene plugs containing reagents for alkalinity, ferric and ferrous iron, sulfide, ammonium and phosphate colorimetric analyses. Filtered pore water samples were preserved in 0.05 N HCl for sulfate analyses or in concentrated sulfuric acid for manganese analyses. Alkalinity, phosphate, ammonium, manganese, Fe(II)/Fe(III), sulfate and sulfide were measured colorimetrically (Table 1). Ammonium was measured on diluted samples to avoid interference from sulfide. The complete set of pore water data is reported elsewhere (Koretsky et al., in prep).

Sediment cores used for microbial culture enumeration and 16S rRNA analyses (this study) and sulfate reduction rate or solid phase analyses (Kostka et al. 2002b) were collected at sites located not more than 0.5 m from the peepers using a stainless-steel wedge-shaped corer to minimize compaction. Sediments were sectioned into 2 cm intervals in a N_2 filled glovebag immediately after collection of the sediment core. Sections were divided into two portions: one portion was used for microbial culture enumeration and 16S rRNA analyses and the other portion was used for sulfate reduction rate measurements and solid phase analyses. Sulfate reduction rates (SRR) and solid phase extractions for poorly crystalline iron oxides, acid

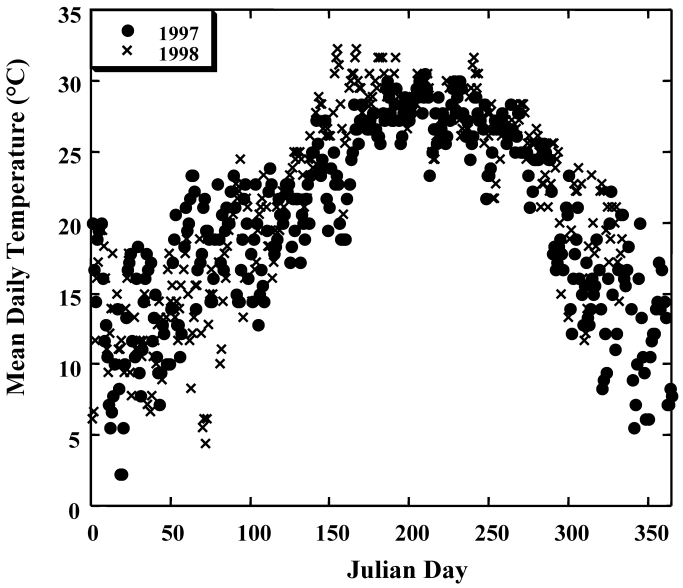

Figure 3. Mean daily air temperature during 1997 and 1998 at Sapelo Island, GA USA. (Source: Southeast Regional Climate Center: http://climate.engr.uga.edu/sapelo_island/daily_90s.html.)

Table 1. Colorimetric pore water analysis methods. Precision is given as the standard deviation of 10 replicate analyses.

Analysis	Method	Precision	Reference
Alkalinity	Bromophenyl Blue	±3% (at 15 mM)	Sarazin et al. (1998)
Phosphate	Molybdate Blue	±3% (at 75 μM)	Greenberg et al. (1992)
Ammonium (NH_3 + NH_4^+)	Indophenol Blue	±6% (at 500 μM)	Grasshoff et al. (1982)
Sulfide	Methylene Blue	±5% (at 0.3 mM)	Grasshoff et al. (1982)
Sulfate	Barium Gelatin Turbidity	±3% (at 20 mM)	Tabatabai (1974)
Fe(II)/Fe(III)	Ferrozine	±5% (at 400 μM)	Viollier et al. (2000)
Manganese	Formaldoxime	±3% (at 75 μM)	Koroleff (1983)

volatile sulfides and chromium reducible sulfides are reported, together with method details for these analyses, in another study (Kostka et al. 2002b).

Aliquots from each 2 cm interval were used for enumeration of culturable anaerobic iron reducing bacteria (FeRB), anaerobic manganese reducing bacteria (MnRB) and aerobic bacteria (AEB). AEB and the complete set of FeRB and MnRB data are reported elsewhere (Koretsky et al., in prep). For enumeration of FeRB, aliquots were serially diluted in anaerobic phosphate buffered saline solution (Myers and Nealson 1988) and spread on synthetic growth media (Lowe et al. 2000) supplemented with Bacto agar (1.5% w/v), NaCl (4% w/v), lactate (15 mM) and Fe(III)-citrate (50 mM). The plates were immediately placed into anaerobic canisters and incubated at room temperature for 1 month before enumerating col-

ony-forming units (CFU). Aliquots for MnRB enumeration were treated similarly, except that 5 mM δ-MnO_2 (synthesized as described by Burnes et al. (1998)) was supplied as the sole terminal electron acceptor (DiChristina and DeLong 1994). All culture enumeration experiments were performed in duplicate, and culture plates that did not contain a terminal electron acceptor were included as controls.

Total nucleic acid was extracted from each 2 cm interval of sediment and used in 16S rRNA hybridization experiments, using whole sediment RNA extraction and subsequent hydridization techniques identical to those described previously (Moran et al. 1993; Lowe et al. 2000). A variety of 16S rRNA-targeted oligonucleotide probes were used in hybridization experiments with total sediment-extracted rRNA. These included probe SRB385, which targets a wide range of SRB within the delta-proteobacteria, as well as probes DSV687 and DSB804, which target the SRB genera *Desulfovibrio* and *Desulfobacter*, respectively (Devereux et al. 1992; Amann et al. 1990, 1992, 1997). Total prokaryotic rRNA was quantified using EUB338 and ARC915, which target the *Bacteria* and *Archaea* domains, respectively (Pace et al. 1986; Amann et al. 1990).

Incubation experiments

Sediments for incubation experiments were collected from the upper 10 cm interval at the ponded marsh site, during April 1999, July 1999 and March 2001, and stored on ice in anaerobic jars for immediate transport to the laboratory. Equal volumes of wet sediment and previously filtered (0.2 μm pore size), N_2-purged and sterilized tidal creek water (collected on site) were mixed together and passed through a 1 mm sieve (to remove plant debris and macrofauna) into a bioreactor. The resulting sediment slurry was stirred at 50 rpm for 2 days under continuous N_2 purging. After homogenization, the sediment slurry was transferred to an anaerobic chamber and dispensed into a series of 15 mL anaerobic Hungate tubes (Belco Glass Inc., Vineland, NJ) with amendments as described in Table 2. Amorphous iron (hydr)oxide (FeOx) was prepared according to Schwertmann and Cornell (2000). Initial measurements (day 0) were completed after addition of amendments. At 2–7 day intervals (see Table 2), two tubes were sacrificed from each set of amendments. The first tube was centrifuged and the supernatant was filtered through a 0.2 μm pore size syringe filter and used for aqueous analyses of pH, alkalinity, sulfate, total dissolved H_2S, Fe^{+2} and Fe^{+3}. Analytical techniques were identical to those used in the field (Table 1). The second tube was used for enumeration of FeRB capable of growing anaerobically on a defined synthetic growth medium with Fe(III) as sole terminal electron acceptor as described above, except that plates were amended with either lactate or acetate (30 mM) and Fe(III)-citrate (50 mM). Plates were incubated anaerobically at room temperature for 14 days in anaerobic canisters (BBL Gas Pak; Beckton Dickinson Co., Cockeysville, MD). A control plate with no terminal electron acceptor was included in each canister.

Table 2. Details of sediment slurry experiments. A sampling interval of, for example, 0, 5, 10 indicates that tubes were sampled at initiation of the experiment and after 5 and 10 days of incubation.

Amendments	Date of Sediment Collection	Initial FeRB Population Size (CFU per mL sediment slurry) at 10, 20 and 30 °C	Sampling Intervals (Days)
Unamended	April 1999	3900 ± 200, 4100 ± 900, 3500 ± 450	0, 4, 7, 10, 14, 18, 21
10 mM Acetate	April 1999	230 ± 300, 2900 ± 1000, 500 ± 100	0, 4, 7, 10, 14, 18, 21
10 mM Lactate	April 1999	4100 ± 500, 3000 ± 400, 2200 ± 300	0, 4, 7, 10, 14, 18, 21
10 mM Lactate	July 1999	840 ± 70, 840 ± 70, 840 ± 70	0, 2, 6, 9, 13, 15, 21
10 mM Lactate 10 mM Molybdate	July 1999	980 ± 30, 980 ± 30, 980 ± 30	0, 2, 6, 9, 13, 15, 21
10 mM Lactate 5 mM FeOx	July 1999	870 ± 40, 870 ± 40, 870 ± 40	0, 2, 6, 9, 13, 15, 21
10 mM Lactate 5 mM FeOx 10 mM Molybdate	July 1999	985 ± 35, 985 ± 35, 985 ± 35	0, 2, 6, 9, 13, 15, 21
10 mM Lactate	March 2001	62000 ± 4200, 62000 ± 4200, 62000 ± 4200	0, 3, 5, 9, 13, 17
10 mM Lactate 10 mM Sulfide	March 2001	4300 ± 9000, 4300 ± 9000, 4300 ± 9000	0, 3, 5, 9, 13, 17
10 mM Lactate 10 mM Molybdate	March 2001	50000 ± 3200, 50000 ± 3200, 50000 ± 3200	0, 3, 5, 9, 13, 17
10 mM Lactate 10 mM Molybdate 10 mM Sulfide	March 2001	43000 ± 10000, 43000 ± 10000, 43000 ± 10000	0, 3, 5, 9, 13, 17

Field results

Depth profiles

A typical set of depth profiles of pore water Mn^{+2}, Fe^{+2}, and H_2S concentrations is shown in Figure 4A. They exhibit the classical geochemical redox stratification with peak Mn^{+2} concentrations at approximately 8 cm depth, followed by peak Fe^{+2} concentrations at approximately 11 cm, and subsequent build-up of dissolved sulfide beginning at approximately 14 cm. However, as seen in Figure 4C and 4D, the highest sulfate reduction rates (SRR), and the highest percentage of SRB-targeted 16S rRNA signals occur within the upper 10 cm of the sediment column, that is, in the oxic to suboxic transition zone characterized by high levels of pore water Mn^{+2} and Fe^{+2} and low dissolved sulfide concentrations. In addition, the highest numbers of culturable FeRB and MnRB are located within the sediment horizon where SRB are most abundant and active (0–10 cm; Figure 4B, 4C & 4D). Thus, the chemical and microbial data point to the presence of active populations of MnRB, FeRB and SRB, coexisting in the upper sediment layer.

Integrated pore water and solid phase geochemistry: seasonal oscillation

Pore water and solid phase geochemical profiles exhibit clear oscillations with season. To facilitate the comparative analysis of seasonal trends among the sites, the concentrations of pore water solutes and microbially reducible Fe(III) are integrated over the upper 20 cm of the sediment column (Figure 5). At all sites and seasons, this depth interval has the highest SRR and the highest levels of culturable MnRB and FeRB. Porosity measurements in this depth interval show no systematic variations with season or depth (Kostka et al. 2002b). Hence, variations in the integrated concentrations are used as direct measures of seasonal fluctuations in the standing pools of pore water solutes.

At the creek bank and levee sites, pools of dissolved Mn^{+2} and Fe^{+2} increase from winter to summer and decrease again during fall (Figure 5A, 5B). Concentrations of Mn^{+2} and Fe^{+2} are much lower at the ponded marsh site and do not exhibit a clear seasonal trend. Sulfide concentrations remain at or below detection at the creek bank site during all seasons. The highest accumulation of sulfide occurs at the ponded marsh site (Figure 5C). During winter, non-zero pore water sulfide concentrations at the levee site are only observed below 20 cm depth. As observed for Fe^{+2} and Mn^{+2} at the creek bank and levee sites, the pool of dissolved sulfide at the ponded marsh site increases from winter to summer and then decreases from summer to fall (Figure 5C). Overall, pore water conditions are progressively more reducing from the creek bank to the interior of the marsh, and from winter to summer.

At the vegetated sites, alkalinity shows a general increase from winter to summer and a decrease from summer to winter (Figure 5D), that is, it follows the seasonal growth cycle of *S. alterniflora* in Sapelo Island saltmarshes (see Figure 2D in Dai and Wiegert (1996)). A similar seasonal cycle of alkalinity is not observed at

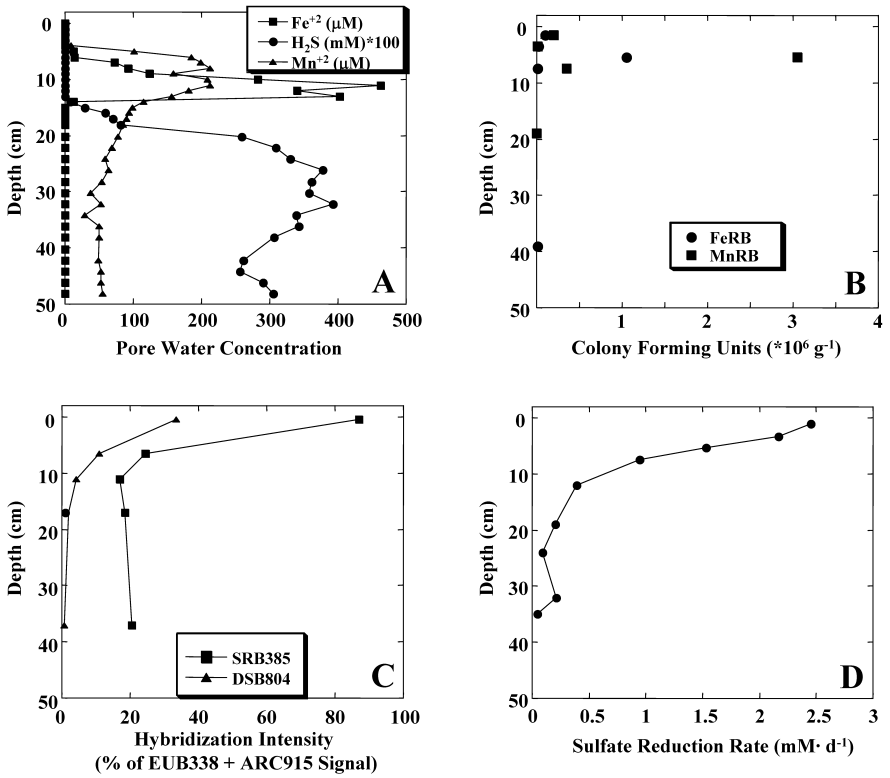

Figure 4. Geochemical and microbial zonation of saltmarsh sediments (levee site, May 1997). FeRB and MnRB were grown with lactate as sole electron donor. Sulfate reduction rates are from Kostka et al. (2002b).

the unvegetated creek bank site. Here alkalinity remains lower than at the other two sites, except in winter.

It has been shown that amorphous Fe(III) (hydr)oxides are preferred by FeRB, and furthermore, that ascorbate extractable Fe(III) (AEF) is a useful proxy for these solid phases in saltmarsh sediments (Lovley and Phillips 1986, 1987; Kostka and Luther 1994; Lowe et al. 2000; Thamdrup 2000; Kostka et al. 2002b). AEF is more abundant at the levee and creek bank sites than at the ponded marsh site (Kostka et al. (2002b); Figure 5E). The levee and creek bank sites show little change in AEF over the three seasons during which measurements were carried out, i.e. winter, spring and summer. At the ponded marsh site, the AEF pool is highest during summer (Figure 5E). During winter, spring and summer, microbially available Fe(III) (hydr)oxides are present in the upper 20 cm of the sediments at all three sites.

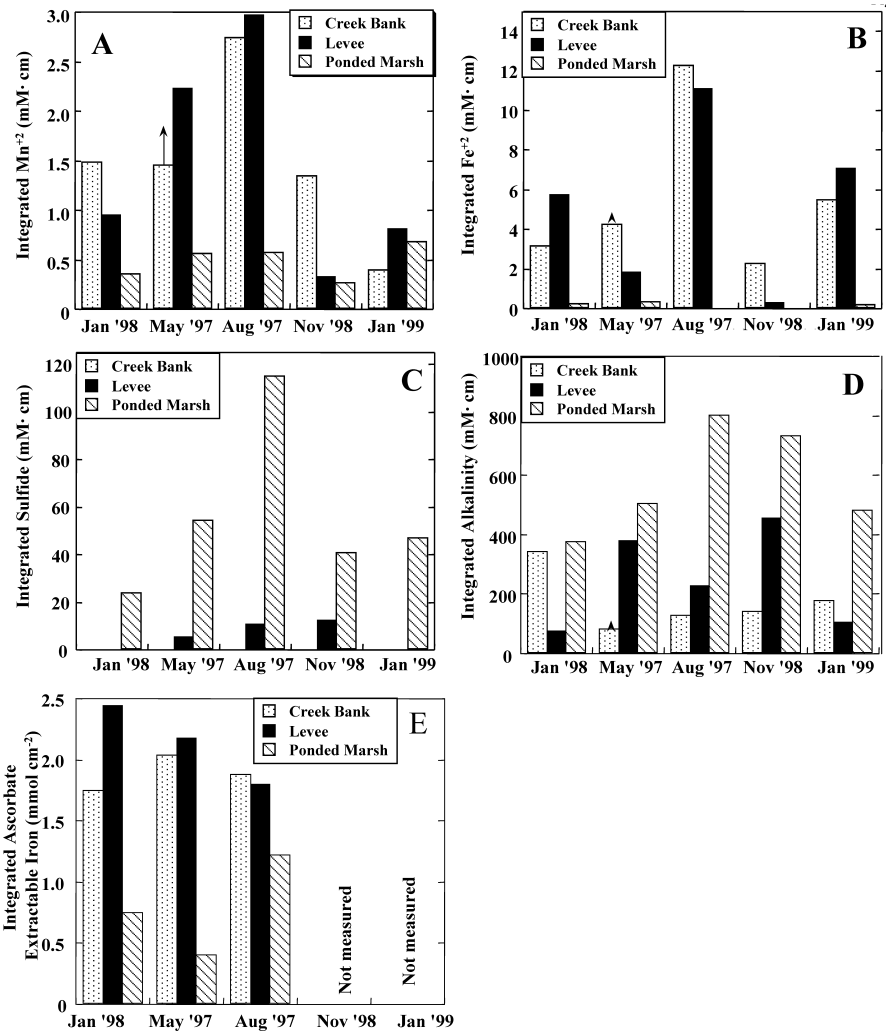

Figure 5. Seasonal pore water and solid phase concentrations at the creek bank, levee and ponded marsh sites integrated from the sediment surface to a depth of 20 cm. No pore water data were measured in the top 5 cm of sediment during May 1997 at the creek bank site; bars correspond to measured data integrated from 5 cm to 20 cm, and arrows indicate approximate integrated levels obtained by extrapolating measured trends to the sediment surface. Ascorbate extractable Fe data are from Kostka et al. (2002b).

Integrated microbial activity and populations: seasonal oscillation

Indicators of microbial population abundance, composition and activity, integrated over the top 20 cm of the sediments, are shown in Figure 6. Significant populations of culturable FeRB are found in the sediments at all three sites (Figure 6A). Their

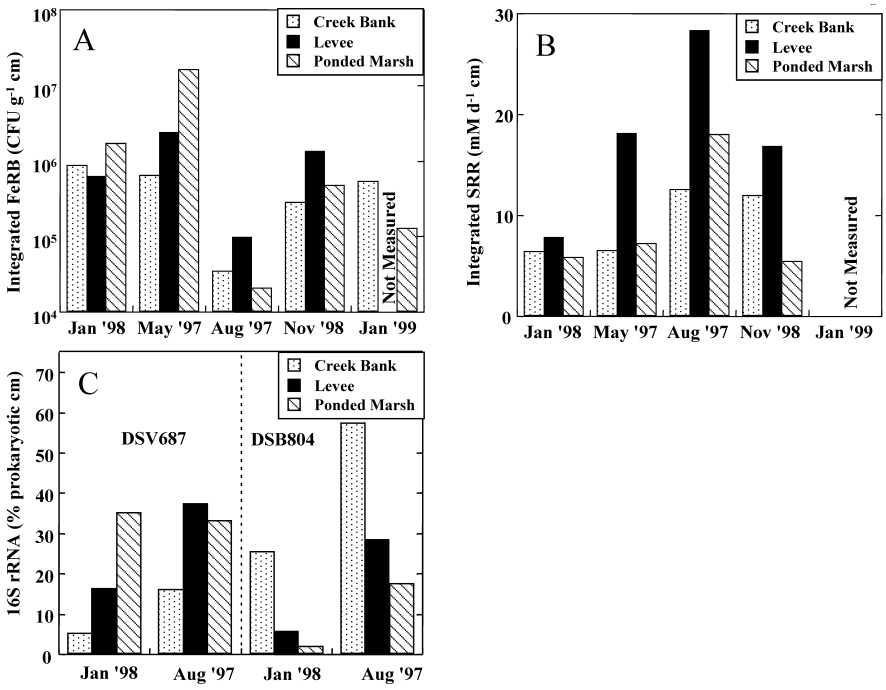

Figure 6. Seasonal indicators of microbial population structure and activity integrated from the sediment-water interface to a depth of 20 cm. SRR were not measured in January 1999; 16S rRNA probing was only carried out in January 1998 and August 1997.

numbers are generally highest in spring, winter and fall; in summer, the culturable FeRB population size drops by more than an order of magnitude at all three sites.

Sulfate reduction rates (SRR) integrated over the upper 20 cm are always greatest at the levee site (Figure 6B). However, it should be noted that SRR depth profiles differ quite significantly among the three sites (see Kostka et al. (2002b)). At the creek bank, there is a slow decrease of SRR with depth, with significant rates measured at 50 cm depth. At the ponded marsh site, the SRR declines rapidly with depth and is negligible below 20 cm due to pore water sulfate depletion. Thus, changing the integration depth from 20 to 50 cm, for example, has a large impact on the integrated SRR values at the creek bank site, but not at the ponded marsh site. Irrespective of the integration depth chosen, however, an identical seasonal pattern of SRR is observed at all three sites: SRR increases from winter to summer, then declines from summer to winter. This trend is also reflected in the *Desulfovibrio* (DSV687) and *Desulfobacter* (DSB804)-targeted rRNA signals, which generally increase in the transition from winter to summer (Figure 6C).

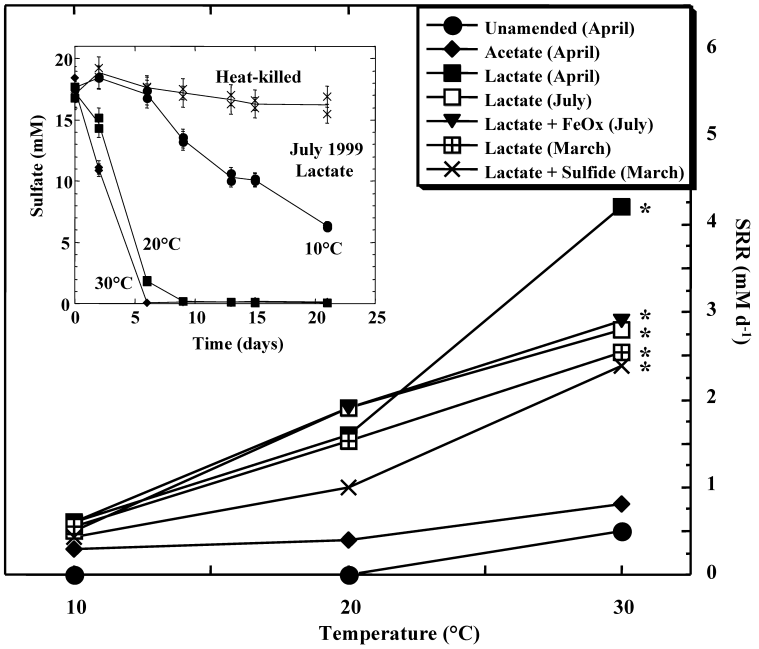

Figure 7. Sulfate reduction rates, calculated from measured sulfate consumption, as a function of temperature and slurry amendments. Stars indicate SRR that may be underestimated due to time resolution of sampling. Inset shows an example of data used to calculate SRR. Replicate analyses of sulfate concentration (mM) are shown as a function of incubation time at 10, 20 and 30° and in heat-killed experiments on sediments collected in July 1999 and amended with 10 mM lactate.

Sediment incubation results

Sulfate reduction

Sulfate reduction rates in sediment incubations are estimated from changes in sulfate concentration measured as a function of time (Figure 7). Temperatures of 10, 20 and 30 °C are representative of conditions encountered in winter, spring and summer, respectively (Figure 3). As shown in Figure 7, SRR increases with increasing temperature. The highest rates in Figure 7 should be regarded as minimum estimates, because of the rapid decreases in sulfate concentration relative to the sampling resolution.

Addition of 10 mM acetate more than doubles SRR relative to unamended samples, and addition of 10 mM lactate results in an order of magnitude increase in SRR. No significant change in SRR is observed with addition of 5 mM amorphous FeOx. Addition of 10 mM dissolved sulfide decreases sulfate reduction rates by 10 to 40%.

Iron reducing bacteria

As the results for lactate and acetate amended plates show similar trends, only lactate data are discussed in detail here. FeRB counts shown in Figure 8 are normalized to the initial number of colony forming units in the slurries to eliminate variations due to the use of sediment batches collected during different seasons (see Table 2). Incubations of unamended sediment slurries at 10, 20 and 30 °C indicate a steady decline in the number of culturable FeRB at 30 °C, that is, at the temperature representative of summer conditions (Figure 8A). Addition of lactate exacerbates the decline with temperature (Figure 8B). Although FeRB appear to rebound during day 14 of the 30 °C experiment, it should be noted that FeRB populations were below the detection limit (BDL) in tubes sampled not only at day 10, but also at days 18 and 21 (data not shown). At a yearly average sediment temperature of 20 °C, addition of 10 mM lactate slightly exacerbates the decline in culturable FeRB (Figure 8D). However, with the addition of molybdate (to inhibit microbial sulfate reduction), the decline in FeRB populations with time is eliminated entirely (Figures 8C, 8D). The addition of sulfide in either the presence or absence of molybdate decreases the size of the FeRB population relative to slurries with no added sulfide (Figures 8E, 8F; see also initial counts in Table 2).

To compare the effects of various treatments, the number of FeRB colony forming units (CFU) is averaged for the duration of each sediment incubation experiment and normalized to the initial population size (Figure 9). While Figure 9 gives a general overview of the response of the culturable FeRB population size to various amendments, it may hide details of temporal changes in population density. For example, the dramatic decrease in FeRB abundance observed at 30 °C in the lactate-amended slurry (Figure 8B) is not reflected as clearly in Figure 9A because of the relatively high FeRB count obtained on day 4.

The inhibiting effect of increased temperature on the propagation of culturable FeRB is clearly seen in Figure 9A. Except at 10 °C, lactate and acetate amendments further enhance the decline in FeRB populations (Figure 9A; also compare 30 °C data in Figures 8A, 8B). Thus, treatments that boost microbial sulfate reduction adversely affect the FeRB populations. Addition of amorphous Fe(III) (hydr)oxides does not influence SRR (Figure 7) and also does not increase culturable FeRB populations (Figure 9B). Addition of molybdate, however, does increase culturable FeRB abundance, especially at 30 °C (Figure 9B). In the presence of molybdate, FeRB population sizes are nearly identical regardless of whether Fe(III) (hydr)oxides are added to the sediment or not (Figure 9B).

Aqueous chemistry

Sulfide concentrations averaged over the time duration of the slurry experiments are shown in Figures 10, 11, and 12. The pool of dissolved sulfide generally increases with increasing temperature, and with addition of acetate, lactate or sulfide (Figure 10A, 12A). At all temperatures, addition of lactate plus Fe(III) (hydr)oxides significantly reduces sulfide accumulation relative to lactate addition alone

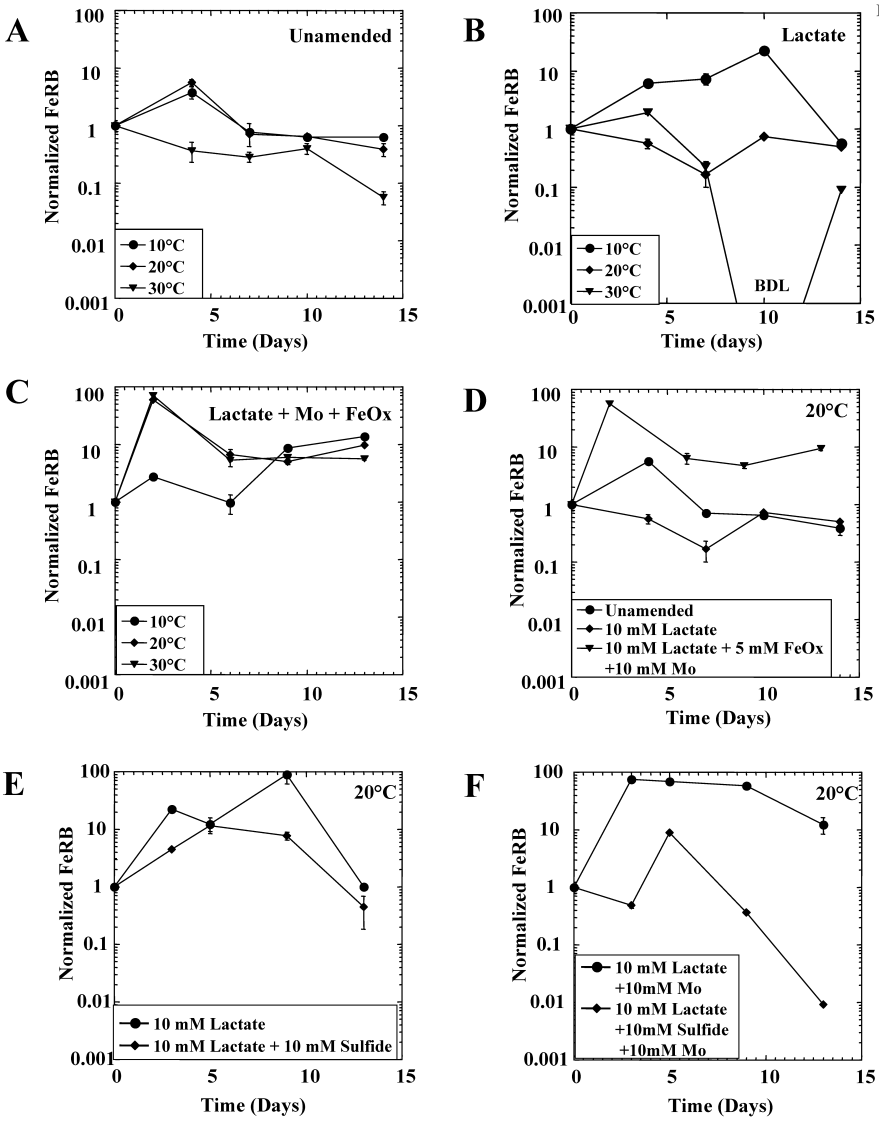

Figure 8. Enumerations of colony-forming units (CFU) of anaerobic FeRB using lactate as the sole carbon source, normalized to the initial FeRB CFU counts (see Table 2). (A) Incubations of sediment collected in April 1999 at 10 °C (circles), 20 °C (diamonds), and 30 °C (triangles). (B) Incubations of sediment collected in April 1999 with 10 mM lactate amendment at 10 °C (circles), 20 °C (diamonds), and 30 °C (triangles). BDL indicates FeRB CFUs were below the detection limit. (C) Incubations of sediment collected in July 1999 and amended with 10 mM lactate, 10 mM molybdate, and 5 mM FeOx at 10 °C (circles), 20 °C (diamonds), and 30 °C (triangles). (D) Incubations at 20 °C of sediment collected in April 1999 with no amendments (circles) or 10 mM lactate addition (diamonds) and sediment collected in July 1999 amended with 10 mM lactate, 10 mM molybdate and 5 mM FeOx (triangles). (E) Incubations at 20 °C of sediment collected in March 2001 amended with 10 mM lactate (circles) or 10 mM lactate with 10 mM sulfide (diamonds). (F) Incubation at 20 °C of sediment collected in March 2001 amended with 10 mM molybdate and 10 mM lactate, in the presence (diamonds) or absence (circles) of 10 mM sulfide.

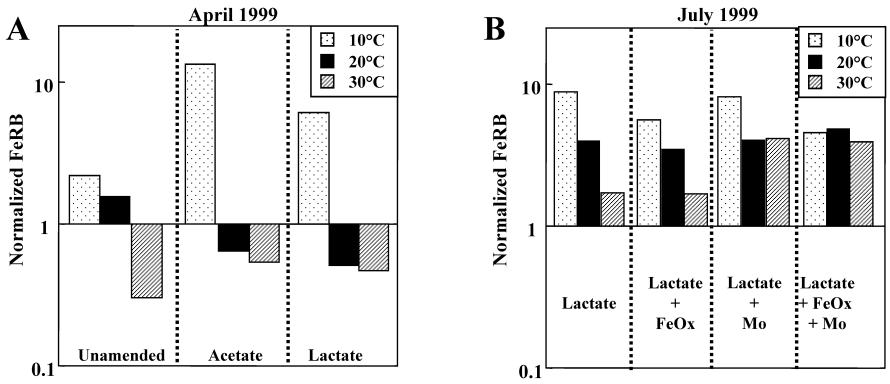

Figure 9. Average population densities of FeRB normalized to initial CFU counts (Table 2) in incubation experiments of sediment collected in (A) April 1999 and (B) July 1999. For comparison with April 1999 data, which was not sampled until day 4 (Table 2), data shown in (B) exclude anomalously high FeRB CFU counts measured on day 2. FeRB populations in heat-killed controls were below detection throughout the experiments. In this and in subsequent figures bars indicate incubation of slurries at 10 °C (stipled), 20 °C (solid) and 30 °C (striped).

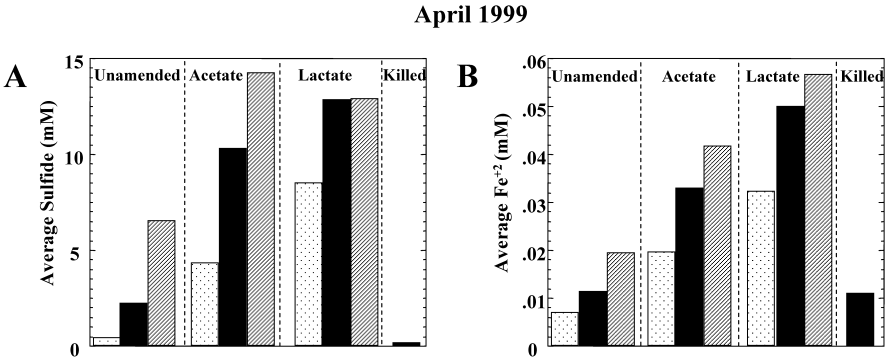

Figure 10. Average concentrations of (A) sulfide (mM) and (B) Fe^{+2} (mM) in incubation experiments with sediment collected in April 1999. The average values were obtained by integrating over the duration of the experiment and dividing by the length of the experiment.

(Figure 11A), indicating reaction of sulfide with Fe(III) (hydr)oxides (e.g., Jacobson (1994); see also discussion below).

Sulfide accumulation in samples with molybdate amendments is similar to that observed for heat killed controls (Figure 11A), with the build-up of sulfide only occurring toward the end of the experiments (data not shown). In experiments with molybdate and sulfide additions, sulfide accumulation correlates inversely with temperature (Figure 12A), decreasing as FeRB population size increases. Like sulfide, dissolved Fe(II) build-up generally increases with increasing temperature (Figures 10B, 11B), except when sulfide and molybdate are added together (Figure 12B). With the addition of molybdate, which inhibits SRB and sulfide production,

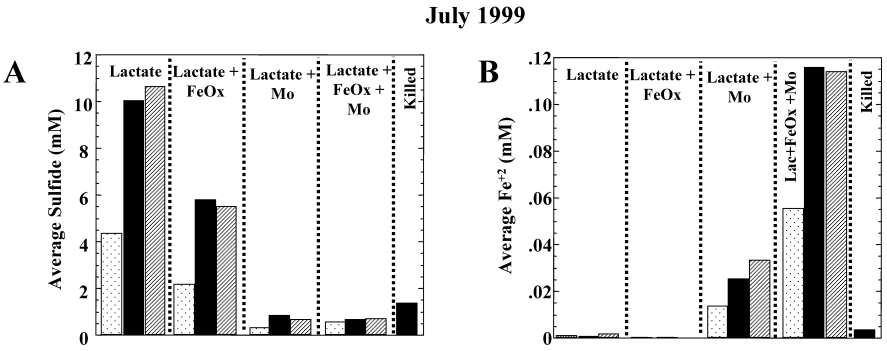

Figure 11. Average concentrations of (A) sulfide (mM) and (B) Fe^{+2} (mM) in incubation experiments with sediment collected in July 1999.

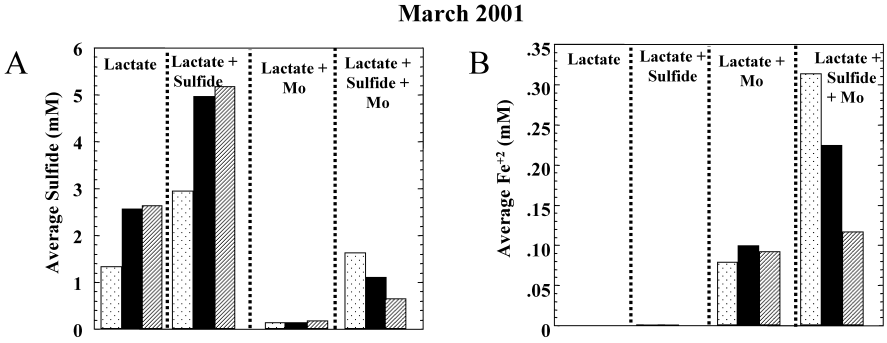

Figure 12. Average concentrations of (A) sulfide (mM) and (B) Fe^{+2} (mM) in incubation experiments with sediment collected in March 2001.

dissolved Fe(II) concentrations always increase relative to amendments with no molybdate added (Figure 11B, 12B).

Discussion

Controls on microbial sulfate reduction

Previous assessments of microbial respiratory pathways in saltmarsh sediments indicate that sulfate reduction is typically the predominant anaerobic organic matter degradation pathway (e.g., Howarth and Teal (1979) and Howarth and Hobbie (1982), Howarth and Giblin (1983), Howes et al. (1984), Howarth and Merkel (1984), King (1988), Hines et al. (1989), Kostka et al. (2002b)). The SRR measured at the sites on Sapelo Island where we collected our data are among the highest values ever reported (Kostka et al. (2002b); Figure 4D), and the SRB-targeted rRNA account for a very large percentage of the total prokaryotic rRNA, especially in the upper 10 cm of sediment (Figure 4C). Direct comparison of total carbon res-

Table 3. Change in alkalinity concentration ratioed to the change in sulfate concentration from the beginning to the end of each sediment slurry experiment.

Experiment	ΔAlkalinity/ΔSulfate at 10 °C, 20 °C, 30 °C
10 mM Lactate (April)	2.6, 2.5, 2.6
10 mM Lactate (July)	1.8, 2.3, 1.8
10 mM Lactate (March)	1.1, 2.6, 2.6

piration rates and sulfate reduction rates at these sites also shows that most organic carbon decomposition is coupled to sulfate reduction (see Table 1 in Kostka et al. (2002b)). The absence of dissolved sulfide in the upper portion of the sediments (Figure 4A), in spite of the high SRR, reflects its rapid removal through oxidation by oxygen or iron and manganese (hydr)oxides (e.g., Aller and Rude (1988) and Myers and Nealson (1988), Burdige and Nealson (1986), Jørgensen (1990), Jørgensen and Bak (1991), Elsgaard and Jørgensen (1992), Canfield and Des Marais (1993), Jacobson (1994), Thamdrup et al. (1994), Urban et al. (1994), von Gunten and Furrer (2000)). Efficient reoxidation of sulfide is also promoted by the intense bioirrigation of the saltmarsh sediments (Meile et al. 2001; Koretsky et al. 2002).

Sulfate reduction rates exhibit greater seasonal variability at the vegetated ponded marsh and levee sites than at the unvegetated creek bank site, while higher SRR are observed at the densely vegetated levee site relative to the more sparsely vegetated ponded marsh site (Figure 6B). The seasonal variations in SRR at the ponded marsh and levee sites further correlate with seasonal changes in aboveground *Spartina* biomass measured at similar sites on Sapelo Island (Dame and Kenny 1986; Dai and Wiegert 1996). Thus, primary production by marsh vegetation appears to exert a major control on the seasonal and spatial trends in SRR. At vegetated sites, roots of actively growing *Spartina* may represent a major source of labile organic carbon for the microbial populations inhabiting the sediments (Hines et al. 1989, 1999; Hines 1991). Sulfate reduction fueled by the release of labile organic compounds by roots has also been reported for other intertidal sedimentary environments (e.g., Isaksen and Finster (1996) and Holmer and Nielsen (1997)).

The incubation experiments in the present study confirm that the availability of labile organic carbon is an important limiting factor for sulfate reduction activity in the saltmarsh sediments: amendments of lactate or acetate result in higher SRR (Figure 7). According to the reaction,

$$3SO^{-2}_{4(aq)} + 2C_3H_5O^{-}_{3(aq)} \rightarrow 3HS^{-}_{(aq)} + 6HCO^{-}_{3(aq)} + H^{+}_{(aq)}, \quad (1)$$

the complete oxidation of lactate by sulfate yields 2.67 moles of alkalinity for each mole of sulfate consumed. The ratios of sulfate consumed to alkalinity produced measured in the slurry incubations amended with lactate only are shown in Table 3. Most ratios are close to the theoretical value of 2.67, as expected if bacterial sulfate reduction dominates heterotrophic activity.

The seasonal oscillations in SRR measured at the field sites, particularly at the vegetated ponded marsh and levee sites, correlate closely with changes in mean atmospheric daily temperature (compare Figures 3 & 6B). As the latter also correlates with primary production by *S. alterniflora*, the effect of temperature on the activity of SRB could be indirectly related to the availability of labile organic matter in the sediments. The sediment incubations, however, demonstrate that temperature also directly influences SRR. When temperature is increased from winter (10 °C) to summer (30 °C) values, sulfate reduction activity increases even when no lactate or acetate is added (Figure 7). Thus, the annual cycles of temperature and primary production together explain the observed seasonal variability of SRR.

As the rates shown in Figure 7 are derived from the decrease in sulfate concentration over time, SRR could be underestimated if sulfide is rapidly reoxidized to sulfate by Fe or Mn (hydr)oxides. However, previous work suggests that reoxidation of sulfide by solid phase Mn or Fe primarily yields polysulfides, thiosulfate, S° or FeS, rather than sulfate (Pyzik and Sommer 1981; Burdige and Nealson 1986; Yao and Millero 1996; von Gunten and Furrer 2000). This agrees with the observation that addition of amorphous Fe(III) (hydr)oxide to the slurries does not change the consumption of sulfate (Figure 7), but significantly reduces the accumulation of dissolved sulfide (Figure 11A).

Controls on microbial iron reduction

The changes in population size of culturable FeRB at the field sites exhibit a very different seasonal dependency compared to sulfate reduction. FeRB populations are lowest during summer and highest during spring (Figure 6A). Thus, temperature and organic matter availability do not appear to be the primary factors controlling the population densities of FeRB in the saltmarsh sediments. Furthermore, the decline in FeRB population during summer does not correlate with a corresponding decrease in Fe(III) availability in the sediments, because AEF, a proxy for bioavailable iron (e.g., Lowe et al. (2000)), is not exhausted at any of the sites in the transition from winter to summer (Figure 5E). The high Fe(II) pore water concentrations at the creek bank and levee sites also indicate active Fe(III) reduction in summer (Figure 5B).

Pore water data alone are not sufficient to determine whether Fe^{+2} is produced via microbial or abiotic Fe(III) reduction. Similarly, variations in culturable FeRB population sizes do not necessarily imply corresponding changes in microbial iron respiration. Nonetheless, the systematic, order-of-magnitude decline of the culturable FeRB populations during summer point to a possible decrease in the relative importance of microbial iron reduction when SRR reaches its maximum value.

Results of the sediment incubation experiments closely parallel the field observations. In the presence of active SRB, growth of FeRB is not stimulated by an increase in temperature or in the availability of organic matter availability or amorphous Fe(III) (hydr)oxides. In fact, as observed in the field, FeRB populations in the experiments decline when temperature increases (Figures 8A, 9A, 9B). Addition of labile organic matter to the sediment slurries sometimes leads to an initial

growth of the culturable FeRB population, but this is followed by a decline in FeRB population size at a rate exceeding that observed in unamended sediments, particularly at 30 °C. The incubation experiments also indicate that microbially available Fe(III) (hydr)oxides are not the primary factor limiting growth of the FeRB; addition of amorphous Fe(III) (hydr)oxide in the presence of active SRB does not increase the FeRB population size (Figure 9B). The laboratory data thus confirm that conditions leading to higher SRR limit the propagation of FeRB.

In the presence of reactive Fe(III) (hydr)oxides, dissimilatory Fe(III) reduction should be an energetically more favorable respiration pathway than sulfate reduction and, hence, FeRB should outcompete SRB for energy substrates. This thermodynamic argument, however, ignores the fact that SRB produce sulfide, which is an effective reductant of Fe(III) (hydr)oxides (Berner 1964; Goldhaber and Kaplan 1974; Pyzik and Sommer 1981; dos Santos Afonso and Stumm 1992; Peiffer et al. 1992; Yao and Millero 1996; von Gunten and Furrer 2000). Thus, rather than through direct competition for available organic matter, SRB may limit microbial Fe(III) reduction by promoting the abiotic reduction of reactive Fe(III) oxides (e.g., Burdige (1993), Jacobson (1994) and Wang and Van Cappellen (1996)).

Chemical data from the incubation experiments further indicate that dissolved sulfide, added to the slurries or produced by SRB, reacts with Fe(III) (hydr)oxides. Addition of Fe(III) (hydr)oxides does not affect SRR (Figure 7) or the population density of FeRB (Figure 9B), but it results in a significantly lower accumulation of dissolved sulfide (Figure 11A). The absence of build-up of dissolved Fe(II) in slurries amended with amorphous Fe(III) (hydr)oxides (Figure 11B), or in experiments in which sulfide is added (Figure 12B), further implies that reaction between dissolved sulfide and Fe(III) (hydr)oxides leads to precipitation of Fe(II) sulfides. When molybdate is added, sulfide production by SRB and therefore precipitation of Fe(II) sulfides should be inhibited. This is consistent with the observed accumulation of dissolved Fe^{+2} in the molybdate-amended slurries (Figures 11B, 12B).

The negative impact of SRB on the propagation of FeRB is eliminated altogether when sulfate reduction is inhibited by molybdate (Figure 8C). That this impact is related to the production of dissolved sulfide is supported by the results from the sediment slurry incubations to which dissolved sulfide is added (Figure 8E, 8F). Furthermore, the accumulation of dissolved Fe(II) in molybdate amended slurries (Figures 11B, 12B) implies that FeRB actively reduce Fe(III) (hydr)oxides when SRB are inhibited. Thus, both chemical and microbial data support a dynamic (kinetic) competition between microbial and chemical iron reduction pathways, which is modulated by the production of sulfide by SRB.

Conclusions

Anaerobic respiration in the saltmarsh sediments of Sapelo Island follows a pronounced seasonal cycle. The rate of sulfate reduction, the predominant pathway of organic matter degradation, is highest in summer and lowest in winter, at the three

sites studied. Slurry incubation experiments provide direct evidence that the activity of SRB correlates positively with temperature as well as with the availability of easily degradable organic compounds. Thus, coincident cycles of air temperature and primary production explain the observed seasonal cycle of sulfate reduction across the marsh.

Pore water (Figures 4, 5B) and solid phase (Figure 5E) data, as well as rate estimates by Kostka et al. (2002b), indicate active turnover of Fe(III) (hydr)oxides at the three sites. However, as sulfate reduction oscillates seasonally in response to variations in temperature and availability of labile organic matter in the sediments, FeRB are forced into a seasonal cycle characterized by a minimum in culturable population density during summer. A consistent behavior is seen in slurry experiments: treatments that enhance SRR, namely higher temperature and labile organic carbon additions, adversely affect growth of FeRB populations. However, when sulfate reduction is inhibited by molybdate, FeRB propagation in slurry experiments no longer depends inversely on temperature. Rather, FeRB are stimulated by additions of lactate and amorphous Fe(III) (hydr)oxides, especially at 30 °C, the temperature representative of summer conditions.

Sulfate reduction inhibits propagation of FeRB via production of dissolved sulfide which chemically reduces bioavailable Fe(III) (hydr)oxides. The sulfide-mediated inhibition of microbial iron reduction is particularly strong in saltmarsh sediments due to the high SRR, and the close proximity of active FeRB and SRB in the upper sediment layers resulting from the numerous roots and burrows that create sharp horizontal redox gradients. This forces a seasonally-oscillating competition for Fe(III) (hydr)oxides between microbial and chemical Fe(III) reduction pathways.

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