Relative contributions of sulfate- and iron(III) reduction to organic matter mineralization and process controls in contrasting habitats of the Georgia saltmarsh

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Abstract

The objectives of this study were to partition out the predominant anaerobic respiration pathways coupled to carbon oxidation and to further elucidate the controls of anaerobic carbon respiration in 3 major saltmarsh habitats at Skidaway Island, GA; the short form of *Spartina alterniflora* (SS), the tall form of *S. alterniflora* (TS), and unvegetated, bioturbated creekbank (CB). Geochemical analysis of pore water and solid phase constituents revealed that the SS site experienced highly reducing conditions with two orders of magnitude higher pore water sulfide inventories (1.884 mmol m^{-2}) than TS (0.003 mmol m^{-2}) and CB (0.005 mmol m^{-2}), respectively. Conversely, reactive Fe(III) inventories at TS (2208 mmol m^{-2}) and CB (2881 mmol m^{-2}) were up to 7-9 times higher than at SS (338 mmol m^{-2}). Incubations and intact core experiments indicated that sulfate reduction accounted for 95% (SS), 37% (TS), and 66% (CB) of total anaerobic respiration.   There was no detectable Fe(III) reduction at SS, while Fe(III) reduction accounted for up to 70 % of carbon oxidation in the 3-6 cm depth interval at TS and 0-3 cm depth of CB, and on average, approximately 55% of carbon oxidation over two-thirds of marsh surface area. Laboratory manipulations provided further evidence for the importance of Fe(III) reduction as the accumulation rates of fermentation products were high when Fe(III) reduction was inhibited by removing the Fe(III) minerals from highly bioturbated CB sediments with higher Fe(III) mineral contents. Anaerobic carbon oxidation, sulfate- and Fe(III)-reduction rates appeared to be highest at the TS site during active plant growth in summer. Overall results suggest that bioturbation by macrofauna is the overriding factor in modulating the pathway of carbon mineralization in the saltmarsh, whereas availability of organic substrates from plants is a key factor in controlling the carbon oxidation rate.

1. Introduction

Microbial respiration (i.e., mineralization of organic matter) represents a transfer of elements from organic matter to inorganic pools (Capone and Kiene, 1988; Jahnke and Craven, 1995). In estuarine environments, benthic microbial respiration releases a substantial amount of inorganic nutrients, which support biological productivity and food web processes in the water column through benthic-pelagic coupling (Bulleid, 1984; Hopkinson et al., 2001; Kemp and Boynton, 1984; Lawrence et al., 2004; Marcus and Boero, 1998; Rocha, 1998). In addition, highly productive salt marshes with net primary productivity exceeding 1,000 g C m^{-2} yr^{-1} (Alongi, 1998; Dame and Kenny, 1986; Schubauer and Hopkinson, 1984) may play a significant role in the outwelling process which supports offshore productivity (Odum, 2000; Valiela et al., 2000). Therefore, quantification of the controls of microbial respiration is crucial to our understanding of the C cycle and biogeochemical dynamics in coastal marine environments.

The combination of an enrichment of organic matter and relatively impermeable clay minerals results in the rapid depletion of oxygen within the top few mm depth of saltmarsh sediments (Furukawa et al., 2004; King, 1988), and thus most organic matter remineralization occurs under anaerobic conditions (Alongi, 1998; Howarth, 1993). The terminal decomposition of organic matter in marine sediments is performed by a variety of respiration processes, and the intensity of each process depends on the availability of organic substrates and terminal electron acceptors (i.e., nitrate, Mn(IV), Fe(III) and sulfate) (Jørgensen, 2000; Thamdrup and Canfield, 2000). Because of the abundance of sulfate in seawater (ca. 25 - 28 mM), sulfate reduction rates have been measured in saltmarshes on the east coast of the U.S. for nearly three decades (Hines et al., 1989; Howarth and Giblin, 1983; Howarth and Teal, 1979; Howes et al., 1984; King, 1988; Kostka et al., 2002a, 2002b), and sulfate reduction is still widely considered to be the predominant pathway of carbon oxidation in saltmarsh sediments (Alongi, 1998; Howarth, 1993). Contrary to the view that saltmarsh sediments are primarily anoxic and sulfidic, the emerging paradigm is that they are a mosaic of temporally and spatially fluctuating redox environments, where oxidants, reductants, and nutrients are rapidly recycled and replenished (Bull and Taillefert, 2001; Dollhopf et al., 2005; Gribsholt et al., 2003; Koretsky et al., 2005). A growing database indicates that saltmarsh sediments also tend to be rich in Fe(III) minerals (Kostka and Luther, 1995; Kostka et al., 2002a, 2002b), allowing Fe(III) reducing bacteria to outcompete sulfate reducing bacteria for carbon substrates thereby dominating anaerobic carbon oxidation, especially in highly vegetated and bioturbated areas of the marsh (Gribsholt et al., 2003; King and Garey, 1999; Koretsky et al., 2003; Kostka et al., 2002b; Lowe et al., 2000).

Salmarshes are exposed to a large number of physical, chemical and biological forcings, resulting in complex biogeochemical cycles. Plant roots and shoots act as a conduit for geochemical exchange, providing a primary source of organic matter (Hines *et al.,* 1989, 1994), and injecting oxygen into subsurface sediments (Dacey and Howes, 1984; Morris and Whiting, 1985). Through their burrowing and feeding activities, macrofauna also act to increase surface area exposed to overlying water and rework surface sediments (Aller, 2001; Kristensen, 2001). Thus, macrobenthic activities increase the supply of reactants for microbially-mediated diagenetic reactions, such as electron acceptors and carbon substrates, while stimulating the removal of toxic metabolites such as dissolved sulfide. However relative significance of those factors in controlling rate and pathways of microbial carbon oxidation has not been delineated extensively in major habitats of the saltmarsh.

Spatial variability and the lack of direct rate measurements over the appropriate spatial scales have confounded ecosystem-wide estimates of carbon cycling in the saltmarsh. Most previous studies have examined a limited spatial area within the marsh and relatively few have incorporated extensive comparisons between marsh habitats delineated by the dominant flowering plant, *Spartina*, and the principal bioturbating organism, the fiddler crab or *Uca* (King, 1988; Kostka *et al.,* 2002a, 2002b). Therefore, the objectives of this study were to: (1) experimentally determine the rates and predominant pathways of microbial respiration coupled to the terminal decomposition of organic matter in sediments across the major habitats of a Georgia saltmarsh; and (2) further test the concept that rapid Fe cycling, facilitated by macrobenthic activities, allows microbial Fe(III) reduction to outcompete sulfate reduction in the presence of high levels of sulfate and Fe(III).

2. Materials and methods

2.1. Study area

The study was conducted at the Saltmarsh Ecosystem Research Facility (SERF) near the Skidaway Institute of Oceanography, Skidaway Island, Georgia in July 2002 (Gribsholt *et al.,* 2003; Kostka *et al.,* 2002b). Sediment temperature ranged from 28 to 31°C, and salinity of the seawater in the tidal creek was 31 psu. The area is dominated by a nearly monospecific stand of smooth cordgrass, *Spartina alterniflora,* although stands of *Juncus romerianus* can be found along the upper fringe of the marsh. The mud fiddler crab (*Uca pugnax*) was the dominant macrofauna responsible for bioturbation in the area (Kostka *et al.,* 2002b). Three distinct saltmarsh habitats were identified according to the abundance of crab burrows, type of vegetation and hydrology (Kostka *et*

al., 2002b): (1) the site covered by the short form of *Spartina alterniflora* (SS) exposed to relatively weak tidal flushing and bioturbation; (2) the site covered by the tall form of *S. alterniflora* (TS) that is abundant along the well-drained levee near a large tidal creek; and (3) unvegetated, but highly bioturbated tidal creek bank (CB). The surface area of the SERF marsh sampled (27,534 km^2) was determined from aerial photographs using the 233m boardwalk as a reference for scale. Surface area of SS, TS and CB sites was 9,062 m^2 (33%), 10,715 m^2 (39%) and 7,757 m^2 (28%), respectively. The average height of *Spartina* was nearly four times greater at TS (93.9 cm) in comparison to SS (26.0 cm), while stem density was higher at SS site (243 stems m^{-2}) than at TS (74 stems m^{-2}) (Smith et al. in preparation). Burrow density at the TS (314 m^{-2}) site was 1.7 and 6 times higher than at CB (184 m^{-2}), and SS (53 m^{-2}) (Smith *et al.,* in preparation). The SERF marsh contained muddy sediments with a porosity (0.72 to 0.88) and density (1.28 to 1.36 g cm^{-3}) that were similar for the three habitats sampled, but organic matter content, as measured by loss on ignition (LOI), was lower at CB (11%) than SS (15%) or TS (15%).

2.2. Experimental design and sample collection

To assess the across-habitat variation in biogeochemical cycling we established six replicate 2 m x 6 m plots in each habitat type. The plots were placed randomly with an area of approximately 10,000 m^2 using a random numbers table. The minimum distance between plots was 10 m. For the purpose of extrapolating to the larger marsh, we assumed the rates measured in our study plots were characteristic of similar habitat types elsewhere in the marsh. For porewater/ solid phase geochemical profiling and sulfate reduction rate measurements, each sampling event required three days, over which we collected a single core from each of 2 plots per habitat and day for a total 18 cores. Cores were analyzed independently from all 6 study plots in each habitat. Sampling was performed randomly at least 5 cm away from plant roots or burrows at all sites. Since the root mass was too dense at the SS site, we could not sample without the roots. This sampling strategy may have underestimated the significance of Fe(III) reduction because Fe(III) reduction in burrow walls and the rhizosphere accounted for almost 100% of C oxidation at these sites (Gribsholt et al 2003). Because of the large amount of analytical work necessary to partition out respiration pathways during rate measurements, only duplicate sampling stations were sampled on a single day for sediment incubations that included direct Fe(III) reduction rate measurements. For the purpose of extrapolating to the larger marsh, we assumed the rates measured in our study plots were characteristic of similar habitat types elsewhere in the marsh. We chose not to analyze the data statistically, but instead to focus on trends showing large differences between sites.

Sediment samples for geochemical analysis were collected using polycarbonate cores (6 cm i.d. x 15 cm length), and cores were immediately sealed with butyl rubber stoppers, and cooled on ice until processed in the laboratory. Within 3 hours of sampling, cores were transferred to a nitrogen filled glove bag, where the sediment was sectioned. The sediment was loaded into polypropylene centrifuge tubes in a N_2-filled glove bag. The tubes were tightly-capped and centrifuged for 15 min. at $5000 \times g$. After reintroduction into the glove bag, pore waters were sampled and filtered through 0.2 µm cellulose acetate syringe filters.

2.3. Pore water analyses

Pore water for the determination of total CO_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. The samples were stored at 4^oC after adding 18 µl of $HgCl_2$ (125 mM) to eliminate the interference of hydrogen sulfude (Lustwerk and Burdige, 1995), and analyzed within a few days of sampling by flow injection with conductivity detection (Hall and Aller, 1992). Dissolved Fe^{2+} in pore water fixed with HCl (0.1N) was determined by colorimetry with a ferrozine solution (Stookey, 1970). Dissolved sulfide was determined on filtered pore water after precipitation with Zn acetate using the methylene blue method (Cline, 1969). Sulfate concentrations were measured in acidified pore water using ion chromatography (Dionex DX-500). Organic acid analyses were carried out on filtered pore water using ion exclusion chromatography with conductivity detection on a Dionex DX-600 ion chromatography. Standard calibration curves were obtained for the following organic acids: oxalate, pyruvate, citrate, malonate, malate, glycolate, formate, lactate, acetate, succinate, isobutyrate, and propionate (detection limits ranged from 1 to 10 µM). Previously Kostka *et al* (2002a) tested the potential contamination of organic acids during filtration, and revealed that less than a few micromolar of any organic acid was produced.

2.4. Solid phase analyses

For determination of solid phase Fe, 6 replicate syringe cores were taken at each site (i.e., 2 cores per site and day), sealed with rubber stopper, and frozen at -20 ^oC until laboratory analysis. In the anaerobic chamber, the cores were sectioned down to 10-cm depth at 2-cm intervals. Wet chemical extraction was used to determine the poorly crystallized Fe(III) oxide minerals. Total Fe was extracted from air-dried sediment in a 0.2 M oxic oxalate solution (pH 3) for 4 hours (Thamdrup and Canfield, 1996), and Fe(II) was extracted in anoxic oxalate (Phillips and Lovley, 1987). Total oxalate-extractable Fe and Fe(II) were determined with ferrozine as described above. Solid Fe(III) was defined as the difference between total Fe and Fe(II). Calibration experiments with pure Fe

phases have confirmed the selectivity of this extraction method towards poorly crystalline Fe phases (Canfield, 1989; Kostka and Luther, 1994). Sediment fixed with Zn acetate from the SO_4^{2-} reduction measurement was used for the determination of total reduced sulfur (TRS), which includes acid volatile sulfide (AVS = FeS + H_2S) and chromium-reducible sulfur (CRS = S^o + FeS_2). Sulfide was determined using single-step distillation with cold 12M HCl and boiling 0.5 M Cr^{2+} solution (Fossing and Jørgensen, 1989).

2.5. Rate measurements

For the measurement of anaerobic carbon mineralization rates, sediment from the 0-6 cm depth interval was collected in July, 2002, and February and September, 2003, using a trowel into a polypropylene bag that was immediately sealed with no head space and kept cool in an ice chest. Within 3 hours of sampling, sediment was homogenized and loaded into 50-ml centrifuge tubes without headspace in a N_2-filled glove bag. The tubes were incubated at in situ temperature in the dark. The tubes were then sacrificed at regular intervals, and the pore waters were extracted by centrifugation and filtered as described above. Anaerobic carbon respiration rates were determined from the accumulation of CO_2 in the pore water as described above. To acquire independent information on the significance of sulfate reduction to anaerobic microbial respiration, sodium molybdate, an inhibitor of sulfate reduction, was added (final conc., 28 mM) to a separate set of the incubation treatments. To measure the Fe(III) reduction rate, the sediment remaining after pore water retrieval from the incubation experiment was homogenized under a N_2 atmosphere, and Fe(II) was extracted in oxalate as described above. Fe(III) reduction rates were determined by the linear regression of Fe(II) concentration with time.

Rates of sulfate reduction were determined by the radiotracer method of Jørgensen (1978). Briefly, six-replicate intact cores (10 cm long with 2 cm i.d.) at each site were acquired (2 cores per day), and located on ice for transport to the laboratory. Two μCi of $^{35}SO_4^{2-}$ was injected into injection port at 1-cm intervals, and cores were incubated for 3 hours at in situ temperature. At termination, the sediment was sliced into sections, fixed in Zn acetate (20%), and frozen until processed in the laboratory. The reduced ^{35}S was recovered by distillation with boiling acidic Cr^{2+} solution according to Fossing and Jørgensen (1989).

2.6. Controls of respiration rates

A series of incubation experiments were further performed at the TS and CB sites to elucidate relative significance of organic and inorganic substrates and electron acceptors (i.e., FeOOH and NO_3^-) in controlling respiration rates. Sediment samples from 0-6 cm depth were collected as described above, and were amended

with following treatments: acetate (f.c., 5 mM), glucose (f.c., 2 mM), nitrate (f.c., 5 mM), phosphate (f.c., 0.2 mM), FeOOH (f.c., 20 mM) and molybdate (f.c., 28 mM), respectively in the N_2 filled globe bag. Total CO_2 produced during the incubation was measured as described above. For the incubation treatments containing Fe(III), amorphous Fe(III) oxyhydroxide was prepared as described by Schwertmann and Cornell (2000) and added during the homogenization step. Reactive Fe(III) content of the sediments was checked before and after treatment using the wet chemical extractions described above.

2.7. Effect of bioturbation in controlling respiration pathways

In order to examine the competition between sulfate and Fe(III) reduction in the absence of any vegetation effects, we performed additional incubations and analyzed for the accumulation of small molecular weight carbon substrates along with CO_2. For this experiment, surface sediments were collected from contrasting areas of the CB habitat: (1) a bioturbated area between crab burrows, where Fe(III) concentrations were high and microbial Fe(III) reduction was previously shown to predominate over carbon oxidation, and (2) a non-bioturbated area where Fe(III) content was low and sulfate reduction predominated (Gribsholt *et al.,* 2003). Six incubation treatments were conducted in duplicate: unamended treatments from each area, molybdate amended treatments from each area, bioturbated sediment amended with hydrogen sulfide + molybdate, non-bioturbated sediment amended with amorphous Fe(III) oxyhydroxide (FeOOH) + molybdate. The assumption is that carbon substrates such as organic acids are reactants for microbial respiration and these products should accumulate when the predominant respiration processes are shut down by specific metabolic inhibitors or if electron acceptor is not available in sufficient amounts. Conversely, fermentation products will not accumulate when respiration processes are active and sufficient electron acceptors are present. Molybdate was added to 28 mM as a specific metabolic inhibitor of sulfate reduction. FeOOH (30 mM) was added to some treatments to alleviate electron acceptor limitation, while in additional treatments, Fe(III) minerals were titrated out of the sediment naturally by adding dissolved sulfide in low millimolar aliquots over a few hour period.

3. Results

3.1. Pore water chemistry

Overall, pore water inventories indicated that SS sediments were more reducing and contained elevated levels of mineralization products in comparison to the TS and CB sites. Inventories of pore water CO_2 and NH_4^+ constituents over the 0-6 cm depth interval were consistently 2-3 times higher at the SS site relative to the TS and

CB sites (Table 1). Pore water CO_2 and NH_4^+ typically displayed a trend of increased concentrations at 3-6 cm depth, especially at the SS site. Accordingly, overall depth integrated inventories of dissolved sulfide at the SS site were approximately two orders of magnitude higher than at TS and CB. Pore water Fe^{2+} accumulated to ten times lower levels at SS in comparison to TS and CB. Acetate concentrations, measured for only 0-3 cm depth interval, were four times higher at the TS site than at the SS and CB sites.

3.2. Solid phase sulfide and iron

Vertical profiles of TRS (total reduced sulfur) revealed clear gradients between the 3 sites (Fig. 1). Depth integrated (0-6 cm) TRS inventories at SS were 2-6 times higher in comparison to TS and CB (Table 2). TRS concentrations decreased with sediment depth to 5 cm at SS, whereas concentrations remained constant or increased with depth at TS and CB (Fig. 1). In contrast to TRS, inventories of reactive Fe(III) at the TS and CB sites (2208-2881 mmol m^{-2}) exceeded the inventory of the SS site (338 mmol m^{-2}) by 6-9 times (Table 2). Vertical distributions of the solid phase Fe at the SS site were constant with depth, and the concentrations were remarkably lower than that of the TS and CB site (Fig. 1). Fe(III) exceeded SO_4^{2-} at TS and CB, but not at the SS site. The majority of Fe and S in saltmarsh sediments is stored in the solid phase. Thus, perhaps the best indication of redox poise in the sediments is the ratio of oxidized Fe(III) minerals to reduced Fe minerals represented by TRS. Using this index, SS sediments were on average 15 to 37 times more reduced.

3.3. Total carbon mineralization rate

A seasonal comparison at the TS site showed that carbon mineralization rates in unamended sediment were highest in July (0.23 mM C hr^{-1}), followed by September (0.13 mM C hr^{-1}) and the lowest rate was observed in February (0.02 mM C hr^{-1}). A comparison between major habitats revealed that carbon mineralization rates measured at two sediment depths in July were 3 to 8 times higher at the vegetated sites (0.14 - 0.41 mM C hr^{-1}) in comparison to the unvegetated site (0.05 - 0.06 mM C hr^{-1}; Table 3), whereas little difference was observed between TS (0.0175 mM C hr^{-1}) and CB (0.0155 mM C hr^{-1}) in February (Table 3). Upon addition of molybdate, a specific metabolic inhibitor of sulfate reduction, C oxidation rates decreased by 95% in incubations of SS sediments, whereas little to no change was observed in incubations of TS or CB sediments (Fig. 2). At TS site, C oxidation rate in samples amended with Mo decreased by 25% at 0-3 cm depth and 42% at 3-6 c m depth (Table 3).

To further test the potential for substrate limitation of microbial respiration in the salt marsh, additional

enrichment incubation experiments were performed at different seasons and across habitats. Respiration rates in sediments amended with carbon substrates (acetate, glucose) were 2 to 4 times higher than in unamended sediments, whereas inorganic nutrients (nitrate and phosphate) or reactive iron did not have a substantial impact on anaerobic mineralization rates (Table 3). Results suggest that anaerobic microbial metabolism at TS site is controlled by the availability of organic substrates.

3.4. Carbon Substrate Turnover

We monitored the concentration of small molecular weight carbon substrates to check for the potential impact of carbon substrates released during homogenization on our respiration rate measurements. In our unamended incubations (represented in Fig. 2), a large accumulation of acetate (2 to 3 mM) was observed in incubations of SS sediments, whereas concentrations from TS or CB incubations were relatively low and constant with time (Fig. 3). Other substrates (lactate, formate, propionate) were detected in low micromolar amounts but these substrates did not accumulate significantly with time. These results indicate that in the SS incubations, high concentrations of fermentation products were released during homogenization of the dense root biomass, and thus CO_2 accumulation rates at SS are artificially elevated. Carbon mineralization rates for the SS site were therefore estimated from the reaction stoichiometry and sulfate reduction rates (Table 4), assuming that 100% of carbon mineralization was due to sulfate reduction. Depth-integrated carbon mineralization rates were thus approximately 5-6 times higher at TS (300 mmol C m^{-2} d^{-1}) as compared to SS (54 mmol C m^{-2} d^{-1}) and CB (64 mmol C m^{-2} d^{-1}).

We conducted further incubation experiments to show that the coupling of anaerobic respiration pathways to carbon turnover can be elucidated by manipulating Fe(III) mineral content or by inhibiting sulfate reduction. Incubations of surface sediments collected from CB in the bioturbated zone between crab burrows, where Fe(III) concentrations were high and microbial Fe(III) reduction was previously shown to predominate over carbon oxidation were contrasted with incubations of sediments collected from a non-bioturbated area of CB at least one meter from any crab burrow, where Fe(III) concentrations were shown to be low (< 5 μmol g^{-1}) and sulfate reduction was previously shown to dominate carbon oxidation (Gribsholt *et al.,* 2003). Only acetate and propionate accumulated to significant amounts amongst all six incubation treatments, indicating their importance as substrates for anaerobic respiration (Table 5). No carbon substrates accumulated in unamended incubations or in the molybdate/Fe(III) treatments of sediments collected from either location (with or without burrows), since either sulfate reduction or Fe(III) reduction was operative in those treatments (Table 5). In incubations of non-bioturbated sediments, carbon substrates only accumulated in response to addition of

molybdate (Table 5), indicating that sulfate reduction predominated over carbon turnover. Conversely, the same carbon substrates accumulated at much higher rates in incubations of bioturbated CB sediments when sulfide as well as molybdate was added in comparison the treatments with molybdate alone, indicating that Fe(III) reduction was more important to carbon substrate turnover in these sediments.

3.5. Rate of sulfate– and Fe(III) reduction

Sulfate reduction rates (SRR) at the SS site showed a rapid decrease with depth (Fig. 4), while the rates remained relatively constant with depth at TS and CB. Depth integrated rates were approximately 2 times higher at TS (46.2 mmol m^{-2} d^{-1}) as compared to SS (25.6 mmol m^{-2} d^{-1}) and CB site (21.1 mmol m^{-2} d^{-1}) (Table 4). Vertical profiles of sulfate reduction rate showed a similar trend to TRS distributions (Fig. 1), indicating that more sulfide accumulated where rates were higher. Fe(III) reduction rates were directly measured as the accumulation of extractable Fe(II) with time in sediment incubations. No Fe(III) reduction was detected in incubations of SS sediments as Fe(II) and total Fe concentration did not change with time in any of the treatments (data not shown). In contrasting sediments from the more heavily bioturbated TS and CB sites, Fe(III) reduction rates were rapid and showed similar trends to those of CO_2 accumulation (Table 4, Fig. 5). Fe (III) reduction rate (FeRR) at TS averaged 0.19 µmol g^{-1} hr^{-1} at 0-3 cm depth interval and 0.26 µmol g^{-1} hr^{-1} at 3-6 cm depth, while the rates at CB were 0.12 µmol g^{-1} hr^{-1} at 0-3 cm and 0.08 µmol g^{-1} hr^{-1} at 3-6 cm. Depth integrated (0 - 6 cm) total Fe(III) reduction rates were 2.5 times higher at TS site (458 mmol m^{-2} d^{-1}) as compared to CB site (181 mmol m^{-2} d^{-1}) (Table 4).

4. Discussion

4.1. Pathways of organic matter mineralization across habitat boundaries

Most studies of anaerobic respiration in saltmarshes on the east coast of the U.S. have been performed in a single habitat, usually vegetated by the short form of *Spartina* (Giblin and Howarth, 1984; Hines *et al.,* 1989; Howarth and Teal, 1979; Howes *et al.,* 1984, 1985; Kostka and Luther, 1995; Lord and Church, 1983; Luther and Church, 1988; Valiela and Teal, 1979). However, we observed that collectively the creek bank and the tall *Spartina* habitats cover two-thirds of marsh surface area at the Saltmarsh Ecosystem Research Facility (SERF), Skidaway Island, Georgia. We further observed that habitat type should have a major influence on the rates and

pathways of organic matter mineralization (Kostka *et al.,* 2002a, 2002b). Because relatively few studies on the carbon cycle in saltmarsh sediments have examined variability across major habitats in detail, ecosystemwide budgets may well have been biased toward measurements from a habitat which does not cover the majority of marsh surface. Therefore, we set out to experimentally partition the predominant anaerobic carbon oxidation pathways in all major habitats of the SERF marsh. Through this analysis, we tested the hypothesis that microbial Fe(III) reduction comprises a substantial carbon oxidation pathway on a marshwide scale.

In this study, we present several lines of evidence which indicate that: (1) anaerobic carbon mineralization pathways largely vary along habitat boundaries; and (2) microbial Fe(III) reduction is a substantial carbon oxidation pathway over the large scale in Georgia saltmarsh sediments. Carbon oxidation pathways showed large differences across the three habitats studied (Table 4). Direct Fe(III) reduction rate measurements yielded no detectable activity at the SS site, whereas microbial Fe(III) reduction accounted for up to 70 % of carbon oxidation in the 3-6 cm depth interval at TS and 0-3 cm depth interval at CB. Microbial Fe(III) reduction accounted for on average 55 % of carbon oxidation over two-thirds of marsh surface area (TS + CB sites) (Table 4). The lack of Fe(III) reduction activity at SS inferred that sulfate reduction dominates over carbon oxidation at this site, and this was corroborated by the fact that CO_2 accumulation was almost completely inhibited by the addition of molybdate, a specific metabolic inhibitor of sulfate reduction (Fig. 2). In contrast, molybdate had much less effect on C oxidation or Fe(III) reduction rates in incubations of TS and CB sediments (Fig. 2, 5). Based on the comparison of intact cores and homogenized sediment incubations, sulfate reduction comprised an average of 95 % of carbon oxidation at the SS site, 37 % at TS, and 66 % at CB over the two depth intervals sampled (Table 4). We extend previous studies (Gribsholt *et al.,* 2003; Kostka *et al.,* 2002a), to show that microbial Fe(III) reduction rates greatly exceed the reduction potential of dissolved sulfide produced by sulfate reduction in habitats that cover two-thirds of marsh surface area. Though previous studies have examined changes in microbial respiration rates across habitat boundaries (King, 1988; King and Wiebe, 1980; Skyring *et al.,* 1979), the corresponding change in carbon oxidation pathways and the marshwide significance of microbial Fe(III) reduction was likely not revealed because of the focus on sulfate reduction rates and the paucity of parallel rate measurements of CO_2 accumulation and Fe(III) reduction in a variety of habitats.

The comparison between independently-measured sulfate reduction rates, Fe(III) reduction rates, and carbon oxidation rates reveals a fairly good mass balance (accounting for 120 to 135% of C equivalents) in 3 out of 4 sets of TS and CB incubations (Table 4). However, in incubations of surface sediments from TS, only 43 % of carbon oxidation equivalents were accounted for by sulfate and Fe(III) reduction rate measurements. Several

reasons can be considered. First, these results imply that other terminal-electron-accepting processes (i.e., denitrification or MnO_2 reduction) could be substantial. Indeed, Bull and Taillefert (2001) observed maxima in pore water Mn at 1-5 cm depth using microelectrodes at the CB site of the SERF marsh, suggesting that Mn reduction is occurring there. Also, humic substances are abundant and may act as an important electron acceptor in the Georgia marsh, as has been suggested for other marshes (Neubauer et al 2005). Further, higher concentrations of acetate, a fermentation product, at the 0-3 cm depth of the TS site (Table 1) implied the potential significance of fermentation in C oxidation. We consider nitrate reduction to be less important to stimulate C oxidation since we observed generally low concentrations of pore water nitrate (usually < 3 μmol cm^{-3}; data not shown) that penetrate to a shallow depth at SERF, and nitrate amendment did not stimulate carbon oxidation rates in our incubations (Table 3).

In addition to the influence of alternate electron acceptors, the results may underscore the uncertainty in comparing parallel rate measurements in sediments that contain a high degree of inherent heterogeneity. For the Fe(III)-rich TS and CB sites, a comparison of carbon oxidation rates with and without molybdate addition would infer that microbial Fe(III) reduction is more important than our mass balance calculations indicate. For example, using the simple comparison of CO_2 accumulation rates to back calculate Fe(III) reduction rates, Fe(III) reduction would comprise nearly 100 % of carbon oxidation at CB (i.e., small change in CO_2 accumulation in presence of molybdate), whereas the rate comparison in Table 3 estimated 65 %. Therefore, while we acknowledge that our comprehensive approach does not account for all iron reduction activity, we have gone with the more conservative estimate for the significance of microbial Fe(III) reduction that is based upon direct rate measurements wherever possible. Using a similar comprehensive approach to this study involving rate measurements and sediment geochemistry, a larger number of studies in subtidal marine sediments have determined that Fe(III) reduction accounts for an average of 25 % of carbon oxidation (Canfield *et al.,* 2005; Thamdrup, 2000). Therefore, our results indicate that the contribution of microbial Fe(III) reduction to carbon cycling on a marshwide basis is higher than that shown for marine subtidal sediments, where the average contribution of Fe(III) reduction is similar to that of organotrophic oxygen respiration, and is only exceeded significantly by sulfate reduction (Thamdrup, 2000).

4.2. Controls of anaerobic respiration pathways.

It is well established that the competition between sulfate and Fe(III) reduction processes in aquatic sedimentary environments depends upon the relative availability of electron acceptors and organic substrates

(Canfield *et al.,* 2005; Megonigal *et al.,* 2004; Roden and Wetzel, 1996). In marine sediments, the contribution of Fe(III) reduction to carbon oxidation shows a strong relationship to the concentration of reactive Fe(III) (Thamdrup, 2000), and this relationship has been quantitatively described using an empirical function developed by Jensen *et al.* (2003). Using this same empirical function, a close coupling between Fe(III) content and the significance of Fe(III) respiration was confirmed in Georgia saltmarsh sediments by Gribsholt *et al.* (2003).

The mechanisms controlling the supply of reactive Fe(III) in marine sediments have not been completely elucidated. Since Fe(III) exists primarily as solid phase oxide minerals, the bulk of studies contend that Fe(III) is supplied to the zone of reduction by sediment mixing, either through bioturbation by macrofauna or through sediment resuspension by waves and currents (Canfield *et al.,* 2005). In addition, the plant rhizosphere may act to recycle Fe (Megonigal *et al.,* 2004; Weiss *et al.,* 2003). In agreement with our earlier studies that were limited to small areas of the marsh (Gribsholt *et al.,* 2003; Kostka *et al.,* 2002a), we propose that in concert with hydrology and to a lesser extent plant effects, fiddler crab burrowing accounts for differences in the partitioning of carbon oxidation pathways across major habitats in saltmarsh sediments. Crab burrows act to amplify geochemical exchange by increasing sediment surface area as well as through reworking of surface sediments (Bertness, 1985; Kristensen and Kostka, 2005; Montague, 1982). Geochemical parameters from the present study support this conclusion over the larger scale. In major habitats (TS, CB) where direct rate measurements showed that Fe(III) reduction was important to carbon oxidation (Table 4), Fe(III) was much more abundant and concentrations generally exceeded the 25 to 30 μmol cm^{-3} threshold over which empirical modeling has predicted Fe(III) reduction should dominate (Fig. 1; Jensen *et al.,* 2003). Fe(III) inventories exceeded sulfate by a factor of 1.5 to 2 at CB and TS, while at SS, the available Fe(III) was 3 times less than that of sulfate (Table 1, 2). Sulfate, in contrast, was never limiting in the SERF marsh as concentrations always exceeded 17 mM and were usually above 25 mM.

Laboratory manipulations of unvegetated sediments from the CB habitat provided further evidence in support of our conclusions that the competition between respiration pathways is likely dependent upon reactive Fe(III) concentration and the presence of burrows (Table 5). In incubations of Fe(III)-rich sediments from the bioturbated area of CB, removal of Fe(III) minerals stimulated a much higher rate of carbon substrate accumulation in comparison to molybdate inhibition of sulfate reduction. In contrast, in sediments sampled from a Fe(III)-poor, non-bioturbated area, significant turnover rates were only measured in the presence of molybdate, and carbon turnover was suppressed by the addition of reactive Fe(III). This suggests that in non-bioturbated CB sediments, dissimilatory Fe(III)-reducing bacteria are limited by low Fe(III) concentrations and

they respond rapidly to Fe(III) amendment by metabolizing fermentation products.

Evidence from radionuclide profiles and modeling studies in saltmarshes along the east coast of the U.S. further support our conclusions. It has long been known that burrowing by fiddler crabs largely determines sediment reworking rates in saltmarsh sediments (Sharma *et al.,* 1987; Vogel et al., 1996). In the North Inlet marsh, South Carolina, McCraith *et al.* (2003) observed that fiddler crabs had a substantial influence on sediment reworking in creekbank habitats where reworking rates were ten times higher than at mid-marsh sites vegetated by short *Spartina*. Furukawa *et al.* (2004) applied a multicomponent reactive transport model to biogeochemical data from the SERF marsh that was constrained by ecological data as well as measured profiles of major redox species. The calculated rate profiles supported the significant difference in the relative importance of Fe(III) and sulfate as terminal electron acceptors between sites with varying degrees of fiddler crab burrowing.

Unlike crab burrowing, the roots of *Spartina* mediate solute and not solid geochemical exchange (Hines, 1991; Mendelssohn and Morris, 2000), and microelectrode profiling has shown that oxygen is depleted within the first few millimeters of *Spartina* roots (Furukawa *et al.,* 2004; Holmer *et al.,* 2002). In addition, though the anatomy of *Spartina* plants allows for the passive and active transport of oxygen into the root zone (Howes and Teal, 1994; Hwang and Morris, 1991), the rate of internal oxygen transport is not sufficient and the roots shift to anaerobic pathways when the external oxygen supply is shut off. Nonetheless, a higher contribution of Fe(III) reduction to C oxidation (ca. 70%) at the root zone (3-6 cm depth) than at the surface layer (0-3 cm) of the TS site indicated that actively growing plants may also play a significant role in regenerating Fe oxide, thereby stimulating Fe(III) reduction (Table 4). Despite the spatially limited influence of O_2 supply via plant in the vegetated sediment, it is plausible to consider that roots are constantly growing into areas and dying; thus, root iron oxides deposited shortly before the soil sample was taken could have remained behind and stimulated Fe reduction. Gribsholt et al (2003) also demonstrated that Fe(III) reduction was dominating C oxidation process at the rhizosphere in the salt marsh. In this study, sediments were collected at least several centimeters from any visible roots, and therefore, our results may underestimate the contribution of Fe(III) reduction in C oxidation process in the TS site.

4.3. Controls of anaerobic carbon respiration.

Overall rate measurements revealed that total C respiration, sulfate- and Fe(III) reduction were highest at TS (Table 3, 4). The results are consistent with previous reports that the leakage of DOC from *Spartina* roots to

rhizosphere enhances microbial activity in the sediment (Hines *et al.,* 1989, 1999). Enhanced C oxidation at the SS site after the artificial release of acetate during sediment mixing (Fig. 2, 3) and lower rate measurements at the unvegetated CB site (Tables 3 and 5, Fig. 4) in addition to the higher respiration rate in the sediment amended with carbon substrates (acetate and glucose) (Table 3) further demonstrated that organic carbon limitation for the anaerobic microbial metabolism is a marsh-wide phenomenon. This is interesting because the saltmarsh is generally considered to be enriched in labile carbon substrates.   The turnover rate of acetate in acetate-amended sediment in Feb. 2003 was 0.034 mM hr^{-1} (data not shown), and was nearly equivalent to the total CO_2 produced (0.063 mM C hr^{-1}) during respiration (Table 3), indicating that acetate was likely the dominant substrate fueling microbial respiration. Hines *et al.* (1994) observed that acetate oxidation rates were small in comparison to sulfate reduction rates in marsh sediment, and certainly additional unidentified carbon substrates are important to anaerobic respiration in the SERF saltmarsh. However, our incubation data indicate that acetate and propionate are important electron donors, in corroboration with microbiological studies showing that acetate-utilizing taxa dominate over other anaerobic respiratory groups in saltmarsh sediments (Hines *et al.,* 1999; Megonigal *et al.,* 2004; Rooney-Varga *et al.,* 1997). Pore water acetate concentrations were also shown to positively correlate with sulfate reduction rates in previous studies of the Georgia marsh (Kostka *et al.,* 2002b). Since the leakage of labile carbon substrates from roots has been linked to the active growth stage of *Spartina* (Hines *et al.,* 1989), vegetation type and parameters regulating plant physiology (i.e., hydrology) are vital in determining spatial variations of anaerobic carbon respiration in the saltmarsh environment. Environmental implications of carbon substrate limitation of microbial respiration may be that the saltmarsh is a significant sink against the external loading of organic compounds, and thus may play a significant role as an environmental buffer between terrestrial and coastal environments.

Acknowledgements

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FIGURE LEGENDS

Fig. 1. Vertical distributions of total reduced sulfur (TRS), solid phase Fe(III) and Fe(II) by site (SS: habitat vegetated by short form of *Spartina alterniflora*, TS: tall form of *Spartina*, CB: unvegetated tidal creek bank)

Fig. 2. Anaerobic carbon respiration rates determined from the accumulation of total CO_2 in incubations of sediments collected from 3 major habitats of the SERF marsh in July, 2002.

Fig. 3. Accumulation of acetate in unamended sediment incubations represented in Fig. 2. No other carbon substrates besides acetate were observed to accumulate in significant concentrations in these incubations. Values represent the average concentration from duplicate incubations.

Fig. 4. Sulfate reduction rates measured using the radiotracer method in intact cores collected from 3 major habitats of the SERF marsh in July, 2002. Values are the mean (± one standard deviation) of six replicate core incubation (see method section).

Fig. 5. Rates of microbial Fe(III) reduction determined from the accumulation Fe(II) in incubations of sediments collected from the TS and CB habitats in July, 2002.

Hyun et al. – Fig. 1

1   **Hyun et al. – Fig. 2**

2

(A) SS (0-3 cm)

(B) SS (3-6 cm)

(C) TS (0-3 cm)

(D) TS (3-6 cm)

(E) CB (0-3 cm)

CB (3-6 cm)

Total CO$_2$ (mM)

Time, hr

Unamended
Mo-amended
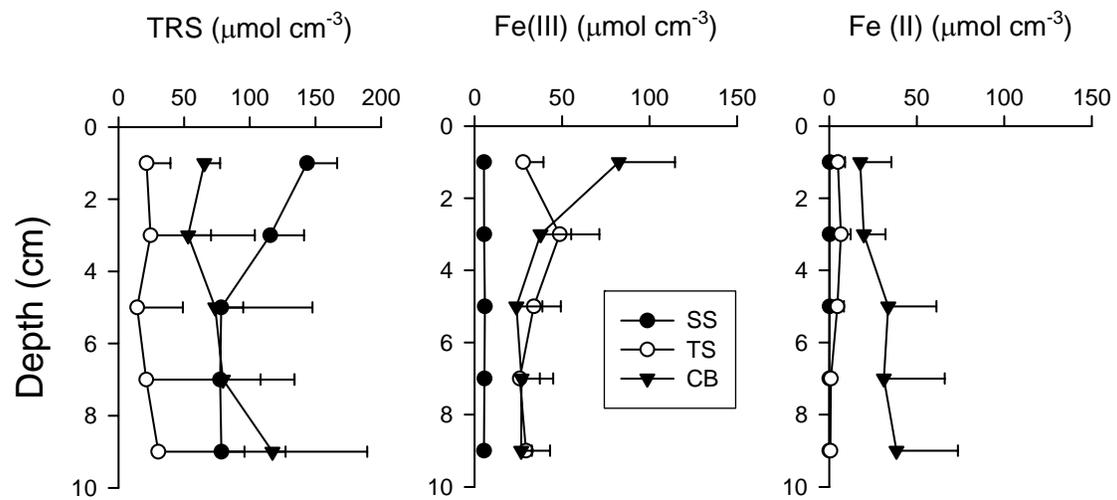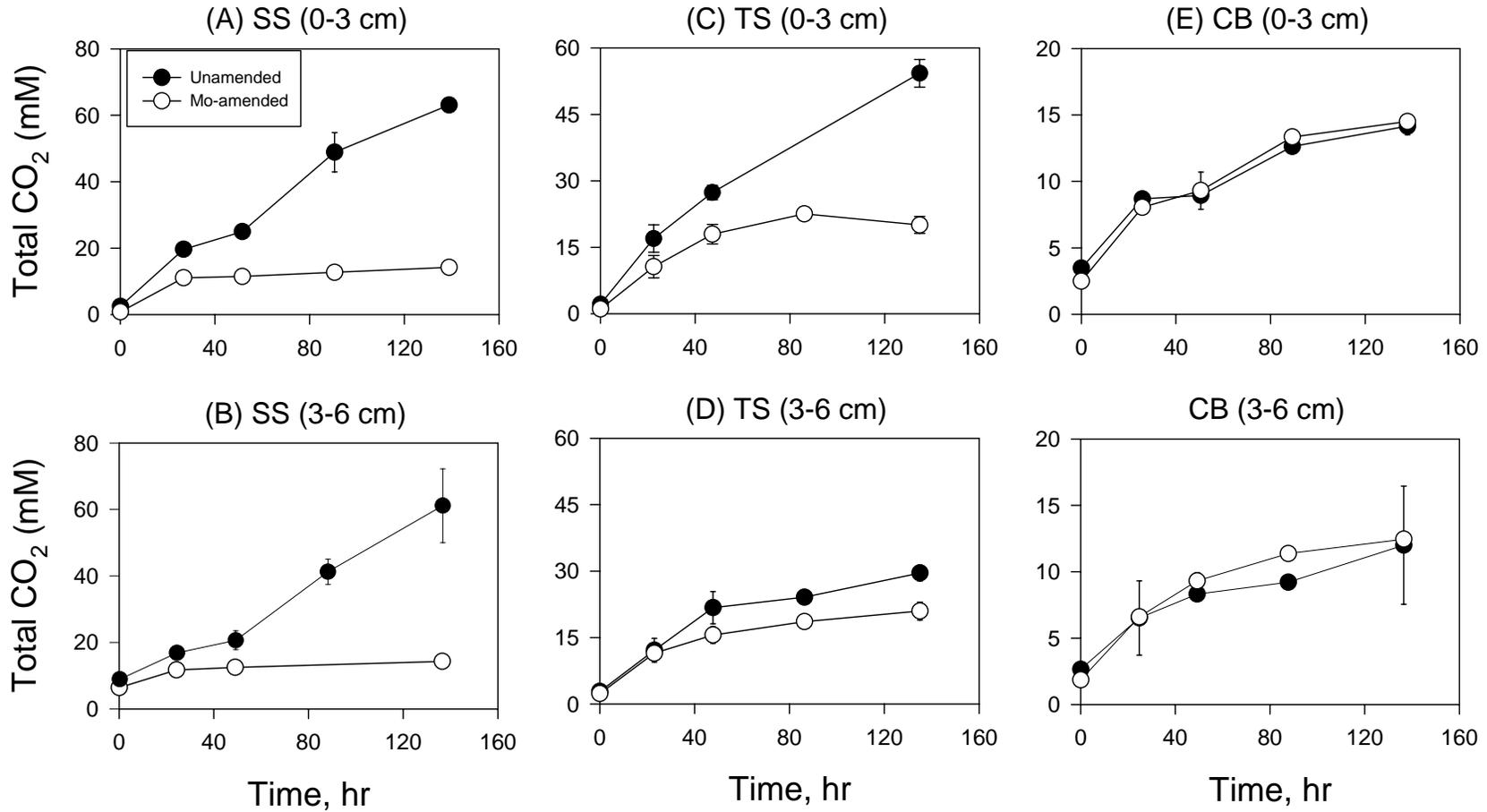
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4

5   **Hyun et al. – Fig. 3**

6
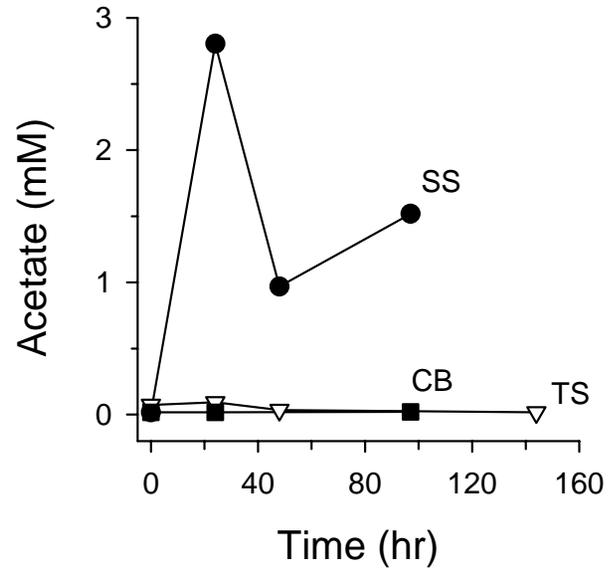
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14 **Hyun et al. – Fig. 4**

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17

Sulfate Reduction Rate (nmol cm^{-3}d^{-1})

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19   **Hyun et al. – Fig. 5**

TS (0-3 cm)

Y = 0.25 X + 43.77
r2 = 0.94

Y = 0.19 X + 41.88
r2 = 0.99

Fe(II), μmol g^{-1}

CB (0-3 cm)

Y = 0.12 X + 32.85
r2 = 0.87

Y = 0.17 X + 18.40
r2 = 0.86

TS (3-6 cm)

Y = 0.26 X + 64.82
r2 = 0.95

Fe(II), μmol g^{-1}

Time (hr)

CB (3-6 cm)

Y = 0.08 X + 34.69
r2 = 0.94

Time (hr)

●— Unamended   ○- Mo-amended

20
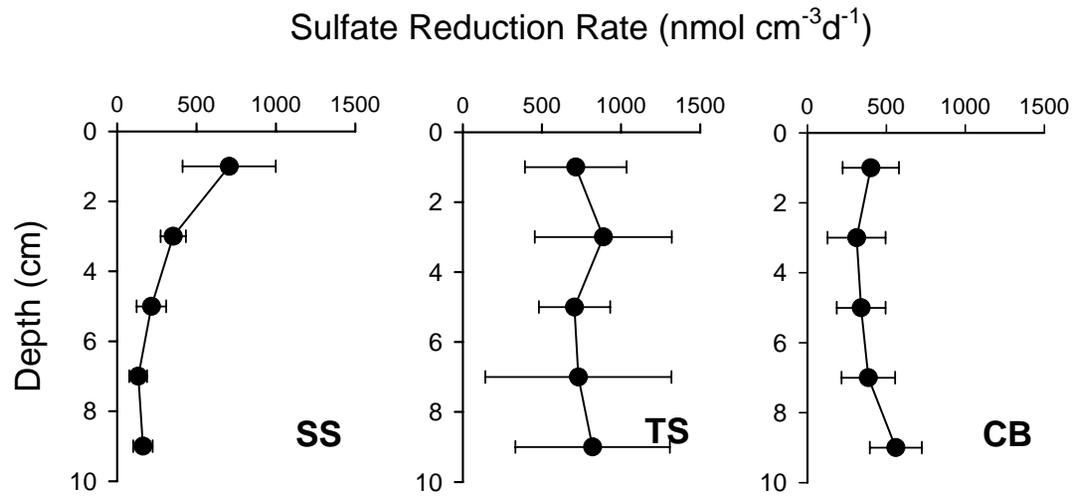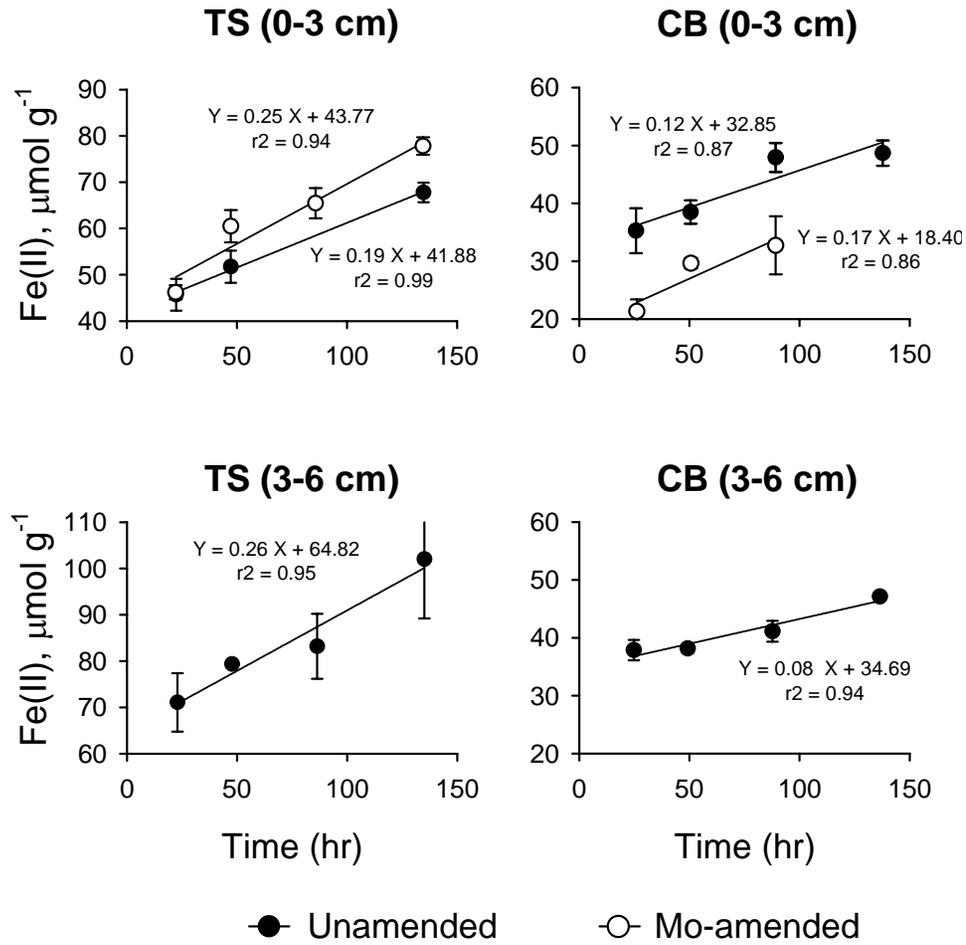
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22   **Hyun et al. – Table 1**

23

24

25   Table 1. Depth-integrated inventories (mmol m^{-2}) of pore water constituents by site

Site	Depth Range (cm)	CO_2	NH_4^+	SO_4^{2-}	HS^-	Fe^{2+}	Acetate
SS	0-3	140	1.92	632	0.212	0.90	0.51
	3-6	299	3.35	463	1.674	0.29	-
TS	0-3	60	0.79	755	0.002	10.78	2.19
	3-6	87	1.41	795	0.002	6.03	-
CB	0-3	73	1.10	690	0.004	5.56	0.51
	3-6	103	1.95	615	0.001	8.57	-

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27 **Hyun et al. – Table 2**

28

29

30 Table 2. Depth-integrated inventories (mmol m^{-2}) of solid phase constituents by site

31 (TRS = total reduced sulfur)

Site	Depth Range (cm)	TRS	Fe(II)	Fe(III)	Total Fe	Fe(III)/TRS
SS	0-3	4,029	7	165	172	0.04
	3-6	2,723	7	173	180	0.06
TS	0-3	672	165	1043	1208	1.55
	3-6	529	161	1165	1326	2.20
CB	0-3	1,839	551	2026	2,577	1.10
	3-6	2,005	873	855	1,728	0.43

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33

34   **Hyun et al. - Table 3**

35   Table 3. Anaerobic carbon oxidation rates measured across the major habitats during different

36   season with various incubation treatments.

Season	Site	Manipulation	Depth range	Respiration Rate (mM C hr^{-1})	r^2
July, 2002	SS*	Unamended	0-3 cm	0.4108	0.9723
		+ Mo	0-3 cm	0.0288	0.9895
		Unamended	3-6 cm	0.4148	0.9825
		+ Mo	3-6 cm	0.0229	0.9994
	TS	Unamended	0-3 cm	0.3257	0.9962
		+ Mo	0-3 cm	0.2418	0.9334
		Unamended	3-6 cm	0.1390	0.8723
		+ Mo	3-6 cm	0.0811	0.9322
	CB	Unamended	0-3 cm	0.0538	0.9353
		+ Mo	0-3 cm	0.0614	0.9275
		Unamended	3-6 cm	0.0461	0.9718
		+ Mo	3-6 cm	0.0499	0.8970
Feb., 2003	TS	Unamended	0-6 cm	0.0175	0.9361
		+ Acetate	0-6 cm	0.0654	0.9291
		+ NO$_3^-$	0-6 cm	0.0158	0.9209
		+ PO$_4^{3-}$	0-6 cm	0.0238	0.9832
	CB	Unamended	0-6 cm	0.0155	0.9462
		+ Acetate	0-6 cm	0.0539	0.9993
		+ NO$_3^-$	0-6 cm	0.0113	0.9737
		+ PO$_4^{3-}$	0-6 cm	0.0170	0.9900
Sept., 2003	TS	Unamended	0-6 cm	0.1266	0.9974
		+ Acetate	0-6 cm	0.3369	0.9897
		+ Glucose	0-6 cm	0.2246	0.9547
		+ NO$_3^-$	0-6 cm	0.0761	0.9380
		+ PO$_4^{3-}$	0-6 cm	0.1088	0.9888
		+ FeOOH	0-6 cm	0.0853	0.9680
		+ Mo	0-6 cm	0.0910	0.9348

37   *artificially stimulated by the release of organic acids during sediment mixing (see text)

38 **Hyun et al. - Table 4**

39

40 Table 4. Partitioning of anaerobic respiration pathways coupled to carbon oxidation (mmol m^{-2} d^{-1}), direct measurement of sulfate reduction

41 (SRR, mmol m^{-2} d^{-1}) and Fe(III) reduction (FeRR, mmol m^{-2} d^{-1}) across major habitats of the Georgia saltmarsh.

Site	Depth range (cm)	CO$_2$ production	SRR	Respiration by Sulfate Red[a]	Total FeRR	Abiotic Fe(III) Red by Sulfate Red[a]	Microbial FeRR	Respiration by Fe(III) Red[a]
SS	0-3	37[b]	17.7	35.4 (≈ 95 %) [c]	-	-	-	-
	3-6	17[b]	7.9	15.8 (≈ 95 %)	-	-	-	-
TS	0-3	210	23.2	46.4 (22.1 %)	195	15.4	180	44.9 (21.4 %)
	3-6	90	23.0	46.0 (51.1 %)	263	15.3	247	61.9 (68.8 %)
CB	0-3	35	11.2	22.3 (63.7 %)	108	7.4	158	25.1 (71.83 %)
	3-6	29	9.9	19.8 (68.5 %)	73	6.6	84	10.2 (57.2 %)

42 [a] stoichiometric equations were used to calculate the partitioning of C mineralization

43 C mineralization by sulfate reduction : $SO_4^{2-} + CH_3COO^- + 2H^+ = 2CO_2 + 2H_2O + HS^-$

44 Abiotic reduction of Fe(III) : $3H_2S + 2FeOOH = 2FeS + S^o + 4H_2O$

45 C mineralization by microbial Fe(III) reduction : $8FeOOH + CH_3COO^- + 17H^+ = 2CO_2 + 14H_2O + 8Fe^{2+}$

46 [b] derived from comparison of CO$_2$ production rates in Mo-amended vs. unamended incubation treatments in Fig 2.

47 [c] parenthesis indicates relative contribution (%) of sulfate reduction and iron(III) reduction to total anaerobic carbon respiration

48   **Hyun et al. – Table 5**
49

50   Table 5. Summary of organic acid accumulation rates from incubations of sediment collected from the CB site

	Fe(III) conc.[a] (μmol cm^{-3})	Manipulation for incubation	Organic acids accumulated[b] (mmol C m^{-2} d^{-1})
CB without burrows	< 5.0	Unamended	0
		+ Mo	16
		+ [Mo & Fe(III)]	0
CB with burrows	25 - 75	Unamended	0
		+ Mo	8
		+ (Mo & HS$^-$)	50

51   [a] Average range of Fe(III) concentrations from depth profiles

52   [b] Calculated as carbon equivalents from regression of acetate and propionate concentrations in

53   duplicate incubations per treatment with time. No other carbon substrates were shown to

54   accumulate to significant levels in the incubations.

55