SEASONAL PATTERNS AND PLANT-MEDIATED CONTROLS OF SUBSURFACE WETLAND BIOGEOCHEMISTRY

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Abstract. In tidal marshes, spatial and temporal variability in the importance of microbial metabolic pathways influences ecosystem-level processes such as soil carbon storage, the regeneration of inorganic nutrients, and the production of atmospherically important trace gases. We measured seasonal changes in rates of microbial Fe(III) reduction, sulfate reduction, and methanogenesis in tidal freshwater and brackish marshes on the Patuxent River, Maryland, USA, and assessed the ability of plant roots to influence these processes by regenerating electron acceptors and supplying electron donors. In both marshes, the importance of microbial Fe(III) reduction was greatest early in the summer and decreased through the study period. Coincident with the seasonal decline in Fe(III) reduction, methanogenesis (freshwater marsh) or sulfate reduction (brackish site) increased in importance. At the brackish marsh, the partitioning of anaerobic carbon metabolism between Fe(III) reduction and sulfate reduction was similar within and below the root zone, suggesting that rhizosphere processes did not control anaerobic metabolism at this site. Instead, seasonal biogeochemical patterns at the brackish marsh were affected by factors such as water table depth and iron–sulfur interactions. At the tidal freshwater site, our results suggest that changes in rates of Fe(III) reduction and methanogenesis were directly affected by plant-mediated processes. In midsummer, Fe(III) reduction accounted for a greater fraction of total anaerobic metabolism in rhizosphere-influenced surface soils than in soils below the root zone. High rates of Fe(III) reduction occurred at the expense of methanogenesis. This study documented strong temporal variations in the outcome of microbial competition for electron donors that ultimately affected the balance between Fe(III) reduction and methanogenesis within tidal freshwater marsh soils. Our data suggested that variations in microbial metabolic pathways were regulated by physiochemical factors at the brackish site and plant activity at the freshwater site. Plant regulation of Fe(III) reduction is a largely unstudied mechanism by which plants influence wetland carbon cycling and greenhouse gas production.

Key words: anaerobic metabolism; iron reduction; methanogenesis; Patuxent River, Maryland; sulfate reduction; tidal brackish marsh; tidal freshwater marsh.

INTRODUCTION

Microbial respiration is a fundamental process that influences the capacity of ecosystems to store soil carbon, mineralize nutrients, and produce greenhouse gases. A central tenet of microbial ecology is that respiration is regulated by supplies of both electron acceptors and electron donors, and by competition between microbial groups for these resources (Hedin et al. 1998). Thermodynamic theory dictates that the outcome of microbial competition for substrates depends on the energetic efficiency of individual metabolic pathways and therefore suggests predictable patterns of microbial activity with changes in the abundance of electron acceptors and donors (Froelich et al. 1979, Megonigal et al. 2004). Plants growing in typically anoxic wetland soils have the potential to increase the abundance of NO3\textsuperscript{−}, Fe(III), SO\textsubscript{4}\textsuperscript{2−}, and other electron acceptors by introducing O\textsubscript{2} via their roots. They are also a major source of organic carbon, a universal electron donor for which heterotrophic microbes compete. Determining how plants influence anaerobic metabolism is essential to understanding how wetlands affect regionally and globally important processes such as carbon sequestration and the production of CH\textsubscript{4}, a greenhouse gas that currently contributes 20% of the anthropogenic radiative forcing.

A classic example of microbial competition for electron donors occurs in river–estuarine systems. Methanogenesis is generally negligible at high salinity because SO\textsubscript{4}\textsuperscript{2−} reducers outcompete methanogens for electron donors, but as SO\textsubscript{4}\textsuperscript{2−} availability decreases toward the head of an estuary, the competitive pressure declines and CH\textsubscript{4} production rates increase (Bartlett et al. 1987, Kelley et al. 1990). Most early studies con-
sidered $SO_4^{2-}$ reduction to be the dominant pathway of anaerobic carbon metabolism in marine systems. However, in the last decade it has been demonstrated that microbial Fe(III) reduction can suppress both $SO_4^{2-}$ reduction (Kostka et al. 2002a) and methanogenesis (Roden and Wetzel 1996, Frenzel et al. 1999), suggesting that Fe(III) reduction has the potential to dominate anaerobic carbon metabolism at both ends of the river–estuarine salinity gradient. In fact, the contribution of different pathways of microbial respiration to carbon metabolism in marine sediments had to be significantly revised to account for the activity of Fe(III)-reducing bacteria (Thamdrup 2000). Several recent studies have examined the interactions between Fe(III) reduction and $SO_4^{2-}$ reduction in salt marsh sediments (Kostka et al. 2002a, b, Gribsholt et al. 2003, Koretsky et al. 2003) and concluded that Fe(III)-reducing bacteria coopt a significant portion of microbial metabolism from $SO_4^{2-}$ reducers. However, few field studies have focused on how these processes vary in time and space, or the role that plants and other factors may play in affecting these processes. The dearth of comparable research in low-salinity tidal wetlands is especially striking since the suppressive effect of Fe(III) reduction on CH$_4$ production has global warming implications (Roden and Wetzel 1996).

We had two overall objectives for this study: (1) to quantify seasonal changes in the proportion of carbon metabolism mediated by Fe(III) reducers, $SO_4^{2-}$ reducers, and methanogens in vegetated tidal freshwater and brackish marshes on the Patuxent River, Maryland, and (2) to understand the degree to which plants influence microbial carbon competition by introducing O$_2$ and organic matter into anaerobic soils. To determine how plant roots affect microbial metabolism, we sampled rhizosphere-influenced and nonrhizosphere-influenced soils at our study sites. We hypothesized that plants would enhance subsurface Fe(III) reduction (at the expense of $SO_4^{2-}$ reduction and methanogenesis) because O$_2$ inputs from roots can regenerate Fe(III) oxides. Furthermore, Fe(III) oxides in the root zone are more amorphous than oxides in bulk soil (Weiss et al. 2004), and both Fe(II)-oxidizing and Fe(III)-reducing bacteria are proportionally more abundant on the root surface than in bulk soil (Weiss et al. 2003). Because roots are sources of labile organic carbon (Hines et al. 1994, Gribsholt and Kristensen 2002), total rates of anaerobic carbon metabolism should be highest in rhizosphere soils. Finally, if rates of plant-mediated O$_2$ and carbon inputs to marsh soils follow patterns of plant production, there should be temporal changes in the competitive interactions between microbial Fe(III) reducers, $SO_4^{2-}$ reducers, and methanogens with subsequent effects on wetland carbon cycling.

**MATERIALS AND METHODS**

**Study region**

We collected samples from two herbaceous intertidal wetlands representing brackish and freshwater end points along the Patuxent River, Maryland, USA. Our tidal freshwater marsh study site at Jug Bay (river km 73; 38.78° N, 76.71° W; see Plate 1) was codominated by *Peltandra virginica* (arrow arum), *Pontederia cordata* (pickerelweed), and *Nuphar luteum* (spatter dock). Salinity at EPA monitoring station TF1.4, which is adjacent to Jug Bay, averaged 0.2 g salt per kg water (range 0–1.3 g/kg) from May to August 2002 (Chesapeake Bay Program water quality database, available online). We also sampled in a 30-ha tidal brackish marsh on the northern end of Jack Bay (river km 25; 38.44° N, 76.60° W). The dominant vegetation at this site included *Spartina alterniflora* (saltmarsh cordgrass), *S. patens* (saltmeadow hay), and *Distichlis spicata* (saltgrass). Salinity in the Patuxent River near the Jack Bay marsh (EPA station LE1.1) averaged 13.5 g salt per kg water (range 12.6–15.2 g/kg) during the study. We did not observe bioturbating organisms such as fiddler crabs or large polychaetes in either marsh.

Experimental design

In June, July, and August 2002, we collected replicate soil cores (≥20 cm depth) in vegetated parts of each marsh using PVC tubes (10–15 cm diameter). The cores were capped on the bottom to minimize O$_2$ leakage into the soil and transported to the lab. In each of the three months, the 8–13 cm deep portion of each core was transferred to a N$_2$-filled glove bag and sampled for biogeochemical rate measurements. Soils from this depth were called “rhizosphere-influenced” soils because this is typically in the middle of the root zone. Our study sites did not contain comparable vegetated and unvegetated areas. In July 2002, we compared shallow (8–13 cm) and deeper (47–52 cm) soils in order to evaluate the ability of plants to affect anaerobic metabolism by leaking oxygen and organic carbon to the soil. In tidal marshes, root biomass is typically greatest in the top 30 cm (Chambers and Fourqurean 1991, Husey and Odum 1992) so soils from 47–52 cm depth should have fewer live roots than soils 8–13 cm deep.

**Biogeochemical rate measurements and chemical analyses**

We measured rates of Fe(III) reduction, $SO_4^{2-}$ reduction, and CH$_4$ production on soils from each marsh. In a N$_2$-filled glove bag, soil sections (8–13 cm deep; also 47–52 cm deep in July) were sliced in half and samples were taken from areas that had not been exposed to air during core processing. Summed metabolism was calculated as Fe(III) reduction + $SO_4^{2-}$ reduction + methanogenesis (all in C units) and does not include unmeasured processes such as denitrification or Mn(IV) reduction. We determined integrated metabolism as the sum of CO$_2$ and CH$_4$ production in August only.

5 (http://www.chesapeakebay.net)
Iron(III) reduction was measured as Fe(II) production in anaerobic soil slurries. Slurries were prepared in serum bottles using 9 mL soil and 9 mL oxygen-free deionized water or artificial seawater (15–20 g salt per kg water), as appropriate for the site. About half of the cores were amended with sodium molybdate (final concentration: 20 mmol/L) to inhibit SO$_4^{2-}$ reduction in order to distinguish between biotic and chemical Fe(III) reduction. A time course of Fe(II) production was determined by daily sampling of the slurries in an anaerobic chamber (COY Products, Grass Lake, Michigan, USA) with an atmosphere of ~3% H$_2$ and 97% N$_2$ for 5–10 days. A ~0.5-mL slurry aliquot was shaken in 10 mL of 0.5 mol/L HCl to extract dissolved and sorbed Fe(II) (Roden and Wetzel 1996). After centrifugation, an aliquot of the HCl extract was added to 2 mL ferrozine and the sample absorbance at 562 nm was used to calculate the concentration of Fe(II) per mass of soil (soil dried at 105°C for >24 h). After sampling, the headspace of each serum bottle was flushed with N$_2$ to remove H$_2$. The Fe(II) accumulation rates were typically linear ($r^2 > 0.8$) for the first six days of the incubation, but often leveled off toward the end of the incubations, suggesting limitation of either reducible Fe(III) or labile C by the end of the incubation. The initial (linear) portions of the Fe(II) vs. time curves were used to calculate rates of Fe(III) reduction. We used a ratio of 1 mole C mineralized to 4 moles Fe(III) reduced to convert Fe(III) reduction rates to carbon units (Roden and Wetzel 1996). Unless otherwise indicated, all reported Fe(III) reduction rates represent only the microbial contribution (i.e., bottles amended with sodium molybdate).

For SO$_4^{2-}$ reduction measurements, 1 or 2 subcores (3 mL volume) were collected from each soil section and sealed in 10 mL plastic syringes. After a 24 h equilibration period, all subcores were injected with 50 or 100 µL of carrier-free $^{35}$SO$_4^{2-}$ (American Radiolabeled Chemicals, St. Louis, Missouri, USA) and incubated at ambient field temperatures (23°C June, 22°C July, 26°C August). Four subcores per sampling date were treated identically except that they were not injected with $^{35}$SO$_4^{2-}$ (experimental blanks). After 24 h, we stopped SO$_4^{2-}$ reduction by immersing the cores in 20% zinc acetate. Sulfate reduction rates were calculated after trapping reduced inorganic sulfur (S$^{2-}$) in a
5% zinc acetate solution following the Cr\(^{3+}\) reduction procedure of Fossing and Jørgensen (1989). Total reduced inorganic sulfur (TRIS) concentrations were measured by titration of the zinc acetate traps. Sulfate reduction rates were converted to units of carbon mineralized using a 2:1 molar ratio of C mineralized per SO\(_4^{2-}\) reduced (Westrich and Berner 1984).

The production of CH\(_4\) (all months) and CO\(_2\) (August only) was measured in sealed serum bottles over periods of 2–10 days. Soil slurries were created as described for Fe(III) reduction (n = 1 or 2 bottles per soil slice). At each of several time points, the headspace was injected with 3 mL of N\(_2\) before removing an equal volume of headspace gas for CH\(_4\) analysis. CO\(_2\) was injected with 3 mL of N\(_2\) before removing an equal volume of headspace gas for CH\(_4\) analysis (i.e., steepest) part of the curve was used. Soil mass-normalized rates were converted to carbon units using a 2:1 molar ratio of C mineralized per CH\(_4\) produced (same as the 1:1 molar ratio of CO\(_2\):CH\(_4\) production reported by Roden and Wetzel [1996]).

Porewater analysis

Two weeks before the July and August cores were collected, diffusion equilibration samplers (“peepers” covered with 0.2 \(\mu\)m Pall Versapor-200 membrane [Pall Corporation, East Hills, New York, USA] were installed (n = 1–2 per marsh). Peepers were removed from the marsh about seven days after core collection (i.e., in early July and early September) and sampled for porewater chemistry. In the field, we fixed Fe\(^{3+}\) in ferrozine and H\(_2\)S in 5% zinc acetate. We subsequently quantified Fe\(^{3+}\) (ferrozine), H\(_2\)S (starch–sodium thiocyanate titrations), SO\(_4^{2-}\) (ion chromatography), and Cl\(^-\) (ion chromatography, used to calculate salinity). An aliquot of peeper water was acidified in a syringe with HCl to convert dissolved inorganic carbon (DIC) to CO\(_2\). Ambient air was drawn into the syringe and shaken vigorously to equilibrate gas concentrations between the air and liquid phases. After discarding the liquid, the gas sample was stored in the syringe until CH\(_4\) and CO\(_2\) analyses. Headspace gas concentrations were converted to porewater CH\(_4\) and DIC concentrations using solubility coefficients calculated from Weiss (1974) and Millero (1995).

Site characterization

Aboveground biomass from each plot was clipped immediately before cores were collected in August. Belowground biomass from the 0–8 cm and 13–20 cm intervals of the August cores was sieved through a 1-mm mesh screen. The 8–13 cm interval, which was used for biogeochemical rate measurements, was not sieved for belowground biomass. On two randomly selected cores per marsh, the total root pool from each depth interval was sorted into live and dead fractions based on the color, texture, and firmness of the roots. Biomass was dried at 70°C for >2 wk prior to weighing. In October 2003, two cores from each marsh were sectioned into 0–5, 5–8, 8–13, 13–18, 25–30, 35–40, and 47–52 cm intervals and used to determine water content, bulk density, and organic matter content (loss on ignition, 16 h at 550°C).

Statistical analysis

Seasonal, marsh-specific, and depth-related differences in biogeochemical processes (raw rates and relative importance of each reaction) and biomass were assessed using \(t\) test and standard least squares models, as appropriate. Statistical significance was set at \(P \leq 0.05\). Analyses were conducted using JMP version 5.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Anaerobic metabolism

Mean anaerobic metabolism rates in Jug Bay freshwater marsh rhizosphere soils ranged from 7.9 to 12.8 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) (dry mass basis), across the months sampled, but there were no significant differences in total rates from month to month (\(P = 0.52\)). In June, Fe(III) reduction dominated anaerobic metabolism at Jug Bay (12.5 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\); 98% of measured metabolism; Fig. 1A, B). As Fe(III) reduction rates declined through the summer, methanogenesis significantly increased from 0.3 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) in June to 10.3 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) in August (from 2% of anaerobic metabolism to 77%). In all months, SO\(_4^{2-}\) reduction accounted for <2% of total anaerobic metabolism (<0.1 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\)) at Jug Bay.

Total anaerobic metabolism was 3- to 11-fold lower (June and July, respectively) at the brackish Jack Bay site (range, 0.7–4.2 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\); Fig. 1C, D) than at Jug Bay. At Jack Bay, the relative importance of SO\(_4^{2-}\) reduction varied temporally (50–95% of total metabolism); these SO\(_4^{2-}\) reduction rates were 4–67 times higher at Jack Bay (0.3–2.7 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) ) than at Jug Bay (Fig. 1). Iron(III) reduction (0.4–1.6 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) ) was of comparable importance to SO\(_4^{2-}\) reduction during June and July, but was responsible for <5% (<0.1 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\) ) of anaerobic carbon mineralization in August. In all months, the rates and importance of methanogenesis at Jack Bay were low (rates, 0–0.04 \(\mu\)mol C g\(^{-1}\) d\(^{-1}\); 0–2% of total). In June, the relative importance of Fe(III) reduction and SO\(_4^{2-}\) reduction at Jack Bay was highly variable (Fig. 1D). This was presumably due to significant intercore variability in terminal electron acceptor availability because total metabolic rates varied by only ~10% between replicate cores.
Fig. 1. (A, C) Seasonal changes in the rates (based on dry mass) and (B, D) relative importance of Fe(III) reduction (solid diamonds), \(\text{SO}_4^{2-}\) reduction (open squares), and methanogenesis (open triangles) during summer 2002. Error bars show \(\pm SE, n = 2\) for June data, \(n = 3-5\) for July and August data. “Percentage of total” is the percentage of total anaerobic metabolism (defined in this article as the sum of Fe(III) reduction, \(\text{SO}_4^{2-}\) reduction, and \(\text{CH}_4\) production, all in carbon units).

During the July sampling event at Jug Bay, mean rates of anaerobic microbial metabolism were consistently higher in rhizosphere soils than deep (nonrhizosphere) soils (Fig. 2A), although the differences (\(\Delta\)) were not significant for Fe(III) reduction (\(\Delta = 3.97 \mu\text{mol C g}^{-1}\text{d}^{-1}; P = 0.07\)) or methanogenesis (\(\Delta = 0.40 \mu\text{mol C g}^{-1}\text{d}^{-1}; P = 0.71\)). However, when expressed as a fraction of total metabolism, Fe(III) reduction was dominant in the rhizosphere (58\% vs. 30\% for shallow and deep soils, respectively; \(P = 0.02\)) and methanogenesis was dominant below the rhizosphere (40\% vs. 69\%; \(P = 0.02\); Fig. 2B). The remaining 1–2\% of anaerobic metabolism was via \(\text{SO}_4^{2-}\) reduction; there were no rhizosphere-related differences in the relative importance of this process at the freshwater marsh in July. At Jack Bay, there were no rhizosphere-related differences in the rates or importance of measured microbial processes in July (Fig. 2).

The Fe(III) reduction rates reported in the previous paragraphs reflect only the microbial contribution to total Fe(III) reduction. By comparing Fe(III) reduction rates between sodium molybdate-amended samples (biotic reduction only) and unamended samples (chemical + biotic reduction), we determined that biological Fe(III) reduction at the Jack Bay brackish marsh accounted for a median of 53\% of all Fe(III) reduction in July (range, 40–133\%; \(n = 5\)) and 39\% in August (range, 31–95\%; \(n = 4\)). In contrast, there were no differences in rates between molybdate-amended and unamended samples from Jug Bay. The difference in the importance of biotic vs. chemical Fe(III) reduction between marshes is consistent with measurements of higher porewater \(\text{H}_2\text{S}\) concentrations (Appendix A) and \(\text{SO}_4^{2-}\) reduction rates (Fig. 1) at the brackish marsh.

At Jug Bay, the rates of anaerobic carbon metabolism estimated by summing all measured microbial respiration pathways were the same as those estimated by summing \(\text{CO}_2\) and \(\text{CH}_4\) production (Fig. 2C). This indicates that we measured the important biogeochemical reduction processes at this marsh and that we used appropriate stoichiometry to convert elemental rates to carbon equivalents. In contrast, the large difference between measured processes and integrated metabolic gas production estimates at Jack Bay (Fig. 2C) suggests that important anaerobic processes in the brackish marsh were underestimated or not measured.

**Porewater profiles**

The porewater concentrations and vertical profiles of the measured carbon (DIC, \(\text{CH}_4\)), iron (Fe\(^{3+}\)), and sulfur species (\(\text{H}_2\text{S}, \text{SO}_4^{2-}\)) varied temporally and spatially between marshes (Appendix A). When averaged across all depths, porewater [DIC] was 2.2–3.7 mmol/L greater in September than in early July (Jug Bay and Jack Bay, respectively; Appendix A). In agreement with the dramatic differences in methanogenesis between the marshes, porewater [\(\text{CH}_4\)] was at least 20-fold lower at Jack Bay than at Jug Bay (July medians, 0.14 vs. 383 \(\mu\text{mol/L}; September, 24 vs. 480 \(\mu\text{mol/L}; Appendix A). Also, porewater [Fe\(^{3+}\)] was lower at the brackish marsh than at the freshwater marsh (range across seasons: Jack Bay, 0.4–125 \(\mu\text{mol/L}; Jug Bay, 130–2729 \(\mu\text{mol/L})

At Jack Bay, near-surface porewater \(\text{H}_2\text{S}\) concentrations in both months were \(\sim 100 \mu\text{mol/L}\) and
steadily increased with depth. Below a depth of ~9 cm (~25 cm in July), H\textsubscript{2}S concentrations increased to >1000 \text{mmol/L} and remained elevated to the bottom of the peeper. At Jug Bay, near-surface H\textsubscript{2}S concentrations were similar to those at Jack Bay, but there were no large changes in porewater [H\textsubscript{2}S] with depth (Appendix A). Median porewater SO\textsubscript{4}\textsuperscript{2-} concentrations were about two orders of magnitude greater at Jack Bay (July, 11.9 mmol/L; September, 6.5 mmol/L) than Jug Bay (<0.03 mmol/L; data not shown). Salinity, which was similar in both months, was higher at Jack Bay than Jug Bay (~14 vs. <0.5 g salt per kg water).

**Site characteristics**

The soils at Jug Bay (freshwater) were mineral whereas those at Jack Bay (brackish) were organic (mean organic content 16\% vs. 70\%, respectively; Appendix B). As a result, Jack Bay had a higher water content (85–90\% of wet mass) and lower bulk density (0.04–0.06 g/cm\textsuperscript{3}) than Jug Bay (65–78\% and 0.08–0.15 g/cm\textsuperscript{3}). At Jug Bay, total reduced inorganic sulfur (TRIS) concentrations at 10 cm depth were lowest in June (11.7 ± 9.5 \text{mmol S/g dry mass}) and increased in July (28.0 ± 2.6 \text{mmol S/g}) and August (31.7 ± 2.1 \text{mmol S/g}; Appendix B). Similarly, TRIS concentrations at Jack Bay increased from June (19.6 ± 1.7 \text{mmol S/g}) to the end of the summer (26.6–29.9 \text{mmol S/g}). At Jug Bay, there was a significant decrease in TRIS from 10 cm to 50 cm depth (8.4 ± 0.6 \text{mmol S/g}), but there were no differences in TRIS with depth at Jack Bay.

There was significantly more belowground biomass (roots + rhizomes) in the surface (0–8 cm) soils at the Jack Bay marsh than at Jug Bay (Table 1). At Jack Bay, root and rhizome biomass were comparable between the 0–8 and 13–20 cm depth intervals. At Jug Bay, there was significantly more belowground biomass in the 13–20 cm interval. At Jug Bay, live roots accounted for 98\% and 78\% of the total root pool (0–8 and 13–20 cm intervals, respectively). At Jack Bay, 94–98\% of the roots were alive, regardless of depth.

**Discussion**

Despite a large volume of research into the physiology of Fe(III)-reducing bacteria (e.g., Lovley and Phillips 1988, Lower et al. 2001), the in situ contribution of these organisms to anaerobic carbon metabolism and nutrient cycling is still poorly understood. We have shown that microbial Fe(III) reduction is potentially the dominant pathway of anaerobic carbon metabolism in tidal freshwater and brackish marshes. The importance of microbial Fe(III) reduction has been demonstrated in a limited number of previous studies of freshwater (Roden and Wetzel 1996, Frenzel et al. 1999) and saline wetlands (Kostka and Luther 1995, Kostka et al. 2002a, Gribsholt et al. 2003). The present study goes further by reporting seasonal variability in
microbial Fe(III) reduction and its impact on other key biogeochemical processes via microbial competition.

In the freshwater and brackish marshes, there were significant changes in the relative importance of Fe(III) reduction at 8–13 cm depth (i.e., the root zone) over the course of three months in the growing season. Because Fe(III)-reducing bacteria are superior competitors for H$_2$ and acetate, the major electron donors used by heterotrophic anaerobic microbes, this seasonal variability necessarily impacts the metabolism of competing microbial populations such as methanogens. In the following section, we argue that seasonal variations in microbial metabolism are caused by the seasonality of plant production in some cases but not others.

**Seasonal patterns in anaerobic biogeochemistry**

For the tidal freshwater marsh at Jug Bay, rates of Fe(III) reduction were greatest in June (Fig. 1A), coincident with the aboveground biomass peak in wetlands dominated by *Peltandra virginica* and *Pontederia cordata* (Neubauer et al. 2000). Furthermore, the decrease in the importance of Fe(III) reduction parallels trends in gross macrophyte photosynthesis in tidal freshwater marshes (e.g., Neubauer et al. 2000: Fig. 6). We suggest that the plant community directly affected Fe(III) reduction through high rates of radial O$_2$ loss (ROL) and Fe(II) oxidation. The continual and rapid regeneration of Fe(III) oxides in the rhizosphere would have supported relatively high rates of Fe(III) reduction earlier in the growing season. This model is supported by the data of Sundby et al. (2003), who found that seasonal cycles of root growth and decay in a salt marsh affected the degree of rhizosphere oxidation and porewater Fe$^{2+}$ availability. Later in the growing season, we suggest that plant senescence and lower rates of ROL caused Fe(III)-reducing bacteria to become limited by a declining pool of labile Fe(III) oxides, allowed methanogens to better compete for electron donors, and increased the importance of methanogenesis during this period. Thus, our data indicate a tight coupling between rates of rhizosphere Fe(II) oxidation and Fe(III) reduction, and suggest that the rapid cycling of iron may influence other biogeochemical pathways.

The seasonal patterns in Fe(III) reduction and SO$_4^{2-}$ reduction at the Jack Bay brackish marsh are more difficult to interpret. Peak aboveground biomass in *Spartina*-dominated marshes generally occurs toward the end of the growing season (e.g., Morris 1988). If coupled seasonal changes in biomass and ROL were influencing anaerobic metabolism at the brackish marsh, as we suggested for the freshwater marsh, we would expect an increase in the importance of Fe(III) reduction throughout our sampling period. However, this is not what we observed (Fig. 1B). One possible explanation is that seasonal changes in flooding (Morris et al. 2002) impacted the redox state of surface soils at the brackish marsh. Mean water levels before the August sampling were 11–14 cm higher than the water levels immediately preceding the June and July sampling events (one week running averages calculated from Annapolis, Maryland, water level data, available online). If the dominant route of Fe(II) oxidation at the brackish marsh was via O$_2$ advection into the soil, higher water table levels could reduce rates of Fe(II) oxidation and therefore affect Fe(III) reduction. This seasonal change in water table depth may also have resulted in greater H$_2$S accumulation in the Jack Bay porewaters late in the growing season (Appendix A). Another possibility is that higher late summer temperatures, in combination with inputs of organic matter from senescing plant material, accelerated rates of SO$_4^{2-}$ reduction (Howarth and Teal 1979, Hines et al. 1989), causing a reduction in Fe(III) availability through either precipitation of Fe–S compounds or chemical reduction of Fe(III) by sulfides, and a seasonal minimum in the population of Fe(III)-reducing bacteria (Koretsky et al. 2003). Determining the exact mechanism(s) responsible for seasonal changes in metabolism at the brackish marsh is difficult since we know the specific pathway for only ~20–30% of the anaerobic decomposition at this site (Fig. 2C; see additional discussion in the Total marsh anaerobic metabolism section).

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### Table 1. Aboveground and belowground biomass sampled in August 2002 from the Jack Bay and Jug Bay tidal marshes, Maryland, USA.

<table>
<thead>
<tr>
<th>Site</th>
<th>Aboveground dry mass (g/m$^2$)</th>
<th>Belowground dry mass (g/dm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live</td>
<td>Dead</td>
</tr>
<tr>
<td>Jack Bay</td>
<td>636.5$^+$ (62.3)</td>
<td>522.0$^+$ (91.2)</td>
</tr>
<tr>
<td>0–8 cm depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–20 cm depth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jug Bay</td>
<td>468.6$^+$ (56.5)</td>
<td>0.0$^+$</td>
</tr>
<tr>
<td>0–8 cm depth</td>
<td></td>
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<tr>
<td>13–20 cm depth</td>
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</table>

Notes: Reported values are means (± SE); $n = 7$ replicate cores for aboveground biomass, $n =$ 5 replicate cores for belowground biomass. Within a column, rows with the same superscript were statistically similar (standard least-squares model with Tukey’s hsd, $P < 0.05$).

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$^6$ (http://co-ops.nos.noaa.gov)
Rhizosphere effects on anaerobic biogeochemistry

Although we hypothesized that the rhizosphere is a site of active redox cycling, it is difficult to directly determine the in situ effects of plant roots on wetland biogeochemistry. Neither of our study sites contained comparable vegetated and unvegetated soils, so we adopted the approach of Frenzel et al. (1999) and compared shallow (rhizosphere influenced) and deep (non-rhizosphere influenced) soils. This approach assumes that depth-dependent rates of microbial activity are mainly due to differences in root activity. There is ample evidence that plant activity explains depth-related changes in labile carbon availability (Hines et al. 1989), soil organic content (Appendix B; Jobbágy and Jackson 2000), and poorly crystalline Fe(III) (Weiss et al. 2004). Factors that vary with depth independently of plant activity include porewater turnover and direct O2 advection into the soil, which we assume were controlled by sampling soils below the water table. This latter assumption was well founded at the water-saturated freshwater site (Jug Bay), but is less certain for the brackish site (Jack Bay), which appeared to have greater variability in water table depths. Depth-related differences in SO4\(^{2-}\) availability would have favored a higher contribution of SO4\(^{2-}\) reduction near the soil surface, but this is not what we observed (Fig. 2B). Inherent differences between rhizosphere and nonrhizosphere soils in terms of absolute rates of microbial respiration were removed by normalizing each process rate to the total anaerobic metabolic rate at a given depth (Fig. 2B). This permitted us to focus on differences in the relative contributions of each pathway with depth, which we interpreted as being driven primarily by variations in root activity.

The comparison of rhizosphere-influenced and nonrhizosphere-influenced soils at Jug Bay supports our hypothesis that radial O2 loss promoted Fe(III) reduction by regenerating Fe(III) oxides. In Jug Bay rhizosphere-influenced soils, we calculated that Fe(III) reduction accounted for 58% of anaerobic metabolism vs. only 30% in nonrhizosphere soils (July data, Fig. 2B). Conversely, methanogenesis was significantly more important in nonrhizosphere soils than in the rhizosphere (69% vs. 40%). Roden and Wetzel (1996) and Frenzel et al. (1999) attributed similar results to competition between Fe(III) reducers and methanogens for electron donors, and this is consistent with thermodynamic theory (Megonigal et al. 2004). The Jug Bay soils are consistently water saturated so that direct penetration of O2 to 10 cm depth is an unlikely mechanism for Fe(III) regeneration. Similarly, porewater turnover at Jug Bay is likely too low (months to years) to explain differences in microbial activity between shallow and deeper soils.

We did not observe rhizosphere-related differences in the relative importance of Fe(III) reduction and SO4\(^{2-}\) reduction at Jack Bay (the brackish site), indicating that chemical or environmental factors, rather than plant biology, were more important at the brackish marsh. Soil extractions in October 2002 indicated that total 0.5 mol/L HCl-extractable Fe concentrations at 10 cm depth were 60-fold greater in the freshwater marsh than the brackish marsh (6 vs. 363 mmol Fe/g dry mass), so the availability of Fe (in either oxidation state) at the brackish marsh may be too low to significantly inhibit SO4\(^{2-}\) reduction. Chemical complexation between iron and reduced sulfur compounds (e.g., FeS and pyrite formation) at the brackish marsh can further limit the availability of Fe for microbial oxidation and reduction. At Jack Bay, advective O2 transport into the soils may be more significant than radial O2 loss (ROL) from *Spartina alterniflora* and other marsh plants in regenerating iron oxides since *S. alterniflora* has low rates of ROL and can suffer from root O2 deficiencies (Mendelssohn et al. 1981). The combination of low available Fe, dynamic Fe–S interactions, and low ROL rates may have reduced the importance of the rhizosphere as a site of Fe-oxide regeneration, and therefore limited rhizosphere Fe(III) reduction at the brackish marsh.

Total marsh anaerobic metabolism

The brackish marsh had far more soil organic matter (Appendix B) and lower rates of microbial respiration than the freshwater marsh (Fig. 1). One explanation is that detritus from the grass-like species at the brackish marsh (*Spartina spp.*, *Distichlis spicata*) was more refractory than that from broad-leaf plants such as *Pellandra virginica* and *Pontederia cordata*, the biomass dominants at the freshwater marsh (Odum and Heywood 1978, Webster and Benfield 1986). These differences in organic matter composition may be more important in affecting metabolic rates than the specific decomposition pathways utilized (e.g., SO4\(^{2-}\) reduction vs. methanogenesis; Kelley et al. 1990). We also suspect that the geomorphologic setting and the high organic content of the Jack Bay marsh leads to greater drainage and higher advective O2 penetration into surface soils during low tide. Thus, much of the initial decomposition at Jack Bay may be mediated by aerobic prokaryotes and fungi (Howes et al. 1984, Benner et al. 1986), processes that were not captured in our anaerobic incubations.

Porewater data support the idea that the surface soils at the Jack Bay marsh were more oxic than those at Jug Bay. For example, the mean DIC concentration in the upper 4 cm at Jack Bay in early September (1.4 mmol/L; Appendix A) was similar to that in tidal flood water (1.2 ± 0.02 mmol/L, *n* = 3), suggesting that the upper soils at Jack Bay were regularly flushed by tidal waters. Flushing with relatively oxic tidal waters would prevent DIC from root and microbial respiration from accumulating in the near-surface soils. At Jug Bay, the concentration difference between near-surface porewaters (2.4–4.1 mmol/L) and tidal flood water (1.3 ±
0.2 mmol/L, n = 3) was much greater, indicating more waterlogging and anaerobiosis. Also, near-surface porewater H2S concentrations were similar in the two marshes (despite greater SO42− reduction at Jack Bay), and may indicate H2S oxidation in well-flushed Jack Bay soils. Thus, the difference in metabolism rates between the Jack Bay and Jug Bay marshes may be a sampling design artifact. If we had measured both aerobic and anaerobic mineralization, we may have reached different conclusions about the comparative rates of total decomposition between these marshes.

These hypotheses cannot explain the differences between summed and integrated metabolism that we measured at Jack Bay (Fig. 2C). Regardless of organic matter lability or the importance of aerobic decomposition, we should have seen similar carbon mineralization rates when comparing calculation techniques, unless our measurements underestimated or missed important anaerobic processes. We have no reasons to suspect our estimates of biological Fe(III) reduction or important anaerobic processes. We have no reasons to suspect our estimates of biological Fe(III) reduction or~

system-scale importance of fermentation as a (non-methanogenic) carbon mineralization pathway has been difficult to assess, largely due to methodological issues and the wide variety of fermentable compounds present in wetland soils. However, since simple organic compounds such as acetate, formate, and propionate can be abundant in wetland porewaters (Hines et al. 1994, Kostka et al. 2002, Vile et al. 2003), we suggest that carbon flow through fermentation may be a significant decomposition pathway in the highly organic Jack Bay soils. Similarly, Vile et al. (2003) reported that SO42− reduction and methanogenesis in a Canadian peatland explained <3% of anaerobic CO2 production and suggested that the majority of anaerobic decomposition at that site was due to the fermentation of low molecular weight soluble organic compounds. An alternate explanation for our data and those of Vile et al. (2003) is that humic acids served as an important electron acceptor. Humic compounds can serve as electron shuttles between metal-reducing microbes and oxidized metals (e.g., Lovley et al. 1996, Weiss et al. 2004), but may also be directly reduced and coupled to the oxidation of organic carbon. Cervantes et al. (2000) estimated that the energy yield from the microbial reduction of AQDS (a model humic acid) is greater than that from methanogenesis and SO42− reduction, but less than that from Fe(III) reduction. Thus, the reduction and recycling of humic compounds derived from the organic soils at the Jack Bay marsh may have mediated a large fraction of metabolism at the expense of SO42− reduction.

**Summary**

In tidal marsh soils, the rates and relative importance of different microbially mediated anaerobic processes can vary along the estuarine continuum (e.g., freshwater vs. brackish sites), temporally during the growing season, and within an individual marsh (e.g., rhizosphere vs. nonrhizosphere soils). For the organic soils of the Jack Bay brackish tidal marsh, we suggested that temporal changes in the activity of different metabolic functional groups during the growing season were driven by changes in environmental factors including Fe−S interactions and water table depth. We did not observe depth-related differences in rates of anaerobic

River sediments are an order of magnitude lower than extractable Fe concentrations (11 µmol Mn/g dry mass vs. 280 µmol Fe/g dry mass; F. Reidel, personal communication), so we suggest that Mn(IV) reduction was unimportant at Jack Bay.

Anaerobic fermentation, which uses organic carbon compounds as both electron acceptors and electron donors, or the use of humic compounds as electron acceptors, may account for a substantial portion of anaerobic metabolism at Jack Bay. Although the role of acetate fermentation as a methanogenic pathway has been well studied (Thebrath et al. 1992, Conrad 1999, Megonigal et al. 2004 and references therein), the ecosystem-scale importance of fermentation as a (non-methanogenic) carbon mineralization pathway has been difficult to assess, largely due to methodological issues and the wide variety of fermentable compounds present in wetland soils. However, since simple organic compounds such as acetate, formate, and propionate can be abundant in wetland porewaters (Hines et al. 1994, Kostka et al. 2002, Vile et al. 2003), we suggest that carbon flow through fermentation may be a significant decomposition pathway in the highly organic Jack Bay soils. Similarly, Vile et al. (2003) reported that SO42− reduction and methanogenesis in a Canadian peatland explained <3% of anaerobic CO2 production and suggested that the majority of anaerobic decomposition at that site was due to the fermentation of low molecular weight soluble organic compounds. An alternate explanation for our data and those of Vile et al. (2003) is that humic acids served as an important electron acceptor. Humic compounds can serve as electron shuttles between metal-reducing microbes and oxidized metals (e.g., Lovley et al. 1996, Weiss et al. 2004), but may also be directly reduced and coupled to the oxidation of organic carbon. Cervantes et al. (2000) estimated that the energy yield from the microbial reduction of AQDS (a model humic acid) is greater than that from methanogenesis and SO42− reduction, but less than that from Fe(III) reduction. Thus, the reduction and recycling of humic compounds derived from the organic soils at the Jack Bay marsh may have mediated a large fraction of metabolism at the expense of SO42− reduction.

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In tidal marsh soils, the rates and relative importance of different microbially mediated anaerobic processes can vary along the estuarine continuum (e.g., freshwater vs. brackish sites), temporally during the growing season, and within an individual marsh (e.g., rhizosphere vs. nonrhizosphere soils). For the organic soils of the Jack Bay brackish tidal marsh, we suggested that temporal changes in the activity of different metabolic functional groups during the growing season were driven by changes in environmental factors including Fe−S interactions and water table depth. We did not observe depth-related differences in rates of anaerobic
metabolism or the importance of Fe(III) reduction vs. SO$_4^{2-}$ reduction, the dominant anaerobic metabolic processes at this site, that would be expected from plant regulation of microbial metabolism.

The dominant anaerobic metabolic processes at the Jug Bay tidal freshwater marsh were Fe(III) reduction and methanogenesis. We reported temporal variability in the importance of Fe(III) reduction and methanogenesis and hypothesized that this variability was directly linked to changes in the biomass or activity of aboveground vegetation, rates of ROL, and Fe(II) oxidation in the rhizosphere. In other words, the ability of plants to oxidize the rhizosphere and influence the rate of Fe(II) oxidation may be critical in controlling rates of Fe(III) reduction in this system. Due to competition between Fe(III) reducers and methanogens for electron donors, spatial (rhizosphere vs. nonrhizosphere) and seasonal changes in rates of Fe(III) reduction ultimately affected the balance between Fe(III) reduction and methanogenesis within the tidal freshwater marsh soils. Thus, plants may play a key role in regulating the relative contributions of anaerobic microbial processes that drive the turnover of organic carbon in wetland soils. A proper test of this hypothesis would require direct manipulations of plant activity, which we did not perform. It remains to be seen if the patterns we observed in this study will hold in tidal freshwater and brackish marshes with different types of vegetation, soils, or hydrology.

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LITERATURE CITED


APPENDIX A

A figure showing porewater chemistry of vegetated soils at the Jug Bay and Jack Bay tidal marshes is available in ESA’s Electronic Data Archive: Ecological Archives E086-183-A1.

APPENDIX B

A figure showing depth profiles of soil bulk density, water content, organic content, and total reduced inorganic sulfur concentration at the tidal marsh study sites is available in ESA’s Electronic Data Archive: Ecological Archives E086-183-A2.