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Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshes

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ABSTRACT

Anaerobic decomposition in wetland soils is carried out by several interacting microbial processes that influence carbon storage and greenhouse gas emissions. To understand the role of wetlands in the global carbon cycle, it is critical to understand how differences in both electron donor (i.e., organic carbon) and terminal electron acceptor (TEA) availability influence anaerobic mineralization of soil organic matter. In this study we manipulated electron donors and acceptors to examine how these factors influence total rates of carbon mineralization and the pathways of microbial respiration (e.g., sulfate reduction versus methanogenesis). Using a field-based reciprocal transplant of soils from brackish and freshwater tidal marshes, in conjunction with laboratory amendments of TEAs, we examined how rates of organic carbon mineralization changed when soils with different carbon contents were exposed to different TEAs. Total mineralization (the sum of $CO_2 + CH_4$ produced) on a per gram soil basis was greater in the brackish marsh soils, which had higher soil organic matter content; however, on a per gram carbon basis, mineralization was greater in the freshwater soils, suggesting that the quality of carbon inputs from the freshwater plants was higher. Overall anaerobic metabolism was higher for both soil types incubated at the brackish site where SO_4^{2-} was the dominant TEA. When soils were amended with TEAs in the laboratory, more thermodynamically favorable respiration pathways typically resulted in greater organic matter mineralization (Fe(III) respiration > SO_4^{2-} reduction > methanogenesis). These results suggest that both electron donors and acceptors play important roles in regulating anaerobic microbial mineralization of soil organic matter.

1. Introduction

Decomposition of organic carbon regulates both the amount of organic material that remains stored in wetland soils, and the amount of mineralized carbon that is released to the atmosphere as greenhouse gases (CO_2 or CH_4). Decomposition often begins with physical fragmentation, proceeds to exoenzyme-mediated hydrolysis of complex organic compounds, and ends with mineralization of simple organic compounds to gases via microbial respiration. This last step in the overall decomposition process – terminal microbial respiration – ultimately requires the flow of electrons from organic matter (electron donors) to one of several terminal electron acceptors (TEAs). Despite the fundamental importance of electron flow to terminal microbial respiration, few studies have explicitly considered the importance of electron donor and acceptor supply in regulating the overall, multi-step process of decomposition.

Many studies have explored the environmental factors that regulate decomposition in wetlands, including: temperature, water-table level, reduction–oxidation potential, and pH (Brinson et al., 1981; McLatchey and Reddy, 1998; Segers, 1998; Whalen, 2005), but there has been little work on the mechanistic control of overall anaerobic mineralization of soil organic matter (as opposed to the relative importance of different TEA pathways) by both electron donor and acceptor supply. The adoption of such an "electron perspective" may be particularly useful in exploring a number of global change scenarios, including increased sulfate (SO_4^{2-}) loading from sea level rise (e.g., Weston et al., 2006), and increased NO_3^- loading from cultural eutrophication (e.g., Vitousek et al., 1997) or acid deposition (e.g., Wieder et al., 1990), all of which can alter both electron donor and acceptor availability in wetland ecosystems. For example, increased salinity caused by sea level rise may cause a change in soil carbon quality (i.e., potential for microbial mineralization) through changes in

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plant species composition (Gough and Grace, 1998; Baldwin et al., 2001; Donnelly and Bertness, 2001). Carbon quality contributes to differences in organic carbon mineralization by controlling the electron donor supply to decomposers (Kelley et al., 1990; Groffman et al., 1991; Schipper et al., 1994).

Concomitantly, sea level rise will also influence the supply of specific TEAs, such as SO_4^{2-}, with uncertain effects on mineralization. It is already well established that the availability of TEAs affects the last (terminal) step of anaerobic decomposition (Capone and Kiene, 1988; Reddy and D'Angelo, 1994; D'Angelo and Reddy, 1999; Megonigal et al., 2004) by acting through interspecific competition for electron donors (acetate and H_2). Indeed, previous work has demonstrated that sea level rise can lead to a shift of electron flow from methanogens to the competitively superior sulfate reducers (Neubauer et al., 2005; Weston et al., 2006). A relatively unexplored question, however, is if this shift in the dominant TEA pathway will influence rates of total anaerobic organic carbon mineralization (i.e., the sum of $CO_2 + CH_4$ produced in anaerobic soil organic matter decomposition; herein "overall mineralization"), and how this pattern interacts with electron donor supply.

The limited evidence available to date is equivocal about whether overall mineralization rates change when microbes use electron acceptors with relatively high free energy yield, such as NO_3^- or SO_4^{2-}, compared to methanogenesis. Among studies that have manipulated the TEA pathway through substrate amendments, some report greater rates of carbon mineralization in the presence of NO_3^- compared to methanogenesis (Pallud et al., 2007; Abell et al., 2009) or SO_4^{2-} compared to methanogenesis (Reddy and Graetz, 1988; Weston et al., 2006; Pallud et al., 2007). However, in a comparison of 10 different wetland soils, D'Angelo and Reddy (1999) did not observe a difference in organic carbon mineralization rates under denitrifying, sulfate-reducing or methanogenic conditions. One possible explanation for this apparent discrepancy is that the importance of TEAs in determining overall anaerobic mineralization is mediated by electron donor availability (e.g., organic carbon quality). It has been demonstrated, for example, that the rate of electron flow from the initial hydrolysis of organic compounds can be faster than electron consumption by subsequent sulfate reduction (Bruchert and Arnosti, 2003). In situations dominated by such an electron "imbalance", the presence of a more energetically favorable TEA may stimulate overall mineralization. By contrast, in soils where the electron source from soil organic matter is in balance with electron sinks from TEAs, overall mineralization rates may be less sensitive to the dominant TEA present. Expressed another way, in order for overall mineralization rates to vary according to the TEA pathway, the final step in the process – microbial respiration via TEA reduction – must ultimately regulate bulk organic matter degradation from beginning to end. There has been limited work on the impacts of different TEAs on overall organic carbon mineralization in soils that differ in organic matter quality.

The primary goal of the present study was to understand how interactions between soil carbon quality (electron donor supply) and site chemistry (electron acceptor supply) influence anaerobic organic matter mineralization in tidal wetland soils. In order to test the influence of different TEA pathways on overall mineralization rates, we manipulated both electron donors and acceptors. By combining a field-based reciprocal transplant of wetland soils and a laboratory-based amendment of TEAs, we examined how rates of carbon mineralization change when soils of different carbon contents experience environments dominated by different electron acceptors. We hypothesized that electron donor quantity (i.e., the amount of soil carbon) would drive overall rates of mineralization, as measured through total carbon mineralization to gases ($CO_2 + CH_4$). We expected that the supply of different TEAs would regulate the terminal step in organic matter mineralization as shown in previous studies, but we tested the additional hypothesis that the dominant TEA pathway would regulate overall mineralization rates as well. Specifically, we hypothesized that overall mineralization rates would increase with the free energy yield of the TEA pathway, normalized to the carbon content of a given soil.

2. Methods

2.1. Study sites for reciprocal transplant

We used reciprocal transplants of bulk soil from two wetlands along an estuarine salinity gradient to investigate the interaction of plant tissue quality and site conditions in controlling organic carbon mineralization. The reciprocal transplant experiment was established in the fall of 2006 at two locations along the Patuxent River, Maryland, USA (Neubauer et al., 2005). The tidal freshwater site (mean salinity 0.2) at Jug Bay (38.78° N, 76.71° W) was dominated by broadleaf emergent vegetation (mainly *Peltandra virginica* (arrow arum), *Pontedaria cordata* (pickerelweed), and *Nuphar luteum* (spatter dock)). The tidal brackish marsh (mean salinity 14) was located on the northern end of Jack Bay (38.44° N, 76.60° W) and the dominant vegetation was *Spartina alterniflora* (saltmarsh cordgrass), *Spartina patens* (saltmeadow hay), and *Distichlis spicata* (salt grass).

2.2. Reciprocal transplant experimental design

Bulk samples of surface (~0–30 cm) soil were collected from vegetated sections of the marsh at Jack Bay (brackish site) on 18 July 2006 and from Jug Bay (freshwater site) on 14 August 2006. The soils were passed through a metal screen (made from 6 mm "¼ inch hardware cloth" mesh) to remove roots and subsequently homogenized and stored at 4 °C until further processing. On 3–4 September 2006, soil from both sites was enclosed in PVC cores consisting of a 10 cm diameter × 8 cm length of PVC pipe covered on each end with landscape fabric (0.5 mm (18 mil) poresize) to allow for interaction of the soils with the surrounding porewater. Twenty cores were created using soil from each site at a final density of 0.30 g cm^{-3} and 0.12 g cm^{-3} (dry weight basis) for Jug Bay and Jack Bay, respectively. Prior to deployment, the cores were stored in a 4 °C incubator. Ten cores were replanted at the site of origin as controls (named either "Fresh" or "Brackish"); the remaining ten were transplanted to the reciprocal site (named either "Fresh@Brackish" or "Brackish@Fresh"). The tops of all cores were buried 10 cm below the soil surface, to ensure they would be below the water table, and oriented to allow for horizontal exchange of porewater through the mesh-covered ends. Cores were deployed at Jack Bay on 26 September 2006 and at Jug Bay on 9 October 2006 during low tides. Although soils from both marshes were collected in vegetated areas, the cores were buried in adjacent mudflats with no vegetation to ensure there would be no plant root impacts on the soil transplants (Fig. 1). Placement in mudflat areas also minimized differences in tidal regimes between the two wetlands and ensured that the cores were consistently inundated. Five replicate cores of each soil type were collected in October 2008 and the remaining five cores were collected in May 2009, yielding incubation periods of 24 and 31 months, respectively. Cores were collected with extra sediment around them to maintain an anaerobic environment, secured in plastic bags, and placed on ice during transport to a laboratory where they were immediately transferred to an anaerobic chamber (Coy Laboratory Products, Inc, Grasslake, MI) with an atmosphere of >95% N_2 and the balance H_2 (to remove molecular oxygen).

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Fig. 1. Example of the experimental set-up with the buried soil cores at Jack Bay, a tidal brackish marsh. Photo: J. Keller.

2.3. Biogeochemical rate measurements and chemical analyses

Preliminary data indicated that nitrate and ferric iron concentrations were too low to support meaningful rates of denitrification or Fe(III) reduction in these soils, largely because these soils were buried at least 10 cm below the surface where there were minimal inputs of oxygen or oxidized forms of nitrogen or iron. The porewater NO_3^- concentration (in mg N L^{-1}) in the porewater of the soils incubated for 31 months was 0.01 for the Fresh soils and 0.45 for the Brackish soils; total Fe extractable with 0.5 M HCl (μmol g^{-1} soil dry weight) was 437.5 for the Fresh soils and 15.0 for the Brackish soils (Neubauer, personal communication). Therefore, on both the 24 and 31 month-incubated soils we measured the rates of the remaining dominant anaerobic metabolic pathways, SO_4^{2-} reduction and CH_4 production, as well as CO_2 production. In the anaerobic chamber, samples were taken from the center of the cores where soil had not been exposed to air during core collection and processing. Subsamples of each core were used to measure SO_4^{2-} reduction and CH_4 production (described below). Organic carbon mineralization via SO_4^{2-} reduction was calculated assuming 2 mol of CO_2 produced per mole SO_4^{2-} reduced; methanogenesis was assumed to yield 1 mol of CO_2 per mole CH_4 produced (Neubauer et al., 2005). Although the relative rates of these two processes were compared to determine the dominant process in each soil type, in most cases we focus on the influence of these processes on rates of total anaerobic mineralization (defined as the sum of $CO_2 + CH_4$ production) in these soils. We also measured soil organic matter content (loss on ignition, 4 h at 550 °C), microbial biomass (Voroney and Winter, 1993) (Table 1), and moisture content from a 20 g subsample dried at 100 °C for 24 h.

For SO_4^{2-} reduction rate measurements, we followed the methods outlined in Mitchell and Gilmour (2008). Briefly, one sub-core (10 mL volume) was collected from each core and immediately injected with 100 μL of carrier-free $^{35}SO_4^{2-}$ that was 1 μCi total activity (Perkin Elmer Life and Analytical Sciences, Inc.) and incubated in the lab (23 °C). After 30 min, we stopped SO_4^{2-} reduction by injecting the cores with 1 mL of 30% (saturated) zinc acetate and immediately put them on dry ice to stop microbial activity. We analyzed sulfate reduction into both acid-volatile sulfur and chromium reducible sulfur and summed them (Fossing and Jorgensen, 1989). Sulfides were trapped in sulfide antioxidant buffer (Brouwer and Murphy, 1994) and analyzed with an ion-specific electrode.

We measured the production of CH_4 and CO_2 in sealed serum bottles over a period of four days. Soil slurries were created using 10–15 mL of field-moist soil and, as appropriate for each site, either 5 mL of oxygen-free deionized water or 5 mL of oxygen-free, one third-strength artificial sea water. Artificial sea water was created from 12 g salt per kg water following the recipe from the Marine Biological Laboratory at Woods Hole, MA (Cavanaugh, 1975), except that we doubled the amount of $MgCl_2$ and left out the $MgSO_4$ to prevent adding additional sulfate. The added water diluted the [SO_4^{2-}] by 25–40%, which may have lowered rates of SO_4^{2-} reduction in samples that were not amended with SO_4^{2-} below field-incubation rates; nonetheless, SO_4^{2-} reduction dominated microbial respiration at the brackish site as expected. Samples were stored in the dark at 20 °C. Headspace gas samples (200 μL) were collected once a day for four days and analyzed for CH_4 using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Kyoto, Japan). CO_2 was measured on additional headspace samples using a LiCor 7000 CO_2/H_2O Analyzer (Lincoln, NE). Linear rates of accumulation of CH_4 and CO_2 ($r^2 > 0.90$) were expressed per day and per gram dry soil.

When we collected the soils incubated for 31 months in the field, we did short-term (four day) and long-term (eight week) amendment experiments with each soil by adding Fe(III) or SO_4^{2-} to stimulate alternative microbial respiratory pathways. Fe(III) was added as ferrihydrite (amorphous Fe) made according to Rabenhorst and Burch (2006). Briefly, we titrated a 0.5 M $FeCl_3$ solution with 1 M KOH until it reached pH 7.5, poured the solution into dialysis tubing with a molecular weight cut off of 6000–8000 Da (Spectra/Por®, Spectrum Laboratories, Inc.), and incubated the tubing in deionized water over several days to remove ions from the solution. To determined the total amorphous iron content of our preparation, we extracted a 0.5 mL aliquot with 0.5 M HCl (1 h), then added 0.25 uL of this solution to 0.25 M hydroxylamine with ferrozine buffer and incubated it in the dark (4 h), and finally measured absorbance at 562 nm on a spectrophotometer (Lovley and Phillips, 1986).

Our short- and long-term incubations used soil slurries as described above, except that we slurried the 10–15 mL of soil with 20 mL of deionized water or one third-strength SO_4^{2-} free artificial sea water, and added Fe(III) or SO_4^{2-} amendments or no amendments for a control. For the short-term incubations, we did a one-time addition of 500 μmol Fe(III) (approximately 3 mL of 167 mM Fe(III) solution) and 128 μmol of SO_4^{2-} (5 mL of 25.5 mM Na_2SO_4), and measured the accumulation of CH_4 and CO_2 in the headspace

Table 1
Mean soil properties for each soil (±1 S.E.) measured on the soils incubated for 31 months in the field. Nitrate data were log transformed prior to the analysis but values presented here are in the original units[†].

Soil property	Fresh	Brackish@Fresh	Brackish	Fresh@Brackish
Bulk density (g cm^{-3})	0.412 (0.002)[a]	0.140 (0.002)[b]	0.16 (0.002)[c]	0.423 (0.005)[a]
% Soil organic matter	17.16 (0.11)[a]	57.82 (0.59)[b]	56.04 (0.29)[c]	17.96 (0.10)[a]
Microbial biomass (μg g^{-1})	4.32 (2.16)[a]	4.54 (2.03)[a]	8.32 (3.72)[a]	15.86 (7.09)[b]
Nitrate (mg N L^{-1})	0.01 (0.00)[a]	0.01 (0.00)[a]	0.46 (0.032)[b]	0.35 (0.08)[b]
Sulfate (mg S L^{-1})	0.03 (0.01)[a]	0.06 (0.01)[a]	93.32 (17.41)[b]	128.15 (34.78)[b]

[†]Letters indicate significant differences (p-value ≤ 0.05).

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for four days. For our long-term incubations, we again added 500 μmol Fe(III) and 128 μmol of SO_4^{2-} to each bottle as in the short-term incubations, the one difference being that in the long-term additions we added these additions once per week for 8 weeks. Then we took weekly measurements of CH_4 and CO_2 in the headspace. We estimated that adding 500 μmol of Fe(III) per week to each serum bottle would prevent Fe(III) limitation. This calculation was made assuming a ratio of 4 mol Fe(III) reduced to 1 mol CO_2 produced (Roden and Wetzel, 1996), the maximum rate of Fe(III) reduction previously measured at these sites ($\sim$7.5 μmol C g^{-1} dry soil d^{-1}), and the measured dry mass:wet mass ratio for our soils (0.31 g g^{-1} for the Fresh soils and 0.13 g g^{-1} for the Brackish soils). Based on prior measurements of sulfate reduction at these two locations which showed that maximum rates were around 6 μmol C g^{-1} dry soil d^{-1} (Neubauer et al., 2005), and assuming the ratio of 1 mol of SO_4^{2-} reduced for every 2 mol of CO_2, 128 μmol of SO_4^{2-} would provide more than enough SO_4^{2-} to stimulate sulfate reduction for one week. All samples were stored in the dark at 20 °C between measurements.

2.4. Statistical analysis

Differences in total organic carbon mineralization between soils were assessed using two-way ANOVA models and Tukey Honestly Significant Differences (HSD) comparisons. We used ANOVAs and pair-wise *t*-tests with Bonferroni corrections for multiple comparisons to examine the differences between the short-term amendment treatments. Data were log transformed when necessary to conform to the assumptions of normality. Statistical significance was set at p-value ≤ 0.05. Analyses were conducted in the statistical software R 2.7.2 (R Development Core Team, 2008).

3. Results

The organic matter content was about 17% in soils originally from the freshwater site and more than 3-fold greater in soils from the brackish site (Table 1). The carbon content of the freshwater soils was not significantly affected by two years of incubation at the brackish site (Fresh site = 17.2% and the Fresh@Brackish site = 18.0%); there was a small, but statistically significant, difference in the organic matter content of brackish marsh soils incubated at the two sites (Brackish@Fresh site = 57.8% and the brackish site = 56.0%; Table 1). Microbial biomass was significantly higher in the Fresh@Brackish soil than all the other soils, but the Brackish soil had 2-fold greater average microbial biomass than either of the soils at the freshwater site (Table 1).

The pronounced difference in soil carbon content between the two sites influenced soil organic carbon mineralization (the sum of $CO_2 + CH_4$ produced). Mineralization on a soil mass basis was greatest in the soils that originally came from the brackish site (Brackish and Brackish@Fresh), reflecting their relatively high soil organic matter content (Fig. 2a and b). The only difference between the two sampling dates was that in October 2008 the two soils from the brackish site had comparable rates of mineralization, while in May 2009 the Brackish@Fresh soil had greater mineralization than the Brackish soil; however, they were both significantly greater than the soils from the freshwater site. These differences in rates of total mineralization per mass of soil indicate that heterotrophic microbial activity increased with soil carbon content, the major source of electron donors.

The trend was opposite when organic carbon mineralization was expressed on a per mass of soil carbon basis. In October 2008, a greater proportion of the soil carbon was respired from freshwater-site soils than brackish-site soils (Fig. 2c). This pattern was not apparent in May

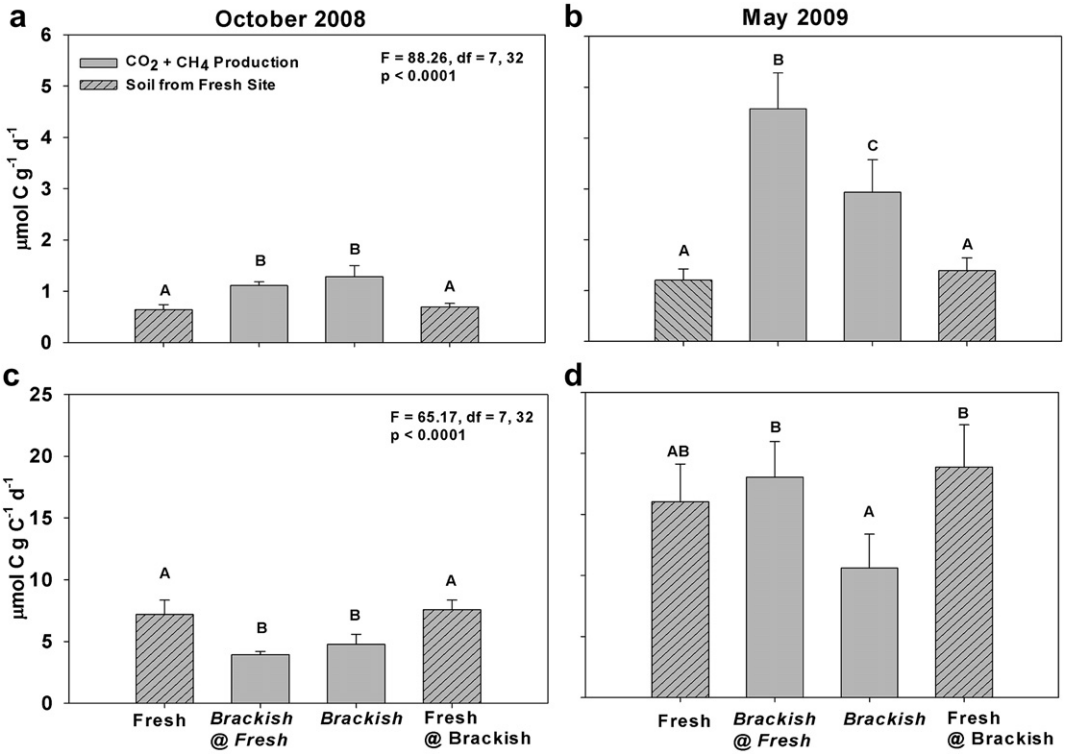

Fig. 2. Total organic carbon mineralization ($CO_2 + CH_4$) per gram soil per day (±1 S.E.) for soils in (a) October 2008 and (b) May 2009, and total mineralization per gram soil carbon per day for soils in (c) October 2008 and (d) May 2009. $N = 5$ replicate cores for all treatments. To facilitate comparison and distinguish the soil origin for each group, hatching indicates soils that came from the fresh site originally while bars without hatching were originally from the brackish site. Data were log transformed for the statistical analysis in order to conform to the assumptions of normality, but the data are displayed in their original units for comparison. Letters indicate a significant difference (p-value < 0.001) between the soils within sampling dates based on two-way ANOVAs and Tukey HSD comparisons. Each soil compared between sampling dates was significantly different (i.e., each soil type in October 2008 was significantly different from the same soil type measured in May 2009).

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Table 2
Relative importance of measured microbial respiration pathways as a percent of the total (Mean ± 1 S.E.) for unamended soils incubated for 31 months in the field. Values add up to 100% because they are based on mineralization rates calculated from direct measurement of these two processes; they do not consider possible unexplained sources of CO_2 or CH_4, such as Fe(III) reduction (see Methods).

% Total anaerobic metabolism	Fresh	Brackish@Fresh	Brackish	Fresh@Brackish
SO_4 reduction	20.3 (6.5)	32.0 (11.0)	97.9 (0.5)	89.0 (3.5)
Methanogenesis	79.7 (6.5)	68.0 (11.0)	2.1 (0.5)	11.0 (3.5)

2009 when rates of mineralization per mass of soil carbon were comparable at the two sites (Fig. 2d). These results suggest that, in addition to carbon quantity, carbon quality of these soils influenced rates of mineralization.

Regardless of soil type, anaerobic carbon mineralization was dominated by methanogenesis at the freshwater site, and by sulfate reduction at the brackish site (Table 2). These differences in the dominant microbial pathways between the sites were driven by terminal electron acceptor availability, with SO_4^{2-} being far more abundant at the brackish site.

In short-term (four day) incubations with different terminal electron acceptor additions, rates of mineralization on a soil-mass basis were lower for all Fe(III) additions than the control soils with no TEA additions. Soils that received SO_4^{2-} additions showed either no change (Fresh and Brackish@Fresh soils) or an increase (Brackish and Fresh@Brackish soils) in mineralization rates (Fig. 3). In long-term (eight week) incubations, overall rates of mineralization were generally higher with Fe(III) additions than SO_4^{2-} additions (Fig. 4). This was true for all but the Fresh@Brackish soils, where rates appeared to be higher with the SO_4^{2-} addition perhaps because the population of sulfate reducers in this transplanted soil was adapted to the higher levels of available SO_4^{2-} at the brackish site. Our results suggest that organic carbon mineralization rates can be influenced by the terminal electron accepting pathway.

4. Discussion

4.1. Role of electron donors: carbon quantity and quality

The purpose of this study was to ascertain the importance of electron donors (soil carbon quantity and quality) and electron acceptors (e.g., Fe(III), SO_4^{2-}) in controlling rates of anaerobic soil carbon matter mineralization. The soils in this experiment differed by over 3-fold in soil organic matter content (Table 1), and organic matter content of soils is a strong regulator of microbial activity and biomass (Groffman et al., 1996; Sutton-Grier et al., 2009). In comparison to the freshwater marsh, the brackish marsh soils contained more soil organic matter, higher microbial biomass, and supported higher rates of mineralization on a per gram basis (Fig. 2a and b). The fact that this difference between soil types persisted when soils were transplanted to a different TEA environment (i.e., freshwater or brackish), indicates that site differences in carbon quantity or quality were more important than TEA availability in regulating overall mineralization rates. Although it was beyond the scope of this study to fully characterize the microbial communities in each soil, the observed differences in biomass and mineralization rates suggest that there were differences in the microbial community structure between the brackish and freshwater soils that persisted even when the soils were transplanted to different environments. We note that the 3-fold difference between sites in soil organic matter content was particularly large in the present experiment, and recognize that additional factors may help explain variation in soil organic matter decay rates when the difference in carbon content is less dramatic.

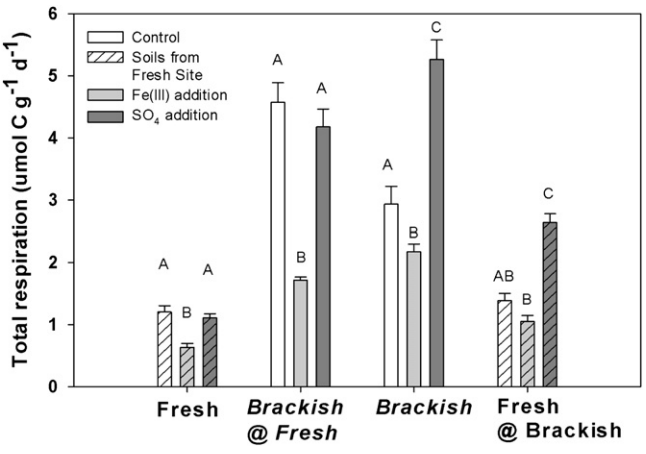

Fig. 3. Rate of organic carbon mineralization ($CO_2 + CH_4$) per gram soil per day for the soils collected after 31 months of incubation in the field and then incubated in the laboratory for four days with different terminal electron acceptors (TEAs); $N = 5$ replicate cores. Hatching indicates soils that came from the fresh site originally while bars without hatching were originally from the brackish site. Letters indicate significant differences within each soil type. Data for the Brackish and Fresh@Brackish soils were log transformed for the statistical analysis but are presented here in their original units.

We determined that the quality of soil carbon also influenced mineralization rates. When we accounted for differences in the carbon content of the soils by expressing mineralization on a carbon mass basis, the freshwater marsh soil supported rates of mineralization that were greater than, or comparable to, the brackish marsh soil (Fig. 2c and d). Even though we were not able to fully characterize the chemical makeup of the organic matter, these differences in mineralization rates suggest that organic carbon compounds in the freshwater soil are more susceptible to microbial mineralization than those in the brackish marsh soil. The pool of labile organic carbon in soils is derived largely from plant inputs, therefore differences in plant community composition and plant ecophysiological traits, such as the C:N ratio of plant tissues, have important effects on the quality of soil carbon pools. The soils from the freshwater site were collected from a part of the marsh in which the dominant plants are broadleaf, emergent vegetation, including *P. virginica* and *P. cordata*, both of which have low shoot C:N ratios (16.3 and 11.5, respectively) (Neubauer et al., 2000), while the vegetation at the brackish site, included *S. alterniflora*, *S. patens*, and *D. spicata*, which have higher C:N ratios than at the freshwater site (28.6, 53.1, and 45, respectively) (Frasco and Good, 1982; Hunter et al., 2008). Carbon inputs from freshwater plants, including *P. virginica* and *P. cordata*, decompose more quickly than brackish or saline species, including *S. alterniflora*, because they have a higher N content (i.e., a lower C:N ratio) and a lower cellulose and lignin content (Odum and Heywood, 1978). The observation that microbial activity on soil carbon mass basis was higher for the soils with higher quality carbon is consistent with a number of previous reports (Groffman et al., 1991; Schipper et al., 1994; Hill and Cardaci, 2004). Several peatland studies also have demonstrated that both CO_2 and CH_4 production are related to the lability of soil organic carbon compounds (Yavitt and Lang, 1990; Bridgham and Richardson, 1992; Crozier et al., 1995), suggesting that carbon quality has a strong influence on rates of greenhouse gas production (Bridgham and Richardson, 1992; Wagner et al., 2005; Reiche et al., 2010).

Our results contrast with a previous study at the same two marshes that found higher rates of organic carbon mineralization on a soil-mass basis at the freshwater marsh site than at the brackish marsh site (Neubauer et al., 2005). There are a number of differences between these two studies that could explain these divergent results. Perhaps most important is the fact that our soils were subjected to decomposition for 31 months without new, labile

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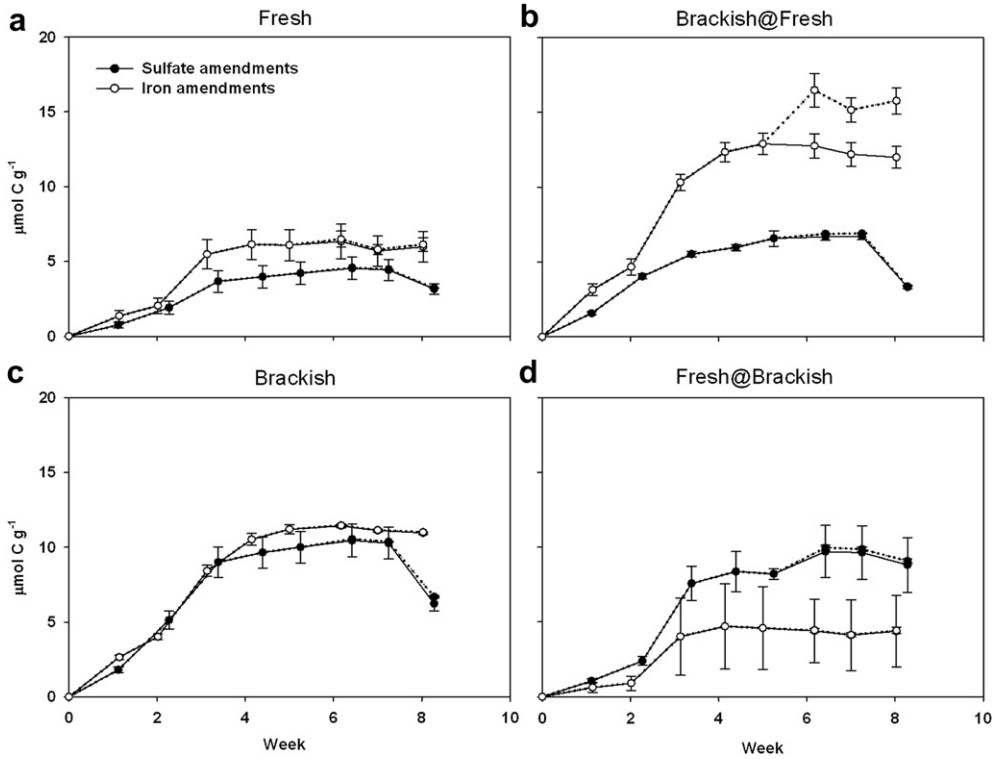

Fig. 4. Cumulative CO_2 or CH_4 (±1 S.E.) accumulated in the headspace of soil incubation bottles in the laboratory. Soil were deployed in the field for 31 months, then incubated for eight weeks in the laboratory with either SO_4 amendments (filled circles) or Fe(III) amendments (open circles). Comparisons are made for (a) Freshwater soils, (b) Brackish@Fresh soils, (c) Brackish soils, and (d) Fresh@Brackish soils ($N = 5$ replicate cores). Solid lines represent mineralization to CO_2. Dotted lines represent methane emitted *in addition* to the CO_2 respired. In most cases there was very little CH_4 produced (<3% of the total carbon respired) except for the Brackish@Fresh soils with the Fe(III) amendments where between week 5 and 6 CH_4 accounted for ~25% of the carbon respired. Values represent accumulated headspace CH_4 or CO_2 over the given incubation period.

plant inputs. Thus, it is likely that the most labile carbon compounds initially present in both soils had been consumed before our incubations were performed. Our results reflect the properties of organic compounds that constitute the largest portion of the soil organic matter pool, those with residence times >1 year.

4.2. Role of electron acceptors

As previously demonstrated, the availability of terminal electron acceptors influences the dominant pathway of microbial respiration. In both soils incubated at the brackish site, sulfate reduction was the dominant microbial pathway while methanogenesis dominated in both soils incubated at the freshwater site (Table 2). The relative contributions of each pathway to overall anaerobic metabolism are consistent with differences in the availability of SO_4^{2-} as an electron acceptor at the two sites.

The more novel and interesting question we addressed is whether differences in the free energy yield of anaerobic microbial respiratory pathways caused differences in soil organic matter mineralization rates. If this is the case, one should observe that mineralization rates increase with the free energy yield of the dominant TEA process. Our results from the reciprocal transplant soil incubations are equivocal on this point (Fig. 2). There was no evidence of increased mineralization with shifts in microbial respiration pathways. Rates of mineralization for both the brackish and freshwater soils were similar whether the soils were incubated in their original location or the reciprocal location (Fig. 2). This occurred despite evidence that the dominant microbial process was strongly controlled by incubation site (Table 2). These results agree with those of D'Angelo and Reddy (1999) who found no significant effect of the dominant TEA process on organic carbon mineralization rates.

By contrast, results from the short-term amendment study provided some evidence that mineralization rates are influenced by short-term variation in TEA availability. At the brackish site, where microbial communities were accustomed to relatively high SO_4^{2-} availability compared to the freshwater site, SO_4^{2-} amendment enhanced mineralization rates compared to controls in both soil types (Fig. 3). At the freshwater site, SO_4^{2-} amendment did not increase mineralization of either soil type. Thus, electron acceptor availability, and perhaps the specific TEA pathway (i.e., SO_4^{2-} reduction versus methanogenesis), influenced short-term (4 d) mineralization rates provided the microbial community was preselected to use the dominant TEA.

A limitation of interpreting our short-term SO_4^{2-} amendment results as an outcome of changing the TEA pathway is the fact that our soils did not respond the same way to the Fe(III) amendment. The fact that the short-term Fe(III) amendment caused a significant suppression of respiration rates is difficult to explain on the basis of changes in terminal electron acceptor availability. It is possible that adding the Fe(III) amendment as an amorphous solid created a coating over surface-attached microorganisms that slowed microbial activity for a period of time. Another possibility is that reduced respiration was caused by the 1-unit decrease in pH in Fe(III)-amended slurries (data not shown; Keller et al. (2009)). A third possibility is that Fe(III) directly inhibited methanogenesis through a mechanism that does not shunt electrons to a CO_2-yielding respiration process, as shown to occur in pure methanogenic cultures (van Bodegom et al., 2004). Our data are consistent with this mechanism because the decrease in total respiration was caused by a decrease in CH_4 respiration and not CO_2 respiration (data not shown).

We do not have sufficient evidence to conclude whether the decrease in respiration rates following short-term amendments of Fe(III) was a meaningful biological response or an artifact of the

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experimental design. However, the long-term amendment experiment did provide limited evidence that Fe(III) amendment can enhance organic carbon mineralization compared to SO_4^{2-} amendment (Fig. 4). The mineralization rates of all but the Fresh@Brackish soil were higher with amorphous Fe(III) addition than SO_4^{2-} addition. This is the predicted response if organic carbon mineralization rates were limited by the energy yield of the TEA pathway.

In anaerobic systems, a consortium of organisms is required to mineralize organic carbon to the inorganic end products CO_2 and CH_4, and competition for electron donors favors pathways with higher energy yield (Megonigal et al., 2004). However, it is unclear whether the terminal step regulates overall rates of anaerobic organic matter mineralization, except for the well known comparison of aerobic and anaerobic processes (e.g., Reddy and Graetz, 1988). The number of previous studies on this topic is limited, and they report equivocal results. One study along an estuarine gradient in the Netherlands determined that anaerobic organic carbon mineralization was greater in wetlands dominated by methanogenesis than in marine-influenced environments with abundant SO_4^{2-} (Middelburg et al., 1996). Similarly, mineralization of barley and diatom particles under sulfate-reducing conditions was greater than, or equal to, mineralization under denitrifying conditions (Kristensen and Holmer, 2001). D'Angelo and Reddy (1999) reported no differences in carbon mineralization in wetland soil incubations as TEA pathways changed. Yet, other studies suggest that the free energy yield of TEAs does influence mineralization rates in anaerobic environments. Weston et al. (2006) reported that a shift from methanogenesis to sulfate reduction in flow-through reactors loaded with tidal freshwater river sediment doubled mineralization rates. Using flow-through reactors and intertidal marsh sediments, Pallud et al. (2007) found that nitrate reduction led to faster rates of organic matter decomposition versus sulfate reduction. In another study, supplying nitrate also led to greater mineralization than in control treatments with no terminal electron acceptor additions (Abell et al., 2009); the authors suggest that this increase occurred because denitrifiers were able to access soil organic carbon that was not available to the fermenting microbial community. The results of our field transplant experiments were also equivocal, but overall they support the hypothesis that the dominant TEA pathway can influence carbon mineralization rates. Differences in the dominant TEA pathway achieved through site placement did not influence rates of mineralization in unamended, short-term incubations. However, results from short-term incubations amended with SO_4^{2-} suggested that organic carbon mineralization rates were higher than in unamended controls, and results from long-term incubations suggested that Fe(III) amendment enhanced mineralization rates compared to SO_4^{2-} amendment. Collectively, it is clear that the question of TEA pathway control on carbon mineralization rates remains largely untested and unresolved, especially with respect to the influence of Fe(III) reduction.

It is also interesting to note that both SO_4^{2-} and Fe(III) additions largely inhibited the less energetically favorable process of CH_4 production in the long-term incubation experiments (Fig. 3). Indeed, CH_4 was not produced until after four weeks of incubation in the Brackish@Fresh soil amended with Fe(III). This soil had the highest observed rate of organic carbon mineralization, which suggests that electron flow may have been sufficient to deplete the Fe(III) amendment, allowing electrons to flow to methanogens (SO_4^{2-} was present in very low concentrations, approximately 0.045 mg S L^{-1}).

5. Summary and conclusions

We demonstrated that both electron donors (organic carbon) and electron acceptors (Fe(III) and SO_4^{2-}) influence rates of microbial organic matter mineralization. With respect to electron donors, organic matter quantity dictated overall rates of mineralization in a comparison of two soils with a 3-fold difference in soil carbon content. But, when we normalized mineralization rates by the soil organic carbon content, mineralization was higher in the soil with the lower carbon content (i.e., freshwater marsh soil), suggesting that the quality of carbon was higher in soils where carbon inputs come from easily decomposable freshwater emergent plants. Evidence of an effect of TEA pathway on carbon mineralization was equivocal. In a field transplant study, there was no conclusive evidence that carbon mineralization was influenced by SO_4^{2-} availability for a given soil type. However, SO_4^{2-} amendments increased short-term (4 d) rates of carbon mineralization compared to controls in both soil types, provided the soils had been incubated at the brackish site, and Fe(III) amendment appeared to enhance mineralization rates compared to SO_4^{2-} amendment in long-term (8 week) lab incubations. Collectively, we interpret our data to suggest that the free energy yield of terminal microbial respiration pathways may influence rates of soil organic matter mineralization (i.e., Fe(III) respiration > SO_4^{2-} reduction > methanogenesis). If so, the terminal pathway would have to be the limiting step in an elaborate series of processes that ultimately reduce complex organic compounds to greenhouse gases. This hypothesis requires more testing because other studies both support and refute our results, and because of its implications for control of greenhouse gas production via organic carbon mineralization in wetlands. For example, factors that influence both electron donors and acceptors, including landscape position, surrounding land use, plant community, and environmental conditions, may need to be modeled in order to predict greenhouse gas emissions from wetlands.

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