RATES OF ANAEROBIC MICROBIAL METABOLISM IN WETLANDS OF DIVERGENT HYDROLOGY ON A GLACIAL LANDSCAPE

Stefanie L. Whitmire[1] and Stephen K. Hamilton[2]
[1]*Universidad de Puerto Rico*
Mayagüez, Departamento de Agronomía y Suelos
PO Box 9030
Mayagüez, Puerto Rico, USA 00681-9030
E-mail: swhitmire@uprm.edu

[2]*W.K.Kellogg Biological Station and Department of Zoology, Michigan State University*
3700 East Gull Lake Drive
Hickory Corners, Michigan, USA 49060

Abstract: Biogeochemical transformations in wetlands impact water quality, nutrient transport across landscapes, and greenhouse gas exchanges with the atmosphere. This study examined anaerobic microbial respiration and methanogenesis in surficial sediments of six wetlands lying on glacial terrain in southwest Michigan, USA. Three of the wetlands were mainly groundwater-fed and three were mainly precipitation-fed. Ambient rates of denitrification, sulfate reduction, iron reduction, methanogenesis, and acetate turnover were measured at each wetland. Ambient denitrification rates were not detectable in any wetland, but denitrifying enzyme activity, measured in two wetlands, indicated that the potential to remove nitrate was higher in a groundwater-fed wetland. Iron reduction was measurable mainly in precipitation-fed wetlands while sulfate reduction was only measurable in the groundwater-fed wetlands. Methanogenesis was measurable in all wetlands, with no differences between wetlands with contrasting water sources, indicating that methanogenesis is important regardless of water source. Acetate turnover rates, which reflect total anaerobic respiration and methanogenesis, were higher in the groundwater-fed wetlands and proportional to the sum of the individual carbon mineralization rates across all wetlands. Even though there was substantial variation in the process rates among these wetlands, the general patterns indicate that water source influences the biogeochemical function of wetlands.

Key Words: anaerobic respiration, denitrification, freshwater wetlands, iron reduction, methanogenesis, sulfate reduction

INTRODUCTION

Wetlands are ecosystems with exceptional ecological and economic values (Costanza et al. 1997). They perform hydrological and biogeochemical functions that affect downstream water quality and hence impact the overall landscape (National Research Council 1995). Anaerobic microbial processes such as denitrification, iron and sulfate reduction, and methanogenesis are key biogeochemical functions of wetlands that extend beyond their roles in the terminal decomposition of organic matter. Denitrification improves water quality by removing excess nitrate (Johnston 1991, Groffman et al. 1996, McLatchey and Reddy 1998). Sulfate reduction is responsible for toxic levels of sulfide in many wetland sediments (Wang and Chapman 1999). Methanogenesis in wetland environments contributes up to half of global emission of CH_4, a potent greenhouse gas, to the atmosphere (Cicerone and Oremland 1988).

Many factors control anaerobic microbial metabolism in wetlands (Fenchel et al. 1998, Megonigal et al. 2004). These include hydrology, temperature, pH, labile carbon availability (Conrad 1996, Segers 1998), and availability of alternative electron acceptors (Lovley and Klug 1982, Yavitt and Lang 1990). It is important to understand these controls on the microbial processes that affect wetland biogeochemistry and how they might change under the influence of human activities. Aerobic respiration is the most energetically favorable terminal catabolic process, but with oxygen depletion in wetland sediments aerobic respiration becomes inhibited and is often followed sequentially by the respiratory reduction of the terminal electron acceptors nitrate (NO_3^-), manganese (Mn(IV)), iron (Fe(III)), and sulfate (SO_4^{-2}). Methanogenesis is the least efficient anaerobic microbial process for the terminal step of organic matter decomposition, and thus it predominates when the inorganic alternative elec-

Table 1. Surface water and sediment characteristics of the study sites. G and P indicate ground water or precipitation as the predominant water sources, respectively, with fraction ground water estimated based on dissolved Mg^{2+}. Surface water and sediment porewater measurements are means from several sampling dates. Organic carbon means are from three replicate cores. NO_3^- concentrations were usually below detection (10–15 µg N/L) in wetland waters. Standard deviations are given in parentheses.

Site (Label)	Area (ha)	Fraction ground water	Surface water conductance (µS/cm; 25°C)	Sediment pH	Sediment % organic matter	Surface water SO_4^{2-} (mg/L)
Loosestrife Fen (G1)	0.4	0.93	520 (34)	6.6 (0.9)	31 (22)	15.6 (8.5)
Kalamazoo River Floodplain (G2)	14.5	0.9	544 (57)	6.5 (0.2)	49 (2.3)	3.51 (0.79)
Turkey Marsh (G3)	3.1	0.71	370 (48)	6.9 (0.6)	61 (9.5)	18.2 (6.0)
Shaw Pond (P1)	0.8	0.38	34 (4)	5.4 (0.2)	46 (17)	2.07 (0.93)
Lux Pond 10 (P2)	0.7	0.05	26 (2)	5.6 (0.1)	7.3 (4.3)	1.88 (0.92)
LTER Kettle (P3)	0.4	0.05	44 (11)	5.3 (0.2)	17 (17)	4.98 (2.30)

tron acceptors that can support anaerobic respiration are not available. Most previous studies of these anaerobic microbial processes have focused on the sediments of lakes, estuaries, and oceans (Lovley and Klug 1982, Kristensen et al. 1994, deGraaf et al. 1996) and on contaminated aquifers (Vroblesky and Chapelle 1994, Chapelle et al. 1996, Landmeyer et al. 2000). Where wetlands have been examined, most studies have dealt with a single ecosystem or process, and comparative studies of multiple processes across sites are uncommon (Groffman et al. 1996, D'Angelo and Reddy 1999, Neubauer et al. 2005).

Hydrology is perhaps the most important factor regulating biogeochemical processes in wetlands generally, yet it has not been considered in the context of anaerobic respiration pathways. Wetlands exist along a gradient of water source from being primarily groundwater fed to primarily precipitation fed (Euliss et al. 2004). Precipitation-fed wetlands tend to be depleted in alternative electron acceptors. Wetlands receiving ground water inputs, either directly or via stream or river flow, usually have higher concentrations of alternative electron acceptors (e.g., Hopkinson 1992), although concentrations will vary from region to region based on soil and bedrock minerals.

Spatial gradients commonly develop in which the sequential replacement of the predominant microbial process occurs with depth or with distance along a hydrological flow path. For example, along a small groundwater-fed stream in southwestern Michigan, Hedin et al. (1998) found that denitrification occurred closest to the emergence of ground water, followed by sulfate reduction and ultimately methanogenesis once nitrate and sulfate were depleted, all over a distance of just a few meters. Similar gradients also occur over time after flooding of wetland soils (Ponnamperuma 1972, D'Angelo and Reddy 1999).

This study examined the rates of terminal processes of anaerobic microbial decomposition across a range of freshwater wetlands lying in close geographic proximity but differing in their predominant water sources. Our hypothesis was that groundwater-fed wetlands would have higher rates of anaerobic decomposition (particularly denitrification and sulfate reduction) than precipitation-fed wetlands, as a result of the continuous inputs of the electron acceptors (e.g., nitrate and sulfate). We predicted that wetlands in which direct precipitation inputs are relatively important would have higher rates of methanogenesis due to the limited supply, episodic inputs, and quick depletion of alternative electron acceptors.

METHODS

Study Sites

The study area is a glacial landscape in southwest Michigan, near the Kellogg Biological Station (KBS) of Michigan State University. This study was conducted in six wetlands, three of which are mainly groundwater-fed (G1, G2, G3) and three others that are mainly precipitation-fed (P1, P2, P3) (Table 1). Detailed site descriptions are found in Whitmire (2003) and Whitmire and Hamilton (2005). The groundwater-fed wetlands include an isolated pond (Turkey Marsh) and two fen wetlands that drain into a nearby stream or river. Ground water discharge in these wetlands was visible as seeps or springs. The precipitation-fed wetlands are isolated depressions that contained shallow standing water. The approximate fraction of ground water in each wetland was ascertained using dissolved magnesium (Mg^{+2}) as a ground water tracer

Table 2.   Sampling dates for each wetland and number of sites sampled for each rate measurement within the wetland. The numbers in parentheses in G3 and P2 are the number of sites sampled in 2001 if different from 2000. All of these measurements were made between May–September.

Wetland	2000	2001	Denitrification	Iron reduction	Sulfate reduction	Methanogenesis	Acetate Turnover
G1		X	2	3	2	3	2
G2	X		3	3	2	3	1
G3	X	X	3 (2)	3	2 (3)	3	2
P1		X	2	3	2	3	2
P2	X	X	3 (2)	3	2	3	1 (2)
P3	X		3	3	2	3	2

(Stauffer 1985). Mg^{+2} is a good indicator of ground water inputs in this setting because: 1) it is little affected by biological uptake, 2) it is not subject to mineral precipitation reactions in these waters, and 3) it is a major weathering product from dissolution of dolomite in the underlying glacial till, and thus enriched in ground water relative to precipitation.

The general chemical composition of each site was characterized with surface water, sediment pore water, and sediment samples. Porewater samples were collected with porewater equilibrators (Whitmire 2003). NO_3^--N and SO_4^{2-} concentrations were analyzed in surface water and porewater using membrane-suppression ion chromatography (Dionex 4200), and dissolved organic carbon (DOC) was analyzed by high-temperature platinum-catalyzed combustion to CO_2 followed by infrared gas analysis (Ionics 1505). Sediment was collected from the top 10 cm of three replicate sediment cores. Percent organic matter was determined by loss on ignition after combusting samples in a muffle furnace at 500°C.

Sediment Sampling

The six wetlands were sampled during June to August of 2000 and 2001, with four wetlands sampled in each year (Table 2). Wetlands G3 and P2 were sampled during both years to assess interannual differences in rate measurements. Within the wet perimeter of the precipitation-fed wetlands, two to three sampling sites were randomly chosen to represent the wetland. In the groundwater-fed wetlands, the sites were aligned in relation to the flow path of water. To avoid the direct influence of roots on sampling and measurements, sites with little or no vascular plant growth were chosen, but in most cases plants occurred within one meter of sampling points. Not all the sites within each wetland were sampled for each process rate measurement.

Soil cores for each process measurement were taken at each sampling site by hand with an acrylic tube (7.6 cm diameter). A particular process rate was measured across all of the wetlands within 1 week to minimize temporal variability and facilitate comparisons among wetlands, with all processes being measured over the summer. Subcores were collected using cut-off plastic syringes from 1-cm holes along the length of the core tube from 0–10 cm beneath the sediment/water interface. These subcores were capped with butyl rubber stoppers, submerged in jars with deoxygenated water, stored on ice in a dark cooler, and immediately transported to the lab. The subcores were extruded into pre-weighed glass vials and flushed with O_2-free N_2 to ensure anoxic conditions in the headspace. Total sediment volumes collected for each assay were as follows: denitrification, 30 mL; iron reduction, 6 mL; sulfate reduction and acetate turnover, 3 mL; methanogenesis, 3 mL. Samples were incubated in the dark at 24°C.

Possible homogenization of sub-centimeter-scale spatial variation by our soil sampling procedure was acceptable within the goals of this study, which focused on comparisons across wetland types.

Rate Measurements

Denitrification. Denitrification rates were measured using a modified acetylene inhibition method (Tiedje et al. 1989). Acetylene blocks the reduction of N_2O to N_2, causing N_2O to accumulate. After flushing the headspace with N_2, acetylene (10 kPa) was added to each jar and the jars were shaken to distribute the acetylene. Gas samples were taken from the headspace at 10, 30, and 60 minutes, placed in over-pressurized, gas-tight serum vials with butyl stoppers, and analyzed as soon as possible on a Hewlett Packard gas chromatograph containing a Porapak-Q packed column and an electron capture detector. Gas samples were stored no more than 1 month; storage tests have indicated that N_2O is stable under these conditions (S.K. Hamilton, unpublished data). Denitrification rates were determined from the change in N_2O during the incubation, divided by the volume of the soil and the incubation time (Groffman et al. 1999).

Denitrification Potential. We measured denitrification potential (enzyme activity) of the sediment by removing nitrogen and carbon limitations for the bacteria, following Groffman et al. (1999), except that we only added 0.05 mM of potassium nitrate to each jar. Gas samples were analyzed as above. This assay was done once during the summer of 2001 at wetlands G3 and P2.

Iron Reduction. Iron reduction rates were determined in wetland sediment cores by measuring the accumulation of dissolved Fe(II) after incubation of 0, 3, and 10 days (Lovley and Phillips 1986a, Roden and Wetzel 1996). There were three replicates for each site within each wetland. Fe(II) was measured using reaction with ferrozine after acid extraction. 0.1 mL of the incubated sediment sample was added to a preweighed vial containing 5 mL of 0.5N HCl. After 1 hour at room temperature, 0.1 mL of this mixture was reacted with 5 mL of ferrozine (1 g/L) in 50 mM HEPES buffer (pH 7). After mixing for 15 seconds, the sample was filtered through a polycarbonate filter (0.2 μm) and its absorbance at 562 nm was read on a spectrophotometer (Stookey 1970). Fe(II) reduction rates were calculated from the linear portion of the Fe(II) vs. time curve.

Sulfate Reduction. Subcores to assay sulfate reduction rates were incubated for 0, 1.5, and 3 hours with 1 μCi of $Na^{35}SO_4$ (American Radiolabeled Chemicals Inc. ARS-105 ^{35}S as sodium sulfate). Microbial activity was stopped by quick freezing the sediment slurry in liquid nitrogen. The samples were acidified with 10 mL of O_2-free 4N HCl to release $H_2^{35}S$, flushed with helium for 30 minutes on a non-metallic flushing manifold with a series of three vials that trapped the $H_2^{35}S$ in 2.5% zinc acetate, and counted on a Wallac scintillation counter with 5 ml of scintillation cocktail. There was generally no detectable $H_2^{35}S$ in the third vial. Residual $^{35}SO_4^{-2}$ was analyzed by filtering 1 mL of the sample slurry, adding 5 mL of scintillation cocktail, and counting the sample. No corrections were made for ^{35}S trapped in organic compounds. The rate of sulfate reduction was calculated following Jorgensen (1978).

Methanogenesis. Methane production rates were determined by measuring the change in the partial pressure of methane (CH_4) in the headspace of sediment slurries over time. Samples were prepared in serum vials as in the assay of iron reduction above. Every 2–3 hours for 8–10 hours, 0.5 mL of the headspace was withdrawn, mixed with 4 mL of He, and injected into a Shimadzu gas chromatograph (GC14A) equipped with thermal conductivity (TCD) and flame ionization (FID) detectors. Methane was

separated with a Molecular Sieve 5A column and a quantified by the FID. Carbon dioxide was separated from the same gas samples by a Porapak-Q packed column and quantified by the TCD. Linear regression analysis was used to calculate rates.

Acetate Turnover. Acetate concentrations were measured to determine the pool size of acetate available to the microorganisms. Bioavailable acetate concentrations were determined based on the enzymatic reaction of acetate with acetyl coenzyme A synthase to produce adenosine monophosphate (AMP) (King 1991). Cores were taken at two sites within each wetland, subcored using 10-mL cut-off syringes, and stored in anaerobic conditions for no more than 2 hours prior to analysis.

To measure acetate turnover, 3-mL replicate subcores were taken at the same sites within a week after the acetate concentrations were determined. Using a method modified from Lovley et al. (1982), the sediment subcore slurries were labeled with 0.2 μCi of [1,2-^{14}C] acetic acid in sodium salt (Amersham, CFA229), and shaken gently to produce a homogeneous distribution of labeled acetate. The slurries were incubated at 23°C for 0, 30, and 60 minutes. The samples were placed in liquid nitrogen to stop microbial activity and kept frozen until analyzed.

The amount of $^{14}CH_4$ and $^{14}CO_2$ produced from the degradation of acetate was determined by passing the gas samples through a CO_2 trap followed by a combustion furnace and another CO_2 trap. Each trap consisted of a series of three scintillation vials containing 3 mL of ethanolamine plus 2 mL of methanol to trap CO_2. To convert all inorganic carbon to CO_2, 1.5 mL of 2M H_2SO_4 was injected into the slurry. The headspace of the slurry was bubbled with O_2-free He to sparge $^{14}CH_4$ and $^{14}CO_2$ out of the slurry solution. The sparged $^{14}CO_2$ was trapped in the first set of vials, while $^{14}CH_4$ continued through a combustion furnace at 850°C to combust $^{14}CH_4$ to $^{14}CO_2$. This $^{14}CO_2$ was trapped in another set of three vials containing ethanolamine and methanol. Prior to liquid scintillation counting, 10 mL of scintillation cocktail was added to the sample. The system was flushed for 10 minutes between each sample. More details are reported in Whitmire (2003).

Statistical Analysis

Differences among wetlands in the various measured rates were assessed by analysis of variance (ANOVA) in SYSTAT (SYSTAT 1998). We analyzed G3 and P2 first to determine if the rates were consistent across years. Because there were no
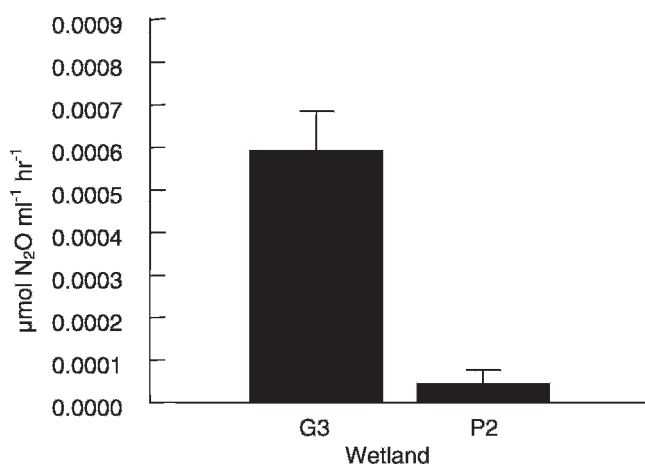
Figure 1. Denitrification potential of one groundwater-fed and one precipitation-fed wetland. The bars are means for three replicate sites within the wetland, with standard deviations.

differences between years, all the wetlands were analyzed together regardless of year. Nested ANOVAs were used to test for differences between wetlands of different water source. Correlations were conducted to examine relationships between anaerobic process measurements and other wetland features (DOC, porewater pH, porewater conductance, percent sediment organic matter).

RESULTS

Denitrification rates were not detected in any wetland in the absence of NO_3^- amendments. However, when nitrate was added to sediment slurries from two of the wetlands, denitrification enzyme activity was observed. There was a higher potential denitrification rate in the groundwater-fed wetland than in the precipitation-fed wetland when nitrate limitation was removed ($p < 0.05$, Figure 1). Caution must be taken, however, when extrapolating this result to all groundwater-fed and precipitation-fed wetlands because measurements of denitrification potentials were made in only one wetland of each category.

Iron reduction was measurable in all of the precipitation-fed wetlands and in one groundwater-fed wetland (G3) (Figure 2A). Although the mean rates in two of the three precipitation-fed wetlands were higher than in the groundwater-fed wetlands, wetlands grouped by predominant water sources were not significantly different ($p = 0.065$).

Sulfate reduction was measurable only in groundwater-fed wetlands and ranged from 0.003 to 0.064 μmol SO_4^{-2}/mL•hr (Figure 2B). There were large differences in sulfate reduction in the ground-

water-fed wetlands, with wetland G1 having sulfate reduction rates nearly three times higher than the other groundwater-fed wetlands. Despite the observation that sulfate reduction rates were variable in the groundwater-fed wetlands, there was no statistical difference in sulfate concentration in porewater (Table 3, $p = 0.24$).

Methanogenesis was the only anaerobic metabolic process detected in all six wetlands. Rates ranged from 0.001 to 0.017 μmol CH_4 /mL•hr (Figure 2C). There were no significant differences between groundwater-fed and precipitation-fed wetlands, but there were significant differences among all wetlands regardless of water source ($p < 0.005$).

Acetate turnover rates in the six wetlands varied from 0.011 to 0.096 μmol /mL•hr (Figure 2D), and the overall average acetate turnover was 0.045 ± 0.027 μmol/mL•hr. Groundwater-fed wetlands had significantly higher turnover rates (average 0.063 ± 0.018 μmol/mL•hr) than precipitation-fed wetlands (average 0.020 ± 0.005 μmol/mL•hr) ($p < 0.005$). There were no significant differences among wetlands with the same water source ($p = 0.21$).

Each anaerobic process rate was converted to the equivalent rate of organic carbon mineralization based on the stoichiometry of the oxidation-reduction reactions (Froelich et al. 1979, Zehnder and Stumm 1988). In the case of methanogenesis, we assumed equimolar production of CH_4 and CO_2 as is typical in freshwater sediments dominated by the acetoclastic methanogenic pathway (Lovley et al. 1982). The carbon mineralization rates for each process were summed to give the total C mineralized for the wetland. In terms of total carbon mineralization by anaerobic metabolism, sulfate reduction was the most important process in groundwater-fed sites and methanogenesis was the most important process in precipitation-fed sites (Figure 3). Even though sulfate reduction was the predominant anaerobic process in groundwater-fed wetlands, it is important to note that methanogenesis still contributed about 20% to 40% of the total in these sites. Iron reduction was most significant in precipitation-fed wetlands, and contributed up to 30% of the total amount of mineralized carbon.

There were few significant relationships between the rate measurements and physical and chemical characteristics of the wetland sediments. Even though organic carbon is often a limiting resource to microorganisms, percent sediment organic carbon and porewater dissolved organic carbon (DOC) concentrations did not correlate with any rate measurement except iron reduction. This is not surprising because the soluble carbon available in pore water is greatest in the surface layers (0–5 cm),
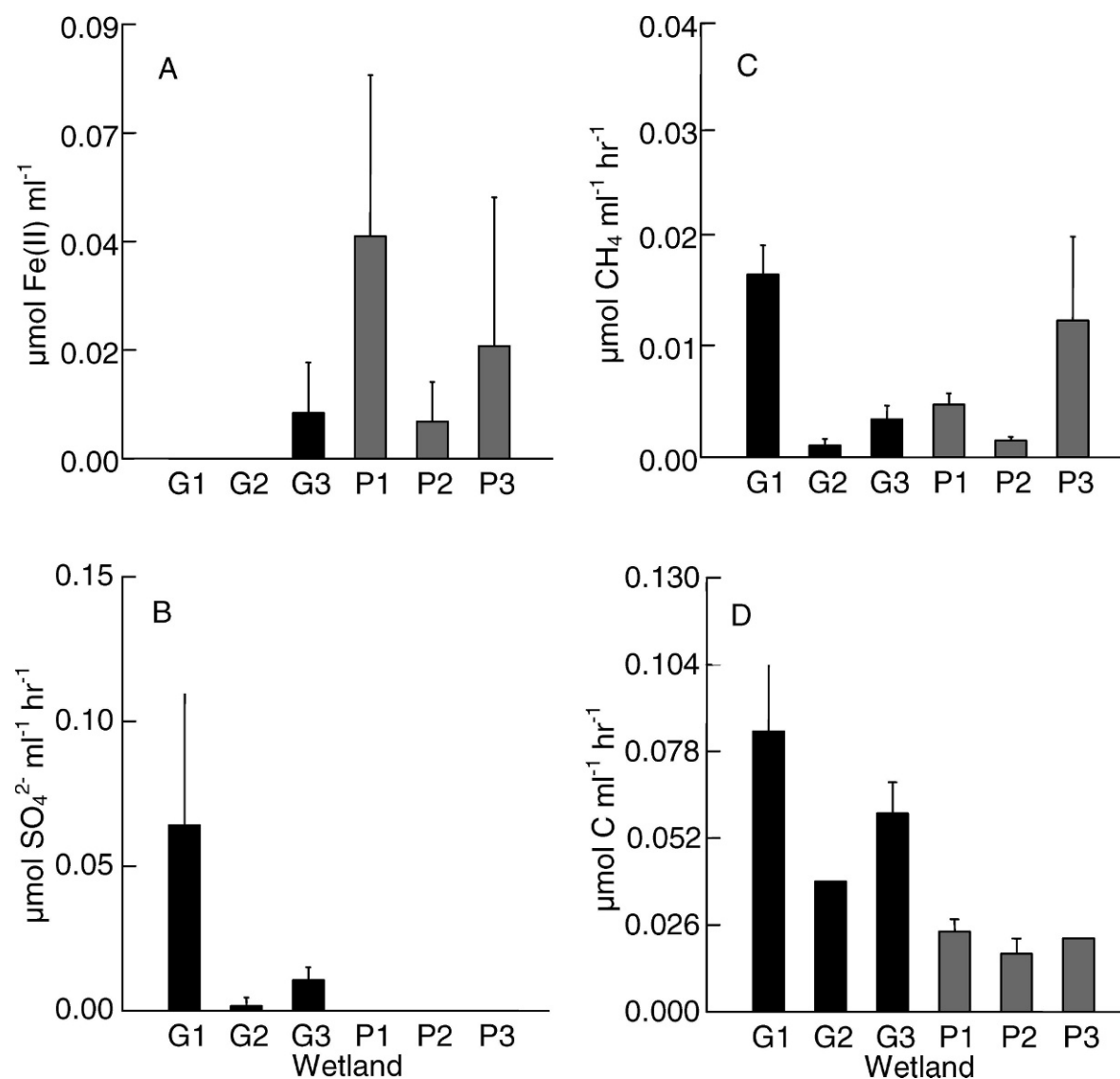
Figure 2. Rates of A) iron reduction, B) sulfate reduction, C) methanogenesis. and D) acetate turnover for six wetlands, three of which were mainly groundwater-fed (G) and three were mainly precipitation-fed (P). Bars show means (with standard deviations) for replicate sites within each wetland. Bars without error bars indicate no within-site replication. Wetlands without data had rates below the limit of detection.

Table 3. Selected physical and chemical characteristics of wetland porewater collected from 0–10 cm depth beneath the sediment-water interface. Each value represents the mean of three replicates ± one standard deviation. (n.d. = not detectable; < 15 µg NO_3^- - N/L or < 0.2 mg DOC/L).

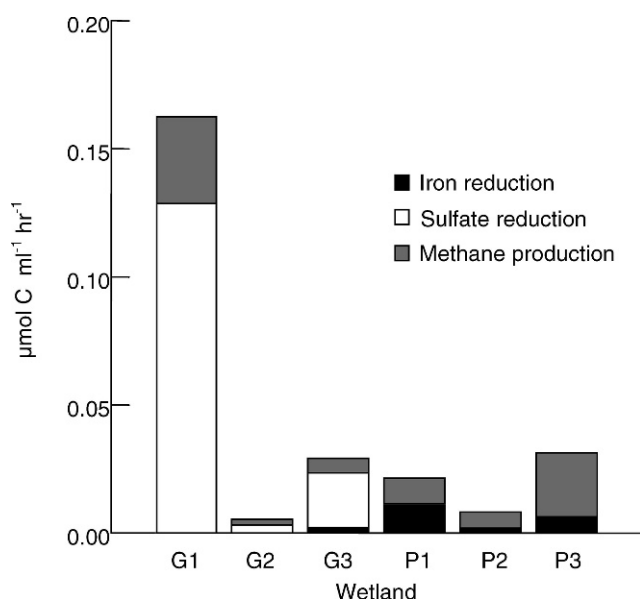| Wetland	pH	NO_3^- - N (mg/L)	SO_4^{2-} (mg/L)	DOC (mg/L)	Acetate (µM)
G1	7.0 (0.1)	n.d.	1.35 (0.2)	11.0 (2.8)	21.8 (4.3)
G2	7.1 (0.5)	n.d.	9.10 (7.0)	n.d.	72.7 (45.6)
G3	6.8 (0.1)	n.d.	4.91 (2.3)	24.8 (6.2)	18.1 (3.1)
P1	5.2 (0.2)	n.d.	0.82 (0.3)	31.7 (5.3)	5.8 (4.0)
P2	6.1 (0.1)	n.d.	0.62 (0.3)	11.1 (1.2)	6.1 (4.6)
P3	5.7 (0.02)	n.d.	1.0 (0.5)	n.d.	21.1 (19.7)

Figure 3. Carbon mineralization rates, based on individual rate assays and assumed stoichiometry of each process, for three mainly groundwater-fed (G) and three mainly precipitation-fed (P) wetlands.

close to the sources of new organic matter (Nedwell 1984), and is often a small proportion of the total DOC.

DISCUSSION

The nature and rates of anaerobic microbial metabolism were variable among the six wetlands, but patterns related to hydrology were evident. Groundwater-fed wetlands had higher rates of anaerobic microbial decomposition than precipitation-fed wetlands (Figures 2 and 3). There are many possible reasons for variation in rates, including differences in productivity that lead to differences in the supply or quality of organic carbon, or that groundwater supplies sulfate to support sulfate reduction, which is a relatively more rapid pathway for decomposition (Weston and Joye 2005). Methanogenesis was ubiquitous, but rates were not different between wetlands with different water sources. Iron reduction was measurable in all three precipitation-fed wetlands but only detected at one groundwater-fed wetland.

Although denitrification was not detected at ambient conditions, high potential denitrification rates were demonstrated by the denitrification enzyme activity assays (Figure 1). In addition, we observed rapid nitrate uptake in push-pull experiments in which nitrate was added directly to the sediment porewaters of these sites (Whitmire and Hamilton 2005). The strong response to added nitrate indicates that nitrate was limiting denitrification in these wetlands, as opposed to labile organic matter. The ground water in southwest Michigan tends to be high in nitrate (2.22 ± 4.0 mg NO_3^--N/L from 22 seeps and springs sampled in 1999–2000; Hamilton, unpublished data); however, nitrate concentrations in surface-water and pore-water samples were rarely above the analytical detection limit (10–15 μg N L^{-1}) in the wetlands studied. Denitrification rates under ambient conditions might not have been detected for a number of reasons. One possibility is that nitrate could have been removed by plant uptake (Groffman et al. 1992). A more likely possibility is that incoming nitrate becomes rapidly depleted by denitrification and other dissimilatory transformations along the edge of the wetland, where most ground water typically enters such depressions. At a riparian interface, Hedin et al. (1998) found that a narrow zone of saturated, organic soils where ground water entered was the most important location for nitrate removal by denitrification. In an estuarine wetland, Tobias et al. (2001) found that almost 90% of nitrate was removed within 50 cm of the wetland-upland boundary. If such rapid removal occurs in these wetlands as well, our sampling points may have been too far from the initial contact of incoming ground water with the wetland sediments, and our sampling dates generally did not coincide with the season of greatest hydrologic inputs that would also be sources of nitrate. Thus, denitrification could be a somewhat more important process in these six wetlands than the ambient rate measurements indicate, but it is spatially and temporally variable.

Sulfate reduction was only measurable in groundwater-fed wetlands, probably because sulfate availability limited the process in precipitation-fed wetlands (Whitmire and Hamilton 2005). The rates measured in these wetlands are lower than those reported in coastal systems (Howarth and Teal 1979), but comparable to sulfate reduction rates in other freshwater wetlands (Yavitt and Lang 1990, Kostka et al. 2002). The mean sulfate concentration from 22 springs and seeps around KBS during the study period was 29.9 ± 18.0 mg/L (Hamilton, unpublished data), compared to 1.94 mg/L found in precipitation during the same time (NADP/NTN). Sulfate concentrations in the porewaters of these wetlands were measurable, but usually lower than in the overlying water (Tables 1 and 3). Precipitation-fed wetlands had lower porewater sulfate concentrations than the groundwater-fed wetlands. Within the groundwater-fed wetlands, wetland G1 had very high rates of sulfate reduction compared to G2 and G3. One site within G1 had a particularly high

sulfate reduction rate, although both sites had higher rates than those found in G2 and G3. One explanation is that wetland G1 could have a larger population of sulfate reducers as a result of more ground water flow. This could result in more continual inputs of sulfate, maintaining sulfate-reducing populations. Ground water flow rates were not measured in this study, but wetland G1 had a visible and constant surface water flow through the wetland driven by inputs from a discrete spring several meters upstream of the sampling points.

Iron reduction generally mineralized less carbon than sulfate reduction or methanogenesis in these soils, contributing 7% to 50% of the total (Figure 3). Iron reduction rates were relatively low, but within the range found in other wetland systems (Lovley and Phillips 1986b, Roden and Wetzel 1996, Neubauer et al. 2005). The soil surface in wetlands G3, P1, and P3 was exposed to air during the study, but wetland P3 became more desiccated than the others. Iron reduction could be more prevalent in the precipitation-fed sites due to occasional drying, which allows $Fe(II)$ to be oxidized back to $Fe(III)$ (Roden and Wetzel 1996), while groundwater-fed wetlands tend to maintain more stable water levels and saturated sediments due to constant ground water inputs. Freshly precipitated $Fe(III)$ oxides support high rates of iron reduction in part because they have a high surface area (Roden 2006). In one experiment, rice fields that were drained once during inundation showed decreased methane production and an increase in the percentage of radiolabeled acetate converted to CO_2 compared to CH_4; this was interpreted as an effect of iron reducers out-competing methanogens in response to an increase in the $Fe(III)$ pool after draining (Kruger et al. 2001). Examining the wet-dry cycles of a wetland may yield important insights into the factors that regulate iron reduction. An alternative hypothesis for regeneration of $Fe(III)$ via oxidation of $Fe(II)$ compounds is O_2 input from the overlying water column or via root oxygen loss (Roden and Wetzel 1996, Mitsch and Gosselink 2000). Our sampling sites were in unvegetated areas, and thus in this study the influence of live roots was assumed to be minimal.

It is generally thought that under anaerobic conditions, methanogenesis is the predominant terminal catabolic process in freshwater wetlands (Mitsch and Gosselink 2000) because freshwaters are generally limited in the availability of electron acceptors. The fact that methane production was significant in both precipitation- and groundwater-fed wetlands suggests that it is an important biogeochemical function regardless of hydrologic category. However, the rates of methanogenesis measured in this study were generally lower than those seen in other freshwater wetlands (Yavitt et al. 1988, Roden and Wetzel 1996, Kruger et al. 2001).

Our observation of methane production in all wetlands is interesting since we found measurable sulfate reduction in the groundwater-fed wetlands and some iron reduction in the precipitation-fed wetlands. Sulfate reducers have been shown to inhibit or outcompete methanogens for carbon sources such as acetate (Lovley et al. 1982, Oremland and Polcin 1982, Lovley and Klug 1983, Westermann and Ahring 1987), and iron reducers can outcompete methanogens in freshwater wetlands (Roden and Wetzel 1996) as well as in rice fields that are either drained or fertilized with ferric iron (Jackel and Schnell 2000, Kruger et al. 2001). However, there could be multiple explanations. Some studies have shown that when there are carbon sources other than acetate that methanogens can utilize, such as methyl amines, methanogens and sulfate reducers can co-occur (Oremland and Polcin 1982, Oremland 1988, Yavitt and Lang 1990). Also our sampling design could explain this co-existence of competing processes if the section of the anoxic zone that was subcored spanned vertical spatial variation in redox conditions (Hunt et al. 1997). The slurries created in the lab would also have disrupted the small-scale sediment structure that can be important to microorganisms (Yavitt and Lang 1990, Hunt et al. 1997).

The terminal process of anaerobic metabolism depends on the activity of fermenting bacteria for supply of substrates, such as acetate (Fenchel et al. 1998). If acetate is the dominant substrate for the terminal anaerobic processes then acetate turnover would reflect anaerobic decomposition rates (Wellsbury and Parkes 1995, deGraaf et al. 1996). We found higher acetate turnover in the groundwater-fed wetlands, supporting our hypothesis that carbon is metabolized at higher rates in wetlands receiving ground water.

Many factors besides hydrology may control anaerobic microbial metabolism. In addition to concentrations of alternative electron acceptors in source waters, temperature, pH, and labile carbon can also be important controls. Sediment temperatures were not significantly different between the precipitation-fed and groundwater-fed wetlands. In this study, all incubations were done at $24°C$ in the lab. This was generally about $4°C$ warmer than temperatures found in-situ. Sediment organic carbon was higher in the groundwater-fed wetlands, but porewater DOC concentrations were variable across wetlands with differing water source. None of these

factors correlated with any of the anaerobic microbial decomposition rates, leading us to believe that they were not responsible for the observed differences in anaerobic microbial decomposition rates.

The importance of pH over the range that we observed as a control on microbial community structure and process rates is poorly understood (Gutknecht et al. 2006). Porewater pH was lower in precipitation-fed compared to groundwater-fed wetlands (5.3–6.1 and 6.6–7.1, respectively) (Table 3), but whether this difference was biologically significant is not certain. More acidic pH than this can slow the overall rate of organic matter decomposition (Goodwin et al. 1988), regulate methanogenesis (Yavitt et al. 2005), and inhibit sulfate reduction (Widdel 1998). In boreal peatlands, where pH often is much lower than in our study, sulfate reduction can still be more important than methanogenesis in carbon mineralization (Vile et al. 2003). There was no correlation between pH and rate measurements, suggesting that pH was not responsible for the trends observed. Methanogenesis had similar rates across all wetlands regardless of pH. However, sulfate reducers have only been active in groundwater-fed wetlands, which were higher in pH.

Seasonal changes can also affect the dominant anaerobic microbial processes. In a study of tidal marsh sediments that varied along a continuum from freshwater to brackish water, Fe(III) reduction was highest at the beginning of the growing season, whereas sulfate reduction or methanogenesis dominated later (Neubauer et al. 2005). In a salt marsh, Hines et al. (2001) found that sulfate reduction rates increased with plant growth and then began to decrease once plant flowering began, probably as a result of carbon leaking from the plant roots.

Since acetate generally is the most important carbon source available to microorganisms carrying out the terminal steps of anaerobic catabolism (Lovley and Klug 1982), comparisons between acetate turnover and total carbon mineralization rates were made by converting each of the anaerobic process rates to carbon mineralization rates using the stoichiometry of the reactions (Froelich et al. 1979, Figure 4). Acetate turnover rates were proportional to anaerobic carbon mineralization rates except in wetland G1, where the sum of the individual mineralization rates was much higher than the carbon mineralization rates estimated from acetate turnover. It is possible that this difference was due to a greater relative importance of other electron donors besides acetate in fueling the terminal step of anaerobic metabolism. Fermentation is only a partial degradation of organic matter
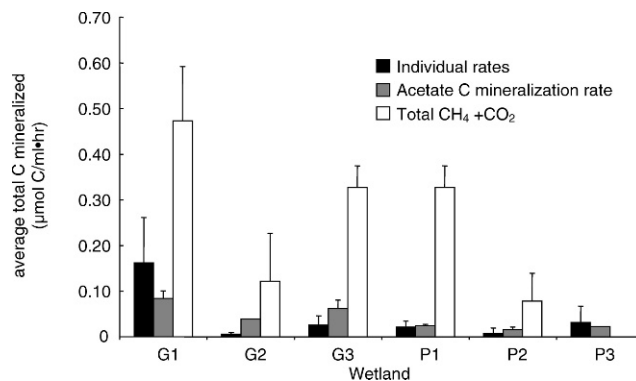

Figure 4. Total carbon mineralization rate estimates based on the sum of individual rates (black bars), acetate turnover rates (grey bars), and total $CO_2 + CH_4$ production (white bars), with standard deviations. Standard deviations could not be calculated for acetate turnover rates at wetlands G2 and P3. Total $CO_2 + CH_4$ could not be calculated for P3.

and acetate is one of the lowest molecular weight products of fermentation, but not the only one (McInerney 1998). Other volatile fatty acids such as propionate, formate, and lactate can also be formed. In a study of lake sediments, lactate turnover rates were higher than acetate turnover rates (Cappenberg and Prins 1974). In a salt marsh, ethanol utilizing sulfate-reducing bacteria were more abundant than the acetate utilizing ones (Hines et al. 2001). Another possibility for the large difference in wetland G1 could be that sulfate reduction was overestimated due to high variance among the replicates, resulting in the greater total carbon mineralization values.

Total $CO_2 + CH_4$ production is another way to examine the total amount of anaerobic metabolism. This estimate assumes that carbon was fully mineralized and did not accumulate as metabolic intermediates during the incubations. Both acetate turnover and anaerobic carbon mineralization rates were much lower than $CO_2 + CH_4$ production (Figure 4). The excess CO_2 could be a result of underestimating all the terminal anaerobic microbial process rates and acetate turnover; however, since acetate turnover and anaerobic carbon mineralization rates are proportional this may not completely explain the difference. Another possibility is that much of the CO_2 production results from carbon flow through fermentation. Vile et al. (2003) reported that sulfate reduction rates exceeded methanogenesis, but together they accounted for only 3% of the total carbon mineralized, with the remainder attributed to fermentation. Similar results were found in a tidal wetland system (Neubauer et al. 2005).

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Comparative studies of multiple processes across sites are uncommon (cf. Groffman et al. 1996, D'Angelo and Reddy 1999), but they are one way to understand hydrologic controls of anaerobic microbial decomposition. Groffman et al. (1996) compared microbial biomass and activity in four different wetland types (defined by hydrology) in eastern New York, finding few differences among the wetland types. Multiple wetlands were examined by D'Angelo and Reddy (1999), who found that electron acceptor and donor availability were the dominant soil factors regulating potential rates of organic carbon mineralization. In that study, wetland soil samples were collected from several places within the United States, dried and stored, and potential terminal anaerobic process rates were determined after maximum reducing conditions were produced upon rewetting in the laboratory. Wetland hydrology was not evaluated in that study, but after electron acceptor amendments they saw a transition of electron flow from methanogenesis to alternative electron acceptors, underscoring the importance of water sources that vary in their composition of electron acceptors.

Anaerobic microbial decomposition plays a critical role in the biogeochemical functioning of natural freshwater wetlands. Even though there was substantial variation in the process rates among the wetlands, we found general patterns that are important for wetland biogeochemistry. Water source influenced the relative importance of anaerobic respiration pathways that mediate organic matter mineralization. In precipitation-fed wetlands, inputs of the electron acceptors nitrate and sulfate occur episodically, while in groundwater-fed wetlands there would be a more continuous supply. Limitation by electron acceptor availability was evident in the denitrification potential measurements, as well as from the rates of disappearance of nitrate and sulfate in those wetlands that we have observed in push-pull experiments (Whitmire and Hamilton 2005). Iron reduction was important in wetlands that have wet-dry cycles. Both iron reduction and sulfate reduction were important enough to attenuate the amount of methane produced in the sediments. Nonetheless, methanogenesis was the dominant process in most of these wetlands. In terms of the overall carbon budgets of these wetlands, anaerobic degradation was relatively more important in the groundwater-fed wetlands, which also support higher vascular plant biomass. The limited supply of electron acceptors in the precipitation-fed wetlands, perhaps in combination with lower plant productivity, could be the reason for their lower rates of total anaerobic metabolism compared to the groundwater-fed wetlands.

ACKNOWLEDGMENTS

LITERATURE CITED

Cappenberg, T. E. and R. A. Prins. 1974. Interrelations between sulfate reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labelled substrates. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 40:457–69.

Chapelle, F. H., P. M. Bradley, D. R. Lovley, and D. A. Vroblesky. 1996. Measuring rates of biodegradation in a contaminated aquifer using field and laboratory methods. Ground Water 34:691–98.

Cicerone, R. J. and R. S. Oremland. 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochemical Cycles 2:299–327.

Conrad, R. 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O and NO). Microbiological Reviews 60:609–40.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Beit. 1997. The value of the world's ecosystem services and natural capital. Nature 387:253–60.

D'Angelo, E. M. and K. R. Reddy. 1999. Regulators of heterotrophic microbial potentials in wetland soils. Soil Biology & Biochemistry 31:815–30.

deGraaf, W., P. Wellsbury, R. J. Parkes, and T. E. Cappenberg. 1996. Comparison of acetate turnover in methanogenic and sulfate-reducing sediments by radiolabeling and stable isotope labeling and by use of specific inhibitors: evidence for isotopic exchange. Applied and Environmental Microbiology 62:772–77.

Euliss, N., J. LaBaugh, L. Fredrickson, D. Mushet, and M. Laubhan. 2004. The wetland continuum: a conceptual framework for interpreting biological studies. Wetlands 24:448–58.

Fenchel, T., G. M. King, and T. H. Blackburn. 1998. Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling, second edition. Academic Press, San Diego, CA, USA.

Froelich, P. N., G. P. Klinkhammer, M. L. Bender, N. A. Luetke, G. R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartman, and V. Maynard. 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et Cosmochimica Acta 43:1075–90.

Goodwin, S., R. Conrad, and J. G. Zeikus. 1988. Influence of pH on microbial hydrogen metabolism in diverse sedimentary ecosystems. Applied and Environmental Microbiology 54:590–93.

Groffman, P. M., A. J. Gold, and R. C. Simmons. 1992. Nitrate dynamics in riparian forests - microbial studies. Journal of Environmental Quality 21:666–71.

Groffman, P. M., G. C. Hanson, E. Kiviat, and G. Stevens. 1996. Variation in microbial biomass and activity in four different wetland types. Soil Science Society of America Journal 60:622–29.

Groffman, P. M., E. A. Holland, D. D. Myrold, G. P. Robertson, and X. Zuo. 1999. Denitrification. p. 272–88. *In* G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins (eds.) Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, NY, USA.

Gutknecht, J. L., R. M. Goodman, and T. C. Balser. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil 289:17–34.

Hedin, L. O., J. C. von Fischer, N. E. Ostrom, B. P. Kennedy, M. G. Brown, and G. P. Robertson. 1998. Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces. Ecology 79:684–703.

Hines, M. E., K. N. Duddleston, and R. P. Kiene. 2001. Carbon flow to acetate and C-1 compounds in northern wetlands. Geophysical Research Letters 28:4251–54.

Hopkinson, C. S. 1992. A comparison of ecosystem dynamics in freshwater wetlands. Estuaries 15:549–62.

Howarth, R. W. and J. M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnology and Oceanography 24:999–1013.

Hunt, R. J., D. P. Krabbenhoft, and M. P. Anderson. 1997. Assessing hydrogeochemical heterogeneity in natural and constructed wetlands. Biogeochemistry 39:271–93.

Jackel, U. and S. Schnell. 2000. Suppression of methane emission from rice paddies by ferric iron fertilization. Soil Biology & Biochemistry 32:1811–14.

Johnston, C. A. 1991. Sediment and nutrient retention by freshwater wetlands: effects on surface water quality. Critical Reviews in Environmental Control 21:491–565.

Jorgensen, B. B. 1978. Comparison of methods for the quantification of bacterial sulfate reduction in coastal marine-sediments. 1. Measurement with radiotracer techniques. Geomicrobiology Journal 1:11–27.

King, G. M. 1991. Measurement of acetate concentrations in marine pore waters by using an enzymatic approach. Applied and Environmental Microbiology 57:3476–81.

Kostka, J. E., A. Roychoudhury, and P. Van Cappellen. 2002. Rates and controls of anaerobic microbial respiration across spatial and temporal gradients in saltmarsh sediments. Biogeochemistry 60:49–76.

Kristensen, E., G. M. King, M. Holmer, G. T. Banta, M. H. Jensen, K. Hansen, and N. Bussarawit. 1994. Sulfate reduction, acetate turnover and carbon metabolism in sediments of the Ao-Nam-Bor Mangrove, Phuket, Thailand. Marine Ecology-Progress Series 109:245–55.

Kruger, M., P. Frenzel, and R. Conrad. 2001. Microbial processes influencing methane emission from rice fields. Global Change Biology 7:49–63.

Landmeyer, J. E., F. H. Chapelle, and P. M. Bradley. 2000. Microbial H_2 cycling does not affect δH_2 values of ground water. Ground Water 38:376–80.

Lovley, D. R., D. F. Dwyer, and M. J. Klug. 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. Applied and Environmental Microbiology 43:1373–79.

Lovley, D. R. and M. J. Klug. 1982. Intermediary metabolism of organic matter in the sediments of a eutrophic lake. Applied and Environmental Microbiology 43:552–60.

Lovley, D. R. and M. J. Klug. 1983. Sulfate reducers can outcompete methanogens at fresh-water sulfate concentrations. Applied and Environmental Microbiology 45:187–92.

Lovley, D. R. and E. J. P. Phillips. 1986a. Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac River. Applied and Environmental Microbiology 52:751–57.

Lovley, D. R. and E. J. P. Phillips. 1986b. Organic-matter mineralization with reduction of ferric iron in anaerobic sediments. Applied and Environmental Microbiology 51:683–89.

McInerney, M. J. 1998. Anaerobic hydrolysis and fermentation of fats and proteins. p. 373–415. *In* A. J. B. Zehnder (ed.) Biology of Anaerobic Microorganisms. John Wiley & Sons, Inc., New York, NY, USA.

McLatchey, G. P. and K. R. Reddy. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. Journal of Environmental Quality 27:1268–74.

Megonigal, J. P., M. E. Hines, and P. T. Visscher. 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. p. 317–424. *In* W. H. Schlesinger (ed.) Biogeochemistry. Elsevier-Pergamon, Oxford, UK.

Mitsch, W. J. and J. G. Gosselink. 2000. Wetlands, third edition. John Wiley & Sons, Inc., New York, NY, USA.

National Research Council. 1995. Wetlands: Characteristics and Boundaries. National Academy Press, Washington, DC, USA.

Nedwell, D. B. 1984. The input and mineralization of organic carbon in anaerobic aquatic sediments. p. 93–131. *In* K. C. Marshall (ed.) Advances in Microbial Ecology. Plenum Press, New York, NY, USA.

Neubauer, S. C., K. Givler, S. K. Valentine, and J. P. Megonigal. 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334–44.

Oremland, R. S. 1988. Biogeochemistry of methanogenic bacteria. p. 641–705. *In* A. J. B. Zehnder (ed.) Biology of Anaerobic Microorganisms. John Wiley & Sons, Inc., New York, NY, USA.

Oremland, R. S. and S. Polcin. 1982. Methanogenesis and sulfate reduction-competitive and noncompetitive substrates in estuarine sediments. Applied and Environmental Microbiology 44:1270–76.

Ponnamperuma, F. N. 1972. The chemistry of submerged soils. p. 29–95. *In* N. C. Brady (ed.) Advances in Agronomy. Academic Press, New York, NY, USA.

Roden, E. E. 2006. Geochemical and microbiological controls on dissimilatory iron reduction. Geoscience 338:456–67.

Roden, E. E. and R. G. Wetzel. 1996. Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnology and Oceanography 41:1733–48.

Segers, R. 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41:23–51.

Stauffer, R. E. 1985. Use of solute tracers released by weathering to estimate groundwater inflow to seepage lakes. Environmental Science & Technology 19:405–11.

Stookey, L. L. 1970. Ferrozine—a new spectrophotometric reagent for iron. Analytical Chemistry 42:779–81.

Tiedje, J. M., S. Simkins, and P. M. Groffman. 1989. Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods. Plant and Soil 115:261–84.

Tobias, C. R., I. C. Anderson, E. A. Canuel, and S. A. Macko. 2001. Nitrogen cycling through a fringing marsh-aquifer ecotone. Marine Ecology-Progress Series 210:25–39.

Vile, M. A., S. D. Bridgham, and R. K. Wieder. 2003. Response of anaerobic carbon mineralization rates to sulfate amendments in a boreal peatland. Ecological Applications 13:720–34.

Vroblesky, D. A. and F. H. Chapelle. 1994. Temporal and spatial changes of terminal electron-accepting processes in a petroleum hydrocarbon-contaminated aquifer and the significance for contaminant biodegradation. Water Resources Research 30:1561–70.

Wang, F. Y. and P. M. Chapman. 1999. Biological implications of sulfide in sediment - a review focusing on sediment toxicity. Environmental Toxicology and Chemistry 18:2526–32.

Wellsbury, P. and R. J. Parkes. 1995. Acetate bioavailability and turnover in an estuarine sediment. FEMS Microbiology Ecology 17:85–94.

Westermann, P. and B. K. Ahring. 1987. Dynamics of methane production, sulfate reduction, and denitrification in a permanently waterlogged alder swamp. Applied and Environmental Microbiology 53:2554–59.

Weston, N. B. and S. B. Joye. 2005. Temperature-driven decoupling of key phases of organic matter degradation in marine sediments. Proceedings of the National Academy of Sciences of the United States of America 102:17036–40.

Whitmire, S. L. 2003. Anaerobic biogeochemical functions of Michigan wetlands and the influence of water source. Ph.D. Dissertation. Michigan State University, Lansing, MI, USA.

Whitmire, S. L. and S. K. Hamilton. 2005. Rapid removal of nitrate and sulfate in freshwater wetland sediments. Journal of Environmental Quality 34:2062–71.

Widdel, F. 1998. Microbiology and ecology of sulfate- and sulfur reducing bacteria. p. 469–585. *In* A. J. B. Zehnder (ed.) Biology of Anaerobic Microorganisms. John Wiley & Sons, Inc., New York, NY, USA.

Yavitt, J. B. and G. E. Lang. 1990. Methane production in contrasting wetland sites - response to organic-chemical components of peat and to sulfate reduction. Geomicrobiology Journal 8:27–46.

Yavitt, J. B., G. E. Lang, and D. M. Downey. 1988. Potential methane production and methane oxidation rates in peatland ecosystems of the Appalachian Mountains, United States. Global Biogeochemical Cycles 2:253–68.

Yavitt, J. B., C. J. Williams, and R. K. Wieder. 2005. Soil chemistry versus environmental controls on production of CH_4 and CO_2 in northern peatlands. European Journal of Soil Science 56:169–78.

Zehnder, A. J. B. and W. Stumm. 1988. Geochemistry and biogeochemistry of anaerobic habitats. p. 1–38. *In* A. J. B. Zehnder (ed.) Biology of Anaerobic Microorganisms. John Wiley & Sons, Inc., New York, NY, USA.