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STEFANIE L. WHITMIRE

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Stephen K Hamilton
Major Professor's Signature

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ANAEROBIC BIOGEOCHEMICAL FUNCTIONS OF MICHIGAN WETLANDS AND THE INFLUENCE OF WATER SOURCE

By

Stefanie L. Whitmire

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ABSTRACT

ANAEROBIC BIOGEOCHEMICAL FUNCTIONS OF MICHIGAN WETLANDS AND THE INFLUENCE OF WATER SOURCE

By

Stefanie L. Whitmire

Biogeochemical transformations in wetlands impact water quality and nutrient transport across landscapes as well as greenhouse gas fluxes. Anaerobic microbial processes, such as denitrification and methanogenesis, play particularly important roles in the biogeochemical functions of wetlands. I explored the influence of anaerobic microbial processes on wetland biogeochemistry in surficial sediments of southwest Michigan wetlands.

I examined porewater chemistry profiles in sediments of 12 wetlands to see if there was evidence for thermodynamic constraints on anaerobic microbial processes and to see if the wetland's water source impacted the porewater chemistry. Although the large spatial and temporal variability in porewater chemistry obscured patterns among wetlands, wetland porewaters were generally depleted in nitrate and sulfate relative to source waters, and the presence of sulfide in porewaters confirmed that sulfate reduction occurred at most sites. Porewater chemistry showed little relationship to water source.

To further investigate anaerobic microbial decomposition in wetland sediments, I measured ambient rates of denitrification, sulfate reduction, iron reduction, methanogenesis and acetate turnover in three groundwater- and three precipitation-fed wetlands. Denitrification was not detected in any wetland. Iron reduction was measurable mainly in precipitation-fed wetlands, while sulfate reduction rates were greater in groundwater-fed wetlands than in precipitation-fed wetlands. Methanogenesis was measurable in all wetlands, with no differences between wetlands with contrasting water sources, indicating that methanogenesis was important irregardless of water source. Acetate turnover rates were comparable to those measured in other productive sediment environments.

Push-pull experiments provided estimates of potential in-situ rates of denitrification and sulfate reduction. All wetlands examined showed the same potential to rapidly remove nitrate and removal was dependent on concentration. Sulfate was also taken up from injected groundwater in all wetlands, but only after nitrate was depleted. The sulfate reduction rates in groundwater-fed wetlands were independent of sulfate concentrations, while the rates in precipitation-fed wetlands were dependent on sulfate concentrations.

Results from these studies indicate that water source can influence the biogeochemical functions of wetlands, but that other factors could influence anaerobic decomposition as well.

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CHAPTER 1

INTRODUCTION TO ANAEROBIC MICROBIAL METABOLISM AND THE BIOGEOCHEMICAL FUNCTIONING OF WETLANDS

Wetlands are increasingly appreciated as ecosystems with exceptional ecological and economic value (Costanza et al. 1997). They perform hydrologic and biogeochemical functions that affect downstream water quality and hence impact the overall landscape (National Research Council 1995). Since wetlands are often situated at points of groundwater recharge or discharge, they affect water quality of ground waters, streams, and most lakes. Wetlands retain sediments and remove excess contaminants and nutrients, such as phosphorus (Gunnars and Blomqvist 1997) and nitrate (Peterjohn and Correll 1984, Johnston et al. 1990), which is especially important in the improvement of water quality in agricultural areas such as the midwestern U.S. On the other hand, sulfate and iron reduction by bacteria in anaerobic environments of wetlands can be problematic for downstream water quality, producing sulfide and ferrous iron, respectively. These compounds can be toxic to plants and animals. Wetlands also trap suspended sediments that would otherwise create problems in downstream waterways.

Anaerobic microbial processes play a central role in the biogeochemical functions of wetlands. Most microbial activity in wetlands occurs under anaerobic conditions because oxygen diffusion through overlying water and saturated sediments is slow and, when present, O_2 is quickly used. Many factors control anaerobic microbial metabolism in wetlands, including hydrology, temperature, pH, carbon availability (Conrad 1996, Segers 1998), and availability of alternate electron acceptors, such as nitrate, ferric iron, and sulfate (Yavitt and Lang 1990). It is important to understand the controls on microbial processes that affect wetland biogeochemistry and how these controls might change under the influence of human activities. By understanding how predominant microbial processes change across a broad suite of wetland ecosystems, we can better understand the underlying factors that regulate the microbial processes, and hence be better able to predict impacts of disturbance and global change on the functions of wetlands.

Natural and constructed wetlands contribute up to 50% of annual CH_4 emission to the atmosphere (Cicerone and Oremland 1988). Wetlands also emit other greenhouse gases, such as nitrous oxide (N_2O) and carbon dioxide (CO_2) (Conrad 1996). These gases are produced by anaerobic microbial metabolism. Since anaerobic microbial respiration plays a role in many of the biogeochemical functions of wetlands, it is important to understand the controls on the nature and rate of microbial activity in wetland environments.

Anaerobic microbial metabolism in wetlands

Anaerobic decomposition is a multi-step process, were fermentation breaks down complex organic matter into simple carbon compounds that are used by other microorganisms in the terminal step of organic matter catobolism. This dissertation addresses the terminal steps in anaerobic decomposition because they are especially important in wetland function.

Oxygen depletion and the consequent reduced conditions are common in wetland waters and ubiquitous in wetland sediments, leading to conditions that favor several forms of anaerobic metabolism by bacteria. Groundwater entering a wetland may pass through organic-rich soil, where O_2 will be entirely consumed by aerobic respiration, and thereafter anaerobic microbial metabolism will commence (Hedin et al. 1998). In anoxic porewaters, nutrients and reduced inorganic substrates increase in concentration. When overlying waters are oxic they produce an oxidized microzone at the sediment-water interface that acts as a barrier to the release of these soluble constituents from the sediments (Wetzel 2001). When anoxic conditions develop in the overlying water the microzone breaks down, releasing solutes such as ammonium, phosphorus and iron from the sediments into the overlying water column. Thus, microbial metabolism influences the biogeochemistry of both the sediment and the overlying waters of wetlands (Schlesinger 1997).

Once oxygen has disappeared from the sediment, anaerobic microbial metabolism begins. Theoretically, anaerobic microbial metabolism is controlled by thermodynamics (Figure 1). The bacteria performing terminal organic matter catabolism are dependent on fermentative bacteria to supply them with simple organic compounds (Chapelle 1993), and thermodynamic calculations predict that those which mediate reactions of greater free energy yield can predominate in anoxic environments (Froelich et al. 1979). Organisms that perform the reactions that yield more energy are favored in a given environment, as long as the electron acceptor is available. As soon as the electron acceptor is depleted, the organisms that perform the reaction with the next highest energy are favored. For instance if nitrate and iron are available, the organisms that perform iron reduction will be inhibited until nitrate is depleted. Each process has implications to biogeochemical function.

Denitrification

Denitrification is an anaerobic microbial process by which nitrate (NO_3^-) is reduced to dinitrogen gases N_2 and N_2O. It is important in the removal of nitrate from wastewater, can decrease nitrate contamination in aquatic systems, and can impact climate because N_2O is a greenhouse gas. Nitrate inputs to wetlands often pulse seasonally due to hydrologic seasonality, but in wetlands, excess nitrate is usually reduced or assimilated by the biota and does not persist long (Conrad 1996), except at low temperatures or in cases where nitrate saturation occurs (Hanson et al. 1994). It has been shown that denitrification is important in the fringe area of wetlands, where surface runoff and groundwater enter the wetland. For example, in a salt marsh 90% of the nitrate was removed within 50 cm of the marsh-upland boundary (Tobias et al. 2001). Denitrification

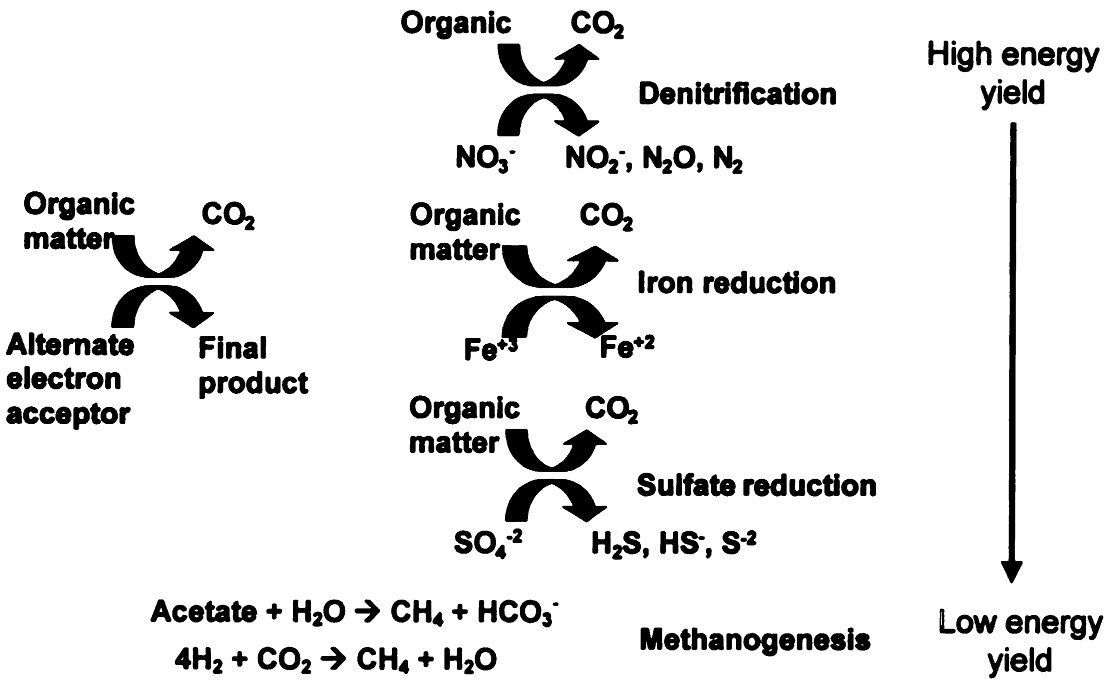

Figure 1: Thermodynamic sequence of the terminal step of anaerobic metabolim. Oxidation-reduction reactions are listed from high energy yield to low energy yield, with methanogenesis last.

has also been extensively studied in riparian areas (Groffman et al. 1992, Johnston 1993, Schipper et al. 1993, Hanson et al. 1994, Hedin et al. 1998),where adjacent wetlands were important in removing nitrate before runoff or discharging groundwater enters streams.

Iron Reduction

Over the course of evolution of life on earth, iron reduction may have been the first globally significant biotic process for the oxidation of organic matter to carbon dioxide (Lovley 1991). If Fe(III) is available in nitrate-depleted anaerobic sediments, then sulfate reduction and methanogenesis are usually inhibited in surface sediments (Sørensen 1982, Lovley 1991, Chapelle and Lovley 1992, Roden and Wetzel 1996). Iron(III) oxides are often abundant in freshwater systems (Lovley 1991), and iron reduction has been shown to mediate as much carbon flow as methane production in the upper 4 cm of sediments in estuarine systems (Lovley and Phillips 1986). The predominance of iron reduction in the shallow sediments may have an important role in the iron cycle of wetlands and influence the cycling of nutrients, like phosphorus. Phosphorus adsorbs to Fe(III) oxyhydroxides, reducing its availability for biotic uptake but releasing it when the Fe(III) is reduced. Iron reduction is also important because Fe(II) produced from Fe(III) reduction can be toxic to plants (Mitsch and Gosselink 2000).

Sulfate reduction

Sulfate reduction is the anaerobic microbial process by which sulfate (SO_4^{2-}) is reduced to hydrogen sulfides (HS^-, H_2S). Sulfur inputs are mainly derived from surface and groundwater flow that drains upland areas, although the proximate source for sulfate in sediments may be organic matter remineralization. Groundwater can continually supply sulfate to the microbial community, allowing sulfate reducers to potentially out-compete methanogens (Lovley and Klug 1983).

Sulfides produced by the microorganisms can be removed from the system by co-precipitating with other ions such as ferrous iron (Fe(II)) to form iron sulfides (FeS and FeS_2). This allows sulfides to be temporarily stored in wetlands and released at a later time or downstream (Evans et al. 1997). For instance, if the sediments dry and oxygen reaches an area with sulfides, reoxidation can occur and the sulfides would be released in the form of sulfate. In wetlands that have frequent wet-dry cycles, this could provide a continual supply of sulfate to sulfate-reducing bacteria, as well as ferric iron (Fe(III)) to iron reducers.

Methanogenesis

Methanogenesis is the dominant terminal process for organic matter degradation when the alternate electron acceptors are not available to support anaerobic respiration (Froelich et al. 1979, Conrad 1996, Fenchel et al. 1998). Methanogenesis is of extraordinary interest because of the large annual contribution of wetlands to atmospheric methane (CH_4), a greenhouse gas (Smagin 2000). The radiative forcing of methane is 20-30 times higher than that of carbon dioxide, so understanding its dynamics is important in assessing global warming. But the increase of CH_4 to the atmosphere in the last few years is not due to natural wetlands, since wetland area has been declining over the last couple of decades.

Methane production is prevalent in wetlands due to the anoxic conditions of the sediments and is achieved by a consortium of bacteria. These include fermentative, syntrophic, acetogenic and methanogenic baceteria. There are 2 main pathways in the formation of methane: 1) through homoacetogens and acetotrophic methanogens, or 2) by syntrophic interactions with H_2-producing bacteria (Conrad 1999). The general reactions for these two methanogenic pathways are:

1) $CH_3COOH \qquad CO_2 + CH_4$

2) $4H_2 + CO_2 \qquad 2\,H_2O + CH_4$

Acetotrophic methanogens produce equal amounts of CO_2 and CH_4, and are thought to play an important role in sulfate-depleted, anaerobic environments (Fenchel et al. 1998). Hydrogenotrophic methanogens, which are autotrophs that use H_2 as an electron donor for the reduction of CO_2, produce only methane.

While freshwater wetlands are an important source for atmospheric CH_4, as much as 80% of the methane produced in the anoxic sediments does not make it to the atmosphere, due to the oxidation of methane by methanotrophs in the oxic surface layer of freshwater wetlands (Conrad 1996, Boon and Lee 1997, King et al. 1990).

Controls on the overall rate of anaerobic metabolism

Wetland soils are usually characterized by high inputs of organic matter resulting from high rates of primary production by the vascular plant community (Reddy and D'Angelo 1997). This organic matter provides the energy necessary for anaerobic fermentation, in which the degradation of complex organic matter is catalyzed by several microbial groups. Complex organic matter, such as plant structural tissue, is oxidized by fermentative bacteria to simple organic acids and hydrogen (H_2). This leads to the formation of simple carbon compounds, like acetate, and H_2. These resources are intermediate products in a complex anaerobic food web that terminates when microorganisms oxidize simple organic substrates using terminal anaerobic processes, such as iron and sulfate reduction and methanogenesis. Hydrogen is used in terminal metabolism by bacteria to reduce inorganic electron acceptors like iron or sulfate, and provides thermodynamic control of bacterially-mediated anaerobic respiration (Hoehler et al. 1998).

The availability of these key intermediaries -- H_2 and acetate -- controls the overall rate of the terminal steps of anaerobic microbial metabolism. Microorganisms associated with different terminal catabolic processes are able to draw down H_2 concentrations to varying degrees (Lovley and Goodwin 1988, Chapelle et al. 1995). Since the production and consumption of H_2 are closely coupled and H_2 has a fast turnover rate, H_2 concentrations provide a potential indicator of which terminal catabolic process is occurring at the time of measurement (Chapelle et al. 1995). The turnover rate of acetate gives an estimate of the total rate of anaerobic microbial metabolism in the system, including both anaerobic respiration and methanogenesis (deGraaf et al. 1996). Vegetation rooted in shallow soils can influence anaerobic microbial respiration by increasing oxygen concentrations in the rooting zone (King 1994, Mendelssohn et al. 1995, Callaway and King 1996, Roden and Wetzel 1996), and by drawing down water levels through evapotranspiration. The local environment around the roots will change from anaerobic to aerobic with the influx of oxygen. This oxygen influx not only supports aerobic respiration but also can cause the oxidation of products of anaerobic metabolism such as H_2S to SO_4, Fe(II) to Fe(III) and CH_4, as well as release constituents bound to metal oxides, such as phosphorus, making them available to plants and microorganisms.

Wetlands in southwest Michigan are abundant and varied, covering about 10-15% of the landscape (Lynch and Waldron 1996). The high diversity of wetlands is linked to high hydrologic diversity. The land around the Kellogg Biological Station is a mosaic of hills and depressions, underlain with a highly permeable glacial till. Wetlands located high on the landscape tend to recharge to the groundwater, while wetlands in low areas receive discharge from groundwater (Winter et al. 1998). There are also wetlands that have groundwater through-flow, with one area receiving groundwater and another recharging to groundwater. Water sources, residence time, and water levels all vary as a function of the local hydrologic and geomorphic setting.

This variation makes the area surrounding KBS an ideal place to do a comparative study addressing how anaerobic microbial metabolism controls biogeochemical function among a set of wetlands of diverse hydrology but located in close proximity, ranging from systems that are mainly groundwater-fed to those that are mainly precipitation-fed.

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CHAPTER 2

POREWATER CHEMISTRY IN WETLANDS ALONG A WATER SOURCE GRADIENT FROM GROUNDWATER TO PRECIPITATION

Introduction

Wetlands are considered ecosystems of disproportionate importance relative to their abundance in the landscape, and their biogeochemical functions are one reason for this importance. Wetlands act as temporary reservoirs for precipitation and runoff and may be located at points of groundwater discharge, and thereby impact water quality (for example by retention of nitrogen). Wetlands are also major natural sources of atmospheric methane, a potent greenhouse gas. Key biogeochemical processes in wetland sediment environments include anaerobic respiration and methanogenesis, terminal steps in the anaerobic degradation of organic matter by bacteria, and these processes tend to be segregated into distinct zones (Froelich et al. 1979, Lovley and Goodwin 1988, Fenchel et al. 1998). These zones are commonly aligned along redox gradients, beginning with denitrification at the most oxidizing end, followed by iron reduction, sulfate reduction and finally methanogenesis. Except for methanogenesis, each of these processes is an oxidation-reduction transformation in which organic matter is oxidized to carbon dioxide and electron acceptors (NO_3, Fe(II) and SO_4) are reduced to final products, and therefore they will not occur in the absence of the necessary electron acceptors. Under conditions of limited labile organic matter, this segregation is based on competitive interactions which in turn are dictated by thermodynamics, since denitrification yields the most energy from organic matter degradation, followed by iron and sulfate reduction and finally methanogenesis. Microorganisms that perform the higher energy-yielding reactions predominate over the others, until the electron acceptors are depleted.

Measurements of the rate of each potential redox reaction provide the most direct determination of which process dominates within a given wetland sediment. However, this is not always possible, and even when it can be done, the labor and cost of making direct rate measurements limit their spatial and temporal resolution. Porewater chemical profiles are often used to identify biogeochemical processes and infer their relative importance in lakes (White et al. 1989, Sinke et al. 1990, Urban et al. 1997, Devito and Hill 1999), and could be used in wetlands for similar purposes.

The objective of this study is to explore the patterns in porewater biogeochemical profiles within wetlands, as well as among wetlands spanning a gradient of water sources from groundwater to precipitation. The measurements I report here – and the processes they potentially indicate – include: NO_3^- (a substrate for denitrification), Fe(II) (a product of Fe reduction), SO_4^{2-} and H_2S (substrate and product of sulfate reduction), and CH_4 (product of methanogenesis). Dissolved CO_2 is also included. Patterns in concentrations provide an indication of biogeochemical processes in these wetland sediments, albeit subject to several caveats, including internal cycling, the assumption of steady state conditions, and spatial and temporal variability (Urban et al. 1997). I analyze the data with three questions in mind: 1) Do vertical profiles provide evidence for thermodynamic constraints on anaerobic microbial processes? 2) What is the spatial and temporal variability in porewater biogeochemistry within a wetland? and 3) Does the wetland's water source impact the porewater biogeochemistry? The water source is hypothesized to matter because precipitation inputs to a wetland are episodic in nature and are acidic with lower concentrations of nitrate and sulfate. Groundwater inputs are more continuous and are more ionically rich, carrying higher concentrations of nitrate and sulfate (Table 1).

Methods

Study Sites

The wetlands in this study occur in the glacial landscape around the Kellogg Biological Station (KBS), an academic unit of Michigan State University, located in southwest Michigan. Wetlands in this region are abundant and cover about 10% of the landscape (Figure 1). The rest of the landscape is covered by agricultural areas, forests, and abandoned fields, with only limited residential development. Wetlands are abundant and varied, with many occurring as isolated, small depressions that lack surface inflows and outflows. I sampled porewater in 13 wetlands during 1999 and resampled 6 of these wetlands during

Table 1: Average concentrations for precipitaiton and groundwater springs. The precipitation means of annual volume weighted means for 1979-96 from the KBS NADP/NTN.

	NO_3-N mg/L	SO_4^{2-} mg/L	pH	alkalinity µeq/L
precipitation	0.42	2.72	4.45	0
groundwater springs	1.73	26.4	7.83	4534

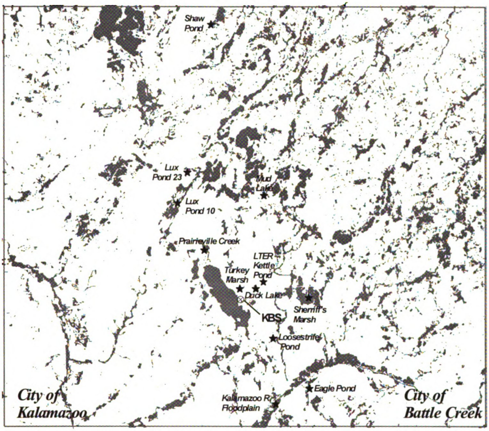


Figure 1: Map of wetlands sampled in southwest Michigan. The W. K. Kellogg Biological Station (KBS) is present for reference.

Table 2: Select wetland characteristics. Surface water and sediment pH measurements are means from all sampling dates. Organic carbon means are from 3 replicate cores, except for wetlands 3, 6-9, and 13 where only one core was taken. Standard deviations are given in parentheses.

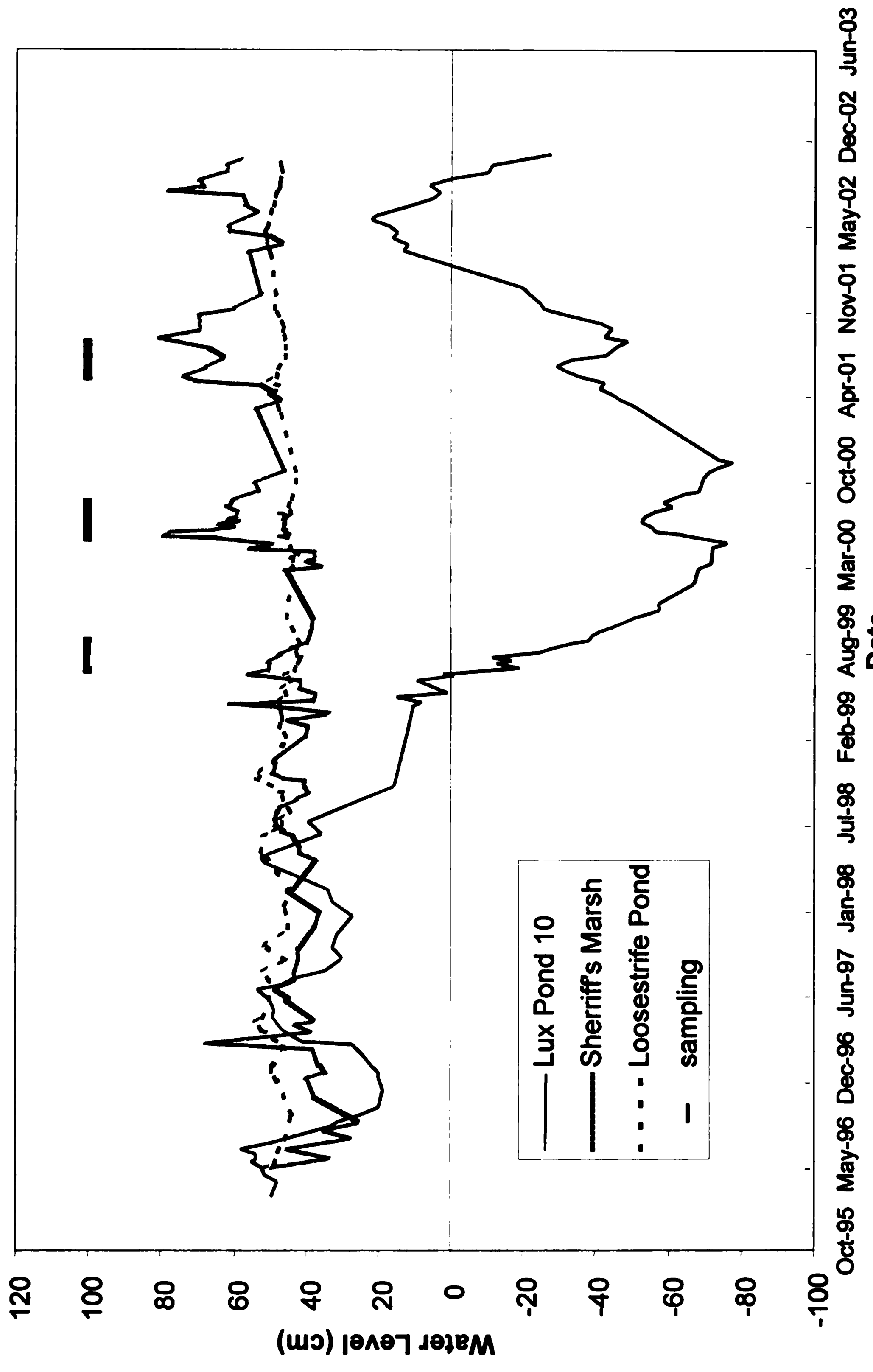
Wetland number	Wetland Name	Wetland Water Source Code	Area ha	NWI Classification	Surface water Conductance (μS cm^{-1})	sediment pH	% organic carbon	surface water SO_4^{2-} (mg L^{-1})	surface water DOC (mg L^{-1})
1	Loosestrife Pond	G	0.4	PUBG	520 (34)	6.6 (0.9)	30.3 (22)	19.1	4.4
2	Kalamazoo River Floodplain	G	14.5	PFO1C	544 (57)	6.5 (0.2)	48.7 (2.3)	6.59	2.5
3	Prairieville Creek	G	46.4	Scrub-Shrub	560	7.3 (0.1)	15.0	31.0	2.2
4	Mud Lake @ Prairieville Creek	G	46.4	Scrub-Shrub	553	6.8 (0.0)	n.a.	32.0	2.3
5	Turkey Marsh	G	3.1	PEM2G	370 (48)	6.9 (0.6)	60.6 (9.5)	13.0	11.4
6	Sheriff's Marsh	G	78.3	PEM	388	6.3 (0.8)	26.6	9.82	4.7
7	Eagle Pond	M	3.5	PEM2G	277	6.9 (0.1)	50.7	5.58	5.9
8	Lux 23	M	1.3	PUBG	218	7.0 (0.1)	11.2	4.24	8.0
9	Duck Lake	M	13.5	PUBG	215	6.3 (0.0)	7.4	2.50	7.6
10	LTER Kettle Pond	P	0.4	POWG	44 (11)	5.3 (0.2)	16.8 (17)		n.a.
11	Shaw Pond	P	0.8	PEMF	34 (4)	5.4 (0.2)	45.9 (17)	1.47	28.9
12	Lux10	P	0.7	PUBG	26 (2)	5.6 (0.1)	7.3 (4.3)	1.09	12.8
13	Mud Lake	P	92.9	Aqautic Bed	37	5.6 (0.2)	50.8	1.11	15.8

2000-2001. Wetland characteristics are summarized in Table 2 and locations are shown in Figure 1. Brief descriptions of each site follow.

Loosestrife Pond is a small groundwater-fed wetland located at the W.K. Kellogg Experimental Forest. This wetland was created by sediment infilling behind a small earthen dam. It was dominated by *Chara* sp. and *Lythrum salicaria* (purple loosestrife), with *Chara* sp. occupying the shallow open water area in about half of the wetland and *L. salicaria* growing on *Carex hystericina* tussocks in the other half. Sampling sites, which were located between tussocks or in the areas where *Chara* was present, were overlain by a few cm of surface water on all sampling dates. The water levels are maintained by an outflow sill and have fluctuated only about 10 cm since 1996 (Figure 2). There is continuous groundwater input from a spring that drives surface water flow across the wetland. This site has been continuously flooded since observations began in 1996.

The Kalamazoo River Floodplain (Floodplain) is a forested floodplain. The section studied is located along the left bank of the river, downstream of the Village of Augusta and within the Fort Custer State Recreation Area. The floodplain study unit is several hundred meters wide and is composed of fine alluvium overlying relatively permeable sand layers. On average, the river floods onto the floodplain one or more times in late winter and early spring for a total duration of about one week per year. The floodplain soils remain saturated for most of the summer due to groundwater seepage from adjacent uplands, which

Figure 2: Water level observations from 1996-2002 for 3 representative wetlands, 2 of which are groundwater-fed (G) and 1 is precipitation-fed (P). The straight solid lines indicate the periods when the sampling was done. Stage data were collected from bi-weekly staff gauge readings at Sherriff's Marsh, Loosestrife Pond and Lux10.


Water Level (cm)
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Oct-95
May-96
Dec-96
Jun-97
Jan-98
Jul-98
Feb-99
Aug-99
Mar-00
Oct-00
Apr-01
Nov-01
May-02
Dec-02
Jun-03
Date
Lux Pond 10
Sherriff's Marsh
Loosestrife Pond
sampling

forms discrete springs in some places where the sandy layers are close to the surface. Surface soils tend to dry somewhat by late summer. Dominant vegetation included a dense growth of emergent, herbaceous plants in areas with more light and a forested canopy in other parts of the floodplain.

Prairieville Creek and Mud Lake at Prairieville Creek are part of a large wetland complex whose outflow becomes a tributary to Gull Lake. Mud Lake is a 46-ha groundwater-fed lake about 1.5 km downstream of the headwaters of Prairieville Creek. It was dominated by submerged aquatic vegetation (particularly *Chara* sp.). The lake was surrounded by dense stands of the exotic *Rhamnus frangula* (glossy buckthorn) on the west, south, and east sides and a sedge meadow on the north side. Groundwater passes through the riparian wetlands and discharges via numerous springs and seeps into Prairieville Creek, which flows through Mud Lake. Water levels in the stream and lake are relatively stable, generally varying 10 cm or less throughout the year. The stream sampling site is the outflow of Mud Lake, where the sediments are sandy with no vegetation.

Turkey Marsh is a small, isolated depressional wetland located just north of the KBS Academic Center and Gull Lake. The wetland has lower-lying areas that usually hold standing water with submersed and emergent vegetation and slightly higher areas covered with dense stands of shrubs (especially *Ilex verticillata*). This wetland's water levels and flooded area have fluctuated considerably over the past few years, ranging from 0 - 50 cm over the sediment surface during the study period. The surface sediments dried out completely during the summer of

2000, well before the porewater measurements were made. During the initial years of higher water levels, the vegetation was mainly composed of a mixture of submersed aquatic plants and *Nuphar advena*, but after drying in 2000 the vegetation changed to include more emergent plants such as *Phalaris arundinacea*, *Carex lacustris*, and *Leersia oryzoides*, with some scattered *Eleocharis* spp. and *Typha latifolia*.

Sherriff's Marsh, a large wetland complex surrounding a sluggish tributary of Augusta Creek, extending across a contiguous area of 276 ha that is bisected by a raised road (45th Street). The vegetation was a mixture of sedge meadows in the wetter areas and forest in varying states of succession in the drier areas; I sampled porewater in the sedge meadow area. The sampling site was located in a central part of the wetland, close to the stream and about 40 m from the road, at a point where the sedge meadow started to form an extensive floating mat. At the sampling point, there was no standing water on the floating mat, but there was about 25 cm of water between the floating mat and the sediment surface. The stream was surrounded by floating mats and the stream bottom had a thick organic layer. Water levels fluctuate in response to precipitation and runoff events. The area has remained flooded since observations began in 1996, and water levels vary about 20 cm seasonally (Figure 2).

Eagle Pond lies in an isolated depression surrounded by deciduous forest in the Fort Custer State Recreation Area, not far from Eagle Lake. The bottom is overlain by about 40 cm of unconsolidated organic sediment. Throughout the summer the water column was filled with dense growths of submersed macrophytes (*Utricularia* sp., *Ceratophyllum* sp.), as well as patches of *Nuphar advena*. The water depth at the sampling point was about 25 cm. Water levels have varied about 60 cm since 1996, but the flooded area has changed little due to the steep sides of the basin.

Lux 23 is a 1.3-ha pond located on the north side of the KBS Lux Arbor Reserve, lying in a depression surrounded by deciduous forest and some conifer plantations. The depression is presently isolated by a dirt road that crosses a narrow junction that once connected Lux 23 to water bodies to the north at high water levels. The water column was filled with *Chara* sp., and in deeper water there are patches of *Nuphar advena*. The sampling point was 2 m from shore in about 25 cm of water. Water levels have varied over ca. 60 cm since 1996 and sediments along the perimeter of the pond were exposed in summer 2000, when water levels were lowest.

Duck Lake is a 13.5-ha lake with a littoral zone that extends about 100 m into the lake. The sampling point was located 10 m from shore in the littoral zone, where the dominant plants included *Nymphaea odorata* and *Brasenia schreberi.* There was 1.5 m of water overlying the organic sediments. Water levels have fluctuated since 1995, but the littoral zone where I sampled has remained flooded throughout this period.

The LTER Kettle Pond lies in a small, isolated depression located on the KBS LTER agricultural research area. The slopes around the pond are covered by deciduous forest and conifer plantations. The pond's plant community was dominated by *Wolffia* and *Lemna* spp. during wet years (1996-1999), but after drying completely in 1999 the basin filled with *Eleocharis obtusa*, *Leersia oryzoides*, and *Phalaris arundinacea*. Standing water was present at the beginning of summer 2000, but the water table dropped to 5 cm below the sediment surface by the middle of the summer. Standing water has persisted since fall 2000.

Lux 10 is located on the KBS Lux Arbor Reserve and is the southeasternmost pond on the property. The pond lies in an isolated depression surrounded by deciduous forest, and a raised dirt road blocks what was once a high-water connection to Lower Crooked Lake. Lux 10 had mainly open water with a few water lilies (*Nuphar advena*) and little submersed aquatic vegetation. The banks were lined by a mix of *Leersia oryzoides*, *Carex lacustris*, *Sagittaria latifolia*, and *Eleocharis* sp. The water levels in Lux 10 dropped during the study, exposing sediments along the edges, but the pond never dried, and the sampling points were located in areas that always had standing water. Since 1996 the water levels have varied over 1.4 m (Figure 2).

Shaw Pond is a 0.8-ha wetland located in an isolated depression west of Otis Lake in the Barry Game State Area, about 22 km north of KBS. This pond is surrounded by forest on sandy soils. The dominant vegetation during wet years was a diverse mix of submersed and emergent aquatic plants (particularly *Nymphaea odorata*, *Nuphar advena*, *Potamogeton* spp.). There were also some emergent grasses and spike rushes that encircle the wetland (*Phalaris arundinacea* and *Eleocharis* spp.), and these species expanded into exposed sediments when water levels were low in 2000. The pond was entirely dry during late-summer 1999 through the summer of 2000, but flooded again in late 2000. During the summers of 2001 and 2002 standing waters were 30 - 75 cm deep. All sampling points remained underwater during the sampling periods of this study, except in 1999. No samples were collected during 2000.

Mud Lake is a 93-ha lake, including 35 ha of wetland, located in Barry Township east of the Lux Arbor Reserve. The wetland occurs on the west side of the lake in an area dominated by *Decodon verticillatus* (swamp loosestrife) and *Chamaedaphne calyculata* (leatherleaf). The sampling point was about 100 m from shore between 2 large patches of these plants, in a wetter area with 15 cm of standing water that contained mostly *Nuphar advena*, *Nymphaea odorata*, and *Brasenia* sp. The unconsolidated organic sediments extended to depths of 50 cm in some places.

Fraction of Groundwater vs. precipitation as water sources

Magnesium concentrations in wetland surface waters were used as a natural tracer of groundwater as a water source compared to precipitation (Stauffer

1985). Dissolved Mg^{2+} is a good indicator of groundwater inputs because it is a major weathering product arising from the dissolution of dolomite in the underlying glacial till in this area, and as a result of equilibration with dolomite the local groundwaters generally contain Mg^{2+} at concentrations of 18-25 mg/L. In contrast to groundwater, Mg^{2+} concentrations are negligible in precipitation (ca. 0.04 mg/L). Magnesium behaves conservatively in wetlands because it is little affected by biological uptake, and it is not subject to significant mineral precipitation reactions. The fraction of groundwater was estimated as a linear mixture between precipitation and local groundwater, as indicated by the closest springs or seeps that could be sampled (Table 3). The wetlands sampled in this study range from receiving 100% of their water from groundwater to less than 10% from groundwater (i.e., greater than 90% from precipitation) (Figure 3). Wetlands with greater than 80% groundwater contributions were classified as groundwater-fed (G), wetlands less than 20% groundwater contributions were classified as precipitation-fed (P), and wetlands between 20-80% were classified as a mixture of groundwater and precipitation (M) (Table 2). Evaporative concentration is not accounted for in this analysis but the ionic proportions in the groundwater-fed sites resemble those of groundwater and do not indicate that they could be precipitation-fed wetlands that experienced an extreme degree of evaporative concentration, raising their Mg^{2+} concentrations close to those of groundwater.

Table 3: Groundwater sampling points used to calculate the fraction of groundwater in each wetland.

Wetland Name	**Groundwater Sample(s)**	**Lab Site Codes**	**Mean Mg^{+2} (mg/L)**
Loosestrife Pond	Loosestrife Pond inflow spring	243	22.0
Kalamazoo River Floodplain	Seep along escarpment of the floodplain	199	20.1
Prairieville Creek	Mean of springs flowing into Prairieville creek	342-343, 345-349	26.0
Mud Lake @ Prairieville Creek	Mean of springs flowing into Prairieville creek	342-343, 345-349	26.0
Turkey Marsh	Spring inflow on the NE end of Turkey Marsh	423	25.5
Sherriff's Marsh	Mean of peripheral springs in Sherriff's Marsh	389-391	20.9
Eagle Pond	Seep along escarpment of Kalamazoo Floodplain	199	20.1
Lux 23	Mean of springs flowing into Prairieville creek	423	25.5
Duck Lake	Spring inflow on the NE end of Turkey Marsh	423	25.5
LTER Kettle Pond	Spring inflow on the NE end of Turkey Marsh	423	25.5
Shaw Pond	Mean of springs flowing into Hasting's Point swamp	151-153	17.9
Lux10	Mean of springs flowing into Prairieville creek	423	25.5
Mud Lake	Mean of springs flowing into Prairieville creek	342-343, 345-349	26.0

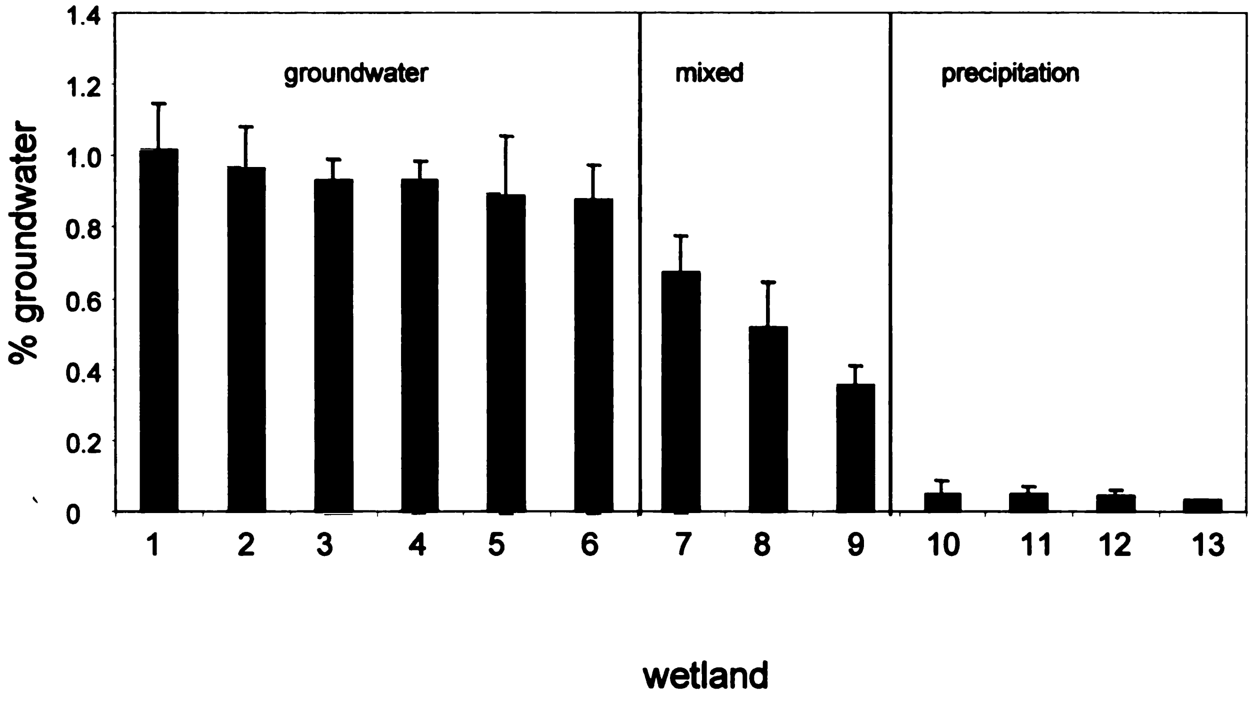


Figure 3: Percentage of the wetland water derived from groundwater (means with standard deviations) calculated using mean Mg^+ concentrations from several samplings between 1998-2001 (number of samplings ranges from 4 to 15, except for Mud Lake that has only 1). Numbers correspond to numbers in Table 2.

Porewater equilibrator design and deployment

Porewater equilibrators (Hesslein 1976) were used to sample the porewaters. The equilibrators are made of acrylic blocks (60 cm x 6.5 cm x 3.8 cm) containing 14 pairs of wells (2.5 cm diameter, 1.5 cm apart, and 13.1 ml volume). The wells are covered with a 2-μm Biotrans® nylon membrane (ICN # BNRG3R) secured in place with a face plate and galvanized bolts.

The equilibrators were assembled underwater to avoid trapping air bubbles in the wells. Once they were assembled, they were placed vertically in water that was sparged for at least 14 hours with He to ensure the water inside the wells was anoxic, and then transported in anoxic water to be deployed in the sediments of the wetlands. They were left in the field for 7 days to allow the water in the wells of the equilibrator to equilibrate with the sediment porewater. All sites of porewater equilibrator deployment were randomly choosen, but selected only if there was at least 10 cm of standing water at the time of deployment. In most cases the porewater equilibrators were centrally located within the wetland, except in Turkey Marsh and the Floodplain where sites were chosen based on existing transects. During 1999, one porewater equilibrator was deployed at one site within each wetland. In 2000 and 2001 duplicate equilibrators were installed at 3 sites within each wetland to allow for extra analyses to be done (NH_4-N, Total Dissolved P, and major cations) (except in Loosestrife Pond where only 2 sites were chosen). These sites were randomly chosen in precipitation-fed wetlands, while the sites within the groundwater-fed wetlands were aligned along the flow path of water based on surface water flow.

Porewater collection from the equilibrators

Upon removing the equilibrator from the sediment, it was kept underwater while sediment was gently washed off. Samples were withdrawn from the equilibrator immediately thereafter to minimize exchange of gases; sampling each equilibrator normally took about 15 minutes. The samples were analyzed immediately upon return to the lab, which was within 3 hours of sampling.

Dissolved CH_4 and CO_2 were measured by withdrawing 5 mL of the water into a 30 mL plastic syringe and sealing the syringe needle with a rubber stopper. In the lab, 15 mL of He at 1 atm was added to the syringe, and the gas-liquid system was vigorously shaken for 1 minute to partition the dissolved CH_4 and CO_2 into the headspace.

The headspace gases were measured with a Shimadzu GC14A gas chromatograph equipped with thermal conductivity (TCD) and flame ionization (FID) detectors and a Shimadzu CR501 integrator. The sample was injected through a 1-mL sample loop, the contents of which were transferred to the carrier gas stream with a Valco 6-port switching valve controlled by the microprocessor. Water vapor was removed by a trap containing Aquasorb® P_2O_5 desiccant. Simultaneous determination of CO_2 and CH_4 was done with two columns and a

Valco series-bypass valve mounted inside the chromatograph oven, which was kept at 70°C. The CO_2 was separated by the 2-m 80/100-mesh Porapak-Q column and shunted directly to the TCD detector. A 2-m column of Molecular Sieve 5A separated CH_4 from the remaining gases (O_2, Ar, N_2), and any water vapor was backflushed out of the system. The CH_4 was quantified by the FID or, if present at >1 % levels, by the TCD. Certified standards of comparable concentrations were used for calibration (Scott Specialty Gases, Inc.).

Water for analysis of anions (Cl^-, NO_3-N and SO_4^{2-}) was collected in 10-mL syringes after removal of subsamples for dissolved gas analysis. Anions were measured using membrane-suppression ion chromatography on a Dionex 4200. Samples were also collected from wells at 3 depths for pH and specific conductance measurement in the lab; pH was measured without exposure of the sample to the atmosphere by injecting the water through tubing to the pH electrode, thereby avoiding loss of dissolved CO_2.

H_2S and Fe(II) were sampled from the other set of wells adjacent to those sampled for dissolved CO_2, CH_4, and anions. All water was withdrawn from each well with a syringe and subsamples were processed immediately in the field. Part of the sample was transferred to 8-mL glass vials for H_2S analysis (see below), keeping the needle underwater as the vial was completely filled and overflowed to minimize aeration and degassing of the sample.

The rest of the sample was dispensed into 15-mL centrifuge tubes containing 7.5 mL of 50mM HEPES buffer with 1 g of ferrozine for Fe(II) analysis (Lovley and Phillips 1987). Fe(II) sample absorbances were subsequently read in the lab using a 1-cm pathlength and 562 nm wavelength; calibration was based on standards made from electrolytic iron.

Once all of the H_2S samples were collected for a particular equilibrator, reagents were added in the field with 1-cc tuberculin syringes: 0.8 mL of amine-acid reagent (0.2 g of amine salt (N,N-dimethyl-p-phenylenediamine oxalate) in 100 mL of 20% HCl) and 0.2 mL of $FeCl_3$ solution (10 g of $FeCl_3$ in 100 mL of concentrated HCl and 400 mL of water). One well from each equilibrator was sacrificed for a blank and injected with 0.8 mL of 20% HCl and 0.2 mL of the $FeCl_3$ solution. All samples were stored in the dark until returning to the lab. The H_2S sample absorbances were read with a 1-cm pathlength at 600 nm. The analysis is largely based on those of Golterman and Clymo (1969) and APHA et al. (1989), in which sulfide reacts with ferric chloride and N,N-dimethyl-*p*-phenylenediamine oxalate to produce methylene blue. The absorbance in a 1-cm cell is multiplied by 37 to give the approximate H_2S concentration in μM (Golterman and Clymo 1969).

During 2000 and 2001, cations, ammonium (NH_4-N), and total dissolved phosphorus (TDP) were collected from a second equilibrator that was deployed next to the first one. The paired wells at each depth were pooled for these analyses, for a total of 7 samples for each profile. Cations were analyzed by flame atomic absorption spectrophotometry, adding lanthanum for the Ca^{2+} and Mg^{2+} measurements to suppress interferences. NH_4-N was measured colorimetrically using a phenylhpochlorite technique (Aminot et al. 1997). TDP was measured by the molybdenum blue colorimetric analysis after persulfate digestion (Valderrama 1981).

Statistical Analyses

Variability within porewater profile

To determine if electron acceptor and final product concentrations varied by depth in a wetland, the porewater profiles were divided into two depth zones: 2-20 cm and 20-40 cm. Only the equilibrator data from 1999 was included in this analysis, when only one site in each wetland was sampled. In each wetland t-tests were performed to determine if the mean concentrations from the 2-20 cm depth range were significantly different from those in the 20-40 cm range.

Spatial variability within wetlands

To look at the horizontal spatial variability within wetlands, equilibrator data from multiple sites sampled in 2000 and 2001 were included. Means for the depth zones of 2-20 and 2-40 cm were used in ANOVAs with zones and sites as factors in each wetland. For this and the following statistical analyses, data were log-transformed to improve normality when necessary.

Temporal variability within wetlands

To look at temporal variation of sediment profiles within wetlands, I examined data from wetlands that had been sampled during more than one year (Loosestrife Pond, Floodplain, TM, Lux10 and Shaw Pond). When I had sampled more than one site within a wetland, the profiles were averaged together to obtain a single mean profile.

Variability between different water sources and among wetlands with the same water source

Data from 1999 were used to examine differences among wetlands with different predominant water sources, classified as precipitation-fed, groundwater-fed, or a mixture (see above). Each wetland was considered a replicate and the sediment porewater profile was averaged. ANOVAs were done using water source as a factor. To look at variability among wetlands within each of the three water source categories, ANOVAs were run with wetland as the factor.

Results

Nitrate was below the detection limit (ca. 15 μg N L^{-1}) in the porewaters of most wetlands and will therefore not be discussed except for specific examples. Porewater sulfate concentrations ranged from less than 1 to over 100 mg L^{-1}, with most wetlands never having over 10 mg L^{-1}. The exceptions are the LTER Kettle Pond that has an average of 33 mg L^{-1} from 20-40 cm, the Floodplain with over 50 mg L^{-1} at some depths between 0-20 cm, and Turkey Marsh with over 50 mg L^{-1} in both zones (Figure 4). Average porewater sulfate concentrations for groundwater-fed wetlands was only slightly higher than for precipitation fed wetlands (7.3 ± 1.5 and 5.5 ± 1.1 mg L^{-1} respectively). Mean surface-water sulfate concentrations ranged from 1.1 – 32 mg L^{-1}, with the groundwater-fed wetlands generally having higher concentrations (Table 2).

Porewater Fe(II) concentrations were fairly consistent across depths in Duck Lake, Eagle Pond, Lux 23, Lux 10, Mud Lake, and Mud Lake at Prairieville Creek, with concentrations generally under 10 mg L^{-1}. Sherriff's Marsh had the highest Fe(II) concentrations (0-20 cm: 35.3 ± 1.1 mg L^{-1}; 20-40 cm: 33.3 ± 1.3 mg $^{-1}$), while the LTER Kettle Pond and Floodplain were also high with Fe(II) concentrations over 10 mg L^{-1} (Figure 5).

Hydrogen sulfide concentrations ranged widely in most wetlands, with Loosestrife Pond, Lux 10, and Turkey Marsh having the greatest range (<5 to >100 mg L^{-1}) (Figure 6). Duck Lake, Sherriff's Marsh, Mud Lake, and Lux 23 all had less than 5 mg L^{-1} across all depths. Mean H_2S concentrations were lower in groundwater-fed than in precipitation-fed wetlands, but they both varied widely (16.5 ± 32 and 25.4 ± 35.7 mg L^{-1} respectively).

Dissolved methane concentrations varied depending on the wetland (Figure 7). A few sites had concentrations below 100 μmol L^{-1} (Floodplain, LTER Kettle

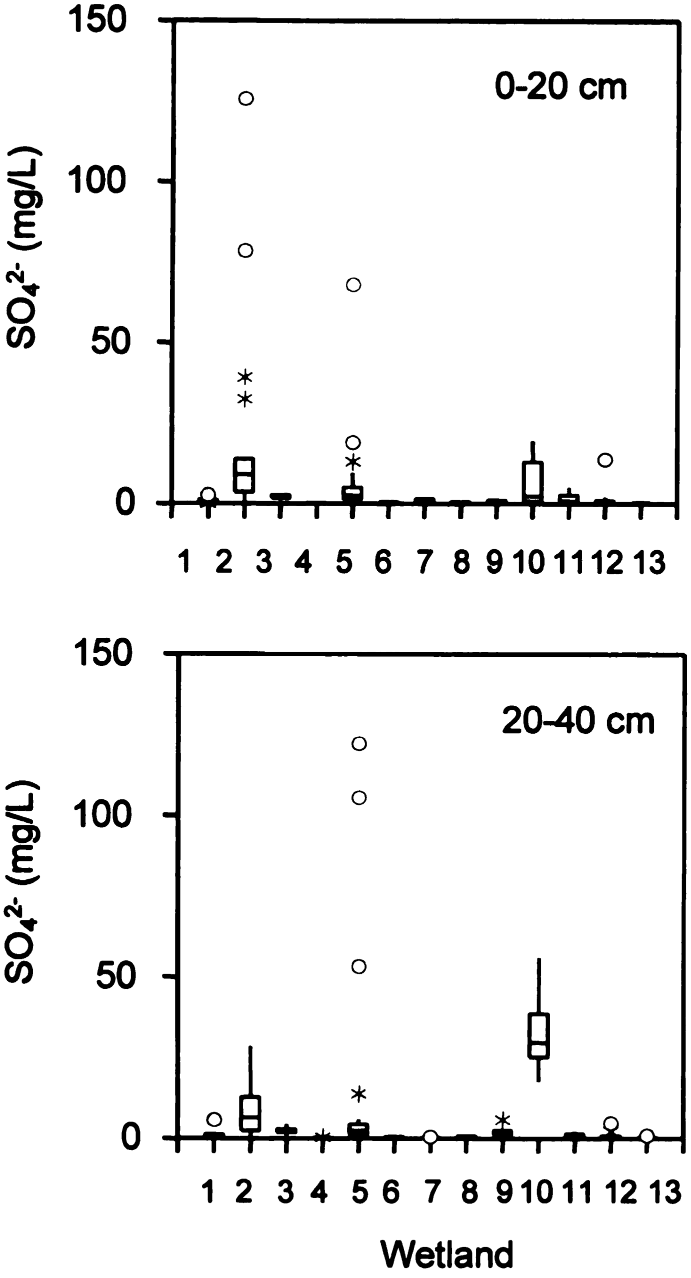


Figure 4: Box plots of sulfate concentrations for each wetland for 0-20 cm (A), and 20-40 cm (B). Data are from 1999-2000. Numbers correspond to wetlands listed in Table 2.

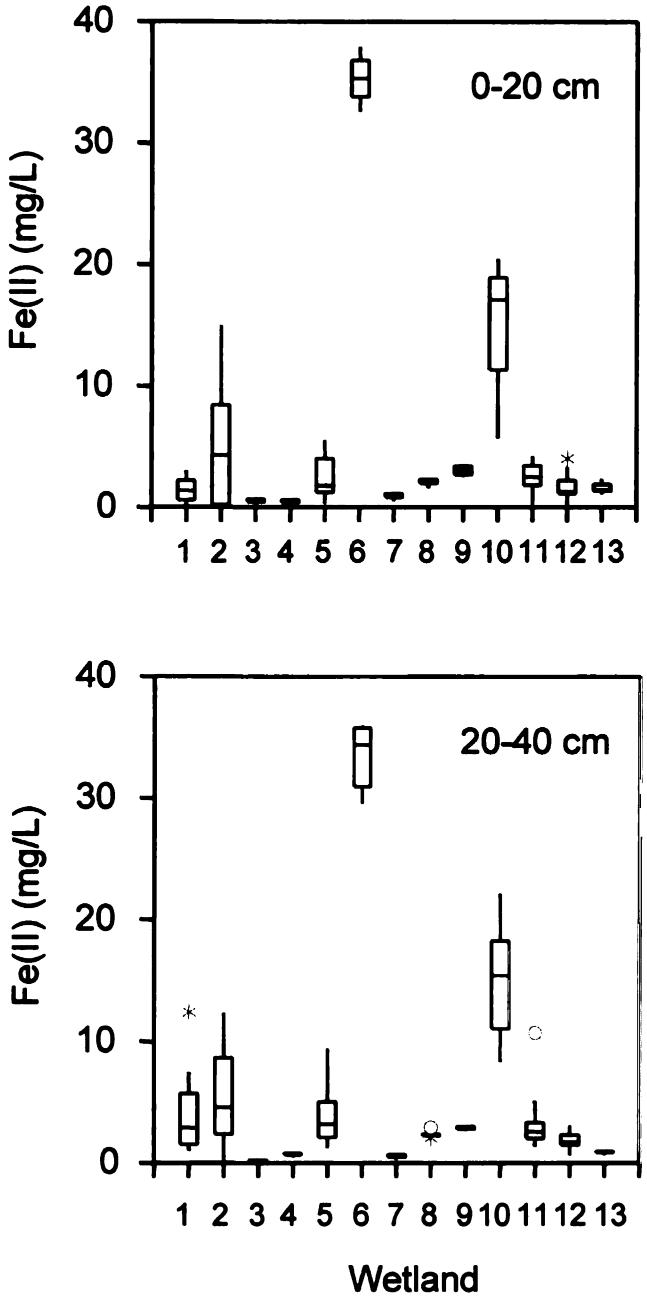


Figure 5: Box plots of porewater iron concentrations for each wetland for 0-20 cm (top graph) and 20-40 cm (bottom graph). Data are from 1999-2000. Numbers correspond to wetlands listed in Table 2.

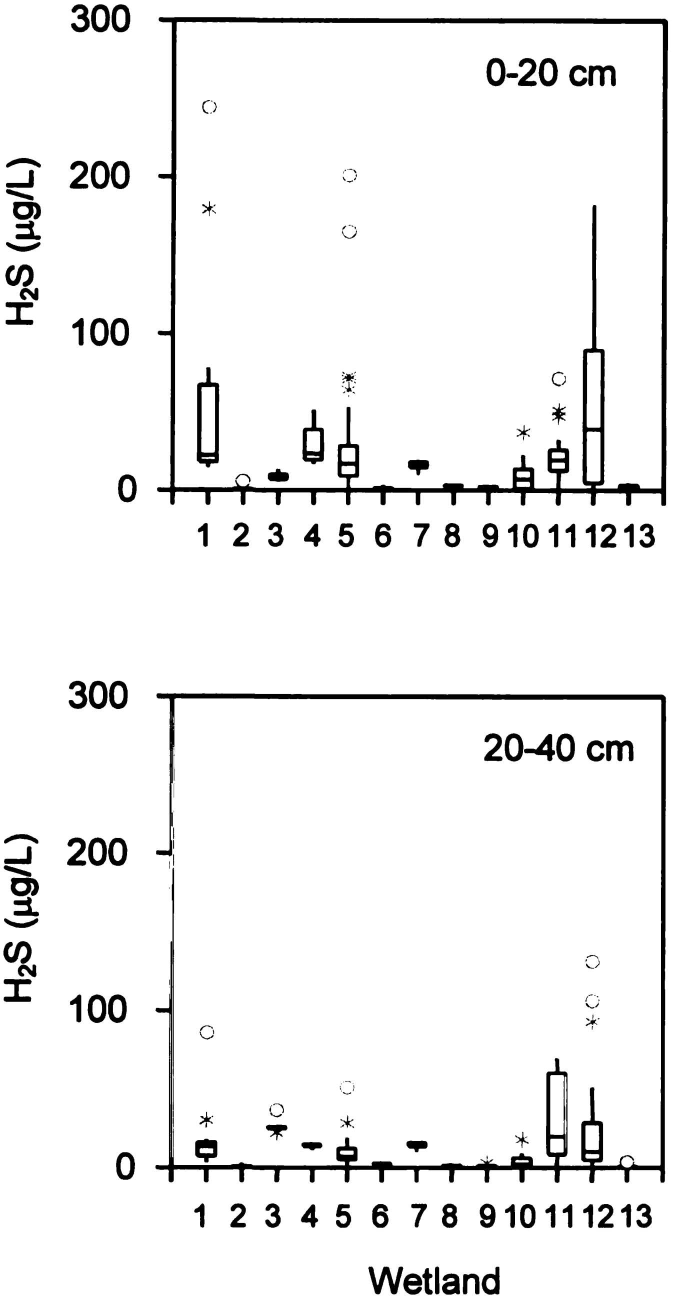


Figure 6: Box plots of porewater hydrogen sulfide concentrations for each wetland for 0-20 cm (A) and 20-40 cm (B). Data are from 1999-2000. Numbers correspond to wetlands listed in Table 2.

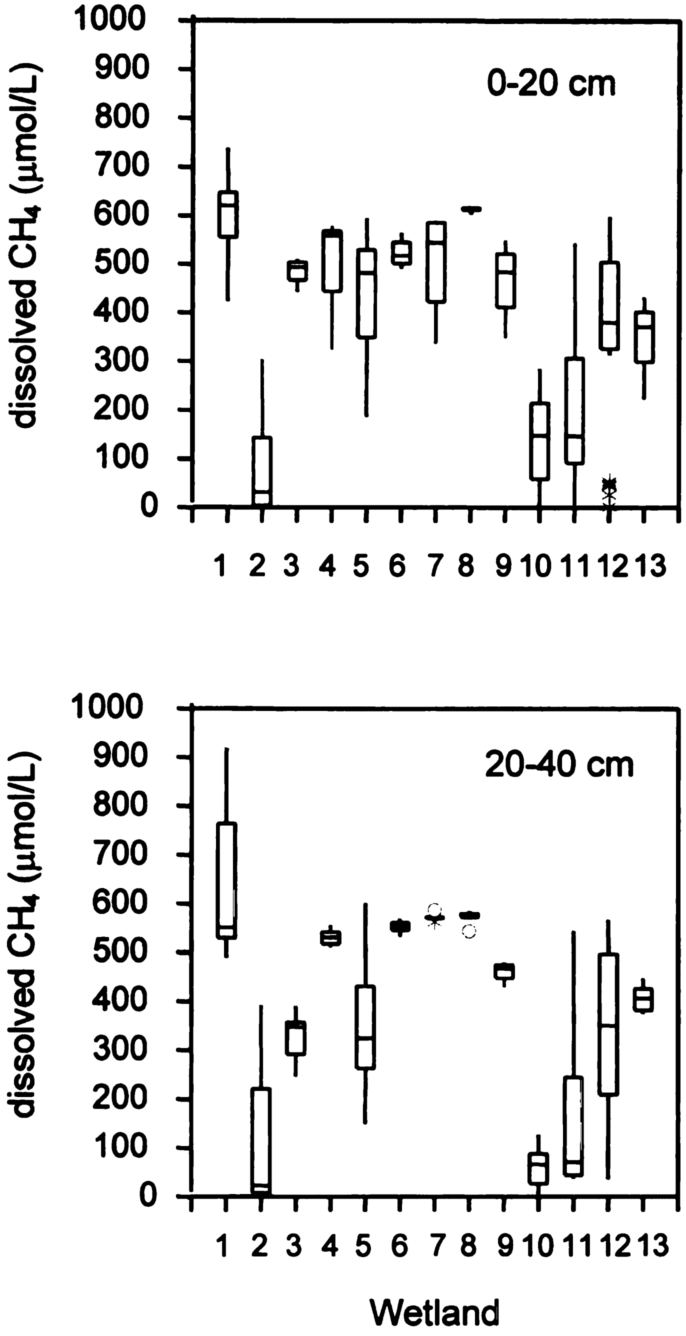


Figure 7: Box plots of porewater dissolved methane concentrations for each wetland for 0-20 cm (A) and 20-40 cm (B). Data are from 1999-2000. Numbers correspond to wetlands listed in Table 2.

Pond, Lux 10 and Shaw Pond), while Loosestrife Pond was the only wetland with greater than 600 μmol L^{-1} of CH_4. CO_2 concentrations were higher than CH_4 and ranged from about 100 to over 6000 μmol L^{-1} (Figure 8). Eagle Pond and Prairieville Creek had the lowest average CO_2 concentrations (1091 ± 199 and 728 ± 45 mg L^{-1} respectively).

Variability within the vertical profile

Each wetland had a unique porewater profile for each solute (Figure 9). In some cases the uppermost 1-2 well pairs were above the sediments, in the open water column, which was generally oxic, and were therefore left out of Figure 9. The wetlands sometimes showed significant differences in the mean concentrations between the 2 depth ranges (0-20 and 20-40 cm). Average Fe(II) concentrations across these zones ranged from less than 1 to 33 mg L^{-1} and generally showed differences between zone 1 and 2 in wetlands toward the low end of the concentration range (Figure 5). In Shaw Pond, Turkey Marsh, and Mud Lake at Prairieville Creek, Fe(II) concentrations were significantly higher from 20-40 cm than from 0-20 cm, while in Eagle Pond and Prairieville Creek the opposite was true.

The LTER Kettle Pond had significantly higher sulfate concentrations from 20-40 cm (Figure 4). Even though Turkey Marsh and the Floodplain had a few high sulfate values (>50 mg L^{-1}), there were no differences detected between the zones. Turkey Marsh, Sherriff's Marsh, Prairieville Creek, Mud Lake at

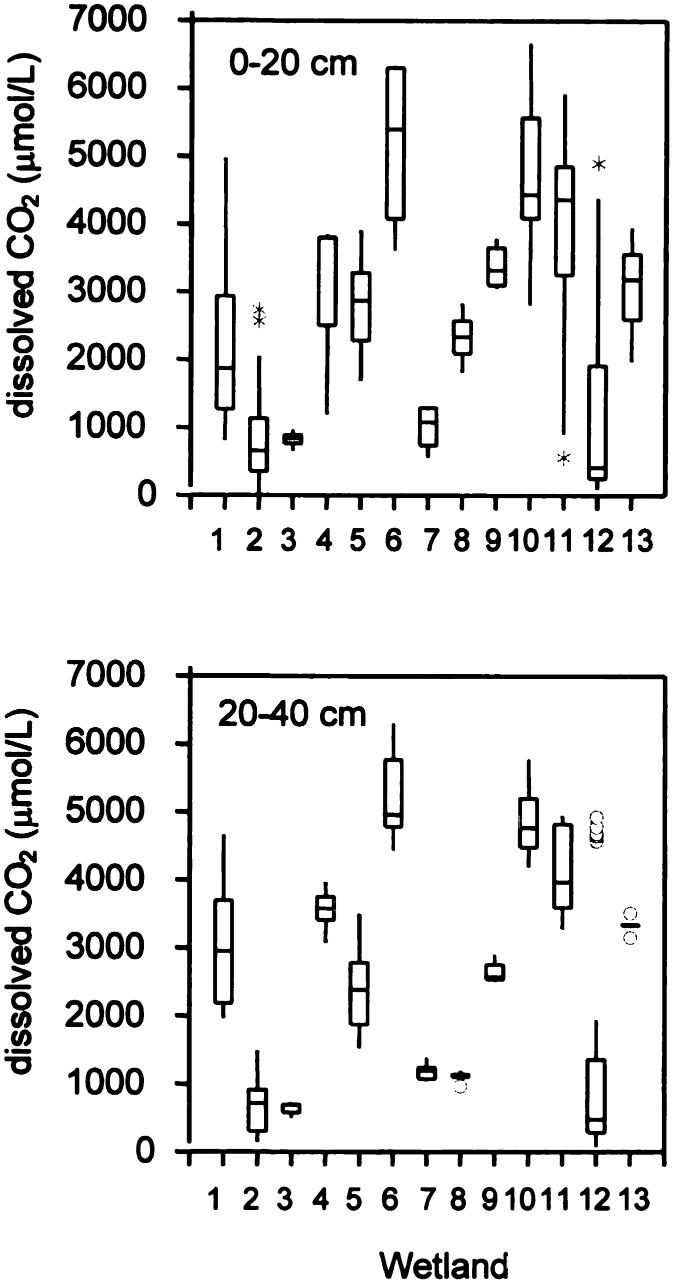


Figure 8: Box plots of porewater carbon dioxide concentrations for each wetland with 0-20 cm (A) and 20-40 cm (B). Data are from 1999-2000. Numbers correspond to wetlands listed in Table 2.

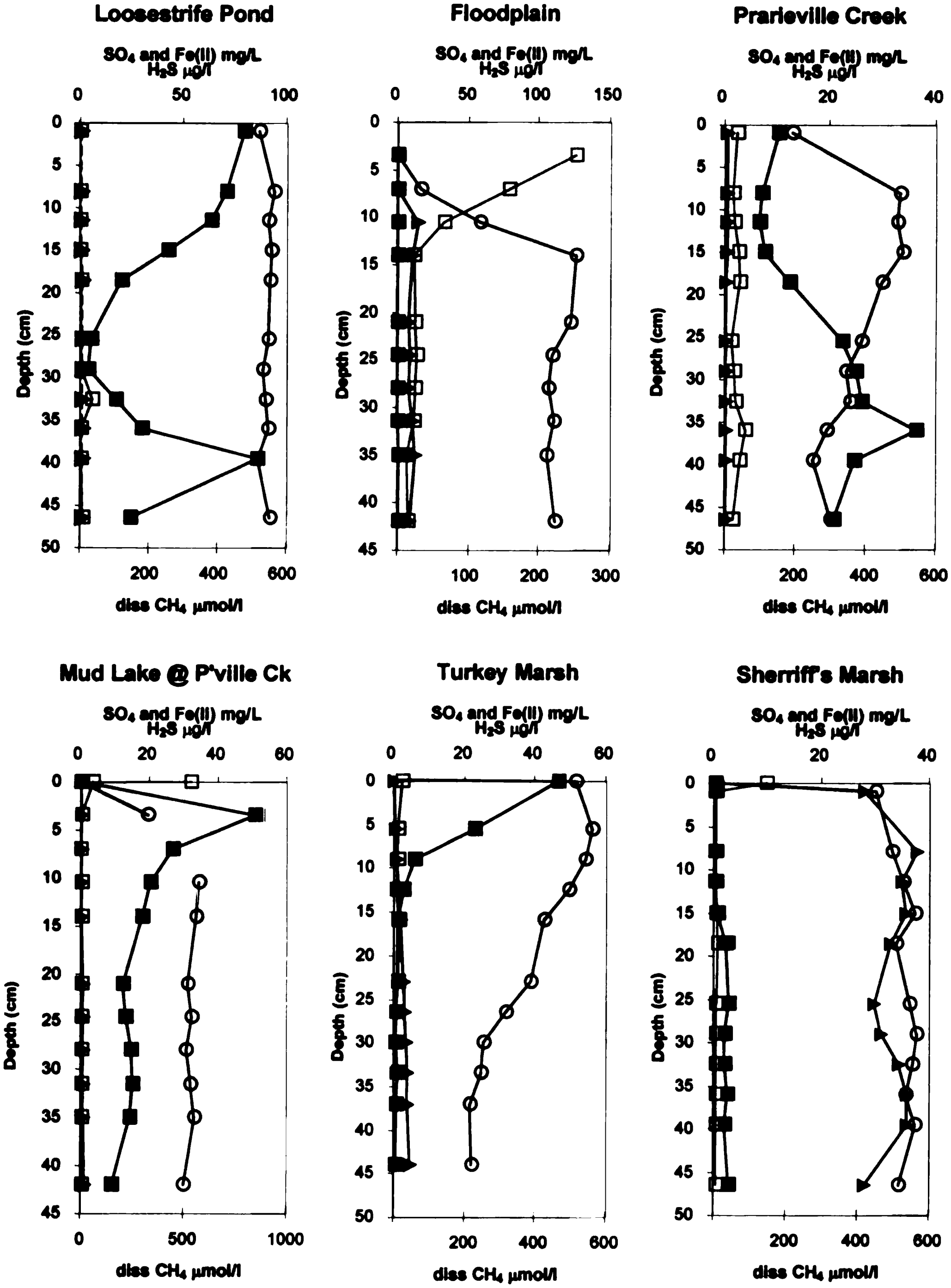


Figure 9: Porewater profiles from 1999 with iron(II) (solid triangles), sulfate (open squares), hydrogen sulfide (solid squares), and methane (open circles). Wells that were above the sediment-water interface (depth set to zero in graph) are not shown here.

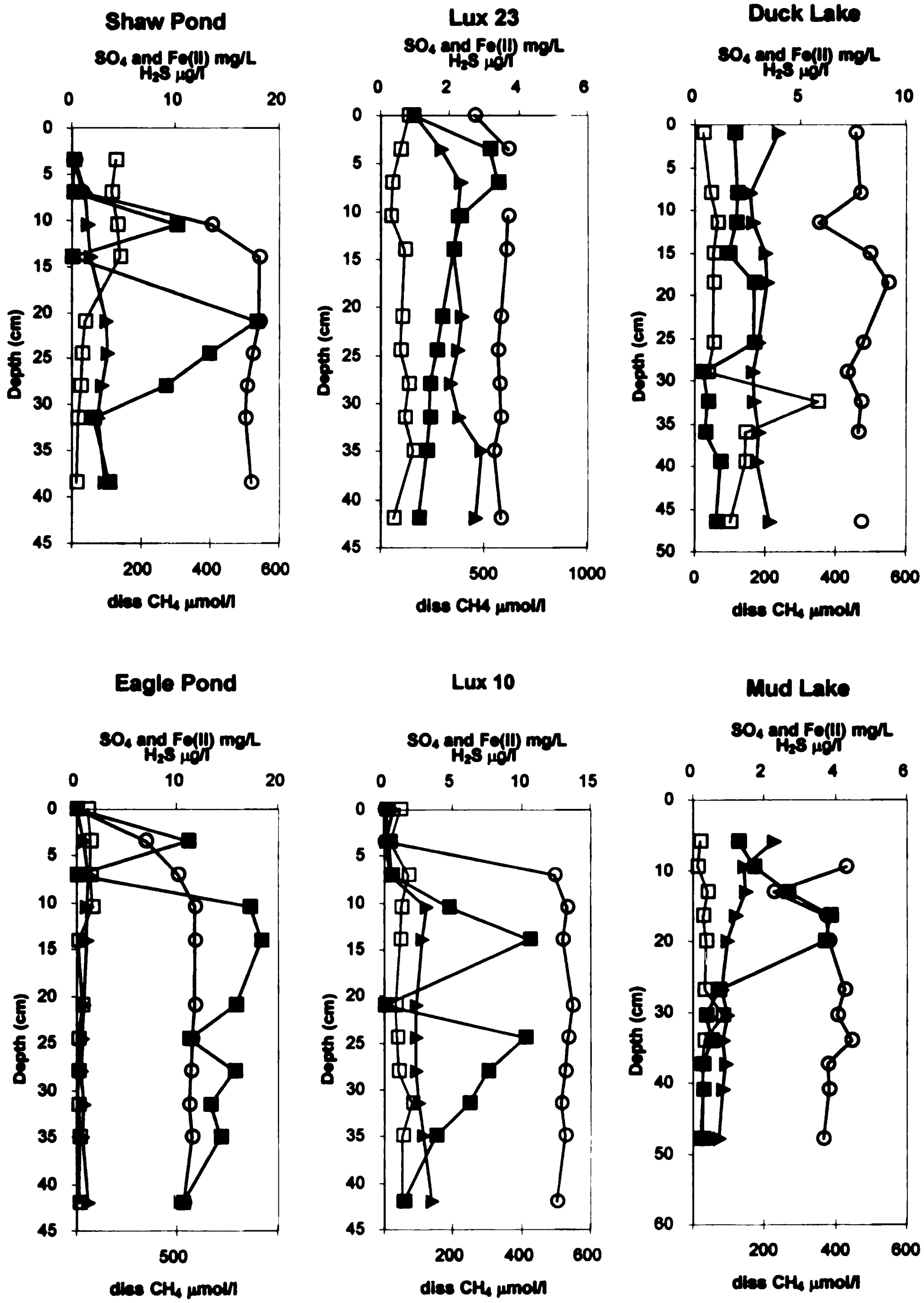


Figure 9 cont.'d.

Prairieville Creek, Lux 23, Loosestrife Pond, and Eagle Pond (all with some groundwater influence) had surface water concentrations that were greater than most of the porewater sulfate concentrations. In Turkey Marsh these surface water concentrations exceeded the mean sulfate concentrations found in local springs (26.4 mg L^{-1}). Hydrogen sulfide concentrations varied from <1 μg L^{-1} to more than 50 μg L^{-1} in the sediment profiles, but only Prairieville Creek and Lux 23 had statistically significant differences between depth zones (Figure 6), and these displayed opposite directions of change.

The wetlands that displayed significant differences in methane and carbon dioxide concentrations between 2-20 and 20-40 cm all had higher concentrations in the upper zone, except for CO_2 in Shaw Pond (Figures 7, 8). Dissolved methane in the wetland sediments was often at or near the limit of its solubility, assuming partial pressures of CH_4 in the 20-80% range. About half of the wetlands had near or above solubilities at 20% CH_4 by volume methane concentrations, with the exception of the Floodplain, the LTER Kettle Pond and Shaw Pond (Figure 10).

Horizontal spatial variability within wetlands

Fe(II) concentrations varied significantly between sites within wetlands in 4 of the 6 wetlands examined ($p<0.05$ for all wetlands) (Figure 11). Sulfate concentrations exhibited different patterns in each wetland. There were differences between sites within 3 wetlands (Figure 12). In the LTER Kettle Pond

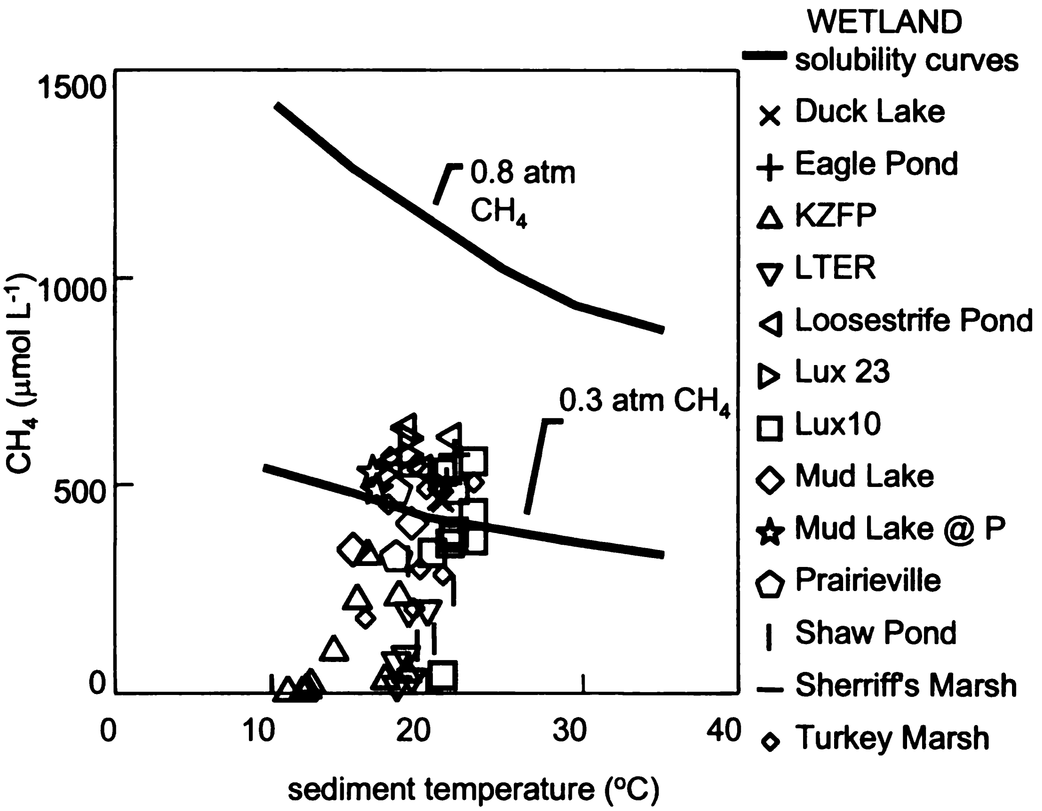


Figure 10: Methane solubility for each wetland. Each point represents the mean of either 0-20 or 20-40 cm for every site within a wetland and year. The solid lines are the solubility curves for 0.3 or 0.8 atm CH_4 from 10-35 °C.

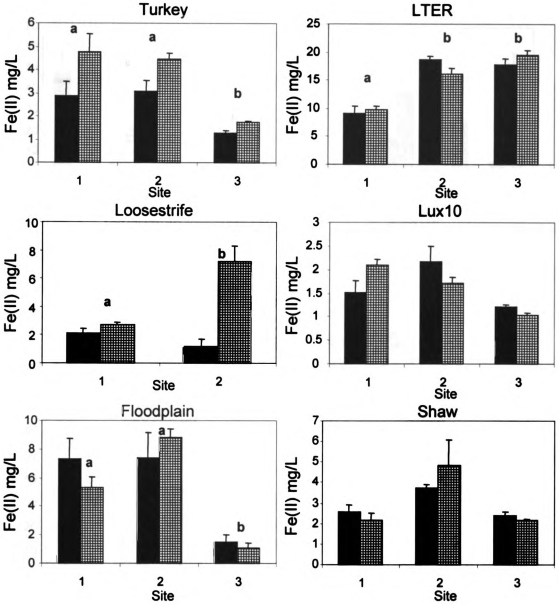


Figure 11: Average Fe(II) concentrations (standard deviations) for each site within wetlands. Samples were taken in 2000 and 2001. Solid boxes are 2-20 cm and checked boxes are 20-40 cm. Lowercase letters indicate significant differences between sites within a wetland, after combining the two depth ranges.

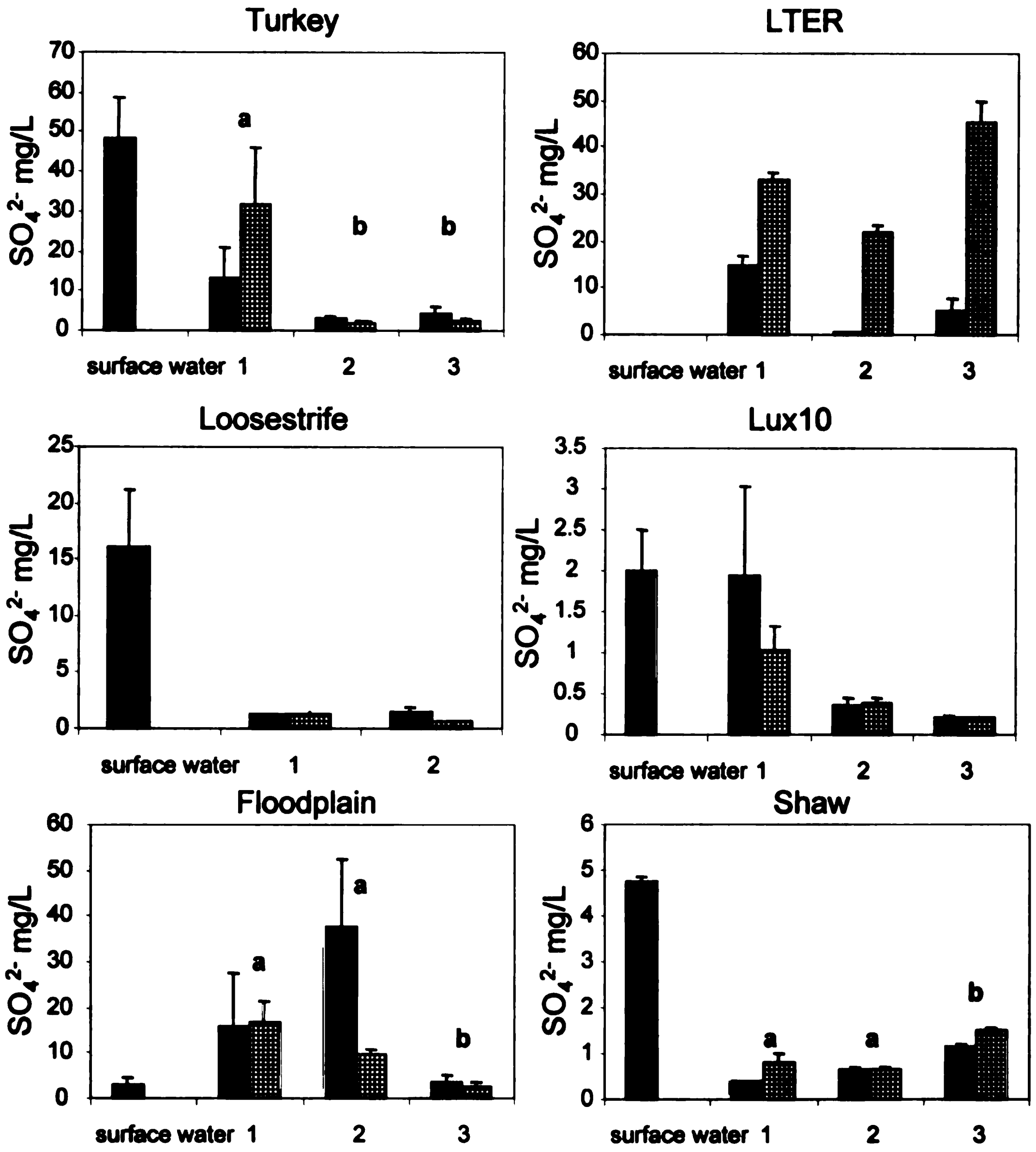


Figure 12: Average sulfate concentrations (standard deviations) for each site within wetlands and surface waters. Samples were taken in 2000 and 2001. Solid boxes are 2-20 cm and checked boxes are 20-40 cm. Lowercase letters indicate significant differences between sites within a wetland, after combining the two depth ranges. There was no surface water collected for the LTER during this sampling.

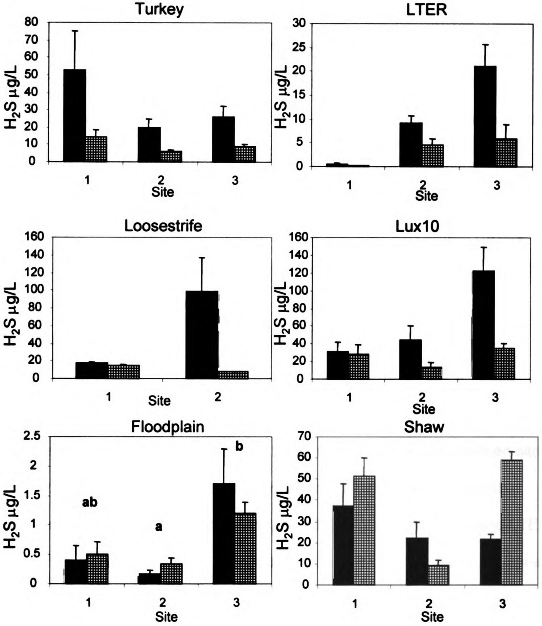


Figure 13: Average H_2S concentrations (standard deviations) for each site within wetlands. Samples were taken in 2000 and 2001. Solid boxes are 2-20 cm and checked boxes are 20-40 cm. Lowercase letters indicate significant differences between sites within a wetland, after combining the two depth ranges.

the deeper zone had higher concentrations of sulfate. Hydrogen sulfide concentrations were generally higher in the shallow zone compared to the deeper zone (Figure 13). Turkey Marsh had statistically significant higher concentrations with shallower depth (p=0.003) but no differences among sites within the wetland. Lux10 and the LTER Kettle Pond had different H_2S concentrations across sites and higher H_2S concentration at shallow depths. The Floodplain had similar concentrations of H_2S with depth, but site 3 had higher overall concentrations (p=0.018). Loosestrife Pond and Shaw Pond had no clear trends in H_2S concentrations but there appears to be significant within-site variation.

Horizontal spatial variability in CH_4 concentrations was common. Lux Pond 10 was the only wetland with no statistically significant differences between sites or depth zones for methane concentration, while Turkey Marsh had variable methane concentrations among sites (p=0.002) (Figure 14). Shaw and LTER ponds had higher methane concentrations from 2-20 cm, but mean methane concentrations varied depending on the site. Loosestrife Pond and the Floodplain had no clear trends, though it appears site variability was important at the Floodplain.

Interannual variability within wetlands

Interannual variation in the porewater profile within a wetland depended on the wetland and the solute being measured. Fe(II) was lower in 1999 than in

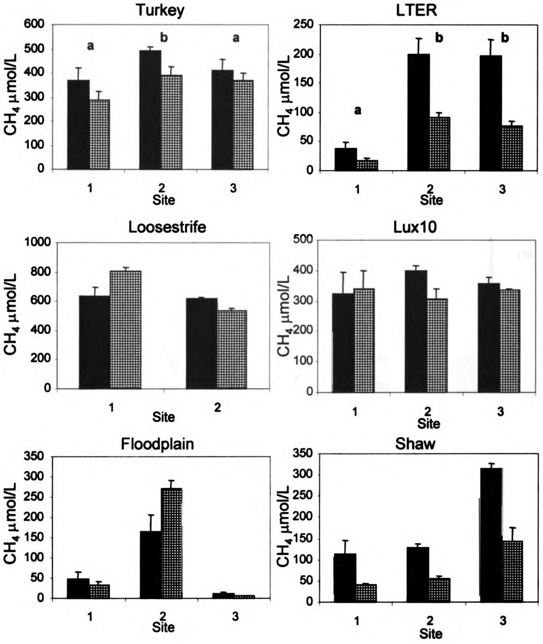


Figure 14: Average CH$_4$ concentrations (standard deviations) for each site within wetlands. Samples were taken in 2000 and 2001. Solid boxes are 2-20 cm and checked boxes are 20-40 cm. Lowercase letters indicate significant differences between sites within a wetland, after combining the two depth ranges.

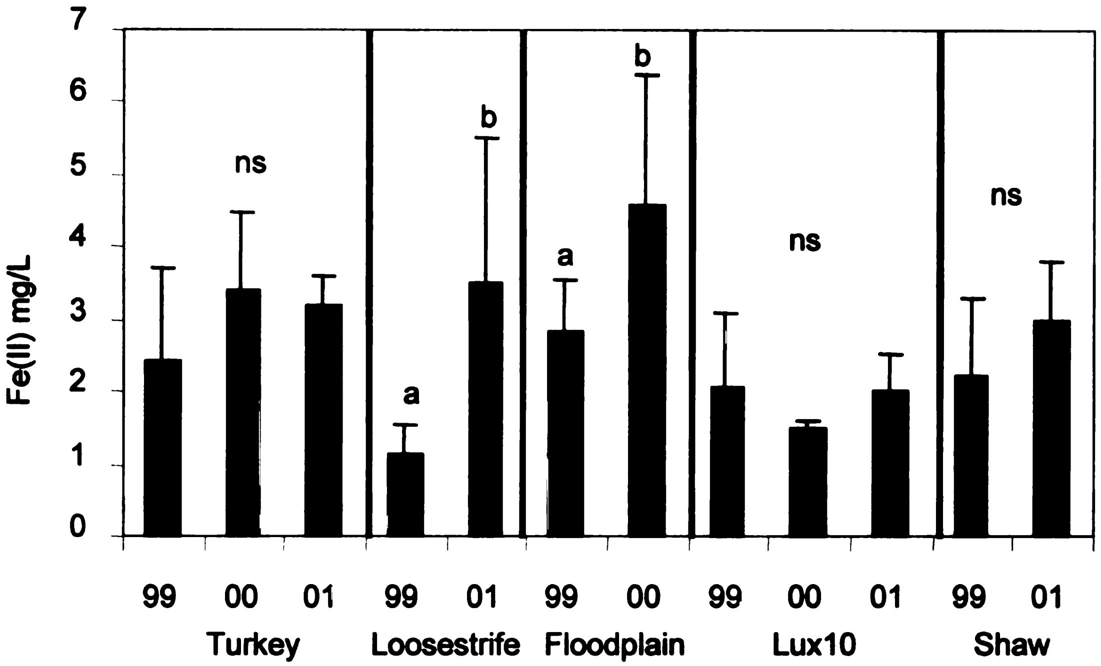


Figure 15: Fe(II) concentrations for porewater profiles measured in different years (means and standard deviations; all depths combined). Significant differences between years within an individual wetland are marked with different letters ($p<0.05$). Wetlands that have no statistically significant differences between years are marked with ns. No comparisons were done between wetlands.

subsequent years in both the Floodplain and Loosestrife Pond ($p=0.009$ and $p<0.001$) (Figure 15). Sulfate concentrations differed between years only in Turkey Marsh and Shaw Pond. In Turkey Marsh, sulfate concentrations in 1999 were lower than in both 2000 and 2001 ($p=0.004$), while Shaw Pond had higher sulfate concentrations in 1999 than 2000 ($p=0.024$) (Figure 16). All wetlands except Loosestrife Pond had different hydrogen sulfide concentrations between years (Figure 17). Methane concentrations were also different between years within each wetland, except for Turkey Marsh (Figure 18). Both Loosestrife Pond and the Floodplain had higher concentrations in 2000 while Lux Pond 10 and Shaw Pond had decreases in methane concentration over time.

Comparison of wetlands with different water sources

After lumping sites into hydrologic categories based on estimated groundwater influence, there were no statistical differences between groundwater-fed, precipitation-fed, or mixed-source wetlands for any solute, although there are some trends (Figure 19). Nitrate was near or below the detection limit in the porewaters of most wetlands (Figure 19A). Methane concentrations were generally high in all wetlands (Figure 19B). The highest hydrogen sulfide concentrations were measured in groundwater-fed sites, although not all such sites were high in H_2S (Figure 19F).

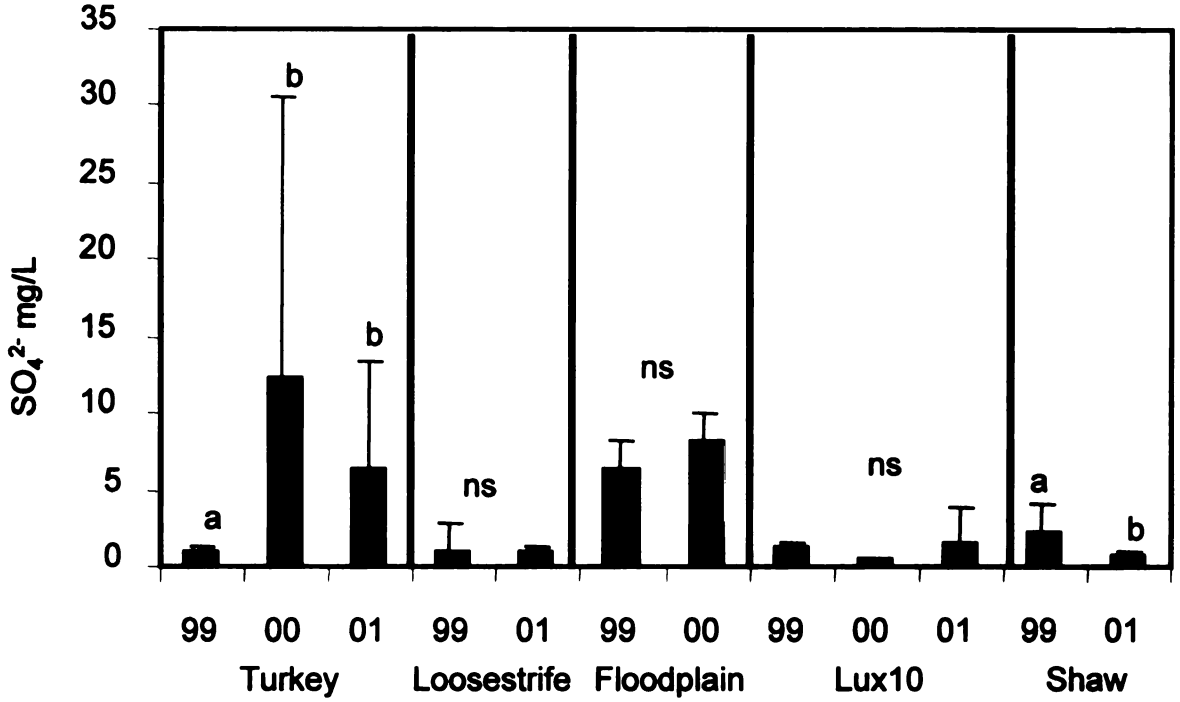


Figure 16: Sulfate concentrations for porewater profiles measured in different years (means and standard deviations; all depths combined). Significant differences between years within an individual wetland are marked with different letters ($p<0.05$). Wetlands that have no statistically significant differences between years are marked with ns. No comparisons were done between wetlands.

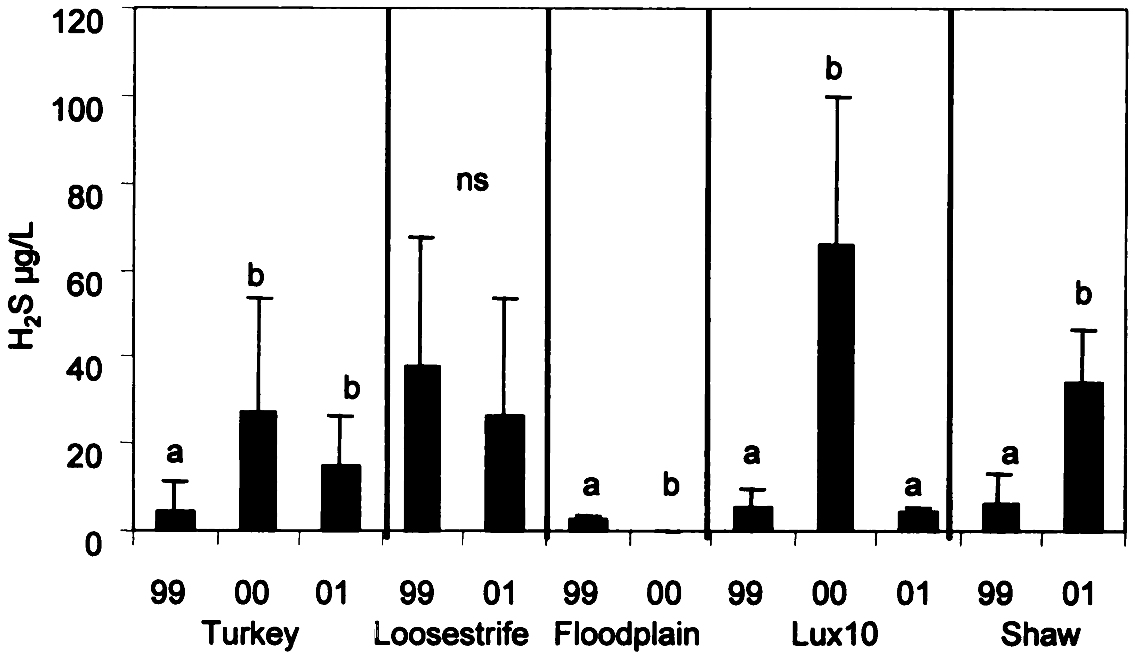


Figure 17: Hydrogen sulfide concentrations for porewater profiles measured in different years (means and standard deviations; all depths combined). Significant differences between years within an individual wetland are marked with different letters ($p<0.05$). Wetlands that have no statistically significant differences between years are marked with ns. No comparisons were done between wetlands.

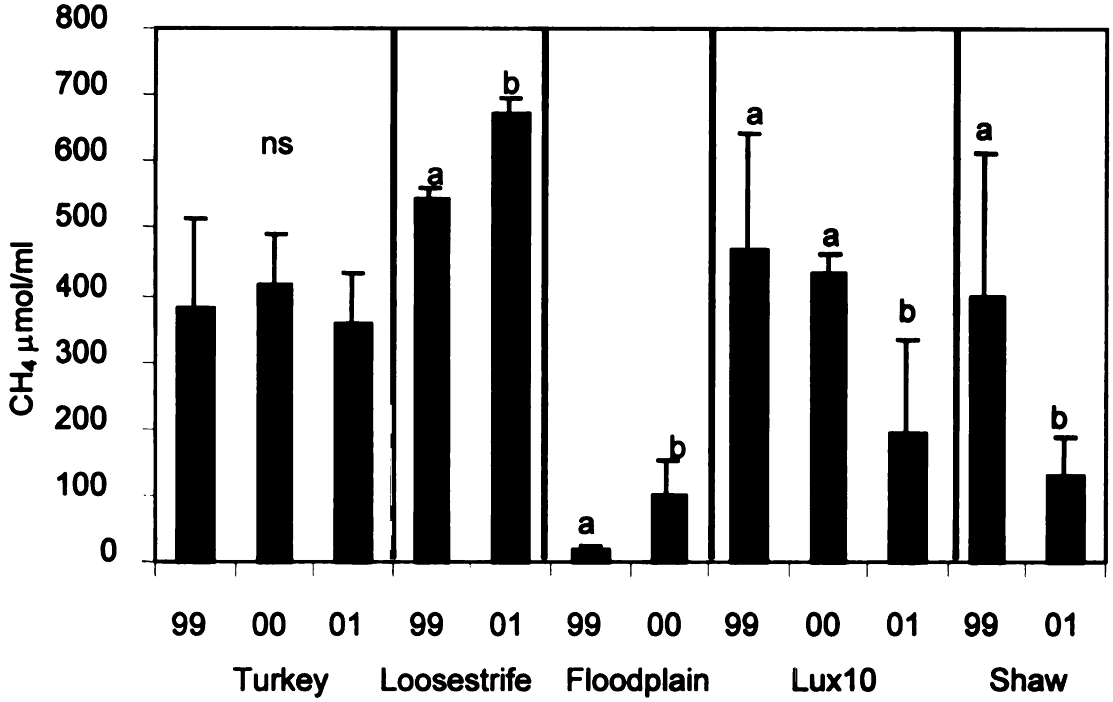


Figure 18: Methane concentrations for porewater profiles measured in different years (means and standard deviations; all depths combined). Significant differences between years within an individual wetland are marked with different letters ($p<0.05$). Wetlands that have no statistically significant differences between years are marked with ns. No comparisons were done between wetlands.

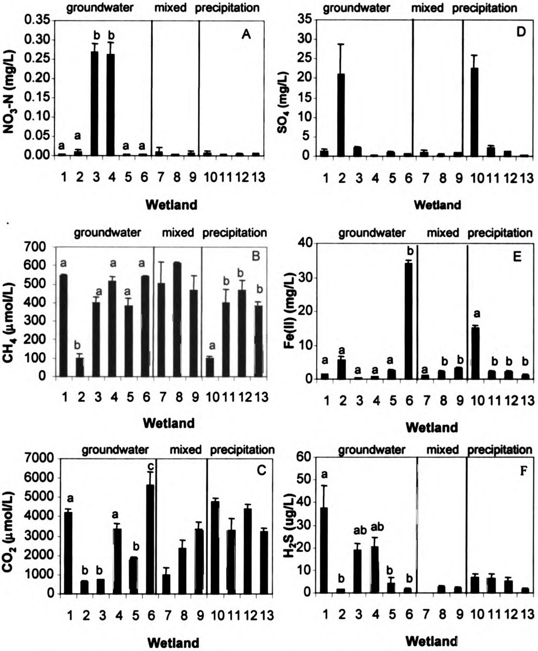


Figure 19: Average porewater concentrations (standard error) of wetlands with different source waters. Data are from 1999. There were no differences between water source categories for any solute. Sites corresponding to wetland numbers are listed in Table 2. Lower case letters indicate wetlands within water source categories that are significantly different at $p<0.05$.

Variability of wetlands within water source categories

Nitrate, Fe(II) and sulfate concentrations were generally low in groundwater-fed wetlands, although for each solute there was one or two wetlands with unusually high concentrations (Figure 19 A, D, E). Methane concentrations were consistent among groundwater-fed wetlands except for the Floodplain, which had mean methane concentrations about 4 times lower than the other wetlands ($p=0.002$) (Figure 19B).

Precipitation-fed wetlands had similar average concentrations for most solutes (Figure 19), with one exception for methane, Fe(II), and sulfate. The LTER Kettle Pond had about 3 times less methane ($p=0.005$) and about 3 times more iron ($p=0.002$) than the other precipitation-fed wetlands (Figure 19 B, E). The LTER Kettle Pond also had more sulfate than the other precipitation-fed wetlands, but this difference is not statistically significant ($p=0.20$) (Figure 19D).

Discussion

Variability within vertical profiles

Vertical patterns in porewater chemistry were inconsistent for each solute, except CH_4. For many of the wetlands sampled in 1999, there were no differences between the shallow and deeper zones for any solute, and there was no difference in sulfate concentration between the 2-20 and 20-40 cm zone for any wetland. The vertical profiles suggest that the processes controlling concentrations of electron acceptors and final products did sometimes show a marked vertical zonation in the uppermost samples (Figure 9), but when lumped into depth zones of 0-20 and 20-40 cm and tested for statistical significance this variation was obscured. For example, the Floodplain had high sulfate concentrations and low methane concentrations near the surface of the sediments. Around 10 cm below the sediment surface, methane concentrations were increasing and sulfate concentrations are decreasing. This is the pattern expected based on thermodynamincs. A similar pattern was found in Mud Lake at Prairieville Creek. In this wetland, H_2S increases as CH_4 decreases. Nevertheless, the relative uniformity of these vertical profiles is surprising. There are two explanations for this. One is that the same processes occur over the entire profile. Another explanation is that the water entering the wetland has been transformed upstream of the sampling location or antecedent to the sampling, and the resultant changes in chemistry have been transported downstream in the flow path or persisted over time.

In wetlands where differences between depth zones were found, some stratification of biogeochemical processes can be inferred. For instance, Prairieville Creek had differences between 2-20 and 20-40 cm for concentrations of Fe(II), H_2S, CH_4, and CO_2 (Figures 4-8). In most instances the 2-20 cm depth range had higher concentrations than the 20-40 cm range, except for H_2S. Fe(II) concentrations were fairly low (only about 0.2 mg L^{-1} difference between the two zones). However, it is possible that iron reduction was occurring in the upper sediments, inhibiting sulfate reduction, while sulfate reduction was occurring in the lower sediments were H_2S is higher. This would support the hypotheses that distinct zones of activity were present. It is interesting that methane was also highest from 2-20 cm. Similar patterns were also seen in Turkey Marsh.

It is possible that the vertical stratification in biogeochemical processes was often not detected due to the coarse resolution of the equilibrators. The 2.5 cm diameter wells integrated concentrations over a large vertical profile, considering that the uppermost cm of the sediment are considered the most biologically active portion of the sediment profile (Lovley and Klug 1986, Lovley and Phillips 1986). It has been suggested that finer resolution sampling would be needed to detect distinct zones in surficial sediment layers (Urban et al. 1997).

Horizontal spatial variability within wetlands

Concentrations of electron acceptors and final products were heterogeneous within wetlands in most cases. This was not unexpected (Hunt et al. 1997). The heterogeneity seen in this study could depend on the variation in groundwater inputs and outputs, the physical structure of the wetland, the inputs of carbon in relation to plant growth, and the sampling scheme within this matrix of complexity.

Fe(II) is a final product of iron reduction, but much of this may remain in association with particulate matter, with perhaps only 1-5% of the Fe(II) accumulating in solution (Lovley 1991, Chapelle 1993). Since Fe(II) is relatively soluble in water, this fraction can move in response to diffusion gradients or flowing water. In wetlands that have groundwater flow, the Fe(II) could be transported downstream, which could influence the measurement I made. Fe(II) that is bound in iron-rich clays, iron sulfides such as pyrite, and iron(II) oxides such as magnetite can be removed from cycling until aerobic conditions are present. This could be the case in precipitation-fed wetlands were seasonal drying occurred, and may be the reason why the LTER Kettle Pond had such high iron concentrations compared to the other wetlands. It has a mineral-clay sediment that completely dried the summer before the equilibrators were deployed, which could have oxidized Fe(II) to Fe(III), which was subsequently available for reduction when anaerobic conditions returned upon later flooding of the dried sediments.

The patterns expected for sulfur are similar to the expected Fe(II) patterns. It was common in groundwater with a range of 15-25 mg L^{-1} in this area, while precipitation had an average of 2.72 mg L^{-1} (NADP/NTN volume-weighted mean for 1979-1996 from KBS station MI26). So in groundwater-fed wetlands there might be more sulfate in areas where groundwater enters the wetland. Turkey Marsh did have higher concentrations of sulfate at the site close to the inflow (site 1), but this pattern did not hold in Loosestrife Pond or the Floodplain, where inflows were also apparent. In Loosestrife Pond the concentrations were low in the porewater at both sites. This could be due to sulfate reduction that occurred before it reached the wetland. The Floodplain did not have a direct flow path, and the exact direction of flow was not measured, though site 3 was close to the outflow and did have the lowest sulfate concentrations. The high concentrations in the surface waters of Turkey Marsh and Loosestrife Pond could be from the oxidation of hydrogen sulfide to sulfate as it was exposed to the aerobic water column, and subsequent evapoconcentration of sulfate by root transpiration in nearby emergent plant stands.

Since precipitation is low in sulfate, high concentrations of sulfate were not expected in precipitation-fed wetlands, and generally were not observed. The LTER Kettle Pond was unique since high concentrations of sulfate were found at depth. Since magnesium can be used as a tracer of groundwater inputs (Stauffer 1985), magnesium concentrations should be high if the sulfate was from groundwater. This was not the case since Mg^{2+} was below 3 mg L^{-1} when the porewaters displayed elevated SO_4^{2-} concentrations (Figure 20). Another possible explanation could be the lack of labile carbon at that depth combined with the severe drying that occurred during 1999, and a clay layer that would retard the movement of water. Since the sediments dried to the point of cracking in that wetland, sulfide as well as Fe(II) compounds could have oxidized. Upon rewetting the iron would be available for microbial reduction, preventing sulfate reduction. Carbon tends to become more refractory with depth in the sediments (Nedwell 1984), limiting anaerobic processes. Since the sediment was clay, the movement of water would be slow, preventing the sulfate from diffusing into the

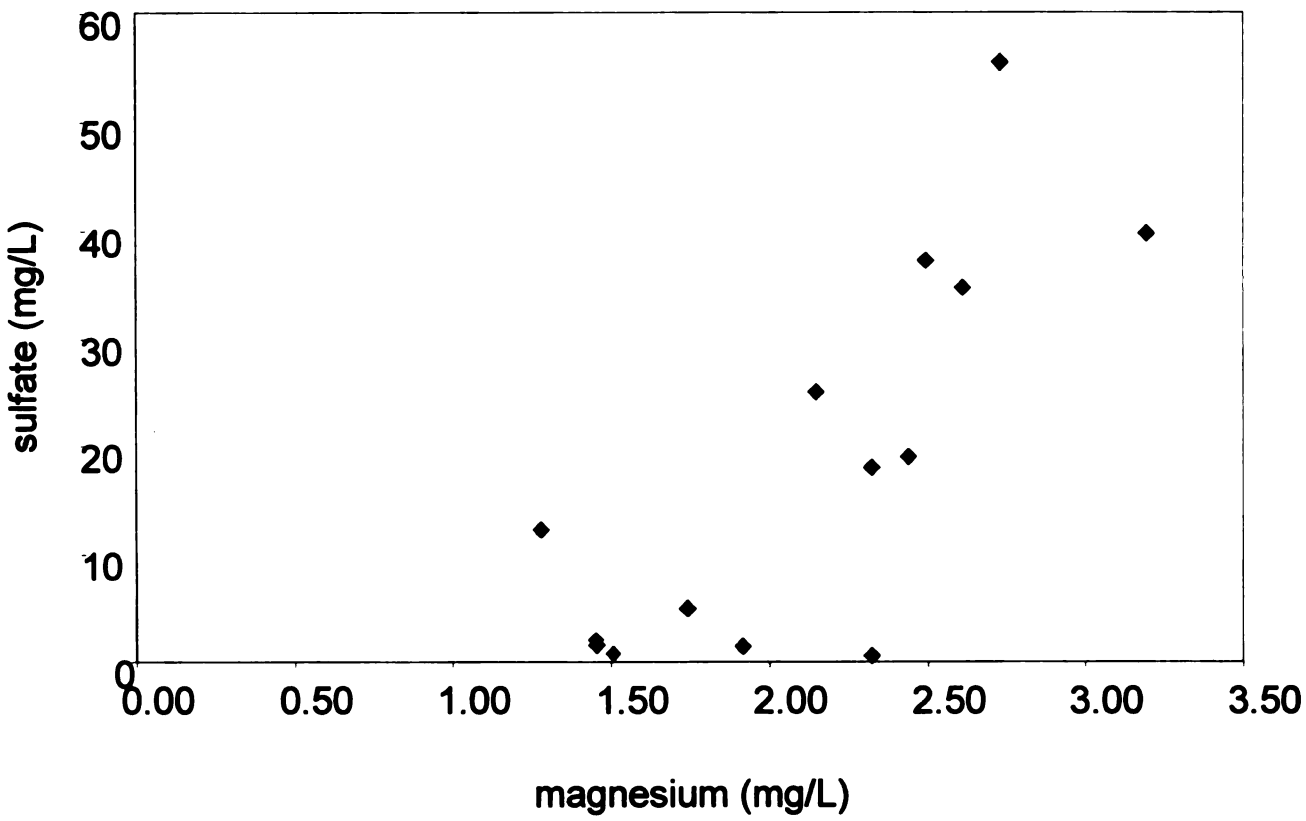


Figure 20: Magnesium and sulfate porewater concentrations from 20-40 cm depth in the LTER Kettle Pond.

shallower sediments and labile carbon from moving down. If carbon is limiting then sulfate reduction might not occur.

Hydrogen sulfide was detected in all wetlands, indicating that sulfate reduction had taken place. However, it is difficult to determine when sulfate reduction took place, and whether it took place at the sampling points or somewhere upstream in the flow path. Since H_2S reacts with Fe(II) to form insoluble ferrous sulfides, it is likely that the concentrations measured would underestimate the amount of sulfate reduction that occurred. If H_2S does not precipitate, it can be transported by advection and diffusion and can be lost to the overlying water and the atmosphere. Since H_2S was detected in all precipitation-fed wetlands, sulfate reduction must have occurred at some point in those wetlands.

Like Fe(II) and H_2S, CH_4 can also be lost away from the site of production by 1) diffusion across the sediment surface into the water column, 2) bubbling to the surface by a process called ebullition, or 3) being transported through the vascular tissues of plants (Schutz et al. 1991). Methane concentrations were at or near solubility in many of the wetlands, indicating that concentrations had reached an upper limit dictated by temperature (Figure 10). As concentrations reach their limit of solubility, further methane production would result in bubble formation and increased loss by ebullition, and this is common especially where emergent plants are sparse or absent (Chanton et al. 1989). This is likely the case in organic-rich sediments like Loosestrife Pond and Turkey Marsh. At the

LTER Kettle Pond, Floodplain, and Shaw Pond, methane concentrations were below solubility (Figure 10), but spatial variability was still important. Dissolved methane that was produced in one area could diffuse away according to concentration gradients through the sediment –water interface. Increasing temperature decreases the solubility of gases and increases the diffusion as well. Thus, CH_4 could diffuse into 0-20 cm from deeper sediments. In vegetated areas, like the Floodplain and Shaw Pond, plants transport methane through their aerenchyma tissue (Dacey and Klug 1979) and the amount of methane that is transported depends of the type of plant (Sebacher et al. 1985). Emergent plants effectively ventilate the sediments by facilitating gas exchange with the atmosphere. This could result in the lower CH_4 concentrations seen in some of these wetlands.

Interannual Variation within Wetlands

Interannual variation was detected in the porewater profiles, but it depended on the wetland and the solute. Like the spatial variation seen within a wetland, it was not surprising that there was some variation from year to year, especially considering the hydrologic changes that took place in the particular years I sampled. During drier years the biogeochemical conditions in wetlands may be affected in various ways, including oxidation of exposed sediments, reduced water flow through wetlands, and dramatic changes in the plant communities (e.g., expansion of emergent plant stands). As discussed above, exposure of sediments to air would oxidize the reduced solutes Fe(II), H_2S, and CH_4. Sulfate was one potential product of sediment oxidation that I measured, and I did observe increases in some sites that appeared to follow sediment oxidation. Turkey Marsh, the Floodplain and Shaw Pond show that this could be occurring (Figure 9).

Some of the interannual differences in the profiles of hydrogen sulfide were very apparent, like in the Floodplain, while others had large changes in concentrations between years. For example, Lux10 had low H_2S concentrations in 1999 and 2001, but they were high in 2000 (Figure 17). 1999 was a dry year and even though Lux10 did not dry completely, there was the potential for hydrogen sulfide to be oxidized to sulfate. This sulfate could then be reduced during 2000. Both Turkey Marsh and Shaw Pond show this trend.

In the case of Fe(II) concentrations it is interesting to note that none of the precipitation-fed wetlands varied among years (Figure 15). Thus, even though Fe(II) can presumably be oxidized during drier years, potentially stimulating iron reduction in wetter years, the same amount of Fe(II) was present over the porewater profile. Since sampling was done only once each growing season, this hypothesized cycle of iron oxidation and reduction could have been missed.

Variability between wetlands with different water sources and among wetlands with the same water source

It was expected that wetlands with contrasting water sources (groundwater vs. precipitation) would show more differences in porewater biogeochemistry due to differences in the chemistry of groundwater and precipitation; however, there were no statistical differences detected when hydrologic categories were compared (Figure 19). Yet, there are some interesting trends. For instance, nitrate was well above detection limits only in groundwater-fed systems. Among these groundwater wetlands, Prairieville Creek and Mud Lake at Prairieville Creek had the highest porewater concentrations of NO_3-N seen in this study. So groundwater-fed wetlands may be more likely to have significant nitrate concentrations in the porewaters. Two sites had elevated sulfate concentrations: the groundwater-fed wetland the Floodplain and the precipitation-fed LTER Kettle Pond, as was discussed above. The Floodplain had high concentrations in the porewaters close to the sediment surface as a result of H_2S being oxidized. The LTER Kettle Pond had high sulfate from 20-40 cm. The highest concentrations of hydrogen sulfide were found among the groundwater-fed wetlands, which varied from wetland to wetland. This makes sense since sulfate concentrations were high in groundwater springs and subsequent reduction could result in an accumulation of H_2S. Even though H_2S was high in groundwater-fed wetlands, precipitation-fed wetlands also had measurable concentrations. Thus, sulfate reduction occurred at these sites even though they receive relatively little sulfate compared to groundwater-fed wetlands.

Fe(II) concentrations were variable. Again, the LTER Kettle Pond had high concentrations, perhaps as a result of complete desiccation the year prior to sampling. Sherriff's Marsh also had high levels of Fe(II), and it is the most extensive wetland I studied. This probably resulted from iron being reduced in the extensive wetland sediments upstream of the sampling point, with no possibility of reoxidation (Chapelle 1993). Thus, Fe(II) may have accumulated in the water as it was transported across the wetland.

Even though methane concentrations were similar in all types of wetlands, the precipitation-fed LTER Kettle Pond and the groundwater-fed Floodplain had lower concentrations than the other wetlands within the same water source category. Both had high Fe(II) and low methane, a potential result of iron reducers outcompeting the methanogens.

To try to understand the relationships between the concentrations on the solutes, pairwise Pearson correlations were performed on the entire data set, using every measurement from 1999-2001. There were few statistically significant results (Table 4). Fe(II) and CO_2 were correlated, suggesting that the iron reduction might be responsible for the high CO_2 concentrations. SO_4 and CH_4, and Fe(II) and H_2S were negatively correlated, supporting the idea of zonation of microbial activity in anaerobic environments. However, the interpretation may be confounded for most of these solutes because 1) several had the potential to be

Table 4: Pairwise Pearson correlation coefficients of all measurements for select porewater chemistry of all wetlands in all years. Values are shown only when significant at $P<0.05$.

	sediment temperature	**conductance**	**NO_3-N**	**SO_4**	**CH_4**	**CO_2**	**Fe(II)**	**H_2S**	**pH**
sediment temperature	1.00								
conductance	NS	1.00							
NO_3-N	NS	NS	1.00						
SO_4	NS	NS	NS	1.00					
CH_4	0.44	NS	NS	-0.33	1.00				
CO_2	NS	NS	NS	NS	NS	1.00			
Fe(II)	NS	NS	NS	0.18	NS	0.50	1.00		
H_2S	NS	NS	NS	NS	NS	-0.19	-0.22	1.00	
pH	NS	0.60	NS	NS	0.66	-0.52	NS	NS	1.00

removed from the porewater by co-precipitation, like Fe(II) and H_2S, 2) degassing can cause the loss of CH_4, CO_2 and H_2S, and 3) concentrations may reflect accumulation from earlier transformations that occurred at the sampling site or upstream of the sampling site.

Conclusion

Wetland porewaters were generally depleted in nitrate and sulfate relative to source waters, suggesting uptake of these ions. The presence of sulfide in porewaters confirms that sulfate reduction occurs at most sites. Methane occurs at concentrations close to its solubility limits in the sediments of most wetlands, indicating that methanogenesis is ubiquitous and likely leads to formation of gas bubbles, creating the potential for ebullition.

Spatial and temporal variability in porewater biogeochemistry is large, and makes conclusions about patterns among wetlands difficult. Porewater chemistry shows little relationship to water source, suggesting that the relative contributions of groundwater and precipitation are not the most important controls on the anaerobic metabolism in these environments, although this conclusion is subject to a number of caveats involving the use of concentrations to infer processes. Later chapters will present more direct evidence regarding anaerobic processes in these wetlands, and that evidence will be compared with the porewater chemistry reported here.

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CHAPTER 3

DIRECT ASSAYS OF RATES OF ANAEROBIC MICROBIAL METABOLISM IN WETLANDS

Introduction

Wetlands are increasingly appreciated as ecosystems with exceptional ecological and economic value (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). Most organic matter produced in wetlands is ultimately degraded by microorganisms, resulting in the release of nutrients that are recycled within the plant community (Wetzel 2001). Anaerobic microbial processes play a central role in the biogeochemical functions of wetlands because water-saturated soils and sediments are generally depleted in oxygen due to high rates of consumption that are not balanced by inputs because of the low solubility and slow diffusion of oxygen in water. Under anaerobic conditions, the terminal processes in organic matter catabolism are particularly important to biogeochemical functions because the processes are coupled with transformations of inorganic compounds, or because they produce methane. Denitrification is a particularly important terminal anaerobic microbial process that contributes to the improvement of water quality by removing excess nitrate (Johnston 1991, Groffman et al. 1996, McLatchey and Reddy 1998). Methanogenesis in wetland environments is also a critical biogeochemical process; natural wetlands contribute up to 50% 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), including hydrology, temperature, pH, labile carbon availability (Conrad 1996, Segers 1998), and availability of alternate 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.

Thermodynamic constraints on microbial metabolism determine the competitive ability of the microorganisms that perform the terminal step in organic matter decomposition, which can occur via either anaerobic respiration or methanogenesis. The thermodynamic constraints are based on the free energy yield of oxidation-reduction (redox) reactions in which an electron donor, such as hydrogen or a low-molecular-weight organic compound, is oxidized and an inorganic electron acceptor, such as nitrate or sulfate, is reduced. Microorganisms harness the free energy released by the redox reaction for maintenance and growth (Conrad 1996, Fenchel et al. 1998). 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 reduction of the terminal electron acceptors nitrate (NO_3^-), manganese (Mn(IV)), iron (Fe(III)), and sulfate (SO_4^{-2}), occurring in decreasing order of free energy yield. Methanogenesis is the least energetically favorable anaerobic microbial process, and occurs after the inorganic alternate electron acceptors have been depleted.

These terminal processes depend on the activity of fermenting bacteria for the supply of substrates, such as acetate and H_2 (Fenchel et al. 1998). Fermentation is an anaerobic process which hydrolyzes polymers into lower molecular weight substrates that, in turn, serve as intermediary compounds fueling terminal anaerobic respiration and methanogenesis. Hydrogen is used in terminal metabolism by bacteria to reduce inorganic electron acceptors like iron or sulfate, and provides thermodynamic control of bacterially-mediated anaerobic respiration (Hoehler et al. 1998). Acetate is considered an important intermediary compound in anaerobic decomposition and potentially the most important carbon source available to anaerobic microorganisms (Lovley and Klug 1982). 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). Acetate turnover is a minimum estimate of the overall rate of anaerobic metabolism because other organic substrates can be important. In addition, much of the degradation of complex organic substrates occurs in fermentative pathways that precede the production of acetate as an intermediary product in anaerobic metabolism.

Differences in the thermodynamic efficiencies of microbially-mediated reactions can be used to predict patterns of anaerobic microbial metabolism in different ecosystems (Lovley and Phillips 1987, Chapelle and Lovley 1992, Hedin et al. 1998, D'Angelo and Reddy 1999). Since anaerobic microbial processes in wetlands are regulated by the availability of terminal electron acceptors, spatial gradients commonly develop in which the sequential replacement of the predominant microbial process occurs with depth or with distance from a hydrological input (Schlesinger 1997). For example, along a small groundwater-fed stream in southwestern Michigan, Hedin et al. (1998) found that denitrification occurred closest to the emergence of groundwater, followed by sulfate reduction and ultimately methanogenesis. Similar gradients could also occur over time after flooding of wetland soils (Ponnamperuma 1972, D'Angelo and Reddy 1999).

Most previous studies of these anaerobic microbial processes have focused on the sediments of lakes, coastal marshes 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, and comparative studies are uncommon (cf. Groffman et al. 1996, D'Angelo and Reddy 1999). Groffman et al. (1996) compared microbial biomass and activity in four different wetland types in eastern New York, finding few differences among the wetland types although there was often considerable variation within wetland types. Hydrology was a key determinant of the wetland types, but was not the focus of that study. In another study where multiple wetlands were examined (D'Angelo and Reddy 1999), 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 later attained upon rewetting in the laboratory. Water source was not evaluated in that study, but there were implications that water source could have been an important control on anaerobic respiration and methanogenesis.

This study will examine differences in 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. I hypothesized that groundwater-fed wetlands will have higher rates of anaerobic decomposition (particularly denitrification and sulfate reduction), as a result of the continuous inputs of the electron acceptors, nitrate and sulfate. Wetlands in which direct precipitation inputs are relatively important were predicted to have higher rates of methanogenesis due to the limited supply, episodic inputs, and quick depletion of alternate electron acceptors found in the comparatively dilute precipitation inputs.

Methods

Study sites

This work is part of a broader research program that examines the hydrological, biogeochemical and ecological diversity of wetlands in southern Michigan. The study area is the glacial landscape around the Kellogg Biological Station (KBS), an academic unit of Michigan State University located in southwest Michigan. This study was conducted in six wetlands; three of which are mainly groundwater-fed wetlands (Loosestrife Pond (G1), Kalamazoo River Floodplain (G2), Turkey Marsh (G3)) and the three others are mainly precipitation-fed (Shaw Pond (P1), Lux10 (P2), LTER Kettle Pond (P3)). Select wetland characteristics are in Table 1 and detailed site descriptions can be found in Chapter 2. Water source was ascertained by calculating the fraction of groundwater in each wetland using magnesium (Mg^{+2}) as a groundwater tracer (Stauffer 1985). Dissolved Mg^{+2} is a good indicator of groundwater inputs because it is little affected by biological uptake, is not subject to mineral precipitation reactions, and is a major weathering product arising from dissolution of dolomite in the underlying glacial till in this area but its concentrations are negligible in precipitation.

Sediment Sampling

The six wetlands were sampled during the summer of 2000 and 2001, with four wetlands sampled in each year. Wetlands G3 and P2 were sampled during both years to assess interannual differences in rate measurements. Within the wetted 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 based on surface water flow. Sites with little or no emergent plant growth were chosen but in most cases plants occurred within one to a few meters. Not all the sites within each wetland were sampled for each process rate measurement (Table 1).

Soil cores for each process measurement were taken by hand with an acrylic core tube (7.6 cm diameter) at the established sites. Subcores were collected from 1 cm holes along the length of the core tube from 0-10 cm depth using cut-off plastic syringes. The subcores were capped with butyl rubber stoppers and immediately transported to the lab submerged in jars with deoxygenated water on ice in a dark cooler. The subcores were extruded into in pre-weighed glass vials. Total sediment volume collected and vial type used for each assay were as follows: denitrification, 30 mL in 0.5-L glass jars; iron reduction, 6 mL in 15-mL glass serum vials; sulfate reduction and acetate turnover, 3 mL in 25-mL glass scintillation vials; methanogenesis, 3 mL in 36-mL glass serum vials. Each vial was thoroughly flushed with O_2-free N_2 to ensure anoxic conditions in the headspace over the sediment subsamples. The samples were incubated at 24^0C.

Table 1: Sampling dates for each wetland and number of sites sampled for the measurement within the wetland. The numbers in parentheses in G3 and P2 are the number of sites sampled in 2001 if it was different from 2000. All of these measurements were made between May –September.

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

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. 4-mL gas samples were taken from the headspace at 10, 30, and 60 minutes and transferred to 2-mL gas vials to yield a pressure inside the vial of 2 atm. Samples were stored no more than 1 month, and then analyzed on a Hewlett Packard gas chromatograph containing a Porapak-Q® packed column and a electron capture detector. Storage tests have indicated the stability of N_2O under these conditions (G.P. Robertson, pers. comm.).

To calculate denitrification rates, the total concentration of N_2O in the jar was determined using Bunsen solubility coefficients to calculate the amount of N_2O in the slurry from the N_2O concentration in the headspace. The rate was determined from the change in the total N_2O over time, divided by the volume of the soil and the incubation time (Groffman et al. 1999).

Denitrification Enzyme Activity Potential

This method measures the potential of the sediment to denitrify by removing nitrogen and carbon limitations for the bacteria. This assay was done once during the summer of 2001 at wetlands G3 and P2. The denitrification assay of

Groffman et al. (1999) was used with the exception of only adding 0.05 mM of potassium nitrate to each jar. 4-mL gas samples were taken from the headspace at 10, 30, and 60 minutes and transferred to 2-mL gas vials. Samples were stored no more than 1 month, and then analyzed on a Hewlett Packard gas chromatograph containing a Porapak-Q® packed column and a electron capture detector.

Iron Reduction

Iron reduction rates were determined in wetland sediment cores by measuring the accumulation of dissolved Fe(II) (Lovley and Phillips 1986a, Roden and Wetzel 1996). The vials were placed in the dark at 23°C and analyzed at 0, 3 and 10 days. There were 3 replicates for each site within the wetland.

Fe(II) accumulation was determined by measurement of Fe(II) before and after incubations of 0-10 days. Fe(II) was measured by adding 0.1 mL of the incubated sediment sample to a preweighed vial containing 5 mL of 0.5 N HCl and reweighing it. After 1 hour at room temperature, 0.1 mL of this mixture was added to 5 mL of ferrozine (1g/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 read on a spectrophotometer at 562 nm (Lovley and Phillips 1986a, Stookey 1970).

The depletion of poorly crystalline Fe(III) was also measured before and after incubation, to provide a check on the Fe(II)-based rates (Lovley 1991, Roden and Wetzel 1996). To determine Fe(III) depletion, another 0.1 mL subsample of the incubated sample was placed in a preweighed serum vial containing 5 mL of 0.25 M hydroxylamine hydrochloride in 0.25 M HCl and reweighed. After 1 hour at room temperature, 0.1 mL of this mixture was added to 5 mL of ferrozine (1g/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 read on a spectrophotometer at 562 nm. Microbially-reducible Fe(III) concentrations are equal to the amount of Fe(II) extracted in HCl minus the Fe(II) extracted in hydroxylamine hydrochloride (Lovley and Phillips 1987).

Sulfate Reduction

Subcores for assays of sulfate reduction rates were extruded into glass scintillation vials and capped with Teflon septa (22/400 TFE/silicon liners, Alltech P/N 95333) and screw caps (Alltech P/N 95331). After flushing with O_2-free N_2, 1μCi of $Na^{35}SO_4$ (American Radiolabeled Chemicals Inc. ARS-105 35S as sodium sulfate) was injected with a glass syringe into each vial and the samples were incubated for 0, 1.5 and 3 hours. Microbial activity was stopped by quick freezing the sediment slurry in liquid nitrogen. After acidifying the samples with 10 mL of O_2-free 4N HCl to release $H_2{}^{35}S$, the samples were processed on a non-metallic flushing train that consisted of a series of 3 vials containing 2.5% zinc acetate. Samples were flushed with helium for 30 minutes and the $H_2{}^{35}S$ gas was trapped in a set of 3 vials. There was generally no detectable $H_2{}^{35}S$ in the third vial. The traps were removed after flushing the sample. 5 mL of Safety Solve® scintillation cocktail was added to each vial, and the samples were counted on a Wallac scintillation counter. 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.

The rate of sulfate reduction (SR: μmol/mL-hr) was calculated as follows (Jorgensen 1978):

$$SR= ([SO_4{}^{-2}] * D * \alpha) / (A * T * C)$$

where $[SO_4{}^{-2}]$ is the moles of sulfate in the sample, D is the number of DPM's as $H_2{}^{35}S$, α is the isotopic fractionation factor, A is the volume of the sediment, T is the incubation time, and C is the DPM's of the added $^{35}SO_4{}^{-2}$.

Methanogenesis

Methane production rates were determined by measuring the change in the concentration of methane (CH_4) in the headspace of sediment slurries over time. Samples were prepared in serum vials as for 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 Shimadzu gas chromatograph containing a

Porapak-Q® packed column and a thermal conductivity detector. Carbon dioxide (CO_2) measurements were also made during this procedure.

To calculate methane production rates, the total concentration of CH_4 in the jar was determined using Bunsen solubility coefficients to calculate the amount of CH_4 in the dissolved phase from the CH_4 concentration in the headspace. The rate was determined from the change in the total CH_4 over time, divided by the volume of the soil and the incubation time.

Acetate Turnover

Determining acetate concentrations is an important step in calculating acetate turnover rates since it yields 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 AMP (King 1991):

$$\text{Acetate} + \text{ATP} + \text{CoA} \xrightarrow{\text{acetyl CoA synthase}} \text{acetyl CoA} + \text{AMP} + \text{PP}$$

Where ATP is adenosine triphosphate, CoA is conenzyme A, AMP is adenosine monophosphate, and PP is 2 phosphate molecules. Cores were taken at 2 sites within each wetland and subcored using 10-mL cut-off syringes. The subcored samples were capped and stored in anaerobic conditions for no more than 2 hours. The subcores were extruded into round bottom 50-mL polyethylene centrifuge tubes, capped and centrifuged at 12,000x g for 10 minutes at 4°C. The supernatant was removed with a sterile glass syringe and filtered into 15-mL polypropylene screw-cap centrifuge vials.

These samples were analyzed immediately by adding 10 μL of each of the following reagents to 1.0 mL of the supernatant: 20 U/mL acetyl CoA synthase (Sigma A1765), 10 mM CoA (Sigma C3144), 10 mM disodium ATP (Sigma A2383), and 200 μg/mL bovine serum albumin (BSA) (Sigma B4287). This gave each sample a final concentration of 100 μM for ATP and CoA. The vials were shaken at 37°C for 1 hour. The acetyl CoA synthase activity was terminated by immersion in a boiling water bath for 2 minutes. Sample blanks contained no CoA. Acetate standard concentrations were 2.5, 5, 10 and 20 μM.

The AMP produced from the enzyme assay was measured on an HPLC with a LC-18-T column (Supelco) and a Brownlee guard column cartridge (RP-18), operated at 1.0 mL/min using 0.1 M KH_2PO_4, at pH 6.0, as the mobile phase under isocratic conditions. An oven temperature of 35°C yielded the best separation of peaks. AMP was detected using a UV detector at an absorption of 260 nm. The sample loop was rinsed with the mobile phase between each injection.

3-mL replicate subcores were taken at the same sites no later than a week after the acetate concentrations were determined. The sediment subcores were extruded into scintillation vials, capped with Teflon stoppers, and flushed 6 times with O_2-free N_2 to ensure that the slurries were anaerobic. The slurries were labeled with 0.9 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 using a series of 3 scintillation vials containing 3 mL of ethanolamine plus 2 mL of methanol to trap CO_2. 1.5 mL of 2-M H_2SO_4 was injected into the slurry to convert all inorganic carbon to CO_2. The headspace of the slurry was bubbled with oxygen-free He to sparge $^{14}CH_4$ and $^{14}CO_2$ out of the slurry solution. The $^{14}CO_2$ was trapped in the first set of vials, while $^{14}CH_4$ passed 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 3 vials containing ethanolamine and methanol. 10 mL of scintillation cocktail was added prior to liquid scintillation counting. The system was flushed for 10 minutes between each sample (Lovley et al. 1982).

Correction for measurement problems in the radiolabeled acetate measurements:

During the summer of 2000, I encountered bioluminescence and discoloration problems with the combination of the $^{14}CO_2$ trapped in ethanolamine and the

Safety Solve® scintillation cocktail. It was determined that Hionic fluor® was a better scintillation cocktail, and it was used instead of Safety Solve® during 2001. To correct the radiocarbon measurements taken during summer 2000, radiolabeled bicarbonate was used as a standard. A 5-mCi ampoule of $Na^{14}CO_3$ was diluted to 20 μCi/L using a dilution solution (0.3 g $NaCO_3$ plus 1 pellet (~0.2 g) NaOH in 1L deionized water) (Strickland & Parsons 1968). A 0.1μCi aliquot was added to each test vial for a total of 5 mL per vial. I added 1 mL of 20% HCl to the vials to release $^{14}CO_2$ which was sparged out of the solution for 7 minutes with helium, and the $^{14}CO_2$ was trapped by passing the gas stream through 3 mL ethanolamine plus 2 mL methanol. Either 10 mL of Safety Solve® or Hionic fluor® scintillation cocktail was added to the vial and the vials counted by liquid scintillation after 24 hours incubation in the dark. Counts from the Safety Solve® cocktail were compared against those using Hionic fluor cocktail to get a conversion factor. I multiplied all measurements from summer 2000 by this conversion factor.

Statistical Analysis

Anaerobic microbial decomposition rates were analyzed using analysis of variance (ANOVA) in SYSTAT (SPSS Inc, 1998). I analyzed G3 and P2 first, to determine if the anaerobic decomposition rates were consistent across years. If there were no interactions, then all the wetlands were analyzed together regardless of year. Only sulfate reduction had significant wetland by date interactions and so separate analyses were run for each year. Nested ANOVAs were used to test for differences between wetlands of different water source.

Results

Denitrification rates were not detected in any wetland in the absence of NO_3^- amendments; however, when nitrate was added to the slurries of 2 of the wetlands, denitrification enzyme activity increased, and later experimental NO_3^- additions in the field showed rapid uptake (Chapter 4). There was a higher potential denitrification rate in the groundwater-fed wetland than in the precipitation-fed wetland when the nitrate limitation was removed ($p<0.05$, Figure 1), although measurements were made in only one wetland of each catagory.

Iron reduction was measurable in all of the precipitation-fed wetlands and in one groundwater-fed wetland (G3) (Figure 2A). Even though the mean rates in 2 of the 3 precipitation-fed wetlands were higher than in the groundwater-fed wetland, 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 0.098 μmol SO_4^{-2} /mL*hr (Figure 2B). There were differences in sulfate reduction in the groundwater-fed wetlands, with wetland G1 having sulfate reduction rates nearly 3 times higher than the other groundwater wetlands.

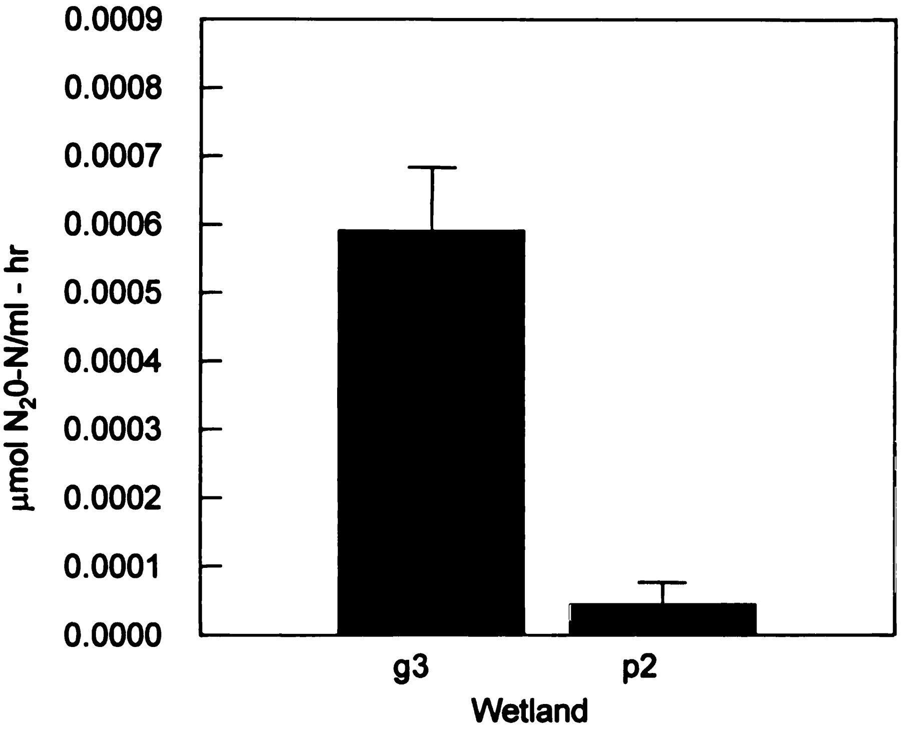

Figure 1: Denitrification potential of one groundwater and one precipitation-fed wetland. The bars represent three replicates from three sites within the wetland, with standard deviations.

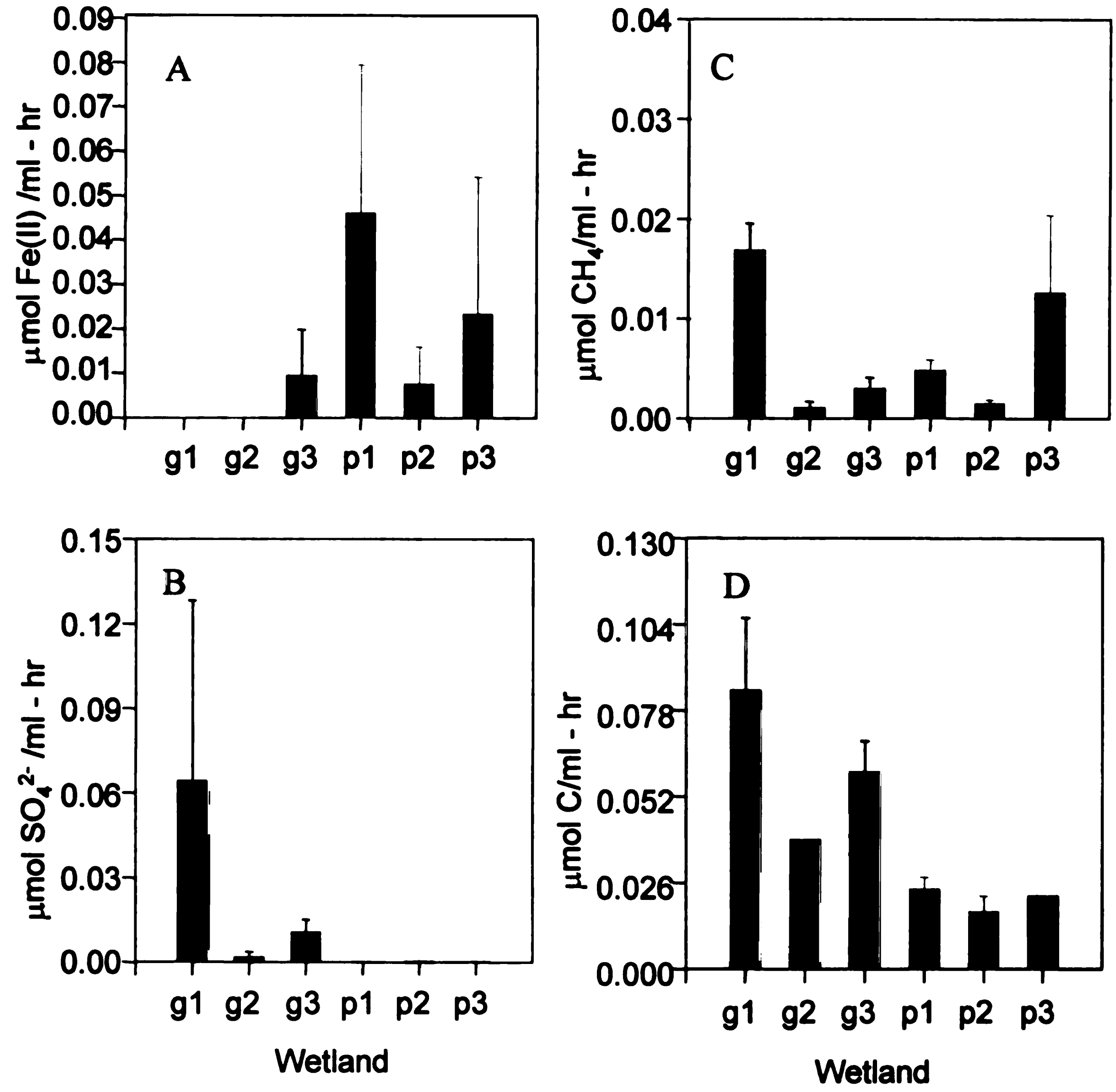

Figure 2: Iron reduction (A), sulfate reduction (B), methanogenesis (C) and acetate turnover (D) rates for 6 wetlands, 3 of which were mainly groundwater-fed (g) and 3 were mainly precipitation fed (p). Wetlands without bars had rates below the limit of detection. Bars show means (with standard deviation) of sites within the wetland, unless error bars are missing because there was no replication.

Table 2: Selected physical and chemical characteristics of wetland porewater from 0-10 cm. Each value represents the mean of 3 replicates ± one standard deviation. n.d. = not detectable.

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)

Despite the observation that sulfate reduction rates were variable in the groundwater-fed wetlands, there was no statistical difference in sulfate concentrations in the porewaters of these groundwater-fed wetlands (Table 2, $p=0.24$).

Methanogenesis was the only anaerobic metabolic process detected in all 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 differences across 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). 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 between wetlands with the same water source ($p=0.21$).

Each process rate was converted to the equivalent rate of organic carbon mineralization based on the stoichiometry of the oxidation-reduction reactions as well as methanogenesis (Froelich et al. 1979, Zehnder and Stumm 1988). In the case of methanogenesis, I assumed equimolar production of CH_4 and CO_2 in these freshwater sediments (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, sulfate reduction is the most important process in groundwater-fed sites and methanogenesis is 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 20-40% 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 (Table 3). Even though organic carbon is often a limiting resource to microorganisms, my measures of particulate organic carbon and porewater DOC did not correlate with any rate measurements, except iron reduction. This is not surprising since total organic carbon has been found to be composed mostly of material that is resistant to anaerobic microbial decomposition, while the soluble available carbon in pore water is greatest in the surface layers (0-5-cm), close to the sources of new organic matter (Nedwell 1984).

Discussion

Anaerobic microbial metabolism was variable among all wetlands, including within wetland water-source categories, but some patterns do emerge from the

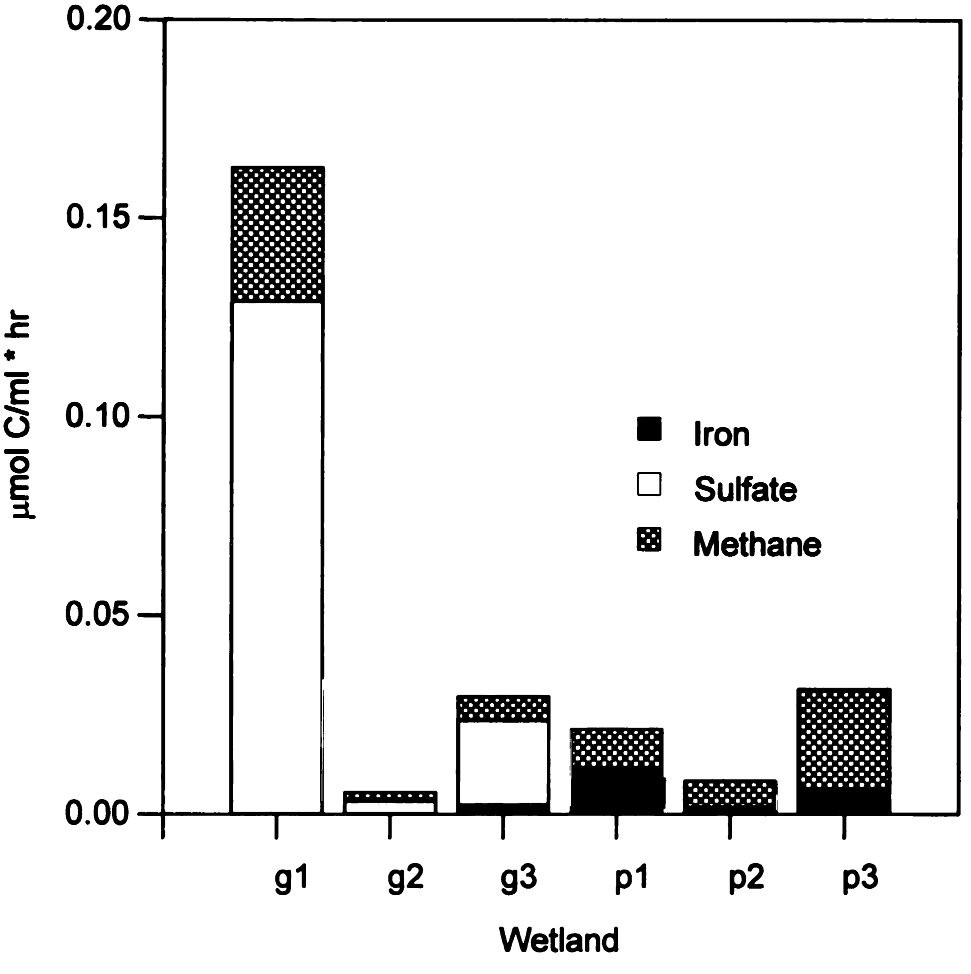

Figure 3: Carbon mineralization rates based on individual rate assays for 3 mainly groundwater-fed (g) and 3 mainly precipitation-fed (p) wetlands.

Table 3: Correlations of anaerobic microbial metabolism with significant p-values in parentheses.

	Iron Reduction	**Sulfate Reduction**	**Methanogenesis**	**Acetate Turnover**
porewater DOC	0.928 (0.072)	NS	NS	NS
% organic carbon	NS	NS	NS	NS
Bulk Density	0.454 (0.039)	NS	NS	NS
Pore-water Conductance	NS	NS	NS	NS
Pore-water pH	-0.413 (0.062)	NS	NS	0.649 (0.012)

data reported here. Groundwater-fed wetlands had higher rates of anaerobic microbial decomposition than precipitation-fed wetlands (Figures 2 and 3). This difference seems to be due to sulfate reduction, since methane production was not different between wetlands with different water sources and iron reduction was not important in the groundwater-fed wetlands.

Even though denitrification was not detected, it is an important wetland biogeochemical process because it results in permanent removal of nitrate, which is a valuable function to maintain good water quality (Johnston 1991, Duncan and Groffman 1994, Groffman and Hanson 1997). The potential for denitrification was present in the wetlands based on denitrification potentials from the acetylene block experiment (Figure 1) and rapid nitrate uptake observed in the push-pull experiments (Chapter 4). This indicates that nitrate was a limiting denitrification in these wetlands, as opposed to labile organic matter. The groundwater in southwest Michigan is known to be high in nitrate (2.22 ± 4.0 mg NO_3^- -N/L from seeps and springs collected between 1999-2000; Hamilton, unpublished data), so it is surprising that denitrification was not detected. Denitrification rates might not have been detected for a number of reasons. For example, nitrate is an important plant nutrient, so it could be removed by plant uptake. Groffman et al. (1992) found low levels of denitrification enzyme activity and microbial biomass during the growing season in a riparian wetland, suggesting that plant uptake was the dominant NO_3^- sink. Another possibility is that incoming nitrate could be rapidly depleted by denitrification along the edge of the wetland. At the soil-stream interface, Hedin et al. (1998) found that a narrow near-stream region was the most important location for NO_3^- removal by denitrification. In another study Tobias et al. (2001) found that almost 90% of nitrate was removed within 50 cm of the wetland-upland boundary in an estuarine wetland. If such rapid removal occurs in my study sites as well, my sampling points may have been too far from the initial contact of incoming groundwater with the wetland sediments, which is generally near the edges of water bodies, and my sampling dates generally did not follow major precipitation inputs. Thus, denitrification could be a more important process in these six wetlands than my measurements indicate.

Based on thermodynamics, after nitrate is depleted, iron reducers can outcompete sulfate reducers and methanogens for carbon substrates (Lovley 1991). Roden and Wetzel (1996) found that iron reduction could mediate a large amount of organic carbon oxidation and suppress CH_4 production in freshwater wetlands found in areas with iron-rich soils. This was due to the regeneration of Fe(III) via oxidation of Fe(II) compounds coupled to O_2 input from the overlying water column and via the plant rhizosphere. Thus, while iron reduction could be an important anaerobic process for iron transformation and mobilization, iron reduction does not seem as important in terms of the amount of carbon mineralized in these particular wetlands. Iron reduction was measurable in all three of the precipitation-fed wetlands, and only in the groundwater wetland G3. These rates are low but within the range found in other wetland systems (Table

Table 4: Summary of Rate Measurements of Iron Reduction

environment	**location**	**depth (cm)**	**rate (nmol cm^{-3} d^{-1})**	**reference**
freshwater wetland	SE US	0-1	3852 (880)	Roden & Wetzel 1996
freshwater wetlands	SE Michigan	0-10	0 – 110.7 (105)	This study
environment		depth	rate (nmol $g_{wetwt}^{-1}d^{-1}$)	reference
coastal lagoon	Denmark	0-5	2880	Sorensen 1982
river, estuary	Maryland	surface	71.3 (126.7)	Lovley & Phillips 1986

4). Wetlands G3, P1 and P3 all dried during the study, with wetland P3 dried to the point were the surface of the sediment cracked, allowing oxidation of the wetland sediments. Iron reduction could be more prevalent in the precipitation-fed sites due to occasional drying which allows Fe(II) compounds to be oxidized back to Fe(III) (Lovley 1991). Later inundation of the wetland returns the sediments to anaerobic conditions and the Fe(III) reducers are able to use the Fe(III) for anaerobic respiration. Examining the wet-dry cycles of a wetland could be important in determining the importance of iron reduction. This is also important because it could initially divert carbon sources away from methanogens, potentially reducing the amount of methane ultimately emitted to the atmosphere. 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, which was interpreted as being due to iron reducers out-competing methanogens (Kruger et al. 2001). Similar results were seen when rice microcosms were fertilized with ferric iron (Jackel and Schnell 2000). However, Bond and Lovley (2002) recently reported that methanogens can indirectly cause the reduction of Fe(III) in the presence of quinone compounds, calling into question the interpretation that Fe(III) suppression of methanogenesis occurs via competition with specialized Fe reducers.

Sulfate reduction was only measurable in groundwater-fed wetlands, probably because sulfate was limiting in precipitation-fed wetlands (Chapter 4). The rates measured in these wetlands are lower than those reported in coastal systems, but comparable to sulfate reduction rates in other freshwater wetlands (Table 5). The mean sulfate concentration from 22 springs and seeps around KBS during the study period was 29.9 ± 18.0 mg/L, compared to 1.94 mg/L found in precipitation during the same time (NADP/NTN). Sulfate concentrations in the porewaters of the wetlands were lower than in source waters. Precipitation-fed wetlands had the lowest porewater concentrations, ranging between 0.7 to 1.9 mg SO_4^{-2}/L. This was lower than the range of porewater concentrations found in groundwater-fed wetlands (2.0-7.1 mg SO_4^{-2}/L). Within the groundwater wetlands, wetland G1 had very high rates of sulfate reduction compared to G2 and G3. One explanation is that wetland G1 could have more sulfate available for the sulfate reducers to use, as a result of higher groundwater flow rates through the wetland. Groundwater 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 point.

It is generally thought that under anaerobic conditions, methanogenesis is the predominant terminal catabolic process in freshwater wetlands (Mitsch and Gosselink 2000), and this is because freshwaters are generally limited in the availability of the electron acceptors nitrate and sulfate. 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

Table 5: Summary of Rate Measurements of Sulfate Reduction

environment	location	depth (cm)	rate (nmol cm^{-3} d^{-1})	reference
salt marsh	Cape Cod	0-20	250 – 6000	Howarth & Teal 1979
intertidal mudflat	San Francisco	surface	243 μmol/flask	Oremland & Polcin 1982
marine sediments, March	Long Island Sound	2-6	104 ± 11	Westrich & Berner 1988
marine sediments, Oct	Long Island Sound	2-6	19.5 ± 2.2	Westrich & Berner 1988
estuary	East Scotland	0-15	537.6	Gibson, Parkes & Herbert 1989
alpine lake	Germany	surface	75 – 1800	Bak & Pfenning 1991
tidal mudflat	Northern Germany	10-15	1970 (34)	Arnosti et al. 1998
Estuary	Denmark	5-10	565 (32)	Arnosti et al. 1998
eutrophic lake sediment	SW Michigan	0-2	3816	Lovley & Klug 1982
oligotrophic lake sediment	SW Michigan	surface	70.68	Lovley & Klug 1983
lake sediment	West Scotland	0-15	12.0	Gibson, Parkes & Herbert 1989
lake sediment	West Scotland	0-15	2.4	Gibson, Parkes & Herbert 1989
profundal lake sediment	Germany	0-8	0 – 1.66	Sass et al. 1997
littoral lake sediment	Germany	0-8	0 – 3	Sass et al. 1997
alder swamp	Denmark	0-10	65.28	Westermanm & Ahring 1987
peatland	West Virginia	0-5	5 – 141	Yavitt & Lang 1990
freshwater wetland	SE US	0-1	0.027 ± 0.002	Roden & Wetzel 1996
wetland associated with creek		0-15	0 – 3000	Kostka et al 2002
freshwater wetlands	SW Michigan	0-10	0 – 153.8	This study

Table 6: Summary of Measurements of Methanogenesis Rates

environment	**location**	**depth (cm)**	**rate (nmol cm^{-3} d^{-1})**	**reference**
peatland	West Virginia	0-10	2.4 (0.72) – 394 (161)	Yavitt, Lang, & Downey 1988
peatland	West Virginia	0-5	3-190	Yavitt & Lang 1990
flooded rice field		0-20	464 (282)	Rothfuss & Conrad 1993
freshwater wetland	SE US	0-1	206 (15)	Roden & Wetzel 1996
freshwater wetlands	SW Michigan	0-10	2.6 – 40.4	This study
environment	**location**	**depth**	**rate (nmol $g_{drywt}^{-1}d^{-1}$)**	**reference**
floodplain	SE Australia	na	72 (21.6)	Sorrell & Boon 1992
rice field 1998	Northern Italy	0-3	360	Krueger, Frenzel, & Conrad 2001
rice field 1999	Northern Italy	0-3	12	Krueger, Frenzel, & Conrad 2001
environment		**depth**	**rate (nmol $g_{wetwt}^{-1}d^{-1}$)**	**reference**
river, estuary	Maryland	surface	75.3 (2.7)	Lovley & Phillips 1986

category. The methanogenic rates measured in this study are generally lower than those seen in other freshwater wetlands (Table 6).

Significant methanogenesis was measured in the simultaneous presence of measurable rates of both iron and sulfate reduction, which should not occur based on thermodynamic considerations alone. This could perhaps be explained if the subcores integrated a lot of small-scale spatial variability, and the subsequent assays would not have allowed enough time for competitive interactions to be fully played out. It has been found that iron reduction in estuarine sediments is limited to depths of 4 cm or less, with the most intense iron reduction taking place in the top 1 cm of the sediment (Lovley and Phillips 1986b). Since my sampling integrated sediment profiles over a 10-cm depth interval, it is likely that any stratification with depth was homogenized by the sampling method.

Even though sulfate concentrations do not seem to be limiting in groundwater-fed wetlands, it is surprising to find sulfate reducers and methanogens, two groups of anaerobic decomposers with different thermodynamic constraints, occurring in the same wetland samples. Since 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), sulfate reduction and methanogenesis were not expected to occur simultaneously. But this could be explained in a couple of ways. 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); other carbon sources were not measured in this study. Another more likely possibility is the same as discussed above for the iron reduction rates. The 0-10 cm section of the anoxic zone that was subcored was too coarse of a scale to differentiate the likely vertical spatial variation in each anaerobic microbial decomposition process (Hunt et al. 1997). Even though there was little variation in sulfate concentrations measured from 0-10 cm in the porewater equilibrators, there was variation in CH_4 concentrations measured with the porewater equilibrators (Chapter 2). The slurries created in the lab may also have disrupted the small-scale sediment structure important to microorganisms (Yavitt and Lang 1990, Hunt et al. 1997).

Despite the possible homogenization of small-sale spatial variation by the sampling procedure, the results presented here still show the importance of iron and sulfate reduction and methanogenesis in terms of the biogeochemical functioning of wetlands. The purpose was not to understand precisely where within the sediment profiles these anaerobic microbial processes were occurring, but to address their importance in overall wetland function, which is why I chose to analyze composite samples from the upper 10 cm of wetland sediments.

Since acetate is considered an important intermediary compound in anaerobic decomposition and potentially the most important carbon source available to microorganisms at the terminus of anaerobic catabolism (Lovley and Klug 1982), comparisons between acetate turnover and total carbon mineralization rates were made by converting all of the anaerobic process rates to carbon mineralization rates using the stochiometry 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 and acetate is one of the lowest molecular weight product of fermentation, but not the only one (McInerney 1998). Other volatile fatty acids such as propionate, formate and lactate can also be formed. In one study of lake sediments, lactate turnover rates were higher than acetate turnover rates (Cappenberg and Prins 1974). Another possibility for the large difference in wetland G1 could be that sulfate reduction was overestimated due to variance within the replicates, resulting in the greater total carbon mineralization values, but I have no reason to suspect this.

Acetate turnover rates support the hypothesis that groundwater-fed wetlands have higher overall rates of anaerobic decomposition. The percent of total

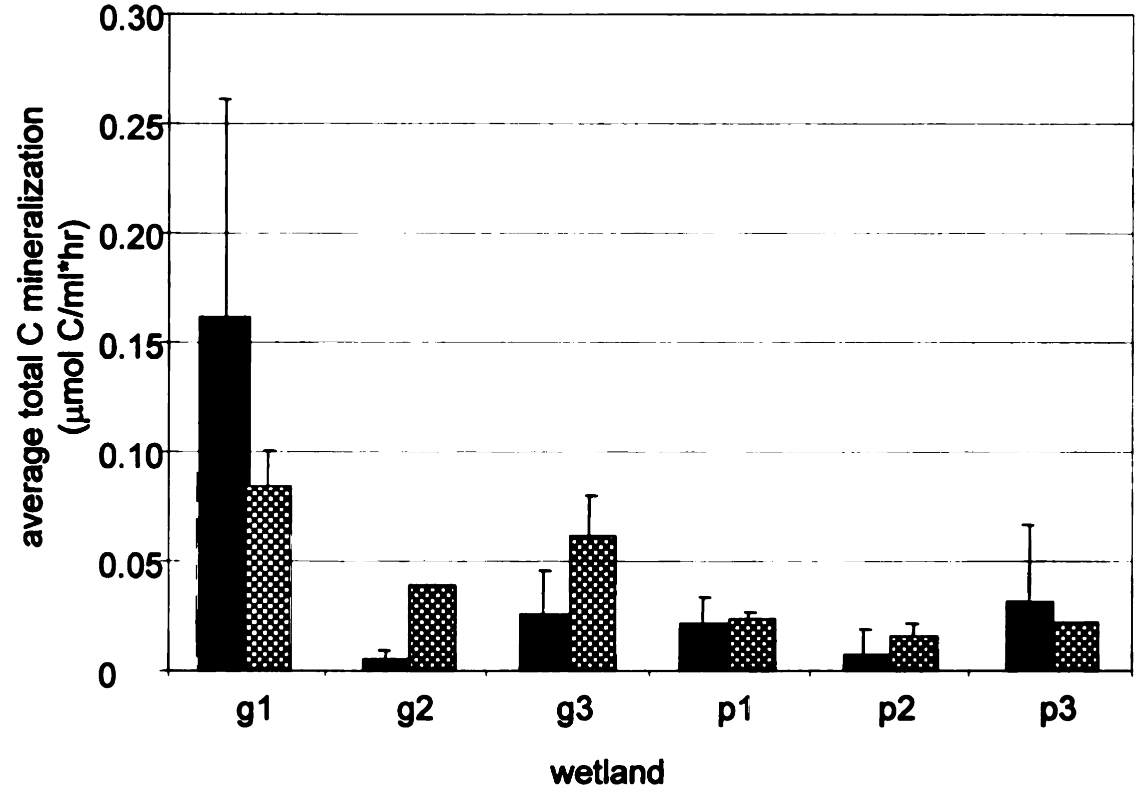

Figure 4: Average C mineralization rate estimates based on the sum of individual rates (solid bars) and acetate turnover rates (checked bars), with standard deviations. Standard deviations could not be calculated for acetate turnover rates at wetlands g2 and p3.

ecosystem production that ultimately degrades through the terminal step of anaerobic decomposition was roughly estimated by dividing acetate turnover by aboveground net primary productivity (ANPP). ANPP values were estimated from a single sampling event at the end of the growing season in 2000, and thus must be regarded with caution; however, they are within the range of values found in the literature for similar systems (Mitsch and Gosselink 2000). The precipitation-fed wetlands had lower ANPP values than the groundwater-fed wetlands (525-626 vs. 600-1000 g m^{-2} y^{-1}, respectively). Based on acetate turnover, the precipitation-fed wetlands have at most 15-25% of their ANPP degraded through the terminal steps of anaerobic decomposition, while the groundwater-fed wetlands have 34-70% of their ANPP degraded that way. These percentages are likely overestimated because they only take into account above-ground productivity of vascular plants and do not include below ground productivity, algal productivity or allochthonous inputs. Nevertheless, the higher percentages in groundwater-fed wetlands are intriguing and suggestive of a positive relationship between vascular plant productivity and the relative importance of anaerobic vs. aerobic degradation of organic matter.

Lower acetate turnover rates in the precipitation-fed wetlands might occur due to inefficient metabolism. One reason could be the lower pH found from 0-10 cm in the precipitation-fed systems compared to groundwater-fed systems (5.2-6.1 vs. 6.8-7.1 respectively) (Table 3). The lower pH would slow fermentation, decreasing the pool of electron donors such as acetate. Acetate concentrations are lower in precipitation-fed wetlands (Table 3). Reduced anaerobic decomposition in precipitation-fed wetlands could lead to organic matter accumulating faster than in groundwater-fed wetlands. However, this depends on the occasional drying that could be more prevalent in precipitation-fed wetlands during dry years. If the system dries then respiration in the upper sediments will switch from anaerobic to aerobic and likely increase considerably.

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, there were general patterns that are important for wetland biogeochemistry. Water source influenced which anaerobic processes would occur. In precipitation-fed wetlands, inputs of the electron acceptors nitrate and sulfate occur episodically, while in groundwater-fed wetlands they are continuously supplied. The electron acceptor limitation was evident in the denitrification potential measurements on subcores in G3 and P2, as well as from the disappearance of nitrate and sulfate in all wetlands included in the push-pull experiments (Chapter 4). Iron reduction was important in wetlands that have wet-dry cycles and these cycles could be important in attenuating the amount of methane released into the atmosphere due to methanogenesis. Methanogenesis was important in all of these wetlands. In terms of the overall carbon budgets of these wetlands, anaerobic degradation seems to be relatively more important in the groundwater-fed wetlands, which also support higher vascular plant biomass. The lower plant productivity and limited supply of electron acceptors in the precipitation-fed wetlands could be the reason for their decreased rates of anaerobic metabolism as compared to the groundwater-fed wetlands.

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CHAPTER 4

ASSESSING DENITRIFICATION AND SULFATE REDUCTION POTENTIAL IN SITU USING SINGLE-WELL PUSH-PULL EXPERIMENTS

Introduction

Wetlands perform important biogeochemical functions that are mediated through anaerobic metabolism by bacteria. Wetlands are often situated at points of groundwater recharge and discharge and hence biogeochemical processes in wetlands can affect downstream water quality, as for example by removing nutrients (Peterjohn and Correll 1984, Tobias et al. 2001b). In addition, wetlands emit greenhouse gases such as methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) that contribute to the regulation of global climate. Several reactions in the terminal step of anaerobic microbial decomposition play a central role in the biogeochemical functions of wetlands, and frequently the nature and rates of these reactions are controlled by thermodynamics (Chapelle 1993).

The saturated nature of wetland sediments hinders oxygen diffusion, so the sediment quickly becomes anaerobic. Thermodynamic constraints on microbial metabolism determine the competitive ability of the microbes that perform the terminal step in organic matter decomposition, which can occur via either anaerobic respiration or methanogenesis. The thermodynamic constraints are based on the free energy yield of oxidation-reduction (redox) reactions in which an electron donor, such as a low-molecular-weight organic compound, is oxidized and an inorganic electron acceptor, such as nitrate or sulfate, is reduced. Microbes harness the free energy released by the redox reaction for maintenance and growth (Conrad 1996, Fenchel et al. 1998). Aerobic respiration is the most energetically favorable reaction, followed in order of decreasing energy yield by denitrification, iron reduction, sulfate reduction, and finally methanogenesis. In light of the escalating problem of nitrogen contamination due to human activities, denitrification is of particular interest because wetlands can be important sites of nitrate removal from water before it reaches groundwater, surface waters, and coastal estuaries and ocean waters.

Groundwater in agricultural landscapes is often enriched in nitrate and sulfate due particularly to fertilizer and animal waste inputs. Rates of loading of nitrate and sulfate to wetlands via precipitation are greatly enhanced due to fossil fuel combustion. To improve our understanding of the potential rates of microbially-mediated removal of nitrate and sulfate, we need better information on what limits these rates in wetland sediments. My direct measurements of in-situ rates found no detectable denitrification and varying rates of sulfate reduction in groundwater-fed wetlands and no detectable denitrification or sulfate reduction in precipitation-fed wetlands (Chapter 3). The fact that denitrification was not detectable even where continuous groundwater inputs are known to occur was surprising since groundwater in the area is high in nitrate, although the sediment porewaters usually showed undetectable levels (Chapter 2).

Many methods have been used to estimate the nature and rates of anaerobic decomposition in sediments, including analysis of geochemical data (Lovley and Goodwin 1988, Lovley et al. 1994), molecular methods (Muyzer and Smalla 1998), and assays in microcosms (Groffman et al. 1996, Roden and Wetzel 1996, D'Angelo and Reddy 1999). These approaches can be affected by disturbance of the natural environment, contamination by oxygen, and introduction of high concentrations of substrate. Laboratory microcosm studies can pose problems because the small sample size can generate high variability, and microcosm rates are often lower than in-situ derived rates (Addy et al. 2002). Collection and transfer of samples to microcosms may also disturb the natural microbial community structure, which could alter rates. In-situ methods used to estimate microbial process rates such as aerobic respiration, denitrification, sulfate reduction and methanogenesis are more representative of subsurface conditions (McGuire et al. 2002). These methods include push-pull tracer experiments, in which a solution containing reactants and amended with a conservative solute tracer is injected into the sediments, then subsequently withdrawn to measure rates of disappearance of the reactant relative to the conservative solute (Istok et al. 1997, Addy et al. 2002, McGuire et al. 2002). This method has proven useful in estimating microbial reaction rates from aquifers (Istok et al. 1997, McGuire et al. 2002), lake sediments (Luthy et al. 2000), and riparian wetlands (Addy et al. 2002).

To determine the potential of these wetlands to remove nitrate and sulfate from inflowing waters via denitrification and sulfate reduction, I experimentally injected groundwater containing nitrate and sulfate into wetland sediments in the field. Through a modification of the push-pull method used to study nitrate and sulfate reduction rates in contaminated aquifers, I have quantified potential denitrification and sulfate reduction in shallow sediments of freshwater wetlands. The experiment involves a "push" and a "pull" phase (Snodgrass and Kitanidis 1998). In the "push" phase, a well-mixed solution containing a reactive solute and a conservative tracer is pushed into the saturated zone of the sediments. The injection solution is dispersed and diluted as it mixes with the native porewater. Concentrations of the reactant may be changed by both microbial activity and dilution, and the dilution occurs by both advection and diffusion. The conservative tracer is used to account for dilution. The "pull" phase begins immediately after the injection solution has been introduced. Consecutive porewater samples are extracted from the same well over time, and the solute concentrations are measured. By comparing the concentrations of the tracer to those of the reactive solute, we can determine the amount of reactive solute depleted by microbial activity. The background concentrations of nitrate and sulfate are measured before the injection and subtracted from measured concentrations during the experiment (although nitrate is normally not detectable).

The push-pull experiments can provide valuable information on the reactions occurring in situ. This study evaluates the biogeochemical changes that occur when a groundwater solution comes into contact with reduced wetland sediment. This is a measurement of the *potential* reaction rates insofar as the groundwater is relatively enriched in nitrate and to a lesser extent sulfate compared to the sediment porewaters, but this enrichment is within the bounds of what local wetland sediments may experience as a result of natural groundwater flow or precipitation events, and thus the rates do not indicate the *maximum* potential under conditions of non-limiting reactants (electron acceptors and donors). My experiments were designed to yield the following information: 1. which reactions can occur in-situ, 2. the order in which the reactions will occur if solutes are introduced simultaneously, and 3. the potential rates of these reactions under realistic conditions.

Methods

Study Sites

The wetlands in this study are all situated in the glacial landscape around the Kellogg Biological Station (KBS), an academic unit of Michigan State University located in southwest Michigan. The study sites include 5 wetlands of various National Wetland Inventory (NWI) classes. Two of the wetlands have groundwater as their main water source (Loosestrife Pond (G1) and Turkey Marsh (G3)) and the other three receive most of their water from precipitation

(Shaw Pond (P1), Lux10 (P2) and LTER Kettle Pond (G3)), as confirmed using dissolved magnesium as a tracer (Chapter 2). The wetlands range in size from 0.4 to 3.1 ha, with most being less than 1 ha (Table 2 in Chapter 2). Two representative sites were chosen within the central wet area each wetland to do these experiments, and these sites coincided with those used in previous microbial rate assays (Chapter 3). The push-pull experiments were performed in relatively wet years (2001-2002) that had been preceded by several relatively dry years (1998-2000), during which many wetlands in the area dried completely (Chapter 2).

Experimental methods

I conducted in-situ push-pull experiments at three of the five wetlands in 2001 and all five wetlands in 2002. To prepare for the push-pull experiment, untreated groundwater was collected from a domestic well at KBS (NO_3^--N = 13.7 mg L^{-1}, SO_4^{2-} = 52.5 mg L^{-1}) and stored at 4°C until the push-pull experiment. The groundwater was amended immediately prior to each experiment with 13-20 mg L^{-1} Br^- (as NaBr) to serve as the conservative solute tracer. I used higher Br^- concentrations (50 mg/L) for wetland g1 to ensure that concentrations would remain measurable in the face of the anticipated advective loss from the high flow at that site.

The push-pull well screens were small stainless steel mesh fuel filters (6 mm i.d., 7.6 cm screen length; American Science & Surplus 4258) attached to about 1 m of Teflon tubing (3.2 mm outside diameter). I installed two wells in each of the five wetlands by inserting them vertically to a depth of 10 cm below the sediment surface (screen depth was 6-13 cm deep after installation) at least 2 days before the push-pull experiment began to ensure an anaerobic environment surrounding the screen. Upon installation, 20 mL of porewater was pulled out of the well to flush and fill the filter and tubing with porewater, thus avoiding oxygenation of the sediment. The sediments at all sites appeared to seal adequately around the well and tubing, and no packing or backfilling was required. From each well, I measured ambient concentrations of NO_3^--N, SO_4^{-2}, Br^-, and dissolved organic carbon (DOC)(see methods below). Sediment temperature was also measured at each well site using a probe that was inserted nearby.

Prior to injection, the groundwater solution was bubbled with He for at least one hour to remove dissolved oxygen. 500 mL of the solution was withdrawn into 60-mL syringes and transported to the wetlands The anoxic solutions were pushed by syringe into the sediment using the wells over the course of 10 -15 minutes (33-50 mL min^{-1}) (Figure 1).

Samples were immediately withdrawn by syringe after the push phase and were periodically withdrawn over time for up to 48 hours after the initial injection. For each sampling, the filter and tubing were first flushed by removing 5 mL of the solution, then 20 mL of the porewater were collected for analysis (total amount of water withdrawn at each time point = 25 mL). The samples were filtered through

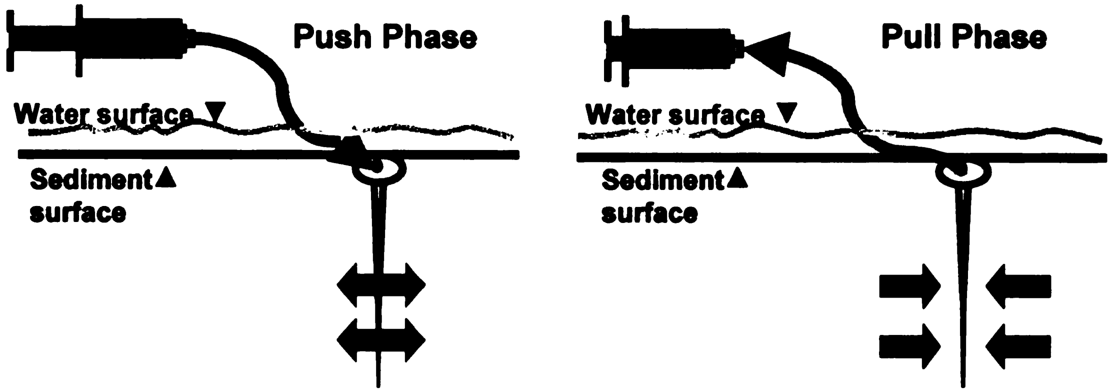

Figure 1. Illustration of the in-situ push-pull method. Groundwater amended with Br^- is pushed into sediments with syringe, incubated in the porewater environment, and later pulled from sediments with the syringe.

0.2-µm membrane (sterile Millex-gs) syringe filters in the field and kept on ice until returning to the lab. Samples were analyzed for Br^-, NO_3^- -N, and SO_4^{2-} using membrane-suppression ion chromatography. Select samples were also analyzed for DOC by high-temperature Platinium-catalyzed combustion to CO_2 followed by infrared gas analysis (Ionics carbon analyzer, model 1550 at 900^0C).

Rate Determination

For each experiment, I calculated nitrate and sulfate uptake rates based on Br^-, NO_3-N and SO_4^{2-} concentrations. I followed the method described by Snodgrass and Kitanidis (1998) for estimating reaction rates from push-pull experiments. Both zero- and first-order rate models were fit to the data and the model with the best fit was selected to determine potential rates; in most cases the choice was obvious.

For zero-order reactions the reactive solute (nitrate or sulfate) concentrations were transformed to remove the effect of dilution as follows:

$$\hat{C}_{reactant}(t) = C^o_{reactant}\,(C_{reactant}(t)/C^o_{reactant} - C_{tracer}(t)/C^o_{tracer} + 1) \qquad (1)$$

where $C^o_{reactant}$ and C^o_{tracer} are the concentrations of the reactive and conservative solutes at injection and $C_{reactant}(t)$ and $C_{tracer}(t)$ are the concentrations measured at time t (Snodgrass and Kitanidis 1998). All concentrations were background-corrected based on the initial sampling prior to injection. The rate of reduction in $C_{reactant}(t)$ (i.e., the uptake rate) is described by the zero-order decay equation:

$$\frac{\partial C_{reac\tan t}(t)}{\partial t} = -\alpha \tag{2}$$

The plot of $\hat{C}_{reactant}(t)$ versus time will fall on a straight line, with the regression slope equal to the reaction rate, if the solute disappears under zero-order kinetics (Snodgrass and Kitanidis 1998).

In wetlands where nitrate and sulfate uptake were better fit to the first-order reaction model, the concentration of the reactant $C_{reactant}(t)$ can be computed using

$$C_{reactant}(t) = C_{tracer}(t)\ e^{-kt} \tag{3}$$

Equation 3 can be rewritten as:

$$\ln\left(\frac{C_{reac\tan t}(t)}{C_{tracer}(t)}\right) = -kt \tag{4}$$

so that the slope of a regression line fit to the plot of $\ln(C_{reactant}(t)/C_{tracer}(t))$ versus time will give an estimate of the first order reaction rate *k*. Since *k* is based on the ratio of $C_{reactant}/C_{tracer}$, complete tracer mass recovery is not necessary to obtain accurate estimates (McGuire et al. 2002).

The analyses described above assume that: 1. the solutes are injected simultaneously in a well-mixed slug; 2. the injection time is short compared to the overall length of the experiment; 3. the dominant processes are advection, dispersion, and constant-coefficient zero- or first-order reactions; 4. the background concentrations of SO_4^{2-}, Br^-, and NO_3-N are negligible or are subtracted from concentrations measured during the experiment; and 5. if flow is heterogeneous then earlier collected samples are more representative of in situ processes than later collected samples. It is reasonable to assume that the injected solution was well-mixed because the solution was well-homogenized prior to injection. The injection time was also short compared to the total length of the experiment. Background concentrations of NO_3 were always negligible, and SO_4 concentrations were corrected for background levels when present. Heterogeneous flow is unlikely to be a significant factor over the small spatial scale and short time course of these experiments.

Test of bromide reactions with sediments

To be sure that there were no significant reactions of the conservative tracer bromide with wetland sediments, I collected sediments from several representative sites in October 2002, injected bromide at concentrations similar to those used in the push-pull experiments, and incubated them in sealed Mason jars. Porewaters were subsampled immediately after the bromide was added and thoroughly mixed, and again 24 hours and 1 week later.

Results

All wetlands had the potential to remove nitrate and sulfate and did so in the order predicted by thermodynamics (nitrate first, followed by sulfate). Nitrate disappeared without any lag time and according to first-order reaction kinetics, except in one case. Once nitrate had been depleted to below detection limits (ca. 15 μg N/L), sulfate uptake was observed, as shown in the example in Figure 2. This sequential uptake pattern was observed in all 5 wetlands. Results of all experiments are summarized in Table 1.

Nitrate depletion occurred as a first-order reaction in all but one case (Table 1). During 2002 in G1, a groundwater-fed wetland, nitrate disappeared by zero-order reaction kinetics, and was left out of statistical analyses comparing first-order reaction rates. The other wetlands varied in their potential to remove nitrate but the differences were not significant (ANOVA, $p=0.076$) (Figure 3). There was no difference between wetlands grouped by groundwater and precipitation water sources ($p=0.373$) (Figure 4).

Unlike nitrate, potential sulfate uptake rates exhibited either zero- or first-order reaction rate kinetics depending on the water source of the wetland. In the groundwater-fed wetlands sulfate uptake was a zero-order reaction, while in

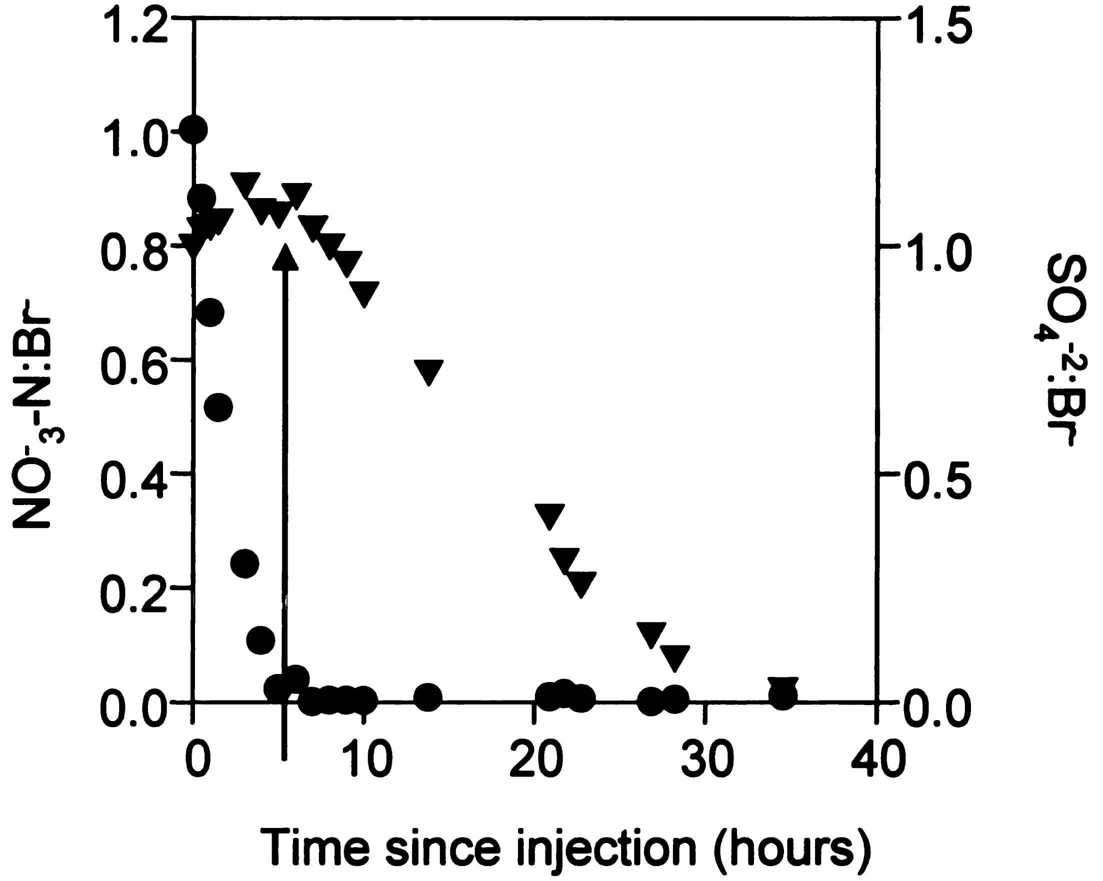

Figure 2. Example of a push-pull experiment from P1 site1. Circles represent NO_3-N:Br mass ratios and triangles represent SO_4:Br mass ratios. The arrow indicates where nitrate has been depleted and sulfate begins to decrease.

Table 1. Means and standard deviations of nitrate depletion rates for replicate sites within a wetland as observed in the push-pull experiments.

Site	NO_3-N k values ($hour^{-1}$)	
	2001	2002
G1	0.50 (0.18)	0.30 (0.03)*
G3	0.15 (0.02)	0.23 (0.10)
P1		0.47 (0.37)
P2	0.25 (0.16)	0.045 (0.04)
P3		0.55 (0.12)

Rate values are from zero-order reactions and the units are mg L^{-1} d^{-1}.

Table 2. Means and standard deviations of sulfate depletion rates for replicate sites within a wetland for push-pull experiments. Zero-order reactions have units of mg L^{-1} d^{-1} and first-order reactions have units of $hour^{-1}$.

	SO_4^{2-} k values (mg L^{-1} d^{-1})		SO_4^{2-} k values ($hour^{-1}$)	
Site	2001	2002	2001	2002
G1	0.15 (0.01)	0.30 (0.14)		
G3	0.25 (0.07)	0.24 (0.014)		
P1				0.057 (0.052)
P2			0.30 (0.05)	0.075 (0.3)
P3				0.12 (0.0)

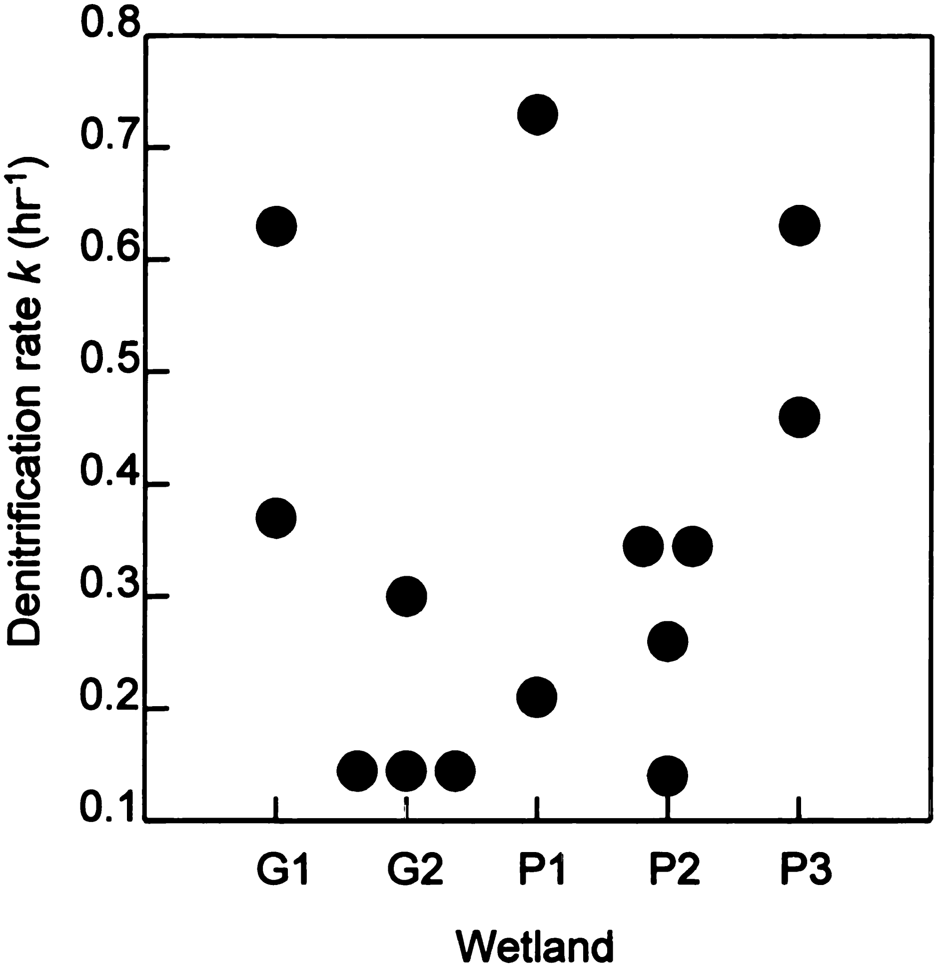

Figure 3. Dot density plot of denitrification rates for groundwater (G) and precipitation (P) wetlands. Each dot represents one push-pull experimental run. 2002 values for Loosestrife Pond were not included since denitrification was a zero-order reaction that year.

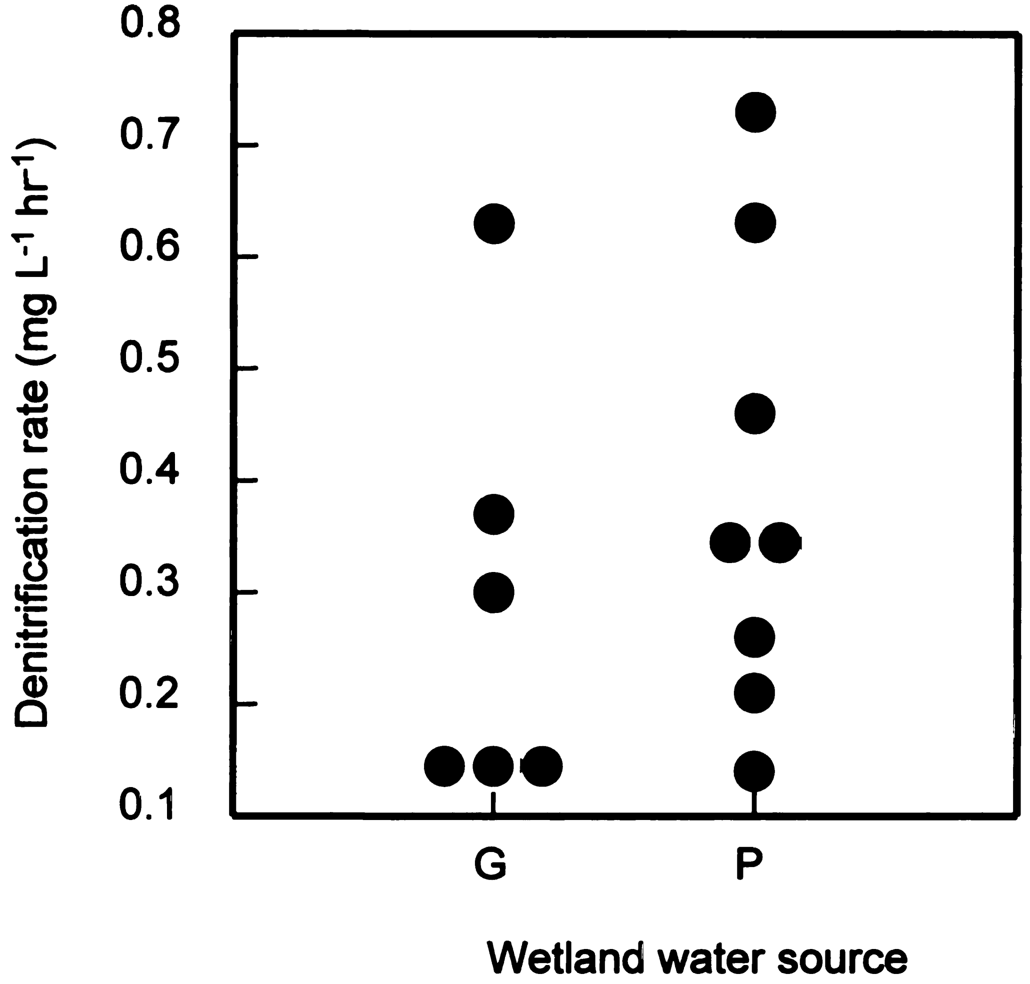

Figure 4. Dot density plot of potential denitrification rates for wetlands with either groundwater (G) or precipitation (P) as their main water source. 2002 values for Loosestrife Pond were not included since denitrification was a zero-order reaction that year.

precipitation-fed wetlands sulfate was removed by first-order reaction kinetics (Table 2). The groundwater-fed wetland G3 had significantly higher rates of sulfate uptake than G1 (p=0.014) (Figure 5), but there were no significant differences between the precipitation-fed wetlands (p=0.177) (Figure 6).

Background concentrations of sulfate are shown in Table 3, together with surface-water concentrations. Porewaters had markedly lower sulfate concentrations than surface waters. Surface-water sulfate concentrations were lower than average groundwater concentrations (Chapter 2), even at sites that were close to 100% groundwater-fed. Nitrate was below detection limits in all background samples and below or close to detection limits in surface waters of these wetlands.

Incubation of representative wetland sediments in sealed jars with bromide added to similar concentrations as used in the push-pull experiments showed no evidence for non-conservative behavior (Table 4). There were no statistical differences between sampling time points. The variation in Table 4 was probably due in part to analytical error associated with the ion chromatography.

Discussion

Caveats concerning the use of bromide as a tracer - Potential problems with the use of bromide as a conservative tracer in push-pull experiments are its potential inhibitory effect on microbial processes (Groffman et al. 1995) and the possibility

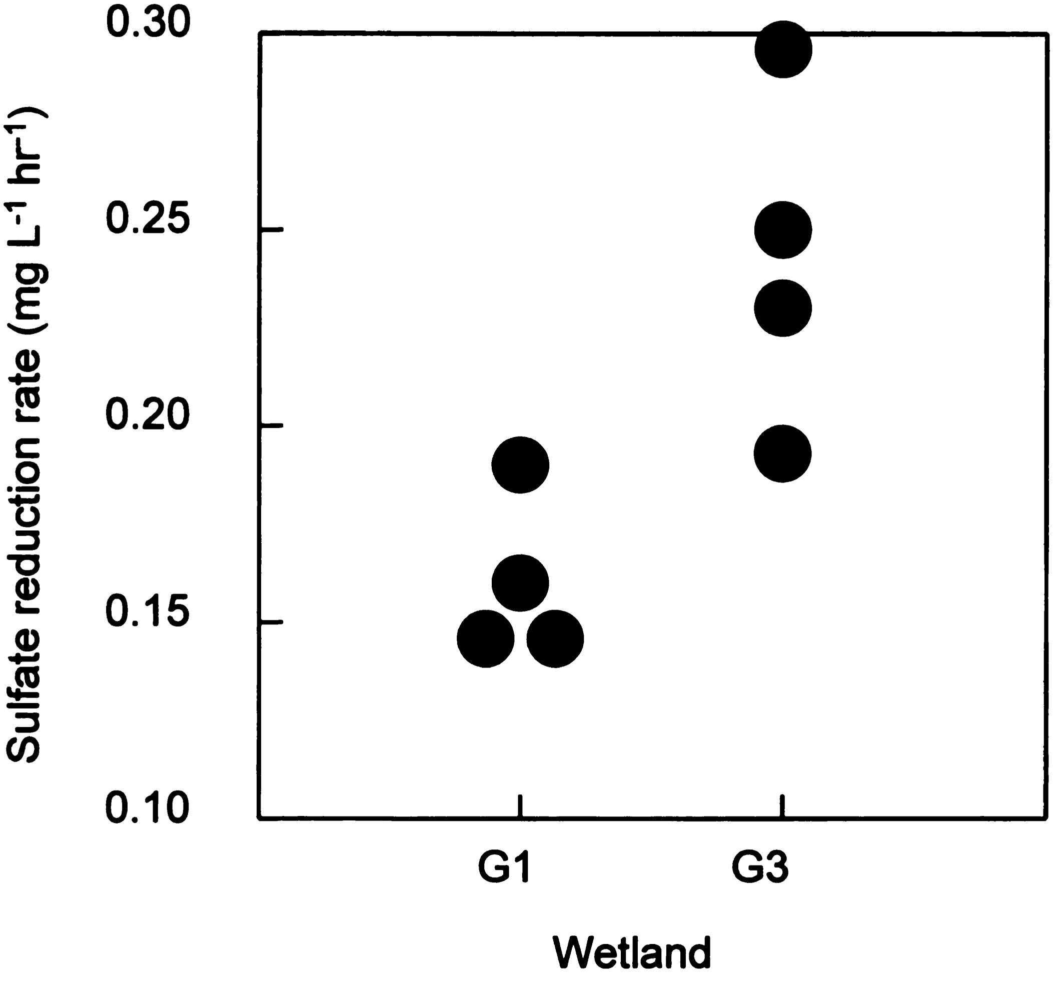

Figure 5. Dot density plot of potential sulfate reduction rates for zero-order reactions. Both wetlands are mainly groundwater-fed. Each dot represents one push-pull experimental run.

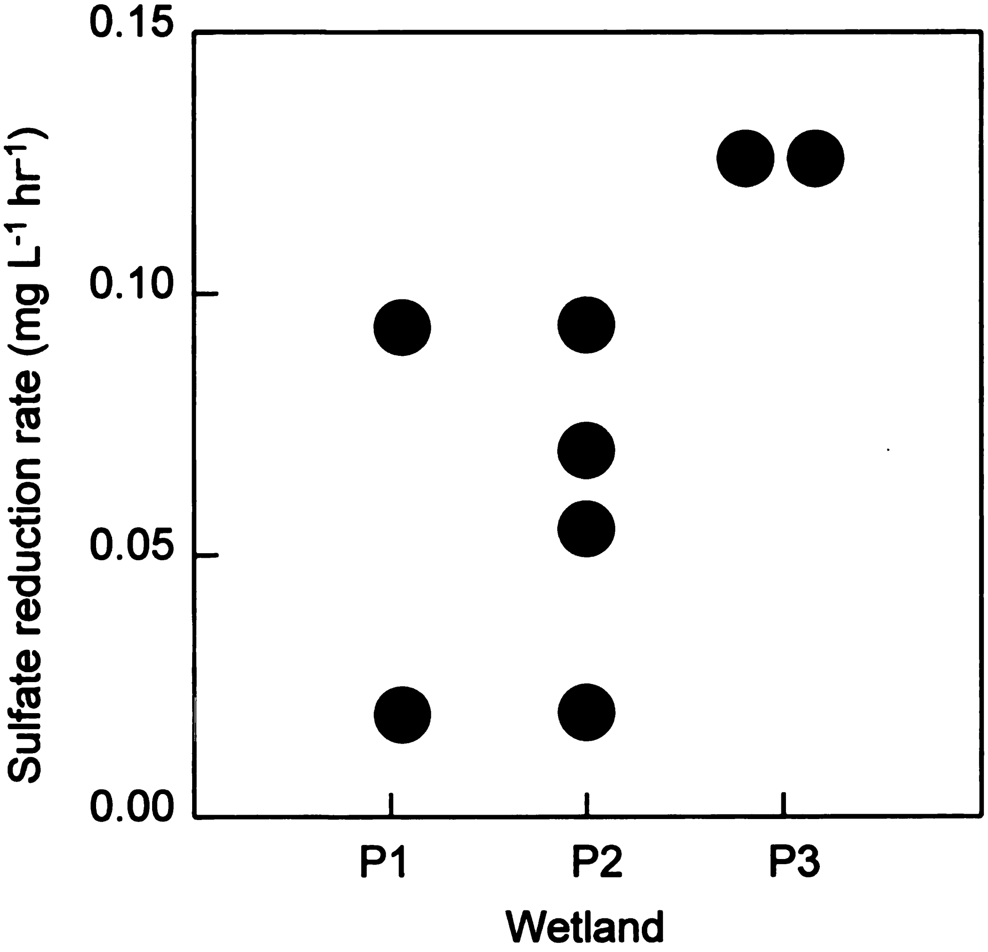

Figure 6. Dot density plot of potential sulfate reduction rates for first-order reactions. These wetlands are mainly precipitation-fed. Each dot represents one push-pull experimental run.

Table 3. Means and standard deviations of background sulfate concentrations by wetland. Porewater means are from replicate sites within each wetland (n=2) and surface water means are from all samples collected in 2001 and 2002 (4 or more samplings).

Site	SO_4^{-2} in porewater prior to injection (mg/L)	SO_4^{-2} in surface water during '01-'02 (mg/L)
G1	0.13 (0.15)	15.6 (8.5)
G3	0.99 (0.71)	18.2 (6.0)
P1	0.28 (0.24)	4.98 (2.30)
P2	0.16 (0.14)	2.07 (0.93)
P3	0.63 (1.18)	1.88 (0.92)

Table 4: Means and standard deviations of bromide concentrations in 3 wetland sediments at the time of addition, and at 24 hours and 1 week after addition to test the stability of bromide in sediment porewaters. There were no statistically significant differences between the time points.

Wetland	Br at beginning (mg/L)	Br at 24 hrs (mg/L)	Br at 1 week (mg/L)
G3	7.0 (0.5)	7.2 (0.3)	7.0 (0.6)
P2	7.4 (0.9)	7.9 (0.4)	8.0 (0.6)
P3	8.8 (0.7)	8.3 (0.6)	8.4 (0.3)

of sediment or plant uptake, which may confound results (Kung 1990, Whitmer et al. 2000). Some inhibitory effects of bromide on microbial processes have been noted at high concentrations of bromide (100 mg L^{-1}), while bromide was added to these wetlands at lower concentrations. Also, I found that nitrate depletion commenced immediately and rates did not increase as the bromide was diluted, suggesting that bromide at the concentrations found in this study was not significantly inhibiting microbial activity.

Sediment uptake was not observed when I incubated saturated sediments in sealed jars with added bromide (Table 4), and thus it was unlikely to occur during the experiments. Although the possibility of plant uptake of bromide cannot be excluded in this study, it is unlikely to be important. In all wetlands except G1, about 50% of the bromide was recovered, and these sites span a wide range of plant densities, including some sites with essentially no roots in the sediments. Since porewater concentrations of bromide are essentially zero at the beginning of the experiment, it is more likely that dispersion (mixing with ambient porewaters) and diffusion away from the injection well explain the partial recovery. Advective loss was especially likely in G1, where surface water flow was visible. Lastly, the potential nitrate and sulfate uptake rates observed in these wetlands were similar to those seen in contaminated aquifers (McGuire et al. 2002), where plant roots are not present.

Nitrate and sulfate uptake - The push-pull experiments demonstrated that all of the wetlands have the potential to take up nitrate and sulfate at concentrations found in groundwater or precipitation inputs. These processes take place in accordance with thermodynamic theory, where terminal electron acceptors are consumed in the order of free-energy yield as organisms compete for labile organic matter. Since denitrification yields more energy, denitrifiers should have a competitive advantage and sulfate reduction should not occur until all of the nitrate has been depleted. All of the push-pull experiments exhibited this response, with sulfate only disappearing after nitrate had been depleted completely (e.g., Figure 2).

This pattern strongly suggests that denitrification is the principal process responsible for nitrate uptake, as opposed to assimilation by plant roots and microbes. Sediment porewaters in these sites were generally very rich in ammonium (between 200 and 1000 μg/L), a preferred nitrogen source for assimilative uptake by microbes, and activity of plant roots was likely limited at most sites because I avoided sampling close to plants to the extent possible. While this was difficult in some sites (e.g., G3 where *Nuphar* rhizomes were ubiquitous), other sites were devoid of nearby vegetation and yet the pattern of nitrate uptake is similar across all sites.

Another explanation for the rapid decrease seen in nitrate concentrations could be dissimilatory nitrate reduction to ammonium (DRNA), where nitrate is reduced to nitrite and then ammonium by fermentative pathways. It is thought to occur only in highly reducing environments capable of maintaining sustained anaerobic metabolism (Tiedje 1988), which might include the sediments of biologically productive wetlands. This process could be in direct competition with denitrification, especially in anoxic saturated sediments (Nijburg et al. 1997). Even though the conditions for DNRA are similar to those for denitrification (reduced environment, available nitrate and labile C), DNRA is thought to be favored in nitrate-limited, carbon rich environments while denitrification is favored when carbon is limited in availability (Kelso et al. 1997, Silver et al. 2001). Since DNRA is a fermentative process, and not a terminal electron accepting process, DNRA should not compete with sulfate reduction. If this is true, then the delayed depletion of sulfate that I observed in all of the wetlands should not occur. In anaerobic rice paddy soils without added carbon, only 15% of the added nitrate was converted to ammonium (Yin et al. 2002). Therefore it does not seem likely that DNRA could explain the nitrate uptake I observed, although further investigation – perhaps using a ^{15}N tracer addition -- would be required to confirm this.

Nitrate depletion commenced immediately in all wetlands, and mostly by first-order reaction kinetics. This indicates that nitrate uptake in these wetlands is limited by nitrate availability. Assuming denitrification is the main uptake process, labile organic matter must be less limiting than nitrate for the bacterial denitrifiers. Yet all of the sediments investigated appear poised to denitrify upon receiving nitrate, indicating that substantial denitrifier populations exist.

The precipitation- and groundwater-fed wetlands had similar potential rates of denitrification. This is unexpected since groundwater in this area is high in nitrate, and two of these wetlands are primarily groundwater-fed. However, nitrate concentrations in surface and subsurface samples have rarely been above the analytical detection limit in any of the wetlands studied. One explanation for the consistently low ambient nitrate concentrations is that nitrate is efficiently removed from inflowing waters upstream of the sampling locations. Many studies in riparian areas have demonstrated that groundwater entering the riparian zone can rapidly lose nitrate by plant uptake and bacterial denitrification (Seitzinger 1988, Simmons et al. 1992, Hill 1996, Hedin et al. 1998). In a one study of a coastal marsh system, nitrate removal was 90% complete within the first 50 cm of the marsh near the upland boundary (Tobias et al. 2001a). Results from another study also suggest that there is a narrow near-stream region that is the most important location for NO_3^- consumption by denitrification (Hedin et al. 1998). Thus, nitrate might be removed prior to water entry into the more central sampling locations in this study. Episodic inputs of nitrate via precipitation falling directly onto the wetland might also be subject to rapid uptake, especially in wetlands with shallow water columns because contact with the sediments is greater, and this removal could occur across the entire sediment area.

The finding that potential denitrification rates are high where nitrate concentrations are evidently low most or all of the time suggests that nitrate is either produced and consumed in a tightly coupled way, or is entering the wetland episodically. Atmospheric deposition is a potentially important episodic nitrate input; the mean NO_3^--N concentration in rain was 1.7 mg L^{-1} (NADP mean for summer 2001, KBS station MI26). However, closely coupled nitrogen remineralization to ammonium, nitrification of ammonium to nitrate, and denitrification within the sediments is also likely, based on other studies. Concurrent measurements of denitrification and sediment-water nitrate flux indicate that nitrate produced in the sediments appears to be the major source for denitrification in most river, lake and coastal marine sediments (Seitzinger 1988).

Sulfate depletion always followed nitrate depletion, but the reaction kinetics were different depending on the water source for the wetland. Sulfate reduction via anaerobic respiration is the most likely process responsible for this uptake, considering its delay relative to nitrate and the fact that plant uptake rarely impacts sulfate concentrations. Groundwater-fed wetlands exhibited zero-order sulfate reduction rates, indicating that rates were independent of sulfate concentrations, perhaps being limited by labile organic carbon instead. G3 had the highest rates of sulfate depletion, although based on earlier measurements using $^{35}SO_4^{2-}$ this wetland did not have the highest ambient rates of sulfate reduction (Chapter 3). Even though G3 had higher rates, sulfate depletion began

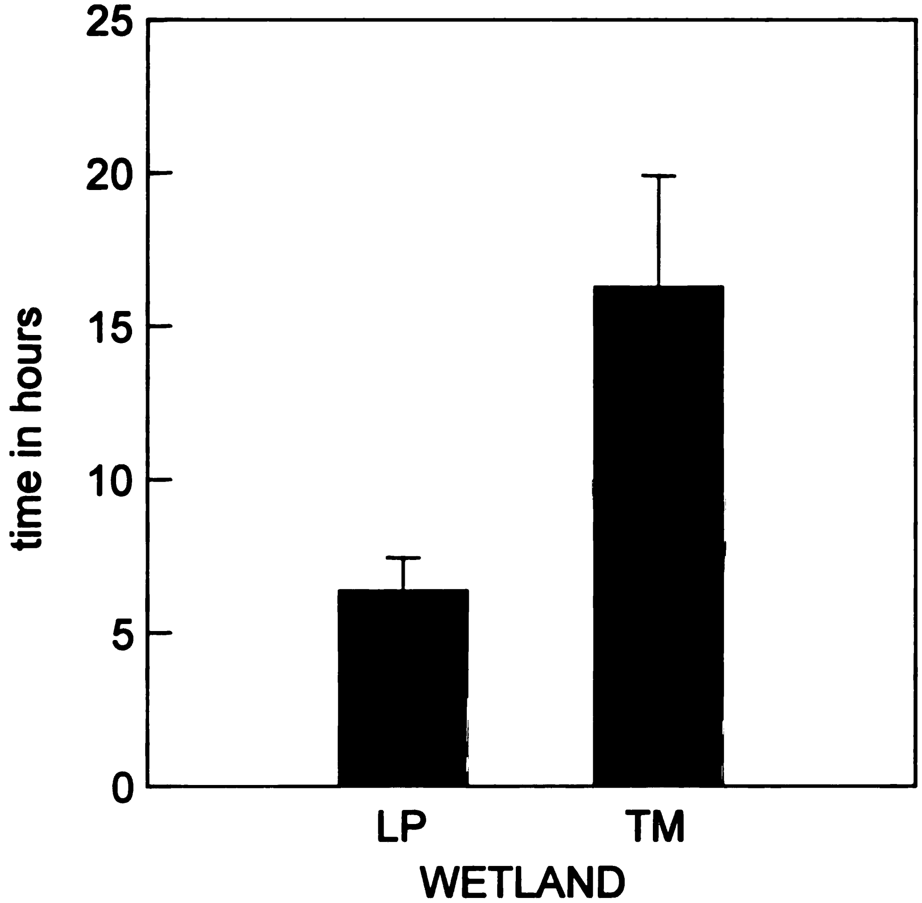

Figure 7: Average time (standard deviation) for sulfate depletion to begin in the groundwater-fed wetlands.

about 10 hours later in G3 than it did in G1 (G3: 16.3 ± 6.1 hours and G1: 6.4 ± 1.8 hours, $p=0.020$) (Figure 7). This is probably due to the fact that nitrate depletion was the slowest in wetland G3; perhaps denitrification inhibited sulfate reduction, preventing sulfate reduction from beginning sooner.

There were no significant differences in potential sulfate reduction among the precipitation-fed wetlands, with an average sulfate reduction rate of 0.074 ± 0.04 hr^{-1} (Figure 6). This shows that sulfate reduction can take place in response to sulfate inputs to precipitation-fed sites, even though ambient rates of sulfate reduction were not measurable in these wetlands (Chapter 3). Microbial sulfate-reducing populations could be maintained in these systems in several ways. First, the mineralization of organic sulfur compounds such as cysteine and methionine could allow sulfate production and consumption to be tightly coupled (Fenchel et al. 1998). Second, sulfate reduction potentials may be high in the precipitation-fed wetlands due to episodic inputs by atmospheric deposition of sulfur compounds – primarily as sulfate. Sulfate deposition is relatively high in southwest Michigan (Berner and Berner 1996). The mean summer sulfate concentration measured in precipitation at KBS for the summer of 2000 and 2001 was 2.1 mg SO_4^{2-} L^{-1} (NADP/NTN station MI26). Average sulfate concentrations in the surface waters of these wetlands vary but are generally higher (Table 3), and hence surface water could provide a source of sulfate to the sediments. Porewaters were always depleted in sulfate relative to the overlying surface waters, suggesting consumption in the sediments. Diffusion of water between overlying water and shallow subsurface sediments could be important in transporting SO_4^{2-} to microorganisms, as has been found in shallow lakes (Kelly and Rudd 1984). Thus, even the microbes in the sediments of the precipitation-fed wetlands could be receiving sulfate. The similar rates of potential sulfate reduction imply that sulfate may be a limiting electron acceptor in all of the precipitation-fed wetlands.

Dissolved organic carbon (DOC) measurements indicate that the DOC pool is diluted following injection into the sediments. In all cases DOC concentrations decrease after injection and then return to concentrations close to those found pre-injection (Table 4). It was not determined how labile the DOC in the injection solution was compared to the sediment porewater, so carbon limitation was not determined, but it seems likely that the injected groundwater did not contain enough labile carbon to stimulate microbial uptake compared to the ambient porewaters.

Obtaining representative rates of in situ microbial processes is often difficult. These push-pull experiments can quantify potential rates of microbial processes such as denitrification and sulfate reduction (Haggerty et al. 1998, Snodgrass and Kitanidis 1998). This study has illustrated how even small, mainly precipitation-fed wetlands have the ability to rapidly remove nitrate and sulfate, even though nitrate and sulfate are present at low concentrations in their porewaters. It is also interesting that all of the wetlands have similar denitrification potentials.

This study reveals some important management implications. All of these wetlands have the potential to improve water quality by removing NO_3^-. This is important since wetlands less than 4 ha are generally not protected from development and the area surrounding KBS is dominated by small depression wetlands (Winter 1996). Destruction or degradation of these wetlands would reduce the cumulative ability of local wetlands across the landscape to take up nitrate in precipitation and local runoff, which is an important ecosystem function for maintaining the quality of human drinking water and downstream aquatic systems. The groundwater-fed wetlands have the potential to remove nitrate in groundwater discharge before it enters streams and lakes. Precipitation-fed wetlands are isolated and have less influence on stream water quality; however, they have the potential to "process" water entering as runoff or precipitation and eventually infiltrating to groundwater systems.

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CHAPTER 5

CONCLUSIONS

Wetlands in southwest Michigan vary in their hydrologic input from being mainly groundwater-fed to mainly precipitation fed. This hydrologic variation may influence the nature and rates of the terminal steps of anaerobic degradation of organic matter by anaerobic respiration or methanogenesis, which are important for biogeochemical functions in wetlands. The influence of anaerobic microbial metabolism on biogeochemical functions of wetlands was examined using porewater chemistry profiles, assays of ambient anaerobic decomposition rates, and experimental determination of in-situ anaerobic processes in a set of wetlands spanning this gradient. Dissolved magnesium served as a natural tracer of groundwater inputs because it has elevated concentrations in groundwater, but is low in precipitation.

Porewaters were generally depleted in nitrate and sulfate relative to source waters, and the presence of the final products H_2S, Fe(II) and CH_4 suggest that sulfate reduction, iron reduction and methanogenesis occurred in all wetlands, though there was large spatial and temporal variability (Chapter 2). Nitrate was below detection (ca. 15 μg N/L) in the wetland sediments.

Ambient levels of denitrification were not measurable, but both the denitrification potentials measured in the lab and the push-pull experiments showed that all of these wetlands have the potential to denitrify over short temporal and spatial scales. Since anaerobic metabolism in these wetlands was not dominated by denitrification, there are important management implications for small isolated wetlands which are often not legally protected. All of these wetlands have the potential to improve water quality by removing NO_3 at the concentrations in local groundwaters. The groundwater-fed wetlands have the potential to remove nitrate in runoff from upland areas or in discharging groundwater before it enters streams and lakes. Precipitation-fed wetlands are often isolated and have less influence on stream water quality; however, they have the potential to buffer the local system from pollutants entering as runoff or precipitation, and they influence the chemistry of infiltrating water that ultimately recharges groundwater.

Wetland sediments were consistently capable of removing sulfate from groundwater and precipitation inputs. Even though groundwater-fed wetlands had higher rates of sulfate reduction than precipitation-fed wetlands (Chapter 3), precipitation-fed wetlands have the potential to remove sulfate, as observed in push-pull experiments. It is likely that sulfate reduction in precipitation-fed wetlands is limited by low sulfate concentrations, shifting the microbial metabolism towards respiration based on other electron acceptors, or methanogenesis.

Iron reduction was measurable mainly in precipitation-fed weltands, but it was not a major process for carbon flow compared to the others measured in this study (Chapter 3). However, iron reduction may be important in wetlands which experience episodic drying.

Methanogenesis is an important process with regard to the overall anaerobic metabolism, even in through-flow wetlands where loading rates of nitrate and sulfate are relatively high and continuous. This suggests that these wetlands will be sources of atmospheric methane irregardless of hyrologic settings. This finding may appear disadvantageous in light of methane's role in global warming. However, considering the value of these wetlands to maintain water quality, store carbon, and provide refuges for species diversity and wildlife, it is clear that these wetlands, although small, can play an important role in the landscape of SW Michigan.

The results of this research raise new questions that would be worthwhile to pursue. First, how does anaerobic metabolism control the biogeochemical functions in the transitional areas from upland to wetland, especially in groundwater-fed systems? Local groundwater is high in nitrate and sulfate, but in the central areas of wetlands nitrate concentrations were always depleted in porewater and ambient levels of denitrification were undetectable. A better understanding of nitrogen cycling could be determined by examining denitrification along transects from uplands to wetland centers, with emphasis on wetland fringes. Second, what is the role of anaerobic microbial metabolism in the overall carbon budgets for wetlands in southwest Michigan? This research, while exploring processes that affect carbon flow, was not an attempt to determine overall carbon budgets in our wetlands. Acetate turnover can estimate the total amount of carbon that flows through anaerobic decomposition, but gives no indication of the amount of primary productivity or organic matter accumulation. In addition, carbon budgets for wetlands typical to this area appear to be absent in the literature, especially for precipitation-fed systems that are not bogs.