Rapid Removal of Nitrate and Sulfate in Freshwater Wetland Sediments

Stefanie L. Whitmire and Stephen K. Hamilton*

ABSTRACT

Anaerobic microbial processes play particularly important roles in the biogeochemical functions of wetlands, affecting water quality, nutrient transport, and greenhouse gas fluxes. This study simultaneously examined nitrate and sulfate removal rates in sediments of five southwestern Michigan wetlands varying in their predominant water sources from ground water to precipitation. Rates were estimated using in situ push–pull experiments, in which 500 mL of anoxic local ground water containing ambient nitrate and sulfate and amended with bromide was injected into the near-surface sediments and subsequently withdrawn over time. All wetlands rapidly depleted nitrate added at ambient ground water concentrations within 5 to 20 h, with the rate dependent on concentration. Sulfate, which was variably present in porewaters, was also removed from injected ground water in all wetlands, but only after nitrate was depleted. The sulfate removal rate in ground water–fed wetlands was independent of concentration, in contrast to rates in precipitation-fed wetlands. Sulfate production was observed in some sites during the period of nitrate removal, suggesting that the added nitrate either stimulated sulfur oxidation, possibly by bacteria that can utilize nitrate as an oxidant, or inhibited sulfate reduction by stimulating denitrification. All wetland sediments examined were consistently capable of removing nitrate and sulfate at concentrations found in ground water and precipitation inputs, over short time and space scales. These results demonstrate how a remarkably small area of wetland sediment can strongly influence water quality, such as in the cases of narrow riparian zones or small isolated wetlands, which may be excluded from legal protection.

WETLANDS are often situated at points of ground water recharge and discharge or along streams and rivers, and thus biogeochemical processes in wetlands can affect downstream water quality, as for example by removing nutrients (Peterjohn and Correll, 1984; Tobias et al., 2001a; Zedler, 2003). Nitrate (NO_3^-) removal by wetlands has received particular attention because of the escalating problem of NO_3^- contamination of drinking water supplies, and the growing recognition that nitrogen (N) pollution of rivers causes eutrophication of marine coastal waters, leading to problems such as harmful algal blooms and oxygen depletion (Howarth et al., 1996; Mitsch et al., 2001). Most of the NO_3^- removal is attributed to denitrification, a form of anaerobic bacterial respiration and a major source of atmospheric nitrous oxide (N_2O), a potent greenhouse gas (Groffman et al., 2000).

The terminal steps of anaerobic microbial decomposition can occur via several alternative processes, including denitrification, sulfate (SO_4^{2-}) reduction, and methanogenesis (Fenchel et al., 1998; Megonigal et al., 2004), and thermodynamic constraints on energy yields determine the competitive ability of the microbes that perform these processes. In anaerobic sediments, denitrification is the most energetically favorable form of respiration; SO_4^{2-} reduction yields less energy and hence tends to occur when NO_3^- is not available. Sulfate removal would thus be expected to occur sequentially after depletion of NO_3^-, unless there are other potential electron acceptors such as iron and manganese, and all of these forms of anaerobic respiration tend to preclude methanogenesis.

Anaerobic respiration in wetlands is potentially stimulated by elevated loading of NO_3^- and SO_4^{2-} as a result of air and water pollution, and the resultant removal of NO_3^- and SO_4^{2-} can help ameliorate this pollution (Kelly and Rudd, 1984; Mitsch et al., 2001; Hey, 2002). Ground water in agricultural landscapes is often enriched in NO_3^- and SO_4^{2-} due largely to fertilizer and animal waste inputs. In addition to ground water inputs, rates of loading of NO_3^- and SO_4^{2-} to wetlands via atmospheric deposition are greatly enhanced due particularly to fossil fuel combustion (Boyer et al., 2002; Mayer et al., 2002). These increased loadings represent a biogeochemical perturbation with interesting consequences for ecosystems, including greater N availability for plant growth, albeit sometimes to the point of toxicity (Fenn et al., 1998), as well as acidification of poorly buffered waters and soils and enhancement of redox transformations, all with multifarious impacts on elemental cycling. Greenhouse gas emissions are also potentially affected by the increased production of N_2O, as well as inhibition of methanogenesis by the competitive superiority of denitrifiers and SO_4^{2-} reducers (Conrad, 1996).

A critical question for wetland management and protection is the role of these anaerobic processes in reducing N and S pollution and thereby providing improved water quality at the landscape level, but our understanding has been limited by the difficulty of measuring in situ process rates and the tendency for investigations to focus on only one of these processes. Many methods have been used to estimate the nature and rates of anaerobic decomposition in sediments, including analysis of dissolved H_2 concentrations (Lovley and Goodwin, 1988; Lovley et al., 1994), molecular methods (Muyzer and Smalla, 1998), lab assays in microcosms (Groffman et al., 1996; Roden and Wetzel, 1996; D'Angelo and Reddy, 1999), and whole-ecosystem isotope tracer studies (Tobias et al., 2001a; Mulholland et al., 2004). Results drawn from these approaches can be affected by distur-

W.K. Kellogg Biological Station and Department of Zoology, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, MI 49060. S.L. Whitmire, current address: Universidad de Puerto Rico, Recinto Universitario de Mayagüez, Departamento de Agronomía y Suelos, PO Box 9030, Mayagüez, PR 00681-9030. Received 2 July 2004. *Corresponding author (Hamilton@kbs.msu.edu).

Abbreviations: DNRA, dissimilatory reduction of nitrate to ammonium; DOC, dissolved organic carbon; KBS, W.K. Kellogg Biological Station.

bance of the natural environment, contamination by oxygen, and introduction of high concentrations of substrate. Whole-ecosystem isotope tracer studies can yield spatially integrated estimates of in situ rates without experimental artifacts, but are costly and would be difficult to perform in many wetland situations. Smaller scale, in situ tracer methods can be used in diverse environments to estimate microbial process rates, and are less apt to alter natural conditions than traditional lab assays. "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, have 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).

The purpose of this study was to investigate rates of removal of NO_3^- and SO_4^{2-} from small wetlands of variable hydrology in the glacial landscape of southwestern Michigan. We employed push–pull tracer experiments to evaluate the removal of NO_3^- and SO_4^{2-} from ground water as it comes into contact with reduced wetland sediment. This is a measurement of the potential reaction rates insofar as the ground water is relatively enriched in nitrate and to a lesser extent sulfate compared to the sediment porewaters. The level of enrichment produced is, however, within the bounds of what wetland sediments may experience as a result of natural ground water flow or precipitation events, and thus the rates do not indicate the maximum potential under conditions of nonlimiting reactants (electron acceptors and donors). The results demonstrate the capacity of wetland sediments to rapidly deplete added NO_3^- and SO_4^{2-}, and the time course of the depletion provides evidence of interactions between N and S cycling in these sediment environments, raising new questions meriting further investigation. The short time and space scales over which removal was observed underscore the potential importance of narrow riparian wetlands along headwater streams and small isolated depressions in providing this ecosystem service; protection and management of such ecosystems may therefore deserve more attention.

MATERIALS AND METHODS

Study Sites

The wetlands in this study lie on the glacial landscape around the W.K. Kellogg Biological Station (KBS), an aca-

demic unit of Michigan State University located in southwestern Michigan between the cities of Kalamazoo and Battle Creek. Wetlands in this region are abundant and cover about 10% of the landscape. These wetlands are embedded in a mosaic of row-crop agricultural areas, forests, and abandoned fields, with only limited residential development. The diverse wetlands in this region include numerous isolated, small depressions that lack surface inflows and outflows but have varying degrees of exchange with the ground water. Most local wetlands receive at least some ground water discharge and many are situated at points of ground water discharge into streams and lakes.

Experiments were performed in five wetlands spanning a range of hydrology and vegetation. Two of the wetlands have ground water as their main water source (Loosestrife Fen and Turkey Marsh), and the other three receive most of their water from precipitation (Shaw Pond, Lux Pond 10, and LTER Kettle Pond), as confirmed using dissolved magnesium as a tracer (Stauffer, 1985; Whitmire, 2003). Wetland characteristics are summarized in Table 1, and brief descriptions of each site follow.

Loosestrife Fen is a small ground water–fed wetland located at the W.K. Kellogg Experimental Forest. This wetland was created by several decades of organic sediment infilling behind a small earthen dam. It was dominated by muskgrass (*Chara* spp.) and purple loosestrife (*Lythrum salicaria* L.), with muskgrass occupying the shallow open water area in about half of the wetland and purple loosestrife growing on bottlebrush sedge (*Carex hystericina* Muhl. ex Willd.) tussocks in the other half. Sampling sites, which were located between tussocks or in the areas where muskgrass was present, were overlain by a few centimeters 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. Ground water enters from a spring that drives surface water flow across the wetland, and this site has been continuously flooded since observations began in 1996. Surrounding uplands are presently used for forestry.

Turkey Marsh is a small, isolated depression 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 common winterberry (*Ilex verticillata* L.). This wetland's water levels and flooded area have fluctuated considerably over the past few years, ranging from 0 to 50 cm over the sediment surface. During the initial years of higher water levels, the vegetation was mainly composed of a mixture of submersed aquatic plants and spatterdock [*Nuphar advena* (Aiton) WT. Aiton], but after drying in 2000 the vegetation changed to include more emergent plants such as reed canarygrass (*Phalaris arundinacea* L.), hairy sedge (*Carex lacustris* Willd.), and rice cutgrass [*Leersia oryzoides* (L.) Sw.], with some scattered spikerushes (*Eleocharis* spp.) and broadleaf cattail (*Typha*

Table 1. Surface water and sediment characteristics of the study sites. Surface water and sediment porewater measurements are means from several sampling dates. Organic carbon means are from three replicate cores. The NO_3^- concentrations were usually below detection (10–15 µg N L^{-1}) in wetland waters. Standard deviations are given in parentheses.

Site	Area	Predominant water source	Surface water conductance (25°C)	Sediment pH	Sediment organic carbon	Surface water SO_4^{2-}	Porewater SO_4^{2-}
	ha		µS cm^{-1}		%	mg L^{-1}	
Loosestrife Fen	0.4	ground water	520 (34)	6.6 (0.9)	31 (22)	15.6 (8.5)	0.13 (0.15)
Turkey Marsh	3.1	ground water	370 (48)	6.9 (0.6)	61 (9.5)	18.2 (6.0)	0.99 (0.71)
LTER Kettle	0.4	precipitation	44 (11)	5.3 (0.2)	17 (17)	4.98 (2.30)	0.28 (0.24)
Shaw Pond	0.8	precipitation	34 (4)	5.4 (0.2)	46 (17)	2.07 (0.93)	0.16 (0.14)
Lux Pond 10	0.7	precipitation	26 (2)	5.6 (0.1)	7.3 (4.3)	1.88 (0.92)	0.63 (1.18)

latifolia L.). Agricultural fields and successional forests surround this site.

The LTER Kettle Pond lies in a small, isolated depression on the KBS Long-Term Ecological Research site. The slopes around the pond are covered by deciduous forest and conifer plantations, and agricultural row crops are grown on surrounding uplands. The pond's plant community was dominated by duckweeds (*Wolffia* and *Lemna* spp.) during wet years (1996–1999), but after drying completely in 1999 the basin filled with blunt spikerush [*Eleocharis obtusa* (Willd.) Schult.], rice cutgrass, and reed canarygrass. 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 Pond 10 is the southeastern-most pond located on the KBS Lux Arbor Reserve. The pond lies in an isolated depression surrounded by deciduous forest. Lux Pond 10 had mainly open water with a few water lilies (spatterdock) and little submersed aquatic vegetation. The banks were lined by a mix of rice cutgrass, hairy sedge, broadleaf arrowhead (*Sagittaria latifolia* Willd.), and spikerushes. The water levels in Lux Pond 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. Successional forests and fields surround this site.

Shaw Pond is an isolated depression west of Otis Lake in the Barry Game State Area, about 22 km north of KBS. This pond is surrounded by deciduous forest on sandy soils. The dominant vegetation during wet years was a diverse mix of submersed and emergent aquatic plants [particularly fragrant water lily (*Nymphaea odorata* Aiton), spatterdock, and pondweeds (*Potamogeton* spp.)]. There were also some emergent grasses and spike rushes that encircle the wetland, 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 to 75 cm deep. All sampling points remained underwater during the sampling periods of this study.

Experimental Procedures

To determine the potential of various wetlands to remove NO_3^- and SO_4^{2-} from inflowing waters via denitrification and sulfate reduction, we injected local ground water containing NO_3^- and SO_4^{2-} into wetland sediments in the field using a modification of the push–pull method previously used to study biogeochemical reaction rates in contaminated aquifers. The injections were done during the summer and thus represent a synoptic survey during the season of mild temperatures and high biological activity. The experimental injection involves a "push" and a "pull" phase (Snodgrass and Kitanidis, 1998). In the "push" phase, a solution containing a reactive solute and a conservative tracer is injected (pushed) into the saturated zone of the sediments. The injection solution is dispersed and diluted as it mixes with the ambient porewater. Concentrations of the reactant may be changed by both microbial activity and dilution, and the dilution occurs by both advection and diffusion. 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. The conservative tracer is used to account for dilution. Comparison of the concentrations of the reactive solute to those of the conservative solute tracer reveals the net flux of the reactive solute.

In situ push–pull experiments were conducted at three of the five wetlands in 2001 and all five wetlands in 2002. Two representative sites were chosen within the central wet area of each wetland, in areas free of plant roots. To prepare the injection solution, untreated ground water was collected from a residential well at KBS (NO_3^-–N = 14–16 mg L^{-1}, SO_4^{2-} = 53–69 mg L^{-1}, dissolved organic carbon [DOC] = 0.6–1.0 mg L^{-1}; analytical methods given below) and stored at 4°C until the push–pull experiment. The ground water was amended immediately before each experiment with 13 to 20 mg L^{-1} Br^- (as NaBr) to serve as a conservative solute tracer. Higher Br^- concentrations (50 mg L^{-1}) were used for Loosestrife Fen to ensure that concentrations would remain measurable in the face of the anticipated advective loss from the higher flow at that site.

The push–pull well screens were small stainless steel mesh filters (6-mm i.d., 7.6-cm screen length; no. 4258; American Science & Surplus, Skokie, IL) attached to about 1 m of Teflon tubing (3.2-mm outside diameter). Wells were inserted vertically to a depth of 10 cm below the sediment surface (screen depth was 6–13 cm deep after installation) at least 2 d before the 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. Porewater was withdrawn to measure ambient concentrations of NO_3^-, SO_4^{-2}, Br^-, and DOC. Sediment temperature profiles were measured at each well site using a probe that was inserted nearby.

Before injection, the ground water solution was sparged with ultrapure He for at least 1 h to remove dissolved oxygen. Five hundred milliliters of the solution was transported to the field in a set of 60-mL syringes. The anoxic solutions were pushed by syringe through the wells and into the sediment over the course of 10 to 15 min (33–50 mL min^{-1}).

Samples were immediately withdrawn by syringe after the push phase and were periodically withdrawn over time for up to 48 h 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 was collected for analysis. The samples were filtered in the field through 0.2-μm membrane (sterile Millex-gs; Millipore, Billerica, MA) syringe filters and cooled to 4°C until analysis. Samples were analyzed for Br^-, NO_3^-, and SO_4^{2-} using membrane-suppression ion chromatography. Selected samples were also analyzed for DOC by high-temperature platinum-catalyzed combustion to CO_2 followed by infrared gas analysis.

For each experiment, we calculated NO_3^- and SO_4^{2-} removal rates based on Br^-, NO_3^-, and SO_4^{2-} concentrations (Snodgrass and Kitanidis, 1998). 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. Linear regressions were performed in SYSTAT 9.0 software (Systat, 1998), and the regression slopes were always significant at $P < 0.05$. For the case of these enzymatically catalyzed reactions, an apparent zero-order fit would represent a reaction that would be first-order at lower concentrations of NO_3^- or SO_4^{2-}, but has become saturated (i.e., limited by some other factor, such as labile organic matter) at higher concentrations of NO_3^- or SO_4^{2-}.

To fit the model for zero-order reactions, the concentrations of the reactive solute (NO_3^- or SO_4^{2-}) were transformed to remove the effect of dilution as follows:

$$\hat{C}_{\text{reactant}}(t) = C_{\text{reactant}}^0 \cdot \left[\left(\frac{C_{\text{reactant}}(t)}{C_{\text{reactant}}^0}\right) - \left(\frac{C_{\text{tracer}}(t)}{C_{\text{tracer}}^0}\right) + 1\right] \quad [1]$$

where C_{reactant}^0 and C_{tracer}^0 are the concentrations of the reactive and conservative solutes at injection and $C_{\text{reactant}}(t)$ and $C_{\text{tracer}}(t)$ are the concentrations measured at time t (Snodgrass and Kitanidis, 1998). All concentrations were background-corrected based on the initial sampling before injection. The rate of decrease in $\hat{C}_{\text{reactant}}(t)$ (i.e., the removal rate) is described by the zero-order decay equation:

$$\frac{\partial \hat{C}_{\text{reactant}}(t)}{\partial t} = -a \quad [2]$$

The plot of $\hat{C}_{\text{reactant}}(t)$ versus time will fall on a straight line, with the linear 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 removal were better fit to the first-order reaction model, the change in concentration of the reactant $C_{\text{reactant}}(t)$ can be modeled using an exponential function:

$$C_{\text{reactant}}(t) = C_{\text{tracer}}(t) \cdot e^{-kt} \quad [3]$$

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

The analyses described above assume that: (i) the solutes are injected simultaneously in a well-mixed slug; (ii) the time required to inject the solution is short compared to the overall length of the experiment; (iii) the dominant processes are advection, dispersion, and constant-coefficient zero- or first-order reactions; (iv) the background concentrations of SO_4^{2-}, Br^-, and NO_3^- are negligible or are subtracted from concentrations measured during the experiment; and (v) if flow is heterogeneous then samples collected earlier are more representative of in situ processes than samples collected later. The injected solution was well-mixed before injection and the injection time was short compared to the total length of the experiment. Background concentrations of NO_3^- were always negligible compared to the ground water, and SO_4^{2-} concentrations were corrected for background levels when they were measurable. Heterogeneous flow is unlikely to be a significant factor over the small spatial scale and short time course of these experiments.

To be sure that there were no significant reactions of the conservative tracer Br^- with wetland sediments, we added Br^- at concentrations similar to those used in the push–pull experiments to sediments from several representative sites in sealed 1-L glass jars. Following addition of the Br^- and thorough mixing, porewaters were subsampled immediately, then after 24 h and 1 wk.

RESULTS

All wetlands had the potential to remove NO_3^- and SO_4^{2-} and did so in the order predicted by thermodynamics (NO_3^- first, followed by SO_4^{2-}). Nitrate disappeared rapidly without any lag time and was depleted to below detection limits (10–15 μg N L^{-1}) within 5 to 20 h (see examples in Fig. 1 and 2). Sulfate removal commenced only after NO_3^- had been depleted in 14 of the 16 experiments done in the five wetlands. In 9 of the 16 experiments, including the example in Fig. 2A, apparent SO_4^{2-}
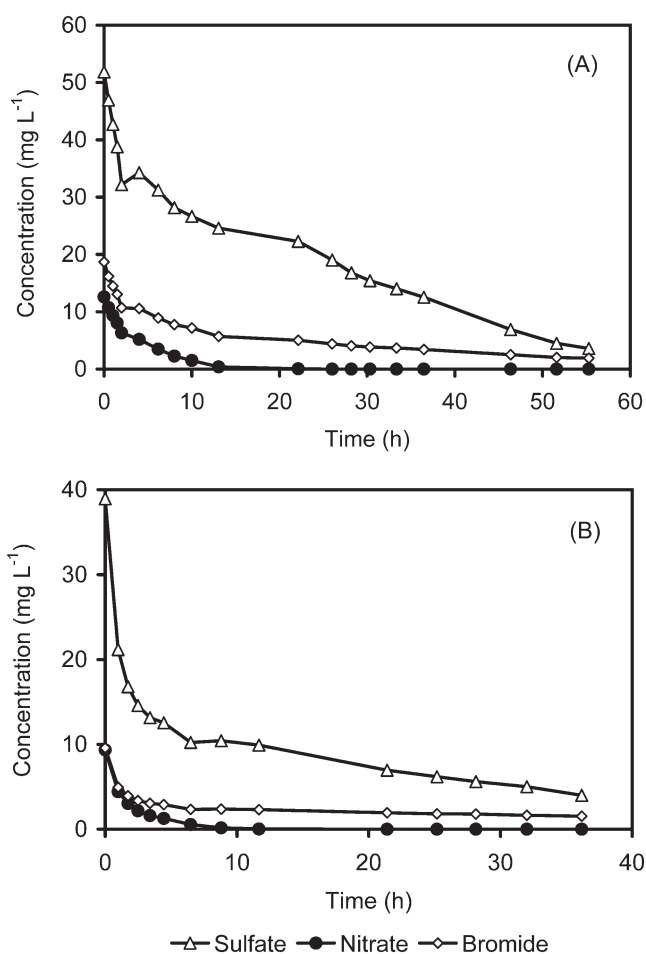

Fig. 1. Reactive (NO_3^- and SO_4^{2-}) and conservative (Br^-) tracer concentrations in two push–pull experiments representing wetlands of contrasting hydrology: (A) ground water–fed Turkey Marsh and (B) precipitation-fed Lux Pond 10.

production was observed, as evidenced by the initial increase in the ratio of SO_4^{2-} to bromide, and this increase always corresponded with the phase of rapid NO_3^- removal. Porewater temperatures at the depth of injection only ranged from 17.5 to 22.3°C and do not explain the observed differences in removal rates among sites.

Nitrate removal occurred as a first-order reaction (i.e., removal was proportional to concentration) in all but one case (Table 2). During 2002, in the ground water–fed Loosestrife Fen, NO_3^- disappeared by zero-order reaction kinetics, and therefore this experiment was left out of statistical analyses comparing first-order reaction rates. The other wetlands varied in their potential to remove NO_3^- but the differences were not significant (ANOVA, $P = 0.076$). There was no difference between wetlands grouped by ground water and precipitation water sources ($P = 0.37$).

Sulfate removal rates exhibited either zero- or first-order reaction rate kinetics depending on the water source of the wetland. In the ground water–fed wetlands, SO_4^{2-} removal was a zero-order reaction (i.e., independent of concentration), while in precipitation-fed wetlands SO_4^{2-} was removed by first-order reaction kinetics (Table 3). The ground water–fed Turkey Marsh
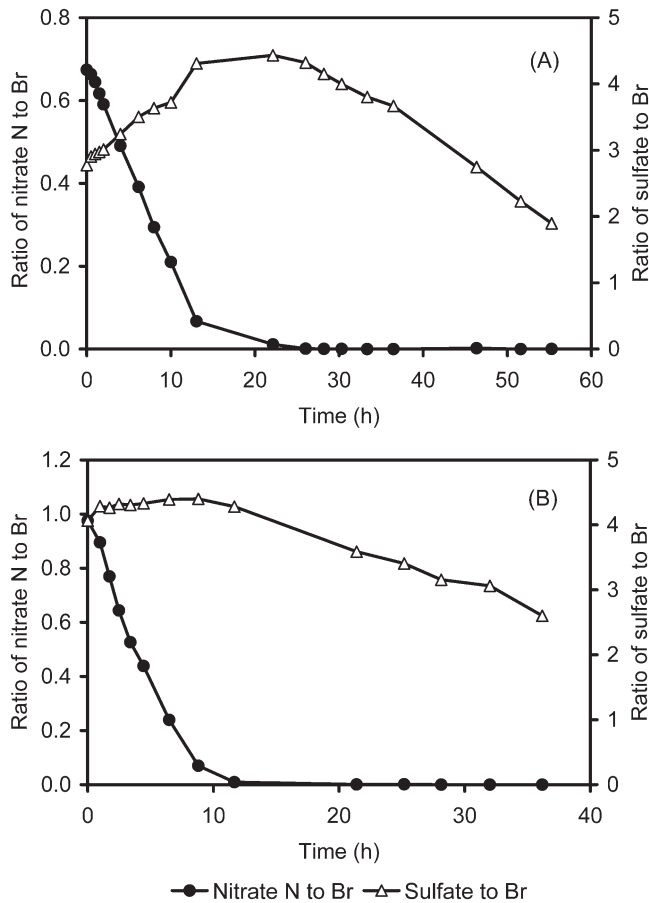
Fig. 2. Ratios of reactant to conservative tracer concentrations in two push–pull experiments representing wetlands of contrasting hydrology: (A) ground water–fed wetland Turkey Marsh and (B) precipitation-fed Lux Pond 10.

had significantly higher rates of SO_4^{2-} removal than ground water–fed Loosestrife Fen ($P = 0.014$), but there were no significant differences among the precipitation-fed wetlands ($P = 0.18$).

Dissolved organic carbon measurements indicate that the porewater DOC pool was diluted on injection of ground water into the sediments (data not shown). In all cases DOC concentrations decreased after injection and then returned to concentrations close to background toward the end of the experiment. We did not determine how labile the DOC in the injection solution was compared to DOC in the sediment porewater, but it seems likely that the injected ground water did not

Table 2. Nitrate removal rates from ground water injected into wetland sediments. Rates displayed first-order kinetics except for Loosestrife Fen in 2002. Values are means (standard deviations) for two sites in each wetland (total of 16 experiments).

Site	NO₃–N k values	
	2001	2002
	h⁻¹	
Loosestrife Fen	0.50 (0.18)	0.30 (0.03)†
Turkey Marsh	0.15 (0.02)	0.23 (0.10)
LTER Kettle Pond		0.47 (0.37)
Shaw Pond	0.25 (0.16)	0.045 (0.04)
Lux Pond 10		0.55 (0.12)

† Rate in mg L⁻¹ h⁻¹, from zero-order reaction model.

Table 3. Sulfate removal rates from ground water injected into wetland sediments. Reaction rates judged to be zero-order are expressed as α values, whereas first-order rates are expressed as k values. Values are means (standard deviations) for two sites in each wetland (total of 16 experiments).

Site	SO₄²⁻ α values		SO₄²⁻ k values	
	2001	2002	2001	2002
	mg L⁻¹ h⁻¹		h⁻¹	
Loosestrife Fen	0.15 (0.01)	0.30 (0.14)		
Turkey Marsh	0.25 (0.07)	0.24 (0.014)		
LTER Kettle Pond				0.057 (0.052)
Shaw Pond			0.30 (0.05)	0.075 (0.3)
Lux Pond 10				0.12 (0.0)

contain enough labile carbon to stimulate microbial uptake compared to the ambient porewaters, which are situated in close proximity to the high biological productivity of wetland plants and algae.

Background concentrations of surface water and porewater SO_4^{2-} (Table 1) show that porewaters had markedly lower SO_4^{2-} concentrations than surface waters, even at sites that were close to 100% ground water–fed. Nitrate was below the ion chromatography detection limit of 10 to 15 μg N L⁻¹ in all background samples, and below or close to this limit in surface waters of these wetlands. An influx of surface water into the samples withdrawn during the experiments would not explain the observed increase in SO_4^{2-} concentrations during the period of NO_3^- removal because concentrations in overlying water were so low. Also, the appearance of the excess SO_4^{2-} does not correspond with the most frequent sampling, which would cause the greatest potential for surface water to be drawn into the sediments.

Incubation of representative wetland sediments in sealed jars with Br⁻ added to similar concentrations as used in the push–pull experiments showed no evidence for nonconservative behavior (Table 4). There were no statistically significant differences between sampling time points. The variation was probably due to imperfectly mixed porewaters in the jars and analytical error associated with the ion chromatography.

DISCUSSION

The push–pull experiments demonstrated that all of the wetlands have the potential to rapidly remove NO_3^- and SO_4^{2-} at concentrations found in ground water or precipitation inputs. Added NO_3^- and SO_4^{2-} were depleted within 5 to 20 h or a few days, respectively, demonstrating that only short residence times (and thus flow paths) are necessary for these sediments to produce

Table 4. Stability of bromide concentrations added to wetland sediments incubated in jars in the laboratory. Data are means from replicate sampling sites within each wetland ($n = 3$) with standard deviations given in parentheses. There were no significant changes over time.

Site	Br⁻		
	At beginning	At 24 h	At 1 wk
	mg L⁻¹		
Turkey Marsh	7.0 (0.5)	7.2 (0.3)	7.0 (0.6)
Shaw Pond	7.4 (0.9)	7.9 (0.4)	8.0 (0.6)
Lux Pond 10	8.8 (0.7)	8.3 (0.6)	8.4 (0.3)

significant improvement in water quality. Nitrate depletion commenced immediately in all wetlands, mostly by first-order reaction kinetics, indicating that NO_3^- removal in these wetlands was limited by NO_3^- availability and that increased NO_3^- inputs would stimulate greater removal. Sulfate removal was evidently not limited by concentration in the ground water–fed sites where continuous SO_4^{2-} inputs occur. The processes responsible for the rapid removal of NO_3^- and SO_4^{2-} warrant further consideration if we are to understand the controlling factors, which in turn determine the capacity of wetlands to remove NO_3^- and SO_4^{2-} as inputs are elevated by pollution, or as the hydrological and ecological characteristics of the wetlands are altered.

Processes of Nitrate Removal

Nitrate and SO_4^{2-} removal appeared to take place in accordance with thermodynamic theory, where terminal electron acceptors are consumed by anaerobic respiration in the order of their free-energy yield as microorganisms compete for labile products of organic matter decomposition. Since denitrification yields more energy in the process of anaerobic respiration, denitrifiers should have a competitive advantage and SO_4^{2-} reduction should be limited until NO_3^- has been depleted. All of the push–pull experiments clearly exhibited this response, with SO_4^{2-} removal only commencing after NO_3^- had been depleted (e.g., Fig. 2).

Microbial transformation is likely to be the principal process responsible for NO_3^- removal, as opposed to assimilation by microbes or plant roots. Sediment porewaters in these sites were generally very rich in ammonium (often between 200 and 1000 μg N L^{-1}), a preferred nitrogen source for assimilative uptake by microbes, and activity of plant roots was likely limited at most sites because to the extent possible we avoided sampling close to plants. While this was difficult in some sites (e.g., Turkey Marsh, where spatterdock rhizomes were abundant), other sites were devoid of nearby vegetation and yet the pattern of NO_3^- removal was similar across all sites. Furthermore, we have found similar uptake rates in intact, root-free sediment cores brought to the laboratory (A.J. Burgin and S.K. Hamilton, unpublished data).

The microbial transformation responsible for NO_3^- removal in wetlands and other saturated sediments is often assumed to be denitrification to N_2 (and N_2O as a byproduct) via anaerobic respiration (respiratory denitrification), in which NO_3^- serves as an alternate electron acceptor for the oxidation of organic matter, and either NO_3^- or labile carbon can be limiting factors. The apparent production of SO_4^{2-} observed in about half of our experiments as NO_3^- was removed suggests that at least part of the NO_3^- removal was somehow linked to SO_4^{2-} production or consumption. This observation is worth further consideration because it could indicate that the NO_3^- removal was not only due to respiratory denitrification, and if at least part of the NO_3^- is converted to ammonium, the removal may not be a permanent N sink.

Two alternative hypotheses could explain the coincidence of NO_3^- removal and SO_4^{2-} production: (i) denitrifiers inhibited SO_4^{2-} reducers by competing for substrate (i.e., H_2), allowing SO_4^{2-} that was continuously produced by oxidation of sulfide to accumulate as long as NO_3^- was available; or (ii) sulfur-oxidizing bacteria utilized the NO_3^- instead of O_2 to oxidize sulfide to elemental S and SO_4^{2-}, thereby producing either ammonium or N_2. The plausibility of the first hypothesis is supported by lab studies demonstrating that SO_4^{2-} reduction can be inhibited by the superior affinity of denitrifiers for H_2 (Klüber and Conrad, 1998). If that were the case, the SO_4^{2-} production we observed would have to reflect reduced SO_4^{2-} consumption in the face of natural rates of SO_4^{2-} influx, perhaps from overlying oxic zones, or in situ production. However, SO_4^{2-} concentrations in overlying waters were low compared to the added ground water, so the SO_4^{2-} may be more likely to have come from sulfide oxidation in the surficial sediment layers. Additional experiments using S isotope tracers would be required to test this hypothesis.

The possibility that sulfur-oxidizing bacteria used NO_3^- to oxidize sulfide or elemental S to SO_4^{2-} is supported by recent research, mostly in marine ecosystems (Fossing et al., 1995; Martin and Brigmon, 1994; Bonin, 1996; Phillipot and Højberg, 1999; Zopfi et al., 2001) but also in fresh waters (Megonigal et al., 2004). It remains uncertain whether S-oxidizing bacteria involved in NO_3^- uptake are denitrifiers (i.e., they reduce NO_3^- to N_2), or produce ammonium in a form of dissimilatory reduction of nitrate to ammonium (DNRA), and there is evidence that they may be able to switch between these pathways (Brunet and Garcia-Gil, 1996; Dannenberg et al., 1992; Otte et al., 1999).

If the S-driven NO_3^- uptake is a form of denitrification, the initial oxidation step may proceed with the following stoichiometry (Fossing et al., 1995):

$$5HS^- + 2NO_3^- + 7H^+ \rightarrow 5S_o + N_2 + 6H_2O$$

The resultant elemental S may be stored in the cells before being oxidized on to SO_4^{2-} in a separate step. Further oxidation of the elemental S to SO_4^{2-} could occur by this reaction (Fossing et al., 1995):

$$5S_o + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+$$
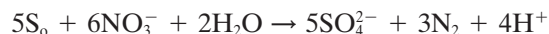
If these two reactions occurred sequentially, the molar ratio of NO_3^- consumed to SO_4^{2-} produced would be 8:5 (=1.6) as in this combined reaction:

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$$
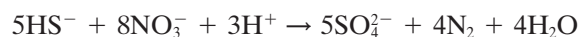
However, over the short time course of our experiments, it is possible that at least some of the sulfide could have been oxidized only to elemental S.

If sulfur-oxidizing bacteria used NO_3^- to carry out DNRA, the N would remain in biologically available form and thus the NO_3^- removal would not be permanent, in contrast to the denitrification reactions given above. Dannenberg et al. (1992) observed molar ratios of SO_4^{2-} production to NO_3^- consumption that were close to unity in cultures of *Desulfovibrio desulfuricans* that produced ammonium from NO_3^- in conjunction with sulfide oxidation. DNRA is generally thought to be sig-

nificant in highly reducing environments capable of maintaining sustained anaerobic metabolism (Tiedje, 1988), which might include the sediments of biologically productive wetlands. DNRA could be in direct competition with denitrification for NO_3^-, especially in anoxic saturated sediments (Nijburg et al., 1997). Even though the conditions for DNRA are similar to those for denitrification (reduced environment, available NO_3^-, and labile organic substrates), 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).

Few studies have examined S-driven DNRA in freshwater systems. Freshwater wetlands are low in S compared to marine systems, but can contain enough to support significant S transformations, as has been demonstrated near our study sites (e.g., Lovley and Klug, 1983). Brunet and Garcia-Gil (1996) found that in the water column of a stratified lake, NH_4^+ production coincided with NO_3^- and H_2S depletion. Dannenberg et al. (1992) showed that freshwater strains of SO_4^{2-} reducing bacteria were better able to perform S-driven DNRA than their marine counterparts. The recent profusion of ^{15}N isotope studies in diverse ecosystems has piqued interest in DNRA as an important ecosystem process because ^{15}N tracer flow from NO_3^- to NH_4^+ has been observed (e.g., Bonin, 1996; Tobias et al., 2001b; Silver et al., 2001; An and Gardner, 2002), although these studies have not elucidated whether the apparent DNRA was linked to S transformations. In contrast, Yin et al. (2002) found that DNRA could not account for most of the NO_3^- removal in freshwater sediments from rice paddies.

The difference between observed concentrations and those expected based on Br^- concentrations indicates the stoichiometry of SO_4^{2-} production compared to NO_3^- removal (Fig. 3). Expected concentrations were calculated as the product of the observed Br^- concentration and the ratio of NO_3^- or SO_4^{2-} to Br^- in the injection solution. Comparison of the mean differences between observed and expected concentrations of NO_3^- and SO_4^{2-} during the sampling period in which NO_3^- was detectable in the porewater indicates the potential importance of S oxidation coupled to NO_3^- removal (Table 5). Assuming the 1.6:1 stoichiometry for NO_3^- removal to SO_4^{2-} production in a denitrifying reaction, S oxidizers could account for 50 and 13% of the total NO_3^- removal in the two experiments, respectively. If on the other hand the reaction is a form of DNRA with 1:1 stoichiometry, S oxidizers could account for 31 and 8% of the total NO_3^- removal. These could be minimum estimates because of the possibility that some of the S oxidation does not proceed completely and appear as SO_4^{2-} in the porewater. Further evaluation of NO_3^- removal linked to S oxidation across a broader range of freshwaters is underway in our laboratory.

Porewater dissolved sulfide concentrations were measured in these wetlands using porewater equilibrators, and the sites with the most marked SO_4^{2-} production had relatively high sulfide concentrations compared to other local wetlands, generally ranging from 10 to 60
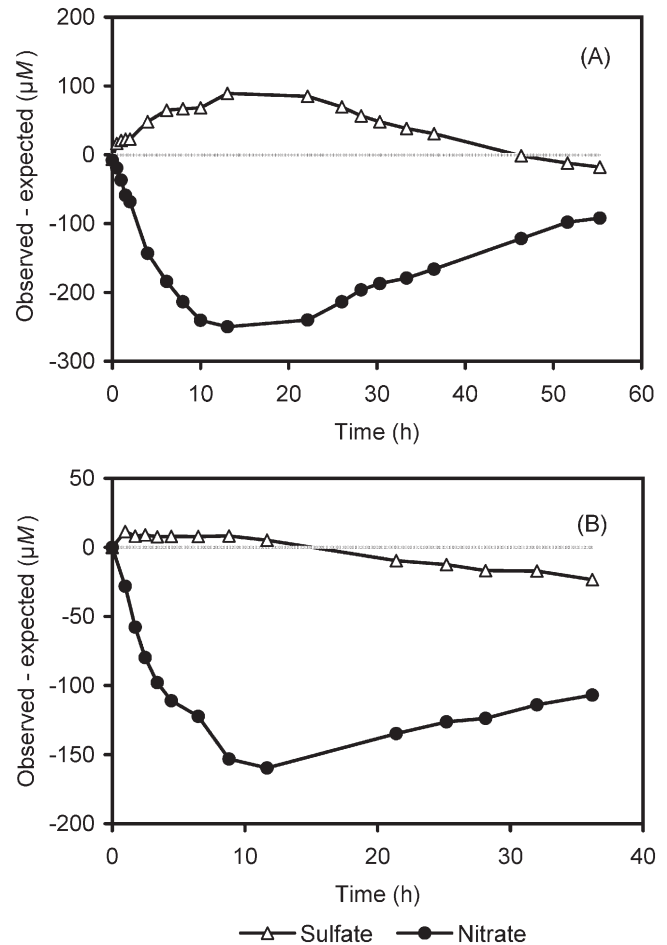

Fig. 3. Comparison of the difference between observed and expected concentrations of NO_3^- and SO_4^{2-} in the two examples of push–pull experiments that were shown in Fig. 1 and 2: (A) ground water–fed Turkey Marsh and (B) precipitation-fed Lux Pond 10.

μM in the upper 20 cm (Whitmire, 2003). Considering the SO_4^{2-} production in units of μM (Fig. 3), these sulfide concentrations cannot account for the apparent production of SO_4^{2-}. However, it is possible that specialized sulfide-oxidizing bacteria can sequester and accumulate NO_3^- to high levels for eventual use as an oxidant, as has been observed in benthic mats of marine S oxidizers (Fossing et al., 1995) and in a constructed wetland reactor with sulfur/limestone columns (Bezbaruah and Zhang, 2003).

Further studies are needed to reach a conclusion about the role of bacterial sulfur oxidation in NO_3^- removal, perhaps utilizing S and N isotopic tracers, but the temporal correspondence between NO_3^- removal and SO_4^{2-} production suggests that S bacteria could be significant in NO_3^- uptake. If so, then the proximate controlling factors could be rather different than if respiratory denitrification were the principal process of NO_3^- removal, and paradoxically, increased SO_4^{2-} loading to wetlands could prove to be linked to larger populations of sulfur-transforming bacteria that would contribute to rapid removal of episodic NO_3^- inputs. If, on the other hand, the observed SO_4^{2-} production proves to be caused by competitive interactions between denitrifiers and SO_4^{2-} reducers, then the increased denitrifi-

Table 5. Stoichiometric comparisons of NO_3^- removal to SO_4^{2-} production for the two examples shown in Fig. 1, 2, and 3. The mean difference between observed and expected concentrations is for the sampling period during which NO_3^- was detectable in the porewater. The % NO_3^- uptake by S oxidizers is estimated assuming either denitrification (yielding a 1.6:1 ratio of NO_3^- removal to SO_4^{2-} production) or dissimilatory reduction of nitrate to ammonium (DNRA) (1:1 ratio); it is not known which of these pathways would prevail.

Site	Observed − expected		NO_3^- uptake by S oxidizers	
	NO_3^-	SO_4^{2-}	1:6 ratio	1:1 ratio
	μ*M*		%	
Turkey Marsh	−160	50	50	31
Lux Pond 10	−101	8.2	13	8

cation caused by NO_3^- pollution may indirectly increase SO_4^{2-} pollution. Thus in either case there would be interesting linkages between N and S cycling that bear on water quality.

Variation in Nitrate Removal Rates among Wetlands

The precipitation- and ground water–fed wetlands had similar potential rates of NO_3^- removal. This is unexpected since concentrations of NO_3^- and SO_4^{2-} are higher in local ground water than in precipitation, and two of these wetlands are primarily ground water–fed. However, NO_3^- concentrations in surface-water and porewater samples were rarely above the analytical detection limit in the wetlands studied. An explanation for the consistently low ambient NO_3^- concentrations is that NO_3^- is efficiently removed from inflowing waters upstream of the sampling locations. Many studies in riparian areas have demonstrated that ground water entering the riparian zone can lose NO_3^- by plant and microbial uptake over short spatial scales (Seitzinger, 1988; Simmons et al., 1992; Hill, 1996; Hedin et al., 1998). In one study of a coastal marsh system, NO_3^- removal was 90% complete within the first 50 cm of the marsh near the upland boundary (Tobias et al., 2001b). Results from another study showed that a narrow riparian zone of a few meters width along a small stream was the most important location for NO_3^- removal (Hedin et al., 1998). Thus, NO_3^- might have been removed before water reached the more central sampling locations in this study. Episodic inputs of NO_3^- via precipitation falling directly onto the wetland might also be subject to rapid removal, especially in wetlands with shallow water columns where contact with the sediments is greater. The finding that potential NO_3^- removal rates are high where NO_3^- concentrations are evidently low most or all of the time suggests that NO_3^- is either produced and consumed in a tightly coupled way, that episodic inputs keep denitrifiers poised to utilize NO_3^-, or that sulfur-transforming bacteria can opportunistically switch to NO_3^- utilization (as discussed above).

Processes and Rates of Sulfate Removal

Sulfate removal always followed NO_3^- depletion, but the reaction kinetics differed depending on the water source for the wetland. Sulfate reduction via anaerobic respiration is the most likely process responsible for this removal, considering its delay relative to NO_3^- and the fact that plant uptake has little impact on SO_4^{2-} concentrations. Ground water–fed wetlands exhibited zero-order SO_4^{2-} removal rates, indicating that rates were independent of SO_4^{2-} concentrations, perhaps due to labile substrate limitation. Turkey Marsh had the highest rates of SO_4^{2-} removal, although based on measurements during a previous year using $^{35}SO_4^{2-}$, this wetland did not have the highest ambient rates of SO_4^{2-} reduction (Whitmire, 2003). Even though Turkey Marsh had higher rates, SO_4^{2-} removal began about 10 h later there than it did in Loosestrife Fen (16.3 ± 6.1 h vs. 6.4 ± 1.8 h, P = 0.020). This is probably because NO_3^- removal was faster in Loosestrife Fen, and SO_4^{2-} removal only commenced after NO_3^- was depleted.

There were no significant differences in potential SO_4^{2-} removal rates among the precipitation-fed wetlands, with an average SO_4^{2-} removal rate of 0.074 ± 0.04 h^{-1} (Table 3). This shows that SO_4^{2-} reduction can take place in response to SO_4^{2-} additions to precipitation-fed sites, even though ambient rates of SO_4^{2-} reduction were not measurable using $^{35}SO_4^{2-}$ in these wetlands (Whitmire, 2003). Microbial sulfate-reducing populations could be maintained in these systems in several ways. First, the in situ mineralization of organic sulfur compounds could allow SO_4^{2-} production and consumption to be tightly coupled (Fenchel et al., 1998). Second, SO_4^{2-} reduction potentials may be high in the precipitation-fed wetlands due to episodic inputs by atmospheric deposition of SO_4^{2-}. Sulfate deposition is relatively high in southwestern Michigan; SO_4^{2-} concentrations in precipitation at KBS average 2.7 mg L^{-1} (National Atmospheric Deposition Program/National Trends Network, 1997). Average SO_4^{2-} concentrations in the surface waters of these wetlands vary but are generally higher (Table 1), and hence surface water could provide a source of SO_4^{2-} to the sediments. Porewaters were always depleted in SO_4^{2-} relative to the overlying surface waters, suggesting consumption in the sediments. Over longer periods, diffusion between overlying water and shallow subsurface sediments could be an important source of SO_4^{2-} to the porewater environment, as has been found in shallow lakes (Kelly and Rudd, 1984).

Caveats Concerning the Bromide Tracer

Caveats concerning the use of Br$^-$ as a conservative tracer in push–pull experiments include its potential inhibitory effect on microbial processes (Groffman et al., 1995) and the possibility of sediment or plant uptake, which may confound results (Kung, 1990; Whitmer et al., 2000). Some inhibitory effects of Br$^-$ on microbial processes have been noted at high concentrations of Br$^-$ (100 mg L^{-1}), but in the experiments reported here Br$^-$ was added at lower concentrations (<20 mg L^{-1} in most cases). Also, the observation that NO_3^- depletion commenced immediately and that rates did not increase as the Br$^-$ was diluted suggests that Br$^-$ at the concen-

trations used in this study did not significantly inhibit microbial activity.

Sediment uptake of Br^- was not observed when we incubated saturated sediments from several wetlands in sealed jars with added Br^- (Table 4), and thus was unlikely to occur during the short-term experiments. Although the possibility of plant uptake of Br^- (and NO_3^- and SO_4^{2-}) cannot be excluded for all of the sites in this study, it is unlikely to be important. In all wetlands except Loosestrife Fen, about 50% of the Br^- was recovered, and these sites span a wide range of plant densities, including sites with essentially no roots in the sediments. We selected areas that were relatively free of plants for the injection sites. Since background porewater concentrations of Br^- were very low, it is more likely that dispersion (mixing with ambient porewaters) and diffusion away from the injection point explain the partial recovery. Advective loss was especially likely in Loosestrife Fen, where surface water flow was visible. Also, the potential NO_3^- and SO_4^{2-} removal rates observed in these wetlands were similar to those seen in contaminated aquifers (McGuire et al., 2002), where plant roots are not present and bacteria are the sole potential cause of biotic uptake or transformation.

Implications for Wetland Management

The rapid rates of NO_3^- and SO_4^{2-} removal demonstrate how very small areas of wetland sediment are capable of improving water quality, and such areas often occur at critical points of water flow between surface and ground water reservoirs. The ground water–fed wetlands have the potential to remove NO_3^- in ground water discharge before it enters streams and lakes. Precipitation-fed wetlands are more isolated and have less influence on surface water quality, but they have the potential to process water entering as runoff or precipitation before it eventually infiltrates to ground water systems, where excessive NO_3^- concentrations are a growing problem. Thus small isolated wetlands and riparian zones of headwater streams appear capable of significant water quality improvement, and if one goal of wetland protection is to preserve and enhance this ecosystem service, protection may need to be extended to these smaller wetland units.

This research underscores the need for further research on the microbial processes responsible for the NO_3^- removal. The ultimate fate of the NO_3^- that is removed remains uncertain because of the possibility that some is converted to ammonium rather than being denitrified, which would retain reactive N in the ecosystem. Seasonal variation in loading of NO_3^- and SO_4^{2-} as well as episodic inputs of these ions to wetlands may affect the nature and efficacy of microbial processes that remove them. In addition, the possible linkages of NO_3^- removal with sulfur transformations may prove important as a mechanism to oxidize reduced sulfur compounds to SO_4^{2-}.

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