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Sulfate reduction and diffusion in sediments of Little Rock Lake, Wisconsin

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Abstract

Rates of sulfate diffusion and reduction were measured in sediments of Little Rock Lake, an oligotrophic, soft-water lake in northern Wisconsin. Laboratory measurements of kinetics of sulfate reduction found half-saturation constants (20–30 μmol liter^{-1}) and Q_{10} values (2.6) similar to values reported in the literature. Sulfate reduction under in situ conditions in sediment cores was limited by sulfate and followed similar uptake kinetics as in laboratory experiments. Some variation in kinetic parameters was evident as a function of location in the lake. No seasonal variation was observed in sulfate reduction rates in the lake sediments, and littoral and pelagic sites exhibited similar rates. Rates of sulfate reduction were much higher than fluxes of sulfate calculated from pore-water profiles. Pore-water profiles also indicated little difference in diffusive fluxes among pelagic and littoral sites and among seasons. The discrepancy between diffusive fluxes and sulfate reduction rates is ascribed to high rates of oxidation of reduced sulfur. Nonlinear rates of sulfate reduction and calculated turnover times of sediment sulfide pools support the hypothesis that sulfide oxidation occurs nearly as rapidly as sulfate reduction.

There is a general perception that sulfate reduction is relatively unimportant for carbon oxidation in freshwater sediments. Although sulfate reduction accounts for 50–100% of anaerobic carbon oxidation in marine systems (e.g. Jørgensen 1989; Howarth 1984; Capone and Kiene 1988), it is thought to account for only 10–30% in hypolimnetic sediments of lakes (e.g. Kelly and Rudd 1984; Kuivila et al. 1989; Ingvorsen and Brock 1982). Even though freshwater sulfate-reducing bacteria have low half-saturation constants for both sulfate and acetate (e.g. Ingvorsen et al. 1981; Schoenheit et al. 1982; Lovley and Klug 1983) that enable them to outcompete methanogens in surface sediments, the relatively low diffusive inputs of sulfate to lake sediments limit the depth over which sulfate reduction can occur (Lovley and Klug 1983). In oligotrophic lakes, diffusive fluxes of sulfate more nearly equal rates of organic matter oxidation, and sulfate reduction is thought to account for a larger fraction of anaerobic carbon oxidation than in eutrophic lakes (Lovley and Klug 1983). The low concentrations of FeS in lake sediments relative to marine sediments (e.g. Berner 1984; Davison 1988; Urban 1994) are interpreted as another indication that little sulfide is produced from sulfate reduction in lakes.

Because rates of sulfate reduction in lake sediments are thought to be limited by diffusive inputs of sulfate (Lovley and Klug 1983), rates of diffusive influx often are equated to gross rates of sulfate reduction. Diffusive inputs of sulfate are low as a result of low concentrations of sulfate; concentrations in lakes (10–500 μmol liter^{-1}) are two to three orders of magnitude lower than in marine systems. Because net diffusive fluxes of sulfate into lake sediments are proportional to lake sulfate concentrations (e.g. Kelly et al. 1987; Baker et al. 1986), sulfate reduction has been modeled as a first-order process with respect to lake sulfate concentrations.

For diffusive fluxes of sulfate into lake sediments to equal gross or total rates of sulfate reduction, other sources of and sinks for sulfate within sediments must be negligible. Landers and Mitchell (1988) demonstrated that formation of sulfate esters could be an important sink for sulfate in sediments. Possible sources for sulfate in sediments include hydrolysis of such sulfate esters and oxidation of reduced

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sulfur. King and Klug (1982) have shown that hydrolysis of sulfate esters provides a negligible contribution of sulfate to sulfate-reducing bacteria in Wintergreen Lake. To date, however, there has been no quantification of the rate of regeneration of sulfate in sediments via sulfide oxidation.

Several recent reports suggest that regeneration of sulfate in sediments may be important. First, rates of sulfide production typically are much higher than rates of reduced-sulfur accumulation in sediments (e.g. Chanton et al. 1987; Berner and Westrich 1985). Second, directly measured gross rates of sulfate reduction (e.g. Dunnette 1989; Kuivila et al. 1989; King and Klug 1982) are much higher than diffusive fluxes of sulfate into lake sediments (Urban 1994). Such high rates of sulfate reduction can be maintained only if another source of sulfate exists besides diffusion from the lake water. Biotic and abiotic reduction of iron and manganese oxides by sulfide can produce polysulfides, elemental sulfur, thiosulfate, polythionates, sulfite, and sulfate (e.g. dos Santos Afonso and Stumm 1992; Pyzik and Sommer 1981; Aller and Rude 1988; Burdige and Nealson 1986). Intermediate oxidation states of sulfur do not accumulate in sediments; if they are formed they must be further oxidized to sulfate or reduced again to sulfide. Rapid rates of sulfide oxidation have been measured in anaerobic marine and lake sediments (Elsgaard and Jørgensen 1992; Jørgensen 1990*b*; Jørgensen and Bak 1990). In the studies of Jørgensen (1990*b*) and Jørgensen and Bak (1990), elemental sulfur and thiosulfate were identified as initial products of sulfide oxidation; subsequent disproportionation and oxidation of both of these products produced sulfate. In the study of Elsgaard and Jørgensen (1992), thiosulfate again was shown to be an important intermediate, but they inferred that a pathway for oxidation of sulfide to sulfate without formation of thiosulfate was also important. The quantitative significance of sulfide oxidation as a source of sulfate in lake sediments has yet to be determined.

The objectives of this study were to determine the rates of sulfate reduction and the controls on this process in sediments of Little Rock Lake, Wisconsin. Rates of sulfate reduction, measured in intact sediment cores under in situ conditions, were compared in littoral and pelagic sediments during different seasons to examine effects of sulfate concentrations, organic carbon availability, and temperature. Responses to temperature and sulfate concentration also were measured in sediment slurries in the laboratory. Diffusive fluxes of sulfate into the sediments were calculated from pore-water profiles measured in multiple locations during all seasons. Results indicate that sulfate reduction is much faster than sulfate diffusion into the sediments, that sulfate reduction is limited by sulfate concentration at any given depth within the sediment, and that seasonal variations in rates of sulfate reduction and diffusion are minor. From these results we infer that reoxidation of sulfide is an important mechanism for maintaining the supply of sulfate in the sediments.

Methods

Site description—Little Rock Lake is a small (17 ha) seepage lake in northern Wisconsin (45°59′N, 89°42′W). The lake has no stream inlets or outlets and receives ~99% of water inputs from direct precipitation and the remainder from groundwater inflow. Consequently, the lake water is very low in dissolved solids (ionic strength = 0.002 mol liter^{-1}) and alkalinity (25 μeq liter^{-1}). The lake has two basins of approximately equal surface area; the north basin has a maximum depth of 10 m (mean depth, 3.8 m) and the south basin has a maximum depth of only 6.3 m (mean depth, 3.1 m). The lake is dimictic and both basins stratify weakly in winter, but the shallowness of the south basin prevents formation of any significant hypolimnion in summer. The small hypolimnion (8% of basin volume) in the north basin does experience oxygen depletion and becomes anoxic in some years. The lake has been the site of an experimental acidification project (Brezonik et al. 1986) since 1983. The two basins were divided from each other at a narrow point with a polyvinyl curtain in 1984. From 1985 to 1991, the north basin was acidified to successively lower pH values (5.6, 5.1, and 4.7 each for 2-yr periods) by adding concentrated sulfuric acid. Experimental acidification caused an increase in sulfate concentration in the north basin from 28 to 70 μM. The south basin received no acid and remained at its initial pH (6.1) and sulfate concentration (28 μM). Additional information on the lim-

nology, sediment, and water chemistry of the lake can be found elsewhere (e.g. Brezonik et al. 1986, 1993; Baker et al. 1989, 1992).

Pore-water profiles—Profiles of sulfate in pore water were measured frequently over the 5-yr period from 1983 to 1988 (Sherman et al. 1994; Weir 1989; Perry 1987). Pore-water equilibrators (Hesslein 1976) were installed at five locations in the lake on multiple occasions. The equilibrators, fitted with a plastic grate to hold them at a fixed depth in the soft pelagic sediments, were left in the lake for 3 weeks. Sites included littoral (1-m water depths in both basins, 3.5 m in the south basin) and pelagic (9 and 7 m in the north basin, 5 m in both basins) sediments. Littoral sediments are 90% sand; the pelagic sediments contain >40% organic matter and have a porosity >90%.

Following retrieval of equilibrators from the lake, pore-water samples were withdrawn and preserved within 45–60 min. Samples for redox-sensitive species (Fe, Mn, H_2S) were removed first, preserved with 0.3 M HCl (metals) or 0.04 M ZnAc (sulfide), and measured within 2 d. Sulfate was measured by ion chromatography, sulfide and dissolved iron by colorimetry (Cline 1969; Am. Public Health Assoc. 1984), and Mn by atomic absorption spectrophotometry with graphite furnace.

Fluxes of sulfate to the sediments were calculated from 37 profiles based on Fick's first law and the assumption that the profiles represent steady state conditions. Diffusion coefficients (from Li and Gregory 1974) were corrected for temperature and porosity (measured by weight loss of sediments upon drying). A discussion of the concentrations and fluxes of all measured ions in these same pore-water profiles is given by Sherman et al. (1994).

Measurement of sulfate reduction in intact sediment cores—To measure gross or instantaneous rates of sulfate reduction, we took short cores from the lake; they were injected with trace amounts of $^{35}SO_4^{2-}$, incubated at lake temperatures for 0–17 h, and analyzed for reduced ^{35}S species (Jørgensen 1978). Cores were collected on three occasions (August, October, and March) to study the influence of season and temperature. The lake was weakly stratified and ice-covered in March, strongly stratified in August, and isothermal in October. Sediment temperatures on these dates were 4, 23, and 11°C, respectively. Cores were taken from 5-m depths in both basins on each occasion. In addition, cores were taken from 0.5- and 8-m depths in the north basin and 1-m depth in the south basin in March and from 0.5-m depth in both basins and 7-m in the north basin in October. To ensure recovery of the intact sediment–water interface, we used small box cores (30 × 30 × 30 cm; Wildco Co.). Immediately upon retrieval of each box core, we inserted 7–21 60-ml plastic syringes (with tip-end cut off) by hand into each box core, simultaneously drawing up on the plunger. After all "minicores" (~10 cm long) had been inserted, the bottoms were capped and the cores retrieved and stored in the dark while enroute to the lab.

In the laboratory, the cores were injected with $^{35}SO_4^{2-}$, incubated at in situ temperatures in the dark, frozen, and later analyzed for various S fractions. The plastic syringes had 5-mm holes at 2-cm intervals filled with silicone sealer to allow injection of ^{35}S, MoO_4^{2-}, acetone, ethanol, or SO_4^{2-}. Cores receiving only $^{35}SO_4^{2-}$ were injected at each port with 0.1 ml of carrier-free $^{35}SO_4^{2-}$ (Amersham) containing 7.4–13.5 nCi ^{35}S. The cores were then incubated in the dark at the appropriate temperature for periods of 0–17 h. Following incubation, the cores were plunged into a bath of dry ice in acetone for 5 min and then stored at −20°C until analyzed (1–10 d later).

A minimum of five minicores was collected from each site. One or two received no $^{35}SO_4^{2-}$ and were analyzed only for pore-water anions (SO_4^{2-}, Cl^-, F^-). One spiked core was frozen immediately ($t = 0$) to test for recovery of $^{35}SO_4^{2-}$. The remaining 3–5 cores were incubated for 0.7–17 h before freezing. In addition, on each sampling date, 2–5 cores were injected with 0.1 ml 0.2 M Na_2S to measure the analytical recovery and to determine whether oxidation occurred during incubation, freezing, or storage. The effects of MoO_4^{2-}, acetate, ethanol, and SO_4^{2-} also were examined on two of the sampling dates. These cores were treated as described above, with the sole exception that the injected solution contained either 0.9 M acetate, 17 M ethanol, 3–17 mM SO_4^{2-}, or 0.9 M MoO_4^{2-} in addition to the $^{35}SO_4^{2-}$. These additions resulted in concentrations in the pore water of 20 mM acetate, 0.4 M ethanol, 70–400 μM SO_4^{2-}, and 20 mM MoO_4^{2-}.

The frozen cores were sectioned into 2-cm

increments, thawed under N_2 in a solution of 2.5 M zinc acetate, then distilled with 20 ml of 1.5 M HCl for 1.5 h at 90°C. The H_2S released into the nitrogen stream was trapped in 20 ml of 0.2 M NaOH. Recovery of Na_2S standards added directly to the distillation flasks was 100%. Recovery of sulfide added to cores before incubation ranged from 55 to 86%, averaging 67%; there was no decrease in recovery with increasing storage time (2 h–2 months). The rates reported below are not corrected for this recovery and hence may underestimate the actual rate of sulfate reduction.

The solution from each trap was diluted to 25 ml, and 2 ml were added to 4 ml of scintillation cocktail in plastic minivials that were subsequently counted on a Beckman LS1800 scintillation counter. Quench corrections were determined from the H number measured for each sample and a quench curve measured separately. Sulfide was measured colorimetrically (Cline 1969) on an additional subsample from the NaOH trap.

After distillation of acid-volatile sulfides (AVS), the samples were centrifuged, decanted, and rinsed twice with 3 M $MgSO_4$. At this point, the sediments were dried at 100°C. The supernatant and rinses were combined and diluted to 100 ml. From this solution, 2 ml were added to 4 ml of scintillation cocktail, and β activity was measured as above. Any incorporation of $^{35}SO_4^{2-}$ into sulfate esters (cf. Landers and Mitchell 1988) that are not hydrolyzed during the acid distillation (cf. Urban and Brezonik 1993) would cause an overestimate of the rate of sulfate reduction. Cores incubated without $^{35}SO_4^{2-}$ were frozen as above, sectioned, and thawed under Ar. Samples were then centrifuged under Ar, and the decanted material was analyzed by ion chromatography (Dionex model 10) for SO_4^{2-}, Cl^-, and F^-.

Laboratory measurements of kinetics of sulfate reduction—The temperature dependence of microbial sulfate reduction was measured in sediment slurries in the laboratory. Surface sediments collected with an Ekman dredge were thoroughly mixed, distributed into vials (20-ml screwcap vials with septa), and purged with nitrogen for 20 min. Vials were then equilibrated at the appropriate temperature for 24 h. At that point, each vial was injected with 0.3 ml of a solution containing 0.2 μCi ml^{-1} $^{35}SO_4^{2-}$ and 10 mM Na_2SO_4. After they were shaken, the vials were incubated in the dark for 0–40 h at temperatures of 4, 10, 15, 23, and 30°C. Four replicates were used at each temperature, together with a control containing molybdate (10 mM). Although vials were not shaken during the incubations, linearity of sulfate reduction with time suggested that diffusion limitation was not important over the incubation times used. Incubations were stopped by immersing vials into a bath of dry ice in acetone. Samples were stored frozen for up to 1 month before analysis. Before analysis, 3 ml of Zn acetate was added to the vials which were then thawed under nitrogen. Samples were then distilled in hot acid (12 ml of 6 M HCl) for 90 min under a stream of N_2. Sulfide was trapped in 0.2 M NaOH which subsequently was made up to a volume of 50 ml. Two milliliters of this solution were added to 4 ml of scintillation cocktail for measurement of $H_2{}^{35}S$. The acid solution was centrifuged, rinsed twice, and the solution plus rinses were made up to a total volume of 100 ml before measurement of $^{35}SO_4^{2-}$ by liquid scintillation counting.

Sediment slurries also were used to examine the dependence of reduction rates on sulfate concentration. Surface sediments from the 5-m site in the north basin were collected with an Ekman dredge and stored at 4°C for 1 week to allow sulfate in the sediments to become depleted. About 10 ml of sediment was placed into 20-ml vials (screwcap vials with septa) which were then capped and purged with N_2 for 30 min. Concentrations of sulfate were adjusted with a 50 mM solution of Na_2SO_4 to range from 5 to 1,000 μM; duplicate measurements were made at each sulfate concentration. After receiving 10 μl of solution containing 6.5 nCi $^{35}SO_4^{2-}$, the vials were incubated for 2 h on a shaker table in the dark at 21°C. Following incubation, the reaction was stopped by immersing the vials in a bath of dry ice in acetone. Samples were thawed under N_2, centrifuged, and both $^{35}SO_4^{2-}$ and SO_4^{2-} were measured in the decanted material.

Two methods were used to calculate rates of sulfate reduction, R (μmol cm^{-3}h^{-1}), from the radiotracer experiments. Typically, rates are calculated according to

$$R = (^{35}S_{\text{reduced}}/^{35}SO_4^{2-}{}_{\text{injected}}) \cdot [SO_4^{2-}] \cdot (\alpha/t). \quad (1)$$

$^{35}S_{reduced}$ (nCi) is the sum of all reduced forms of ^{35}S recovered, $^{35}SO_4^{2-}{}_{injected}$ (nCi) the amount of radiotracer injected, $[SO_4^{2-}]$ the sulfate concentration (μmol cm^{-3}) in the sample, α the isotope fractionation factor, and t the incubation time (e.g. Jørgensen 1978; Howarth and Teal 1979; Fossing and Jørgensen 1989). The isotope fractionation factor (1.03–1.06; Jørgensen 1978) was neglected in this study, as in many others, because the uncertainties in all other measurements far exceed this correction factor. Use of this equation is based on the assumptions that sulfate uptake follows Monod kinetics, that sulfate concentrations remain constant during incubation, that the fraction of ^{35}S reduced is small, and that there is no back oxidation of reduced ^{35}S (e.g. Hobbie 1973; Howarth and Merkel 1984). In the present study, this equation was used only when these conditions were thought to exist (viz. in the laboratory kinetic assays with high concentrations of added SO_4^{2-}).

As discussed below, the above assumptions were not met in the assays with intact sediment cores. In brief incubations (15–60 min), large fractions (5–40%) of the ^{35}S were reduced even though sulfate concentrations appeared to remain constant. Furthermore, production of reduced ^{35}S was not linear with time. Nonlinearity was attributed to reoxidation of the reduced ^{35}S (discussed below). The usual model had to be modified to account for this reoxidation as follows:

$$d([^{35}SO_4^{2-}])/dt = \{-V_f/(K_m + [SO_4])\} \cdot [SO_4^{2-}] \cdot SA_{OX} + \{V_b/(K_{mb} + [S_{red}])\} \cdot [S_{red}] \cdot SA_{red}. \quad (2)$$

V_f and V_b are the forward and back rate constants, K_m and K_{mb} are the half-saturation constants for the forward and reverse reactions, $[S_{red}]$ is the concentration of reduced sulfur species undergoing reoxidation, and SA_{OX} and SA_{red} are the specific activities in the oxidized and reduced pools of S. Integration of this isotope dilution equation for the case of Monod kinetics does not yield expressions readily applicable to the experimental protocol we followed (cf. Blackburn 1979). Consequently, we used a simpler approach—examining initial reaction rates. At the start of all experiments, the specific activity of the reduced-sulfur pools is zero, and the second term in Eq. 2 can be dropped. Because sulfate concentrations remained constant during the course of incubations, Eq. 2 was further simplified to first-order kinetics:

$$d([^{35}SO_4^{2-}])/dt = -k_f \cdot [SO_4^{2-}] \cdot SA_{OX}. \quad (3)$$

k_f is a first-order rate constant equal to $V_f/(K_m + [SO_4^{2-}])$. Integration of this expression yields

$$\ln([^{35}SO_4^{2-}]/[^{35}SO_4^{2-}{}_{injected}]) = -k \cdot t. \quad (4)$$

Because of the large fraction of ^{35}S reduced, rate constants could be obtained as the slopes of plots of $\ln([^{35}SO_4^{2-}]/[^{35}SO_4^{2-}{}_{injected}])$ vs. time. Because of the formation of end products other than AVS, decreases in $^{35}SO_4^{2-}$ were used rather than formation of [^{35}S]AVS to calculate rate constants. Sulfate reduction rates were calculated as the rate constant times the sulfate concentration (Eq. 3).

Results

Sulfate profiles from pore-water equilibrators—All profiles of sulfate in pore water showed depletion of sulfate below the sediment surface (*see* figure 3 of Sherman et al. 1994). Over a depth interval of 1–10 cm, concentrations of sulfate decreased from ambient lake values (13–45 μmol liter^{-1}) to concentrations between 2 and 14 μmol liter^{-1}. Among the 33 profiles, seven showed peaks in sulfate at or below the sediment surface.

Diffusive fluxes calculated by application of Fick's law ranged from 0 to 4.9 μmol m^{-2} h^{-1} (Table 1). The average of all fluxes was 1.5 ± 0.94 μmol m^{-2} h^{-1} (mean ± SD, $n = 33$). An average deviation of 22% was observed in fluxes from three pairs of replicate profiles measured on one sampling date. There were no statistically significant differences in the magnitude of fluxes among different sites (Table 1). At a given site, fluxes were higher in summer than in winter and spring. Also, the depth at which sulfate depletion began in pore-water profiles changed seasonally; depletion began higher above the sediment surface in summer than in winter (Sherman et al. 1994). The factor accounting for the most variance (20%) in the magnitude of the fluxes was the sulfate concentration in the overlying water column (Fig. 1A). Linear regression (flux = $0.38 + 0.04 \cdot [SO_4^{2-}]$) yields a rate constant of 0.37 ± 0.16 (SE, $n = 33$) m yr^{-1}. Temperature accounted for an additional 10% of the variance in fluxes (Fig. 1B). The activation energy

Table 1. Summary of sulfate diffusion rates measured with pore-water equilibrators in Little Rock Lake.

Site (water depth)	No. of measurements	Flux (μmol m^{-2}h^{-1}) Range	Flux (μmol m^{-2}h^{-1}) Mean	[SO_4^{2-}]* (μmol liter^{-1}) Range
Littoral				
South basin (1 m)	3	1.3–3.7	2.2	22–28
South basin (3.5 m)	3	1.3–1.8	1.5	25–28
North basin (1 m)	3	1.0–1.5	1.3	30–33
Pelagic				
South basin (5 m)	7	0.7–2.2	1.4	12–27
North basin (5 m)	7	0.5–4.9	1.6	24–40
North basin (7 m)	4	0.5–2.4	1.5	12–35
North basin (9 m)	6	0–1.9	1.2	15–50

* Sulfate concentration 1–5 cm above the sediment surface.

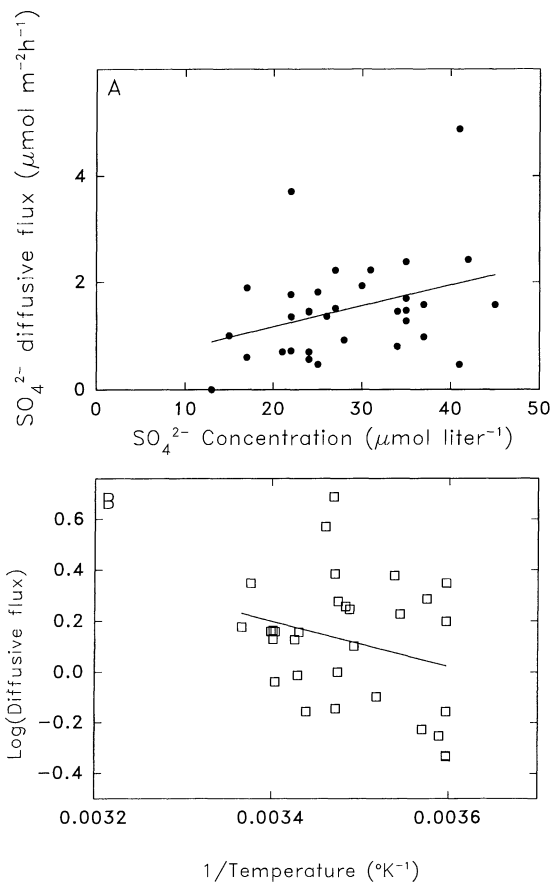

Fig. 1. A. Rates of sulfate diffusion were positively correlated with concentrations of sulfate in the lake water overlying the sediments. Stepwise multiple linear regression analysis indicated that this factor accounted for 20% of the variance in diffusion rates. B. Diffusion rates also appeared to be a function of temperature. Stepwise multiple regressions indicated that temperature accounted for 10% of the variance in diffusion rates. An Arrhenius plot (fluxes were normalized to sulfate concentrations to give rate constants) shows much scatter but indicates that the activation energy for the rate-controlling process was only 5 kcal mol^{-1}.

calculated from an Arrhenius plot of rate constants (flux/concentration) vs. inverse temperature (significant at $P = 0.05$) was only 20.9 kJ mol^{-1}, corresponding to a Q_{10} of < 1.5. The residual fluxes not explained by sulfate concentrations and temperature were inversely related to site depth, but stepwise multiple regressions indicated this factor accounted for only 5% of the variance in fluxes.

Sulfur distribution and reduction in intact sediment cores—Sulfate profiles in pore waters of the short syringe cores (Fig. 2) provide important confirmation that artifacts do not cause errors in the measured rates of sulfate reduction (cf. Kelly and Rudd 1984). Profiles showed that sulfate was depleted in the sediments at all sites, with minor seasonal differences but consistent differences between sites. Differences among sites in the north basin were not readily apparent, but consistent differences were observed in the south basin. Concentrations of sulfate were always lower in all core increments at the south basin 5-m site than at other sites. In contrast, sulfate concentrations and inventories were higher at the south basin littoral site in both October and March. In general, profiles looked similar to profiles measured with pore-water equilibrators; concentrations decreased from 25–40 μmol liter^{-1} in the lake water to 5–20 μmol liter^{-1} within 5 cm of the sediment surface. Similarity of the profiles in Fig. 2 to profiles from pore-water equilibrators indicates that insertion of the minicores did not cause movement of SO_4^{2-} from the overlying water into the sediments. Increased concentrations in the bottom section of some cores may reflect oxidation of reduced sulfur as a result of exposure of this section to air during capping of the syringes. In general, sulfate concentrations between 5- and 10-cm depth in the minicores (3–20 μM) were similar to those measured in pore-water equilibrators. Oxidation of reduced S or hydrolysis of organic S may have caused these concentrations to be overestimated; the similarity between minicores and pore-water equilibrators despite vastly different handling strategies suggests that

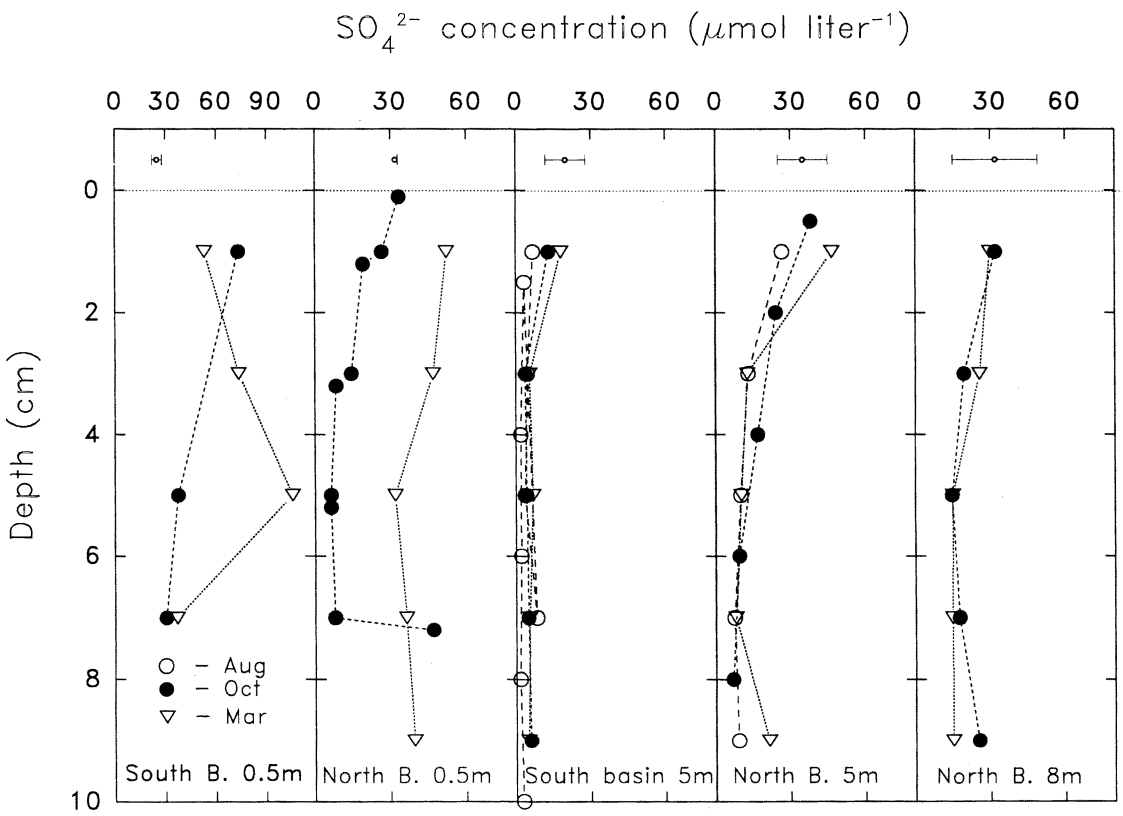

Fig. 2. Pore-water profiles of sulfate in the intact cores used for measurement of sulfate reduction rates were similar to profiles obtained with pore-water equilibrators. At the 5-m site in the south basin, concentrations in the cores consistently were lower than concentrations in the equilibrators. Seasonal trends were evident only at the littoral sites. The concentrations shown for the overlying water represent the mean and range of all values recorded with pore-water equilibrators at these sites.

such effects may not be important. Overestimation of sulfate concentrations at these depths could cause an overestimate of the rates of sulfate reduction.

Inventories of AVS measured in all short cores used for measurement of sulfate reduction did not show the expected seasonal and spatial trends (Table 2). Concentrations of AVS generally were constant with depth in the cores or showed a subsurface maximum between 2 and 4 cm. Inventories were lowest in the south basin littoral site but were similar in all pelagic sites independent of water depth. The north basin littoral site was in a sheltered bay, and the sediments contained considerable leaf litter as well as higher AVS content than the south basin littoral site. Seasonally, lowest inventories were observed in August; inventories in March were comparable to or even higher than inventories in October (Table 2).

Reduction of $^{35}SO_4^{2-}$ to [^{35}S]AVS occurred in all cores, but calculation of rates of reduc-

Table 2. Inventories (mmol S m^{-2}) of AVS in sediments of Little Rock Lake. (Not measured—nm.)

Site (water depth)	Aug	Oct	Mar
	(mean±SD)		
Littoral			
South basin	nm	2.4±1.4	1.8±1.2
North basin	nm	29±11	47±5
Pelagic			
South basin (5 m)	4.6±0.7	19±5.4	22±1.8
North basin (5 m)	8.0±0.9	26±4.0	25±6.4
North basin (8 m)	nm	19±2.4	37±7.6

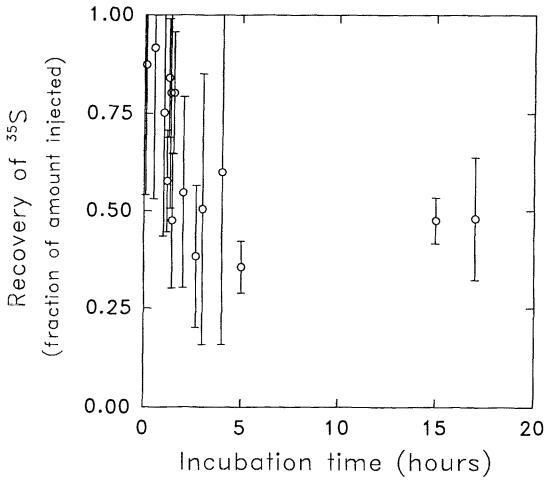

Fig. 3. Recovery of ^{35}S from intact cores decreased with increasing time of incubation. Shown here are the averages (O) and standard deviations (error bars) for all samples (all dates, all sites, all depth intervals). Recovery in controls with molybdate (not shown here) was 100% even after 4 h of incubation, but recovery in time-0 samples was only 87%.

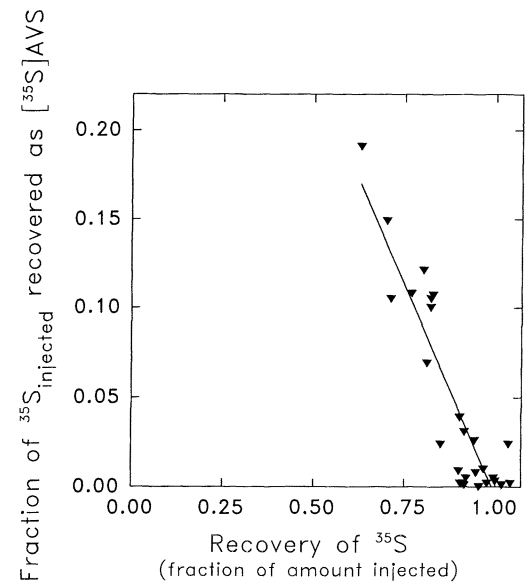

Fig. 5. Recovery (as a fraction of that injected) of ^{35}S as AVS decreased with decreasing total recovery of ^{35}S (i.e. [^{35}S]AVS + $^{35}SO_4^{2-}$). Decreasing recovery of ^{35}S is thought to result from formation of other end products besides AVS. The rate of formation of these other end products was proportional to the formation of AVS. Shown here are recoveries (per core) for all minicores in March.

tion is problematic. Calculation of rates must take into account the formation of end products other than AVS and the relatively large conversion of ^{35}S. Recovery of ^{35}S generally was <100% and decreased with increasing incubation time (Fig. 3). Failure to recover 100% of the ^{35}S within the AVS and SO_4^{2-} pools probably resulted from formation of end products other than [^{35}S]AVS. Incorporation of $^{35}SO_4^{2-}$ into organic sulfur compounds (both sulfate esters and carbon-bonded sulfur), elemental sulfur, and pyrite has been observed previously in sediments of Little Rock Lake (Urban and Brezonik 1993; Baker et al. 1989) as well as other lakes (Fossing and Jørgensen 1989; Landers and Mitchell 1988; Rudd et al. 1986*a*). The distributions of [^{35}S]AVS in the cores (Fig. 4) generally exhibited the same pattern; activities were lowest in the topmost increment and increased with depth. Formation of other end products appeared to be proportional to the formation of [^{35}S]AVS (Fig. 5) and also increased with depth in the cores. Sulfate reduction rates based only on appearance of [^{35}S]AVS may, therefore, underestimate the total rate of sulfate transformation.

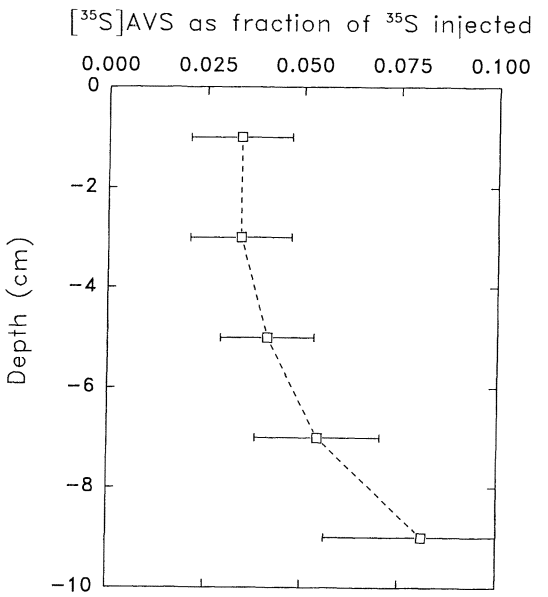

Fig. 4. Depth profiles of [^{35}S]AVS generally showed increasing activities with depth. Shown here are the average and SE (n = 27) for all cores incubated longer than 25 min in March. Decreased activity toward the surface results from increased concentrations of SO_4^{2-}.

If either first-order or Monod kinetics apply to the uptake of unlabeled SO_4^{2-}, the disappearance of $^{35}SO_4^{2-}$ should be first order with

respect to concentrations of $^{35}SO_4^{2-}$ provided that the concentration of unlabeled SO_4^{2-} does not change during the experiment. Analysis of pore-water profiles of SO_4^{2-} before and after incubations showed no decrease in sulfate concentrations, although an increase of 20% was observed in some cases. Plots of $\ln(^{35}SO_4^{2-}{}_{rec}/{}^{35}SO_4^{2-}{}_{inj})$ and $\ln([^{35}S]AVS/{}^{35}SO_4^{2-}{}_{inj})$ vs. time generally appeared linear for the first 1–3 h of incubation and leveled off at longer incubation times (Fig. 6; $^{35}SO_4^{2-}{}_{inj}$ is the amount of $^{35}SO_4^{2-}$ injected in each increment, and $^{35}SO_4^{2-}{}_{rec}$ is the amount of $^{35}SO_4^{2-}$ recovered). Rate constants (k, % h^{-1}) were calculated by linear regression as the slope of such plots for all incubation times <2 h. Sulfate reduction rates were calculated as this slope multiplied by the sulfate concentration at each depth. Areal rates (μmol m^{-2} d^{-1}) were calculated for each core as the sum of the products of rate multiplied by depth for each increment.

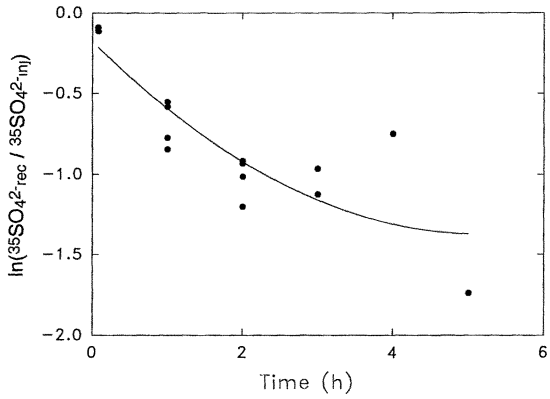

Fig. 6. Rates of sulfate reduction appeared constant for only the first 1–3 h. A line (based on nonlinear regression through the data) is given to help show the trend. Rates were calculated as the product of sulfate concentration times the slope of plots of $\ln(^{35}SO_4^{2-}{}_{rec}/{}^{35}SO_4^{2-}{}_{inj})$ vs. time for times <2 h.

Intercepts of the regressions, corresponding to the fraction of label recoverable as $^{35}SO_4^{2-}$ at "time 0," varied widely (range 88.7–114%) but averaged nearly 100% (mean, 98%). These intercepts were significantly higher than the fraction of ^{35}S recovered in time-0 controls (avg ± SD = 87.4±10.2%). Time-0 controls exhibited significant formation of [^{35}S]AVS as well (0.1–10.5%; avg ± SD = 2.1±2.9%). Controls incubated with molybdate, a specific inhibitor of dissimilatory sulfate reduction, exhibited little conversion of $^{35}SO_4^{2-}$ to [^{35}S]AVS (0.26±0.17%; mean ± SD), and recovery of ^{35}S (AVS + SO_4^{2-}) averaged 101±2%. Although addition of MoO_4^{2-} might also competitively inhibit sorption of SO_4^{2-}, the analytical methods used would have recovered all $^{35}SO_4^{2-}$ adsorbed on Fe or Al oxides as well as any $^{35}SO_4^{2-}$ reversibly adsorbed on other sites. We think that loss of $^{35}SO_4$ in time-0 samples reflected the rapid rate of biological sulfate transformation. Such transformation was completely inhibited by MoO_4^{2-} that was added simultaneously with $^{35}SO_4^{2-}$ but was significant in the time (up to 5 min) that elapsed between injection of ^{35}S and immersion into the dry ice bath for the time-0 controls.

Rates of sulfate reduction ranged from 0 to 8.8 nmol cm^{-3} h^{-1}, and areal rates ranged from 29 to 218 μmol m^{-2} h^{-1}. Rates in March were higher than rates in either August or October (Table 3). All sites were generally similar except the north basin 5-m site, which exhibited higher rates on each date. Addition of sulfate to intact cores stimulated reduction rates at all depths except the surface sample (Fig. 7). Injection of acetate and ethanol had no effect on reduction rates (data not shown).

Laboratory studies of sulfate reduction kinetics—Laboratory measurements indicated that sulfate reduction rates were strongly influenced by temperature (Fig. 8). Rates of sulfate reduction observed in these experiments (0.02–0.45 nmol cm^{-3} h^{-1}) were much lower than those observed in the intact cores; despite apparent linearity of reduction with time, some diffusion limitation may have occurred. A temperature optimum was not observed in the temperature range 4–30°C; rates appeared to increase exponentially with temperature. The plot of log(rate) vs. inverse temperature indicates that the activation energy may not have been constant over the entire temperature range. Use of all data points yielded a Q_{10} value of 2.6. This value is higher than would be expected if diffusion were the sole factor controlling observed rates but may underestimate the microbial response to temperature if diffusion limitation did affect the rates. Previous studies have indicated the temperature dependence of sulfate reduction is even more pronounced (Q_{10} 2.9–3.6; Nielsen 1987; Ingvorsen et al. 1981; Jørgensen 1977).

Laboratory experiments indicated that sul-

Table 3. Summary of rates of sulfate reduction (μmol S $m^{-2}h^{-1}$) measured with ^{35}S in intact sediment cores. (Not measured—nm.)

Site (water depth)	Aug	Oct	Mar
	(mean±SE)		
Littoral			
South basin	nm	29±7.8	73±9.2
North basin	nm	76±6.0	88±10.0
Pelagic			
South basin (5 m)	74±21	40±6.9	70±26
North basin (5 m)	64±8.5	146±26	218±109
North basin (8 m)	nm	47±22	61±19

fate reduction followed Monod type kinetics (Fig. 9). Rates of reduction in these experiments (1–7 nmol cm^{-3} h^{-1}) were comparable to rates observed in intact cores with similar sulfate concentrations. In these experiments only the decrease in $^{35}SO_4^{2-}$ activity was measured rather than formation of reduced end products; formation of ester sulfates or sorption of sulfate on the sediments could, therefore, have led to an overestimate of rates. Rinsing of sediments with $MgCl_2$ did not release additional ^{35}S into solution, however; hence, we believe sorption was negligible. The half-saturation constant calculated from these experiments (20 μmol $liter^{-1}$) is within the range (10–70 μmol $liter^{-1}$) reported in the literature for freshwater sulfate-reducing bacteria (Smith and Klug 1981; Ingvorsen et al. 1981; Lovley and Klug 1986).

Discussion

Sulfate diffusion—Diffusion of sulfate into lake sediments as a result of microbial sulfate reduction occurs even in soft-water lakes with low sulfate concentrations (e.g. Rudd et al. 1986*b*; Cook et al. 1987). Diffusive fluxes and

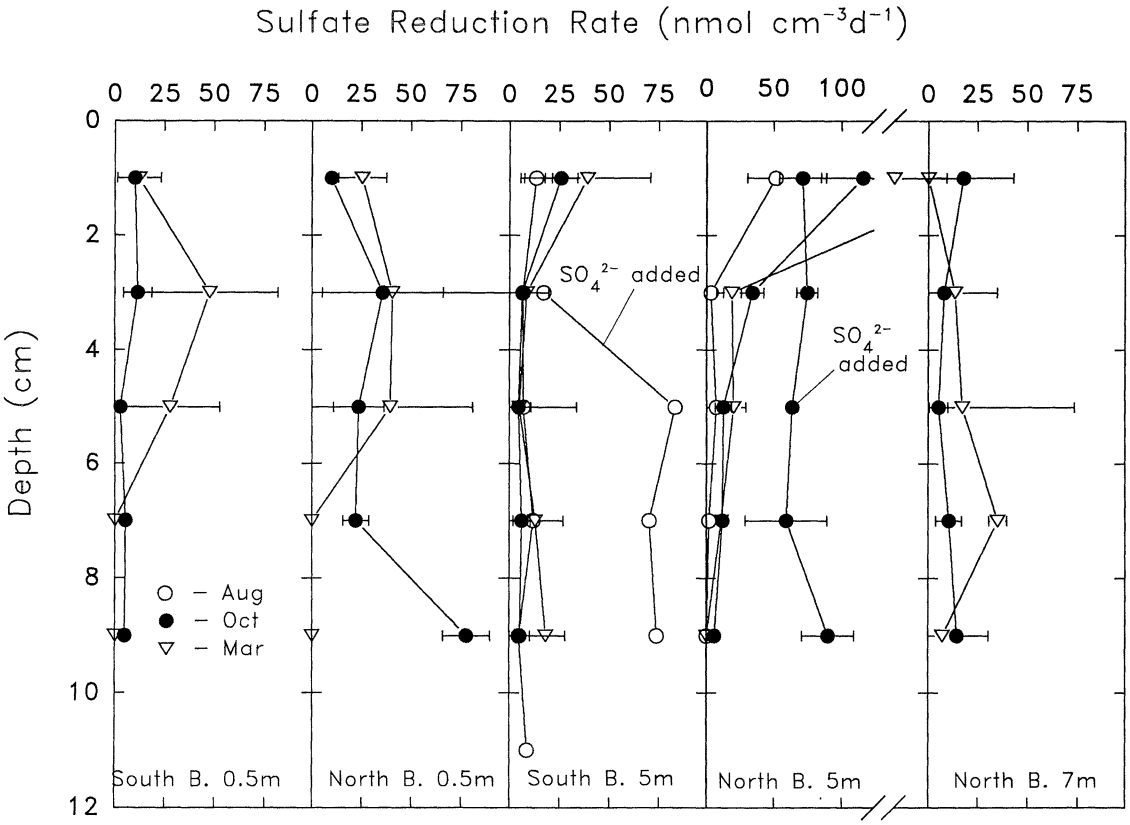

Fig. 7. Rates of sulfate reduction were generally similar at all sites and showed no consistent seasonal trends. In pelagic sediments, rates generally were highest at the surface and decreased with depth. Addition of sulfate (equal concentrations were added at both 5-m sites) stimulated reduction rates in all depth increments except the topmost increment.

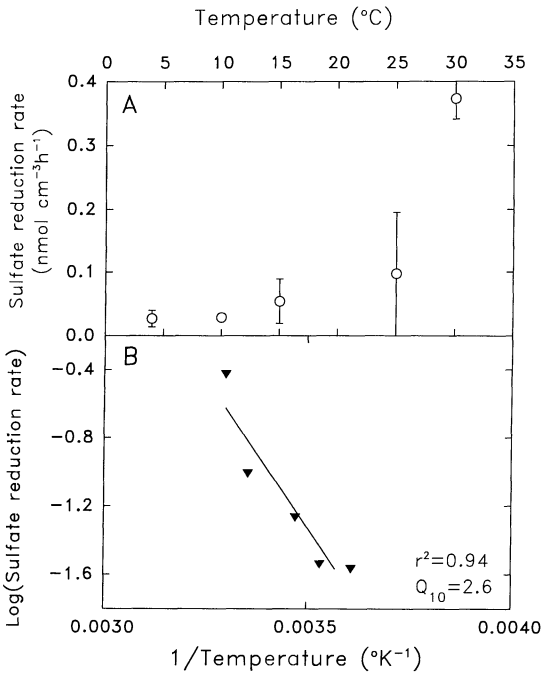

Fig. 8. A. Rates of sulfate reduction measured in sediment slurries in the laboratory showed large responses to changes in temperature. However, rates were much lower than those measured in intact cores. B. An Arrhenius plot of the data (sulfate concentration was equal at all temperatures) indicated that the activation energy may not have been constant over the entire temperature range. A regression with all data points yields a Q_{10} value of only 2.6, lower than many reported in the literature.

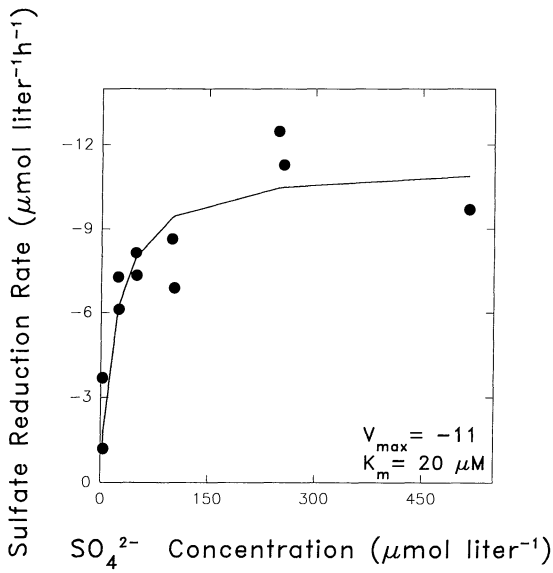

Fig. 9. Sulfate reduction rates measured in sediment slurries in the laboratory (23°C) exhibited Monod-type kinetics with a half-saturation constant (K_m) of 20 μmol liter^{-1} and a maximum rate of 11 μmol liter^{-1} h^{-1}. Constants were determined by nonlinear least-squares regression (line shown) through the data.

net retention of sulfate in sediments of numerous lakes have been shown to be a function of lake sulfate concentrations (e.g. Kelly et al. 1987; Baker et al. 1986; Urban 1994). This study also indicated that much of the variability in diffusive fluxes in a single lake was due to fluctuations in concentrations of sulfate (Fig. 1A). Both the diffusive fluxes and the proportionality constant to lake sulfate concentration measured in this study were similar to those previously reported. Fluxes reported here for Little Rock Lake (0.5–4.9 μmol m^{-2} h^{-1}) are at the low end of the range (1.4–10.4) reported for 14 soft-water lakes by Rudd et al. (1986*b*). The 10-fold range reported here for a single lake points to the need for multiple measurements to determine a lakewide average. The average transfer coefficient (determined as the slope of a plot of flux vs. lake sulfate concentration) for Little Rock Lake was 0.37 m yr^{-1}. This value is similar to the value of 0.36 m yr^{-1} reported by Kelly et al. (1987) as the average for 11 lakes, although Kelly et al. multiplied their measured diffusive fluxes by 0.5 to account for oxidation of sulfide (Rudd et al. 1986*a*). Baker et al. (1986) reported a slightly higher value (0.46 m yr^{-1}) based on ion budgets for 14 lakes.

Comparison of the diffusive fluxes reported here with other measurements of sulfur retention in Little Rock Lake indicates that porewater profiles provide a reasonable estimate of net sulfur retention resulting from sulfate reduction in sediments. Limnocorrals in littoral regions of the lake exhibited sulfate losses of 13 μmol m^{-2} d^{-1} (Perry et al. 1986), and enclosures in pelagic regions had losses of 170 μmol m^{-2} d^{-1} (L. Baker unpubl.); these losses compare favorably to the diffusive fluxes of sulfate of 12–118 μmol m^{-2} d^{-1} reported here. Lakewide rates of sulfur accumulation in sediment cores have been reported to be 29 mmol m^{-2} yr^{-1} (Baker et al. 1992); when corrected for the accumulation of seston sulfur in sediments (9 mmol m^{-2} yr^{-1}; Baker et al. 1989), the resulting accumulation rate of microbially reduced sulfur (20 mmol m^{-2} yr^{-1}) is a little

higher than the extrapolated mean diffusive flux of sulfate (13 mmol m^{-2} yr^{-1}).

Our study indicates that diffusive fluxes of sulfate are regulated largely by physical parameters affecting diffusion. Although Sherman et al. (1994) demonstrated that diffusive fluxes did vary seasonally (rates were lower in winter than in summer), and depletion of sulfate appeared to begin deeper in sediments in winter, our analysis suggests that these differences can be explained largely by effects of temperature on the kinetics of diffusion. Rates of diffusion exhibit small changes in response to temperature compared to biological processes; Li and Gregory (1974) demonstrated that rates of sulfate diffusion change by a factor of only 1.7 between 5 and 23.7°C. Rates of biological processes would be expected to vary by a factor of ~4 over this temperature range. From the activation energy derived above (cf. Fig. 1B), we calculate that diffusive fluxes of sulfate in Little Rock Lake varied only by a factor of 1.8 over the same temperature range.

If the data from 1983 to 1987 are examined together, diffusive fluxes of sulfate did not show systematic spatial variations in the lake. Neither pairwise comparison of pore-water profiles from the same water depths in both basins (Baker et al. 1989) nor comparison of all sites in both basins over this entire period revealed any statistically significant differences, because this time period includes preacidification and early results of the experimental acidification. Experimental acidification did increase sulfate concentrations in the north basin (from 26 to 60 μmol $liter^{-1}$; Brezonik et al. 1993) and did increase the net rate of sulfur storage in the sediments of the north basin (Sampson et al. 1994; Brezonik et al. 1993). The variability in pore-water fluxes (20% average deviation among fluxes from replicate, adjacent pore-water profiles) was too great for a statistically significant difference to be observed in diffusive fluxes of sulfate among the two basins over the time period encompassed by this report. Nevertheless, without the increase in sulfate concentrations caused by the experimental acidification, the dependence of diffusive fluxes on sulfate concentrations (Fig. 1A) would not have been observed.

No difference was found between diffusive fluxes from littoral and pelagic sites. If focusing of labile organic matter to deep parts of the lake controlled rates of sulfate reduction (Carignan and Lean 1991), an increase in diffusive fluxes with water depth should have been observed. Although the organic matter content of littoral sediments is low (2–10%) relative to pelagic sediments (20–60%) in Little Rock Lake, the organic matter content per unit volume is similar in both sediment types (26–136 vs. 14–42 mg cm^{-3}; *see also* Rudd et al. 1986*b*). Hence, the lack of spatial trends may not demonstrate that sulfate diffusive fluxes are independent of organic C availability.

Diffusive fluxes were proportional to concentrations of sulfate in lake water. In the past, this has been interpreted to indicate that sulfate-reducing bacteria are substrate limited. However, it will be demonstrated below that lake sulfate concentrations affect the physical process of diffusion rather than biological rates of sulfate reduction.

Microbial sulfate reduction—This study demonstrates that very high rates of sulfate reduction occur even in sediments of oligotrophic lakes with low sulfate concentrations. Rates measured in this study (29–218 μmol m^{-2} h^{-1} or 0–8.8 nmol cm^{-3} h^{-1}) are comparable to those measured in both hard-water and eutrophic lakes (Table 4). Data are too few for a definitive comparison, but they do suggest that rates of sulfate reduction in lake sediments are not limited either by lake sulfate concentrations or by availability of organic matter. Surprisingly, rates of sulfate reduction in lakes are comparable to rates for many marine systems (Table 4), where concentrations of sulfate are 2–3 orders of magnitude higher.

Measured rates of sulfate reduction were much higher than diffusive fluxes of sulfate into sediments of Little Rock Lake. The maximum diffusive flux (4.9 μmol m^{-2} h^{-1}) was only a sixth of the lowest rate of sulfate reduction (29 μmol m^{-2} h^{-1}), and the average diffusive flux (1.5 μmol m^{-2} h^{-1}) equaled only 2% of the average rate of sulfate reduction (82 μmol m^{-2} h^{-1}). This apparent anomaly is not unique to Little Rock Lake but is true of every lake in which both processes have been measured. Sulfate diffusive fluxes in Lake Lugano (1.4–7.8 μmol m^{-2} h^{-1}; Lazzaretti and Hanselmann 1992) are only 2–7% of the rates of sulfate reduction (Sorokin 1975). Discrepancies in other lakes range from a factor of 3 in Lake Washington (Kuivila et al. 1989) to 64

Table 4. Reported rates of sulfate reduction measured with ^{35}S.

Location	$[SO_4^{2-}]$ (μmol liter^{-1})	Sulfate reduction rate (mmol m^{-2}d^{-1})	Sulfate reduction rate (nmol cm^{-3}h^{-1})	Reference
Lakes				
Little Rock				
Littoral	6–62	0–15	0–30	This study
Pelagic	6–62	0–12	0–70	This study
Wintergreen	208	15.3	19	Smith and Klug 1981
Lawrence			3.0	Lovley and Klug 1986
Mendota	83–220	100–220	3–23	Ingvorsen et al. 1981
Third Sister			0–4.2	Dunnette 1989
Washington	105	0.12	0.07	Kuivila et al. 1989
Maggiore	646	5.5	0.2–7.9	Sorokin 1975
Lugano	742	3.3	1.6–2.9	Sorokin 1975
Faro	3,600	0.1–3.3	0.2–1.8	Sorokin and Donato 1975
Braband		354		Jørgensen 1990*a*
Sempach	114	66		Urban 1994
Greifen	140	118		Urban unpubl.
Marine sediments				
Danish coast		1.0–20		Jørgensen 1982
Limfjorden		13		Jørgensen 1977
Nova Scotia		17.6		Hargrave and Phillips 1981
Salt marshes				
Cape Lookout Bight		34		Chanton et al. 1987
Sapelo Island		70		Howarth and Giblin 1983
Sippewissett		206		Howarth and Teal 1979

in Lake Mendota (Ingvorsen et al. 1981) and 200 in Greifensee (Urban unpubl.).

Rates of sulfate reduction much higher than diffusive inputs of sulfate to sediments can be maintained only if an additional source of sulfate exists. Two possible sources of sulfate include hydrolysis of sulfate esters and reoxidation of reduced sulfur to sulfate. King and Klug (1982) showed that supply of sulfate esters to the sediments of Wintergreen Lake amounted to only 4% of the rate of sulfate reduction. Only 42% of the sulfate esters were estimated to be hydrolyzed in this lake. In Little Rock Lake, deposition of sulfate esters amounts to only 16.5 μmol m^{-2} d^{-1} (Baker et al. 1989), $<1\%$ of the rate of sulfate reduction. By inference, rates of oxidation of reduced sulfur must nearly equal rates of sulfate reduction.

Two other observations support the conclusion that rates of oxidation of reduced sulfur are rapid. First, the turnover time of AVS is short (1–27 d). Turnover times may be calculated as the inventory of AVS (Table 2) divided by the rate of AVS production (60–90% of the rates of sulfate reduction in Table 3). This calculation does not necessarily mean that the entire pool of AVS is transformed this rapidly, but at least a portion of it is short lived in the sediments. Existing data are inadequate to show how much of the AVS is reoxidized to sulfate and how much is transformed to other reduced end products (pyrite or reduced organic sulfur compounds; cf. Baker et al. 1989; Urban and Brezonik 1993). Pools of pyrite are much larger at all sites than pools of AVS (Baker et al. 1992). A minimum estimate of the pyrite turnover time can be made by assuming that all AVS is converted to pyrite; this assumption yields a turnover time of 50–400 d. The upper 10 cm of sediment has accumulated over the past 40 yr (Baker et al. 1992), so turnover times of 50–400 d imply that formation and oxidation of pyrite (as well as AVS) are fast relative to accumulation.

Nonlinearity of sulfate reduction rates (Fig. 6) also might be an indication of rapid oxidation of reduced sulfur. Decreasing rates of reduction with increasing incubation time (Fig. 10) might result from increasing rates of ^{35}S oxidation as the pool of reduced ^{35}S increased with time. Rates based on disappearance of $^{35}SO_4^{2-}$ as well as rates based on appearance

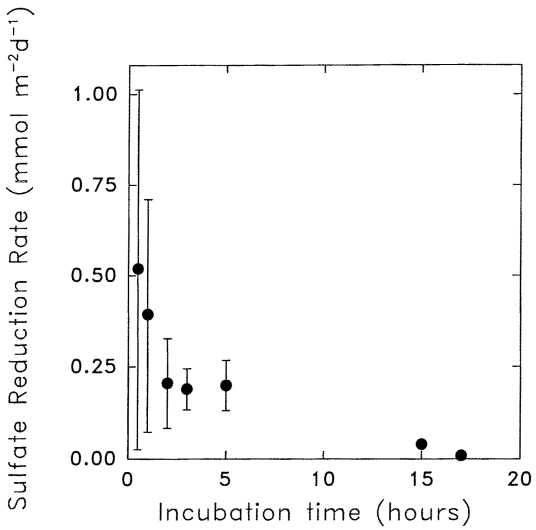

Fig. 10. Sulfate reduction rates calculated after the method of Fossing and Jørgensen (1989) decreased with increasing incubation time. Rates were calculated as the sum of $([^{35}S]AVS/^{35}SO_4^{2-}{}_{inj})\cdot[SO_4]\cdot(Z/t)$ for each core, where Z is the depth increment for each core section. Decreasing rates with time are thought to result from rapid reoxidation of reduced ^{35}S.

of [^{35}S]AVS decreased with time; transformation of [^{35}S]AVS to pyrite cannot be the sole explanation.

Without rapid reoxidation of reduced sulfur to sulfate the inventory of sulfate in the pore waters would be depleted quickly. Inventories of sulfate in the top 10 cm of sediment ranged from 1,000 to 4,000 μmol m^{-2}. Turnover times (inventory/reduction rate) for these pools ranged from 6 to 30 h. Because up to 24 h elapsed between core retrieval from the lake and incubation with ^{35}S, depletion of sulfate should have been observed in the pore waters of the short cores relative to concentrations in pore-water equilibrators unless regeneration of sulfate was rapid. Not only is diffusion too slow to resupply sulfate at this rate, but the pool of available sulfate in lake water above the sediments in the minicores was too small to supply all of the sulfate reduced during core incubations. Only at the 5-m site in the south basin was such depletion observed. Furthermore, no depletion of sulfate was observed after incubation times of 4 h. These observations suggest that sulfate concentrations in pore waters are in steady state; rates of influx from diffusion of lake water and regeneration through oxidation of reduced sulfur equal rates of reduction. Higher steady state concentrations of sulfate in pore waters from littoral vs. pelagic sites (Fig. 2) may indicate that the ratio of sulfate supply (diffusion plus sulfide oxidation) to sulfate reduction is higher in littoral sites. The lower AVS inventory in the south basin littoral site relative to pelagic sites supports this interpretation.

Although our data seem consistent with rapid reoxidation of sulfide, we have no measurements of either sulfide oxidation rates or consumption rates of possible electron acceptors. Rates of sulfide oxidation measured recently in marine sediments were found to be a fourth as fast as rates of simultaneous sulfate reduction (Elsgaard and Jørgensen 1992). In recent work, we have shown that sulfide is oxidized to sulfate in anaerobic lake sediments (Urban unpubl.), but it remains unclear what electron acceptors are involved. Oxygen is the probable electron acceptor in the upper 1 cm, and Fe oxides may be important below this depth. In sediments of Little Rock Lake, concentrations of Mn are only a tenth those of Fe (Weir 1989). Pore-water profiles indicate that Fe reduction occurs throughout the zone of sulfate reduction (Sherman et al. 1994), but the rates are not known. The inventory of Fe oxides between 3- and 10-cm depth in the sediments would be adequate to oxidize all H_2S produced in this zone for only ~70 d. Prolonged anoxic oxidation of sulfide also was observed in the study of Elsgaard and Jørgensen (1992) despite a similarly inadequate supply of Fe and Mn oxides. We do not yet know if this apparent lack of electron acceptors indicates that the rates of sulfate reduction and inferred rates of sulfide oxidation have been overestimated or if it points to involvement of unidentified electron acceptors (organic matter or HCO_3^-).

Rates of sulfate reduction within the sediments were limited by sulfate concentrations in the pore waters. Laboratory experiments indicated that reduction of sulfate followed Monod kinetics with a half-saturation constant of 20 μmol liter^{-1} (Fig. 9). Rates of reduction in intact cores also were limited by sulfate concentrations. Injection of sulfate into intact cores resulted in increased rates of sulfate reduction (Fig. 7). Injection of similar concentrations of sulfate into intact sediment cores from two

sites resulted in nearly equal rates of reduction throughout the sediment profiles. These rates (75 nmol cm^{-3} d^{-1}) are lower than the rates observed at comparable sulfate concentrations (100 μM) in the laboratory kinetic assays with mixed sediments (190 nmol cm^{-3} d^{-1}; Fig. 9); lower rates may result from lower microbial populations (hence lower V_{max}) in the intact cores than in the surface sediments used in the laboratory assays. A plot of reduction rate vs. sulfate concentrations at each depth interval suggests that sulfate reduction followed Monod kinetics in the intact cores as well (Fig. 11). We might have expected that bacterial densities would change with depth in the sediments and that half-saturation constants, population densities, or carbon availability might vary among different sites. Indeed, the composite diagram with data from all sites and all depths (Fig. 11A) reveals no consistent trends and may indicate that no single factor is limiting at all sites. However, similar trends are observed for both 5-m sites (Fig. 11B). Nonlinear regression yielded an estimate of the half-saturation constant (28 μM) for these sites similar to that obtained in the laboratory assays (20 μM), but V_{max} was lower for the intact cores (100 vs. 264 nmol cm^{-3} d^{-1}).

These results help to clarify the response of diffusion rates to lake-water sulfate concentrations (Fig. 1A). Previously it has been suggested that this relationship results from the substrate-limited state of the sulfate-reducing bacteria (Baker et al. 1986; Kelly et al. 1987). However, it is apparent that diffusion provides a relatively small fraction of the sulfate supply to the sulfate-reducing bacteria; reoxidation of reduced sulfur is quantitatively more important in maintaining the steady state concentrations in the pore waters. Comparison of diffusive fluxes $\{F(\mu\text{mol m}^{-2}\text{ h}^{-1}) = 0.38 + 0.04 \cdot [SO_4]$; Fig. 1A$\}$ with rates of sulfate reduction $\{R$ (μmol liter^{-1} h^{-1}) $= V_{max} \cdot [SO_4]/(K_m + [SO_4])\}$ indicates that sulfate reduction in a sediment layer 0.2 mm thick could consume sulfate at the rate at which it diffuses into the sediments. Both the measured profiles of sulfate reduction (Fig. 7) and the sulfate gradients extending over several centimeters (Fig. 2) indicate that sulfate reduction is not confined to such a narrow zone. Hence, microbial kinetics alone cannot explain the dependence of diffusive fluxes on sulfate concentrations. Rather, the balance of sulfate reduction and sulfate regeneration creates a sink for sulfate in the sediments whose magnitude is independent of lake sulfate concentration. Increasing sulfate concentrations in the water above the sediments will, however, create a larger concentration gradient and enhance the diffusive flux.

Rates of sulfate reduction will be enhanced only if sulfate concentrations in the pore waters are increased. Experimental addition of sulfate to Little Rock Lake did increase net diffusion of sulfate into the sediments (Brezonik et al. 1993; Sampson et al. 1994). However, concentrations of sulfate in the pore waters were increased only after the sediments had lost their buffering capacity and became acidified (Sampson et al. 1994). Increasing sulfate concentrations in lake waters per se will not necessarily affect rates of sulfate reduction.

This study did not reveal any significant differences in rates of sulfate reduction in littoral and pelagic sediments. Previous investigations also have pointed to the importance of sulfate diffusion into littoral sediments low in organic C content (Cook et al. 1986; Rudd et al. 1986*b*; Sherman et al. 1994). Comparability of rates in littoral and pelagic sediments supports the calculations of Cook et al. (1986) that indicate that organic matter is not limiting sulfate reduction even in littoral sediments. Lack of response of sulfate reduction rates in both littoral and pelagic sediments to additions of acetate and ethanol also supports the hypothesis that sulfate reduction is not carbon limited in these lake sediments. Sulfur retention in sediments has been shown to be affected strongly by sediment organic matter content (Stauffer 1991; Giblin et al. 1991; Urban 1994); however, this may result from effects of organic matter on reoxidation rates of reduced sulfur rather than on rates of reduction. This hypothesis may be supported by the lower burden of sulfide in littoral sediments compared to pelagic sites (Table 2); reoxidation of sulfur may be more complete in sediments low in organic C.

Lack of seasonal trends in rates of sulfate reduction (cf. Table 3) would seem to contradict the laboratory studies and literature reports that indicate that sulfate-reducing bacteria exhibit large responses to temperature changes. Other studies also have indicated that rates of sulfate reduction in lakes do not show large seasonal variations (King and Klug 1982;

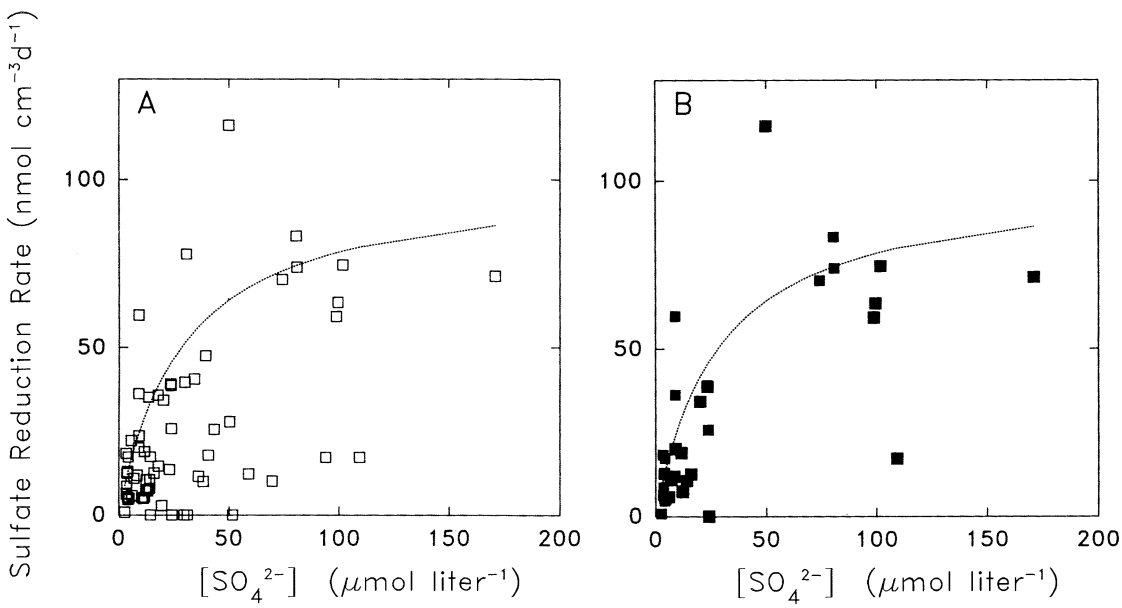

Fig. 11. A. Data for all sites, all dates, and all depths indicated no clear relationship between rates of sulfate reduction in the intact cores and sulfate concentrations at each depth increment. B. Data from the two 5-m sites did appear to follow Monod kinetics with a half-saturation constant (28 μM) similar to that determined in laboratory experiments. The curves shown in both panels are based on kinetic constants determined with sediment slurries in the laboratory (*see Fig. 9*).

Ingvorsen et al. 1981). Results of Rudd et al. (1990) and Kling et al. (1991) indicate that oxygen penetration and oxidation of reduced sulfur are enhanced during winter and at times of lake overturn. Enhanced rates of oxidation of reduced sulfur might enhance sulfate supply and stimulate sulfate reduction rates during colder months, offsetting the decrease in rates induced by lower temperatures.

Our results contradict several accepted limnological paradigms. The first of these is that sulfate reduction is relatively unimportant for carbon oxidation in lake sediments. As discussed above, rates in lakes appear to be comparable to rates in salt-water environments. This comparability implies that sulfate reduction may account for a much larger fraction of anaerobic carbon respiration than previously thought (*see* Capone and Kiene 1988). Based on net rates of sulfate reduction (i.e. rates of sulfate diffusion into sediments), one would conclude that sulfate reduction accounted for <1% of total carbon oxidation in the sediments of Little Rock Lake; the majority (90%) is accounted for by aerobic respiration and the remainder (10%) by methanogenesis (data from Weir 1989). However, the high rates of sulfate reduction measured in this study indicate that sulfate reduction can account for 35% of total carbon oxidation (78% of anaerobic carbon oxidation). Aerobic respiration accounts for only 55% of the total; the remaining oxygen consumption is due (perhaps indirectly) to reoxidation of reduced sulfur. Sweerts et al. (1991) also have shown that aerobic respiration accounts for a relatively small fraction of the sediment oxygen demand. Our study suggests that sulfate reduction is important both for carbon turnover and oxygen consumption within lakes.

Our study also would seem to contradict the accepted paradigm that electron acceptors are used in the order of decreasing free energy yield (Stumm and Morgan 1981; Zehnder and Stumm 1988). This paradigm is based, in part, on the assumption that organisms obtaining more energy from available substrates will grow faster and thereby outcompete organisms using less efficient modes of respiration. However, as mentioned above, much of the oxygen consumption may not be due to aerobic respiration but to oxidation of reduced inorganic

substances (sulfide); anaerobic organisms may have a method for outcompeting the energetically favored aerobes. Furthermore, the rapid rates of sulfide oxidation that are inferred from this work can occur only if oxidants are close at hand; diffusion over distances $\gtrsim 1$ mm could not support these rates. Because much sulfate reduction and sulfide oxidation takes place below 1-cm depth in the sediments, iron and manganese oxides rather than oxygen are the likely oxidants for the sulfide. Similar observations have been made in marine sediments (Jørgensen 1990*b*; cf. Elsgaard and Jørgensen 1992). This implies that reduction of Mn(IV), Fe(III), and SO_4^{2-} co-occur in the same zone in sediments.

Finally, retention of sulfur in sediments must be understood in light of this evidence of rapid sulfur oxidation and reduction in sediments. Sulfur in sediments has two sources: organic sulfur from seston and sulfate that diffuses from the water column. It has been argued previously that factors affecting rates of sulfate reduction (e.g. carbon supply, sulfate availability) control rates of diffusion and hence rates of sulfur retention in sediments (e.g. Kelly et al. 1987; Giblin et al. 1990, 1991; Cook and Kelly 1992). This study shows that it is incorrect to equate rates of sulfate diffusion with rates of sulfate reduction. If rates of sulfate reduction are much higher than rates of sulfate diffusion into sediments, then sulfate reduction per se cannot be the control on rates of diffusion. Rather, diffusion is the net result of the competing processes of sulfate reduction and reduced sulfur oxidation. Hence, effects on sulfur retention of carbon supply, lake sulfate concentration, and lake mixing must be understood in terms of their effects on these two competing processes. Lake mixing and carbon supply may have a larger effect on oxidation of reduced sulfur than on rates of sulfate reduction.

Rates of accumulation of sulfur in sediments depend on the balance between inputs (seston deposition, sulfate diffusion) and recycling. In many lakes, increases in rates of sulfur accumulation are smaller than would be predicted to have resulted from the increased sulfate concentrations caused by acid deposition in these lakes (Mitchell et al. 1988; Norton et al. 1988). The disparity between predicted diffusive fluxes and long-term storage suggested by the studies of Mitchell et al. (1988) and Norton et al. (1988) may indicate that episodic or seasonal oxidation of reduced sulfur (e.g. Rudd et al. 1990; Kling et al. 1991) plays a large role in regulating net sulfur storage over time periods of years. Our study demonstrated that even over short time periods (hours to weeks), diffusive fluxes of sulfate are regulated by the balance between rates of reduction and oxidation.

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