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Effects of sulfate concentration in the overlying water on sulfate reduction and sulfur storage in lake sediments

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Abstract. We investigated the effects of sulfate concentration on sulfate reduction and net S storage in lake sediments using ^{34}S as a tracer. The water overlying intact sediment cores from the hypolimnion of Mares Pond, MA, was replaced with two $Na_2{}^{34}SO_4$ solutions at either ambient (70 μM) or elevated (260 μM) sulfate concentrations. The $\delta^{34}S$ of the added sulfate was 4974 ‰. Over two months, the net sulfate reduction rate in the ambient sulfate treatment was zero, while the net rate for the high sulfate treatment was 140 μmoles/m^2/d. The water overlying the cores was kept under oxic conditions and the sediment received no fresh carbon inputs, thus the net rate reported may underestimate the *in situ* rate. Gross sulfate reduction rates calculated by isotope dilution were approximately 350 μmoles/m^2/d for both treatments. While the calculation of gross sulfate reduction rates in intact sediment cores can be complicated by differential diffusion of ^{34}S and ^{32}S, isotopic fractionation, and the possible formation of ester sulfates, we believe these effects to be small. The results suggest that sulfate reduction is not strongly sulfate-limited in Mares Pond. The difference in net sulfate reduction rates between treatments resulted from a decrease in sulfide oxidation and suggests the importance of reoxidation in controlling net S storage in lake sediments. In both treatments the CRS and organic S fractions were measurably labelled in ^{34}S. Below the sediment surface, the CRS fraction was the more heavily labelled storage product for reduced sulfides.

Introduction

It has generally been assumed that increasing sulfate concentrations in lakes should result in higher rates of sulfate reduction in sediments (Kelly & Rudd 1984; Holdren et al. 1984; Carignan 1985). Increased sulfate reduction rates have been measured in lakes and enclosures experimentally acidified with sulfuric acid (Cook & Schindler 1983; Kelly & Rudd 1984). Further, increases in total S concentrations in lake sediments have been linked to historical increases in sulfate deposition due to acid precipitation (Fry 1986; Giblin et al. 1990; Mitchell et al. 1990). However, Giblin et al. (unpublished data) found no increase in total S in intact sediment cores from Mountain Lake, Nova Scotia, that were incubated for one year with overlying water containing 5000 μM sulfate (sulfate levels are currently 20 μM in this lake).

In the past, researchers have associated increases in S storage with increases

in sulfate reduction (Gorham et al. 1974; Nriagu 1984; Mitchell et al. 1988). However, sulfur storage is the net result of two competing processes, gross sulfate reduction and sulfide oxidation. Sulfide oxidation has been shown to be an important process in both freshwater and marine sediments. Kling et al. (1991) found rapid reoxidation of seasonally stored S from anoxic Mares Pond sediments upon overturn. In coastal marine sediments, Jorgensen (1977) found that 90% of sulfides produced via sulfate reduction were quickly reoxidized. Hence, to predict sulfur storage we must know how sulfate reduction and sulfide oxidation change with increasing sulfate concentrations.

Sulfide oxidation rates may strongly depend upon the form in which the sulfide is found in the sediments (Rudd et al. 1986a). Sulfides can react with both Fe and organic C in sediments (Cook 1981; Kelly & Rudd 1984; Nriagu & Soon 1985; Rudd et al. 1986a), although there has been some discussion as to which potential sink is more important in lake sediments. Some studies have found that carbon-bonded S is the primary long term storage product for sulfides formed via sulfate reduction (Rudd et al. 1986a; Landers & Mitchell 1988). Rudd et al. (1986a) found that carbon bonded sulfur was less subject to oxidation than iron sulfides. In contrast, other studies have found that inorganic reduced S is the most important from in which sulfides are stored (Carignan & Tessier 1988; Giblin et al. 1990). One difference between these studies is that Rudd et al. (1986a) carried out their experiments on epilimnetic sediments, while Giblin et al. (1990) and Carignan & Tessier (1988) primarily studied hypolimnetic sediments from lakes experiencing hypolimnetic oxygen deficits. Giblin et al. (1990) have suggested that the form in which sulfides are buried in lake sediments is regulated by interactions between lake water sulfate and oxygen concentrations, organic C inputs to sediments, and Fe availability.

The objective of our study was to examine the effects of sulfate concentration in the overlying water on gross sulfate reduction, sulfide oxidation, and net S storage in lake sediments. We report the results of a laboratory experiment in which ^{34}S-enriched sulfate was added to the overlying water and used as a tracer. Use of the stable isotope ^{34}S as a tracer enabled us to measure both the gross and net sulfate reduction rates in intact sediment cores by isotope dilution. By measuring ^{34}S changes in sediments we were able to examine the relative importance of carbon-bonded S and inorganic-reduced S as sinks for reduced sulfides.

Methods

Sampling Site: Mares Pond is a kettle pond located 5 km inland on Cape Cod, Massachusetts. Rich (1980) classified Mares Pond a mesotrophic based on hypolimnetic oxygen deficits. The pH of epilimnetic water in Mares Pond ranges from 6.6–6.9 (Kling et al. 1991). The maximum depth of the pond is

17.2 m and the surface area is 11.3 ha. For a description of sediment chemistry see Giblin et al. (1990) and Kling et al. (1991).

Eight microcosm sediment cores (microcosm: 15.5 cm diameter, 50 cm tall) were taken by SCUBA divers from 17 m on August 1, 1991. The hypolimnion was oxic (4 ppm O_2) when the cores were removed. The cores were taken to a sediment depth of about 20 cm. The intact cores were brought back to the laboratory and placed in an insulated water tank held at 10 °C (lake bottom water temperature was 8 °C). Special tops (Dornblaser et al. 1989) were then fitted on the core tubes to measure total oxygen consumption in the sediment and overlying water. Oxygen consumption rates were calculated by measuring the decline in O_2 concentrations in the overlying water over a period of several days. Once the respiration measurements were completed, filtered samples (0.45 μM) from the overlying water of all cores were taken for sulfate and $\delta^{34}S_{SO4}$ analysis. Then two cores were harvested for initial measurements of porewater and sediment chemistry. The cores were sectioned under N_2 in 1 cm intervals to 2 cm, 2 cm intervals from 2–10 cm, and 4 cm intervals from 10–18 cm. The sections were homogenized, and aliquots of mud were taken for total S, chromium-reducible S (CRS), and $\delta^{34}S_{CRS}$ measurements. Porewater sulfate and sulfide samples were obtained by centrifugation of a separate aliquot which was kept under N_2 until analysis.

The remaining six cores were split into two treatments based on the respiration data such that the average respiration rate for each treatment was similar (Table 1). The overlying water from each core was removed and replaced with sodium sulfate solutions made up in distilled water. The solutions had sulfate concentrations of either 70 μM or 260 μM. The surface sulfate concentration of Mares Pond was approximately 70 μM, thus the first treatment represented ambient conditions. Each treatment solution was enriched with $^{34}SO_4$ to give an initial $\delta^{34}S$ value of 4974 ‰ ($^{34}S/^{32}S = 0.27$) in the sulfate of the overlying water. Initial water samples were taken for sulfate and $\delta^{34}S_{SO4}$, and the overlying water was sampled every 2–5 days thereafter for the duration of the experiment. The overlying water of all cores was replaced with fresh sodium sulfate solution on day 17. After one month two cores from each treatment were harvested and sectioned as described previously. In addition, oven-dried sediments from Core D (70 μM sulfate treatment) and Core F (260 μM sulfate treatment) were rinsed once with 10 mM KCl and three times with distilled water to remove adsorbed sulfate. These rinsed sediments were oven-dried and analyzed for total S, $\delta^{34}S_{TOTS}$, and $\delta^{34}S_{CRS}$. The remaining two cores received one additional water change on day 45, and were harvested and sectioned on day 55.

Laboratory Analyses (for details see Giblin et al. 1990): Bulk density and water content were determined by weighing a known volume of mud and drying it in an oven at 55 °C. Total S was determined on similarly oven-dried samples with a LECO SC-32 Sulfur Determinator and LECO coal calibration standards. Duplicate samples had an average coefficient of variation of 1.1%

of the mean (N = 9 pairs). Chromium-reducible S (CRS), which includes Fe monosulfides, FeS_2, and elemental S, was determined on freeze-dried mud by heating in an acid $Cr(II)Cl_2$ solution (Howarth & Merkel 1984). Liberated sulfides were precipitated in a Zn acetate trap and measured colorimetrically. Sulfate samples were bubbled with N_2 to remove sulfides prior to analysis by ion chromatography (Dionex 2010i). Organic S was calculated by subtracting CRS from total S.

Alkalinity (Alk) was determined by potentiometric titration with 0.16N H_2SO_4. Titration endpoints were determined by the Gran method (Stumm & Morgan 1981).

Isotope Analyses: Total S isotopic composition of sediments was determined by first converting S to sulfate and then precipitating the sulfate with barium chloride to $BaSO_4$ (White et al. 1989). Oven-dried sediments (200–300 mg) were mixed in a 1:10 ratio with powdered KNO_3. The mixture was vacuum sealed in large Pyrex tubes (22 mm O.D., 16 inch long) and combusted in a muffle furnace as follows: 190 °C overnight, 350 °C for two hours, 450 °C for two hours, and 580 °C for two hours. After cooling overnight, the tube bottoms were frozen in liquid nitrogen to prevent an explosion when they were opened. Tubes were then cracked open, thawed, and the contents were digested in 0.1 N HCl. The leachate was then filtered (#4 Whatman) to remove coarse particulates (20–25 μm pore size). The filtered solution was acidified with 6 M HCl and heated to boiling, at which point the solution received a dropwise addition of 3 ml of 10% $BaCl_2$. Boiling continued for twenty minutes; then the solution was allowed to cool overnight. The $BaSO_4$ precipitate was recovered onto Whatman #42 filter paper (2.5 μm pore size) and combusted for twenty minutes at 250 °C and thirty minutes at 850 °C. The precipitate was then decomposed to SO_2 with V_2O_5 for mass spectrometric analysis (Yanagisawa & Sakai 1983). Sediment samples that were highly enriched isotopically due to the ^{34}S spike were diluted with an SO_2 standard gas of known mass and isotopic content ($\delta^{34}S$ = +3.05 ‰) to minimize contamination of the mass spectrometer.

Sulfate in water samples was also precipitated with barium, filtered onto Whatman #42 filter paper, ashed, and decomposed to SO_2 as described above. Samples that were highly enriched isotopically due to the ^{34}S spike were diluted with a known amount of 10 μM sulfate solution ($\delta^{34}S$ = –3.4 ‰) to minimize contamination of the mass spectrometer.

Reduced S from CRS analysis was captured as a zinc sulfide precipitate in zinc acetate traps. The acetate was rinsed from the precipitate with distilled water, and then the precipitate was decomposed to SO_2 with V_2O_5. Pyrite standards analyzed by the CRS procedure were found to be within 0.5 ‰ of pyrite standards analyzed directly by the sealed tube method described above (Mean = 7.0 ‰, n = 12).

All S isotopic determinations were measured on a Finnigan MAT 251 isotope ratio mass spectrometer (except $\delta^{34}S_{org}$, which was calculated by

difference). Samples were measured relative to the Canyon Diablo Troilite standard and calculated as

$$\delta^{34}S\ (‰) = [(R_{sample}/R_{std}) - 1] * 10^3 \quad (1)$$

where $R = {}^{34}S:{}^{32}S$. Duplicate samples varied by < 0.2 ‰.

A mass balance approach was used to back calculate actual isotope values from the diluted samples that were run on the mass spectrometer. In the equation

$$(M_s)({}^{34}S_s) + (M_d)({}^{34}S_d) = (M_{ds})({}^{34}S_{ds}) \quad (2)$$

M stands for mass of S and the subscripts s, d, and ds stand for sample, diluent, and diluted sample, respectively. ${}^{34}S_d$ and ${}^{34}S_{ds}$ are determined from a linear relationship between $\delta^{34}S$ (measured) and ${}^{34}S$ (Peterson & Fry 1987). Since M_s, M_d, and M_{ds} are known, ${}^{34}S_s$ can then be calculated. In addition, one must calculate ${}^{32}S_s$ based upon proportional changes in abundance which are due to the isotopic enrichment. The total S pool is represented by the sum of its isotopes:

$$S = {}^{32}S + {}^{33}S + {}^{34}S + {}^{36}S \quad (3)$$

At the natural abundance level of 0 ‰, the relative proportions of the isotopes are: ${}^{32}S = 0.94941$, ${}^{33}S = 0.00769$, ${}^{34}S = 0.04273$, and ${}^{36}S = 0.00017$ (${}^{36}S$ is small enough to be insignificant for the purposes of our calculations). When the S pool is enriched in ${}^{34}S$, the relative abundances of the other isotopes decrease accordingly. Although ${}^{32}S_s$ can be estimated by the change in ${}^{34}S$, a small correction must be made to take into account the change in ${}^{33}S$ as well. Once this proportional correction has been made, equation (1) can be solved for $\delta^{34}S$ of the enriched samples.

Determination of Sulfate Reduction Rates: We used isotope dilution equations (Blackburn 1979; Bowden 1984) to calculate gross and net rates of sulfate reduction. The sulfate concentration (C) in the overlying water at any time (t) can be described by the following equation:

$$C(t) = C(0) + (r_p - r_c)t$$

where $C(t)$ = sulfate concentration at time t
$C(0)$ = sulfate concentration at time 0
r_p = gross production rate of sulfate
r_c = gross consumption rate of sulfate

Here production and consumption are assumed to be gross sulfide oxidation and gross sulfate reduction, respectively. If there is no change in the sulfate concentration over time, $C(t) = C(0)$, and thus the quantity $(r_p - r_c)$, the net

sulfate reduction rate, is zero. In this case, the gross sulfate reduction rate can be determined by the rate of decrease in the fraction of ^{34}S in the overlying water with time relative to natural abundance levels in the sediment.

Results and discussion

Overlying water: Sulfate concentrations in the ambient (70 µM) sulfate treatment did not change over the course of the experiment (Fig. 1a), indicating that the net sulfate reduction rate was zero for this treatment. In contrast to the concentration of sulfate, the fraction of ^{34}S in the sulfate dropped significantly between water changes, indicating a significant turnover of sulfate in the overlying water (Fig. 1b). The decrease in $^{34}S_{SO4}$ relative to natural abundance levels is a function of the gross sulfate reduction rate (Fig. 2). The average gross sulfate reduction rate (r_c) for the three 70 µM

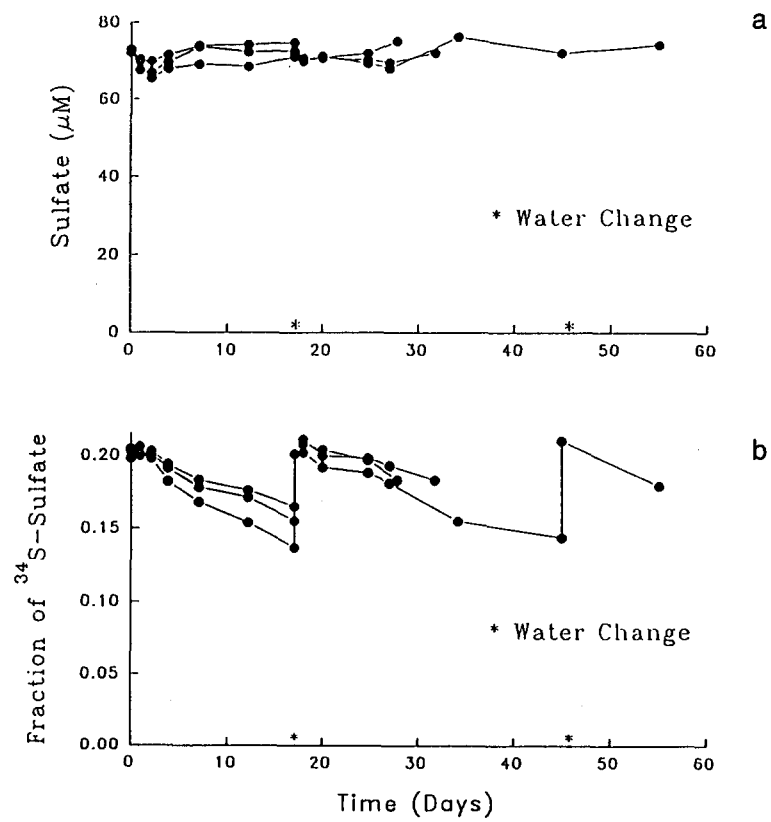

Fig. 1. Mares Pond microcosm Experiment: 70 µM Sulfate Treatment. a) Sulfate concentration (µM) and b) the fraction of ^{34}S in the overlying water. * indicates complete change of overlying water.

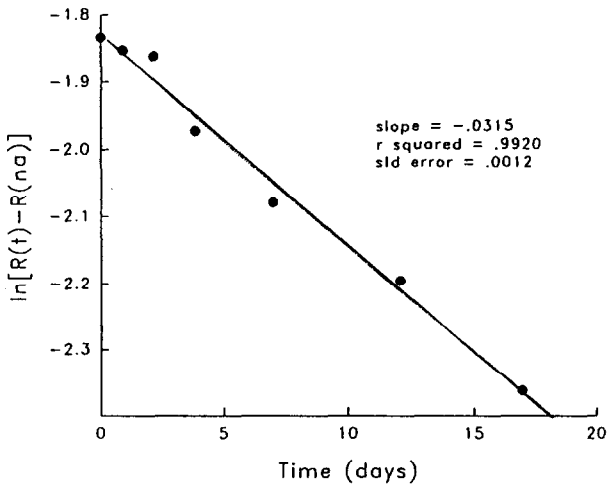

Fig. 2. Mares Pond Microcosm Experiment. Isotope dilution plot for Core E in 70 µM Sulfate Treatment. R(t) = relative abundance of ^{34}S in overlying water at time t. R(NA) = natural abundance of ^{34}S in Mares Pond sediment, taken to be 7 ‰ (Fry, unpublished data).

sulfate treatment cores was 352 ± 96 (SE) µmoles/m^2/d (Table 1). Because there was no net sulfur storage the sulfide oxidation rate (r_p), must be equal to the gross sulfate reduction rate, i.e. 352 ± 96 (SE) µmoles/m^2/d (Table 1).

In the 260 µM sulfate treatment, sulfate concentrations decreased between water changes (Fig. 3a). The fraction of ^{34}S in the overlying water of these cores did not decrease as much as in the 70 µM sulfate treatment cores due to the larger sulfate pool size (Fig. 3b). However, the decrease in ^{34}S was sufficient to apply isotope dilution equations to the data. Using the methods of Bowden (1984), the gross sulfate reduction rate (r_c) in the high sulfate treatment was 342 ± 91 (SE) µmoles/m^2/d (Table 1), which was not statistically different from the rate calculated for the ambient sulfate treatment. Increasing sulfate concentrations well above ambient levels did not appear to increase gross sulfate reduction rates as calculated by isotope dilution. Thus, the results suggest that gross sulfate reduction is not sulfate limited in Mares Pond.

As calculated by isotope dilution, the high sulfate treatment had a gross oxidation rate (r_p) = 202 ± 46 (SE) µmoles/m^2/d. The difference between the rates of reduction and oxidation suggests that a small amount of net sulfate reduction is occurring ($r_c - r_p$ = 140 ± 45 (SE) µmoles/m^2/d). Another way to calculate the net sulfate reduction rate is by mass balance, where "sulfate disappearance" is the measured disappearance of sulfate from the overlying water, and the "increase in porewater storage" is the difference between the porewater sulfate inventory at the end of the experiment and that at the beginning. The net sulfate reduction rate is then calculated by subtracting the increase in porewater storage from the sulfate disappearance. We did observe

Table 1. Comparison of rate data between initial and treatment cores. O_2 uptake was measured prior to start of experiment. r_p (gross sulfate production rate) and r_c (gross sulfate consumption rate) calculated by isotope dilution. SO_4^{2-} disappearance is from the overlying water. Net sulfate reduction rate is calculated by mass balance. Alkalinity gain is measured in overlying water. Errors reported are standard errors.

Treatment/ Core	O_2 uptake (mmoles/m^2/d)	Time harvested (days)	r_p (μmoles/m^2/d)	r_c (μmoles/m^2/day)	SO_4^{2-} disappearance (μmoles/m^2/day)	Net sulfate reduction rate (μmoles/m^2/day)	Alkalinity gain (μequiv./m^2/d)
Initial							
A	8.57	0					
B	9.27	0					
70 μM SO_4^{2-}							
C	8.75	28	256	256	-47.3	–	443
D	9.26	32	317	317	0.5	–	271
E	13.20	55	483	483	1.5	–	251
	$\bar{x} = 10.07 \pm 1.99$		$\bar{x} = 352 \pm 96$	$\bar{x} = 352 \pm 96$	$\bar{x} = 15.1 \pm 22.8$		$\bar{x} = 322 \pm 86$
260 μM SO_4^{2-}							
F	10.28	28	170	330	473	123	970
G	11.29	32	267	459	551	214	748
H	9.41	55	170	237	323	179	322
	$\bar{x} = 10.33 \pm 0.77$		$\bar{x} = 202 \pm 46$	$\bar{x} = 342 \pm 91$	$\bar{x} = 449 \pm 95$	$\bar{x} = 172 \pm 37$	$\bar{x} = 680 \pm 269$

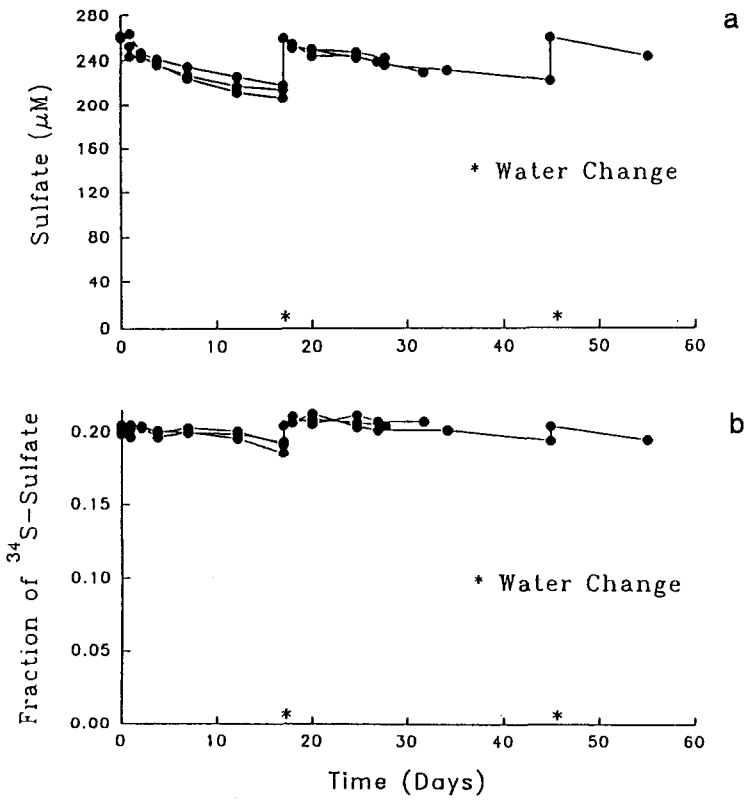

Fig. 3. Mares Pond Microcosm Experiment: 260 μM Sulfate Treatment. a) Sulfate concentration (μM) and b) the fraction of ^{34}S in the overlying water. * indicates complete change of overlying water.

sulfate disappearance from the overlying water (Fig. 3a, Table 1). Subtracting the increase in porewater storage (data not shown) from the sulfate disappearance we calculate a net sulfate reduction rate of 172 ± 37 (SE) μmoles/m^2/d (Table 1). Thus the net sulfate reduction rate calculated by mass balance compares well to the net rate calculated by isotope dilution. The alkalinity data (Table 1) supports our net sulfate reduction rate calculations. Alkalinity is generated and consumed from a variety of processes, including sulfate reduction and sulfide oxidation. The reduction of sulfate to sulfide generates two equivalents of alkalinity for every equivalent of sulfate consumed. Table 1 shows that the difference in alkalinity generation between the high and low sulfate treatments (358 μequivalents/m^2/d) is almost exactly what would be predicted from the net sulfate reduction rates calculated by isotope dilution and by mass balance. However, as explained above, the net sulfate reduction in the high sulfate treatment appears to have resulted not

from an increase in gross sulfate reduction but from a decrease in sulfide oxidation (Table 1).

In our experiment gross sulfate reduction did not increase with increasing sulfate concentration. This suggests that the sulfate reducing bacteria in the sediments of Mares Pond are strongly carbon limited rather than sulfate limited. It is more difficult to think of mechanisms which would explain the decrease in sulfide oxidation we observed. We would expect that a smaller percentage of the reduced sulfur would be oxidized if the sulfate reducing zone extended deeper into the sediment when sulfate concentrations in the overlying water were increased. However, this should increase the total zone where sulfate reduction is occurring and hence increase the gross sulfate reduction rate. In the following paragraphs we discuss various sources of error and speculate on the reason for the observed increase in sulfur storage.

One explanation for our results might be that the assumptions behind our isotope dilution calculations are in error. We assumed that the S being oxidized has a $^{34}S/^{32}S$ ratio equal to that of the bulk sediment. Given the short time scale of this experiment, the bulk pool of ^{34}S in the sediment would not change appreciably. However, it is possible that the most recently reduced S would be most subject to reoxidation. The linearity of the data in Figure 2 suggests that the $^{34}S/^{32}S$ ratio of the S being oxidized did not noticeably change over the time scale of this experiment. Thus, the assumption is probably valid.

Another issue is whether the differential diffusion of ^{34}S and ^{32}S into the sediment affects the calculated rates of sulfate reduction. The molecular diffusion coefficients of ^{34}S and ^{32}S in aqueous solution are similar, but the concentration gradients of the two isotopes were initially different in our experiment as a result of the ^{34}S enrichment. Chanton et al. (1987), studying S isotopes in anoxic marine sediments, concluded that isotopes diffuse in response to their individual concentration gradients. The enrichment of ^{34}S in the overlying water of the ambient treatment cores changed the relative abundance of each isotope such that there was an initial concentration gradient in ^{34}S directed into the sediment and a gradient in ^{32}S directed out of the sediment. If the sulfate reducing bacteria in the sediment initially consumed more ^{34}S than ^{32}S due to different concentration gradients, and the calculation of gross sulfate reduction is based on the decrease in the fraction of ^{34}S in the overlying water, then our calculated sulfate reduction rate is an upper limit.

We measured the $^{34}S/^{32}S$ ratios in the porewater at the end of the experiment. The ratios of $^{34}S/^{32}S$ in the porewater were similar to the overlying water, confirming the assertion of Chanton et al. (1987) that differential diffusion occurs in response to specific gradients. However in this experiment, overestimation of the sulfate reduction rate due to differential diffusion is believed to be small, since the amount of sulfate diffusion required to equilibrate the initial porewater concentration imbalance was less than 10% of the total sulfate uptake over the course of the experiment. Further, our calculations of the rates of diffusion of the isotopes show that most of the concentration gradient

imbalance is corrected within seven days. Here again, the linearity of the dilution data over eighteen days (plotted for Core E in Fig. 2) shows that diffusion is not the major cause of ^{34}S-sulfate dilution from the overlying water of these cores.

In the high sulfate (260 μM) treatment, the initial concentration gradients for ^{34}S and ^{32}S were both directed into the sediment. As with the ambient sulfate treatment, if the initial consumption of ^{34}S was greater than ^{32}S then our calculated rate of sulfate reduction is an upper limit. But again, the amount of sulfate diffusion required to equilibrate the initial concentration imbalance was less than 10% of the total sulfate uptake. Thus, any overestimation of the calculated sulfate reduction rate is believed to be small.

An additional consideration is whether isotopic fractionation during sulfate reduction plays a major role in determining the fate of ^{34}S and ^{32}S in this system. Sulfate reduction in lake sediments may produce sulfides that are depleted in ^{34}S relative to ^{32}S. The size of the depletion is typically in the range of 2–20 ‰ (Fry 1986). However, the sediments in the upper 10 cm of a Mares Pond core (Fry, unpublished data) had $\delta^{34}S$ values only 1–3 ‰ lower than lake sulfate $\delta^{34}S$ values, indicating that isotopic fractionation during sulfate reduction in Mares Pond sediments is small. Further, a majority of field and laboratory studies have reported little or no fractionation (0–5 ‰) associated with the oxidation of sulfides (Toran & Harris 1989).

Finally there is the issue as to whether or not the sulfate losses and gains are actually due to oxidative and reductive processes. The formation or mineralization of ester sulfates would also change the sulfur balance. Studies on lake sediments usually show some net mineralization of sulfate esters within sediments (David & Mitchell 1985), and this would lead to an overestimate of our gross sulfide oxidation rate. However, this would not cause us to underestimate gross sulfate reduction rates at higher sulfate concentrations. In addition, we found that the CRS fraction of the sediment was more heavily labelled than organic sulfur at all depths except in the top cm. CRS can only be formed through dissimilatory S reduction while organic S may be formed through both assimilatory and dissimilatory processes. The greater labelling we observed in the CRS fraction (shown below) demonstrates that dissimilatory S reduction is more important than assimilatory processes in this experiment.

In summary, we feel that the rates we have calculated are not artifacts of our experiments. The net sulfur storage we measured by mass balance is also in close agreement with the difference between the gross sulfate reduction rates and the gross sulfide oxidation rates we calculated by isotope dilution lending further support to the calculations. We offer two possible explanations as to why we observed an increase in sulfur storage in the absence in an increase in gross sulfate reduction rates. The first is that core to core differences were large enough to obscure a real stimulation in the gross sulfate reduction rates with higher sulfate concentrations. The treatment means were not significantly different but the standard errors of the treatment means were

about 90 μmoles/m^2/d (Table 1), nearly 30% of the mean. The second is that sulfide oxidation decreased in the high sulfate treatment because in the high sulfate treatment sulfate penetrated deeper into the sediments. This allowed sulfate reducing bacteria to intercept reduced substrates diffusing up from depth deeper in the sediment than in the low sulfate treatment. By intercepting the substrates deeper, the sulfate reduction zone moves down to a zone where there was less sulfide oxidation but the gross sulfate reduction rate remained unchanged. The sediment data presented below confirms that the zone of the sulfur storage was deeper in the high sulfate treatment but the importance of reduced substrates diffusing up from below is speculative.

Comparison to in-situ rates: Kling et al. (1991) determined *in situ* rates of net sulfate reduction for Mares Pond sediments by measuring the net loss of sulfate from the water column when the hypolimnion was anoxic. Their *in situ* rate of sulfate loss of 680 μmoles/m^2/d greatly exceeds our zero net rate in the 70 μM treatment indicating that anoxic conditions greatly decrease sulfide oxidation. However, not all reduced sulfides produced from sulfate reduction will be permanently stored. Kling et al. (1991) reported substantial rates of sulfide oxidation when the overlying water of Mares Pond cores was aerated to simulate turnover. Giblin et al. (1990) reported that the long term (since 1900) inorganic S burial rate for Mares Pond was approximately 30 μmoles/m^2/d. Thus, oxidation of reduced S is an important control on the storage of S in Mares Pond sediments in the natural environment as well as in our experiments.

The net rate of sulfate reduction reported by Kling et al. (1991) exceeds our gross rate by a factor of two. Since their measurements were taken when the hypolimnion was anoxic there should be little sulfide oxidation, and their net rate of sulfate reduction should equal our gross rate. The difference in the reported rates may reflect the lack of fresh carbon inputs to our microcosms over the course of the experiment, or the difference in the amounts of carbon available to sulfate-reducing bacteria when the overlying water is oxic. Both explanations support the hypothesis that sulfate reduction in these sediments can be limited by carbon supply.

Sediments: The CRS fraction of the sediments was measurably labelled in ^{34}S in cores from both sulfate treatments (Fig. 4). In all cores the most highly labelled sediments were found between 1–2 cm. The depth of the label extended down to approximately 9 cm, showing that gross sulfate reduction was occurring down to this depth. After the first month of the experiment, the amount of ^{34}S in the CRS fraction at depths below 4 cm was greater in the high sulfate treatment (Fig. 4). It may be that increasing the sulfate concentration increases S burial in the long term by pushing the zone of sulfate reduction deeper into the sediment where oxidation is less important.

Constructing a complete mass balance for all the ^{34}S in this system was not possible. The net reduction of sulfate during the experiment (Table 1) was very small (< 1%) compared to the total sulfur inventories in the cores (Table 2). There is a high natural background concentration (4.2%) of ^{34}S in

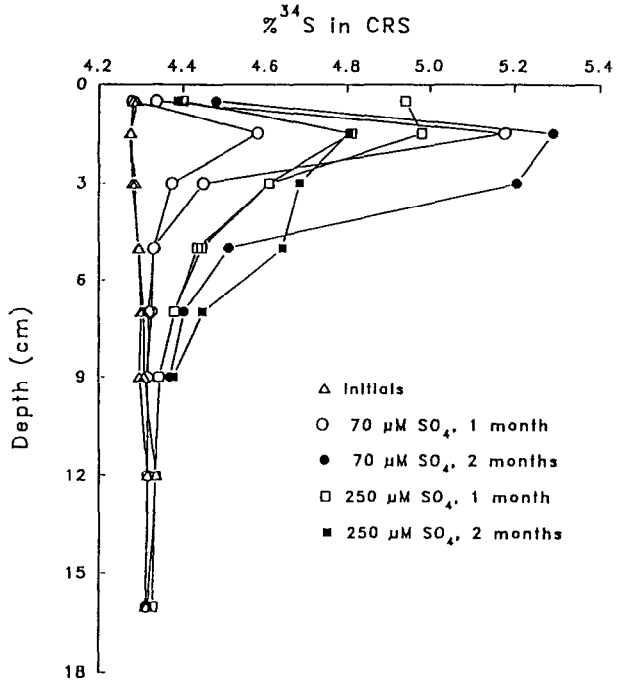

Fig. 4. Mares Pond Microcosm Experiment. $\%^{34}S$ in CRS fraction in sediments.

Table 2. Time of harvest and sediment inventories for CRS and $CR^{34}S$. Errors reported are standard errors.

Core	Time harvest (days)	CRS inventory 0–10 cm ($mmoles/m^2$)	$CR^{34}S$ inventory 0–10 cm ($mmoles/m^2$)
Initial			
A	0	822	35.3
B	0	840	36.1
		$\bar{x} = 831 \pm 9$	$\bar{x} = 35.7 \pm 0.4$
70 μM SO_4^{2-}			
C	28	984	42.8
D	32	813	35.5
E	55	676	30.4
		$\bar{x} = 824 \pm 126$	$\bar{x} = 36.2 \pm 5.1$
260 μM SO_4^{2-}			
F	28	833	36.9
G	32	965	43.0
H	55	1053	47.8
		$\bar{x} = 951 \pm 90$	$\bar{x} = 42.6 \pm 4.5$

the sediments. Although we could clearly see differences in the incorporation of label into the sediments between the treatments, core to core differences in total sulfur, CRS content, and the bulk density made it impossible to measure differences in the total ^{34}S inventory (illustrated with CRS in Table 2).

In spite of the fact that we could not complete a rigorous mass balance of all the ^{34}S in the experiment we could use ^{34}S to measure the relative importance of sulfur incorporation into CRS and organic S. In the surface sediments of both the ambient and high sulfate treatments, CRS and organic S are equally labelled with ^{34}S (Table 3). Both fractions are highly labelled compared to average surface (0–3 cm) $\delta^{34}S$ values measured by Kling et al. (1991) for a core from Mares Pond ($\delta^{34}S_{CRS} = -3.2$, $\delta^{34}S_{ORG} = 10.4$). However, because CRS makes up only about 20% of the total sulfur at this depth, organic sulfur is the major sulfide sink in the surface one cm of the sediments.

Table 3. $\delta^{34}S$ (‰) of total sulfur (TOTS), chromium-reducible sulfur (CRS), and organic sulfur (ORG) for the ambient sulfate treatment (70 μM, Core D) and high sulfate treatment (260 μM, Core F). Dried sediment was rinsed to remove sulfate prior to determination of total sulfur. Organic sulfur values were calculated by mass balance.

Depth (cm)	$\delta^{34}S_{TOTS}$	$\delta^{34}S_{CRS}$	$\delta^{34}S_{ORG}$
Ambient SO_4^{2-}			
0–1	20.4	15.8	21.2
1–2	50.5	223.1	-2.9
2–4	20.7	42.6	2.8
4–6	12.3	14.0	10.3
6–8	10.9	11.4	10.4
High SO_4^{2-}			
0–1	29.8	30.8	28.5
1–2	63.9	131.9	45.5
2–4	53.8	82.1	40.8
4–6	28.2	39.5	24.6
6–8	20.2	26.6	18.1

Below 1 cm, the CRS fraction is the more heavily labelled storage product in both treatments of this experiment, and the 1–2 cm section is the most highly labelled. At these depths CRS makes up nearly half of the total sulfur pool making CRS the more important sulfide sink for the sediments overall.

Conclusions

The experiment presented here demonstrated how an understanding of sulfide oxidation is critical to a thorough understanding of S dynamics in lake sediments. Increasing the sulfate concentration in water overlying Mares Pond sediments increased the net sulfate reduction rate, not by increasing the gross

sulfate reduction rate but by decreasing the rate of sulfide oxidation. The results suggest that some other factor, such as carbon, may limit gross sulfate reduction in Mares Pond sediments. Higher sulfate concentrations may increase net sulfur storage in the long term by pushing the zone of sulfate reduction deeper into the sediment, where reduced sulfides are less susceptible to oxidation. ^{34}S-labelled sulfate also proved to be useful as a tracer in determining the fate of sulfur in Mares Pond sediments. At the end of the experiment both the CRS and organic S fractions were labelled. At both sulfate concentrations, CRS and organic S were nearly equally labelled near the surface but CRS was more highly labelled below 1 cm.

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