

## RESPONSE OF ANAEROBIC CARBON MINERALIZATION RATES TO SULFATE AMENDMENTS IN A BOREAL PEATLAND

MELANIE A. VILE,<sup>1,3</sup> SCOTT D. BRIDGHAM,<sup>1,4</sup> AND R. KELMAN WIEDER<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556 USA

<sup>2</sup>Department of Biology, Villanova University, Villanova, Pennsylvania 19085 USA

**Abstract.** A small body of research suggests that dissimilatory sulfate reduction can affect the carbon balance of peatlands, yet this has not been tested widely, despite the fact that peatlands contain approximately one-third of the global soil carbon pool. Here we evaluate the role of dissimilatory sulfate reduction in a site that receives low atmospheric sulfur deposition. We hypothesized that, in peatlands receiving low sulfate inputs, methane production should dominate anaerobic carbon mineralization. We further hypothesized that with sulfate amendments, anaerobic carbon mineralization could show an overall increase if terminal carbon mineralization in unamended peat is limited by an inadequate supply of electron acceptors. To test these hypotheses, we delineated six 1-m<sup>2</sup> plots in an ombrotrophic, boreal peatland in central Alberta, Canada (Bleak Lake Bog), which receives <2 mmol S·m<sup>-2</sup>·yr<sup>-1</sup>. Three of the plots were amended with sulfate (78 mmol S·m<sup>-2</sup>·yr<sup>-1</sup>). We measured anaerobic rates of sulfate reduction, CH<sub>4</sub> production, and CO<sub>2</sub> production. In opposition to our hypotheses, sulfate amendments did not increase rates of sulfate reduction, increase CO<sub>2</sub> production, or decrease CH<sub>4</sub> production over a 2-yr period, but did increase both sulfate pool sizes and residence times. Despite low rates of sulfate reduction compared to other freshwater wetlands, daily average sulfate reduction (1.7 mmol/m<sup>2</sup>) exceeded regional annual inputs of atmospheric sulfate deposition (1.6 mmol/m<sup>2</sup>). Between 77% and 99% of reduced sulfate was incorporated into the carbon-bonded sulfur pool, which turns over slowly. The slow turnover rate and comparatively low sulfate reduction rates may be related to the low iron content of the bog peat. Additionally, sulfate reduction initially was sulfate limited in the control plots, but with sulfur amendments, the sulfate limitation was removed; sulfate reduction appeared to be limited by some other factor, possibly labile carbon. Dissimilatory sulfate reduction was more important to total anaerobic carbon mineralization than methane production, although neither process dominated overall anaerobic carbon mineralization (<2% of total). Fermentation appeared to be the dominant anaerobic carbon mineralization pathway at Bleak Lake Bog, yet the mechanisms of how this process affects the carbon balance of peatlands has never been evaluated. Overall, our results suggest that, in at least the short term, soil carbon turnover in peatlands will not be enhanced by increased atmospheric sulfur deposition. If we want to understand the controlling factors on soil carbon storage in sites like Bleak Lake Bog, we should begin to examine anaerobic carbon mineralization via fermentation pathways.

**Key words:** carbon; CH<sub>4</sub> production; CO<sub>2</sub> production; dissimilatory sulfate reduction; ombrotrophic; peatlands; Sphagnum; sulfur.

### INTRODUCTION

Peatlands cover large areas of the earth's land surface (346–500 × 10<sup>6</sup> ha) (Gorham 1995, Bridgham et al. 2000), and contain one-third of the global soil carbon pool (~500 Pg C) (Gorham 1995). With such a large portion of the global soil carbon pool in peatlands, a shift in the balance between net primary productivity and decomposition under a changing climate could po-

tentially result in peatlands becoming a net source of atmospheric carbon (Moore et al. 1998). As with most ecological systems, carbon mineralization rates within peatlands exhibit considerable temporal and spatial variability on a variety of different scales (e.g., Waddington and Roulet 1996, Bridgham et al. 1998, Updegraff et al. 1998, 2001, Rustad et al. 2000). Consequently, quantifying the present role of peatlands in the global carbon cycle is challenging. Even more difficult is predicting peatland carbon cycling responses under a changing climate (Moore et al. 1998, Kirschbaum 2000, Wieder 2001). Although the need for better information about microbially mediated carbon cycling dynamics in peatlands has been realized for over a decade (cf. Gorham 1991), we still have only a rudimentary understanding of the considerable heterogeneity of peatland microbial processes (Gorham 1995,

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<sup>3</sup> Present address: Department of Ecology and Evolutionary Biology, 106A Guyot Hall, Princeton University, Princeton, New Jersey 08544, USA. E-mail: mvile@princeton.edu

<sup>4</sup> Present address: Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, Oregon 97403-5289 USA.

Bridgman et al. 1998). Dissimilatory sulfate reduction is one such process that can affect carbon cycling in peatlands, yet it has not been studied extensively in these systems, despite their importance in the global carbon cycle.

The importance of dissimilatory sulfate reduction to anaerobic carbon mineralization, carbon balance, and energy flow has been demonstrated widely in salt marshes and marine sediments; sulfate reduction is the dominant anaerobic carbon mineralization pathway in those systems (Howarth and Teal 1979, 1980, Howarth and Jorgenson 1984). A few studies have examined sulfate reduction in freshwater peat, and have found that sulfate reduction can play a major role in anaerobic carbon mineralization (e.g., Wieder et al. 1990). Despite the small sulfate pool size, reported rates of sulfate reduction in freshwater wetlands overlap with the lower end of rates typically reported for salt marshes (Spratt et al. 1987, Wieder and Lang 1988, Wieder et al. 1990). This overlap is facilitated by the rapid turnover of sulfate between reduced and oxidized inorganic forms (Wieder and Lang 1988).

Few studies have determined rates of sulfate reduction in freshwater peat (i.e., Spratt et al. 1987, Wieder and Lang 1988, Nedwell and Watson 1995), and these studies have been conducted in sites that receive moderate inputs of atmospheric sulfur deposition (i.e., 31–78 mmol S·m<sup>-2</sup>·yr<sup>-1</sup>). The incidence of high sulfate inputs through acid precipitation, coupled with the unexpected importance of sulfate reduction in freshwater peatlands, led Wieder et al. (1990) to suggest a new hypothesis. Peatlands receiving low inputs of atmospheric sulfate should exhibit a predominance of methanogenesis over sulfate reduction if all other electron acceptors are in low supply. However, if such peatlands become subjected to high rates of sulfur deposition, sulfate reduction could be enhanced as an anaerobic carbon mineralization pathway, at the expense of methanogenesis.

The main objective of this study was to determine the contribution of sulfate reduction to anaerobic carbon mineralization in a peatland that receives low atmospheric sulfur deposition. If methane production is the dominant anaerobic carbon mineralization pathway at this site, then sulfate amendments should lead to an increase in the contribution of sulfate reduction to anaerobic carbon mineralization. The consequences for total carbon dioxide (CO<sub>2</sub>) production are less certain. If in the natural system, organic carbon compounds are efficiently mineralized to CO<sub>2</sub>, then adding sulfate might be expected to alter the relative importance of different terminal carbon mineralization pathways without a corresponding change in total CO<sub>2</sub> production. However, if mineralization of carbon compounds is limited by the availability of electron acceptors, then addition of sulfate could increase CO<sub>2</sub> production.

In this manuscript, we examine the role of atmospheric sulfur deposition on sulfur biogeochemistry

and anaerobic carbon mineralization in peat from Bleak Lake Bog, central Alberta, Canada. Specifically, we consider sulfur cycling in terms of sulfur incorporation into both inorganic and organic fractions via dissimilatory sulfate reduction, and the proportion of total carbon mineralization via the sulfate reduction and methane production pathways.

## METHODS

### *Study site*

Bleak Lake Bog (54°41' N, 113°28' W, elevation 625 m) is an ombrotrophic bog within a larger peatland complex located in the Sub-humid Low Ecoclimatic Region of central Alberta, Canada (Ecoregions Working Group 1989). The area is characterized by warm summers and cold, snowy winters (Vitt et al. 1995). Total annual precipitation is ~490 mm, with greatest precipitation in June and July. The average mean annual temperature is 1.4°C, and the frost-free period is ~120 d (Szumigalski and Bayley 1996). Annual atmospheric sulfur deposition is <1.6 mmol/m<sup>2</sup> (McDonald et al. 1996).

The topography of Bleak Lake Bog is characterized by forested hummocks and lawns (Vitt et al. 1995). The vegetation of the forested portion of the bog is composed of scattered *Picea mariana* and a layer of ericaceous shrubs, *Ledum groenlandicum*, *Vaccinium oxycoccus*, *Smilacina trifolia*, and *Rubus chamaemorus* (Szumigalski and Bayley 1996). The lawns consist of large, dry hummocks interspersed with smaller, wetter hollows. Hummocks are vegetated predominantly by the moss *Sphagnum fuscum*, while hollows are vegetated by *Sphagnum angustifolium* and *Sphagnum magellanicum* (Vitt et al. 1995, Szumigalski and Bayley 1996).

The hummock peat surface is typically between 0.3 and 0.5 m above the water table, and surface water pH ranges from 3.4 to 4.2 (Szumigalski and Bayley 1996). In August 1996 and July 1997, the water table was ~15 and 10 cm below the peat surface, respectively, and pore water pH was 4.2 on both sampling dates (M. A. Vile, *personal observation*). Even above the water table, water contents in peat are typically >90% water on either a wet mass (cf. Fig. 1) or volume basis, and thousands of percent on a dry mass basis. As a result, the existence and persistence of anaerobic microsites within the peat above the water table can sustain anaerobic processes.

### *Experimental setup and peat core collection*

In June 1996, we established six plots (1.0 m<sup>2</sup>) in a sparsely forested lawn portion of the bog to minimize the effect of shading from trees and to avoid difficulty in coring due to the presence of tree roots. The plots were characterized by nearly complete *Sphagnum* coverage. The experimental design consisted of three replicate plots randomly assigned each to a control treat-

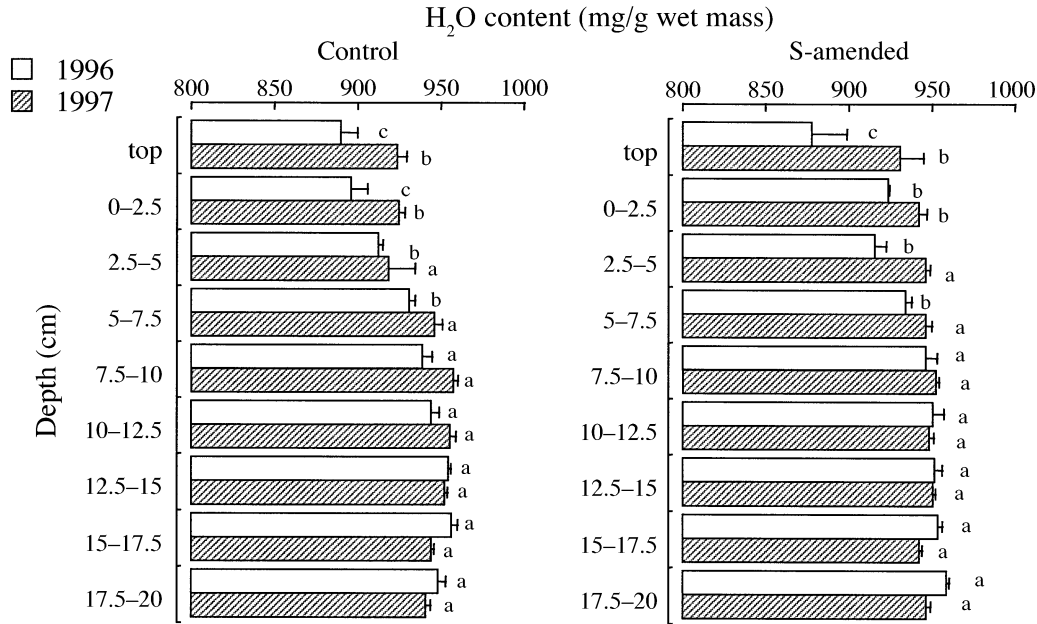


FIG. 1. Water content as a function of depth for the control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations + 1 SE (core determination  $\times$  3 subreplicates per core). Means with different lowercase letters represent significant ( $P < 0.05$ ) differences.

ment and to a sulfur-amended treatment. The sulfur-amended plots received  $\text{Na}_2\text{SO}_4$  at a rate of  $80.5 \text{ mmol SO}_4^{-2}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$  to simulate deposition of atmospherically derived sulfate in a moderately polluted region.

We added  $\text{Na}_2\text{SO}_4$  to 12 L of bog water collected from a water hole located  $\sim 15 \text{ m}$  from the plots, and sprinkled the solution uniformly over each plot; control plots received bog water only. Field application of S to the plots in 1996 was made approximately once every two weeks from 25 June through 15 September, and in 1997, from 15 May through 15 September. On 8 August 1996 and 30 July 1997, we collected two peat cores from each plot using 10 cm diameter, 20 cm long polyvinyl chloride (PVC) cylinders. One core was used to determine rates of sulfate reduction, while the other core was used to determine anaerobic  $\text{CO}_2$  and  $\text{CH}_4$  production. Care was taken to ensure minimal compaction of peat during core extraction. If in any case compaction was  $>2 \text{ cm}$ , cores were abandoned and another attempt at extraction was made. We capped the cores and transported them to the Meanook Biological Field Station where cores were immediately labeled with carrier-free  $^{35}\text{SO}_4^{2-}$  for determination of sulfate reduction rates and incubated anaerobically for determination of  $\text{CO}_2$  and  $\text{CH}_4$  production rates.

#### Rates of sulfate reduction

Rates of sulfate reduction were calculated as the proportion of added  $^{35}\text{SO}_4^{2-}$  incorporated into both the reduced inorganic sulfur (RIS) and the carbon-bonded sulfur (CBS) fractions. The RIS fraction includes  $\text{H}_2\text{S}$ ,  $\text{FeS}$ ,  $\text{FeS}_2$ , and  $\text{S}^0$ , while the CBS fraction includes

organic matter containing a C-S bond. The dominant end product of sulfate reduction in marine systems, where both sulfate (concentrations typically  $>40 \text{ mmol/L}$ ) and ferrous iron ( $\text{Fe}^{2+}$ ) are abundant, is the RIS fraction. Incorporation into the CBS fraction contributes minimally to the total rate (Howarth and Teal 1979, Wieder and Lang 1988). In freshwater wetlands, however, where concentrations of sulfate can be quite low (typically  $<15 \mu\text{mol/L}$ ), and where in some cases ferrous iron concentrations are low, rates of sulfate reduction may be underestimated substantially if incorporation into the CBS fraction is not determined (Wieder et al. 1990).

Rates of sulfate reduction were determined by radiolabeling the cores with  $^{35}\text{SO}_4^{2-}$ . While still housed inside the PVC pipe, we removed the bottom end caps, and allowed the gravitational pore water to drain. Once drained, we replaced the end caps, and sprinkled the cores with  $\sim 7.8 \text{ MBq } ^{35}\text{S}$  as  $\text{Na}_2\text{SO}_4$  in roughly the same volume of water that was drained [i.e., 222 mL distilled, deionized water (DDW)] using a plastic wash bottle. We incubated the cores for 48 h at  $20^\circ\text{C}$  in 1996 (24 h in 1997), and stopped the reaction by surrounding the cores with dry ice. Once frozen, we sectioned each peat core into 2.5 cm depth increments using a band saw, and each depth increment was further divided into six subsections. Three of the six subsamples of each depth increment were used to determine both the RIS and CBS fractions and their  $^{35}\text{S}$  activities. The remaining three subsections of each depth increment were used to determine the  $\text{SO}_4^{2-}$  pool size and a dry mass to wet mass conversion factor. The top sections of cores

incubated for rates of sulfate reduction were variable in height (i.e., 0.5–3 cm), and comprised the living, green portion of *Sphagnum* mosses and vascular plants. This section was removed from each core prior to chemical determination of rates (i.e., after labeling), and is referred to as “top” in figures for sulfate pool size, rates of sulfate reduction, and residence time.

We determined the reduced inorganic sulfur (RIS) fraction and its  $^{35}\text{S}$  activity using  $\text{Cr}^{2+}$  reduction in a Johnson-Nishita apparatus (Johnson and Nishita 1952). Inorganic sulfur ( $\text{H}_2\text{S} + \text{S}^0 + \text{FeS} + \text{FeS}_2$ ) is quantitatively and specifically converted to  $\text{H}_2\text{S}$ , trapped in zinc acetate solution and quantified by iodometric titration (Zhabina and Volkov 1978, Howarth and Jorgensen 1984, Wieder and Lang 1988). Prior to carrying out the iodometric titration, we added duplicate 1-mL aliquots of the zinc acetate trapping solution to 10 mL of Scint BD scintillation cocktail for determination of  $^{35}\text{S}$  activity by liquid scintillation. We used appropriate quench correction and found quenching to be negligible. This protocol allowed for determination of both the reduced inorganic  $^{35}\text{S}$  in a single peat sample and the total reduced inorganic sulfur pool.

Following  $\text{Cr}^{2+}$  reduction, the peat was rinsed to remove unreacted  $^{35}\text{SO}_4^{2-}$ . Subsequently, the remaining solid peat sample was subjected to Eschka's procedure, a method for quantifying total sulfur (ASTM 1982). Because we had already removed all inorganic forms of sulfur (RIS and  $\text{SO}_4^{2-}$ ) from the peat, any radioactivity found in the Eschka's filtrate was attributed to  $^{35}\text{S}$  that had become incorporated into the CBS fraction. We calculated the total dissimilatory sulfate reduction end products as the sum of the RIS and CBS fractions.

Assimilatory sulfate reduction can also result in the reduction of sulfate to sulfide, with the latter incorporated into organic compounds, mainly in sulfur-containing amino acids, in both microbes and plants. Given that it is not possible to distinguish between assimilatory and dissimilatory sulfate reduction, we feel confident in assuming that our rates are the result of dissimilatory sulfate reduction for the following reasons. First, the amount of sulfate utilized by sulfate reducers for dissimilatory purposes far exceeds the amount used for cell synthesis (Widdel 1988). For every 100 g of RIS that sulfate-reducing bacteria form, 14 g of cell dry mass are synthesized. With a sulfur content of 1.3% measured in sulfate reducers, synthesis of the indicated amount of cell mass would require only 0.2 g of  $\text{H}_2\text{S}$  (Widdel 1988). Second, from measurements of sulfur concentration (0.8 mg/g of dry mass, Turetsky and Wieder 2000) in *Sphagnum* plants, the dominant cover species at Bleak Lake Bog, and estimates of net primary productivity at the site (NPP, 288 g organic matter  $\text{m}^{-2}\cdot\text{yr}^{-1}$ ; Wieder 2001), we estimate that assimilatory reduction could account for a maximum of 1.4–3.5% of our measured rates.

To calculate the dissolved  $\text{SO}_4^{2-}$  pool size, we placed the three separate subsamples individually into bea-

kers. We added 100 mL of DDW to each subsample, stirred the peat slurry occasionally, and after 1 h, removed 10 mL of the peat slurry using a syringe. We filtered the slurry through syringes fitted with 1.0- $\mu\text{m}$  glass fiber filters. To determine the  $^{35}\text{S}$  proportion of the total  $\text{SO}_4^{2-}$  pool size, we removed two separate 1.0-mL aliquots from the above filtrate and placed them into two separate scintillation vials, each containing 10 mL of Scint BD scintillation cocktail. Sulfur-35 sulfate was determined by liquid scintillation. We determined  $\text{SO}_4^{2-}$  concentration by ion chromatography on the remaining filtrate (Dionex Model 2010, AS4A column; Dionex Corporation, Marlton, New Jersey, USA).

Rates of sulfate reduction were calculated as the proportion of added  $^{35}\text{SO}_4^{2-}$  recovered as both RIS and CBS times the initial dissolved sulfate pool size, times a factor of 1.06 to account for discrimination against the heavier  $^{35}\text{S}$  isotope (Wieder et al. 1990).

#### *Caveats regarding the whole-core sulfate-reduction methodology*

All previous studies (e.g., Wieder and Lang 1988, Spratt and Morgan 1990) that have examined dissimilatory rates of sulfate reduction in freshwater *Sphagnum*-derived peat have done so on small (4–10 g of wet peat) subsamples under a pure anaerobic environment. The whole-core approach we adopted attempted to minimize disturbances to microbial communities and to preserve the nature of the original physico-chemical environment. We acknowledge that adding the radioactive label in distilled water that was in equilibrium with atmospheric gases may have disturbed the redox status of individual peat cores, at least temporarily. We decided not to add purged (i.e.,  $\text{O}_2$ -free) water for the following reason. Bleak Lake Bog is an ombrotrophic peatland, with all nutrients and water entering the peatland via wet and dry deposition. Rainwater, in equilibrium with atmospheric gases, enters the peat via the surface, trickling down the peat column until reaching the top of the permanently saturated water table. As a result, with every rain event, peatlands are exposed to rainwater in equilibrium with atmospheric  $\text{O}_2$ . In this regard, our labeled-water addition mimicked a typical rain event.

Prior to adding our labeled water, we needed to drain the gravitational water in individual cores and replace it with labeled water to ensure the distribution of  $^{35}\text{SO}_4^{2-}$  label to the core bottom. (Data presented in this paper show that we were successful in distributing the label to the bottom of the core.) *Sphagnum*-derived peat has a notoriously high water-holding capacity and low hydraulic conductivity (Boelter 1965, 1969). The 222 mL of water drained from each core (and immediately replaced) represents only ~15% of the estimated 1.5 L of water initially present in each core (10 cm diameter, 20 cm deep, 95% water by volume). Our whole-core approach was intended to maximize the preser-

vation of the natural environment while incubating cores for sulfate reduction using  $^{35}\text{SO}_4^{2-}$ .

#### *Rates of carbon dioxide and methane production*

To determine production of  $\text{CO}_2$  and  $\text{CH}_4$ , the green *Sphagnum* layer was removed from each core so that rates of  $\text{CO}_2$  production were not confounded by either  $\text{CO}_2$  fixation via photosynthesis or  $\text{CO}_2$  release from live aboveground plant respiration. Subsequently, we sectioned each core into 5 cm depth increments (without removing roots), and then sectioned each depth increment into quarters. We placed three of the peat quarters separately into 1.0-L airtight jars to incubate the peat anaerobically, while we oven-dried the fourth quarter of peat to determine a wet-to-dry mass conversion factor. We sampled headspace gas via a rubber septum in the lid of each jar. At the beginning of the experiment, we alternately evacuated and flushed each jar repeatedly with  $\text{O}_2$ -free  $\text{N}_2$  for 15–20 min to remove oxygen from any potentially existing microsites, and to create an anaerobic environment. The peat quarters were incubated anaerobically in the dark for 48 h at 20°C in 1996 (24 h in 1997). After the incubation period, we removed two separate 5.0-mL subsamples of headspace gas using a syringe and needle, and injected the gas samples individually into 4.0-mL vacutainer tubes. These gas samples were transported to the University of Notre Dame where concentrations of  $\text{CO}_2$  and  $\text{CH}_4$  were determined on a Varian model 3600 (Varian Incorporated, Walnut Creek, California, USA) gas chromatograph with thermal conductivity and flame ionization detectors, respectively, after separation on a Poropak-Q column (Varian Incorporated). Dissolution of  $\text{CH}_4$  and  $\text{CO}_2$  into the aqueous phase was accounted for by Henry's Law constants and sample pH (Stumm and Morgan 1981).

#### *Statistical analyses*

A  $2 \times 2 \times 9$  factorial nested repeated measures analysis of variance (rmANOVA) was used to determine if there was a significant difference in rates of sulfate reduction or  $\text{CO}_2$  production by examining between-subject effects (of S amendment treatment, depth, and the interaction between treatment and depth), and within-subjects effects (of time, the repeated factor, and all associated interactions). For each depth interval within a replicate core, there were three subreplicates, which we treated as nested within each depth and treatment combination. At each depth, we report mean values that represent an average of nine determinations  $\pm$  one standard error (3 subreplicates  $\times$  3 replicate cores). Because  $\text{CH}_4$  production in the second year of fertilization was below detection, a  $2 \times 9$  factorial nested ANOVA was used to determine if there was a significant difference between treatment, depth, and the interaction between treatment and depth with the 1996  $\text{CH}_4$  production data only. Fisher's test of Least Significant Difference (LSD) was used to make

a posteriori comparisons for all ANOVAs. To determine if sulfate-reducing bacteria were sulfate limited, correlations of sulfate reduction rates with  $\text{SO}_4^{2-}$  pool sizes were evaluated with Spearman's rank correlation test (SAS 1996). To further evaluate the relationship between sulfate reduction rates and  $\text{SO}_4^{2-}$  pool size between treatments and years, linear regression analyses were performed. In plots of sulfate reduction rate as a function of sulfate pool size, an analysis of covariance (ANCOVA) was used to determine if there was a significant difference between the slopes for the different treatments (i.e., control vs. S-amended) for different years (1996 vs. 1997) (SAS 1996). Comparison of sulfate turnover times was accomplished using a  $2 \times 9$  factorial ANOVA separately for each year to determine if there was a significant difference between control and sulfur-amended plots. For these data, we were concerned only with differences between treatments rather than the combination of treatment and year.

## RESULTS

### *Sulfate pool size*

Sulfate amendments increased the sulfate pool size between 2 and 3.5 times over the 2-yr duration of the experiment (Fig. 2). In both 1996 and 1997, the mean sulfate pool size in the sulfur-amended plots (187 nmol/cm<sup>3</sup> and 160 nmol/cm<sup>3</sup>, respectively) was significantly greater than in the control plots (53 nmol/cm<sup>3</sup> and 87 nmol/cm<sup>3</sup>, respectively) ( $P = 0.0001$ , Fig. 2).

### *Rates of sulfate reduction based on incorporation into RIS*

There was a significant three-way interaction among treatment, depth, and year based on incorporation of reduced S into the RIS fraction ( $P = 0.0078$ ; Table 1, Fig. 3). The nature of the interaction indicated that in the control plots, rates of sulfate reduction were greater in 1997 than in 1996 for some but not all depths, and in 1997 rates were greater in surface peat than at other depths. In contrast, in the sulfur-amended plots, rates of sulfate reduction did not significantly differ between years or with depth (Fig. 3). Differences between control and sulfur-amended plots were evident only at certain depths (i.e., top, 0–2.5 cm, 2.5–5 cm, 12.5–20 cm) in 1997, and in the top depth section in 1996 (Fig. 3). Integrated rates of sulfate reduction to a depth of 20 cm, based on incorporation into the RIS fraction, were between 3 and 10 times higher in the control cores than in the sulfur-amended cores for 1996 and 1997 (Table 2).

### *Rates of sulfate reduction based on incorporation into the CBS fraction*

There was a significant interaction between treatment and depth based on incorporation of sulfur into the CBS fraction, as well as a significant year effect (Table 1,

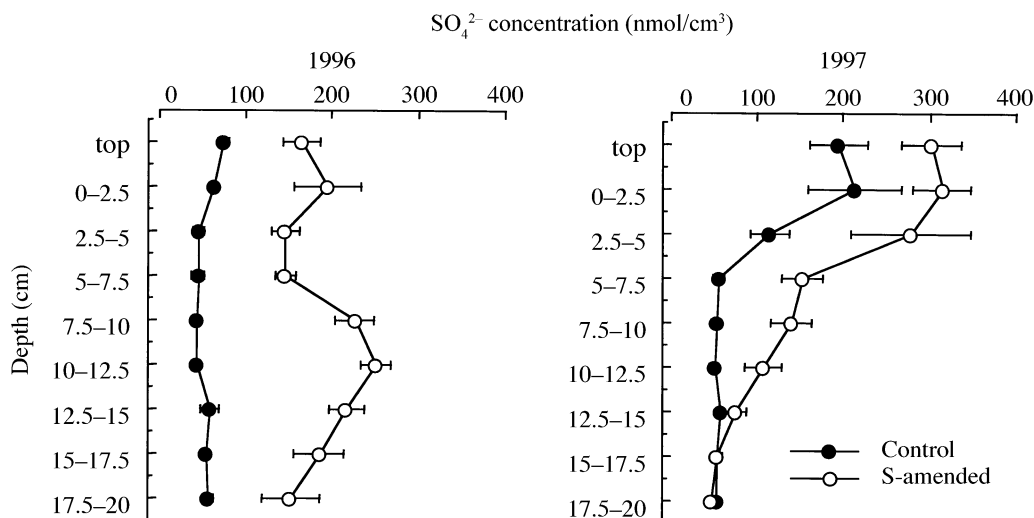


FIG. 2. Sulfate pool size as a function of depth for the control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations  $\pm 1$  SE (3 core determination  $\times$  3 subreplicates per core).

Fig. 4). The nature of the interaction between treatment and depth indicates significantly greater rates of sulfate reduction in the control cores for surface (top 5 cm) sections only. Rates of sulfate reduction did not differ between control and sulfur-amended cores for remaining depth sections with the exception of the 12.5–15 cm section, where rates of sulfate reduction were greater in the sulfur-amended cores than in the control cores (Fig. 4). A significant year effect, consistent across the treatment  $\times$  depth interaction, reflects greater mean ( $\pm 1$  SE) rates of sulfate reduction in 1997 ( $9.3 \pm 0.5$  nmol $\cdot$ cm $^{-3}\cdot$ d $^{-1}$ ) than in 1996 ( $5.8 \pm 0.8$  nmol $\cdot$ cm $^{-3}\cdot$ d $^{-1}$ ) (Fig. 4). Integrated rates of sulfate reduction based on incorporation into a CBS end product, to a depth of 20 cm, were greater in control cores in 1997 than in sulfur-amended cores in 1997, while minimal differences in integrated rates of sulfate reduction resulted between treatments in 1996 (Table 2). These results suggest that sulfur additions did not increase the rate of incorporation of S into the CBS pool via sulfate reduction.

*Total rates of sulfate reduction based on incorporation into both the RIS and CBS fraction*

Total rates of sulfate reduction followed the same pattern as those for the CBS fraction because  $>90\%$  of the radioactive  $^{35}\text{S}$  label had become incorporated in the CBS fraction. There was a significant interaction between treatment and depth, and a significant year effect (Table 1, Fig. 5). The nature of the interaction between treatment and depth indicates significantly greater rates of sulfate reduction in the control cores than in S-amended cores for surficial (top 5 cm) sections only. Rates of sulfate reduction did not differ between control and sulfur-amended cores for remaining depth sections with the exception of the 12.5–15

cm section, where rates of sulfate reduction were greater in the sulfur-amended cores than in the control cores (Fig. 5). A significant year effect, consistent across the treatment  $\times$  depth interaction, reflects greater rates of sulfate reduction in 1997 ( $9.6 \pm 0.8$  nmol $\cdot$ cm $^{-3}\cdot$ d $^{-1}$ ) than in 1996 ( $5.9 \pm 0.5$  nmol $\cdot$ cm $^{-3}\cdot$ d $^{-1}$ ). Integrated rates of sulfate reduction, to a depth of 20 cm, were similar for control cores and sulfur-amended cores in 1996. In contrast, greater integrated rates occurred in the control plots in 1997 (Table 2).

Sulfate reduction rates into both the RIS and CBS fractions were significantly ( $P = 0.0001$ ) positively correlated with sulfate pool size for both treatments when combined over the 2-yr duration of the study (Spearman's rho values of  $\rho = 0.460$  and  $\rho = 0.373$  for the control [ $n = 142$ ] and sulfur-amended plots [ $n = 146$ ], respectively). Assuming that sulfate reduction rates are dependent on  $\text{SO}_4^{2-}$  pool size, we further evaluated this relationship using linear regression to show that the predictive value was very low in the sulfur-amended plots ( $R^2 = 0.037$ ; Fig. 6). Analysis of covariance (ANCOVA) indicated that within each treatment, the slopes of regressions of sulfate reduction rate as a function of sulfate pool size did not differ between years (control plots: ANCOVA,  $P = 0.2509$ ,  $n = 142$ ; sulfur-amended plots: ANCOVA,  $P = 0.0805$ ,  $n = 146$ ) (Fig. 6). The slope for the control cores was significantly greater than the slope for the sulfur-amended plots with data from 1996 and 1997 combined (ANCOVA,  $P = 0.0001$ ) (Fig. 6).

Residence times (days) for the sulfate pool were calculated as the inverse of the proportion of added  $^{35}\text{SO}_4^{2-}$  incorporated into both the RIS and CBS pool during the 24- or 48-h incubations. Residence times were significantly longer for the sulfur-amended plots than for the control plots in both years in surface depths, but

TABLE 1. Repeated-measures ANOVA summary statistics for rates of sulfate reduction and CO<sub>2</sub> production.

Source	df	Type III ss	Mean square	F	P†
Rates of sulfate reduction based on incorporation into the RIS fraction					
Tests of hypotheses for between-subject effects					
Treatment	1	11.151	11.151	13.08	<b>0.0009</b>
Depth	8	10.296	1.287	1.51	0.1884
Treatment × depth	8	10.239	1.2799	1.50	0.1913
Replicate (treatment × depth)	36	30.695	0.8527		
Univariate tests of hypotheses for within-subjects effects					
Time	1	2.9102	2.9102	29.10	<b>0.0001</b>
Treatment × time	1	1.9294	1.9294	19.29	<b>0.0001</b>
Depth × time	8	2.0986	0.2623	2.62	<b>0.0092</b>
Treatment × depth × time	8	2.1486	0.2686	2.69	<b>0.0078</b>
Error	216	21.6005	0.1000		
Total	287	91.364			
Rates of sulfate reduction based on incorporation into the CBS fraction					
Tests of hypotheses for between-subject effects					
Treatment	1	138.5	138.5	1.09	0.3039
Depth	8	1522.9	190.4	1.50	0.1934
Treatment × depth	8	2288.2	286.0	2.25	<b>0.0465</b>
Replicate (treatment × depth)	36	4583.3	127.3		
Univariate tests of hypotheses for within-subjects effects					
Time	1	810.3	810.3	21.25	<b>0.0001</b>
Treatment × time	1	62.7	62.7	1.64	0.2012
Depth × time	8	367.9	46.0	1.21	0.2967
Treatment × depth × time	8	246.3	30.8	0.81	0.5968
Error	216	8238.4	36.1		
Total	287	17 597.7			
Rates of sulfate reduction based on incorporation into both the RIS and CBS fractions					
Tests of hypotheses for between-subject effects					
Treatment	1	228.3	228.3	1.99	0.2024
Depth	8	1765.0	220.6	1.63	0.1508
Treatment × depth	8	2548.7	318.6	2.35	<b>0.0379</b>
Replicate (treatment × depth)	36	4874.9	135.4		
Univariate tests of hypotheses for within-subjects effects					
Time	1	910.3	910.3	23.47	<b>0.0001</b>
Treatment × time	1	86.6	86.6	2.23	0.1366
Depth × time	8	366.5	45.8	1.18	0.3115
Treatment × depth × time	8	247.7	31.0	0.80	0.6048
Error	216	8378.1	38.8		
Total	287	19 026.3			
Rates of CO <sub>2</sub> production					
Tests of hypotheses for between-subject effects					
Treatment	1	937.8	937.8	1.80	0.1984
Depth	3	7269.6	2423.2	4.65	<b>0.0160</b>
Treatment × depth	3	252.6	84.2	0.16	0.9206
Replicate (treatment × depth)	16	8335.6		521.0	
Univariate tests of hypotheses for within-subjects effects					
Time	1	430.4	430.4	1.99	0.1614
Treatment × time	1	1519.7	1519.7	7.02	<b>0.0092</b>
Depth × time	3	1159.9	386.7	1.79	0.1540
Treatment × depth × time	3	27.0	9.0	0.04	0.9887
Error	112	24 252.4	216.5		
Total	143	44 185.2			

† Significant values ( $P < 0.05$ ) in boldface.

exhibited no difference at 5–7.5 cm depth in 1997, and no difference at depths below 12.5–15 cm for both years (Fig. 7) (ANOVA, significant treatment × depth interaction,  $P = 0.0061$  and  $0.0013$  for 1996 and 1997, respectively). Slower turnover times in the sulfur-amended plots indicate dynamic cycling of sulfate an-

ions in the control plots, a finding that we did not anticipate.

#### Rates of carbon dioxide and methane production

Rates of CO<sub>2</sub> production had a significant depth effect, as well as a significant interaction between treat-

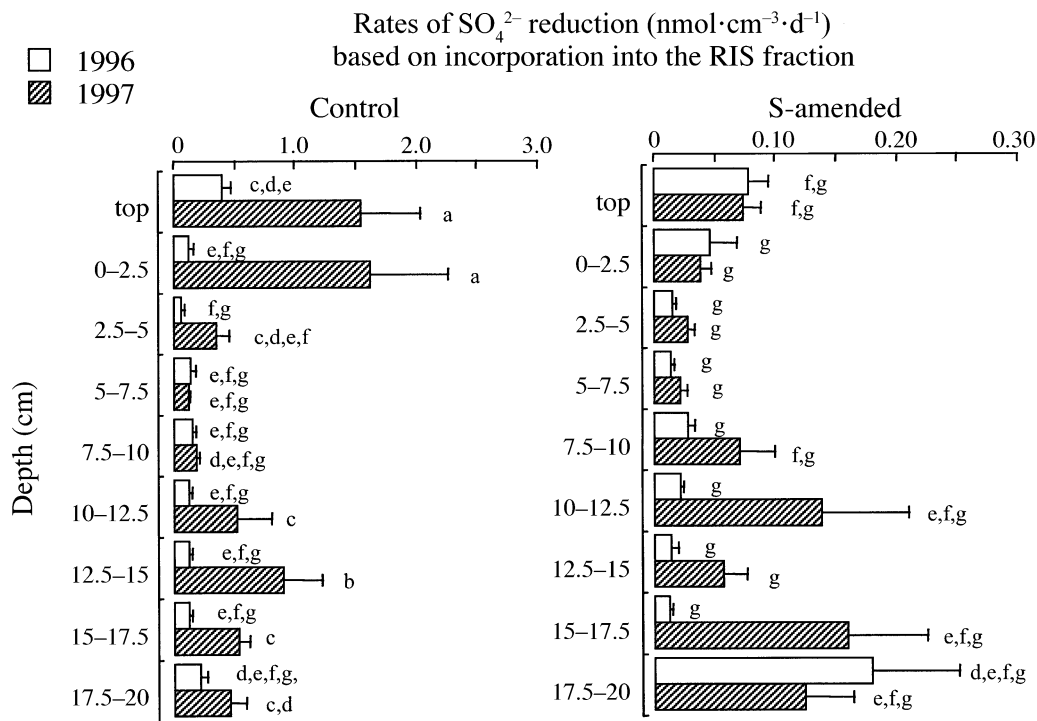


FIG. 3. Rates of sulfate reduction as a function of depth based on incorporation into the reduced inorganic sulfur pool (RIS) for control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations + 1 SE (3 core determination  $\times$  3 subreplicates per core). Means with different lowercase letters represent significant ( $P < 0.05$ ) differences.

ment and time (Table 1, Fig. 8). Rates of  $\text{CO}_2$  production were greater in the 0–5 cm depth section for both control and sulfur-amended plots. The nature of the treatment  $\times$  time interaction indicates significantly greater rates of  $\text{CO}_2$  production in the control cores in 1996 ( $1030 \pm 67 \text{ nmol}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$ ) than in 1997 ( $791 \pm 67 \text{ nmol}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$ ). The sulfur-amended cores did not exhibit significantly greater rates of  $\text{CO}_2$  production in 1997 ( $824 \pm 74 \text{ nmol}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$ ) than in 1996 ( $751 \pm 66 \text{ nmol}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$ ) (Fig. 8). Methane production is reported for 1996 only as measurements were below detection for 1997. In 1996, there was no significant treatment effect ( $P = 0.7342$ ), depth effect ( $P = 0.1376$ ), nor any significant interaction between depth and treatment ( $P = 0.7025$ ). Mean ( $\pm 1 \text{ SE}$ ) rates of  $\text{CH}_4$  production for the control and sulfur-amended plots were  $0.48 \pm 0.05$  and  $0.46 \pm 0.03 \text{ nmol}\cdot\text{cm}^{-3}\cdot\text{d}^{-1}$ , respectively.

Total daily integrated rates of  $\text{CO}_2$  production for the top 20 cm of peat ranged from 113 to 705  $\text{mmol}/\text{m}^2$ , with mean daily values for control plots in 1996 and 1997 of  $206 \pm 13$  and  $474 \pm 113 \text{ mmol}/\text{m}^2$ , respectively, and mean daily values for sulfur-amended plots in 1996 and 1997 of  $150 \pm 20$  and  $495 \pm 137 \text{ mmol}/\text{m}^2$ , respectively. Total daily integrated rates of  $\text{CH}_4$  production for the top 20 cm of peat in 1996 ranged from 72 to 116  $\mu\text{mol}/\text{m}^2$ , with mean daily values for

control and sulfur-amended cores of  $95 \pm 12$  and  $91 \pm 13 \mu\text{mol}/\text{m}^2$ , respectively.

## DISCUSSION

### Sulfur cycling at Bleak Lake Bog

In the top 20 cm of peat, mean integrated rates of sulfate reduction ranged from 0.8 to 4.3  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ , as compared to rates at other *Sphagnum*-dominated peat bogs: 1.7–6.3  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for the New Jersey Pine Barrens (top 25–30 cm; Spratt et al. 1987), 1.1–16.2  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for southern Scotland peats (top 30 cm; Nedwell and Watson 1995), and 9–34  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for West Virginia peat (top 30 cm; Wieder and Lang 1988). Rates of sulfate reduction in peatlands can be compared to rates of 250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for lake sediments (Kelly and Rudd 1984, Rudd et al. 1986), 2–15  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for coastal marine sediments (Howarth and Jorgensen 1984), and 25–420  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$  for salt marsh peat (Howarth and Teal 1979, King et al. 1985).

Although sulfate reduction rates at Bleak Lake Bog are at the low end of the ranges for most ecosystems, the values are within the range reported for other bogs, and higher than what we would have predicted given the low rate of atmospheric sulfate deposition at Bleak Lake Bog. The ultimate source of sulfur to Bleak Lake Bog is atmospheric deposition, but the magnitude of

TABLE 2. Rates of sulfate reduction converted to the cumulative amount occurring to a 20-cm depth beneath a given surface area of Bleak Lake Bog peat.

Sulfate reduction end product	Rates of sulfate reduction ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ )			
	Control		Sulfur-amended	
	1996	1997	1996	1997
RIS	$0.04 \pm 0.01$	$0.17 \pm 0.06$	$0.01 \pm 0.003$	$0.02 \pm 0.006$
CBS	$1.27 \pm 0.31$	$2.69 \pm 0.80$	$1.32 \pm 0.25$	$1.69 \pm 0.54$
Total	$1.30 \pm 0.32$	$2.84 \pm 0.86$	$1.33 \pm 0.26$	$1.70 \pm 0.55$

Note: Data are reported as means  $\pm$  1 SE;  $n = 3$ .

sulfate reduction at Bleak Lake Bog far exceeds inputs of atmospheric sulfate. Annual sulfate deposition to the site is  $1.6 \text{ mmol}/\text{m}^2$  (McDonald et al. 1996, Jendro 1998), whereas total *daily* sulfate reduction in the top 20 cm of peat, averaged for both treatments for both years, was  $1.74 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ . Consequently, rates of sulfate reduction at Bleak Lake Bog can only be sustained through the continual replenishment of the dissolved sulfate pool by RIS oxidation, a hypothesis put forth for other peat bogs (e.g., Wieder and Lang 1988). Concentrations of RIS, measured in Bleak Lake Bog peat by  $\text{Cr}^{2+}$  reduction and iodometric titration, were below detection limits, suggesting that the RIS pool does not accumulate to any substantial extent. Given that we obtained measurable incorporation of the  $^{35}\text{S}$

label into the RIS fraction, but a negligible RIS pool size, rapid oxidation of the RIS pool must be occurring.

Rates of sulfate reduction typically have been calculated based on incorporation into the RIS fraction alone (Spratt et al. 1987, Spratt and Morgan 1990, Nedwell and Watson 1995, Watson and Nedwell 1998). Wieder and Lang (1988) showed that 16–18% of the labeled sulfur became incorporated into the CBS pool, suggesting that sulfate reduction rates will be underestimated if the CBS end products are not included in the determination. As is evident in peat from Bleak Lake Bog, failing to include incorporation of reduced S into the CBS fraction would have grossly underestimated rates of sulfate reduction, as the CBS fraction contained between 77 and 95% of the total short-term

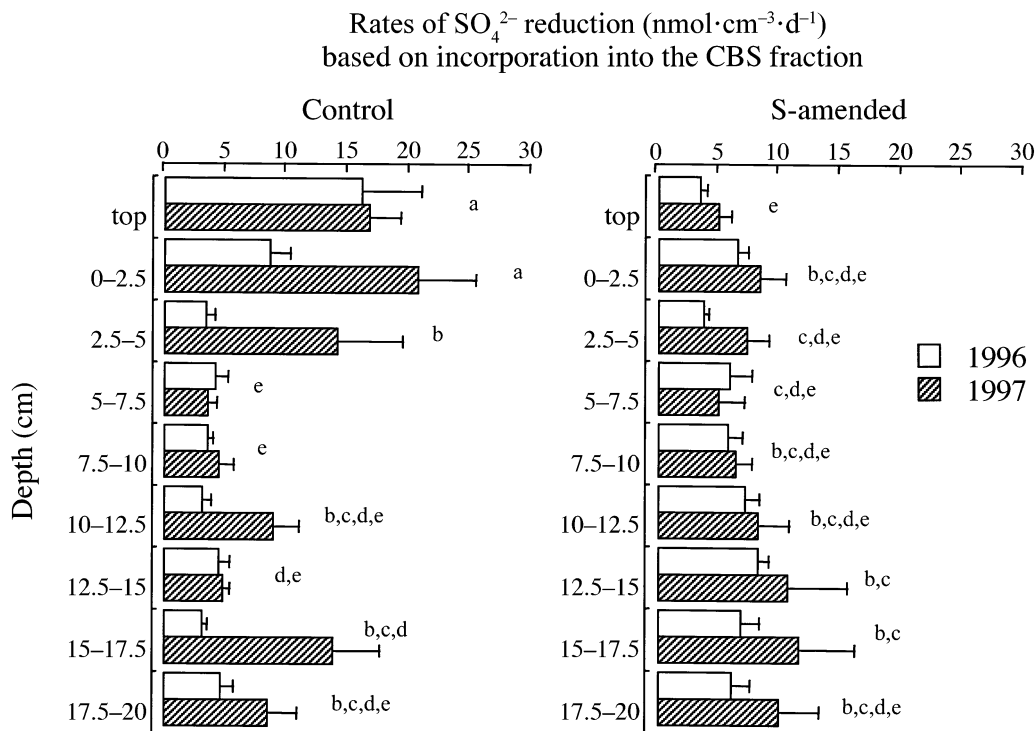


FIG. 4. Rates of sulfate reduction as a function of depth based on incorporation into the carbon-bonded sulfur pool (CBS) for control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations  $\pm$  1 SE (3 core determination  $\times$  3 subreplicates per core). Means with different lowercase letters represent significant ( $P < 0.05$ ) differences.

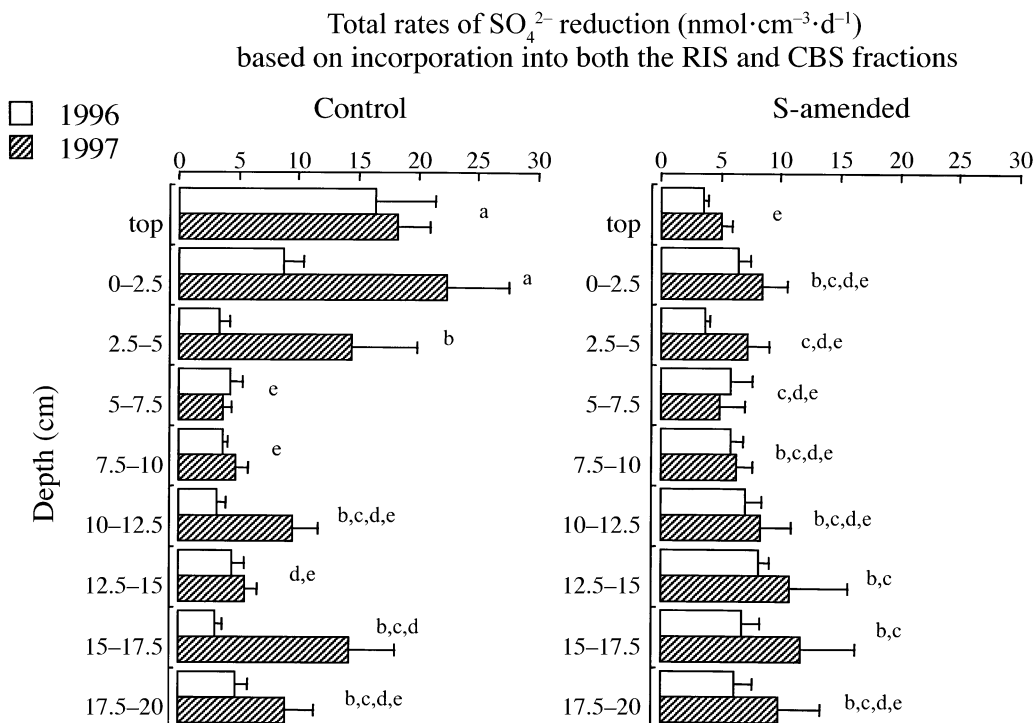


FIG. 5. Total rates of sulfate reduction as a function of depth based on incorporation into both the reduced inorganic sulfur pool (RIS) and the carbon-bonded sulfur pool (CBS) for control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations + 1 SE (3 core determination  $\times$  3 subreplicates per core). Means with different lowercase letters represent significant ( $P < 0.05$ ) differences.

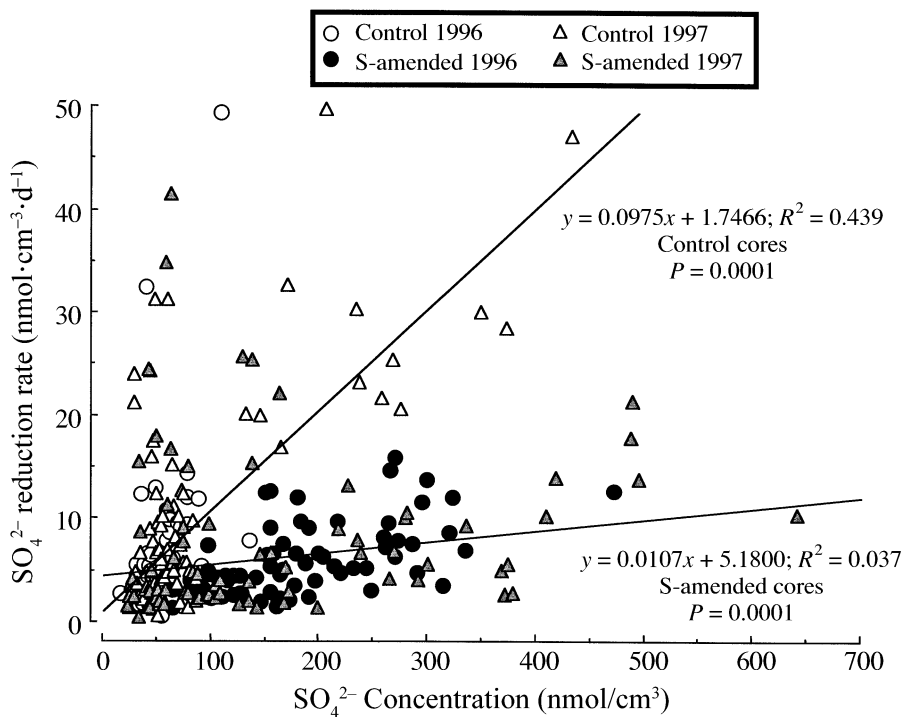


FIG. 6. Regressions of sulfate reduction rate on sulfate pool size for control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of three subsamples from each depth from each core.

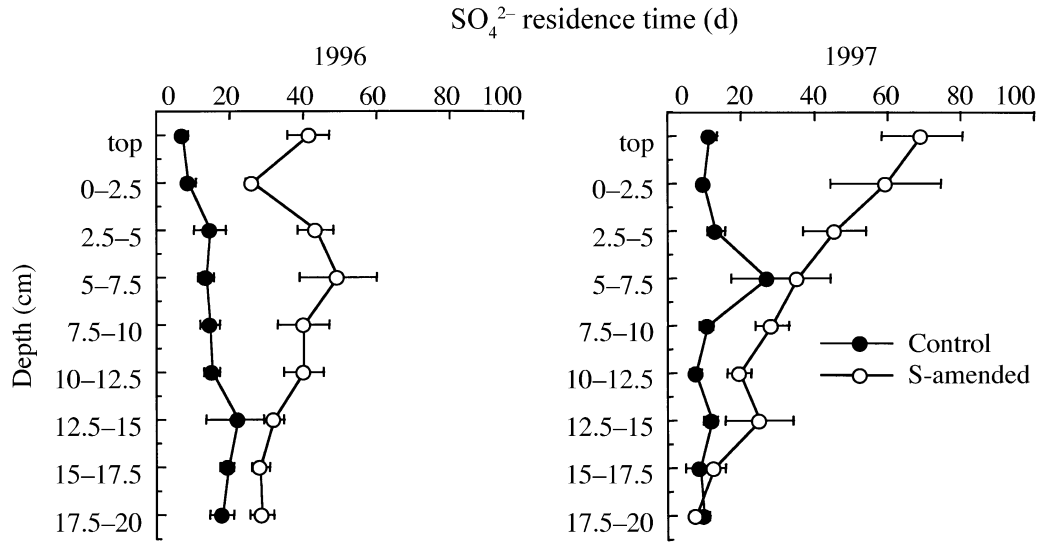


FIG. 7. Sulfate pool size turnover rate for control and sulfur-amended plots in 1996 and 1997. Each value represents the mean of nine determinations  $\pm$  1 SE (3 core determination  $\times$  3 subreplicates per core).

(i.e., 24–48 h) end product of dissimilatory sulfate reduction (Fig. 5). In contrast to previous studies, at Bleak Lake Bog the CBS end product is dominant.

Such a large proportion of sulfate reduction end product becoming incorporated into the relatively stable CBS pool (residence time is on the order of years to decades) (Wieder and Lang 1988) could partially explain the low rates of sulfate reduction observed in this study. All previous studies that have looked at sulfate reduction in freshwater peat have shown that the major short-term end product of sulfate reduction

is RIS, such as FeS and FeS<sub>2</sub> (Spratt et al. 1987, Wieder and Lang 1988, Spratt and Morgan 1990, Wieder et al. 1990). Regardless of the short-term fate of added <sup>35</sup>S, the CBS pool is substantially larger than the RIS pool in freshwater peatlands (Brown 1985, Spratt et al. 1987, Wieder and Lang 1988, Spratt and Morgan 1990, Banerjee and Chapman 1996). Because the CBS pool turns over much less rapidly than the RIS pool (Wieder and Lang 1988), sulfate reduction end products become sequestered, and cannot cycle out of this pool as quickly as end products in the RIS pool. Sulfate replenish-

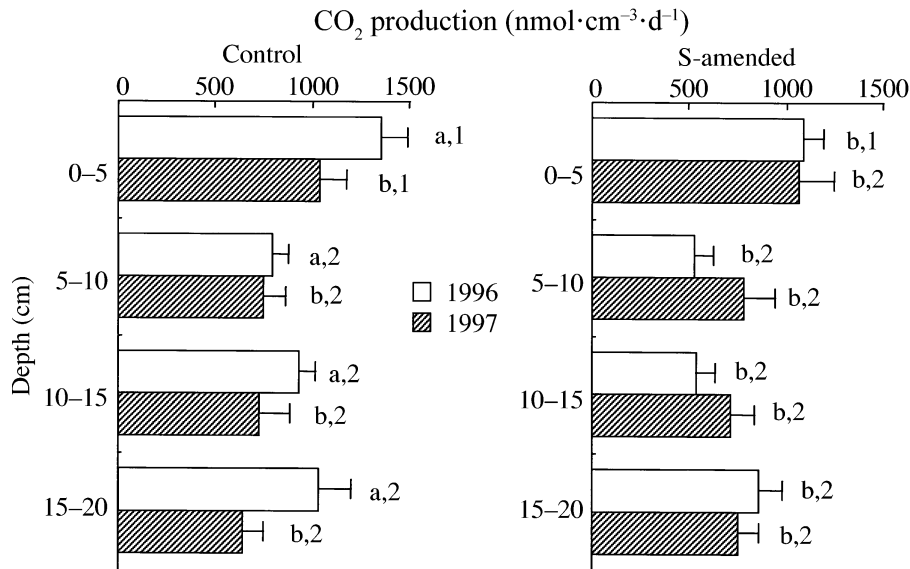


FIG. 8. Rates of carbon dioxide production as a function of depth for control and sulfur-amended plots in 1996 and 1997. Lowercase letters represent the nature of the interaction between treatment and time, and numbers illustrate the depth effect. Each value represents the mean of nine determinations  $\pm$  1 SE (3 core determination  $\times$  3 subreplicates per core). Means with different lowercase letters and numbers represent significant ( $P < 0.5$ ) differences.

ment is slowed, thus lowering rates of dissimilatory sulfate reduction.

The scarcity of reduced iron ( $\text{Fe}^{2+}$ ) at Bleak Lake Bog is another factor that could contribute to our low rates of sulfate reduction. The relative abundance of the inorganic sulfur fractions in peats when the RIS pool is the dominant short-term end product generally is in the order  $\text{FeS}_2 > \text{FeS} > \text{S}^0 > \text{SO}_4^{2-}$  (e.g., Wieder and Lang 1988). Hydrogen sulfide gas, formed as a short-term end product of sulfate reduction, can react rapidly with organic matter, producing CBS, unless reduced iron is present, in which case  $\text{H}_2\text{S}$  can rapidly form  $\text{FeS}$  or  $\text{FeS}_2$  (Casagrande et al. 1979, Brown 1985, Wieder and Lang 1988). In peat from Bleak Lake Bog, dissolved iron concentrations are low ( $< 0.4$  mg/L) (Vitt et al. 1995), which also could explain why the major end product of sulfate reduction is CBS and not RIS (Figs. 3–5).

Other studies have found that the continued reoxidation of  $\text{FeS}$  and  $\text{FeS}_2$  and concomitant reduction of  $\text{SO}_4^{2-}$  allowed for the overlap in rates of sulfate reduction between both salt marshes and coastal marine sediments with freshwater peatlands (Spratt et al. 1987, Wieder and Lang 1988). When this occurs, sulfur cycles through the reduced and oxidized inorganic sulfur pools several times before it either leaves the peatland or becomes part of the relatively recalcitrant CBS pool (Wieder et al. 1990). This cycling process suggests that sulfate reduction may be more important to anaerobic C mineralization than inputs from atmospheric deposition might indicate. In contrast to other peatlands where sulfate reduction is dominated by fluxes through the RIS pool, in Bleak Lake Bog, sulfur cycling is dominated by fluxes into the CBS pool. Moreover, sulfur amendments do not appear to shift the flux of sulfur cycling into the RIS pool, as we had expected, suggesting possible Fe limitation (Figs. 3–5).

Mean  $\text{SO}_4^{2-}$  concentrations in interstitial peat water for Bleak Lake Bog for the control and sulfur-amended plots were 70 and 173  $\mu\text{mol/L}$ , respectively (Fig. 2). These values are well below  $\text{SO}_4^{2-}$  saturation concentrations of 300 to 3000  $\mu\text{mol/L}$  reported for sulfate-reducing bacteria (Singleton 1993), but are within the range of sulfate concentrations reported for other freshwater peatlands (Wieder and Lang 1988, Spratt and Morgan 1990, Nedwell and Watson 1995, Watson and Nedwell 1998). Based on the sulfate requirements of dissimilatory sulfate-reducing bacteria, these results suggest that sulfate-reducing bacteria at Bleak Lake Bog may have been  $\text{SO}_4^{2-}$  limited even in the sulfur-amended plots. However, the relationship between rates of sulfate reduction and sulfate concentrations in peat suggests otherwise. In plots of sulfate reduction rate as a function of sulfate pool size, the slopes for the control plots (Fig. 6) were much steeper than the slopes for the sulfur-amended plots, further suggesting that sulfate-reducing bacteria initially were sulfate limited. The flatness of the slopes (relative to control plots) for

the sulfur-amended plots suggests that over time (i.e., probably 1–2 months), sulfate-reducing bacteria may have become limited by some other factor, possibly labile carbon. Although sulfate-reducing bacteria are surrounded by an abundant source of carbon in peat, this carbon is highly recalcitrant (Bridgham et al. 1998). Hence, the labile carbon pool (e.g., acetate and lactate) may be in short supply, and could become limiting, as diverse microbial groups would be competing for these carbon sources.

Sulfate amendments resulted in larger pool sizes and longer residence times for sulfate. If bacteria were solely sulfate limited, sulfate additions should result in greater rates of sulfate reduction, which possibly could lead to no change in sulfate pool size and no change in sulfate residence time. In contrast, with added sulfate we saw no increase in sulfate reduction rate, despite increases in both the sulfate pool size and sulfate residence time. Apparently, little added sulfate was required to overcome the sulfur limitation present in control plots.

Our findings lead us to propose the following scenario with regard to sulfur cycling at Bleak Lake Bog. Because between 77% and 99% of the radioactively labeled  $\text{SO}_4^{2-}$  becomes incorporated into the CBS pool, and this pool has a much longer residence time than the RIS pool (Fig. 7, cf. Wieder and Lang 1988), dissimilatory sulfate reduction at Bleak Lake Bog is much slower than in more minerotrophic peatlands (e.g., Wieder and Lang 1988), but not any less dynamic. Sulfate-reducing bacteria can cycle on a daily basis the quantity of sulfate supplied annually in atmospheric deposition. Because the RIS pool was below detection, the 1–23% of sulfate incorporated into the RIS fraction must be cycling rapidly (Fig. 7), which is a finding similar to other *Sphagnum* peat bogs (e.g., Wieder and Lang 1988, Spratt and Morgan 1990). After reoxidation of the RIS pool, sulfate-reducing bacteria can continue to reduce sulfate. Sulfate-reducing bacteria most likely are sulfate limited, but upon amendment with sulfate become limited by the supply of labile carbon or by some other factor.

Temperature is an additional factor that can influence rates of sulfate reduction; rates of sulfate reduction increase with increasing temperature (Spratt et al. 1987, Wieder and Lang 1988, Nedwell and Watson 1995). Moreover, net primary production (NPP) and decomposition drive carbon accumulation in peatlands (Gorham 1995, Wieder 2001), and are temperature-dependent processes as well (Kirschbaum 2000). At Bleak Lake Bog, mean annual temperature is 1.4°C (Szumigalski and Bayley 1996), presumably resulting in lower NPP and rates of decomposition than in peatlands at lower latitudes, where rates of sulfate reduction are higher (e.g., Wieder and Lang 1988, Spratt and Morgan 1990). Additionally, incubation temperatures in this study were between 5° and 10°C lower than in other studies where rates of sulfate reduction were also high-

er (e.g., Wieder and Lang 1988, Spratt and Morgan 1990). It is therefore most likely that a combination of sulfate, temperature, reduced iron, and labile carbon are limiting sulfate reduction in peatlands at high latitudes in regions with low atmospheric sulfur inputs.

#### *Carbon balance of Bleak Lake Bog*

Prevailing concepts hold that methane production should predominate as the most important pathway of anaerobic carbon flow in peatlands receiving low rates of atmospheric sulfur deposition (cf. Rogers and Whitman 1991). If methanogenesis were to predominate over sulfate reduction as the major anaerobic carbon mineralization pathway, in theory, the importance of  $\text{CH}_4$  production should decrease as  $\text{SO}_4^{2-}$  concentrations increase (Wieder et al. 1990, Nedwell and Watson 1995). In 1996, there was no significant difference in  $\text{CH}_4$  production between the control and sulfur-amended plots, and in 1997,  $\text{CH}_4$  production was undetectable, suggesting that sulfate amendments did not inhibit  $\text{CH}_4$  production as expected (Lovely and Klug 1983, Fauque 1995). Watson and Nedwell (1998) found that increasing  $\text{SO}_4^{2-}$  concentrations to 100  $\mu\text{mol/L}$  in peat samples did not significantly affect  $\text{CH}_4$  production rates compared to controls, although increasing  $\text{SO}_4^{2-}$  concentrations to 1000  $\mu\text{mol/L}$  significantly decreased methane production rates. We applied sulfate in eight applications, each of which was designed to increase the concentration of sulfate to 1000  $\mu\text{mol/L}$ , yet instantaneous sulfate concentrations in the peat were <300  $\mu\text{mol/L}$ , suggesting biotic uptake of ~70% of applied sulfate, dilution, and/or leaching loss from the plots.

We expected to see significantly greater  $\text{CO}_2$  production in the sulfur-amended plots relative to control plots. We found greater  $\text{CO}_2$  production in control plots than in sulfur-amended plots in 1996, but no significant difference in 1997 (Fig. 8), indicating that, at least in peat from Bleak Lake Bog, sulfur amendments neither increased anaerobic carbon flow through the sulfur pathway, nor increased total anaerobic carbon mineralization. These results suggest that the carbon balance of peatlands will not likely be substantially affected by increasing atmospheric sulfur deposition, at least in the short term (i.e., two years).

We converted rates of sulfate reduction and methane production into stoichiometric equivalents of carbon to determine which process was more important in terms of anaerobic carbon mineralization. Stoichiometrically, reduction of one mole of  $\text{SO}_4^{2-}$  yields two moles of mineralized C as  $\text{CO}_2$ , but production of one mole of  $\text{CH}_4$  produces one mole of C (if acetate is used; Conrad 1989). For all plots, both sulfate reduction and methane production accounted for a small percentage of the total anaerobic carbon flow (<2% and 0.05%, respectively). However, sulfate reduction was more important than methanogenesis, which is contrary to the current paradigm that methanogenesis should be more important

in low  $\text{SO}_4^{2-}$  environments. Moreover, the small percentage of anaerobic carbon flow attributed to both sulfate reduction and methanogenesis leaves >97% of total anaerobic carbon mineralization unattributed to a specific functional microbial pathway. Although  $\text{CO}_2$  production could have been somewhat inflated due to root respiration, our interpretation of these results does not change.

The dominant anaerobic carbon mineralization at Bleak Lake Bog remains unknown, but could be attributed to fermentation of soluble organic compounds with low molecular mass. The products of fermentation reactions are low molecular mass dissolved organic compounds (DOC) that may be quite important in the ecological functioning of peatlands. Concentrations of DOC are high in porewaters of peatlands, and the low pH (~4) of peatlands is predominantly due to organic acid production (Gorham et al. 1985, Verry and Urban 1993, Gorham 1995). DOC is an important component of aquatic ecosystems, and wetlands are the predominant source of DOC to streams and lakes (Kaplan and Newbold 1993, Schiff et al. 1998). Detectable, but low (<1 mg/L) concentrations of acetate, propionate, pyruvate, oxalate, formate, and tartrate were measured in bogs of continental, western Canada, yet production and degradation of these compounds remains poorly characterized in peatlands (J. A. Navaratnam, unpublished data). Clearly, if we want to understand the controlling factors on soil carbon storage in sites like Bleak Lake Bog, which is typical of western Canadian bogs, we should begin to examine anaerobic carbon mineralization via fermentation pathways.

#### *Conclusions*

In peat from Bleak Lake Bog, at least in the short term, anaerobic carbon metabolism is not affected by simulated atmospheric sulfur deposition. Sulfur amendments did not increase rates of sulfate reduction, increase  $\text{CO}_2$  production, or decrease  $\text{CH}_4$  production over a 2-yr period, although both  $\text{SO}_4^{2-}$  concentrations and  $\text{SO}_4^{2-}$  residence time did increase. These results agree well with studies looking at the impact of nitrogen and sulfur fertilizations on the carbon balance of peat from Bleak Lake Bog (Jendro 1998). Alternatively, it has been shown that rates of sulfate reduction increase with increasing rates of regional atmospheric sulfur deposition (M. A. Vile, unpublished data), suggesting that much longer time scales are needed to cause substantial shifts in the importance of particular anaerobic carbon mineralization pathways, at least in *Sphagnum*-dominated peatlands. In this study, between 77% and 99% of reduced sulfate was incorporated into the CBS pool, which has not been shown previously. Low Fe contents in Bleak Lake Bog peat most likely are responsible for the slow (i.e., >24–48 h) turnover rate of sulfate, little incorporation into the RIS pool, and hence low rates of sulfate reduction. These results suggest that in addition to sulfate in the control plots,

and labile carbon in the sulfur-amended plots, sulfate reduction also may be indirectly Fe limited. We hypothesize that higher Fe availability in more minerotrophic wetlands will lead to greater sulfate reduction rates and a proportionally greater influence of sulfate reduction in anaerobic carbon cycling. Overall, it appears that (in order of increasing importance) sulfate concentrations, available Fe concentrations, and labile carbon control sulfate reduction rates in freshwater peatlands. Fermentation appears to be the dominant anaerobic carbon mineralization pathway at Bleak Lake Bog, and how this process affects the carbon balance of peatlands remains to be evaluated.

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