

# Assessing sulfate and carbon controls on net methylmercury production in peatlands: An in situ mesocosm approach

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## Abstract

The transformation of atmospherically deposited inorganic Hg to the toxic, organic form methylmercury (MeHg) is of serious ecological concern because MeHg accumulates in aquatic biota, including fish. Research has shown that the Hg methylation reaction is dependent on the availability of SO<sub>4</sub> (as an electron acceptor) because SO<sub>4</sub>-reducing bacteria (SRB) mediate the biotic methylation of Hg. Much less research has investigated the possible organic C limitations to Hg methylation (i.e. from the perspective of the electron donor). Although peatlands are long-term stores of organic C, the C derived from peatland vegetation is of questionable microbial lability. This research investigated how both SO<sub>4</sub> and organic C control net MeHg production using a controlled factorial addition design in 44 in situ peatland mesocosms. Two levels of SO<sub>4</sub> addition and energetic-equivalent additions (i.e. same number of electrons) of a number of organic C sources were used including glucose, acetate, lactate, coniferous litter leachate, and deciduous litter leachate. This study supports previous research demonstrating the stimulation of MeHg production from SO<sub>4</sub> input alone (~200 pg/L/day). None of the additions of organic C alone resulted in significant MeHg production. The combined addition of SO<sub>4</sub> and some organic C sources resulted in considerably more MeHg production (~500 pg/L/day) than did the addition of SO<sub>4</sub> alone, demonstrating that the highest levels of MeHg production can be expected only where fluxes of both SO<sub>4</sub> and organic C are delivered concurrently. When compared to a number of pore water samples taken from two nearby peatlands, MeHg concentrations resulting from the combined addition of SO<sub>4</sub> and organic C in this study were similar to MeHg “hot spots” found near the upland–peatland interface. The formation of MeHg “hot spots” at the upland–peatland interface may be dependent on concurrent inputs of SO<sub>4</sub> and organic C in runoff from the adjacent upland hillslopes.

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## 1. Introduction

Mercury is a potent neurotoxin (Ratcliffe et al., 1996) and human exposure to Hg is primarily through the consumption of contaminated fish (Fitzgerald and Clarkson, 1991). Although atmospherically-deposited inorganic Hg (Hg(0) and

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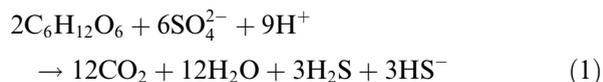
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Hg(II) is the principal non-point source of Hg to “pristine” ecosystems, it is the transformation of inorganic Hg into methylmercury (MeHg), an organic form that accumulates in aquatic biota (Bloom, 1992) that is of greatest concern. Atmospheric sources of MeHg are generally insufficient to account for the MeHg in aquatic biota such as fish (Gilmour and Henry, 1991; Rudd, 1995).

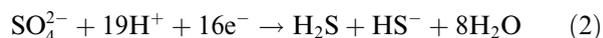
The transformation of inorganic Hg to MeHg is dominantly a biologically-mediated process (Berman and Bartha, 1986). Sulfate-reducing bacteria (SRB) are the principal microbiological methylators of inorganic Hg (Compeau and Bartha, 1985; Gilmour et al., 1992), but recent research has also shown that Fe-reducing bacteria may be important in Fe-rich freshwater sediments (Fleming et al., 2006). Due to the mediation of Hg methylation by anaerobic bacteria, the reaction is redox-sensitive (Compeau and Bartha, 1984; DeLaune et al., 2004) and therefore tends to occur in low dissolved O<sub>2</sub> environments such as lake-bottom sediment (Winfrey and Rudd, 1990), the anoxic hypolimnion of lakes (Eckley et al., 2005) and different types of wetlands (St. Louis et al., 1994; Hurley et al., 1995; Krabbenhoft et al., 1995; Branfireun et al., 1996; Galloway and Branfireun, 2004). Branfireun et al. (1996) and Branfireun and Roulet (2002) found distinct zones in a peatland where high MeHg concentrations in pore water corresponded with areas of groundwater discharge. Branfireun and Roulet (2002) hypothesized that these zones of groundwater discharge were important both in maintaining anoxic conditions for obligate SRB and in delivering SO<sub>4</sub> to the bacteria to support the methylation of Hg.

Several mesocosm-scale studies have been carried out to investigate the causal relationships between added SO<sub>4</sub> and the production of MeHg in wetland soils (Branfireun et al., 1999, 2001; Harmon et al., 2004). These studies have been in response to findings of increased Hg contamination of fish in lakes that are affected by atmospheric SO<sub>4</sub> and Hg deposition, and runoff from surrounding wetlands. Branfireun et al. (1999, 2001) found that additions of SO<sub>4</sub> to peatland mesocosms in both short- and long-term experiments resulted in significantly increased MeHg concentrations in pore waters. Harmon et al. (2004) found similar increases in pore water MeHg concentrations in model constructed wetland mesocosms amended with SO<sub>4</sub>. In a recent study where SO<sub>4</sub> was added to half of a peatland, Jeremiason et al. (2006)

found that this addition resulted in a 3 times increase in pore water concentrations of MeHg and a 2.3 times increase in MeHg flux from the peatland during the late spring. Studies such as this have concluded that additions of SO<sub>4</sub> stimulate the reduction of SO<sub>4</sub> to sulfide by SRB, as in the following equation:



wherein the inorganic and organic redox half-reactions (assuming glucose as the electron donor) are:



and that this stimulation of SO<sub>4</sub> reduction has similarly stimulated Hg methylation (King et al., 1999). What has not been investigated in previous work is whether C supply through the organic half-reaction (Eq. (3)) may metabolically limit SO<sub>4</sub> reduction and thus Hg methylation in presumably labile C-rich wetlands.

Many wetlands, but especially peatlands, are long-term C-accumulating ecosystems that are important to the global C cycle (Belyea and Clymo, 2001). Although peatlands may accumulate meters of C-rich peat, much of the accumulated peat is hundreds to thousands of years old. Pools of C may be large, but the pools of labile C in peatlands are proportionately small (Updegraff et al., 1995; Bridgham et al., 1998). Chemical reactions in peatlands thus may be controlled not by the mass of C, but rather by the relative availability of labile C to microbial communities.

In upland–peatland catchments of northern Minnesota, one mechanism whereby a relatively labile supply of C (for example, from forest litterfall) may be delivered to a peatland is via upland runoff to the upland–peatland interface. Kolka et al. (2001) found that the annual flux of total organic C (TOC) via upland runoff to a bog in northern Minnesota was 1.10 g/m<sup>2</sup> of upland area, most of this flux occurring in the spring and fall. Since the flux of TOC from the upland hillslope is focused only into the peatland margin, this area receives fluxes of TOC in considerable excess of other peatland areas where TOC is delivered only via throughfall or in situ soil degradation.

The main purpose of this study was to simulate fluxes that may occur from an upland hillslope into a peatland and to determine which combination of

SO<sub>4</sub> and/or labile C would result in the greatest net production of MeHg in peatland pore water. It was hypothesized that the greatest net production of MeHg would occur with the combined addition of both SO<sub>4</sub> and a labile C compound. Several different types of organic C were added to the mesocosms in this study (glucose, acetate, lactate, deciduous leaf litter leachate, and coniferous leaf litter leachate)

to both differentiate between natural sources of C substrate and to differentiate between the favored substrates of different groups of SRB. Since previous research has shown that acetate-using SRB are potent methylators of Hg (King et al., 2000), it was hypothesized that experiments involving the addition of acetate would result in the highest production of MeHg.

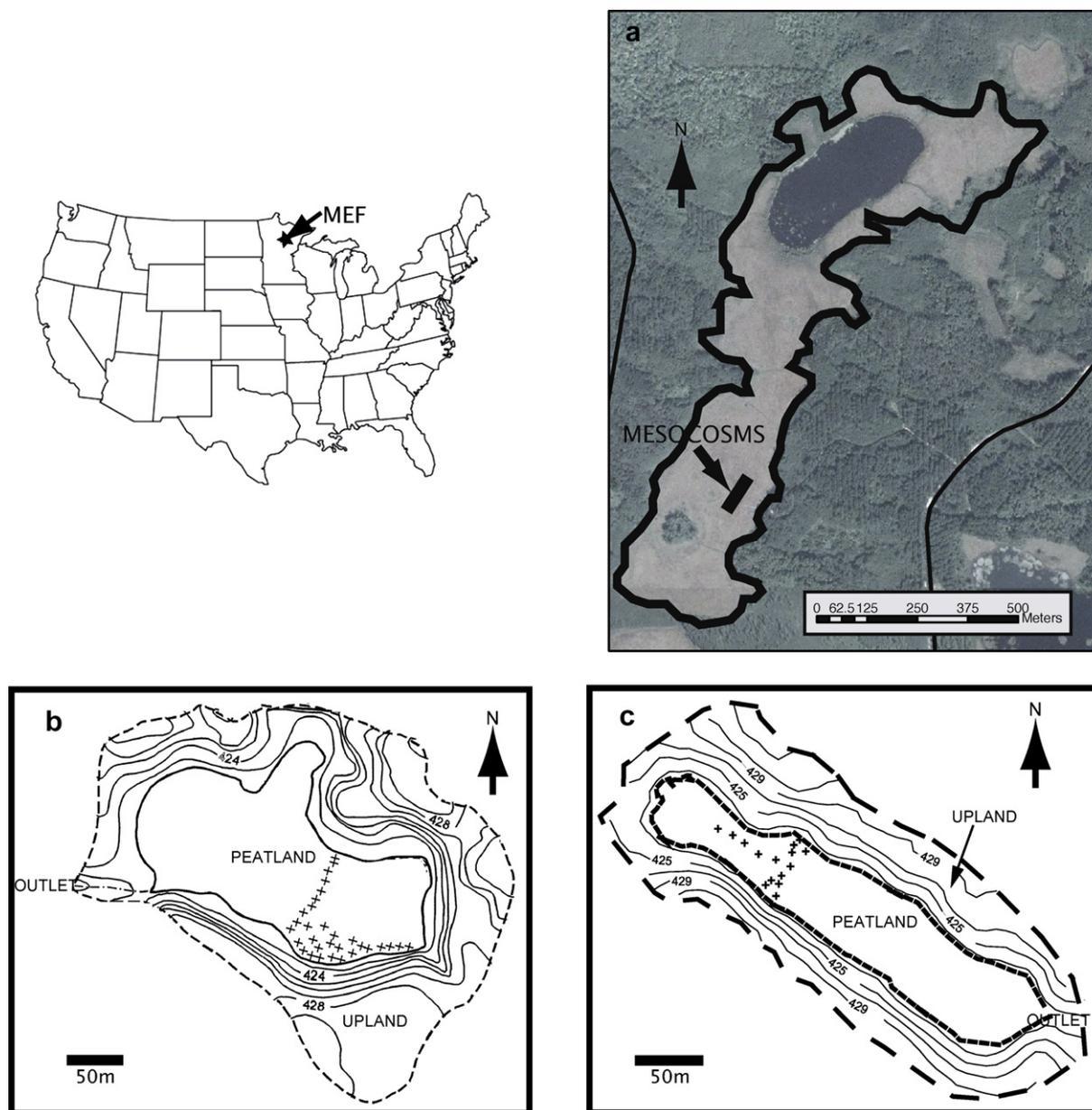


Fig. 1. The study area, located at the Marcell Experimental Forest (MEF) in north-central Minnesota. The principal study site was (a) Bog Lake Bog (aerial photograph; peatland outlined in dark black). Peatland pore waters were also sampled in two nearby peatlands: (b) S2 and (c) S6. “+” denote pore water sampling points.

## 2. Study area

This study took place in Bog Lake Bog, a 10 ha ombrotrophic bog at the Marcell Experimental Forest (MEF; 47°32'N, 93°28'W), NE of Grand Rapids, in north-central Minnesota (Fig. 1). The MEF is a US Forest Service site encompassing several experimental watersheds. Monitoring of hydrological and climatic data, as well as environmental field research has taken place there since 1959. MEF is an operating site of both the National Atmospheric Deposition Program (NADP), which monitors general atmospheric deposition chemistry, and the Mercury Deposition Network (MDN), which monitors the atmospheric deposition of Hg.

Generally, the glaciated terrain of the MEF is typical of the western Great Lakes region and is characterized by rolling upland topography with numerous small lakes and peatlands. Yavitt et al. (2000) have previously reported on the characteristics of the Bog Lake Bog site. Briefly, peat soils are derived from sphagnum mosses (dominated by *Sphagnum majus*), ericaceous shrubs (dominated by *Chamedaphne calyculata*) and scattered sedges (*Carex* species). Some stunted black spruce (*Picea mariana*) and tamarack (*Larix laricina*) are found across the bog. The microtopography of the bog is characterized by hummocks, hollows, and lawns, but is visually more topographically and vegetatively homogenous than other peatlands within the MEF. It was for this reason that Bog Lake Bog was chosen as the study site.

In this paper, results from the mesocosm study are compared to natural MeHg and THg concentrations in two other peatlands within the MEF, S2 and S6 (Fig. 1). Briefly, peatland S2 is a 3.0 ha peatland surrounded by 6.2 ha of mineral upland soils and peatland S6 is a 2.4 ha peatland surrounded by 6.5 ha of mineral upland soils. Vegetation is similar in the two peatlands and dominated by a mat of *Sphagnum* species mosses, with an overstory of *P. mariana*.

Climate at the MEF is characterized as sub-humid continental. For the period from 1961 to 2000 (US Forest Service, unpublished data), the average annual air temperature was 3 °C, with average January and July air temperatures of –14 °C and 19 °C, respectively. Average annual precipitation is 785 mm, 75% of which occurs during the snow-free period. This study took place from June 6 to 12, 2005. For the duration of the actual exper-

imental additions, light rain occurred sporadically, totaling 18 mm and the average air temperature over the same period was 15 °C.

## 3. Methods

### 3.1. Mesocosm installation

In an area approximately 50 m from the edge of Bog Lake Bog, 46 open-ended plywood boxes (side dimensions: 60 × 60 × 60 cm) were installed in rows on either side of two boardwalks. The mesocosms were installed in the bog by cutting a 60 × 60 cm square in the peat using a long hand saw and pushing the boxes into this square until only approximately 5 cm of each box remained above the ground surface. Previous coring at the site showed that the boundary between the more fibric, less decomposed surface peats (acrotelm) and the very decomposed undifferentiated humic peats (catotelm) was at a depth of approximately 40 cm. Given the very low hydraulic conductivity of well decomposed peat, frames were installed deep enough into this layer to ensure that there would be no inter-mesocosm interaction of pore water during the experiments. The mesocosms were installed in uniform, topographically flat areas (lawns). Large hummocks and hollows were avoided since previous work has shown that MeHg concentrations in pore waters can differ between hummocks, hollows and lawns (Branfireun, 2005).

The installation of the mesocosms was completed in the summer of 2004 and was left for one full year before experimentation began to ensure that: (1) vegetation within the mesocosms was unaffected by the installation, (2) the installation of the mesocosms would not change the ambient levels of MeHg or THg in the bog pore water, and (3) ambient pore water concentrations of MeHg and THg could be determined before the experiment to ensure initial similarity among the experimental treatments. Through visual observation and the comparison of samples taken in the summer of 2004 and samples taken immediately prior to the start of this study, it was confirmed that the vegetation was unaffected and that MeHg and THg concentrations did not change as a result of the installation process (data not shown). Two mesocosms were excluded prior to the start of this study due to consistently higher MeHg concentrations in pore waters than the others, leaving 44 mesocosms for use in this study.

### 3.2. Experimental design

To explore the controls that different amounts of  $\text{SO}_4$  and labile C may have on the production of MeHg, a controlled factorial addition design was applied (Table 1) involving two different loads of  $\text{SO}_4$  (4 times and 10 times the average annual atmospheric deposition) and energetic-equivalent loads of glucose, acetate, and lactate (i.e. equal number of electron donors to electron acceptors). All three C compounds have been used in previous studies to investigate the effect of different organic electron donors on  $\text{SO}_4$  reduction activity (i.e. Maillacheruvu et al., 1993; Song et al., 1998; King et al., 2000; Karnachuk et al., 2005). Acetate and lactate were also chosen as electron donors that might allow differentiation between the two major groups of SRB: acetate-oxidizing and non-acetate oxidizing (Postgate, 1984). No additional Hg was deliberately added to the experimental mesocosms, however the additions involving deciduous and coniferous leaf litter leachate did contain some Hg (see end of this section). The amount of  $\text{SO}_4$  deposited from the atmosphere was calculated using precipitation chemistry data from the NADP site (mean  $\text{SO}_4$  concentration in precipitation between January 2000 and August 2004 was 0.79 mg/L) at the MEF and scaling it to the 0.036 m<sup>2</sup> mesocosms using average annual rainfall (785 mm). It was decided to add 4 times the average annual atmospheric deposition of  $\text{SO}_4$  as the smaller addition because of similarities to a whole-peatland  $\text{SO}_4$  addition experiment occurring in another peatland at the MEF, which added the same amount (see Jeremiason et al., 2006). Four times the annual atmospheric deposition of  $\text{SO}_4$  at the MEF is approximately equivalent to current atmospheric loading in the northeastern United States (National Atmospheric Deposition Program, 2006). All chemicals were diluted to 2 L using deionized water, which is the equivalent volume of a modest rainfall (5.6 mm). This volume of water ensured

delivery of solutes through the surface vegetation to the saturated zone. Equal applications of these quantities of  $\text{SO}_4$  and/or C were made to duplicate mesocosms using an acid-rinsed plastic watering can. An equivalent volume of deionized water was added to 4 control mesocosms.

In addition to these synthetic C compounds, two natural sources of mixed organic C were added. Prior to the onset of the experiment, litterfall from an aspen upland (deciduous litter) and a red pine upland (coniferous litter) were collected in separate 20 L pails. Each pail was filled with deionized water, covered, and left for 3 days at 20 °C. After 3 days, the contents were filtered using ashed 0.7 µm glass fiber filters and a sample of the leachate was taken for the determination of MeHg and total Hg (THg) concentration as well as the concentration of dissolved organic C (DOC) and major ions. The remaining leachate was added to the appropriate mesocosms within 2 h. Since chemical concentrations could not be established beforehand, 1 L of leachate was diluted to 2 L with deionized water and added to each appropriate mesocosm as explained in Table 1. It was later determined that additions involving deciduous leaf litter leachate contained the following chemical masses: THg: 28.6 ng, MeHg: 0.22 ng, DOC: 55 mg,  $\text{SO}_4$ : 2.6 mg. Additions involving coniferous leachate contained the following chemical masses: THg: 46.0 ng, MeHg: 0.19 ng, DOC: 84 mg,  $\text{SO}_4$ : 2.6 mg.

### 3.3. Pore water sampling

Ultra-clean trace metal protocols were used at all times for the preparation of sampling equipment and for the sampling of water in the field. Mesocosm pore waters were sampled 24 h prior to the addition of  $\text{SO}_4$  and/or C and then 24, 48 and 72 h following the additions. Previous work by Branfireun et al. (1999) and Mitchell (2007) indicated peak MeHg production occurred within 2 days.

Table 1  
Controlled factorial addition experimental design

	No carbon	4X equiv. <sup>a</sup> acetate	10X equiv. acetate	4X equiv. lactate	10X equiv. lactate	4X equiv. glucose	10X equiv. glucose	Deciduous leachate	Coniferous leachate
No sulfate	4 <sup>b</sup>	2	2	2	2	2	2	2	2
4X sulfate	2	2	n.i. <sup>c</sup>	2	n.i.	2	n.i.	2	2
10X sulfate	2	n.i.	2	n.i.	2	n.i.	2	2	2

<sup>a</sup> Equiv. refers to an energetic-equivalent (same number of electrons) load.

<sup>b</sup> All numbers represent replicate experiments completed and reported in this paper.

<sup>c</sup> “n.i.” indicates that experiments involving these combinations were not investigated.

To sample pore waters from each mesocosm, a Teflon piezometer with a 5 cm perforated end was pushed into the peat surface and a sample integrated from 2.5 to 7.5 cm below the water table was pulled using a peristaltic pump equipped with Teflon tubing. In an effort to amalgamate the small-scale spatial variability in each mesocosm, at each sampling time, the piezometer was pushed into 5 random places and the samples were combined into one Teflon transfer vessel. The contents of the transfer vessel were then filtered (0.7  $\mu\text{m}$  ashed glass fiber filter) in the field using a peristaltic pump with Teflon tubing and an in-line Teflon filter holder. The filtered sample was collected in a PETG bottle for analysis of MeHg and THg concentrations and separate HDPE bottles for analysis of DOC and major ion concentrations. All samples were kept in a cooler on ice until transport back to the laboratory. Sample bottles for Hg analysis were double bagged and upon return to the laboratory, acidified to 0.5% with concentrated ultrapure HCl. All samples were subsequently kept cool and in the dark until analysis.

To compare the results of the mesocosm experiments to the natural spatial variability observed in other peatlands, pore waters were sampled in two nearby peatlands at the MEF, S2 and S6. Pore waters were collected using identical sampling methods in transects spanning from the upland–peatland interface to the center of each peatland in early October 2004. Thirty-two samples were collected in peatland S2 and 15 samples were collected in peatland S6.

### 3.4. Analytical methods

THg and MeHg analyses were performed in a Class 100 cleanroom at the University of Toronto by US Environmental Protection Agency (USEPA) Methods 1630 (USEPA, 2001) and 1631 (USEPA, 2002). MeHg concentration was determined by aqueous phase ethylation (Bloom, 1989) and cold vapour atomic fluorescence spectroscopy (CVAFS) following distillation (Horvat et al., 1993; Olson et al., 1997). Briefly, the sample distillate was ethylated with Na tetraethylborate, buffered with Na acetate, and purged with  $\text{N}_2$  onto glass traps filled with Tenax®. The Tenax® trap was then heated in a stream of Ar, the Hg stream was speciated on a gas chromatography column, combusted to  $\text{Hg}^0$  using a pyrolytic column, and detected on a Tekran® 2500 by CVAFS. Recovery of a methylmercury spike was  $100 \pm 13\%$  ( $n = 28$ ), replication of

duplicates was  $12 \pm 9\%$  ( $n = 15$  pairs), and the detection limit, calculated as  $3\sigma$  of distillate blanks, was 0.06 ng/L ( $n = 15$ ).

THg concentration in water was determined using a Tekran® model 2600 CVAFS mercury detector with automated sampler. The day prior to analysis, 1 mL of BrCl was added to 40 mL of sample. Analysis was by CVAFS with two-stage gold trap amalgamation and reduction by  $\text{SnCl}_2$ . Recovery of a mercury spike was  $104 \pm 12\%$  ( $n = 14$ ), replication of duplicates was  $3.3 \pm 2.8\%$  ( $n = 33$  pairs), and the detection limit was 0.17 ng/L ( $n = 24$ ).

Major ions were analyzed on a model DX 500 Dionex® ion chromatography system with a self-regenerating suppressor. Dissolved organic C was analyzed on a Shimadzu® 5050 Total Organic Carbon Analyzer, employing high temperature (680 °C) catalytic combustion at McGill University (see Fraser et al., 2001). Three to 6 injections of each sample were performed until coefficients of variation were less than 5%.

### 3.5. Statistical methods

To determine differences in net MeHg production between treatments, analyses of covariance to test for parallelism (using the  $F$  test) were completed. These analyses, as well as tests for the significance ( $p < 0.05$ ) of linear relationships were completed using STATISTICA® (Statsoft Inc., Tulsa, OK) software.

## 4. Results and discussion

### 4.1. Initial conditions

Considering the spatial heterogeneity that is often inherent in peatland pore waters because of microtopographical influences (Branfireun, 2005), there was an acceptably low range in pore water concentrations of MeHg, THg, DOC and  $\text{SO}_4$  (Table 2) among the mesocosms prior to commencing the experiment. For example, all MeHg concentrations were less than 510 pg/L whereas Branfireun (2005) found that pore water concentrations were between less than 1000 pg/L in deep hollows and beneath hummocks to over 3500 pg/L in shallow hollows. Furthermore, the variability in pore water chemistry was considerably less than that observed in the other nearby studied peatlands (see Table 3). There was very little difference in soil temperatures in the mesocosms prior to  $\text{SO}_4$  and/or C addi-

Table 2  
Initial chemical concentrations of pore water in peatland mesocosms

	[MeHg] (pg/L)	[THg] (ng/L)	% MeHg	[DOC] (mg/L)	[SO <sub>4</sub> ] (mg/L)	T <sub>5 cm</sub> (°C)	T <sub>15 cm</sub> (°C)
Mean	260	3.42	8.0	21.6	0.07	19.8	15.2
Median	240	3.24	7.5	23.4	0.06	19.9	15.3
σ	90	1.10	2.9	10.8	0.08	0.9	0.5
Range	90–510	1.91–6.04	3.0–16	7.6–39.9	<d.l. <sup>a</sup> –0.53	18.0–21.3	14.6–15.9
n	44	44	44	44	44	44	44

<sup>a</sup> d.l.: lower detection limit.

tions with near surface (5 cm depth) temperature approximately 5 °C warmer than at 15 cm depth. The initial chemical conditions in all mesocosms were therefore similar.

#### 4.2. Experimental controls

Mesocosms to which only deionized water was added showed neither significant net MeHg production nor a significant increase in %-MeHg during the experiment (Fig. 2). Net changes in MeHg concentration, %-MeHg, DOC concentration, and SO<sub>4</sub> concentration over time were all close to zero. All *r*<sup>2</sup> values were very low (<0.01) and none of the relationships over time were significantly greater than zero (*p* > 0.05). The authors were thus confident that differences observed in other treatments would be the result of that specific treatment (i.e. a cause–effect relationship) and not the result of possible interferences.

#### 4.3. Effect of carbon addition

No significant increases in MeHg concentration or %-MeHg over time were observed as a result of the C additions (*p* > 0.05; Fig. 3). The addition of a considerable amount of litter-derived THg in the deciduous leachate treatments (28.6 ng) and the coniferous leachate treatments (46.0 ng) did not result in any significant production of MeHg. It is

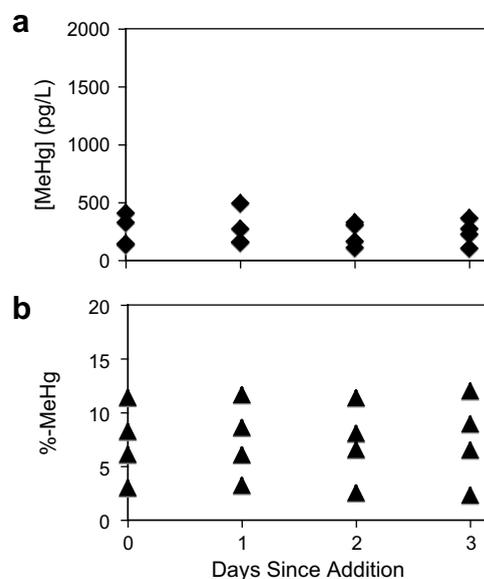


Fig. 2. Changes in (a) [MeHg] and (b) %-MeHg over time in 4 replicate control mesocosms.

clear that a straightforward labile C limitation on Hg methylation under otherwise natural conditions in this peatland does not exist. Furthermore, assuming that the inorganic Hg introduced with the leaf litter leachates is at least in part bioavailable, Hg methylation in this peatland does not appear to be strongly controlled by the availability of inorganic Hg.

Table 3  
Pore water chemistry in two nearby peatlands at the Marcell Experimental Forest

	Peatland S2					Peatland S6				
	[MeHg] (pg/L)	[THg] (ng/L)	% MeHg	[DOC] (mg/L)	[SO <sub>4</sub> ] (mg/L)	[MeHg] (pg/L)	[THg] (ng/L)	% MeHg	[DOC] (mg/L)	[SO <sub>4</sub> ] (mg/L)
Mean	660	8.72	8.4	62.2	1.6	550	8.80	7.0	50.8	1.6
Median	620	8.19	6.8	62.0	1.3	320	7.16	5.2	47.1	0.99
σ	460	3.96	6.0	13.7	0.95	360	4.74	5.1	15.9	1.8
Range	100–1990	2.61–21.3	1.6–23.3	40.3–90.6	0.48–4.6	190–1420	4.23–22.2	2.7–19.0	30.4–81.7	0.28–7.5
n	32	32	32	31	32	15	15	15	15	14

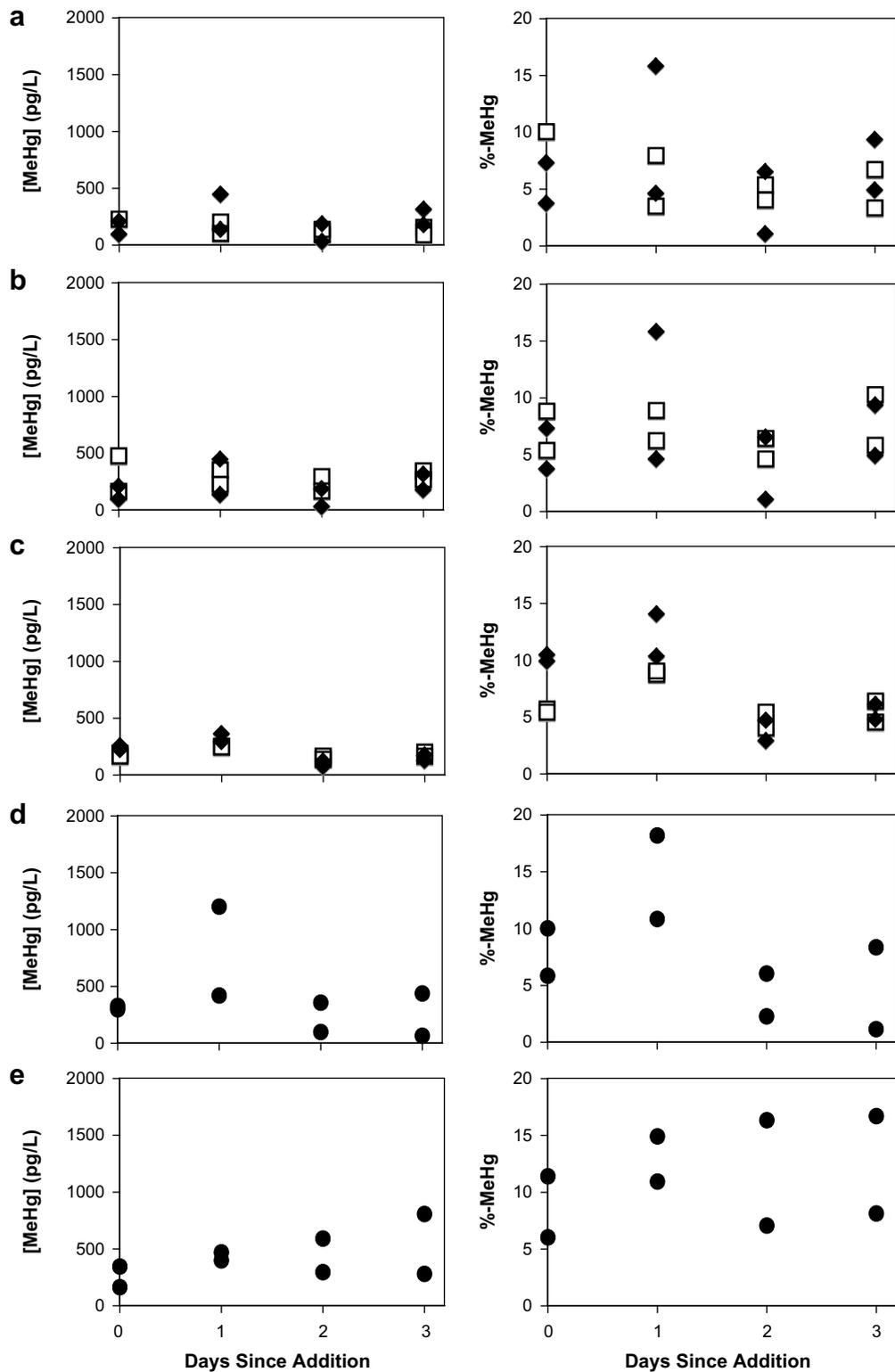


Fig. 3. Changes over time in [MeHg] (left) and %-MeHg (right) resulting from the addition of (a) glucose, (b) acetate, (c) lactate, (d) coniferous forest litter leachate, and (e) deciduous forest litter leachate. For (a) through (c), 4 times energetic-equivalent C addition ( $\blacklozenge$ ) and 10 times energetic equivalent C addition ( $\square$ ). None of the graphs show significant linear trends ( $p > 0.05$ ).

Previous research has shown relationships between additions of C and MeHg production, but direct links with C mineralization have not been clear. Hall et al. (2004) demonstrated that the addition of birch leaves and jack pine needles to lake mesocosms resulted in considerable production of new MeHg, but that the rates of MeHg production were not proportional to C decomposition and therefore not directly related to C metabolism. Balogh et al. (2002) found that MeHg concentrations in runoff from a small agricultural stream in Minnesota increased dramatically as a result of leaf-fall events. Considering the results from the present study, it is not believed that additions of labile C alone can increase the methylation of Hg in peatlands, perhaps because peatlands have a higher buffering capacity for other effects of C degradation, such as changes to pH and anoxia, compared to other aquatic ecosystems.

#### 4.4. Effect of sulfate addition

The addition of  $\text{SO}_4$  resulted in a considerable increase in both the net production of MeHg (170–240 pg/L/day) and changes in %-MeHg (3.6 to 4.6%/day; Fig. 4). All linear relationships over time were significant ( $p < 0.05$ ) except for the

change in %-MeHg over time following the addition of 4 times the annual atmospheric deposition of  $\text{SO}_4$  ( $p = 0.127$ , Fig. 4a-right), likely due to the variability in THg concentration over time. Rates of net MeHg production were not significantly different between  $\text{SO}_4$  addition treatments ( $F_{[1,12]} = 0.459$ ,  $p = 0.511$ ). Rates of net MeHg production were significantly higher than both the controls ( $F_{[2,26]} = 7.782$ ,  $p = 0.002$ ) and the C additions ( $F_{[9,59]} = 3.098$ ,  $p = 0.004$ ). The change in %-MeHg between  $\text{SO}_4$  addition treatments was not significantly different ( $F_{[1,12]} = 0.127$ ,  $p = 0.728$ ). The change in %-MeHg over time was also significantly greater than both the controls ( $F_{[2,26]} = 3.471$ ,  $p = 0.046$ ) and C additions ( $F_{[9,59]} = 2.855$ ,  $p = 0.007$ ). Thus, the addition of  $\text{SO}_4$  results in both significantly greater net production of MeHg and a significantly greater increase in %-MeHg when compared to controls or the addition of just organic C. Higher pore water  $\text{SO}_4$  concentrations were apparent in the 10-times  $\text{SO}_4$  treatment (Table 4). Although the 4-times  $\text{SO}_4$  treatment appeared to result in more rapid production of MeHg than the 10-times  $\text{SO}_4$  treatment, the amount of  $\text{SO}_4$  added had no statistically significant effect on net MeHg production or change in %-MeHg.

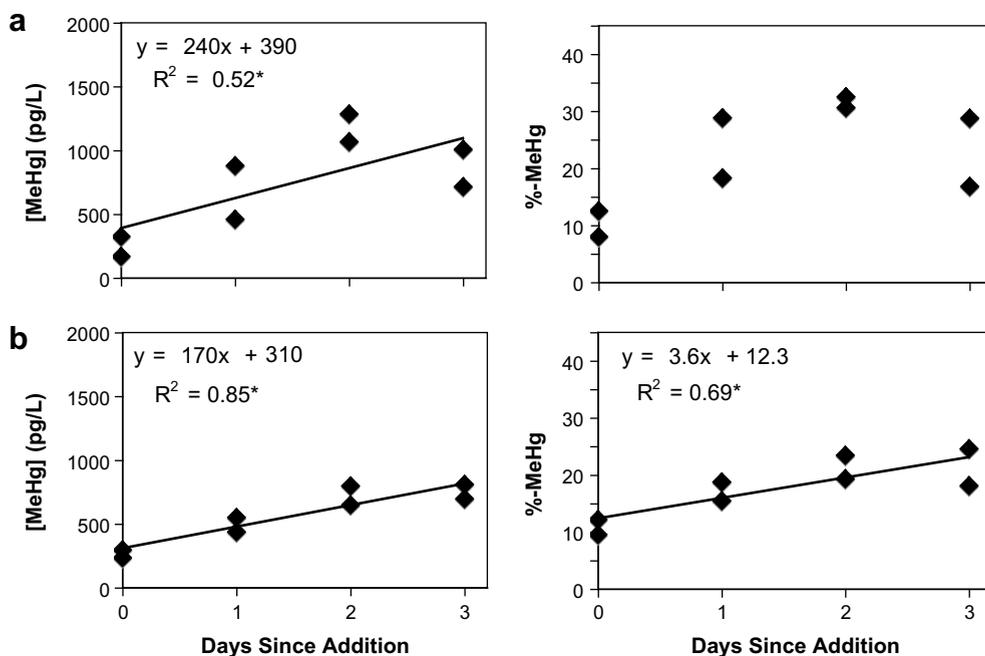


Fig. 4. Changes over time in [MeHg] (left) and %-MeHg (right) resulting from the addition of (a) 4 times, and (b) 10 times, annual atmospheric deposition of  $\text{SO}_4$ . Note the change in the y-axis scale compared to previous figures for the %-MeHg graphs. \*Indicates a significant linear trend ( $p < 0.05$ ).

Table 4  
Changes in pore water sulfate and dissolved organic carbon (DOC) concentrations during experiments (values are mean  $\pm$  standard deviation)

Treatment	Pre-addition [SO <sub>4</sub> ] (mg/L) <sup>a,b</sup>	Post-addition [SO <sub>4</sub> ] (mg/L) <sup>c,d</sup>	Pre-addition [DOC] (mg/L) <sup>a,b</sup>	Post-addition [DOC] (mg/L) <sup>c,d</sup>
Control	0.02 $\pm$ 0.02	0.40 $\pm$ 0.57	24.6 $\pm$ 8.6	26.3 $\pm$ 3.8
4X glucose <sup>e</sup>	0.03 $\pm$ 0.04	0.11 $\pm$ 0.03	15.3 $\pm$ 11.0	25.8 $\pm$ 3.2
10X glucose	<d.l. <sup>g</sup>	0.12 $\pm$ 0.03	7.5 $\pm$ 0.8	20.1 $\pm$ 8.4
4X acetate	0.07 $\pm$ 0.01	0.44 $\pm$ 0.66	25.9 $\pm$ 0.5	35.9 $\pm$ 6.6
10X acetate	0.03 $\pm$ 0.03	0.32 $\pm$ 0.64	16.8 $\pm$ 13.1	30.8 $\pm$ 8.6
4X lactate	0.09 $\pm$ 0.04	0.20 $\pm$ 0.12	24.9 $\pm$ 0.5	22.8 $\pm$ 3.9
10X lactate	0.03 $\pm$ 0.04	0.46 $\pm$ 0.65	23.8 $\pm$ 0.5	24.2 $\pm$ 3.2
Conif. leachate	0.04 $\pm$ 0.02	0.18 $\pm$ 0.20	35.0 $\pm$ 6.9	34.6 $\pm$ 4.3
Decid. leachate	0.05 $\pm$ 0.00	0.30 $\pm$ 0.16	24.0 $\pm$ 5.1	27.3 $\pm$ 2.6
4X sulfate <sup>f</sup>	0.13 $\pm$ 0.14	6.7 $\pm$ 1.7	24.4 $\pm$ 1.0	24.7 $\pm$ 2.4
10X sulfate	0.02 $\pm$ 0.03	26.0 $\pm$ 10.8	25.2 $\pm$ 1.3	27.7 $\pm$ 3.5
4X sulfate + Glucose	0.06 $\pm$ 0.01	11.9 $\pm$ 3.2	26.0 $\pm$ 5.8	27.1 $\pm$ 3.5
10X sulfate + Glucose	0.07 $\pm$ 0.03	34.6 $\pm$ 9.2	29.4 $\pm$ 8.0	32.4 $\pm$ 7.0
4X sulfate + Acetate	0.07 $\pm$ 0.01	11.4 $\pm$ 6.3	31.9 $\pm$ 0.2	28.8 $\pm$ 2.5
10X Sulfate + acetate	0.08 $\pm$ 0.00	34.2 $\pm$ 7.1	25.5 $\pm$ 3.8	26.3 $\pm$ 2.1
4X Sulfate + lactate	0.05 $\pm$ 0.01	1.9 $\pm$ 1.5	24.3 $\pm$ 3.1	22.4 $\pm$ 4.6
10X Sulfate + lactate	0.10 $\pm$ 0.04	0.15 $\pm$ 0.05	28.1 $\pm$ 2.4	28.0 $\pm$ 3.6
4X sulfate + Conif. leachate	0.04 $\pm$ 0.02	10.9 $\pm$ 4.4	29.8 $\pm$ 9.4	36.5 $\pm$ 8.5
10X sulfate + conif. leachate	0.06 $\pm$ 0.01	46.6 $\pm$ 7.5	30.4 $\pm$ 4.1	38.9 $\pm$ 4.1
4X sulfate + decid. leachate	0.05 $\pm$ 0.02	6.0 $\pm$ 0.55	23.0 $\pm$ 0.2	25.1 $\pm$ 3.9
10X sulfate + decid. leachate	0.06 $\pm$ 0.01	43.2 $\pm$ 5.6	32.2 $\pm$ 1.6	30.3 $\pm$ 6.5

<sup>a</sup>  $n = 4$  for controls.

<sup>b</sup>  $n = 2$  for all other treatments.

<sup>c</sup>  $n = 12$  for controls.

<sup>d</sup>  $n = 6$  for all other treatments.

<sup>e</sup> In carbon treatments, 4X and 10X refer to 4- and 10-times energetic equivalent loads of organic C (same number of electrons) with respect to scenarios of 4- and 10-times annual atmospheric deposition of SO<sub>4</sub>.

<sup>f</sup> In sulfate treatments, 4X and 10X refer to 4- and 10-times annual atmospheric deposition of SO<sub>4</sub>.

<sup>g</sup> d.l. = lower detection limit.

The range in MeHg concentrations (initial concentrations of  $\sim$ 200 pg/L, increasing up to 1290 pg/L) in pore water as a result of SO<sub>4</sub> addition alone was consistent with results from previous mesocosm-scale studies. In short-term SO<sub>4</sub> addition experiments such as this one, Branfireun et al. (1999) found that pore water MeHg concentrations increased from approximately 200 pg/L to as high as 9190 pg/L following SO<sub>4</sub> addition to a peatland mesocosm in northwestern Ontario. In longer-term experiments, repeated additions of SO<sub>4</sub> to peatland mesocosms in northern Sweden changed MeHg concentrations in pore water from approximately 330 pg/L to approximately 1900 pg/L (Branfireun et al., 2001). In constructed wetland mesocosms, Harmon et al. (2004) found that the mean pore water MeHg concentration in SO<sub>4</sub>-amended mesocosms (1700 pg/L) was considerably higher than in control mesocosms (500 pg/L).

The insignificant difference in MeHg production between the two levels of SO<sub>4</sub> addition demon-

strates that greater inputs of SO<sub>4</sub> do not necessarily result in a proportionally larger production of MeHg. This finding is consistent with that of Branfireun et al. (1999) and Harmon et al. (2004). Larger inputs of SO<sub>4</sub> may have more strongly stimulated the demethylation of MeHg by SRB (Oremland et al., 1991) in the 10 times SO<sub>4</sub> treatment, however, these mechanisms have not been elucidated in the literature. Another reason may be the precipitation of dissolved Hg in pore water due to the formation of insoluble sulfide–Hg complexes, rendering the Hg less bioavailable (see Gilmore et al., 1992; Benoit et al., 1998). A limited number of samples analyzed for pore water dissolved sulfide concentration (data not shown) showed that sulfide concentrations did not increase above 3  $\mu$ M as a result of the SO<sub>4</sub> additions. These concentrations are low enough to possibly enhance Hg methylation through the production of neutral HgS<sup>0</sup>, which may be more bioavailable to methylating bacteria (Benoit et al., 1999). It is also possible

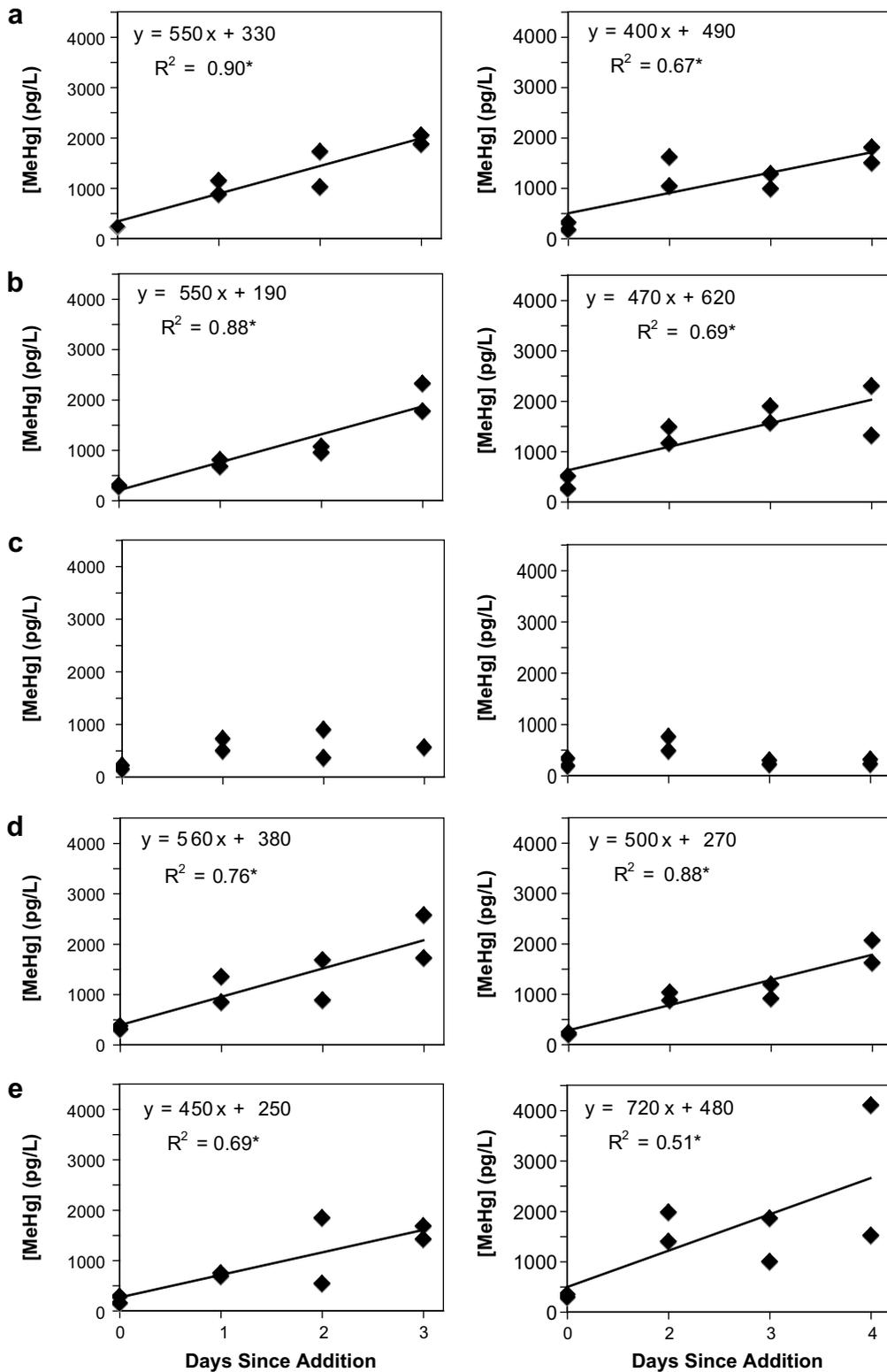


Fig. 5. Changes in [MeHg] over time resulting from the combined addition of 4-times (left) and 10-times (right) annual atmospheric deposition of  $\text{SO}_4$  and energetic-equivalent (equal number of electrons) doses of (a) glucose, (b) acetate, (c) lactate, (d) coniferous forest litter leachate, and (e) deciduous forest litter leachate. Note the change in the y-axis scale compared to previous figures. \*Indicates a significant linear trend ( $p < 0.05$ ).

that the maximum rate of Hg methylation is not fully realized with increased  $\text{SO}_4$  because of organic substrate limitations to the  $\text{SO}_4$  reduction reaction. Sulfate may no longer be a limiting reactant to respiration in SRB when more than 4-times annual atmospheric  $\text{SO}_4$  deposition is added. Thus, if enough  $\text{SO}_4$  is present in the peat pore waters, the availability of labile C may control the production of MeHg. Even though peatlands are high-C environments, this hypothesis has been alluded to in previous research (Branfireun et al., 1999 and Harmon et al., 2004). No research has yet demonstrated this possibility with respect to the production of MeHg in peatlands, therefore this hypothesis is explored in the following section.

#### 4.5. Effect of combined sulfate and carbon addition

For most, but not all treatments, the combined addition of  $\text{SO}_4$  and organic C resulted in considerably higher net production of MeHg (400 to 720 pg/L/day; Fig. 5) than for additions of  $\text{SO}_4$  alone. All linear relationships between the change in MeHg concentration and time (net MeHg production) were statistically significant ( $p < 0.05$ ) except for both of the additions involving lactate ( $p = 0.17$  to  $0.60$ ). Statistically, the following combined additions resulted in a significantly higher rate of net MeHg production as compared to  $\text{SO}_4$ -only additions: 4-times  $\text{SO}_4$  and coniferous leachate ( $F_{[2,18]} = 5.139$ ,  $p = 0.017$ ), 10-times  $\text{SO}_4$  and coniferous leachate ( $F_{[2,18]} = 6.065$ ,  $p = 0.010$ ), 4-times  $\text{SO}_4$  and equivalent glucose ( $F_{[2,18]} = 8.068$ ,  $p = 0.003$ ), and 4-times  $\text{SO}_4$  and equivalent acetate ( $F_{[2,18]} = 7.669$ ,  $p = 0.004$ ). All of the 4-times  $\text{SO}_4$  and energetic-equivalent organic C treatments were statistically indistinguishable from the 10-times  $\text{SO}_4$  and equivalent organic C treatments ( $p > 0.05$ ). The 4-times  $\text{SO}_4$  and equivalent glucose, acetate, or coniferous leachate additions all resulted in similar, and the highest, rates of net MeHg production ( $\sim 550$  pg/L/day).

For the trend in %-MeHg over time (Fig. 6), all linear relationships were significant except for both treatments involving lactate ( $p = 0.29$  to  $0.43$ ), the 4-times  $\text{SO}_4$  and coniferous leachate treatment ( $p = 0.059$ ), and the 10-times  $\text{SO}_4$  and deciduous leachate treatment ( $p = 0.103$ ). This is largely due to the variability in THg concentrations. These treatments also had greater variability in  $\text{SO}_4$  and DOC concentrations (Table 4). It is still clear that

without adding Hg to these systems, the net MeHg production was significant.

Since the change in %-MeHg over time for the 4-times  $\text{SO}_4$  treatment did not result in a statistically significant relationship (Fig. 4), the combined  $\text{SO}_4$  and organic C additions were compared only to the 10-times  $\text{SO}_4$  treatment. Of the combined  $\text{SO}_4$  and C treatments, only the 4-times  $\text{SO}_4$  and deciduous leachate ( $F_{[1,12]} = 4.899$ ,  $p = 0.047$ ) and the 4-times  $\text{SO}_4$  and glucose ( $F_{[1,12]} = 8.911$ ,  $p = 0.011$ ) treatments resulted in a significantly higher rate of increase in %-MeHg compared to the 10-times  $\text{SO}_4$  treatment. All other treatments had rates that were statistically indistinguishable from the 10-times  $\text{SO}_4$  treatment. The 4-times  $\text{SO}_4$  and energetic-equivalent C treatments were also not significantly different from their corresponding 10-times  $\text{SO}_4$  and energetic-equivalent C treatments.

The variability observed in some of the experiments could be due to a number of factors including small-scale spatial variability in zones of anoxia and microbial community composition. Although there is variability between duplicate mesocosms as a result of the combined  $\text{SO}_4$  and C additions in some of the experiments, the overall response is clear. The combined addition of  $\text{SO}_4$  and some forms of organic C results in greater net production of MeHg than does the addition of  $\text{SO}_4$  alone. With elevated runoff or atmospheric loading of  $\text{SO}_4$  to peatlands, the most rapid rates of net MeHg production will be realized where there are coincident inputs of labile organic C.

While the combined addition of  $\text{SO}_4$  and labile C has undoubtedly fueled the heterotrophic metabolism of SRB, it also remains possible that these additions may have affected a larger microbial community. Very little research has been conducted on how different microbial populations interact in a community to support the methylation of Hg. A more diverse community of microbes (including methanogens and SRB) may have been stimulated through these chemical additions, which has led to a considerably greater net production of MeHg. Pak and Bartha (1998) found that SRB and methanogens can methylate Hg in cultures through interspecies H transfer, but most research on Hg methylation has been species- or function-specific. A community response may have been responsible for the high rates of net MeHg production in these combined additions, however, results from the C-only additions (Fig. 3) do not support this.

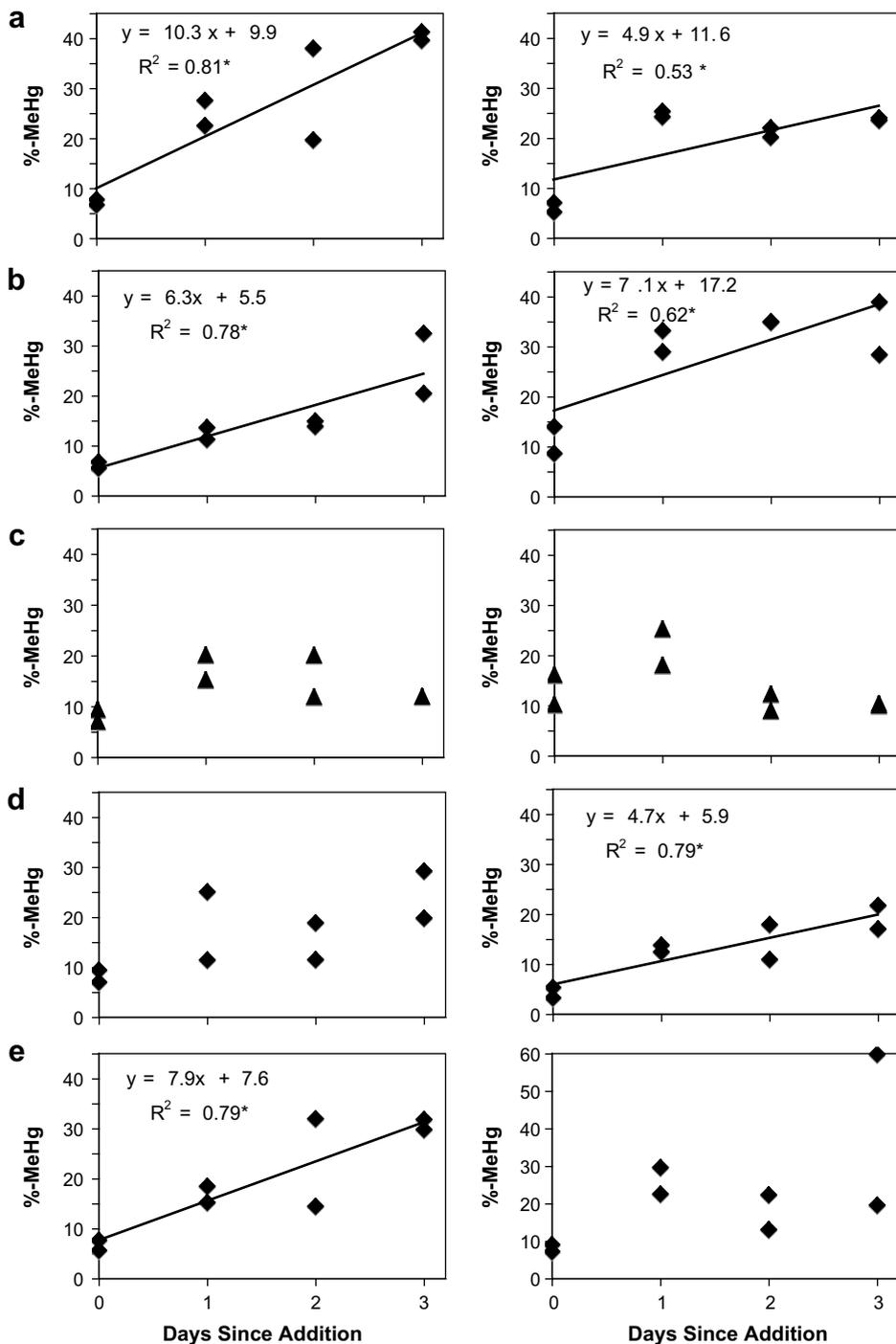


Fig. 6. Changes in %-MeHg over time resulting from the combined addition of 4-times (left) and 10-times (right) annual atmospheric deposition of SO<sub>4</sub> and energetic-equivalent (equal number of electrons) doses of (a) glucose, (b) acetate, (c) lactate, (d) coniferous forest litter leachate, and (e) deciduous forest litter leachate. \*Indicates a significant linear trend ( $p < 0.05$ ).

The similar and high rates of net MeHg production under the 4-times SO<sub>4</sub> and equivalent C (except lactate) treatments support hypotheses that non-

acetate oxidizing SRB (as stimulated by lactate) methylate Hg at slower rates than do other SRB. Alternatively, MeHg demethylation may be supported

by the addition of lactate. For bacteria capable of methylating Hg, there is no substrate discrimination between glucose, acetate or natural sources of organic C.

The lower combined loads of SO<sub>4</sub> and organic C generally resulted in greater net production of MeHg than did the higher combined loads of SO<sub>4</sub> and organic C. It is unlikely that this was the result of the formation of “unavailable” Hg–sulfide complexes, since the available sulfide data (data not shown) indicated that the highest production of dissolved sulfide occurred in the mesocosms of highest net MeHg production. It can be alternatively hypothesized that higher loading of SO<sub>4</sub> and C more strongly stimulated the demethylation of Hg. This may have been due to the stimulation of other microbial communities through the addition of organic C, such as methanogenic Archaea, which are known MeHg demethylators (Oremland et al., 1991). The formation of MeHg production “hot spots” may thus not always be controlled by the simple delivery of limiting reactants to a favorable biogeochemical zone as McClain et al. (2003) note for biogeochemical “hot spots”. In the case of MeHg production, the balance between these reactants is important. MeHg production “hot spots” will be more strongly controlled by intermediate loads of SO<sub>4</sub> and labile C, loads that swing the balance between Hg methylation and MeHg demethylation further toward the side of Hg methylation.

### 5. Significance to mercury methylation in peatlands and conclusions

The major implication of the research described here is that the formation of a MeHg “hot spot” (Mitchell et al., 2008) is dependant on the flux of both SO<sub>4</sub> and a labile source of C to a zone of persistent anoxia. This observation may be useful in predicting where MeHg “hot spots” may occur and how the effects of both climate and land-use change may affect the methylation of Hg in peatlands.

The highest MeHg concentrations and the largest %-MeHg in two other nearby peatlands (Table 3) occurred at the interface zone between the upland and the peatland. Both the elevated MeHg concentrations and %-MeHg in pore waters observed as a result of combined SO<sub>4</sub> and C additions have serious ecological implications because concentrations and %-MeHg were similar to the highest observations made in the other nearby peatlands (Table

3). From the knowledge gained from this mesocosm-scale study, it is hypothesized that MeHg “hot spots” occur at the upland–peatland interface for 3 reasons:

- (1) This area receives inputs of SO<sub>4</sub> and C in considerable excess of atmospheric and in situ sources as a result of upland runoff to the peatland margin. The amount of runoff would be site-specific, but likely related to upland subcatchment area and soil and vegetation characteristics.
- (2) The upland–peatland interface zone tends to be less nutrient-limited and may be the primary reason why greater vegetation diversity and overall biomass are maintained here. This denser vegetation may increase the amount of C delivered to the area via throughfall, litter-fall, and plant senescence and subsequently provide the boost to in situ SO<sub>4</sub> reduction that results in greater Hg methylation.
- (3) The upland–peatland interface zone had clearly more decomposed surface peat than did the peatland interior. This leads the authors to hypothesize that the interface zone has higher rates of C mineralization and thus may be providing a useable C substrate in situ that is resulting in higher mercury methylation.

The methylation of Hg resulting from all of these scenarios may be enhanced because of ecosystem perturbations such as climate change and changes in forest cover. The possible effects of climate change are not entirely clear. With increasing global temperatures and elevated atmospheric CO<sub>2</sub> concentrations, the concurrent effects of expected lower water tables in peatlands (Gorham, 1991) and increased plant productivity (Drake et al., 1997) on peatland hydrology and biogeochemistry make it difficult to predict how the Hg cycle may be affected. This requires further research. The likely effects of forest management practices, in particular upland clear-cutting, on the production of MeHg in peatlands are much clearer. The removal of upland trees would decrease evapotranspiration, leading to increased upland runoff (Hornbeck et al., 1993). An increase in upland runoff would augment the flux of sulfate and carbon into the peatland (as simulated in this study), which stimulates MeHg production. Clear-cutting of upland trees may also increase the flux of water from the entire watershed (Verry

et al., 1983), leading to more MeHg being exported from the basin where it may contaminate downstream aquatic ecosystems. It does, however, remain unknown how long such a scenario might continue. This is an area of continuing research.

Research is ongoing to examine how the observations from this study extend to the spatial and temporal patterns of MeHg production in peatlands. Further research is necessary to examine the links between the kinetics and temporal stability of MeHg production and the hydrological conditions and timing necessary for the export of MeHg from peatlands. More explicit studies on the microbial communities responsible for Hg methylation and MeHg demethylation in peatlands are required. This is especially true in light of the general observation that the area of peatland used in this study was situated far from areas of upland derived SO<sub>4</sub> and C input, yet responded rapidly to these inputs. Finally, this work should be expanded to other ecosystems and/or wetlands where inputs of SO<sub>4</sub> and labile C differ.

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