

# Sulfate Addition Increases Methylmercury Production in an Experimental Wetland

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Atmospheric mercury is the dominant Hg source to fish in northern Minnesota and elsewhere. However, atmospherically derived Hg must be methylated prior to accumulating in fish. Sulfate-reducing bacteria are thought to be the primary methylators of Hg in the environment. Previous laboratory and field mesocosm studies have demonstrated an increase in methylmercury (MeHg) levels in sediment and peatland porewaters following additions of sulfate. In the current ecosystem-scale study, sulfate was added to half of an experimental wetland at the Marcell Experimental Forest located in northeastern Minnesota, increasing annual sulfate load by approximately four times relative to the control half of the wetland. Sulfate was added on four separate occasions during 2002 and delivered via a sprinkler system constructed on the southeast half (1.0 ha) of the S6 experimental wetland. MeHg levels were monitored in porewater and in outflow from the wetland. Prior to the first sulfate addition, MeHg concentrations (filtered, 0.7  $\mu\text{m}$ ) were not statistically different between the control ( $0.47 \pm 0.10 \text{ ng L}^{-1}$ ,  $n = 12$ ; mean  $\pm$  one standard error) and experimental  $0.52 \pm 0.05 \text{ ng L}^{-1}$ ,  $n = 18$ ) halves. Following the first addition in May 2002, MeHg porewater concentrations increased to  $1.63 \pm 0.27 \text{ ng L}^{-1}$  two weeks after the addition, a 3-fold increase. Subsequent additions in July and September 2002 did not raise porewater MeHg, but the applied sulfate was not observed in porewaters 24 h after addition. MeHg concentrations in outflow from the wetland also increased leading to an estimated 2.4 $\times$  increase of MeHg flux from the wetland.

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Our results demonstrate enhanced methylation and increased MeHg concentrations within the wetland and in outflow from the wetland suggesting that decreasing sulfate deposition rates would lower MeHg export from wetlands.

## Introduction

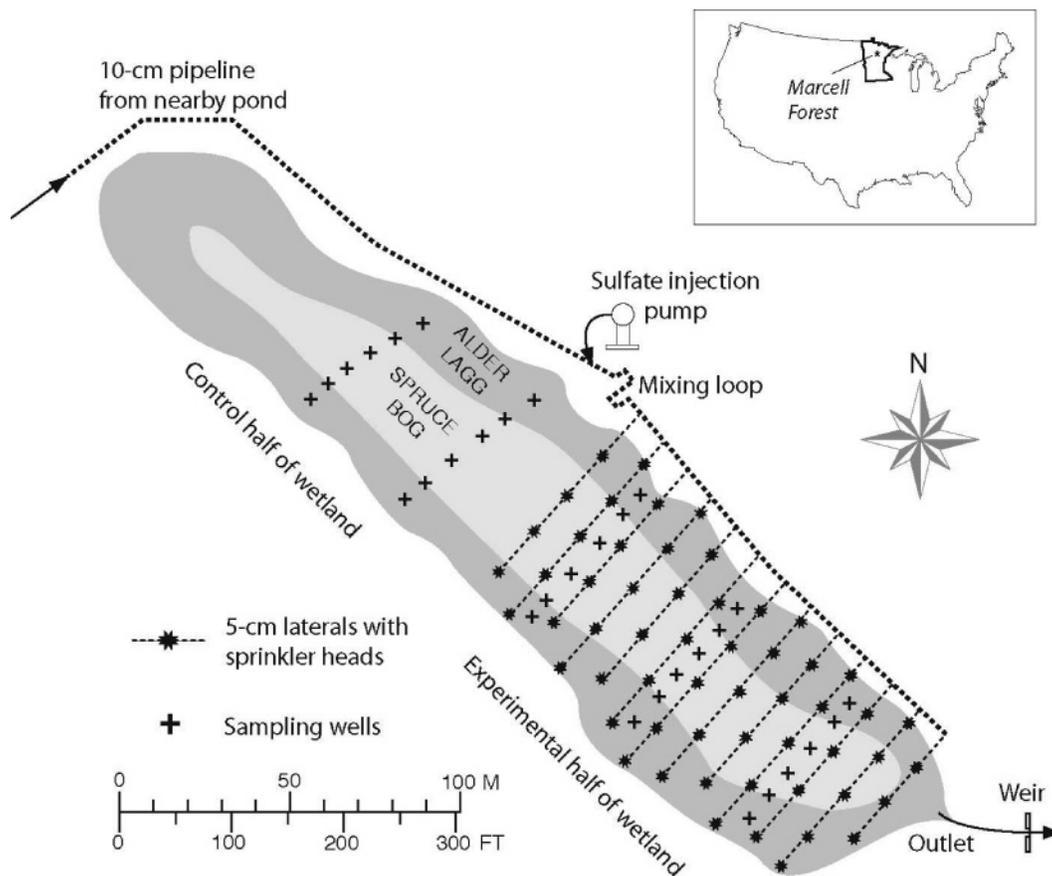
Efforts to reduce mercury (Hg) emissions in Minnesota and throughout the rest of the world assume change in atmospheric deposition of Hg will ultimately result in a proportional change of methylmercury (MeHg) concentrations in fish, all other things being constant. Accordingly, it is thought that fish now have mercury concentrations that are 3–4 times greater than natural (preindustrial) levels, because there is strong evidence that atmospheric Hg deposition is currently 3–4 times greater than natural rates (1–6). However, the proportion of Hg that is methylated and bioaccumulated in fish may not have been constant in some aquatic systems over that time period. Higher than expected Hg concentrations in fish may be the result of increased sulfate deposition to sulfate-poor ecosystems, where sulfate availability controls the activity of the bacteria that methylate Hg. A comparison of museum fish from the 1930s collected from low alkalinity lakes in northern Minnesota and fish collected from the same lakes in the 1980s indicated a 10-fold increase in Hg concentrations (7), consistent with the sulfate-enhancement hypothesis.

Hg methylation in natural systems is primarily by sulfate-reducing bacteria in sediments (8–11) and in wetlands (12–16), but has also been observed in floating macrophytes and periphyton (17). Wetlands, being a major source of MeHg to waters where fish exist (18–21), represent a critical link between atmospheric Hg deposition and accumulation of MeHg in aquatic food chains. The objective of this study is to determine if enhanced sulfate loads elevate MeHg levels in a sub-boreal *Sphagnum*/conifer wetland. Previous studies conducted in the laboratory and in field microcosms demonstrate a link between increased sulfate reduction rates and enhanced Hg methylation (8, 12). In this study, we artificially increased sulfate loads to an experimental wetland to examine the impact of increased sulfate deposition on Hg methylation at the watershed scale.

## Material and Methods

**Site Description.** The United States Department of Agriculture Forest Service Marcell Experimental Forest (MEF; Figure 1) is an 890 ha tract of land located 40 km north of Grand Rapids, Minnesota (47°32'N, 93°28'W). The experimental site, wetland S6, is one of seven small watersheds that have been used for long-term study of forest hydrology and Hg cycling at the MEF (22–26). Climatic and hydrologic data have been collected continuously at monitoring stations since 1959. Two peatland/upland forest watersheds have been instrumented and studied in detail, including hydrology (27, 28), nutrient cycling and behavior (29, 30), and release of organic carbon and acidity (31). A National Atmospheric Deposition Program (NADP) site has been operating at Marcell since 1978 and the first Mercury Deposition Network (MDN) station began operation at the MEF in 1992 (32, 33). Hydrologic monitoring and other related research continues at the MEF.

The landscape of the MEF is typical of morainic landscapes in the western Great Lakes region. The S6 watershed contains an elongate 2.0 ha mature black spruce (*Picea mariana*) and



**FIGURE 1.** The S6 wetland in the Marcell Experimental Forest, northern Minnesota. The irrigation system consists of ~360 m of 10-cm diameter PVC pipe running adjacent to the north side of the S6 wetland. From this main line, thirteen 5-cm diameter laterals, spaced 14 m apart, extend across the experimental half of the wetland. Adjustable sprinkler heads spaced at 16-m intervals along each lateral operate with a spray radius of approximately 8–9 m and rotate on 0.6-m vertical risers. Wells for sampling peat pore waters are arrayed along five transects, each consisting of two lagg wells, two bog wells, and two “transition” wells between the bog and the lagg.

tamarack (*Larix laricina*) wetland. The S6 wetland (Figure 1) is characterized by an alder (*Alnus rugosa*) lagg (a zone of higher pH at the contact with mineral-soil uplands) encircling the slightly raised spruce/*Sphagnum* bog. Outflow from the S6 watershed (pH = 4.9 ± 0.7) has been monitored with a 120° V-notch weir since 1964 (34). The 6.9 ha upland was clear-cut in 1980 to convert the upland from predominantly aspen (*Populus tremula*) to white spruce (*Picea glauca*) and red pine (*Pinus resinosa*).

**Sulfate Additions.** Sulfate was added to the experimental half of the S6 wetland in five simulated rainfall events (6–10 mm) from November 2001 through October 2002 by means of a PVC irrigation system (35) constructed in 2001 (Figure 1). The system consists of ~360 m of 10-cm diameter PVC pipe running adjacent to the north side of the wetland. From this main line, thirteen 5-cm diameter laterals, spaced 14 m apart, extend across the experimental half of the wetland. Adjustable sprinkler heads spaced at 16 m intervals along each lateral operate with a spray radius of approximately 8–9 m and rotate on 0.6 m risers. Valves installed on each lateral allowed flow rates to be maintained to operate sprinkler heads at the desired radius. The PVC pipes were glued together at most joints, but flexible hosing at several joints allows for temperature contraction and expansion. Source water for the system was drawn from a dilute (conductivity ~10  $\mu\text{S cm}^{-1}$ ), low mercury (<1 ng L<sup>-1</sup>), rain-fed pond, and a concentrated sodium sulfate solution was injected into the main line resulting in sulfate concentrations in the irrigation water of ~200 mg L<sup>-1</sup>. A mixing loop after the injection point ensured a homogeneous sulfate solution. When the desired amount of sulfate had been added, a 1-mm

rainfall equivalent cleared the lines and “washed” the sulfate off plant surfaces and into the peat porewaters. The 2002 sulfate load delivered by the irrigation system was 32 kg ha<sup>-1</sup>, equivalent to approximately four times current annual atmospheric deposition and similar to atmospheric sulfate deposition in the northeastern United States (32, 33). The sulfate load was seasonally distributed based on historical sulfate deposition rates. Lithium bromide was used as a hydrologic tracer, but it appears to be nonconservative, and was not as useful as hoped.

**Field Sampling.** Filtered water samples were collected from 30 peat wells 1 day prior to, and 1, 3, 5, 7, 14, 28, and 56 days following, each sulfate addition. The wells were situated along 5 transects designated as experimental (ET1, ET2, and ET3) or control (CT2 and CT3). Each transect consisted of 6 wells: 2 lagg wells (one each in the N and S lags), 2 bog wells, and 2 transition wells. The bog wells were located in the raised black spruce area of the wetland, the lagg wells were in the alder lagg, and the transition wells were located between the lagg and raised bog portions of the wetland. Unfiltered samples were collected at the S6 and nearby S7a outlet weirs every two weeks and whenever peat well sampling occurred. All mercury samples were collected in acid-cleaned 125 mL Teflon bottles using established protocols (24). Peat wells were designed to integrate peat porewater from the surface of the water table down to about 25 cm and by design collected porewater from depths corresponding to greatest hydraulic conductivity. Peat wells consisted of acid-cleaned 5-cm diameter PVC pipes cut to a length of 45 cm and driven approximately 35 cm into the peat. Approximately 40 holes (0.65-cm diameter) were drilled

into the wells to allow porewater to flow freely. A 2.5-cm diameter, finely slotted, acid-cleaned PVC Geoprobe screen, capped on the bottom, was inserted into each well and wells were capped between samplings. Samples were drawn from inside the Geoprobe screen with a hand pump and filtered through 0.7  $\mu\text{m}$  ashed glass fiber filters. Field duplicates and blanks constituted approximately 20% of all samples collected. Experimental results from the November 2001 and October 2002 additions are not presented in this paper because many of the sample wells froze shortly after sulfate additions. Outflows from sampled watersheds were measured at 120° V-notch weirs with individually calibrated stage–discharge relations and hourly stage readings (S7a) or a continuous strip-chart recorder (S6).

**Laboratory Methods.** Accepted clean methods were utilized throughout the collection and analysis of mercury and methylmercury samples. Samples analyzed for total mercury were first oxidized with 0.2 N bromine monochloride, neutralized with hydroxylamine, and then analyzed using the stannous chloride/cold vapor atomic fluorescence spectroscopic (CVAFS) method (24, 36). Analysis of MeHg was performed using the aqueous distillation/CVAFS method (37, 38). Briefly, following distillation, water samples were ethylated with sodium tetraethylborate, purged with nitrogen and collected on Tenax TA (Alltech 60–80 mesh) traps. Hg species were thermally desorbed from the Tenax in an argon stream and separated on an OV-1 chromatographic column, converted to elemental mercury in a pyrolytic column, and analyzed on a Tekran 2400 CVAFS. Lab duplicates and performance standards were routinely analyzed as part of the quality assurance plan. Sulfate and other anions were measured by ion chromatography (Dionex ICS 2000), while cations were measured with ICP-MS (ThermoFinnigan PQ ExCell).

## Results and Discussion

**Porewater MeHg Concentrations.** Dramatic increases in porewater MeHg concentrations were observed following the May 22, 2002 sulfate addition (Figure 2a). One day prior to the addition (Day –1), MeHg levels in the peat porewaters were not significantly different ( $p = 0.62$ ) in the control ( $0.47 \pm 0.10 \text{ ng L}^{-1}$ ,  $n = 12$ ; mean  $\pm$  one standard error) versus the experimental ( $0.52 \pm 0.05 \text{ ng L}^{-1}$ ,  $n = 18$ ) half of the wetland (Figure 2a). In the period between the May and July additions, MeHg porewater levels in the experimental half increased and remained elevated, while the control half exhibited no statistically significant change relative to Day –1. All MeHg concentrations in the experimental half were statistically higher than those of Day –1 at  $p < 0.05$  except for Day 56 ( $p = 0.13$ ). Porewater MeHg levels in the experimental half were also higher than the control half at  $p < 0.05$  except for Day 1 ( $p = 0.06$ ), demonstrating that the sulfate addition elevated MeHg levels after the May addition and, relative to the control half, maintained them for an extended period of time. Total Hg levels were similar between the experimental and control halves at this time; however, the fraction of total Hg occurring as MeHg increased after the May sulfate addition and remained elevated (Figure 2b). In addition, other water chemistry parameters (cations, anions, pH, and DOC) unimpacted by the sulfate addition behaved similarly between the experimental and control halves.

Changes in MeHg levels in the experimental half were inversely related to sulfate concentration in the peat porewaters in the first four sampling dates following the May addition (Figure 2a). Sulfate levels were undetectable at Day –1 in both the control and experimental halves. Following the May addition the average sulfate concentration increased to  $1.09 \pm 0.33 \text{ mg L}^{-1}$  ( $n = 18$ ) at Day 1 in the experimental half of the wetland and remained undetectable in the control half. As the sulfate reducing bacteria utilized the added sulfate,

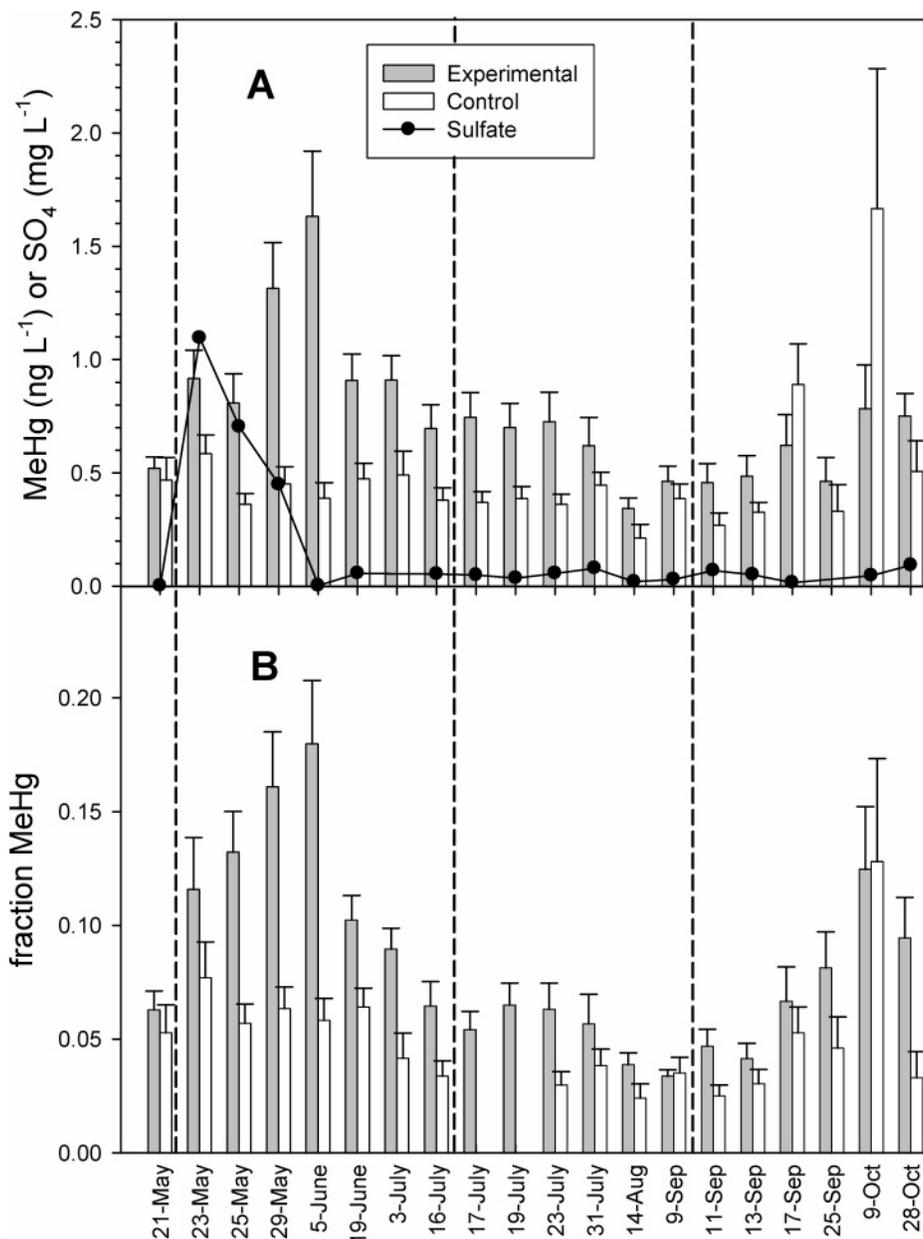
levels began to drop gradually, until sulfate was undetectable again on June 5 (Day 14) and porewater MeHg concentrations were at a local maximum,  $1.63 \pm 0.27 \text{ ng L}^{-1}$  ( $n = 18$ ). Following June 5 and prior to the July addition, sulfate levels across the wetland were detectable, but lower in the control half, although not statistically ( $p > 0.05$ ). The average sulfate concentration in the control during 2002 was  $0.02 \pm 0.01 \text{ mg L}^{-1}$ .

MeHg levels decreased after the June 5 maximum, but not back to the pre-addition levels. Net methylation (methylation – demethylation) was apparently enhanced in the experimental half of the wetland by the addition of sulfate. Two possible mechanisms for sustaining the elevated MeHg concentrations include the creation of a larger biologically available sulfur pool (14, 39, 40) or an increase in sulfate-reducing bacteria that methylate mercury.

The current study employed a large number of sampling wells collecting depth-integrated porewaters dispersed over a large area (2.0 ha). The large scale and experimental design makes it difficult to compare to other studies. However, similar studies done at smaller scales and at specific depth intervals were conducted in the Experimental Lakes Area (ELA), Canada (12) and in Degero Stomyr in northern Sweden (14). In the current study, MeHg porewater concentrations increased by a factor of 3 (from  $0.52 \pm 0.05 \text{ ng L}^{-1}$  to  $1.63 \pm 0.27 \text{ ng L}^{-1}$ ) two weeks after a 4 $\times$  increase in sulfate load (Figure 2a). Branfireun et al. (12) reported MeHg increases of up to 10 $\times$  following a 20 $\times$  increase in sulfate load to an experimental mesocosm (0.16 m<sup>2</sup>) in a poor fen peatland at ELA. A 2 $\times$  increase in sulfate load at the ELA study site resulted in a 3–4-fold increase in MeHg levels (12). The ELA study was conducted over 5 days and in most cases MeHg in the porewaters returned to pre-addition levels. The study in Sweden (14) examined MeHg in porewaters from sedge peatland microcosms (4 m<sup>2</sup>) dosed with sulfate for three years. A MeHg increase of approximately 5 $\times$  was reported in the mesocosm receiving an  $\sim 7\times$  increase in sulfate load.

Rain events influence MeHg levels in S6 not only by supplying sulfate, nutrients, and mercury, but also by transporting added sulfate within the wetland or flushing it from the wetland. The first rainfall after the spring addition—12 mm on May 28 and 17 mm on May 29—was not substantial enough to flush the added sulfate from the wetland. Indeed, the estimated sulfate load transported from the wetland was only 0.36 kg from May 21–June 5 compared to the added sulfate of 14.3 kg. An extremely large rain event (208 mm) occurred on June 22–24, preceded by a smaller event (36 mm) on June 18–19, resulting in record flows from S6 (Figure 3b). The amount of sulfate transported from the wetland at this time was 4.3 kg, still a relatively small amount compared to what was added. Despite this extreme hydrologic event, MeHg in the porewaters of the experimental half of the wetland exceeded those in the controls.

Contrary to expectations from the May sulfate application, MeHg concentrations did not increase in peat porewaters following the July and initially after the September sulfate additions (Figure 2). Moreover, there was no observed increase in porewater sulfate in the experimental peat wells, even 1 day after the applications. However, MeHg concentrations remained elevated in the experimental half relative to the control until late September. The most likely explanation for this seasonal contrast is temperature, which plays a key role in controlling sulfate reduction and methylation/demethylation rates. At the time of the May addition peat temperatures (as measured at the nearby S2 wetland, 0.4 km away), were still quite cool (4.5 °C at 5 cm), the bog having thawed only weeks before, and the added sulfate persisted for two weeks and changes in MeHg were observed. Peat temperatures increased slowly to above 16 °C by the time of the July addition and were still at 15 °C for the third addition

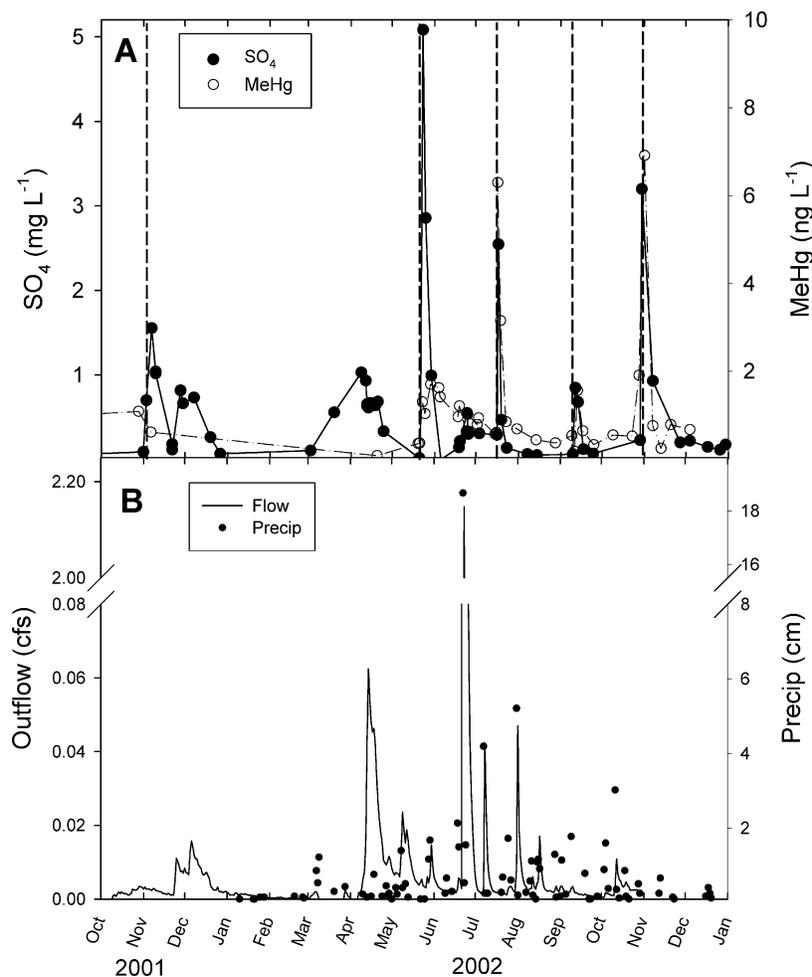


**FIGURE 2. (A) MeHg concentrations ( $\pm 1$  standard error) in pore waters from control and experimental peat wells and sulfate concentrations in experimental peat wells only; sulfate was generally below detection ( $< 0.01 \text{ mg L}^{-1}$ ) in the control wells. Each dotted line represents a sulfate application. (B) The fraction of total Hg existing as MeHg in control and experimental peat wells.**

in early September. The warm late-summer peat temperatures likely led to very high sulfate reduction rates such that much of the added sulfate may have been consumed within 24 h (the first sampling day) following the July and September applications. Some of the sulfate may have also been entrained in the more abundant vegetation during the summer additions.

A subsequent decrease in peat temperature and outflow in late September/early October coincided with more variable MeHg concentrations and the control half actually exceeding MeHg levels in the experimental half on a few days, but these differences are not statistically significant (Figure 2). Currently, we cannot explain these observations, but they appear independent of the sulfate addition. The limited MeHg results from after the October 2002 addition (not presented because of extensive well freeze-up) were also highly variable and

may be related to decreases in temperature. A few of these samples had MeHg concentrations exceeding  $10 \text{ ng L}^{-1}$ , however they could not be independently verified by additional late season field collections. Decreased temperatures might have contributed to the increase in MeHg concentrations, but other factors including Hg deposition through litterfall or possibly organic matter oxidation owing to late-season water-level fluctuations could have played a role. Litterfall, which begins in mid-September, is an important component of the total Hg flux to the Marcell wetlands, contributing nearly twice the Hg delivered by wet deposition alone (41, 42). Water level in the wetland was decreasing at this time creating relatively stagnant conditions. Flow from S6 decreased substantially in September 2002 with only a few small rain events (Figure 3b). With the decline in water level, labile organic matter in the surface peat may have been oxidized releasing bound mercury as well as sulfate to the dissolved phase.



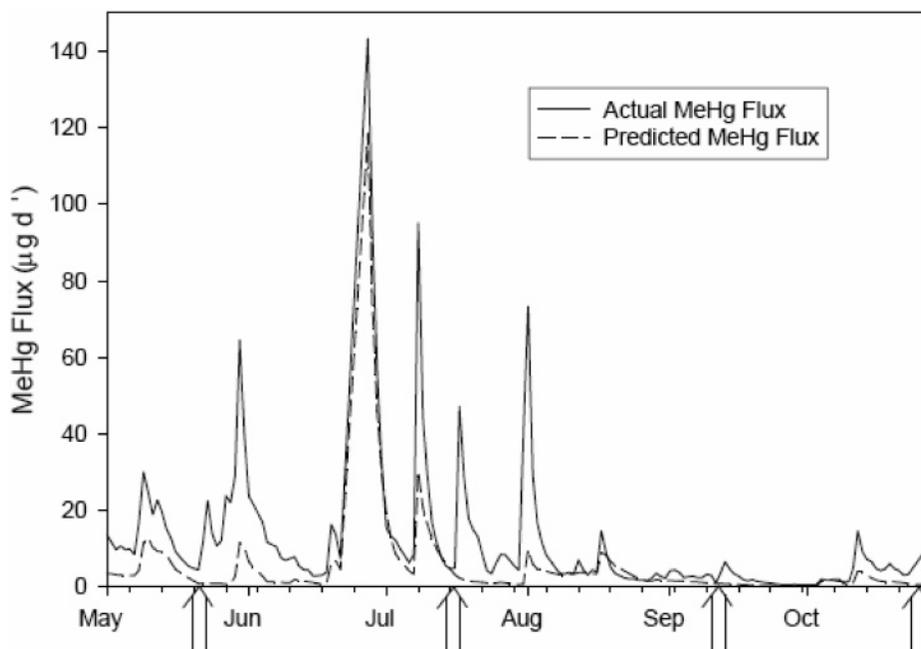
**FIGURE 3. (A) MeHg and sulfate concentrations in the outflow from the S6 wetland. (B) Hydrologic outflow and precipitation events at S6. Flows were measured by chart recorder at the S6 weir (in operation since 1964), and precipitation was measured with a rain gauge located near the west end of the S6 wetland.**

**MeHg Export from S6.** MeHg and sulfate concentrations increased at the S6 weir following each sulfate addition (Figure 3a), although the timing of the increases varied over the course of the experiment. Elevated concentrations observed at the weir after the July and September additions are in contrast to the peat wells where increases in sulfate or MeHg were not observed (but MeHg remained elevated relative to the control). Higher sulfate concentrations persisted at the weir following the May and late October additions, consistent with the peat well trends. A small pool impounded behind the weir likely contributed to these trends. Although sulfate was not added directly to the pool, some sulfate flowed into it within hours of each addition, increasing sulfate concentrations. Sulfate levels at the weir then declined over time as the pool was flushed by additional sulfate-depleted water from the wetland. For example, in May the flushing rate,  $k_f$ , of the weir pool was  $1.37 \text{ d}^{-1}$ , ( $k_f = \text{flow}/\text{volume}$ ). The observed first-order loss of sulfate from the pool,  $k_{\text{obs}}$  ( $0.27 \text{ d}^{-1}$ ), from Day 1 to Day 7 was significantly less than  $k_f$  indicating a substantial flow of sulfate from the wetland to the weir pool. Sulfate levels in the peat porewaters were elevated at this time (Figure 3). In contrast, pool flushing rates following the July ( $0.48 \text{ d}^{-1}$ ) and September ( $0.33 \text{ d}^{-1}$ ) additions, were similar to  $k_{\text{obs}}$  for July ( $0.59 \text{ d}^{-1}$ ) and September ( $0.37 \text{ d}^{-1}$ ) suggesting that a pulse of sulfate was introduced to the weir pool within hours after these additions and then simply flushed out. Presumably due to high sulfate reduction rates or the sulfate never reaching the water table, sulfate in peat

porewaters was insignificant during July and September and thus outflow of sulfate from the wetland to the pool was insignificant at this time. Water chemistry samples were not taken frequently enough following the October 2002 addition to calculate  $k_{\text{obs}}$  accurately.

MeHg trends at the weir closely track those for sulfate (Figure 3a). Following the May addition, MeHg concentration gradually increased at the weir, similar to the peat porewaters (Figure 2). The concentrations at the weir and in the peat porewaters were also similar at this time indicating that the peat porewaters were supplying the MeHg flowing over the weir. However, following the July and September additions, MeHg concentrations at the weir spiked immediately after each addition and the weir concentrations exceeded peat porewater concentrations. It is not clear if these spikes were due to high levels of MeHg flowing from the wetland or MeHg formation in the weir pool itself. However, based on the flushing rate of the pool, it appears that the dominant loss process for sulfate was flushing and that sulfate reduction in the weir pool was negligible.

Empirically modeled MeHg export from S6 without sulfate addition was compared to measured MeHg export in 2002. The observed daily MeHg export exceeded the predicted MeHg export during periods immediately following sulfate additions. To model MeHg export from S6 in the absence of sulfate additions, data from 2001 (prior to the 2002 sulfate additions to S6) showed a strong correlation between flows at the S6 weir and a nearby wetland weir, S7a ( $r^2 = 0.71$ ).



**FIGURE 4.** Actual and predicted fluxes of MeHg from the S6 wetland for 2002. The predicted flux is that which would have occurred in the absence of sulfate addition and is based on a correlation of 2001 (pretreatment) MeHg fluxes from S6 with those from a nearby reference wetland, S7a (see text). Arrows indicate experimental sulfate applications.

Furthermore, MeHg export from S7a was correlated to MeHg export from S6 in 2001

$$\log \text{Flux}_{S6} = 1.23 \times \log \text{Flux}_{S7a} - 1.62 \quad (r^2 = 0.77 \text{ in } 2001) \quad (1)$$

where  $\text{Flux}_{S6}$  ( $\mu\text{g d}^{-1}$ ) is the measured MeHg flux out of wetland S6 and  $\text{Flux}_{S7a}$  ( $\mu\text{g d}^{-1}$ ) is the measured flux out of wetland S7a.  $\text{Flux}_{S6}$  and  $\text{Flux}_{S7a}$  are daily fluxes determined from average daily flows measured at the weirs and MeHg concentrations interpolated between sampling dates (see Supporting Information). In 2001, the weirs were sampled biweekly and in 2002 additional samples were collected from the weir at S6 corresponding to each porewater sampling date. Using eq 1, the MeHg flux for May through October 2002 that would have come from S6 in the absence of sulfate addition was estimated and compared to the actual flux (Figure 4). Excluding the high flow values from the June 22–24 storm event and the unusually high MeHg concentration observed the day after the October 2002 addition (including these values yields an even greater enhancement), the MeHg flux observed in 2002 ( $1780 \mu\text{g MeHg}$ ) was more than two times greater (144%) than would have occurred without sulfate addition ( $730 \mu\text{g MeHg}$ ).

In this study, enhanced MeHg concentrations were observed in the experimental peat porewaters and in the flow from the S6 wetland following sulfate addition. Enhanced MeHg concentrations were not observed in peat porewaters following the July and September additions, but the added sulfate did not increase porewater sulfate concentrations due to either rapid sulfate utilization or entrainment in overlying vegetation. Not all MeHg and sulfate trends observed can be readily explained in this initial year of sulfate addition, but sulfate addition enhanced MeHg concentrations in most cases, despite the fact that our addition of sulfur was negligible relative to the sulfur pool in the upper 30 cm of peat. At no point in the study were there any indications that the sulfate load decreased methylation as has been observed in the past in lake enclosures (43). The most likely explanation for these observations is that biologically available sulfur is a limiting factor in this system for the methylating bacteria. The addition

of the limiting factor, sulfate, increased MeHg levels and may have increased the biologically active sulfur pool in S6. One possible implication of this study is that historic increases in atmospheric sulfate deposition (now on the decline) may have enhanced contemporary MeHg production and export from wetlands, contributing to widespread mercury contamination of aquatic food chains. It follows that decreases in sulfate deposition could result in less export of MeHg from wetlands and possibly result in lower MeHg levels in fish.

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### Supporting Information Available

Additional plots and further information on methods related to eq 1 and Figure 4 used to estimate enhanced export of MeHg from the S6 wetland. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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