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The stimulation of methylmercury production by decomposition of flooded birch leaves and jack pine needles

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Abstract. The link between methylmercury (MeHg) production and decomposition of flooded organic matter was examined using an enclosure experiment. Six plastic enclosures were filled with lake water containing low concentrations of dissolved organic carbon (146 $\mu mol\,L^{-1}$) and MeHg (0.02 $ng\,L^{-1}$) and anchored in a lake at the Experimental Lakes Area, northwestern Ontario. Either fresh birch leaves, fresh jack pine needles, or no plant tissues at all were added to enclosures. Birch leaves decomposed $\sim$2.4 times faster than jack pine needles as measured by the total carbon decomposition by-products produced in enclosures over time. However, measured net MeHg production in enclosures containing birch leaves (0.35 ± 0.05 ng per g carbon added) was five times lower than in the enclosures containing jack pine needles (1.94 ± 0.28 ng per g carbon added). These results showed that MeHg production is not solely related to rates of organic matter decomposition, and that increases in MeHg associated with flooded birch leaves and jack pine needles resulted from the production of new MeHg as opposed to leaching of MeHg already in the plant tissues during decomposition.

Introduction

Concentrations of the neurotoxin methylmercury (MeHg; CH_3Hg^+) in fish in reservoirs often exceed Health Canada consumption guidelines of 0.5 $\mu g\,g^{-1}$ wet mass (Brouard et al. 1994; Scruton et al. 1994; Bodaly and Fudge 1999). Reservoirs are also sources of the greenhouse gases carbon dioxide (CO_2) and methane (CH_4) to the atmosphere due to aerobic and anaerobic microbial mineralization of organic carbon in flooded soils and vegetation (Rudd et al. 1993; Duchemin et al. 1995; St. Louis et al. 2000; Matthews et al. in press). Decomposition of flooded organic matter in reservoirs has been hypothesized to fuel the production of MeHg, which bioaccumulates in aquatic food webs, resulting in fish with elevated Hg concentrations.

A number of studies utilizing field (Roulet et al. 2000, 2001; Balogh et al. 2002; Porvari et al. 2003), litterbag (Heyes et al. 1998; Hall and St. Louis submitted), mesocosm (Hecky et al. 1991), and laboratory (Porvari and Verta 1995; Thérien and Morrison 1999) approaches have shown that inundation of plant tissue results in increased MeHg concentrations in surrounding water and biota. Whole ecosystem flooding experiments at the Experimental Lakes Area (ELA) in northwestern Ontario,

Canada, examining changes in the biogeochemical cycling of carbon and Hg in response to reservoir creation (Kelly et al. 1997; Hall et al. in press; St. Louis et al. in press), have also demonstrated that decomposition of flooded organic matter in reservoirs resulted in increased MeHg concentrations in food web organisms. These findings have spurred debate focusing on two possible mechanisms that may cause these elevated MeHg levels.

The first mechanism is the simple leaching during decomposition of MeHg already present in flooded organic matter. As a result, the rate at which MeHg enters the food web may be directly proportional to the rate at which organic matter is mineralized. Although total Hg (THg) and MeHg concentrations in boreal plants are typically very low (Moore et al. 1995; Munthe et al. 1995; Rasmussen 1995), litterfall has been shown to be an important input of MeHg and THg to forest floors (St. Louis et al. 2001). MeHg in organic matter has been associated with low molecular weight water-soluble fulvic acids (Reddy and Aiken 2001), and therefore may be more leachable than inorganic forms of Hg (Hultberg and Munthe 2001). Under this scenario, the quantity of MeHg that could enter the reservoir food web would never exceed what was stored in organic matter prior to flooding.

The second mechanism involves the production of new MeHg following reservoir creation (Kelly et al. 1997). This biomethylation of inorganic Hg depends on the metabolism of the methylating organisms (e.g., sulphate reducing bacteria; SRB) in anaerobic zones of reservoirs, as well as the amount of substrates, such as HgII, small organic compounds, and sulphate (SO_4^{2-}) bioavailable for methylation metabolism. Rates of Hg methylation may therefore be directly proportional to rates of microbial mineralization of organic carbon because products of decomposition are important substrates for Hg methylation. The extent of Hg methylation may be affected following flooding by changes in environmental factors that have been shown to directly affect methylation, such as pH (Winfrey and Rudd 1990; Gilmour and Henry 1991), temperature (Bodaly et al. 1993), selenium (Se) concentrations (Fjeld and Rognerud 1993; Rudd and Turner 1983; Turner and Rudd 1983), and anoxia. In this scenario, the amount of MeHg available for bioaccumulation can far exceed what was initially there in organic matter, and the production of new MeHg can persist for long periods of time following flooding.

Although past studies have shown that flooding organic carbon results in increases in MeHg in water and biota, these studies were unable to determine if increases in MeHg were due to leaching or new MeHg production, nor did they concurrently measure increases in MeHg and decomposition by-products of flooded plant tissue. Here we describe an enclosure experiment in which we simultaneously quantify the production of MeHg and decomposition by-products following inundation of two plant tissues (birch (*Betula papyrifera*) leaves and jack pine (*Pinus banksiana*) needles) found in the boreal ecoregion where northern reservoirs are commonly created. We hypothesized that: (1) there will be production of new MeHg associated with the decomposition of flooded plant tissues, and (2) rates of MeHg production will be directly proportional to rates of decomposition.

Methods

Experimental set up

Six rigid, opaque plastic enclosures were placed randomly in a raft floating in a sheltered area of an oligotrophic lake at the ELA (Lake 240) for 53 days in July 2000. Each enclosure was filled with $\sim$420 L of water from Lake 240, which contained low concentrations of both dissolved organic carbon (146 μmol L^{-1}) and MeHg (0.02 ng L^{-1}), leaving a headspace of $\sim$75 L (Figure 1). Two enclosures each received 300 g of either fresh birch leaves or fresh jack pine needles hand picked while wearing vinyl gloves. Two enclosures contained only lake water. Please see Figure 1 for further details of enclosure setup.

Sampling protocol and analytical methods

By-products of decomposition

Carbon in plants. To quantify the amount of carbon added as plant tissue to each treatment enclosure, carbon content of freeze-dried birch leaves and jack pine needles was analyzed at the beginning and end of the experiment using an Exeter Analytical Model 440 Elemental Analyzer at the University of Alberta Limnology Laboratory. The relationship between fresh and dry weights (d.w.) was determined by weighing sub-samples of plant tissue before and after freeze-drying. The mass of carbon added to each enclosure was determined by multiplying the dry weight of plant tissue by the % carbon content.

Inorganic carbon in water and enclosure headspace. We measured increases in concentrations of dissolved inorganic carbon (DIC) and dissolved CH_4 (by-products of organic carbon mineralization) in enclosures over time. The opaque enclosures prevented any photosynthetic assimilation of CO_2 produced during mineralization of plant tissue. Samples for dissolved gas analyses were collected twice a week. Just prior to sampling, the water in each enclosure was mixed for 20 min by pumping bottom water into the headspace using high volume water pumps.

Dissolved gas samples were collected in evacuated Wheaton bottles of known volume ($\sim$160 mL) containing 8.9 g of KCl and backfilled with 10 mL of ultra-high purity (UHP) nitrogen (N_2) to maintain a headspace. Concentrated phosphoric acid of 0.5 mL was injected into each sample to convert all bicarbonate to CO_2. A volume of Lake 240 water was injected back into enclosures to replace that removed in samples. Enclosure headspace samples were collected into evacuated 60 mL Wheaton bottles through a septum in the lid using double-ended needles.

Samples containing dissolved gases were shaken on a wrist action shaker for 10 min prior to analysis to equilibrate dissolved CO_2 and CH_4 with the N_2 headspace. A 0.2 mL sample of headspace from the water samples, as well as the enclosure headspace, was injected into a Varian Model 3800 gas chromatograph

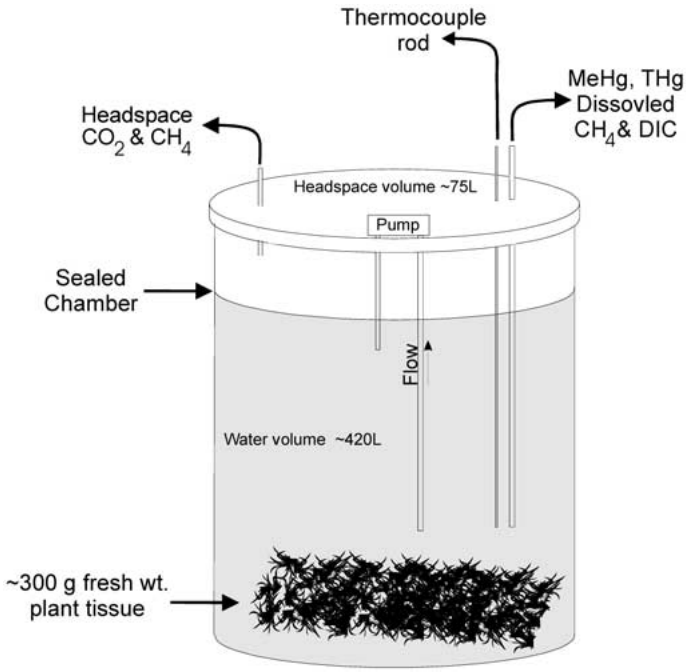

Figure 1. A cross-section of an enclosure containing plant tissue. Each enclosure was ∼75 cm wide and 115 cm deep, and filled with ∼420 L of whole lake water leaving a ∼75 L headspace. Lids for each enclosure contained a Teflon tube with an acid cleaned nitex mesh filter for water sampling and a Constantan copper–nickel thermocouple rod attached to a Campbell Scientific datalogger for monitoring water temperatures. Lids were attached to enclosures using silicone caulking. The water in each enclosure was mixed for 20 minutes prior to sampling by pumping bottom water into the headspace using high volume water pumps equipped with acid-rinsed Teflon gears and fittings (St. Louis et al. 2003).

(GC) using a Hamilton pressure lock syringe. Gas samples were analyzed using a flame ionization detector at 250 °C and UHP hydrogen as a carrier gas. Chromatographic separation was achieved through a Hayes Sep D column at 80 °C. A ruthenium methanizer converted CO_2 to CH_4. Standards (Praxair; Linde-Union Carbide) ranging from 20 to 19,900 CO_2 ppm and 1.6 to 78.1 CH_4 ppm, were used to generate calibration curves ($r^2 > 0.98$) before and after each set of samples. Peak areas were integrated using Star WorkStation analytical software. CO_2 and CH_4 were converted to concentrations ($\mu mol\,L^{-1}$) using Henry's Law (Hamilton et al. 1994), correcting for temperature and barometric pressure differences between sample collection and gas analysis.

Water chemistry including organic carbon. To characterize differences in the chemical composition of the water in the enclosures, water samples for general chemistry were taken in Nalgene polypropylene bottles from each enclosure at the

beginning and end of the experiment. Samples were analyzed at the ELA Aquatic Analytical Chemistry Laboratory for parameters that might affect potential Hg methylation in aquatic systems, such as pH, alkalinity, and dissolved ions (Cl^-, SO_4^{2-}, K^+, Mg^{2+}, Ca^{2+}, and Fe^{2+}) (Stainton et al. 1977). Other products of decomposition, such as dissolved and suspended nutrients, were also measured. Suspended carbon and dissolved organic carbon (DOC) concentrations were divided by the amount of carbon added as plant tissues to standardize differences in suspended carbon and DOC among treatments.

Total carbon pools. We estimated rates of decomposition of plant tissues by calculating the total amount of carbon produced in each enclosure. The total carbon pool dissolved in the water in each enclosure was calculated by multiplying DIC, CH_4-C, DOC, and suspended C concentrations by the water volume in each set of enclosures. The total inorganic carbon pool in the headspace was calculated by multiplying CO_2 and CH_4-C concentrations by the headspace volume. The average total carbon pool in enclosures containing only water was subtracted from the pool in enclosures containing birch leaves or jack pine needles and divided by the amount of carbon in plant tissues added to determine standardized carbon production among treatments. Inorganic carbon pools (DIC, CO_2, and CH_4) were calculated for each sampling date, whereas total carbon including DOC and suspended C were calculated at the end of the experiment only.

Mercury

Mercury in plants. Both MeHg and THg concentrations were measured in plant tissues to: (1) assess the amount of MeHg and THg added to each enclosure at the beginning of the experiment, and (2) determine if there was a net increase in MeHg in the plant tissues over the course of the experiment. A sub-sample of plant tissues added to the enclosures was freeze-dried and ground using an acid-rinsed stainless steel coffee grinder. At the end of the experiment, after removing the majority of the water from enclosures, a portion of the decomposed plant tissue was collected by pouring the remaining water through a 500 µm mesh. Plant samples were analyzed for concentrations of MeHg and THg at Flett Research Ltd. (Winnipeg, MB). Five to six milligrams of freeze-dried plant tissues was digested in 300 µL KOH/MeOH overnight at 75 °C and analyzed for MeHg using cold vapor atomic fluorescence spectrophotometry (CVAFS) after ethylation (Bloom 1989; Horvat et al. 1993; Liang et al. 1994). For THg analysis, plant tissues were first digested in 1 HNO_3:2.5 H_2SO_4 for 6 h at 250 °C. Digests were cooled, diluted with ultra-clean distilled water, and BrCl was added to maintain all Hg species in solution as HgII. Following digestion, samples were analyzed using CVAFS (Bloom and Crecelius 1983; Bloom and Fitzgerald 1988). Detection limits were 0.25 ng g^{-1} for MeHg and 1 ng g^{-1} for THg.

The mass of MeHg and THg added to enclosures in fresh plant tissues was calculated by multiplying the dry weight of tissues by the concentration of MeHg and THg. The decomposed plant tissue in the enclosures (especially birch leaves) became suspended in the water column over the course of the experiment, and only

a small amount could be collected at the end of the experiment to determine concentrations of MeHg and THg. Unfortunately, final plant weight could not be determined and a final mass of MeHg in the decomposing plant tissues could not be measured directly. We therefore used a THg mass balance budget to estimate final plant weights assuming that, because the enclosures were closed systems, the initial and final mass of THg in the water and plants in each enclosure did not change over the course of the experiment even though some THg may have leached from plant tissues during decomposition. We solved for the final THg mass in plant tissues as follows (known quantities are italicized):

$$(\textit{Initial THg mass in water}) + (\textit{initial THg mass in plant tissues}) = (\textit{Final THg mass in water}) + (\text{final THg mass in plant tissues}),$$

where the THg mass in water was calculated by multiplying the volume of the enclosures by the concentration of THg in the water at the beginning and end of the experiment, and the initial THg mass in plant tissues was calculated by multiplying the concentration of THg in birch leaves and jack pine needles by the amount of plant tissue added on a dry weight basis to each enclosure. We then solved for the final weight of plant tissues:

$$\text{Final weight of plant tissues} = \frac{\textit{Final THg mass in plant tissues}}{\textit{Final THg concentration in plant tissues}}$$

The final plant weights were multiplied by final MeHg concentrations in plants to determine the MeHg mass in jack pine needles and birch leaves at the end of the experiment.

Mercury in water. Unfiltered enclosure water was collected for MeHg and THg analyses through Teflon lines attached to a high volume water pump fitted with acid rinsed Teflon gears. Samples were pumped into ultra-clean 125 or 250 mL Teflon bottles using clean-hands–dirty-hands sampling protocol (St. Louis et al. 1996). Flett Research Ltd. analyzed unfiltered water samples for MeHg and THg concentrations. Twenty percent of samples were taken in duplicate. MeHg samples were frozen until analysis and THg samples were preserved using trace metal grade HCl (to 1% of total sample volume). Samples were analyzed for MeHg using CVAFS after distillation (Horvat et al. 1993) and aqueous phase ethylation (Bloom 1989) (method detection limits = $0.015\,\text{ng}\,\text{L}^{-1}$ at a blank level of 0.02–$0.03\,\text{ng}\,\text{L}^{-1}$). Total Hg samples taken in the first 2 weeks and at the end of the experiment were analyzed by using CVAFS as described in Bloom et al. (1988) (method detection limit of $0.1\,\text{ng}\,\text{L}^{-1}$ at a blank level of 0.3–$0.4\,\text{ng}\,\text{L}^{-1}$). The total MeHg mass in the water in each enclosure at the beginning and end of the experiment was calculated by multiplying MeHg concentrations by the water volume in enclosures.

Net increases in methylmercury mass. The final total mass of MeHg in each enclosure was calculated by adding the mass of MeHg in the plant tissues to the

mass of MeHg in the water. The net increase in MeHg mass in treatment enclosures was calculated as follows:

(Final MeHg mass in water and plant tissues)
– (initial MeHg mass in water and plant tissues)
– (average increase in MeHg mass in water in control enclosures).

A standardized rate of MeHg production per mass of carbon added in plant tissue was determined by dividing the final net increase in MeHg mass by the mass of carbon in plant tissues added.

Results

By-products of decomposition

Carbon in plants

The percent dry weight and carbon content of fresh jack pine needles ($49.1 \pm 3.7\%$ and $52.0 \pm 0.1\%$ carbon, respectively; Table 1) were greater than those in fresh birch leaves ($34.2 \pm 3.0\%$ and $49.3 \pm 0.5\%$ carbon, respectively). As a result, there was 1.5 times more carbon added to the enclosures containing jack pine needles (76.6 ± 5.9 g carbon) than to the enclosures containing birch leaves (50.7 ± 4.9 g carbon; Table 1).

Inorganic carbon in water and enclosure headspace

We initially sealed the headspace of the enclosures from the atmosphere with silicone caulking under the lids. However, this seal was broken sometime between Days 15 and 33. On Day 33, when the leak was detected, enclosures were resealed with additional silicone caulking. Despite the leaks, however, it was evident that birch leaves decomposed faster than jack pine needles. The average concentrations of DIC in treatment enclosures began to increase immediately after plant tissues were added, and were always higher than average DIC concentrations in the enclosures containing only water (Figure 2(A)). Average DIC concentrations in enclosures containing birch leaves exceeded those in enclosures with jack pine needles after Day 33 (ANOVA; $p = 0.002$; $F = 82.75$), reaching a maximum of $1190\ \mu mol\ L^{-1}$ at the end of the experiment. Average DIC concentrations in enclosures containing jack pine needles increased at the beginning of the experiment, but plateaued at concentrations between 500 and $600\ \mu mol\ L^{-1}$ from Day 19 on. Average DIC concentrations in enclosures containing only water (range = 128–$240\ \mu mol\ L^{-1}$) did not increase over time (Figure 2(A)). By Day 29, average headspace CO_2 concentrations were significantly higher in enclosures containing birch leaves compared to those containing jack pine needles (ANOVA; $p = 0.006$, $F = 43.33$). Increases in CO_2 concentrations in the headspace of the jack pine barrels ranged from 15 to over $250\ \mu mol\ L^{-1}$ and were less pronounced than increases in enclosures containing birch leaves, which reached concentrations of

Table 1. Weight and % carbon content in plant tissues[a].

	Fresh plant tissue added (g)	Equivalent dried mass added[b] (g)	%C in dried plants at the start of the experiment	C added (g)	% C in dried plants at end of experiment
Birch leaves	300 ± 0 (300, 300)	103 ± 8.9 (94, 112)	49.3 ± 0.46 (48.9, 49.8)	50.7 ± 4.9 (45.8, 55.5)	55.35 ± 0.06 (55.3, 55.4)
Jack pine needles	300 ± 0 (300, 300)	147 ± 11.1 (136, 158)	52.0 ± 0.08 (51.9, 52.1)	76.6 ± 5.9 (70.7, 82.5)	53.8 ± 0.04 (53.7, 53.8)

[a]Values for individual enclosures are presented in parentheses.
[b]Calculated using ratio of fresh weight to dry weight ratios.

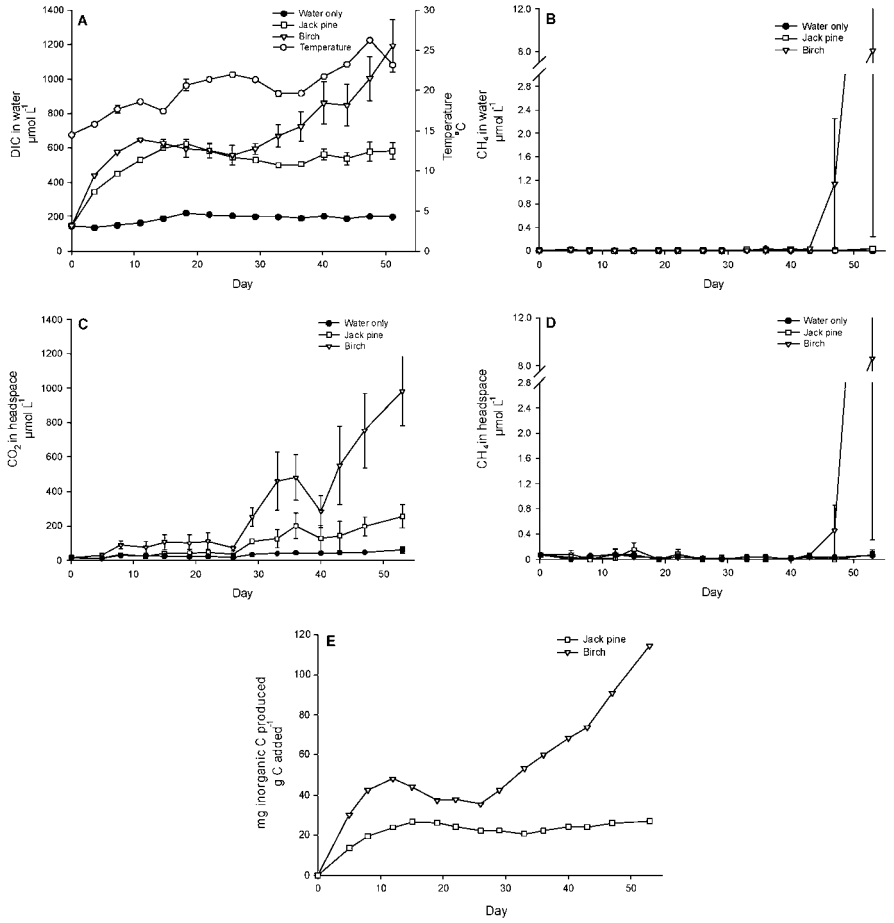

Figure 2. Concentrations of (A) dissolved inorganic carbon (DIC) and (B) methane (CH_4) in unfiltered water and (C) carbon dioxide (CO_2) and (D) CH_4 in the headspace from enclosures containing jack pine needles, birch leaves, and water only. (E) Total mass of inorganic carbon produced in unfiltered water per gram of carbon added as either jack pine needles or birch leaves. Total inorganic carbon produced was calculated by subtracting the C produced in the control enclosures from the total C in unfiltered water and headspace of each enclosure and dividing by the mass of C in dried plants at the beginning of the experiment (Table 1). Inorganic C production per g C added was calculated for each enclosure and then averaged.

980 µmol L^{-1} by the end of the experiment (Figure 2(B)). Average headspace CO_2 concentrations in the control barrel never exceeded 65 µmol L^{-1}. There was virtually no CH_4 detected in either the enclosure water or headspace, with the exception of one containing birch leaves, which had elevated CH_4 concentrations in both the water and the headspace after Day 43 of the experiment (Figure 2(C) and (D)).

Table 2. Concentrations of chemical constituents in water from enclosures at the beginning and end of the enclosure experiment.

Chemical Parameter	Initial	Final		
		Birch	Jack pine	Control
Nutrients[a]				
DOC ($\mu mol\,L^{-1}$)	585 ± 15	1650 ± 240	1620 ± 50	595 ± 5
TDN ($\mu g\,L^{-1}$)	310 ± 45	1045 ± 425	425 ± 15	310 ± 5
NH_4^+ ($\mu g\,L^{-1}$)	32 ± 19	1215 ± 1195	20 ± 2	59 ± 24
NO_2^- ($\mu g\,L^{-1}$)	0 ± 1	0	0	0
NO_3^{2-} ($\mu g\,L^{-1}$)	1 ± 2	1 ± 0	0	3 ± 2
TDP ($\mu g\ L^{-1}$)	3.5 ± 0.5	7.0 ± 3.0	5.5 ± 3.5	8.0 ± 0
Suspended C ($\mu g\,L^{-1}$)	610 ± 50	6895 ± 455	3580 ± 1320	435 ± 125
Suspended N ($\mu g\,L^{-1}$)	49 ± 4	1188 ± 114	478 ± 153	47 ± 15
Suspended P ($\mu g\,L^{-1}$)	4 ± 0	240 ± 21	73 ± 26	3 ± 1
Chlorophyll *a* ($\mu g\,L^{-1}$)	1.90 ± 0.66	0.19 ± 0.08	0.14 ± 0.05	0.08 ± 0.02
Physical parameters				
pH	7.23 ± 0.02	5.70 ± 0.06	5.84 ± 0	6.61 ± 0.13
Alkalinity ($\mu eq\,L^{-1}$)	138.5 ± 1.5	374.5 ± 6.5	214.5 ± 7.5	140.0 ± 3.0
Conductivity ($\mu S\,cm^{-1}$)	25.5 ± 0.5	45.0 ± 3.0	29.5 ± 0.5	26.5 ± 0.5
Anions				
Cl^- ($mg\,L^{-1}$)	0.28 ± 0.6	0.35 ± 0.015	0.47 ± 0.1	0.35 ± 0.03
SO_4^{2-} ($mg\,L^{-1}$)	2.74 ± 0.33	0.94 ± 0.01	0.38 ± 0.09	2.79 ± 0.04
Cations				
Fe^{2+} ($mg\,L^{-1}$)	0.05 ± 0.01	0.07 ± 0.03	0.01 ± 0.06	0.01 ± 0.02
Ca^{2+} ($mg\,L^{-1}$)	2.34 ± 0.09	3.49 ± 0.03	2.26 ± 0.02	2.41 ± 0.01
Na^+ ($mg\,L^{-1}$)	0.92 ± 0.01	0.94 ± 0	0.92 ± 0.01	0.90 ± 0.01
Mg^{2+} ($mg\,L^{-1}$)	0.76 ± 0.02	1.44 ± 0.05	0.98 ± 0.01	0.76 ± 0.01
K^+ ($mg\,L^{-1}$)	0.43 ± 0.01	2.36 ± 0.04	1.59 ± 0.04	0.43 ± 0.01

[a]DOC = dissolved organic carbon; TDN = total dissolved N; TDP = total dissolved P.

Water chemistry including organic carbon

Water chemistry data also suggested that birch leaves decomposed faster than jack pine needles. Concentrations of total suspended and dissolved nutrients and some cations (Ca^{2+}, K^+, Mg^{2+}) at the end of the experiment were higher in enclosures containing birch leaves than in enclosures containing jack pine needles (Table 2). Concentrations of SO_4 in the water of enclosures with jack pine needles were lower than enclosures with birch leaves at the end of the experiment (Table 2), possibly suggesting greater rates of SO_4 reduction there than in enclosures with birch leaves. pH was lower in the treatment enclosures compared to the control enclosures (Table 2) and alkalinity was higher in enclosures containing jack pine needles than in enclosures with birch leaves. There were no changes in dissolved concentrations of Na^+ (0.90–0.94 $mg\,L^{-1}$), Fe^{+2} (0.01–0.07 $mg\ L^{-1}$), and Cl^- (0.28–0.47 $mg\ L^{-1}$) among enclosures over the duration of the experiment (Table 2). There were no differences in DOC concentrations in enclosures with birch leaves compared to those with jack pine needles, however, the average suspended carbon concentration

was 1.9 times higher in enclosures with birch leaves compare to those in enclosures with jack pine needles (Table 2).

Total carbon pools

The amount of inorganic carbon produced (i.e., mineralized) per g carbon in plant tissue was 1.4–3.9 times higher in enclosures with birch leaves than in enclosures with jack pine needles (Figure 2(E)). Flooded birch leaves produced a maximum of 97.5 mg inorganic carbon per g carbon added by the final day of the experiment (Figure 2(E)). Flooded jack pine needles produced a maximum of 26.5 mg inorganic carbon per g carbon added by Day 12. After Day 12, it appears that mineralization ceased because the amount of inorganic carbon produced per g carbon added remained relatively constant between 19.6 and 26.0 mg for the remainder of the experiment. Because enclosures were not properly sealed for a period of approximately 18 days in the middle of the experiment, a portion of the CO_2 and CH_4 produced was lost to the atmosphere. If the amount of leakage was dependent on the rates of inorganic C production, we would have observed greater loss of gaseous C from the enclosure containing birch leaves, which supports other observations of increased decomposition in flooded birch leaves compare to jack pine needles.

At the end of the experiment, the average DOC pools were similar among the treatment enclosures, however average suspended carbon pools were 1.9 times greater in the enclosures with birch leaves (Figure 3(A)). The total carbon pool, including all inorganic and organic forms of carbon, was 1.3 times greater in the enclosures with birch leaves compared to those containing jack pine needles. Once carbon pools were standardized to the amount of carbon added as plant tissue, the enclosures with birch leaves produced 269 mg per g C added as plants, which was 2.4 times greater than the total carbon produced per g C added as plants in the enclosures with jack pine needles (110 mg per g C added; Figure 3(B)).

Mercury

Mercury in plants

MeHg concentrations in fresh birch leaves ($0.36\ ng\ g^{-1}$) were 3.6 times higher than in jack pine needles ($0.10\ ng\ g^{-1}$). THg concentrations in fresh birch leaves ($9.31\ ng\ g^{-1}$) were 1.3 times lower than in jack pine needles ($12.26\ ng\ g^{-1}$; Table 3). At the end of the experiment, average MeHg and THg concentrations in birch leaves ($0.69 \pm 0.28\ ng\ MeHg\ g^{-1}$, $45.28 \pm 9.86\ ng\ THg\ g^{-1}$) were ~ 2 fold higher than jack pine needles ($0.32 \pm 0.15\ ng\ MeHg\ g^{-1}$, $19.01 \pm 3.47\ ng\ THg\ g^{-1}$; Table 3).

The percent THg that was MeHg (%MeHg) in fresh plant tissues was initially much higher in birch leaves (3.87%) than in jack pine needles (0.82%). %MeHg in birch leaves decreased over the course of the experiment to $1.45 \pm 0.29\%$ while %MeHg in jack pine needles increased to $1.70 \pm 0.23\%$ (Table 3). At the end of the experiment, there was no difference in %MeHg between tissue types (Table 3).

The jack pine needles (final modeled mass = 82.5 and 118.6 g d.w.) lost $31.7 \pm 12.2\%$ of their original mass, whereas the birch leaves (final modeled

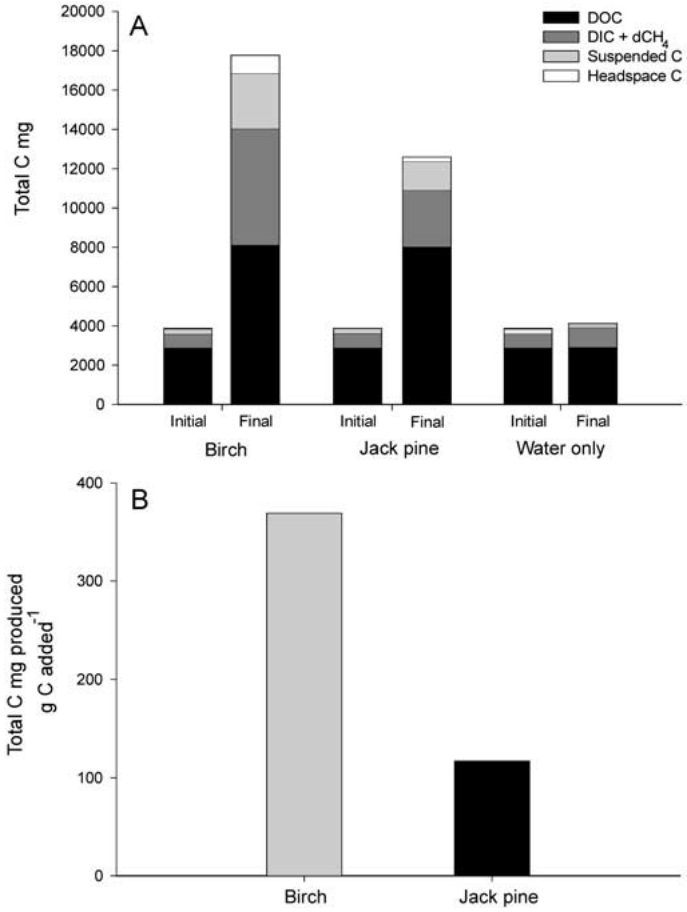

Figure 3. (A) Initial and final average total carbon pools in enclosures including DOC, DIC, dissolved methane (dCH_4), suspended carbon, and carbon in enclosure headspace. (B) Total carbon produced (total carbon pools minus carbon pools in control enclosures) at the end of the 53-day experiment, standardized to the amount of carbon added in plant tissue.

Table 3. Concentrations of methylmercury (MeHg) and total mercury (THg), and percent THg that was MeHg in plant tissues[a].

	Birch leaves			Jack pine needles		
	THg ($ng\,g^{-1}$ d.w.)	MeHg ($ng\,g^{-1}$ d.w.)	%MeHg	THg ($ng\,g^{-1}$ d.w.)	MeHg ($ng\,g^{-1}$ d.w.)	%MeHg
Initial	9.31	0.36	3.87	12.26	0.10	0.82
Final	45.28 ± 9.86	0.69 ± 0.28	1.45 ± 0.29	19.01 ± 3.47	0.32 ± 0.15	1.70 ± 0.23
	(55.13, 35.42)	(0.96, 0.41)	(1.74, 1.16)	(22.49, 15.54)	(0.33, 0.30)	(1.47, 1.93)

[a]Values for individual enclosures are presented in parentheses.

mass = 18.8 and 34.2 g d.w.) lost 74.2 ± 7.5% of their mass by the end of the 53-day experiment. But the calculation of plant mass at the end of the experiment was only an estimate. However, decomposition as indicated by the dissolved inorganic carbon concentrations was 1.4–3.9 times faster in flooded birch leaves than in flooded jack pine needles. This is similar to differences in final mass of plant tissues modeled using THg concentrations, which show that birch leaves decomposed approximately 2.4 times faster than jack pine needles.

The modeled final mass of MeHg in jack pine needles (31.4 ± 4.2 ng MeHg) was ∼2 times greater than the modeled final MeHg mass of birch leaves (16.1 ± 2.0 ng MeHg) (Table 4).

Mercury in water

Average concentrations of MeHg in water were significantly higher in enclosures with jack pine needles (0.42 ± 0.06 ng L^{-1}) than in enclosures with birch leaves (0.20 ± 0.01 ng L^{-1}) at the end of the experiment (ANOVA; $p = 0.004$, $F = 57.90$; Tukey's test $p = 0.021$) (Figure 4(A)). Average concentrations of MeHg in water in enclosures with plant tissues were significantly higher than concentrations in enclosures with only water by Day 29 (ANOVA; $p = 0.002$, $F = 88.51$) and remained so until the end of the experiment.

Average THg concentrations in water decreased slightly at the beginning of the experiment; however, by the end of the experiment, THg concentrations were not significantly different from initial concentrations (ANOVA; $p = 0.515$, $F = 0.84$) (Figure 4(B)). In all enclosures with plant tissues, the %MeHg in water was greater at the end of the experiment compared to the beginning (Figure 4(C)). The %MeHg in water flooding jack pine needles (23.4 ± 3.6%) was 1.7 times higher than in water flooding birch leaves (13.5 ± 2.5%) at the end of the experiment, and 3.3 times higher than in enclosures containing only water (7.1 ± 1.0%).

At the end of the experiment the mass of MeHg in the water in enclosures with jack pine needles (175.0 ± 25.8 ng) was ∼2 times greater than in the water of enclosures with birch leaves (81.2 ± 4.50 ng) (Table 4). There was a 5-fold increase in MeHg mass (42.8 ± 0.39 ng) in enclosures with only water over the duration of the experiment.

Net increases in methylmercury mass

The mass of MeHg in water was similar among enclosures at the beginning of the experiment (∼8 ng; Table 4). However, there was more MeHg added to enclosures in birch leaves (36.8 ± 0.1 ng) than in jack pine needles (14.9 ± 0.1 ng) (Table 4). As a result, the initial total MeHg mass in the enclosures with birch leaves (45.1 ± 0.1 ng) was ∼2 times greater than in the enclosures with jack pine needles (23.1 ± 0.1 ng) (Figure 5(A)). At the end of the experiment, total mass of MeHg in water and plant tissues was 206.4 ± 21.6 ng and 97.3 ± 2.5 ng for enclosures with jack pine needles and birch leaves, respectively (Figure 5(A)).

The average *total net increase* of MeHg mass in the jack pine needles treatment (148.6 ± 21.7 ng) exceeded that in the birch leaves treatment (17.6 ± 2.63 ng) by over 8-fold, despite greater initial MeHg mass in the birch treatment (Table 4) and higher rates of decomposition. The average ng MeHg produced per g carbon added

Table 4. Methylmercury (MeHg) mass in unfiltered water and plant tissue at the beginning and end of the experiment[a].

	Initial MeHg (ng)			Final MeHg (ng)			Net MeHg increase[c]
	Plants	Water	Total	Plants[b]	Water	Total	
Birch	36.8 ± 0.1	8.20 ± 0.4	45.1 ± 0.1	16.1 ± 2.0	81.2 ± 4.5	97.3 ± 2.5	17.6 ± 2.6
	(37.0, 36.7)	(8.1, 8.2)	(45.1, 45.0)	(18.1, 14.0)	(76.7, 85.7)	(94.8, 99.8)	(15.0, 20.1)
Jack pine	14.9 ± 0.1	8.2 ± 0	23.1 ± 0.1	31.4 ± 4.2	175.0 ± 25.8	206 ± 21.2	148.6 ± 21.7
	(14.7, 15.0)	(8.2, 8.2)	(23.0, 23.2)	(27.2, 35.6)	(201, 149)	(228, 185)	(170.3, 126.9)
Control	0	8.2 ± 0.1	8.2 ± 0.1	0	42.8 ± 0.40	42.8 ± 0.4	
		(8.32, 8.1)	(8.3, 8.1)		(42.5, 43.2)	(42.5, 43.2)	

[a]Values for individual enclosures are presented in parentheses.

[b]Final plant MeHg mass values were calculated from modeled final plant weights (see text).

[c](Final MeHg mass in water and plants – initial MeHg mass in water and plants) – (average increase in MeHg mass in water in control enclosures).

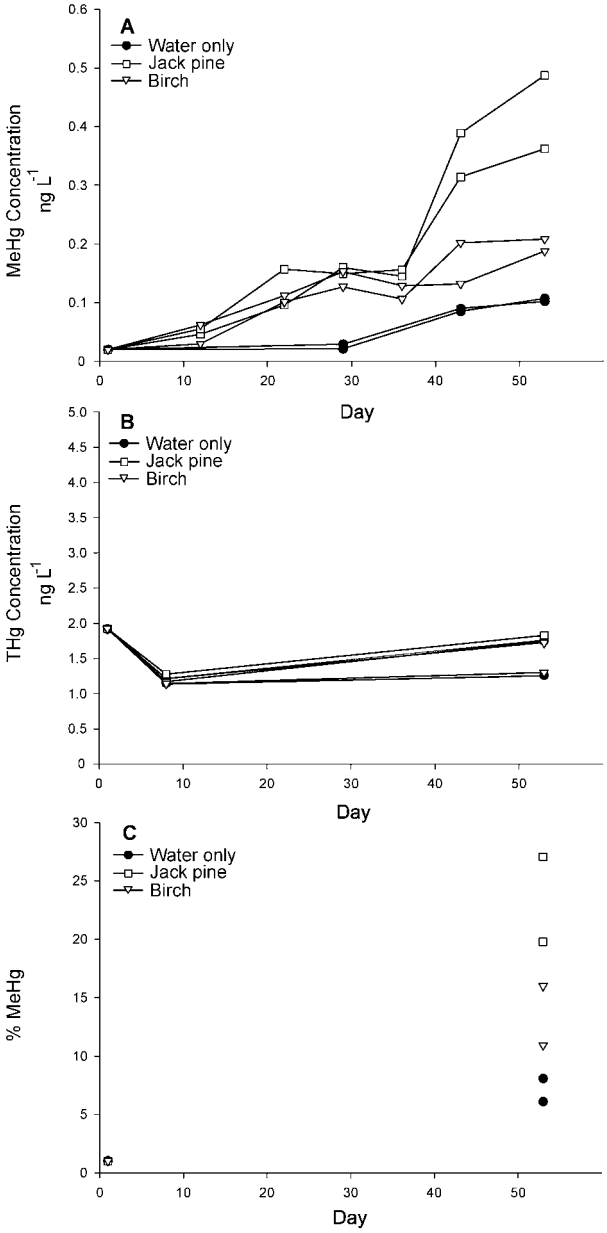

Figure 4. (A) Methylmercury (MeHg) concentrations in unfiltered water from enclosures containing jack pine needles, birch leaves, and water. (B) Total mercury (THg) concentrations in unfiltered water from enclosures containing jack pine needles, birch leaves, and water. (C) The percent of THg that was MeHg (%MeHg) in unfiltered water.

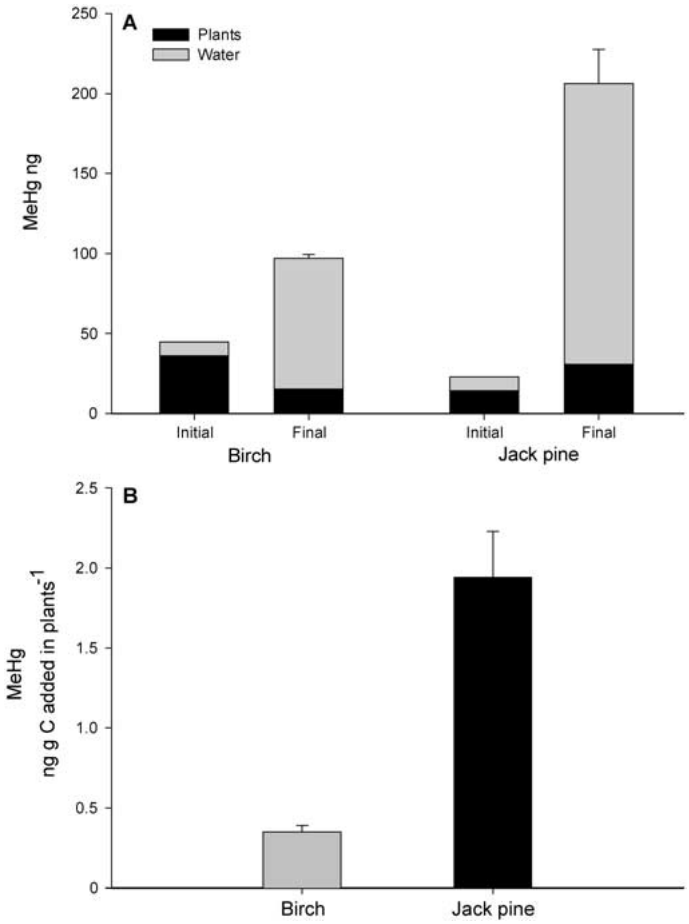

Figure 5. (A) Total initial and final methylmercury (MeHg) mass in unfiltered water (including MeHg mass in water in control enclosures) and plants in treatment enclosures (also see Table 3). (B) Net mass of MeHg produced per mass of carbon added as either jack pine needles or birch leaves. Net MeHg production was calculated dividing net MeHg increases (Table 3) by the mass of C in dried plants at the beginning of the experiment (Table 1). MeHg production per g C added was calculated for each enclosure and then averaged.

in plant tissues, as calculated by dividing the net MeHg increase in each enclosure by the amount of C added in dried plants, was almost five times higher in enclosures containing jack pine needles (1.94 ± 0.28 ng) compared to enclosures containing birch leaves (0.35 ± 0.05 ng) (Figure 5(B)).

Discussion

This study addressed two hypotheses: (1) there will be production of new MeHg associated with the decomposition of flooded plant tissues, and (2) rates of MeHg

production will be directly proportional to rates of decomposition. The increases in MeHg mass clearly show that net methylation was stimulated by flooding jack pine needles and birch leaves. However, there was eight times less MeHg production associated with flooded birch leaves compared to flooded jack pine needles, despite birch leaves decomposing $\sim$2.4 times faster than jack pine needles. While our data support our first hypothesis that there was production of new MeHg associated with the decomposition of flooded plant tissues (as opposed to just leaching of MeHg already present in tissues), the data did not support our second hypothesis that rates of Hg methylation are directly proportional to rates of organic carbon mineralization.

Decomposition

Decomposition of flooded birch leaves was $\sim$2.4 times greater than flooded jack pine needles as indicated by modeled mass loss of tissues, concentrations of DIC, DOC, and suspended carbon, as well as concentrations of other products of decomposition such as suspended and dissolved nutrients. The large increases in DIC and low pH in the water column suggest that the majority of inorganic C in the water column existed as dissolved CO_2 (Hutchinson 1957), however, there was also a likely increase in the amount of organic acids released from the plant tissues during decomposition. Increases in alkalinity in water containing birch leaves and jack pine needles were likely due to the release of positively charged species (NH_4^+ and base cations) from plant tissue during decomposition, coupled with the decrease in anions due to increased rates of SO_4 reduction (Dillon et al. 1997). Studies in streams in Spain (López et al. 2001) and in the Swedish boreal ecoregion (Haapala et al. 2001) also found that birch (*Betula* sp.) leaves decomposed more rapidly than other plant tissues such as Monterey pine (*Pinus radiata*), *Eucalyptus nitens*, ray grass (*Lolium perenne*), and willow (*Salix* sp.) leaves when placed in water.

Unfortunately, we did not measure dissolved oxygen or redox potential in our enclosures, however, concentrations of dissolved oxygen would most likely have been negatively correlated with decomposition and therefore lower in the enclosures with birch leaves. The general absence of CH_4 in the water and headspace of our enclosures also indicates that large zones of anoxia did not form and that decomposition in the water column was aerobic. O_2 was required for aerobic decomposition, which indicates that there was a substantial leak of O_2 into the enclosures. However, we cannot estimate the O_2 in the water column because the enclosures were re-sealed and we cannot be certain that the enclosure headspace was at equilibrium with the atmosphere.

Methylmercury production versus leaching

The amount of MeHg added to the enclosures as birch leaves was almost double that added to enclosures as jack pine needles, yet at the end of the experiment, the

mass of MeHg in water flooding jack pine needles was 3.4 times higher than MeHg mass in water flooding birch leaves. When the net measured production of MeHg was standardized to the amount of carbon added in plants, the amount of MeHg produced per g carbon added in the jack pine treatments (1.9 ± 0.3 ng) exceeded that in birch treatments (0.4 ± 0.1 ng) by five times over the course of the experiment. An increase in the %MeHg often indicates increased net methylation rates within aquatic systems (Kelly et al. 1995; Rudd 1995). The %MeHg in all of our enclosures increased by the end of the experiment, but the average %MeHg in water containing jack pine needles was almost double that in the water containing birch leaves. If leaching had been the primary source of MeHg to water in our enclosures, the mass of MeHg in the water in the treatment enclosures would not have exceeded the initial mass of MeHg in the enclosures.

Why did we observe more methylation associated with the decomposition of jack pine needles than with the decomposition of birch leaves?

Our experimental results support the hypothesis that there was production of new MeHg as a result of the decomposition of flooded plant tissues as opposed to leaching from decomposing flooded organic matter. The results of our study are consistent with other studies that examined MeHg increases in coniferous needles compared to deciduous leaves and grasses. Studies in the South Indian Lake reservoir in northern Manitoba demonstrated that when spruce (*Picea mariana*) boughs were added to enclosures containing perch, THg concentrations in these perch were greater than in those held in enclosures to which prairie sod and moss-peat were added (Hecky et al. 1991). Black spruce needles sampled from litterbags placed in an experimentally flooded wetland at the ELA exhibited an increase of 800% of original MeHg mass, compared to increases of 630% of original mass in *Sphagnum fuscum* moss and 50% of original mass in sedge grass (*Carex rostrata*) stalks (Heyes et al. 1998). Our results do not support our hypothesis of higher production of MeHg in the more easily decomposable birch leaves relative to jack pine needles.

We propose three possible explanations as to why there was greater production of MeHg associated with flooded jack pine needles compared to flooded birch leaves: (1) the bioavailability of HgII (i.e., the ability of HgII to pass through microbial cell walls into methylating organisms) differed among treatments; (2) environmental factors and biogeochemical processes affecting methylation may have differed among enclosures, and/or (3) rates of demethylation differed among treatments.

Bioavailability of HgII

Differences in MeHg production associated with decomposing plant tissues may be attributable to differing ability of HgII to enter methylating organisms. This *bioavailability* could be affected by differences in concentrations of DOC, which can bind to HgII resulting in Hg complexes that are, because of either ionic nature or size, unable to cross cell membranes (Benoit et al. 2001; Haitzer et al. 2002).

Barkay et al. (1997) measured the bioavailability of HgII using a genetically altered bacterium that produced light when HgII crossed the cell membrane (Selifonova et al. 1993). They found a negative relationship between the amount of DOC present and HgII bioavailability. In our study, at the end of the experiment there was 1.5 times more DOC in enclosures with birch leaves than in enclosures with jack pine needles. This possibly resulted in more inhibition of methylation in the birch leaves compared to jack pine needle treatments. The type and quality of DOC (not measured in our study) may also have been an important factor.

Environmental factors affecting methylation

In our study, higher net MeHg production in the enclosures containing jack pine needles could indicate that environmental factors favored methylation there over enclosures with flooded birch leaves. Stimulation of MeHg production has been found in environments with low pH (Winfrey et al. 1990; Gilmour et al. 1991), increased temperature (Bodaly et al. 1993), decreased Se concentrations (Rudd et al. 1983; Turner et al. 1983; Fjeld et al. 1993), low redox potential (i.e., anoxia) (Compeau and Bartha 1984; Björnberg et al. 1988), and increased SO_4 concentrations (Compeau and Bartha 1985; Gilmour et al. 1992; Branfireun et al. 1999).

The pH in water in both treatment enclosures was lower than in enclosures containing only water (Table 2), indicative of decomposition of flooded plant tissue (Schlesinger 1997). However, differences in average pH between treatments with birch leaves (5.7 ± 0.06) and jack pine needles (5.85 ± 0) were negligible. There were also no differences in water temperatures among enclosures (Figure 2). This indicated that neither pH nor water temperature were important factors in differing methylation rates. Concentrations of Se were not measured in our enclosures, however other studies have shown that Se concentrations are generally extremely low at the ELA (V. Palace and R.A. Bodaly, unpublished data), and therefore unlikely to suppress methylation.

Laboratory studies have shown that methylating SRB perform best in low redox environments (Compeau et al. 1984; Björnberg et al. 1988; Regnell and Tunlid 1991; Pak and Bartha 1998), and rates of methylation measured in vessels containing flooded organic matter were higher under anaerobic conditions than aerobic conditions (Porvari et al. 1995). Despite the aerobic water column, anoxia could have occurred in micro-zones surrounding plant tissues while the enclosures sat undisturbed between sampling periods. Jack pine needles did not break into small pieces and float in the water column as the birch leaves did, and therefore anaerobic micro-zones were more likely to occur among jack pine needles sitting on the bottom of the enclosures, than in pieces of birch leaves floating in the aerobic water column.

Methylation of HgII has been shown to be stimulated by inputs of SO_4 to boreal peatlands (Branfireun et al. 1999), and freshwater (Gilmour et al. 1992) and estuarine (Compeau et al. 1985) sediments, as might be expected if SRB are the environmentally relevant methylating bacteria. At the end of our experiment, concentrations of SO_4 in the water of enclosures with jack pine needles were lower than enclosures with birch leaves. Alkalinity in water containing jack pine needles was much higher than in water containing birch leaves. Both of these findings

suggest more active SO_4 reduction, and hence HgII methylation, in enclosures with jack pine needles than in enclosures with birch leaves, supporting our hypothesis that anaerobic micro-zones may have formed around the jack pine needles.

Demethylation
Rates of methylation and demethylation cannot be measured directly without adding tracer mercury species (Ramlal et al. 1986; Hintlemann et al. 2000), and as a result, we are only able to determine *net* production of MeHg using a mass budget approach in the enclosures. Differences in the measured net amount of MeHg produced among treatments could be attributed to differences in demethylation rates. For example, if the organic carbon added to the birch enclosures stimulated rates of HgII methylation, but also enhanced rates of demethylation, we would expect to see less *net* methylation in the flooded birch leaves compared to flooded jack pine needles. It is possible that rates of both methylation and demethylation were increased in the enclosures containing birch leaves, resulting in lower *net* methylation in the enclosures containing birch leaves compared to those with jack pine needles where possibly only HgII methylation was stimulated.

Summary

The addition of fresh jack pine needles and birch leaves to our enclosures stimulated the production of new MeHg. The results of our experiment corroborate laboratory, microcosm, and field studies examining increases in MeHg concentrations in water inundating plant tissue (Hecky et al. 1991; Porvari et al. 1995; Thérien et al. 1999; Balogh et al. 2002) and support our hypothesis that increased MeHg observed in flooded environments is due to the production of new MeHg and not the leaching of MeHg already present in flooded organic matter. There was no direct link between rates of decomposition and rates of measured net MeHg production. Differences in MeHg production associated with flooded birch leaves and jack pine needles could be due to differences in environmental factors affecting HgII bioavailability (e.g., binding to DOC), SO_4 reduction rates, and/or rates of demethylation. Our study suggests that the amount of organic carbon stored in a reservoir prior to flooding is not a good predictor of the extent of future MeHg increases. Reservoirs created by flooding upland forest that contains relatively less organic carbon stores may result in contamination of reservoir fisheries equal to, or exceeding, reservoirs created over wetland areas with very large organic carbon stores. Our study also suggests that litterfall inputs of jack pine needles into lakes and streams could stimulate Hg methylation more than litterfall inputs of birch leaves during the annual fall senescence.

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