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Potential Hg methylation and MeHg demethylation rates related to the nutrient status of different boreal wetlands

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Abstract Despite methylmercury (MeHg) production in boreal wetlands being a research focus for decades, little is known about factors in control of methylation and demethylation rates and the effect of wetland type. This is the first study reporting potential Hg methylation (k_m) and MeHg demethylation rate constants (k_d) in boreal wetland soils. Seven wetlands situated in northern and southern Sweden were characterized by climatic parameters, nutrient status (e.g. type of vegetation, pH, C/N ratio, specific UV-absorption), iron and sulfur biogeochemistry. Based on nutrient status, the wetlands were divided into three groups; (I) three northern, nutrient poor fens, (II) a nutrient gradient ranging from an ombrotrophic bog to a fen with intermediate nutrient status, and (III) southern, more nutrient rich sites including two mesotrophic wetlands and one alder (*Alnus*) forest swamp. The k_m/k_d ratio in general followed %MeHg in soil and both measures were highest at the fen site with intermediate nutrient status. Northern nutrient poor fens and the ombrotrophic bog showed intermediate values of %MeHg and k_m/k_d. The two mesotrophic wetlands showed the lowest %MeHg and k_m/k_d, whereas the alder swamp had high k_m and k_d, resulting in an intermediate k_m/k_d and %MeHg. Molybdate addition experiments suggest that net MeHg production was mainly caused by the activity of sulfate reducing bacteria. A comparison with other studies, show that k_m and %MeHg in boreal freshwater wetlands in general are higher than in other environments. Our results support previous suggestions that the highest MeHg net production in boreal landscapes is to be found in fens with an intermediate nutrient status.

Keywords Methylmercury · Mercury · Wetlands · Methylation · Demethylation

Introduction

Methyl mercury (MeHg) is an intensively studied compound with toxic, persistent and bioaccumulating properties (Fitzgerald and Clarkson 1991). The main route of MeHg exposure to humans is fish, while transformations of the compound encompass several phases, e.g. soil and water (Selin 2009). Therefore

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our understanding of MeHg net production and cycling in the terrestrial and aquatic environments is of fundamental importance.

A number of studies have reported that boreal wetlands are net sources of MeHg; e.g. large scale field manipulation experiments (St. Louis et al. 2004), input–output budget studies (St. Louis et al. 1996; Selvendiran et al. 2008), small-scale manipulations (Mitchell et al. 2008a) and measurements of inorganic mercury (Hg) and MeHg concentrations in pore water (Branfireun and Roulet 2002; Mitchell et al. 2008b). It has been suggested that "poor-fen" types of boreal wetlands are landscape level "hot-spots" for MeHg net production (St. Louis et al. 1996; Mitchell et al. 2008a), as compared to e.g. ombrotrophic types of wetlands. If the perspective is expanded to include more productive wetlands, studies in the Florida Everglades have shown that methylation rates (and %MeHg in soils) decrease along a nutrient gradient from intermediate productive pristine areas in the south, to nutrient rich areas influenced by agriculture in the north (Gilmour et al. 1998). Also demethylation rates decreased along the same gradient (Marvin-Dipasquale and Oremland 1998). Taken together, these studies may suggest that intermediate or relatively "nutrient-poor" conditions favor a net production of MeHg, maintaining high steady-state concentrations of MeHg (as reflected by %MeHg) in wetland soils.

Factors in control of the observed pattern of MeHg net production variability among different types of wetlands are largely unknown. In boreal wetlands, the physiological effect of low pH on bacteria, the availability of sulfate and quality of organic matter as electron acceptors and donors, respectively, for sulfate reducing bacteria (SRB) have been emphasized (Barkay et al. 1997; St. Louis et al. 2004; Mitchell et al. 2008a, b). In more eutrophic environments, such as the Florida Everglades, sulfate is not limiting and the availability of Hg for methylating bacteria (Benoit et al. 1999) and negative effects of sulfide (covarying with sulfate and other nutrients) on bacterial activity (Gilmour et al. 1998) seem to be more important for MeHg net production. In salt marshes, availability of low molecular mass organics exuded by plants (Windham-Myers et al. 2009) and the activity of iron reducing bacteria (FeRB) (Mitchell and Gilmour 2008) have been linked to the net production of MeHg.

Despite much focus on boreal wetlands as sources for MeHg, there is, to our knowledge, no study in which methylation and demethylation rates (and rate constants) have been reported for boreal wetland soils. There are also very few reports on %MeHg in soil from boreal wetlands (Skyllberg et al. 2003). The %MeHg in soil—note the difference from %MeHg in pore water which is highly influenced by the abundance and composition of dissolved ligands for MeHg and Hg complexation (Skyllberg 2008)—has been shown to be a good proxy for in situ net methylation in sediments (Benoit et al. 2003; Drott et al. 2008a). In this study we relate short term (by means of laboratory incubation experiments) and long term (using %MeHg in soil as a proxy) net MeHg production to nutrient status and biogeochemical properties of seven boreal wetlands situated in northern and southern Sweden.

Material and methods

Site descriptions

Seven Swedish, boreal wetlands situated within predominantly forested watersheds were selected for this study. The central object of each site was defined as the central, low-lying area with the groundwater level within 10 cm of the soil surface at high-flow periods (Fig. 1). Below follows a brief description of the sites, divided into three subsets. A more detailed description of the vegetation is given as supplementary material.

Subset (I): northern sites

The watersheds of the three northern wetland sites are closely situated in a very flat area covered by sandy glacial till developed from gneissic bedrock. Soils are dominated by Spodosols and Histosols (Soil Survey Staff 2010). The wetland part of all three sites have a similar type of less nutrient demanding *Sphagnum* and *Carex* dominated vegetation, but they differ in the proportions of forested and open wetlands and area of open water in their central objects (Table 1).

The central object of site Storkälsmyran (SKM) is a 2.0 ha forested, riparian zone wetland developed in connection to a stream which is receiving water from an upstream located semi-open wetland and forested

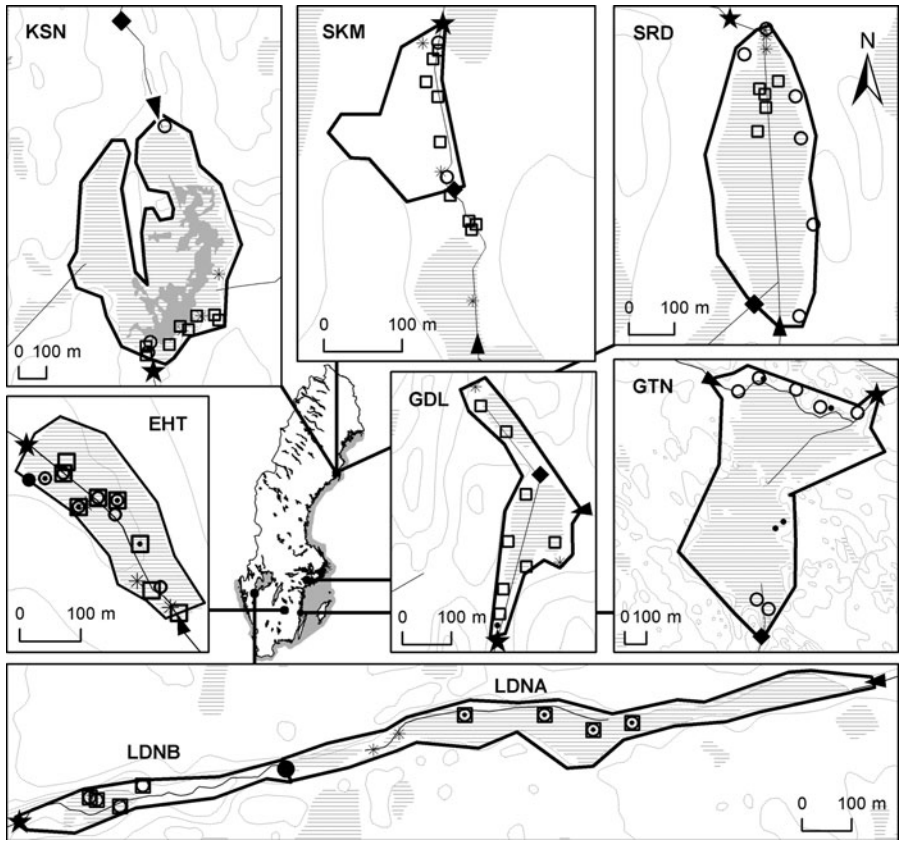

Fig. 1 Map of sites. Stream sampling points are denoted *solid triangle* (indicating flow direction, main inlet), *solid diamond* (inlet), *solid circle* (outlet) and *solid star* (main outlet). Soil samples are denoted *small solid circle* (2006), *open circle* (2007), *open square* (2008) and *eight spoked asterisk* (2009). Central wetland objects are *circled with a solid black line*. Streams are marked by *thin black lines*. *Solid grey* areas denote open water areas and striped areas denote wetland areas. Faint dotted topographic contours illustrate 5 m intervals

Table 1 Site descriptions

Wetland	Coordinates		Catchment area (ha)	Central object area (ha)	Areal % of central object[a]	Average temp (°C)[b]		Temp. sum (°C)[c]	Prec. (mm)[d]	%Snow[e]
	Long	Lat				Jan	July			
SKM	63°56′52″	20°38′48″	47.2	2.0	11, 89, 0	−4.8	15.3	2007	699 ± 38	25 ± 2
SRD	63°57′37″	20°40′24″	120	24	30, 70, 0					
KSN	63°57′8″	20°38′13″	101	34	23, 59, 18					
LDNA	58°19′58″	12°30′46″	90.8	7,7	27, 73, 0	−0.1	17.2	2766	923 ± 91	11 ± 5
LDNB	58°19′46″	12°29′7.5″	108	2,6	72, 28, 0					
EHT	57°34′15″	15°12′5.0″	58.5	4,2	100, 0, 0	−1.7	16.6	2500	714 ± 137	8 ± 4
GTN	57°27′20″	16°34′53″	2271	56	31, 69, 0	−0.2	17.9	2880	669 ± 27	7 ± 1
GDL	58°59′22″	17°12′53″	36.4	4,3	0, 26, 74	−1.0	17.8	2707	626 ± 95	14 ± 4

[a] % of forested wetland, open wetland and open water of the central object

[b] Mean annual air temperature (°C) ± SD during 2006–2009

[c] Mean annual sum ± SD of air temperatures exceeding 5°C in 2006–2009

[d] Mean annual precipitation ± SD in 2007–2009

[e] The annual mass% contribution from snow to the total wet deposition ± SD in 2007–2009

uplands. The stream was sampled on 21 occasions at three locations situated 200 m upstream, and at the inlet and outlet of the central object. Ten soil samples were collected in pockets of peat soil prior to flooding (2007) and in the flooded part of the central object (2008 and 2009).

The central object of site Sjöarödd (SRD) is a 24 ha open wetland with an average peat thickness of 2–3 m, lacking areas of open clear-water. Two inlet streams and the outlet were sampled on 20 occasions. During the same period, 13 soil samples were collected in the northern part and along the eastern part of the wetland. In June 2008 two ditches were dug along the edges of the wetland and 30 cm of peat was excavated from a 2 ha area near the outlet. This caused increased drainage and a doubled concentration of sulfate in the outlet stream.

The central object of site Kroksjön (KSN) is a 34 ha open wetland having 18% of open clear-water. Samples were taken at the outlet and in two inlet streams on 21 occasions. During the same period 14 soil samples were collected along the eastern part of the wetland. In May 2008 a dam was built in the outlet, increasing the height of the water surface by in average 30 cm at the outlet.

Subset (II) and (III): southern sites

Subset (II) comprises a wetland gradient at Långedalen (LDN), a narrow valley formed in highly crystalline diabase bedrock at the table mountain Hunneberg (70 m.a.s.l.). From the bedrock a sandy till has developed. The central object consists of an artificially drained open, ombrotrophic bog (site LDNA) of 7.7 ha (including 1.7 ha of forested edges of the wetland) and a down-stream located mixed bog-fen (site LDNB) of 2.6 ha. A stream flows in the middle of the valley and it was sampled at the inlet and in the outlets of LDNA and LDNB on 16 occasions. In May 2009 two dams were built; one upstream the open bog and one downstream the bog, creating new flooded areas upstream and in the lower end of LDNA after spring snow-melt and autumn rains. In total 14 soils samples were taken at LDNA and 9 at LDNB.

Subset (III) consists of three wetlands; EHT, GTN and GDL. The central object of site Edshult (EHT) is 4.2 ha covered by a productive *Alnus glutinosa* swamp. It receives runoff from a 8.4 ha upstream located drained bog (sparsely covered by >100 years-old *Pinus silvestris* trees) and 37 ha of drained peaty soil (Histosols and Humic Spodosols) covered by 40–50 years-old *Picea abies* plantations and ~3 ha of a 4-years-old clear-cut. As a consequence, highly acidic and natural organic matter (NOM) rich water enters the *Alnus* swamp. The inlet and the two outlets were sampled on 17 occasions and 21 soils samples were taken in the central object.

The central object of site Gästern (GTN) is a formerly eutrophic lake that after several drainage operations, the last in the 1920s, has changed into a mesotrophic wetland with no open water. In October 2006 the lake was restored by damming the outlet, thereby increasing the water level. The watershed is by far the largest of our sites (27 km^2). After the dam was built in 2006, the water level was kept high enough to create open water corresponding to more than half of the total area of the former lake most of the season. The inlet to the central object and the outlet (at the dam) was sampled on 19 occasions and 11 soils samples were taken.

The central object of site Grundsdal (GDL) is an artificial wetland, created in spring 2007 by flooding 4.3 ha of a former pasture that after abandonment was not possible to reforest because of clayey-silty soils and frosty conditions. GDL is dominated by open water. The two inlets and the outlet were sampled on 17 occasions and 11 soil samples were taken.

Sampling and sample preparation

Soils were sampled in the central wetland objects, in some cases concentrated to an area close to the outlet (Fig. 1). A 0–10 cm soil core with a diameter of 10.5 cm was taken by a steel peat corer with a cutting edge. The zero level of the core was defined by the ground water table at the sampling occasion, maximizing the link between biogeochemistry in soil samples and wetland runoff. In order to minimize effects due to varying water levels by season and restoration actions (damming or drainage), sample points were adjusted to locations where the water table was within centimeters of the soil surface. Soil samples were collected in November 2006, September 2007 and 2008 and May 2009. Bulky roots, sticks and largely fresh plant materials were removed from the surface layer of the sample. During 2006 and 2007, the soil core was immediately put in a 1 L plastic bucket. The bucket was filled to the top with soil (leaving no headspace), sealed with a rubber lid and insulating tape to minimize oxidation of the soil. In 2008 and 2009, double plastic zip-lock bags were used and the air was pressed out before sealing the

bags. All samples were kept dark and cool on ice during transport and until sample preparation.

Soil samples were processed as soon as possible, in order to avoid changes during storage. During large sampling campaigns, the maximum storage time (in darkness at 4°C) was 2 weeks. All subsequent handling of soil samples was done in a glove-box filled with N_2 (g). The top 2 cm of soil was removed from the sample container in case it had been oxidized. Dissolved H_2S and pH were measured using a H_2S microsensor (Unisense, Denmark) and a Mettler Toledo InLab 412 electrode coupled to a MA 120 ion meter (Mettler Toledo, USA), at 3–5 different positions and depths in the sample container. After homogenization, approximately 50 g was withdrawn for analysis of total concentrations of N, C, S as well as Hg solid phase speciation analysis and incubation studies (see below). For extraction of pore water, subsamples were transferred to 100 ml centrifugation tubes, tightly sealed by tape and centrifuged (Beckman Coulter, USA) for 30 min at 7000 rpm. In order to facilitate subsequent filtration, the centrifugation was repeated with the supernatant in 50 ml centrifuge tubes. The supernatant was collected in 20 ml PVC-free, sterile plastic syringes (Beckton Dickinson, Spain) and filtered through 0.45 µm non-pyrogenic, sterile filters (Sarstedt, Germany) in the glove-box. The filtered pore water was divided into subsamples analyzed for total Hg, MeHg, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), specific UV-absorbance (SUVA) at 254 nm, Fe(II)/(III), major anions and metal cations. Pore water subsamples used for total Hg and metal cation analyses were acidified by addition of 1 M HCl (aq.). Subsamples to be analyzed for Fe(II)/Fe(III) were stored in darkness in the glove-box until analysis, to reduce the risk of oxidation.

Stream water samples were collected at the main in- and outlets of the wetland sites in 125 or 150 ml acid-washed Teflon bottles (Hg and MeHg) and 250 ml HDPE-bottles (ancillary chemistry). Bottles were rinsed three times with stream water before they were filled to the top and tightly sealed, thereby limiting oxidation. All water samples were kept cool and dark until sample preparation. Stream water samples from the southern sites were transported by regular post-service, causing a lag of 2–3 days between sampling and sample preparation. pH was measured in the laboratory using a Mettler Toledo InLab Science electrode connected to a SevenMulti Ion meter (Mettler Toledo, USA). Samples were filtered in ambient air using 0.45 µm filters and plastic syringes. The filtered water was analyzed for the same parameters as the pore water, except Fe(II)/Fe(III).

Determination of MeHg in water

An aqueous species specific isotopically enriched standard, i.e. $Me^{200}HgCl$ (96.41%), prepared in-house (Snell et al. 2000) from ^{200}HgO (ORNL, Oak Ridge, TN, USA) was added to water and pore water samples immediately after sample preparation. The sample was left to equilibrate for at least 24 h at 4°C in tightly sealed 50 ml centrifuge tubes. MeHg was derivatized using sodium tetraethyl borate and acetate-buffer before purging for 9 min with He (g) at a flow of 100 ml/min and collecting the derivatised MeHg on tenax adsorption tubes (Lambertsson and Bjorn 2004). The preconcentrated sample was analyzed by thermal desorption onto a GC-ICPMS (Agilent 6890N GC, Agilent 7500 ICPMS, Agilent Technologies, USA) (Larsson and Frech 2003). Concentrations of MeHg were determined using isotope dilution analysis. For every batch of samples triplicate blanks were analyzed and blank correction was performed.

Incubation experiments and determination of MeHg in soil

Approximately 10 g of soil was weighted (3 decimal digits) in two 50 ml centrifuge tubes. Aqueous, species specific, isotopically enriched standards of $Me^{204}HgCl$ (98.11%) and $^{201}Hg(NO_3)_2$ (98.11%) corresponding to 10% of the total MeHg and Hg concentrations, were added to each tube. One tube denoted T < 1 h, was immediately put in the freezer. The other tube, denoted T48, was left to incubate in darkness and room temperature (21 ± 1°C) for 48 h, after which also this sample was put in the freezer until extraction and analyses. All steps prior to, as well as the actual incubation, were done in a glove-box filled with N_2 (g). The frozen samples were thawed and extracted with $KBr/CuSO_4/H_2SO_4/CH_2Cl_2$. Isotopically enriched $Me^{200}HgCl$ (96.41%) was added to the samples as internal standard prior to the solid–liquid extraction. Ethylation, preconcentration and

analysis were done in the same way as for MeHg determination in water samples described above. Equations described in Qvarnstrom and Frech (2002) were used to calculate concentrations of ambient MeHg, and final $Me^{201}Hg$ and $Me^{204}Hg$ concentrations. The protocol for incubation experiments and determination of MeHg in soil described above corresponds to the method in Lambertsson et al. (2001) with two exceptions. Due to the heterogeneity of the wetland samples, it was necessary to divide them in two tubes before adding isotopically enriched standards in order to increase the possibility of equal distribution of the standards. In 2007, $\sim$2 g N_2-purged water from the stream draining each wetland was added to the northern wetland samples, to facilitate the mixing of tracer. Amounts of transformed ^{201}Hg were used to calculate potential methylation rates (ng g^{-1} day^{-1}) by Eq. 1:

$$\text{Potential methylation rate } \left(\text{ng g}^{-1}\,\text{day}^{-1}\right) = \left(\left[\text{Me}^{201}\text{Hg}\right]_{\text{T48}} - \left[\text{Me}^{201}\text{Hg}\right]_{\text{T}<1}\right)/2 \quad (1)$$

Using the determined value of $[Me^{201}Hg]_{T<1,}$ or setting $[Me^{201}Hg]_{T<1}$ to its theoretical value of 0, gave similar results. However, in order to remove variability caused by some obviously anomalous $[Me^{201}Hg]_{T<1}$ values, the latter approach was consistently used for all incubations. Potential methylation rate constants (k_m, day^{-1}) were calculated by dividing the potential rate by the mass of added tracer (g g^{-1}). Potential demethylation rates were calculated based on mass of transformed tracer $Me^{204}Hg$ by Eq. 2, and the potential demethylation rate constant (k_d) was calculated by Eq. 3:

$$\text{Potential demethylation rate } \left(\text{ng g}^{-1}\,\text{day}^{-1}\right) = \left(\left[\text{Me}^{204}\text{Hg}\right]_{\text{T}<1} - \left[\text{Me}^{204}\text{Hg}\right]_{\text{T48}}\right)/2 \quad (2)$$

$$k_d\ \left(\text{day}^{-1}\right) = -1 * \ln\left(\left[\text{Me}^{204}\text{Hg}\right]_{\text{tracer}} - \left(\left[\text{Me}^{204}\text{Hg}\right]_{\text{T}<1} - \left[\text{Me}^{204}\text{Hg}\right]_{\text{T48}}\right)\right) - \ln\left(\left[\text{Me}^{204}\text{Hg}\right]_{\text{tracer}}\right) \quad (3)$$

To determine the contribution from the activity of SRB to methylation and demethylation processes, soil samples collected in May 2009 were added molybdate (Mo) during incubation experiments. To avoid causing a general inhibition of bacteria owing to large excess additions (Fleming et al. 2006), two levels of Mo were added corresponding to 100% ("low level") and 500% ("high level") of sulfate concentrations in pore water solution.

Total Hg determinations

The total Hg content in soils was determined by solid combustion atomic absorption spectrometry using a LECO AMA 254 mercury analyzer (Altec, Czech Republic). Instrument blanks were analyzed in triplicates before the analysis of the certified reference material IAEA-433 (International Atomic Energy Agency). Results for the CRM were accepted if they were within 7% of the certified values. Soil samples were analyzed and corrected for blank values and the CRM-result. In stream and pore water total Hg was determined following the US EPA method 1631. In brief, the sample was preserved with concentrated HCl (aq.). Before analysis all Hg was oxidized to Hg(II) using BrCl. A sequential reduction allowed the destruction of halogens. After the final reduction to Hg^0 using $SnCl_2$, the sample was purged and Hg^0 collected on a gold trap. After thermal desorption the Hg^0 was injected to a cold-vapor atomic fluorescence spectrophotometer (CV-AFS) (US EPA 2002).

Spectrophotometric analyses

For all spectrophotometric analyses a 1 cm quartz cell and a UV/VIS 920 spectrophotometer (GBC Scientific Equipment, Australia) were used.

SUVA

Absorbance of aromatic functional groups in water and pore water samples at 254 nm was determined and corrected for the absorbance by total dissolved Fe, in agreement with Weishaar et al. (2003). The reported specific UV-absorbance (SUVA) reflects the aromaticity of natural organic matter.

Fe(II)/Fe(III)

An aliquot of 300 µl of ferrozine reagent was added to 3 ml of filtered pore water sample in the glovebox. After correction of the small absorbance of Fe(III), the absorbance of Fe(II) was measured at 562 nm following the description in Viollier et al. (2000).

Additional chemical analyses in water

Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were analyzed using a Shimadzu TOC-5000 analyser (Schimadzu Company, Japan) and concentrations of NO_3^-, Cl^- and SO_4^{2-} were analyzed using anion-exchange HPLC with conductivity detection (IC-Dionex 4000i, Dionex Corporation, USA). Concentrations of Ca, Fe, Mg, Mn, Na and total S were determined by ICP-OES (Spectro Ciros Vision, Spectro Analytical Instruments, Germany).

Meteorological data

Meteorological data were provided by the Swedish meteorological and hydrological institute (SMHI). This includes data on temperature and precipitation measured at stations within 50 km from the investigated wetland sites. For the northern sites SKM, SRD and KSN, the stations used were Umeå airport and Bredoret D for temperature and precipitation respectively. For the southern sites, stations used for temperature and precipitation data were Vänersborg (LDN), Prästkulla and Värne (EHT), Oskarshamn and Kråkemåla (GTN), and Södertälje and Gnesta (GDL), respectively.

Data analysis

For statistical processing of the data, PASW Statistics 18 (SPSS Inc., USA) was used. All data was checked for normality and homogeneous variation using Shapiro-Wilkinson test and Levene statistics. Non-normally distributed data were log transformed before undergoing statistical tests. One-way ANOVA was used to determine significant differences among sites in the complete data set, followed by pair-wise comparison between sites using post hoc Tukey's test. When data were non-normally distributed with inhomogeneous variation, the non-parametric Welch and Brown-Forsythe's tests were used followed by Games-Howell's test (Zar 1996).

Results

Acidity, nutrient status and electron acceptors

The sites are divided into three groups based on climate/geography, acidity, nutrient status and ancillary chemistry. Below follows a summary of soil and stream biogeochemical parameters, complemented by more details and calculated statistical differences in the supplementary material. Soil and stream data presented are average (pooled) results for all 3 years 2007–2009. Despite some inter-annual variability, the relative differences among sites were in general sustained among years (data not shown).

The three northern sites of subset (I) (SKM, SRD and KSN) are all acidic (pH 4.3–4.8 in soil pore water, Fig. 2a), nutrient poor (low in Ca, Mg, Mn, nitrate, Table A, supplementary material) *Sphagnum-Carex* dominated wetlands differing in the composition of forested/riparian zone, open wetland and clear-water (Table 1). Subset (II) is the LDN site, representing a gradient from an acidic (pH 4.6 in soil pore water), nutrient poor *Sphagnum* bog (LDNA) to a slightly more nutrient rich *Carex* fen (LDNB) with a pH of 5.1 in soil pore water. Subset (III) includes the three more nutrient rich southern sites; a moderately nutrient rich *A. glutinosa* dominated swamp (EHT), a mesotrophic wetland (GTN) and an artificially constructed wetland in a nutrient poor forest landscape (GDL).

SUVA, a well-documented proxy for the aromaticity of DOM (Weishaar et al. 2003), was above 4.0 in all streams with a pH below 5.5 (Table A, Supplementary material). Thus, as expected, acid streams also had high aromaticity of DOM. Streams draining the two most nutrient rich wetlands (GTN and GDL) showed the lowest SUVA, indicative of a higher quality of organic matter as potential electron donors for bacteria at these sites. The relatively high SUVA at the relatively productive EHT is explained by large import of humic rich water from the upstream located bog.

A lower productivity at the northern sites and the bog-end of site LDN (LDNA) is also reflected by the higher C/N ratios at these sites, as compared to C/N ratios at the fen (LDNB) and at sites EHT, GTN and GDL (Fig. 2f). The same trend was evident for concentrations of parameters related to alkalinity (Mg, Ca, DIC), which were higher at EHT, GTN and GDL (Table A, supplementary material).

Concentrations of the potential electron acceptors NO_3^-, Mn, Fe and SO_4^{2-} were in general lower at the less productive sites; SKM, SRD, KSN and LDNA (Fig. 2b, c, Table A, supplementary material). At EHT nitrate concentrations were markedly higher

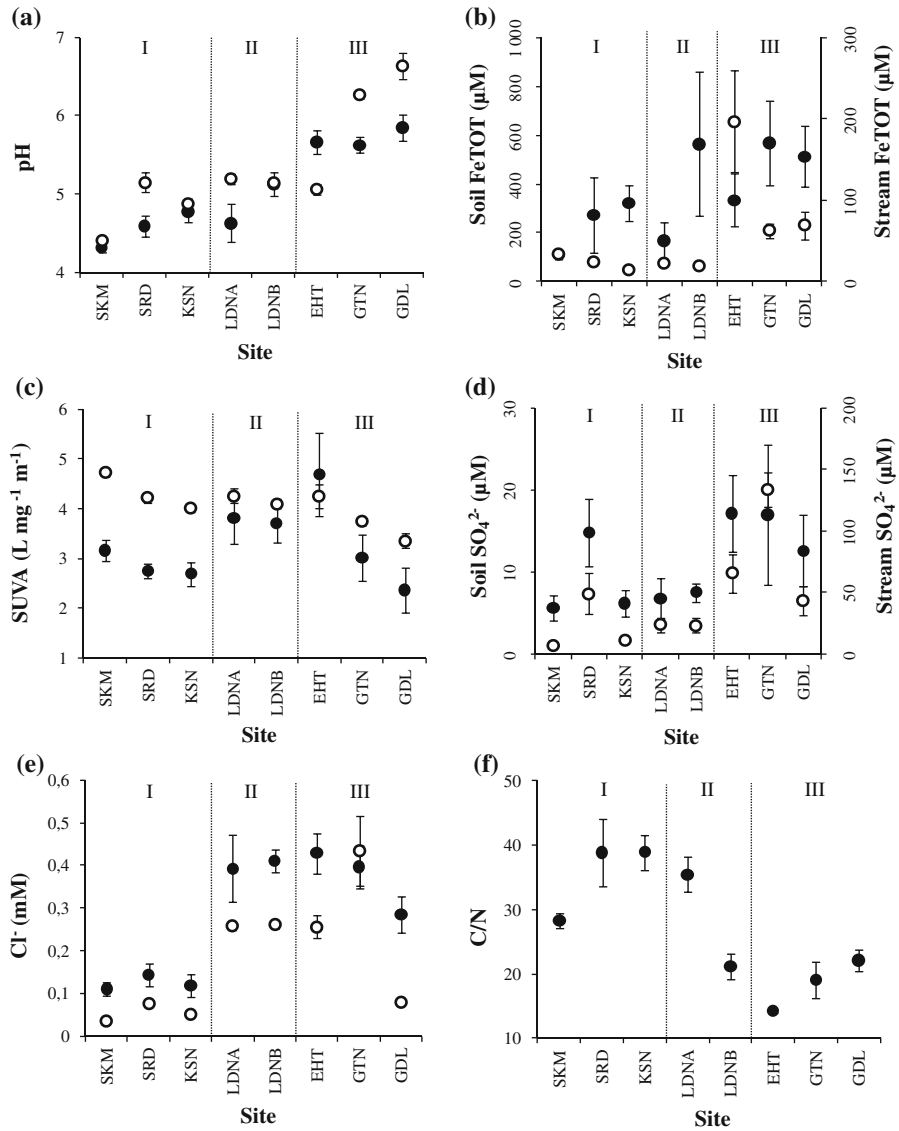

Fig. 2 Mean **a** pH, **b** Fe, **c** SUVA, **d** SO_4^{2-}, **e** Cl^- in soil pore water (*solid symbols*) and stream waters (*open symbols*) and **f** C/N-ratios in wetland soils. Roman numbers designate groups of wetlands; northern nutrient poor sites (I), nutrient gradient LDN (II) and southern nutrient richer sites (III). Groups are separated by *vertical dashed lines*. *Error bars* denote SE for all samples from 2007 to 2009

than at the other sites, which may be explained by the N fixing ability of actinomycetes in symbiosis with *A. glutinosa*. Total Fe and sulfate were highest in the streams draining the most productive sites EHT, GTN and GDL (Fig. 2b, d). The relatively high concentrations and large standard error of SO_4^{2-} at site SRD (Fig. 2d), reflects an oxidation of sulfides and export of sulfate after artificial drainage of the site in 2008. Salinity, represented by Cl, followed a geographical pattern along north–south and west–east gradients, with lower salinity at increasing distance to the Atlantic Ocean (Fig. 2e).

When it comes to the products of Fe and sulfate reduction, a higher concentration of sulfide at GTN and GDL than at the northern riparian wetland SKM was the only significant ($p < 0.05$) difference observed in pore waters (Fig. 3). Inorganic sulfide was detected in most samples, but the concentration [$(S(-II) = [H_2S] + [HS^-]$) only occasionally exceeded 5 µM. During sampling H_2S went off from most soil samples (as recognized by its characteristic smell), indicating that the reported concentrations of sulfides likely were underestimates. In all soil pore waters, Fe(II) was the dominating form comprising 50–80%

of total Fe, indicating suboxic to anoxic conditions in most soils.

Concentrations of MeHg and Hg in soils

The site average, total concentration of Hg in the wetland soils varied from 113 to 287 ng g^{-1} on a dry mass basis (Fig. 4a). Total Hg at site EHT was significantly higher (287 ± 28.3 ng g^{-1} dw) than all other sites except SKM, KSN and LDNA (Games-Howell's test, $p < 0.005$). Concentrations of MeHg varied more among sites (3.5–21 ng MeHg g^{-1} dw, with a mean of 6.7 ng g^{-1} dw) than total Hg (Fig. 4a). There was no significant correlation between MeHg and total Hg among sites. Soil MeHg at LDNB was significantly higher than at all other sites except LDNA and EHT (Games-Howell's test, $p < 0.05$). Total Hg and MeHg expressed in relation to dry mass or organic C showed the same trend among sites as absolute concentrations.

%MeHg, potential methylation and demethylation rates

%MeHg (of total Hg in soil) can be regarded an estimate of a longer term In Situ net production of MeHg, if the total pool of Hg in soil is considered to reflect the available source of Hg for methylating organisms (Drott et al. 2008a). Site LDNB had significantly higher %MeHg (16 ± 3.1%) than all other sites except LDNA (Tukey's test, $p < 0.05$, Fig. 4c) and the mesotrophic wetland GTN showed significantly lower %MeHg (2.3 ± 0.51%) than all other sites (Tukey's test, $p < 0.05$). Thus, LDNB and GTN showed the highest and lowest In Situ net MeHg production, respectively.

The potential methylation rate constant (k_m) is a measure of the short-term MeHg production. The only significant difference among sites was a higher k_m at EHT, as compared to SKM, KSN and LDNA (Tukey's test, $p < 0.01$, Fig. 4b). Because of a substantial variability among samples and sampling occasions, significant differences are difficult to establish using a reasonable amount of samples. Nevertheless, of the northern sites the open wetland SRD had a higher k_m than the forested, riparian stream wetland SKM and the open water wetland KSN. Along the nutrient gradient, the fen-part LDNB had a higher k_m than the upstream bog-part LDNA, even if the difference was not significant ($p = 0.15$). Of the most nutritious wetlands, the two most productive ones; GTN and GDL, showed similar and intermediate k_m. Potential methylation rates (ng g^{-1} dw day^{-1}) and rate constants (k_m, day^{-1}) showed similar trends when comparing sites (Table B, supplementary material).

The potential demethylation rate (Table B, supplementary material) showed a large variation among samples within sites, several of which showing no detected demethylation. It is well-known from previous studies that demethylation rates are very sensitive to small variations in the amount of MeHg tracer added. Enough MeHg is needed to detect demethylation, but too high concentrations may cause toxic effects. In some environments demethylation rates and k_d may even increase with the addition of MeHg (Drott et al. 2008b). Because $Me^{204}Hg$ was added to correspond to 10% of ambient MeHg, absolute concentrations differed substantially among sites. To account for these differences, k_d should be a better basis for comparing sites than absolute demethylation rates. Among sites, a higher k_d at

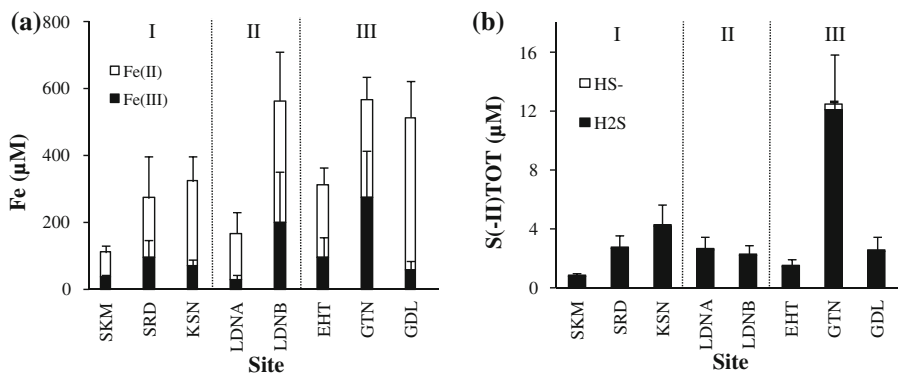

Fig. 3 **a** Total Fe and **b** inorganic sulfide concentrations S(−II) = $[H_2S] + [HS^-]$ in soil pore waters. *Error bars* denote SE for all samples from 2007 to 2009

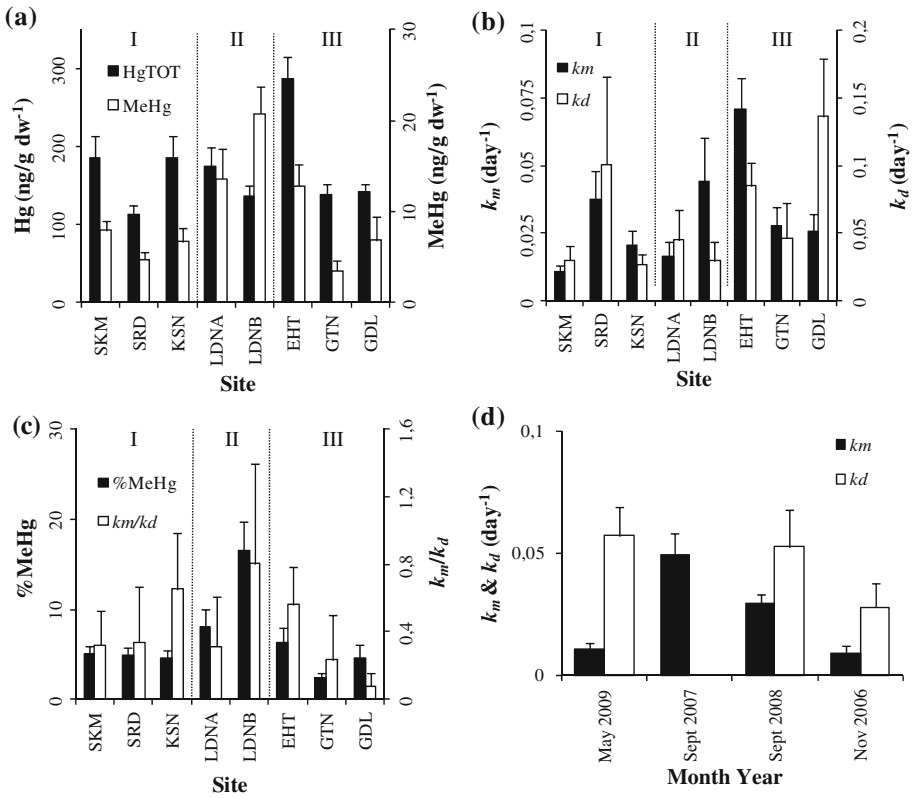

Fig. 4 Concentrations of **a** total Hg and MeHg, **b** potential methylation and demethylation rate constants (k_m and k_d), **c** k_m/k_d and %MeHg in soils. *Error bars* represent SE for all samples from 2007 to 2009, except for data on k_m and k_d for GTN which only include samples taken in 2006. **d** Seasonal and annual variations in mean k_m including all soil samples. In September 2007 demethylation rates were below detection because of too small isotope tracer additions. *Error bars* denote SE for all samples from 2008 to 2009

EHT as compared to SKM and KSN was the only significant difference ($p < 0.05$, Fig 4b).

%MeHg in soils is a net result of production (methylation) and degradation (demethylation), and it may therefore be compared to the quotient, k_m/k_d. Note that due to the effect of different tracer additions and differences in their bioavailabilities absolute values of k_m and k_d, and their quotient, cannot be used for quantitative comparisons. It may, however, be useful to compare trends in %MeHg and in the k_m/k_d quotient among sites (Fig. 4c). Trends in %MeHg and k_m/k_d among sites roughly follow each other, illustrating that the steady-state or longer-term In Situ net MeHg production (%MeHg) is related to the short-term production and degradation (k_m/k_d ratio), as determined in the laboratory.

Values of %MeHg and k_m/k_d were quite similar for all three northern sites (subset (I), Fig. 4c), despite some differences in absolute numbers of k_m and k_d (Fig. 4b). Notably, a high k_m in the open wetland SRD was counteracted by a relatively high k_d. At the bog-fen site LDN, both %MeHg and k_m/k_d were higher in the fen (LDNB) than in the bog (LDNA). The k_m was higher at LDNB whereas k_d was quite similar at both sites, suggesting that the higher %MeHg at LDNB was explained by a significantly higher methylation. At the *Alnus* swamp (EHT), moderate %MeHg in soils can be explained by high k_m values being cancelled out by similarly high k_d values (Fig. 4b–c). Notably, the two most nutrient rich sites, GDL and GTN, had the lowest k_m/k_d ratios and %MeHg of all sites (even if %MeHg at GDL was similar in size as at the three northern sites). At GTN both k_m and k_d were in the mid-range of the sites in this study, whereas site GDL showed the highest demethylation rate constant of all sites.

To investigate the seasonal variation of k_m and k_d data were pooled into sampling occasions, irrespective of site (Fig. 4d). The k_m in September 2007 and 2008 (average 0.05 and 0.03 day^{-1}, respectively) were significantly higher than in May 2009 (0.01 day^{-1}) and November 2006 (0.01 day^{-1}) (Tukey's test, $p < 0.008$). The demethylation rate constant k_d, showed no significant seasonal differences.

Molybdate addition experiments

Because data on concentrations of sulfate were taken from previous years sampling, Mo additions corresponded to 16–200% and 60–870% of the actual sulfate concentrations at low and high additions, respectively. As a consequence of Mo addition, k_m decreased by 14–96% in all soils (Fig. 5a). Due to the limited number and heterogenic character of the samples taken within a site, no significant differences could be established among controls and the two levels of Mo additions. The clear and logical trend however demonstrate that SRB were responsible for the majority of the Hg methylation at most sites. In general, there was no major difference in reduction of k_m between the lower and the higher level of added Mo, showing that SRB activity was almost completely inhibited already at the lowest additions. The smallest effect on k_m (decrease of 22 ± 24% and 14 ± 25% for "low" and "high level" respectively) was observed at the riparian zone wetland SKM, which also had the lowest k_m in absolute terms of all sites. At all other sites, Mo addition decreased the MeHg production by more than 50%. Because of large variations in k_d, a clear effect from Mo additions on k_d was only observed at the northern, nutrient poor sites SKM and KSN (Fig. 5b).

Discussion

Mercury concentrations and seasonal methylation/demethylation rates

The average concentrations of total Hg in the wetland soils of this study are in the range of concentrations reported from other boreal wetland soils, e.g. 65.8–186.6 ng g^{-1} (Liu et al. 2003), 193–260 ng g^{-1} (Warner et al. 2003) and 16–347 ng g^{-1} (Selvendiran et al. 2008). Similar to other studies, the concentration of MeHg varied more among sites than total Hg. Average concentrations of MeHg in this study are in the high end of previous reports from boreal wetlands; 0.16–1.9 ng g^{-1} (Liu et al. 2003), 0.35–0.64 ng g^{-1} (Warner et al. 2003), 0.1–7.8 ng g^{-1} (Selvendiran et al. 2008) and 0.1–13.4 ng g^{-1} (Skyllberg et al. 2003). %MeHg in our wetlands ranged from 2.3 to 17%, which is on a similar level as reported from a forested, riparian zone wetland along a stream in northern Sweden; 0.4–17.2% (Skyllberg et al. 2003). In most other studies %MeHg values have been lower, such as 0.2–1.2% in a Chinese bog (Liu et al. 2003) and 0.01–1.0% in a Canadian high Arctic wetland (Loseto et al. 2004).

The seasonal variation in k_m (Fig. 4d) revealed the highest numbers in September. Plausible explanations are fairly high temperatures and availability of newly deposited above- and belowground litter and dead microbial material, potentially available as electron donors for methylating bacteria. Several studies have shown that organic matter quantity and quality is one of the major factors controlling net MeHg production in soils and sediments (Drott et al. 2007, 2008a; Windham-Myers et al. 2009; Lambertsson and Nilsson 2006). Lower k_m values in spring and late

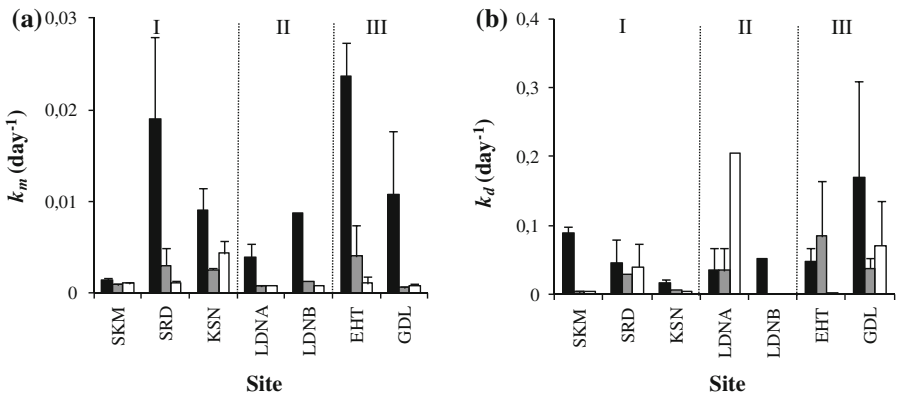

Fig. 5 Potential methylation rate constant k_m (**a**) and demethylation rate constant k_d (**b**) for soil samples with no added Mo (*black bars*), "low level" of added Mo (*grey bars*) and "high level" of added Mo (*white bars*). *Error bars* denote SE for triplicate samples collected in May 2009

fall can be explained by the early succession of bacterial community (May), and by low temperatures and a likely depletion of high quality electron donors (November). Similar seasonal patterns have been found in other studies, e.g. in an eutrophic gradient across the Florida Everglades (Gilmour et al. 1998) and in a boreal forest humic lake sediment (Skyllberg, unpublished data).

Differences in k_m, k_d and %MeHg among boreal freshwater wetlands

Despite differences in the hydrological characteristics of the three northern wetlands (SKM, SRD, KSN), they show a quite similar net formation of MeHg; both in short-term (k_m/k_d) and in longer term (%MeHg) perspectives. Of the southern sites, the bog-end LDNA of site LDN, had quite similar acidity and nutrient status as the northern sites. The k_m/k_d ratio for LDNA is on the lower end of the northern sites, whereas the %MeHg is slightly higher. Thus, it seems that the conditions for methylation and demethylation is quite similar at all low pH nutrient poor sites in this study, independent of their hydrology and geographical location (climate).

The higher %MeHg, k_m and k_m/k_d at the fen site LDNB, as compared to the upstream bog site LDNA, indicate that the fen stimulates a higher MeHg net production, both in the short and long-term. The higher pH and higher nutrient status, as reflected by a more nutrient demanding vegetation and a lower C/N-ratio, together with a slightly lower SUVA, suggest that bacteria at LDNB are provided with more easily degradable organic matter. Similar differences in biodegradability of organic matter related to general nutrient status was observed between a fen and a bog in Juneau, Alaska (Fellman et al. 2008). Thus, a higher availability of electron donors for methylating bacteria is the most apparent factor explaining higher %MeHg, k_m and k_m/k_d at LDNB than LDNA. This observation is in agreement with reports on wetlands dominated by zones of groundwater discharge, e.g. poor fen sites, rather than by zones of groundwater recharge, e.g. bog sites, being "hot-spots" for Hg methylation in boreal wetland areas, on the basis of mass balance calculations (St. Louis et al. 1996) and %MeHg in stream waters (Branfireun and Roulet 2002; Mitchell et al. 2008b).

Of the three more nutrient rich, southern sites, the alder swamp EHT had values of %MeHg and k_m/k_d quite similar to the northern sites. The difference being that both the k_m and k_d were high at EHT (Fig. 4b). The mercury biogeochemistry at EHT is likely influenced by both the productive, lush vegetation in the *A. glutinosa* stand, its litter giving rise to high quality organic matter electron donors, and by the acidic water input from the upstream located bog and drained peatlands. This input explains the high SUVA, Fe and SO_4^{2-} (Fig. 2), high DOC concentrations (in average 350 and 109 mg l^{-1}, pore and stream water respectively, Table A, supplementary material) and the high concentrations of total Hg at EHT (Fig. 4a). As a consequence of this availability of potential electron donors and acceptors, k_m was the highest and k_d among the highest of all sites. Obviously the conditions at this site stimulated both methylation and demethylation reactions.

The two most nutrient rich sites, GTN and GDL, showed intermediate k_m values, but because of high k_d values the k_m/k_d ratio were the lowest of all sites (Fig. 4c). The %MeHg was correspondingly low for GTN, whereas %MeHg at GDL was somewhat larger. Together with the observation from EHT, these observations suggest that demethylation reactions are relatively more expressed at sites with a higher pH, lower C/N ratio and relatively high nutrient status (including Fe and SO_4^{2-}). It can also be noted that four out of the five sites with the highest k_m (SRD, EHT, GTN, GDL) also showed the highest k_d. This indicates that conditions that stimulate methylation reactions also stimulate demethylation reactions. The only exception is the fen site LDNB, at which a high methylation rate was not associated with a high demethylation rate. The overall conclusion is that MeHg net production shows an optimum, not at the poorest and not at the richest sites, but at sites with an intermediate nutrient status.

The addition of Mo during incubation did not reveal any clear differences among the wetlands regarding which group of microorganisms are responsible for methylation of Hg in the wetlands. The experiment did, however, clearly illustrate that most Hg methylation in the wetlands of this study is conducted by SRB. At site SKM, Mo had less effect. This may be explained by the small amount of added Mo in relation to ambient sulfate (16.5 and 72.4% for "low" and "high level", respectively) at SKM. However,

similarly low levels of Mo addition relative to the ambient sulfate concentration (15.7–37.5 "low level") resulted in a decreased k_m of 80–90% at all southern sites. Therefore, it is possible that bacteria not inhibited by Mo, such as FeRB, contributed substantially to the Hg methylation at SKM. This is further supported by the low sulfide concentrations at SKM (Fig. 3) and the influence of potentially Fe(III) rich water transported through the mineral soils along the riparian zone wetland. Inhibition of SRB by Mo could indirectly favour the activity of methanogens, which are known to demethylate MeHg but not methylate Hg^{2+} (Oremland et al. 1991; Pak and Bartha 1998). This may explain the increase in k_d at site LDNA with added Mo, but it would need verification by the addition of BES as inhibitor of methanogens.

Differences among freshwater, estuarine and marine environments

To put this study into a larger perspective and identify environments with high potential to produce MeHg, a qualitative comparison of k_m, k_d and %MeHg with data from the literature is presented in Table C (supplementary material). It should be noted that quantitative comparisons of k_m and k_d is complicated by the fact that measured rates are valid only under certain conditions, e.g. at a specific temperature, incubation time and ambient concentrations of Hg and MeHg (Merritt and Amirbahman 2009).

Despite freshwater wetlands being identified as environments supporting net MeHg production, there are only a handful reports on k_m and k_d data (Table C, supplementary material). In the Florida Everglades, k_m values and %MeHg both decreased with increasing productivity and sulfate concentrations along a gradient from the pristine freshwater wetlands in the southern part to the more productive northern end (Gilmour et al. 1998). This pattern is similar to the high-productivity end of the sites in this study; with higher k_m and %MeHg in the fen LDNB as compared to the more productive sites EHT, GTN and GDL. As in our boreal wetlands, methylation rates in the Everglades were lowest during the cold season (December) and SRB were highly responsible for MeHg production as shown by Mo inhibition (Gilmour et al. 1998). No increase in MeHg production was noted after addition of sulfate, suggesting no limitation of electron acceptors. In the highly productive freshwater wetlands at Yolo Bypass in California, k_m was markedly higher than in our study, and %MeHg was lower. This may indicate a high demethylation rate, which, however, was not determined (Windham-Myers et al. 2009).

A number of studies report k_m and k_d in sediments from marine and estuarine environments (Table C, supplementary material). Higher k_m in freshwater than in brackish water sediments in Sweden (Drott et al. 2007), was linked to a higher availability of easily degradable organic matter (as reflected by a lower C/N ratio) and higher water temperatures in the freshwaters (situated in the same area as the southern wetlands of this study). In general, freshwater wetlands show higher k_m and %MeHg than most salt marshes, estuarine and marine sediments (Fitzgerald et al. 2007; Roulet et al. 2001). The reason for these differences is unclear, one factor could be salinity. It has been demonstrated that methylation rates in sediments generally decrease with increasing salinity under anoxic conditions (Compeau and Bartha 1987), whereas at more oxygenic conditions salinity played less of a role. The decrease in methylation rates with increasing salinity (often positively correlated sulfide concentrations) have been explained by a change in speciation towards negative forms of Hg-chloride species (Barkay et al. 1997) or by a change in the speciation of Hg-sulfide species (Benoit et al. 1999). These explanations are still unsubstantiated, given the very low concentrations of Hg-chlorides in natural environments and the uncertainty about the size of the stability constant for the formation of HgS^0(aq.) (Skyllberg 2008).

pH and build-up of sulfide have also been shown to affect mainly the physiology of bacteria and their uptake of Hg. Low pH stimulates methylation (Kelly et al. 2003; Golding et al. 2008) and high concentrations of sulfide hamper the activity of SRB (Reis et al. 1992; Compeau and Bartha 1987; Gilmour et al. 1992). Some studies also indicate that FeRB, which activity often is higher in freshwater environments, contribute to methylation of Hg (Fleming et al. 2006; Kerin et al. 2006). In other studies, environments with reduction of Fe(III), including experimental addition of Fe(II), have been shown to be associated with lower methylation rates (Warner et al. 2003; Mehrotra et al. 2003; Mehrotra and Sedlak 2005; Windham-Myers et al. 2009).

Reported k_d values are generally lower in freshwater wetlands (Table C, supplementary material).

Similar to observations across sites and with depth in terrestrial and brackish water systems, k_d expresses no clear general trends along salinity gradients (Drott et al. 2008b; Lambertsson and Nilsson 2006). This suggests that variations in methylation rates rather than demethylation rates determine the net formation of MeHg in most environments, as reported in several studies (Drott et al. 2008a; Hammerschmidt and Fitzgerald 2006).

Conclusion

The generally higher net methylation of Hg in freshwater wetlands as compared to salt marshes, estuaries and marine environments may be explained by several factors. In this study, we show that boreal wetlands with low to intermediate nutrient status have the highest net production of MeHg, as reflected by potential methylation and demethylation rates and %MeHg in soil. We suggest that an improved quality of organic matter as electron donors for bacteria and nutrient status in fens, as compared to ombrotrophic bog-type of wetlands favours methylation over demethylation, whereas a further increase in nutrient status and the concurrent change in alkalinity (increased pH) favours demethylation over methylation reactions. Additional research focusing on factors associated with nutrient status as a key factor in Hg cycling would help elucidate the role of wetlands in MeHg production and degradation.

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