Title

The influence of permanently submerged macrophytes on sediment mercury distribution, mobility and methylation potential in a brackish Norwegian fjord

Authors

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Abstract

Macrophytes are shown to affect the microbial activity in different aqueous environments, with an altering of the sediment cycling of mercury (Hg) as a potential effect. Here, we investigated how a meadow with permanently submerged macrophytes in a contaminated brackish fjord in southern Norway influenced the conditions for sulfate reducing microbial activity, the methyl-Hg (MeHg) production and the availability of MeHg. Historically discharged Hg from a chlor-alkali plant (60-80 tons, 1947-1987) was evident through high Hg concentrations (492 mg Tot-Hg kg^{-1}, 269 µg MeHg kg^{-1}) in intermediate sediment depths (10-20 cm) outside of the meadow, with reduced concentrations within the meadow. Natural recovery of the fjord was revealed by lower sediment surface concentrations (1.9-15.5 mg Tot-Hg kg^{-1}, 1.3-3.2 µg MeHg kg^{-1}). Within the meadow, vertical gradients of sediment hydrogen sulfide (H_2S) E_h and pH suggested microbial sulfate reduction in 2-5 cm depths, coinciding with peak values of relative MeHg levels (0.5 % MeHg). We assume that MeHg production rates was stimulated by the supply and availability of organic carbon, microbial activity and a sulfide oxidizing agent (e.g. O_2) within the rhizosphere. Following this, % MeHg in sediment (0-5 cm) within the meadow was approximately 10x higher compared to outside the meadow. Further, enhanced availability of MeHg within the meadow was demonstrated by significantly higher fluxes (p<0.01) from sediment to overlying water (0.1-0.6 ng m^{-2} d^{-1}) compared to sediment without macrophytes (0.02-0.2 ng m^{-2} d^{-1}). Considering the productivity and species richness typical for such habitats, submerged macrophyte meadows

located within legacy Hg contaminated sediment sites may constitute important entry points for MeHg into food webs.

Keywords

Gunneklevfjorden, *Potamogeton crispus*, sulfate, methyl mercury, microbial activity, rhizosphere

1. Introduction

Mercury (Hg) is a global pollutant known to bio accumulate and magnify through food webs, primarily as the methylated, organic and readily bioavailable form methyl mercury (MeHg)(Morel et al., 1998). MeHg is a neurotoxin and has potential harmful effects on humans and animals (WHO, 1991). On a global scale, rivers are estimated as the source of approximately 30 % of Hg to open ocean, of which more than 80 % is estimated to be buried in deltas and estuaries (Amos et al., 2014). Anaerobic estuarine and coastal marine environments are considered important sites for bacterial transformation of inorganic Hg into MeHg (Donaldson et al., 2010, Hollweg et al., 2009, Lehnherr, 2014, Lehnherr et al., 2011). Sulfate reducing bacteria (SRB) are commonly cited as key methylating microbes, particularly in saline and brackish systems where sulfate is abundant (Compeau and Bartha, 1985, King et al., 2000, Merritt and Amirbahman, 2009). Recent research have suggested that the ability to produce MeHg may be more broadly distributed among microbes than previously recognized (Parks et al., 2013, Podar et al., 2015). Microbial MeHg

production may indirectly be controlled by factors affecting microbial community composition in general and SRB activity in specific (King et al., 2001, Ullrich et al., 2001), such as temperature, pH, redox potential (E_h) gradients, sulfur (S) and availability of labile organic matter (Drott et al., 2008, Hollweg et al., 2009, Lehnherr et al., 2012b, Schartup et al., 2013a). Recently, studies have documented that not only the presence but also species composition of submerged macrophytes may determine microbial abundance and affect microbial activity (Cosio et al., 2014, Regier et al., 2012). The importance of vegetated areas for the production of MeHg have been demonstrated in agricultural wetlands, freshwater lakes, rivers and saltmarshes in southern temperate or tropical zones (Bravo et al., 2014, Krabbenhoft et al., 1998, Lehnherr et al., 2012a). However, knowledge is lacking for the influence of macrophytes on sediment MeHg production, flux from sediment and the subsequent potential for bioaccumulation of Hg within heavily contaminated areas. Macrophytes comprise a vast diversity of aquatic organisms in a wide range of littoral ecosystems (Cosio et al., 2014, Noges et al., 2010) and provide important and numerous microhabitats for aquatic organisms, offering shelter, substrate and food. Hence, the MeHg production within submerged macrophyte meadows could be of significant importance for bioavailability of Hg and uptake of MeHg into food webs.

In this study, we wanted to assess the impact of permanently submerged macrophytes on methylation and re-distribution of Hg in the sediments of Gunneklevfjorden, a brackish fjord in southern Norway which has been the recipient

for discharges of Hg from a chlor-alkali plant for 40 years (Skei, 1978a). Mercury-cell chlor-alkali plants have been identified as major sources of Hg releases to the environment (Bravo et al., 2014, Ullrich et al., 2007). Even though the contamination of the fjord has been well known for decades (Skei, 1989), the abundance of MeHg and the role of the permanently submerged macrophytes in the production of MeHg has not previously been investigated. During 2013-2015 several investigations were undertaken to develop a remediation action plan intended to reduce the ecosystem risk from Hg and other contaminants. We hypothesized that the macrophytes create favorable conditions for anaerobic microbial activity with subsequent production of MeHg. To address the hypothesis, we examined spatial variation in total Hg (Tot-Hg) and MeHg concentrations in sediment and pore water, as well as the flux of Hg from sediments to overlying water.

2. Materials and methods

2.1 Study site

Our study site, Gunneklevfjorden, is a 0.7 km^2, shallow (max depth 11 m) fjord located in the temperate zone of Norway (Figure 1), connected to the urban impacted river Skienselva in the north and to the industrialized fjord Frierfjorden in the south. Tidal fluctuations are small (20-30 cm) and water exchange occurs mainly through the narrow and 3.5m deep channel in the north, resulting in typical surface waters salinity of 1-5 PSU whereas bottom waters may range 5-20 PSU (Molvær, 1989, Molvær, 1979). The concentration of Hg and other contaminants in the river is

generally low compared to the water mass of the Gunneklevfjorden and a recent mass-balance has estimated an annual export of 0.5 kg Hg from the Gunneklevfjorden to Skienselva and Frierfjorden (Olsen et al., 2015).

Since early 1900, the Gunneklevfjorden has been severely influenced by anthropogenic activities and discharges from a range of industrial sources (Skei, 1989). Between 1947 and 1987 the fjord suffered from discharges of approximately 60-80 tons of Hg, of which 20-30 tons are estimated to be stored in the sediments within the fjord (Skei, 1978b).

The Gunneklevfjord hosts a permanently submerged meadow of macrophytes (Figure 1) dominated by *Potamogeton crispus* (curly-leaf pondweed), which constitutes an important habitat within the fjord. The meadow is classified to be of national importance due to its size (>0.01 km^2) and the presence of the threatened species *Zannichella palustris* (horned pondweed) (Mjelde, 2015). Maximum depth for growth of macrophytes in Gunneklevfjorden was observed to be 2.5-3.0 m during the investigation period. Root penetration depth is known to vary depending on the macrophyte species and is typically 0-15 cm for the *P. crispus* (Wang, 2013), defining the rhizosphere.

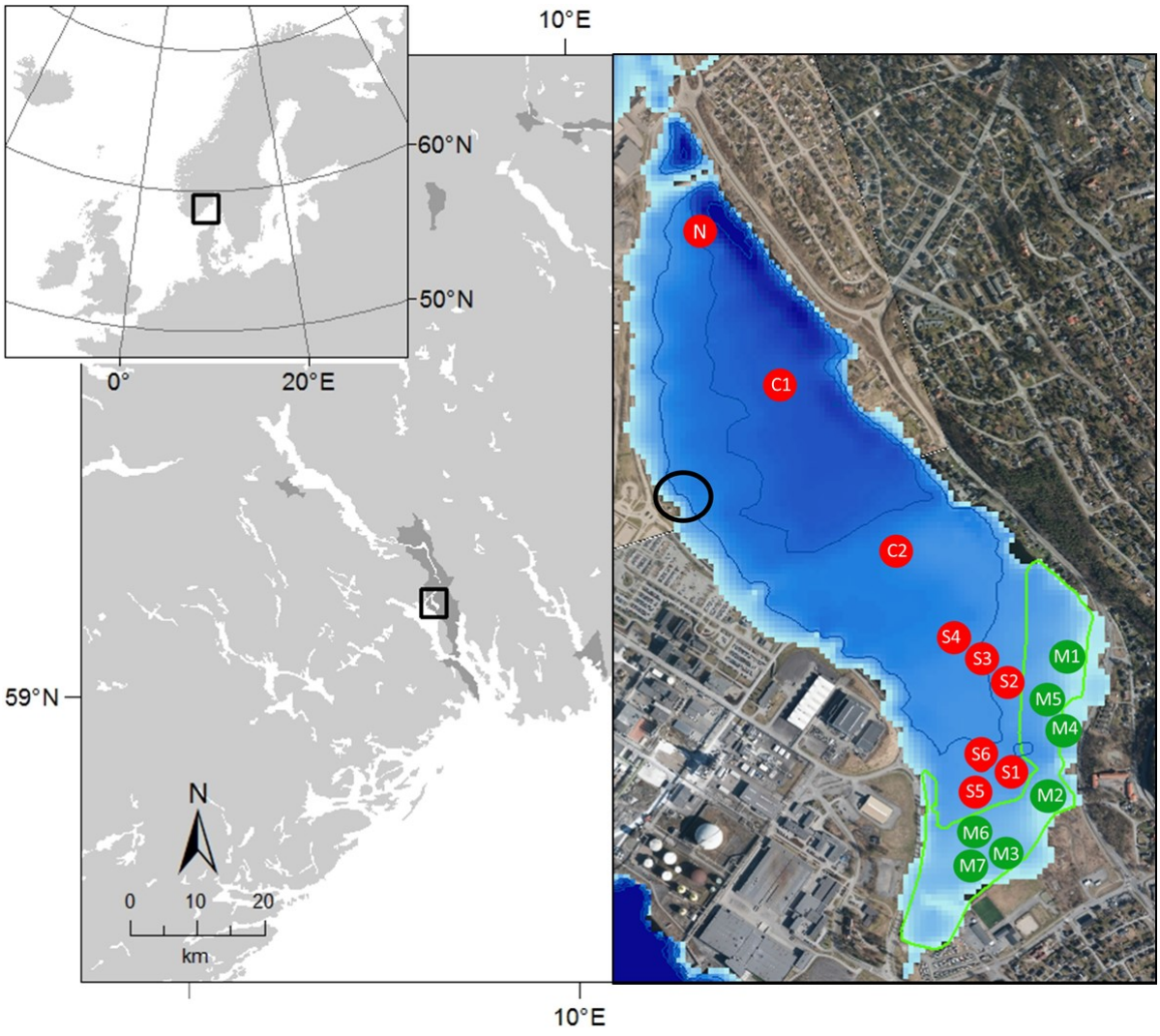

Figure 1. Map of Gunneklevfjorden, Norway, with sampling sites outside the meadow (N, C1, C2, S1- S6) and within the meadow (M1-M7) during 2013-2015. The point of discharge of Hg (1947-1987) is marked with a circle.

2.2 Core and grab sampling of sediments

This paper is based on sediment and pore water samples collected through several independent investigations during 2013 – 2015, making up a total of 321 samples for various analysis (Table 1). Duplicate sediment cores (Core I and Core II) were

collected with a Niemistö core sampler at three sites outside the meadow ("North" (N), "Center1" (C1) and "Center2" (C2)), and one single core was collected within the meadow (site "Meadow1" (M1)) (Figure 1). The cores were cut in 1 cm thick slices down to 5 cm, in 2 cm thick slices from 5 to 15 cm, and then in 5 cm slices for the rest of the core. Core I was used for analyses of grain size (fraction <63 μm) and concentrations of Tot-Hg, MeHg and TOC. Core II (and Core I in M1) was used for direct electrode measurements of pH, E_h and sulfide during the sectioning of the sediment. In addition, Core II was used for ^{210}Pb-dating by DHI Denmark (Eek and Slinde, 2015).

The electrode measurements were done using an electrode assembly with separate sensors for pH (standard glass combination electrode), E_h (Radiometer P101 platinum electrode) and S^2-ions (Radiometer F1212S sulphide ion selective electrode). In field, standard IUPAC buffers of pH 4, 7 and 9 calibrated the pH electrode. Both samples and standards were measured at *in situ* temperatures close to 10˚C.

The concentration of hydrogen sulphide ($[\Sigma H_2S] = [S^{2-}] + [HS^-] + [H_2S]$) was calculated using the measured potential on the sulphide electrode and pH (Boulègue, 1978, Schaanning et al., 1997). The term pS (= $-\log[\Sigma H_2S]$) is preferred to denote H_2S concentrations (mol L^{-1}) calculated from electrode measurements performed directly in untreated sediment samples (Aller, 1978).

Additionally 51 sediment samples for analyses of Tot-Hg and MeHg were collected within and outside the meadow using an Ekman grab (Table 1). All sediment samples were immediately frozen in airtight containers after sampling and stored frozen (-20˚C) until analyses.

Table 1. The total number of sediment samples (n=309) and pore water samples (n=12) collected for analyses of Tot-Hg, MeHg, TOC and electrode measurements (pH, E_h and pS) at different sites in the Gunneklevfjorden during 2013-2015.

	Water depth (m)	**Number of cores/max core depth (cm)**	**Number of core subsamples (slices) for analysis**				**Number of grab subsamples (0-5 cm) for analysis**		**Number of pore water subsamples from grabs**	
Sampling sites			**Tot-Hg**	**MeHg**	**TOC**	**pH, pS, E_h**	**Tot-Hg**	**MeHg**	**Tot-Hg**	**MeHg**
Outside the meadow										
North (N), core	10	2/65	18	18	9	17				
Center1 (C1), core	6	2/65	18	18	9	18				
Center2 (C2), core	5	2/55	17	17	9	16				
South1 (S1), grab	3.1						14	5		
South2 (S2), grab	3.4				1		1	1		
South3 (S3), grab	3.5				1		1	1	1	1
South4 (S4), grab	3.1				1		1	1	1	1
South5 (S5), grab	3.2				1		1	1	1	1
South6 (S6), grab	3.8				1		1	1		
Within the meadow										
Meadow1 (M1), core	2.5	1/18	10	10	7	9				
Meadow2 (M2), grab	1.7						14	8		
Meadow3 (M3), grab	2.0						14	7		
Meadow4 (M4), grab	1.5				1		1	1	1	1
Meadow5 (M5), grab	2.7				1		1	1	1	1
Meadow6 (M6), grab	1.4				1		1	1	1	1
Meadow7 (M7), grab	2.6				1		1	1		
Total number of data points			63	63	43	60	51	29	6	6

2.3 Pore water extraction and calculation of partition coefficients

From 6 grab samples, sediment was subsampled, immediately placed in airtight containers and kept undisturbed, dark and cool (~ 4˚C) until pore water extraction within 24 hours after sampling. Pore water was extracted by centrifugation at 7000

rpm for 30 minutes and filtering the supernatant through 0.45 µm membrane filter to remove remaining particles. Samples for MeHg analysis were preserved with 1 ml 37% hydrochloric acid (HCl) (Braaten et al., 2014, USEPA, 1998). All pore water samples were stored frozen (-20˚C) until analyzed.

Partition coefficients (K_d) were calculated from concentrations of Tot-Hg and MeHg in sediment (0-5 cm) and pore water based on equation (1), and presented as Log(K_d):

$$Log(K_d)=Log(C_{sediment}/C_{pore\ water}) \quad (1)$$

where

$C_{sediment}$ = Concentration of Tot-Hg (µg/kg) or MeHg (µg/kg) in 0-5 cm sediment

$C_{pore\ water}$=Concentration of Tot-Hg (µg/L) or MeHg (µg/L) in pore water from 0-5 cm sediment

2.4 Flux measurements

To measure the flux of Hg from sediment to overlying waters, triplicate box core samples (0.1m^2, 30-40 cm deep) were collected outside (S1) and within the meadow (M1) simultaneously with the cores (Figure 1). The sampling and mesocosm set-up were done in accordance with well established procedures which provides large box-core samples with undisturbed vertical layering and little loss of benthic organisms (Berge et al., 1986, Näslund et al., 2011, Schaanning et al., 2008, Trannum et al., 2011, Trannum et al., 2010). In the mesocosm, the box-cores were submerged

to the rim in a water bath holding 10°C and with continuous flow of brackish water (5-10 PSU). The overlying water in each core was continuously exchanged with separate flows of 0.5-1 ml L^{-1} of the same water source. An airlift system described in Josefsson et al. (2012) was applied in each core to maintain a well-mixed and oxic overlying water. The source water and the water above the sediment were sampled in separate bottles (1 L) after 2 months and again after 3 months for analysis of Tot-Hg and MeHg. HCl was immediately added to the bottles for MeHg analysis. The water samples were kept cool (~4°C) and analyzed within 4 weeks from sampling.

The flux (F) from sediment to overlying water was calculated by the following equation (2):

$$F=(C_o-C_i)Q/A \quad (2)$$

where

C_i = Concentration of Tot-Hg or MeHg in common source water

C_O=Concentration of Tot-Hg or MeHg in outlet water from each box core

Q= Water flow rate through each box core

A= Box core area (0.1 m^2)

Concentrations of MeHg were frequently below the detection limit (DL; 0.02 ng L^{-1}) both in source water and in the overlying water. Nevertheless, concentrations (C_i) as low as 0.001 ng L^{-1} were reported at both measuring occasions and were used for flux calculations. The mean flow of 0.7 ml min^{-1} and a C_O corresponding to the DL of

0.02 ng L^{-1} would yield a flux of 0.2 ng m^{-2} d^{-1}. Accordingly, this was considered as the detection limit for the MeHg fluxes reported.

2.5 Chemical analysis

Analysis of Tot-Hg in sediment was performed at the University College of Southeast Norway by a Lumex 915M instrument with a PYRO 915 pyrolysis unit (DL 0.5 µg kg-1). MeHg in sediments was analyzed according to Bloom et al. (1997) and USEPA (1998). The method includes leaching with potassium bromide (KBr; 18 %), sulfuric acid (H_2SO_4; 5 %) and copper sulfate ($CuSO_4$; 1M), extraction into dichloromethane (DCM) and back extraction into distilled water before heating (70 °C for 5 hours). Determination of MeHg in sediment was done with aqueous ethylation, purge and trap, and detection with cold vapor atomic fluorescence spectrometry (CVAFS). An automated system was then used for analysis of MeHg (Brooks Rand Labs MERX automated systems with Model III AFS Detector). Analysis of a MeHg certified reference material (CRM, ERM-CC580; estuarine sediment) was within the reported range (75 ± 4 ng g^{-1}). The DL for MeHg was 20 pg g^{-1}. TOC was analyzed in 43 of the sediment samples (Table 1) using a Phoenix 8000 TOC-TC analyzer, following standard method NS-ISO 8245 .

Analyses for Tot-Hg and MeHg in water and pore water were based on USEPA Methods 1631 (USEPA, 2002) and 1630 (USEPA, 1998), respectively. Tot-Hg was determined by oxidation, purge and trap, and detected with CVAFS (DL 0.1 ng L^{-1}), and MeHg was determined by distillation, aqueous ethylation, purge and trap, and

detected by CVAFS (DL 0.02 ng L^{-1}). Precision of duplicate samples was < 10% for Tot-Hg and < 20% for MeHg. Recovery of blank spikes and matrix spikes were 80-120 % for MeHg and 90-110 % for Tot-Hg.

2.6 Statistical analysis

All statistical analyses were done using the statistical language and software environment R, version 3.1.0 (Team, 2014). The vertical distribution of Hg concentrations was explored by plotting the sediment depth profiles measured in the cores from site N, C1, C2, and M1. All data were further explored by test of correlation to reveal candidates for explanatory variables for Tot-Hg and MeHg in sediments. Shapiro test revealed normality for Tot-Hg and MeHg in sediment and for Tot-Hg in pore water without transformation, whereas normality was violated for MeHg in pore water. However, log-transformation did not improve normality for MeHg in pore water, so non-parametric tests were used for this parameter. ANOVA was used for testing the difference between within and outside the meadow in Tot-Hg and MeHg in the upper 5 cm of sediment, whereas ANCOVA tested for presence of macrophytes as a significant predictor for MeHg in sediment. Samples below the DL were set to DL /2 for the statistical analysis. This was relevant only for results of MeHg in 4 of the 20 pore water samples (S2-4).

3. Results

3.1 Biogeochemical characteristics

Large horizontal and vertical variations were observed with regard to biogeochemical characteristics (Figure 2). Within the meadow (M1), a distinct TOC maximum (52.4 µg C mg^{-1} dw), high concentrations of sulfide (pS=3.8) and low values of E_h (-59mV) were found in 2-5 cm depth. Two pH minima (6.5 and 6.6) at about 2.5 and 4.5 cm depth, corresponded closely with the upper and lower boundary of the layer with high sulfide concentrations.

Outside the meadow (C1 and C2) concentrations of TOC were high and the pH reached anomalous high values (>10). Anomalously high E_h values were recorded in the northern part (N) reaching more than 500 mV, compared to E_h in the range -295 to 170mV at C1, C2 and in the meadow (M1). Sulfide was lacking at site N, and varied from pS=1.8 (~16 mM) in 55 cm sediment depth at site C1 to pS >15 in 12 cm depth within the meadow (M1).

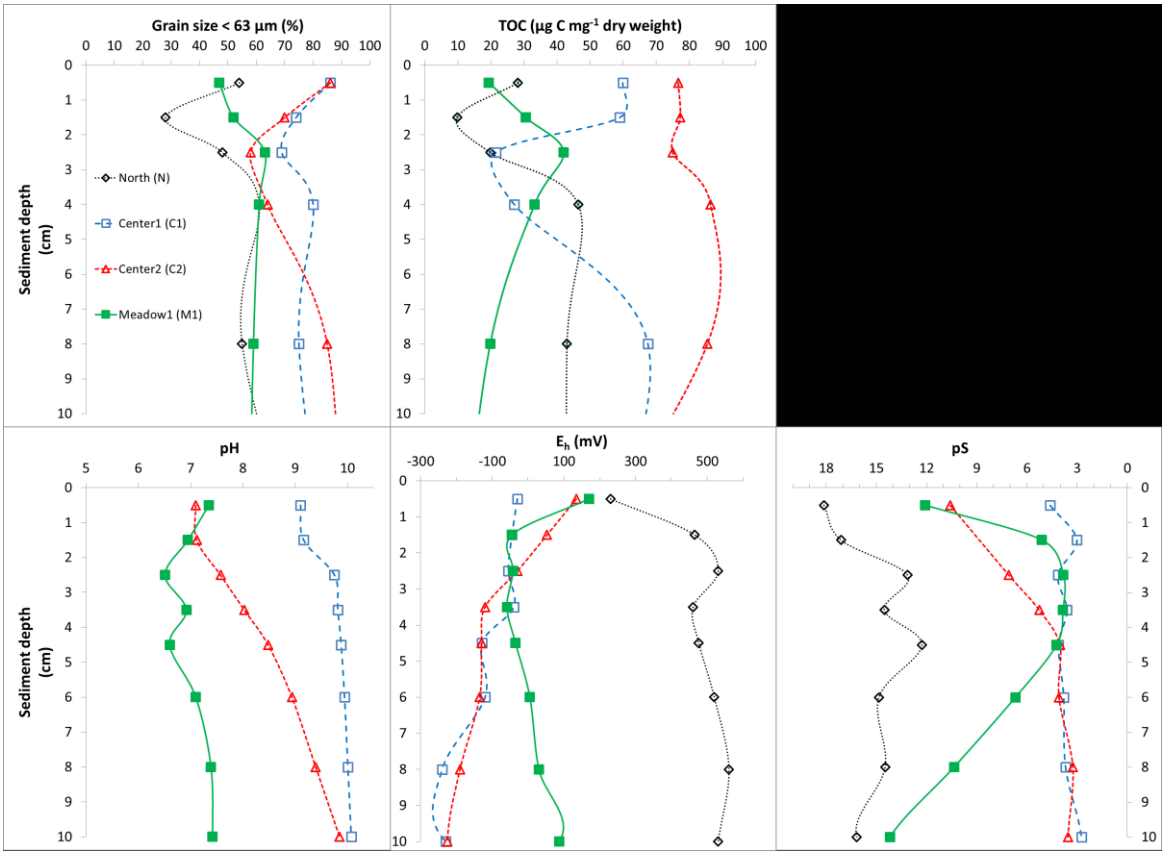

Figure 2. Details (0-10 cm) of the vertical distribution of A) relative proportion (%) of fine grain (<63 µm); B) TOC (µg C mg^{-1} dw); C) pH; D) E_h (mV) and E) pS (-log(ΣH_2S)), measured in sediment cores from outside the meadow (N=black, C1=blue, C2=red) and within the meadow (M1=green). pH was not measured at site N. Note values in reverse order for pS.

3.2 Sediment concentrations

Tot-Hg and MeHg concentrations in sediment ranged from 0.24 to 142 mg Tot-Hg kg^{-1} dw and from 0.03 to 26.4 µg MeHg kg^{-1} dw (Table 2). Concentrations of both Tot-Hg and MeHg increased from the surface and down to peak concentrations found at 10-15 cm sediment depth outside the meadow (maximum 491 mg Tot-Hg

kg^{-1} dw and 268 µg MeHg kg^{-1} dw), and at 8 cm (19 mg Tot-Hg kg^{-1} dw) and 4-6 cm (33 µg MeHg kg^{-1} dw) within the meadow (Figure 3). Within the meadow, the methylated fraction of Hg reached 0.5 % MeHg at 4-6 cm depth, which was approximately 10-fold and significantly higher ($p<0.001$) compared to any of the other sampling sites (% MeHg <0.1) when comparing the upper 10 cm of the sediment depth profiles.

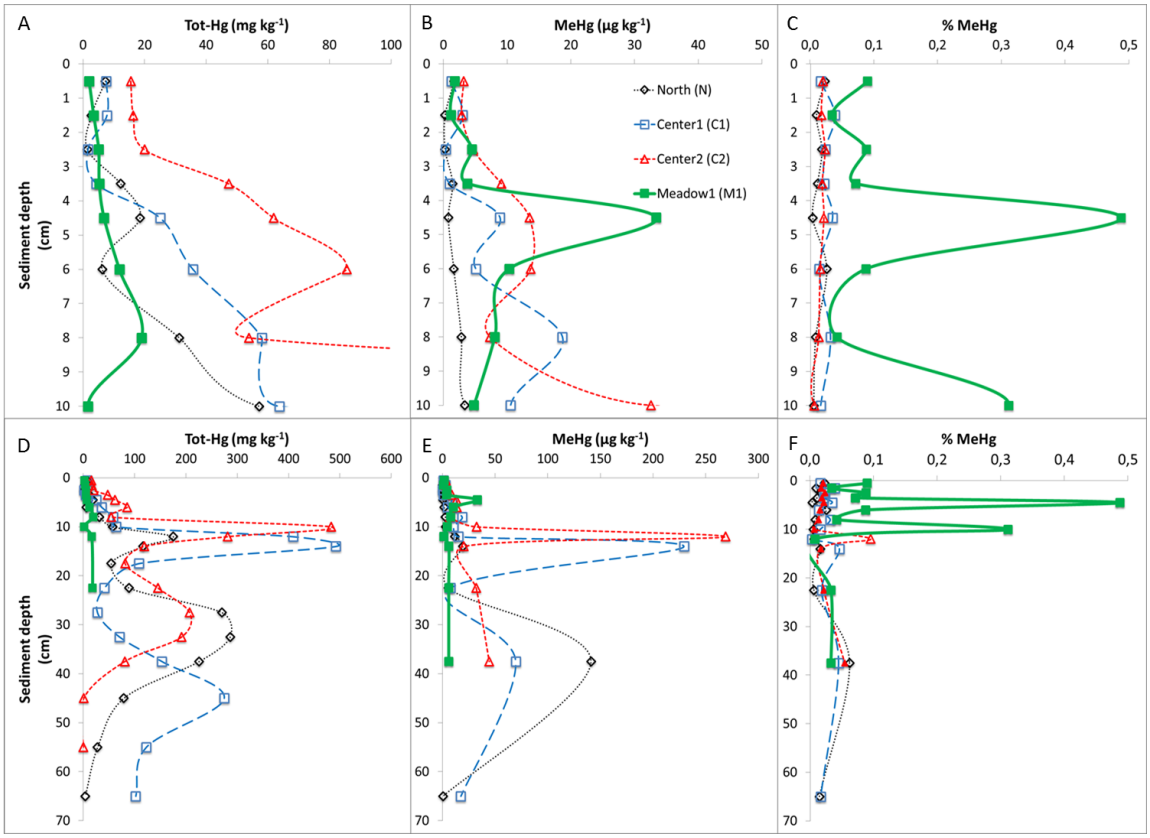

Figure 3. Vertical sediment profiles of Tot-Hg (mg kg^{-1} dw), MeHg (µg kg^{-1} dw) and % MeHg in 0-10 cm (A-C) and in the entire core (D-F), based on three core samples from outside the meadow (N=black, C1=blue and C2=red) and one from within the meadow (M1=green). Note different scales.

In the upper 5 cm of sediments where macrophyte roots are believed to impact sediment geochemistry, Tot-Hg and MeHg were significantly higher ($p<0.05$) outside the meadow compared to within (Figure 4), and so were TOC. However, four very high values of % MeHg (ranging 0.1-0.4 % MeHg) were observed within the meadow, and mean concentration was higher (0.07 % MeHg) than outside (0.02 MeHg).

Table 2. Mean±sd of sediment and pore water concentrations (0-5 cm) of Tot-Hg and MeHg based on pooled data from single grab samples. Log (K_d) is calculated according to equation (1).

	Tot-Hg			**MeHg**		
Site	**Sediment (mg kg^{-1} dw)**	**Pore water (µg l^{-1})**	**Log(K_d) (l kg^{-1})**	**Sediment (µg kg^{-1}dw)**	**Pore water (µg l^{-1})**	**Log(K_d) (l kg^{-1})**
Outside the meadow						
mean±sd	54.2±35.2	0.9±0.5	4.8±0.6	14.6±9.1	0.01±0	3.1±0.2
n	22	3	3	13	3	3
Within the meadow						
mean±sd	9.5±11.9	0.6±0.9	4.9±0.6	3.4±4.5	0.09±0.08	2.05±0.5
n	33	3	3	20	3	3

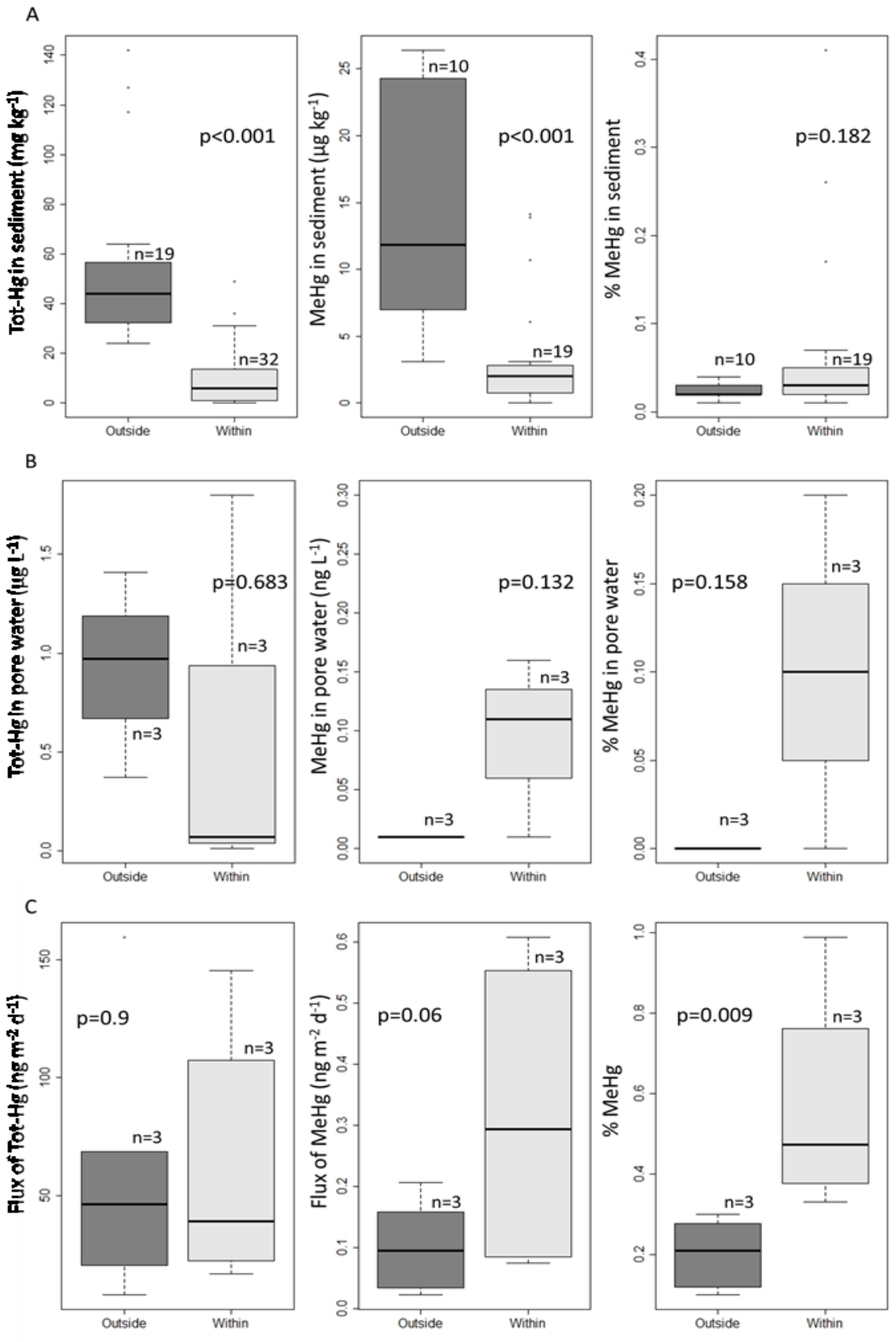
A
Tot-Hg in sediment (mg kg-1)
p<0.001
n=19
n=32
Outside
Within
MeHg in sediment (µg kg-1)
n=10
p<0.001
n=19
Outside
Within
% MeHg in sediment
p=0.182
n=10
n=19
Outside
Within
B
Tot-Hg in pore water (µg L-1)
p=0.683
n=3
n=3
Outside
Within
MeHg in pore water (ng L-1)
p=0.132
n=3
n=3
Outside
Within
% MeHg in pore water
p=0.158
n=3
n=3
Outside
Within
C
Flux of Tot-Hg (ng m-2 d-1)
p=0.9
n=3
n=3
Outside
Within
Flux of MeHg (ng m-2 d-1)
p=0.06
n=3
n=3
Outside
Within
% MeHg
p=0.009
n=3
n=3
Outside
Within

Figure 4. Tot-Hg (left) and MeHg (center) concentrations in 0-5 cm within and outside the meadow in A) sediments and B) pore water, and C) the fluxes of Tot-Hg (left) and MeHg (center). The right-hand figures show the proportion of Tot-Hg present as MeHg (% MeHg). The boxes present the median value and 50 % of the data.

Close correlation was found between concentrations of Tot-Hg and MeHg (Figure 3) in the upper 5 cm of sediments ($r=0.93$) making Tot-Hg a significant predictor ($p<0.001$) for MeHg in sediment when including all samples. However, Tot-Hg was not a significant parameter for MeHg ($p= 0.09$) within the meadow. Further, within the meadow Tot-Hg and MeHg in the sediments were closely correlated with TOC ($r=0.96$, $p<0.01$) (Figure 5), whereas outside the meadow the correlation was not significant ($p= 0.22$). Accordingly, linear regression models calculated for Tot-Hg and MeHg in sediment within the meadow including only concentrations of TOC (Figure 5) explained 93 % ($r^2=0.93$) and 92 % ($r^2=0.92$) of the variance in the dataset, respectively.

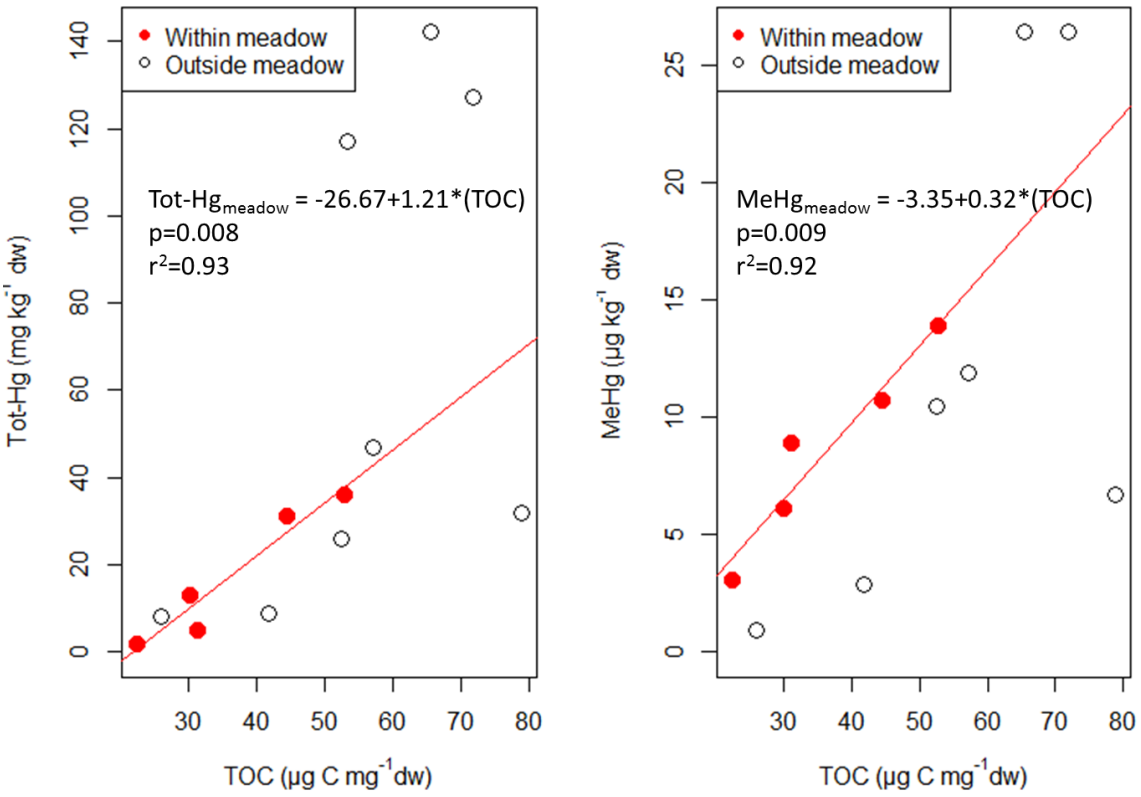

Figure 5. Concentrations of Tot-Hg (left) and MeHg (right) as a function of TOC in sediment (0-5 cm). Correlations were significant only within the meadow ($p<0.05$) and fitted regression lines are based on data from within the meadow only.

3.3 Pore water concentrations

Pore water concentrations of Tot-Hg reached higher concentrations outside the meadow than within (mean 0.9 µg L^{-1} and 0.6 µg L^{-1}, respectively), whereas concentrations of MeHg reached higher values within the meadow than outside (mean 0.09 µg l^{-1} and 0.01 µg l^{-1}, respectively). However, the differences were not significant at the 95% significance level, probably due to few data points and large

variance. The % MeHg within the meadow reached a maximum of 0.24 % whereas outside the meadow the maximum was 0.003 % MeHg, and correlation between concentrations of Tot-Hg and MeHg in pore water was low when including all samples (r=-0.009, p=0.98). The calculated partitioning coefficient for MeHg ($K_{d,MeHg}$) was typically 1-3 orders of magnitude lower than the corresponding coefficient for Tot-Hg ($K_{d, TotHg}$) (Table 2). Within the meadow Log($K_{d,MeHg}$) was significantly lower than outside (p=0.01), whereas insignificant difference was calculated for Log($K_{d,Tot-Hg}$).

3.4 Flux of Hg from sediment

The flux of Tot-Hg from sediment (Figure 4) was not significantly different (p=0.91) within the meadow compared to (17-145 ng m^{-2} d^{-1}) outside (8-160 ng m^{-2} d^{-1}). However, within the meadow there was a significant (p<0.05) higher Tot-Hg flux - to - sediment concentration ratio, whereas the Tot-Hg flux - to - pore water concentration ratio was not significantly different from outside (p=0.43). The flux of MeHg and the % MeHg in the flux from sediment to water was significantly higher (p=0.06 and p=0.009, respectively) within the meadow (0.1-0.6 ng MeHg m^{-2} d^{-1} and 0.3-1.0 %MeHg with mean ± sd 0.31 ± 0.24) compared to outside the meadow (0.02 - 0.2 ng MeHg m^{-2} d^{-1} and 0.1-0.3 % MeHg with mean ± sd 0.1 ± 0.08). Further, there was a significantly higher MeHg flux - to – MeHg sediment concentration ratio within the meadow (p=0.01), indicating that there are other mechanisms controlling the flux of MeHg within the meadow in addition to sediment concentration of MeHg,

whereas the MeHg flux – to – MeHg pore water concentration ratio was slightly higher outside the meadow compared to within (p=0.07).

4. Discussion

4.1 Evidence for enhanced microbial activity within the meadow

Outside the meadow, the natural processes appeared to be biased by the industrial discharges and anthropogenic disturbances, which most probably is responsible for the anomalies observed in the sediment depth profiles from outside the meadow (Figure 2). According to ^{210}Pb-analysis (Eek and Slinde, 2015), the upper 10-25 cm of the sediment includes the period of mercury discharges from the chlor-alkali plant (1947-1987). The wide range of pH in these sediments has later been confirmed by microelectrode measurements within the 0-5 cm layer, which showed pH from 8.0 to 10.8 at sampling site S1 and 4.4-7.8 at site M1 (Schaanning et al., 2014). The low pH may be due to readily available degradable organic matter (Schaanning and Kupka Hansen, 2005). The anomalously high E_h values recorded at site N was consistent with the lack of H_2S, which is normally associated with positive E_h values, though E_h>500 mV is not normally found in natural sediments and may result from a persistent influence from electroactive redox compounds discharged from industrial processes (e.g. Cl_2/Cl^-, Mg/Mg^{2+}).

Although H_2S is present in sediment outside the meadow at levels similar to those found in the meadow, the vertical profiles of pS as well as E_h are very different (Figure 2). This, together with the higher methylation efficiency within the meadow

(Figure 3) indicates that different mechanisms are controlling the MeHg production within and outside the meadow. The low E_h and pS values at about 2-5 cm depth within the meadow indicates sulfate reduction and production of H_2S in this depth, supporting the hypothesis that the macrophytes create favorable conditions for anaerobic sulfate reducing microbial activity, though sulfate reduction was not measured directly. The conditions in sediments outside the meadow seem to be heavily influenced by post-deposition chemical processes rather than natural processes. Within the meadow, the distinct TOC maximum in 2-5 cm depth may be explained by organic carbon exudates from plant roots into the rhizosphere, as described by others (Cosio et al., 2014). Exudates may be a major contributor to labile carbon for microbial processes feeding bacterial activity and carbon degradation via sulfate-reduction (Gilmour and Riedel, 1995) to an extent that dissolved sulfide accumulates in the pore water. The H_2S production caused by degradation of organic matter via sulfate reduction ($CH_2O + ½\ SO_4^{2-} = HCO_3^- + ½\ H_2S$) will buffer the pH at about 7.0. Numerous reactions between sulfide and various oxidizing agents (e.g. O_2, NO_3 and Fe- and Mn- oxide minerals) may occur to remove the H_2S produced, and produce the acid required to explain the pH-minima observed on either side of the high sulfide zone (e.g. $HS^- + O_2 = SO_4^{2-} + H^+$). The striking similarity of the two pH minima at about 2.5 and 4.5 cm in M1 would not be expected if labile electron acceptors like O_2 and NO_3 were supplied by diffusion from the overlying water. Therefore, active release of O_2 from the plants on both sides of the sulfide production maximum is a simple way of explaining the symmetry of both the pH and the pS profiles across this layer. Thus, the observed biogeochemical

profiles can be reasonably explained by plant roots injection of labile carbon, and an electron acceptor such as O_2 (or NO_3), at sediment depths between 2 and 5 cm. This supports previous findings that macrophyte roots in frequently submerged habitats (e.g. wetlands, bogs) provide pathways that introduce O_2 into the sediment column, thereby enhancing the depth of the redox transition zone (Aldridge and Ganf, 2003), and affecting the biogeochemical cycling of sulfur in sediments.

4.2 Enhanced MeHg production in the rhizosphere

The maximum Hg concentrations across all sampling sites were found outside the meadow at site C1, reaching 492 mg Tot-Hg kg^{-1} dw and 269 µg MeHg kg^{-1}dw in 14 cm sediment depth (Figure 3). These values are in the same order of magnitude as concentrations reported from other chlor-alkali plant sites (Ullrich et al., 2007), and demonstrates the role of the sediment as a sink for Hg discharges. Distance from the previous point of discharge (Figure1) and water circulation patterns are probable explaining factors for the differences in surface sediment concentrations of Tot-Hg between outside and within the meadow (Figure 4). In addition, we suggest a possible "barrier effect" from the macrophytes, as the *P.crispus* in Gunneklevfjorden has been observed to create a dense meadow with stems reaching from the seabed to the water surface. This may have the potential to limit the water flow through the meadow and thereby reducing the supply of particles into the meadow area. Earlier studies have reported that macrophytes tend to enhance sedimentation rates and reduce resuspension (Garcia et al., 2003, Le Hir et al., 2007, Vereecken et al., 2006,

Wetzel, 2001). Hence, it appears that the impact from macrophytes on sedimentation rates may be site specific.

The decrease in Hg concentration from peak values in the sediment depth profiles towards the sediment surface seen both within and outside the meadow, probably reflects elimination of discharges and sedimentation of cleaner particles after the chlor-alkali plant was closed down in 1987. Within the meadow the peak Tot-Hg value was more shallow than outside the meadow, probably modified with slightly slower sediment growth in the meadow due to higher bioturbation and different sedimentation or compaction processes, as discussed above.

Enhanced MeHg production within the meadow was most clearly evidenced by solid-phase MeHg and % MeHg (Figure 3B, 3C), with peak MeHg concentrations (4-6 cm) coinciding with the low pH and sulfide production layer (Figure 2C, 2E), indicating that this is a dynamic maximum related to processes that may be controlled by the rhizosphere system. These results are complimentary to empirical evidence that high concentrations of sulfide inhibit the bioavailability and subsequently the Hg methylation at high sulfide concentrations through a range of mechanisms, such as precipitation of HgS (Gilmour et al., 1998, Benoit et al., 2001, Langer et al., 2001, Jay et al., 2002, Drott et al., 2007, Skyllberg et al., 2006, Han et al., 2007, Liu et al., 2009, Zhang et al., 2012, Graham et al., 2012), though the vertical distribution pattern provided no evidence for accumulation of Tot-Hg in this layer (Figure 3A). However, the roots themselves might act as a storage site for Hg (Olsen

et al., 2016). Higher MeHg is often observed in ecosystems and sediment with low pH (Golding et al., 2007, St. Louis et al., 1996) and methylating microorganisms have been found to be favored at lower pH (Rubec, 2003, Winch et al., 2008), indicating that related microbial processes may causes both (low pH and higher MeHg).

The enhanced MeHg production within the meadow seen in the sediment depth profiles was not reflected in the surficial sediments when comparing between the two contrasting environments (Figure 4), though the methylated fraction (% MeHg) may be a better indication on favorable conditions for the methylation process than absolute MeHg concentrations, which tend to be proportional to Tot-Hg. The slightly enhanced value of % MeHg within the meadow (Figure 4-1C), and the coinciding sediment depth (2-5 cm) for the peak % MeHg value (Figure 3C) and the sulfide production layer (Figure 2E), strongly suggest that this is also the depth at which MeHg enter the sediment. The enrichment of TOC within the meadow at approximately the same depth (Figure 2B), indicated that TOC might be an important factor explaining the presence of MeHg. The influence of TOC on MeHg production was supported by the correlation between TOC and MeHg within the meadow (Figure 5). Most likely, the MeHg is produced within the rhizosphere itself, favored by input of organic carbon, high microbial activity and availability of other electron acceptors such as O_2 or NO_3. This is complimentary to other studies that have concluded on the sediment-water interface as the primary methylation site (Bravo et al., 2014), and supports that macrophytes in brackish waters stimulate MeHg production, as has previously been suggested for other habitats (Aldridge and

Ganf, 2003, Canario and Vale, 2012). Outside the meadow, the lack of correlation between MeHg and TOC indicates that MeHg production is not stimulated by high concentrations of TOC alone (Figure 2B). Tot-Hg being the only predictor for MeHg in sediments has also been found in other studies of estuarine sediments (Schartup et al., 2013b). Thus, we suggest that the production of MeHg within the meadow as well as outside is based on legacy Hg as a source, whereas the geochemical conditions within the meadow are heavily impacted by macrophyte-induced geochemistry and bears little evidence of the industrial history.

4.3 The impact of macrophytes on availability of Hg

Enhanced % MeHg in pore water within the meadow compared to outside was consistent with enhanced production of MeHg, and was in accordance with previous observations from both marine and freshwater vegetated areas (He et al., 2007, Hines et al., 2000, Ullrich et al., 2001). The observed low K_d-values for MeHg within the meadow (Table 2) most likely is a result of higher MeHg production rates overruling slow equilibrium kinetics, though pore water concentrations may also be influenced by shorter-term changes in solubility or adsorption processes unrelated to microbial MeHg production (Hammerschmidt et al., 2008, Sunderland et al., 2006, Jonsson et al., 2009). A greater partitioning (low K_d) into pore water in the presence of high sulfide concentrations has been reported for both inorganic Hg and MeHg (Bailey et al., 2017, Bloom et al., 1999, Merritt and Amirbahman, 2007, Hammerschmidt et al., 2008, Jonsson et al., 2009), though this does not explain the difference in solubility of MeHg within and outside the meadow since sulfide was

equally high in the two areas. Despite a small data-set for pore water and subsequent K_d calculations, and the inherent risk for biased pore water results due to oxidation of pore water samples, our estimated K_d values are in the same order of magnitude as found in other studies (Lyon et al., 1997).

Accordingly, the higher flux of MeHg within the meadow compared to outside (Figure 4C) was consistent with higher solubility of MeHg in the meadow (Log $K_{d,MeHg}$ = 2.6) compared to outside (Log $K_{d,MeHg}$ = 3.5) (Table 2). Hence, MeHg may perhaps escape from the sediments before being bound to the sediment particles. The combination of enhanced % MeHg in pore water and enhanced solubility of MeHg within the meadow may contribute to explain the enhanced flux of Tot-Hg relative to sediment concentration from within the meadow (Figure 4C-1), as there is a weaker binding of MeHg to particles compared to Tot-Hg, demonstrated by lower K_d-values for MeHg than for Tot-Hg (Table 2). In addition, the formation of soluble organic complexes or compounds other than MeHg would be expected from the higher availability of organic carbon in the rhizosphere (Ndungu et al., 2016). Also, higher abundance of benthic organisms and subsequent bioturbation within the meadow could explain the higher flux-to-sediment ratio of both Tot-Hg and MeHg compared to outside. Bioturbation will enhance mixing between pore water and overlying water (Hollweg et al., 2009) and lower the pore water concentrations which are in a dynamic steady state between dissolution and loss by diffusion to the overlying water. Further, this could explain the nearly significantly lower ($p=0.07$) flux – to – pore water MeHg concentration ratio within the meadow compared to

outside, despite higher flux of MeHg within the meadow. The median flux values for within and outside the meadow (0.20 ng m^{-2} d^{-1} and 0.09 ng m^{-2} d^{-1}, respectively) up-scaled to the respective sediment surface areas showed that the meadow which constitutes approximately 10 % of the area represents approximately 25 % of the daily flux of MeHg from sediment to water (20.3 µg d^{-1} and 59.4 µg d^{-1}, respectively).

5. Conclusions

We assume that MeHg production rates within the meadow was stimulated by the supply and availability of organic carbon, microbial activity and a sulfide oxidizing agent (e.g. O_2). Thus, higher MeHg production rates combined with increased bioturbation are probably the most important factors enhancing the release of MeHg from sediments vegetated by macrophytes, as suggested by others (Cosio et al., 2014, Hollweg et al., 2009, Lehnherr, 2014).

No comparable flux data has been found in the peer-reviewed literature for contaminated, brackish, temperate waters, demonstrating the novelty of this study. However, our flux values were generally lower than reported for MeHg during spring and summer in temperate wetlands and lakes (Krabbenhoft et al., 1998, Lehnherr et al., 2012a), but this comparison may not be relevant without taking into consideration a possible seasonality in MeHg production (Bloom et al., 2004, Gosnell et al., 2016, Merritt and Amirbahman, 2008). The functioning of the macrophytes in supplying O_2 (or other electron acceptors) and TOC to the rhizosphere, probably implies that seasonal fluctuations in biomass production,

including macrophyte growth, results in seasonal fluctuations of MeHg production in vegetated habitats.

This study supports the hypothesis that macrophytes promote a microenvironment favorable for microbial processes typically found in redox transition zones, such as SRB activity and subsequent sulfate reduction, stimulating the production of MeHg. Hence, submerged macrophyte meadows located within Hg contaminated sediment sites may enhance the availability of Hg to aquatic food chains. Thus, productive meadows may represent important entry points for food webs, compared to presumably less productive non-meadow areas. How important this entry point is will be determined by several factors including biota feeding strategy and time spent within the meadow. Because porewater sulfide affects both MeHg production and MeHg partitioning, factors that control porewater sulfide are critical to understanding Hg cycling. The MeHg production and availability in macrophyte habitats located within legacy Hg contaminated sites needs more attention in future studies, with regard to both quantitative scaling and the factors controlling the methylation process.

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