Biogeosciences, 9, 493–507, 2012
www.biogeosciences.net/9/493/2012/
doi:10.5194/bg-9-493-2012

Biogeosciences

Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils

T. Frohne[1], J. Rinklebe[1], U. Langer[2], G. Du Laing[3], S. Mothes[4], and R. Wennrich[4]

[1]University of Wuppertal, Department D, Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany
[2]State Office for Environmental Protection of Saxony-Anhalt, Department of Soil Protection, Reideburger Straße 47, 06116 Halle/Saale, Germany
[3]Ghent University, Faculty of Bioscience Engineering, Coupure Links 653, 9000 Gent, Belgium
[4]UFZ Helmholtz Centre for Environmental Research, Department of Analytical Chemistry, Permoserstraße 15, 04318 Leipzig, Germany

Correspondence to: J. Rinklebe (rinklebe@uni-wuppertal.de)

Received: 27 June 2011 – Published in Biogeosciences Discuss.: 2 September 2011
Revised: 23 December 2011 – Accepted: 9 January 2012 – Published: 26 January 2012

Abstract. An automated biogeochemical microcosm system allowing controlled variation of redox potential (E_H) in soil suspensions was used to assess the effect of various factors on the mobility of mercury (Hg) as well as on the methylation of Hg in two contaminated floodplain soils with different Hg concentrations (approximately 5 mg Hg kg^{-1} and >30 mg Hg kg^{-1}). The experiment was conducted under stepwise variation from reducing (approximately −350 mV at pH 5) to oxidizing conditions (approximately 600 mV at pH 5). Results of phospholipid fatty acids (PLFA) analysis indicate the occurrence of sulfate reducing bacteria (SRB) such as *Desulfobacter* species (10Me16:0, cy17:0, 10Me18:0, cy19:0) or *Desulfovibrio* species (18:2ω6,9), which are considered to promote Hg methylation. The products of the methylation process are lipophilic, highly toxic methyl mercury species such as the monomethyl mercury ion [$MeHg^+$], which is named as MeHg here. The ln(MeHg/Hg_t) ratio is assumed to reflect the net production of monomethyl mercury normalized to total dissolved Hg (Hg_t) concentration. This ratio increases with rising dissolved organic carbon (DOC) to Hg_t ratio (ln(DOC/Hg_t) ratio) ($R^2 = 0.39$, $p < 0.0001$, $n = 63$) whereas the relation between ln(MeHg/Hg_t) ratio and lnDOC is weaker ($R^2 = 0.09$; $p < 0.05$; $n = 63$). In conclusion, the DOC/Hg_t ratio might be a more important factor for the Hg net methylation than DOC alone in the current study. Redox variations seem to affect the biogeochemical behavior of dissolved inorganic Hg species and MeHg indirectly through related changes in DOC, sulfur cycle, and microbial community structure whereas E_H and pH values, as well as concentration of dissolved Fe^{3+}/Fe^{2+} and Cl^- seem to play subordinate roles in Hg mobilization and methylation under our experimental conditions.

1 Introduction

Mercury (Hg) is one of the most hazardous heavy metals, posing a risk to humans and environment (e.g. Wolfe et al., 1998; Gibicar et al., 2006; Bergeron et al., 2011). It is distributed widespread all over the world and can be found in various environmental compartments such as floodplains (e.g. Devai et al., 2005; Overesch et al., 2007; Rinklebe et al., 2009). Many floodplain soils have accumulated large amounts of Hg as a result of atmospheric deposition or through transport from the watershed (e.g. Boening, 2000; During et al., 2009; Rinklebe et al., 2010). Large floodplain areas along the Wupper River (Germany) are heavily polluted with Hg due to the discharge of waste originating from textile industry, particularly from dye factories, and metal industry during the last centuries.

The mobility, bioavailability, ecological and toxicological effects of Hg are strongly dependent on its chemical speciation (Ullrich et al., 2001). Methylation of inorganic Hg is an important process, which can fundamentally change its mobility, bioavailability, and toxicity (Boening, 2000). The

products of this methylation process are lipophilic, highly toxic methyl mercury species such as dimethyl mercury [Me_2Hg] or the monomethyl mercury ion [$MeHg^+$], which is named as MeHg in the following. Both methyl mercury species exhibit a significant risk to humans and wildlife due to its neurotoxicity and tendency to accumulate in the food chain (Wolfe et al., 1998; Boening, 2000; King et al., 2002; Li et al., 2010).

Generally, the mobility and methylation of Hg in frequently flooded soils is determined by a range of factors, such as redox potential (E_H), pH, dissolved organic carbon (DOC), sulphur (S), chloride (Cl^-), iron (Fe), and total dissolved Hg (Hg_t) content (e.g. Skyllberg et al., 2003; DeLaune et al., 2004; Sunderland et al., 2006). Dissolved organic carbon interacts strongly with Hg by the formation of Hg-DOC complexes (e.g. Ravichandran, 2004; Khwaja et al., 2006; Feyte et al., 2010). The high affinity of Hg to DOC can partly be attributed to the binding of Hg with reactive sulfur groups in the hydrophobic acid fraction of DOC (Karlsson and Skyllberg, 2003; Shanley et al., 2008).

Mercury immobilization can be induced under anoxic conditions due to the formation of hardly soluble Hg sulfides (e.g. Skyllberg et al., 2003; Du Laing et al., 2009). Sulfides (S^{2-}) are generated through sulfate (SO_4^{2-}) reduction, which is mainly catalyzed by microorganisms. Sulfate reducing bacteria (SRB) (e.g. *Desulfovibrio desulfuricans*, *Desulfobulbus proprionicus*) mediate the formation of S^{2-} as a result of respiration processes that require SO_4^{2-} as a terminal electron acceptor (King et al., 2002). During Hg methylation, microorganisms increase their resistance to Hg by rendering the Hg^{2+} ion ineffective in disturbing the normal biochemical processes within the cell (Boening, 2000). This methylation process has been found to be mainly conducted by SRB and Fe reducing bacteria (Compeau and Bartha, 1985; Macalady et al., 2000; Fleming et al., 2006; Merritt and Amirbahman, 2009). Furthermore, it might be possible that other organisms such as aerobic bacteria, fungi, and seaweed may play a role in Hg methylation as suggested for tropical environments and mangrove wetlands (Coelho-Souza et al., 2006; Wu et al., 2011). The microbial community composition in soils and sediments can be characterized by the analysis of phospholipid fatty acids (PLFA) (e.g. Macalady et al., 2000; Rinklebe and Langer, 2006; Langer and Rinklebe, 2009). This method allows identifying the presence of SRB (Taylor and Parkes, 1985; Coleman et al., 1993; Macalady et al., 2000).

Iron can influence the dynamics of Hg in soils. For instance, Fe(hydr)oxides are able to adsorb Hg; thus, they can act as important Hg sinks (Fernandez-Martinez et al., 2006; Harris-Hellal et al., 2011). Moreover, the mobility and methylation of Hg can be influenced by Cl^-, for example through the formation of Hg chloride or MeHg-Cl complexes (Davis et al., 1997; Skyllberg et al., 2003).

Although the presence of Hg and MeHg in the environment has been frequently documented (e.g. van Faassen, 1975; Boening, 2000; Agusa et al., 2005; Devai et al., 2005; Gibicar et al., 2006), mechanistic experiments aimed to study the redox-induced mobilization and immobilization of Hg and MeHg as well as information on biogeochemical factors affecting the methylation rate of Hg in floodplain soils are very scarce up to date.

Thus, our aim was to assess the impact of E_H, pH, DOC, SO_4^{2-}, Fe, and Cl^- on the mobility and methylation of Hg in two floodplain soils with different Hg contamination levels (approximately 5 and $>30\,mg\,Hg\,kg^{-1}$, respectively) under acidic to neutral pH conditions. Therefore, we used an automatic biogeochemical microcosm system allowing establishing definite, computer-controlled redox conditions in soil suspensions.

2 Materials and methods

2.1 Study site

Soil samples were collected from two floodplain soils (Wupper 1 = W1; Wupper 2 = W2) at the lower course of the Wupper River (Germany) close to the confluence into the Rhine River (Fig. 1). The study sites are located about 15–20 km to the north of Cologne, Germany, near the town Leverkusen, (W1: 51°4′0.48″ N, 6°59′0.77″ E; W2: 51°5′4.1″ N, 7°0′12.61″ E). The distance between the two study sites is about 2 km. The long term average annual precipitation is approximately 800 mm and the long term average annual air temperature is 10.8 °C (DWD, 2009). The geological parent material consists of sediments of the Rhine River ("Niederrheinische Bucht"), which is predominantly shale from Devonian origin ("Rheinisches Schiefergebirge"). The study sites are used as grassland and are flooded seasonally by the Wupper River, usually in springtime (Wupperverband, 2009). The Wupper River is approximately 115 km in longitude with an average gradient of 0.4 %. The discharge averages $15.4\,m^3\,s^{-1}$. The catchment area of the Wupper River comprises $814\,km^2$. Both soils are classified as Eutric Fluvisols (IUSS-ISRIC-FAO, 2006).

2.2 Sampling, pre-treatment, and analysis of bulk soil

Soil samples were collected from the genetic A-horizons (0–10 cm for W1; 0–32 cm for W2). Soil sampling was performed in four replicates of about 1 kg each which were merged to one sample. For chemical analysis, soil material was homogenized, air-dried, and sieved to <2 mm. Subsamples were ground in an agate disc mill. Physico-chemical soil properties were determined according to standard methods (Schlichting et al., 1995). Total C (C_t) and total N (N_t) were determined via dry combustion and thermal conductivity detection using a C/N/S-Analyzer (Vario EL Heraeus, Analytik Jena, Germany). A C-MAT 550 (Stroehlein, Germany) was used to measure inorganic C by dry combustion and IR-detection. Soil organic C was calculated as the difference

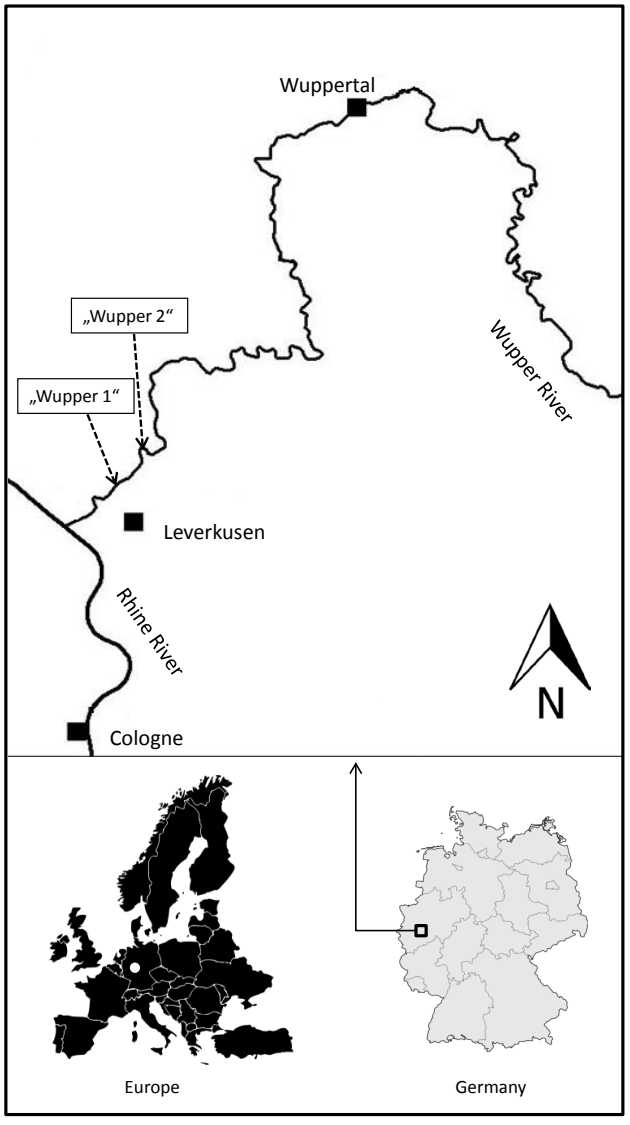


Fig. 1. Study site.

between C_t and inorganic C. Particle-size distribution was measured using the pipette sampling technique by wet sieving and sedimentation (Blume et al., 2000). Total metal concentrations of the soil were quantified after digestion using aqua regia (37 % HCl + 65 % HNO_3, volume ratio 3:1) ignoring the immobile silica-bound fraction. Total Hg was analyzed by a cold vapor atomic absorption spectrometer (FIMS 400, Perkin Elmer, USA). The calibration range was 0.5–20 $\mu g\,l^{-1}$. The dissolved samples were appropriate diluted for this calibration range using 0.01 M nitric acid. Analytical accuracy was achieved by the use of certified reference material (IAEA 405, IAEA 433, NIST 2709, and LGC-6139). Inductively coupled plasma atomic emission detection (Ciros CCD, Spectro, Germany) was used for determination of Fe. For the determination of total S, the soil was compressed to pellets and S was measured by energy dispersive X-ray spectroscopy (XLAB 2000, Spectro, Germany). The calibration (using wax pellets) was done in the concentration range 0.0150–15 $g\,kg^{-1}$. The following 15 reference materials had been used: GBW 7309–7312, GBW 7409–7411, LKSD 1, LKSD 4, NIST 2704, and NIST 2710.

For the soil microbial analysis (PLFA), fresh soil samples were sieved to <2 mm and thereafter frozen at −20 °C. After storage, samples were allowed to thaw at 4 °C for one day and 4 h at 20 °C before analysis. Phospholipid extraction and PLFA analysis were performed following the standard procedure described by White et al. (1979) and Frostegård et al. (1991). Lipids were extracted with a modified single-phase mixture chloroform-methanol-citrate buffer (1:2:0.8 v/v/v) (Bligh and Dyer, 1959). The resulting lipid material was fractionated into neutral lipids, glycolipids, and polar lipids by a silica-bonded phase column. The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert et al., 1985). Samples were analyzed by gas chromatography/mass spectroscopy using a Hewlett-Packard 6890 series gas chromatograph with a HP-5MS column (60.0 m length, 0.25 mm internal diameter, coated with a cross-linked 5 % phenyl methyl rubber phase with a film thickness of 0.25 µm) interfaced to an Agilent 5973 mass selective detector. The resulting chromatograms were evaluated by mass spectra, retention times, and nonadecanoic acid methyl ester (19:0) as the internal standard (N-5377, Sigma Chemical, Inc.). The analytical quality was confirmed by the repeated analysis of a standard bacterial acid methyl ester mix and a 37-component FAME mix (47080-U and 47885-U, Supelco, USA). PLFA were designated using the nomenclature described by Feng et al. (2003). More details regarding the method of PLFA analyses can be found in Rinklebe and Langer (2006) and Langer and Rinklebe (2009).

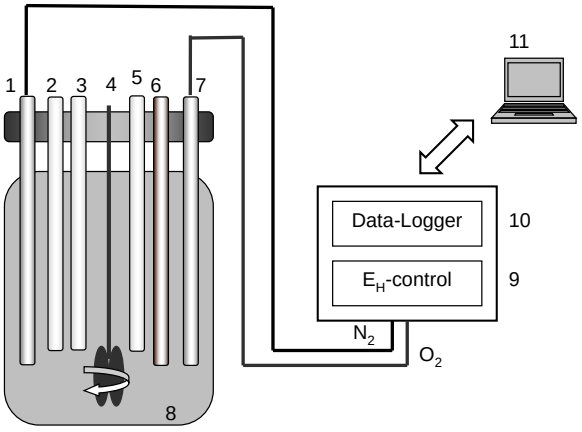
2.3 Redox experiment

2.3.1 Biogeochemical microcosm system

Flooding events were simulated using an automated biogeochemical microcosm system in the laboratory (Fig. 2). This system allows establishing pre-defined redox conditions in soil suspensions by flushing them with nitrogen (N_2) or oxygen (O_2). Thus, it is possible to study the effect of E_H almost independent from other parameters. Recently this system was described in detail by Yu and Rinklebe (2011) and successfully used in previous studies for the investigation of trace gases (Yu et al., 2007), for the quantification of mercury emissions (Rinklebe et al., 2010), and for the determination of the dynamics of trace metals (Rupp et al., 2010; Frohne et al., 2011). The current study was conducted in four independent trials for each soil. The microcosms (MCs) were filled with 200 g air-dried soil and 1600 ml deionized water. Homogenous conditions were reached by stirring the slurry

continuously. Redox potential, pH, and temperature in each MC were monitored every ten minutes by electrodes (Meinsberger Elektroden, Germany) and stored by a data logger. The pH values of MC 8 and the E_H values of MC 1 after approximately 800 h incubation could not be monitored due to an error of the electrodes. The measured redox potential values were normalized to pH 5, because the mean pH during the experiment was around 5 for both soils. Thus, the corrected values will be referred to as "E_{HatpH5}" in the following.

Straw and glucose were added to each MC to provide an additional source of organic matter for microorganisms. As a result, levels of E_{HatpH5} decreased (Fig. 4). This process was accelerated by continuously flushing the MCs with N_2 for several days. When lowest E_H values were reached, the first sample was taken from each MC. Thereafter, E_H-values were increased in steps of approximately 100 mV by adding O_2. Thereby, E_H was kept within the set E_H-windows $\pm$10–20 mV around the aimed value by supplying O_2 or N_2 automatically when the outer limits of the E_H-windows were exceeded. Redox potential was maintained for approximately 24 h within each window and afterwards set to the next window. Sampling was conducted approximately 24 h after reaching each new E_H-window (Fig. 4). The soil/water ratio remained the same during the experiment. After achieving the highest E_H levels, N_2 was added to lower E_H again.



The soil microcosm system:

(1) dispersion tube for N_2
(2) redox potential- (E_H) electrode
(3) pH electrode
(4) stirrer
(5) thermometer
(6) sampling tube
(7) dispersion tube for O_2
(8) glass vessel
(9) automatic E_H regulation by N_2 and O_2 valves
(10) data logger (E_H, pH, temperature)
(11) PC control for datalogger and valve system

Fig. 2. Biogeochemical microcosm setup.

2.3.2 Sample preparation, sub-sampling, and storage during the redox experiment

The slurry in the MCs was sampled using a plastic syringe connected with a PTFE tube. The slurry samples were immediately centrifuged for 15 min at 3000 rpm. Afterwards, the supernatants were filtered through a 0.45 µm Millipore membrane (Whatman Inc., Maidstone, UK) under N_2-atmosphere. Thereafter, the filtrate (defined as the soluble fraction) was aliquoted to subsamples for subsequent analysis. For measuring Hg_t, the first 10 ml subsample was preserved with 200 µl 0.2 M bromine monochloride solution (BrCl) and stored at 8 °C in bottles of acid rinsed borosilicate glass with PTFE-lined caps. A second subsample (8 ml) was stored in acid rinsed glass bottles at 8 °C for the analysis of MeHg. Another 10 ml subsample was stabilized by addition of 400 µl 65 % HNO_3 for analysis of total Fe and total S. Another subsample (10 ml) was stored at −20 °C and gradually thawed for the determination of DOC, Cl^-, and SO_4^{2-}.

2.3.3 Chemical analyses of the redox experiment samples

Total Hg was measured with cold vapor atomic fluorescence spectrometry (CV-AFS) (mercur duo plus, Analytik Jena, Germany). Mercury standard solutions were prepared by diluting mercury standard solution 1000 mg l^{-1}Hg (CertiPur, Merck) with deionized water. A 7-point calibration curve including a blank sample was used for sample analysis. An intern reference sample was analyzed after every 10 samples to check the instrument drift. The drift was satisfying for all measurements. The detection limit was 10 ng l^{-1}. The relative standard deviation of repeated measurements was below 3 % for all samples.

The analyses of MeHg in the subsample were conducted by gas chromatography with atomic emission detection (GC-AED). An amount of 2 ml of the sample was spiked with 4 ml buffer solution (pH 4.5) and 20 µl Na-propylborat solution (2 % in THF). The solution was stirred for 10 min. The Hg species were enriched from the aqueous phase by solid phase microextraction (SPME) in the headspace mode. Analytes were enriched onto a 100 µm polydimethylsiloxane (PDMS) – fibre for 30 min at a temperature of 30 °C. The prepared samples were stored at 15 °C until measurement. Samples were processed automatically by a multipurpose sampler (MPS2, Gerstel, Mülheim, Germany) combined with a gas chromatograph Hewlett-Packard 6890 (Agilent, Waldbronn, Germany) and a microwave-induced plasma atomic emission detector jas 2350 (jas GmbH, Moers, Germany). Thermal desorption was carried out directly in the injector of the gas chromatograph for 1 min at 200 °C. The analyses were carried out using an HP1 column (25 m × 0.32 mm × 0.17 µm) and He as carrier gas. Injection was performed in splitless

mode and the oven was programmed from 40 °C (2 min) to 280 °C at 25 °C min^{-1}. Reagent gases for the AED were O_2 and H_2, the make-up gas flow (He) was 130 ml min^{-1}. The Hg emission line 254 nm was monitored. Only monomethyl mercury ($MeHg^+$) – and no dimetyhl mercury (Me_2Hg) – could be detected. The detection limit for $MeHg^+$ was 0.8 ng Hg l^{-1}. The calibration range was 1–100 ng Hg l^{-1}. Quality control was carried out using following reference materials: IAEA 405, IAEA 433, and CRM 462. Total Fe and S were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) (Ultima 2, Horiba Scientific, Unterhaching, Germany). A four-point calibration was conducted by diluting single standard and multi element solutions (CertiPur, Merck) with deionized water. Analysis was conducted in three replications. The relative standard deviation of replicate analysis was below 5 %.

Dissolved organic carbon was measured after 2-point calibration with a TOC-analyzer (TOC-V_E, Shimadzu, Kyoto, Japan). Measurement was performed in two replications for each sample. The detection limit was 1 mg l^{-1}. Sulfate and Cl^- were determined using an ion chromatograph (Personal IC 790, Metrohm, Filderstadt, Germany) with a Metrosep A Supp 4 - column. The detection limit was 0.03 mg l^{-1} for both ions.

2.4 Calculations and statistical analysis

Mean values of E_H and pH data measured every 10 min originating from 3, 6, 12, and 24 h periods prior to sampling were calculated. Values below the detection limit were excluded for the statistical analyses. Thereafter correlation and regression analyses between Hg_t and MeHg on the one hand and E_H, pH, DOC, SO_4^{2-}, Cl^-, Fe, and S on the other hand were conducted. Relations between MeHg/Hg_t and DOC/Hg_t on one hand and DOC and Hg_t on the other hand were also calculated. Mean E_H and pH values of the 6 h period prior to sampling were used because they revealed the highest regression coefficients in most cases. Correlation analysis was conducted by SPSS 19. ORIGIN 6.0 was used to calculate regressions and descriptive statistics. For regression analyses, the naturally logarithmised values (ln) of Hg_t, MeHg, DOC, Cl^-, SO_4^{2-}, and Fe were taken, because the range of the values differed for several orders of magnitude. According to Fowler et al. (2006), the strength of the correlations was categorized in our study as follows: $r < 0.20$ (corresponds to the coefficient of determination $R^2 < 0.04$) represent very weak correlations; r between 0.20 and 0.39 (R^2 between 0.04 and 0.15) weak correlations; r between 0.4 and 0.69 (R^2 between 0.16 and 0.48) modest correlations; and $r > 0.69$ ($R^2 > 0.48$) strong correlations.

3 Results

3.1 Properties of the bulk soils

Selected properties of the studied bulk soils are provided in Table 1. The soils of W1 and W2 mainly consist of sand and silt. The content of organic carbon is relatively high and the contents of inorganic carbon are 0.01 % (W1) and 0.005 % (W2). The pH is slightly acidic to neutral. Soil W1 is contaminated with approximately 5 mg kg^{-1} Hg, whereas soil W2 is higher contaminated and contains >30 mg Hg kg^{-1} (Table 1). For both soils, concentrations of Hg exceed the action value of 2 mg Hg kg^{-1} set by the German Federal Soil Protection Ordinance (BBodSchV, 1999).

The results of PLFA analysis of the bulk soil are shown in Fig. 3. A total number of 26 PLFA (W1) and 20 PLFA (W2), respectively, were found in the soils. Here, those fatty acids which were previously identified as possible biomarkers for SRB are of particular interest. The fatty acids 15:0, 10Me16:0, cy17:0, 18:2ω6,9, 10Me18:0, and cy19:0 can serve as biomarkers for SRB (Taylor and Parkes, 1983; Kohring et al., 1994; Macalady et al., 2000). The fatty acids 10Me16:0 and cy19:0 revealed the highest values whereas cy17:0 and 10Me18:0 showed intermediate values in both soils. The PLFA 15:0 and 18:2ω6,9 were low concentrated in the studied soils.

3.2 Redox experiment

The variations (mean, median, and range) of the measured parameters during the E_H experiment are provided in Table 2. The range was 0.09–8.27 µg l^{-1} for Hg_t and 1.3–101 ng 1^{-1}. for MeHg. The mean values were 0.99 µg l^{-1} for Hg_t and 14 ng l^{-1} for MeHg. The E_H ranges from −335–601 mV (all data). The pH ranges from 4.1–7.2 (all data) with mean values around 5 for both soils. During the experiment, E_H and pH reveal a very weak significant negative correlation ($R^2 = 0.02$; $p < 0.01$; $n = 47,941$). The development of E_{HatpH5} measured in the slurry every 10 min during the experiment and concentrations of Hg_t in the soluble fraction at the sampling time are given in Fig. 4. The lowest E_{HatpH5} levels were around −150 and 0 mV in MCs 1–4 (W1) and between −100 and −350 mV in MCs 5–8 (W2). The highest E_{HatpH5} levels were around 500 mV for W1 and 500–600 mV for W2. The development of pH measured in the slurry every 10 min and the values of MeHg in the soluble fraction at the sampling points during the experiment are given in Fig. 5. The mean initial pH was 7.0 ± 0.2 for W1 (MCs 1–4) and 5.7 ± 0.3 for W2 (MCs 5–8). The pH dropped rapidly in all MCs to values between 4 and 5. Afterwards, the pH slightly increased in all MCs when increasing the E_H stepwise. A relationship between Hg_t and $E_{H,}$ or between pH and MeHg in the course of the experiment is not obvious (Figs. 4 and 5).

The ln(MeHg/Hg_t) ratio revealed a modest positive relationship with ln(DOC/Hg_t) ($R^2 = 0.39$; $p < 0.0001$; $n = 63$)

Table 1. Selected properties of the studied soils (C_{org}: organic carbon, N_t: total nitrogen) total metal concentrations (aqua regia soluble), and total sulfur (S) of the studied bulk soils Wupper 1 (W1) and Wupper 2 (W2).

Soil	Depth [cm]	Texture [%]			C_{org}	N_t	$C_{org}N_t^{-1}$	Fe	Hg	S
		Sand 0.063–2 mm	Silt 0.002–0.063 mm	Clay <0.002 mm	[%]			[g kg $^{-1}$]	[mg kg $^{-1}$]	
W1	0–10	44	48	8	6.2	0.4	15.6	34	5.2	2060
W2	0–32	55	36	9	7.9	0.4	19.8	49	31.5	2669

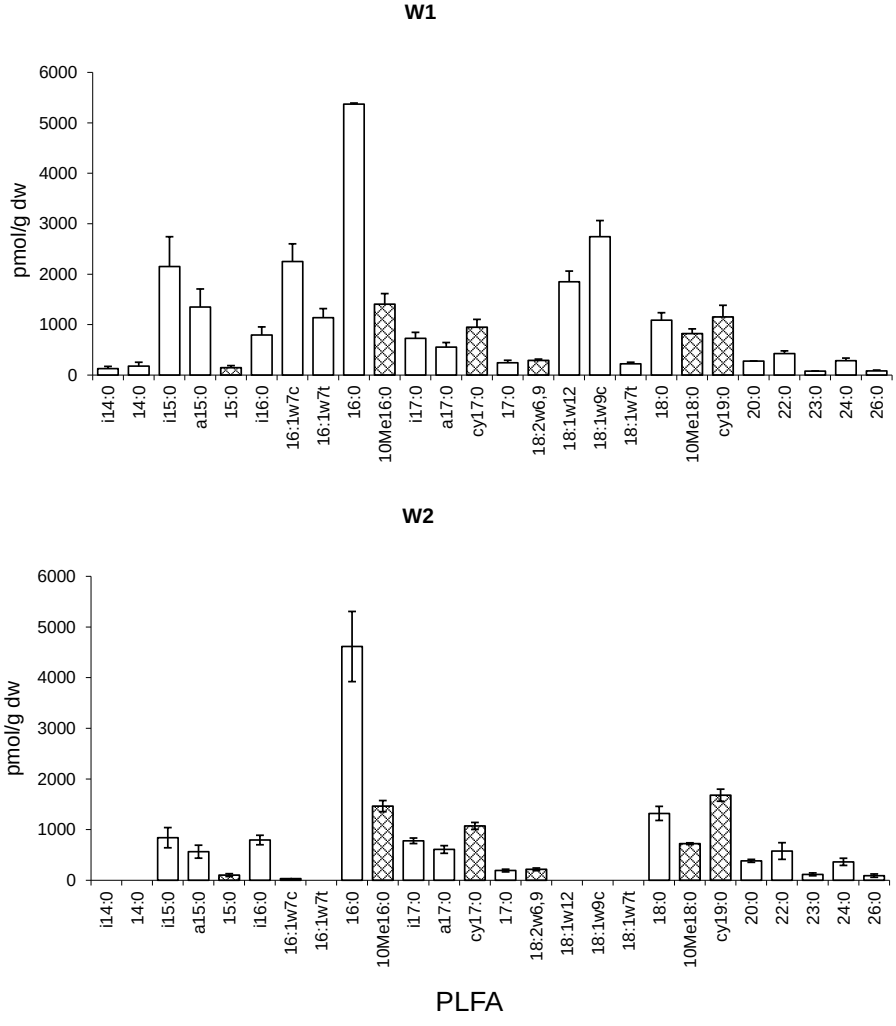


Fig. 3. Dry weight (dw) of phospholipid fatty acids (PLFA) in the bulk soils Wupper 1 (W1) and Wupper 2 (W2). Biomarkers for sulfate reducing bacteria are cross hatched.

(Fig. 6). There was a weaker negative relationship between ln(MeHg/Hg_t) and lnHg_t ($R^2 = 0.18$; $p < 0.001$; $n = 63$) and a weak positive relation between ln(MeHg/Hg_t) and lnDOC ($R^2 = 0.09$; $p < 0.05$; $n = 63$). LnHg_t values were correlated with lnMeHg values and regression analysis showed a modest positive relationship ($R^2 = 0.16$; $p < 0.005$; $n = 63$). Results of the regression analysis between lnDOC, lnCl^-, lnSO_4^{2-}, $E_{H,}$ lnFe, pH and lnHg_t on one hand and lnMeHg in the soluble fraction on the other hand are provided in Table 3. With the increase of Hg_t and MeHg, Cl^-, SO_4^{2-}, and

Table 2. Variations (mean, median, range) of concentrations of elements and compounds in the soluble fraction as well as pH, and redox potential (E_H) in the slurry.

		Mean	Median	Range	n
Hg_t	[µg l^{-1}]	0.99	0.49	0.09–8.27	65
MeHg	[ng l^{-1}]	14	5.8	1.3–101	65
E_H(6h)[1,3]	[mV]	230	297	−332–577	67
E_H(all data)[2,3]		239	319	−335–601	53 553
pH (6h)[1]		5.0	5.0	4.4–6.2	67
pH (all data)[2]		5.5	5.2	4.1–7.2	47 941
DOC	[mg l^{-1}]	2096	1989	1082–4463	67
SO_4^{2-}		32.0	8.6	2.2–223	67
Cl^-		584	144	72–3896	67
S		2.1	2.0	0.7–7.7	67
Fe		199	114	0.2–553	67

[1] means of data 6 h before sampling
[2] data measured every 10 min during the experiment
[3] E_H corrected to pH 5 (see Materials and Methods)

DOC in the soluble fraction increased. These correlations were strong for MeHg and DOC and modest for the other parameters. Iron was positively related to MeHg but not to Hg_t. Values of pH revealed a modest negative relation to MeHg and no correlation to Hg_t (Table 3). Values of E_H (Table 3) and S (data not given) are not related to Hg_t or MeHg. Values of Cl^- and SO_4^{2-} in the soluble fraction were weakly associated with E_{HatpH5} (linear relationship with $R^2 = 0.15$; $p < 0.01$; $n = 67$ for Cl^- and curved relationship $R^2 = 0.13$; $p < 0.05$; $n = 67$ for SO_4^{2-} respectively). Iron contents in the soluble fraction revealed a modest negative relationship to E_{HatpH5} ($R^2 = 0.33$; $p < 0.001$; $n = 67$). Contents of DOC did not have a significant relationship with E_{HatpH5} (data not shown).

4 Discussion

4.1 Direct impact of E_H and pH on the mobility and methylation of Hg

The biogeochemical behavior and the dynamics of Hg and MeHg under changing redox conditions are affected by various factors. Our original hypothesis was that systematic changes of E_H from anaerobic to aerobic conditions should have a considerable impact on the methylation of Hg since it has been reported that MeHg increases with decreasing E_H (DeLaune et al., 2004; Sunderland et al., 2006). On the other hand, Ullrich et al. (2001) stated that anaerobic conditions might favor the reduction from Hg^{2+} to hardly soluble Hg^0, which in turn may reduce Hg mobility and Hg methylation because of reduced bioavailability. However, in the current study, a direct impact of E_H on Hg_t or MeHg concentrations could not be detected (Figs. 4 and 5; Table 3). The variations of Hg_t values during the experiment seem to be almost independent from E_H variations (Fig. 4). Wallschläger et al. (1998) found consistent with our results, that the mobility of Hg is less influenced by changing redox conditions. Hintelmann and Wilken (1995) also reported that absolute E_H might not be the most important factor regulating Hg methylation activity in anoxic sediments. The results of the current study appear to confirm those assumptions.

Redox potential has also an effect on the pH. Generally it is well established that pH increases during reduction because reduction processes consume protons (e.g. Yu et al., 2007). Accordingly, a similar behavior was generally observed in our study. Literature data on effects of pH on the mobility and methylation of Hg are contradictory. Some authors found enhanced mobility and methylation of Hg at low pH (Boening, 2000; Ullrich et al., 2001; Wu et al., 2011). This was attributed to the fact that DOC is more positively charged at low pH and therefore has weaker tendencies to form complexes with Hg, enhancing its availability for methylating bacteria (Ravichandran, 2004). This process could have occurred in the current study as well and might contribute to explain the modest negative relationship between MeHg and pH (Table 3). In contrast, low pH can decrease Hg methylation in anoxic sediments, maybe due to the suppression of bacterial activity at low pH (Gilmour and Henry, 1991). However, the results presented here show a less clear effect of pH on Hg_t and on MeHg. Although the relationship between MeHg and pH is modest (Table 3), a mutual development between MeHg and pH is not obvious (Fig. 5) suggesting that additional factors are needed to explain MeHg variations. Accordingly, Wallschläger et al. (1996) have shown that the influence of pH on the solubility of Hg is relatively low compared to other metals (e.g. Cd, Ni, Co, Zn, Cu, Pb). The direct impact of E_H on the behavior of Hg and MeHg seems to be very weak in our study. Instead, indirect effects of E_H and pH on the mobility and methylation of Hg through E_H or pH related changes of other determining factors such as concentrations of DOC, Fe, Cl^-, and SO_4^{2-} should be more important under our experimental conditions.

4.2 Impacts of DOC, Fe, Cl^-, and SO_4^{2-} on the mobility and methylation of Hg

Total mercury and MeHg concentrations were positively related to DOC concentrations in the current experiment whereas this relationship is stronger between MeHg and DOC (Table 3). Similar results have been obtained by other authors (Covelli et al., 2009; Obrist et al., 2009; Feyte et al., 2010) since Hg and MeHg tend to form complexes with organic carbon. The interaction between DOC and Hg_t respectively MeHg can partly be attributed to the binding of Hg with reactive S groups in the dissolved organic molecules, especially in the hydrophobic acid fraction of DOC (Karlsson

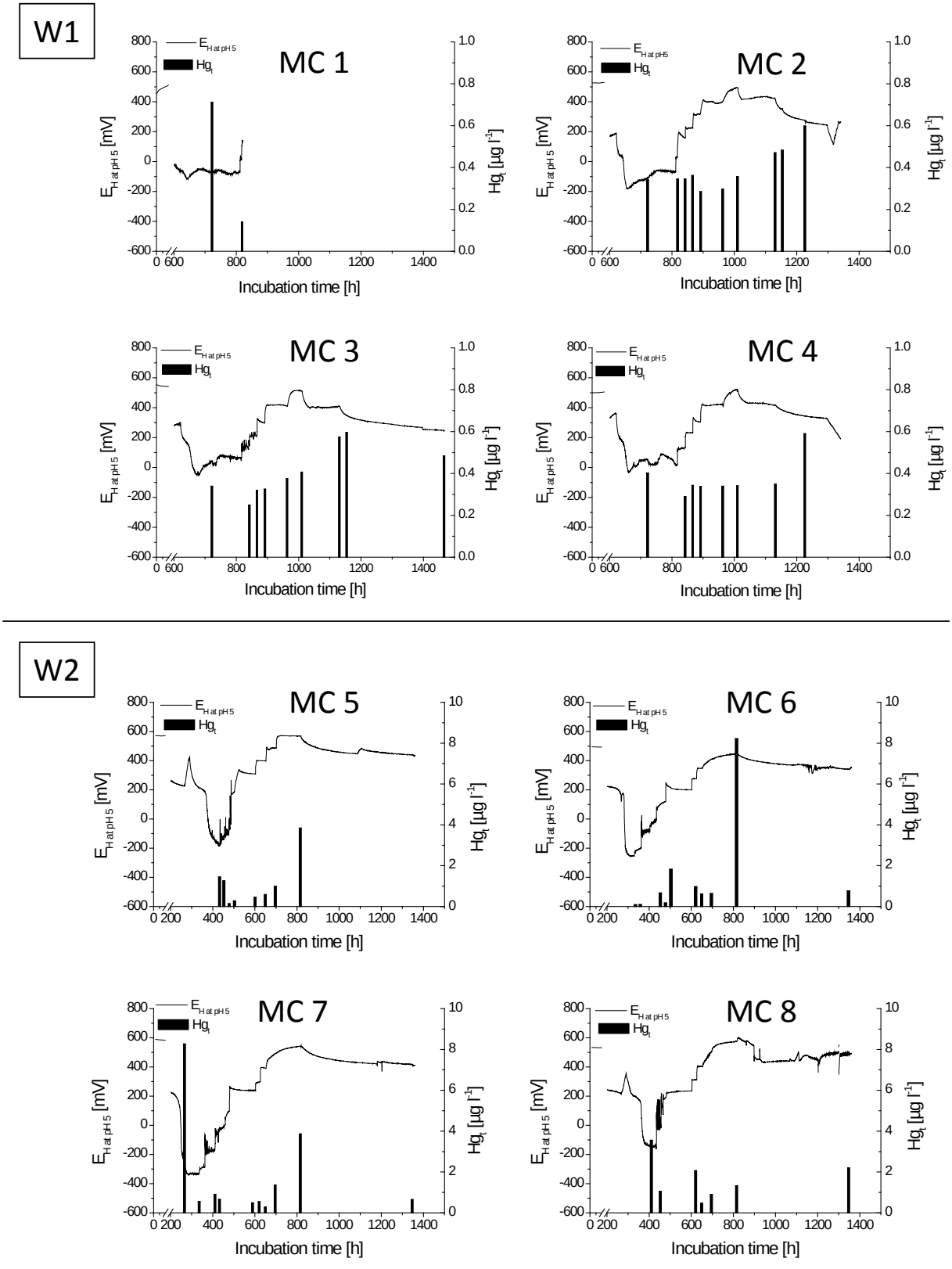


Fig. 4. E_{HatpH5} measured in the slurry every 10 min and Hg_t concentrations in the soluble fraction during the experiment for each microcosm (MC) (Wupper 1 = W1, Wupper 2 = W2).

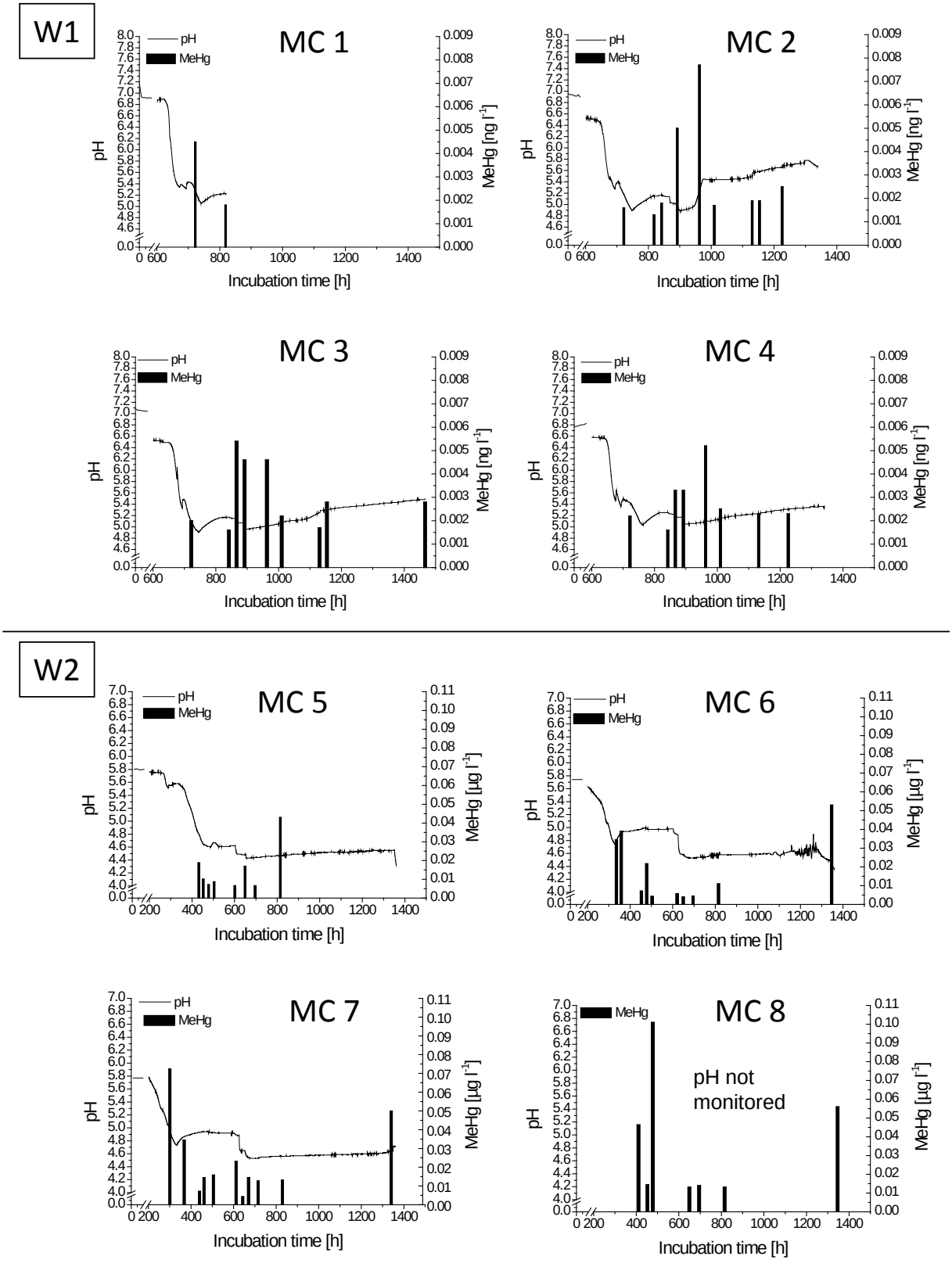


Fig. 5. Development of pH measured in the slurry every 10 min and MeHg concentrations in the soluble fraction during the experiment for each microcosm (MC) (Wupper 1 = W1, Wupper 2 = W2).

Table 3. Regressions between total mercury (Hg_t) resp. methyl mercury (MeHg) vs. anions (Cl^-, SO_4^{2-}), DOC, E_{HatpH5}, Fe, and pH in the soluble fraction. (+) positive relationship; (-) negative relationship; ns = not significant with $p \geq 0.05$.

		$lnHg_t$	lnMeHg
lnDOC	regression equation	Y = 7.674 + 0.209 X	Y = 8.739 + 0.236 X
	R^2	**0.26 (+)**	**0.53 (+)**
	p	<0.0001	<0.0001
	n	65	65
$lnSO_4^{2-}$	regression equation	Y = 2.956 + 0.697 X	Y = 4.962 + 0.473 X
	R^2	**0.28 (+)**	**0.20 (+)**
	p	<0.0001	<0.0005
	n	65	65
lnCl-	regression equation	Y = 5.900 + 0.687 X	Y = 7.716 + 0.433 X
	R^2	**0.29 (+)**	**0.18 (+)**
	p	<0.0001	<0.001
	n	65	65
lnFe	regression equation	Y = 3.979–0.330 X	Y = 6.720 + 0.516 X
	R^2	0.02 (ns)	**0.08 (+)**
	p	0.236	<0.05
	n	65	65
pH	regression equation	Y = 4.957–0.0626 X	Y = 4.367–0.125 X
	R^2	0.03 (ns)	**0.17 (-)**
	p	0.19	<0.001
	n	65	65
E_{HatpH5}	regression equation	Y = 249.201 + 29.990 X	Y = −4.765–8.038E-4 X
	R^2	0.01 (ns)	0.03 (ns)
	p	0.36	0.19
	n	65	65

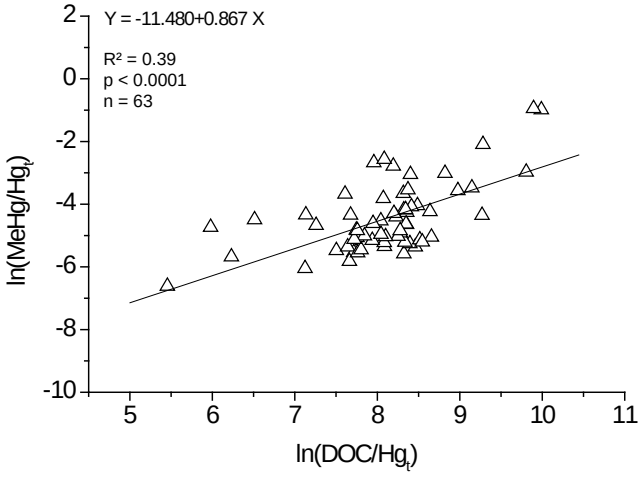


Fig. 6. Relation $ln(MeHg/Hg_t)$ vs. $ln(DOC/Hg_t)$ in the soluble fraction.

and Skyllberg, 2003; Ravichandran, 2004; Khwaja et al., 2006; Shanley et al., 2008).

In addition to the impact of DOC due to a complexation of Hg_t and MeHg, a positive relationship between $ln(MeHg/Hg_t)$ and $ln(DOC/Hg_t)$ was found in our study (Fig. 6). The $MeHg/Hg_t$ ratio is assumed to reflect the net production of MeHg normalized to the Hg concentration or the methylation efficiency respectively (Shanley et al., 2005; Skyllberg et al., 2007). Low $MeHg/Hg_t$ ratios can be due to low Hg methylation or to high MeHg demethylation rates (Remy et al., 2006). Thus, increasing DOC/Hg_t ratio might have favored Hg net methylation or decreased demethylation in the current study. One reason for rising DOC/Hg_t ratio might be increasing DOC concentrations. In this case, increasing DOC could have promoted Hg net methylation or depressed demethylation in our study. Other studies have shown that high DOC contents can promote Hg methylation by enhanced SRB activity (see below), since DOC can serve as an important carbon source for bacteria (Davis et al., 1997; Ullrich et al., 2001; Lambertsson and Nilsson, 2006). Furthermore, DOC can contribute to abiotic methylation of

Hg by donating methyl groups (Weber, 1993). However, abiotic methylation seems to be a process of minor importance compared to biological methylation (Avramescu et al., 2011). The positive relationship between ln(MeHg/Hg_t) and ln(DOC/Hg_t) seems to be important in our experiment. In contrast, the relationship between ln(MeHg/Hg_t) and lnDOC is weak, indicating that DOC alone might be a weaker factor in determining the Hg net methylation as also found by Skyllberg et al. (2003). Instead, the DOC/Hg_t ratio seems to play a more important role for Hg net methylation.

We also observed a negative relationship between ln(MeHg/Hg_t) and lnHg_t. A positive relationship was found between lnHg_t and lnMeHg as also reported by Sunderland et al. (2006) and Ouddane et al. (2008). Thus, increasing Hg_t concentrations seem to have an inhibitory effect on Hg net methylation but may lead to higher total MeHg concentrations. High Hg concentrations can generally affect soil microorganisms in different ways. First, it is reported that high Hg_t contents can exhibit toxic effects on methylating bacteria resulting in a depression of MeHg production (Ullrich et al., 2001). Secondly, microorganisms in Hg contaminated soils can be well adapted to Hg stress. This adaption can favor the selection of Hg tolerant bacteria in soils (Oliveira et al., 2010; Ruggiero et al., 2011). As a consequence, demethylation can be stimulated at high inorganic Hg concentrations by Hg tolerant bacteria which lead to reductive demethylation of MeHg (Schäfer et al., 2004). Bacterial Hg resistance is encoded by the mercury resistance (*mer)* operon encoding proteins that act amongst other factors in mercury detoxification. Most Hg resistant isolates contain *merB* (organomercury lyase), *merA* (mercuric reductase), *merP*, and *merR* genes (Lapanje et al., 2010; Ruggiero et al., 2011). Both toxic effects of Hg_t on methylating bacteria and the occurrence of Hg tolerant bacteria might have occurred in the current study in parallel. However, the correlations between Hg_t, MeHg, and MeHg/Hg_t are relatively low indicating that Hg_t appears to have limited utility as a predictor of Hg net methylation and MeHg concentrations. This is in good agreement with Ouddane et al. (2008) who indicates that the production of MeHg is dependent on other parameters such as SRB in addition to total Hg concentrations in sediments with high Hg methylation activity.

Concentrations of MeHg and Hg_t in the soluble fraction can also be influenced by the redox cycling of S, which is abundant in both soils (Table 1). The relationships between Hg_t and SO_4^{2-}, and between MeHg and SO_4^{2-} are moderate (Table 3) what might indicate that both Hg_t and MeHg may be linked to the sulfur cycle. Brümmer (1974) mentioned that sulfides are generated from sulfates below E_H −50 mV at pH 7 (corresponds to E_H 68 mV at pH 5). As the E_{HatpH5} values fell below 68 mV in all MCs during the incubation (Fig. 4), the formation of sulfides is most likely in our experiment. The soil slurries turned black and developed a typical odor with decreasing E_H, which also points towards the formation of sulfides. We did observe a correlation between E_{HatpH5} and SO_4^{2-}. However, this relationship is weak and not linear. Relationships between S_t and E_{HatpH5}, Hg_t, or MeHg could not be found. One reason for that can be the rapid internal cycling of S, which makes SO_4^{2-} concentrations a poor indicator for SO_4^{2-} reduction rates (Koretsky et al., 2007; Yu et al., 2007). When the concentration of reduced inorganic S reaches a certain value the solubility and speciation of Hg^{2+} may be controlled by the precipitation of insoluble HgS or the formation of charged polysulfide Hg-complexes as previously reported by several authors (e.g. Davis, 1997; Benoit et al., 2001; Du Laing et al., 2009). This may result in decreasing Hg concentrations in the dissolved phase when sulfates are removed from the dissolved phase upon reduction to sulfides. On the other hand, this may also result in the solubilisation of mercury upon oxidation of sulfides to sulfates under oxic conditions. Both processes may explain the positive correlations we observed between dissolved SO_4^{2-} and Hg_t and MeHg concentrations. The formation of HgS at low E_H can also decrease the availability of Hg^{2+} for methylation, consequently reducing MeHg production (Ullrich et al., 2001; Han et al., 2008). In contrast, reducing conditions can promote microbial mediated S reduction, which in turn can increase Hg methylation (Duran et al., 2008). In addition, high sulfide concentrations in marine environments containing organic matter seem to promote the uptake of Hg^{2+} by methylating bacteria such as sulfate reducing bacteria (SRB) maybe due to enhanced Hg bioavailability in mixed DOM-Hg-S complexes (Benoit et al., 2001; Sunderland et al., 2006). Sulfate reducing bacteria have been identified to be the principal methylators of inorganic Hg in sediments (Compeau and Bartha, 1985; King et al., 2002). The range of bacterial activity is large due to the variation in quantity and quality of organic matter, abundance of SRB, temperature, and SO_4^{2-} availability (Pallud and van Capellen, 2006). Various PLFA have been frequently used as biomarkers (e.g. SRB) to describe the microbial community structure in different environments (Taylor and Parkes, 1985; Coleman et al., 1993; Macalady et al., 2000; Wegener et al., 2008). The PLFA which might indicate the presence of *Desulfobacter* are 10Me16:0, cy17:0, 10Me18:0, and cy19:0 (Kohring et al., 1994). Those PLFA were abundant in both studied soils (Fig. 3). The fatty acids 10Me16:0 and 10Me18:0 might serve as indicators for *Desulfobacter* and additionally for actinomycetes (Taylor and Parkes, 1983; Frostegård, 1993). The polyunsaturated fatty acid 18:2ω6,9 might indicate the occurrence of *Desulfovibrio* (Macalady et al., 2000) or fungi (Frostegård, 1993). *Desulfobulbus* species are characterized by unbranched fatty acids such as 15:0 (Taylor and Parkes, 1983), which is widely distributed among different bacterial taxa (Macalady et al., 2000). Iron reducing bacteria such as *Geobacter* species are also able to methylate mercury in pure cultures at rates comparable to *Desulfobulbus* (Fleming et al, 2006; Kerin et al., 2006; Windham-Myers et al., 2009). Additionally, Avramescu et al. (2011) reported that

Fe reduction through Fe reducing bacteria might decrease demethylation. According to Kohring et al. (1994), *Geobacter metallireducens* mainly consists of the PLFA 16:1ω7c and 16:0, which are abundant in our soils (Fig. 3). These PLFA can also be found in many other organisms (Zelles, 1997). However, we might speculate that Fe reducing bacteria could be present in our soils and therefore might also contribute to Hg methylation in the current study. The soil W1 contains a higher quantity of PLFA compared to W2, both in numbers (W1 = 26; W2 = 20) and in total PLFA biomass (W1 $\approx$ 27 000 pmol g^{-1}; W2 $\approx$ 17 000 pmol g^{-1}). The number of PLFA which are related to SRB is 6 for both soils and the total biomass of these PLFA is approximately 5000 pmol g^{-1} for both soils. As measured by the total PLFA biomass, W1 contains 19 % PLFA related to SRB whereas W2 contains 29 % PLFA related to SRB. This indicates that SRB are more dominant at site W2. These values lead to the assumption that the relatively high Hg concentrations at the W2 site seem to have no direct toxic effect on SRB.

The statistical relationships between Hg_t and Cl^- as well as between MeHg and Cl^- are modest (Table 3) indicating that interrelations of these parameters might exist. Chloride can influence Hg speciation due to the competition of Cl^- with Hg_t and MeHg for binding sites of soil particles, which can reduce Hg adsorption onto soil particles and promote the release of Hg into the aquatic phase (Yin et al., 1997; Liu et al., 2009). This process probably occurred in our study. Moreover, the behavior of Hg in the soluble fraction can partly be affected by the formation of Hg-Cl-complexes, which is relevant at E_{HatpH5} >500 mV (Davis, 1997). Under the mostly acidic pH conditions which occurred in the current study, the formation of partly water soluble $HgCl_2$ as well as nearly water insoluble Hg_2Cl_2 is possible (Davis, 1997; Ullrich et al., 2001). Generally, in solution [MeHg]Cl is formed in the presence of Cl^- (Skyllberg et al., 2003). Thus, rising Hg_t and MeHg concentrations with increasing Cl^- concentrations in our study might indicate the formation of Hg-Cl compounds probably mostly under aerobic conditions (Davis et al., 1997; Takeno, 2005). The weak relationship between E_{HatpH5} and Cl^- reveals that Cl^- concentrations are not decisively influenced by E_H.

In general, Fe(hydr)oxides are able to adsorb Hg to a certain extent (Fernandez-Martinez et al., 2006; Liu et al., 2009; Harris-Hellal et al., 2011). Moreover, Mehrotra and Sedlak (2005) explained decreased mercury methylation in anoxic wetland slurries upon amendment of Fe(II) by reduced availability of Hg for methylation due to the formation of FeS which subsequently decreased the pool of bioavailable neutral mercury-sulfide species. Hollweg et al. (2009) indicate that Hg interacts with inorganic sulfur ligands in FeS complexes decreasing the bioavailability of Hg. In the current study, both soils contain considerable amounts of Fe (Table 1) and Fe(hydr)oxides should precipitate at high E_H which is confirmed by the negative relationship between Fe in the soluble fraction and E_{HatpH5}. However, no evidence was found in this study that Hg_t was linked to the Fe cycle whereas MeHg showed a weak relationship to Fe (Table 3). This may be attributed to the fact that DOC competes with Fe(hydr)oxides for binding Hg and MeHg (Feyte et al., 2010) and the DOC contents were high in our study. Additionally, pH might be an important factor in this context, because Hg is only preferentially sorbed to Fe(hydr)oxides in the neutral-alkaline pH-range (Ullrich et al., 2001). In summary, redox variations seem to affect the concentrations of dissolved Hg_t and MeHg indirectly through related changes in DOC, sulfur cycle, and microbial interaction and community structure whereas E_H and pH values, as well as concentration of dissolved Fe and Cl^- seem to play subordinate roles in Hg mobilization and methylation under our experimental conditions.

5 Conclusions

In our E_H experiment the ln(DOC/Hg_t) ratio is positively correlated to net MeHg production. This indicates that the ln(DOC/Hg_t) ratio seems to play an important role for the Hg net methylation. Dissolved organic carbon itself can mobilize Hg and MeHg due to the formation of soluble complexes. Mercury methylation also seems to be linked to the S chemistry while the influence of Fe and Cl^- on Hg methylation and speciation seems to be weak in our study. However, the methylation of Hg seems to be affected by the soil microbial community. On the one hand, Hg methylation might be favored by reducing conditions through enhanced microbial activity such as SRB bacteria, as indicated by the presence of the respective PLFA biomarkers. On the other hand, reducing conditions might lead to the formation of hardly available HgS what might contribute to a decrease of MeHg production. In conclusion, future studies on the fate of mercury in wetland soils should include silty and clayey soil material and should focus on the specific role of the soil microbial community structure.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/493/2012/bg-9-493-2012-supplement.pdf.

Acknowledgements. The authors thank M. Braun, S. Czickus, and C. Vandenhirtz for their technical assistance. Furthermore, we thank T. Labatzke and A. Becker (Analytik Jena AG) for their support in mercury analysis.

Edited by: S. Bouillon

References

Agusa, T., Kunito, T., Iwata, H., Monirith, I., Tana, T. S., Subramanian, A., and Tanabe, S.: Mercury contamination in human hair and fish from Cambodia: levels, specific accumulation and risk assessment, Environ. Pollut., 134, 79–86, 2005.

Avramescu, M.-L., Yumvihoze, E., Hintelmann, H., Ridal, J., Fortin, D., and Lean, D. R. S.: Biogeochemical factors influencing net mercury methylation in contaminated freshwater sediments from the St. Lawrence River in Cornwall, Ontario, Canada, Sci. Tot. Environ., 409, 968–978, 2011.

BBodSchV: Directive of the execution of the federal protection act (Federal soil protection and hazardous waste directive – BBodSchV) (in German), Bundesgesetzblatt I, 1554–1582, 1999.

Benoit, J. M., Gilmour, C. C., and Mason, R. P.: The influence of sulfide on solid phase mercury bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3), Environ. Sci. Technol., 35, 127–132, 2001.

Bergeron, C. M., Hopkins, W. A., Todd, B. D., Hepner, M. J., and Unrine, J. M.: Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae, Environ. Sci. Technol., 45, 3781–3787, 2011.

Bligh, E. and Dyer, W.: A rapid method of total lipid extraction and purification, Can. J. Physiol. Pharmacol., 37, 911–917, 1959.

Blume, H.-P., Deller, B., Furtmann, K., Leschber, R., Paetz, A., and Wilke, B.-M.: Handbuch der Bodenuntersuchung, Bd. 1 bis 7. DIN Vorschriften, Wiley-VCH, Weinheim, 2000.

Boening, D. W.: Ecological effects, transport, and fate of mercury: a general review, Chemosphere, 40, 1335–1351, 2000.

Brümmer, G.: Redoxpotentiale und Redoxprozesse von Mangan-, Eisen- und Schwefelverbindungen in hydromorphen Böden und Sedimenten, Geoderma, 12, 207–222, 1974.

Coelho-Souza, S. A., Guimaraes, J. R. D., Mauro, J. B. N., Miranda, M. R., and Azevedo, S. M. F. O.: Mercury methylation and bacterial activity associated to tropical phytoplankton, Sci. Tot. Environ., 364, 188–199, 2006.

Coleman, M. L., Hedrick, D. B., Lovley, D. R., White, D. C., and Pye, K.: Reduction of Fe(III) in sediments by sulfate-reducing bacteria, Nature, 361, 436–438, 1993.

Compeau, G. C. and Bartha, R.: Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sediment, Appl. Environ. Microb., 50, 498–502, 1985.

Covelli, S., Acquavita, A., Piani, R., Predonzani, S., and De Vittor, C.: Recent contamination of mercury in an estuarine environment (Marano lagoon, Northern Adriatic, Italy), Estuar. Coast. Shelf S., 82, 273–284, 2009.

Davis, A., Bloom, N. S., and Que Hee, S. S.: The environmental geochemistry and bioaccessibility of mercury in soils and sediments: A review, Risk Anal., 17, 557–569, 1997.

DeLaune, R. D., Jugsujinda, A., Devai, I., and Patrick Jr., W. H.: Relationship of sediment redox conditions to methyl mercury in surface sediment of Louisiana lakes, J. Environ. Sci. Heal. A, 39, 1925–1933, 2004.

Devai, I., Patrick, W. H., Neue, H.-U., DeLaune, R. D., Kongchum, M., and Rinklebe, J.: Methyl Mercury and Heavy Metal Content in Soils of Rivers Saale and Elbe (Germany), Anal. Lett., 38, 1037–1048, 2005.

Du Laing, G., Rinklebe, J., and Vandecasteele, B.: Trace metal behaviour in estuarine and riverine floodplain soils and sediments: a review, Sci. Tot. Environ., 407, 3972–3985, 2009.

Duran, R., Ranchou-Peyruse, M., Menuet, V., Monperrus, M., Bareille, G., Goni, M. S., Salvado, J. C., Amouroux, D., Guyoneaud, R., Donard, O. F. X., and Caumette, P.: Mercury methylation by a microbial community from sediments of the Adour Estuary (Bay of Biscay, France), Environ. Pollut., 156, 951–958, 2008.

During, A., Rinklebe, J., Böhme, F., Wennrich, R., Stärk, H.-J., Mothes, S., Du Laing, G., Schulz, E., and Neue, H.-U.: Mercury Volatilization from Three Floodplain Soils at the Central Elbe River (Germany), Soil Sediment Contam.: Int. J., 18, 429–444, 2009.

DWD (Deutscher Wetterdienst): Wetter und Klima - Deutscher Wetterdienst - Klimadaten, http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&pageLabel=_wdwww_klima_umwelt_klimadaten_deutschland&T82002gsbDocumentPath=Navigation{%}FOeffentlichkeit{%}2FKlima_Umwelt{%}2FKlimadaten{%}2Fkldaten_kostenfrei{%}2Fausgabe_mittelwerte_node.html_nnn{%}3Dtrue, Cited 2 December 2009.

Feng, Y., Motta, A., Reeves, D., Burmester, C., van Santen, E., and Osborne, J.: Soil microbial communities under conventional-till and no-till continuous cotton systems, Soil Biol. Biochem., 35, 1693–1703, 2003.

Fernandez-Martinez, R., Loredo, J., Ordonez, A., and Rucandio, M. I.: Physicochemical characterization and mercury speciation of particle-size soil fractions from an abandoned mining area in Mieres, Asturias (Spain), Environ. Pollut., 142, 217–226, 2006.

Feyte, S., Tessier, A., Gobeil, C., and Cossa, D.: In situ adsorption of mercury, methylmercury and other elements by iron oxyhydroxides and organic matter in lake sediments, Appl. Geochem., 25, 984-995, 2010.

Fleming, E. J., Mack, E. E., Green, P. G., and Nelson, D. C.: Mercury Methylation from Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-Reducing Bacterium, Appl. Environ. Microb., 72, 457–464, 2006.

Fowler, J., Cohen, L., and Jarvis, P.: Practical Statistics for Field Biology, Wiley, Chichester, 2006.

Frohne, T., Rinklebe, J., Diaz-Bone, R. A., and Du Laing, G.: Controlled variation of redox conditions in a floodplain soil: Impact on metal mobilization and biomethylation of arsenic and antimony, Geoderma, 160, 414–424, 2011.

Frostegård, A., Tunlid, A., and Bååth, E.: Microbial biomass measured as total lipid phosphate in soils of different organic content, J. Microbiol. Meth., 14, 151–163, 1991.

Frostegård, A., Tunlid, A., and Bååth, E.: Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals, Appl. Environ. Microb., 59, 3605–3617, 1993.

Gibicar, D., Horvat, M., Nakou, S., Sarafidou, J., and Yager, J.: Pilot study of intrauterine exposure to methylmercury in Eastern Aegean islands, Greece, Sci. Tot. Environ., 367, 586–595, 2006.

Gilmour, C. C. and Henry, E. A.: Mercury methylation in aquatic systems affected by acid deposition, Environ. Pollut., 71, 131–169, 1991.

Guckert, J. B., Antworth, C. P., Nichols, P. D., and White, D. C.: Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in procaryotic community structure of estuarine sediments, FEMS Microbiol. Ecol., 31, 147–158, 1985.

Han, S., Obraztsova, A., Pretto, P., Deheyn, D. D., Gieskes, J., and Tebo, B. M.: Sulfide and iron control on mercury speciation in anoxic estuarine sediment slurries, Mar. Chem., 111, 214–220, 2008.

Harris-Hellal, J., Grimaldi, M., Garnier-Zarli, E., and Bousserrhine, N.: Mercury mobilization by chemical and microbial iron oxide reduction in soils of French Guyana, Biogeochemistry, 103, 223–234, 2011.

Hintelmann, H. and Wilken, R.-D.: Levels of total mercury and methylmercury compounds in sediments of the polluted Elbe River: influence of seasonally and spatially varying environmental factors, Sci. Tot. Environ., 166, 1–10, 1995.

Hollweg, T. A., Gilmour, C. C., and Mason, R. P.: Methylmercury production in sediments of Chesapeake Bay and the mid-Atlantic continental margin, Mar. Chem., 114, 86–101, 2009.

IUSS-ISRIC-FAO: World reference base for soil resources. A framework for international classification, correlation and communication, World Soil Resources Reports, 103, 128 S., Rome (FAO), 2006.

Karlsson, T. and Skyllberg, U.: Bonding of ppb levels of methyl mercury to reduced sulfur groups in soil organic matter, Environ. Sci. Technol., 37, 4912–4918, 2003.

Kerin, E. J., Gilmour, C. C., Roden, E., Suzuki, M. T., Coates, J. D., and Mason, R. P.: Mercury Methylation by Dissimilatory Iron-Reducing Bacteria, Appl. Environ. Microb., 72, 7919–7921, 2006.

Khwaja, A. R., Bloom, P. R., and Brezonik, P. L.: Binding Constants of Divalent Mercury (Hg^{2+}) in Soil Humic Acids and Soil Organic Matter Environ. Sci. Technol., 40, 844–849, 2006.

King, J. K., Harmon, S. M., Fu, T. T., and Gladden, J. B.: Mercury removal, methylmercury formation, and sulfate-reducing bacteria profiles in wetland mesocosms, Chemosphere, 46, 859–870, 2002.

Kohring, L. L., Ringelberg, D. B., Devereux, R., Stahl, D. A., Mittelman, M. W., and White, D. C.: Comparison of phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA sequence similarities among dissimilatory sulfate-reducing bacteria, FEMS Microbiol. Lett., 119, 303–308, 1994.

Koretsky, C. M., Haveman, M., Beuving, L., Cuellar, A., Shattuck, T., and Wagner, M.: Spatial variation of redox and trace metal geochemistry in a minerotrophic fen, Biogeochemistry, 86, 33–62, 2007.

Lambertsson, L. and Nilsson, M.: Organic material: The primary control on mercury methylation and ambient methyl mercury concentrations in estuarine sediments, Environ. Sci. Technol., 40, 1822–1829, 2006.

Langer, U. and Rinklebe, J.: Lipid biomarkers for assessment of microbial communities in floodplain soils of the Elbe River (Germany), Wetlands, 29, 353–362, 2009.

Lapanje, A., Zrimec, A., Drobne, D., and Rupnik, M.: Long-term Hg pollution-induced structural shifts of bacterial community in the terrestrial isopod (Porcellio scaber) gut, Environ. Pollut., 158, 3186–3193, 2010.

Li, P., Feng, X., and Qiu, G.: Methylmercury exposure and health effects from rice and fish consumption: A review, Int. J. Environ. Res. Public Health, 7, 2666–2691, 2010.

Liu, G., Cai, Y., Mao, Y., Scheidt, D., Kalla, P., Richards, J., Scinto, L. J., Tachiev, G., Roelant, D., and Appleby, C.: Spatial variability in mercury cycling and relevant biogeochemical controls in the Florida Everglades, Environ. Sci. Technol., 43, 4361–4366, 2009.

Macalady, J., Mack, E. E., Nelson, D. C., and Scow, K. M.: Sediment Microbial Community Structure and Mercury Methylation in Mercury-Polluted Clear Lake, California, Appl. Environ. Microb., 66, 1479–1488, 2000.

Mehrotra, A. S. and Sedlak, D. L.: Decrease in net mercury methylation rates following iron amendment to anoxic wetland sediment slurries, Environ. Sci. Technol., 39, 2564–2570, 2005.

Merritt, K. A. and Amirbahman, A.: Mercury methylation dynamics in estuarine and coastal marine environments - A critical review, Earth-Science reviews, 96, 54–86, 2009.

Obrist, D., Johnson, D. W., and Lindberg, S. E.: Mercury concentrations and pools in four Sierra Nevada forest sites, and relationships to organic carbon and nitrogen, Biogeosciences, 6, 765–777, doi:10.5194/bg-6-765-2009, 2009.

Ouddane, B., Mikac, N., Cundy, A. B., Quillet, L., and Fischer, J. C.: A comparative study of mercury distribution and methylation in mudflats from two microtidal estuaries: The Seine (France) and the Medway (United Kingdom), Appl. Geochem., 23, 618–631, 2008.

Oliveira, A., Pampulha, M. E., Neto, A. C., and Almeida, A. C.: Mercury tolerant diazotrophic bacteria in a long-term contaminated soil, Geoderma, 154, 359–363, 2010.

Overesch, M., Rinklebe, J., Broll, G., and Neue, H.-U.: Metals and arsenic in soils and corresponding vegetation at Central Elbe river floodplains (Germany), Env. Poll., 145, 800–812, 2007.

Pallud, C. and Van Capellen, P.: Kinetics of microbial sulfate reduction in estuarine sediments, Geochim. Cosmochim. Ac., 70, 1148–1162, 2006.

Ravichandran, M.: Interactions between mercury and dissolved organic matter-a review, Chemosphere, 55, 319–331, 2004.

Remy, S., Prudent, P., and Probst, J.-L.: Mercury speciation in soils of the industrialised Thur River catchment (Alsace, France), Appl. Geochem., 21, 1855–1867, 2006.

Rinklebe, J. and Langer, U.: Microbial diversity in three floodplain soils at the Elbe River (Germany), Soil Biol. Biochem., 38, 2144–2151, 2006.

Rinklebe, J., During, A., Overesch, M., Wennrich, R., Stärk, H.-J., Mothes, S., and Neue, H.-U.: Optimization of the simple field method to determine mercury volatilization from soils – Examples of 13 sites in floodplain ecosystems at the Elbe River (Germany), Ecol. Eng., 35, 319–328, 2009.

Rinklebe, J., During, A., Overesch, M., Du Laing, G., Wennrich, R., Stärk, H.-J., and Mothes, S.: Dynamics of mercury fluxes and their controlling factors in large Hg-polluted floodplain areas, Environ. Pollut., 158, 308–318, 2010.

Ruggiero, P., Terzano, R., Spagnuolo, M., Cavalca, L., Colombo, M., Andreoni, V., Rao, M. A., Perucci, P., and Monaci, E.: Hg bioavailability and impact on bacterial communities in a long-term polluted soil, J. Environ. Monitor., 13, 145–156, 2011.

Rupp, H., Rinklebe, J., Bolze, S., and Meissner, R.: A scale-depended approach to study pollution control processes in wetland soils using three different techniques, Ecol. Eng., 36, 1439–1447, 2010.

Schäfer, J. K., Yagi, J., Reinfelder, J. R., Cardona, T., Ellickson, K. M., Tel-Or, S., and Barkay, T.: Role of the Bacterial Organomercury Lyase (MerB) in Controlling Methylmercury Accumulation in Mercury-Contaminated Natural Waters, Environ. Sci. Tech-

nol., 38, 4304–4311, 2004.
Schlichting, E., Blume, H.-P., and Stahr, K.: Bodenkundliches Praktikum, Blackwell, Berlin, 1995.
Shanley, J. B., Kamman, N. C., Clair, T. A., and Chalmers, A.: Physical controls on total and methylmercury concentrations in streams and lakes of the northeastern USA, Ecotox., 14, 125–134, 2005.
Shanley, J. B., Mast, M. A., Campbell, D. H., Aiken, G. R., Krabbenhoft, D. P., Hunt, R. J., Walker, J. F., Schuster, P. F., Chalmers, A., Aulenbach, B. T., Peters, N. E., Marvin-DiPasquale, M., Clow, D. W., and Shafer, M. M.: Comparison of total mercury and methylmercury cycling at five sites using the small watershed approach, Environ. Pollut., 154, 143–154, 2008.
Skyllberg, U., Qian, J., Frech, W., Xia, K., and Bleam, W. F.: Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment, Biogeochemistry , 64, 53–76, 2003.
Skyllberg, U., Drott, A., Lambertsson, L., Björn, E., Karlsson, T., Johnson, T., Heinemo, S. A., and Holmström, H.: Net methylmercury production as a basis for improved risk assessment of mercury-contaminated sediments, Ambio, 36, 437–442, 2007.
Sunderland, E. M., Gobas, F. A. P. C., Branfireun, B. A., and Heyes, A.: Environmental controls on the speciation and distribution of mercury in coastal sediments, Mar. Chem., 102, 111–123, 2006.
Takeno, N.: Atlas of Eh-pH diagrams, Intercomparison of thermodynamic databases, Geological Survey of Japan Open File Report, 419, http://www.gsj.jp/GDB/openfile/files/no0419/openfile419e.pdf, cited 08 June 2011, 2005.
Taylor, J. and Parkes, R. J.: The Cellular Fatty Acids of the Sulphate-reducing Bacteria Desulfobacter sp., Desulfobulbus sp. and Desulfovibvio desulfuvicans, J. Gen. Microbiol., 129, 3303–3309, 1983.
Taylor, J. and Parkes, R. J.: Identifying Different Populations of Sulphate-reducing Bacteria within Marine Sediment Systems, Using Fatty Acid Biomarkers, J. Gen. Microbiol., 131, 631–642, 1985.
Ullrich, S. M., Tanton, T. W., and Abdrashitova, S. A.: Mercury in the aquatic environment: A review of factors affecting methylation, Crit. Rev. Env. Sci. Tech., 31, 241–293, 2001.
Van Faassen, H. G.: Short communication - Methylation of mercury compounds in soil, sediment and sewage-sludge samples, Plant Soil, 44, 505–509, 1975.
Wallschläger, D., Desai, M. V. M., and Wilken, R.-D.: The role of humic substances in the aqueous mobilization of mercury from contaminated floodplain soils, Water Air Soil Poll., 90, 507–520, 1996.
Wallschläger, D., Desai, M. V. M., Spengler, M., Carvalhinho Windmöller, C., and Wilken, R. D.: How humic substances dominate mercury geochemistry in contaminated floodplain soils and sediments, J. Environ. Qual., 27, 1044–1054, 1998.
Weber, J. H.: Review of possible paths for abiotic methylation of mercury (II) in the aquatic environment, Chemosphere, 26, 2063–2077, 1993.
Wegener, G., Shovitri, M., Knittel, K., Niemann, H., Hovland, M., and Boetius, A.: Biogeochemical processes and microbial diversity of the Gullfaks and Tommeliten methane seeps (Northern North Sea), Biogeosciences, 5, 1127–1144, doi:10.5194/bg-5-1127-2008, 2008.
White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbie, R. J.: Determination of the sedimentary microbial biomass by extractable lipid phosphate, Oecologia, 40, 51–62, 1979.
Windham-Myers, L., Marvin DiPasquale, M., Krabbenhoft, D. P., Agee, J., and Cox, M. H.: Experimental removal of wetland emergent vegetation leads to decreased methylmercury production in surface sediment, J. Geophys. Res., 114, G00C05, doi:10.1029/2008JG000815, 2009.
Wolfe, M. F., Schwarzbach, S., and Sulaiman, R. A.: Effects of mercury on wildlife: A comprehensive review, Environ. Toxicol. Chem., 17, 146–160, 1998.
Wu, H., Ding, Z., Liu, Y., Liu, J., Yan, H., Pan, J., Li, L., Lin, H., Lin, G., and Lu, H.: Methylmercury and sulfate-reducing bacteria in mangrove sediments from Jiulong River Estuary, China, J. Environ. Sci., 23, 14–21, 2011.
Wupperverband: FluGGS: FlussgebietsGeoinformationsSystem der Wupperverbandes, http://fluggs.wupperverband.de/internet/initParams.do, Cited 15 Dec 2009.
Yin, Y., Allen, H. E., Huang, C. P., and Sanders, P. F.: Effects of pH, chloride and calcium(II) on adsorption of monomethylmercury by soils, Environ. Toxicol. Chem., 16, 2457–2462, 1997.
Yu, K. and Rinklebe, J.: Advancement in soil microcosm apparatus for biogeochemical research, Ecol. Eng., 37, 2071–2075, 2011.
Yu, K., Böhme, F., Rinklebe, J., Neue, H.-U., and DeLaune, R. D.: Major biogeochemical processes in soils - A microcosm incubation from reducing to oxidizing conditions, Soil Sci.Soc. Am. J., 71, 1406–1417, 2007.
Zelles, L.: Phospholipid fatty acid profiles in selected members of soil microbial communities, Chemosphere, 35, 275–294, 1997.