Mercury mobility and methylmercury formation in a contaminated agricultural flood plain: Influence of flooding and manure addition.

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Abstract

The fate and the methylation of mercury (Hg) in the terrestrial environment are still poorly understood and although the main drivers of release and methylation of mercury in soils are known (low redox potential and microbial carbon availability) their interactions are not well understood. This is of concern since many agriculturally used floodplains, where the recurring flooding and agricultural practices (e.g. manure amendments) may have an impact on the fate and the biomethylation of Hg, are at the same time Hg-contaminated. In this study, we modified and validated existing methods to extract and analyze methylmercury (MeHg) by HPLC-ICP-MS in soils and we assessed the Hg and MeHg concentrations in three fields situated in a Hg polluted agricultural floodplain. Further, we incubated the top soil from the three studied fields for 11 days under flooded conditions in presence or absence of 2 mass % of cow manure, a common agricultural amendment in the area. Total Hg and MeHg concentrations ranged from <limit of detection (LOD, 0.012 mg/kg) to 28.2 mg/kg and from 1.2 to 7.8 µg/kg respectively. Hg was released to the soil solution after 12 hours with a maximum between day 2 and day 7. MeHg levels in the soil solution were < LOD although it was found in the soil before and after the incubation. The addition of cow manure to saturated soils led to an increase in the MeHg concentrations of the soil solid phase by up to fivefold to a maximum of 26.4 ± 1.1 µg/kg (n=3). Our study demonstrates

that the combination of low redox potential because of flooding with common agricultural practices such as the amendment of manures enhances the formation of toxic MeHg.

Introduction

Sources of mercury (Hg) to the environment can be both natural (e.g., volcanoes) and anthropogenic (e.g., chemical industry, fossil fuel burning, incineration of waste or the extraction of metallic gold from alluvial washings)[1,2]. The most important uses of Hg in the chemical industry are the chloralkali and acetaldehyde production, where Hg serves as an electrode and a catalyst respectively[3]. Such activities can cause severe Hg pollution to the aquatic and terrestrial ecosystems with risks of mass poisoning, the Minamata catastrophe in Japan in the 1950s being a prime example[4]. There, the unintentional formation and release of monomethylmercury (MeHg), a potent neurotoxin, in the local marine bay and MeHg biomagnification in the food web due to its lipophilic nature[5], caused the death of almost 1000 people. This was due to the high levels of MeHg in fish and shellfish combined with a fish-rich diet of the local people[4,6,7]. Because of the catastrophe in Minamata, research focused on Hg biomethylation, first in the aquatic environment and later in the terrestrial environment with a focus on paddy rice and forest soils[8]. However the drivers behind Hg behavior and its methylation in soils are not well understood[3,9–11].

In soils, Hg mobility and methylation are mainly governed by the redox potential (Eh), dissolved/soil organic matter (DOM/SOM), sulfur (S) and selenium (Se) concentrations, pH and both the presence of iron (Fe-) and manganese (Mn-)oxyhydroxides[12–15]. MeHg is assumed to be mainly formed by sulfate-reducing bacteria (SRB), Fe-reducing bacteria (FeRB) and methanogens under anoxic conditions from dissolved inorganic mercury (Hg^{2+})[9,16–18]. Further, it is agreed, that Hg methylation depends on the speciation of both dissolved and solid-phase inorganic Hg^{2+} rather than the total Hg pool[9,19,20]. DOM plays a key role in Hg methylation and mobility, since on one hand it serves as a carbon (C) source and an electron donor for Hg methylators and it was shown to influence the Hg bioavailability by complexing Hg^{2+} [11,21–23]. The affinity of Hg to both DOM and SOM is well documented. In organic molecules, Hg shows the highest affinity to thiol functional groups but also carboxyl and amino groups are complexing Hg[12,13,24,25]. Recent work showed that also DOM quality (e.g. molecular weight or chemical composition originating from different sources) plays

an important role in Hg bioavailability and methylation[26–28]. Experiments of Zhao and co-workers[23] showed that the presence of DOM inhibited Hg methylation by an Fe-reducing bacterium, but enhanced Hg methylation by a sulfate-reducing bacterium. In the latter study, it was suspected that thiol functional groups in DOM and on the cell's surfaces compete for Hg. Fe and Mn oxy-hydroxides are important sorbents of Hg and organic molecules. Consequently, organo-Hg complexes may sorb on the mineral surfaces and be subsequently released during reductive dissolution of the oxy-hydroxides[29–31]. Recent work showed that mineral bound OM-Hg and POM-Hg can act as a direct Hg source for Hg methylators[32]. Se also seems to impact methylation by limiting Hg availability due to the formation of Se-Hg complexes and mercuric selenide if selenide is present[11]. Fundamental questions about the biomethylation itself such as the mechanism of Hg uptake by methylating microorganisms and the biochemical pathway by which MeHg is produced still remain unanswered[33].

Recurring reducing conditions and OM supply are the main methylation promoters in soils. For instance, ecosystem-scale studies reported higher MeHg in forest soils after flooding as a result of the change to anoxic conditions and increased DOM supply through decomposing vegetation[34–36]. Another experiment in rice paddies showed that flooding increased the Hg bioavailability and methylation[37], while soil incubation experiments reported Hg mobilization and methylation in flooded soils[38,39]. The addition of organic amendments, such as rice straw, in paddy soils increased the methylation rate of Hg, likely because it provided additional DOM as C source for the methylating organisms[40,41] The application of mineral fertilizers can also increase the methylation rate of Hg in agricultural systems, because it enhances the microbial activity[42]. In forest soils, fertilizers seem to enhance methylation as a result of increasing pH and microbial activity[43].

There is still a lack of experimental studies that investigate the influence of flooding and common agricultural practices, such as manure addition, on the behavior of Hg although agriculture on Hg-contaminated floodplain soils might present substantial risks to human health. Decreasing Eh in soils leads to Hg^{2+} mobilization into the soil solution as a result of the reductive dissolution of Mn- and Fe-oxyhydroxides and associated release of adsorbed or occluded Hg[3,44–49]. The few existing studies on the topic focused on the influence of water logging in forest soils[50,51] and flooding and

OM concentrations in rice paddies[40–42,52–54], while studies on temperate agricultural fields have been scarce[38,55], although Hg-contaminated sites can often be found in floodplains[56].

Here, we investigated the release and the methylation of Hg in agricultural floodplain soils contaminated by an acetaldehyde-producing company in order to better characterize the release and methylation of Hg and assess the potential future threat that Hg in agriculturally used Hg-contaminated floodplain soils represents. To do so, we modified and validated a fast extraction and analysis technique for MeHg in soils using HPLC-ICP-MS. Then, we determined the horizontal and vertical spatial distribution of Hg and MeHg in soil. Thirdly, we conducted an incubation experiment with soils from the same three fields to study the influence of flooding and manure amendment on Hg release and methylation.

Methods

Reagents

During the whole study, ultrapure water from a laboratory water system (MilliQ, ≥18.2MΩ resistivity, Merck Millipore Inc., Burlington, MA, USA) was used for extractants and standard solutions, whereas a central system supplied deionized (DI) water for the incubation experiment and the various washing solutions. MeHg standard solutions were prepared by dissolving methylmercury(II)chloride (Sigma-Aldrich, Buchs, CH) in a small volume of methanol and then in ultrapure water. Multi-element standard (ICPMS-71A, Inorganic Ventures, Christianburg VA, US), and Hg standard (Fluka, Buchs, CH) were used for the total element analyses. Extractions were conducted with hydrochloric acid (HCl, 35%, Supra quality) from Roth (Karlsruhe, DE) and Dichloromethane (DCM, pro analysi) from Fisher Scientific (Loughborough, UK). The HPLC mobile phase (98 % of 0.1 % w/v L-cysteine + 0.1 % L-cysteine·HCl·H_2O (pH = 2.3) and 2 % methanol) was prepared with L-cysteine (Merck KGaA, Darmstadt, DE), L-cysteine·HCl·H_2O (VWR Chemicals, Leuven, BE) and HPLC grade methanol (Merck Millipore Inc., Burlington, MA, USA). Nitric acid (HNO_3, 69 %, Supra quality) from Roth and hydrogen peroxide (H_2O_2, Suprapur quality) from Fluka were used for total digestions.

Study sites and soil sampling

We sampled soils (all Fluvisols[57]) in three fields from an agricultural floodplain contaminated with Hg originating from an acetaldehyde-producing company. The investigated fields are situated in Switzerland, in the Rhone Valley, between the cities of Visp and Raron. Between the 1930s and the 1970s, the company released Hg in a discharge canal linked to the Rhone river which then flows into the Lake Geneva. This canal was periodically cleaned, and the sediments were disseminated in the valley, including the surrounding agricultural fields[58]. The three sampling sites were situated along this canal which is called the Grossgrundkanal. They were chosen because they fulfilled the following conditions: they were (I) situated in the floodplain of the valley, (II) agriculturally used, and (III) contaminated with Hg (preliminary data obtained from the Agency for the Environment of the Canton Valais) . All three soils have a silt loam texture, neutral to slightly alkaline pH, and a low to average organic carbon concentration in the topsoil (Tab. 1). At each site, soil cores of 50 cm depth were taken with a Pürckhauer corer (diameter 2 cm). Cores were drilled in triplicate at four distinct distances (5, 10, 20, 40 m) from the canal. For sampling, the plant cover was removed, and the core was divided into five depth intervals: 0-10, 10-20, 20-30, 30-40 and 40-50 cm. At each site, the three samples of the same depth and distance were pooled to result in a total of 60 composite samples. In addition to the soil cores, composite topsoil samples (0-10 cm, 1-2 kg) were collected at a distance of 5 m from the canal at each sampling site. These topsoil samples were used for the incubation experiment and kept fresh in a closed plastic bag (no drying) in the freezer at -20°C prior to incubation. The soil core samples were first frozen at -20 °C just after sampling and then freeze-dried for approximately 48h prior to extraction and analysis[59]. Dry samples were sieved to <2 mm, homogenized and ground manually with a ceramic mortar.

Soil characterization

For pH measurement, 10 g of soil was suspended in 25 mL of 0.01 M $CaCl_2$. Measurements were conducted 2 hours after equilibration. Soil moisture content was determined by weighing the soil before and after oven-drying and bulk density by collecting soil samples in the field in 100-cm^3 stainless steel rings. The loss on ignition (LOI) technique (550 °C for two hours muffle furnace) was used to determine the organic matter concentration. The C and N concentrations were measured before and after the measurement of LOI to determine total and inorganic C (and organic

C as the difference between total and inorganic C) using an elemental analyzer (vario El cube, Elementar Analysensysteme, Langenselbold, Germany). DOC in soil solution was measured with a vario TOC cube (Elementar Analysensysteme, Langenselbold, Germany). Eh and pH were measured with an Eh electrode (SenTix ORP, WTW, Dinslaken, Germany) and a pH electrode (SenTix 21, WTW, Dinslaken, Germany) connected to a pH meter (pH 330i, WTW). For grain size analyses soil samples were first treated with H_2O_2 in order to remove organic matter and then dispersed in a solution of 22 mM sodium carbonate and 18 mM sodium hexametaphosphate. before the particle-size composition was measured on a MasterSizer 2000 (Malvern Panalytical Ltd., Malvern, UK). The concentration of sulfate (SO_4^{2-}) in soil solution was measured using ion chromatography (ICS-900, Dionex, Sunnyvale CA, USA).

Microwave-Assisted Digestion for Total Concentrations

Each sample from the soil cores as well as a representative aliquot of the topsoil used for incubation were digested using a microwave-assisted technique (Ethos contFlow 1600, milestone, Shelton CT, US) with 8 mL of 69 % HNO_3 and 2 mL 30 % H_2O_2 for 0.1 g of soil. For each of the three sites, two samples were digested in triplicates. For quality control, a Certified Reference Material (CRM, NIST San Joaquin Soil 2709a) and a blank sample were included in each microwave run. After digestion, each sample was brought to 20 mL with ultrapure water.

Soil extractions for MeHg

Typically, MeHg extraction and analysis was conducted on the soils before and after incubation and on a subset of samples from the cores (5 and 10 m distance to canal, 0-10, 10-20, 20-30 cm depth). Commonly, MeHg in soils and sediments only accounts for a small fraction of the total Hg concentration (<1%)[9]. Hence, there was a need for a selective extraction for MeHg. Therefore, we adapted the method developed by Brombach and coworkers in order to make it compatible with HPLC-ICP-MS measurement[60]. Briefly, 50 mg of sample was suspended in 5 mL of 35 % HCl and 5 mL ultrapure water in a 20 mL borosilicate glass vial (Wheaton, Milleville, NJ, UK). After 30 min of ultra-sonication, the vial was centrifuged for 3 min at 680g (3500 rpm) and the supernatant transferred into a 50-mL separating funnel. Then, the lipophilic organic Hg was extracted two times by shaking with DCM in a separating funnel (2 x 4 mL). The two DCM solution thus obtained

were combined in a new 20 mL borosilicate glass vial. To prevent contamination, the separating funnels were washed by shaking first with a 10% HNO_3 solution, and then a 10% HCl solution, and DI water after each acid wash. The main modification we made to the Brombach et al.[60] method is that we used the HPLC mobile phase (L-cystein solution) for the back extraction in order to make it compatible with a HPLC-ICP-MS measurement. In detail, 2 mL of the HPLC mobile phase were added to the DCM extract and the DCM was evaporated with a constant flow of N_2 by using a FlexiVap™ at 50°C (GlasCol®, Terre Haute, IN, USA). The remaining L-cysteine solution was then weighed to determine its exact volume. The extracts were stored at 4°C and analyzed within 48 hours. The method was tested using a CRM (ERM-CC580, estuarine sediment) and blank extractions with only the reagents and no soil sample.

Incubation Experiment

Two days before the start of the experiment, the soil samples were thawed in a fridge at 4°C and moist sieved < 2 mm. The three prepared soils were then incubated in triplicate (80 g each) in 9 Erlenmeyer flasks (250 mL). Another 9 identical samples were incubated with 2 mass% additional OM (78.4 g soil and 1.6 g OM). The added OM was commercially available cow dung manure, which was dry and finely chopped (Hauert HBG Dünger AG, Grossaffoltern, Switzerland CH). The manure's Hg content was analyzed prior to the experiment (18.4 ± 6.9 µg/kg). All 18 Erlenmeyer flasks were then flooded with 120 mL of DI water. Each Erlenmeyer was equipped with a rhizon sampler connected to a syringe needle (Rhizon flex 5 cm, 0.15 µm pore size, Rhizosphere Research Products, Wageningen, NL) in order to sample soil solution and was then covered with parafilm. These microcosms were placed in a growth chamber (Binder, Tuttlingen, DE) at a constant temperature of 18°C (mean summer temperature of the study site) and a relative air humidity of 60 %. The incubation experiment lasted for 11 days.

The soil solutions were sampled after 6 h, 12 h, 1, 2, 3, 5, 7, 9 and 11 days. Samples were retrieved by applying a vacuum on the syringe needle in the form of a 5 mL vacutainer (Plastipak, Becton Dickinson, Franklin Lakes, NJ, USA). Since the soil solution passed through the rhizon sampler (pore size = 0.15 µm), no further filtering was necessary. At each sampling point, four different subsamples were taken (1: for total Hg and multi-element measurement, diluted in 1 % HNO_3 and

193 0.5 % HCl; 2: for Hg speciation diluted in the HPLC mobile phase; 3: for dissolved organic carbon
194 (DOC) and dissolved organic nitrogen (DON) measurements diluted in ultrapure water, 4: for ion
195 chromatography IC). Eh and pH values were measured immediately by using a 5th aliquot of the
196 soil solution. All samples were stored in a fridge at 4 °C prior to analyses.

197 ***Measurement of metal and MeHg concentrations***

198 Total metal concentrations (Hg, Fe, Mn) in soil digests and soil solutions were determined using
199 Inductively-Coupled Plasma Mass Spectrometry (ICP-MS 7700x ICP-MS, Agilent Technologies,
200 Waldbronn, Germany). The instrument's settings are shown in Tab. S1. Indium served as an
201 internal standard (*m/z* 115) and was continuously mixed with the sample through a T-piece. Hg
202 was measured at *m/z* 201. Details about the special washout for Hg to avoid a memory effect can
203 be found in Tab. S2. Hg standards and samples were diluted with a mixture of 1% HNO_3 and 0.5%
204 HCl. Standards and samples for all other elements were diluted in 1% HNO_3.
205 MeHg concentrations in soil extracts and soil solutions were determined by connecting a High-
206 Pressure Liquid Chromatograph (HPLC 1200 Series, Agilent Technologies, Santa Clara, CA, USA) to
207 the ICP-MS. The details of the method, initially suggested by Hight & Cheng[61] and optimized by
208 Sannac & Chen[62], are presented in Tab. S1. Briefly, the mobile phase (98 % of 0.1 % w/v L-cysteine
209 + 0.1 % L-cysteine·HCl·H2O (pH = 2.3) and 2 % methanol) was set to a flow rate of 1 mL/min. The
210 calibration standards for MeHg measurements were prepared daily by diluting the MeHg stock
211 solution with the HPLC mobile phase.

212 # Results and Discussion

213 ***MeHg extraction and analysis method***

214 Combining the modified MeHg extraction method of Brombach et al.[60] with the HPLC method based
215 on Hight & Cheng[61] led to satisfying results. The recovery for MeHg in the CRM (ERM-CC580: 75 ±
216 4 µg/kg MeHg) was 102 ± 5 % (n= 11). The LOD for MeHg was 1 µg/kg for soil or sediment samples
217 and 10 ng/L for water samples (no extraction step). This LOD is slightly lower than the ones of
218 other comparable methods listed in the review by Jagtap & Maher[63]. To further validate the
219 method, a batch of homogeneous soil samples from our study area was split in two identical sets.

One set was extracted in our laboratory and the other was sent to a commercial laboratory (Brooks Applied Labs, Bothell, WA, USA) which used the US EPA 1630 method with Ethylation, Purge & Trap, GC-Pyrolysis-CVAFS[55]. Fig. 1 illustrates a generally good match of the two methods. However, a paired t-test ($p < 0.001$) confirmed slightly higher concentrations with the method used in our laboratory. This can be explained by the fact that our recovery was consistently in the upper range compared to the recovery obtained by spiking soils using the US EPA 1630 method (102 ± 5% for CRM using our method vs. 77-106% for spiked samples using US EPA 1630). However, the Intraclass Correlation Coefficient (ICC): 0.9562, showed that the method we used is in good agreement with the conventional method for samples in this concentration range. The modified method represents an alternative for laboratories that are not equipped with specific analyzers since it uses HPLC-ICP-MS (ICP-MS can even be replaced by the cheaper AFS). Finally, although the LOD is higher for this method, 16 samples a day can be extracted and the run time for each sample is 4 min.This makes it interesting for risk assessment of large areas where many samples should be processed and thus opens the way to high throughput analysis of MeHg in soils.

Hg and MeHg concentrations in soil

Hg concentrations (total Hg if not mentioned otherwise) in the 60 soil samples showed values from below the LOD (0.012 mg/kg) up to 28.2 mg/kg. All three sites showed the highest Hg concentrations in the top 30 cm and closest to the canal. Two gradients of decreasing Hg concentration were prominent: with increasing i) distance from the canal and ii) soil depth (Fig. 2). These two gradients confirmed the reported past activities, when dredged sediments from the canal were deposited at its shore on the fields before further distribution for soil improvement or leveling soils in the surroundings[58]. Agricultural activities such as ploughing might have carried highly contaminated material from the shore further away from the canal and deeper into the soils[58]. The concentrations were all above the Swiss Remediation Threshold value of 2 mg/kg between 0 and 40 cm up to 10 m from the canal. For Site B at 20 m distance from the canal, the concentrations exceeded this value also down to 40 cm while at Site A only down to 20 cm. No samples exceeded this value at 40 m from the canal. Only few samples had Hg concentrations below the Swiss Investigation Threshold value of 0.5 mg/kg and these samples originated from below 40

248 cm and at least 10 m away from the canal, except for Site C which exhibited lower concentrations
249 than the other two sites..
250 Correlation analyses per site, distance, and depth between Hg concentrations and the percentage
251 of the clay fraction showed positive relationships (Tab. 2, Fig. S1). The clay fraction became less
252 important for the Hg concentrations in soil with distance and depth. Furthermore, a one-way
253 ANOVA showed that Site C had a significantly lower clay ($p < 0.001$) and a significantly lower Hg
254 concentration ($p < 0.05$) than sites A and B. In soils, Hg concentration and the clay grain size
255 fraction are usually linked in soils depleted in organic matter, since Hg adsorption increases with
256 smaller particle size (clay mineral content) and increasing specific surface area[64]. The weaker
257 relationship between clay and Hg concentrations in the less Hg-contaminated samples can be
258 explained by the mixing of strongly and little Hg-loaded clay originating from the dredged
259 sediments and the background soil, respectively. We suggest two possible explanations for the
260 lower Hg concentrations at Site C. (i) It is known that the canal sediments have a silty clay texture
261 while natural soils in the study area consist rather of silty sand[65] indicating that probably less
262 sediment from the canal was amended to Site C. (ii) The soil at Site C contained less clay and thus
263 fewer sorption sites for Hg and therefore some of the initial Hg input was leached or volatilized.
264 We measured MeHg concentrations in the soil samples with the highest Hg concentrations at each
265 site, i.e. next to the canal (5 m) and in the plough horizon (0-30 cm). The 18 selected samples
266 showed MeHg concentrations from 1.2 to 7.8 µg/kg (Fig. 3). It is interesting to note that Site C had
267 lower Hg but not lower MeHg concentrations than Sites A and B. Consequently, Site C showed the
268 highest MeHg contribution to total Hg with a percentage of up to 0.084% (Tab. 3). Our results
269 demonstrate that the MeHg concentration does not depend on total Hg concentrations in soils. We
270 speculate instead that the MeHg/Hg concentration ratios might be related with bioavailable Hg
271 concentrations and the composition and activity of the microbial community. The MeHg
272 concentrations as well as the MeHg/Hg ratios in our study soils were in the same range as reported
273 by Gilli et al.[55] in the same region, at a chlor-alkali industrial site in Italy by Cattani et al.[66], or in the
274 floodplain of a former Hg mining area in China by Wang et al.[37]. However, Skyllberg et al.[67] reported
275 a MeHg contribution to the total Hg concentrations of 17% in unpolluted boreal peat soil. High

MeHg/Hg ratios might only be possible if total Hg in the soil is at a natural low level and soil conditions favor methylation.

Incubation: Hg release into soil solution

The soils used for the incubation experiment had initial Hg concentrations at Sites A, B and C of 28.0 ± 1.0, 26.7 ± 0.4 and 5.2 ± 0.1 mg/kg, respectively. The Hg release into the soil solution of the microcosms differed slightly between Site A and the Sites B and C showing a similar Hg release. Overall, there was an initial increase in dissolved Hg up to 30 to 40 µg/L within 2-5 days for microcosms of sites B and C and within 7 days for microcosm of Site A with OM. In all microcosms, the Hg release to the soil solution took place earlier and was more pronounced when OM was added (Fig. 4 a,b). However, soils of Site A released only Hg if treated with OM. Also, Eh of Site A remained higher in the second half of the incubation (Fig. S2 c,d), which was also accompanied by a smaller release of Fe and Mn into the soil solution (Fig. 4 d,e). Apart from the incubation of Site A without OM, the Hg concentrations in the soil solution increased until around day 3 in every microcosm. After that, the Hg concentration decreased to background values (after 6h). This pattern, as well as the amount of released Hg from soil to soil solution (< 1 %) are consistent with other microcosm studies[39,49,55,68,69]. Of interest is the similar maximum amount of Hg being released to the soil solution in all OM-containing microcosms (Site A: 38.9 µg/L, Site B: 33.8 µg/L and Site C: 23.1 µg/L, Fig. 4) although the soil from Site C contained five times less Hg than the other two sites. The initial increase of dissolved Hg in the soil solution may be attributed to the dissolution of weakly bound Hg that is mobilized through mechanisms such as desorption from phyllosilicate and Fe and Mn (oxyhydr)oxide surfaces, dispersion of colloids < 0.15 µm, cation exchange or mobilization as organo-Hg complexes by dissolved organic matter[13,24,25,68]. Furthermore, it can be the result of the reductive dissolution of Mn- and Fe-(oxyhydr)oxides-bound Hg[47]. Indeed, our data shows that in every microcosm Mn- and Fe-(oxyhydr)oxides reduction occurred, as reflected by the increase in total dissolved Fe and Mn concentrations (Fig. 4c, d, e, f). These two redox-sensitive metals are theoretically reduced at potentials of 450 mV for Mn and at 150 mV for Fe, which we did not measure for the bulk material, because the used Pt electrode detects an average Eh value of a larger soil volume, but which are likely reached at some microsites. Interestingly, the courses of Mn concentrations, but not Fe, in the soil solution of

305 microcosms from sites B and C (with and without OM addition) correlated well with the increase
306 of Hg concentrations in soil solution (Fig. 4 a,b,c,d and Fig. S3). Grigg et al.[70] observed a correlation
307 between Mn and Hg concentrations during sequential extraction of contaminated soils in the
308 region suggesting that a fraction of soil Hg was associated with Mn (oxyhdr)oxides. It is therefore
309 likely that in this soil, Mn-(oxyhydr)oxide dissolution governed the Hg release, thus making Mn-
310 (oxyhydr)oxide the primary binding site for Hg and a Hg sink under oxic conditions. This is further
311 confirmed by the microcosms from Site A without OM addition, which have no notable Hg release
312 and constantly lower Mn concentrations than the soil solutions from sites B and C. Fe release
313 consistently took place after the Hg concentrations started to diminish (Fig. 4 e, f). It is therefore
314 unlikely that Fe-(oxyhydr)oxide dissolution plays an essential role for the Hg release in the studied
315 floodplain soil. Although the reductive dissolution of Mn-(oxyhydr)oxides seemed to play an
316 important role for the Hg release into soil solution, it is not possible to completely exclude a role
317 of organo-complexation of Hg by dissolved organic matter, since we observed a strong release of
318 DOC into soil solution in the first days of the incubation (Fig. 4 g, h).
319 Other studies also observed a decrease of Hg concentrations in the soil solution after reaching a
320 maximum[49,55,68]. However, the exact mechanisms which took place in our experiment are difficult
321 to elucidate. Possible explanations are 1.) re-adsorption of Hg to clay minerals after the latter had
322 lost some of their DOC cover, 2.) Hg co-precipitation with sulfide nanoparticle aggregates[49]which
323 may not have passed our rhizon sampler (pore size of 0.15 µm) or 3.) the adsorption of Hg^{2+} onto
324 the SOM functional groups (thiol, carboxyl and amino groups) which are well known to sorb Hg^{2+}
325 [12,13,24,25].
326 Dissolved Hg in the soil solution is potentially available for microbes which can methylate Hg to
327 MeHg or reduce Hg^{2+} to Hg^0. The latter mechanism is a common detoxification mechanism of
328 bacteria in such environments[71,72]. Since Hg^0 is volatile, it may have exited the microcosm. Frossard
329 et al.[73] showed the presence of the bacterial detoxification genes (merA gene) in soils situated at a
330 distance of about 150 meters from our study sites However, it is unlikely that sufficient Hg^{2+} was
331 reduced to Hg^0 to solely explain the decrease in dissolved Hg in our microcosms.

Incubation: MeHg formation in the soil

MeHg was not detectable in the soil solution throughout the incubation period of 11 days during which the soil was kept flooded (LOD: 10 ng/L). This is to be expected since the calculated concentrations for MeHg in soil solution are mainly below LOD when using common soil-water partitioning coefficients ($\log(Kd_{MeHg}) = [1.3, 3.8]$)[74]. MeHg was, however, measured in the soil before and after the incubation (Fig. 5). Before incubation, the microcosms of Site A, B and C had MeHg concentrations of 3.5 ± 0.3, 6.1 ± 0.5 and 4.3 ± 0.4 µg/kg respectively. After the experiment, concentrations increased up to 26.4 ± 1.1 µg/kg at Site B and 21.6 ± 2.2 µg/ kg at Site C which corresponded to a fivefold increase. While there were only minor changes in microcosms without OM treatment, the increase in the MeHg concentrations in microcosms with 2 % OM was significant (*Z*-test: $p < 0.001$). In the microcosms with 2% OM, the MeHg contribution to total Hg concentrations increased during the experiment from 0.04% to 0.2%. The maximum increase of this percentage from 0.08% to 0.49% occurred in one microcosm from Site C. In sediments, the threshold of 1% is rarely exceeded due to the cyclical nature of the methylation process (methylation and demethylation) and the often-observed inverse relationship of MeHg production with Hg concentrations[9]. However, there are some reported exceptions for unpolluted soils. Indeed, Skyllberg et al.[67] found MeHg contribution to the total Hg concentrations of between 1.2% and 17.2% in peaty stream bank soils of a pristine boreal forest.

It is likely that the significant increase of MeHg concentrations in the solid soil of the microcosms with 2% OM is related to their higher bioavailable OM concentration (measured as DOC concentration) and their lower Eh (Fig. 4 and Fig. S2). DOC serves as a C and energy source for microbes and influences the microbial activity[21]. Higher microbial activity lowers the redox potential because O_2 is consumed[75]. As a consequence, the higher DOC concentrations might have caused greater Hg desorption which in turn increased the availability of Hg for methylation. However, Hg in the soil solution is not necessarily available for methylating bacteria because organo-Hg complexes might be too big for bacterial uptake[9,76]. It is accepted that anoxic conditions tend to favor Hg methylation, whereas oxic conditions seem to promote demethylation[9]. The different physico-chemical conditions together with possible differences in the composition of the microbial community at Site A seem to have inhibited methylation or promoted demethylation

compared to Sites B and C, although the release of Hg in soil solution was the highest at Site A (Fig. 4). The fact that MeHg concentrations in microcosms of Sites B and C were similar (Site B: 26.4 ± 1.1 µg/kg and Site C: 21.6 ± 2.2 µg/kg Fig. 5), despite having very different soil Hg concentrations, is interesting. We suggest that Hg methylation is more related to the Hg concentration in the soil solution than in the solid soil (Fig. 4).

Different studies have reported an enhanced Hg methylation due to flooding in terrestrial ecosystems. For example, Wang et al.[37] found significantly lower methylation rates and a lower number of sulfur-reducing bacteria if a Hg-polluted paddy soil was kept aerobic instead of flooded in incubation experiments. This highlights that the redox milieu is a key factor controlling Hg methylation. Furthermore, the decomposing vegetation also plays a role under flooded conditions[34–36]. Commonly, ecosystem-scale studies also show higher MeHg concentrations in soils and peat after flooding. Rolfhus et al.[36] found a 52-fold increase of MeHg concentrations due to flooding. Further, 86 % of the produced MeHg remained after 9 years of non-flooded conditions. This indicates that MeHg can be more persistent than previously thought and could explain the levels of MeHg found in the oxic soils of the three investigated fields (Fig. 3, Tab. 3).

Studies that investigate the effect of agricultural manure amendment (or OM addition in general) on the Hg methylation are rare. However, the similarity of our results to the study by Liu et al.[40] are interesting. The latter authors found in an incubation experiment a two times higher MeHg concentration if Hg-contaminated paddy soil samples were treated with rice straw. However, our MeHg concentrations were up to ten times higher and the effect within the treatments was more pronounced, possibly because animal manure introduces more readily available C and more nutrients than rice straw.

Conclusion

A selective extraction method is crucial in order to measure MeHg in soil samples in an effective way using HPLC. The modified extraction method for lipophilic organic mercury with subsequent analysis using HPLC coupled to ICP-MS presented here showed an excellent recovery of MeHg from standard reference materials at a high precision. Furthermore, the comparison with a conventional

388 method showed a good fit of results for our samples. This method shows great potential and should
389 be further improved to lower the LOD and increase sample throughput.
390 In the soils of the three studied fields situated in a polluted agricultural floodplain, the two
391 observed gradients in Hg concentrations (with increasing soil depth and distance from the canal,
392 from which the Hg pollution originated) confirmed the historical canal maintenance practices
393 which resulted in the redistribution of dredged sediments to the soils next to the canal. In higher
394 polluted soil samples (close to the canal and 0-30 cm depth) we measured MeHg concentrations
395 between 1.2 to 7.8 µg/kg. However, in contrast to total Hg, no spatial MeHg gradient was found.
396 The MeHg/Hg concentration ratio was always below 0.1%, which agrees with other studies of Hg
397 polluted soils[37,55,66].
398 We observed a strong Hg release into the soil solution within a few days during an incubation
399 experiment with the flooded Hg-polluted soils, even in less contaminated soils such as Site C. In
400 OM (cow dung) treated microcosms, a part of the bioavailable Hg^{2+} in the soil solution was
401 methylated during the experiment. By the end of the experiment, OM-treated soils showed a 2 to
402 5 times higher MeHg concentration than before the experiment. This demonstrates that upon
403 flooding, Hg can be remobilized as well as methylated and therefore can become available to plants
404 and soil-dwelling organisms. Further, these results show that the potential release of Hg and its
405 subsequent methylation does not depend on the total Hg concentration in soil and that even less
406 contaminated sites could pose a similar environmental risk as the more polluted ones. It is
407 important to realize that common agricultural practices such as manure amendment, can provoke
408 increased methylation rates if the soil is at least episodically saturated. More experimental studies
409 are needed to assess the risks associated with different agricultural practices such as manure,
410 slurry and sewage sludge amendments as well as straw return on fields, especially in Hg-polluted
411 floodplain soils.

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Electronic supplementary information (ESI)

ESI is available is the form of a .doc file

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Tab. 1. Soil properties of the investigated fields (mean ± sd). Soil properties refer to the average of 5 depth layers (10 cm layers, 0-50 cm) if not mentioned otherwise.

	Site A	Site B	Site C
Grown crop	maize (harvested)	wheat (harvested)	grass (cut)
GPS coordinates	46.30210N 7.81182E	46.30089N 7.81949E	46.30040N 7.82237E
pH	7.4-8.1	7.6-8.0	7.3-7.8
Texture:	Silt loam	Silt loam	Silt loam
Sand (%, *n* = 9)	18.0 ± 1.5	23.1 ± 7.3	26.4 ± 6.2
Silt (%, *n* = 9)	69.1 ± 1.5	64.5 ± 4.4	64.8 ± 4.2
Clay (%, *n* = 9)	12.9 ± 1.5	12.4 ± 3.3	8.9 ± 2.2
Moisture content (%, n = 20)	25.6 ± 1.1	25.6 ± 1.8	27.0 ± 1.8
Bulk density (g/cm^3, n = 3)	1.2 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
Organic C concentration (mass %, 0 – 20 cm)	1.9 ± 0.2 (*n*=7)	1.7 ± 0.2 (*n* = 8)	2.0 ± 0.3 (*n* = 8)

Tab. 2. Correlation coefficients and error probabilities of clay content (%) with Hg concentration (mg/kg). n.s. is not significant.

	p	**Pearson correlation coefficient R**
Site		
A	n.s.	0.61
B	< 0.01	0.85
C	n.s.	0.37
Distance		
5 m	< 0.001	0.92
10 m	< 0.01	0.85
20 m	0.05	0.66
Depth		
0 cm	< 0.05	0.73
20 cm	< 0.05	0.77
40 cm	n.s.	0.53

655 **Tab. 3.** Concentrations of total Hg and MeHg and the MeHg/Hg ratio for the soil cores at 5 and 10
656 m distance from the canal and down to 30 cm (mean ± sd). The first number in the sample name
657 denotes the distance from the canal (m), the second number soil depth (cm).

Sample	Hg [mg/kg]	MeHg [µg/kg]	MeHg/Hg [%]
5 m distance			
A5-0	20.0 ± 0.7	2.5 ± 0.5	0.013
A5-10	19.6 ± 1.1	2.6 ± 0.5	0.013
A5-20	17.0 ± 1.0	1.9 ± 0.4	0.011
B5-0	23.7 ± 0.5	1.8 ± 0.2	0.008
B5-10	28.2 ± 0.6	7.8 ± 0.9	0.028
B5-20	19.6 ± 0.4	2.1 ± 0.3	0.011
C5-0	6.8 ± 0.3	2.6 ± 0.2	0.039
C5-10	7.2 ± 0.3	3.2 ± 0.3	0.044
C5-20	3.2 ± 0.1	1.2 ± 0.1	0.037
10 m distance			
A10-0	12.6 ± 0.7	2.6 ± 0.6	0.021
A10-10	14.0 ± 0.8	2.6 ± 0.6	0.019
A10-20	12.7 ± 0.7	3.0 ± 0.6	0.023
B10-0	27.5 ± 0.6	2.4 ± 0.3	0.009
B10-10	26.5 ± 0.5	2.0 ± 0.2	0.008
B10-20	20.1 ± 0.4	2.4 ± 0.3	0.012
C10-0	4.1 ± 0.2	2.4 ± 0.2	0.060
C10-10	3.9 ± 0.2	2.0 ± 0.2	0.051
C10-20	2.6 ± 0.1	2.2 ± 0.2	0.084

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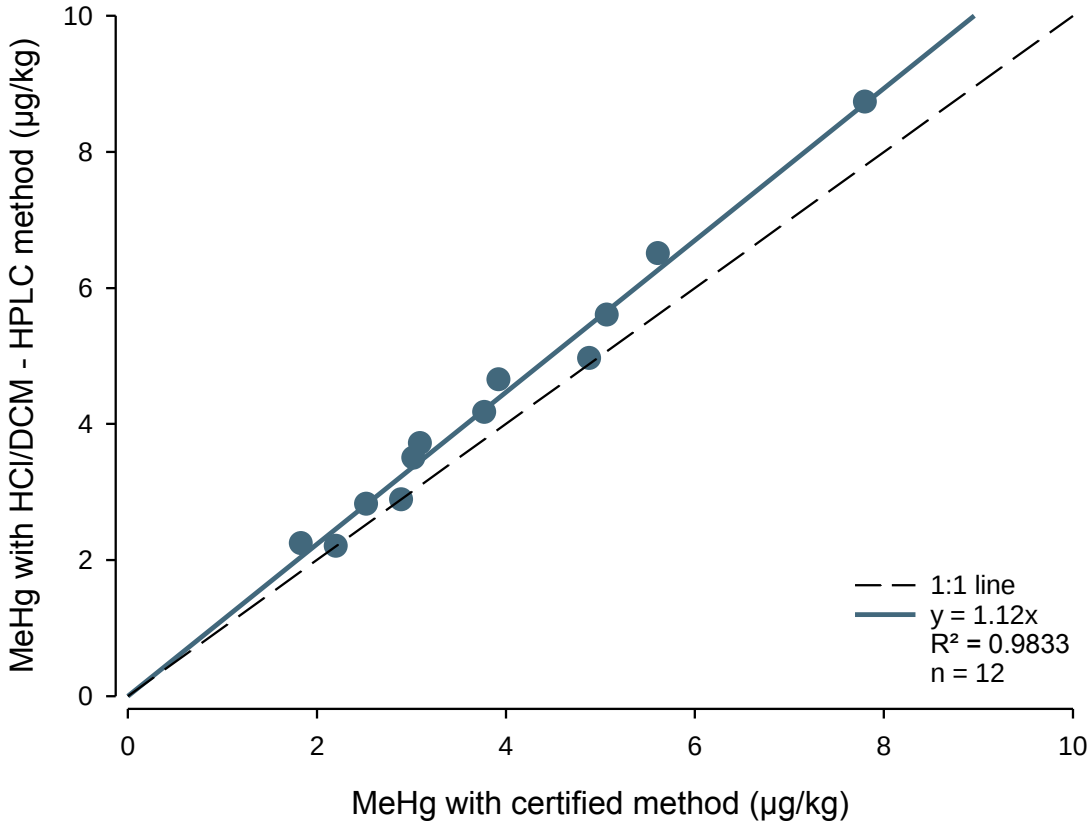

660

661 **Fig. 1.** Comparison of MeHg concentrations obtained from our modified extraction coupled to HPLC-

662 ICP-MS measurement method with the certified method US EPA 1630.

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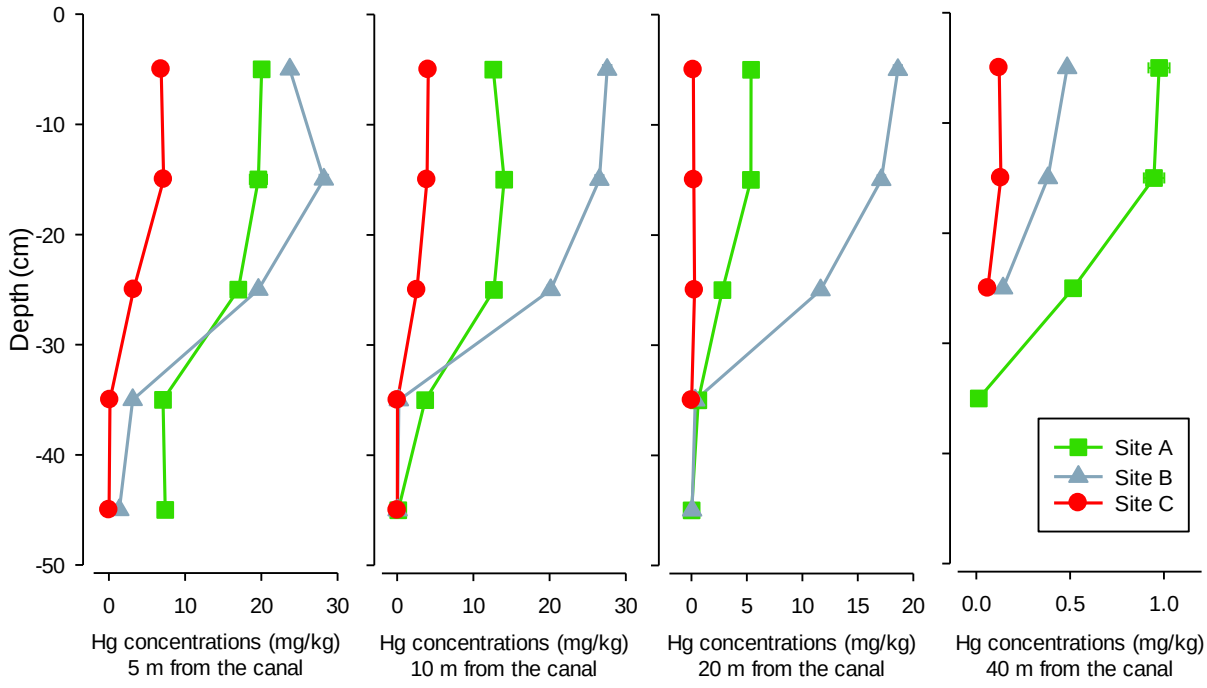
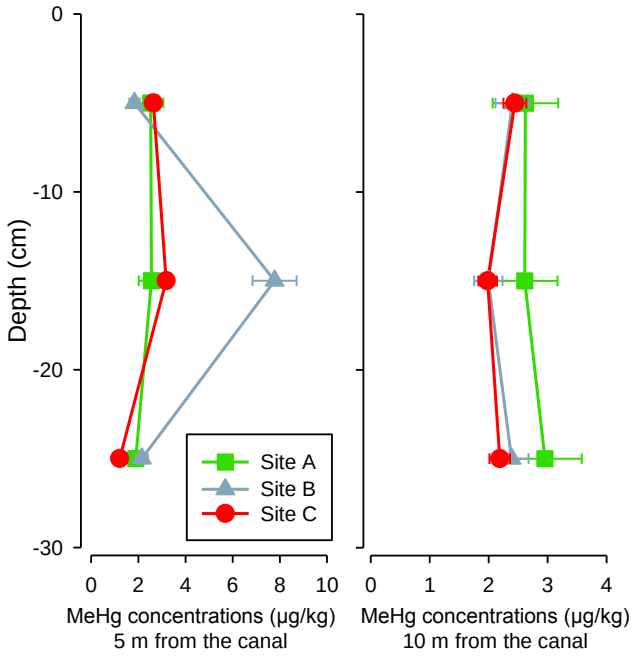


672

673 **Fig. 2.** Vertical distribution of total Hg concentrations in soils at three sites in the Valais, Switzerland

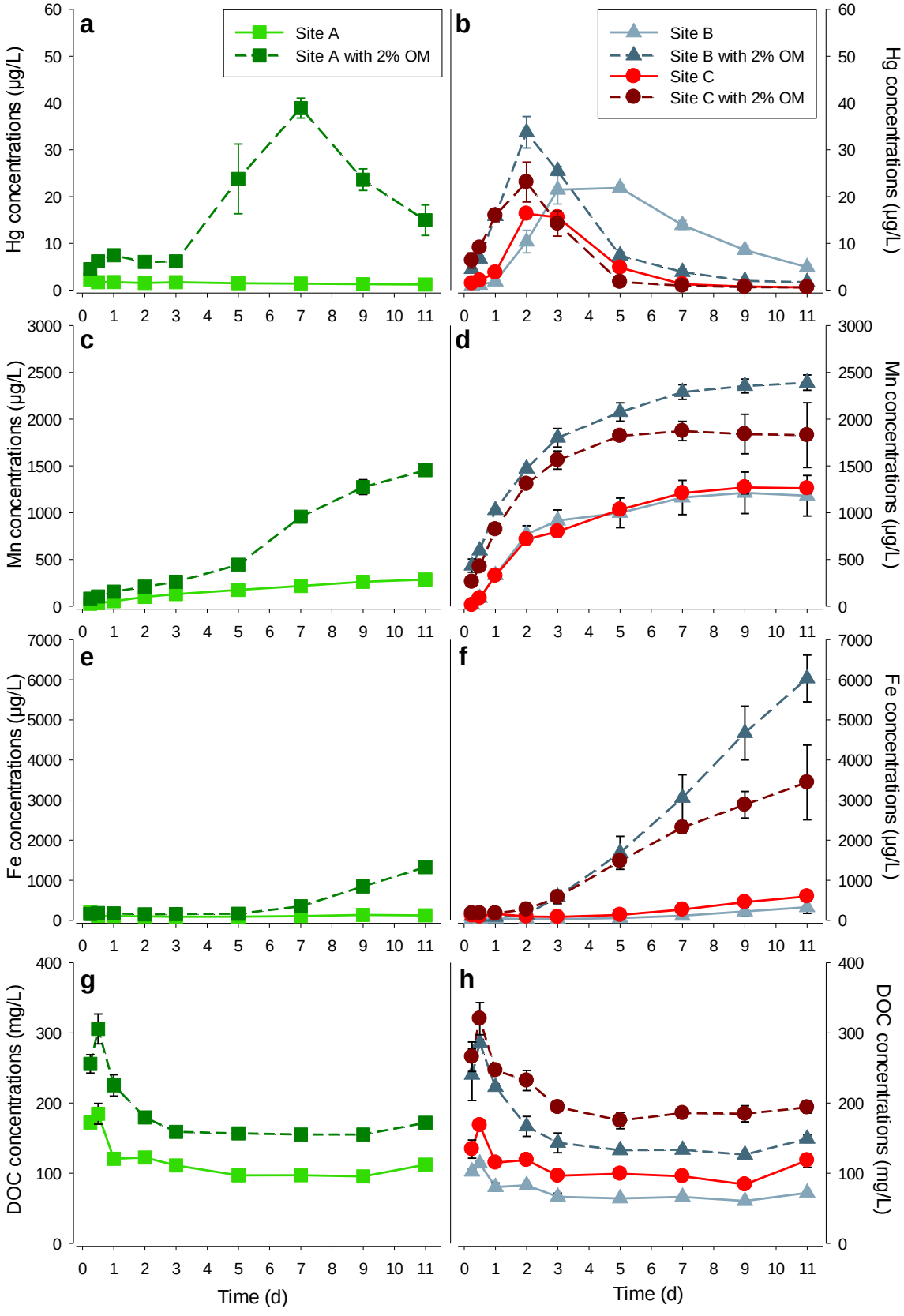
674 (mean ± sd, *n* = 3).



675

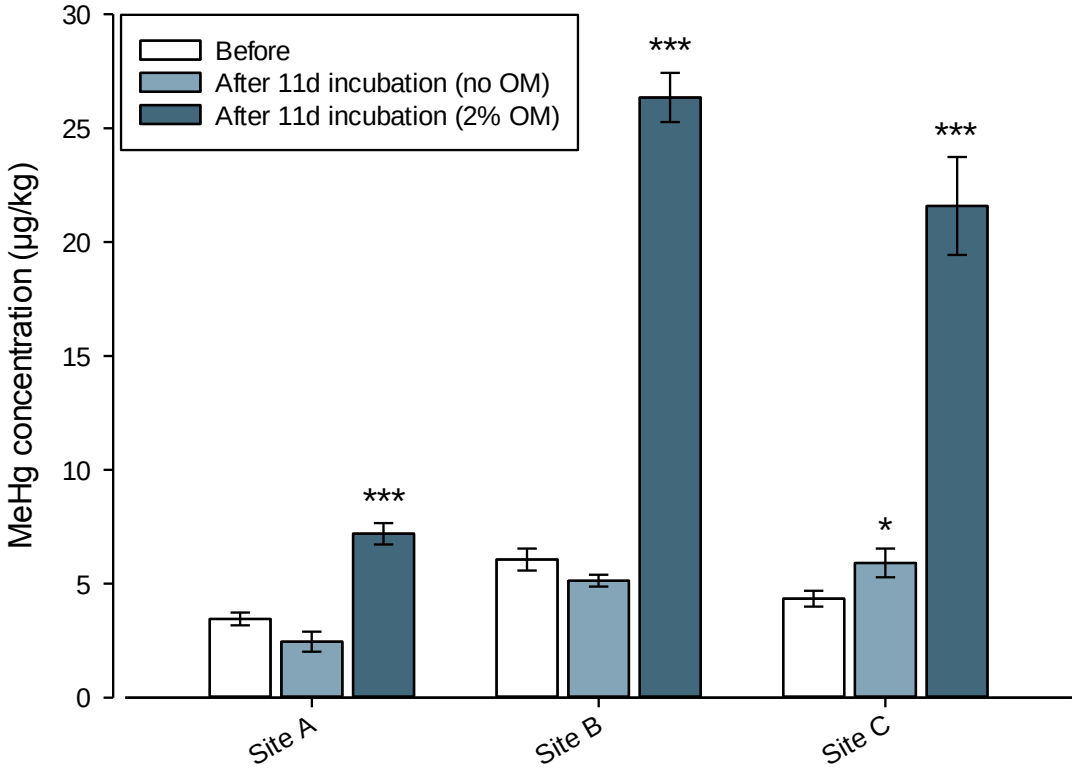
676 **Fig. 3.** Vertical distribution of MeHg concentrations in soils at three sites in the Valais, Switzerland

677 (mean ± sd, *n* = 3).


a
b
c
d
e
f
g
h
Site A
Site A with 2% OM
Site B
Site B with 2% OM
Site C
Site C with 2% OM
Hg concentrations (µg/L)
Mn concentrations (µg/L)
Fe concentrations (µg/L)
DOC concentrations (mg/L)
Time (d)

678

679 **Fig. 4.** Hg (a,b), Mn (c,d), Fe (e,f) and DOC (g,h) concentrations in soil solution during the incubation
680 experiment. The left panels (a,c,e,g) represent Site A. The right panels (b,d,f,h) represent Sites B
681 and C (mean ± sd, *n* = 3 separate microcosms).



682

683 **Fig. 5.** MeHg concentrations in soils before incubation (white), after incubation without OM (cow
684 dung) addition (light blue) and after incubation with 2% OM added (dark blue). Asterisks denotes
685 a significant difference (*Z*-test) between the MeHg concentrations before and after incubation (*:
686 $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

687

688