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Methylmercury in lake bed soils during re-flooding of an Appalachian reservoir in the northeastern USA

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Abstract

Mercury methylation, where inorganic mercury (Hg) is converted to methylmercury (MeHg), can increase in soils when flooded. While effects of the initial flooding of soils on MeHg production have been well studied, less is known about impacts of re-flooding on MeHg production. Lake Perez, an impounded recreational reservoir in the Appalachian Highlands, was completely drained then re-filled 7 years later. We use a combination of chemical, soil physical, and microbial data to quantify changes in MeHg before and after re-flooding of the lakebed. Portions that were transiently saturated due to pluvial flooding had the highest pre-flooded MeHg concentrations. When the lake was re-flooded, concentrations of MeHg in subaqueous soils increased by a factor of 2.74 (+174%) on average. Substantial variability was observed among the sampling sites, with smaller increases in MeHg at sites subjected to seasonal flooding during periods when the reservoir was drained. The increase of soil MeHg after re-flooding was lower in this study compared to studies that evaluated soil MeHg after initial flooding, indicating that re-flooding of a former lake bed caused a smaller response in MeHg production compared to initial flooding of terrestrial land. This study advances understanding of the environmental impact of impounded reservoirs.

1. Introduction

Artificial impoundments creating lakes or reservoirs are developed for many environmental or socio-economic purposes, including hydropower, wastewater treatment, flood control, and recreation. Flooding of terrestrial land might, in turn, influence the biogeochemical status of the soils and can alter the cycling of certain elements, including mercury (Hg). The methylation of Hg from inorganic forms to organic methylmercury (MeHg) and the accumulation of MeHg in food webs has been observed to increase as a consequence of flooding (Tremblay and Lucotte 1997, Porvari 1998, Tremblay *et al* 1998, Bodaly *et al* 2004, Hall *et al* 2005, Hall *et al* 2009). Because MeHg is toxic to the nervous system, it can pose a health risk for humans and wildlife, and it accumulates and biomagnifies in the food web. Concentrations of Hg in fish within inland waters in the USA (Scudder *et al* 2009) and other hemi-boreal regions (Depew *et al* 2013, Braaten *et al* 2019) are frequently above the U.S. EPA tissue residue criterion of 0.3 $\mu g\, g^{-1}$ Hg (wet weight for Hg in fish tissue) that are deemed as potentially harmful for humans consuming fish (US EPA 2001). Since reservoir creation and associated flooding of terrestrial soils is one of the most common anthropogenic manipulations of freshwater aquatic ecosystems (Hsu-Kim *et al* 2018), it is of great importance to understand flooding impacts on ecosystems and how to mitigate negative effects when

reservoirs are created and managed. Mailman *et al* (2006) have articulated several potential strategies to mitigate high levels of Hg in fish in hydroelectric reservoirs and lakes, such as burning before flooding, removing vegetation, capping bottom sediment, phosphorus addition, and site selection. We focus in this study on the latter, evaluating how re-flooding of a drained lake bed influences the production of MeHg in lake bed soils.

Soils in subaerial environments become subaqueous and shift from a largely oxidized to a largely anoxic, or reduced, environment after flooding (Erich *et al* 2010). This may increase the transformation of inorganic Hg(II) to MeHg by biotic methylation that occurs in anoxic or suboxic environments. Microorganisms within the groups of sulfur-reducing bacteria (Gilmour *et al* 1992, King *et al* 2001), iron-reducing bacteria (Fleming et al 2005), methanogenic archaea (Wood *et al* 1968, Hamelin *et al* 2011) as well as syntrophic and acetogenic bacteria (Gilmour *et al* 2013, Hu *et al* 2020) among others include taxa known to methylate Hg. Upon flooding, a reduced redox environment can form, and organic matter can also accumulate from decomposing plant materials, both favoring Hg methylating microorganisms (Tjerngren *et al* 2012, Levanoni *et al* 2015). Biotic Hg methylation is further influenced by redox conditions, pH, and temperature (Ullrich *et al* 2001) as well as the availability of electron acceptors, such as sulfate or iron(III) and mercury ligands, e.g. mercury-sulfide (HgS) species that can cross the cell membrane of Hg methylation microorganisms (Hsu-Kim *et al* 2013). Over time, the change in soil environment from subaerial to subaqueous may change the chemical speciation of elements such as Fe, Mn and S, the soil structure, and organic matter accumulation (Erich *et al* 2010), all of which may influence the rate of Hg methylation.

Several studies have detected elevated MeHg concentrations in water and biota after flooding from hydroelectric dam construction (Bodaly *et al* 2007, Trembley *et al* Tremblay and Lucotte 1997), experimental reservoirs (St Louis *et al* 2004, Hall *et al* 2005, Anderson 2011), and beaver ponds (Driscoll *et al* 1995, Driscoll *et al* 1998, Roy *et al* 2009, Levanoni *et al* 2015). Experimental flooding in northwest Ontario detected an initial 8-fold increase of MeHg in the water column (Hall and Louis 2004). The dramatic initial increase declined 2 years after flooding but remained elevated during the first 9 years (St. Louis *et al* 2004). Experimental flooding in field (Kelly *et al* 1997) and laboratory environment (Porvari and Verta 1995) suggested elevated MeHg to be mainly attributed to new Hg methylation in the sediments and not just mobilization of the MeHg pool in the flooded soil/peat. Larger reservoirs, like hydroelectric dams in Canada (Mucci *et al* 1995, Lucotte *et al* 1999) and Finland (Porvari 1998), where erosion may have caused resuspension of MeHg from flooded soils, have been found to result in elevated fish Hg concentrations that may persist for decades (Bodaly *et al* 2007, Anderson 2011).. Less of a response on fish Hg concentrations in some large reservoirs in China suggest that low carbon content in flooded soils can limits new formation of MeHg (Larssen 2010, Yao *et al* 2011, Li *et al* 2015).

While several studies have demonstrated that newly established impoundments can act as a source of MeHg, less is known about how Hg methylation is affected by re-flooding of former lake beds. A relatively common phenomenon in constructed reservoirs is a dam failure that causes the impoundment to lose water, which is followed by reservoir re-filling after repair. Impoundments may also be emptied and restored due to changes in priority in the communities or maintenance of dam constructions. Studies in beaver ponds (Levanoni *et al* 2015) and forest timber-harvest soils (Kronberg *et al* 2016) suggest that soils experiencing initial flooding are more prone to cause elevated MeHg production compared to persistently or former waterlogged soils. We focus on the effect of lake re-flooding on MeHg production.

High rates of wet- and dry- atmospheric Hg deposition in the Northern Appalachian Mountain region of the eastern United States have led to an accumulation of anthropogenic Hg in vegetation, soils, and aquatic food webs (Yu *et al* 2014, Risch *et al* 2017). The objective of this study was to quantify the effect of re-flooding on net MeHg production in lake bed soils, focusing on Lake Perez, in central Pennsylvania, USA. Lake Perez was first flooded in 1960 to support public recreation, was emptied in 2007 due to a dam failure, and was re-filled in 2013–2014 after the dam was repaired. We hypothesized that re-flooding of the dry lake bottom would increase MeHg production and result in greater MeHg inventories in soils. However, during the years when the lake was drained, the lower portion of the lake bed was subjected to local pluvial flooding caused by precipitation events. Thus, we hypothesized that the increase in MeHg production would be smaller in this area of local flooding in the lake. We use a combination of chemical, soil physical, and microbial data to quantify changes in MeHg before and after re-flooding of the lake bed. Redoximorphic features in drained lake soils were characterized by identifying the presence of depletions and concentrations in the dry lake bed prior to flooding. In addition to measuring the MeHg/THg concentrations in the dry lake bed soils, one of the gene clusters required for Hg methylation, *hgc*A, was analyzed by DNA sequencing techniques to evaluate the presence of Hg methylating microorganisms at the different sites of the drained impoundment. The question of whether re-flooded soils cause less of a response on MeHg production compared to initial flooded soil is important to consider when constructing lakes, reservoirs, or dams for hydroelectric operations, particularly if the impoundments will be used for public recreational fishing, as in the case of our study lake. Further, this case study of the complete draining and re-flooding of a reservoir presents a rare, natural experiment that can provide new insights to Hg dynamics in impounded reservoirs.

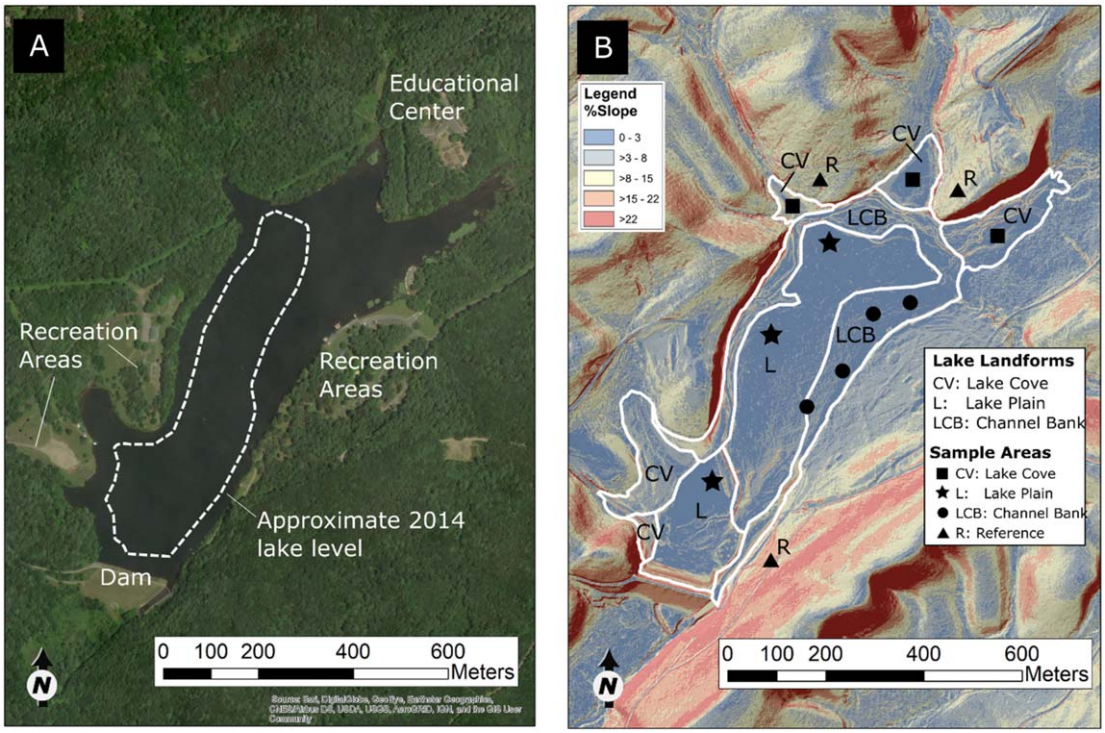

Figure 1. Lake Perez is located in Stone Valley, amidst the Ridge and Valley Physiographic Province of central Pennsylvania, USA. (A) shows an aerial image of the lake. The lake bed was dry during the first sampling in August 2013, was partially flooded during the second sampling in August 2014 (boundary shown), and was almost entirely flooded during the third sampling in November 2014. (B) shows slope categories of the lake bed as well as sampling areas. Three sampling sites were dispersed around each point on the map, 10 m apart from each other. Thus there were 48 sampling sites over the Lake Perez lake bed and on shore-line soils.

2. Methods

2.1. Site description

Lake Perez is a 0.29 km^2 impounded recreational reservoir located in the Stone Valley Forest of central Pennsylvania (40° 39′48″N 77° 54′52″W), within the Susquehanna Shale Hills critical zone observatory. The lake and its forested watershed are located within the Ridge and Valley physiographic province of the Appalachian Highlands in the eastern USA. There are two major inlets to the lake, including Shaver's Creek and a smaller unnamed stream that merges with Shaver's Creek in the lake area (figure 1). The lake was completely drained in 2007 to repair a structural deficiency in the dam's spillway. The main stream channel was passed through a culvert under the dam during re-construction between 2007 and 2013, and the lake bed was dry during this period. The slow and continuous re-filling of the lake via recharge from the inlet tributaries began in November 2013, and the lake was filled by December 2014.

While bare soils were present in some areas of the dry lake bed, ground vegetation was dominated by grasses, forbs, mosses, and some shrub and young tree cover. Some ground vegetation was present over almost the whole lake bed, but the lower lake bed (L sites) that was subjected to seasonal pluvial flooding, had sparse vegetation mainly consisting of forbs, mosses, and grasses. Some areas of exposed soils were also present in the lower lake bed. Bedrock under the lake bed and along its edge is comprised of Silurian age shale and siltstone (Berg *et al* 1980). Shore-line soils consist of Fragiudults (Ernest soil series), Fragiaqualfs (Brinkerton soil series), Dysterudepts (Berks, Weikert or Calvin soil series), Hapluidalfs (Edom soil series), and Endoaquepts (Atkins soil series). Lake bed soils were mapped per Erich and Drohan (2012) and Soil Survey Staff (Staff 2017).

2.2. Soil sampling

We sampled the lake bed soils to quantify soil characteristics and MeHg concentrations before and after re-flooding. Soil sampling was conducted on three occasions: one pre-flooding when the whole lake bed was dry in August 2013, one intermediate flooding when about half of the lake bed was flooded in August 2014, and one post-flooding occasion when the whole lake was flooded in November 2014 (figure 1).

Samples were collected using a soil core auger in the dry lake bed, and a Russian auger was used to collect samples from a boat after flooding. The inner undisturbed parts of the soil core were collected using acid washed disposable spoons in trace cleaned glass bottles or centrifuge tubes (for THg and MeHg), or Ziplock bags (for

total nitrogen and total carbon). Samples were placed on dry ice in a cooler until reaching the laboratory where they were stored in a −18 °C (subaerial soils from first sampling) or −80 °C (subaqueous and subaerial soils from second and third sampling) freezer. All soils in the dry lake bed were Entisols.

Lake bed soils were collected in three geomorphic units: the lake plain (L) that is the deepest lake bottom, the lake channel bank (LCB) that is located closer to the shore-line, and the lake cove (CV) that is located in three bay areas of the lake. To account for the potentially confounding influence of seasonal variations in MeHg not associated with flooding, on-shore reference soils were sampled (R sites) during each sampling event (figure 1) (Dystrudepts of the Berks and Weikert soil series). In each of these four sampling areas, 9–12 sites were sampled in 3–4 clusters within each geomorphic unit (figure 1). While the clusters were distributed, the 3 samples within each cluster were sampled 10 m apart from each other. Although the distances between the sampling sites within the clusters were less than between clusters, all samples (n = 39) were treated as independent samples as soil chemistry is often highly heterogeneous at a smaller scale. The upper centimeters of the A horizon were sampled for THg and MeHg analysis on all three sampling occasions (n = 117), and total nitrogen (TN) and total carbon (TC) were determined on the first sampling occasion at all sites (n = 39). Samples for microbiological analyses were only collected at selected sites during the second (n = 25) and third (n = 29) sample occasion. The samples for microbial analysis were selected to cover all areas (CV, LCB, L, and R) with more samples from the lake bed (CV, LCB and L) than from the upland soils (R).

2.3. Soil analyses

Soils and redoximorphic features were described according to Schoeneberger *et al* (2012). Field soil texture was determined by staff who had calibrated to laboratory samples (Thien 1979). Laboratory particle size analyses were conducted per the pipette method with pre-treatments (Gee and Bauder 1986). THg and MeHg soil samples were freeze-dried and homogenized before analyzes. Freeze-drying, homogenization and analyses of MeHg and THg were carried out by the USGS Mercury Research Laboratory in Middleton, WI following the US EPA method 7473 (US EPA 2007). The method includes a thermal decomposition step, followed by amalgamation and detection by atomic absorption spectrophotometry. Reproducibility and accuracy of the measurements were checked by replicating samples and reference standards. TC and TN were analyzed at the Environmental and Agricultural Testing Service laboratory at North Carolina State University, using a Perkin Elmer 2400 to determine total elemental carbon and nitrogen by combustion.

2.4. Microbiological analyses

Microbial DNA was extracted from soil samples following manufacturer's protocol using the FastDNA® SPIN Kit for Soil (MP Biomedicals), and the extracted DNA was analyzed for purity and quantity with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Parks *et al* (2013) identified two gene clusters that are required for Hg methylation, *hgcA* and *hgcB*. In order to detect the presence or absence of Hg methylators in this study, the *hgc*A gene was amplified using an established *hgc*A primer set, *hgc*A_261F (CGGCATCAAYGTCTGGTGYGC) and *hgc*A_912R (GTGTAGGGGGTGCAGCCSGTRWARKT) (Schaefer *et al* 2014).[48] *hgc*A was amplified in 50 μl reaction system using GoTaq® Green Master Mix (Promega), and PCR conditions followed Schaefer (2014), which included an initial denaturation of 2 min at 98 °C followed by 35 cycles (10 s at 96 °C, 30 s 60 °C, and 45 s at 72 °C). PCR was performed on a Bio-Rad MyCycler™ thermal cycler (Bio-Rad). Amplification products were verified by gel electrophoresis on a 1% agarose gel stained with GelRed™ Nucleic Acid Gel Stain (Biotium) for 50–60 min at 90V in 0.5X TBE buffer using 10 or 20 μl of PCR reaction and 1 μl of 100 bp Plus ladder (Gold Biotechnology). Gels were analyzed using a Bio-Rad gel imager with ImageLab software to identify the expected 651 bp *hgc*A band (Figure S2).

2.5. Statistics

A mixed model was used to test if MeHg concentrations and %MeHg were significantly different before and after flooding. In addition to the variable flooding status (Unflooded and Flooded sites), specific area of the lake bed (CV, LCB, and L) was also used as fixed factor, and sample occasion (First, Second, and Third) as a repeated structure. A one-way ANOVA was used to evaluate possible differences in soil MeHg and %MeHg between the geomorphological areas (CV, LCB, and L) during pre-flooding conditions (first sampling occasion). A Dunnet test was used for post hoc multiple pairwise comparisons between the areas. A one-way ANOVA was also used to compare the MeHg concentrations in samples where the hgcA gene was detected or not. All statistical analyses were conducted on data normalized using log-transformation. All statistical analyses were carried out using JMP software (SAS JMP® Version 15 (2020)) with an alpha of 0.05.

2.6. Review table

To place our study in context with others evaluating soil MeHg before and after flooding (e.g., caused by lake or wetland creation, hydropower, or beaver activities), we searched Web of Science™ on 28 October 2020 for peer-reviewed literature using 'dam', 'damming', 'impoundment', 'reservoir' or 'flooding' as title keywords (TI). As topic keywords (TS) we used 'flooding', 'creation', 'restoration', 'construction', 'mercury', 'methylmercury', 'sediments' or 'soils'. The search summaries are as follow: 'TI = (dam OR damming OR impoundment OR reservoir OR pond OR flooding) and TS = (flooding OR Creation OR restoration OR Construction) and TS = (Mercury OR Methylmercury) and TS = (Sediments OR Soils)'. We restricted the search to only include peer-reviewed articles in English. The Web of Science™ search resulted in 107 references. These were manually sorted to find the studies relevant to the conditions in our study using the following criteria: 1) studies are field studies evaluating the change in bottom sediment MeHg (not filtered particles in water phase) after flooding by relating flooded soils either to natural lake sediments or to pre-flooded conditions or by comparing seasonal flooding with permanently inundated water; 2) studies are not from tropical environments; 3) studies are not from marine waters; and 4) studies are not from flooded rice paddy soils. Studies that met these criteria (n = 8) are included in table 1.

3. Results

3.1. Redox concentrations and depletion colors in dry lake bed

Redox concentrations and depletion colors were present during the first sampling occasion in the former gley sediments of the dry lake bed and in all areas of the lake bed, suggesting oxidation/reduction events (table S1, figure S1). In the upper A horizon, the abundance of redox concentrations and depletion colors were highest and their range was from common (LCB and CV) and few (L). However, soil characterization was also conducted to 50 cm (horizon A1-B3), and in the lower horizons all areas of the lake bed had redox concentration and depletion colors in the range from non to common (table S1) abundance. Redox concentrations and depletion colors were, however, never present at the R sites.

3.2. THg and MeHg in soils prior to flooding

Topsoil (O horizon) mean THg concentrations in the L, CV, LCB and R areas during pre-flooding conditions ranged between 78.7–95.0 ng g^{-1}, and mean carbon content ranged between 2.1%–4.3%C. There were no statistically significant differences (ANOVA, $p < 0.05$) between the L, CV, LCB and R areas in THg concentrations or %C (table S2). The mean carbon content over the whole lake bed (L, CV and LCB sites) was 3.2%C. In contrast, whereas the mean THg concentrations were quite similar between the areas, the MeHg concentrations and the percent of THg present as MeHg (%MeHg) in the O horizon significantly differed between the sites during pre-flooding conditions (ANOVA, $p < 0.05$). During the first sampling occasion when the whole lake bed was dry, the mean MeHg concentrations (±SD) and %MeHg (±SD) were higher in the L sites (1.30 ± 0.55 ng g^{-1} and $1.59 \pm 97\%$) compared to the LCB (0.55 ± 0.26 ng g^{-1} and $0.68 \pm 0.24\%$), and CV (0.37 ± 0.12 ng g^{-1} and $0.44 \pm 0.12\%$) sites (table S2).

3.3. Soil MeHg after flooding

The MeHg concentrations and %MeHg at the R sites were similar over the three sampling occasions with mean MeHg concentrations (±SD) of 0.21 ± 0.13 ng g^{-1}, 0.20 ± 0.12 and 0.22 ± 0.17 and mean %MeHg (±SD) of $0.37 \pm 0.25\%$, $0.29 \pm 0.16\%$ and $0.36 \pm 0.21\%$ for the first, second and third sample occasion, respectively (table S2). The fact that there were no significant differences in MeHg in the R sites outside of the lake between the three sampling dates supported the notion that external forcing such as temperature differences between sampling periods did not play a strong role. The lack of significant differences in MeHg in the R sites also allows for a comparison of MeHg concentrations and %MeHg in soils from pre-flooded (sampling occasion 1), intermediate flooded (sampling occasion 2) and post-flooded (sampling occasion 3) conditions without requiring a correction for seasonal effects. Concentrations of MeHg and % MeHg of THg increased significantly by flooding (Mixed model, $p < 0.05$). The concentrations of MeHg increased by an average of 174% and the percent MeHg of THg increased by an average of 158% after flooding (figure 2). The mean post-flooding concentration of MeHg over the whole lake bed (L, CV and LCB sites) was 1.96 n g^{-1}. By contrast, the THg concentrations were not significantly affected by flooding. Both concentrations of MeHg and % MeHg were also significantly different between the geomorphologic areas, with highest values in the L sites and lowest in the CV sites (figure 2). Although there was a general increase in MeHg after flooding, the sites responded quite differently to flooding. While the MeHg concentrations increased by 189% in CV and 181% in LCB sites, the increase was only 75% in the L sites (figure 2a). Also, the relative increase of %MeHg was much higher in CV (164%) and LCB (135%) sites compared to L (76%) sites (figure 2b).

Table 1. Flooding effects on soil MeHg concentrations or mercury methylation rate constants (K_m) in our study compared to previous studies.

Reference	Flooding effect on MeHg	Flooding effect on methylation rate (K_m) or %MeHg	Flooded soils related to	Years since flooding	Initial flooding?	Medium	Purpose of flooding	Geographic Region
Our study	174%		Pre-flooded soils	1 month-1yr	No	Upper (5 cm) sediments	Lake restoration	USA, Pennsylvania
St Louis *et al* 2004 and Hall *et al* 2005	872%		pre-flooded peat cores	2 yrs	Yes	peat cores 0–60 cm	Experimental flooding	Canada, Ontario
St Louis *et al* 2004	85%		pre-flooded peat cores	5 yrs	Yes	peat cores 0–60 cm	Experimental flooding	Canada, Ontario
St Louis *et al* 2004	224%		pre-flooded peat cores	9 yrs	Yes	peat cores 0–60 cm	Experimental flooding	Canada, Ontario
Hall *et al* 2005	900%–7000%		Pre-flooded soils	1–3 yrs	Yes	Flooded soils (humic and mineral) of three sites with high, intermediate and low carbon	Experimental flooding	Canada, Ontario
Kainz and Lucotte 2002, downstream LA-40	Up to 1000%		Natural lake sediments	3 yrs	Yes	Sediment at sediment-water interphase	Hydroelectric	Canada, Quebec
Ortega *et al* 2018	No effect	220% (K_m)	pond older than 10 yrs	0–10 yrs	Varies	Upper (2 cm) sediments	Beavers	Sweden
Eckley *et al* 2017 (Eckley *et al* 2015)	280%		Sediments of permanently inundated water	Seasonally flooded since 1942	Seasonal repeated	Upper (2 cm) sediments	Flood-control	USA, Oregon
Xiang *et al* 2018, Liu *et al* 2020		around 240%	Sediments of permanently inundated water and non-inundated soils	Seasonally flooded soils	Seasonal repeated	Upper 0–20 cm soils/sediments	Hydroelectric	China, Three Gorges Reservoir

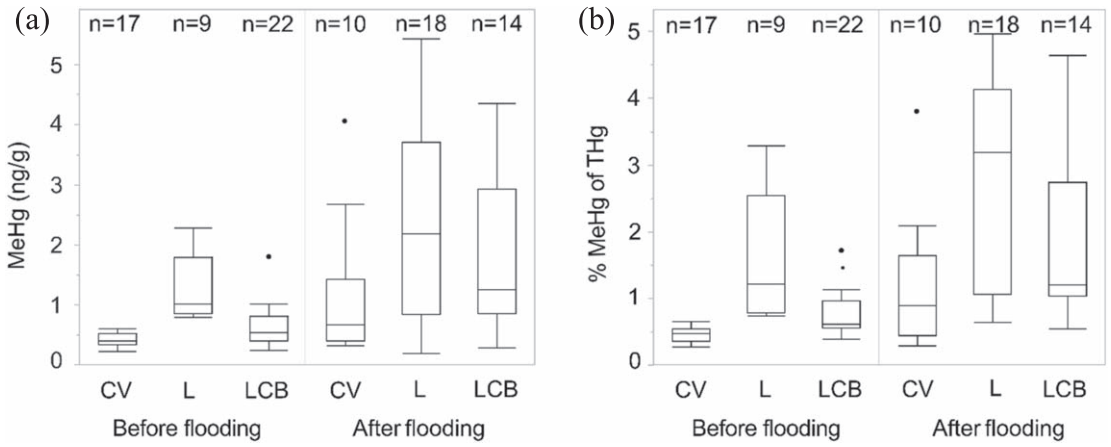

Figure 2. Concentration of MeHg (a) and percent MeHg of THg (b) in soil samples from the different lake regions (CV: lake cove, L: lake plain, and LCB: lake channel bank) before and after flooding.

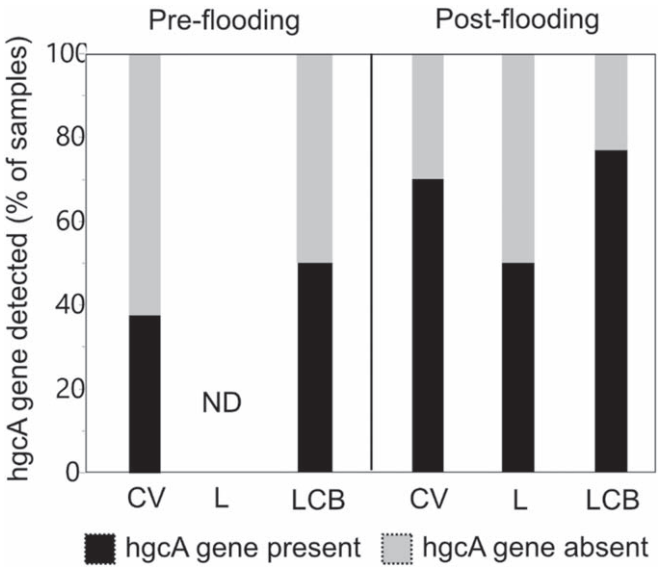

Figure 3. The percentage of the samples where the hgcA gene (based on primers from Schaefer *et al* 2014) were detected from the different lake regions (CV: lake cove, L: lake plain, and LCB: lake channel bank) before and after the sampling sites were flooded. ND: no data.

3.4. Presence of hgcA genes

Median MeHg concentrations were higher in samples where the *hgc*A gene was detected (1.17 ng g^{-1} and 1.35 ng g^{-1}) compared to not detected (0.42 ng g^{-1} and 1.10 ng g^{-1}) (CV and LCB sites, respectively); however, differences were only significant in CV sites (ANOVA, $p > 0.05$). No significant difference was found in MeHg concentration between the L samples where the *hgc*A were present (2.30 ng g^{-1}) and not present (2.92 ng g^{-1}). Furthermore, the *hgc*A genes were detected more frequently in samples from post-flooded soils (70% and 77%) compared to pre-flooded soils (38% and 50%) in the CV and LCB sites (figure 3, table S3). No samples were collected at the L sites during pre-flooding conditions. The *hgc*A gene was detected in 50% of the samples from the flooded L sites.

3.5. Comparison with other studies

Our Web of Science^{TM} search resulted in 8 previous studies evaluating subaqueous soil concentrations of MeHg after flooding, re-flooding or seasonal flooding of soils. The purpose or reason for this flooding varied from hydroelectric operations, beaver activities, flood control ponds to experimental flooding. The increase in MeHg concentrations observed in our study (174%) is in the lower range relative to previous studies, especially compared to those that evaluated recent and initial flooding (table 1). Initial flooding generally caused manifold increases in MeHg concentrations when measured within 0–3 years after flooding (800%–7000%) (Kainz and

Lucotte 2002, St Louis *et al* 2004, Hall *et al* 2005). Seasonal flooding in USA (Eckley *et al* 2015, Eckley *et al* 2017) and China (Xiang *et al* 2018, Liu *et al* 2020) has been associated with smaller increases in MeHg (<300%). The increase of MeHg from seasonal flooding (Eckley *et al* 2015, Eckley *et al* 2017) and the degree of elevated MeHg still present 9 years after flooding in Ontario (St. Louis *et al* 2004) were in the same range as the numbers of elevated MeHg in subaqueous soils measured in the present study.

4. Discussion

Lake Perez was completely drained for nearly 7 years before its dam structure was repaired and the lake re-filled. This provided an unprecedented opportunity to explore the impacts on soil MeHg production during re-flooding. Some low-lying areas of the drained lake bed had been frequently flooded and transiently saturated during precipitation events (L sampling areas), while other upland and well-drained areas of the lake bed had remained largely dry (CV and LCB sampling areas).

Prior to the re-flooding of the dry lake bed, soil concentrations of MeHg were approximately twice as high in L sites as compared to CV and LCB sites, suggesting that the seasonal flooding may have built up high MeHg in soils of the L sites due to in situ production, assuming low or negligible sedimentation rates. Seasonally flooded reservoirs have been identified to cause higher Hg methylation rates compared to sediments of permanently inundated environments, and where the Hg methylation might be favored by sulfur cycling between reduced and oxidized forms when water-levels are fluctuating (Eckley *et al* 2017, Xiang *et al* 2018, Liu *et al* 2020). However, due to possibly more reduced conditions in the deeper part of the lake, the lower lake bed may also have had higher MeHg prior to the lake being emptied in 2007. The presence of redox concentrations and depletions in all lake areas (CV, LCB and L sites) suggests that reduction and oxidation events have occurred across the lake bed and not just in the deep bottom (L) sediments. In a reducing environment (which these colors indicate), it is most likely that MeHg was produced by various anaerobic microorganisms capable of methylating Hg, such as SRB, IRB and methanogenic archaea.

After the dam was repaired, re-flooding Lake Perez caused lake bed soil MeHg *concentrations* to increase by 174% and the *percent* MeHg (of THg) to increase by 158%, on average, across the sampling sites. Previous studies have also found that MeHg production in soils increases after flooding (Hall *et al* 2005, Anderson 2011, Eckley *et al* 2017), though the increase we observed was in the lower range from that of former studies (table 1). Flooding may cause more reduced conditions where Hg methylating microorganisms may establish, but it can also initiate many biogeochemical processes that influence the prerequisites for Hg methylation, for example by influencing the solubility of Hg and MeHg adsorbed to soil particles (Skyllberg *et al* 2003, Eckley *et al* 2017) and changing the cycling of sulfur (Eckley *et al* 2015) and organic carbon (Ortega *et al* 2018). Flooding may also mobilize elements from the terrestrial vegetation to sediments (Naiman *et al* 1994).

Mixed models suggest that the variation in MeHg concentrations and %MeHg at this study site was explained by the effect of flooding, but also by differences between lake areas. The reason that lake area is important in these mixed models is likely attributed to the high pre-flooding MeHg concentrations and %MeHg in the L sites compared to other sites. However, the areas of the lake bed responded differently to flooding (figure 2), and the increases of MeHg and %MeHg after flooding in the CV and LCB sites were approximately double that of the L sites. In accordance with our hypothesis, the higher response in the CV and LCB sites indicate that the lack of seasonal flooding during the 7 years between emptying and refilling the lake, caused a larger response in these sites.

The *hgc*A gene was detected in fewer samples from L sites (50%) compared to the CV and LCB (70%–80%) sites; this is consistent with the idea that high MeHg concentrations at L sites during post-flooding were due to the long term accumulation of MeHg rather than to higher rates of production by Hg methylating microbes. This is further supported by the fact that MeHg concentrations increased by a smaller amount after large-scale flooding at the L (75%) sites compared to the CV (189%) and LCB (181%) sites (figure 2). In the CV and LCB sites the *hgc*A gene was detected more frequently (70%–80% of the sites) after flooding than before flooding (38%–50% of the sites), indicating either that populations of Hg methylating microorganisms may become established after flooding, or that flooding conditions stimulate Hg methylation by resident populations. However, more efforts are needed to explore the population dynamics of microorganisms correlated to MeHg production. Furthermore, the Hg methylation in terrestrial habitats have been shown to not only be linked to the communities of microorganisms carrying the *hgc*A gene, but also to the presence and activity of non-mercury methylating communities, likely providing growth substrate for the *hgc*A-carrying microorganisms (Liu *et al* 2019, Xu *et al* 2019).

There are several possible reasons why Hg methylating microorganisms might not have established at the same rate in the L sites, and why MeHg did not increase as much as in CV and LCB, in these sites after flooding. This could be due to: (1) reduced conditions already present before permanent flooding and the possible lack of

oxidized electron acceptors such as sulfate or iron (III); or (2) less vegetation compared to other sites that could fuel the Hg methylating microorganisms with fresh organic carbon. More terrestrial vegetation, as found in the CV and LCB sites (as judged by visible observations during sampling), might have increased the availability of fresh organic carbon sources and nutrients. Seasonal flooding at L sites caused the vegetation to be comparatively sparse, containing only grasses, forbs, mosses and some areas of exposed soils. Calder *et al* (2016) and Meng *et al* (2016) found a strong relation between post-flooding MeHg concentrations and the organic carbon content of the flooded soils. Organic carbon quality and quantity have been found to determine the flooding effect on MeHg (Calder *et al* 2016, Ortega *et al* 2018). As there was no difference in %C in the CV, LCB and L areas, the carbon content could not explain the higher response in CV and LCB sites compared to L sites. However, flooded vegetation could influence the quality of organic carbon. Ortega *et al* (2018) found the Hg methylation rate to be positively influenced by fresh humic substances from flooded soils, as well as *in situ* production of algal-derived organic matter, triggered by possible elevated nutrient availability in the sediments. We thereby suggest that both (1) and (2) contributes to the higher response on MeHg in CV and LCB sites compared to L sites after large scale re-flooding.

Results show that re-flooding of a lake bed that had been dry for approximately 7 years provided the right conditions to stimulate Hg methylation, or could cause a re-establishment of Hg methylating microorganisms, where re-flooding increased net MeHg production in lake bed soils. The magnitude of the MeHg increase observed in this study was lower than observed in former studies evaluating impacts of initial flooding of terrestrial soils (table 1), suggesting that initial flooding may be more prone to increased MeHg production than areas that have been previously flooded. This notion is further supported by results comparing areas of the lake bed that were subject to frequently pluvial flooding to areas of the lake bed that had been largely dry for 7 years. Similar results have been found for MeHg in water when comparing new and re-colonized beaver ponds (Levanoni *et al* 2015). Further, fish Hg concentrations have been found to be higher in reservoirs where water levels have been fluctuating more and sediments have been exposed for longer time periods, likely due to a combination of re-oxidation and vegetation establishment that may promote MeHg production when water levels rise again (Sorensen *et al* 2005, Larson *et al* 2014). Soils that are persistently or formerly water-logged might already have undergone several oxidation/reduction events that could have lessened the effect of flooding on MeHg production.

In addition to the fact that Lake Perez was not initially flooded, the lower response of MeHg observed in our study compared to other flooding studies could also be influenced by the low organic carbon content in the topsoils of Lake Perez (3.2% C) (table 1). Inundation at very large hydroelectric dams in China (e.g. the Three Gorges Reservoir) resulted in lower increases in fish Hg concentrations (Li *et al* 2015) compared to other large hydroelectric dams in Canada (Lucotte *et al* 1999) and Finland (Porvari 1998). The lack of a more pronounced effect in some Chinese reservoirs might be due to the lower carbon content limiting new production of MeHg in flooded soils (Larssen 2010, Yao *et al* 2011, Li *et al* 2015). We used the relatively low percent soil carbon values measured at our study site as input to a linear relationship presented by Calder *et al* (2016) relating sediment MeHg concentrations and %C, to explore the potential post-flooding MeHg concentrations attributed to carbon. The average post-flooding concentrations of MeHg observed at our study site (1.96 ng g^{-1}) were slightly lower than the predicted values. This supports the notion that Hg methylation declines after an extended period of previous flooding.

Because frequent flooding caused continuously high MeHg concentrations in the soils of Lake Perez, areas that were transiently flooded during precipitation events had higher soil concentrations of MeHg prior to the re-flooding of the dry lake bed, compared to areas that had been largely dry. Terrestrial areas subject to initial flooding (or in this case, flooding after a many years of no water cover) were more prone to increase MeHg net production compared to sites that were frequently flooded. Though the flooding of the initially dry sites may have a stronger effect on the rate of new production of MeHg, the flooding of the initially transiently-saturated sites may have stronger downstream effects since MeHg that accumulated over time in sediment may be released to the downstream water bodies after flooding.

5. Conclusions

Our study revealed how the re-flooding of a drained reservoir caused the increased production of MeHg in lake bed soils. The results suggest that the former flooding status may be of high importance for how the soil MeHg level responds to flooding, which should be explored in further case studies in other environmental settings. Changes in biogeochemical cycling of Hg and production of MeHg in lake bed soils are important when considering the potential for bioaccumulation of MeHg in aquatic life and the food chain. These results contribute to understanding of the environmental impact of impounded lakes and reservoirs.

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Data availability statement

The data that support the findings of this study are openly available in Eklöf *et al* (2021), at the following URL: https://www.hydroshare.org/resource/79093ce25c4745359bbc24be70fcd77b/.

The supporting information includes the following, as referred to in the text above: Table S1: Soil characterization data and water content for the sampling sites prior to flooding; Table S2: Mean concentrations of THg and MeHg and percentage THg that is MeHg from the three sampling occasions, and percentage nitrogen and carbon from the first sampling occasion; Table S3: Genomic DNA extraction data, hgcA content, and sampling conditions; Figure S1: Photo of sample from pre-flooded soil where redox concentration and depletion colors have appeared in the formerly gley sediments, suggesting intense oxidation/reduction events; and Figure S2: Agarose gel electrophoresis with PCR amplication products of soil samples using the hgcA primer pair from Schaefer *et al* (2014).

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