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Effects of vegetation on methylmercury concentrations and loads in a mercury contaminated floodplain

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ABSTRACT

The Yolo Bypass (YB) is a large flood conveyance system designed to protect the city of Sacramento, California, USA from flooding when the Sacramento River approaches flood stage. The Sacramento River watershed and YB are a source of methylmercury (MeHg) to downstream habitat as a result of historic mercury (Hg) and gold mining practices. In the dry season, the YB is extensively farmed and grazed. However, depending on the water year, the floodplain may remain inundated for months. Our experiments focused on the role of pasture land and decomposing vegetation as a source of MeHg during extensive periods of floodplain flooding. Decomposing vegetation, rather than sediment, was identified as the principal source of filter passing MeHg (fMeHg) within the floodplain. The decomposing vegetation provided a substrate for microbial methylation of inorganic Hg contained within the plants. In replicated flooded mesocosm experiments, MeHg concentrations increased from 2.78 to 31.0 ng g^{-1} dw and 3.41 to 56.8 ng g^{-1} dw in decomposing vegetation. In field collections, the concentrations of MeHg in vegetation increased from preflood levels of 2.78 to 45.4 ng g^{-1} dw after 17 weeks of flooding. The importance of vegetation was shown in laboratory experiments where there was a positive correlation between the amount of fMeHg in water and the amount of vegetation added. These results also provide Hg concentration data for an important functional type of vegetation, grasses, and fill a data gap that contributed to uncertainties with regards to the role of vegetation in Hg cycling.

1. Introduction

Between 1850 and 1980, California was the nation's leading producer of mercury (Hg) averaging about 100 million kg per year with approximately 12 million kg used in the Sierra Nevada gold fields in the mid to late 1800s to enhance the recovery of gold in hydraulic placer and hardrock mining operations (Churchill, 2000). It is estimated that approximately 4.5 million Kg of Hg was lost to the environment from hydraulic gold mining operations and approximately 1.3 million kg was lost at hardrock mines. Today, historical gold mines are a significant source of Hg to the California Sacramento-San Joaquin Delta (Delta), the largest freshwater estuary on the west coast of North America. Widespread Hg contamination in fish, sediment and water in the Delta has led to health advisories posted in the Delta recommending no consumption of large striped bass and limited consumption of other sports fish (Wiener et al., 2003; Davis et al., 2008). Elevated concentrations of methylmercury (MeHg) in fish tissue also represent a hazard to piscivorous wildlife (Ackerman et al., 2007; Wiener et al., 2003).

The California Central Valley Regional Water Quality Control Board adopted a Basin Plan Amendment, and a Total Maximum Daily Load (TMDL) as part of a strategy to resolve Hg impairment from different sources in the Delta (Wood et al., 2010). Included in the plan are fish tissue objectives allocating maximum annual average MeHg loads from different sources, such as open waters created through flooding, and different regions of the Delta to achieve these fish tissue objectives and protect human consumption of sports fish. The plan uses a phased, adaptive management approach, allowing regulated entities to conduct studies to determine how and if the regulated entity can attain the load allocation in the TMDL.

The Yolo Bypass floodplain (YB) and upstream watershed is required to reduce its exports of MeHg by 78 % (Wood et al., 2010). Under high floodwater conditions, when the Sacramento River overflows into the YB, the floodplain is one of the major sources of MeHg to the Delta. Winter flooding mass balance studies, conducted by the California

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Department of Water Resources (DWR), isolated the total MeHg mass produced within the YB by calculating the mass contributions from all major inputs into the YB and the mass exports from the YB. Mass balance calculations from their study estimated the upper YB watershed, under winter flooding conditions, exports to the Delta approximately 37 % of the total MeHg load contributed by the Sacramento River itself (DWR, 2020). This finding is similar to those by Foe et al., 2008, however, the reason for this contribution was not explored.

Given the YB's contribution of MeHg load to the Delta, it is important to identify the contributing factors associated with MeHg production within the floodplain. In turn, understanding the factors driving MeHg production within the YB could provide managers and regulators with approaches to achieve the TMDL allocation. Suggested management practices to reduce MeHg production in the YB floodplain include: 1) amending soil with iron or other amendments; 2) reducing upstream inputs from historical Hg mines; and 3) recirculating or increasing residence time of drainage water (McCord and Heim, 2015). Unfortunately, many of these alternatives are not practical for the YB.

Worldwide, other studies have identified MeHg and total Hg contamination to floodplains from upstream Hg mines; for example studies in Europe (Frohne et al., 2012; Beckers et al., 2019; Žagar et al., 2006), studies in Canada (Emmerton et al., 2013; Eckley et al., 2017), studies in USA (Flanders et al., 2010; Bradley et al., 2010, 2011; Lazareva et al., 2019; Marvin-DiPasquale et al., 2014; Wang et al., 2020), studies in South America (Roach et al., 2013; Desrochers et al., 2015; Guimaraes et al., 2000; Roulet et al., 2000), and a study in South Africa (Kading et al., 2009).

Our work focused on the relative contribution of decomposing vegetation to MeHg generation within the YB floodplain. We chose to evaluate this source because, when flooded, the largest inundated land use is pasture devoted to cattle grazing (DWR, 2020). The contribution of decomposing vegetation to MeHg production has been documented in both newly flooded reservoirs and wetlands (see for example, Balogh et al., 2002; Bodaly et al., 1984; Branfireun, 2000; Roulet et al., 2000, 2001; Gustin et al., 2006; Windham-Myers et al., 2009, 2014) and it follows that a similar result could occur with flooding of YB pasture land. We hypothesize that, an increase of above ground vegetation biomass results in an increase of MeHg released to overlying floodplain

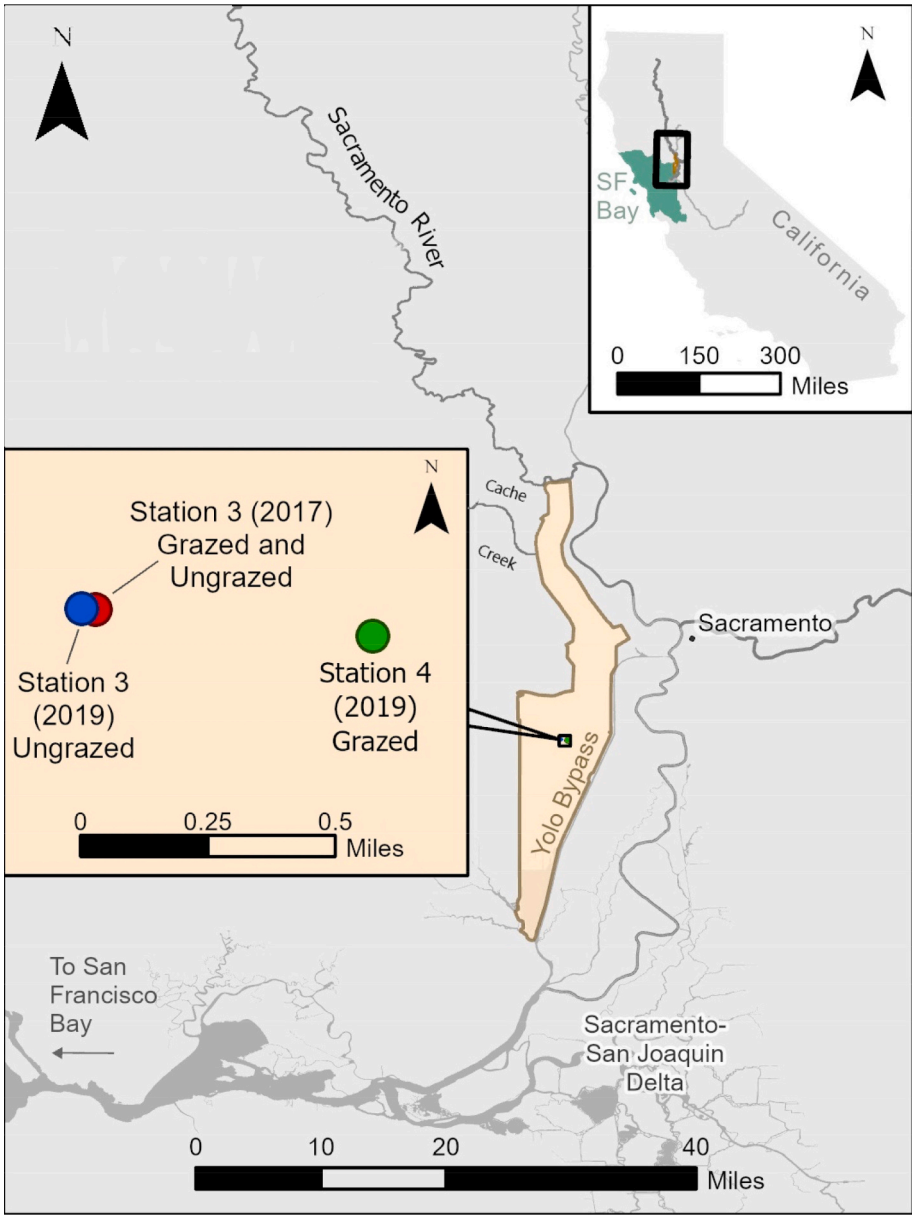

Fig. 1. Study area and sample locations.

waters.

Using mesocosm and laboratory settings, our main objectives were to: 1) address the role of live vegetation, decomposing vegetation, and sediments in the internal production and cycling of MeHg in the YB and 2) assess two commonly used land management practices in the YB-grazing and disking- that reduce surface vegetation as a control method to mitigate MeHg. Mesocosm experiments were used to investigate the effect ungrazed, grazed, and disked vegetation had on MeHg. A microcosm experiment with controlled masses of vegetation and sediment was conducted in the laboratory to examine relationship between sediments, vegetation, and MeHg. The laboratory approach allowed us to isolate and focus on the importance of sediment versus vegetation and whether there was a relationship between the amount of vegetation biomass and fMeHg, relationships that could potentially be obscured by the ambient heterogeneity associated with soils and vegetation excavated for our mesocosm experiments.

2. Material and methods

2.1. Geographic and sample setting

The study area and sampling locations for mesocosm material are shown in Fig. 1. The YB is a narrow but long 2.4 × 10^2 km^2 highly vegetated flood water conveyance area used for farming and a wildlife refuge. The YB is flooded during high flow winter storms to protect the City of Sacramento and surrounding areas from flooding. On average the Yolo Bypass floods 7 out of 10 y with inundation occurring roughly between October and April (US Bureau of Reclamation, 2022). When fully inundated YB carries 80 % or more of Sacramento River flood flows southward to the Delta. During wet year winters in Northern California, the hydrology is dominated by flood flows where the YB floodplain can be inundated for weeks to months. Additional descriptive information on the YB is found in *Supplementary material S-1*.

2.2. Mesocosm experiments

Mesocosms were used to simulate flooding of YB pasture land and measure MeHg in overlying water over time for the following controlled treatments: 1) ungrazed pasture, 2) grazed pasture, and 3) disked pasture (*Supplementary material S-2*). The grazed and ungrazed treatments consisted of flooding mesocosms with intact sod collected from grazed and ungrazed pastures with no additional alterations. Disking is an agricultural term referring to cutting the soil and burying part of the vegetation residue with a disk or harrow. The disked treatment simulated the flooding of disked pasture. Water only controls consisted of replicate mesocosm containers filled with water, but no sod.

Mesocosm studies were conducted November 2017 through January 2018 (five weeks) and February through March 2019 (4 weeks) at DWR's Bryte Laboratory, located adjacent to the YB in West Sacramento, California. To ensure that sample treatments experienced similar winter temperature fluctuations as floodwaters, mesocosm experiments were conducted outdoors under a protective tent (2017 experiment), or within an uninsulated metal shelter with a fan continuously introducing ambient air (2019 experiment).

At the time of sample collection, ungrazed stations consisted of dead rye grass, *Lolium perenne*, with some newly sprouted rye grass from early winter rains. In 2017, the vegetation composition of the grazed and ungrazed sample plots appeared similar, with visibly reduced biomass of the vegetation in the grazed samples. However, in 2019, the vegetation in the grazed sample area consisted primarily of newly sprouted rye grass and clover, *Trifolium*, while the ungrazed location contained a mixture of dead and newly sprouted vegetation.

To create mesocosms intact sod (containing sediment with undisturbed, rooted vegetation) was collected from an actively cattle grazed YB pasture (Fig. 1, Station 4). Sod for ungrazed and disked treatments was collected from adjoining pasture where cattle had been excluded from grazing by fencing (Fig. 1, Station 3). To simulate disking of a field consistent with the depth of much of the disking done by tractor in the YB, a shovel was used to break apart and turn vegetation under the surface uniformly to a depth of 15 cm. Roots and vegetation were broken and mixed thoroughly before adding to the mesocosm containers (*Supplementary material S-3*). Mesocosms were filled with water slowly, as to not disturb sod, to a water depth of 23 cm (*Supplementary material S-4*). After flooding mesocosms the soil making up the sod became saturated sediment by definition.

For the first mesocosm experiment, sod for the grazed and ungrazed treatments was excavated to a depth of 10 cm and placed into precleaned rectangular 18 × 30 (L × W) cm polyethylene bins which were placed in precleaned 28 × 53 (L × W) cm mesocosms and filled with 34 L of water pretested to confirm low levels of fMeHg. Mesocosm size was increased to 38 × 84 cm and 74 L of water for the second experiment and replication of treatments was increase from 3 to 5.

Water in the mesocosms was changed twice a week, on days 3 and 7 to keep MeHg and other constituent levels in mesocosms from building up to unrealistic concentrations in comparison to levels found in natural flood waters. Mesocosm waters dissolved organic carbon (DOC) target was ≤10 mg L^{-1}, as previous sampling had determined that organic carbon content of flood waters was usually ≤10 mg L^{-1} (DWR, 2020). Commercial, aquarium trade, aerators were used to ensure that dissolved oxygen in overlying water was >90 % saturation. Mesocosms were aerated continuously to simulate oxygenated floodwater conditions (DWR, 2020). Water temperature was 9.0 ± 2.2 (mean ± standard deviation) for 2017 and 10.3 ± 1.1 (mean ± standard deviation) for 2019. These temperatures were within the range found in YB floodwaters (10–16 degrees C).

Water samples for the determination of fMeHg and DOC were collected weekly (2017) and biweekly (2019) from mesocosms using Gelman 0.45-micron capsule filter in line with a portable peristaltic pump with pre-cleaned Teflon and C flex tubing.

Surface vegetation biomass were determined for grazed and ungrazed pastures by clipping vegetation flush with soil surface in 0.25 or 0.50 m^2 quadrants. Clippings were collected, dried in the laboratory at room temperature, and weighed when dry. Vegetation was analyzed to determine total Hg (THg) and MeHg concentrations. In addition, decomposing vegetation was collected, for determination of THg and MeHg, from pasture after a 120-day flood event. Litter bags (1 mm nylon mesh), precleaned with hydrochloric acid (HCl) and rinsed, filled with 2 g of dried rye grass were placed in the ungrazed mesocosms. Litter bags were retrieved at the end of the experiment and the bags with partially decomposed material were freeze dried, weighed, and analyzed for THg and MeHg. Mass of MeHg in vegetation was calculated by multiplying the concentration of MeHg by the vegetation mass for both initial and final samples. Vegetation pool of MeHg was calculated from biomass survey data, MeHg concentrations and total pasture area for the YB. In addition, THg and MeHg concentrations were measured in 0–2 cm depth sediment for ungrazed and disked treatments.

2.3. Microcosm experiment

A laboratory microcosm experiment was designed to isolate the effect of sediment and vegetation biomass on fMeHg concentrations over time. To control the variability between treatments, homogenized soil and one species of dried plant (*Lolium perenne*) was used. Five treatments with five replicates for each treatment were prepared in 1 L beakers by adding varying amounts of vegetation to a constant amount of soil. Treatments tested were as follows: 1) control water; 2) sediment only; 3) vegetation only; 4) sediment plus 2.5 g vegetation; 5) sediment plus 5 g vegetation; 6) sediment plus 10 g vegetation.

Soil for the experiment was collected in October 2017 from Site 3 (Fig. 1) and kept dry until use. Soil was sieved to 1 mm and packed into a pre-cleaned 5 cm × 5 cm glass dishes to a depth of 2 cm. Starting soil MeHg concentrations were measured. Vegetation masses were chosen to

simulate intensive grazing pressure (low biomass) to no grazing pressure (high biomass) and represented the range of vegetation biomass measured from field surveys in the YB. THg and MeHg concentrations were determined for vegetation used in experiment. Vegetation was added to precleaned litter bags and secured to glass dishes at bottom of beakers. Soil and vegetation were flooded by slowly adding 800 mL of pretested low fMeHg tap water resulting in saturated sediments and vegetation with overlying water (*Supplementary material S-5*). Overlying water was kept oxic by aerating with commercial, aquarium trade, aerators. Temperature was kept constant at 12–14 °C.

To simulate several months of extended flooding which is what occurs during major floods in the YB, the experiment was conducted for 8 weeks beginning in October 2018 and finishing in December 2018. Beaker water was completely exchanged every 3–4 days with pretested low fMeHg water to keep DOC and other water quality parameters similar to those found under ambient conditions. Water samples were collected immediately prior to a water change and analyzed for fMeHg and DOC. Litter bags were retrieved at the end of the experiment and the bags with partially decomposed material were freeze dried, weighed, and analyzed for THg and MeHg. At week 8 sediment was collected from the sediment plus 10 g vegetation and sediment only treatments for MeHg analysis.

2.4. Sample analysis

Analysis of THg, MeHg in water, sediment, and vegetation was conducted by the Marine Pollution Studies Laboratory at Moss Landing Marine Laboratory (MLML). Filtered and non-filtered water samples were acidified within 24 h of collection and acidified to 0.5 % using Baker 12 N reagent grade HCl and kept refrigerated at 4 °C prior to analysis. Methylmercury analysis followed a modified version of the U.S. Environmental Protection Agency (EPA) method 1630, which is described in detail in Horvat et al. (1993). Vegetation samples were digested in 25 % potassium hydroxide methanol reagent followed by distillation and analysis as described for MeHg water samples. Sediment and vegetation THg concentrations were determined using a Milestone DMA-80 direct mercury analyzer. Dissolved organic carbon sample collection and analysis followed EPA 415.1 and was measured by Bryte Laboratory.

2.5. Quality assurance and quality control

Analyses of samples were conducted in batches of 20, which included method blanks, laboratory duplicate, matrix spikes, matrix spike duplicates, and laboratory control spike or certified reference material. Average QA results for matrix spikes, matrix spike duplicates, laboratory control spikes, and laboratory duplicates met or exceeded QA goals (*Supplementary material S-6*). Two individual matrix spikes out of 160 were out of control limits but could not be rerun due to lack of volume. All samples were analyzed within method-required holding times.

2.6. Statistical analysis

All statistical analyses were performed using R Statistical Software (v3.6.0 and v4.0.5, R Core Team, 2021). For parametric analyses, raw or ln transformed data were checked for assumptions of normality using the Shapiro-Wilk test for normality of residuals and the Levene's test for assumptions of homogeneity of variance. Non-parametric tests were used for data not meeting parametric assumptions.

2.6.1. Mesocosm experiments

A 2-way ANOVA on ln transformed data was used followed by post-hoc Tukey Honestly Significant Difference (HSD) tests (1st mesocosm experiment). A 2-way ANOVA of ln transformed data detected a significant interaction effect, therefore a single Tukey HSD test on time and treatments was not conducted. Instead, several 1-way ANOVAs were conducted on ln transformed data using an adjusted family error rate followed by Tukey HSD post-hoc tests (2nd mesocosm experiment).

2.6.2. Microcosm experiment

A Kruskal-Wallis rank sum test was conducted on the raw data followed by either a post hoc Tukey HSD test or a Dunn's multiple comparison test on ranked data.

3. Results

3.1. Mesocosm experiments

Soil, disked and ungrazed mesocosm sediment THg and MeHg concentrations and MeHg as a percent of THg are shown in *Supplementary material S-7*. Average THg and MeHg concentrations of preflood soil were 0.150 ± 0.008 μg g^{-1} dry weight (dw) and 2.69 ± 1.46 ng g^{-1} dw respectively. Pasture soil characteristics of percent loss on ignition (LOI), percent fines (PF), and grain size (GS) were measured and reported by Work and Schoellhamer (2018) as part of a larger effort studying erosion of YB soils. Pasture soils had LOI, PF, and GS values of >10 to 20 %, 61 to 90 %, and 78 to 16 μm respectively (Work and Schoellhamer, 2018). Average sediment THg concentrations remained relatively constant in flooded mesocosms with increased variability in ungrazed relative to disked treatments. Sediment MeHg concentrations in disked mesocosms were uniform while MeHg concentrations in ungrazed mesocosms were a factor of 2.5 times higher, at week 5, than starting soil concentration. Percent MeHg in sediment ranged from 0.877 to 4.12 % with highest values observed in ungrazed mesocosms.

Vegetation THg and MeHg concentrations, and MeHg as a percent of THg are shown in *Supplementary material S-7*. Preflood vegetation THg concentration was 0.020 ± 0.012 μg g^{-1} dw and increased to 0.134 ± 0.025 μg g^{-1} dw week 5. Vegetation MeHg concentration increased from 2.78 ± 1.51 ng g^{-1} dw, for preflood samples, to 31.0 ± 5.89 ng g^{-1} dw week 5. Preflood vegetation MeHg as percent of THg was 17.9 ± 12.0 % and decomposing vegetation was 23.6 ± 6.46 %. At completion of the second mesocosm experiment, decomposing vegetation average THg and MeHg concentrations were 0.041 ± 0.002 μg g^{-1} dw and 56.8 ± 11.9 ng g^{-1} dw respectively. Average vegetation MeHg as percent THg was 138.6 ± 36.6 %. Decomposing vegetation, collected from pasture after a 120 day flood, had THg concentrations of 0.704 ± 0.580 μg g^{-1} dw (non-irrigated pasture) and 0.252 ± 0.080 μg g^{-1} dw (irrigated pasture). Decomposing vegetation concentrations of MeHg were 36.4 ± 4.88 ng g^{-1} dw (non-irrigated pasture) and 45.4 ± 36.2 ng g^{-1} dw (irrigated pasture) post flood.

Surface vegetation biomass estimates for ungrazed and grazed pasture are listed in *Supplementary material S-8*. Average ungrazed pasture biomass was 465 ± 52 g m^{-2} and average grazed pasture biomass was 234 ± 66 g m^{-2}. Biomass for the estimated 70.6 km^2 of YB pasture land, including both ungrazed and grazed pasture, was 2.4 × 10^7 kg. The estimated pools of MeHg in YB pasture vegetation is listed in *Supplementary material S-9*. Average mass of MeHg in preflood vegetation was estimated to be 73 ± 8 g. Post flood decomposing vegetation pool of MeHg was between 602 and 1363 g with an average of 935 ± 299 g. For a 120 day flood the potential MeHg load from decomposing vegetation in YB pasture was 8 ± 3 g day^{-1}. This estimate was based on the assumption that vegetation is decomposed and transported downstream during large flood events. Field observation of a rye grass field before and after the 120-day 2017 flood support this assumption as almost all the rye grass was absent after the flood (*Supplementary material S-10*). It is important to point out that the data collected for estimating the mass of MeHg in decomposing vegetation is limited in that biomass was determined only 6 times and the variability in the biomass studies was large (CV = 39 %), all of which leads to increased uncertainty.

For each weekly incubation period (weeks 2, 3 and 5), water column fMeHg for 3 treatments (disked, ungrazed, and grazed) are given in

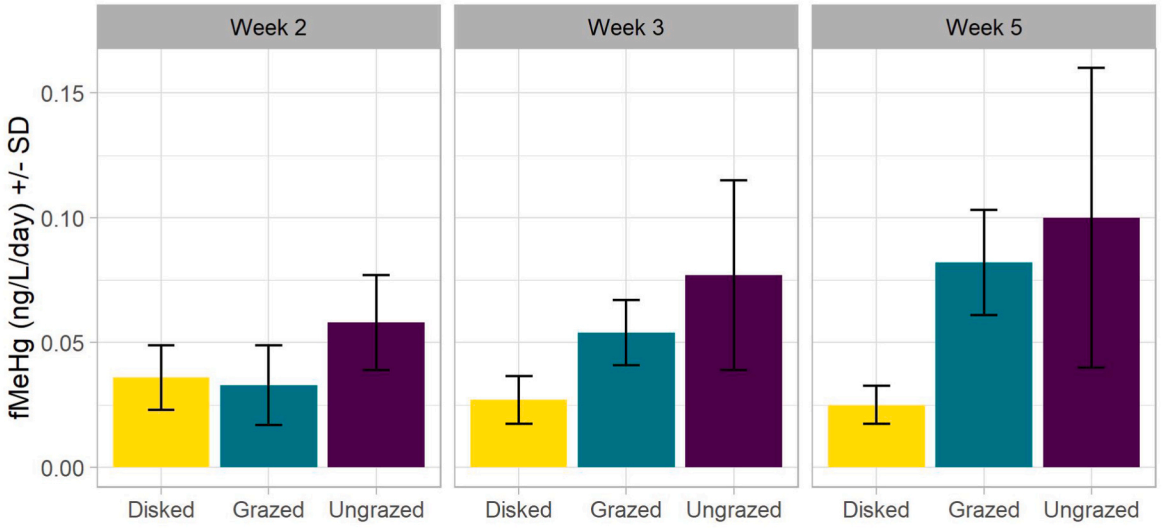

Fig. 2. Mean and standard deviation of fMeHg (ng L^{-1} day^{-1}) for disked, grazed, and ungrazed treatments ($n = 3$) at week 2, 3, and 5 in first mesocosm experiment. Control waters (not shown) were all less than the detection limit of 0.013 ng L^{-1}.

Fig. 2 and *Supplementary material S-11*. Control waters were all below the detection limit of 0.013 ng L^{-1} MeHg throughout the experiment.

For grazed treatment, fMeHg increased with inundation time; however, this pattern was not observed with the disked treatment or ungrazed treatment (large uncertainty) (Fig. 3). At week 2, fMeHg in the grazed treatment averaged 0.033 ng L^{-1} day^{-1} and increased to 0.054 ng L^{-1} day^{-1} at week 3 and 0.082 ng L^{-1} day^{-1} at week 5 (Fig. 2 and *Supplementary material S-11)*. We observed no fMeHg change with inundation time in the disked treatment containing no surface vegetation (Fig. 2). At week 2, fMeHg in the disked treatment was 0.036 ng L^{-1} day^{-1}. At week 5, fMeHg in the disked treatment was 0.024 ng L^{-1} day^{-1}.

The 2-way analysis of variance (ANOVA) analysis on the natural log transformed data indicated that there were significant differences between treatments. Tukey multiple comparison tests indicated the grazed and ungrazed treatments both had significantly higher fMeHg than the Disked treatment ($p < 0.025$, and <0.001; respectively). There were no significant differences in fMeHg between grazed and ungrazed treatments (Tukey multiple comparison test, $p < 024$).

For all treatments, cumulative mass of fMeHg in overlying water increased with time (*Supplementary material S*-12). Regression coefficients were 15.2 to 20.3 ng $week^{-1}$ for grazed and ungrazed, respectively, and 6.48 ng $week^{-1}$ for disked (*Supplementary material S*-12). For all treatments, the correlations between cumulative fMeHg masses and incubation time were significant ($r^2 = 0.68$ to 0.86, $p <$ 0.05).

The rate of change in fMeHg concentration (ng L^{-1} day^{-1}) for disked, grazed, and ungrazed treatments during the second mesocosm experiment are given in Fig. 3 and *Supplementary material S-13*.

The disked treatment showed no trend; fMeHg concentrations in overlying water in weeks 2 and 4 were 0.115 and 0.128 ng L^{-1} day^{-1}, respectively. The grazed treatment had the highest response; fMeHg increased from 0.155 to 0.505 ng L^{-1} day^{-1} between weeks 2 and 4. The ungrazed treatment was intermediate in response between the grazed and disked treatments, increasing from 0.088 to 0.298 ng L^{-1} day^{-1} between weeks 2 and 4.

A 2-way ANOVA on natural log transformed data indicated a significant interaction effect between the sampling events and land management practices ($p < 0.01$). Therefore, one-way ANOVA analyses were used on natural log transformed data to assess significant differences

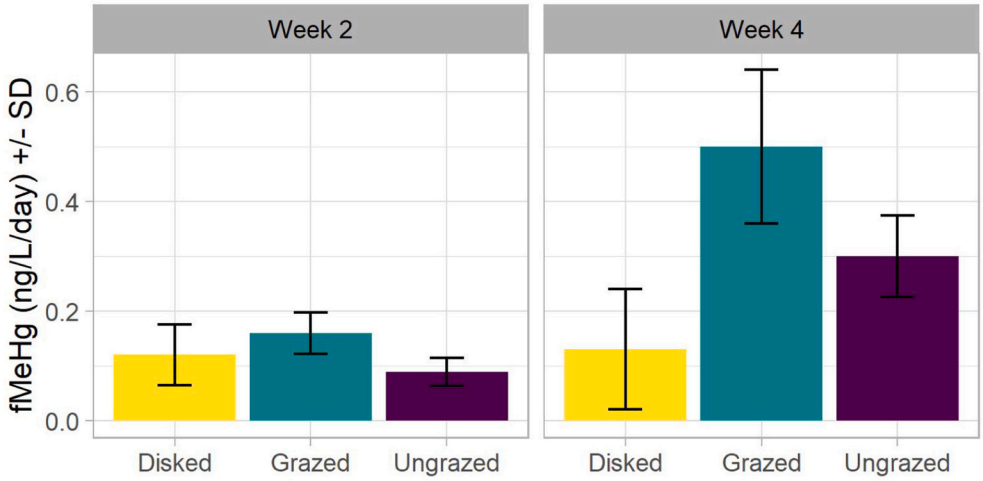

Fig. 3. Mean and standard deviation of fMeHg (ng L^{-1} day^{-1}) for disked, grazed, and ungrazed treatments ($n = 5$) at week 2 and 4 in second mesocosm experiment. Control waters (not shown) were all less than the detection limit of 0.013 ng L^{-1}.

between treatments for each week. When separated by sample week, in week 2 no significant differences in fMeHg were detected between treatments. In week 4, both the grazed and ungrazed treatments had significantly higher fMeHg than the disked treatment (Tukey multiple comparison tests $p < 0.001$, $p < 0.025$; respectively). Although week 4 fMeHg in the grazed treatment was measurably higher than the ungrazed treatment, there was no significant difference in fMeHg between the grazed and ungrazed treatments (Tukey multiple comparison test, $p = 0.2$). Seasonal offset as a factor influencing results between the two mesocosm experiments, due to ambient status of the sod at the time of collection, cannot be ruled out. In addition, an increase in replication for the second mesocosm experiment likely reduced uncertainty in ungrazed treatment.

3.2. Microcosm experiment

The microcosm experiment soil and sediment MeHg concentrations are shown in *Supplementary material S-14*. Average dry sieved soil MeHg concentration was 4.56 ± 0.18 ng g^{-1} dw. Sediment MeHg concentrations collected at end of experiment from sediment plus 10 g vegetation and sediment only treatments were 4.96 ± 0.17 and 4.74 ± 0.19 ng g^{-1} dw respectively.

Vegetation THg and MeHg concentrations and MeHg as a percent of THg are shown in *Supplementary material S-14*. Average THg and MeHg concentrations in dry vegetation prior to start of microcosm experiment were 0.073 ± 0.024 μg g^{-1} dw and 3.41 ± 0.33 ng g^{-1} dw respectively. Average decomposing vegetation THg concentrations in from vegetation plus sediment and vegetation only treatments were 0.098 ± 0.021 and 0.117 ± 0.026 μg g^{-1} dw respectively. Decomposing vegetation MeHg concentrations in vegetation plus sediment and vegetation only treatments were 25.1 ± 2.04 and 26.4 ± 4.75 ng g^{-1} dw respectively. Percent MeHg in dry vegetation was 4.70 % and increased to 25.6 and 22.5 % in decomposing vegetation from vegetation plus sediment and vegetation only treatments respectively.

Aqueous results from the laboratory microcosm experiment are listed in Table 1. At week 4 and 8, fMeHg (ng L^{-1} day^{-1}) from the vegetation-only treatments at medium and high levels of biomass were 5 to 10 times higher than the sediment (with no vegetation) treatment. The sediment only treatment was significantly different from the vegetation only treatments at medium and high levels of biomass at both 4 and 8 weeks exposure (Kruskall Wallis test ($p < 0.01$) followed by post hoc Tukey test on ranks ($p < 0.01$).

At weeks 4 and 8, treatments with sediment plus vegetation (medium and high levels of biomass) were 10 to 15 times higher than treatments with sediment only. The sediment only treatment was significantly different from the treatments of sediment plus vegetation at medium and high levels of biomass at both weeks 4 and 8 (Kruskall Wallis test ($p < 0.01$) followed by post hoc Dunn's test ($p < 0.05$).

Supplementary material S-15 shows fMeHg in overlying water with time for varying masses of vegetation: (sediment-only with 0 g vegetation added and sediment plus vegetation with 2.5, 5, and 10 g vegetation added). A positive correlation was observed between biomass added and fMeHg ($r^2 = 0.70$: $p < 0.01$, week 4 and $r^2 = 0.56$; $p < 0.01$, week 8), illustrating that the higher the mass of vegetation present, the greater the rate of change in fMeHg concentration.

4. Discussion

YB floodplain pasture soil and sediment Hg concentrations exceed 0.100 μg g^{-1}, a value that has been considered a "background" concentration (Obrist et al., 2016). The floodplain elevated Hg concentrations are related to proximity and connectivity to upstream precious metal mining activities (Wood et al., 2010) as well as atmospheric deposition (Obrist et al., 2016). In a synthesis of Hg in the western United States data for important functional types of vegetation, such as grasses, were largely lacking and contributed to uncertainties with regards to the role of vegetation in Hg cycling (Obrist et al., 2016). YB pasture above ground vegetation Hg concentrations reported here fill this data gap and provide a range of THg and MeHg concentrations in both preflood and decomposing grass vegetation.

There is significant literature evidence that vegetation increases or enhances microbial MeHg production in certain environments. For example, decomposing plant litter affects MeHg cycling in a boreal poor fen (Branfireun, 2000). Depositing Bull Rush stems and leaves over Bull Rush beds increases MeHg production (King et al., 2002). Burning or removing vegetation before new reservoir filling decreased MeHg in the water after the lake was filled (Mailman and Bodaly, 2006; Mailman et al., 2006). There is also evidence from both the laboratory and field studies that annual wetting of leaf litter produces MeHg that can be transported downstream after the first winter rainfall (Heyes et al., 1998; Hecky et al., 1991; Hall and St. Louis, 2004; Hall et al., 2004). Root zones in wetlands have also been implicated in higher organic matter and increased MeHg production; and removal of plants decreased MeHg production (Windham-Myers et al., 2009, 2014).

Our study provides multiple lines of evidence to support the hypothesis that decomposing plants are an important factor driving the production and release of fMeHg to overlying water during floodplain inundation. The microcosm experiment showed that the greater the mass of decomposing vegetation, the greater the production of fMeHg and release of fMeHg to overlying water, highlighting that the amount of vegetation is likely important in controlling fMeHg in overlying waters during inundation of the floodplain. The results from microcosm and mesocosm experiments showed that sediment devoid of surface vegetation or with vegetation disked into the sediment produced significantly less fMeHg than the vegetated treatments, again highlighting the importance of decomposing vegetation in addition to sediment controlling fMeHg concentrations. This relationship is not surprising and most likely reflects that fact that microbial activity for the breakdown of vegetation and Hg methylation increases with the greater abundance of organic matter. This occurs both through the greater abundance of organic matter "fuel" for microbial activity and concomitantly the greater abundance of inorganic Hg in the vegetation available for microbial methylation of Hg.

There were significant differences between the ungrazed and disked treatments. These investigations show that decaying vegetation alone can result in the significant microbial production and release of fMeHg

Table 1
Mean and standard deviation fMeHg (ng L^{-1} day^{-1}) for seven treatments ($n = 5$) at 2, 4 and 8 weeks of incubation.

Treatment	Week 2			Week 4			Week 8		
	fMeHg (ng L^{-1} day^{-1})			fMeHg (ng L^{-1} day^{-1})			fMeHg (ng L^{-1} day^{-1})		
Biomass Level	Low (2.5 g)	Medium (5 g)	High (10 g)	Low (2.5 g)	Medium (5 g)	High (10 g)	Low (2.5 g)	Medium (5 g)	High (10 g)
Sediment and vegetation	0.076 ± 0.021	0.194 ± 0.061	0.367 ± 0.112	0.100 ± 0.044	0.252 ± 0.113	0.546 ± 0.256	0.076 ± 0.032	0.253 ± 0.107	0.418 ± 0.274
Vegetation only	0.004 ± 0.001	0.083 ± 0.079	0.149 ± 0.037	0.005 ± 0.002	0.125 ± 0.124	0.223 ± 0.274	0.003 ± 0.009	0.143 ± 0.088	0.372 ± 0.183
Sediment only	0.007 ± 0.003			0.024 ± 0.016			0.023 ± 0.011		
Control	0.008 ± 0.003			0.007 ± 0.006			0.006 ± 0.006		

to solution compared to sediment alone. And, moreover, MeHg production becomes substantially enhanced when sediment is also present. These results are consistent with observations that microbial production of MeHg can occur in oxic waters associated with anoxic microzones on particles and surfaces (Gascón et al., 2016; Balzer et al., 2023; Bouchet et al., 2018). It is also well established that recently inundated vegetation undergoing microbial decay results in enhancing MeHg in the overlying water through microbially-mediated MeHg production processes.

Our experimental results suggest that most of the MeHg found associated with vegetation in this study was produced by microbially-meditated processes within the vegetation or biofilms during the decomposition process. We hypothesize that this increase in plant MeHg levels likely results from two processes: (1) the decrease in plant biomass as decomposition continues and (2) an enhancement in microbial activity as the decay process converts the particulate organic carbon in the plant to low molecular weight organics, fueling the microbial Hg methylating processes.

The increase in MeHg concentration and mass in decomposing pasture vegetation observed in this study have been observed in other types of vegetation as well. Similar results have been found by Heyes et al. (1998) who found the mass of MeHg in decomposing pine needles and moss increased by approximately 700 and 500 % and Hall and St. Louis (2004) who found large increases in MeHg concentrations and masses in upland and flooded landscapes after decomposition. For example, alder and bunchberry plants increased 200 times, birch and blueberries increased 35 times and jackpine, bryophytes, and lichen increased 3–14 times.

Our experimental results provide evidence that decomposing vegetation plays an important role in the observed fMeHg generated within the floodplain. This importance can be shown by comparing the predicted MeHg in vegetation from our experiments with the loads calculated directly from the concentrations and flows of water entering and leaving the YB and the required load reductions mandated for the YB. Direct calculations of winter 2017 fMeHg load from the YB, calculated using a mass balance approach, was 1692 g yr^{-1} (DWR, 2020), while the reduction in MeHg required by the regulatory agencies for the YB is 833 g yr^{-1} (Wood et al., 2010). Although our replicates and number of experiments were limited, the similarity between experimental results (average of 8 ± 3 g day^{-1}; *Supplementary material S-9*) and direct measurements of YB mass balance suggests that at least part of the net increase in MeHg observed in the YB Mass Balance studies could be accounted for by the release of MeHg from decomposing vegetation. Even though our experimental results are scaled up from small mesocosms and were not measured directly in the field, the similarity between experimental and calculated mass loads from direct measurements, suggests that; a) the contribution of MeHg in decomposing vegetation in the YB should not be overlooked when evaluating sources of MeHg to a floodplain system, and that, specifically, in the YB, vegetation in pastures may have sufficient MeHg mass to account for a significant portion of the net internal increase in MeHg loads developed within the flooded YB itself, suggesting that the floodplain is more than a neutral transport system conveying loads into the Delta from its various tributaries, but is an active contributor to the MeHg loads exported downstream to the Delta and b) controlling biomass could make a positive impact to the required load reduction of fMeHg loads exiting the YB.

5. Conclusion

Our results demonstrate that decomposing vegetation is a significant factor in enhancing microbial MeHg production and release to overlying waters during floodplain inundation. Microcosm and mesocosm experiments confirm that the amount of decomposing vegetation plays a crucial role in controlling MeHg concentrations, as organic matter serves as fuel for microbial activity and Hg methylation. Our study also provides crucial Hg concentration data for grasses, an essential functional type of vegetation, filling a data gap that previously contributed to uncertainties surrounding the role of vegetation in Hg cycling. By comparing experimental results with direct measurements from the floodplain, we show the potential significance of MeHg released from decomposing vegetation in contributing to net increases in MeHg loads within the YB floodplain system. These findings contribute to our understanding of Hg dynamics in contaminated floodplains and provide a valuable basis for future research and management strategies aimed at mitigating MeHg contamination and protecting downstream habitats in the YB and similar floodplain systems worldwide.

CRediT authorship contribution statement

Wesley A. Heim: Project administration, Supervision, Conceptualization, Investigation, Writing – review & editing. **David Bosworth:** Conceptualization, Investigation, Writing – review & editing. **Carol DiGiorgio:** Project administration, Supervision, Conceptualization, Investigation, Writing – review & editing. **Mark Stephenson:** Conceptualization, Investigation, Writing – review & editing. **Gary Gill:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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