Mercury transformation and release differs with depth and time in a contaminated riparian soil during simulated flooding

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15 **ABSTRACT**

16 Riparian soils are an important environment in the transport of mercury in rivers and wetlands,
17 but the biogeochemical factors controlling mercury dynamics under transient redox conditions in these
18 soils are not well understood. Mercury release and transformations in the O_a and underlying A horizons
19 of a contaminated riparian soil were characterized in microcosms and an intact soil core under
20 saturation conditions. Pore water dynamics of total mercury (Hg_T), methylmercury (MeHg), and
21 dissolved gaseous mercury (Hg⁰_(aq)) along with selected anions, major elements, and trace metals were
22 characterized across redox transitions during 36 d of flooding in microcosms. Next, Hg_T dynamics were
23 characterized over successive flooding (17 d), drying (28 d), and flooding (36 d) periods in the intact
24 core. The observed mercury dynamics exhibit depth and temporal variability. At the onset of flooding in
25 microcosms (1-3 d), mercury in the O_a horizon soil, present as a combination of ionic mercury (Hg(II))
26 bound to thiol groups in the soil organic matter (SOM) and nanoparticulate metacinnabar (β-HgS), was
27 mobilized with organic matter of high molecular weight. Subsequently, under anoxic conditions, pore
28 water Hg_T declined coincident with sulfate (3-11 d) and the proportion of nanoparticulate β-HgS in the
29 O_a horizon soil increased slightly. Redox oscillations in the intact O_a horizon soil exhausted the mobile
30 mercury pool associated with organic matter. In contrast, mercury in the A horizon soil, present
31 predominantly as nanoparticulate β-HgS, was mobilized primarily as Hg⁰_(aq) under strongly reducing
32 conditions (5-18 d). The concentration of Hg⁰_(aq) under dark reducing conditions correlated positively
33 with byproducts of dissimilatory metal reduction ($\Sigma(\text{Fe, Mn})$). Mercury dynamics in intact A horizon soil
34 were consistent over two periods of flooding, indicating that nanoparticulate β-HgS was an accessible
35 pool of mobile mercury over recurrent reducing conditions. The concentration of MeHg increased with
36 flooding time in both the O_a and A horizon pore waters. Temporal changes in pore water constituents

(iron, manganese, sulfate, inorganic carbon, headspace methane) all implicate microbial control of redox transitions. The mobilization of mercury in multiple forms, including Hg_T associated with organic matter, MeHg , and $\text{Hg}_{(aq)}^0$, to pore waters during periodic soil flooding may contribute to mercury releases to adjacent surface waters and the recycling of the legacy mercury to the atmosphere.

1. INTRODUCTION

Mercury released from mining and industrial operations often accumulates in riparian soils, which are important environments for mercury cycling at the interface between terrestrial and aquatic ecosystems. At the confluence of hydrologic flow paths, riparian soils exhibit dynamic biogeochemical and hydrologic conditions recognized to control the mobilization of mercury and other constituents (e.g., organic matter) to adjacent surface waters (Vidon et al., 2010). The fluctuation of soil redox state in response to soil saturation (e.g., overbank flooding, precipitation events), in particular, is hypothesized to be the primary control on the sequestration of mercury in soils as metacinnabar ($\beta\text{-HgS}$) (Barnett et al., 1997) and the formation and release of methylmercury (MeHg) (Balogh et al., 2004; Regnell et al., 2009; Barringer et al., 2010), the form of mercury that bioaccumulates in organisms (Mason et al., 1996). As a result of counteracting processes, it is unclear if transient redox conditions from periodic soil flooding increase or decrease mercury mobility and bioavailability. An improved biogeochemical understanding of mercury mobilization and transformation in riparian soils is needed to predict the long-term fate of mercury in terrestrial and aquatic environments, particularly under scenarios of contaminant remediation and land or water use changes.

Divalent mercury (Hg(II)) usually predominates in contaminated soils (Biester et al., 2002; Slowey et al., 2005; Southworth et al., 2010) and is retained or mobilized by competing interactions with

organic matter and inorganic sulfide. The speciation of Hg(II) recently deposited in organic-rich soils is dominated by complexation with thiol groups in soil organic matter (SOM) (Skylberg et al., 2006; Nagy et al., 2011). Similarly, complexation of Hg(II) with thiol groups in dissolved organic matter (DOM) (Haitzer et al., 2002) in soil pore waters is thought to be responsible for the co-mobilization of mercury with DOM from riparian soils to streams (Mierle and Ingram, 1991; Shanley et al., 2008; Dittman et al., 2009; Brigham et al., 2009; Dittman et al., 2010). In particular, the hydrophobic organic acid fraction of DOM, compared to other DOM fractions, contains greater thiol group content (Haitzer et al., 2003) and has been observed to correlate positively with the filter-passing total mercury (Hg_T) concentration in streams (Shanley et al., 2008; Dittman et al., 2009; Dittman et al., 2010). DOM of greater hydrophobic quality can be mobilized by transport processes (e.g., lateral flow in saturated soils) (Aiken and Cotsaris, 1995; Sebestyen et al., 2008) and biogeochemical reactions (e.g., dissolution of Mn(III,IV) and Fe(III) (hydr)oxides) (Chin et al., 1998; Grybos et al., 2009). Additionally, the aging of Hg(II) complexed to DOM under oxic conditions results in conversion of thiol bound Hg(II) to nanoparticulate β -HgS over week-to-month time scales (Manceau et al., 2015), an abiogenic mechanism that may explain the immobilization of older mercury in oxic non-flooded soils (Hintelmann et al., 2002).

The formation of β -HgS is often ascribed to a biogenic pathway in flooded soils (Barnett et al., 1997). The depletion of molecular oxygen in saturated soils results in microbial respiration of alternative terminal electron acceptors, most notably sulfate (SO_4^{2-}). Inorganic sulfide, the byproduct of dissimilatory sulfate reduction, outcompetes organic matter for Hg(II), which results in the formation of poorly soluble β -HgS (Gerbig et al., 2011; Graham et al., 2012). Authigenesis of β -HgS in contaminated riparian soil has been attributed to sulfate reduction during previous flooding episodes (Barnett et al., 1997). The view that this β -HgS is a recalcitrant immobile sink for mercury has been challenged by studies demonstrating that nanoparticulate β -HgS formed in the presence of DOM and free sulfide is both stabilized in solution and bioavailable to methylating organisms (Zhang et al., 2012; Graham et al.,

2012; Pham et al., 2014). Dissolved organic matter stabilizes β -HgS particles as small as 3-5 nm in diameter by slowing particle growth rate (Deonarine and Hsu-Kim, 2009) and limiting particle size and crystallinity (Ravichandran et al., 1999; Gerbig et al., 2011). Previous laboratory studies of flooded soils demonstrate the mobilization of other trace metals from soils as nanoparticulate sulfide minerals (Weber et al., 2009a) and the co-mobilization of mercury with copper (Hofacker et al., 2013).

Transient redox conditions as a result of periodic soil flooding also influence the formation of bioavailable MeHg and dissolved gaseous mercury ($\text{Hg}^0_{(\text{aq})}$), the latter controlling the flux of mercury from terrestrial and aquatic ecosystems to the atmosphere. Sulfate-reducing bacteria and other anaerobic microorganisms convert Hg(II) to MeHg (Gilmour et al., 2013; Podar et al., 2015). In-stream measurements of MeHg during high flow events have been observed to coincide with low concentrations of molecular oxygen and increased concentrations of manganese and iron (Balogh et al., 2004; Regnell et al., 2009; Barringer et al., 2010), which suggests that the hydrologic flushing of reduced riparian soils may be the source of MeHg. Furthermore, several models that describe MeHg concentrations in streams use parameters based on riparian zone hydrology (i.e., hydrologic flow path, water table depth) (Burns et al., 2014; Eklöf et al., 2015). Under dark anoxic conditions, Hg(II) can also be reduced to $\text{Hg}^0_{(\text{aq})}$ through biotic (e.g., dissimilatory metal-reducing bacteria) (Wiatrowski et al., 2006; Hu et al., 2013a) and abiotic pathways (e.g., redox-active DOM groups, aqueous Fe(II), Fe(II)-bearing minerals) (Allard and Arsenie, 1991; Wiatrowski et al., 2009; Gu et al., 2011; Zheng et al., 2012; Bone et al., 2014). The formation of $\text{Hg}^0_{(\text{aq})}$ as a result of soil flooding increases mercury emissions from soils (Obrist et al., 2010), but $\text{Hg}^0_{(\text{aq})}$ can also be oxidized and methylated by anaerobic bacteria (Colombo et al., 2013; Hu et al., 2013b). It is unclear if nanoparticulate β -HgS in riparian soils is a source of mercury for MeHg or $\text{Hg}^0_{(\text{aq})}$ formation.

Sites of legacy mercury contamination show a mixture of mercury species in soils based on X-ray absorption spectroscopy (Kim et al., 2003; Manceau et al., 2015), chemical extractions (Slowey et al.,

2005; Southworth et al., 2010), and thermo-desorption (Biester et al., 2002). Furthermore, the distribution of mercury species in soil can vary with soil depth down to 30 cm and between soil horizons (Manceau et al., 2015). In general, contamination can span depths corresponding to several soil horizons that differ in organic matter content, mineralogical composition, and microbial community structure (Biester et al., 2002; Hansel et al., 2008; Southworth et al., 2010). Based on the factors controlling mercury transformation and mobilization discussed above, we hypothesize that biogeochemical controls on mercury dynamics in riparian soils differ between soil horizons due to unique soil mercury speciation and pore water biogeochemistry.

Here we describe results of a study designed to identify the dominant biogeochemical processes controlling mercury transformation and release dynamics from contaminated riparian soils during flooding. Riparian soils were collected downstream from Oak Ridge, Tennessee, USA. First, mercury release dynamics were evaluated in microcosm flooding experiments with soils from two soil horizons that define a contaminated soil profile: the organic-rich O_a horizon and underlying A horizon. Soil mercury speciation was quantified by high-resolution X-ray absorption near-edge structure (HR-XANES) spectroscopy (Manceau et al., 2015, 2016). Pore water concentrations of Hg_T, MeHg, and Hg⁰_(aq) were quantified over 36 d of flooding. Next, the mobilization of mercury from O_a and A horizon soil to pore waters was characterized in intact soils over two consecutive flooding periods of 17 and 36 d. The results demonstrate spatial and temporal variability in the biogeochemical factors controlling mercury dynamics during flooding along a contaminated soil profile.

2. EXPERIMENTAL

2.1. Sample Location, Soil Collection, and Soil Processing

Riparian floodplain soils were collected 1 m from the bank of East Fork Poplar Creek, Tennessee (N 35°57.959; W 084°21.570; ± 7 m), a watershed affected by historic mercury contamination (Brooks and Southworth, 2011). The sample location is located approximately 17 km downstream from the U.S. Department of Energy Y-12 National Security Complex (Fig. EA-1) and had not been subject to soil remediation (Southworth et al., 2010). The discharge of mercury, primarily as dissolved Hg(II), at the headwaters of the creek between 1950 and 1963 resulted in the contamination of the watershed (Brooks and Southworth, 2011). Today, contaminated riparian soils are recognized as the primary source of mercury to East Fork Poplar Creek surface waters during precipitation events and high flow conditions (Southworth et al., 2010; Riscassi et al., 2015).

To establish soil horizons and the mercury depth profile, a soil pit was dug and samples were collected at the surface (0-5 cm) to a maximum depth of 65 cm at 10 cm intervals in April, 2010. Field observations made during soil collection are provided in Table EA-1. Samples were shipped overnight to Boulder, CO. In the laboratory, soils were freeze-dried, sieved to ≤ 0.71 mm using a stainless steel sieve to remove large rocks and organic debris, and homogenized by mortar and pestle. Soils used in both microcosm and intact soil core flooding experiments were collected as intact soil cores in October, 2010. Soil structure and stratigraphy were not altered from field conditions. Intact soil cores (20 cm diameter, 30 cm height) were collected by excavating around the desired soil (Fig. EA-2a), placing a 2 mm-thick perfluoroalkoxy Teflon® bag over the soil core, placing polyvinyl chloride pipe (25.4 cm inside diameter, 30 cm tall) over the Teflon® bag (Fig. EA-2b), and filling the annular space with polyurethane expanding foam. Redoximorphic features (e.g., gray clay, orange mottling) were observed at ≥ 40 cm depth, which indicated an oxic/anoxic redox transition. Soil cores were shipped overnight to Boulder, CO, and stored

at 4 °C until use. For microcosm flooding experiments, intact cores were sectioned in the lab under an ambient atmosphere into the O_a (0-5 cm depth) and A (20-30 cm depth) soil horizons. Sectioned bulk soils were air dried and sieved to ≤ 2 mm using a stainless steel sieve to remove large rocks and organic debris.

2.2. Materials

High purity water (≥ 18 MΩ cm resistivity), trace metal-grade acids, and ultrahigh purity gases were used in this study. Borosilicate glass jars (I-Chem 200 series) with Teflon®-lined caps were used for the storage of soils, as vessels for microcosms, and for storage of aqueous samples for mercury and DOM analyses. Glassware was cleaned by soaking in acid (solution of 10% HCl and 10% HNO₃) for 24 h, rinsing three times with high purity water, and baking at 450 °C for 4.5 h. Samples for metal and ion analyses were stored in acid-cleaned (solution of 10% HCl and 10% HNO₃) low-density polyethylene bottles. Lysimeters were constructed of borosilicate glass with sintered-glass tips with a 10-16 µm pore-size cutoff (6 mm diameter, 110 mm length; EcoTech). Lysimeters were connected to fluorinated ethylene propylene Teflon® tubing without the use of organic adhesives to prevent DOM contamination (Siemens and Kaupenjohann, 2003). The lysimeter and Teflon® tubing had a dead volume of 2.7 mL.

2.3. Soil Characterization

Soils (≤ 0.71 mm) used to establish soil horizons and the mercury depth profile were analyzed for total organic matter content by loss on ignition (LOI) (550 °C for 2 h; Dean, 1974) and total mercury concentration by thermal desorption and atomic absorption spectroscopy detection (DMA-80 Direct Mercury Analyzer, Milestone). Total mercury was quantified using a calibration curve prepared with certified reference materials from the National Institute of Standards and Technology (NIST; 1630a, 2702) and National Research Council Canada (TH-2, WQB-1, WQB-3). The dry bulk density of the soil core was estimated to be 1.23 g (cm)⁻³. The following analyses were performed on bulk O_a (0-5 cm

depth) and A horizon (20-30 cm depth) soil (≤ 2 mm) used in microcosm flooding experiments. Dry bulk density was estimated based on dry soil mass and the volume of the sectioned soil core (Section 2.1). Soil pH was measured in a 0.01 M CaCl_2 solution at a 1:5 solid-to-liquid ratio. Total organic matter content was quantified by LOI. Mineralogical composition was quantified by powder X-ray diffraction (XRD; Siemens Kristalloflex 805; Eberl, 2003). Oriented XRD slides were used to identify clay mineralogy using glycol solvation and heating at 550 °C. XRD spectra were modeled using RockJock (Eberl, 2003); the degree of fit for all samples was below 0.10. Effective cation exchange capacity (*ECEC*) was quantified in unbuffered 0.1 M BaCl_2 after a 2 h extraction at a 1:60 solid-to-liquid ratio (Hendershot and Duquette, 1986). The *ECEC* was determined as the sum of the BaCl_2 -extractable cations using the following equation:

$$ECEC = \sum([i] * z_i) \quad (1)$$

where $[i]$ is the concentration of cations (Mn^{2+} , Fe^{2+} , Mg^{2+} , Ca^{2+} , Al^{3+} , Na^+ , K^+) and z_i is the charge of i .

The assumption that iron and manganese were divalent had a negligible effect on *ECEC* values ($< 0.5\%$).

Major and trace elemental composition was determined by acid digestion (100 mg soil, 3.5 mL HCl, 1.5 mL HF, 2 mL HNO_3 ; Farrell et al., 1980) followed by inductively coupled plasma atomic emission spectrometry (ICP-AES; Thermo Scientific, ARL 3410+) and inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, SCIEX Elan DRC-e), respectively. Total mercury concentrations were quantified by aqua regia digestion (200 mg soil, 9 mL HCl, 3 mL HNO_3), preservation with 1% volume/volume (v/v) 0.2 M BrCl , and aqueous analysis using SnCl_2 reduction, dual amalgamation, and cold vapor atomic fluorescence spectroscopy (CVAFS) detection (Tekran 2600) following EPA method 1631. Calibration standards of Hg_T were prepared from NIST standard reference material 3133. The deviation in element and total mercury concentrations between digestion duplicates was $\leq 5\%$ and $\leq 2\%$, respectively. All concentrations are reported on a dry mass basis.

2.4. Microcosm Flooding Experiments and Pore Water Analyses

Microcosm flooding experiments were performed in the dark at 25 ± 2 °C in an anoxic glovebox (filled with 5% H₂, 95% N₂; O₂ < 0.1 ppm). Water was deaerated by purging with helium for 1 h. O_a or A horizon soil (50 g) was placed in 125 mL borosilicate glass jars and high purity water was added to a solid-to-liquid ratio of 1 kg L⁻¹. Microcosms were turned end-over-end for 3 min to ensure thorough wetting of soils. Lysimeters were inserted vertically into the vessel and secured with the tip about 1.5 cm above the bottom of the vessel (Fig. 1a). Independent microcosms ($n = 28$ for both O_a and A horizon soil) were sampled over 36 d. The gas inside the glovebox was exchanged every 3 d. Experimental blanks ($n = 2$) contained only high purity water. Additional microcosm systems ($n = 2$ for both O_a and A horizon soil) were assembled identically to those described above in 100 mL acid-cleaned serum bottles capped with rubber septa. Methane concentrations in the headspace of these microcosms were measured over 36 d of flooding to qualitatively identify methanogenic conditions. Details on headspace methane analyses are provided in the Electronic Annex.

Microcosm pore water samples were collected by connecting a syringe to lysimeters and drawing samples at a rate of about 0.6 mL min⁻¹. First, 4 mL of pore water were collected, the syringe was disconnected while maintaining vacuum pressure in the lysimeter, and the sample was expelled for pH and redox potential ($E_{\text{Ag/AgCl}}$) measurements. The syringe was reconnected to the lysimeter and about 16 mL were collected for aqueous analyses. The flow chart provided in Fig. EA-3 outlines the processing and analyses performed on microcosm pore water samples. Pore water pH and $E_{\text{Ag/AgCl}}$ measurements were made with pH (Slimtrode, Hamilton Company) and oxidation-reduction potential (ORP; Orion Thermo Scientific) electrodes, respectively, on undiluted, unfiltered pore waters immediately after collection in an anoxic glovebox. The pH and ORP electrodes were calibrated daily (pH 4, 7, and 10 buffers and a $E_{\text{Ag/AgCl}}$ +220 mV ORP standard (Thermo Scientific), respectively). Redox potential measurements were converted from the Ag/AgCl reference electrode to the standard hydrogen

220 electrode (E_H) by adding 200 mV (25 °C). Pore water samples (about 16 mL) were diluted to a total
221 volume of about 95 mL with deaerated high purity water. Unless otherwise specified, analyses were
222 performed on 22 pore water samples for both O_a and A horizon microcosms collected between 0 and 36
223 d of flooding. Measurements of MeHg, $Hg^0_{(aq)}$, sulfide, total iron, and Fe(II) were made at the following
224 times: 1, 3, 7, 14, 28, and 36 d ($n = 6$ for both O_a and A horizon soil). Unfiltered ($< 10\text{-}16\text{ }\mu\text{m}$; based on
225 lysimeter pore-size cutoff) solutions were analyzed for Hg_T , MeHg, $Hg^0_{(aq)}$, and major and trace elements.
226 Diluted pore water samples were filtered (0.2 μm Supor®, Pall Life Science), discarding the first 5 mL of
227 filtrate, and analyzed for Hg_T , MeHg, major and trace elements, anions, dissolved inorganic carbon (DIC),
228 dissolved organic carbon (DOC), ultraviolet and visible light (UV-vis) absorption, total iron and Fe(II), and
229 sulfide. Particulate concentrations ($0.2\text{ }\mu\text{m} \leq X < 10\text{-}16\text{ }\mu\text{m}$) were determined as the difference between
230 unfiltered ($< 10\text{-}16\text{ }\mu\text{m}$) and filtered ($\leq 0.2\text{ }\mu\text{m}$) concentrations.

231 Samples for Hg_T analysis were preserved with 1% v/v 0.2 M BrCl and measured by SnCl_2
232 reduction, dual amalgamation, and CVAFS detection (average daily detection limit (DDL) of 2.5 pM
233 determined at three times the standard deviation of the 2.5 pM calibration standard ($n = 6$); relative
234 percent deviation between duplicate analyses averaged 1.1%). Microcosm blanks ($n = 2$) did not show
235 appreciable levels of Hg_T ($< 36\text{ pM}$ filter-passing Hg_T) after 36 d compared to soil-containing microcosms.
236 The average Hg_T concentration measured in experimental blanks at 36 d was subtracted from sample
237 concentrations and represented $< 1\%$ of the Hg_T in all cases. MeHg samples were preserved with 1% v/v
238 6 M HCl, stored in the dark, and shipped to the U.S. Geological Survey Wisconsin Water Science Center
239 for analysis by isotope dilution. Approximately 0.1 pmol of isotopically enriched Me^{199}Hg and 1 mL of
240 1.6 M copper sulfate solution were added to 40 mL aliquots for MeHg analysis. Ambient MeHg was
241 determined by relating the ratio of added Me^{199}Hg to Me^{202}Hg after distillation, aqueous phase
242 ethylation, trapping on Tenax, isothermic gas chromatography separation, and detection by an
243 automated MeHg analytical system (MERX, Brooks-Rand) coupled to an ICP-MS (ELAN 9000, Perkin

Elmer) (average DDL of 2.8 pM). MeHg spikes of natural isotopic abundance showed an average of 108% recovery ($n = 6$). The relative percent deviation between duplicate MeHg analyses averaged 5.5%. $\text{Hg}_{(\text{aq})}^0$ samples were processed within 10 min of sample collection in an anoxic glovebox to minimize $\text{Hg}_{(\text{aq})}^0$ loss by volatilization or oxidation (Lindberg et al., 2000). Unfiltered, diluted pore water (50 mL) was purged in the dark with argon for 30 min (flow rate of 100 mL min^{-1} ; purge gas volume (water volume) $^{-1} = 60 \text{ L L}^{-1}$) (Lindberg et al., 2000) in 125 mL acid-cleaned gas wash bottles onto pre-cleaned gold-coated sand traps. A gold trap was placed in-line prior to gas wash bottles to further clean argon of mercury. Gold traps were capped, stored in zip lock bags, and shipped overnight to the U.S. Geological Survey Wisconsin Water Science Center for immediate analysis by thermal desorption and ICP-MS detection (ELAN 9000, Perkin Elmer). The concentration of $\text{Hg}_{(\text{aq})}^0$ in a microcosm blank ($n = 1$) incubated for 36 d, quantified at 0.04 nM, was subtracted from sample concentrations and represented $\leq 4\%$ of the $\text{Hg}_{(\text{aq})}^0$ quantified in all cases. Bubbler blanks, containing only 50 mL high purity water, averaged 1 pM ($n = 3$). The fractions of filter-passing Hg_T as $\text{Hg}_{(\text{aq})}^0$, filter-passing Hg_T as MeHg, and particulate Hg_T as MeHg were determined based on Hg_T measurements made on the same microcosm pore water sample.

Major element (Fe, Al, Si, Mn, Na, K, Mg, Ca) and trace metal (Cu, Ni, Pb, Sr, Zn) concentrations were determined on samples preserved with 1% v/v 15.7 M HNO_3 by ICP-AES and ICP-MS, respectively. One outlier in measured filter-passing zinc ($0.9 \mu\text{M}$) and nickel ($7.14 \mu\text{M}$) concentrations in the O_a horizon microcosm sampled at 14 d was omitted. Inorganic anions (Cl^- , NO_3^- , SO_4^{2-}) and organic acids (acetate, formate, propanoate) were quantified by ion chromatography (IC; Dionex IonPac® AS14A column). Total iron and Fe(II) were quantified by a ferrozine assay on samples preserved with 1% v/v 6 M HCl (To et al., 1999); Fe(III) was determined as the difference between total iron and Fe(II). Sulfide was measured immediately after sample collection using a methylene blue assay (Hach 2700 spectrophotometer; dilution-corrected detection limit of $0.4 \mu\text{M}$). DIC concentrations were determined by alkalinity titration (Barringer and Johnson, 1996). DOC concentrations were determined by persulfate

oxidation (OI 700 carbon analyzer) and corrected for carbon from quantified organic acids. Experimental blanks did not show appreciable levels of DOC (< 0.02 mM) after 36 d.

The redox state of samples for UV-vis absorption measurements was maintained prior to analysis to minimize optical interference by Fe(III) (Poulin et al., 2014). Cuvettes were filled in a glovebox, capped, and brought out for immediate spectrometric analysis. UV-vis absorption spectra were measured from 190-800 nm using a spectrophotometer (Agilent Technologies, model 8453) and a 1 cm quartz cuvette. Sample spectra were measured with respect to a blank spectrum containing high purity water. Decadic absorbance values were converted to Napierian absorption coefficients as follows:

$$\alpha_{\lambda} = 2.303A_{\lambda}/l \quad (2)$$

where α_{λ} is the Napierian absorption coefficient (cm^{-1}), A_{λ} is the decadal absorbance, and l is the cell path length (cm). Spectral slopes were calculated by fitting Equation 3 to the Napierian absorption spectra between wavelengths of 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-400}$):

$$\alpha_{\lambda} = \alpha_{\lambda_{ref}} e^{-S(\lambda - \lambda_{ref})} \quad (3)$$

where α_{λ} is the Napierian absorption coefficient at the specified wavelength, $\alpha_{\lambda_{ref}}$ is the Napierian absorption coefficient at the reference wavelength, and S is the slope fitting parameter (Green and Blough, 1994). The spectral slope ratio (S_R) was defined as $S_{275-295} : S_{350-400}$ and has been inversely correlated to DOM molecular weight (Helms et al., 2008). Laboratory addition experiments confirmed that Mn(II) did not interfere with S_R measurements (details provided in the Electronic Annex, Fig. EA-4).

Changes in the pore water ionic strength were estimated in Visual MINTEQ (Gustafsson, 2014) at the measured pH and included the following constituents: K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Fe^{2+} , Al^{3+} , Cl^- , NO_3^- , SiO_4^{2-} , SO_4^{2-} , acetate, CO_3^{2-} , and DOC. Contributions of DOC to ionic strength were estimated using the NICA-Donnan model assuming 50% of the DOC to be fulvic acid; this assumption was varied between

10%-100% to evaluate the sensitivity of ionic strength estimates to changes in organic matter composition.

2.5. Intact Soil Core Flooding Experiments and Pore Water Analyses

The goal of this experiment was twofold: (1) to investigate whether the same processes control mercury dynamics in intact, undisturbed soil matrices as in loose, sieved soil, and, (2) to assess the importance of sequential flooding periods on mercury release behavior. Experimental details are provided in the Electronic Annex. Briefly, lysimeters were inserted into the soil core to sample O_a (5 cm below soil surface) and A horizon pore waters (23 cm below the soil surface) (Fig. 1b, Fig. EA-2c). The soil core was flooded with high purity water from the bottom (Lewis and Sjöström, 2010). A constant water height was maintained about 3 cm above the soil surface using a Mariotte bottle. Pore waters were sampled over 17 d of flooding, at which point the soil core was drained from the bottom and exposed to air for 28 d. Following the drained period, the soil core was flooded for another 36 d. Pore water samples were collected by connecting lysimeters to N_2 -filled acid-cleaned serum bottles in which a vacuum was maintained and processed immediately in an anoxic glovebox. Pore water samples were diluted with deaerated high purity water. Because of limited sample volume, pore water samples were analyzed for a selected set of parameters that included filter-passing Hg_T , DOC, DIC, and anions (Cl^- , NO_3^- , SO_4^{2-}) using methods described in Section 2.4.

2.6. Mercury Speciation in Microcosm Soils

Mercury speciation in O_a and A horizon soil prior to flooding ($t = 0$ d) was determined previously (Manceau et al., 2015). In this study, we report spectroscopic analysis of O_a horizon soil recovered from microcosm incubations after 36 d of flooding for comparison ($t = 36$ d). Soil was recovered from microcosms immediately after pore water sampling, homogenized, frozen under a N_2 atmosphere, freeze-dried, and immediately placed under a N_2 headspace. The clay-size fraction was isolated to obtain

sufficient mercury concentration for HR-XANES analysis, which is detailed in the Electronic Annex. Samples were prepared as pressed pellets and cooled to 10 K using a liquid He cryostat in order to minimize radiation damage (e.g., X-ray photoreduction of Hg(II) to Hg⁰). A detailed account of HR-XANES spectra collection, measured at the mercury L₃-edge, is provided in the Electronic Annex. Briefly, 20 sweeps were made of each sample to improve the signal-to-noise ratio, moving the sample by a few hundred microns between each sweep to access unexposed fresh material. The standard deviation (σ) of the average signal was calculated at each energy point of the spectrum. Spectra were normalized to unity at $E = 12360$ eV and modeled using linear combination fitting (LCF) with the following model spectra: Hg(II)- and MeHg-complexed to Elliott soil humic acid (ESHA), β -HgS, cinnabar (α -HgS), and elemental mercury (Hg⁰). Details on the preparation of Hg(II)-SOM, MeHg-SOM, β -HgS, and α -HgS reference materials and LCF approach can be found in Manceau et al. (2015). Details on the preparation of the Hg⁰ reference material and the estimated detection limit for Hg⁰ in soils are provided in the Electronic Annex (Fig. EA-5). As shown previously, and reproduced in Fig. EA-6, the spectra of synthetic nanoparticulate β -HgS and the A horizon soil show excellent agreement and can be used indifferently to model nanoparticulate β -HgS in O_a horizon soil (Manceau et al., 2015). Therefore, the A horizon spectrum was used as an internal reference for nanoparticulate β -HgS in LCF modeling of O_a horizon spectra.

In addition, two soil heating assays and a mercury leach test were performed to evaluate if appreciable levels of Hg⁰, too low to be detected by spectroscopy, were present prior to flooding ($t = 0$ d) (details in the Electronic Annex, Table EA-2). Briefly, O_a and A horizon soils were heated at 80 °C for up to 8 h (Sladek and Gustin, 2003) and at 100 °C for 0.5 h (Biester et al., 2002) and changes in soil total mercury concentration were quantified; Hg⁰ is expected to volatilize from soils at a temperature ≥ 80 °C. Next, the concentration of total mercury released to oxic and deaerated high-purity water was quantified using the solid:liquid ratio and equilibration times outlined by Bloom et al. (2003).

2.7. Construction of pH – E_H Stability Diagrams

Thermodynamic models of soil pore waters were constructed using the Act2 module of The Geochemist's Workbench (v. 10.0.3) (2014) at 25 °C. The speciation of mercury and sulfur were modeled as a function of pH and E_H using equilibrium constants for reactions listed in Table EA-3. System parameters were selected to best represent the studied pore waters. Models of O_a and A horizon pore waters used $\sum \text{Hg} = 100 \text{ nM}$, which was assigned based on approximate maximal filter-passing Hg_T concentrations observed in microcosm pore waters (described in Section 3.3). For each model, the total sulfur content was input as the average sulfate concentration measured in microcosm pore water samples upon soil inundation ($\sum S_{O_a \text{ horizon}} = 0.40 \text{ mM}$, $\sum S_{A \text{ horizon}} = 0.24 \text{ mM}$). Chloride was included using the average measured concentration in microcosm pore water samples ($\sum \text{Cl}_{O_a \text{ horizon}} = 0.24 \text{ mM}$, $\sum \text{Cl}_{A \text{ horizon}} = 0.34 \text{ mM}$) (data not shown). Metacinnabar was allowed to precipitate using a solubility expression of bulk material ($\text{Hg}^{2+} + \text{HS}^- = \beta\text{-HgS}_{(s)} + \text{H}^+$; $\log K = 36.8$) (Drott et al., 2013) despite the previous observation that these soils contain nanoparticulate $\beta\text{-HgS}$ (Fig. EA-6) (Manceau et al., 2015); therefore, the E_H-pH analysis does not account for the effect of particle size on mineral solubility (Navrotsky et al., 2008). Elemental mercury was allowed to form as $\text{Hg}^0_{(aq)}$. Complexation between Hg(II) and organic thiol groups in SOM and DOM were represented by a two-coordinate complex ($\text{Hg}^{2+} + \text{RS}_2^{2-} = \text{Hg}(\text{RS}_2)$; $\log K_{\text{Hg-L}} = 28.5$) (Haitzer et al., 2002). Mercury binding site concentrations in O_a and A horizon soils ($\text{RS}_2^{2-}_{(\text{SOM})}$) were estimated at 0.4 and 0.2 mmol (kg soil)⁻¹, respectively, assuming a strong binding site density of $5 \times 10^{-9} \text{ mol (mg SOM)}^{-1}$ (Haitzer et al., 2002) and the average soil organic matter content (Table 1). Likewise, the concentration of strong binding sites in DOM ($\text{RS}_2^{2-}_{(\text{DOM})}$) in O_a and A horizon pore waters were estimated at 3.0 and 0.6 μM , respectively, assuming the same strong binding site density ($5 \times 10^{-9} \text{ mol (mg DOM)}^{-1}$) and the average DOC concentration in pore waters (corrected for quantified organic acids and assuming carbon is 50% of DOM). Aqueous Hg(II)-chloride (HgCl^+ , $\text{HgCl}_2^0_{(aq)}$, HgCl_3^- , HgCl_4^{2-}), -hydroxide (HgOH^+ , $\text{Hg}(\text{OH})_2^0_{(aq)}$, $\text{Hg}(\text{OH})_3^-$), and -sulfide complexes ($\text{Hg}(\text{SH})_2^0_{(aq)}$, HgS_2H^- , HgS_2^{2-})

were included in the model for completeness, but proved to have little effect on the resulting speciation diagrams.

3. RESULTS

3.1. Soil Characterization

Compositional properties of O_a (0-5 cm) and A (20-30 cm) horizon soils used in microcosm experiments are presented in Table 1. Soil horizons were designated based on field observations (Table EA-1) and soil characteristics. Fig. EA-7 presents total mercury and organic matter depth profiles. The O_a horizon soil contained more organic matter, less mercury, and fewer clay minerals in comparison to A horizon soil. In both soils, quartz and K-feldspar were the main non-clay minerals, and illite, kaolinite, and vermiculite were the dominant clay minerals. Goethite was the only Fe(III) oxyhydroxide detected and it accounted for $\leq 0.7\%$ by weight in each soil. Soil mercury concentrations were elevated in the O_a horizon ($72.8 \mu\text{mol kg}^{-1}$) and A horizon ($338 \mu\text{mol kg}^{-1}$) relative to uncontaminated soils (Obrist et al., 2011), showed a maximum between 10-25 cm depth, and substantially decreased below 35 cm depth (Fig. EA-7a). Mercury was concentrated in the clay-size fraction ($< 2 \mu\text{m}$) (239 and $952 \mu\text{mol kg}^{-1}$ in O_a and A horizon, respectively) (Table 1). Other chalcophile metals (copper, lead, zinc) and nickel were also present in the soils at appreciable levels (Table 1).

3.2. Microcosms: Pore Water Chemistry

In the microcosms, the dynamics of dissolved redox pairs predominantly followed that predicted based on the sequential reduction of terminal electron acceptors (Kirk, 2004). Although atmospheric oxygen was not present at the onset of soil flooding, the dynamics of terminal electron acceptors occurred at similar flooding times to those observed in microcosm experiments conducted in the

presence of atmospheric oxygen (Weber et al., 2009a; Hofacker et al., 2013) (Fig. 2). Pore water E_H dropped immediately following soil inundation (Fig. 2a, 2h) and nitrate levels were always below the detection limit in both soils ($< 0.8 \mu\text{M}$; data not shown). The release of manganese and iron to pore waters (Fig. 2b, 2i) is assumed to arise from the reductive dissolution of Mn(III,IV) and Fe(III) (hydr)oxides and contribute to the increase in pore water pH with flooding time (Fig. 2a, 2h) (Kirk, 2004). Filter-passing iron was confirmed to be Fe(II) by ferrozine assay (data not shown). The appearance of Fe(II) in pore water samples occurred concurrently with sulfate reduction in O_a horizon microcosms (Fig. 2b, 2c), but followed sulfate reduction in A horizon microcosms (Fig. 2i, 2j). Pore water sulfate was exhausted after 11 and 13 d in the O_a and A horizon systems, respectively (Fig. 2c, 2j). Filter-passing sulfide concentrations were at or below the detection limit ($\leq 0.4 \mu\text{M}$) for all samples except in O_a horizon pore water after 3 d, where sulfide was measured at $0.8 \mu\text{M}$ (data not shown); this result suggests that sulfide was scavenged in solid phases. Methanogenic conditions, as indicated by increased headspace methane concentrations, developed at ≥ 18 and ≥ 24 d in the O_a and A horizon soils, respectively (Fig. 2d, 2k). DIC concentrations increased linearly over the experiment (Fig. 2e, 2l) presumably as a result of microbial respiration. Concentrations of alkaline earth metals (calcium, magnesium, and strontium) increased with flooding time (Fig. EA-8) likely due to cation competition by Fe(II) and Mn(II) for soil exchange sites (Kirk, 2004; Weber et al., 2009b).

Pore water DOC concentrations (total DOC after subtracting for acetate-carbon) increased with flooding time (Fig. 2f, 2m) likely a result of increased solubility of DOM at higher pH and co-release of DOM associated with Mn(III,IV) and Fe(III) hydr(oxides) during reductive dissolution (Chin et al., 1998; Oste et al., 2002; Grybos et al., 2009). In both O_a and A horizon soils, S_R rapidly decreased between 1-2 d (Fig. 2g, 2n) and slowly decreased for the remaining duration of the experiment. The sharp decrease in S_R observed between 1-2 d indicates a shift to DOM of higher molecular weight (Helms et al., 2008), whereas the gradual decrease in S_R with increased flooding time is likely caused by the biodegradation

of DOM (Wickland et al., 2007). The accumulation of acetate by acetogenesis was observed in O_a horizon pore waters between 1-18 d flooding time followed by acetate depletion between 18-30 d (Fig. 2f). The onset of methanogenesis, as indicated by the increase in headspace methane (Fig. 2d), was attributed to the depletion of acetate (Yao and Conrad, 1999; Kirk, 2004). In A horizon pore waters, acetate was depleted within 5 d of flooding. Formate and propanoate were not detected in any sample. Altogether, O_a horizon microcosms developed reducing conditions earlier and exhibited a delay in the depletion of labile organic carbon (acetate) in comparison to A horizon microcosms, both of which likely result from the greater abundance of organic substrate in O_a compared to A horizon soils (Yao and Conrad, 1999) and its resulting influence on bacterial and archaeal community structure (Hansel et al., 2008). Based on concentrations of above mentioned filter-passing constituents, the ionic strengths of O_a and A horizon pore waters were estimated to increase from 12 to 31 mM and 3.0 to 9.7 mM between 1-36 d, respectively (Fig. EA-8d, EA-8h).

The mobilization of copper, zinc, and nickel to pore waters as a result of soil flooding was also observed. In both soils, concentrations of filter-passing copper were greatest at the onset of flooding and decreased with flooding time, whereas particulate copper concentrations increased between 1-5 d and decreased between 7-14 d (Fig. 3a, 3e). Filter-passing concentrations of zinc were similar to those of filter-passing copper (Fig. 3b, 3f), while filter-passing nickel levels increased with increased flooding time (Fig. 3c, 3g). Particulate zinc and nickel were negligible. Other chalcophile metals observed to influence multi-metal contaminant mobility in saturated soils (e.g., lead) (Weber et al., 2009b) were below detection limits (< 0.9 nM).

3.3. Microcosms: Pore Water Mercury Dynamics

The behavior of total mercury in O_a horizon microcosms showed three distinct stages (Fig. 4a). Filter-passing Hg_T rapidly increased between 1-2 d (stage 1), decreased between 2-7 d concurrent with

the period of sulfate reduction (Fig. 2c) (stage 2), and persisted at low levels (about 4 nM) between 11-36 d (stage 3). The dynamics of particulate Hg_T were similar to those of filter-passing Hg_T (Fig. 4a). Levels of $Hg^0_{(aq)}$ were low over the entire flooding period aside from a small increase at 3 d (Fig. 4b, Fig. EA-9a). Concentrations of filter-passing MeHg increased between 1-25 d and slightly decreased between 25-36 d (Fig. 4c). MeHg represented a moderate to low percentage of filter-passing Hg_T at 1 and 3 d (1.4% and 0.2%, respectively), but it accounted for a high percentage of Hg_T at 14 and 25 d (8.8% and 9.2%, respectively). In contrast, concentrations of particulate MeHg (Fig. 4c) mainly mimicked those of particulate Hg_T (Fig. 4a) and accounted for < 0.2% of particulate Hg_T at all sample times.

Mercury release dynamics in A horizon microcosms also displayed three stages, but contrast with those described above for O_a horizon systems. Filter-passing Hg_T concentrations were low in A horizon pore waters at the onset of flooding (≤ 3 d) (stage 1), exhibited an increase of about 18-fold between 5-18 d (stage 2), and reached a plateau between 24-36 d (Fig. 4d) (stage 3). Levels of particulate Hg_T showed greater variability in comparison to filter-passing Hg_T at early flooding times, but showed a trend of increasing concentration with flooding time similar to those observed for filter-passing Hg_T (Fig. 4d). Trends in $Hg^0_{(aq)}$ were similar to those of filter-passing Hg_T (Fig. 4e, Fig. EA-9b). Assuming that the $Hg^0_{(aq)}$ readily purged from unfiltered pore water samples (described in Section 2.4) was not associated with particles $\geq 0.2 \mu m$, $Hg^0_{(aq)}$ accounted for a progressively greater fraction of filter-passing Hg_T with flooding time. The fraction of filter-passing Hg_T as $Hg^0_{(aq)}$ was 7%, 30%, 77%, and 82% at 1, 7, 25, and 36 d, respectively (Fig. EA-9b). Concentrations of filter-passing and particulate MeHg exhibited similar trends to those of Hg_T , with concentrations increasing with flooding time (Fig. 4f). In contrast to O_a horizon soils, MeHg accounted for < 0.5% of filter-passing and particulate Hg_T at all sample times.

3.4. Mercury Speciation in Microcosm Soils

Changes in mercury speciation of O_a horizon soil in response to flooding were quantified using HR-XANES spectroscopy. Fig. 5a presents spectra of the Hg(II)-SOM reference, Hg⁰ reference, A horizon soil ($t = 0$ d; used as an internal nanoparticulate β -HgS reference), O_a horizon soil prior to flooding experiments ($t = 0$ d), and O_a horizon soil after 36 d flooding ($t = 36$ d). The A horizon spectrum was modeled as $100 \pm 3\%$ nanoparticulate β -HgS (Fig. EA-6) (Manceau et al., 2015). Spectra of O_a horizon soil were accurately modeled by LCF using two reference spectra: Hg(II) bound to thiol groups in SOM (Hg(II)-SOM) and nanoparticulate β -HgS. Prior to flooding, the O_a horizon spectrum was modeled as a linear combination of 23% Hg(II)-SOM and 77% nanoparticulate β -HgS ($2\sigma = 6\%$). After 36 d of flooding, the O_a horizon spectrum showed a small decrease in the proportion of Hg(II)-SOM (20%) accompanied by an increase in β -HgS (80%) ($2\sigma = 6\%$). The shift in speciation to less Hg(II)-SOM is detected by the decrease in the sharp Hg(II)-SOM peak at 12.2792 keV that results from Hg(II) linearly coordinated to two thiol ligands and the broad Hg(II)-SOM peak at about 12.2890 keV (Fig. 5b) (Manceau et al., 2015). Statistical analysis of spectra in the 12.2792 and 12.2890 keV regions shows that the difference is significant at the 1σ level (3%); therefore, a shift in mercury speciation caused by flooding the O_a horizon soil was detected but was minor. Hg⁰ was not detected in HR-XANES spectra of O_a and A horizon soils (Fig. EA-5) or by the soil heating assays and mercury leach test (Table EA-2), confirming that neither soil contained appreciable levels of Hg⁰ at $t = 0$ d.

3.5. Intact Soil Core: Pore Water Chemistry

A subset of analyses that included nitrate, sulfate, DOC, and DIC were performed on pore waters collected from the intact soil core over the two sequential flooding periods (Fig. EA-10). In pore waters sampled from the O_a horizon, nitrate concentrations were at or below the detection limit (0.8 μ M) during flooding period 1 (0-17 d) (Fig. EA-10a). Sulfate reduction occurred in O_a pore waters between 2-15 d of flooding period 1. The soil core was drained, exposed to air for 28 d, and flooded again for 36 d.

Both nitrate and sulfate levels were elevated at the onset of the flooding period 2 (45-80 d) and decreased with flooding time presumably by microbial reduction (Fig. EA-10a, EA-10b). Elevated concentrations of nitrate and sulfate, observed at the onset of flooding period 2, were likely generated through aerobic nitrification (Reddy et al., 1984) and abiotic sulfide oxidation (Burton et al., 2009); this result suggests the thorough oxidation of the soil core during the drained period. In comparison to the microcosms (Fig. 2c, 2j), sulfate concentrations in the intact soil pore waters were greater at the onset of flooding and persisted at later flooding times (Fig. EA-10b), which suggests the rates of anaerobic respiration in intact soils were slower. Levels of DIC and DOC increased in intact soil pore waters with increased flooding time during both flooding periods (Fig. EA-10c, EA-10d). The concentration of DOC released to O_a horizon pore water was lower at the onset of flooding period 2 compared to flooding period 1 (Fig. EA-10d). Relative differences in DIC and DOC concentrations between O_a and A horizon pore waters were comparable to results from microcosms.

3.6. Intact Soil Core: Mercury Release Dynamics

During flooding period 1, mercury release dynamics in saturated intact soils mirrored those observed in microcosms. In the O_a horizon, concentrations of filter-passing Hg_T increased rapidly following soil inundation with a peak concentration observed at 4 d (Fig. 4g). A gradual decline in filter-passing Hg_T was observed between 4-17 d coincident with the decrease in sulfate (Fig. EA-10b). In the A horizon, filter-passing Hg_T concentrations were low at the onset of flooding period 1 and increased approximately 6-fold between 7-16 d. During flooding period 2, filter-passing Hg_T increased slowly between 46-79 d in the O_a horizon (Fig. 4g), which was in contrast to mercury release behavior during flooding period 1. In the A horizon, mercury dynamics during flooding period 2 were similar to flooding period 1 and increasing to a plateau between 52-79 d (Fig. 4h). Concentrations of filter-passing Hg_T were 1.3-2 orders of magnitude lower in pore water samples collected from intact soils in comparison to microcosms (Fig. 4). Mercury release curves for intact soils were defined by multiple data points and the

average total volume of pore water sampled at any time represented $\leq 1.2\%$ of the estimated total pore volume, which suggests that trends in filter-passing Hg_T were not an artifact of the sequential sampling of pore waters from intact soils.

4. DISCUSSION

4.1 E_H – pH Diagrams of Soils

Distinct differences in mercury transformation and release dynamics in O_a and A horizon soils during flooding reveals spatial and temporal variability in the biogeochemical controls on contaminant mobilization along the soil profile. The results are interpreted in part using E_H -pH diagrams (Fig. 6) that show the equilibrium configuration of dominant aqueous, gaseous, and solid mercury species expected in the microcosms. The E_H -pH diagrams (Fig. 6) include three mercury species: Hg(II) bound to SOM (modeled as $\text{Hg(RS}_2\text{)}_{\text{SOM}}$), Hg(II) as metacinnabar ($\beta\text{-HgS}_{(s)}$), and $\text{Hg}^0_{(\text{aq})}$. $\text{Hg(RS}_2\text{)}_{\text{SOM}}$ dominates at high E_H , the boundary between $\text{Hg(RS}_2\text{)}_{\text{SOM}}$ and $\beta\text{-HgS}_{(s)}$ coincides with the boundary of the SO_4^{2-} - $\text{H}_2\text{S/HS}^-$ field of dominance, and $\text{Hg}^0_{(\text{aq})}$ dominates both above and below the $\beta\text{-HgS}_{(s)}$ field at conditions of low E_H and high pH.

The measured E_H and pH of O_a horizon microcosm pore waters over the course of soil flooding were primarily within the $\text{Hg(RS}_2\text{)}_{\text{SOM}}$ field over the first 2 d (stage 1), were primarily within the $\beta\text{-HgS}_{(s)}$ field over 2-7 d (stage 2), and overlapped the $\beta\text{-HgS}_{(s)}/\text{Hg}^0_{(\text{aq})}$ boundary over 11-36 d (stage 3) (Fig. 6a). Therefore, the E_H -pH diagram suggests that mercury speciation in the O_a horizon changed with increased flooding time from Hg(II) binding to thiol groups in SOM to $\beta\text{-HgS}$ precipitation to $\text{Hg}^0_{(\text{aq})}$ production. In A horizon microcosms, the measured E_H and pH were within the $\text{Hg(RS}_2\text{)}_{\text{SOM}}$ field over the first 2 d, the $\text{Hg}^0_{(\text{aq})}$ field over 2-7 d, and the $\beta\text{-HgS}_{(s)}$ field over 11-36 d (Fig. 6b). This suggests that mercury speciation

in the A horizon changed with increased flooding time from Hg(II) binding to thiol groups in SOM to $\text{Hg}^0_{(\text{aq})}$ production to $\beta\text{-HgS}$ precipitation. Considering these E_{H} -pH diagrams, and recognizing that such diagrams do not account for kinetic factors influencing mercury speciation, the processes governing mercury dynamics in O_a and A horizon soils are examined in the following sections.

4.2 O_a Horizon: Mercury Mobilization with Organic Matter and Sequestration as $\beta\text{-HgS}$

Based on results from the microcosm experiments, the observed rapid mobilization of Hg_T to O_a horizon pore waters was likely coupled to the release of organic matter. Mercury mobilization was coincident with a sharp decrease in S_R (Fig. 2g, 4a) indicating a shift to DOM of greater molecular weight, a property that co-varies with aromaticity and hydrophobicity (Chin et al., 1994). The hydrophobic organic acid fraction, compared to other DOM fractions, has greater thiol group content (Haitzer et al., 2003), exhibits enhanced reactivity with respect to mercuric sulfides (Ravichandran et al., 1998; Waples et al., 2005; Slowey, 2010), and preferentially sorbs to (hydr)oxide mineral surfaces (Meier et al., 1999). The onset of reductive dissolution of Mn(III,IV) and Fe(III) (hydr)oxides between 1-2 d may be responsible for the mobilization of DOM of greater hydrophobic quality (Chin et al., 1998; Grybos et al., 2009). Prior to flooding, Hg(II)-SOM accounted for $23 \pm 6\%$ of the total mercury in O_a horizon soil (Fig. 5a), which supports the hypothesis of mercury mobilization with organic matter. Furthermore, the measured E_{H} and pH were primarily within the $\text{Hg}(\text{RS}_2)_{\text{SOM}}$ field of dominance during mercury mobilization (stage 1) (Fig. 4a, 6a). Mobilized filter-passing Hg_T was likely Hg(II) complexed by DOM thiol groups (Haitzer et al., 2002), but could also be nanoparticulate $\beta\text{-HgS}$ associated with DOM that pass a $0.2 \mu\text{m}$ filter (Ravichandran et al., 1999; Deonaraine and Hsu-Kim, 2009; Gerbig et al., 2011; Zhang et al., 2012; Pham et al., 2014). Although we cannot rule out the possibility that mercury mobilization was a result of flooding the soil with low ionic strength water, the mobilization of mercuric sulfide colloids by low ionic strength water has been observed only over min to h time scales in packed column

experiments (Slowey et al., 2005), which would not explain the increase in filter-passing and particulate Hg_T at > 1 d (Fig. 4a).

The decrease in filter-passing Hg_T between 3-7 d (stage 2) (Fig. 4a) is explained by the sequestration of mercury as authigenic nanoparticulate $\beta\text{-HgS}$ during a period of sulfate reduction (Fig. 2c). This hypothesis is supported by (1) HR-XANES results that identify a modest increase in the fraction of mercury as nanoparticulate $\beta\text{-HgS}$ as a result of soil flooding (Fig. 5) and (2) overlap between the measured pH and E_H and the $\beta\text{-HgS}$ field of dominance between 3-7 d (Fig. 6a). Assuming mercury was mobilized as Hg(II) complexed to DOM, the sulfide concentration measured at 3 d ($0.8\ \mu\text{M}$) is sufficient for sulfide to outcompete DOM thiol groups for Hg(II) (Gerbig et al., 2011; Graham et al., 2012). The formation of $\beta\text{-HgS}$ in O_a horizon pore water may be responsible for the delayed maximum in particulate Hg_T relative to filter-passing Hg_T (Fig. 4a). The increase in pore water ionic strength with increased flooding time (Fig. EA-8d) may have also contributed to the decrease in Hg_T by promoting particle deposition (Weber et al., 2009a). The possibility that $\beta\text{-HgS}$ formed through interactions with other metal sulfides is discussed in Section 4.5. Persistent Hg_T at nanomolar concentration after the depletion of pore water sulfate (11-36 d) was likely due to the stabilization of nanoparticulate $\beta\text{-HgS}$ by DOM (Deonarine and Hsu-Kim, 2009; Slowey, 2010; Gerbig et al., 2011).

Assuming that filter-passing and particulate Hg_T released to pore waters was sequestered in soils as $\beta\text{-HgS}$ ($1.7\ \mu\text{M}\ \text{Hg}_T$ released between 1-11 d; Fig. 4a), the fraction of $\beta\text{-HgS}$ in O_a horizon soil is predicted to have increased by 2.3%, which is in good agreement with the observed 3% increase in $\beta\text{-HgS}$ quantified by HR-XANES analysis (Fig. 5). Under the assumption that all SOM-bound Hg(II) was available to react with sulfide at the onset of flooding ($17\ \mu\text{mol}\ \text{kg}^{-1}$), there was a 23-fold molar excess of sulfate to Hg(II) -SOM. Despite this excess, mercury was only partially sequestered as $\beta\text{-HgS}$ ($80 \pm 6\%$) after 36 d of flooding. A limit in the proportion of mercury as nanoparticulate $\beta\text{-HgS}$ formed in soil under

conditions of sulfate reduction, which we also observed in the conversion of Hg(II)-SOM to nanoparticulate β -HgS in the absence of sulfide (Manceau et al., 2015), may be explained by the inaccessibility of SOM-bound Hg(II) to sulfide, slow reaction kinetics between sulfide and Hg(II) in soils, or the scavenging of sulfide by other chalcophile metals (discussed in Section 4.5). Nonetheless, the observed formation of authigenic β -HgS simultaneous with sulfate reduction supports inferences drawn from field and laboratory studies of soils and sediments that undergo periods of anoxia (Barnett et al., 1997; Wolfenden et al., 2005).

Trends for mercury release in the O_a horizon of intact soils during flooding period 1 (Fig. 4g) agree with trends from the microcosms (Fig. 4a). These results substantiate that mercury mobility in undisturbed surface soils is also controlled by mobilization with organic matter, which is supported by a field study of East Fork Poplar Creek (Riscassi et al., 2015). Furthermore, mercury immobilization is controlled by the formation of β -HgS during sulfate reduction. In comparison to the microcosms (Fig. 2c), anaerobic conditions developed at a slower rate in the intact soils as evidenced by sulfate dynamics (Fig. EA-10b), which are reflected in slower kinetics of mercury release and immobilization (Fig. 4a, 4g).

Our results identifying mercury mobilization with organic matter support field observations of strong correlations between stream concentrations of filter-passing Hg_T and the hydrophobic organic acid fraction of DOM during high flow events (Shanley et al., 2008; Dittman et al., 2009; Dittman et al., 2010). Watershed-scale observations of this phenomenon have been attributed to the mobilization of DOM of greater hydrophobic quality in surface soils by transport-driven processes (e.g., lateral flow in saturated soils) (Aiken and Cotsaris, 1995; Sebestyen et al., 2008). Our findings further suggest that biogeochemical factors in saturated soils, namely the reductive dissolution of Mn(III,IV) and Fe(III) (hydr)oxides, also play an important role in the mobilization of mercury with hydrophobic DOM.

Mercury mobilization trends were not reproduced over two successive flooding periods within the O_a horizon of intact soils (Fig. 4g). The formation of nanoparticulate β-HgS during sulfate-reducing conditions in flooding period 1 likely depleted the labile mercury pool associated with SOM. This inference is supported by similar mercury release trends of the O_a horizon soil during flooding period 2 and those of the A horizon soil in which nanoparticulate β-HgS dominates mercury speciation (Fig. 4g, 4h). It is also possible that mercury release during flooding period 2 was influenced by the lower concentration of DOC released at the onset of flooding (Fig. EA-10d), or the irreversible deposition of mercury during flooding period 1. The source of labile mercury in the O_a horizon soil initially is unclear, but may be explained by the oxidative dissolution of nanoparticulate β-HgS by molecular oxygen or DOM, as suggested by studies conducted with bulk mercuric sulfide (Ravichandran et al., 1998; Barnett et al., 2001, Waples et al., 2005). Reducing conditions in surface soils may ultimately decrease mercury mobility by depleting mobile mercury pools associated with SOM.

4.3 A horizon: Hg⁰_(aq) Formation in Soil Containing Nanoparticulate β-HgS

The dynamics of mercury release from the A horizon soil were primarily controlled by Hg⁰_(aq) formation during and following the period of sulfate reduction. Hg⁰ was not present at measureable levels in A horizon soil prior to flooding (Table EA-2, Fig. 5a), which indicates that Hg⁰_(aq) was formed during soil flooding. An abiotic explanation for Hg⁰_(aq) formation is provided based on the measured pH and E_H of A horizon microcosms. Experimental measurements show Hg⁰_(aq) accumulation in pore waters after 7 d (Fig. 4e), which is in fair agreement with the overlap of the measured pH and E_H and the Hg⁰_(aq) field between 2-7 d (Fig. 6b). However, the greatest increase in the concentration of Hg⁰_(aq) occurred between 7-14 d (Fig. 4e) when the measured pH and E_H overlapped with the β-HgS field (Fig. 6b). Disagreement between the thermodynamic model and observed persistence of Hg⁰_(aq) in pore waters after 7 d (Fig. 4) may be a result of the model input value for sulfur (details in Section 2.7). Pore water sulfate was depleted between 11-14 d (Fig. 2j), which closely overlaps in time with the transition of

measured pH and E_H from the $Hg^0_{(aq)}$ field (2-7 d) to that of β -HgS (11-36 d) (Fig. 6b). Sulfide was not detected in pore waters at a concentration above 0.4 μ M over the entire 36 d of flooding; therefore, the E_H -pH analysis does not take into account the absence of available sulfur beyond 14 d. Excluding sulfur in the thermodynamic model predicts the predominance of $Hg^0_{(aq)}$ between 2-36 d based on the measured pH and E_H , which may explain the observed presence of $Hg^0_{(aq)}$ between 7-36 d (Fig. 4e). It is also possible that this discrepancy is because measured E_H values do not reflect redox equilibrium (Lindberg and Runnells, 1984), there is uncertainty in model inputs such as the equilibrium constant for β -HgS (details in Section 2.7), or that $Hg^0_{(aq)}$ formation was influenced by kinetic factors like the slow binding of Hg(II) to strong organic matter binding sites (Gasper et al., 2007; Jiskra et al., 2014) or the influence of DOM quantity and quality on the rate of formation, rate of dissolution, size, and crystallinity of nanoparticulate β -HgS (Deonarine and Hsu-Kim, 2009; Slowey, 2010; Gerbig et al., 2011).

The formation of $Hg^0_{(aq)}$ under the given experimental conditions is not easily explained by known processes assuming that Hg(II) was released from β -HgS and subsequently reduced to $Hg^0_{(aq)}$, and there is no evidence in the literature for reduction of Hg(II) in bulk mercuric sulfides to $Hg^0_{(aq)}$ (Ravichandran et al., 1998; Brandon et al., 2001). Mercury mobilization was observed under anoxic, sulfide-limited conditions ($\leq 10^{-6}$ M); therefore, Hg(II) release from β -HgS did not occur through oxidative dissolution by molecular oxygen (Barnett et al., 2001) or redox-active DOM functional groups (Waples et al., 2005), ligand-promoted dissolution by sulfide (reported for sulfide $\geq 10^{-4}$ M) (Paquette and Helz, 1995; Ravichandran et al., 1998), or interactions with aerobic sulfur-oxidizing bacteria (Jew et al., 2014). Likewise, the formation of aqueous Hg(II)-polysulfide complexes (e.g., HgS_xSH^-), proposed to increase the solubility of β -HgS (Paquette and Helz, 1997; Jay et al., 2000), does not explain mercury mobilization. Polysulfides (S_x^{2-}), which are formed by reactions between sulfide and elemental sulfur (S^0), require molecular oxygen or abundant Fe(III) (hydr)oxide to facilitate S^0 formation (Poulton et al., 2004), neither of which were present in the microcosms (Section 3.1).

One possible explanation for $\text{Hg}_{(\text{aq})}^0$ formation under reducing conditions is that nanoparticulate β -HgS is labile to anaerobic microbial redox pathways yielding $\text{Hg}_{(\text{aq})}^0$. It is well-established that poorly ordered, nanoparticulate β -HgS formed in the presence of DOM is bioavailable to methylating organisms (Zhang et al., 2012; Graham et al., 2012; Pham et al., 2014). It is reasonable to expect that nanoparticulate β -HgS is also bioavailable to other transformation processes, specifically the reduction of Hg(II) to $\text{Hg}_{(\text{aq})}^0$ by dissimilatory metal-reducing bacteria (Wiatrowski et al., 2006; Hu et al., 2013a). Dissimilatory metal-reducing bacteria (e.g., *Geobacter sulfurreducens* PCA) can directly reduce a range of major (Fe(III), Mn(IV)) and trace (Hg(II), U(VI)) elements (Lovley et al., 1993; Wiatrowski et al., 2006; Hu et al., 2013a) and, unlike the mercury resistance operon (*mer*) (Barkay et al., 2003), Hg(II) reduction occurs in anoxic environments at sub-micromolar concentrations of mercury similar to experimental pore waters (Wiatrowski et al., 2006; Hu et al., 2013a). Hg(II) reduction by dissimilatory metal-reducing bacteria is supported by a correlation between byproducts of dissimilatory metal reduction ($\Sigma(\text{Fe}, \text{Mn})$) and $\text{Hg}_{(\text{aq})}^0$ ($R^2 = 0.80$; $n = 6$) in A horizon pore waters (Fig. 7a). A strong positive correlation is also observed between $\Sigma(\text{Fe}, \text{Mn})$ and filter-passing Hg_T ($R^2 = 0.73$; $n = 22$; Fig. 7b), which was predominantly $\text{Hg}_{(\text{aq})}^0$ at late flooding times and showed release dynamics similar to that of $\text{Hg}_{(\text{aq})}^0$ (Fig. 4d, 4e). Dissimilatory metal-reducing bacteria capable of Hg(II) reduction (*Geobacter sulfurreducens* PCA) have been identified in soils within the East Fork Poplar Creek watershed (Mosher et al., 2012) and reported Hg(II) reduction rates are sufficient to account for the production of $\text{Hg}_{(\text{aq})}^0$ in microcosms (Hu et al., 2013a). The plateau in $\text{Hg}_{(\text{aq})}^0$ at late flooding time was not limited by $\text{Hg}_{(\text{aq})}^0$ solubility (about 0.28 μM ; Schuster, 1991) and may be explained by availability of Mn(III,IV) and Fe(III) (hydr)oxides to dissimilatory metal-reducing bacteria. The occurrence of this process in the A horizon microcosms, but not in the O_a horizon microcosms, may be a result of spatial heterogeneity in microbial community structure in the soil profile (Hansel et al., 2008). It is also possible that $\text{Hg}_{(\text{aq})}^0$ formed through abiotic Hg(II) reduction pathways involving Fe(II) species (aqueous Fe(II), Fe(II)-bearing minerals) (Wiatrowski et al., 2009;

Amirbahman et al., 2013; Bone et al., 2014) or redox-active DOM functional groups (Allard and Arsenie, 1991; Gu et al., 2011; Zheng et al., 2012).

Competitive complexation of Hg(II) by DOM thiol groups is recognized to limit Hg(II) reduction (Gu et al., 2011; Hu et al., 2013a) regardless of whether or not Hg(II) reduction occurred through a biotic or abiotic pathway. In the A horizon pore waters, we estimate that DOM thiol groups exceeded filter-passing Hg_T by at least a factor of 5 over the entire microcosm experiment assuming that the DOM was 50% carbon and had a strong Hg(II) binding site density of $5 \times 10^{-9} \text{ mol (mg DOM)}^{-1}$ (Haitzer et al., 2002). Observed Hg(II) reduction, despite sufficient Hg(II) complexing capacity of pore water DOM, may reflect that the Hg(II) that was reduced was in a different form than strong complexes with DOM (e.g., nanoparticulate β -HgS) or that the formation rate of strong Hg(II)-DOM complexes was slow (Gasper et al., 2007; Jiskra et al., 2014) in comparison to Hg(II) reduction (Gu et al., 2011; Zheng et al., 2012; Hu et al., 2013a). DOM thiol groups have also been proposed to facilitate the oxidation of $Hg^0_{(aq)}$ to Hg(II) under reducing conditions (Gu et al., 2011; Zheng et al., 2012). In our experiments, the long-term stability of $Hg^0_{(aq)}$ in pore waters in the presence of abundant DOM thiol groups shows that ligand-induced oxidation of $Hg^0_{(aq)}$ is not a dominant process under these E_H -pH conditions. Future work is needed to evaluate the reactivity of nanoparticulate β -HgS with respect to Hg(II) reduction pathways, particularly in terms of rate-limiting processes including formation, dissolution, and aging of β -HgS nanoparticles (Slowey, 2010; Zhang et al., 2012; Graham et al., 2012; Pham et al., 2014).

Our findings reveal that mercury was mobilized as both particulate Hg_T and $Hg^0_{(aq)}$ from the A horizon soil in which nanoparticulate β -HgS dominated soil mercury speciation. Results suggest $Hg^0_{(aq)}$ was formed by biotically-mediated Hg(II) reduction, which has been inferred in anoxic soils and sediments (Peretyazhko et al., 2006; Obrist et al., 2010). This reduction pathway may explain the elevated levels of $Hg^0_{(aq)}$ observed in stream sediments within the same watershed (Brooks and Southworth, 2011). The production of $Hg^0_{(aq)}$ during periodic flooding periods likely contributes to

atmospheric mercury fluxes from the East Fork Poplar Creek watershed, estimated at 1-10 kg y⁻¹ (Lindberg et al., 1995), and may represent an important process enhancing the recycling of legacy mercury to the atmosphere. It is unclear if Hg⁰_(aq) would also form by a similar mechanism in uncontaminated soils containing nanoparticulate β-HgS. Mercury release dynamics were consistent between microcosms and intact soils and replicated over successive redox oscillations in intact soils (Fig. 4), which confirms that nanoparticulate β-HgS in soils is continually accessible to pathways yielding Hg⁰_(aq). Therefore, soils containing nanoparticulate β-HgS may represent a significant pool of mercury that can be mobilized as Hg⁰_(aq) to adjacent surface water and the atmosphere under strongly reducing conditions.

4.4. Mercury Methylation in Riparian Soils

Methylmercury (MeHg) was produced within or mobilized from both flooded soils under reducing conditions with trends in MeHg largely following those of Hg_T (Fig. 4). Anaerobic microorganisms can methylate mercury in the form of nanoparticulate β-HgS (Zhang et al., 2012; Graham et al., 2012; Pham et al., 2014) and Hg⁰_(aq) (Colombo et al., 2013; Hu et al., 2013b). However, formation of Hg⁰_(aq) is known to limit biotic mercury methylation (Barkay et al., 2003; Lin et al., 2014) and reported rates of Hg⁰_(aq) methylation are slower than those for aqueous Hg(II) and nanoparticulate β-HgS species (Zhang et al., 2012; Hu et al., 2013b; Pham et al., 2014), which may explain the smaller fraction of MeHg as part of the filter-passing Hg_T in the A horizon (≤ 0.5%) versus the O_a horizon pore waters (≤ 9%). MeHg dynamics may also be explained by the methylation of other mercury species, such as Hg(II) complexed to DOM or associated with soils (Jonsson et al., 2012), or the release of MeHg from soils during flooding and stabilization in pore waters by DOM complexation (Porvari and Verta, 1995). Microbially-mediated MeHg demethylation may explain the decrease in filter-passing MeHg at late flooding times in O_a horizon pore waters (Marvin-DiPasquale et al., 2000).

Flooded soils are recognized as important sites for MeHg formation (Porvari and Verta, 1995). We observed increases in pore water MeHg as a result of flooding across a contaminated riparian soil profile containing Hg(II) bound to thiol groups in SOM and nanoparticulate β -HgS, the latter traditionally considered a recalcitrant sink for mercury in the environment. The rapid increase in pore water MeHg as a result of soil flooding and elevated fraction of filter-passing Hg_T as MeHg in O_a horizon pore waters ($\leq 9\%$) suggests that surface soils are a more important source of MeHg to adjacent surface waters than underlying soils. Our results corroborate watershed-scale studies of contaminated and uncontaminated sites that identify reduced riparian soils as important sources of MeHg to adjacent surface waters (Balogh et al., 2004; Shanley et al., 2008; Brigham et al., 2009; Regnell et al., 2009; Barringer et al., 2010; Southworth et al., 2010), and support models that use riparian zone hydrology (i.e., hydrologic flow path, water table depth) to predict MeHg concentrations in streams (Burns et al., 2014; Eklöf et al., 2015). MeHg levels were greatest under iron- and sulfate-reducing conditions, which adds evidence that the flushing of reduced riparian soils is responsible for episodic fluxes of MeHg into streams that coincide with reduced stream water chemistry.

4.5. Multi-Metal Contaminant Dynamics

Several studies demonstrate the co-mobilization and co-sequestration of trace metals in flooded soils (Weber et al., 2009a; Weber et al., 2009b; Hofacker et al., 2013). Here, mercury dynamics are interpreted independently of the dynamics of other trace metals because (1) the studied soils contain mercury at concentrations 2-3 orders of magnitude greater than uncontaminated soils (Obrist et al., 2011) and (2) the interactions of Hg(II) with DOM and sulfide are exceptionally strong relative to other trace metals. For example, mercury has been shown to be mobilized at the onset of soil flooding through the incorporation of elemental mercury (Hg^0) into metallic copper (Cu^0) nanoparticles in soils with a lower molar Hg/Cu ratio ($1.4\text{-}1.8 \times 10^{-3}$) (Hofacker et al., 2013) than soils in this study ($1.2\text{-}2.7 \times 10^{-1}$). The analysis of nanoparticles using X-ray absorption spectroscopy by Hofacker et al. (2013)

show Hg^0 atoms exclusively surrounded by Cu^0 atoms at a molar Hg/Cu ratio of about 0.002. Although trends reported here of particulate Hg_T and copper ($> 0.2 \mu\text{m}$) in the O_a horizon (Fig. 3a, 4a) show good agreement with those reported by Hofacker et al. (2013) of colloidal Hg_T and copper ($> 0.025 \mu\text{m}$), the molar Hg/Cu ratios in O_a horizon pore waters show the preferential release of mercury over copper (molar Hg/Cu ratio > 1.0) (Fig. 3d). This result casts doubt on the feasibility of sequestering mercury inside metallic copper nanoparticles at equimolar concentration. Nevertheless, our results on copper dynamics (Fig. 3a, 3e) in the O_a and A horizon are in good agreement with those in other floodplain soils that attribute copper mobilization to the formation of metallic copper nanoparticles (Weber et al., 2009a, Hofacker et al., 2013).

The sequestration of mercury by co-precipitation and sorption to other metal sulfides has also been observed (Wolfenden et al., 2005; Jeong et al., 2007; Skyllberg and Drott, 2010; Hofacker et al., 2013). Reactions between Hg(II) and FeS can facilitate the sequestration of mercury as $\beta\text{-HgS}$ (Jeong et al., 2007; Skyllberg and Drott, 2010) and were considered in the interpretation of mercury dynamics in the O_a horizon. However, such reactions are unlikely in the O_a horizon soil because Hg(II) is expected to associate with the SOM (79.6 g kg^{-1}) rather than FeS formed from sulfate reduction (maximum of about 0.4 g kg^{-1} FeS assuming all sulfate was converted to FeS ; FeS:SOM mass ratio $\leq 0.5\%$) (Skyllberg and Drott, 2010). This comparison assumes a uniform Hg(II) binding site density in SOM in the O_a horizon and peat soils used by Skyllberg and Drott (2010). Nevertheless, the sequestration of mercury and other chalcophile metals as sulfide precipitates was indirectly coupled because of sulfate limitation. Assuming all metals remained as cations during soil flooding, the molar summation of all prominent chalcophile metals in O_a horizon soil ($\Sigma(\text{Cu, Zn, Pb, Ni, Hg(II)-SOM})$) exceeded available sulfate by a factor of 7 (Table 1), which suggests that there may not have been sufficient sulfur to sequester all chalcophile metals as sulfide minerals. Competition between mercury and other mobilized chalcophile metals (copper, zinc, nickel, iron) for biogenic sulfide is largely controlled by relative differences in sulfide

mineral solubility and metal concentration. For comparison, β -HgS ($\text{Hg}^{2+} + \text{HS}^- = \beta\text{-HgS}_{(s)} + \text{H}^+$; $\log K = 36.8$) is about 14, 25, 31, and 33 orders of magnitude less soluble than CuS ($\text{Cu}^{2+} + \text{HS}^- = \text{CuS}_{(s)} + \text{H}^+$; $\log K = 22.3$), ZnS ($\text{Zn}^{2+} + \text{HS}^- = \text{ZnS}_{(s)} + \text{H}^+$; $\log K = 11.5$), NiS ($\text{Ni}^{2+} + \text{HS}^- = \text{NiS}_{(s)} + \text{H}^+$; $\log K = 5.6$), and FeS, respectively ($\text{Fe}^{2+} + \text{HS}^- = \text{FeS}_{(s)} + \text{H}^+$; $\log K = 3.6$) (Allison et al., 1990; Drott et al., 2013). Differences in sulfide mineral solubility and insufficient sulfide were likely responsible for the near-complete immobilization of mercury during sulfate reduction in O_a horizon microcosms (Fig. 4a), and, therefore, the persistence of elevated copper, zinc, nickel, and iron in pore waters at late flooding times (Fig. 3), which corroborates findings of Weber et al. (2009b). Although we cannot exclude the possibility that mercury adsorbed to or co-precipitated with other metal sulfides (e.g., CuS) resulting in β -HgS formation in the O_a horizon (Hofacker et al., 2013), these mechanisms are more likely in soils with lower mercury concentrations.

5. CONCLUSIONS

The results of this study demonstrate distinct temporal differences in mercury transformation and release dynamics between soil horizons separated by about 20 cm in the soil profile. The organic-rich surface soil (O_a horizon) contained a mercury pool that was readily mobilized along with organic matter upon soil flooding and sequestered as nanoparticulate β -HgS under sulfate-reducing conditions. In the underlying A horizon soil in which mercury was primarily present as nanoparticulate β -HgS, the formation of $\text{Hg}^0_{(\text{aq})}$ predominantly controlled mercury mobilization. The formation of $\text{Hg}^0_{(\text{aq})}$ in the A horizon soil indicates that authigenic nanoparticulate β -HgS in soil is labile to transformations that increase the mobilization of legacy mercury to the atmosphere. Successive redox oscillations in intact soils exhausted the mobile mercury pool associated with organic matter in the O_a horizon, but did not influence mercury release dynamics in the A horizon. The accumulation of MeHg in pore waters during

soil flooding suggests that reduced riparian soils are an important source of MeHg to neighboring surface waters.

Mercury mobilization from riparian soils to adjacent streams depends on both biogeochemical and transport processes. This study demonstrates that biogeochemical controls on mercury dynamics are consistent between flooding experiments conducted with sieved, air-dried soils and undisturbed, intact soils. Reducing conditions developed at slower rates in flooded intact soils compared to microcosms, likely a result of the physical, chemical, and biologic heterogeneity in intact soils. Slower reaction rates in intact soils were reflected in the kinetics of mercury dynamics. Our results suggest that the flushing of reduced riparian soil pore waters to adjacent streams may contribute to episodic fluxes in Hg_T and MeHg during high-flow events. More work is needed to evaluate coupled biogeochemical- and transport-driven processes that control mercury mobilization from intact soils under realistic hydrologic regimes, with the aim of integrating these phenomena into quantitative models describing mercury fluxes and mobilization behavior.

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Tables:

Table 1. Characterization of O_a and A horizon soils (≤ 2 mm sieved) used in microcosm experiments. Concentrations are presented on a dry mass basis.

	O _a horizon	A horizon
Depth	0 – 5 cm	20 – 30 cm
Bulk Density (g cm ⁻³)	0.94	1.62
Non-Clay Mineralogy (weight %)	Quartz (65.3 %), K-feldspar (6.1 %)	Quartz (64.0 %), K-feldspar (5.6 %)
Clay Mineralogy (weight %)	Illite (11.8 %), Kaolinite (1.8 %), Vermiculite (1.7 %)	Illite (16.1 %), Kaolinite (3.0 %), Vermiculite (1.0 %)
pH _{CaCl2}	6.5	5.9
ECEC (mmol _c kg ⁻¹)	176	84.7
Organic Matter (g kg ⁻¹)	79.6	38.8
Major Elements^a (% oxide)		
SiO ₂	79.9	77.5
Al ₂ O ₃	6.9	8.0
Fe ₂ O ₃	2.6	3.0
K ₂ O	1.6	1.7
MgO	0.4	0.5
CaO	0.6	0.3
MnO	0.1	0.1
Trace Metals^a (μmol kg⁻¹)		
Hg (bulk soil; ≤ 2 mm)	72.8	338
Hg (clay-size fraction; ≤ 2 μm)	239 ^b	952
Cu	635	1268
Ni	420	693
Pb	150	209
Zn	1807	1831
Se	< 13	≤ 13

^aMean of digestion duplicates; relative percent deviation was ≤ 5%. ^bValue based on a single digestion due to limited sample mass.

Figure Captions:

Fig. 1. Diagram representations of the (a) microcosm and (b) intact soil core flooding apparatus.

Fig. 2. Changes in conditions within microcosms containing O_a (left panel; plots a-g) and A horizon (right panel; plots h-n) soil over 36 d flooding that includes pore water pH and redox potential (E_H), pore water concentrations of filter-passing iron (Fe), manganese (Mn), sulfate (SO₄²⁻), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), acetate, and spectral slope ratio (S_R) and headspace concentrations of methane. Note that the y-axis scale bars are different between plots of headspace methane (d, k), DIC (e, l), and DOC and acetate (f, m). Each point in time is from an individual microcosm.

Fig. 3. Changes in filter-passing (open symbols) and particulate (closed symbols) concentrations of copper (Cu), zinc (Zn), and nickel (Ni) and the Cu/Hg molar ratio of O_a (left panel, plots a-d) and A horizon microcosm pore water samples (right panel, plots e-h) collected over 36 d flooding. The dashed line in plots d and h indicate the molar ratio of Hg/Cu in soils. Each point in time is from an individual microcosm.

Fig. 4. Mercury dynamics in O_a (plots a-c) and A horizon (plots d-f) microcosm pore waters over 36 d flooding that includes concentrations of filter-passing and particulate total mercury (Hg_T) and methylmercury (MeHg), and dissolved gaseous mercury (Hg⁰_(aq)). Stages 1-3 above plots a and d reflect filter-passing Hg_T dynamics. Plots g and h present changes in filter-passing Hg_T of intact soil core pore waters collected from the O_a (g) and A soil horizons (h), respectively, over two flooding periods (0-17 and 45-80 d). The gray region indicates the period where the soil core was drained (17-45 d). Note that y-axis scale bars are different between plots g and h. Lines are plot between data points to guide the eye. Filter-passing and particulate Hg_T concentrations of microcosm pore waters used for Hg⁰_(aq) (plots b, e) and MeHg quantification (plots c, f) are presented in Fig. EA-9.

Fig. 5. Mercury speciation in clay-size fraction of soils by HR-XANES spectroscopy. (a) Spectra for Hg(II)-SOM using Elliott soil humic acid (ESHA), Hg^0 , A horizon prior to flooding ($t = 0$ d), O_a horizon soil prior to flooding ($t = 0$ d) (black), and O_a horizon soil after 36 d flooding ($t = 36$ d) (black). Linear least-square fit (blue) to the O_a horizon $t = 0$ d sample with 77 ± 6 mol% nanoparticulate metacinnabar ($\beta\text{-HgS}$; represented by the A horizon) and 23 ± 6 mol% Hg(II)-SOM, where 6% is 2σ . Linear least-square fit (blue) to the O_a horizon $t = 36$ d sample with 80 ± 6 mol% nanoparticulate $\beta\text{-HgS}$ (represented by the A horizon) and 20 ± 6 mol% Hg(II)-SOM. (b) A comparison of O_a horizon spectra at $t = 0$ d (black) and $t = 36$ d (red) showing a minor but detectable shift (equal to $\pm 1\sigma$) in mercury speciation to more nanoparticulate $\beta\text{-HgS}$ (indicated with black arrows). All spectra were collected at 10 K (He temperature). The normalized sum-squared residual (NSS) is the normalized difference between two spectra. HR-XANES spectra of A horizon and O_a horizon $t = 0$ d are from Manceau et al. (2015).

Fig. 6. E_{H} -pH diagrams showing the measured pH and E_{H} of (a) O_a and (b) A horizon pore water samples presented over the prominent mercury and sulfur species. The stability of mercury species, sulfur species, and water are shown with solid black lines, dashed gray lines, and dashed black lines, respectively. Open (stage 1), gray-filled (stage 2), and black-filled (stage 3) symbols correspond to stages in O_a and A horizon filter-passing total mercury (Hg_{T}) dynamics presented in Fig. 4a and 4d, respectively. Concentrations of model constituents can be found in Section 2.7.

Fig. 7. The linear correlations between (a) dissolved gaseous mercury ($\text{Hg}^0_{(\text{aq})}$) and (b) filter-passing total mercury (Hg_{T}) and the sum of filter-passing iron and manganese ($\sum(\text{Fe}, \text{Mn})$) in A horizon pore waters of microcosms.