

**Response to:****Comment on bg-2020-466 – Brett Poulin*****Referee Comment:***

The study titled “Mercury mobility, colloid formation and methylation in a polluted fluvisol as affected by manure application and flooding draining cycle” aims to identify the release dynamics of Hg in two soils under two conditions (with and without manure) over two flooding periods. Two soils were characterized and incubated in laboratory microcosms with synthetic rainwater and with/without manure over two flooding cycles. Pore water was documented at numerous points over the two flooding periods, and measured for total Hg, metals, anions, cations, DOC, and pH and Eh. Colloids were collected at 3 time points during each of the 2 flooding periods, and AF4 measurements determine the size distribution and some elemental composition (Hg, Cu, Fe, Mn, carbon). Methylmercury was quantified in the soil a 4 time points between t=0 and t=final conditions.

Overall, the study documents some nice results from the incubation experiment that test the effects of soil properties and manure addition. The study design and methods are well done, and I agree with the majority of the conclusions. However, my main comments are about the presentation of the work and ways to improve the clarity in presentation. I have itemized general comments and specific comments that should be addressed by the authors before considering this work for publication. The authors are encouraged to edit the manuscript thoroughly for editorial clarity. I did not identify all the sentences and statements that were unclear, but have listed some editorial comments in the Specific Comments section below.

***Author Response:***

Thank you very much for the constructive and thorough comments on our study. These greatly contribute to the improvement of the manuscript and we edited it accordingly to better clarify the presentation of the results and outcomes of our study.

***Referee Comment:***

The importance of sulfate reduction should be revisited in this paper, as inorganic sulfide will scavenge pore water Hg(II) and result in authigenic formation of  $\beta$ -HgS. There is very little to no discussion of the decrease in sulfate concentrations in the microcosms, which indicates sulfate reduction and is a key biogeochemical transformation that can result in Hg partitioning back to the soils. Figure S7 in the SI shows very high levels of sulfate at the start of the experiments (150-1000 mg/L) and drastic decreases in concentration with flooding time.

***Author Response:***

We agree with the reviewer and have now thoroughly discussed the importance of sulphate reduction in the manuscript. However, due to the limitations described in the manuscript (gradient between artificial rainwater on the surface and soil solution, as seen for  $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{Na}^+$ ), we are introducing the use of the molar ratios of  $\text{SO}_4^{2-}$  to  $\text{Cl}^-$  ( $[\text{SO}_4^{2-}]:[\text{Cl}^-]$ ) to monitor sulphate reduction. During sulphate reduction, sulphate to chloride ratios will decrease as in geochemical terms  $\text{Cl}^-$  is a conservative species.

**Referee Comment:**

For the presentation of the Microcosm results, and figure presentation, I recommend the authors (1) use the redox ladder to guide the initial presentation, (2) consider discussing the Hg release dynamics in terms of “stages” or periods of time describing trends in the concentrations, and (3) detail the release dynamics of the other metals separately. Regarding item 1 of the redox active elements, in Lines 247-255, there is no mention of Fe or sulfate and all pertinent constituents (nitrate, Mn, Fe, sulfate) should be presented together in a single figure (at present, the reader has to look to the SI and main text figures). The observation that reductive dissolution of Fe wasn't observed in Flooding period 1 is still a result that needed to be stated, and there is no mention of the decrease in sulfate from ~1000mg/L to 500 mg/L in flooding period 1 of the HMLC incubations. Regarding item #2 of the Hg release dynamics, on Lines 274-278, you may consider revising to describe the release dynamics in ‘stages’. “Concentrations of Hg were low between X-X days (phase 1), increased to a maximum at 4 days (phase 2), and decreased between 4 and 14 days (Phase 3).” These same ‘stages’ could be references when describing the colloidal data. Regarding the third item on other metal contaminants, the study presents data on diverse metals (Cu, and all metals in Figure S8), but Cu is the only metal discussed. The authors need to discuss the data they present in all figures, otherwise it is unclear why those data are presented in the first place. I commend the authors for a nice study and recognize that presenting the various non-metal metal data is challenging.

**Author Response:**

We thank the referee for these constructive suggestions. Regarding item 1.) We drew a figure including  $\text{NO}_3^-$ , Mn, Fe and  $[\text{SO}_4^{2-}]:[\text{Cl}^-]$  together with DOC in on figure and discussed soil reduction, including Fe reduction and sulphate reduction. Due to the already mentioned experimental limitations we used  $[\text{SO}_4^{2-}]:[\text{Cl}^-]$  to monitor sulphate reduction in the experiment. Regarding item 2.) We drew a new figure including HgT concentrations for the different filtrates and introduced Phases as suggested. These Phases were also subsequently used in the discussion of the Hg colloids. Regarding item 3.) To introduce and discuss the dynamics of all measured trace metals in our experiment would be beyond the scope of this manuscript. However, the importance of metallic Cu was demonstrated earlier in soils with high Cu:Hg ratios (<https://pubs.acs.org/doi/10.1021/es4010976>). We acknowledge the referees view that what is displayed should be discussed and excluded parameters which were not discussed in the manuscript.

As suggested, the new figures are highlight 1.) redox processes, 2.) Hg measurements, 3.) trace elements. They are attached at the end of this document.

**Referee Comment:**

Have the authors considered including an analysis that estimates if the thiol content of the DOM was exceeded in their experiments, to contrast with the soil analysis (Lines 339-345)? The strong binding site capacity of DOM has been quantified to be ~5 nmol/mg DOM (<https://pubs.acs.org/doi/10.1021/es025699i>). They can assume DOC is 50% of the DOM, and compare the strong binding site capacity of the DOM to the total Hg concentration < 0.02  $\mu\text{m}$ .

Further, they could estimate how the addition of manure changed the Hg binding state (saturated vs unsaturated) in both the soil and pore water.

**Author Response:**

We did not measure thiol content of the DOM. However, this would be a good way to better characterize the DOM in future experiments.

We used the suggested factors to calculate the saturation of Hg and included this into the discussion. However, we did not conduct a geochemical modelling where we included aqueous  $\text{Cl}^-$  and sulphide species. Therefore, these estimates have to be taken with caution.

**Referee Comment:**

As a reader, it will improve clarity if you spell out the various soils and treatments. I had to go back repeatedly to the methods to remind myself what the various acronyms meant (“HMLC”). This is important because of the two soils and two treatments (control vs manure).

**Author Response:**

We acknowledge and share the referees view, that abbreviations are not an optimal way to discuss the data. However, we think that for displaying and presenting the data in Figures, Methods and Results this practice is more accessible.

**Referee Comment:**

Line 28 – consider deleting “again”.

**Author Response:**

Accepted.

**Referee Comment:**

Line 30 – consider “formation and aggregation” of ...

**Author Response:**

Changed to “formation and aggregation”

**Referee Comment:**

Line 46-47 – There is “cinnabar” in the environment, but in the form of mineral deposits or associated with mercury mining activities. In the vast majority of environments, mercuric sulfide is present as authigenic nanoparticulate metacinnabar ( $\beta$ -HgS nano).

**Author Response:**

Changed to “..associated with  $\text{FeS}(s)$  or found as cinnabar ( $\text{HgS}(s)$ ) or meta-cinnabar ( $\beta$ - $\text{HgS}(s)$ ).” For clarification.

**Referee Comment:**

Line 59 – consider deleting “e.g.”.

**Author Response:**

Accepted

**Referee Comment:**

Line 62-63 – consider expanding to include the microbial process. At present, it reads awkward because Hofacker 2013 and 2015 are referenced but it is somewhat unclear what the 2015 study contributed.

**Author Response:**

Accepted. Added: “[...]which were shown to be formed by bacteria (*Clostridium* species) (Hofacker et al., 2015)”

**Referee Comment:**

Line 66 – The first reports of DOM facilitating the dissolution of HgS were by <https://pubs.acs.org/doi/10.1021/es9804058>. This study should be cited.

**Author Response:**

Accepted and added.

**Referee Comment:**

Line 66-68; here you reference “altering the reaction kinetics of HgS(s) formation”, in which case you should cite Ravichandran et al., 1999 and Deonaraine and Hsu-Kim, 2009.

**Author Response:**

Accepted and added

**Referee Comment:**

Line 72-74; Ligand exchange is important, but in the vast majority of systems there is an excess of strong thiols binding sites in the DOM for all the Hg(II). This was first documented by <https://pubs.acs.org/doi/10.1021/es025699i> and should be integrated into this sentence.

**Author Response:**

Haitzer et al. 2002 suggest that the lipophile fraction of DOM sampled from an aquatic system contain high amount excess of thiols sites. We integrated this reference in this paragraph and added a sentence about high density of strong binding sites in high molecular weight NOM.

**Referee Comment:**

Lines 66-70 and 430-431; the authors need to cite primary literature that document how DOM controls the nanocrystalline structure of  $\beta$ -HgS particles, which is a key property influencing the bioavailability of mercury under sulfidic conditions. Two paper that should be considered due to conditions that closely represent natural conditions include <https://pubs.acs.org/doi/abs/10.1021/es201837h> and <https://pubs.acs.org/doi/10.1021/acs.est.7b02687>.

**Author Response:**

We considered the two articles which report experimental evidence of meta-cinnabar formation close to our conditions. This and the decrease in  $[\text{SO}_4^{2-}][\text{Cl}^-]$  further supported our hypotheses of HgS formation in the manure added runs.

**Referee Comment:**

Line 88; the authors may consider also looking at a recent paper on OM amendments to mine tailings. <https://www.sciencedirect.com/science/article/pii/S0269749120370585>

**Author Response:**

Thank you for this input we Cited this paper in the introduction and added it in the discussion.

**Referee Comment:**

Line 92-93; The authors are encouraged to revise this sentence, as it could be improved to highlight the various environments where methylation is prominent (riparian zones, saturated soils etc) because of the redox conditions.

**Author Response:**

Accepted. Changed to: "Environments of elevated Hg methylation (riparian zone, estuary) are also places of elevated NOM degradation and mineralization due to temporal changes in redox conditions."

**Referee Comment:**

Lines 108-2213; somewhere in this paragraph it should be mentioned that "microcosm experiments" were carried out.

**Author Response:**

Accepted.

**Referee Comment:**

Line 112 – revised to “0.02 and 10  $\mu\text{m}$ ” or an equivalent term. At present, “0.02/10  $\mu\text{m}$ ” is a fraction and doesn’t make sense to me.

**Author Response:**

We agree, this may be confusing. Changed to “0.02 and 10  $\mu\text{m}$ ”

**Referee Comment:**

Line 132: First, this sub-header should read “Microcosm Experiments”. It is confusing to call them “incubations” when later you refer to them as microcosms – please be consistent and in all instances state “microcosms”. Second, in a section below you detail the “Incubation experiment blanks” but those are not detailed in this section, and they should be.

**Author Response:**

Changed. First, we renamed the section added the details ask for in the section “2.2 Microcosm Experiments”. Second, we added information about blanks in “2.5 Soil solution sampling and analyses”. “[...]Incubation experiment blanks were taken by sampling MilliQ water through from an empty 1 L borosilicate aspirator bottle 3 times throughout the experiment.[...]”.

**Referee Comment:**

Line 135-136 – revised to “After the initial incubation period soils were used in the flooding and draining experiments, which were conducted in 1 L borosilicate glass aspirator bottles (Fig. S2).” It would appear Fig. S2 should be called out.

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 137- revise to “Microcosm experiments were performed in experimental triplicate....”

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 141-142; revise “were” to “was”; rainwater is singular.

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 146; I presume you mean “remove any remaining air bubbles...”

**Author Response:**

Yes, this is correct. Accepted and changed.

**Referee Comment:**

Lines 192-195; consider revising to “At each sampling time, sample splits were preserved without further filtration (<10um) and filtered at 0.02 µm (add filter details). Additionally, at 2,5 and 9 days an additional sample split was filtered at 0.45 µm for colloid characterization.” What was the filter type for the 0.45 um filter?

**Author Response:**

We used a Polytetrafluoroethylene Hydrophilic syringe filters (BGB, Boeckten, Switzerland) 0.45µm filters. We added details about 0.45µm filters in this section.

**Referee Comment:**

Line 102 – DOC should be reported in units of mg/L, for consistency with incubation results.

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 206 – revise to “filtered” fractions. And, it is not common to use “suffix” to describe a subscript, which is what is presented for each term.

**Author Response:**

Thank you for this suggestion. We revised to “filtered” fractions and replaced “suffix” with “subscript”.

**Referee Comment:**

Line 231 – revise to “0.5% HCl and 1.0% HNO<sub>3</sub>”.

**Author Response:**

Changed to “1.0 % HNO<sub>3</sub> and 0.5 % HCl”

**Referee Comment:**

Line 249 and 275 – consider revision to “NO<sub>3</sub>- depletion” or “exhausted”.

**Author Response:**

Accepted. We changed “reduction” to “depletion”.

**Referee Comment:**

Line 260-262; this sentence doesn't make sense and needs revision.

**Author Response:**

This sentence was revised in the scope of revising the results section and figures. We drew new figures including  $\text{NO}_3^-$ , Mn, Fe and  $[\text{SO}_4^{2-}]:[\text{Cl}^-]$  together with DOC and discussed soil reduction in detail.

**Referee Comment:**

Line 261 – when describing concentrations in the text, the same units should be used as presented in the figure. Figure 2 presents Mn in units of mg/L.

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 270 – The reader probably won't remember the "cornfield soil" is the HMLC soils. See my comment above to just spell out the soil type. Consider revising "throughout the experiment" to "over both flooding periods".

**Author Response:**

We acknowledge and share the referees view, that abbreviations are not an optimal way to discuss the data. Spelling out the soil types would be confusing, as the two soil samples only differ in land use and not in soil type. As it was not the goal of this study to compare land use types. We arrived to the conclusion that naming the soils after their mercury content and organic carbon content would be a more approachable practice. Thus, we keep the abbreviations as they are now.

**Referee Comment:**

Figure 4 caption; it is entirely unclear what is meant by "Details on the deconvolution procedure are provided in the supplement".

**Author Response:**

Accepted. Added: "The fractograms of all analysed time points are shown in the supplement" (Figs. S9-S12).

**Referee Comment:**

Figure 4 – should the y-axis label for the top panel indicate "particulate" and should state "total Hg".

**Author Response:**



Accepted and added.  $HgT_{<0.45\mu m}$ . We did not add “particulate”. In most soil science studies samples filtered at  $0.45\mu m$  are considered “dissolved”. Instead of confusing the reader with various terms (... $0.45\mu m$  = dissolved ;  $0.02\mu m$  = truly dissolved ;  $<1kDa$  =...), we included the cut off sizes as a subscript.

**Referee Comment:**

Section 3.2 – Consider finding locations in this section to emphasis that you’re looking at time points across the two flooding events. Visually, the size proportion of Hg species data look interesting as they show trends in the first flooding period and little change after that.

**Author Response:**

As you suggested we assigned phases and used them to indicate that we are looking the evolution of colloidal Hg through time in the discussion.

**Referee Comment:**

Section 3.3; the sub-header title should specify this is for the ‘soil’.

**Author Response:**

Accepted and changed.

**Referee Comment:**

Line 329-330; I don’t agree with this conclusion regarding the association of Hg to particulate Mn. In looking at Figure 2, the relative proportion of particulate Mn and Hg decreases with flooding time, but their overall concentration is still low. It is more likely that Hg is release from the soil. The decomposition (and solubilization) of OC in the soils can also release Hg. The pore water DOC concentration is reflecting both release and utilization of DOC, so may not necessarily capture the role of DOC on the Hg mobilization due to carbon mineralization.

**Author Response:**

Thank you for this input. We can not conclusively say that this is the only process responsible for the increase in dissolved Hg. But especially during Mn reduction the oxidation of NOM compounds can be pronounced. We added references giving evidence of enhanced NOM degradation during Mn oxide reduction and discussed this as an alternative process. Mn reductive dissolution was driving both the release of Hg sorbed to Mn-hydroxides and the degradation and mineralisation of Hg-NOM.

**Referee Comment:**

Lines 346-349 – can you discount the possibility that soil heterogeneity could have contributed to the observed variability?

**Author Response:**

We are convinced that the homogenisation procedure used (Section 2.1) and amount of fresh soil used for an incubation (ca. 500g) made a soil heterogeneity effect unlikely.

**Referee Comment:**

Lines 367-368 – the pore water data strongly support that sulphate reduction is occurring, which show drastic decreases in the concentration of sulphate with increased flooding time. In microcosms of this nature, several biogeochemical processes are occurring simultaneously and the Eh of the system isn't sufficient to assess if sulphate reduction is or is not occurring. My assessment is that it is more likely that sulfate reduction resulted in the re-association of porewater Hg with soils, compared to the NOM complexation.

**Author Response:**

Sulphate concentrations were not directly indicative of the onset of sulphate reduction. This is due to a chemical gradient between supernatant rain water and soils solution demonstrated by the continuous decrease in concentration of conservative ions ( $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ) (Sect. 4.4). To monitor sulphate reduction, we therefore now added the molar ratios of  $\text{SO}_4^{2-}$  to  $\text{Cl}^-$ . During sulphate reduction, sulphate to chloride ratios will decrease as in geochemical terms  $\text{Cl}^-$  is a conservative species.

We agree that the Eh measured through a suction cup is not a sufficient measure in complex soil system due to the range of redox presents simultaneously in different pore sizes. We are convinced that sulphate reduction takes place within the incubators. And that Hg is scavenged or reassociated by the formation of  $\beta\text{-HgS}$  in soils. However, one cannot conclusively tell if either this or the complexation NOM is the governing process, we therefore discuss both hypothesis in the discussion.

**Referee Comment:**

Line 378 – Poulin et al 2016 shows distinct  $\text{Hg}(0)$  formation in contaminated soil incubations, and should be cited here.

**Author Response:**

Accepted and Cited.

**Referee Comment:**

Lines 380 – one would need citations for the sentence on abiotic vs biotic reduction.

**Author Response:**

We revised this paragraph and added citations for both biotic and abiotic reduction in the dark. UV-light reduction does not apply to our experiments, as they were conducted in the dark.

**Referee Comment:**

Lines 436-438 – one interpretation is that the soils had been subject to period soil flooding that contributed to mercury methylation.

**Author Response:**

This is a valuable point made. We did not monitor the flooding history prior to the sampling. Therefore, we discussed the possibility of a flooding event which induced MeHg levels prior to the field sampling.

***Referee Comment:***

Lines 444-445; could the higher microbial activity be the result of addition of labile carbon? The author should consider highlighting here the diversity of microbial communities that can methylate Hg, as is provided in the Introduction. Sulphate reducing bacteria, metal reducing bacteria, and fermenters are possible contributors to mercury methylation.

***Author Response:***

A higher microbial activity could indeed be the result of addition of labile carbon in the form of manure. We revised this paragraph and highlighted the diversity of Hg methylators using the references given in the introduction.

***Referee Comment:***

Figure 2 – the y-axis labels are very hard to read, and would be nearly impossible to read in print form. Consider re-working the figures as I suggest above, where all terminal electron acceptor processes and DOC are included in a single figure, then all Hg measurements, then all other trace metals.

***Author Response:***

We understand and support the input, created new figures and increased the figure sizes. In the new version of the manuscript three separate figures are presented per soil, highlighting redox processes, Hg measurements, trace elements. They are attached at the end of this document.

We excluded a selection of measured trace metals in the article as the discussion of all the measured trace metals is beyond the scope of this manuscript.

***Referee Comment:***

SI Specific Comments:

Line 7 – this figure should be revised to state “total Hg” when total Hg is measured. This needs to be fixed in all cases in text and figures, in the main text and SI.

***Author Response:***

Accepted and changed for all the figures and in the text.

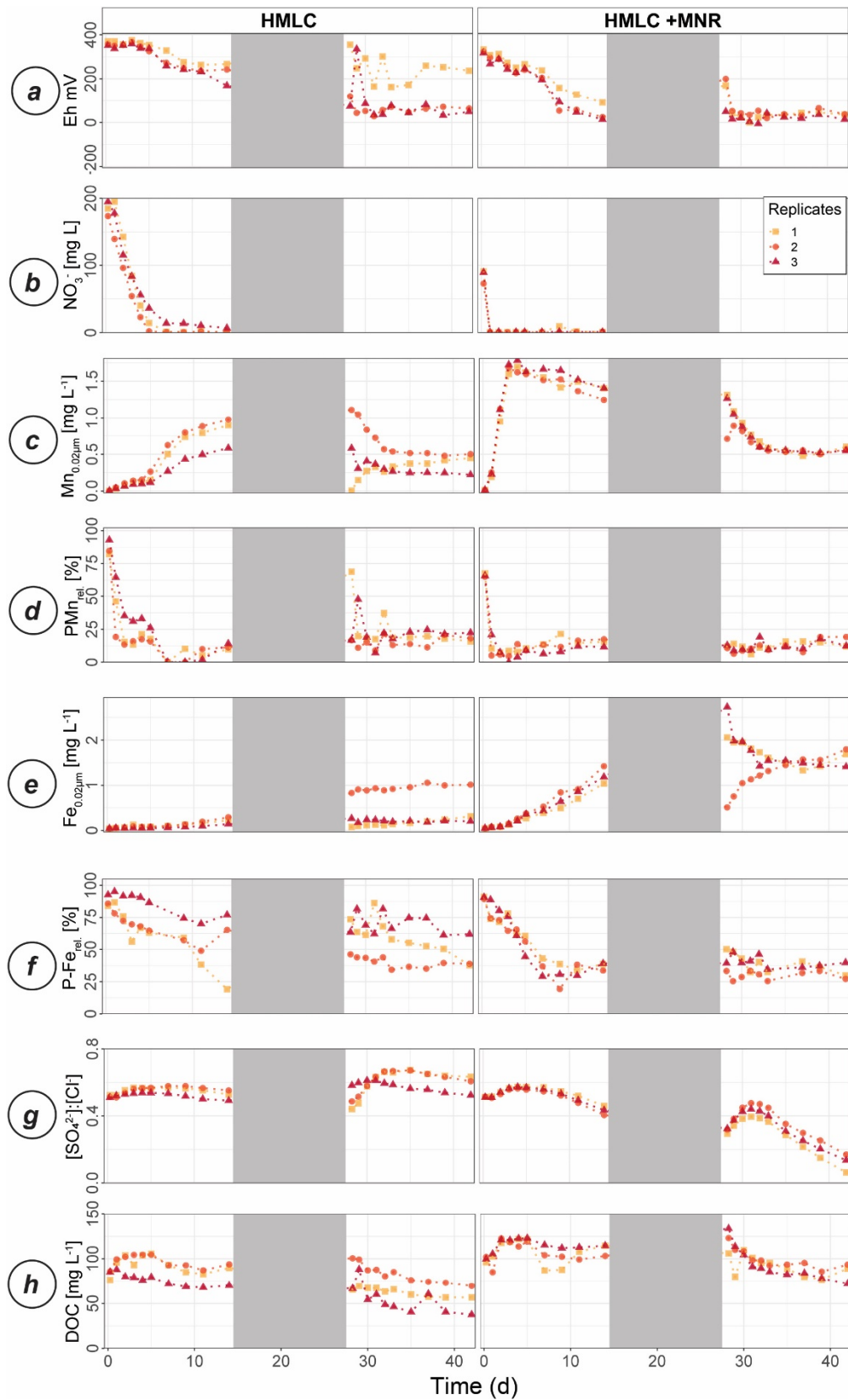


Figure 1 Soil solution dynamics in cornfield soil (HMLC) incubations for redox potential (a), redox reactive elements (Mn, PMn, Fe, P-Fe,  $[SO_4^{2-}]:[Cl^-]$ ) (b-f) and dissolved organic carbon (h). Lines between points were plotted to improve readability. The gray area indicates the drained period.

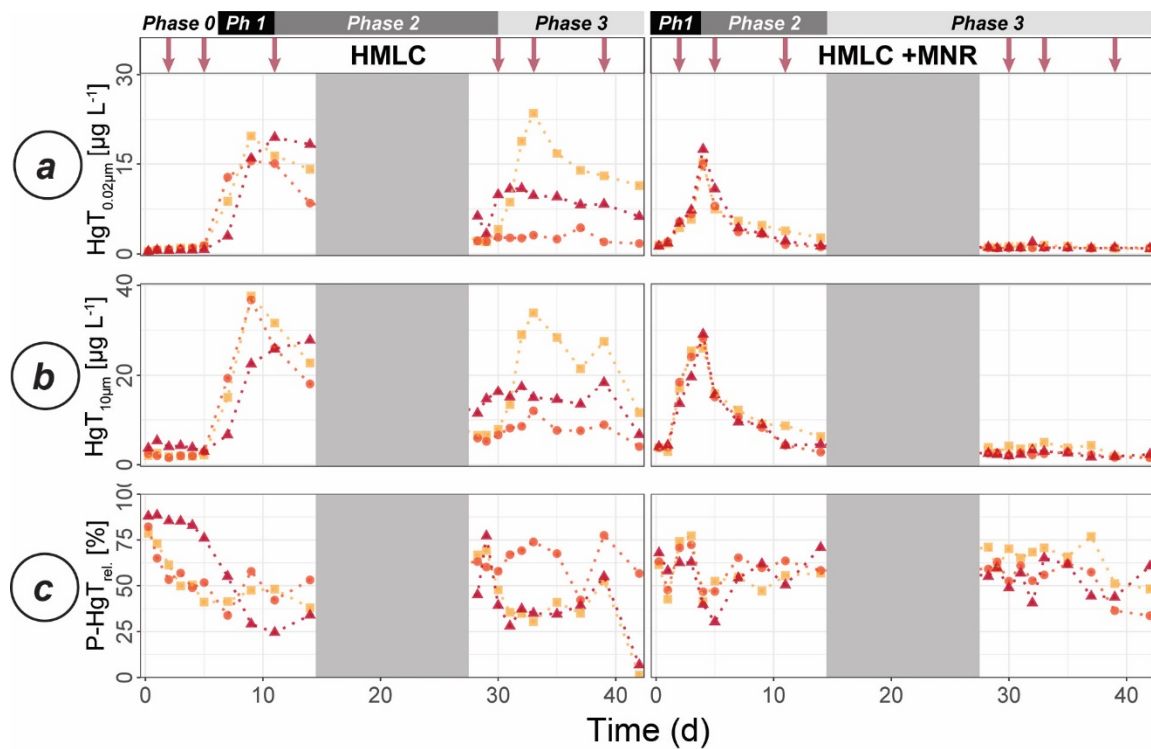


Figure 2 Soil solution dynamics in cornfield soil (HMLC) incubations for Hg (a-c) subdivided in phases (0-3). Lines between points were plotted to improve readability. The gray area indicates the drained period. Red arrows indicate sampling days for AF4-ICP-MS analyses.

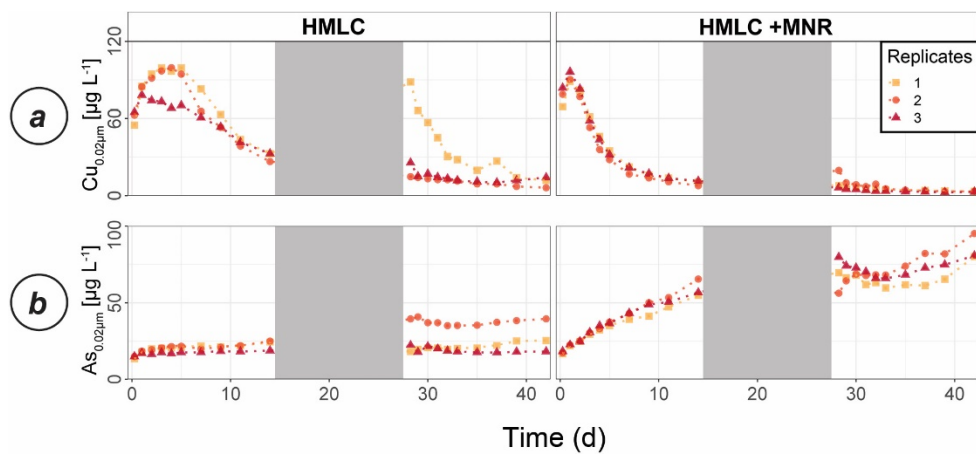


Figure 3 Soil solution dynamics in cornfield soil (HMLC) incubations for Cu (a) and As (b). Lines between points were plotted to improve readability. The gray area indicates the drained period.

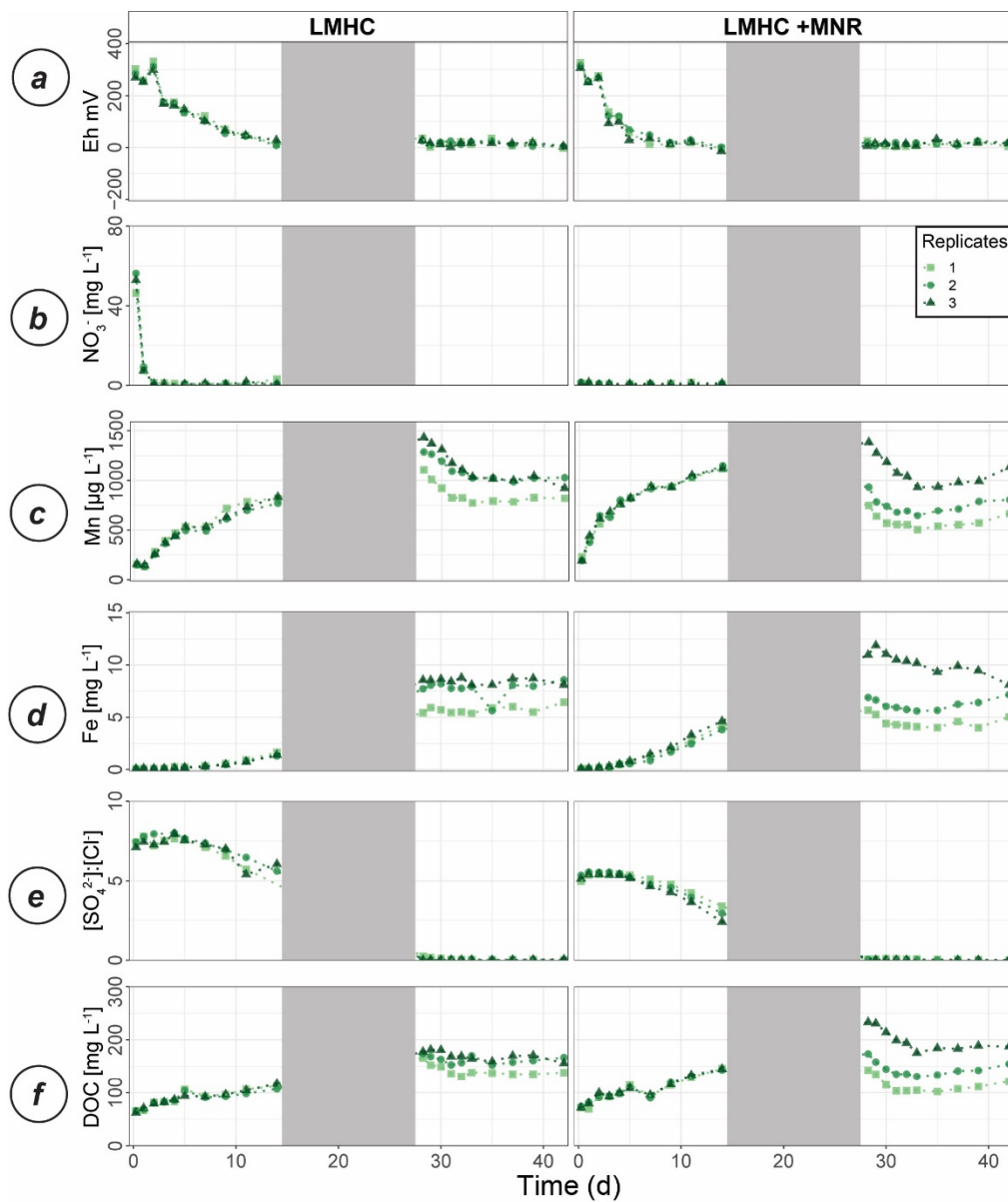


Figure 4 Soil solution dynamics in pasture field soil (LMHC) incubations for redox potential (a), redox reactive elements (Mn, PMn, Fe, P-Fe,  $[\text{SO}_4^{2-}]:[\text{Cl}^-]$ ) (b-f) and dissolved organic carbon (h). Lines between points were plotted to improve readability. The gray area indicates the drained period.



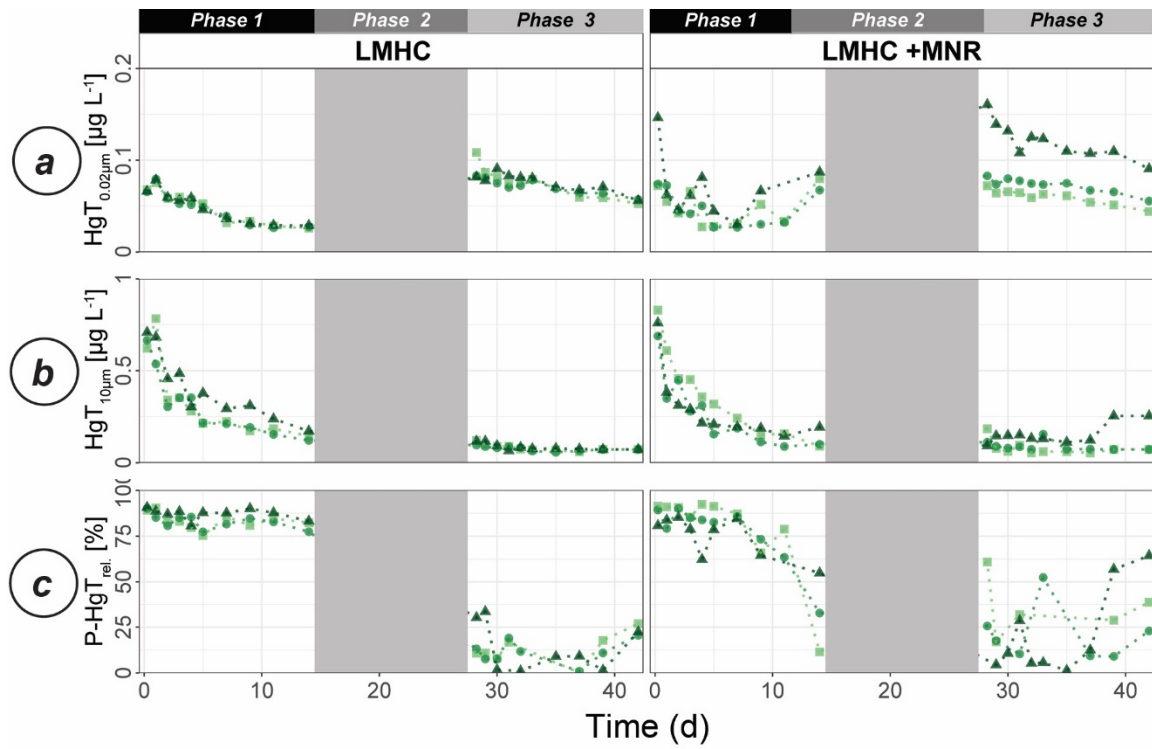


Figure 5 Soil solution dynamics in pasture field soil (LMHC) incubations for Hg (a-c) subdivided in phases (1-3). Lines between points were plotted to improve readability. The gray area indicates the drained period.

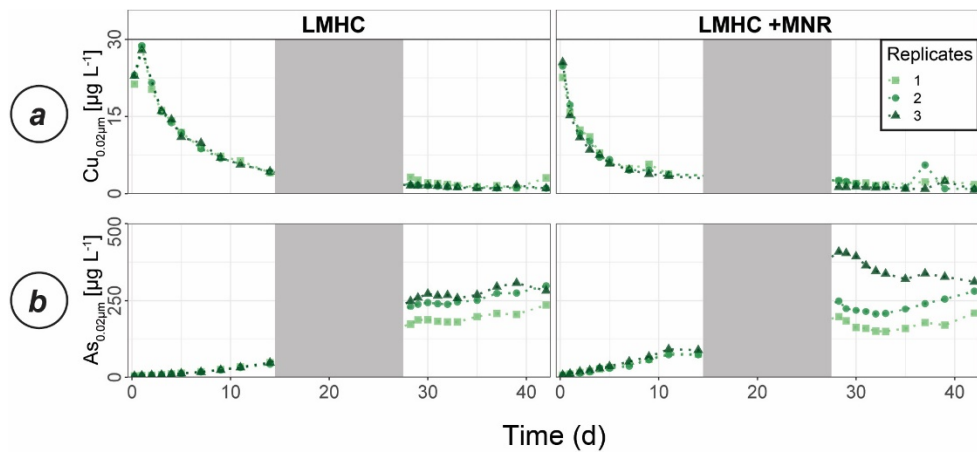


Figure 6 Soil solution dynamics in pasture field soil (LMHC) incubations for Cu (a) and As (b). Lines between points were plotted to improve readability. The gray area indicates the drained period.