Response to:

Comment on bg-2020-466 - Amrika Deonarine

Referee Comment:

Very interesting data set on Hg-colloids and Hg methylation during flooding events! Agree with R1 comment on highlighting the novel and unexpected results in this study. Be careful though with overstating your conclusions, particularly with respect to HgS(s) formation (does the redox data support sulphate reduction/sulphide production?) and microbial activity (not measured)

Author Response:

We thank the A.D. for the interest in our study and appreciate the detailed comments. Please find the responses to you inputs.

Referee Comment:

Section 2.2: Was there a control for the manure only? AF4, Hg and MeHg data might be interesting for comparison.

Author Response:

We did not preform a microcosm experiment for manure only. However, Manure's Hg (45 ng g^{-1}) and MeHg (<0.02 ng g^{-1}) concentrations are given in Table 1. These levels are likely too low for a AF4 run. A MeHg and iHg spiking to the manure and a subsequent AF4 measurement would be indeed interesting and allow for comparison.

Referee Comment:

Line 144: Could you clarify what one application of the manure was?

Author Response:

We acknowledge the need of further clarification and added in the supplement:

"One application of liquid manure (0.6 % (w/w)) represents the recommended minimal application of 0.67 t km^{-2} following the principles of fertilization of agricultural crops in Switzerland (Richner and Sinaj, 2017). This calculation assumes an affected soil depth of 10 cm and soil bulk density of 1.2 g cm^{-3}. This value is in the range of bulk density of soils from this area previously measured in our lab."

Referee Comment:

Line 241: There is a correction which can be made for the Fe contribution to SUVA. See

Poulin, B., et al. (2014). "Effects of iron on optical properties of dissolved organic matter." Environ. Sci. Technol. 48: 10098-10106.

Author Response:

We acknowledge the provided reference. However, the mentioned correction can not be applied.

The correction proposed by Poulin and co-authors is made for SUVA batch measurements. In our case, colloidal Fe can be both Fe(II) or Fe(III) (but not truly dissolved, as it is removed during the injection). We did not monitor Fe oxidation state of colloids during AF4 run and a the extrapolation of bulk Fe(II) and Fe(III) over the size spectrum would not be expedient.

Referee Comment:

Line 242: What exactly do you mean by “associated”? Which wavelengths were run for the humic-like fluorophores? Why was FLD run – what does it provide in addition to SUVA?

Author Response:

We have removed "associated" and replaced by "co-eluted". Fluorescence is more specific for online detection of humic substances-like components at an Excitation WL = 270 nm and an Emission WL = 460 nm, and allow to distinguish from protein-like components (Excitation WL = 280 nm and an Emission WL = 350 nm). Using AF4-FLD-UVD most of the studies agreed with lower size-range distribution of fluorogenic components compared to absorbing components, certainly due to the overlap absorbance of slightly higher sized iron-clusters or small ion colloids.

Referee Comment:

Line 320: Why do you think this fraction consists of HgS colloids?

Author Response:

In this paragraph we do not discuss colloid fractions. We assume the comment refers to Line 302. As stated in the text the Hg colloid fraction is neither overlap with NOM nor signals of the ICP-MS (e.g. Fe, Mn, Cu). Therefore, we hypothesise that this signal might originate from a HgS species (likely nanoparticulate meta cinnabar). The relative increase of this fraction was observed at the onset of sulphate reduction in the HMLC +MRN MC.

Referee Comment:

Line 322: Consider including values here.

Author Response:

Since the values can be found in the Table 3, we did not think it necessary to repeat them here.

Referee Comment:

Line 340: Red-S and Hg concentrations should be expressed in mol/g. Also, Hg can complex with other functional groups such as O-containing functional groups in OM. How does this fit into the competition scenario between OM and Mn oxides?

Is it possible considering the pH and pzc of Mn oxides that Hg can adsorb to the surface? Are there any other studies which have reported Hg- Mn interactions?

Author Response:

Thank you for this interesting input. Early lab experiments report the adsorption capacity for Hg^{2+} on MnO2 surfaces to be 15 mmol kg^{-1} over a wide range of pH (5-11)(P. Thanabalasingam and W. Pickering, 1985). This is in accord with the experimentally assessed pzc of MnO_2 of (4.9-4.1) (Miyittah et al., 2016). Considering these references, the presence of 493 ± 21 mg kg^{-1} Mn in the HMLC soil and an oversaturation of strong binding sites of SOM in our soil, sorption of Hg on MnO2 is likely.

Referee Comment:

Line 357: Have you considered that OM can directly reduce Mn oxides or act as an electron shuttle?

Author Response:

Direct reduction of Mn oxides is a possible mechanism and was added here.

Referee Comment:

Line 367: There are many minerals which form black precipitates. Is there geochemical modeling data or XRD data to support this? Does the redox data support sulphide production?

Author Response:

Unfortunately, we could neither consider geochemical modelling nor XRD as suitable to identify the precipitates due to the following reasons.

1.) XRD is a very powerful technique for characterisation of crystal structures of solid materials. However, characterisation of nano particles and solids with low crystallinity require a high sample purity (consider: https://doi.org/10.1021/acsnano.9b05157). This fact makes is rather difficult to characterize the newly formed phases (likely semi-crystalline phases) by XRD.
We analysed bulk soils before and after the experiment using XRD. Like this it was only possible to characterize the major crystal phases of the soil matrix (see manuscript: section 2.4).
In our opinion, the analyses of the black precipitates in question would require a very sensitive and time-intensive purification procedure when using our available XRD techniques and was beyond the scope of the study.

2.) Further, we did not consider conducting aqueous geochemical modelling of Hg species. Here, the characterization of the DOM is crucial to get a good picture. Unfortunately, we did not further characterize DOM.

Concerning the redox data:
In incubators where manure was added we observed a vast decrease in Eh. It ranged between 100 and 0 mV at the time of the formation of the "black precipitates". Earlier incubation studies observed the onset of sulphate reduction already a 0mV. It is assumed that the bulk Eh measurements of the sampled pore water do not entirely reflect the redox conditions in the different pores and aggregates of the soil microcosm. Further, we used a flow-through system to measure Eh of the soil solution and oxidation of the sampled water between Microcosm and Eh probe cannot be out ruled although highly unlikely since the device is specifically made for this

purpose. Despite the rather high Eh measurements, we are convinced sulfate reduction and meta cinnabar precipitation is taking place. Now, we introduced [SO_4^{2-}]:[Cl^-]to monitor sulphate reduction. Sulphate concentrations were not directly indicative of the onset of sulphate reduction. This is due to a chemical gradient between supernatant rainwater and soils solution demonstrated by the continuous decrease in concentration of conservative ions (Cl^-, Na^+, K^+) (Sect. 4.4).

Referee Comment:

Line 368: Is there a reference for the formation of sulphide minerals in meso- and micropores?

Author Response:

With this line we wanted to emphasize that redox potential is not an exact measure for ongoing redox processes. Eh measurement may only be used qualitatively in complex soil systems.

Referee Comment:

Line 386: Does the redox data support sulphide production?

Author Response:

Despite the rather high Eh measurements, we are convinced sulphate reduction and meta cinnabar precipitation is taking place. Now, we introduced [SO_4^{2-}]:[Cl^-] to monitor sulphate reduction. Sulphate concentrations were not directly indicative of the onset of sulphate reduction. This is due to a chemical gradient between supernatant rainwater and soils solution demonstrated by the continuous decrease in concentration of conservative ions (Cl^-, Na^+, K^+) (Sect. 4.4).

See comments on L367

Referee Comment:

Line 406-408: This is very interesting. How do you think this relates to the decrease in Hg-0.02 um after 4 days for HMLC+MNR?

Author Response:

In case of HMLC+MNR, the Hg colloidal distribution was dominated by the presence of larger fractions (30 - 450 nm). Manure addition facilitates the soil reduction process and is a source of POM and larger NOM aggregates. As an effect of manure addition, Hg adsorb to larger NOM-aggreagtes and/or form nano particulate meta-cinnabar.

Referee Comment:

Line 404: Complexation is driven by thermodynamics and not necessarily by ligand concentration. Geochem modelling might help support this statement on chloride complexation.

Author Response:

Cl is present at extremely high concentrations. As we show in Fig. S6 and S7 both K and Cl are in the range of 0.8 g L^{-1} and 0.5 g L^{-1} in soil solution at the beginning of the incubation. The ionic strength of this solution is (surprisingly) close to brackish waters. We assume that K and Cl concentrations would have decreased upon the next rain events in the area given that K and Cl are conservative elements which are highly soluble and marginally interact with high specific surface minerals. This points towards an inorganic fertilizer application before the field sampling.

Although the binding of Hg to NOM might be favorable, an addition of such high amounts of KCl in NOM poor soil might still influence Hg speciation in the upper most cm of the soil column.

We could not consider conducting aqueous geochemical modelling of Hg species because the characterization of the DOM is crucial to get a good picture ande could not further characterize DOM.

Referee Comment:

Line 415: What is the fraction of the total Hg in the “small pool”?

Author Response:

Unfortunately, we did not use methods to analyse the solid speciation of our soil (sequential extraction procedures, or EXAFS) However, we estimated that we sampled approximately (0.02 % of the soils HgT) during the whole experiment. Therefore, the proportion of Hg mobilized from soil matrix seams relatively low, compared to its total content.

Referee Comment:

Line 425: Does the redox data support sulphide production?

Author Response:

See answer to the comment above, on L367

Referee Comment:

Line 442: What were the concentrations of bioavailable Hg?

Author Response:

Thank you for this question. We agree that bioavailability is more complex than a chemical measurement, no total chemical measurement can account for bioavailability. Some tools available can relate potential bioavailability, like DGT measurements (Ndu et al., 2018). Following this it is expected that higher truly dissolved Hg could be more bioavailable than other size fractions. However, the study also showed that filter passing <0.2 µm filter passing Hg might not be a good measure for bioavailability.

We included this in the discussion about bioavailable concentration and did not give concentrations, as this would be too farfetched.

Referee Comment:

Figure 3: Is there Fe data?

Author Response:

We added concentrations of [SO_4^{2-}]:[Cl^-] and Fe to the Figures.

A complete dataset of this study is accessible in the data repository ZENODO http://doi.org/10.5281/zenodo.4058676 Fe data is also included there as well.

Referee Comment:

Figure 4: Consider revising the legend labels to be more descriptive of the different fractions. There is overlap between the 6-25, 6-450 and 30-450 nm size fractions, which makes interpreting the Hg proportion data difficult (sum to 100%).

Author Response:

The deconvolution of the fractograms included an intermediate fraction of Hg bearing colloids ranging between d_h = 6 nm and d_h = 450 nm depending on the sample. This fraction was added to refine the fractogram fittings and indicates that this population overlap a more polydisperse Hg particle population. The sum of colloid fractions was in accord with the total Hg in the filter fraction of 0.45µm filtrates.

Referee Comment:

Table 3: Be careful with de/methylation. What you are quantifying is a decrease in net methylation and not necessarily demethylation processes.

Author Response:

This is right. In the new version we consider this remark. We replaced methylation and demethylation with “Net MeHg production” when discussing our results.

References

Miyittah, M. K., Tsyawo, F. W., Kumah, K. K., Stanley, C. D., and Rechcigl, J. E.: Suitability of Two Methods for Determination of Point of Zero Charge (PZC) of Adsorbents in Soils, Communications in Soil Science and Plant Analysis, 47, 101–111, doi:10.1080/00103624.2015.1108434, 2016.

Ndu, U., Christensen, G. A., Rivera, N. A., Gionfriddo, C. M., Deshusses, M. A., Elias, D. A., and Hsu-Kim, H.: Quantification of Mercury Bioavailability for Methylation Using Diffusive Gradient in Thin-Film Samplers, Environmental science & technology, 52, 8521–8529, doi:10.1021/acs.est.8b00647, 2018.

P. Thanabalasingam and W. Pickering: Sorption of mercury(II) by manganese(IV) oxide, Environmental Pollution Series B, Chemical and Physical, 10, 115–128, 1985.