

*Replies to the interactive comment on “Effects of extraction conditions on the redox properties of soil organic matter (SOM) and its ability to stimulate microbial iron(III) mineral reduction by electron shuttling”.*

This paper compares how different extraction methods influence the composition of soil organic matter (SOM) derived from the process. They compared SOM extracted by neutral pH water and mediated by alkaline extraction followed by acid precipitation (the standard approach used to delineate soil humic and fulvic acids) under oxic and anoxic conditions. The authors determined carbon recovered, specific UV absorbance @ 254 nm (SUVA), and most importantly the exchangeable electron capacity (EEC) as well as electron accepting and donating capacities (EAC and EDC). The manuscript is well-organized, and easy to read (even though there are a couple of typos that spell checker did not catch). The most important contribution, however, is the electrochemical analyses that were conducted, which makes this paper really unique. There are a few major issues that I have, and specific comments are below.

We would like to thank the reviewer for going through our manuscript and providing useful suggestions to help us to improve the manuscript. We also appreciate the overall positive response and in particular his/her appreciation of the electrochemical analyses of the SOM extracts. We will go through the manuscript carefully and incorporate all suggestions and comments.

1. The SUVA data seems fine for the water extracted SOM and fall well within the range of values reported by others (e.g., Weishaar et al., 2003). However, the FA and HA alkaline extraction conducted anoxically were off the charts and many factors higher than the highest value reported by Weishaar et al., 2003. These numbers appear unrealistic and could be due to the presence of iron (both (II) and (III)) in the extracts that reached 3 mM. Given that Weishaar et al., reported iron interference (they use Fe(III) as an example, but

noted that Fe(II) can also interfere) at levels of only a few mg/L (or 10's of  $\mu\text{M}$ ) this could be a positive interference to their SUVA data.

We agree with the reviewer that the  $\text{SUVA}_{254}$  values measured in our study, especially for the HA isolated from the water-extracted OM ( $0.207 \text{ L mg}^{-1} \text{ C cm}^{-1}$ ), are almost one order of magnitude higher than the reported  $\text{SUVA}_{254}$  values for HA chemically extracted from Coal Creek soil in Weishaar et al., 2003 ( $0.039 \text{ mg}^{-1} \text{ C cm}^{-1}$ ) (Weishaar et al., 2003), and also higher than the typical  $\text{SUVA}_{254}$  values of HA analyzed in many other studies (Beckett et al., 1987; Chen et al., 2003; Fox et al., 2017). However, the  $\text{SUVA}_{254}$  of all our FA extracts range from 0.017 to  $0.042 \text{ L mg}^{-1} \text{ C cm}^{-1}$ , and these values are in line with previous studies (Beckett et al., 1987; Chen et al., 2003; Fox et al., 2017).

One reason for the higher  $\text{SUVA}_{254}$  values for the HA isolated from water-extracted SOM under anoxic conditions in our study compared to others, in addition to the differences in the soils from which the HA were extracted, as suggested by the reviewer, could be the presence of Fe(II) and Fe(III). As shown by Weishaar et al. (2003), the presence of  $4 \text{ mg L}^{-1}$  Fe(III) showed an absorbance value about  $0.65 \text{ cm}^{-1}$  at 254 nm wavelength, and the absorbance increased with increasing Fe(III) concentrations. Based on this study, we can hypothesize that also in our case the presence of Fe(III) influenced the measured  $\text{SUVA}_{254}$  value of the HA isolated from the water-extracted SOM under anoxic conditions. The Fe(III) concentration in the HA isolated from water-extracted SOM under anoxic condition was  $33 \mu\text{mol L}^{-1}$ . Please note that we did not use the concentration of  $3 \text{ mmol L}^{-1}$  as suggested by the reviewer, because  $3 \text{ mmol L}^{-1}$  was the Fe(II) concentration determined right after the anoxic water extraction of SOM. However, all of the samples were passed through 0.45 mm syringe filters under oxic conditions

before the SUVA analyses. Therefore, a large amount of the Fe(II) was oxidized to Fe(III) and removed as particulate Fe(III) by the filtration, as explained in the manuscript **line 196-199, page 7**. The remaining Fe(II) and Fe(III) in the samples were analyzed and shown in Table S3 and the Fe(III) concentration for HA isolated from the water-extracted SOM under anoxic condition was  $33 \mu\text{mol L}^{-1}$ .

$$33 \mu\text{mol L}^{-1} \times 56 \text{ g mol}^{-1} = 1.848 \text{ mg L}^{-1}$$

According to Weishaar et al. (2003),  $1.848 \text{ mg L}^{-1}$  Fe(III) has an absorbance value of  $0.15 \text{ cm}^{-1}$  at 254 nm wavelength. With the additional  $28 \mu\text{mol L}^{-1}$  Fe(II), the contribution of iron to the measured  $\text{SUVA}_{254}$  value should be even higher. Therefore, we would like to add one sentence in the results section 3.1, **line 203, page 7**, as “*A previous study showed that  $4 \text{ mg L}^{-1}$  Fe(III) yielded an absorbance value of  $0.65 \text{ cm}^{-1}$  at 254 nm wavelength (Weishaar et al., 2003). Therefore, we believe that the high  $\text{SUVA}_{254}$  value of HA isolated from the water-extractable OM (please note, this is the term used to replace ‘water-extracted SOM’ as suggested by reviewer 1) compared to  $\text{SUVA}_{254}$  values of HA shown in previous studies could be caused by the presence of Fe(II) and Fe(III) in the sample due to the microbial Fe(III) reduction that occurred under the anoxic extraction conditions.*”

2. There was only passing mention of the NMR and fluorescence data. Why wasn't this data more prominently discussed in the paper (as opposed to a glancing mention in the SI)? For example, how does the EEM data “confirm higher contents of aromatic carbon” (the explanation in the SI caption was inadequate)? Further, the relatively smaller differences in NMR determined aromaticity between anoxically extracted vs oxic extraction SOM is not reflected in the much larger (order of magnitude) spread observed for SUVA (see above). Further, the EEMs from Figure S2 look really odd and I suspect that this caused by the really

high DOC levels used by the authors (100 mg/L!). At those levels inner- filter-effects will become dominant as the solution will be optically dense to the point where inner-filter corrections will likely no longer work. Typically, fluorescence EEMs are collected at much lower (nearly two orders of magnitude) DOC concentrations to minimize inner-filter-effects (see papers by Stedmon et al., in L and O). Thus, because the data is likely improperly collected I would simply eliminate it from the discussion.

We would like to thank the reviewer for the comments and suggestions. First, we would like to clarify that the concentration of all samples used for the EEM analysis was not 100 mg C L<sup>-1</sup>. In the manuscript, line 113, page 4, it says “*Freeze-dried SOM/FA/HA powders were dissolved in Milli-Q water (pH 7) at a concentration of 100 mg C L<sup>-1</sup> and the solutions were agitated for 12 h at 300 rpm at room temperature, samples were then filtered through 0.45 mm syringe filter (mixed cellulose ester (MCE, Millipore, Germany)). For fluorescence analyses, samples were prepared by stepwise dilution of extract solution with Milli-Q water (pH 7) until absorbance values of 0.300 at 254 nm wavelength were reached*”. Therefore, after the stepwise dilution, the final concentration of samples used for the EEM analyses was much lower than 100 mg C L<sup>-1</sup> and inner-filter effects can be neglected.

However, as the reviewer pointed out, we did not draw any conclusions directly from the EEM data. As also explained in the reply letter to reviewer 1, we did this because there are many debates about whether, to any extent, the EEM spectra can reflect the redox state and the aromaticity of the OM samples (Fimmen et al., 2007; Maurer et al., 2010). Since only very briefly mentioned the results of the EEM spectra and since leaving the EEMs out completely will not impact the conclusion of the manuscript at all, as

suggested by the reviewer, we would like to completely remove the EEM results from our manuscript.

Regarding the NMR, as we commented already in the reply letter to reviewer 1, we would like to show in the revised manuscript the NMR spectra of all samples in the supporting information and add the aromaticity values of the OM extracts calculated from the NMR analyses to Table 1.

Finally, regarding the reviewer question “why the very distinct difference between different OM extracts as shown by the SUVA results could not be seen in the calculated aromaticity from NMR”: in previous studies that applied NMR to characterize the aromaticity of OM, the differences between different OM samples are usually in the range of 10%. For example, in the study of Lorenz et al. (2006), seven different OM samples extracted from different sampling sites were analyzed, and the aromaticity of these samples ranged from 21-32%. Inbar and co-authors compared the aromaticity of native SOM and the same SOM after 147 days of composting, and the aromaticity only changed from 35% to 37% (Inbar et al., 1990). In our study, all of the OM extracts, although extracted in different ways, were from the same soil. Therefore, we believe that, for example, the 4% difference in the aromaticity between chemically-extracted HA under oxic and chemically-extracted HA under anoxic condition does indicate a potential difference in the aromaticity of these two samples. Moreover, although the differences of the aromaticity among different OM samples calculated from NMR are not as significant as the differences of  $SUVA_{254}$  values among different samples, the aromaticity of SOM/HA/FA extracted under anoxic conditions was higher than the aromaticity of SOM/HA/FA extracted under oxic conditions. Furthermore, under anoxic conditions, FA

and HA isolated from the water-extracted SOM (water-extractable OM) both have higher aromaticity than the water-extractable OM itself. Therefore, our NMR results are perfectly in line with the  $SUVA_{254}$  results and the electron exchange capacity analysis of the OM samples and can be used to support and strength our argument that the chemical extraction and the presence of oxygen impacts the aromaticity thus the redox activity of the SOM extracts.

3. I think the discussion regarding the comparison between Suwannee River reverse osmosis dissolved organic matter (DOM) to the fulvic acid fraction isolated by XAD-8 chromatography (as opposed to acid precipitation) does not add any value to the paper because you are basically comparing apples and oranges (i.e., SOM vs. aquatic DOM). The methods are totally different from alkaline and neutral extraction and there are no mineral phases involved. The authors can delete the entire discussion and it will not affect the conclusions or the quality of this paper.

Following the reviewer's suggestion, we would like to remove this part of the discussion: **line 326-343, page 12.**

4. While the authors point to several studies demonstrating correlations between DOC and Fe(II) formed from the dissolution of iron oxides in batch incubation studies, evidence for this relationship has also been reported in benthic pore waters. See papers by Burdige (et al.), Chin (et al.), plus many others. I think showing that this phenomenon occurs in real aquatic systems strengthens the arguments put forth by the authors for this paper.

As suggested by the reviewer, to strengthen our argument of the correlation between DOC and Fe(III) mineral dissolution, we would like to add one sentence as follows (at **line 285, page 10**):

*“In-situ monitoring of the DOC flux in pore water of marine sediment or freshwater wetland also suggested an increase in DOC with increasing amount of microbial iron(III) mineral reduction (Burdige et al., 1992; Burdige et al., 1999; Chin et al., 1998)”.*

## References

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