

Dear Professor Vanclooster:

We are pleased to submit our newly revised manuscript “Technical note: Effects of iron(II) on fluorescence properties of dissolved organic matter at circumneutral pH” as well as a tracked changes version of the document. We are grateful for the constructive comments of Referee 3 and are thankful to them for agreeing to review the revised manuscript and assess the changes that we made in response to Referee 1 and Referee 2’s comments. Our detailed point-by-point responses to their comments are available below. The Referee’s comments are in black Arial font, our responses are in purple Times New Roman, and our revised text is in blue Times New Roman.

Thank you for considering our revised manuscript.

Sincerely,



Cara C. M. Manning on behalf of coauthors

Comments from Referee 3:

Overall, the revisions performed by the authors are acknowledged. However, some issues still require further clarification:

1. The effect of the added SO₄ ions is still not accounted for. Could SO₄ ions in the added concentration affect the obtained fluorescence spectra and indices? Is the spiked solution comparable to the naturally-occurring high-Fe(II) concentration water in the deltaic aquifer? This needs to be addressed (either by referencing previous research or experimentally).

Thanks for this suggestion to clarify the potential effects of SO₄²⁻ addition via the FeSO₄(H₂O)₇ spike and compare the ion concentrations in the spiked solution to the natural conditions in the aquifer. We note that Poulin (2014) also prepared iron stock solutions with FeSO₄(H₂O)₇ and reported a significant quenching effect caused by the binding of Fe(II). The stock solution was taken from the deep layer of the aquifer, where we previously measured the ambient concentration of SO₄²⁻ was 71 mg/L (Jia, 2015). Therefore, as we increased Fe(II) from 1.3 to 306 mg/L (a factor of 240 increase), the SO₄²⁻ likely increased from approximately 71 to 595 mg/L (a factor of 8 increase). In our revised text below, we demonstrate that the Fe, SO₄²⁻ and Cl concentrations in the experimental spiked solution are similar to the natural conditions occurring in the aquifer. We therefore believe that the dominant effect we observed was the effect of addition of Fe(II).

Revised section 3.2 (new text in **bold**):

“An Fe spiking solution of 1000 mg/L Fe(II) was prepared with FeSO₄(H₂O)₇, **following Poulin et al. (2014)**, using the DOM stock solution so that spiking with Fe(II) would not

change the overall concentration of DOM. ... **Previous analyses of water from W3-14 via ICP-OES (Jia, 2015) indicated the SO_4^{2-} concentration was 71 mg/L and Cl^- concentration was 1670 mg/L. Therefore, as the Fe(II) concentration was increased by a factor of 240 (from 1.3 to 306 mg/L), the SO_4^{2-} concentration only increased by a factor of 8 (from approximately 71 to 595 mg/L). We therefore expect that the dominant effect observed through this addition experiment is the effect of increasing Fe(II), rather than the effect of increasing SO_4^{2-} and/or total anions. The anion and cation concentrations in the experimental spiked solution were similar to the natural conditions occurring in the aquifer. For example, for the depths with Fe^{2+} from 50–435 mg/L, the range in SO_4^{2-} was 13–600 mg/L and range in Cl^- was 50–9600 mg/L (Jia, 2015)."**

2. Since the water sample used in this work was artificially spiked with an Fe(II) salt, a comparison between the highest concentration sample and the naturally-occurring high-Fe(II) concentration water in the deltaic aquifer would significantly strengthen the paper.

Agreed. As mentioned in the response to point 1 above, we have added a comparison between the natural and spiked waters to the manuscript. The thesis of Jia (2015) presents a full geochemical characterization of the Kidd II aquifer, including EEM spectra of the natural water samples (thesis figure 4.36). We plan to submit a site characterization paper separately, and decided it would be best to first publish a technical note describing and validating our approach of using EEM spectra in waters with high Fe(II), which we can then reference in the site characterization paper.

3. In the description of the FI (lines 239-243) the authors clearly state that the value of the index indicates the source of the organic matter. The following paragraph describes a change in FI caused by the increase in Fe(II) concentrations. However, the addition of Fe(II) clearly did not change the source of the organic matter in the system. This can be confusing to the reader and thus should be clarified and properly explained.

Thanks, we have clarified this description.

Changed to (new text in **bold**):

"In the absence of fluorescence quenching by other dissolved constituents, high values of FI (approximately 1.80) indicate that DOM is derived from extracellular microbial activity, whereas low values of FI (approximately 1.20) suggest that DOM comes from terrestrial plant and soil organic matter (Cory & McKnight, 2005)."

4. The last section of the introduction (lines 79-92) is very long and contains a description that is more suitable for the 'Methodology' section. I recommend shortening this section significantly.

We have shortened this section; it is now three lines long instead of 13. We realized that the text is repetitive to points already in the methodology, section 3.3 (line 177-189), so we eliminated some text in the introduction and referenced section 3.3.

Revised text in introduction (new text in **bold**):

“We identified the degree of quenching by Fe(II) based on the excitation-emission matrix (EEMs) regions and peaks. In this study we fit EEM spectra to a previously derived 13-component PARAFAC model (Cory & McKnight, 2005); **see section 3.3 for further details.**”

5. In line 281 the authors state that the residuals of the PARAFAC model are plotted in the supporting information. However, I wasn't able to find a supporting information file in your submission which makes the evaluation of this part difficult (I did find the online datasets, but these contain only .txt files and no plotted data).

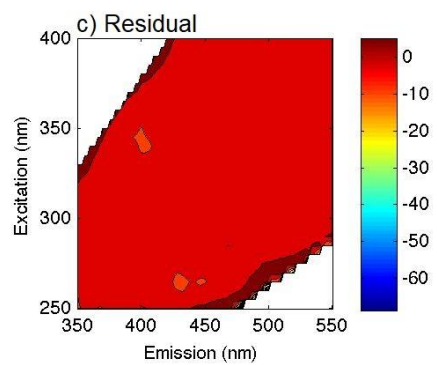
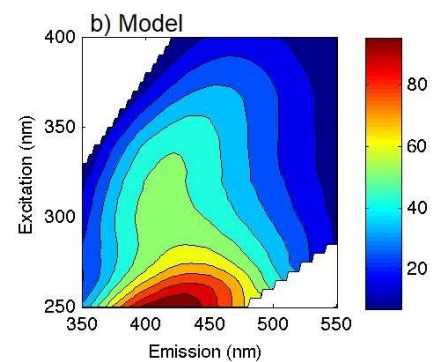
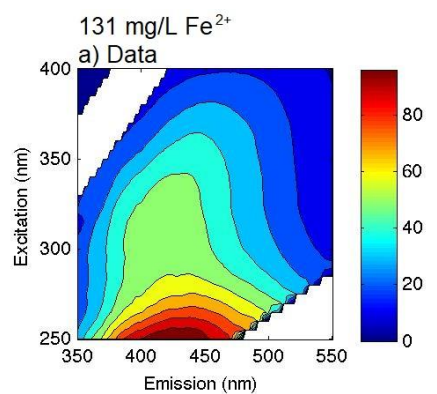
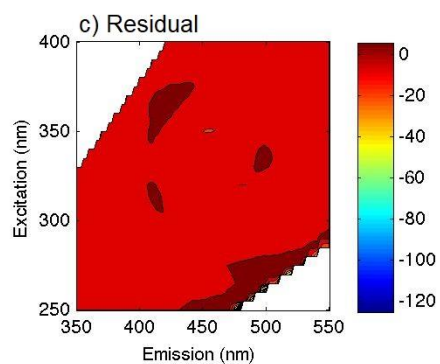
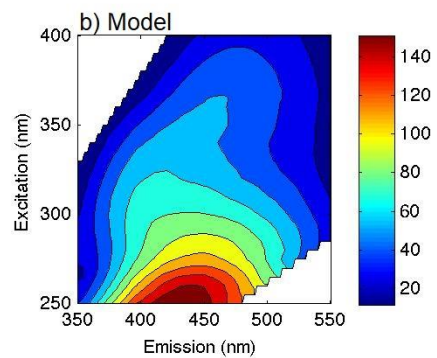
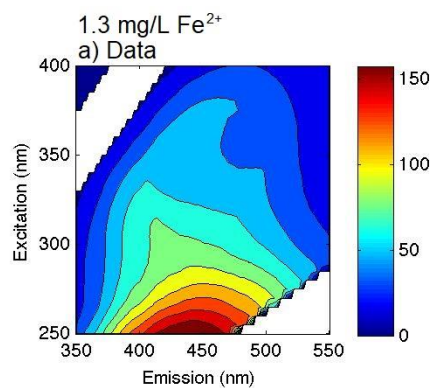
We apologize for the confusion caused by our inaccurate statement that the residuals of the PARAFAC model are plotted in the supporting information. We had considered adding these plots but ultimately decided against it. We have removed this statement from the revised manuscript.

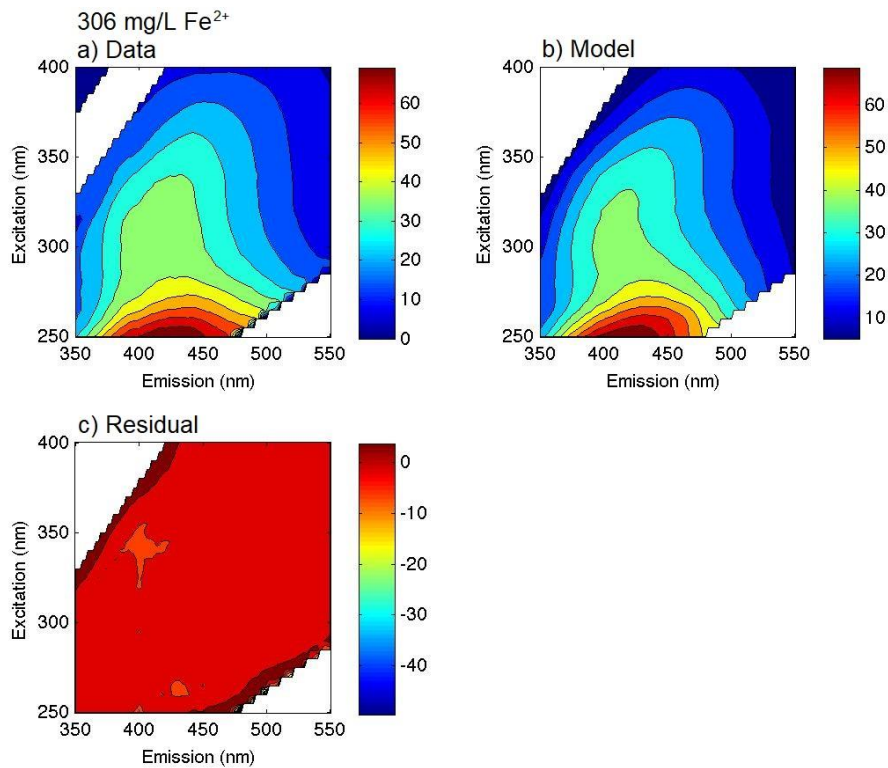
To provide you with confidence in our approach of applying the Cory & McKnight (2005) PARAFAC model and to demonstrate that the residuals were negligible, we have provided plots of the data, model and residuals below for three of the samples (1, 131, and 306 mg/L Fe²⁺). The values in the colorbar of each figure represent the fluorescence intensity in Raman Units for a given excitation and emission wavelength.

These figures are consistent with our statements on line 181 of the revised manuscript: “To ensure that this 13-component model adequately represented the fluorescent organic matter characteristics within the sample set, the residual fluorescence remaining after the model was applied were plotted and analysed. No systematic residuals were found after fitting the EEMs to the PARAFAC model, suggesting that the model was able to represent the samples, and that Fe(II) additions did not significantly change the structure of fluorophores in the groundwater stock solution from the Kidd 2 site.”

We decided against including these figures in a supporting information file because they were a different visualization of the data than the Figure 2 in the main manuscript and we thought that it may cause confusion to show two different visualizations of the EEMs. We generated these figures in 2013 and did not archive the output values for the model and residuals. We found that it was too difficult to exactly reproduce all the steps to generate the residuals and re-plot the data 7 years after the original analysis was performed, and therefore we decided against including plots of the residuals in the supporting information.

We believe that the figures below will convince you that no systematic residuals are present when fitting our sample EEM data to the Cory & McKnight (2005) model.





6. The description of the main experiment and the aquifer location in line 48 appears again in the context of the objective (lines 71-76). This is an unnecessary repetition. I recommend omitting lines 48-50. The text in lines 46-48 can be joint to the following paragraph.

We agree that lines 48-50 and 71-76 are repetitive. As suggested, we removed lines 48-50 (“In this study, an Fe(II) addition experiment was performed to assess the quenching effect of Fe(II) in groundwater samples from a deltaic aquifer in Richmond, British Columbia, Canada, where natural Fe(II) concentrations reach over 300 mg/L (5.4 mM).”) and combined lines 46-48 into the following paragraph.

7. The ‘Conclusions’ section currently fits the description of a summary.

Changed section title from “Conclusions” to “Summary”.

8. I find the separation between the results and the discussion unnecessary. However, I leave that to the authors’ consideration.

We decided to maintain the separation between results and discussion. The results section is more structured (containing 4 subsections, each on a different) whereas the discussion goes through the results holistically in a single section

Technical comments:

1. Line 37: consider omitting the word 'Additionally'.

Agreed, omitted.

2. Section 3.3: this section described data analysis rather than sample analysis. I recommend moving the first two lines (148-149) to the previous paragraph that describes the specifics of the Fe(II) addition experiment and changing the title to 'Data analysis'.

Agreed, we have changed the section 3.3 title from "Sample analysis" to "Fluorescence data acquisition and analysis." Lines 148-149 which describe the Fe(II) concentration determination are moved to the previous section 3.2 which we have changed from "Fe(II) addition experiment" to "Fe(II) addition experiment and concentration determination."

3. Line 137: 'An Fe spiking...' instead of 'A Fe spiking...' (same as you correctly wrote in line 49).

Done.

4. Line 154: 'collected at a bandpass of 5 nm' instead of 'collected at a bandpass at 5 nm'.

Done.

5. Line 165: omit 'then'.

Omitted.

6. Line 174: change '=' to 'of'.

Done.

7. Section 4.1: the title of the section is awkwardly phrased. I recommend changing it to either 'The effect of Fe-quenching on EEM fluorescence' or at least 'Fe-quenching effect on EEM fluorescence' (same comment stands for section 4.2).

Agreed, we've changed the section 4.1 title to "The effect of Fe(II) quenching on EEM fluorescence" and changed the section 4.2 title to "The effect of Fe(II) quenching on PARAFAC modeling and component distribution"

8. Line 218: add references.

Agreed, changed as described below.

Original text: “Coble (1996; 1990) identified five primary peaks from a visual inspection of EEMs, including humic-like Peaks A, C, and M; and protein-like Peaks B and T. The peaks are believed to be linked to the organic matter properties, and have been used for fluorescence comparisons in numerous studies. Coble (1996; 1990) identified five primary peaks from a visual inspection of EEMs, including humic-like Peaks A, C, and M; and protein-like Peaks B and T.”

Revised text: “Many studies have characterized fluorescence properties of waters based on the primary peaks in EEM spectra, identified by visual inspection and/or multivariate data analysis (Chen et al., 2003; McKnight et al., 2001; Murphy et al., 2013; Shen et al., 2020; Stedmon et al., 2003; Stedmon & Bro, 2008). The positions of these peaks are believed to be linked to the organic matter properties. Coble (1996; 1990) identified five primary peaks from a visual inspection of EEMs, including humic-like Peaks A, C, and M; and protein-like Peaks B and T.”

9. Line 226: ‘Although a linear relationship...’ instead of ‘Although the linear relationship...’.

Changed.

10. Line 251 ‘they’ instead of ‘he’ (the cited paper has multiple authors).

Changed.

11. Line 269: ‘Similarly’ instead of ‘Similar’.

Changed from “Similar to HIX” to “Similar to trends for HIX” on line 269. Analogous correction performed on line 220.

12. Line 278: omit the word ‘yet’.

Omitted.

Thank you again for taking the time to provide very helpful feedback on our revised manuscript and to acknowledge the changes we made in response to the other reviewers.