

Interactive comment on “Ferrihydrite associated organic matter (OM) stimulates reduction by *Shewanella oneidensis* MR-1 and a complex microbial consortia” by Rebecca E. Cooper et al.

Anonymous Referee #2

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General Comments:

The manuscript is well written and the main points come across easily. In the introduction, the authors recognize a number of studies that analyze the influence of organic matter (OM) on Fe(III) reduction kinetics and Fe-mineral transformations. These studies have been carried out under abiotic conditions or with pure cultures of well-known Fe-reducers such as *Geobacter* and *Shewanella*. This study focuses on (1) defining how a natural microbial consortium influences the reduction of Fe(III)-OM complexes and (2) determining microbial community changes under anaerobic Fe-reducing conditions. These findings will significantly impact our understanding of Fe-OM interactions

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under environmentally relevant conditions.

Specific Comments:

Introduction Line 33: should be “The majority of which is dispersed..” Line 44: should be “The coprecipitation of OM with Fe results in. . .” Line 49-51: This sentence is confusing. It seems like you are trying to make two separate arguments (1) coprecipitated ferrihydrite has different properties than pure ferrihydrite, and (2) coprecipitated ferrihydrite has different reactivity compared to ferrihydrite with sorbed OC. Maybe split into two sentences? Line 74-93: This paragraph has important information that you reply on in your discussion. Lines 94-97 clearly state the overall findings, but I found it a bit difficult to follow lines 74-93. Line 101: should be “is the dominant electron accepting process”

Methods Line 110-112: This first sentence is confusing. Line 117: what is Corg? Line 129: This first sentence seems like it should be last sentence of the previous paragraph. Line 130-133: These sentences are out of place. I don't think you need to talk about DNA extraction or PCR here. Maybe move them to the “DNA extractions” and “Quantitative PCR” sections. Line 146: Move this up so that the reader knows that you performed the forest floor extract solutions. Line 149-156: You may want to add this paragraph to a section called “Preparation of pure cultures”. Line 156: I'm not sure why you write about “Defined freshwater medium” here if you also have a much more detailed description in lines 162-167. Line 176: If you are not showing any data (not even in the supplemental information), then why mention that you did this? 179: Did you also collect XRD spectra of the starting materials? Line 199: move this sentence to the end so that it follows the order in which things were done. It will also make it easier for the reader to understand that you did the PCR and prepared the libraries yourself and just did the sequencing at the LGC Genomic GmbH. Line 224: Did you normalize the data using qiime or any other method? If you did, you should mention this because it is important when comparing the abundance of different taxa across various samples.

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Results The order of these sections has a great flow. However, I would consider merging the Results and Discussion sections into one section. This will avoid many repetitive statements. Section 3.1: The reduction rates stated in this paragraph are not the same as the ones in Table 1. Section 3.3: When reading this section and flipping to the figures, I found it difficult to determine if I was looking at the correct figure. Please double check the Figure numbers that you refer to in the text. For example, in line 285 you mention beta diversity and Figure 5b and I think you meant to reference Figure 6b.

Discussion Line 373: Add reference. Line 394: “:” should be “.” Line 404: Add reference. Line 405: Add reference. Line 435: Have you considered removing singletons and see what the data looks like? Section 4.3: Include mineral formulas and consider citing the following paper: Influence of Coprecipitated Organic Matter on Fe²⁺(aq)-Catalyzed Transformation of Ferrihydrite: Implications for Carbon Dynamics Chunmei Chen, Ravi Kukkadapu, and Donald L. Sparks Environmental Science & Technology 2015 49 (18), 10927-10936 DOI: 10.1021/acs.est.5b02448 Line 480: Where is the evidence of this finding? Maybe consider placing this information in the section about mineral composition. Line 500-503: This last sentence is not clear.

References Consider citing more recent studies such as: Influence of Coprecipitated Organic Matter on Fe²⁺(aq)-Catalyzed Transformation of Ferrihydrite: Implications for Carbon Dynamics Chunmei Chen, Ravi Kukkadapu, and Donald L. Sparks Environmental Science & Technology 2015 49 (18), 10927-10936 DOI: 10.1021/acs.est.5b02448

Dinesh Adhikari, Qian Zhao, Kamol Das, Jacqueline Mejia, Rixiang Huang, Xilong Wang, Simon R. Poulson, Yuanzhi Tang, Eric E. Roden, Yu Yang, Dynamics of ferrihydrite-bound organic carbon during microbial Fe reduction, *Geochimica et Cosmochimica Acta*, Volume 212, 2017, Pages 221-233, ISSN 0016-7037, <http://dx.doi.org/10.1016/j.gca.2017.06.017>. (<http://www.sciencedirect.com/science/article/pii/S0016703717303666>)

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Weinan Pan, Jinjun Kan, Shreeram Inamdar, Chunmei Chen, Donald Sparks, Dissimilatory microbial iron reduction release DOC (dissolved organic carbon) from carbon-ferrihydrite association, *Soil Biology and Biochemistry*, Volume 103, 2016, Pages 232-240, ISSN 0038-0717, <http://dx.doi.org/10.1016/j.soilbio.2016.08.026>. (<http://www.sciencedirect.com/science/article/pii/S0038071716302115>) Keywords: Carbon sequestration; DOC-Mineral stability; *Shewanella oneidensis* MR-1; Dissimilatory iron reduction; Anaerobic conditions

Figures Consider merging Figures 1 and 2 into a 4-panel Figure. This will allow the reader to compare the results obtained from pure cultures and natural inoculum.

Consider merging Figures 3 and 4 into a 4-panel Figure (make sure the y-axis is the same for all). This will allow the reader to compare the results obtained from pure cultures and natural inoculum. You may also want to consider changing T0, T middle and Tend to day 0, day X and day 288. This will keep the axis consistent with the other figures.

Figure 5C. The results illustrated in this Figure were not clear to me. Perhaps state the taxonomic level of the x-axis? Or add different shapes to differentiate between CFh vs Fh and AFh vs Fh?

Figure 6. I understand the importance of using various alpha diversity parameters. However, I am not sure illustrating them all in a main figure is necessary. Perhaps choose one parameters and talk about that one in the paper. The rest could go in the supplemental materials.

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