

**Reviewer 1, Comment 1 (RC1.1).** This is an interesting system in that it has overlapping S, N, Fe, and C cycles all in the anoxic portion of the water column. This is unusual, b/c in many systems at least one of these is functionally absent, a minor component, or undergoes minimal redox transformations because of the dominance of (an)other component(s).

**DP1.1:** The reviewer has kindly provided us with a relevant synthesis of the importance of our observations in this lake system, and it has been incorporated in the abstract and as introductory statement to a new section 4.7, titled “The unbalanced aqueous redox system in Lake Medard: synthesis”. This introductory paragraph reads:

“The newly formed Lake Medard has overlapping S, N, Fe, and C cycles occurring in the anoxic portion of the water column. This is unusual in natural redox stabilized meromictic lakes where at least one of these cycles is functionally diminished or undergoes minimal redox transformations because of the dominance of (an)other component(s). Such stable condition favour alternation of two bistable states that are driven by feedback reactions in turn determined by the organic carbon content of the system. Accordingly, ferruginous conditions shall occur in low productivity, organic poor systems; whilst sulfide-rich, methanogenic conditions dominate in high productivity systems (Antler et al., 2019; van de Velde et al., 2021). [...]”

**RC1.2.** The paper is mostly observational, but I think that’s fine, because the authors have developed a nice model for the processes occurring that’s shown in Figure 8. The paper presents a lot of results. I almost wish the authors could tie everything together a little more succinctly.

**DP1.2.** We have streamlined few sections of the manuscript while keeping our aim, which is to provide a complete account of current biogeochemical conditions in a transitional redox system. We have in all possible instances shortened and synthesized our results. But, please note that we had to include the Methods, as per reviewer 3 suggestion, in the main text. This adds to the overall length of the revised MS.

**RC1.3.** I think Figure 8 gives a nice summary of the processes involved. Maybe a little more time in the discussion focused on this model and a little less on the “paleo” implications would help the reader synthesize all of the observations into the process model.

**DP1.3.** As per reviewer suggestion we now dedicated a section (4.7, see DP1. Above) to better explain our biogeochemical model, also providing a synthesized view of the system based on our observations and interpretations. Reviewer 3, however, commented (RC3.25) on the “paleo” implications being quite enjoyable a section. So, we have chosen to keep it as it is (preprint version).

**RC1. 4.** Below, I provide some specific comments and suggestions that I hope can help improve the manuscript.

**DP1.4.** We sincerely thank the reviewer for her/his attention to detail while reviewing our draft/preprint; the comments and suggestion kindly provided were addressed as described below.

**RC1. 5.** Ln. 11. Do the authors mean “reductive Fe(III) dissolution”?

**DP1.5.** Yes, missing “I” is now added (Ln. 11)

**RC1. 6.** Ln. 16. “sustained” how?

**DP1.6.** Here we referred to a continuous genetic potential for anoxic sulfide oxidation. For clarity, the wording in the abstract has been modified as follow (Lns. 21-3): “[...] Yet, the planktonic microbial succession across the nitrogenous and ferruginous zones also indicates genetic potential for chemolithotrophic sulfur oxidation **in the anoxic portion of the bottom water column [...]**”.

**RC1. 7.** Ln. 17-18. What is the electron acceptor for sulfide oxidation? And if sulfide is oxidized all the way to sulfate, at which point does the sulfur disproportionation happen?

**DP1.7.** We considered that this information was adequately developed (within the length-limitations of the abstract section) in Lns. 19-21 (now 25-26): “Near and at the anoxic sediment-water interface, vigorous sulfur cycling, can be fuelled by ferric and manganic particulate matter and redeposited siderite stocks.” There are no further changes regarding this reviewer’s comment.

**RC1. 8.** Ln. 104. Change to “DNA extraction and MiSeq”

**DP1.8.** This has changed as per the Methods section now being part of the main text. Yet, please note that Ln. 120 now reads: “[...] environmental DNA (eDNA) extraction followed by MiSeq Illumina 16S rRNA gene amplicon sequencing [...]”

**RC1. 9.** Ln. 105. Do you mean ICM-MS? Does mass spec work for specific ions? I don’t think this is an adequate description of the analytical methods.

**DP1.9.** We thank the reviewer for noticing an omission/ and error in our description of analytical methods. Ion concentrations were determined using HP-LC. The following lines (Lns. 121-22) were added to address this flaw kindly noticed by the reviewer: “[...] (ii) mass determinations of cations (iron, manganese, potassium, sodium, magnesium and calcium); (iii) high pressure liquid chromatography for concentrations of chlorine, sulfate, nitrate, ammonium and phosphate anions, and VFA abundances; [...]”. Further details on how the measurements were carried out are in Sect. 3.1.5, subtitled “Ions, ammonia, and VFAs concentration analyses”.

**RC1. 10.** I’m also a little bit leery of putting the entire description of nucleic acid-based microbial community analysis in the supplement.

**DP1.10.** The methods, including the specific set of analyses indicated by the reviewer, are now part of the main text.

**RC1. 11.** Ln. 106. Change to “measurement of dissolved”

**DP1.11.** Done as per reviewer suggestion (Ln. 124).

**RC1. 12. Figure 2[b]:** Can the authors make the axes the same on panels above and below the redoxcline? My first impression was that there was no difference between any of the organic acids, but there are actually rather dramatic differences.

**DP1.12.** Fig. 2b has been modified as per reviewer suggestion to better highlight the change in VFAs concentration recorded above and below the redox aqueous interface.

**RC1. 13.** Ln. 150. VFAs were a minor fraction of the total DOC. What is likely the rest? How labile might it be, and how does that inform the biogeochemical model?

**DP1.13.** The main components supporting microbial growth are simple mono- and oligomers that are only present in nM concentrations. We determined VFAs concentrations as they act as electron donor and or C source to heterotrophic microorganism that could influence the observed hydrochemical processes. As per this request of the reviewer, the composition of the DOC pool is inferred as follow (Lns. 357-60): “[...] DOC is generally comprised of relatively high molecular weight organic compounds (not quantified here), such as cellular exudates from alive and senescent planktonic microorganisms (e.g., algae, protists, bacteria) and their degradation products. Probably present in solution were also soluble humic substances derived from the biological breakdown of refractory organic matter (i.e., lignite particles) in the sediment (Petrasch et al., 2018). [...]”

**RC1. 14.** Ln. 167-168. Wouldn’t sulfate reduction induce increase in pH? You’re producing carbonate alkalinity and reducing a strong acid (sulfate) to a weak acid (sulfide).

**DP1.14.** Usually it is the expected effect. But, MSR has been experimentally shown to decrease pH when lactate is the electron donor. Our annotation speculates on such an effect potentially occurring here as exhaustion of lactate is linked to the increased number of OTUs functionally associated with  $\text{SO}_4^{2-}$  reduction in the monimolimnion. The following line address

further the speculative assertion (Lns. 378-79): “Therefore, the complete (oxidation to CO<sub>2</sub>) and incomplete (to acetate) oxidation of lactate by MSR could be a factor contributing to the slight decrease in pH in the monimolimnion (see Gallagher et al., 2012). [...]”

Gallagher K.L. Kading T. Braissant O. Dupraz C. Visscher P.T.: Inside the alkalinity engine: The role of electron donors in the organomineralization potential of sulfate-reducing bacteria: *Geobiology* 10, 518–530, 2012.

**RC1. 15.** Ln. 221. Please be consistent in including the charge for nitrate

**DP1.15.** Charge of nitrate is now constantly stated in all instances where it appears on the text.

**RC1. 16.** Ln. 223. Please change “sequenced” to “detected”

**DP1.16.** Done.

**RC1. 17.** Ln. 232. By “abundance peak” do you mean maximal relative abundance?

**DP1.17.** Yes, as in revised Ln. 456: “The maximal relative abundance of an *Azospira*-like microorganism (95 % similarity) coincides with the peak of relative maximal abundance of members of the Gallionellaceae family at 49 to 50 m depth (Fig. 5a, Supplement 1). [...]”

**RC1. 18.** Ln. 210-256. Did the authors try to quantify nitrite? If there are nitrogen transformations occurring in this system, I would expect it to be important, and perhaps the ultimate oxidant for Fe<sup>2+</sup> in reactions 1 and 2.

**DP1.17.** The reviewer is right, nitrite could be expected to increase concentrations towards the anoxic part of the water column and exert an important control in reactions leading to Fe oxidation. Nitrite role, however, remains to be further tested experimentally or by specialized sampling/analytical protocols that can resolve reactive N availability and transformations occurring in the natural lab under examination. In our case, increasing Cl<sup>-</sup> concentrations with increasing depth hindered an accurate profiling of nitrite.

The revised text now informs the reader what anions were considered (please see DP1.9). Also, the following text was added as preamble to presenting reactions 1 to 3: “These cycles in the aqueous system under consideration are likely interlinked throughout microbial mediation in the generalized Reactions (1-3), but nitrite may as well be a relevant oxidant : [...]”

**RC1. 19.** Ln. 282. Are the authors referring to Fe(II) oxidation by Mn(III/IV)? Please clarify.

**DP1.19.** Yes. As per reaction 4, iron is oxidized and Mn is reduced. It can also be seen as Fe(II) as reductant of Mn(III,IV). To clarify on this note, we modified the text to read “[...] Divalent iron [...]” (Ln. 497).

**RC1. 20.** Ln. 293. Please change “[Fe]” to “Fe concentration”; also here and throughout, please check tense agreement.

**DP1.20.** Changed as per reviewer suggestions. Sentence tense agreement also revised thoroughly though the text.

**RC1. 21.** Ln. 301. I don’t know about this. Attributing metabolism when you only have 91% similarity is tough.

**DP1.21.** True. The offending sentence is now removed.

**RC1. 22.** Ln. 329. Please remove “significantly”

**DP1.22.** Done.

**RC1. 23.** Ln. 336 and throughout this section. Why does diversity matter. Wouldn’t relative abundance be more informative with respect to S transformations? There could be a whole lot of diversity of sulfate reducers, but they’re only a minor fraction of the community.

**DP1.23.** We have clarified what we referred here as diversity as follow (Ln. 555): “[...] low number of taxonomic groups, and Ln. 576: “[...] a more diverse sulfur-respiring bacterial

population (Fig. 5c). This was dominated by many relatively rare taxa and a few abundant lineages [...]"

The issues of considering relative abundance alone as a control of relevant hydrochemical transformations include potential biases induced by sampling processing. The information provided can therefore only inform us on changes in conditions that allow, for instance rare taxa to better thrive, or additional MSRs to be detected with the protocols implemented. We have added the following text to add additional context to this matter (Ln. 59-61): "Despite limitations linked to quantitative biases introduced, for example, during sample DNA extraction, PCR amplification, and uneven coverage of universal primers across phylogenetic groups, the sequencing of amplified fragments of prokaryote rRNA genes can provide insights useful for ecological deductions (see Piwosz et al., 2020) [...]"

Piwosz, K., Shabarova, T., Pernthaler, J., Posch, T., Šimek, K., Porcal, P., and Salcher, M. M.: Bacterial and Eukaryotic Small-Subunit Amplicon Data Do Not Provide a Quantitative Picture of Microbial Communities, but They Are Reliable in the Context of Ecological Interpretations, 5, 2020.

**RC1. 24.** A later use of the term "diversity" leads me to believe the authors are referring to diversity of S metabolisms (e.g., oxidation, disproportionation, reduction of different redox states, etc.), but I'm not sure. Please revisit the use of this term and clarify.

**DP1.24.** The use of the term has been revisited and clarified as indicated above.

**RC1. 25. Ln. 372-374:** If there's evidence of S metabolizing organisms and some aqueous chemical evidence of S transformations, why no change in  $\delta^{34}\text{S}$ -sulfate?

**DP1.25.** This question of the reviewer is answered in Lns. 615-19 of the revised text: "The intracellular isotope exchange of sulfite with anoxic ambient waters has been proven to produce an oxidized  $\text{SO}_4^{2-}$  product that is enriched in  $^{18}\text{O}$  relative to precursory thiosulfate and/or sulfite. This enrichment displays only a minor change, if any, in its corresponding S isotope composition (e.g., Böttcher et al., 2005; Johnston et al., 2014; Bertran et al., 2020; see Table 1)." (Lns. 398-401 of the preprint). But please note that the following lines were added as a closing statement of the section 4.4.1 (Lns. 619-24) to account for an increase observed at depth 48: "In line with this assertion, at the monimolimnion we observed in dissolved sulfate a negligible sulfur isotope fractionation accompanying the recorded fractionation of oxygen isotope. Yet, we registered a small, but significant reverse sulfur isotope effect (+2.2 ‰) at the upper hypolimnion (Fig. 6a: 48 m depth). This isotope effect could be ascribed either to abiotic or biotic oxidation processes of intermediate S species occurring at that level of the water column (see Zerkle et al., 2016, their table 1). [...]"

**RC1. 26.** Reactions 4-6. Is Mn-dependent S disproportionation from the Böttcher et al. 2001 paper? What about the siderite-dependent disproportionation? I am unsure how this reaction might occur.

**DP1.26.** Yes.  $\text{FeCO}_3$ : i.e., dissolution of siderite by excess  $\text{H}^+$  increases the availability of Fe(II) that scavenges by-product  $\text{H}_2\text{S}$  to sustain disproportioning bacterial growth (Thamdrup et al., 1993). The missing relevant references describing these disproportionation mechanisms were added (Ln. 613) to provide further context (i.e., half reactions).

**RC1. 27.** Ln. 520, 534, 559. Why are these minerals italicized?

**DP1.27.** These are now subsections comprising section 4.6.

**DP1.28 [Final Statement, acknowledge]:** We sincerely thank the reviewer for a thorough revision of our preprint that has contributed to its improvement aimed at final publication.