Reviewer 2

Reviewer 2, Comment 1(RC2.1). [Article]: is well-written and summarizes all principal aspect of the pit lake, as well as the importance of the study and how in general was conducted. There were few specific comments that I would the authors to take into consideration.

DP2.1. We sincerely thank the reviewer for her valuable time, the many suggestions generously given that—together with her constructive criticism—have significantly improved the MS for final publication.

RC2.2. [Methods section]: is nicely organized in subsections as supplementary information (S1), but there were specific details that would enhance the reproducibility of the applied methods.

DP2.2. Missing details that were missing are now added. For example, on sub-sampling tasks; HP-LC, etc. Also, please note that the methods section is now part of the main text as per the R3 request: RC3.11.

RC2.3. [Results and Discussion]: the authors did a good job discussing all the results and its significance. This section requires more work specially with respect to enhancing the clarity of the figures and their description in the text.

DP2.3. The figures have been further processed for clarity, for example we added in all profiles the hypo and monimolimnion, etc, we improve colour palettes used for genomics figure, revised captions, etc.

RC2.4a. It was not clear to me when was the lake flooded, 2005 according to figure 1a? If so, please state this in line 43 where it is written: "This newly formed,…". Do you have supporting info of when did the lake become meromictic, or how long has it been meromictic?

DP2.4a. Information and a reference to relevant published work that fill this gap kindly highlighted by the reviewer, have been added (Lns. 88-9): "[…] The filing of the former mine open-cast mine with river water started in 2010 and was reportedly completed in 2016 (Kovar et al., 2016). […]"

Also, Lns. 103-04): "[…] Water column stratification has been observed since 2009, when environmental monitoring of the hydrological system begun (e.g., Medová et al., 2015) […]"

Medová, H., Přikryl, I., Zapomnělová, E., and Pechar, L.: Effect of Postmining Waters on Cyanobacterial Photosynthesis, Water Environment Research 87, 180–190, 2015.

RC2. 3b. Do the meromictic conditions of the lake vary seasonally? Please present supporting information about this too.

DP2.4b. Yes. To address the reviewer request we revised the MS, Lns. 350-53, to now read: "Short-lived changes in redox potential of about 150 mV in the bottom water column were recently considered by Umbría-Salinas et al. (2021). These changes have effects on water column speciation (Fig. B1, Appendix B), and affect the partitioning of several redox sensitive metals that bind to reactive iron phases in the upper sediments (Umbría-Salinas et al. 2021, for details)."

Also, the revised Fig. B1 now shows the Eh-pH variability that we evaluated in the HAZMAT paper.

RC2. 5. Line 95: the authors talk about the present oligotrophic stratified conditions of the lake as a topic sentence. First, I would like to see physico-chemical profiles at this point to ease the understanding of such conditions for the reader. I also would like the authors to describe in more detail such conditions in all stratified layers. Finally, the last sentence of the paragraph, starting in line 100, deserved more written description too.

DP2.5. A call to Fig 2a, describing details on stratification of the bottom water column, is now added to the lines indicated by the reviewer (now Ln. 96-97). The reference to the oligotrophic state was deleted for being extemporary at this point, as kindly noticed by the reviewer.

Additional description on the Pourbaix diagrams in Fig. B1 was added (Ln. 108-09).: "[…]. The stability diagrams show that under the current aqueous physicochemical conditions, colloidal Fe oxyhydroxides form in the bottom anoxic waters (Fig. B1)."

RC2. 6a. Methods In line 13 [S1], when the authors refer to ~11 mL water samples, is 11 mL the aliquot amount referred to in line 11?

DP2.6a. The description provided is clear on this sampling step (now Lns. 188-121): Aliquots of the lake water collected were immediately transferred from the sampler to pre-cleaned (i.e., rinsed with ddH_2O and oven-burned 550 ºC), 12 mL exetainer septum capped vials (Labco) pre-filled with He(g) and 1mL NaCl oversaturated solution (40%) for CH_4, or 1 mL 85% phosphoric acid for ΣCO_2. On board, the vials were filled with ~11 mL water samples […]". No other changes were implemented regarding this query, but please note that Methods is now moved to the main text, its extended section 3.

RC2. 6b. In addition, how many samples were collected? Were they collected along depth? where exactly?

DP2.6b. Lns. 115-19 now clarify on this important matter: "[…] Lake Medard (from 47 to 55 m depth) in its central location (Fig. 1a, star). The probing resolution was 1 m above and below the O_2 minimum zone and 0.5 m at the redoxcline. Water column samples (n = 8 and 4 replicates) were collected in November 2019 using a Ruttner sampler with a capacity of 1.7 L. Flushing/rinsing of the sampling device with distilled water (dH_2O) was performed between samples. A total of eighth samples were taken at 47, 48, 48.5, 49, 50, 52, 54 and 55 m depth. Replicate samples were taken at 47, 48.5, 50 and 54 m depth."

Also, Ln 135: "[…] A total of 11 water samples (i.e., 47 to 54 m depth and replicates), each consisting of 1 L water, […]"

RC2.6c. In section SM1.1.3, please clarify the number of samples taken. The same applies for SM1.1.4 and include information about samples from which depths (or layers) were considered for the cation and ion concentration analyses.

DP2.6c. Please refer to DP2.6b, above.

RC2.6d. In section1.1.5, please clarify the following questions: were the 11 water samples (line 29), the same as described in section SM1.1.3? If so? why eleven? does this number include biological replicates? are these only from two depths? it is important to clarify, where these samples were taken along depth. More details of the PCR and sequencing protocol would benefit future researchers and reproducibility of the methods.

DP2.6d. Please refer to DP2.6b.

RC2.6d. Were the mineralogical (SM1.2.2), isotopic (SM1.2.3), and SEM (SM1.2.4) analyses applied to all sliced sediments?

DP2.6e. Yes. The intro paragraph to section 3.2 (Methods applied to solid phases) reads:

"3.2 Sediment samples

We also sampled the upper anoxic sediment column to a depth of ~8 cm. The mineralogy of these sediments was qualitatively and semi-quantitatively assessed via X-ray diffraction (XRD). The $\delta^{34}S$ and $\delta^{18}O$ of gypsum ($CaSO_4 \cdot 2H_2O$), $\delta^{13}C$ of siderite ($FeCO_3$), and $\delta^{34}S$ isotope values of pyrite (FeS_2) from these sediments were also measured. Scanning electron microscopy aided by electron dispersive spectrometry (SEM-EDS) was used for textural analyses focused on the S- and/or Fe-bearing phases. In addition, a sequential extraction scheme (after Poulton et al., 2004; Goldberg et al., 2012) was conducted to characterize the sedimentary partitioning of reactive Fe and Mn fractions. Further details follow […]"

RC2.7 Results and Discussion, Subsection 4.1: I am little confused about what is shown in Figure 2a. What is happening above 48 m? To what depth are you referring to? depth of the lake water, or depth of the whole lake? Based on what is presented in Figure 2a, I interpret

that the depth of the water column is only ~10 m? I am sorry if it is not that obvious to me, but it might be worth to clarify.

DP2.7. To address the lack of clarity kindly pointed out by the reviewer, the caption has been also modified as follow: "[…] Lake Medard in its central sampling location, which has a maximum depth of 56 m (a) […].". Also, all figures portraying a depth profile now read, in their vertical axis, "Water column depth"

RC2.8 Ln 121: the authors refer to several previous profiles of the lake. Do you have previous profiles? Are they published somewhere? or included in the supp info?

DP2.8. Yes. Ln. 311, reference added: "[…] Petrash et al. (2018)".

RC2.9 Ln. 135: "The hydrochemically different monimolimnion persists in the deepest depressions of the lakebed throughout the year; although with slight variations in the monitored Eh range that could be accompanied by minor (±1 m) shifts in the vertical position of the redoxcline." Can you show profiles of this on the supp info?

DP2.9. Short-lived Eh-Ph variations are now shown in Fig. B1 as per this request of the reviewer. Please note that a publication dedicated to evaluating this observation and their implications is given as reference: Umbria-Salinas et al. (2021).

RC2.10 [caption of figure 2], authors refer to dysoxic (n=4) and anoxic (n=3): at which depth were these samples collected, respectively?

DP2.10. Caption to Fig 2b was modified as follow: "[…] quantified in the dysoxic (n= 4, 48 m depth) and anoxic (n= 3; 54 depth) waters of Lake Medard […]"

RC2.11: Authors included a separating line referring to the redoxcline in Fig2b. Does this mean that the upper part of Fig 2b corresponds to the myxo-hypolimnion and the lower part to the monimolimnion? If so, please clarify it in the figure too. In addition, what are the red crosses? Could you also include an explanation in the caption?

DP2.11. Fig. 2b has been re-produced for clarity as per this important request of the reviewer.

RC2.12 [Section 4.2]: In line 150, the given DOC concentrations correspond to an average value of the 7 samples refer in figure 2b?

DP2.12. The average of all of the samples (and replicates) collected in the bottom waters. The text now reads (Ln 356): "The average of measured DOC concentrations in the bottom waters sampled is 1,050 ± 500 µM."

RC2.13. "A six- to ten-fold increase in concentrations of acetate, oxalate, and formate occurred towards the increasingly saline and O2-depleted bottom waters." This might be better to visualize in a profile. Could you please include one in the supp info?

DP2.13. No. A depth-profile is, unfortunately, not available for VFAs concentrations. This was the undesirable consequence of sample/replicate losses while fine-tunning/adapting the measuring conditions to the concentrations of multiple ions present, notably the increasing Cl^- concentrations towards the bottom.

RC2.14. In line 163, when authors referred to "[ΣCO2] were inversely correlated with the δ13C values", are they referring to figure 3d.

DP2.14. Referred to the profiles showed in Figs 3a-b (now clarified in Ln. 375). in

RC2.14. Paragraph starting in line 169 should have included a reference to Table 1 somewhere.

DP2.14. The edited section, now starting in Ln. 380, includes few references to Table 1, where appropriate.

RC2.15. About Figure 3d referred in line 189, I thought this figure was referring to the water samples. Please, clarify or correct accordingly.

DP2.14. The flux we referred in Fig. 3d and text is from the sediments to the water column. To clarify, Ln. 393 now reads: "[…] The range of estimated isotopic C values of the CO_2 flux from the sediments to the water column is between -3.0 and -4.2 ‰ (Fig. 3d). […]"

RC2.15 Section 4.3: Colours in Fig 5 must be changed. In 5a, there are two yellows, two greens, two light blues corresponding to different organisms, making it hard to interpret the figure and correlated with the written text. Fig 5b is even harder to differentiate colors and organisms.

DP2.15. Colour palettes used in the revised Fig. 5 were updated as per this relevant request of the reviewer.

RC2.16. While describing the microbial community, authors should be more quantitative (avoid low or high and refer to percentage). How much does "increases significantly" or "the abundance peak" mean? In addition, please be specific if what is shown in Fig 5. corresponds to normalized abundance in percentage with respect to the whole community or only among each microbial group shown separately in a b and c.

DP2.16. We did not implement quantitative PCR, in consequence the values are described for ecological interpretations only, e.g., *there is an increase in relative proportions {for a given OTU} from z1 to z2*. The value of amplicon reads do not constitute a definite measure of the *real* composition of the community, and limitations of 16S rDNA analyses has been discussed intensively in the microbial ecology literature, and we now cite a recent work that nicely clarifies the general current view on this matter (Ln. 59-61 at the introduction section): "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)."

RC2.17. In section 4.3.2, the subtitle refers to dissolved Mn and Fe, are they total concentrations? otherwise please be specific and in accordance with what is shown in Fig 4b: MnII and FeII.

DP2.17. The subtitle now reads (Ln. 493): "4.3.2 Dissolved divalent manganese and iron, and the iron-utilizing prokaryotes"

RC2.18. In addition, do you have concentrations of Mn(IV)? How do authors know Mn(IV) is settling down from the upper layer? Or Fe(II) is difussing upwards?

DP2.18. These conflicting assertions are, in our opinion, valid educated guesses based on the well-known geochemical behaviour of Mn and Fe in redox interfaces and across concentration gradients, such as depicted in Figure 4 and numerically described in Table 1 (please see classic references cited: Davidson 1993; Loveley and Phillips, 1988).

No particulate metal concentrations profiles were produced as part of this study.

RC2.19. In line 291, do authors have mineralogical evidence of this fact" "Dissolved phosphate is re-complexed back onto nanocrystalline and amorphous ferrihydrite-like phases precipitating at the redoxcline." The same comment for mackinawite mentioned in line 295.

DP2.19. Phosphate: We have presented (in Table 1) geochemical evidence for phosphate solubilization occurring in the monimolimnion (i.e., increasing dissolved phosphate concentrations towards the lakebed), with a decreased concentration trend towards the redoxcline that is, on the other hand, indicative of complexation of the oxyanion via biotic and/or abiotic amorphous iron oxides formation at the oxycline (this is portrayed in Fig. 8).

Mackinawite: this is the prevalent metastable precursor of pyrite, and it is rather difficult to quantify in most practical cases, particularly when such cases involve field sampling. However, to comply with the query by the reviewer—regarding inferred monosulfide precipitation, we have added the following text (Ln.554-55): "[…] circumstantial evidence for FeS precipitation, with another being δ^{56}Fe values that increased across the redoxcline and towards the SWI

(Petrash et al., 2022)". On this matter, further information was provided (Ln 851-54): "[…] results from an undergoing $\delta^{56}Fe$ systematics study (Petrash et al., 2022) firmly support co-recycling of Fe and S. An increase in the relative proportion of dissolved ^{56}Fe towards the lakebed ($\delta^{56}Fe$ = +0.12 ± 0.05 ‰;) is then ascribed to precipitation of monosulfides, whilst precipitation of oxyhydroxides at the redoxcline leads to depletion of the residual dissolved Fe(II) in heavy isotopes at the redoxcline ($\delta^{56}Fe$ = -1.77 ± 0.03) (e.g., Busigny et al., 2014).

RC2.20. In line 303, when referring to *Pseudomonas* spp., do authors have any control showing that *Pseudomonas* was not part of the extraction kits, or sampling material?

DP2.20. Yes, controls had not enough extractable DNA for PCR. However, should the flaw suggested by the reviewer be the case, we would not expect to have contamination by this spp. affecting only one, intermediate sample and its replicate, but not the neighbouring samples. *Pseudomonas* was also identified in Petrash et al. (2018) in other sampling location, but using different isolation kit. In consequence, we are confident in *Pseudomonas* spp. being a relevant player in the metal respiring community near the redoxcline.

RC2.21. In 310, include a reference for "anoxygenic phototrophic and nitrate reducing species (*Magnetospirillum* and *Ferrigenium*; Fig. 5b, Supplement 2)", and "*Azospira*-like species."

DP2.21. Done. Ln 533. Also please note that the Supplement referred is now 1.

RC2.22. In line 323, when referring to the peak of *Geobacter,* include where specifically and how much?

DP2.22. The text indicated by the reviewer has been modified as follow (now Ln. 543): "[…] within the monimolimnion, at about 54 m depth." Again (as per DP2.16, above), we have no qPCR data, and we used universal primers that are not specific for Geobacteracea. The universal primers, however, did amplify a relatively higher abundance of *Geobacter* that iincidentally coincides with the peak of iron reduction. Importantly, if of relevance for the reader, please note that the Krona chart in [now] Supplement 1, interactively produces the # reads for any of the bacteria or archaea present in each sample/ or replicate that we evaluated.

RC2.23. There are some names of organisms in Fig 5b that are not mentioned in the text. Should you better remove them from the figure and include them, as other less abundant taxa, or mentioned them in the text.

DP2.23. Non-mentioned organisms in Fig 5b are now removed from Fig. 5b, as per this relevant suggestion of the reviewer.

RC2.24. In line 345, in Fig 5c is only as *Thioalkalivibrio...*should you add the species name too as you did in the text?

DP2.24. "*[…] paradoxus"* added (Ln. 566).

RC2.25. In line 349, authors mentioned "The abundance of *S. hydrogenivorans* increases in parallel to a decrease in the *T. paradoxus*-like bacterium, which suggests that the latter may be at a disadvantage and limited by organic C fixation under the specific hydrochemical conditions prevailing at the redoxcline" With the current colors in Figure 5, it is difficult to see what you are showing in the text.

DP2.25. True. The palette was updated. Also, please note that numerical values are in the Krona chart provided as supplement.

RC2.26 Ln. 360: which bar corresponds to *D. acetoxidans* in Figure 5c. The same comment for *Desulfaticacillum* in line 365 and *Sulfitobacter* in line 366.

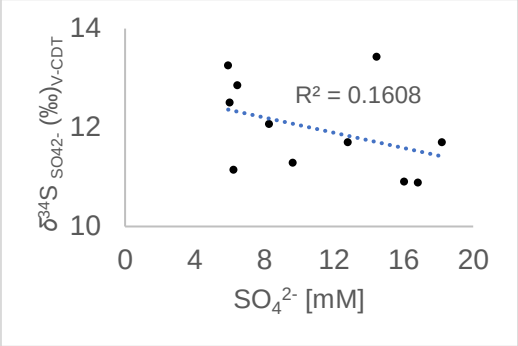
DP2.26. Please see DP2.25.

RC2.27. In general, with the current colors in Fig 5, it is difficult to agree with the conclusions stated by the authors in section 4.3

DP2.27. Please see DP2.25.

RC2.28 Section 4.4 Ln 374: do authors have values to support the "weak correlation"?

DP2.28. The supporting values are listed in Table 1. The R^2 (~0.16) from an attempt for linear correlation of such values (shown below only), is now provided in the text (Ln. 595).



RC2.29: A reference is needed for the following statement: "It is also within the range observed in studies of S disproportionation reactions generally proceeding under anoxic conditions" in line 383.

DP2.29. True. Relevant references now added (Ln. 604): "[…] observed in studies of S disproportionation reactions generally proceeding under anoxic conditions (e.g., Böttcher et al., 2001, 2005)"

RC2.30: Reference needed for the examples given in line 409.

DP2.30. There are no "examples" given in line 409 of the preprint, but stable isotope measurements conducted in the acidic drainage shown in Fig. 1. These are plotted as well in Fig. 6, which now with colours better allow distinguishing them.

RC2.31. Section 4.5: Be more quantitative with respect to sentences like in line 445: "…. increase slightly towards the bottom of our 8 cm depth core but their abundance, relative to total iron, decreases downwards" or 451: "a significant increase…"

DP2.31. Quantities are listed/detailed in Table 2, but we have deleted qualificatives such a 'significant' or 'slightly', while referencing changes listed by depth (cm) in Table 2.

RC2.32. Ln. 454: a Sect. 4.6.3 is referred but not found in the current version of the manuscript.

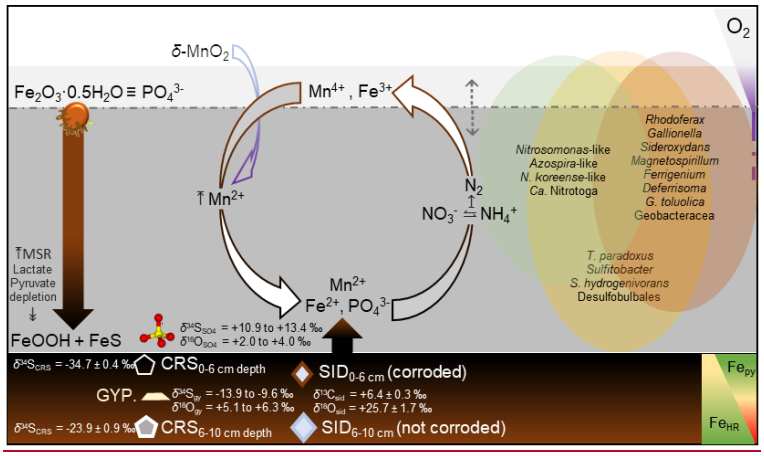
DP2.32. Section 4.6.1 is now referred (there was a typo), Ln. 425.

RC2.33. Section. 4.7, Ln. 595, name which "scarce examples" authors are referring, as well as in line 596: add reference and name which lakes

DP2.33. This request of the reviewer has been addressed as follow (now Lns. 873-76): "[…] the scarce examples of redox stratified marine basins existing today (i.e., Black Sea, Cariaco Basin; Lyons et al., 2009), nor in the few natural mesotrophic to eutrophic meromictic lakes that are presumedly analogues to redox stratified ancient oceans (see Koeksoy et al., 2015)."

RC2.34. About figure 8: Nice figure but there are some improvements to be done: 1) a legend is required to understand symbols and colors in the figure. 2) add a depth profile and names of each layer. 3) why is it necessary the venn diagrams for the microbes, what each color of the circles mean? Add the biogeochemical role of each microbial group included in the figure.

DP2.34a. The caption of the figure has been simplified, and to address further the chosen grouping representing fuctionalities we added further information. The caption now reads:



"Figure 8. Scheme summarizing the speciation and stable isotopes ranges of sulfur-bearing phases (pyrite, S^0, CRS; gypsum, GY) and siderite (SID) and the biogeochemical cycling mechanisms likely operating in the redox stratified Lake Medard and its SWI. (Background colours as in Fig. 2) The prokaryote groups depicted represent nitrate, iron and sulfur utilizing species identified via 16S gene amplicon sequencing (see text for details)."

Note also that, as suggested by the reviewer, additional information was added to the edited version of the figure.

DP2.36. The reviewer kindly pointed out also a list of typos, repeated text, etc. These minor issues indicated by the reviewer in the preprint, were all addressed. Also, sentence tense agreement also revised thoroughly through the text.