Reviewer 3 (Dr. Alexandra A. Phillips)

RC3.1 Cutting jargon: this paper is strongest when integrating results across their interdisciplinary dataset. However, the paper is in many places (namely the abstract, introduction, and final discussion section) unnecessarily complex and loses the non-expert reader. The authors should revisit these parts of the manuscript with an eye for unnecessary jargon - places they will lose interested geobiologists in a complicated explanation of geology, for example. In places where jargon is unavoidable, offering a few more definitions to the reader will help broaden the readership of this really interesting interdisciplinary study.

DP3.1. As per this relevant reviewer's suggestion, we have revisited and edited the text for the sake of simplicity, explained limnological-specific jargon, and simplified the text whenever possible. For example (Lns. 48-50): "[…] Natural lakes that display permanent stagnation and marked redox gradients in their water column are termed meromictic.[…]"

Also, Ln. 55: "[…] This newly formed, lacustrine system features low nutrient contents (i.e., it is oligotrophic), […]"

We also simplified our study site description, which now has no jargon related to the Miocene rift stage, fault mineralization, and paleolake development.

RC3.2 Improved figures: The figures should be reworked with a goal of consistency. Much of the results/discussion center around different zones of the lake's water column, but it is difficult to orient yourself across the many figures. Figure 2a panel 4 does a nice job showing the mixolimnion, hypolimnion, and monimolimnion - I think it would be helpful to see these zones in all the figures - either as the different shades of gray like in the Eh figure or with dashed lines and labels. A few of the figures also appear low resolution - namely, figure 5, 6, and 7. Other comments specific to each figure can be seen below.

DP3.2. We have carefully implemented this relevant suggestion to all the figures where a water column profile is shown. These now consistently display the redox stratification. All figures are 300 dpi, portable network graphics.

RC3.3. Separating results and discussion: It was difficult as a reader to follow the results and discussion section - I wanted to already be acquainted with much of the data before seeing it synthesized. I found myself jumping back and forth across the sections often. My suggestion to the authors is to split the results and discussion and focus the results to be a succinct section, with extraneous details moved to the SI.

DP3.3. We tried implementing this suggestion prior to submission, and again now—to comply with the reviewer suggestion, but our feeling is that it detrimentally affects the intended integration of microbial ecology and geochemistry, also the importance of certain geological (e.g., mineralogical) observations for geochemical/ ecological interpretations. Then, the MS ends being even more disseminated and sections largerly unbridged. Therefore, we prefer keeping the manuscript as a Results and Discussion-type report and did not fulfil this suggestion of the reviewer.

RC3.4. Some parts felt too long and should be more succinct, while other parts begged for more detailed discussion

DP3.4. We have shortened some sections (e.g., Sects. 2 and 4.2.2), while adding additional discussion of results, where presumed desirable (e.g., Lns. 915-20): "Our observation could make the case for niche differentiation linked to high loads of dissolved metal concentrations conferring a competitive advantage to these archaea under suboxic conditions (e.g., Gwak et al., 2019). Alternatively, the NH_3-oxidizing archaea sequenced predominantly in the suboxic waters possess a yet to be explored tolerance to anoxia (see Mußmann et al., 2011). For instance, *Ca.* Nitrosocaldus encodes a pyruvate:ferredoxin oxidoreductase that is rather uncommon among aerobic ammonia oxidizers (Daebeler et al., 2018), but which is used by most anaerobes to catalyze the decarboxylation of pyruvate to form acetyl-coenzyme A (Chabrière et. al., 1999)."

RC3.5. More methods: Currently, [in the main text] there are not enough details for someone to replicate any of the work or think critically about advantages or disadvantages for any method. Much of the SI methods section should be moved to the main text.

DP3.5. Methods are now moved to the main text as per reviewer's request. Also, missing information on HP-LC was added (see DP3.11f, below), and all additional editions suggested by the reviewer in RC3.11 were incorporated.

RC3.6. [Abstract]: I would recommend moving the last few lines on the importance of the study more broadly to earlier in the abstract, perhaps as the second sentence, and then expanding/clarifying the point in the current first sentence that this geochemical situation is an unusual but exciting case study

DP3.6. We implemented the abstract editions kindly offered by the reviewer.

RC3.7 [Introduction] Line 32: It would be helpful for those less familiar with limnology terms to define meromictic briefly, perhaps simply as "indefinitely stratified" or something similar

DP3.7. Done, please see DP3.1.

RC3.8 Line 33: "common sulfate deficiency" feels unnecessarily complicated, do you mean low in sulfate or absent of sulfate?

DP3.8. Yes. The revised text (Ln. 44) now reads: "but low sulfate concentrations"

RC3.9 Line 39: If possible, I think it would be helpful to add one more sentence about the importance/relevance to paleo-studies - the connection to me right now is a little weak so would be great to strengthen that point a little more - how exactly do they better inform Precambrian Ocean redox stratification models?

DP3.9. Done. Lns. 51-53 now reads: "[...]. They constitute natural labs that permit constraining master aqueous geochemical variables pertaining to iron mineralization and, thus, are relevant to disentangle key aspects on the deposition of ancient iron formations"

RC3.10 Line 47: Would be helpful to include a mention of the lake's pH as well (anywhere in this introduction)

DP3.10. Done, Lns. 54-56 now read, "[…] Ferruginous water columns that also contain elevated dissolved sulfate concentrations are not uncommon in acidic shallow pit lakes (e.g., Denimal et al., 2005; Trettin et al., 2007), and have also been reported from the pH neutralized post-mining Lake Medard in NW Czechia (Petrash et al., 2018; Fig. 1a)."

RC3.11a [Methods]: I think a majority of the SI details should be moved to the main text - because of this I also have line edit suggestions for the SI methods.

DP3.11a. Please see 'DP3.5' above. We thank the reviewer for this relevant suggestion.

RC3.11b. Water sampling: what depths were sampled? Did those change based on the physiological parameters prior to water sampling?

DP3.11b. Methods now clearly state the water depths sampled (Ln. 224-229): "[…] 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 eight 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."

RC3.11c. Line 11: were the exetainers cleaned prior to sampling?

DP3.11c. Yes, this is stated in Ln. 301-02 of the methods sections that now read: "[…] from the sampler to pre-cleaned (i.e., three-times rinsed with ddH_2O and oven-dried at 550 ºC), 12 mL exetainer septum capped vials (Labco), […]".

RC3.11d. Line 13: define PES

DP3.11d. PES acronym definition is now in Ln. 276 (its first appearance): "[…] using sterile high flow, 28 mm diameter, polyethersulfone (PES) filters to remove particles >0.45 µm […].

RC3.11e. Line 22: please define which anions and cations

DP3.11e. Defined as per this important suggestion kindly provided by the reviewer (Lns. 230-34): "[…] (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 in the bottom water column; […]".

RC3.11f. Line 70: more details are needed on the IC method used for the VFA analysis - what is the run time, column used, etc?

DP3.11f. More details added to Methods, Ln 288-299 now reads:

"3.1.5 Ions, ammonia, and VFAs concentration analyses

Ions and ammonia concentrations were measured in filtered, unacidified water sample aliquots using high pressure liquid chromatography (HP-LC) on a Dionex IC25 IC + Eluent Generator EG40 instrument at the Biology Centre of the Czech Academy of Sciences, Ceske Budejovice. We used a Dionex IonPac AS11-HC ion exchange column (2x250 mm) that permits resolving analytes in our complex sample matrices in a single run (45 min) by using separation of inorganic anions via a large-loop injection on a microbore (2 mm) isocratic pump. Ammonium ion detection/quantification was achieved via a fluorescence detector after the post-column derivatization. A combined stock calibration standard solution featuring environmentally relevant anions ratios was used for determining their concentrations and was prepared from corresponding analytical-reagent grade sodium salts. VFAs (Volatile fatty acids) were measured on the same instrument. To optimize and calibrate the method for VFA analyses, and determine the limits of detections, we used stock standard mixtures of IC grade formate, oxalate, acetate, lactate, pyruvate, and butyrate standards for preparing working saline stocks solutions. Detection limits were better than 60 ppb for lactate and oxalate, and 200 ppb for byturate, formate, and acetate."

RC3.11g. Line 77: How much 5M NaOH was added?

DP3.11g. Information missing was added (Ln. 329): "[…] then 50 mL of 5 M NaOH were added. […]"

RC3.11h. Line 87: should be "quantified gravimetrically" - also can you clarify what you mean here? Just weighed?

DP3.11h. Edited to "weighted" (Ln 340).

RC3.11i. Line 147: Sort of unusual to report 3 sigma, maybe just report 2 sigma as you did earlier to be consistent

DP3.11i. Typo deleted, it corresponded to preliminary results considered in an earlier draft.

RC3.12. Supplement figure 3: this appears very pixelated on my download; can you make sure the figure has high enough resolution?

DP3.12. A higher resolution printout of the PREEQC results for mineral's SI is now provided in Supplement 2.

RC3.13. Line 119: awkward to start the sentence with "Figure 2a"

DP3.13. Sentence corrected as per this request of the reviewer. The offending line now reads (Lns. 423-29): "Physicochemical parameters measured in the dysoxic to anoxic bottom waters at the time of sampling (November 2019) are shown in Fig. 2a […]"

RC3.14. Line 150: can you elaborate on the DOC concentrations? What does that tell you about the system - is it typical or unusual? A little more discussion would be great, especially because you mention in the abstract that SR may be limited by low amounts of metabolizable OC

DP3.14. Done, the section now states the following in its first paragraph:

"The average of measured DOC concentrations in the bottom waters sampled is 1,050 ± 500 µM. This range of values was higher than observed in the bottom waters of meromictic lakes such as or Matano (< 100 µM; Crowe et al., 2008), or Pavin (300 ± 100 µM; Viollier et al., 1995). 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 (Petrash et al., 2018). VFAs are linear short-chain aliphatic mono-carboxylate compounds produced during anaerobic degradation of the organic compounds referred above. They serve as C sources and electron donors for the planktonic microbial heterotrophy and were therefore quantified here. VFAs in the bottom waters […]"

RC3.15. Line 155: what is your hypothesis for the change in VFA concentration in the different layers of the lake? Can you relate this more explicitly to your 16S data at all?

DP3.15. We have related changes in VFA with the microbial ecology for example in Lns. 701-04:

"Although speculative, it is possible that microbial sulfate reduction (MSR) is responsible for the observed lactate depletion in the bottom waters. Therefore, the complete (oxidation to CO_2) 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). […]"

Also, Lns. 1075-79, i.e., with regard to *Desulfobulbus* more likely disproportionating S0, instead of thriving organotrophically: "Pyruvate, as lactate, was found below our detection limits across the bottom water column where sequences distantly related to *D. propionicus* (91 % similarity in 428bp) appeared to be particularly abundant (Fig. 5c; Supplement 1).

RC3.16. Line 159: I think a mention of pH should come much earlier, at least very early in results, if not hinted at in introduction - my initial assumption from hearing about a post-mining lake would be to expect really acidic conditions, so saying that the pH is closer to 7-8 earlier would be helpful.

DP3.16. As per reviewer suggestion, pH is mentioned in then introduction (DP3.10). Also, early in the results (Sect. 4.1, 1st paragraph, now Lns. 430-37 reads): "The pH in the hypolimnion was ~8.2 and decreased moderately downwards, reaching 7.4 ± 0.2 units near the anoxic SWI."

RC3.17 Line 163: Please put some of the d13C values in the text, such as an average or range

DP3..17 Done. Lns 702-03: […]. The $\delta^{13}C$ values are in the range +0.2 to -4.1, and were directly correlated with the dissolved sulfate concentrations [SO_4^{2-}] (Table 1), […]

RC3.18. This discussion on 4.2.2 on total DIC seems very lengthy compared to the other results sections and could be shortened for readability and to better emphasize the main points.

DP3.18. The discussion on total DIC has been now streamlined, shortened some 20 % (it begins in Ln 697).

RC3.19a. Line 171: Please change "d13C signatures" here and elsewhere to d13C values.

DP3.19a. Done. We now referred to values regarding this isotope system.

RC3.19b Line 189: Instead of “estimated isotopic C signature of the CO2” say either estimated C isotope composition or estimated d13C value

DP3.19b. Edited as per reviewer suggestions (Ln. 733).

RC3.20 Line 370: the title of 4.4.1 is awkward, maybe “Isotopic evidence”

DP3.20. The sub-section titles were edited as per reviewer’s suggestion. The specific title now reads: “4.4.1 A proxy for disproportionation” (Ln. 1118)

RC3.21. Line 373: I would suggest avoiding "heavier" and just stick with "enriched in 18O"

DP3.21. Done, Ln. 1121.

RC3.22 Line 375: a number itself can't be narrow, so maybe change to something like "the bottom waters had a narrow range of d18O values: X to Y"

DP3.22. Text edited as per reviewer suggestion, Ln. 1124: “The ambient bottom waters had a narrow $\delta^{18}O_{H2O}$ range of values: -6.1 to 6.7 ‰ […]”

RC3.23 Line 410: you say that for the initial sulfate composition it is reasonable to assume its similar to the nearby acidic drainage and the pit lake before flooding - the second seems more reasonable to me but you dont report those values in the text? What are those?

DP3.23. Those values were reported in the compilation made in Fig B2 (Appendix B).

RC3.24. Line 574: extra parenthesis dangling - sentence is also not grammatically correct, so should be fixed

DP3.24. Corrected Lns. 1484-85: “[…] could be ascribed to incomplete microbial sulfate reduction, with an additional open system oxidative sulfur cycling also being probably active”

RC3.25 Line 595: more references needed here, overall really enjoyed this section on the paleo implications!

DP3.25. The reference provided, Lyons et al., 2009, is a review. But the text now reads (Ln. 1638): “[…] existing today (i.e., Black Sea, Cariaco Basin; Lyons et al., 2009, and refences therein), […]”

RC3.26 Figure 1: I really enjoyed this figure! Especially nice to see the lake from 2005 to 2020. The figure caption has a call out to panels b and c, but not to panel a, so that should be added.

DP3.26. The figure’s caption is now corrected with regard to missing information.

RC3.27 Figure 2: In part b I think the main idea is to compare the VFA concentrations above and below the redoxcline - its currently hard to see that difference and the relative differences between other VFAs - it would be more clear to show these on all the same plot - so instead of 6 separate figures just one figure

DP3.27. The figure’s caption is now corrected as per DP3.26. The revised Fig. 2b now shows, in the background, the stratification defined by the Eh gradients.

RC3.28 Table 1: I’m a little concerned by the errors in the ammonium measurements - especially that surface sample, where the error is larger than the measured value - is there also a reason why phosphate doesn’t have concentration brackets - maybe just an error?

DP3.28. We thank the reviewer for her attention to details that allowed pointing out our mistake in data transcription to Table 1. Based on our replicates, errors in HC-LC data for ammonia, and in other anions simultaneously measured, are now correctly stated and do coincide to what was shown in Fig. 4a.

RC3.29 Figure 3: For panel A can you change it to mM so that the range is not to 12000 in the axis? Also needs more tic marks to see values in between, panel c also needs further tic marks for sulfate concentration. In the figure caption there is a CO2 that needs the 2 to be subscripted and "value" needs to be added after d13C.

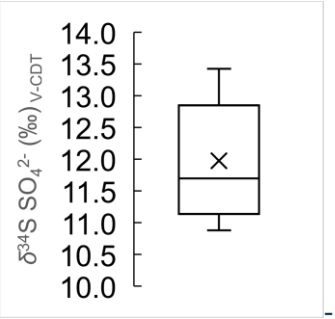
DP3.29. The figure edition requested in this query were implemented.

RC3.30 Figure 5: this figure is pretty hard to read with the colors as they are - I would think about the main point you are trying to make with this figure - instead of having the arrow for the redoxycline. I would make the edit I suggested earlier for all figures, having different shades of grey boxes in the background - its hard to compare the abundance of different microbes against each other because the scale changes across panels as well.

DP3.30. Figure 5 was modified as per reviewer's request. We conserved, however, the variable scales as they do directly convey information on the relative abundances of the functional groups that we considered.

RC3.31 Figure 6: there is one data point for d34S of sulfate that is much higher value - at ~13.5 permil? Is this an outlier?

DP3.31. Concerning such value, effectively it is an outlier (see boxplot below), the revised text (Ln. 1193-96) now reads: "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 accruing at this level of the water column (see Zerkle et al., 2016, their table 1)."



RC3.32. Should be mentioned in text - 6c would be a bit easier to see if the symbols were also colored if possible

DP3.32. Figure 6c now has coloured markers as per reviewer's suggestion.

RC3.33. I hope these comments were helpful and they assist in improving what is already a really interesting manuscript.

DP3.33 Final Statement, acknowledgement: We sincerely thank Dr. Phillips for suggestions, her time and dedication to this review, and for rising concerns that helped us improving the revised manuscript. Dr. Alexandra Phillips provided a clear and detailed review. We very much appreciate her attention to details, sound suggestions, and constructive criticism. Above we described how we addressed her comments, and in a single case where we disagreed (splitting results and discussion section, i.e., RC3.3), we explained why.