

Biogeosciences Discuss., referee comment RC3
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Comment on bg-2021-253

Alexandra Phillips (Referee)

Referee comment on "Aqueous system-level processes and prokaryote assemblages in the ferruginous and sulfate-rich bottom waters of a post-mining lake" by Daniel A. Petrash et al., *Biogeosciences Discuss.*, <https://doi.org/10.5194/bg-2021-253-RC3>, 2021

Petrash et al. presents a largely observational study of a post-mining, low nutrient lake with a unique geochemical setting that may be helpful as an analogue for nutrient cycling in the ancient ocean. They combine geological, biological, and chemical data for their investigation of both the anoxic water column and upper sediments.

Overall, the paper is well written and the results very interesting. I believe the paper should be accepted following a few larger edits that I think will improve the manuscript:

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.

2) Improved figures: The figures should be reworked with a goal of consistency across figures. Much of the results/discussion centers around different zones of the lake's water column, but it is difficult to orient yourself across the many figures. Figure 1a 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.

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. Some parts felt too long and should be more succinct, while other parts begged for more detailed discussion. 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. Then the discussion section can focus on bridging the many types of data together, perhaps starting with Figure 8 which is a nice schematic for the entire ecosystem. I think this will help the readability of the manuscript.

4) More methods: Currently, 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.

Section specific comments:

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

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
- Line 33: "common sulfate deficiency" feels unnecessarily complicated, do you mean low in sulfate or absent of sulfate?
- 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?
- Line 47: Would be helpful to include a mention of the lake's pH as well (anywhere in this introduction)

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:

- Water sampling: what depths were sampled? Did those change based on the physiological parameters prior to water sampling?
- Line 11: were the exetainers cleaned prior to sampling?
- Line 13: define PES
- Line 22: please define which anions and cations
- Line 70: more details are needed on the IC method used for the VFA analysis - what is the run time, column used, etc?
- Line 77: How much 5M NaOH was added?
- Line 87: should be "quantified gravimetrically" - also can you clarify what you mean here? Just weighed?
- Line 147: Sort of unusual to report 3 sigma, maybe just report 2 sigma as you did earlier to be consistent
- Line 159: there appears to be a typo with an extra (- maybe a missing reference?
- Supplement figure 3: this appears very pixelated on my download, can you make sure the figure has high enough resolution?

Results and Discussion:

- Line 119: awkward to start the sentence with "Figure 2a"
- 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
- 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?
- 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
- Line 163: Please put some of the d13C values in the text, such as an average or range
- 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
- Line 171: Please change "d13C signatures" here and elsewhere to d13C values
- Line 189: Instead of "estimated isotopic C signature of the CO2" say either estimated C isotope composition or estimated d13C value
- Line 370: the title of 4.4.1 is awkward, maybe "Isotopic evidence"
- Line 373: I would suggest avoiding "heavier" and just stick with "enriched in 18O"
- 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"
- 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?
- Line 574: extra parenthesis dangling - sentence is also not grammatically correct, so should be fixed

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

Figures/Tables

- 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.
- 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
- 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?
- 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.
- 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 redoxcline 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.
- Figure 6: there is one data point for d34S of sulfate that is much higher value - at ~13.5 permil? Is this an outlier? Should be mentioned in text - 6c would be a bit easier to see if the symbols were also colored if possible

I hope these comments were helpful and they assist in improving what is already a really interesting manuscript. This is my first time reviewing an article and I very much enjoyed it. Sincerely, Dr Alexandra A Phillips