

Biogeosciences Discuss., referee comment RC2  
<https://doi.org/10.5194/bg-2021-253-RC2>, 2021  
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## **Comment on bg-2021-253**

Anonymous Referee #2

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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-RC2>, 2021

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### **Overall quality of the preprint ("general comments")**

#### Introduction

It 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 considerations.

#### Methods

This section is nicely organized in subsections as supplementary information (S1), but there were specific details that would enhance the reproducibility of the applied methods.

## 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.

### **Individual scientific questions/issues ("specific comments")**

#### introduction

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

In 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.

Methods

From S1:

In line 13, when the authors refer to ~11 mL water samples, is 11 mL the aliquot amount referred to in line 11? In addition, how many samples were collected?, were they collected along depth? where exactly?

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.

In section 1.1.5, 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.

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

## 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.

In line 121, authors refer to several previous profiles of the lake. Do you have previous profiles? Are they published somewhere? or included in the supp info?

In line 135, authors wrote: "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 ( $\pm 1$  m) shifts in the vertical position of the redoxcline." Can you show profiles of this on the supp info?

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

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?

## Section 4.2

In line 150, the given DOC concentrations correspond to an average value of the 7 samples refer in figure 2b?

Authors said: "A six- to ten-fold increase in concentrations of acetate, oxalate, and formate occurred towards the increasingly saline and O<sub>2</sub>-depleted bottom waters." This might be better to visualize in a profile. Could you please include one in the supp info?

In line 163, when authors refered to "[ΣCO<sub>2</sub>] were inversely correlated with the δ<sup>13</sup>C values", are they referring to figure 3d.

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

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

### Section 4.3

Colors 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.

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.

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. 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?

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.

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?

In 310, include a reference for "anoxygenic phototrophic and nitrate

Reducing species (*Magnetospirillum* and *Ferrigenium*; Fig. 5b, Supplement 2), and *Azospira*-like species."

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

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.

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

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.

In line 360, which bar corresponds to *D. acetoxidans* in Figure 5c. The same comment for *Desulfaticacillum* in line 365 and *Sulfitobacter* in line 366.

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

Section 4.4

In line 374, do authors have values to support the "weak correlation"?

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.

Reference needed for the examples given in line 409.

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..."

In line 454, a Sect. 4.6.3 is referred but not found in the current version of the manuscript.

Section. 4.7

In line 595, name which "scarce examples" authors are referring, as well as in line 596: add reference and name which lakes

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 the each layer. 3) why is it necessary the venn diagrams for the microbes, what each color of the circles mean? Add the the biogeochemical role of each microbial group included in the figure.

### **Technical corrections: typing errors, etc**

Introduction

Figure 1. Please describe parts b and c in the caption of the figure.

Methods SM1

Line 126, correct format accordingly.

## Results and discussion

Line 119: change this sentence by a better topic sentence that summarizes the results presented in this paragraph instead of starting the sentence with Figure 2a shows....

In line 158, I think it is Figure 3a

In line 209, "with functional annotations on the planktonic prokaryote community" is duplicated.

In line 349, include % after 97.

In line 370,  $\delta^{18}\text{O}_{\text{SO}_4}$  is as  $\delta^{18}\text{O}_{\text{SO}_4}$ , please keep it one way or the other but be consistent.

In Fig. 6a-b include what CDT and V-SMOW mean.

When referring to reactions, authors use Rcs or Rs, use one way or the other but be consistent.

Transform the first sentence starting in line 403 into a topic sentence. Avoid starting a paragraph with Figure.. shows...., this is also the case in line 438.

In figure 7, use the same acronyms in the figure as referred in the text.

Line 516, replace Like for Lake.

Line 637, found duplicated: near the anoxic sediment-water interface near the anoxic sediment-water interface

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-253/bg-2021-253-RC2-supplement.pdf>