Biogeosciences Discussions

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Reply on RC1

Jiaying Abby Guo et al.

Author comment on "Investigating the effect of nickel concentration on phytoplankton growth to assess potential side-effects of ocean alkalinity enhancement" by Jiaying Abby Guo et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-312-AC1, 2022

Dear referees,

Thank you for your comments on my manuscript. We appreciate the time and effort that you have dedicated to providing your valuable feedback on our manuscript. Here are our point-by-point responses to these comments and concerns.

Comments from the reviewer 1

Comment: Guo and colleagues present an interesting and well-written manuscript about the lack of sensitivity of a wide array of phytoplankton species to changes in Ni. This work is timely given the recent intensification of interest in CDR and the threat of Ni toxicity when olivine and other minerals are added to enhance alkalinity. Overall, I believe that this manuscript is suitable for publication in its current state. I appreciate that the authors clearly articulate the uncertainties and ambiguities involved in extrapolating culture experiments with EDTA to open ocean conditions.

Response: We thank Reviewer 1 for their kind comments.

Comment: My criticisms at this stage might be filed as suggestions, but I hope the authors consider them, even if they are not required to do so. First, there are several instances, especially in the abstract, discussion, and conclusion, where qualitative terms (e.g. ”mildly” “not very pronounced”) are used to describe results without any kind of quantitative description or definition afterward. It would be helpful in most of these cases to describe the scale of the (lack of) effect: (e.g. < 10 % change), either as a parenthetical or in a following sentence. The authors explain how small differences in growth rate can quickly result in significant population shifts so clarifying what the authors perceive as mild/small/not very much will be important as future work expands on this research. Similarly, there is a trend in the results section to focus on p-values, without really describing the range of values measured.

Response: We agree with Reviewer 1 on the raised issues and have revised the manuscript to specifically describe the scale of the Ni effects and the range of values measured quantitatively.

Comment: Second, the authors should consider removing Section 4.1.3 or integrating it with other sections of the discussion. As is, the information here is not very relevant to the results and does not really investigate the presence or absence of Ni enzymes in the species investigated. This would seem to require evidence from genomic sequencing or database searches of the organisms used or close relatives. Certainly, the word "link" in the subsection title seems misapplied.

Response: We agree with the Reviewer's comment and we have integrated this paragraph into section 4.1.1 - Dependency of Ni sensitivity on nitrogen sources.

Comment: Lastly, and least consequentially, the aspect ratio of figure 2 seems to potentially overemphasize the lack of variation found in the data. It occurred to me that more square panels might be a more neutral presentation of the same results.

Response: Thank you – we agree. We have expanded the y-axis and narrowed the x-axis so that the response of each species is more obvious. We have also increased the font size of labels to make them easier to read.

Comment: Below are a few line notes that mostly reinforce the points above. I think the authors have done a very good job here, so there are the final fixes I'd recommend before this is published.

Line 23: "mildly" might be replaced by a quantitative statement based on percent change

Response: We have changed the manuscript following this recommendation.

Comment: Line 27 (and elsewhere): its important to clarify here that EDTA is "synthetic" to avoid confusion that EDTA experiments are direct analogs for natural organic ligands.

Response: Agreed. We have clarified this where necessary including in the abstract.

Comment: Line 50: what does "quality" mean in this context? Would "identity" or "composition" be more clear here?

Response: Yes, we agree. We now state "composition" instead of "quality".

Comment: 73: The mention of nitrogenase here seems abrupt. Perhaps another sentence of introduction is warranted here. Note also that Ni is an essential part of hydrogenase enzymes used by some N2-fixers., e.g Tuo et al. 2020 in L&O.

Response: We agree and have revised the manuscript accordingly. Specifically, adding sentences to explain the Ni's biological role: "For N_2-fixers, nitrogenase is a key enzyme for dinitrogen (N_2) fixation. Since nitrogenase can be inactivated by reactive oxygen species, such as O_2^-, Ni-SOD is indirectly involved in nitrogen fixation process in cyanobacteria. In addition, hydrogen gas (H_2) is generated as a by-product in the nitrogen fixation process, and Ni is an essential part of the hydrogenase enzymes regulating H_2 metabolism used by some N_2-fixers (Tuo, et al. 2020). So, Ni plays an important part in N_2 fixation. ".

Comment: 87: please provide more information about the Synechococcus sp. strain used. The Synechococcus phylogeny can be very confusing/misleading so details regarding strain and/or ecotype membership are essential if others seek to reproduce this work. I was not able to identify the strain based on web searches alone or on the ANACC culture collection webpage.

Response: *Synechococcus* sp. (CS-205) is in sub-cluster 5.2 and pigment type 1 (only

phycocyanin). We have added this information. This strain has been genetically characterized and the dataset is evaluated with a paper to be submitted soon but it is unfortunately not published yet. The information about CS-205 in GenBank is:

SUBID	BioProject	BioSample	**Accession**	Organism
SUB9829021	PRJNA736758	SAMN19663758	**JAHLZB000000000**	Synechococcus sp. CS-205

Comment: 90: MilliQ does not specify the grade of water. This should be 18.2 mega-ohm cm-1 grade water.

Response: We now state the grade of the water.

Comment: 157: it might be reassuring to comment on how the visual minteq calculations differ from the recommended Ni' vs. dNi values of Sunda et al. (2005) in the Algal Culturing Techniques text.

Response: Thanks for your suggestion. Sunda et al. (2005) does not provide a recommendation for nickel (Ni), as it is not typically added to Aquil seawater medium (see their Table 4.6). To quote Sunda et al. (2005) (relevant text in boldface): "Nickel is the only nutrient metal that reacts at slower rates than iron (Morel and Hering 1993), **but nickel is necessary only for cultures growing on urea as a nitrogen source** (because urease is a nickel enzyme); the problem of nickel buffering is thus rarely encountered (Price and Morel 1991)." The assumption made when making Aquil seawater medium is that the background nickel concentration is sufficient to meet the metabolic nickel demands of phytoplankton when they are not grown on urea.

TABLE 4.6 Composition of major and minor nutrient enrichments in metal-replete Aquil*. The metal nutrients and vitamins are prepared as separate stock solutions. The major nutrients may be prepared separately or mixed with the SOW salts.

Nutrient	*Final concentration (M)*	*Log [M']*
Major nutrients		
P—$NaH_2PO_4 \cdot H_2O$	1×10^{-5}	
N—$NaNO_3$	1×10^{-4}	
Si—$Na_2SiO_3 \cdot 9H_2O$	1×10^{-4}	
Metal/metalloid nutrients*		
Fe—$FeCl_3 \cdot 6H_2O$	1.00×10^{-6}	—
Zn—$ZnSO_4 \cdot 7H_2O$	7.97×10^{-8}	−10.93
Mn—$MnCl_2 \cdot 4H_2O$	1.21×10^{-7}	−8.03
Co—$CoCl_2 \cdot 6H_2O$	5.03×10^{-8}	−10.77
Cu—$CuSO_4 \cdot 5H_2O$	1.96×10^{-8}	−12.63
$Na_2MoO_4 \cdot 2H_2O$	1.00×10^{-7}	−7.00
Na_2SeO_3	1.00×10^{-8}	−8.00
Vitamins		
B_{12}	3.96×10^{-10}	
Biotin	2.50×10^{-9}	
Thiamine	2.96×10^{-7}	

Metal/metalloid nutrients are prepared with EDTA (see text).

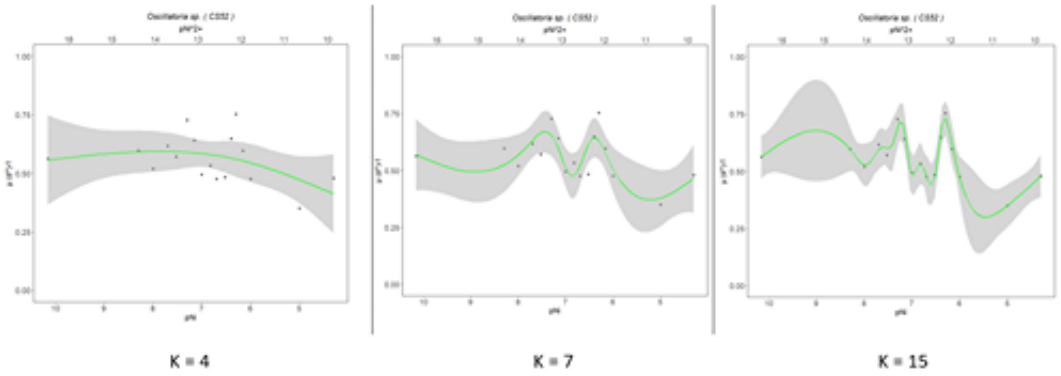
However, our pNi range (where pNi = -log[Ni^{2+}]) is similar to that of Price and Morel (1991), who grew the diatom *Thalassiosira weissflogii* in Aquil media containing either EDTA or DTPA. Price and Morel (1991) used a pNi range of 9 to 14 compared to our range of 6 to 16. We have added the protocol for Visual MINTEQ calculations to the Appendix.

Comment: Figure 1: can the species used in panel b and c be named here?

Response: We have added the species names in panels b and c.

Comment: 212: an additional explanatory sentence for the k-value here might be needed. It seems like the decision whether a function is over vs. under-fitting remains somewhat arbitrary?

Response: We have added further explanation here. The choice of k-value depended on the fitted curve result and was adjusted visually to fit the data. If the k-value is too small, the fitted curve will be close to a straight line and will ignore the trend of Ni effects; if the k-value is too large, the fitted curve will exaggerate the change of Ni effects.



K = 4 K = 7 K = 15

Comment: 221: can it be state what the concentration of ligands is assumed to be, 0?

Response: Thank you for pointing this out. Yes, in this experiment we assumed the ligand concentration in the Southern Ocean seawater media is 0 and the Minteq calculation is based on this assumption. We have revised this sentence to include this information.

Comment: 222: should be "largely"

Response: Thank you. We have changed the sentence for improved clarity.

Comment: 313: phrases like "not very pronounced" would be better if replaced by quantitative statements, e.g. <10%.

Response: We have changed these phrases to quantitative statements.

Comment: 317: same thing re: "were smaller". Similar issue on 435: "low"

Response: We have changed these statements to be quantitative.

Comment: 429: Im not aware of specific evidence suggesting that strong Ni ligands will be able to outcompete natural Fe-binding ligands. Perhaps changing "this would" to "this may" would allow for more uncertainty here.

Response: Thank you for this suggestion. We have adopted this change.

Reference:

Price, N. M. and Morel, F. M. M.: Colimitation of phytoplankton growth by nickel and nitrogen, Limnol. Oceanogr.,36, 1071-1077, https://doi.org/10.4319/lo.1991.36.6.1071, 1991.

Sunda, W. G., Price, N. M., and Morel, F. M. Trace metal ion buffers and their use in culture studies. Algal culturing techniques, 4, 35-63, 2005.

Cheers,

Jiaying Abby Guo