

Biogeosciences Discuss., author comment AC3
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Reply on CC1

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-AC3>, 2022

Dear Guillemette Gandon, Mathis Gillio, Lubin Grosbuis, Zuzi Koscikova, and Louise Litrico,

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 science community

Comment: The paper has an interesting topic, clearly identifying the knowledge gap and highlighting the importance of the study.

In the *Introduction*, a bit more clarification could be added to how increased ocean alkalinity can increase ocean capacity to store more CO₂ (Line 40).

Additionally, there is no smooth transition to the questions – there could be a connection between the mentioned two functions of Ni (L64-70) and the research questions (L77-79). We suggest an additional explanation of how these functions can affect your hypotheses.

Response: Thank you for your comment. We have added more information as requested.

Comment: Similarly, a deeper explanation of why seawater from 15 m from the Southern Ocean was chosen could be included in the *Introduction* or beginning of *Materials and Methods* to improve the accessibility to a broader readership.

Response: Thank you for pointing this out. We used the Southern Ocean seawater from 15m because this seawater contains relatively low trace metal and organic ligand concentrations and thus, it can be a good comparison to our Aquil media which has a high ligand (EDTA) concentration. We have clarified this and refer to the article from Boye, et al. (2001) in the text.

Comment: The paper is missing an explanation for the choice of phytoplankton species used as well as unequal representation of functional groups (3, 4, 3, and 1 for each group respectively). Could you elaborate on this?

Response: Thank you. We have added an explanation for the choice of species in the Materials and Methods section.

Comment: We would recommend restructuring some parts of the *Materials and Methods* section and bringing essential explanations at the beginning, such as explaining EDTA usage and the choice of seawater from the Southern Ocean from 15 m deep.

Response: Thank you for your suggestions. We have revised the manuscript accordingly. Specifically, we added a starting sentence "EDTA binds with metal ions and helps the dissolution of metal ions to create a nutrient replete medium." in line 139.

Comment: Similarly, limitations could be more fully discussed in the *Discussion* section, such as using an average medium of all ocean compositions to portray the variation in nickel's effect on each species (mentioned in 2.1 part) or unpredicted light cycle (L109-114).

Response: Thank you for pointing these limitations out. The limitations of our experiment with respect to media, species, and light cycle were mentioned in the manuscript as you mentioned, but in our opinion these are not the main limitations of our research. Other more concerning limitations were discussed instead, such as ligands (Line 413-421) and nitrogen source used in the experiment (4.1.1).

Comment: Although the figures (Figure 3 and 4) are straightforward, it would be reasonable, and probably improve clarity, to group them into 4 phytoplankton groups. Original figures could be still presented in *Appendix*.

We would also recommend developing more on the species-specific response as it is one of the paper's main findings. More information about studies' observation for each species and its implications can help.

Response: We agree with the comment that more species-specific discussion may be needed and we have therefore used statistical descriptions in the Results and Discussion. As for the figures, each sub-figure has 17 data points and if we plotted them according to their functional group this would reduce the clarity of the figures. 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: Line 48: Can you please clarify "appropriate scale"?

Response: Yes, we now use statistical descriptions in the Results and Discussion sections.

Comment: Line 69-70: Please, can you explain how the increase of Ni concentration with depth affects phytoplankton?

Response: Yes, we have revised the description to make it clearer.

Comment: Line 89-91: Please provide the chemical composition of Aquil medium or a link to its definition. (Could be in Appendix).

Response: Thank you for your suggestions. We will have added details about the Aquil media in the Appendix.

Comment: Line 139: Deeper explanation of EDTA usage should be at the beginning of the *Materials and Methods* section.

Response: We have provided a clearer rationale for using EDTA at the beginning of this

paragraph (Line139).

Comment: Line 184: Please, clarify if it was a standard sample size of phytoplankton samples added to the FRR fluorometry cuvette.

Response: Thank you. Yes, 5ml of samples were added for measurement each time. We have added this information in the text.

Comment: Line 188-193: Could you clarify the choice of channels for the fluorometry? Why did you use 3? A short explanation of why you chose these would be helpful. Similarly, you could clarify how these channels coincide with the photosynthetic pigments across the phytoplankton function groups.

Response: We measured fluorescence on three channels but only used the data from two channels (A and C) because the results from channels A and B were virtually indistinguishable. We polished this sentence: "Channel A (450nm) (and B) preferentially stimulates photochemistry in diatoms, haptophytes, and dinoflagellates because of the presence of chlorophyll a in their cells while channel C (450 and 624nm) has an extra wavelength for the stimulation of phycobilin which is commonly present in cyanobacteria (Roy et al., 2011)."

Comment: Line 232: "Every strain was able to grow in all Ni concentrations in Aquil media for at least 3 batch cycles" – should be moved to the *Materials and Methods* section.

Line 252: Again, the part about an additional experiment should be moved to the *Materials and Methods* section.

Section 3.3 (Line 304-307): Restructuring the paragraph with a better opening statement would be helpful. The paragraph could start with specific species instead of "Like in Aquil media...". Moreover, additional information about the actual comparison between Aquil media and the natural seawater media is needed to increase the value of the paragraph.

Line 304: "*P. tricornutum* growing in the natural Southern Ocean seawater media..." – this should be explained earlier in the text with the definition of natural Southern Ocean seawater media.

Response: Thank you! We have revised the manuscript as suggested.

Comment: Line 311: Restructuring the statement: "...do not have a strong effect." As it seems there is an effect for some species, we recommend using instead "effect varies for species" and/or "some species are affected while others not."

Line 312, Line 327: Restructuring the statement "only a few" and "most species". Please, use real numbers instead.

Response: Thank you for your suggestions. We have used statistical / quantitative descriptions here to describe the Ni effect.

Comment: Line 310, Line 326: Consistency in terms with the identical meaning: "FRR fluorescence" and "photosynthesis performance".

Response: Thank you. Firstly, FRR fluorometry and photosynthetic performance are not identical in meaning: the first is the method and the second is the measure. "Photosynthesis performance" means both F_v/F_m and σ_{PSII} results in our manuscript.

Comment: Line 329-330: It could be helpful to add some more literature to support the statement, as there are only 2 species (*Oscillatoria* sp. and *Synechococcus* sp.) common to both Glass and Dupont (2017) study and your study.

Line 388: Please, provide more than one reference for the statement "earlier studies".

Response: Thank you for your comments. We have added references Oliveira and Anita (1986) and Dupont et al., (2008) to support this statement.

Comment: Line 412: Argument about generalising the findings more widely to natural communities of phytoplankton seems too strong. The paper touches on the issue of different climate regions (in the paper are only phytoplankton species from temperate regions) and community competition very slightly.

Response: We have revised our description: "As the tested species cover a relatively wide range of taxa, it may be assumed that our findings can be generalised more widely to natural communities of phytoplankton in the temperate region". We agree that more research on this topic is needed to draw confident conclusions.

Comment: Line 414-418: Doesn't Ocean seawater media naturally contain other organic ligands? Couldn't these have been quantified?

Response: There is little information about concentrations and types of Ni-binding organic ligands in the Southern Ocean because these ligands occur at very low concentrations within a highly complex mixture of organic matter (Boiteau et al., 2016). Take Fe-binding organic ligands as examples: the characterized types of Fe-binding organic ligands were different in various studies due to the diverse measuring protocol, and the concentrations of these ligands in the Southern Ocean varied from 0.72 to 12.3 nmol/L (Nolting et al., 1998; Boye et al. 2001; Buck et al., 2010). What we can say for certain is that the Southern Ocean seawater we used in the experiment has a lower organic ligand concentration than the Aquil media (100 µmol/L EDTA).

Comment: Appendix A; Line 456: Please, add some clarification for the calculations statement: "The free ion concentrations were calculated based on the total ion concentrations together with the added concentration"

Response: Thank you for your suggestions. We will change the sentence to "The free ion concentrations were calculated based on the background total ion concentrations together with the added concentration".

Reference:

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Boye, M., Berg, C. M. G. V. D., Jong, J. T. M. D., Leach, H., Croot, P., and Baar, H. J. W. D.: Organic complexation of iron in the Southern Ocean. *Deep-Sea Res. Pt. I*, 48, 1477 -

1497, [https://doi.org/10.1016/S0967-0637\(00\)00099-6](https://doi.org/10.1016/S0967-0637(00)00099-6), 2001.

Nolting, R.F., Gerringa, L.J.A., Swagerman, M.J.W., Timmermans K.R., and Baar, H.J.W.: Fe (III) speciation in the high nutrient, low chlorophyll Pacific region of the Southern Ocean, *Mar Chem.*, 62, 335-352, [https://doi.org/10.1016/S0304-4203\(98\)00046-2](https://doi.org/10.1016/S0304-4203(98)00046-2), 1998.

Roy, S., Llewellyn, C., Egeland, E., & Johnsen, G. (Eds.). *Phytoplankton Pigments: Characterization, Chemotaxonomy and Applications in Oceanography* (Cambridge Environmental Chemistry Series). Cambridge: Cambridge University Press, doi:10.1017/CBO9780511732263, 2011.

Cheers,

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