RC2: 'Comment on bg-2023-130', Anonymous Referee #2, 04 Oct 2023 reply

The manuscript by Xin et al. presents results on the response of important phytoplankton to increased Ni concentrations in the context of olivine-based ocean alkalinity enhancement. This study is novel, timely, and the methods, analyses, and interpretation seem to be scientifically sound. I recommend publication, but kindly ask the authors to consider the following questions and comments when revising their manuscript.

Line 39: "…co-benefit of mitigating ocean acidification…" – state that this would especially occur at OAE deployment sites, prior to seawater equilibration with CO_2.

Response: Thank you for the above suggestion. We agree that OAE has the co-benefit of mitigating OA at the deployment sites. However, we respectfully disagree that this happens prior to seawater equilibration with CO_2 as the pH is still a bit higher than the pre-OAE application levels after equilibration. We modified the text accordingly. *"OAE, in addition to enhancing the buffering capacity of seawater, has the co-benefit of mitigating ocean acidification at the deployment sites (Köhler et al., 2010)."*

Lines 41-42: Consider adding a brief discussion regarding the merits of using olivine over other proposed OAE minerals here.

Response: We revised the text*: "Among the most recognized alkaline minerals, olivine rocks have gained considerable attention due to their relative fast weathering rate, wide availability, and low cost (Schuiling and Krijgsman, 2006; Hartmann et al., 2013)."*

Lines 55-56: Why did the authors select these three organisms for their study? Are they meant to be functional group representatives? If so, a brief note regarding the importance of the functional groups they represent would be useful.

Response: We provided a brief introduction to explain their importance as follows: "*In this study, we examined the impacts of Ni on three representative marine phytoplankton species: the diatom Thalassiosira weissflogii, the dinoflagellate Amphidinium carterae, and the coccolithophore Emiliania huxleyi. These species were selected as they represent the three dominant functional groups of phytoplankton."*

Lines 70-80: This section would benefit from the inclusion of additional information. What were the experimental timeframes and why were they selected? Although timeframes are shown in Figs 2c, 3c, and 4c, discussing them here would be helpful. The experimental timeframe for the *A. carterae* experiment appears to be two days longer than that of the *E. huxleyi* and *T. weissflogii* experiments – what was the reason for this? For how many generations was each species grown? The replication strategy is also not clear to me. Were samples collected from triplicate independent replicates at each sampling point? Finally, I would suggest editing this section's heading – the heading implies that methods used for determining species responses will be presented, but it seems to focus more on the experimental design; section 2.3 seems to focus more on methods used to determine species response.

Response: Thank you for the above suggestion. We rephrased the text accordingly. The changes are reported in the following lines:

Line 83: We have changed the heading of 2.2 to "Experimental setup" to remove the overlap with the heading of 2.3.

Lines 85: Experiments were performed in triplicate independent 75 mL falcon flasks for each of the Ni concentrations.

Line 87: Samples (1 mL) of each flask were collected into sterile 2 mL microtubes.
Lines 89–95: To minimize the impact of changes in the carbonate chemistry of the medium induced by cellular metabolism, the phytoplankton biomass at the harvest time should

consume less than 5% of the total dissolved inorganic carbon (Zondervan et al., 2002). The maximum density was determined based on the cell carbon quota of the tested species. Accordingly, in this study, the maximum cell densities of A. carterae, T. weissflogii, and E. huxleyi never exceeded 12000 cells mL^{-1}, 15000 cells mL^{-1}, and 130000 cells mL^{-1}, respectively (Zondervan et al., 2002; Olenina et al., 2006).
Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., and Niemkiewicz, E.: Biovolumes and size-classes of phytoplankton in the Baltic Sea HELCOM Balt, Sea Environ. Proc. No. 106, 144 pp., 2006.
Zondervan I., Rost B. & Riebesell U.: Effect of CO_2 concentration on the PIC/POC ratio in the coccolithophore Emiliania huxleyi grown under light-limiting conditions and different daylengths, J. Exp. Mar. Biol. Ecol., 272, 55-70, https://doi.org/10.1016/S0022-0981(02)00037-0*, 2002.*
Lines 75-76: "…between 9:00 a.m. and 10:00 a.m.…" – to avoid the effect of the photocycle…
Response: We have rephrased the sentence based on the suggestion as follows*: "Samples were always collected at the same time of day between 9:00 a.m. and 10:00 a.m. to avoid an effect of the photocycle."*

Lines 94-95: Consider rewording this sentence for clarity and to define IC50 (e.g. – "The inhibitory concentration [IC50], at which growth is inhibited by 50%, was…").
Response: We have rephrased the sentence as follows*: "The half maximal inhibitory concentration (IC50), at which the growth rate is inhibited by 50%, was determined from the dose-response curve."*

Table 1: As the measured and free Ni^{2+} concentrations presented in Table 1 are from triplicate measurements, standard deviations be included here.
Response: The standard deviations are now provided in Table 1 in the revised version of the manuscript.

Figs. 2a-b, 3a-b, and 4a-b: The data points are a bit hard to read, especially at lower Ni concentrations.
Response: We are sorry for this inconvenience. We added a table (Table S1) with all growth rate values in the supplementary.

Fig. 2c, 3c, 4c: Data points are missing error bars. Additionally, units should be provided for log-transformed cell densities.
Response: The error bars are always available. If not visible, error bars are smaller than symbols. We specified this in the caption of the graphs.
The units have been added as cell density (log10 cells mL^{-1}).

Lines 139-140: "A similar trend…" – this sentence does not flow well.
Response: We have rephrased the sentence as follows: *"The cell densities and growth rates of E. huxleyi increased with the addition of Ni up to 3.89 µmol L^{-1}. At 0.9 µmol L^{-1} Ni, the maximum cell density with a 63 % increase was observed ($p < 0.01$). The growth rate increased by about 11 %, but this value is not statistically significant ($p = 0.07$)."*

Line **143-144: I find this interpretation a bit confusing: the authors note that *E. huxleyi* growth was inhibited after day 3 upon being exposed to Ni, pointing to Fig. 3c, but – in**

some treatments – log cell densities appear to be higher than the control after day 3. Clarifying which treatment level is being referred to would reduce ambiguity.
Response: Thank you for the above suggestion. We have rephrased this paragraph as follows: *"With the increase in Ni concentration after 15.3 µmol L^{-1} Ni, the density started to decrease and the growth rate was inhibited. From 15.3 to 78.6 µmol L^{-1} Ni, the cell densities decreased significantly between 57-72 % compared to the control ($p < 0.05$; Fig. 3a). The decrease in growth rate reached up to 24 % at the highest Ni concentration compared to the control (Fig. 3b). The growth variance of E. huxleyi started on day 3 after being exposed to Ni (Fig. 3c)."*

Line 153-154: Here, too, clarification about which treatment level is being referred to would be helpful.
Response: We have rephrased the paragraph as follows: *"The cell densities of T. weissflogii remained relatively stable until 8.0 µmol L^{-1} Ni, after which the densities started to decrease significantly ($p < 0.05$; Fig. 4a). Similarly, the growth rates of T. weissflogii remained relatively stable until 40.9 µmol L^{-1} Ni, above which the growth rates started to decrease significantly ($p < 0.05$; Fig. 4b). After being exposed to Ni, T. weissflogii reacted immediately from day 2 onwards (Fig. 4c)."*

Line 164: Please consider removing "Up to today,". Consider the following as a potential alternative: "Trace metals are required by phytoplankton for numerous physiological processes and biochemical reactions; however, it is difficult to disentangle the distinct role of each element."
Response: We have rephrased the sentence following the reviewer's suggestion.

Line 168: Wouldn't "nutrient-enriched media" be a better choice here since f/2 media was used?
Response: We agree that f/2 media could be considered as nutrient-enriched media and changed the "nitrate-enriched" to "nutrient-enriched".

Lines 193-200: The authors should also discuss the observed increase in *E. huxleyi* specific growth rates at low Ni^{2+} concentrations. What are the proposed hypotheses that explain this increase?
Response: Thank you for the above suggestion. We have added the possible explanation for the enhancement in *E. huxleyi* growth also based on another reviewer's comment as follows: *"Interestingly, we observed an enhancement in the cell densities of E. huxleyi at low Ni concentrations. Ni serves as a necessary micronutrient to the Ni-containing enzyme urease in phytoplankton when the primary nitrogen source is urea (Price and Morel,1991). However, this does not apply to our study. To the best of our knowledge, there are no clear reports indicating the positive effects of nickel as a nutrient when nitrate serves as the nitrogen source. One possible explanation might be that the introduction of low-dose toxins prompted an increased rate of cell division, a phenomenon known as hormesis. Studies on various phytoplankton groups revealed a similar dose-response pattern, where low doses exhibited beneficial effects and high doses led to toxicity. In these investigations, hormesis was attributed to low increased levels of Cd (Brand et al., 1986) and Cu (Brand et al., 1986; Pérez et al., 2006; Yang et al., 2019). This interpretation differs from the notion of metal limitation. Considering Ni, a slight increase in concentrations positively impacted multiple chlorophyll fluorescence parameters associated with photosynthesis in terrestrial plants, which was explained as a hormetic response (Moustakas et al., 2022). Another potential explanation is that Ni may, to some extent, contribute to the functionality of superoxide dismutase enzymes which are vital components in an organism's defense against oxidative*

stress (Sunda 2012). Nevertheless, this growth alteration should not be dismissed, as it could indirectly impact the competitive dynamics within ecosystems containing multiple phytoplankton species."

Brand, L. E., Sunda, W. G., and Guillard, R. R.: Reduction of marine phytoplankton reproduction rates by copper and cadmium, J. Exp. Mar. Biol. Ecol., 96, 225-250, https://doi.org/10.1016/0022-0981(86)90205-4, 1986.
Moustakas, M., Moustaka, J. and Sperdouli, I.: Hormesis in photosystem II: a mechanistic understanding, Curr. Opin. Toxicol., 29, 57-64, https://doi.org/10.1016/j.cotox.2022.02.003, 2022.
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.
Pérez, P., Estévez-Blanco, P., Beiras, R. and Fernández, E.: Effect of copper on the photochemical efficiency, growth, and chlorophyll a biomass of natural phytoplankton assemblages, Environ. Toxicol. Chem., 25, 137-143, https://doi.org/10.1897/04-392R1.1, 2006.
Sunda, W.G.: Feedback interactions between trace metal nutrients and phytoplankton in the ocean, Front. Microbiol., 3, 204, https://doi.org/10.3389/fmicb.2012.00204, 2012.

Yang, T., Chen, Y., Zhou, S. and Li, H.: Impacts of aerosol copper on marine phytoplankton: A review, Atmosphere, 10, 414, https://doi.org/10.3390/atmos10070414, 2019.

Line 226-227: If space permits, it would be useful to discuss the range of Ni concentrations found in olivine, especially within the context of the Ni concentrations selected for this study (Simkin and Smith [1970] could be useful for this).
Response: Thank you for the above suggestion. We have included the discussion regarding the range of Ni concentrations. We added the discussion in lines 204-212 for a better continuity as follows: *"Basic and ultrabasic rocks, which are widely recognized source minerals for OAE, would introduce high amounts of Ni into seawater during mineral dissolution (Renforth, 2019). A wide range of Ni content in olivine (0-0.44 wt%) suggests that the Ni release is source-dependent (Simkin and Smith, 1970). In a previous batch reaction experiment using forsterite olivine sand with 0.26 wt% Ni, an increase of 100 µmol L^{-1} alkalinity was associated with a parallel increase of approximately 3 µmol L^{-1} dissolved Ni during the non-stoichiometric dissolution process (Montserrat et al., 2017). According to these results, the concentration of released Ni could potentially reach the highest concentration tested in this study with a doubling of the current ocean alkalinity level, e.g. at the point source of alkalinity release."*

Simkin, T. and Smith, J. V.: Minor-element distribution in olivine, J. Geol., 78, 304–325, https://doi.org/10.1086/627519, 1970.

Line 231: LC50 should be defined here.
Response: We rephrased the sentence and added an explanation for LC50 to remove the ambiguity.
"*For example, certain diatom species with low IC50 and copepod species with LC50 (concentration expected to be lethal to 50 % of the tested organisms), might be susceptible to the released nickel in the context of OAE (see Table 2)."*

Lines 252-253: Limestone and its derivatives are unlikely to be metal-free (e.g., Gabe and Rodella, 1999).
Response: We have rephrased the sentence following the reviewer's suggestion: *"For OAE applications, minerals containing less heavy metals, such as quicklime produced from limestone, could also be considered (Gabe and Rodella, 1999; Šiler et al, 2018)."*

Line 256: Consider including a note about the slow dissolution times observed for olivine as well (e.g., Fuhr et al., 2022).
Response: We added the discussion regarding the slow dissolution time of olivine as follows: *"In addition, its economic costs for extraction and transportation are relatively low, and the duration required for dissolution is shorter compared to olivine (Caserini et al., 2022; Fuhr et al., 2022)."*
Fuhr, M., Geilert, S., Schmidt, M., Liebetrau, V., Vogt, C., Ledwig, B., and Wallmann, K.: Kinetics of olivine weathering in seawater: an experimental study, Front. Clim., 4, 39, https://doi.org/10.3389/fclim.2022.831587, 2022.
Lines 262-273: Based on their results, what do the authors suggest for future experiments?
Response: We added the discussion based on the results as follows: *"Future studies focusing on the taxonomical shift in natural communities and on incorporation and potential bioaccumulation of Ni in different species under cumulative Ni are foreseen to provide a more comprehensive understanding of the potential effects and risks of metal release associated with OAE."*