Associate Editor: Patricia Grasse

L478: The authors state that they observed potential iron precipitation. Did the authors also observe other precipitates in the experiment? Is it possible that Ni also precipitated during the experiment? How do trace metal concentrations change with time?

a. **We observed a reddish-brown amorphous precipitate consistent with iron (oxy)hydroxide precipitation, but not any other precipitates. It is certainly possible that some Ni could have co-precipitated with the iron, as this is known to happen. However, we did not make measurements of dissolved metals in our experiments and so do not know how Ni concentrations may have changed over time. We added the following text to the paper on line 483 to address this issue:**

   "It is possible that other metals including Ni may have co-precipitated with the iron, as has been documented in other aquatic systems (Laxen 1985). If so, this would lower dissolved Ni levels, a process that could also occur during olivine deployments in the ocean."

   Laxen D.P.H., 1985. Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions: The role of humic substances, Water Research 19(10): 1229-1236.

I saw that a new version was already uploaded to the BioRxiv server.
Please also provide a marked-up manuscript version highlighting the changes

a. **Uploaded**

I would appreciate if the author could upload a table (possibly as xlsx. file) with all measurement parameters.

a. **The data and table of parameters are included in the data file that we submitted in the open-source repository, Zenodo, and the link is provided in the "Data Availability" statement at the end of the manuscript. We have updated the statement to say that both the data and the parameters are included in this excel file. "All data and parameters can be found at https://zenodo.org/record/8157750." (line 739)**

RC1: Lennart Bach

Hutchins et al. investigate how phytoplankton could respond to Ocean Alkalinity Enhancement via olivine. The methods do not use natural olivine minerals but simulate a "cocktail" of dissolution products using chemicals. The research is rock solid and an important addition to the currently sparse literature needed to evaluate if OAE could be a

significant risk to the environment. I hope my comments are helpful for refining this manuscript.

Comments:

Line 41: The IPCC as such evaluates but does not call for action.

a. **We adjusted the text to reflect the urgency of the climate crisis within IPCC reports (lines 41-43).**

Line 62: I think it is important to clarify here that there are no negative physiological effects observed. However, we cannot exclude that negative ecological/biogeochemical effects occur on these and other phytoplankton. It is addressed in the last sentence but then this sentence should be clarified accordingly.

a. **We have adjusted the text accordingly (lines 62-65).**

Line 71: The Bach et al reference may not be the appropriate one in this specific case. Perhaps cite IPCC AR6 here.

a. **Inserted IPCC AR6 citation (line 73)**

Line 74: The Renforth and Henderson reference may not be the right reference here. Perhaps cite Rogelj et al., 2018, or the latest IPCC chapter where CDR was called “unavoidable” for the first time.

a. **Inserted the Rogelj et al., 2018 citation (line 76)**

Line 77: Better cite Caserini et al., 2022 (GBC paper) and Taylor et al., 2016 (Nature CC) here.

a. **Inserted Caserini et al. 2022 and Taylor et al. 2016 (lines 78-79)**

Line 79: I suggest citing Oelkers et al., 2018 here (the review on olivine dissolution).

a. **Inserted Oelkers et al 2018 (line 80).**

Line 85: Perhaps also cite Hauck et al., 2016 here (the modelling paper on OAE with Si and Fe additions).

a. **Inserted Hauck et al. 2016 citation (line 96).**

Line 180: What were background concentrations of the relevant trace metals?

Background dissolved metals were not measured in the natural seawater used to make culture medium, but presumably were very low relative to amounts

added to the medium in the experiments (for instance, 3.36 uM added Fe in the 100% OL). In comparison, we have measured several thousand times less (< 1nM dFe) in our similar cleanly collected seawater from the SPOT time series site in the past where this seawater was collected using trace metal clean results. We added text and a reference to say this (lines 189 - 192).

Line 192 and below: this was already said in the previous section.

a. **These sentences have now been removed.**

Line 264: what sort of filters were used for BSi?

a. **Pall Life Science 0.6 um polycarbonate filters, 25 mm diameter. We have added this to the text (line 273).**

Line 289: Were normality and equal variance checked and always the case? Was data transformed in case they were not? A more complete description is needed here.

a. **We used F tests to verify equality of variance, and this was supported for our data in all cases. We also used the Shapiro Wilk test for significant departures from normality, which was not the case for any of our data. We have now added this information to the Methods on lines 312-316.**

Results: In the section about diatoms it would be good to call the medium OL100 or something like this to make it easier for the reader that the highest OL concentration was investigated here.

a. **In the diatom section, we added "100%" before every "OL" term to denote the highest OL concentration and to be consistent with the "100% OL" terms in the subsequent figures in the manuscript.**

Figs. 1/2/3: Does the fact that all species grow best in ACM where no Fe, Co, Ni was added mean that ACM was TM contaminated? Is there data on the background Fe concentrations? I am asking because I would assume that Fe limitation in ACM would depress growth much more.

a. **We appreciate this note as our methods were not clearly articulated here. The ACM contained normal replete Aquil culture medium levels of nutrients and trace metals, including 250 nM Fe and 50 nM Co along with 25 uM EDTA (but no added Ni or Cr), which is why the cultures grew very well, as we intended in this positive control treatment. We have now clarified this by re-writing the methods describing the ACM and OL medium formulation on lines 178-183, and have amended the text in several places to refer to "positive control ACM"**

to remind readers that this is our comparative benchmark for optimum growth conditions.

Fig. S1: Why is this a supplement figure and not Nitzschia? I would put both in the main text.

a. **We have now combined Fig. 1 and Fig. S1.**

Fig. S3: A shows rates but then the unit is PIC/POC?

a. **Thank you for catching this, the figure legend was in error here. We have fixed the figure legends and the respective y-axes accordingly. Also to note, the figure is now Fig S2.**

Line 331: The conclusion that dissolution products are not toxic to 'diatoms' are not justified based on the two species investigated here. The authors should be very specific and not generalize much beyond what was studied here. Also, the framing that olivine provides essential nutrients is true but this could also be considered as eutrophication/species composition changes in a more ecological context (in contrast to the physiological perspective taken here). So, balancing the pros and cons would be good.

a. **We have modified the manuscript throughout to focus our conclusions on the specific species studied, and restrict our comments to predictions based on these model isolates that will need to be tested by further work (e.g., lines 573-576). We've also broadened our conclusions to recognize that ecological consequences need to be considered (e.g. lines 728-732).**

Line 33 something: It may be worth highlighting the POC production increase in Ehux from 10 to 30%.

a. **We changed the text to include this observation on lines 412-413.**

Line 372: Same comment on generalization as expressed for line 331.

a. **Adjusted text to be specific to this study (lines 424-427).**

Line 440+ something: I was not convinced by the conclusion of the cyanobacteria section. It seems to neither apply for non-N2-fixing nor for N2-fixing cyanos. First, Synechococcus is extremely fertilized by dissolved olivine, far more than the diatoms and Ehux. Second, Croccosphaera is fertilized too. It is not growing/fixing as well as in the ACM but ACM is possibly their favourite medium and therefore hard to match. So, the question is to what extent your control represents their real world environment or rather some sort of "paradise"? Third, the delivery of olivine does provide trace metals (unless particles scavenge trace metals (does not apply here) or it triggers some sort of excess TM precipitation). So, I am very nervous about the conclusion that olivine will not affect cyanos

in an ocean environment. If there is more Fe (or other TMs) around than in the case of no olivine-OAE then this is still likely to have effects, even if the physiological outcome presented here does not detect it. I think much more caution must be taken on this conclusion. Importantly, physiological results must be considered as such, acknowledging that results may be different in natural setting.

a. **To address this comment, we added text to point out that Crocosphaera benefited more from the OL than Trichodesmium, although it still achieved only a quarter as much growth as in ACM without EDTA (lines 490-516). The reviewer is correct, ACM is a replete medium and all of the phytoplankton grew very well in it- this is as we intended, since the ACM served as a positive growth control for comparative purposes. We agree that replete conditions like this are probably rare in nature, but our intention was not to do an ecologically realistic treatment here, but rather to provide a needed experimental control. We also added text to the end of this paragraph pointing out that these are physiological results, and they will eventually need to be put into a more realistic ecological context (lines 531-537).**

Line 491: Generally agree with this statement although toxicity cannot be excluded for Tricho (noting that the EDTA may have mitigated the toxicity).

a. **Indeed, we were concerned about this too but believe our follow-up experiments shown in Fig. 4 fairly well ruled out metal toxicity to Trichodesmium being alleviated by the EDTA additions. The only metals present in the OL were Fe, Ni, Co and Cr. We used literature results to show that the amounts of Ni and Co we added were very unlikely to be toxic to Tricho, and our experiments showed the presence or absence of Cr (without EDTA) made no difference to growth, whereas the presence or absence of EDTA alone did make all the difference (Fig. 4B). This suggests to us that the growth-stimulating effect of EDTA had to have come from solubilizing the Fe and making it more bioavailable. However, we have adjusted the language in this sentence to reflect that we did not observe a toxic effect rather than the leachate not being toxic in general (line 573).**

Line 531: How was “robust frustule development” determined? SEM? Please clarify.

a. **Frustule development was assessed using the biogenic silica (BSi) measurements, normalized to POC. Note that cellular Si:C ratios were as high in the 100% OL as in the ACM for both Nitzschia (Fig 1C) and Ditylum (Fig 1F), suggesting normal, robust frustule development. We added text to qualify this statement (line 613-614).**

Line 534: Albright et al did not find an increase in coral calcification, just an increase in net reef calcification (also possible via reduced dissolution). You could also refer to the study by Gore et al. 2019 who measured increased red algae calcification under OAE.

a. **The text has been adjusted, and the Gore et al. 2019 citation has been added (lines 617-618).**

Line 540: Unclear why your results is true for many phytoplankton. It may be useful to stress again that you investigated physiological responses, while ecological changes can also be induced indirectly (e.g. via changing competition).

a. **We would suggest that since our results consistently showed exposure to olivine dissolution products was either positive or neutral for all six very taxonomically diverse species of phytoplankton, there are many other species that would also not exhibit negative responses to olivine products exposure.**

   We agree about needing more research on possible ecological changes, and to address this issue we added this sentence to the Abstract on lines 64-67: "Future studies can shed light on long-term eco-evolutionary responses to olivine exposure and on the potential effects that marine microbes may in turn have on olivine dissolution rates and regional biogeochemistry.". We also emphasized this in the Conclusions section on lines 640-643: "It is important to note that our experiments focused on physiological responses, while further work will be needed to explore the possibility of indirect effects on important ecological factors such as predation or competition.", and on lines 728-732 in the final paragraph: "Future research directions may include longer term experiments with prokaryotes and natural microbial communities to expand our understanding of olivine exposure on important taxa that help drive biogeochemical cycling in the oceans, particularly experiments to test for ecological effects on processes like competition and trophic interactions at the community and ecosystem levels."

Line 565: Agree that within your results it appears that all tested phytoplankton benefit physiologically from olivine-based OAE. The hypothesis posted in our 2019 paper with the green and white ocean was coming from the perspective that nutrients will limit marine productivity. This factor is not considered in N and P replete cultures as yours. So, the issue with your conclusion may be that it applies for cultures but not so much for environments where competition for nutrients is key. In other words, the question appears to be who benefits relatively most. The one benefitting least would be the loser, even if growing faster under olivine fertilization. As such I think your conclusion of a "green AND white ocean" is not necessarily transferable to the competitive ocean environment.

a. **We agree, and added qualifying text to this paragraph saying that our conclusion is only valid for the nutrient-replete conditions of our laboratory experiments, and that outcomes under nutrient-limited conditions in the ocean might be quite different (lines 659-662).**

Line 585: This conclusion is not logical to me. If you fertilize everything but N2 fixers then you deplete fixed N faster, thereby creating the niche for N2 fixers faster. So, you just speed up the uptake but do not affect the competitiveness of N2 fixers (all very theoretical of course).

a. **We agree this sentence was quite speculative, and to avoid confusion about ecological outcomes of olivine additions for N2 fixers versus other phytoplankton (which are after all unclear at present), we removed it from the paper.**

Line 593: This is very interesting. Does it mean that you expect that olivine does Fe-fertilize Tricho under natural conditions? The sentence implies just that but no statement is being made.

a. **We think predicting that bacterial siderophores will mobilize enough Fe from olivine applications to fertilize Trichodesmium under natural conditions is a bit of a bridge too far at this point, so we'd prefer to leave the text as is, just pointing out that our cultures may have lacked some of the natural processes that can provide Trichodesmium with otherwise refractory sources of Fe in the ocean.**

Line 605: Do you mean "fertilization" instead of limitation? Agree with this conclusion for the coastal ocean but what about the open ocean?

a. **Our results suggested high levels of precipitated Fe from olivine dissolution might not be available to the two N_2 fixers, which is why we used the word limitation. The reviewer raises an interesting point between coastal and open ocean systems. Considering the many other chemical factors at play in situ along with the fact that Trichodesmium colonies can be found in either coastal or open ocean systems, we feel we do not have sufficient evidence to speculate further in terms of Fe limitation or fertilization for open ocean. We originally spoke in terms of coastal systems as this is where all of the focus is placed for coastal enhanced weathering applications. That said, enhanced weathering applications may ultimately being conducted in open ocean settings in the future. Hence, we have modified these sentences to shift the frame towards a broader context in terms of both iron nutritional status and general oceanic applications. "In nature, *Trichodesmium* is also likely to occur mostly as colonies, and so may have access to additional siderophore-bound iron,**

including from both naturally-occurring supplies as well as potentially from any oceanic olivine applications. Thus, changes in the iron nutritional status of N_2 fixers due to marine olivine additions may not occur in the real ocean." (lines 698-701).

Line 607: I wonder if growth inhibition is the right word here. Isn't it rather that something was missing rather than something was inhibiting?

a. **We agree, and changed this text on line 702-703 to say: "...The reduced growth rates of the diazotrophs on our synthetic OL…"**

Line 633: I think this conclusion with respect to fisheries is premature. Differential stimulation likely means shift in communities, which could be good, bad, or neutral for fish production. Furthermore, it remains unclear if there is some sort of bioaccumulation and perhaps even biomagnification of problematic dissolution products. I strongly suggest removing this conclusion towards fisheries.

a. **We agree with the reviewer and have removed the fisheries text.**

Reviewer 2: Anonymous

1. Except the cyanobacteria, is there any additional information about these cultures? For the diatoms, only genus-level designation is provided. Was any sequencing performed to ID them, e.g. 18S? Is there a strain ID from a culture collection? Where were they isolated from? I suggest that the authors include all of this information.
    a. **We have now added detailed information on the species names, strain numbers (where available) and geographic origins of the phytoplankton isolates (lines 167-175). The diatom Ditylum brightwellii was identified by 18S sequencing after isolation by T. Rynearson, and the Synechoccus isolate was identified through whole genome sequencing by J. Zheng; the rest of the strain IDs were provided by the Bigelow Culture Collection where they were obtained.**
2. Overall, I suggest that the authors clarify the results of the statistical tests. At times, it is quite clear in the text, e.g. paragraph starting at line 305, and other times it is not, e.g. lines 324-325 and 337. The authors could consider using letters on the plots to denote significant differences among means.
    a. **We have added symbols to denote levels of significance to the figures, although because we did several types of experiments, letters won't work for all of them. We added this text to the statistics section of the Methods to explain the symbols we added (lines 314-321): "For experiments with only one or two OL treatments and the ACM control, graphs are presented with each treatment marked with a letter denoting significant differences at the $p < 0.05$ level from each of the other treatments. For experiments such as OL dilution series**

with many treatments (7 in this case), clear visualization of differences with all other treatments using letters is not feasible. For these experiments, significant differences in the OL treatments relative to the ACM positive control are indicated by asterisks (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$).” We also added the precise p values for the various experiments at appropriate places in the text.

3. SI Fig 3 and Lines 367-371 – For the plot, can this be split up more? Are the bars on the left side of the plot for growth rates and the ones on the right for PIC/POC ratios? It is confusing when presented this way. Are the growth rates and PIC/POC ratios truly “very similar” as stated on lines 367-369? They are nearly significantly different (p=0.05 and p=0.07), and if I am interpreting the plot correctly, they look somewhat different, particularly the PIC/POC ratios.
    a. **We agree this was confusing on SI Fig. 3 (now SI Fig. 2), so to address this issue we put a vertical line down the middle of each panel to separate the two halves, and also marked the two bars on the right in Si Fig A and B (the PIC/POC ratios, as the reviewer surmised) with a different fill pattern. We also changed the figure legend to clarify this. We changed our description of these data in the text as follows (lines 417-422): “*Emiliana* specific growth rates were slightly higher in the OL than in the ACM (p = 0.05), while cellular particulate inorganic:particulate organic carbon ratios (PIC:POC, mol:mol) were not significantly different in the two treatments (p= 0.07, Supp. Fig. 2A). Likewise, POC-specific fixation rates (p=0.04; TC h^{-1}) were slightly higher in the OL than in the ACM treatments, while PIC:POC fixation ratios were the same (p=0.07, Supp. Fig. 2B).”**
4. Title and abstract line 49 – The authors did not really test “phytoplankton groups,” rather they tested single species representatives of important groups. I suggest that the authors alter this language to be more accurate.
    a. **We agree that our results showed model species were not affected negatively by olivine additions, not entire phytoplankton major taxonomic groups. We have changed the title and abstract to say “species” instead of groups, and edited the text throughout the paper to refer specifically to our isolates rather than their major taxonomic affiliations. We agree our results will need to be tested in additional experiments with other species before larger generalizations can be made.**
5. Line 100 – change “will affect” to “may affect”
    a. **Changed (line 101).**

6. Line 250-260 – Are the growth rates in the plots shown from both microscopic counts and chlorophyll *a*? This is a bit unclear to me.
    a. **This methods text was incorrect, actually we did not present the growth rates calculated from microscopic cell counts as we only had these analyses for some of the experiments. Those microscopy-based rates we did have were nearly identical to the chlorophyll-based rates. To be consistent throughout, all growth rates shown are based on chlorophyll. We removed the methods text about cell count-derived growth rates, and added text to say they were all chlorophyll-based on lines 259.**

Lines 337-366 – PIC:POC levels in Ehux were higher at 50-100% OL but also quite high at 0% OL. Do the authors have any thoughts as to why this occurred?

    b. **This is the result of the PIC/POC production values being a ratio, rather than an absolute value. At 0%OL, CO2 fixation into POC was reduced quite a bit more than fixation into PIC , and so the preferentially lowered denominator resulted in a higher ratio (though still not as high as in the highest OL concentrations). We added some text to point this out on lines 408-414.**
7. Line 537 – change “N2-fixing cyanobacteria” to “Trichodesmium and Crocosphaera” Others, i.e. symbionts, were not examined here.
    a. **Changed (lines 621).**
8. Fig 1 – I believe that it would be easier to see the results if the panels from Supplementary Fig. 1 are also shown in Fig. 1. The direct comparison between the two diatoms would be nice to see.
    a. **Fig. 1 and Fig. S1 have now been combined.**
9. Fig 6 – the spacing between days is not even, i.e., there is the same amount of space between days 1 and 2 and 4 and 6 which makes the change over time more difficult to interpret.
    a. **We agree, and we changed the X-axis in Fig. 6 as suggested.**
10. Lines 575-578 – I disagree that diatoms can access precipitated Fe oxides. The reductive Fe uptake mechanism mentioned is for siderophore uptake (Coale et al 2019 PNAS). Kazamia et al 2018 which the authors also mention refers to endocytosis of siderophores as well.
    a. **We appreciate this point by the reviewer. Clearly both diatoms were able to access considerable Fe somehow, despite it being present as newly precipitated Fe oxides from the saturated levels added in the OL. This was the only Fe source available to them, and they grew very well on it. There is evidence that freshly precipitated colloidal Fe hydroxides can serve as an Fe source, although this initial bioavailability declines rapidly as the precipitates age. We also agree with the reviewer that the presence of bacteria in the cultures could have provided siderophore-solubilized Fe to the diatom ferric reductase uptake**

systems. Accordingly, we added more nuanced text and two new references in this section as follows: "In accordance with their well-studied reductive Fe uptake systems (Morrissey and Bowler, 2012), there is evidence that diatoms can access Fe to some degree from freshly precipitated amorphous colloidal Fe hydroxides (like those in our experiments), although the bioavailability of Fe precipitates declines quickly as the hydroxides age and acquire a more crystalline structure (Yoshida et al. 2006). Alternately, the precipitated Fe in our experiments could have become available to the phytoplankton ferric reductases via solubilization by siderophores produced by bacteria in our non-axenic cultures (Coale et al. 2019), and diatoms can even potentially take up Fe through endocytosis in some cases (Kazamia et al., 2018). (lines 668-676).

11. Line 604 – I believe "siderophore-bound iron" would be more appropriate than "microbiome-provided"
    a. **Changed (line 699).**