**Response to comments from Reviewer 1**

We appreciate the reviewer's positive feedback and thank them for their time and effort. We have addressed each of the comments below, with our responses introduced in italics and labelled as *Authors' Response (AR)*.

## General Comments:

The paper by Schneider et al. investigates the ecological and chemical consequences of non-CO2-equilibrated Ocean Alkalinity Enhancement (OAE) using a mesocosm experiment in a temperate fjord. The study's overarching hypothesis is grounded in existing literature, particularly the "white vs. green ocean" framework (Bach et al., 2019), and addresses known gaps in empirical OAE studies. Specifically, it aims to test whether varying levels of added total alkalinity (TA) using silicate- and calcium-based minerals alter carbonate chemistry, air-sea CO2 fluxes, calcification, net community production (NCP), and zooplankton respiration. This hypothesis is both relevant and timely, as most OAE research remains conceptual or model-based. Some explicit strengths, use of Gafar et al.'s (2018) CaCO3 Production Potential (CCPP) bridges cellular physiology with mesocosm-scale data. Estimation of zooplankton respiration as the difference between oxygen-based and DIC-based NCP is innovative and revealing.

## Specific Comments:

**2. Experimental Design and Methodological Soundness**

The methodology needs to be improved and supplemented with the method description (and results) currently missing.

- One of the biggest limitations is that there was only one direct DIC sampling point for validation introduces uncertainty into TA-pH derived DIC estimates. Such uncertainties have likely propagated through time but that has not been addressed in the paper. In addition, NCP is derived from changes in DIC — so uncertainty compounds over time. This needs to be addressed and evaluated.

*AR: We agree that it is a limitation of the study to only have 10 direct DIC measurements, from which a correction of measured pH across the gradient could be obtained, which then was used throughout the study to calculate DIC. However, the correction at the highest pH level of 8.5 is 0.03 pH units (see Fig. S2 in original supplement) which, in turn, translates to a calculated DIC offset of about 25 µmol $kg^{-1}$ (only ~1.2%). The fact that DIC remained relatively stable in the days following the TA addition—consistent with expectations for this oligotrophic phase of the experiment—gives us confidence that the DIC estimates based on corrected pH measurements are reliable. Furthermore, even if there were a tendency for increasing offsets in calculated pH in the higher TA treatments, this would hardly affect our NCP calculations, as they are based on relative change over time. Finally, the effects observed in NCP are only mineral-type, which would not change if all values are skewed. We will include these clarifications in a separate paragraph in the discussion.*

- Methodology of how preparing different feedstock solutions is not described in sufficient details. Artificial separation of silicate and TA effects may not represent real-world OAE mineral

additions. How were the concentrations of all the feedstocks measured to assure that the concentrations at the end were correct?

*AR: The preparation of the feedstock solutions is laid out in lines 106-111. We will add further information, such as that the Ca and Mg solutions, as well as the NaOH were all prepared in individual 20 L of deionized water and then added to the respective mesocosms. Mg and Ca were not measured, as deemed a rather small change of only a few percent, given the large natural background concentrations (~49.8 and 9.6 mmol $kg^{-1}$at salinity 33, respectively). However, the increase in silicate and TA by NaOH was confirmed by direct measurements in the mesocosms right after the additions. Concerning the fact that artificial separation of silicate and TA may not represent real-world mineral additions, even at the lowest TA addition of 150 µmol $kg^{-1}$, the silicate addition for a correct olivine stoichiometry would have resulted in an increase of silicate by 37.5 µmol $kg^{-1}$. This is more than an order of magnitude larger than what is considered to be limiting for diatom growth. Furthermore, only about 10 mol $kg^{-1}$ of silicate was taken up until the end of the experiment, meaning that in any case, silicate concentrations would have been non-limiting throughout the experiment in all mesocosms. We will add this additional information to the methods section.*

- How much NO3-, Si and Si(OH)4 and Ca2+ were added, mention specific numbers. Why was NO3- added to up to 4umol/kg, which is at least 4-5 times higher than in a fjord, creating completely artificial conditions for those communities inhabiting fjord? And subsequently, how do you know that this is a natural response of the communities acclimatized to low nutrient levels, instead of artificial response that might be out of scope if OAE without the added nutrients would happen? Can you decouple this effect somehow and include this in the discussion and results section?

*AR: We will add the information that Ca and Mg were added in a 1:2 ratio to TA. Concerning the nutrient additions, we will refer to Ferderer et al., 2024 for further and specific details. When it comes to upwelling events bringing nutrients to the surface, these are not uncommon in the study area and similar nutrient additions have been carried out in a number of past mesocosm studies there, e.g., Schulz et al. 2008, Schulz et al. 2017. The latter study has also highlighted that nutrient additions halfway through an experiment tend to amplify otherwise difficult to detect differences in community composition/biogeochemical element cycling. We will add this information to the discussion.*

- What depth was N2O taken, up to 20 m or the surface, not clear from the text.

*AR: $N_2O$ sub-samples were taken from the IWS (Integrating water samplers), which integrated water from 0-20 m depth. We consider this is sufficiently clear between lines 129 and 132 (section 2.4).*

- Respiration measurement description is missing.

*AR: If the referee refers to Zooplankton respiration, we consider this to fit better in the discussion section, as it came only as a result of comparing our DIC-derived NCP with oxygen-derived NCP from a different publication. No measurements were performed.*

- The description or the reference to the flow cytometry analyses is missing.

*AR: We will add a brief description of the Flowcytometry analysis.*

**3. Data Collection and Analytical Approach**
**Analytical concerns:**

- The pH measurements required dye corrections due to potential impurity artifacts—highlighting the fragility of spectrophotometric pH at high alkalinity. Can you comment and revise?

*AR: We wouldn't say that spectrophotometric pH is fragile, since the change in absolute values was only 0.03 pH units at the highest measured pH of 8.5 (see response above). Such pH dependent offsets using unpurified dyes, even when trying to apply corrections have been described previously (e.g., Douglas & Byrne 2017). It highlights the advantage to over-determine the carbonate system by measuring 3 rather than only 2 parameters for cross-validation. We will add the reference to the appropriate methods section.*

- Assumption of 1:1 O2:C ratio in NCP calculation may oversimplify complex respiration dynamics. How do you rectify this? In which range does this ratio hold? Could that be different for the respiration of the micro vs large zooplankton (above 280um)?

*AR: Indeed, the reviewer is correct that the trends we see in calculated zooplankton respiration could also be the result of changes in the respiratory, as well as the photosynthetic quotient. We will discuss this in more detail in the revised version of our manuscript.*

- Data on respiration is missing entirely.

*AR: Please see previous reply to comment.*

- The large variability of DIC upon the nutrient addition is overwhelming and not well explained, also not matching the trends in the other parameters. Provide better explanation.

*AR: The authors do not agree with this statement. As explained in the first paragraph of the discussion, the DIC decrease is due to primary productivity being stimulated by the nutrient addition. The fact that this is difficult to detect in other parameters than the nutrients themselves is that they are either hardly affected, i.e., TA, or only slightly, e.g., pH and pCO2, which are difficult to spot due to the rather large initial treatment differences as opposed to a more uniform DIC.*

- Where did you take the 95% for full equilibration from?

*AR: This is based on a simply forward calculation, assuming average gas exchange rates determined in our study and calculating how long it would take for a 95% equilibration. The 95% threshold was chosen to provide a more realistic estimate of the equilibration time, as the process follows an exponential pattern and reaching a true 100% is then virtually impossible.*

- No coccolithophore or diatom data presented??? It is literally impossible to draw some of the results and conclusion in this paper unless there is data available for this.

*AR: Coccolithophore data is available in the Supplement, FS 5. We will highlight this more prominently in the main text. For diatoms, we only have BSi data as a proxy that can be correlated to each treatment. It will be added in the appendix as well.*

- Are there any taxonomic or metagenomic assessments to resolve zooplankton community and why the decision on cutting it at 280um?

*AR: No taxonomic or metagenomic data is available to resolve RZ. We didn't decide on the cutting at 280um, this cut comes from the methodology applied in Marín-Samper et. al, 2024. 280 µm have been found to being a good compromise to not exclude too much of the natural community and at the same time ensure reproducibility between replicate incubations.*

**3. Results and Interpretation**

**Calcification:** Coccolithophore calcification followed an optimum curve relative to pCO2, with a peak around 250 µatm and suppression at extremes. However, in the figure S4a, calcification is below 0 for the two highest treatments, which is not explained anywhere in the text. Does this indicate dissolution. Even less severe treatments are still just hardly above 0, especially before the addition of nutrient part, which signifies lack of calcification overall, and only just happening in the first three treatments. How does this align with the NCP, can you correlate? And how does it align with the CALC, could it have any effect on the TA? Is this species-specific, could it be due to any other calcifiers (not just the autotrophs)? In general, the drawback of this is also that no other potential calcifiers have been implicated in the CALC, only the autotrophs. Are there any data available to support this, or discount for the impact of zooplankton on the CALC?

*AR: Having calcification hovering around the 0 line or being negative is most likely related to the inherent uncertainty stemming from a mass balance involving four measurements with their individual uncertainties (TA, salinity, nitrate and phosphate). However, the fact that we find an optimum curve suggests that despite these uncertainties, the overarching pattern is preserved when calculating cumulative calcification for the entire experiment. We will mention this in the discussion. Concerning CALC and NCP, both are derived considering changes in TA (see Eqs. 5 and 6). This in turn also means that calcification by any organisms is captured by this method. The fact, however, that the cumulative calcification correlated well with cumulative coccolithophore counts (Suppl. Fig. S5) suggests that they were the dominant calcifiers in our experiment.*

**Net Community Production (NCP):** NCP was significantly higher in silicate treatments post-fertilization, with no direct pCO2 effect. But the effect of the pH was not investigated and should be included in ANOVA. Also, why is NCP related to Si and not to Ca2+ treatment- again, data on diatoms and phytoplankton are absolutely essential, otherwise this all on the level of inferences.

*AR: Given the set-up of this experiment, pH and $pCO_2$ are intimately correlated, i.e., there is a quasi-linear relationship of proton concentration and pCO2. So, either one of them could have been chosen, but we decided to stick to the one parameter that is relevant to all aspects of the manuscript. We will add this piece of information. Concerning the Si/Ca question, there is a relatively large background of $Ca^{2+}$ in seawater, meaning that our additions change concentrations only by a few percent (0.8 – 3.1%). In contrast Si is a macronutrient needed by a particular group of producers and that changed in between the two treatments by several orders of magnitude. Hence, Si seems to be behind the mineral-effect on NCP rather than Ca. Even though we do not have data on diatoms, BSi data will also be added to the Supplement as a proxy.*

Also, how does Chla correlate with NCP and Calcification (Figure S4a-c and S1f)?

*AR: While there is a reasonable correlation between daily changes in NCP and chlorophyll standing stocks, as one would expect, the latter cannot be compared to calculated calcification, as what is shown here are cumulative changes not daily rates.*

**Zooplankton Respiration:** Respiration declined with decreasing pCO2 and was lower in Si treatments, but in general, this aspect is largely underexplored and insufficiently presented. Much more effort needs to be put in explaining respiration data and how it links to suggested trophic-level complexity. Present the data on respiration beyond 2 days, 2-day data is insufficient, compare the pre and post nutrient treatment.

*AR: Data on zooplankton respiration is cumulative. Hence, all values throughout the entire experiment are factored in when we take the cumulative mean for the last 2 days. Also, comparing RZ pre and post nutrient addition, it appears that there is a consistent trend. We will make this clearer in the text.*

<u>The results of respiration are also fundamental in explaining some of the effects and should be put in the Results, not Discussion, section.</u>

*AR: As explained previously, we consider zooplankton respiration to better fit in the discussion and would like to keep it there, as it resulted from comparing to and discussing a dataset from another publication. We will make the text clearer regarding this topic.*

**Discussion:**
In general, this study is really divided in two parts:

- Pre-nutrient treatment that is represented of the fjord environment under OAE and post-nutrient that is NO LONGER representative of the oligotrophic fjord conditions, whereby the used communities were not acclimated to such increases in nutrients and is just a mesocosm trial of OAE with nutrients. In such systems, the communities and species could react completely

differently than under such artificial conditions. This aspect is now touched upon in the results and discussion and I would like the authors to fully dedicate the effort on the potential confounding effects due to such nutrient addition and how different the fjord system response to OAE would be if such strong nutrient artificial addition was not present- Fco2 still high, but NPC insignificant, what about respiration etc?

*AR: Thank you for the suggestion. We will broaden the discussion on this topic and add supplementary figures on responses in the two phases as well as the whole experiment. We will also include a summary table (exemplified as follows).*

| **Parameter** | **Phase 1** | **Phase 2** | **Phases 1+2** |
|---|---|---|---|
| cCALC | Gradient only, optimum curve | Gradient only, optimum curve | Gradient only, optimum curve |
| $cNCP_{DIC}$ | Gradient only | Mineral only | Mineral only |
| cRZ | Gradient only | None | Gradient and Mineral, no interaction |
| cBSi | Mineral only | Mineral only | Mineral only |

- In addition, no evaluation of the longer-term dynamics to capture seasonal or successional effects is presented.

*AR: Extrapolating our six weeks results to obtain longer-term dynamics will be difficult as to the unknown of seasonal and successional variability.*

REFERENCES

Douglas, N. K., Byrne, R. H., Achieving accurate spectrophotometric pH measurements using unpurified meta-cresol purple, Mar. Chem., 190:66.72 (2017).

Ferderer, A., Schulz, K. G., Riebesell, U., Baker, K. G., Chase, Z., Bach, L. T., Investigating the effect of silicate- and calcium-based ocean alkalinity enhancement on diatom silicification, Biogeosci., 21:2777–2794 (2024).

Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyeröfer, M., Müller, M. N., Egge, J. K., Nejstgaard, J. C., Neill, C., Wohlers, J., Zöllner, E., Build-up and decline of organic matter during PeECE III, Biogeosci., 5:707–718 (2008).

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel, A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A. J., Sswat, M., Riebesell, U., Phytoplankton blooms at increasing levels of atmospheric carbon dioxide: Experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes, Frontiers, Mar. Sci., 4:64 (2017).