RC2: Anonymous Referee #2

General Comments:

I read with pleasure the paper: "Assessing the impacts of simulated Ocean Alkalinity Enhancement on viability and growth of near-shore species of phytoplankton" by J. Oberlander and coauthors. This manuscript is of high interest for the assessment of OAE. The author digs into the available data set from the previous study that investigated the impact of increased pH on phytoplankton (outside the context of OAE) and performs culture experiments on two species, Thalassiosira pseudonana and Diacronema lutheri with two different approaches (batch culture and semi-continuous) to trace the (very) short impact of increase pH and the long(er) impact in the context of OAE.
That said, I think the manuscript needs significant revision before it is suitable for publication.

Major Comments:

It was hard to follow the structure of the article. Some chapters and subchapters do not follow the standard structure of an article to the point that methodologies, statistics analyses and results are mixed.

We agree that a major reorganization of the Methods would be helpful to the reader. We propose to address this by consolidating all of the experimental methods into a new, separate section, with the differences in set-up between the different experiments tabulated for ease of comparison. The table would include the experimental ID, treatment (alkalization as concentration and duration of exposure), culture volume, presence/absence of aeration, measurement parameters and frequency. The revised Methods would also include a better explanation of the rationale for picking the studies compared in Section 2: a literature survey in which our search terms included "phytoplankton AND (alkalinization OR "high pH")". Studies found in the search were gated to include those in which cultures were maintained under pH drift conditions, with culture medium ensuring that the media would have DIC:DIN below the Redfield Ratio, and with time-series (growth curves) of phytoplankton abundance and pH.
(Reviewer 1, Comment 16)

The analysis of previous studies is almost at the beginning of the article, but it is not discussed adequately in the discussion and conclusion chapters. Some literature is omitted throughout the article. What about other studies made for ocean acidification that tested however higher pH values e.g. Bach et al., 2015? Or some of the Riebesell et al., studies on coccolithophore?

With respect to the analysis of prior studies in the Discussion and Conclusion, we will work to correct this by including a paragraph outlining the conclusions from the analysis as well as discussion further explaining the connection with OAE.
In regard to studies omitted in the analysis, we focused primarily on laboratory studies that included direct relationships between increasing pH and the concentration of cells or growth rate of the phytoplankton. The studies included in this analysis had this data easily available in their papers (*i.e.*, figures or reported values), and due to time constraints contacting authors to request access to data that was not included was not feasible. We agree that the work conducted by Bach, Riebesell, and many others on ocean acidification is valuable to understanding the potential impacts of OAE. We note that Bach et al. (2015) does include pH and growth rate data for *C. pelagicus*, however because the focus of his study was CO_2 it did not appear in our literature search for prior studies investigating elevated pH where our search terms included "phytoplankton AND (alkalinization OR "high pH")". We believe that for the purposes of this manuscript, which this analysis was a single section, that focusing on a subset of the literature that is

consistent in terms of experimental approach and reporting gains in the simplicity of the comparison what it loses in breadth.

In the discussions previous articles related to the response of phytoplankton to the perturbation induced by OAE are not mentioned (e.g. Gately et al., 2023) and in general many references are missing throughout the text.

The same point was made by Reviewer 1. We propose to reference Gately et al. (2023), Iglesias-Rodríguez et al. (2023), Guo et al. (2023), Paul et al. (2024), Hutchins et al. (2023), Ferderer et al. (2022), and Subhas et al. (2022) for studies of the impact of OAE on phytoplankton, and Schulz et al. (2023), Moras et al. (2022), and Hartmann et al. (2023) for the efficiency of OAE and the impacts of calcite precipitation

Specific Comments:

Abstract

The abstract should be strongly revised. On top of some inaccuracies that I will report in the following lines, the reasons why studying the impact of high pH on phytoplankton is lost within the lines. Moreover, the study is based on two specific species that are important components of the phytoplankton community in specific marine contexts (i.e. near-shore, temperate waters). The final statement (lines 18-19) is therefore too general and misleading. The author should consider the findings of this study and not generalise their results to other taxonomic groups. The analysis of the prior study is lacking in the abstract even if it's an important part of this study. It should be mentioned.

The same problems were noted by Reviewer 1 in comments 1-4 and 30, our proposed changes are as follows:

- Rewording lines 8-9: "One proposed NET is Ocean Alkalinity Enhancement (OAE), in which artificially raising the alkalinity favours formation of bicarbonate from CO_2, leading to a decrease in the partial pressure of CO_2 in the water, and a subsequent invasion of atmospheric resulting in net sequestration of atmospheric carbon."
- Rewording lines 11-12: "The potential impacts of OAE were assessed through an analysis of prior studies investigating the effects of elevated pH on phytoplankton growth rates in pH-drift experiments and by experimentally assessing the potential impact of short-term elevation of pH on the viability and subsequent growth rates of two representative near-shore species of phytoplankton."
  Rewording line 16: "However, there was a significant decrease in growth rates with long-term (8 days) exposure to elevated pH."
- Deleting lines 18-19
- Addition of the following: "The analysis of prior studies indicates wide variability in the growth response to elevated pH within and between taxonomic groups, with about 50% of species expected to not be impacted by pH increase expected unequilibrated mineral-based OAE. To the extent that the growth responses reflect (largely unreported) parallel reductions in DIC availability, the susceptibility may be reduced for OAE in which CO2 in-gassing is not prevented."

1. Lines 7-8: technologies used twice.

Suggested rewording: “In response, new tools are being developed…”

2. Line 9: OAE is not only mimicking but is enhancing/accelerating the process. Moreover, it should be made more explicit why CO2 is ultimately sequestered from the atmosphere. The link is missing.

   Please see the response above to the general abstract comments for the suggested revision to lines 8-9.

3. Lines 10-11: As mentioned before, the aim of the study and the need to understand the impact of increased pH on primary producers should be better expressed.

   Proposed rewording:
   “The aim of this study was to investigate the impact of simulated OAE, through the alteration of pH, on phytoplankton representative of the spring and fall blooms in near-shore, temperate waters. The potential impacts were assessed through 1) an analysis of prior studies investigating the effects of progressively elevated pH on phytoplankton growth rates, and 2) by experimentally assessing the potential impact of elevated pH on the viability and growth rates of two representative near-shore species.”

Introduction
In general: many references are missing throughout the whole introduction. Just a few examples: at line 36 after “drawdown”; at line 37 after “dominates”. There’s a huge literature to cite in the whole introduction that is completely missing.

Please see the response to the final Major Comment, and the below specific comments.

1. Lines 22-27 The first lines are out of topic. It seems more like an introduction to a thesis than an article. I suggest to find another way to introduce the study. This paragraph seems non-correlated to the next one.

   The reviewer is correct to infer that the manuscript arose from a MS thesis. Suggested rewording: “Climate change has become one of the most pressing problems facing us as a society, with atmospheric carbon dioxide (CO_2) concentrations steadily increasing over the past 250 years (Dlugokencky and Tans, 2018). This led to the signing of the Paris Agreement in 2015, with the agreed upon goal to keep the global average increase in temperature below 2 °C (*United Nations Framework Convention on Climate Change*, 2015). It is widely acknowledged, however, that reducing emissions will not be enough to meet this goal and carbon dioxide removal (CDR) will be needed. In fact, many of the IPCC scenarios that comply with the Paris Agreement regulations require as much as 10-20 Gt of CO_2 removal per year (Honegger and Reiner, 2018). To achieve this ambitious removal target Negative Emissions Technologies (NETs) will be needed.”

2. Line 24: Galland et al., 2012: I guess many more references can be added here.

   Please see the response to Introduction comment 1 above.

3. Line 29: I disagree. NETs are not developed to combat rising atmCO2. See also recent literature on the need to reduce CO2 emission and on the minor role of CDR in this context. Please delete the first part of this sentence or try to put it in a different context.

   Suggest deleting this sentence.

4. Line 31: here and through the text take care about wording. Is OAE going to restore the pH and the carbonate system to their natural state? Why then study the impact of high pH if you consider OAE as a "restoring process"? This is a provocative question. I think this way of summarising the process is incorrect.

   Suggested rewording:
   "Ocean Alkalinity Enhancement (OAE) is one promising NET that involves anthropogenically raising the alkalinity, and as a result the pH, of a parcel of water causing the partial pressure of the CO_2 in that water to decrease. This change leads to either the uptake of CO_2 from the atmosphere or a reduction in the release of CO_2 from the ocean, depending on the initial air-sea gradient. Both scenarios result in a theoretical net reduction of atmospheric CO_2 and storage in the form of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions in the ocean (Oschlies et al., 2023)." (Reviewer 1, Comment 5)

5. Line 33-38 there are different ways to apply OAE. Not all of them change the pH (i.e. increase the pH). In an equilibrated OAE for example, DIC is increasing while pH is rather stable. Please be cautious with this description and try to rephrase it. In the whole article, CO2 limitation is also barely mentioned.

   Suggested rewording:
   "There are currently several different methods of OAE in development, including mineral- and electrochemical-based methods, with deployment from vessels, through preexisting outfalls, or from placement on beaches. The focus of this study is the mineral-based approach from preexisting outfalls, implementation of which is likely to occur through addition of unequilibrated hydroxide minerals (OH^-) to the coastal surface ocean." (Reviewer 1, Comment 7)

6. Line 41: I would rephrase this part since at the moment we don't know how the regulations might change. There are ongoing projects that are trying to evaluate efficiency, efficacy and env. impact in the open ocean (ship). I would mind the words again and avoid saying: "almost certainly" I would suggest the authors refer to your possible case scenarios instead (i.e. land-based, coastal release of TA).

   Agreed. We suggest removing this paragraph in favour of the statement included in the above comment (Introduction 5, above).

7. Line 46: I would delete "especially" since the impact at different trophic levels is equally important

   Suggested rewording: substitute "including" for "especially".

8. Lines 49-64: some sentences are disconnected. Line 53-54: what does that mean? Why in this context do you think is important? References are missing and a big range of pH is put in the loop. This sentence it's a bit of a: "and then what?" sentence.

   Suggested rewording for lines 53-56:
   "The reason for the low concentration of dissolved CO_2 usable by phytoplankton is well illustrated in a Bjerrum plot (*e.g.*, Zeebe and Wolf-Gladrow, 2001) which shows bicarbonate's dominance of the inorganic carbon species at pH values of 6 – 9. Although CO_2 is the substrate for Rubisco, the prevalence of bicarbonate underlies a strong selective pressure among phytoplankton for the ability to utilize CO_2 in a carbon concentrating mechanisms (CCM). This is a trait observed across taxonomic groups (Colman et al., 2002; Nimer et al., 1997; Beardall et al., 2020). Different CCMs facilitate uptake of CO_2 by its active transport across the cell membrane and/or by uptake of HCO_3^- through anion exchange, followed by its conversion to CO_2 by carbonic anhydrase (Coleman et al., 2002l Nimer et al., 1997; Beardall et al., 2020). Taxonomic differences in the energetic costs of different CCMs (Raven et al., 2014), in the pH optima of different forms of carbonic anhydrase (Idrees et al., 2017; Supuran, 2023), and in the specificity of different forms of Rubisco for CO_2 vs O_2 (Iñiguez et al., 2020) suggest that alkalization has the potential to alter community growth rates or to cause shifts in taxonomic structure within mixed assemblages."
   In regard to the missing references, please see the response to Comment 9, below.

9. Lines 56-64: almost no references. They should be added.

   Suggest adding the following references:
   - Line 60 following "…gradient to function (Beardall and Raven, 2016)."
   - Line 61 following "…or have carbonic anhydrase (Beardall et al., 1976; Raven and Hurd, 2012; Raven et al., 2014; Raven et al., 2017)."

10. Line 66: it is not only Hansen, 2002. There's a lot of literature on the response of phytoplankton to changes in pH in the optic of OA studies. Some of them tested also higher pH values!

    Suggested rewording for: "(Hansen, 2002)" to "(see Supplement 1; Bach et al., 2015; Langer et al., 2006)."

11. Line 71: the aim of the study should be better clarified and underlined and not with an "en passant" sentence like this one.

    Suggested rewording:
    Delete the final sentence on lines 70-71 and modify lines 73-77 as "The study addressed potential impacts of OAE via the response of phytoplankton growth rates, viability, and photosynthetic competence via responses to elevated pH. First, published data were fitted to a model of growth to quantify the effect of progressively rising pH on the growth rates of a range of cultures phytoplankton. Second, the viability, growth rates, and photosynthetic competence (as F_v/F_m) were measured for two representative near-shore phytoplankton species, the diatom *Thalassiosira pseudonana* and the prymnesiophyte *Diacronema lutheri* (formerly *Pavlova lutheri*), following exposure to short- (10 minutes) and long-term (8 days) elevated pH."

Literature Review & Data Digitization

1. Line 83: Since the literature review is not only based on Hansen 2022, this way of citing the studies is not correct. Please refer to the table in the supplementary. On top of that: why other studies that tested high pH on diatoms and or coccolithophores were excluded? The choice of the considered study is not clear to me.

   It is not immediately clear how the citation is inappropriate in this context as there is no specific citation on line 83. We proposed to clarify the criteria used to select the studies that were included in Section 2, as suggested below:
   A revised Methods section which would also include a better explanation of the rationale for picking the studies compared in Section 2: a literature survey in which our search terms included “phytoplankton AND (alkalinization OR "high pH")”. Studies found in the search were gated to include those in which cultures were maintained under pH drift conditions, with culture medium ensuring that the media would have DIC:DIN below the Redfield Ratio, and with time-series (growth curves) of phytoplankton abundance and pH. (Reviewer 1, Comment 16)

Examining the impact of prolonged, elevated pH on phytoplankton with and without DIC resupply

1. Line 174: The description of OAE is incorrect. First, there are different ways to apply OAE (equilibrated and non-equilibrated). DIC in a non-equilibrated approach is rather stable at the very beginning.

   Suggested rewording:
   “Most of the studies analyzed in Section 1 did not permit CO_2 in-gassing, as they were conducted using a closed-bottle, batch-culturing method. However, for OAE to successfully function as a NET, in-gassing of CO_2 is required to increase the DIC pool.” (Reviewer 1, Comment 13)

2. Line 192-194: This is more a method than a description of the results.

   These details can be moved to a proposed revised Methods section as described in response to the first Major Comment.

3. It’s not clear what this paragraph is about.

   We are unsure to which paragraph the reviewer is referencing in this comment.

4. I don’t know if this is the right paragraph but the way pH and the other carbonate chemistry parameters are not clear to me. What did you measure? DIC or TA on top of pH? And how?

   For this experiment, DIC and pH were measured, and the remaining carbonate chemistry parameters were calculated using CO2SYS (Lewis and Wallace, 1998). This would be stated explicitly in the proposed revision of the Methods.

5. Lines 195-196: I strongly disagree with this statement. I would tone it down to the specific case that you are considering.

Suggested rewording:
"These results demonstrate that the test organism, the biological responses to alkalization depend in large part on CO_2 in-gassing, so the response to mineral-based OAE and subsequent in-gassing could not necessarily be inferred from pH drift experiments."

Assessing the effects of short- and long-term alkalization on viability, growth, and photosynthetic competence in two coastal phytoplankton

I found the whole of chapter 4 quite hard to follow. Methods and results are mixed in a way that makes the reading quite challenging. I strongly encouraged the authors to rethink the structure of the whole chapter 4 to make it easier to follow by the reader.

We propose to consolidate the methods used for the different sections of the paper into a single section. Please see the response to the first Major Comment.

Discussion

1. Line 305-306: the first sentence of the Discussion is out of topic as mentioned by the way by the authors. Why mention it as the first sentence MRV if it is unrelated to the aim of the study?

   Suggested rewording for the first two sentences (lines 305-307):
   "Where OAE is based on non-equilibrated alkalization, it will result in a rise in both alkalinity and pH. We have tested potential responses through a combination of data analysis and experimental manipulations, under conditions in which the pH increase is accompanied by DIC drawdown and in conditions in which the two are largely decoupled."

2. The very first sentence mentions the increase in pH. However, OAE could be applied in different ways (not only with the addition of NaOH) that could impact less the ecosystem. I mean the equilibrated approach that would not induce an increase in pH but in DIC. On top of that, even if the aim of the study is the impact of high pH on phytoplankton, what about CO2 limitation?

   Please see the response to Discussion Comment 1, above.

3. Line 308: a variation from 8.2-8.3 is it by the way in the natural range of variation in a marine context, meaning that phytoplankton is by definition able to cope with this (and even bigger) pH variations. Should we then be surprised that this shift is not making any impact on the studied species?

   Suggested rewording:
   "This result is not unsurprising considering the natural variations of pH in a marine system tend to fluctuate much more than this (Oberlander, 2023)."

4. Line 315: with cautions in which sense and why?

   Suggested rewording:
   "This suggests that evaluation of unequilibrated OAE in the context of pH-drift cultures should be completed with careful consideration of the associated changes in the DIC availability."

5. In general, if OAE is applied in a non-equilibrated way as in the case of NaOH solutions, the authors should consider that equilibration will take time. Therefore, the perturbation of the carbonate chemistry could last relatively longer in the water

   We propose a comprehensive review of the manuscript to ensure that specific details regarding unequilibrated OAE are explained thoroughly.

6. Line 310- 315: I strongly disagree. Equilibration could take time to happen. It is not such a fast process at least not in every oceanic/marine context. Ingassing could take longer, therefore, the pH-drift effect must be considered. I value the long/semi-continuous studies that should give us information on the long(er) response of the phytoplankton. But I disagree with the limited value of short(er) batch experiments that within some minutes/hours/days can give us a hint of how a species responds to the carbonate chemistry perturbation induced by increased TA

   Suggested rewording:
   "The mechanism and potential impacts of OAE allow for CO_2 invasion to restore the equilibrium concentration of CO_2 after the conversion of existing CO_2 into bicarbonate. Where the approach is not calibrated to raise alkalinity without perturbing pH, OAE will result is a rise in pH with adjustment of the DIC speciation towards bicarbonate and carbonate, followed by in-gassing of CO_2 to restore the equilibrium. If in-gassing is restricted, the rise in pH will be accompanied by a drawdown in DIC if there is an excess of photosynthesis versus respiration. Comparisons of alkalized cultures with high DIC (semi-continuous cultures or aerated batch cultures) and those in which there was a drawdown (sealed pH drift cultures) show that in some cases there is no difference in the resulting growth rates (the dinoflagellates *Ceratium furca* and *C. fusus*; Figure 2) but in others it is pronounced (the congeneric dinoflagellate *C. tripos* and the diatom *Thalassiosira pseudonana*; Figures 2 and 3). The potential effects of OAE are therefore likely to depend on the degree to which changes in pH and DIC are decoupled."

7. Line 318: references missing after others

   Suggested rewording:
   "The analysis of prior studies of pH-dependant growth rates indicates that dinoflagellates are statistical outliers among the taxonomic groups and may be more sensitive to alkalization than most others (Table 1; Supplement 1)."

8. Line 321: what is likely to be a high mean?

   Suggested rewording:
   "…summer assemblages in nearby Mariager Fjord when pH could reach a up to a value of 9 between the months of May and August (Hansen, 2002)."

9. Line 322: compared to what? (i.e. which group?)

   Suggested rewording:
   "Their higher sensitivity to raise pH, when compared with the other species investigated in Table 1 and Figure 1, might also reflect fundamental…"

10. Line 336: this is the authors' idea since there are studies there that are now trying to apply OAE simulating the release from a ship in the open waters. I would suggest that the authors will use the opportunity to relate their study to the specific case of coastal water and/or basin OAE.

    Suggested rewording:
    "A last reason for caution in extrapolating the pH-drift responses lies with the most probable scenario for conducting unequilibrated OAE in coastal waters. Discharge would occur in the nearshore in this scenario, likely into strong lateral flow environments."

11. Line 355: London protocol see comment above

    Suggested rewording removes reference to the London Protocol (see response to Discussion Comment 10, above).

12. Line 342-345: how accurate are these pH numbers? How were they calculated?

    These values were calculated using the growth rate, corresponding initial pH, and Equation 2 from the manuscript. Suggested rewording:
    "The threshold values for reductions in growth rate with chronic exposure were 8.59±0.059 and 8.68±0.199 for *T. pseudonana* and *D. lutheri*, respectively."

13. In the discussion, most of the studies on the impact of OAE on phytoplankton are not mentioned. The article is lacking in putting its results into a broader context considering studies like Gately et al, 2023.

    Please see the response to the final Major Comment above.

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