

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Aaron Ferderer et al.

Author comment on "Assessing the influence of ocean alkalinity enhancement on a coastal phytoplankton community" by Aaron Ferderer et al., Biogeosciences Discuss.,
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Dear referee,

Thank you for your comments on our manuscript. We appreciate the time and effort you have dedicated to providing valuable feedback on our manuscript. Here are our point-by-point responses to your comments.

Comment 1: The first reservation I have is the duration of the reported experiment, which appears to have been too long given the size of the bottle. The experiment lasted for more than 20 days. Did the authors consider possible biases in species composition because of the bottom effect? I am not convinced that it is possible to interpret with confidence biology data obtained after 10 days of experimentation, and any conclusion drawn about changes in phytoplankton communities based on such data may well be erroneous. The bottle effect is well known and can seriously bias results. The authors should provide convincing evidence that this effect has not influenced their results.

Response 1: The reviewer addresses a fundamental limitation of all experimental ecological studies with enclosures. Bottle effects always affect the outcome in experimental ecological studies, regardless of the size of the container (Carpenter, 1996; Duarte et al., 1997). Importantly, however, there is no evidence to suggest that a specific duration (e.g. 10 days as suggested here) is an ideal timeframe to minimise bottle effects (Duarte et al., 1997). From our experience, even very large and sophisticated floating mesocosms enclosing ~50,000 L of natural seawater induce 'bottle effects' right from the beginning (day 1) as evidenced from much stronger fluctuations in phytoplankton communities inside the mesocosms than in the surrounding seawater (see figure 3 in Bach et al., 2019). Thus, we disagree that 20 days is too long as there is no basis for such an argument. Twenty days was chosen as we were able to cover a nutrient-induced phytoplankton bloom and the subsequent post-bloom phase with sufficient data points to obtain meaningful insights. With regards to biases in the community due to bottle effects, as noted above, this is a general problem and we therefore agree that bottle effects can drive changes in community composition (as is discussed in length in Bach and Taucher, 2019). However, these bottle effects occur in all micro and mesocosms. Thus, we implicitly account for them so that the comparison between control and treatment are still valid. Or in other words, while there may be deviations from natural plankton successions due to bottle effects, the conclusions drawn from the comparisons between the control and treatments are still technically sound. We will add a clarification to the manuscript to account for the reviewer's valid concern: "Nevertheless, despite some potential

advantages, we acknowledge and are fully aware that our microcosm setup cannot reproduce the full physical (or chemical/biological) complexity of nature (Carpenter, 1996). Enclosures of any type will very likely induce so-called bottle effects (Bach and Taucher, 2019), which can alter the observed community succession and therefore affect the transferability of the outcome to natural (non-enclosed) communities (Carpenter, 1996). While this is a general limitation of these kind of experimental studies, we stress that bottle effects would occur in all replicates so that the comparison between control and treatments (as done in our study) is valid.”

Comment 2: The second reservation I have is the validity of phytoplankton composition data obtained after the complete depletion of nutrients. Nearly all the N and P was depleted at day 6 (Si was depleted at day 8), but the authors reported phytoplankton composition changes for 20 days after nutrients had completely run out. I think the effects of alkalinity increase on phytoplankton composition should be evaluated under the ample nutrient conditions. Once the nutrients were depleted the phytoplankton would have been affected by both alkalinity change and nutrient constraints. These two variables may have in combination contributed to phytoplankton composition change. The authors should explain how they differentiated the effect of alkalinity change from the effects of nutrient constraints.

Response 2: The goal of this experiment was to observe changes in the phytoplankton community over various stages of a bloom, including when inorganic nutrients were depleted, in excess and declining. We emphasise that phytoplankton are nutrient-limited for most of the seasonal cycle. Thus, assessing the effects of OAE on communities under nutrient-depleted conditions is at least equally as important as nutrient-replete conditions. It is important to keep in mind that we initially enclosed the same water with similar nutrient concentrations (with random, non-systematic variation). We agree that differences in plankton communities later in the experiment may have been induced by the different (residual) nutrient concentrations after the bloom. However, these different concentrations were then a result of the treatments, established on the first day of the study. For example, the equilibrated OAE treatment had a pronounced effect on silicate drawdown. It is possible that this difference in silicate concentration had a downstream effect on the community in the post-bloom phase. However, the silicate difference is a result of the treatment and thus ultimately an important effect of OAE, transmitted by changes in nutrient drawdown. To account for this valid concern raised by the reviewer we will add the following clarification “The aim of this experiment was to assess the influence of alkalinity enhancement on the various stages of a spring bloom. This included periods at which nutrients were in excess, declining, and depleted. The effect of nutrient depletion on the phytoplankton community in the absence of enhanced alkalinity was observable in the control treatment. However, it is possible that OAE treatments affected nutrient drawdown during the bloom so that differential nutrient concentrations in the post-bloom phase amplified the emerging differences between the control and OAE treatments.”

Comment 3: My third reservation is the validity of the three experimental treatments used. Case 1 was a control (no alkalinity change); Case 2 involved an alkalinity increase without equilibration with atmosphere CO₂ and so an initial pH > 8.6; and Case 3 involved an alkalinity increase and equilibration with atmospheric CO₂, and so an initial pH was closer to the pH of the control. I am concerned about the design of the treatment cases 2 and 3. The goal of this experiment was to assess the effects of alkalinity increase on phytoplankton composition and elemental ratios during photosynthesis. To assess this the initial pH in cases 2 and 3 and the control should have been the same or at least similar. The authors needed to make sure that the effect of pH on phytoplankton composition and

elemental uptake ratios in their experiment was minimized. I suspect that the difference in results between Case 1 versus Case 2 or Case 3 may have been because of pH differences rather than the alkalinity differences. In the reported experimental setting, the differences among the three cases might have arisen from pH differences rather than the alkalinity differences or have been affected by their interaction. This is potentially a major problem with the study and interpretation of its results, so the authors should provide appropriate justification for their experimental design.

Response 3: Ocean Alkalinity Enhancement entails a variety of changes in the marine carbonate system, even though the increase in alkalinity is its name-giving feature. OAE leads to an increase in pH and decrease in $[\text{CO}_2]$ with these changes being much more pronounced when not accounting for an immediate invasion of atmospheric CO_2 into the water column (as in the equilibrated treatment). In carbonate chemistry equilibrium, it is impossible to establish the same pH and TA between two treatments when DIC is different between them (Zeebe and Wolf-Gladrow, 2001). As such it is not possible nor the goal of this experiment to manipulate alkalinity independently of pH. We did not report the initial pH of the microcosms (before alkalinity addition) here as it is assumed that all microcosms had the same initial pH as the water was taken from the same location over a ~30-minute time period. This is confirmed by the initial pH of the three control microcosms which varied in pH by 0.01 units. Based on this fact, pH across the three treatments before alkalinity addition was likely the same. The reviewer also states, "In the reported experimental setting, the differences among the three cases might have arisen from pH differences rather than the alkalinity differences or have been affected by their interaction". We agree, it is almost certain that the differences observed in our experiment were due to the divergence in pH and CO_2 among microcosms after the addition of alkalinity. Alkalinity itself does not affect biology as it is a chemical concept (Bach et al., 2019a) however the major concern surrounding OAE is the changes in carbonate chemistry associated with the increasing alkalinity (pH, CO_2). Assessing the effect of changes in pH and CO_2 on a natural phytoplankton community as a result of increased alkalinity is the primary aim of this manuscript. This has been discussed explicitly e.g. in section 4.1.3.

Comment 4: My fourth reservation concerns the C:N ratio. The authors measured the C:N ratio throughout the experiment, presumably to enable investigation of the effect of the three treatments on the elemental C:N ratio. If this is the case, only those measurements made under conditions of ample N and P availability are relevant. Once nutrients were depleted, nutrient limitation would more strongly constrain the C:N ratio rather than any change in alkalinity. Although the C:N ratio under the nutrient depleted conditions deviated considerably from the Redfield ratio, its impact is likely to have been minimal as the uptake of C and N by phytoplankton under ample nutrient conditions would have far exceeded those under the depleted conditions.

Response 4: We think that it is important to record and show this for the entire experiment and not selectively for only the major bloom phase because plankton communities (and the biogeochemical processes they drive) experience nutrient-depleted conditions for most of the seasonal cycle. Our main argument against the reviewer's reservation is the same as in our reply to comment 2. While C:N stoichiometry may have been affected by nutrient depletion, the temporal differences in nutrient depletion itself was in some cases (i.e. where significant) a treatment effect. Thus, OAE can have influence on C:N indirectly by having first-order effects on nutrient cycling. It is therefore very important to not only consider nutrient-replete stages of a bloom in these types of experiments but also nutrient-deplete stages. To clarify this concern, we will add the following statement to the results where post-bloom C:N data is mentioned: "Differences in the drawdown of inorganic nutrients, particularly PO_4^{3-} and Si(OH)_4 (Fig. 4) may have

enabled or amplified differences in organic matter stoichiometry, which developed in the post-bloom period. However, it is important to keep in mind that such developments (when significant) were ultimately caused by the treatments, even if they are indirectly induced by direct effects on nutrient drawdown that occurred earlier in the experiment.”

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