We thank the reviewers for their thoughtful and insightful comments on the manuscript. We have provided our responses in blue text and included some proposed changes to the wording of the main text/captions in *green italicized text* below.

Reviewer 2 (Citation: https://doi.org/10.5194/egusphere-2025-871-RC2)

General Comments:

This study presents the results from a microcosm experiment conducted to follow a natural phytoplankton bloom in Chesapeake Bay, using various methodological approaches (including 18S rRNA gene analysis and an NPZ model). The introduction in well-written, with a clear focus and a plausible research gap and objective. The methods are very thoroughly described and good to follow. All figures are done very nicely and informatively. I added some comments throughout the methods and introduction to consider. For the discussion, I agree with reviewer #1 that the depletion of nutrients for terminating the bloom should receive a bit more thought (as scratched upon in lines 351-355) and I added some additional comments.

Thank you for your thoughtful feedback! We agree with both reviewers that the study would benefit from a deeper discussion of the role of nutrient limitation in our microcosm experiment. We have detailed in our responses how we will be addressing nutrient depletion.

METHODS

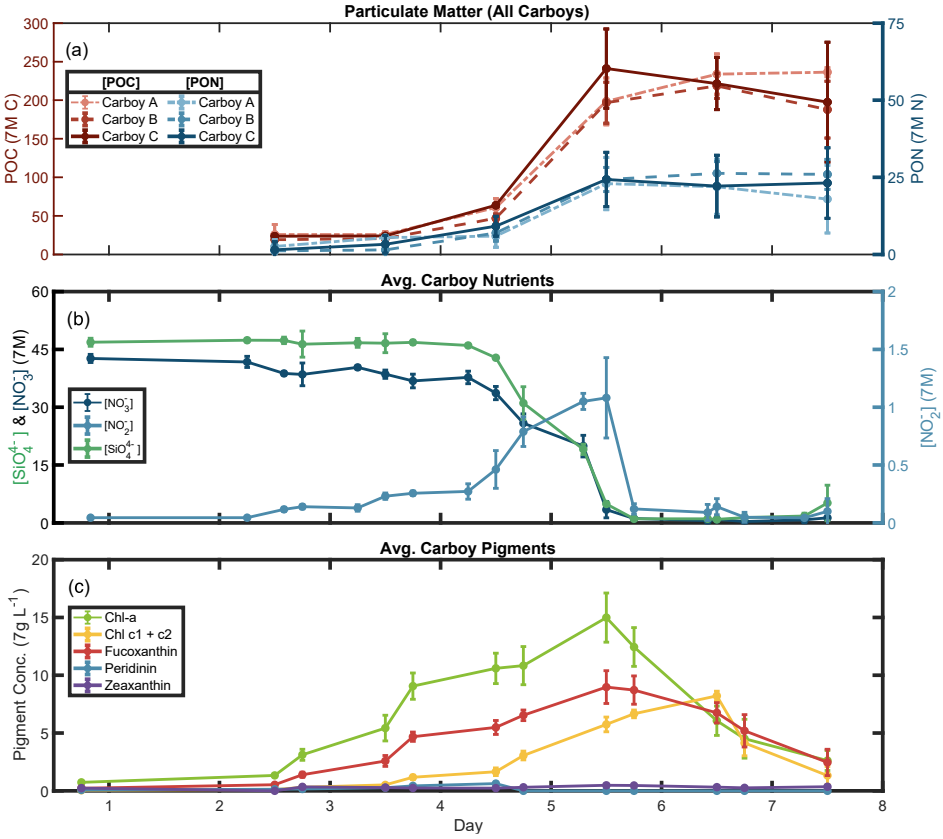
Line 87-89: How were the added concentrations of nitrogen, silica and phosphorus chosen? The reasoning for their supply ratio is clear with the follow-up sentence, but I am wondering about their concentration.

40 µM NO_3^- was chosen to promote a diatom bloom, as well as to represent the nutrient loading to which Chesapeake Bay is exposed. Silica and phosphorus concentrations were chosen to provide appropriate nutrient ratios. Lower nutrient concentrations can promote cyanobacterial or dinoflagellate blooms over a diatom bloom (Adolf et al., 2006; Conley and Malone, 1992; Huang et al., 2020). While 40 µM NO_3^- is a little high compared to historical ambient nutrient concentrations at the mouth of the bay, it is well within the range of observed nutrients and blooming concentrations observed throughout the main stem of Chesapeake Bay (Harding et al., 2019, fig. 4g-i; Malone et al., 1996, fig. 6).

Line 100 and line 165: Why were pigment and chlorophyll measurements only started/shown on day 2? In the discussion, it says (in lines 396-398): "The variability in the timing of the bloom peak may be due to minor differences in the starting community that each carboy received, as seen in the dissimilarity present between replicate inoculum samples despite being filtered from the same stock of water." To get an own impression of this, it would be informative to see day 0 data for all carboys.

Reviewer #1 had the same query and a more detailed explanation is presented in our response to their comments. Pigment samples were not collected from individual carboys until day 2 because we expected pigment concentrations to be below detection early in the bloom and did not have enough water in the experimental incubations to allow filtration of a larger volume to increase

assay sensitivity. Instead, we have added pseudo day 0 pigment data to figure 1 (as shown below).



Regarding lines 396-398, this is referring the beta diversity analysis of 18S-based community composition seen in figure 4g. We thank the reviewer for pointing out that this needs clarification and will add a reference to Fig. 4g here.

Lines 104-105: The DNA samples at 12:00 were collected for all carboys? Maybe to make it clearer add that it was all carboys in that case. How did you select the days on which you additionally sampled carboy C i.e., what additional information do you gain from those additional days?

Thank you to both reviewers for pointing out the confusing wording here. We will add clarification that duplicate inoculation samples were collected for day 0, each carboy was sampled daily at ~noon, and that carboy C was additionally sampled for duplicates on certain days. We have provided proposed changes in our response to reviewer #1.

Due to constraints in sequencing capacity, we selected three relatively high biomass samples around the expected bloom transition and peak, where we expected the greatest variability. This allowed us to investigate how the magnitude of carboy-to-carboy community variability compared to potentially patchy sampling within a carboy.

Line 165: What is the assumption of a 1:100 biomass ratio of zooplankton to phytoplankton based on? Is there any literature on this that can be referenced here?

The 1:100 biomass ratio was chosen based on the assumption that the concentration of zooplankton would be comparatively low at the start of the bloom due to the 210 µm inoculum pre-filter, which would have removed large zooplankton while allowing most phytoplankton to pass through. We note that 1:100 is just the average ratio used for initialization, as the N_{NO3}, N_{Phyto}, and N_{Zoo} each varied ± 20% across model iterations.

Line 192: Which alpha diversity metric was calculated and why?

Both OTU richness and Shannon index (H) were used as alpha diversity metrics to compare our data to previous PDR studies, as the observed PDR can vary depending on if/how a diversity measure accounts for evenness. We did not initially think it was necessary to specify this in the methodology, as we often refer to general trends in alpha diversity in the results and discussion. We can add clarification in the methodology for the alpha diversity metrics used.

e.g. Line 192 → "For relative abundance and alpha diversity analyses (OTU richness and Shannon index), carboy C duplicates…"

RESULTS

Fig. 1: Why are nutrients and pigments only shown for carboy 3? I suggest including the other carboys as well. As written the text and as also seen in Fig S1, the carboys indeed behave quite similarly. But with only showing one carboy, it always seems a bit suspicious to me at first.

Thank you to both reviewers for pointing this out. Figure 1 has been updated to show carboy averages for nutrients and pigments (shown above).

Lines 221-222: I think, it is not 100% correct to say that phosphate followed a similar pattern as the other nutrients. In the next half sentence, it is already stated that different from the other nutrients, phosphate gradually decreased while the other nutrients stayed rather constant until day 4 and then rapidly decreased. Consider rephrasing the start of the sentence.

The description of phosphate concentration patterns will be reworded.

Line 223 → "Phosphate (PO_4^{3-}) concentrations decreased more consistently between days 1 and 5 (Fig. S2) from an initial average concentration of 4.0 ± 0.4 µM to 0.6 ± 0.1 µM by 18:00 day 5."

Fig. 1: Chlorophyll c which is present in the Figure 1 is not mentioned in the text, although all other pigments are mentioned. Please clarify.

Variable ratios of Chl-c to other pigments throughout the bloom can be indicative of a community shift (e.g. Chl-a to Chl-c ratio changes during the late bloom). This will be noted in the text.

Line 236 → "Pigment accumulation trends along with the concurrent consumption of SiO_4^{4-} indicate that a diatom bloom occurred, and the late–bloom decrease in Chl–a:chlorophyll c may indicate a shift in phytoplankton community following the bloom peak (Dursun et al., 2021). Additionally, the decoupling between Chl–a and POM concentrations in the late bloom resulted in a large range of POC:Chl–a ratios."

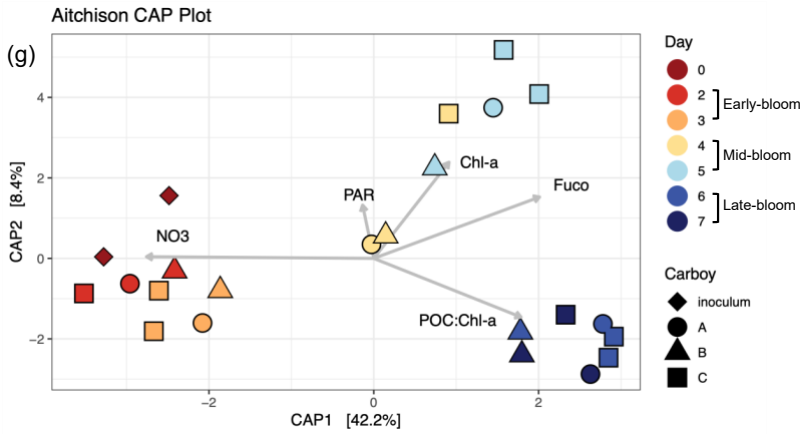
Lines 265-269: This part can be moved to the methods.

We had initially included this short paragraph to give a brief review of how OTUs were defined in our study and provide an explanation for why metazoan sequences were removed from the results presented in this section. This information is also provided with greater detail in the methodology. If the reviewers feel that this information does not need to be repeated in the results, we can remove it.

Fig. 4: Just leaving this comment here with knowing this is hard to change. While reading the paragraph from lines 274-281, I realized I am not able to tell the classes in Fig. 4 apart myself due to very similar colors. As I said, I am just leaving this here as a note.

We appreciate this note and the understanding from our reviewers. While we found this color palette to have the best color variability while remaining accessible to those with color vision deficiencies, we are open to suggestions for different color palettes.

Fig. 4: The color of the inoculum shapes is hardly visible. I know the color in this case is redundant with the shape, but maybe changing the shape to a diamond would help here.



Thank you for pointing this out. The symbol for inoculum samples will be updated as shown above so that the colors are more visible.

DISCUSSION

Line 347-348; line 354-355: These two sentences read a bit like they open a line of reasoning but close it again without giving it enough credit. While the first one says that a depletion of nutrients on day 5 led to the bloom's demise, the last sentence says that factors other than nutrient limitation need to be considered for the bloom's termination. This leaves the question open why the nutrient limitation that is mentioned in the first part is not further discussed.

We agree with both reviewers that the role of nutrient depletion in the bloom's demise should be discussed more deeply. We will add discussion (proposed changes provided above in our response to reviewer #1) of transient nutrient limitation based on the kinetics- and stoichiometry-based thresholding, as well as partial nutrient limitation estimated by the NPZ model.

Line 433: When H was already lower than other studies, but not as low as expected during a bloom, were the other studies that are referenced here not during a bloom? Whether they measure H during a bloom or not already makes quite a difference, as also mentioned in the discussion.

The Wang et al. (2024) and Cram et al. (2024) studies did not specifically target blooms, but were chosen for comparison because they reported on 18S-based analyses of the whole eukaryotic community in the main stem of the bay. Cram et al. (2024) sampled throughout Chesapeake Bay during the summer, while Wang et al. (2024) sampled along both spatial and seasonal gradients. While Wang et al. (2024) included samples which may have been collected during seasonal blooms, they found no statistical difference in H across seasons (Wang et al., fig. 3) and rarely observed H < 3 (Wang et al., fig. 4).

Line 476-477: This statement needs some references, I think, even though some are mentioned before in the text.

We will add another citation for Smith 2007 and clarify that this is for aquatic systems.

SMALL CORRECTION:

Line 536: "(c) particulate organic carbon (POC) and …" instead of "particulate organic (c) carbon (POC)" in description of Fig. 3.

Thank you! We will update the wording.

*Fig. 3 caption → "**Figure 3:** … and (c) particulate organic carbon (POC) and (d) particulate organic nitrogen (PON) concentrations. …"*

References

Adolf, J. E., Yeager, C. L., Miller, W. D., Mallonee, M. E., and Harding, L. W.: Environmental forcing of phytoplankton floral composition, biomass, and primary productivity in Chesapeake Bay, USA, Estuar. Coast. Shelf Sci., 67, 108–122, https://doi.org/10.1016/j.ecss.2005.11.030, 2006.

Conley, D. and Malone, T.: Annual cycle of dissolved silicate in Chesapeake bay: implications for the production and fate of phytoplankton biomass, Mar. Ecol. Prog. Ser., 81, 121–128, https://doi.org/10.3354/meps081121, 1992.

Cram, J. A., Hollins, A., McCarty, A. J., Martinez, G., Cui, M., Gomes, M. L., and Fuchsman, C. A.: Microbial diversity and abundance vary along salinity, oxygen, and particle size gradients in the Chesapeake Bay, Environ. Microbiol., 26, e16557, https://doi.org/10.1111/1462-2920.16557, 2024.

Dursun, F., Tas, S., and Ediger, D.: Assessment of phytoplankton group composition in the Golden Horn Estuary (Sea of Marmara, Turkey) determined with pigments measured by HPLC-CHEMTAX analyses and microscopy, J. Mar. Biol. Assoc. U. K., 101, 649–665, https://doi.org/10.1017/S0025315421000631, 2021.

Harding, L. W., Mallonee, M. E., Perry, E. S., Miller, W. D., Adolf, J. E., Gallegos, C. L., and Paerl, H. W.: Long-term trends, current status, and transitions of water quality in Chesapeake Bay, Sci. Rep., 9, 6709, https://doi.org/10.1038/s41598-019-43036-6, 2019.

Huang, K., Feng, Q., Zhang, Y., Ou, L., Cen, J., Lu, S., and Qi, Y.: Comparative uptake and assimilation of nitrate, ammonium, and urea by dinoflagellate Karenia mikimotoi and diatom

Skeletonema costatum s.l. in the coastal waters of the East China Sea, Mar. Pollut. Bull., 155, 111200, https://doi.org/10.1016/j.marpolbul.2020.111200, 2020.

Malone, T. C., Conley, D. J., Fisher, T. R., Glibert, P. M., Harding, L. W., and Sellner, K. G.: Scales of Nutrient-Limited Phytoplankton Productivity in Chesapeake Bay, Estuaries, 19, 371, https://doi.org/10.2307/1352457, 1996.

Wang, H., Liu, F., Wang, M., Bettarel, Y., Eissler, Y., Chen, F., and Kan, J.: Planktonic eukaryotes in the Chesapeake Bay: contrasting responses of abundant and rare taxa to estuarine gradients, Microbiol. Spectr., 12, e04048-23, https://doi.org/10.1128/spectrum.04048-23, 2024.