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.

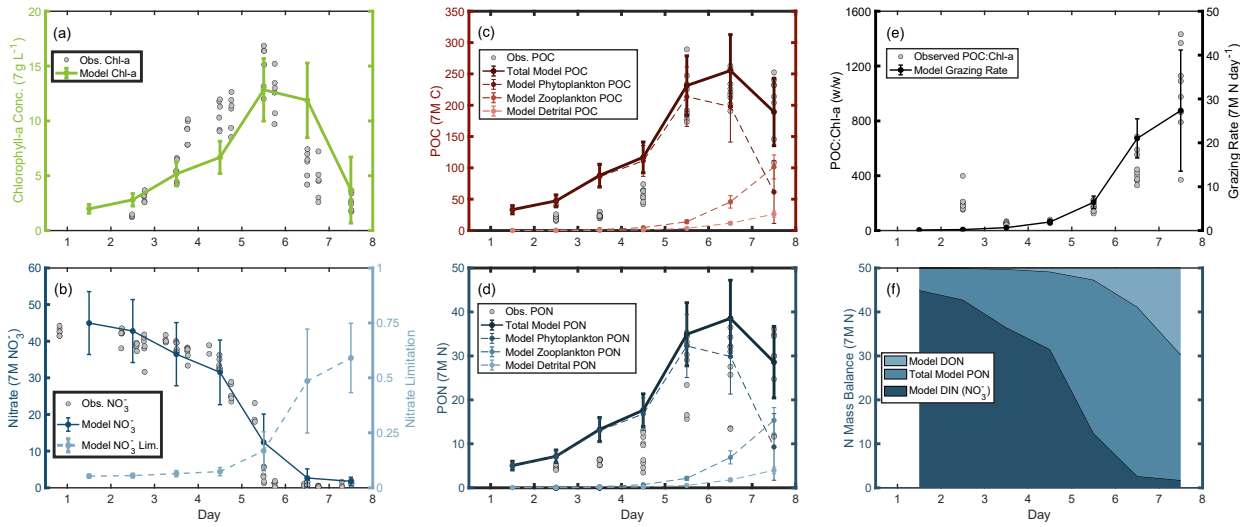
Reviewer 1 (Citation: https://doi.org/10.5194/egusphere-2025-871-RC1**)**

General Comments:

This study conducted a microcosm experiment using a natural Chesapeake Bay phytoplankton community to evaluate bloom dynamics over time. The authors present a thorough and well-written manuscript which captures trends in diversity, particulates, and stoichiometry during their 8-day incubation. Their data clearly shows bloom initiation and termination, as well as a negative productivity-diversity relationship, and provides well thought out explanations for each of these observations. However, there is a strong focus on grazing as the cause of bloom termination, which seems to be driven by results from the COBALT simulation. Though there is some support for this conclusion, I would urge the authors to be more cautious as I don't believe there is sufficient data on grazing (rates, community etc.) to conclude that grazing was the main driver of bloom termination, though it may have played a role. Conversely, there seems to be a lot of data to support the impact of nutrient depletion on bloom dynamics (uptake rates, POC:Chl etc.), but this explanation was not fully explored. At elevated summer temperatures and in a closed system, nutrient depletion is likely to have contributed, and these factors should be considered in greater depth. Additionally, the authors could also discuss how a closed-system, like the one presented here, might compare to dynamics in the open ocean.

Overall, this study was compelling and well-constructed, incorporating diverse methodology, from DNA to an NPZ model, in order to evaluate phytoplankton bloom dynamics. In particular, this study provides valuable insight into how phytoplankton diversity changes throughout a bloom. Some additional discussion could make this study even more broadly applicable.

Thank you for your kind words and valuable input! We agree that the study would benefit from a deeper discussion of the role of nutrient limitation in our microcosm experiment. One analysis that we will add is the modeled nitrate limitation. The N_{lim} parameter (eqn. S6) determines the amount of phytoplankton growth permitted by the available nutrients as a fraction of potential growth, where $N_{lim} = 1$ indicates no nitrate limitation and $N_{lim} = 0$ indicates complete limitation. Therefore 1- N_{lim} represents the limitation and has been added to figure 3b as below.



We had initially anticipated that nutrient depletion would be the driver of the bloom's demise because the 210 µm inoculum pre-filter was meant to prevent large grazers. However, we came to suspect grazing as a factor due to the observed "gap" in N mass balance (prior to modeling), leading us to use the model to investigate that possibility further. We have detailed in our responses below other ways which we will be addressing nutrient depletion. We will also add a note on the impacts of a closed system experiment to section 4.6.

Specific Comments:

The methods are described well and are generally easy to follow.

Line 91: Were nutrients ever replenished in these microcosms or just spiked initially?

We will add clarification that the incubations did not receive any further nutrient additions.

Line 91 → "Carboys were incubated for 8 days … without further nutrient additions in an on-deck…"

Line 93: What was the exact temperature during the deck-board incubations?

The temperature data were presented in figure S7, but not previously discussed in the main text. We will add a sentence to the beginning of the results (line 212) stating the range of light and temperature measurements

*Line 93 → "Light (photosynthetically active radiation; PAR) and temperature displayed typical diel cycles during the incubations ranging 22.5-29°C, with average daily temperature$_{max}$ = 28.0 °C and average daily PAR$_{max}$ = 77.2Wm^{-2} (median = 59.3 Wm^{-2}) (**Fig. S7**). Daytime PAR was lowest on day 3 and reached a maximum of 242.8 Wm^{-2} at 14:00 on day 7."*

We will also clarify in the methods (line 93) that the continuous measurements were only taken between 18:00 on day 1 and 18:00 on day 7. Fig S7 will be updated to show this temporal range and the caption will be updated to clarify data averaging.

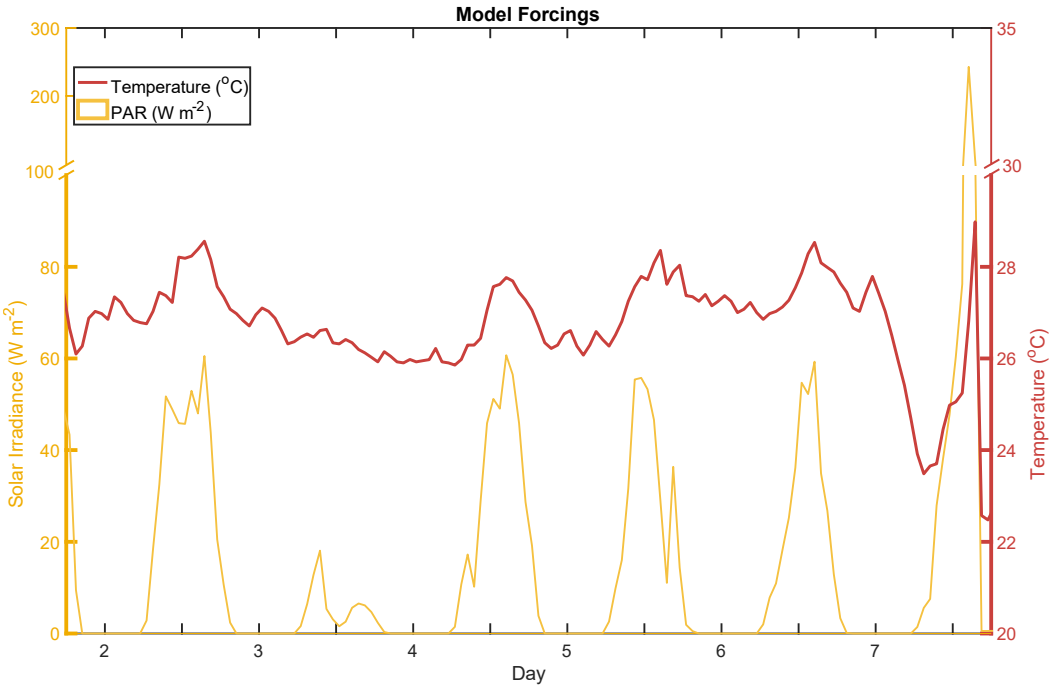


*Fig. S7 caption → "**Figure S7: Light and temperature.** Continuous light (yellow shaded region) and temperature (red line) measurements are plotted for days 1-7 of the microcosm experiment. Duplicate loggers were combined and measurements were averaged along hourly intervals (e.g. the value plotted for 12:00 is the average of all measurements taken between 11:30 and 12:30 from both data loggers). Note scale change above the y-axis break."*

Line 105: This section is unclear. As I understand, DNA analyses were conducted for all carboys and technical duplicates were done for carboy C only. However, this paragraph suggests that DNA was only analyzed from carboy C. Please reword so this can be clarified.

Thank you for bringing this to our attention. This will be clarified in the methodology.

Line 105 → "DNA samples were taken from each carboy concurrently with the 12:00 pigment and nutrient samples. Additional duplicates were collected on select days for carboy C and two additional DNA samples were collected from the surface water inoculum."

Line 157: Is it reasonable to include a medium-sized copepod when these were filtered out from the microcosm (i.e. how big is the model grazer)? Why not a small zooplankton (e.g. small copepod or heterotrophic dinoflagellate)? How might this have impacted modeled grazing rates or grazing preferences?

The medium sized zooplankton (copepods) in the model represent the parameters of ~200–2,000 µm equivalent spherical diameter zooplankton (Stock et al., 2020). Given that the inoculum was pre-filtered with a 210 µm mesh, but the DNA analysis found Maxillopoda spp. and *Acartia tonsa* (which can grow much larger than 200 µm), we suspect that the zooplankton community started small and grew over the course of the bloom. Alternatively, the small zooplankton class have a higher/faster ingestion rate, and would likely have resulted in a shorter bloom with lower peak chlorophyll. Therefore, the medium size class was selected as an appropriate estimate of the dominant grazer activity.

While it might be more realistic to have used a range of phytoplankton and zooplankton sizes, the goal of this study was to test if a simplified NPZ model could replicate the observations and provide general insight on potential community dynamics.

Line 165: Did this assumption of phytoplankton biomass include an estimate of growth from day 1 to 2? What was the reasoning for not measuring chlorophyll on day 1?

The model day 1 phytoplankton biomass did not explicitly include growth between days 1 and 2. Instead, an average concentration of N_{Phyto} = 5 µmol kg^{-1} ± 20% was chosen to balance an initial phytoplankton population which was large enough to trigger a bloom, but small enough to roughly match day 2 chlorophyll concentrations.

Pigment samples were not collected on day one because we expected pigment concentrations to be below detection within the first 24 hours of incubation, so we did not take time zero or day 1 samples given our sample volume restrictions. As a result, we don't have individual carboy data for that day. However, a pseudo day 0 pigment analysis has been added to figure 1 (now showing the avg. pigment data across carboys). Two day 0 samples were produced by filtering ~1L each of a 10% dilution of inoculum in 0.3um filtered media immediately after inoculation and without receiving any additional nutrients. These details will be added to the methodology and clarified in the figure caption.

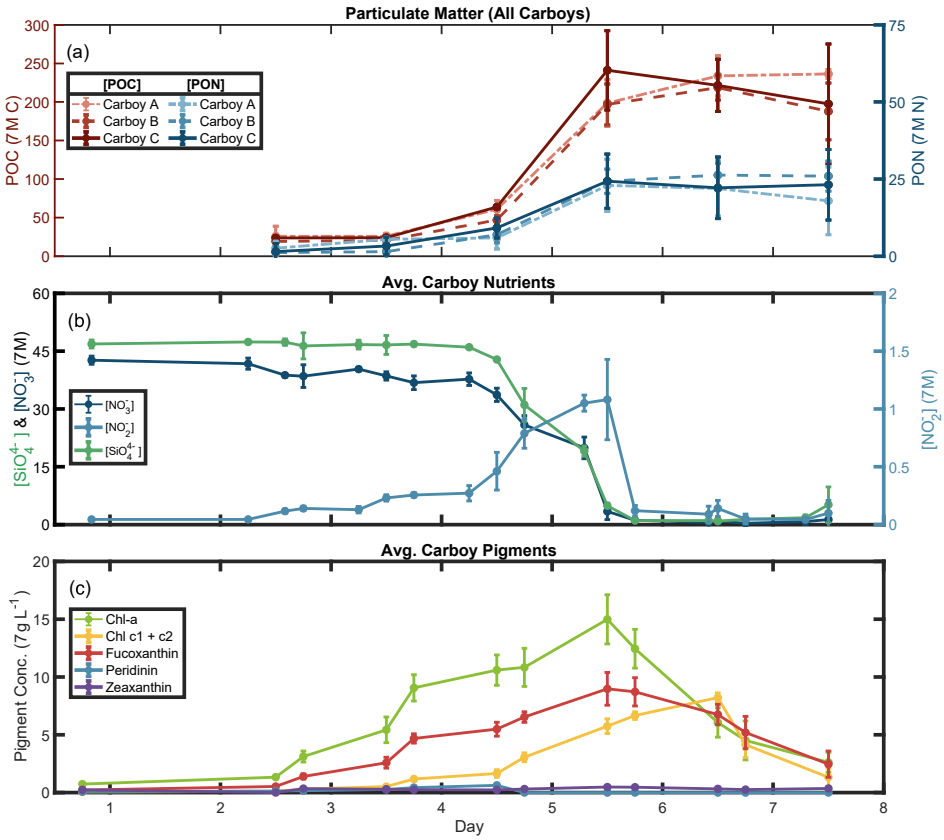


*Fig. 1 caption → "**Figure 1: Biogeochemical data indicate a bloom.** (a) Particulate organic carbon (POC) and nitrogen (PON) concentrations for all carboys over the course of the bloom simulation. POC is in red/pink, PON is in blue, and line type denotes carboy. Error bars in (a)*

represent the standard deviation of sample replicates. Average carboy concentrations are shown for (b) nutrients and (c) pigments (data for individual carboys are presented in Fig. S1). (b) Nutrient measurements during the bloom simulation are presented as dark blue lines for nitrate ($[NO_3^-]$), light blue lines for nitrite ($[NO_2^-]$), and purple lines for silicate ($[SiO_4^{4-}]$). (c) Pigment concentrations for chlorophyll a (Chl-a), chlorophyll c (Chl c1 + c2), and diagnostic pigments for diatoms (fucoxanthin), dinoflagellates (peridinin), and cyanobacteria (zeaxanthin). Error bars in (b, c) represent the standard deviation between carboys. Day 0 pigment concentration is from a separate 10% inoculation "dilution" (described in the methodology), rather than individual carboys. Time is shown as days since carboy inoculation where day 1 begins at 00:00 following inoculation. The grey shaded region indicates the peak bloom (~noon day 5)."

Figure 1. This figure is nicely laid out to illustrate dynamics in the microcosm. It might be worth considering to use an average across all carboys, as in panel a, rather than just focusing on carboy C. Alternatively, figure S1 could be substituted here.

We agree with the reviewer that carboy averages are more appropriate for nutrient and pigment concentrations and figure 1 has been updated accordingly.

Line 240. The POC:Chl increases on day 6 are also consistent with phytoplankton becoming nutrient limited. See Jakobsen and Markager (2016), L&O or Arteaga et al. (2016), Glob. Biogeochem. Cycles. Generally, under replete conditions, phytoplankton cells tend to allocate greater resources to chlorophyll synthesis and growth, resulting in an inverse C:Chl – nutrient relationship. So, these trends could be a nutrient limitation signal as well as a grazing signal.

Thank you for your input and additional references. The relationship between nutrient availability and phytoplankton POC:Chl-a was a factor we considered and we do believe that it played a role in both the low ratios observed during the peak bloom and high ratios toward the end of the bloom. However, ratios >1000 are unlikely to be due solely to variable phytoplankton POC:Chl-a. This will be clarified in the discussion section (line ~362), but we provide additional detail below.

Figure 3e may have also caused confusion because it previously incorrectly displayed maximum POC:Chl-a < 250 even though ratios exceed 1000 (as in fig S3d-f). This has been fixed!

Model outputs from Arteaga et al. (2016, fig. 9) and Behrenfeld et al. (2005, fig. 2, 3) predict maximum phytoplankton POC:Chl-a of ~200 and ~300, respectively, and only in very low productivity regions and seasons, with nearly no available nutrients. Additionally, observation-based studies have found maximum values < 350 (Jakobsen and Markager, 2016, and the references therein), and diatom culture experiments (Laws and Bannister, 1980, from Behrenfeld et al. 2005 fig 3c) reported maximum POC:Chl-a < 500 in their highest nutrient stress and lowest growth rate conditions. Therefore, while phytoplankton-specific POC:Chl-a may have increased during the late-bloom, an increase non-phytoplankton biomass was also necessary to explain the observed ratios.

We will add to line 240 that phytoplankton-specific POC:Chl-a may also play a role in the high late-bloom ratios and clarify in the discussion (line ~363) why non-phytoplankton biomass must also be accumulating.

Line 240 → "Late bloom ratios were much higher, reaching an average POC:Chl–a > 1000 on day 7, with maximum values of up to 1258.6 ± 247.2 in carboy B (Fig. S3d–f), suggesting the accumulation of non–phytoplankton biomass in the late bloom and a potential shift in phytoplankton POC:Chl–a."

Line 276: How do you compare the low peridinin concentration with the high dinoflagellate relative abundance?

We were also initially surprised by the high relative abundance of dinoflagellates in the absence of peridinin. However, the primary dinoflagellates present in the DNA data were most closely related to *Karenia mikimotoi*, which do not use peridinin as their primary pigment. Based on additional accessory pigment analysis, we determined that the relative abundance of dinoflagellates was a result of high gene copy numbers (line 414-425). We go into more detail on the pigment analysis in the supporting material (Supporting analysis, lines S149-169).

Figure 4g – This figure is great. It shows changes over time, biological replication, and drivers all in one figure.

Thank you!

Line 352-355: This is a bit confusing. There only needs to be one limiting nutrient to cause a bloom decline. Here, the authors present evidence for a limiting nutrient on days 5 and 6, which is consistent with when the bloom crashes. It seems nutrients are being prematurely dismissed, but I would argue that they should be given greater focus and discussion in this manuscript.

We agree that the phrasing, especially "consistently limiting" was a bit confusing. We will update the text to note that nutrients may have been transiently limiting following the Liang et al. (2019) thresholds – i.e. met criteria for limitation in one sample, but not the following timepoint and not in multiple carboys at the same time, with the exception of the two timepoints already listed in the main text.

Additional discussion of nutrient's role in the bloom's demise will be added here, both in terms of the threshold-based limitation and the model estimations of nitrate limitation (added to fig 3).

Line 352 → However, following the combined kinetics– and stoichiometry–based thresholds outlined in Liang et al. (2019), nutrients were only transiently limiting during the latter half of the bloom (Table S7). There were only two timepoints when nutrients were limited in more than one carboy: SiO_4^{4-} on the evening of day 5 and NO_3^- on the evening of day 6, and neither of these nutrients were limited in back–to–back samples. Additional analysis of the NPZ model revealed that nutrients likely became partially limiting following the bloom peak. Modeled nutrient limitation did not match the timing of threshold–based limitation, but did reach an average maximum of ~60% at the end of the blooming period (Fig. 3b). The combination of incomplete or transient nutrient limitation and consistently high POM during the late–bloom indicate that factors other than nutrient availability likely contributed to the bloom decline; the potential role of grazing is further investigated below.

Line 363 – Again, POC:Chl can also be a sign of nutrient limitation. Please review the above references.

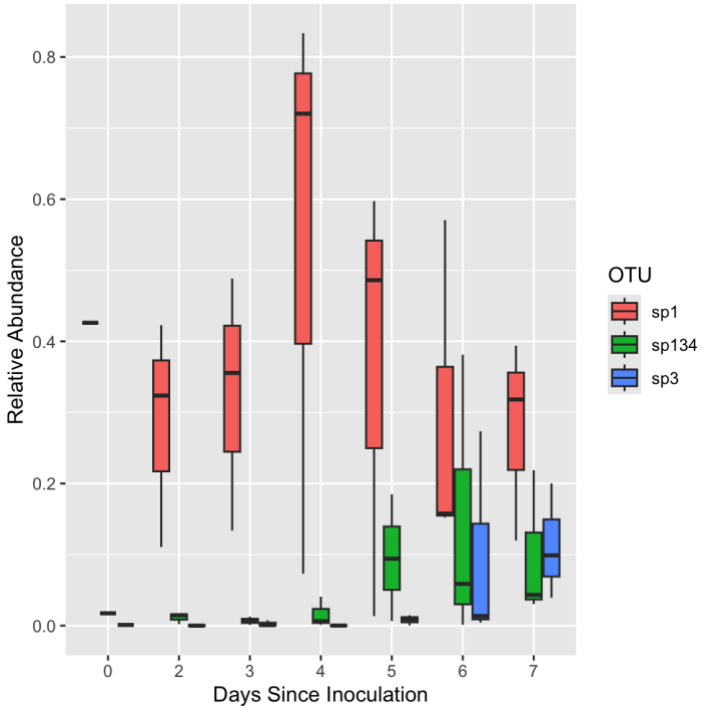
We agree that nutrient limitation likely led to increased phytoplankton POC:Chl-a during the late-bloom, but must conclude that an accumulation of non-phytoplankton biomass was also necessary to explain the observed POC:Chl-a > 1000. As noted above, we will add additional clarification for our reasoning.

Line 362 → "In contrast to the low peak bloom POC:Chl–a, the extreme POC:Chl–a values observed at the end of the carboy incubations far exceeded both the average phytoplankton POC:Chl–a of ~40–90 observed in Chesapeake Bay (Cerco 2000 and the references therein) and previous observations of maximum phytoplankton POC:Chl–a <500 due to nutrient limitation (e.g.; Jakobsen and Markager, 2016; Laws and Bannister, 1980), with average day 7 values over 10–fold greater than expected for the region. Low available nutrients during the late–bloom may have contributed to an increase in POC:Chl–a, however high total community POC:Chl–a is primarily influenced by the ratio of phytoplankton POC to zooplankton and detrital POC (Banse 1977)."

Line 365: Could these metazoan sequences result from copepod detritus?

It is possible that a portion of the metazoan sequences were from detritus or external DNA. However, if metazoan DNA was primarily present detritus, we would expect the relative abundance of all metazoan OTUs to be highest at the beginning and decrease over time, as the DNA is degraded and the POM of living organisms increased. Instead, our 3 arthropod OTUs have distinct relative abundance patterns – peaking at different points in the bloom.

In the figure below, sp1 is a Maxillopoda spp., sp3 is *Acartia tonsa*, and sp134 is a combination of "other" arthropods.



Lines 470-477: It may also be worth noting that this incubation was a closed system design and thus likely is unable to capture all the diversity patterns that exist in an open system. A closed

system prevents both immigration/emigration and nutrient replenishment which could have impacts on diversity metrics.

We agree that micro- and mesocosm experiment alpha diversity is bounded by the organisms present in the initial inoculation. This will be noted in the text.

Line 472 → "The issue of regionality may occur because a range of several orders of magnitude … because taxa appear more cosmopolitan at small scales (Smith 2007). The latter effect may be heightened in micro- and mesocosm experiments where diversity is bounded on both ends by the inoculum community. It is also true for many generalist microbes regardless of study scale and may also explain why …"

Line 497: While grazing may have contributed significantly to the observed trends, I still think its important not to discount the role that nutrients may have had on bloom termination. This is briefly stated on lines 509-511, but could be expanded on throughout.

Agreed; the impact of nutrient limitation will also be noted in the conclusion.

Technical Corrections:

Line 36: Maybe "silica cell walls" instead of "silica shells?"

We will correct "shells" to "cell walls."

Line 336-337: This could be moved to the results.

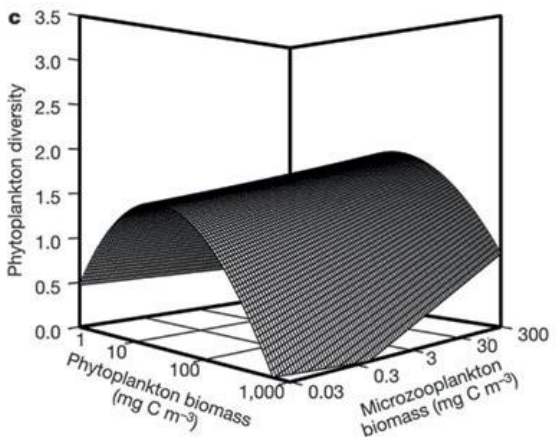
Thank you for pointing this out. We will move the significance values (Kruskal-Wallis p-values) to the results section (line 252).

References: Sal et al 2013 (Figure 7) is missing from the references.

Thank you for catching this. The citation will be added to the references!

Line 470: It's difficult to distinguish the unimodal relationship in the global dataset. Could the points be made transparent (in R, use 'alpha'), to help visualize the density of points?

We will clarify in the main text that the canonical unimodal PDR is defined not only by well delineated curves, but also by clusters of points bounded by a unimodal curve (Smith, 2007). As noted in Skácelová & Lepš (2014), "biomass can be important in regulating the upper limit of diversity, whereas at all the biomass values, extremely species poor communities are found." We will also note that the well delineated unimodal curve in Irigoien et. al. (2004) showed that phytoplankton- or zooplankton-specific diversity was a function of both phytoplankton and zooplankton biomass as separate parameters. Collapsing Irigoien et al.'s 3D model (2004, fig2c shown below) into two dimensions broadens the range of expected diversity at a given biomass.



Line 470 → "While a unimodal PDR, either expressed as a unimodal curve or a cloud of points whose upper bound is defined by a unimodal curve (Skácelová and Lepš, 2014; Smith, 2007), may be observed in regional studies, it may not apply to local diversity patterns, which can appear monotonic (Rosenzweig, 1992). This can be seen when comparing the combined global and local data as a whole to individual localized experiments (Fig. 7a,b). Excluding the much higher 18S-derived diversity of this study, the upper bounds of all microscopy-derived data clearly displayed unimodal patterns and the data fit flatter, though still significant, unimodal curves. Additionally, Irigoien et. al. (2004) showed that phytoplankton- or zooplankton-specific diversity was a function of both phytoplankton and zooplankton biomass as separate parameters, making the unimodal PDR curve of combined factors less distinctly defined. Contrastingly, the individual localized micro- and mesocosm studies displayed both positive and negative monotonic PDRs. The issue of regionality…all commonly reported in aquatic microbial PDR studies (Graham and Duda, 2011; Skácelová and Lepš, 2014; Smith, 2007). It is important to note that the potential diversity at any given time in incubation studies is bounded by the organisms present in the inoculum. However, aside from unimodal…natural aquatic microbial communities (Smith, 2007)."

We can also add a quadratic regression curve fitted to all microscopy-derived diversity data, to figure 7a,b by modifying the equation outlined in Irigoien et al. (2004). We will note in the caption and the main text which data is included in the curve, and in the caption that it is representative of 3D model simplified to 2 dimensions. The colors and alpha in figure 7 will be changed to make visualization clearer and the colors figure S6 will be changed for consistency.

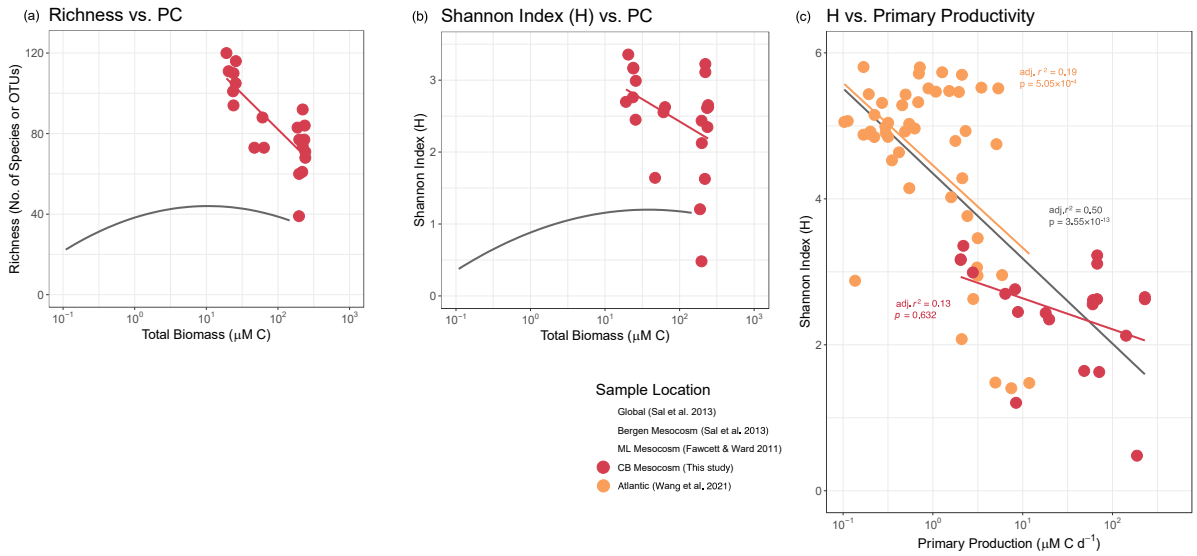


Fig. 7 caption → "Figure 7: Productivity-Diversity Relationship (PDR). Several studies were compared using 3 different PDRs. (a,b) The global (Sal et al., 2013) and the Moss Landing (ML, Fawcett and Ward 2011) diversity data were obtained via microscopy and the Bergen mesocosm data was extracted from the global dataset. (c) The Atlantic (Wang et al., 2021) and Chesapeake Bay (CB, this study) diversity data were calculated from 18S-based OTUs. The Atlantic Net Community Production rates were converted to Primary Production rates according to Li & Cassar (2016, eq. 7). Quadratic regression curves, representing a 2D simplification of the Irigoien et al. (2004) 3D model, and linear regressions are plotted for select datasets. Shaded regions around regressions indicate the 95 % confidence interval and the adjusted r^2 and model significance are listed in the respective color of a given dataset. Grey regressions indicate combined datasets. The grey curves in (a, b) are fitted to all microscopy-based diversity data and the grey line in (c) is the linear regression for the combined Atlantic and Chesapeake Bay datasets."

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