We would like to thank the reviewer for the comprehensive reviews for our paper (MS No.: egusphere-2026-2917). We have gone over all the issues raised by the reviewer and revised the manuscript accordingly. These comments provided much assistance with reshaping and clarifying the manuscript. We hereby present point-by-point answers to the issues raised by the reviewers. Our answers are in blue.

Anonymous Referee #2

The authors present a detailed examination of carbon cycling in the upper 100 m of the water column in the oligotrophic Gulf of Aqaba across late spring to late summer, using a series of incubation experiments. They conclude that stratification-driven nutrient limitation in the euphotic zone leads to rapid recycling of photosynthetically produced organic carbon, with a large fraction of this carbon rapidly shunted to the dissolved pool. They further situate these findings within the context of climate-driven upper-ocean stratification and associated declines in surface production, and call for additional work extending to longer time periods and more oligotrophic regions.

Reply: We thank the reviewer for his/her positive view of our manuscript.

The manuscript reads well, although a key methodological concern is the use of GF/F filtration to partition 14C-labeled carbon into particulate versus dissolved fractions, given that the study's own premise predicts a shift toward smaller cell sizes under stratification and nutrient limitation. GF/F has a pore size of ~0.7 µm and is known to under-retain picoplankton in this size class, particularly Prochlorococcus-like cyanobacteria (~0.6 µm), which means a fraction of labeled carbon residing in intact small cells could pass into the filtrate and be misclassified as dissolved organic carbon rather than particulate biomass. Because this artifact would bias the partitioning in the same direction as the paper's central claim, it is difficult to distinguish a genuine increase in extracellular release from an artifact of size-selective filter breakthrough as the community shifts toward smaller cells.

Reply: Several methodological precautions and empirical considerations confirm that the observed shift toward higher PP_{DOC} fraction during stratification represents a genuine biological signal rather than a filtration artifact:

1. Studies evaluating picoplankton retention on GF/F filters show that they retain the vast majority of *Prochlorococcus* cells under gentle vacuum pressure. For example, in an axenic *Prochlorococcus marinus* MED4 culture, Bertilsson et al. (2003; L&O) found ~95% capture on Whatman GF/F filters under low vacuum (the same as we used). Additionally, although not directly addresses *Prochlorococcus* but more broadly phytoplankton, Chavez et al., (1995 L&O) reported that GF/F and 0.2 µm membrane filters recovered equivalent total chlorophyll *a* in oligotrophic open-ocean samples, suggesting that GF/F filtration is not likely to cause a significant material loss under similar sampling conditions.
2. If PP_{DOC} increases were an artifact of *Prochlorococcus* breakthrough, we would expect PP_{DOC} percentages to peak strictly when *Prochlorococcus* numerical dominance was highest (that is, >80 m around the DCM). Instead, the relative increase in PP_{DOC} and PER aligns with physiological stress indicators (nutrient exhaustion) rather than cell density shifts alone.

3. GF/F filtration has been the standard approach for PP measurements for several decades and has been applied extensively across a wide range of marine systems, including the GoA (e.g., Riech et al., 2024 that summarize a decade of monthly measurements). We therefore chose to retain this methodology to ensure consistency with the large body of existing literature and to facilitate direct comparison of our results with previous studies. Importantly, similar methodological approaches have also been used in published studies from other oceanic regions. For example, POC production has been quantified as the fraction retained on APFF glass-fiber filters, which are operationally comparable to GF/F filters, while DOC production was measured in the corresponding glass-fiber filtrate (Teira et al., 2001 MEPS). Thus, our approach is consistent with established and previously published methodologies for partitioning primary production between particulate and dissolved fractions.
4. To ensure cellular integrity and prevent both cell breakthrough and rupture during filtration, all samples were filtered under low vacuum pressure of 50 mmHg.

We clarified this in the revised text in sections 2.3 (Methods) and 4.1 (Discussion):

"...Filtration was performed under gentle vacuum pressure to minimize cell disruption, intracellular carbon release, and cell breakthrough. Although GF/F filters have a nominal pore size of ~0.7 µm, their depth-filtration properties allow efficient retention of small picocyanobacterial (e.g., *Prochlorococcus*) under low-pressure filtration conditions (Bertilsson et al., 2003)..." (Lines 113-17).

"...We acknowledge that the nominal ~0.7 µm pore size of GF/F filters may raise concerns regarding the potential passage of small picoplankton, particularly *Prochlorococcus*. However, previous studies indicate that cell breakthrough is minimal under low-vacuum filtration conditions. For example, Bertilsson et al., (2003) showed that ~95% of cells in an axenic culture of *Prochlorococcus marinus* MED4 were retained on Whatman GF/F filters under low vacuum, comparable to the conditions and filters used in our study. In addition, although not specifically addressing *Prochlorococcus*, Chavez et al., (1995) reported equivalent recovery of total chlorophyll a using GF/F and 0.2 µm membrane filters in oligotrophic open-ocean samples, suggesting that GF/F filtration is unlikely to result in substantial loss of phytoplankton biomass (and thus production) under similar conditions. Moreover, the observed seasonal increase in PER (Figure 2D) was associated with changes in nutrient availability and microbial metabolism rather than simply tracking chlorophyll biomass. These observations therefore support the interpretation that the increase in extracellular release reflects a physiological response rather than an artifact of size-selective filtration..." (Lines 439-452).

Furthermore, this concern is compounded by another potential sources of error in the 14C-PER approach that are not addressed in the manuscript, for example, possible adsorption of labeled dissolved or colloidal material onto the glass fiber matrix (which would bias in the opposite direction, inflating the particulate fraction). Given that the paper's mechanistic conclusion rests entirely on this filter-based partitioning, I would recommend the authors either validate their GF/F separation against a defined 0.2 µm membrane filter (or a size-fractionation series) in this system, or explicitly address why filter breakthrough of small cells cannot account for the observed DOC-dominant signal.

Reply: We agree that filter-based separations may involve inherent trade-offs, including both cell breakthrough (discussed above) and the adsorption of ^{14}C-labeled dissolved or colloidal organic matter onto the glass fiber matrix. Nevertheless, the fact that we observe a clear, statistically significant shift toward elevated PP_{DOC} and PER during summer stratification despite any potential filter adsorption of labeled DOC demonstrates that the extracellular release signal is robust and unlikely to present a significant adsorption bias.

"...Additionally, abiotic retention or adsorption of ^{14}C-labeled dissolved organic matter onto the glass-fiber matrix may lead to biased PP_{POC} and, consequently, of PER (López-Sandoval et al., 2018; Maske and Garcia-Mendoza, 1994). Nevertheless, the shift toward elevated PP_{DOC} and PER as summer stratification progress, despite this potential matrix adsorption, suggest that enhanced extracellular release is a genuine physiological feature of nutrient-stressed autotrophs in the study area rather than a filtration artifact..." (Lines 452-458).

Below are some minor comments:

Line 118, describe how inorganic carbon is removed.

Reply: Acidification drops the sample pH below 2, shifting the carbonate equilibrium so that all remaining dissolved inorganic carbon ($H^{14}CO_3^-$) is converted into $^{14}CO_2$ gas. The samples were then left open in a radioactive fume hood overnight to allow the $^{14}CO_2$ gas to fully outgas into the atmosphere, ensuring only organic carbon remained in solution.

"...Filters were rinsed with 5 ml of sterile-filtered seawater collected from the same site (<0.22 µm) and the samples were subsequently acidified with 50 µl of 37% hydrochloric acid (HCl, lowering pH to <2) and left open in a fume hood overnight to allow conversion of all DIC to pCO_2 and complete degassing and removal of inorganic carbon as $^{14}CO_2$..." (Lines 119-122).

Line 128, what's "bicarbonate evaporation"?

Reply: Removed.

Line 180, what type of filters are used for nutrient sample collection. Some of the NOx values look suspicious, i.e., the high values in 20 and 40 m in June and August, respectively.

Reed, M.H., Strope, E.K., Cremona, F., Myers, J.A., Newell, S.E. and McCarthy, M.J., 2023. Effects of filtration timing and pore size on measured nutrient concentrations in environmental water samples. Limnology and Oceanography: Methods, 21, 1-12.

Reply: Nutrient samples were collected directly without prefiltration and frozen immediately until analysis. The localized elevated Nox values represent isolated outliers. Similar sporadic NOx concentrations have been previously reported in the Gulf of Aqaba and (e.g., Meeder et al., 2012; Reich et al., 2024). Importantly, these isolated points do not significantly affect the seasonal patterns or the depth-integrated NOx inventories presented in Table 1. We have updated Section 2.2 in the Methods to state that samples were unfiltered and added a brief note in the Results regarding these outlier values and their negligible effect on integrated inventories.

"...Samples were collected in acid-washed plastic Falcon tubes without prefiltration and kept frozen at -20 °C until analysis within a few months..." (Lines 183-184).

"...The sporadic elevated NOx concentrations (e.g., 20 m in June and 40 m in August, Figure 1B) likely represent outlying measurements associated with the analysis of unfiltered and freezed samples (Krom et al., 2005; Reed et al., 2023). Similar sporadic NOx peaks were previously reported in the Gulf of Aqaba using similar methodology (Meeder et al., 2012; Reich et al., 2024; Rahav et al., 2026). Importantly, these values have a negligible effect on the depth-integrated NOx inventories (Table 1) and do not alter the broader seasonal patterns..." (Lines 228-234).

Line 212, add MLD in the nutrient plots to show the relative position. Also it would be useful to show actual euphotic zone, not simply 0-100 m.

Reply: We now include the MLD in Figure 1A and refer the reader to Table 1 for additional details. The euphotic zone in the Gulf of Aqaba typically extends to ~100 m during summer, although seasonal and interannual variability has been reported with the 1% PAR depth reaching ~110 m during summer (Stambler, 2006; Dishon et al., 2011). During our study, irradiance at 100 m corresponded to 0.5-1.8% of surface PAR. This information has now been added to Table 1 and the Results: "...Photosynthetically active radiation (PAR) ranged from 1200-1950 µmol quanta m^{-2} s^{-1} at the surface and declined exponentially with depth, reaching <20 µmol quanta m^{-2} s^{-1} at 100 m, equivalent to 0.5-1.8% of surface irradiance (Table 1)..." (Lines 221-224).

Line 251, remove "to".

Reply: Corrected.

Line 358-360, incomplete sentence.

Reply: Corrected. The sentence now reads: "...PP_{DOC} remains concentrated in the upper water column and is relatively stable over the season, while PER increases both with depth and over time, reaching its highest values during periods of strongest stratification (Figure 2)..." (Lines 375-378).