10 August 2026

We would like to thank the reviewers for the comprehensive reviews for our paper (MS No.: egusphere-2026-2917). We have gone over all the issues raised by the reviewers 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 #1,

This manuscript presents a valuable dataset on microbial carbon cycling during the stratified season in the Gulf of Aqaba. By combining measurements of particulate and dissolved primary production, bacterial production, community respiration, and ancillary environmental parameters, the authors provide a useful perspective on how seasonal stratification may alter carbon allocation between particulate and dissolved pathways in an oligotrophic marine system. The study addresses an important question and the dataset is unique. I found the manuscript generally well organized and suitable for publication after revision. However, several aspects of the analyses and interpretations require clarification and some conclusions should be more cautiously framed.

Reply: We thank the reviewer for his/her positive view of our manuscript. Please see below descriptions of the revisions made to address all comments in full.

1. One of the main conclusions of the manuscript is that increasing stratification shifts carbon cycling toward dissolved pathways and enhances microbial recycling. Since both the percentage of PER and BGE were calculated, it would be informative to directly examine whether these two quantities are related. For example, is BGE higher when a larger fraction of newly fixed carbon is released as dissolved organic carbon? A simple correlation analysis between PER and BGE could provide additional support for the proposed coupling between dissolved carbon release and bacterial carbon utilization.

Reply: We agree with the reviewer that testing the relationship between PER and BGE is a logical step. However, PER and BGE were not statistically correlated across the euphotic water column ($r=0.106$, $p=0.59$). This lack of correlation is primarily driven by opposing vertical gradients, where PER typically increases with depth near the base of the euphotic zone (Fig. 2D), while BGE usually peaks in warmer surface waters (Fig. 3C). Additionally, PER reflects the proportion of fixed carbon exudation rather than its C:N:P stoichiometry or molecular lability; under nutrient stress, carbon-rich exudates are often rapidly respired (overflow metabolism) rather than converted into bacterial biomass. Nevertheless, absolute rates of PP_{DOC} correlated significantly with BP ($r=0.50$, $p<0.01$, Fig. 4), confirming that newly exuded dissolved organic carbon serves as an important substrate fueling heterotrophic biomass production in this system.

We have added a brief clarification regarding the relationship between PER, PP_{DOC}, and BGE in Section 4.1:

“...Under sustained stratification, reduced nutrient supply constrains autotrophic growth while promoting DOC release and accumulation, thereby maintaining BR even as primary production declines (Hansell et al., 2009; Hansell, 2013). Interestingly, while absolute rates of PP_{DOC} significantly correlate with bacterial production (r=0.50, p<0.01; Figure 4), the PER does not exhibit a statistically significant correlation with BGE across the water column (r=0.106, p=0.59). This decoupling is primarily driven by opposing vertical gradients, as PER increases toward the base of the euphotic zone while BGE peaks in warmer surface layers, as well as potential bacterial overflow metabolism where carbon-rich exudates are preferentially respired rather than assimilated into biomass. Nevertheless, the low BGE observed (Table 1, Figure 3C) further support the view that most of the consumed carbon is respired rather than converted into biomass, a characteristic of strongly recycling systems in oligotrophic ocean settings (del Giorgio and Cole, 1998)....” (Lines 504-416).

2. The manuscript interprets the observed BGE values as characteristic of strongly recycling oligotrophic systems. While this interpretation is reasonable, recent studies from other oligotrophic environments have reported comparatively high BGE values despite nutrient limitation. For example, recent work conducted in the Taiwan Strait found relatively elevated BGE under oligotrophic conditions (Liu et al., 2026; JGR-Oceans). It would be useful to discuss these contrasting observations. In particular, what factors might explain why oligotrophic systems sometimes exhibit low BGE and sometimes relatively high BGE? A brief discussion placing the Gulf of Aqaba observations within this broader context would enhance the manuscript.

Reply: We thank the reviewer for highlighting this important context and bringing the recent findings from Liu et al. (2026) to our attention. We have updated Section 4.1 to explicitly discuss these contrasting observations, placing our findings within this broader context and citing Liu et al. (2026). In short, BGE may vary across oligotrophic environments depending on a combination of organic matter quality/lability, water temperature, physical oceanographic setting, availability of micro-nutrients and other environmental factors. For example, higher BGE in oligotrophic waters may occur when heterotrophic bacteria have access to labile organic matter or nutrient-rich substrates through micro-scale spatial patchiness, rapid biological turnover, and episodic physical events. In contrast, the severe nutrient depletion in the GoA during summer stratification leads to the exudation of carbon-rich, nutrient-poor DOM, forcing bacteria to respire excess carbon via overflow metabolism to acquire scarce nutrients, which likely lowers BGE. Furthermore, the warm surface temperatures in the GoA (~25-28 °C) elevate basal metabolic costs, favoring respiration over cell growth. Additionally, regions like the Taiwan Strait experience dynamic physical mixing, terrestrial runoff, or episodic upwelling and Kuroshio intrusions that supply inorganic nutrients and labile DOM to sustain elevated BGE. In contrast, the summer water column in the Gulf of Aqaba is an enclosed, highly stratified basin isolated from major deep-water nutrient inputs or runoff (apart from episodic desert dust inputs).

The following text was added: “...These low growth efficiencies are consistent with the energetic constraints typical of highly stratified oligotrophic waters, indicating that bacterial carbon use was directed mainly toward respiration rather than growth, with much of the organic carbon rapidly returned to dissolved inorganic form (del Giorgio and Cole, 1998). While low BGE is widely observed in unproductive open-ocean settings, a recent study from the oligotrophic Taiwan Strait reported elevated BGE values (Liu et al., 2026). Such contrasts suggest that oligotrophy alone does not dictate bacterial metabolic efficiency. Instead, BGE may be regulated by the substrate stoichiometry and lability, thermal regimes, and different physical oceanographic settings. In systems exhibiting higher BGE such as the Taiwan Strait, heterotrophic bacteria may access labile dissolved organic matter or nutrient-rich substrates through micro-scale spatial patchiness (e.g., phycosphere exudates, viral lysis), rapid biological turnover, or episodic physical events like upwelling, terrestrial runoff, and Kuroshio intrusions (Liu et al., 2026). In contrast, in the hyper-oligotrophic, warm, and highly isolated summer water column of the Gulf of Aqaba, severe nutrient limitation likely promotes the exudation of carbon-rich, nutrient-poor dissolved organic matter. This forces heterotrophic bacteria to respire excess carbon via overflow metabolism to meet their nutritional demands while sustaining high basal metabolic costs, thereby maintaining low to moderate BGE...” (Lines 422-438).

3. Some statements need to be clarified.

Lines 352-356: I found this interpretation difficult to follow. If chlorophyll biomass becomes increasingly concentrated in the DCM, it does not necessarily imply “low standing stocks.” Could lower bacterial consumption at DCM explain this pattern? The authors should clarify what they mean by “maintaining low standing stocks but operating at reduced photosynthetic efficiency” and explain the mechanistic basis for this decoupling more explicitly.

Reply: As in many oligotrophic ocean regions, the DCM in the GoA represents a deep photoacclimation maximum (DAM) rather than a deep biomass maximum (DBM). Accordingly, the DCM in the study area is characterized by high *Prochlorococcus* abundance (e.g., Lindell et al., 1995, L&O) but relatively low total primary production (e.g., Riech et al., 2024, L&O). Under these low-irradiance conditions, cells increase their intracellular chlorophyll content to enhance light harvesting rather than proportionally increasing carbon biomass. Furthermore, as the reviewer suggests, the pronounced decline in heterotrophic bacterial production with depth (Figure 3A) likely reduces microbial carbon consumption and associated loss rates, allowing *Prochlorococcus* and other photoacclimated cells to persist at the DCM despite relatively low carbon fixation rates. We clarified the section accordingly:

“...This decoupling between biomass and productivity is consistent with a community persisting under chronic low light and low nutrient availability (Marañón et al., 2010), representing a deep photo-acclimation maximum (DAM) rather than a deep biomass maximum (DBM) as found in some other oligotrophic settings (Hodges and Rudnick, 2004; Mignot et al., 2014). Additionally, the sharp decline in heterotrophic BP with depth (Figure 3A) likely reduces microbial loss rates, thereby allowing photo-acclimated phytoplankton at the DCM (mainly *Prochlorococcus*, Lindell and Post, 1995; Reich et al., 2024) to persist despite low carbon fixation rates. In other words, the high chlorophyll does not imply high biomass but rather high chlorophyll content per cell…” (Lines 366-374).

Lines 381-386: The contrast with Devresse et al. (2022) is currently confusing. Earlier in the paragraph, semi-labile DOC is presented as a reservoir that buffers temporal mismatches between production and consumption. However, in the ETNA example, semi-labile DOC appears to support a production-driven carbon cycle. Please clarify whether the semi-labile DOC in these two cases represents accumulated DOC pools versus freshly produced DOC associated with contemporaneous primary production.

Reply: In the Eastern Tropical North Atlantic (Devresse et al., 2022), high rates of contemporaneous PP_{DOC} directly supply freshly exuded labile organic carbon that exceeds BCD, resulting in heterotrophic activity tightly coupled to daily autotrophic production (a production-driven regime). In contrast, in the Gulf of Aqaba during summer, daily PP is apparently too low and 'fresh' exudated PP_{DOC} supply alone is insufficient to support community metabolic demand. Consequently, heterotrophic activity relies increasingly on the accumulated standing stock of semi-labile DOC built up over seasonal timescales, alongside internally recycled organic matter (a recycling-driven regime).

We revised the sentence for a better clarity:

"...In contrast, a recent study in the Eastern Tropical North Atlantic reported that BP and BR closely follow PP_{TOT}, with PP_{DOC} supply often exceeded bacterial carbon demand (Devresse et al., 2022). There, heterotrophic activity was largely controlled by and coupled to contemporaneous production, resulting in a production-driven carbon cycle..." (Lines 398-402).

Lines 418-420: If nutrients are rapidly taken up by microorganisms, biological processes are clearly influencing their distributions.

Reply: We agree. We revised the sentence: "...The absence of strong linear correlations between ambient nutrient concentrations and biological rates reflects the rapid uptake capacity of nutrient-starved microbial communities. This rapid biological drawdown maintains nutrient pools near detection limits, effectively masking statistical correlation..." (Lines 474-477).

Lines 424-427: Earlier results indicate that PER values are also relatively high near the DCM. Could the pronounced DCM observed during stratification primarily reflect photoacclimation rather than true biomass accumulation? Therefore, it is not obvious why carbon fixed at the DCM should preferentially contribute to biomass accumulation and export rather than dissolved pathways.

Reply: We have removed the sentence to avoid confusion.

Minor comments

Please define all abbreviations when they first appear in the manuscript. For example, DOC and silicic acid [Si(OH)4] should be introduced with their full names before abbreviations are used.

Reply: Corrected (e.g., GF/Fs, $Si(OH)_4$, DOC, CTD, HCl, TCA, CO_2).

Lines 80–88: The knowledge gap regarding the partitioning of newly fixed carbon and its connection to heterotrophic metabolism is introduced twice in slightly different wording. Streamlining this section would improve readability and sharpen the study objectives.

Reply: Agree. We revised the paragraph to avoid redundancy: "...Despite extensive characterization of the physical and chemical seasonal cycles in the Gulf of Aqaba (Biton and Gildor, 2011), and the recognized dominance of microbial recycling in oligotrophic waters (e.g., low f-ratios, Alothman et al., 2025), the corresponding shifts in microbial carbon processing, particularly the partitioning of the carbon fixed during the stratified season between particulate matter, dissolved carbon released from cells, and heterotrophic utilization of this carbon, remain insufficiently resolved..." (Lines 80-85).

Line 251: The phrase "in June–September to (Figure 2B)" appears incomplete and should be corrected.

Reply: Corrected (removed the word "to").

Line 256: "in the area" are rather vague. Please specify this region.

Reply: Corrected: "in the area" was changed to "in the Gulf of Aqaba".

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).