01 May 2026

RC1

We would like to thank the reviewer for the comprehensive and critical reviews for our paper now entitled: *"Ammonia and nitrite oxidation in the upper euphotic zone of the oligotrophic Red Sea"* (manuscript ID: egusphere-2026-855). We have gone over all of 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.

Summary:

This paper aims to assess the contribution of ammonium and nitrite oxidation to nitrogen cycling in the sunlit surface ocean of the Gulf of Aqaba during late spring and summer.

They report that they measure the lowest integrated ammonia and nitrite oxidation rates ever measured in oligotrophic waters, however their rate data is difficult to interpret without reporting limits of detection for their rate measurements. The low contribution of nitrification to total dark carbon fixation in the surface ocean is interesting in the context of Bayer et al 2025's paper showing that ammonia oxidizers don't account for all the DIC fixed in the deep ocean.

General:

This paper is very clearly written and easy to read. The reporting of nitrification rates from the GoA is interesting and would help understand how nitrification works in oligotrophic systems. The connection to total carbon fixation and the "missing" fixed carbon is relevant to current questions. Broadly, more detail is required about the methods and caveats of the data collected, and the discussion could include more about how these collected data can and cannot clarify how nitrification works in the upper 100m of the GOA.

Reply: We thank the reviewer for the constructive and insightful comments. We have revised the manuscript to more explicitly address methodological constraints/caveats and the interpretative scope of our data. We believe these revisions substantially improved both the transparency of the methodological framework and the interpretation of nitrification dynamics in the GoA and in oligotrophic systems in general.

The caveats of the experimental design need to be clearly discussed. For example, a 24hr incubation can run into issues of substrate regeneration and/or grazer activity etc. I am curious how the 20nM NH4 15N tracer addition relates to your measured NH4 concentrations. It can be quite hard to add tracer at low levels (<10%) in oligotrophic systems without perturbing the rates, and understanding these rates in the context of kinetics is interesting since even with these N additions the reported rates are low. Even with 30-50% added NH4, the rates are all still low, and similarly low? Does that mean the AOA community is not really substrate limited? Does the rate remain low because there are so few AOA there? Please describe this design and its repercussions further.

Reply: We revised the manuscript to more explicitly address the caveats associated with our experimental design. In regards to the comment provided here, we now clarify the relationship between the tracer addition and the low ambient NH_4^+ concentrations in the study area, noting that this represents a moderate enrichment (typically ~30-50%) and may influence measured rates. We further emphasize that the reported rates should be interpreted as potential rates rather than strictly in situ rates.

The following paragraph was thus added to the M&M:

"*…samples were amended with ^{15}N-labeled ammonium chloride ($^{15}NH_4Cl$, >98 atom %; Cambridge Isotope Laboratories) at a concentration of ~20 nmol L^{-1} which is sufficient to yield a quantifiable signal while potentially introducing some degree of tracer perturbation (discussed below)*…" (Lines 115-118).

"*...Note that for the ammonia oxidation rates we added tracer additions which correspond to 30-50% of the ambient NH_4^+ concentrations. While we aimed to minimize substrate perturbation, such additions are inherently challenging in ultra-oligotrophic systems, where even low absolute tracer concentrations can represent a substantial fraction of the ambient pool (Zheng et al., 2020). Consequently, the reported rates should be considered as potential rates under moderately enriched conditions rather than strictly in situ rates (Dodds and Jones, 1987). Additionally, incubations were conducted over 24 h, which may allow for processes such as ammonia regeneration, microbial turnover, and grazing to influence substrate availability and isotopic dilution. Although $HgCl_2$-poisoned controls and parallel measurements were used to account for abiotic and background signals, these incubations cannot fully resolve short-term dynamics or transient coupling between regeneration and oxidation processes. These methodological constraints are inherent to low-rate measurements in oligotrophic systems (Ward, 1985) and should be considered when interpreting the results*..." (Lines 158-170).

We have also expanded the discussion to address the implications of observing consistently low ammonium oxidation rates despite this enrichment. We now explicitly consider that factors such as light inhibition and oligotrophic adaptation likely constrain rates in the upper euphotic zone.

"*…a key consideration in interpreting the measured rates is the relative magnitude of the $^{15}NH_4^+$ tracer addition compared to ambient substrate concentrations. In the upper euphotic zone, where NH_4^+ concentrations were often near detection limits (Figure 1), the addition of ~20 nmol L^{-1} (tracer) represented a substantial enrichment of the available pool. Under such conditions, if ammonia oxidation were strongly substrate-limited, one might expect a measurable stimulation of rates. However, the observed rates remained consistently low across depths and sampling periods (Table 1; Figure 3), even under these 'enriched' conditions. This suggests that factors other than immediate substrate availability exert primary control over ammonia oxidation in the upper waters of the GoA. These may include low abundances of ammonia-oxidizing archaea (Aizawa et al., 2023; Smith et al., 2016), strong light inhibition in surface waters (Figure 1B), or physiological constraints associated with oligotrophic adaptation (Yin et al., 2024; Zhou et al., 2024). Rather than indicating the absence of substrate limitation per se, our results imply that ammonia oxidation operates under a combination of*

ecological and environmental constraints that limit its overall contribution to nitrogen cycling in this system…" (Lines 354-368).

Finally, we discuss the limitations of 24 h incubations, including potential substrate regeneration and microbial interactions, and how these may affect rate estimates.

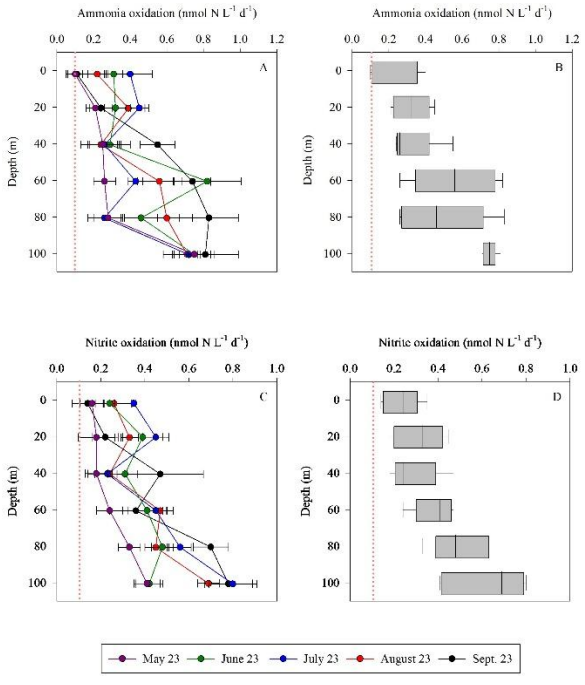
"*...Moreover, the use of 24 h incubations introduce additional uncertainty, as internal recycling of ammonium and microbial interactions may partially decouple measured rates from instantaneous in situ conditions. Therefore, the rates reported here are interpreted as conservative estimates of nitrification potential in the upper euphotic zone...*" (Lines 368-371).

I believe the limit of detection for all rates are needed, especially when reporting such low oxidation rates. These values are very near zero and difficult to interpret. Measurements below detection limit should be noted on figure of NO3 measurements.

Reply: We thank the reviewer for highlighting this important point, particularly given the low oxidation rates reported. We have revised the manuscript to explicitly define detection thresholds based on parallel $HgCl_2$-killed controls. The rate detection limit was calculated as the mean killed-control rate plus three standard deviations, corresponding conservatively to ~0.1 nmol N L^{-1} d^{-1}. Rates at or below this threshold are now treated as indistinguishable from background signal and are interpreted accordingly in the R&D.

The following text was added to the M&M: "*...Rates in the 'mercury-killed' controls were typically negligible relative to the 'live' bottles (usually <0.05 nmol N L^{-1} d^{-1}). The resulting detection limit, which was defined as the mean killed-control rate plus three standard deviations, corresponded to 0.1 nmol N L^{-1} d^{-1}. Rates below this threshold were considered indistinguishable from background signal and are interpreted as below detection...*" (Lines 139-143).

We also revised Figure 3 and show the detection limit:



Ammonia oxidation rates (and ammonia concentrations) are often highest below the deep chl max in other systems and previous research shows fairly abrupt rates increases that correspond to archaeal gene counts, but ore offset from peak ammonium concentrations (eg Beman et al 2012). Does your correlation between NH4 concentration and oxidation rate tell you something about how ammonium supply in the surface (above the deep chl max) behaves differently from deeper in the euphotic zone?

Reply: We examined the relationship between NH_4^+ and ammonia oxidation rates across the sampled depths. While a positive relationship between NH_4^+ concentration and ammonia oxidation rates exists, it is relatively weak overall (Pearson r≈0.30). In contrast, the relationship between depth and ammonia oxidation rates is substantially stronger (r≈0.75), suggesting that depth-related environmental gradients exert a greater influence on the observed variability in oxidation rates than substrate concentration alone. When examined by depth intervals (0-50 m vs. 50-100 m), the relationship between NH_4^+ concentration and ammonia oxidation rates is very weak in the upper 50 m (r≈0.23) but becomes somewhat stronger below 50 m (r≈0.41). This suggests that in surface waters above the DCM, ammonium more tightly coupled to rapid biological recycling and competition with phytoplankton and heterotrophic microbes, resulting in a weak coupling. In contrast, in deeper euphotic layers where light inhibition is substantially reduced, NH_4^+ availability appears to play a greater role, though still small. Overall, these results indicate that ammonia oxidation in the upper water column of the GoA is more strongly structured by depth-dependent factors (e.g., light) than by ambient NH_4^+ concentrations. This is consistent with previous studies (e.g., Beman et al., 2012), which show that nitrification maxima are often decoupled from NH_4^+ concentration peaks and instead reflect the distribution and activity of ammonia-oxidizing microorganisms.

We added the following paragraph to the R&D: "*...To assess substrate control, we examined the relationship between NH_4^+ concentration and ammonia oxidation rates across depths. This relationship was weak overall (Pearson, r≈0.30), whereas rates were more strongly associated with depth (r≈0.75), indicating a dominant role of depth-related gradients (Figure S1, Table S1). When examined by depth intervals (0-50 m vs. 50-100 m), the NH_4^+–oxidation relationship was weak in the upper 50 m (r≈0.23) and stronger >50 m (r≈0.41) (Figure S1, Table S1). This suggests that rapid recycling and competitive uptake weaken NH_4^+– oxidation rate coupling in surface waters, whereas reduced light inhibition at depth allows a somewhat greater influence of NH_4^+. These results are consistent with previous studies showing that nitrification maxima are often decoupled from NH_4^+ peaks and instead reflect depth-dependent ecological structuring (Beman et al., 2012)...*" (Lines 343-353).

I don't think you can strongly conclude that much about phytoplankton influence on the N cycling. You only have carbon based photosynthetic rates and chl a data. There is room for discussion around this though, and you could discuss the need to co-measure phytoplankton and nitrifiers in future studies.

Reply: We have revised the manuscript to moderate our interpretation and clarify that phytoplankton influence on N cycling is inferred from carbon-based proxies. We now frame these relationships more cautiously and emphasize that the observed patterns are consistent with, but do not demonstrate, coupling between phytoplankton activity and nitrification. We

also added a statement highlighting the need for future studies to co-measure phytoplankton dynamics, nitrogen uptake rates, and nitrifier abundance/activity in order to better resolve these interactions.

The following text was added: "*...while variations in ammonia oxidation rates broadly co-occurred with changes in primary production and chlorophyll a, these relationships should be interpreted with caution. In this study, phytoplankton activity was assessed using carbon-based proxies, and no direct measurements of nitrogen uptake or community composition were conducted. Therefore, any inferred coupling between phytoplankton dynamics and nitrification remains indirect. The observed patterns are consistent with the expectation that phytoplankton influence the availability and cycling of regenerated nitrogen, but do not allow us to disentangle the relative roles of substrate competition, regeneration, or microbial community structure. Future studies that combine measurements of phytoplankton nitrogen demand, ammonium regeneration, and nitrifier abundance and activity will be required to directly resolve these interactions in oligotrophic systems...*" (Lines 493-503).

We also added in the conclusion section the following text: "*...while our results suggest a potential linkage between phytoplankton activity and nitrogen cycling, this inference is based on carbon-derived proxies (primary production and chlorophyll.a) rather than direct measurements of species-specific nitrogen uptake or microbial community composition using genetic markers. Resolving this coupling will require future studies that simultaneously quantify phytoplankton nitrogen demand, ammonium regeneration, and nitrifier abundance and activity...*" (Lines 525-530).

Are the rates you see at 5m statistically different from 60-80m? Other papers suggest the rates are uniformly low in the upper water before an abrupt increase (eg Beman 2012 Fig 6) vs other papers that show a less abrupt decline in rates more like your data (eg. Travis et al 2023 Fig 4). Its curious why rates are not uniform through the N-depleted surface. How deep is the mix layer?

Reply: We have now added the mixed layer depth (MLD) information to Table 1. During the study period, the MLD ranged from ~45 m in May to ~15 m in August, indicating progressive stratification of the upper water column. We also clarified that ammonia oxidation rates did not show an abrupt subsurface increase comparable to that reported in some systems, but rather a more gradual depth-related pattern. This suggests that nitrification in the upper GoA remained low throughout much of the N-depleted surface layer, with modest increases at depth likely reflecting reduced light inhibition and/or more 'favorable' conditions for nitrifiers below the upper mixed layer. We now discuss this pattern in relation to previous studies showing either abrupt or more gradual vertical increases in ammonia oxidation rates.

The following text was added: "*...During the study period, the mixed layer depth shoaled from ~45 m in May to ~15 m in August (Table 1), reflecting progressive seasonal stratification. The vertical pattern of ammonia oxidation (Figure 3) suggests that rates remained low throughout the strongly illuminated upper water column, while modest increases at 60–80 m likely reflected reduced light inhibition and/or more favorable conditions for nitrifier activity below the mixed layer. Thus, unlike systems where ammonia oxidation increases sharply below the*

deep chlorophyll maximum, nitrification in the GoA appears to follow a more gradual depth-related response during stratified conditions..." (Lines 313-320).

You will need to discuss how the data was summarized into your pearson correlation chart with a section in the methods. Correlations of 1 are a perfect fit, and many of your relationships look close to 1 or -1 on the color scale. I suggest showing the regression data in the supplement. I'm assuming you aggregated all the profiles, and did one linear regression, but this feels a bit misleading. Be very careful when interpreting correlations because so many things co-vary but are not necessary causal. The limited depths of this dataset (0-100) makes it harder to draw comparisons to datasets that work down to the typical nitrification maxima (below the chl max).

Reply: We have revised the methods to explicitly describe how the correlation matrix was constructed (please see the new subsection 2.5). Additionally, we added two tables in the supporting information displaying the full descriptive statistics which now accompany Figure 4. Briefly, Pearson correlations were calculated using all individual observations across depths and sampling dates, without prior averaging by profile. We acknowledge that this approach may inflate correlations due to co-variation with depth and other shared gradients. To improve transparency, we now include the underlying regression relationships in the supplement (Figure S1), allowing direct visualization of data structure and variability. We have also revised the discussion to more cautiously interpret these correlations, emphasizing that they reflect co-variation rather than causality. Finally, we acknowledge that our dataset is limited to the upper 100 m and does not extend to the deeper nitrification maxima observed in other systems. We have added text to clarify that this depth limitation restricts direct comparison with studies resolving full water-column nitrification structure and that the reported relationships primarily reflect processes within the upper euphotic zone.

The following text was added:

Methods (subsection 2.5): *"...Pairwise relationships between environmental variables and process rates were evaluated using Pearson correlation coefficients calculated across all individual observations, including all sampled depths (0-100 m) and stations. No prior averaging by depth or profile was applied. Because many variables co-vary with depth and season, these correlations should be interpreted as measures of co-variation rather than independent or causal relationships. Full Pearson correlation statistics (r, r^2, p-values) are provided in Supplementary Tables S1 and S2. Statistical analyses were performed using Python..."* (Lines 214-220).

R&D: *"...We note that correlation analysis should be interpreted with caution. Many parameters considered here co-vary with depth (e.g., PAR, chlorophyll.a) and seasonal stratification (mixed layer depth), which can produce strong apparent relationships without implying direct mechanistic coupling. Furthermore, as the dataset is restricted to the upper 100 m, it does not capture the full vertical structure of nitrification, including deeper maxima often observed below the deep chlorophyll maximum. Accordingly, these correlations primarily reflect processes operating within the upper euphotic zone and should not be extrapolated beyond this depth range..."* (Lines 486-493).

Supplementary information:

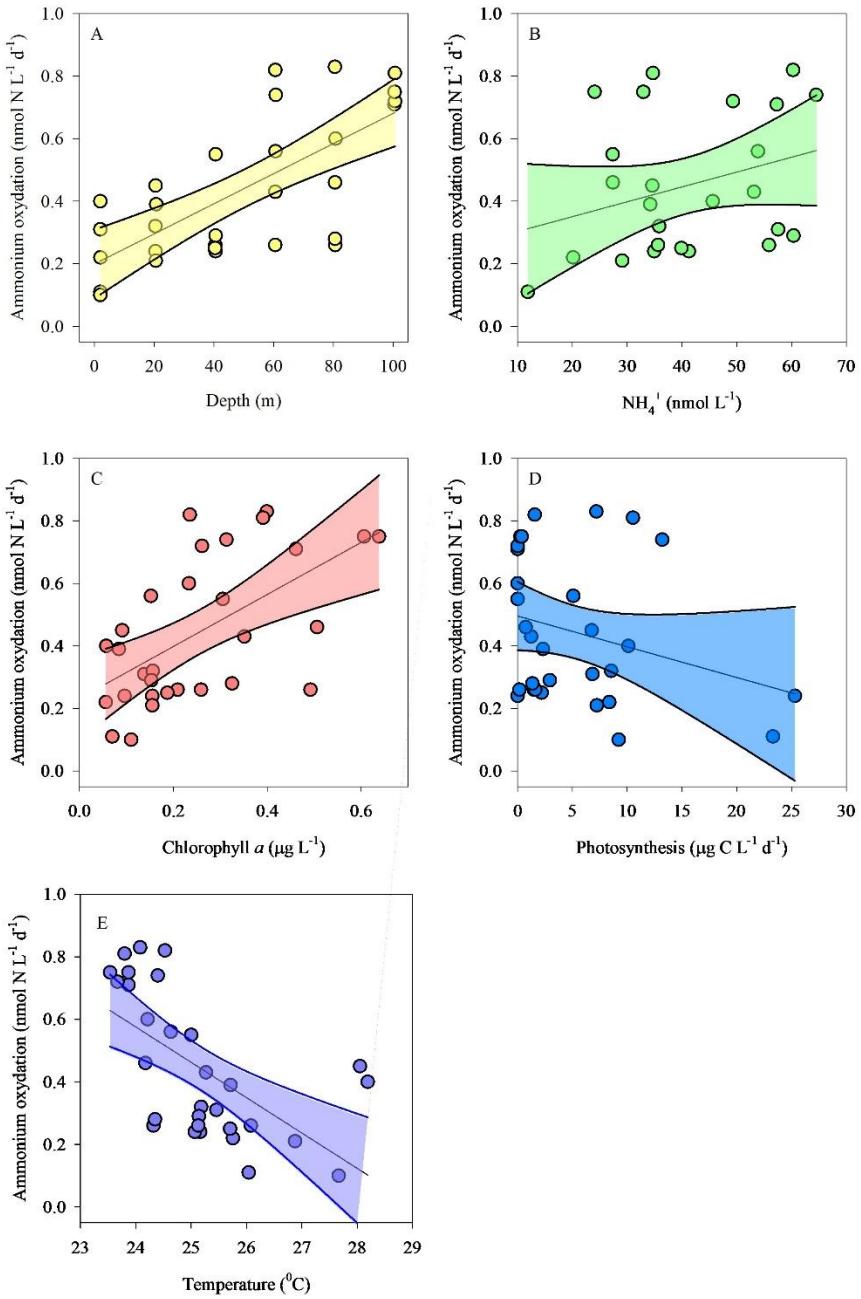


Figure S1. *Visual representation of the relationships between key environmental variables and ammonia oxidation rates across all samples (0-100 m). Data shown is ammonia oxidation rates vs. (A) depth (r=0.72, p<0.001), (B) NH_4^+ (r=0.30, p=0.14), (C) chlorophyll a (r=0.59, p=0.001), (D) photosynthesis (r=0.28, p=0.139) and (E) temperature (r=0.61, p=0.01). The black line shows the linear fit and the background area signifies the 95% confidence interval. For the full descriptive statistics see Tables S1 and S2.*

Additionally, we now present the full correlation statistics in Supplementary Tables S1 and S2 and refer to these table for further reading in Figure 4 legends (Lines 509-510).

Table S1: Pearson correlation statistics between environmental variables and ammonium oxidation rates. Correlation coefficients (r), coefficients of determination (r^2), sample size (n), and corresponding p-values are shown for pairwise relationships calculated across all observations (0-100 m, all cruises). Correlations reflect co-variation among variables and do not imply causation.

Variable	**n**	**r**	**r^2**	**p-value**
Pressure	30	0.719	0.517	<0.001
Temperature	30	-0.612	0.375	<0.001
Salinity	30	-0.297	0.088	0.111
Density	30	0.629	0.395	<0.001
Oxygen	30	0.266	0.071	0.155
PAR	30	-0.486	0.236	0.007
pH	29	0.008	~0.000	0.969
NH_4	25	0.303	0.092	0.141
NO_2	30	0.554	0.306	0.002
NO_3	30	0.294	0.087	0.115
PO_4	30	-0.117	0.014	0.536
Chl-a	30	0.591	0.349	<0.001
Photosynthesis	30	-0.277	0.077	0.139
DCF	29	0.178	0.032	0.356

Table S2: Pearson correlation statistics between environmental variables and nitrite oxidation rates. Correlation coefficients (r), coefficients of determination (r^2), sample size (n), and corresponding p-values are shown for pairwise relationships calculated across all observations (0-100 m, all cruises). Correlations reflect co-variation among variables and do not imply causation.

Variable	**n**	**r**	**r^2**	**p-value**
Pressure	30	0.728	0.531	<0.001
Temperature	30	-0.574	0.329	<0.001
Salinity	30	-0.152	0.023	0.424
Density	30	0.620	0.384	<0.001
Oxygen	30	0.329	0.108	0.076
PAR	30	-0.494	0.244	0.006
pH	29	0.020	~0.000	0.920
NH_4	25	0.286	0.082	0.165
NO_2	30	0.443	0.197	0.014
NO_3	30	0.449	0.202	0.013
PO_4	30	-0.301	0.091	0.106
Chl-a	30	0.541	0.292	0.002
Photosynthesis	30	-0.319	0.102	0.086
DCF	29	0.494	0.244	0.006

Something about the missing carbon should probably be in the conclusion.

Reply: Text added to the conclusions as suggested: "*...while our results suggest a potential linkage between phytoplankton activity and nitrogen cycling, this inference is based on carbon-derived proxies (primary production and chlorophyll.a) rather than direct measurements of species-specific nitrogen uptake or microbial community composition using genetic markers. Resolving this coupling will require future studies that simultaneously quantify phytoplankton nitrogen demand, ammonium regeneration, and nitrifier abundance and activity...*" (Lines 525-530).

Specific line comments:

Line 21: I suggest reversing the order of the clauses in this sentence (ie suppressed rates first, increasing with depth second)

Reply: Sentence revised as suggested: "*Overall, rates were low in the highest-irradiance surface waters and increased with depth*" (Lines 21-22).

Line 22: I'm not sure the rates were "suppressed" because you didn't experimentally test if light inhibited the rates. You have measured low rates at high light levels.

Reply: Corrected - please see the previous remark.

Line 22: Are you comparing integrated rates to the Tang database? Your last figure is comparing the per L rates….

Reply: This statement reflects that the rates measured here (either volumetric or depth integrated) are low compared to those reported for other oligotrophic systems.

Line 26: <2% of chemoautotrophic activity or DFC? Define chemoautotrophic activity as dark carbon fixation? Or not, because DCF includes heterotrophic activity too.... please clarify. Referring to anaplerosis here feels like a jump. Maybe clarify the sentence so it is clear you are talking about carbon now.

Reply: Sentence revised: "*...Ammonia and nitrite oxidation together supported <2% of dark carbon fixation rates, suggesting other processes, not accounted for, drive this chemoautotrophic activity...*" (Lines 25-26).

Line 104: Is this your detection limit, or what Meeder reported in their data?

Reply: It's the same in this case. We used the same methodology and same lab to run these samples.

Line 109: With a 24 hour incubation some discussion about the potential caveats on your rates measurements is warranted. Daylong incubations in sunlit surface waters will likely capture uptake by phytoplankton as well as release of nitrite (even if chl is low, prochlorococcus and synechococcus are active as seen by your photosynthesis rates). Al-Qutob et al 2002 and Travis et al 2024 both document release of nitrite by phytoplankton via nitrate reduction which might dilute your labeled nitrite pool over 24 hours and lead to underestimates of ammonia oxidation

and nitrite oxidation. You could use simple modeling to estimate how these dilutions with nitrite would influence your calculated rates.

Reply: We acknowledge that 24 h incubations in sunlit waters may include the production of unlabelled nitrite via phytoplankton nitrate reduction, which could dilute the $^{15}NO_2$ tracer pool and lead to an underestimation of ammonia and nitrite oxidation rates. We have revised the manuscript to explicitly state this potential source of isotope dilution. Having said that, while it is possible to estimate this effect using simple models, doing so would require assumptions about *in situ* nitrite production rates that were not directly measured in this study and may vary substantially across systems. We therefore believe it is not advisable to quantitatively model this process. Instead, we emphasize that any such dilution would bias our rate estimates conservatively (i.e., toward underestimation) and therefore does not alter our main conclusion that nitrification rates in the upper euphotic zone are low.

The following text was added: "*...Another potential cavate arising from the 24 h incubation is the potential production of unlabelled nitrite via phytoplankton nitrate reduction (e.g., Travis et al., 2024) thereby diluting the $^{15}NO_2^-$ pool leading to an underestimation of both ammonia and nitrite oxidation rates. In the present study, however, primary production and ambient nitrite concentrations were low, suggesting that this effect was likely limited in magnitude...*" (Lines 170-175).

Line 123: What are your detection limits here?

Reply: Information added (please refer to our detailed reply above).

Line 187: Without a clear vertical trend feels contradictory to your later correlation with ammonia oxidations rates that appear to have a clear vertical increase, especially the summary in Fig 3B.

Reply: Sentence revised: *"...Concentrations of NH_4^+ ranged from undetectable to 65 nmol L^{-1}...*" (Lines 232-233).

Line 194: Ko et al. does not seem like the best reference for nitrite release from phytoplankton. Depending on whether you suggest nitrite release solely from light limitation or from co-occurring access to NO3 at depth, one of these references may be more appropriate (Collos, 1998; Kiefer et al., 1976; Lomas and Glibert, 2000; Lomas et al., 2000; Sciandra and Amara, 1994; Vaccaro and Ryther, 1960; Wada and Hattori, 1971; Berube et al., 2023). You may also consider that nitrite is released under high light too (see Travis et al 2024 nitrate reduction rates, and original idea in Lomas and Glibert 1999).

Reply: We thank the reviewer and have revised the text to incorporate two of the suggested references (Berube et al., 2023; Collos, 1998) instead of Ko et al.

Line 203: Confusing sentence here. Do you mean below the depths you measured? Or below ~20m? All your DIN measurements are similarly nano-molar levels.

Reply: Sentence removed.

206: Where are the chlorophyll maxima relative to where you stopped sampling at 100m? What is the mix layer depth?

Reply: Data on the mixed layer depth was added to Table 1. We do not have the full chlorophyll profile due to logistical constrains but based on previous observations in the study area the DCM is typically located at ~80-120 m during summertime (Data source: the National Monitoring Program).

234: Fig 1. Some of your nitrate and nitrite concentrations appear to be below your detection limits. It would also help to have the X axes start at 0.

Reply: Corrected as suggested.

Line 237: Photosynthesis rate in carbon units converted to nitrogen requirement with a Redfield approximation (C:N ~7) suggests your phytoplankton community a <20m is using 70-100 nmol N L-1 d-1. This would suggest to me that there might be competition for N substrates at these depths...Mackey et al 2011 has ammonia oxidation rate vs ammonia uptake rates that show 20x higher ammonia uptake rates in this system.

Reply: Converting primary production to nitrogen demand suggests that phytoplankton requirements in surface waters may substantially exceed the measured nitrification rates, implying potential competition for reduced nitrogen substrates. Having said that, we stress that this inference is based on carbon-derived estimates of phytoplankton demand rather than direct measurements of N uptake, and should therefore be interpreted cautiously. Nevertheless, previous studies (e.g., Mackey et al., 2011) support the view that ammonium uptake can greatly exceed nitrification rates in oligotrophic surface waters.

We added the following text: "*...Converting photosynthesis to nitrogen demand using Redfield stoichiometry (C:N ≈ 6.6) suggests that phytoplankton nitrogen requirements in surface waters may substantially exceed the measured nitrification rates. This implies that regenerated nitrogen, including ammonium, is rapidly consumed, potentially limiting its availability for ammonia-oxidizing microorganisms. However, this inference is based on carbon-derived estimates of phytoplankton demand rather than direct measurements of nitrogen uptake and should therefore be interpreted cautiously. Nevertheless, previous studies indicate that ammonia uptake can greatly exceed nitrification rates in oligotrophic surface waters (Mackey et al., 2011)...*" (Lines 335-342).

Line 244: You mention light inhibition controlling the ammonia oxidation rates, but what is the abundance of the nitrifiers? Can you distinguish between AOX communities that have low abundance, vs communities that are more abundant but have inhibited rates per cell? Typically amoA genes abundance is near zero in the surface and leads to near zero rates of ammonia oxidation (eg. Santoro 2013, Beman 2012)

Reply: Indeed, in the absence of direct measurements of nitrifier abundance (e.g., *amoA* gene counts) we cannot distinguish whether the low ammonia oxidation rates observed in surface waters reflect low population size or reduced per-cell activity. This issue is already brought up in the manuscript. We also refer to previous studies showing that *amoA* gene abundance is often minimal in surface waters, consistent with very low nitrification rates.

"*...the absence of measurements of nitrifier abundance preclude us from distinguishing whether low bulk rates reflect low population size or potentially high per-cell activity...*" (Lines 522-524).

Line 256: High chlorophyll is often associated with increased NH4 levels, which would enhance substrate-dependent ammonia oxidation rates. Phytoplankton uptake of ammonium can still occur. I don't think you have data justifying that phytoplankton are not consuming available N in this low-N system. Prochlorococcus and syn are both present and capable of taking up NH4 (and possibly NO2). Especially since your incubation were carried out in ambient light. Do you have any dark bottle incubations? Attempting to prevent photosynthesis would get closer to a nitrifer-only rate.

Reply: Incubations were conducted under ambient light conditions and therefore represent net rates integrated over the natural community, rather than nitrifier-only activity. While we did not perform dark bottle incubations specifically targeting nitrifier-only rates, we did quantify DCF, which constrains chemoautotrophic carbon assimilation under dark conditions. These measurements support the interpretation that chemoautotrophic activity, including that of nitrifiers, is low in the upper euphotic zone.

Line 258: Can you explain further why lack of correlation between chl and Nh4 means there is no competition between nitrifiers and phytoplankton for nh4?

Reply: Sentence revised for clarity: "*...the lack of correlation between chlorophyll a and ammonia (R^2=0.003) does not preclude competition, but instead likely reflects rapid recycling and tight coupling between ammonium production and uptake...*" (Lines 303-305).

Line 272: Higher sensitivity of nitrite oxidizing bacteria to light was done in studies comparing nitrite oxidizing bacteria to ammonia oxidizing bacteria (Guerrero and Jones, 1996; Olson, 1981). Since we now understand AOA to dominate ammonia oxidation in the ocean, we're not entirely sure this is true in situ. For example, see Travis et al 2024 where nitrite oxidation rates appeared to be less inhibited by increasing light compared to the co-occurring ammonia oxidizing community. Sources of ammonia substrate to fuel ammonia oxidation may just be coming from more shallow sources (decaying phytoplankton blooms), while nitrite substrate could be produced via ammonia oxidation across deeper depths.

Reply: We agree that attributing the deeper nitrite oxidation maximum solely to greater light sensitivity of nitrite oxidizers may not represent the full story, particularly given that earlier comparisons were based on ammonia-oxidizing bacteria rather than the archaeal communities that dominate in the ocean. We have therefore revised the sentence to interpret the observed depth offset in terms of substrate availability and vertical decoupling between ammonia and nitrite oxidation, while not excluding a potential role for light.

"...Nitrite oxidation maxima (~100 m) were deeper than those of ammonia oxidation (~60 m). This vertical offset may reflect differences in substrate supply and the decoupling of ammonia and nitrite oxidation along the water column in addition to differential sensitivity to light (Wan et al., 2021). For example, ammonium supply may be more closely linked to shallower

regeneration processes, whereas nitrite can accumulate and persist at greater depths (Travis et al., 2024)..." (Lines 325-330).

Line 274: I think this conclusion is too broad. Meeder and Mackey both show that nitrite concentration and inventory varies significantly across season/bloom. Your dataset is focused on May-Sept, and thus misses the deep winter mix layer and spring bloom where you see nitrite accumulate. I suggest being more specific, and say that your comparable rates means there is low net nitrite production coming from any mismatch in the two steps of nitrification during these seasons.

Reply: Indeed, our dataset is essentially limited to the summer season (most oligotrophic period) and does not capture seasonal dynamics such as winter mixing or spring bloom conditions, during which nitrite accumulation can be more pronounced. We have revised the sentence accordingly to clarify that the comparable ammonia and nitrite oxidation rates observed here imply low net nitrite production resulting from limited decoupling between the two steps of nitrification during the period sampled.

"*...Our results demonstrate that ammonia and nitrite oxidation occurred at comparable rates, which is consistent with the typically low concentrations of NO_2^- observed in the GoA during summertime (Figure 1D, Meeder et al., 2012), and consistent with the low net accumulation of NO_2^- resulting from limited decoupling between the two steps of nitrification*..." (Lines 331-334).

Line 282: Could some of the carbon fixed be from urea oxidation by ammonia oxidizers? (Wan et al 2024)

Reply: sentence revised: "*...DCF is widely thought to be dominated by ammonia and nitrite oxidation, as these metabolic processes provide energy that, in turn, support chemoautotrophic CO_2 assimilation (Middelburg, 2011), although additional pathways such as urea oxidation by ammonia oxidizers may also contribute (Wan et al., 2024)*..." (Lines 386-389).

Line 306: remove one "also"

Reply: Corrected.

Line 318: I would be careful with interpreting these correlations as surprising. The warmest temperature have very low NH4 so rates are likely responding to substrate regardless of temperature. Please show the regressions.

Reply: Sentence revised: "*...Temperature correlated negatively with ammonia and nitrite oxidation rates (Figure 4, $r=0.61$, $p<0.01$), likely reflects substrate limitation rather than a direct temperature effect...*" (Lines 438-440).

The relationship between ammonium oxidation and temperature is now shown in the Supplement (Figure S1E). Note that Figure S1 does not present the full correlation matrix presented in Figure 4 but instead includes only key variables identified during the revision.

Line 329: I'm wondering about this correlation between NH4 and oxidation rates. Classically, high nh4 concentrations occur because of phytoplankton bloom/decay (ie nh4 accumulates

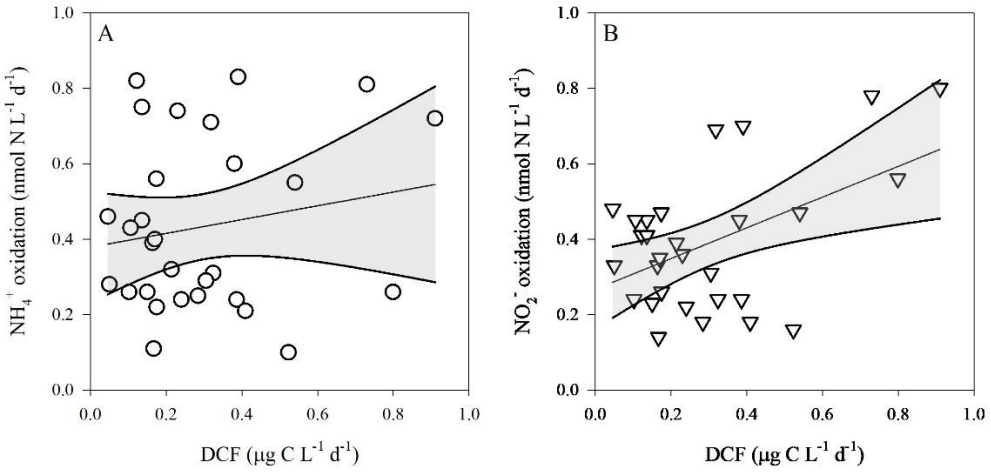
below a bloom) and are often ~10m offset from peak ammonia oxidation. My guess is that the near-surface is more "stable" and down towards the chlorophyll max experiences more variability in substrate and the responding AOA community abundance and then the rates. See

Reply: We agree that the observed correlations between substrate concentrations and oxidation rates should be interpreted cautiously and may reflect co-variation with depth and ecosystem structure in addition (or despite) to a direct substrate control. We have revised the manuscript accordingly to soften the interpretation and to acknowledge that variability near and below the chlorophyll maximum, including bloom-related processes, may influence both substrate availability and nitrifier activity.

The sentence now reads: "*...In agreement with this line of thought, substrate availability was positively correlated with ammonia oxidation (NH_4^+, NO_2^-) and nitrite oxidation (NO_2^-, NO_3^-), highlighting the substrate-dependent nature of nitrification. Alternatively, these relationships may reflect co-variation with depth and associated environmental gradients, rather than direct substrate control alone (discussed below)...*" (Line 449-453).

Line 353: The expectation that nitrification would be a large component of DCF and then the fact that it is not, is one of the more interesting parts of the paper. I'd like to see the regression figure of this data.

Reply: As requested, please see below the regression plots between DCF and ammonium oxidation (A) or nitrite oxidation (B). In both cases, although the correlation coefficients were positive (r>0), the relationships were weak and not statistically significant (p>0.05). More information about the correlations is now provided in the revised text. Additional information on the Person correlations between ammonium or nitrite oxidation vs. environmental/ancillary variables is now provided in Tables S1 and S2 (please see above).



"*...although both ammonia and nitrite oxidation were positively coupled with DCF (r=0.49 and 0.17, respectively), the correlations were not statistically significant (p>0.05) and is in line with the overall low contribution of these processes to DCF...*" (Lines 478-481).

Line 355: Wouldn't other archaeal and bacterial community members also be possible contributers to DCF? Heterotrophic DIC fixation? Urea oxidation? I think there is room for more discussion about that else could be happening here.

Reply: We agree that additional microbial pathways, including heterotrophic inorganic carbon assimilation and other chemoautotrophic processes may contribute to DCF. We have revised the manuscript to broaden this interpretation accordingly. However, a detailed characterization of the relative contributions of these pathways is beyond the scope of the present study, which focuses primarily on nitrification rates. We therefore limit the discussion to acknowledging these potential contributions without further elaboration.

"*...This suggests that additional pathways such as anaplerotic processes may contribute to DCF (Dijkhuizen and Harder, 1984; Erb, 2011), as well as other chemoautotrophic metabolisms beyond nitrification such as urea oxidation, sulfur oxidation and iron oxidation (Arandia-Gorostidi et al., 2024; Dang and Chen, 2017), while the contribution of ammonia and nitrite oxidation to total DCF is low (Table 1*)..." (Lines 481-485).

Fig 4: Need to see the regressions.

Reply: As requested, descriptive statistics for the relationships between ammonium oxidation (Table S1) and nitrite oxidation (Table S2) and the ancillary environmental variables (e.g., temperature, salinity, chlorophyll, nutrients) are now provided in full in the supplementary information Tables S1 &S2 (please see above).

Line 368: Your ammonium concentrations are low, but can you really say the low rates are caused directly by low substate? You mention trace metals and light. There is no data on abundance of archaea, per cell rates could be high?

Reply: We revised the conclusion section to avoid a direct causal statement and instead frame the observed low rates as being consistent with low substrate availability, while also acknowledging additional constraints. As the reviewer stated, in the absence of measurements of nitrifier abundance (e.g., ammonia-oxidizing archaea), we cannot distinguish whether low bulk rates reflect low population size or potentially elevated per-cell activity. This limitation is now explicitly stated in the revised manuscript.

"*...The reason for the low rates in GoA is attributed to the low substrate availability during summertime (Figure 1C-E) but likely reflect a combination of environmental and ecological constraints rather than a single controlling factor*..." (Lines 516-518).

"*...Moreover, the absence of measurements of nitrifier abundance preclude us from distinguishing whether low bulk rates reflect low population size or potentially high per-cell activity*..." (Lines 522-524).

372: I think the global compilation includes many oligotrophic datapoints, or at least low NH4. You could pull out a subset of the global compilation from systems that are closer to the Gulf of Aqaba. I'm not sure how relevant it is to plot your data with the super high southern ocean data…

Reply: The sentence removed altogether during revision.

Line 376+: Do you think we need to go out and measure more really low nitrification rates? I guess Mackey et al 2011's spring ammonia oxidation rates was much higher, so maybe you'd expect to see higher nitrification in the spring.

Reply: Additional measurements should not simply focus on reproducing low nitrification rates but rather capture their variability across seasons and environmental conditions. This is already reflected in the text, which emphasizes the need to resolve temporal variability, including periods of enhanced nitrification associated with mixing and bloom conditions, rather than focusing solely on low summertime rates.

I think your data could suggest measuring other things to find what fixes the missing inorganic carbon. You didn't mention the carbon stuff in your conclusion.

Reply: We revised the conclusion section to acknowledge that the observed DCF signal cannot be fully attributed to nitrification alone and likely includes contributions from additional microbial pathways. We now highlight the need for future studies to combine rate measurements with complementary approaches (e.g., microbial community composition, alternative metabolic pathways) to better constrain the sources of inorganic carbon fixation in the GoA and in other oligotrophic systems.

"*...Furthermore, the contribution of nitrification to DCF appears to be limited, suggesting that additional microbial pathways contribute to inorganic carbon fixation in this system. Constraining these contributions will require future studies that integrate rate measurements with microbial community and metabolic analyses to better resolve the sources of DCF in oligotrophic waters...*" (Lines 539-543).

Line 415: Just use the 2025 reviewed version?

Reply: Corrected.