01 May 2026

RC2

We would like to thank the reviewer for the comprehensive review of our manuscript now entitled "*Ammonia and nitrite oxidation in the upper euphotic zone of the oligotrophic Red Sea*" (MS No.: egusphere-2026-855). We have addressed all comments in full and revised the manuscript accordingly. These suggestions have significantly improved the clarity and structure of the manuscript.

Below, we provide detailed, point-by-point responses. Our replies are indicated in blue.

The upper ocean nitrification is certainly a field that attract great interest from marine biogeochemist, which could relate to new production and carbon cycle. This paper suggests a negligible contribution of nitrifiers to Red Sea euphotic zone dark carbon cycle. Generally, it can be a potential piece of contribution, but I think there are some concerns regarding to methods and discussion:

Reply: We have carefully and comprehensively revised the manuscript in response to the reviewer's comments (as well as those of Reviewer RC1). All suggestions, edits, and concerns have been fully addressed.

Major concerns:

My personal experience suggests that one of the difficulties of euphotic zone nitrification in such oligotrophic regions is to measure the product isotope. The usual demanding N amount for denitrifier or chemical methods is 10-20 nmol N (or ~5 nmol for higher 15N ratio). In the context of low nitrate/nitrite level ~10 nmol/L, it will require 100 mL water to only achieve 1 nmol N in IRMS analysis (usually means large deviation between samples). The usual denitrifier bottle is max for 20 mL water injection. I am very confused about how to obtain isotopic results in such a low N concentration. Of course, now there are some more advanced mass spectra or protocols for lower N amount, but authors didn't mention any of these in the methods (Line 119-122).

Reply: We agree that measuring isotope enrichment in ultra-oligotrophic systems such as the GoA is analytically challenging due to the low ambient concentrations of dissolved inorganic nitrogen. We would like to clarify that seawater incubations were conducted in 1 L bottles (not 0.5 L as mistakenly stated in the original manuscript), which allowed us to collect sufficient sample volume to ensured sufficient N mass for reliable IRMS detection, even at the low ambient summertime concentrations in the GoA. We note that recent methodological modifications for isotopic analysis of low-concentration nitrite may provide enhanced sensitivity (i.e., Jiang et al., 2026 Analytical Chemistry), yet it does not indicate that the rate measurements presented here are skewed. We have revised the methods section to clarify the incubation volume and to better describe the analytical approach used for isotope quantification and their potential caveats.

"*...Note that in order to ensure sufficient nitrogen mass for isotopic analysis under low ambient nutrient concentrations, relatively large incubation volumes (1 L) were used, and a substantial* *fraction of the filtrate was processed for isotope measurements. The azide and denitrifier methods employed here are well established for the analysis of low-concentration nitrite and nitrate pools and allow reliable detection of ^{15}N enrichment in oligotrophic systems (Buchwald et al., 2018)…"* (Lines 127-132).

"*…Recent methodological modifications involving anion-exchange resin enrichment coupled with azide reduction for low-concentration nitrite isotope analysis were suggested to enhanced analytical sensitivity (Jiang et al., 2026); however, the established approaches used here are widely applied in oligotrophic systems and were sufficient for robust detection of ^{15}N enrichment in the present study…*" (Lines 132-136).

Another usual way to deal with this is adding carrier 14N into analysis bottles. But it would cause another serious detection limit problem, such a low rate with carriers 14N will means very small variation in isotopic signal. I didn't see any data or figure to support the detectable of end product and, thus, the rate.

Reply: As stated, the use of a ^{14}N carrier is sometimes applied to increase the nitrogen mass for isotopic analysis. However, for low-rate processes such as those measured here, the addition of carrier nitrogen would substantially dilute the isotopic signal and reduce the sensitivity of detecting small changes in ^{15}N enrichments. For this reason, no carrier was added in our analyses. Instead, we relied on relatively large incubation volumes and robust (and commonly used) analytical method, which allowed sufficient accumulation of labelled product for detection and reproducibility with other studies. The detection limit (0.1 nmol N L^{-1} d^{-1}) is now explicitly reported and supported by the clear separation between live samples and $HgCl_2$-killed controls. In the revised Figure 3, this detection limit is also indicated, demonstrating that the measured signals are above background variability and therefore analytically robust.

The incubation could be a potential problem. The high PP will also mean a quick turnover in the DIN system. This is even true under dark incubation, since N uptake is usually less sensitive to light and higher dark N uptake can be present. The nM level of N can be perturbated significantly, e.g., regeneration will dilute 15NH3 and/or uptake can eat all the 15N signals in product. The authors should provide evidence to suggest their system is valid for rate measurement in 1 day timescale, usually time series and carrier protection of product. The authors can also provide the end concentration of nitrite/nitrate compared with t0, which are usually simultaneously obtained with isotope ratio measurement.

Reply: We agree that 24 h incubations in oligotrophic systems may be influenced by rapid nutrient turnover, including regeneration and uptake processes that can affect isotopic signals. These processes could, theoretically, dilute the ^{15}N substrate pool or reduce the accumulation of labelled products. At the same time, in ultra-oligotrophic environments such as the GoA, long incubations are often required to allow sufficient accumulation of labelled product for detection, given the low biomass and expected low nitrification rates. Shorter (e.g., hourly) incubations, while minimizing turnover effects, may fail to produce detectable signals and therefore introduce their own limitations. Moreover, the clear separation between live samples and $HgCl_2$-killed controls allowed us to define a conservative detection limit, and the measured rates fall within the range reported for similarly oligotrophic systems (e.g., Figure 5).

Importantly, any dilution of the isotopic signal due to regeneration or uptake would bias the rate estimates conservatively (i.e., toward underestimation).

We have revised text to more clearly acknowledge these caveats and to emphasize that the reported rates should be interpreted as conservative estimates of nitrification activity over the incubation period.

"*...in oligotrophic systems, rapid recycling of dissolved inorganic nitrogen can influence both substrate availability (Christie-Oleza et al., 2017) and isotopic enrichment during incubation experiments (Stukel, 2020). Thus, processes such as ammonium regeneration and microbial uptake may dilute the ^{15}N substrate pool or reduce accumulation of labelled products (Braun et al., 2018). However, we surmise that any such processes, if occurred here, would tend to reduce the apparent isotopic enrichment and thus bias rate estimates toward underestimation. Another possible limitation regards the uncertainty in low nutrient concentrations in the GoA (most notably within the upper mixed layer depth) that may propagate into rate calculations, as substrate concentrations are explicitly included in the rate equations (see equations 1-3). However, such uncertainty affects absolute rate estimates proportionally and does not alter the overall interpretation of low nitrification activity. Accordingly, the reported rates should be considered conservative estimates of nitrification activity over the incubation period...*" (Lines 371-383).

Generally, the methods and validity of results are the basis to further discuss the environmental significance.

Reply: We thank the reviewer for this comment and agree that a robust methodological framework is essential for interpreting environmental significance. We have revised the manuscript to further clarify the methodological approach, explicitly acknowledge its limitations, and ensure that interpretations are appropriately constrained by the data.

Some minor suggestions:

Title: Line 1 Ammonia oxidation is more commonly used in the field (e.g., Bayer et al., 2025, nature geosciences; Ward 2008 etc.), instead of ammonium oxidation

Reply: Corrected throughout.

Line 43 nitrogen instead of N, or define before

Reply: Corrected.

Line 64 using PAR to define euphotic zone is a more common way or simply say upper ocean.

Reply: Sentence revised: "*...we report ammonia and nitrite oxidation rates in the upper euphotic zone (surface and down to ~100 m, representing 100% to ~0.5-1.8% of surface irradiance, respectively) of the Gulf of Aqaba...*" (Lines 64-66).

Line 95 precision of ~20 nmol L-1 may be not enough for low concentration of nitrite/nitrate in this case. As substrate concentration has a proportional impact on the rate calculation. Some note should be added in discussion. I will suggest adding example standard working curves and precision test of N species in the appendix in this case.

Reply: Nutrient analyses were performed following standard protocols with established calibration procedures and quality controls. While full calibration curves are not presented here, the analytical performance (including precision and detection limits) falls within the expected range for oligotrophic systems and is consistent with previous studies. Given that the primary uncertainty affects absolute concentration values, any associated impact on rate calculations would propagate proportionally and does not alter the overall interpretation of low nitrification activity. We revised the manuscript to clarify this point:

"...Note that calibration and quality control procedures were carried out during nutrient measurements. The analytical precision and detection limits were within the expected range for oligotrophic seawater measurements..." (Lines 106-108).

"*...Another possible limitation regards the uncertainty in low nutrient concentrations in the GoA (most notably within the upper mixed layer depth) that may propagate into rate calculations, as substrate concentrations are explicitly included in the rate equations (see equations 1-3). However, such uncertainty affects absolute rate estimates proportionally and does not alter the overall interpretation of low nitrification activity. Accordingly, the reported rates should be considered conservative estimates of nitrification activity over the incubation period...*" (Lines 377-383).

Line 100 low concentration measurement may be affected by blank effect, that is the water preparing standard working curve may contain NH3 blank which affect the final results.

Reply: Procedural blanks were routinely measured and subtracted from sample signals to avoid bias in reported concentrations. This step is a standard practice in low-level nutrient measurements and ensure that reported concentrations are not skewed by background contamination. We have ample experience in this kind of measurements in oligotrophic systems (e.g., the eastern Mediterranean Sea which is even more challenging).

We added: "*...Procedural blanks were routinely measured and subtracted from sample signals to account for background contamination...*" (Lines 105-106).

Line 112-114 The adding concentration may stimulate the rate, especially when natural substrate level is low.

Reply: The section was revised to reflect this potential cavate:

"*…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)...”* (Lines 158-163).

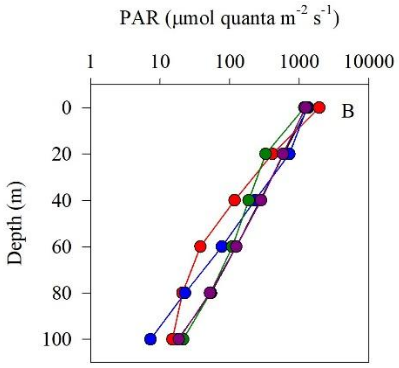
Line 119-122 see my major concerns The author omitted the nitrite removal protocol in nitrite oxidation rate measurement.

Reply: Please refer to our detailed reply above.

Line 234 PAR can be present in log and % scale and mark the 1% or 0.1% light level.

Reply: We now present the irradiance in log scale (i.e., revised Figure 1B). The % of surface irradiance at 100 m depth are also reported in lines 228-229.

Revised Figure 1B:



Line 237 typo in June 253

Reply: Corrected.

Line 281-line 308 Line 354 I think DCF in the upper sunlit ocean is somehow differ with aphotic ocean setting. Even under dark, phytoplankton can significantly contribute to DCF. Some discussion will be helpful. Also, some heterotrophs can contribute to DCF.

Baltar, F. and Herndl, G. J.: Ideas and perspectives: Is dark carbon fixation relevant for oceanic primary production estimates?, Biogeosciences, 16, 3793–3799, https://doi.org/10.5194/bg-16-3793-2019, 2019.

Li W. K. W., Irwin B. D., Dickie P. M., (1993), Dark fixation of 14C: Variations related to biomass and productivity of phytoplankton and bacteria, Limnology and Oceanography, 38, doi: 10.4319/lo.1993.38.3.0483.

Morris Ian, Yentsch Clarice M., Yentsch Charles S., (1971), RELATIONSHIP BETWEEN LIGHT CARBON DIOXIDE FIXATION AND DARK CARBON DIOXIDE FIXATION BY MARINE ALGAE, Limnology and Oceanography, 6, doi: 10.4319/lo.1971.16.6.0854.

Reply: We agree with the reviewer and revised the discussion accordingly. At the same time, because the present study focuses on ammonia and nitrite oxidation, we limit the expanded DCF discussion to its relevance for interpreting nitrification-linked carbon fixation rather than developing DCF as a central theme.

“*...DCF in the sunlit ocean should not be interpreted solely as nitrification-driven chemoautotrophy. Even under dark incubation conditions, inorganic carbon fixation may include contributions from phytoplankton-associated dark metabolism, heterotrophic inorganic carbon assimilation, and other microbial pathways (Baltar and Herndl, 2019; Reich et al., 2026). A recent 10-year analysis from the GoA (same study site) showed that DCF is a persistent but variable component of carbon cycling, contributing substantially to total autotrophic carbon fixation (Reich et al., 2024). Therefore, while our data suggests that ammonia and nitrite oxidation contribute only a minor fraction of total DCF, the remaining DCF signal likely reflects multiple unresolved microbial processes (Reich et al., 2025)...*” (Lines 414-422).

Line 318 Relation does not necessarily relate to causation, the rate correlated with depth and PAR. And the temperature just covaries with depth and rate. I don’t see it as a surprise

Reply: We agree with the reviewer and the sentence was revised accordingly: “*...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).

We also added the following text: “*...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...”* (Lines 217-218).

“...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...” (Lines 486-489).

Line 345-346 Phytoplankton biomass is not necessary represented by Chl a, especially in the light-decreasing depth context, photo acclimation can change the relationship.

Reply: We now clarify that the observed relationship likely reflects conditions at the DCM, where elevated chlorophyll *a* may result from photo-acclimation rather than increased biomass, and to avoid overinterpreting this signal in terms of biomass alone.

“*...This pattern likely reflects conditions near the deep chlorophyll maximum (~80-100 m), where chlorophyll a is elevated due to photo-acclimation under low light conditions rather than strictly higher biomass (Cornec et al., 2021; Fennel and Boss, 2003; Scofield et al., 2020). In these depths, reduced irradiance and enhanced organic matter turnover may promote ammonium regeneration, providing substrate that supports nitrification...*” (Lines 469-473).

Line 388 Figure 5. More explanation of data selection criteria should be clarified. For examples, how to filter euphotic zone, 100 m can be quite deep for coastal ocean, but too shallow for gyre. I will suggest authors to classify the regions to add some depth of discussion. I am personally very interested about, e.g., where has highest euphotic zone nitrification, where is low, why?

Reply: The data compilation presented in Figure 5 was derived from the recent excellent synthesis by Tang et al. (Earth Syst. Sci. Data, 15, 5039–5077, 2023), with the aim to place the rates measured in this study within the context of previously reported open-ocean observations. Accordingly, we included only offshore euphotic measurements as defined in the original studies. We acknowledge that the definition and depth of the euphotic zone can vary across oceanic regions, and we have clarified this point in the revised manuscript. However, a more detailed classification by region or water type (e.g., coastal, gyre systems) is beyond the scope of the present study, which focuses on regional measurements in the GoA. We now explicitly state this limitation and note that variability in euphotic depth and ecosystem structure likely contributes to the spread in reported rates across systems.

"*...Data include only offshore euphotic-zone measurements as defined in the original studies. We note that the depth and definition of the euphotic zone vary among regions, which may contribute to variability in reported rates...*" (Lines 551-553).