BG Discussion: Reply to Reviewer 1 Comments (RC1)

Thank you for your time in reviewing this manuscript. We appreciate your feedback and your efforts to improve this work.

This manuscript addresses scientific questions within the scope of Biogeosciences. It tackles the problem of how light influences the relative rates of four microbial processes involved in nitrite cycling in the eastern tropical North Pacific, and as such presents some novel data. The primary nitrite maximum and the base of the euphotic zone was sampled and then, using experimental incubations with 15N labeled substrates, the production of nitrite due to microbial ammonia oxidation and phytoplankton nitrate reduction were measured along with nitrite consumption by nitrite oxidation and nitrite uptake. The conclusion reached were that net nitrite production from these 4 processes was highest in dark treatments and that ammonia oxidation was the dominant process contributing to the net nitrite. The authors say that light may modulate nitrite accumulation in the PNM.

As they describe , historically the nitrite in the PNM has been thought to be due to both phytoplankton that take up and reduce nitrate in the light and then when they sink into the dark release nitrite, and an imbalance of the two steps of microbial nitrification as the nitrite oxidizers are more light-sensitive than the ammonia oxidizers. Apparently, few studies have directly measured the individual steps or processes of nitrite cycling in field collected communities (as is done in this paper) but have inferred the relative rates from microbial cultures. This paper supports the differential responses to light of the 2 steps of nitrification, and dark promoting the highest net nitrite production. This paper says that both microbial and phytoplankton processes occur, but that ammonia oxidation dominates the nitrite cycling (“a critical nitrite production mechanism”) and can occur in light up to 25% of surface PAR, although it tends to decrease with light treatments The effect of light on microbial nitrite reduction was not clear-cut and the authors determined that phytoplankton could be both net nitrite producers and consumers, although at one station there were significant contributions from nitrate reduction.

A few comments and concerns- although the overall presentation is clear and the language fluent, the visuals are extremely hard to read, especially Figure 6 – the different shaded of black and grey ae challenging to discern and I would recommend using maybe colors or patterned approach (e.g. stripes). Fig 6d - lines cannot be discerned. The symbol legends and tic labels are very hard to read as font is so small. The map can only be read if you enlarge the figure on the screen, not much good when as a pdf. Fig. 7- make the symbols

larger? And again, the lines are very faded- could these be black instead of grey? I used Tables S1 and S2 a lot when reading the paper so they should be included in the main manuscript. If tight on number of figs and tables, might incorporate Figure 3 with Figure 2- I used both together when reading.

Thank you for your figure suggestions. I have replotted Fig. 6 with higher contrast greyscale and patterned bar plots.

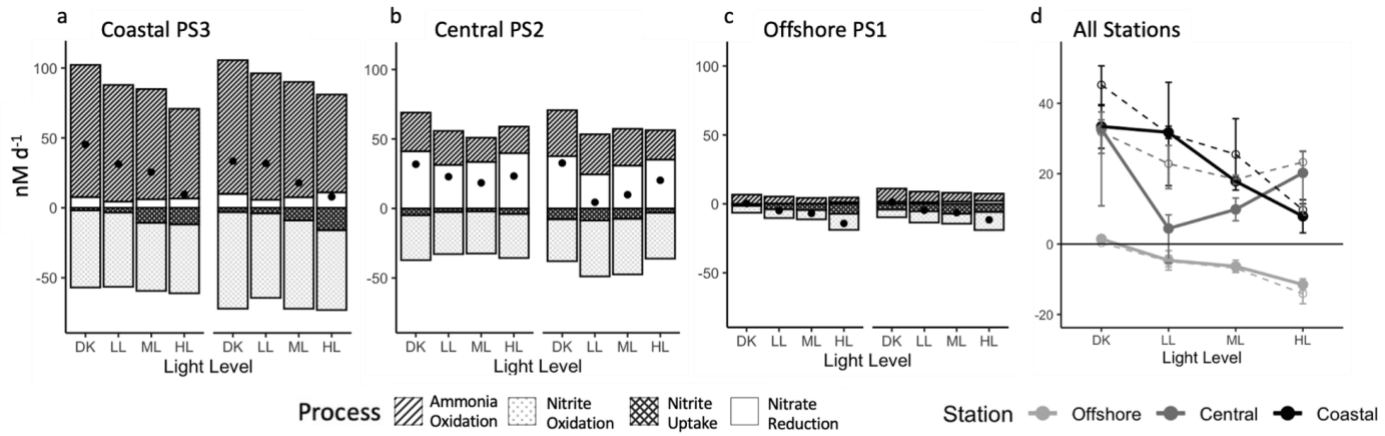
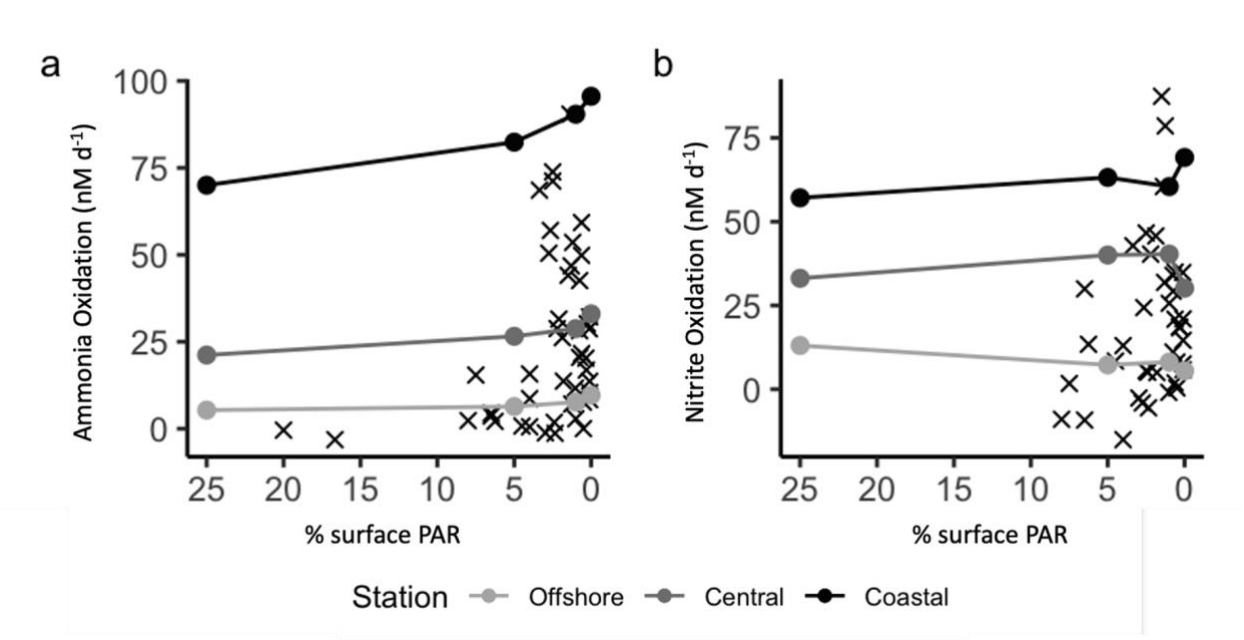


Figure 7 font and shape sizes have been increased and higher contrast greyscale has been used for the experimental lines. The station colors are now consistent with Figure 6.



I've left Figure 2 and 3 separate, because combining them onto Figure 2 leads to much smaller individual panels on the multipanel plot and a large white space. The map size will be increased, and Table S1 and S2 will be moved to the main manuscript as Table 2 and Table 3 respectively.

It would be great to have a small conceptual diagram with the four nitrite cycling processes plus the phytoplankton 15N uptake (Fig 3) to summarize the results, with maybe size of arrows indicating response to light. This diagram could also be used to present a simple mass balance.

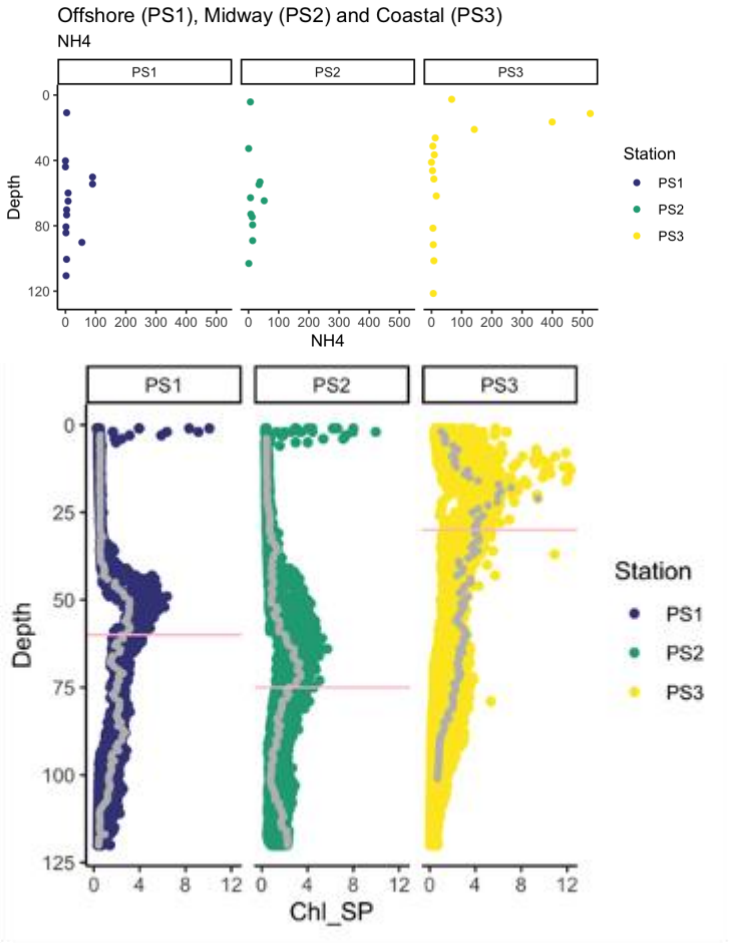
Thank you for this suggestion. We will add a conceptual diagram next to the map.

I am not a microbial nitrite cycling specialist but one concern I have is where the ammonium comes from in the ETNP to feed the ammonia oxidation. I was hoping that with the emphasis on microbial ammonium oxidation providing the nitrite for accumulation in the PNM, this source would be discussed. The only ammonium data was that in Tables S1 and S2. Maybe I am oversimplifying but at the rates described, the initial nM levels of ammonium available (Tables S1 and S2) would all be gone on the order of hours unless the ammonium was replaced from somewhere else- but from where- grazing?

Thank you for highlighting the importance of source ammonium for these processes. Rates of ammonium regeneration were not directly measured in this study, but other literature measurements suggest rates near the PNM (~1% PAR) on the order of 25-60 nM d^{-1} (Clark et al. 2005, EGU abstract). Dickson and Wheeler (1995) off the Oregon coast measured rates of >400 nM d^{-1} in the surface ocean. In the Atlantic Ocean, Clark et al. (2008) measured ammonium regeneration rates up to 160 nM d^{-1}, which was nearly 10x the associated nitrification rates. This suggests that regenerated ammonium can be supplied in excess of loss processes. The range in ammonium regeneration rates from the literature suggest direct measurements in this region would be helpful in better understanding the local turnover. Unfortunately we did not make direct measurements of ammonium regeneration in this study.

Ammonium profiles tend to have many local maxima through the surface layer. Our ammonium data from this cruise is minimal (only 3 discrete profiles, not measured every cast). However the ammonium maxima tend to line up with PNM and Chlorophyll maxima in a predictable vertically stratified pattern, suggesting classic ammonium source from phytoplankton decomposition/grazing. The persistence of the ammonium accumulation

below the chlorophyll maxima does hint that any ammonium sources are also fairly persistent and likely have rates either equivalent or greater than the measured consumption processes.



I think this mismatch may come from the methodology of using saturating levels of 15N substrate to measure the rates - ammonia is at the nM level and the 15N additions are 10 times the ambient concentrations. This may be lead to an overestimate of the ammonia oxidation being carried out, as these data offer the optimal potential of maximal ammonia oxidation. This is less of a problem for the nitrate and nitrite where ambient levels are uM, so adding 200 nM to measure nitrate reduction is more like adding the trace levels and is more realistic of the ambient situation. I realize the authors describe their rationale for using uniform 200 nM tracer additions and this would not impact the light treatment study as all treatments were given the same. But this approach will likely stimulate rates and overestimate ammonia oxidation relative to the nitrate reduction tracer data that was obtained with trace level tracer additions. This would also explain why in Fig 7 most the

experimental ammonium oxidation values are so much higher than the ambient measurements. Then this should be mentioned in the discussion more, and the emphasis on ammonia oxidation relative to phytoplankton nitrate reduction and nitrate uptake put into context.

Yes, you are correct in noting the potential for nitrogen additions to be more/less influential based on the ambient nitrogen concentrations present in the source water for each experiment. Ammonium ^{15}N additions (200 nM) are often a much larger percentage of the ambient ammonium pool compared to the ambient nitrite and nitrate. Ammonia oxidation kinetics work by Xu. et al (2019) showed that rate measurements in the subtropical western North Pacific were increased 3x with a 20 nM ^{15}N addition (starting NH_4 = 29 nM), with the caveat that initial absolute rates maxed out at 0.48 nM d^{-1} (V_{max}) which indicates a significantly different community of ammonia oxidizers than our region of study. Work by Horak et al. (2013) with field communities from the Hood Canal, WA also showed increases in rates up to 6 nM d^{-1} due to 300 nM ^{15}N spike concentrations (starting NH_4 = 50 nM). While the ^{15}N spike addition doubled the absolute rate, again rates observed in the ETNP region (our study area) can typically be much higher (>20 nM d^{-1}).

In work by Beman et al. (2013) from the ETNP region, a uniform 42 nM ^{15}N spike was used to measure ammonia oxidation rates with ambient ammonium concentration at the ammonium maxima reaching up to 200 nM, and their peak ambient ammonia oxidation rates at each station ranged from ~ 35 to 120 nM d^{-1}. These rates are similar in range to the ambient rates measured using our 200 nM ^{15}N spike methods (this manuscript and Travis et al. 2023), suggesting that the percentage of ^{15}N added may not influence the variation in rates as much as the variation in archaeal community across stations. However, these rate measurements are both likely to be potential rates (enhanced by the ^{15}N addition to an unknown degree). While ammonium additions were typically a larger percentage of the ambient ammonium (compared to ^{15}N-nitrate spikes), since we do not have corresponding kinetics experiments we cannot determine the relative enhancement of each process (e.g. ammonium oxidation vs nitrate reduction). It is likely that each microbial community responds to substrate increases to differing degrees. This caveat will be more clearly noted in the discussion.

Although the paper is focused on the influence of irradiance, the question of where the nitrite in the PNM is always in the background and this emphasis on ammonia oxidation from experimental saturated uptake values may be a bit misleading; the phytoplankton nitrate uptake rates (Fig 3) suggest that phytoplankton may still be important, even if the

direct 15NO3 to 15NO2 rates (nitrate reduction) measured with trace isotope do not seem sufficiently high.

Yes, we agree with the nuanced interpretation that nitrate reduction may still be an important contributing process for nitrite production under some conditions. These situations may be slightly obscured by the tendency for our ^{15}N spikes to enhance measured ammonia oxidation rates more than measured nitrate reduction rates. We will try to highlight this point more clearly in the discussion.

On positive note, the methods and assumptions were clearly outlined, the results both in the supplementary and main body supported their interpretations and the number of references were appropriate. Amount and quality of the supplementary material was appropriate although Figure S1 should be increased in size and a vertical profile of ammonium should be provided for context.

Figure S1 will be increased in size, and ammonium data will be added into the supplement.