Biogeosciences Discussions

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Nicole M. Travis et al.

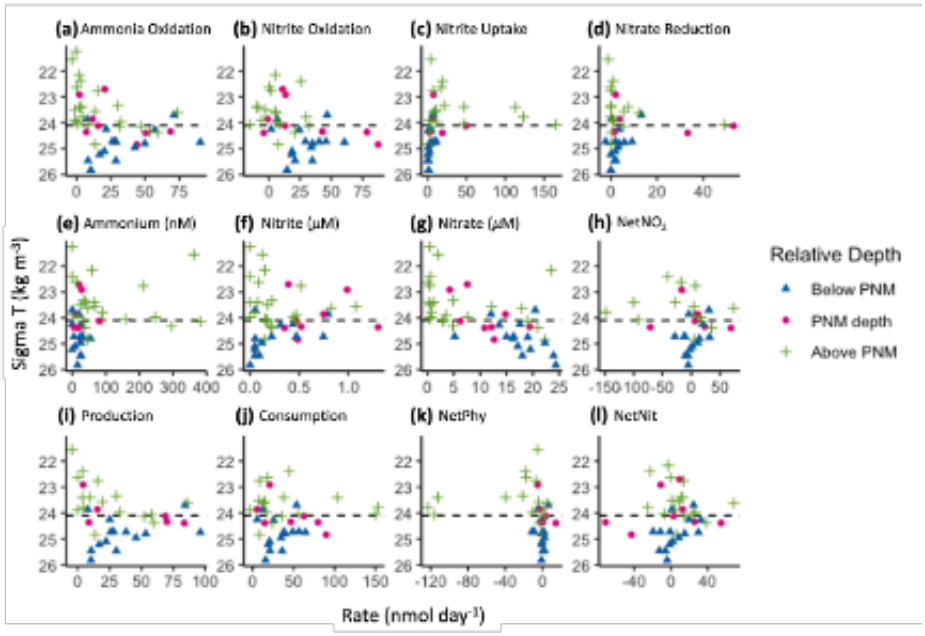
Author comment on "Nitrite cycling in the primary nitrite maxima of the eastern tropical North Pacific" by Nicole M. Travis et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-137-AC1, 2022

1.2 Major comments

1) The paired light-dark incubation is one of the strengths of the present study. Given that most previous studies used dark incubation for nitrification rate measurement and light incubation to quantify phytoplankton-associated processes rates, the paired light-dark incubation should inform more comprehensive and accurate rates by integrating the rates derived in both conditions. The present manuscript is unclear how the daily rate is derived and whether the results from both light-dark incubations have been incorporated? Meanwhile, comparing the light and dark incubation rates would also help assess the diel rhythm of NO2- cycling in the sunlit ocean.

All the rates used to calculate daily rates in the original manuscript were from incubations at near-in-situ light levels, which in most cases was the low light (LL) tank (~1% PAR). The exceptions were from shallow depths where the medium light or high light incubation tanks were closer to the ambient light levels at source water collection depths. Each of these in-situ light levels has a paired dark incubation. The daily rates (nM/d) reported in the original manuscript for ammonia oxidation, nitrite oxidation and nitrate reduction were calculated using hourly rates from the ambient light treatments (incubation length of 8 hours starting near dawn) multiplied across 24 hrs, and did not incorporate the dark-incubated rates. Therefore if dark nitrification rates are higher than light incubated rates, the previously presented daily nitrification rates would be underestimated. Conversely, if dark rates of nitrate reduction are lower than the light incubations, the previously presented daily phytoplankton rates would be an overestimate. Nitrite uptake rates were measured from a 24 hour incubation period that captured a full day:night cycle, and therefore represent a true daily rate.

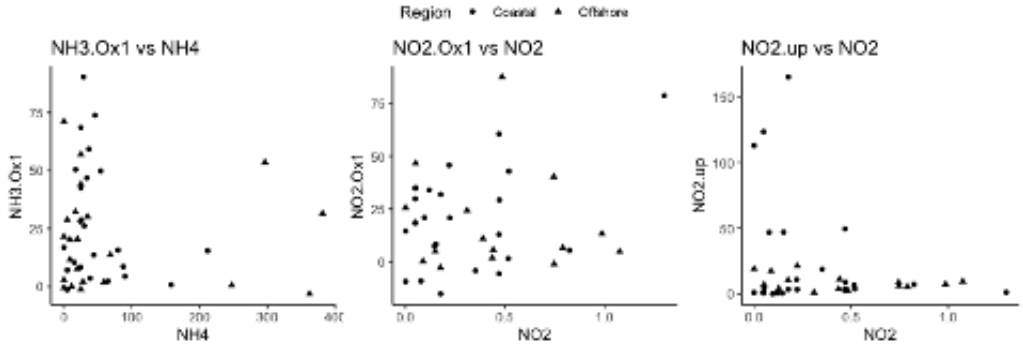
In response to the reviewer question, we used the paired dark:light incubated samples to calculate new daily rates using a simple assumption of a 12 hr:12 hr daily cycle. Some dark incubation data were not available for nitrate reduction measurements in 2016, so those daily rates were calculated using 24 hr light incubated rates. Daily nitrite uptake rates were not recalculated due to the 24 hr incubation time mentioned above. Figure 4 (revised below) and the rate data supplement (Table S1) will be updated to include dark rates where available, and the new calculated daily rates.



This correction had minimal impact on the overall patterns seen in the nitrification rates, because dark and light incubations did not differ significantly. This is an interesting finding in itself, and the average daily rates using 12 hr:12 hr daily cycle vs 24 hr low light daily cycle for ammonia oxidation and nitrite oxidation were 23.0 vs 23.3, and 18.9 vs 19.1 nM/day, respectively.

2) I have concerns about using the gross rates derived from the high tracer enrichment (i.e., 200 nM). Because the NO2- concentration drops sharply outside the PNM, and the NH4+ concentration appears to be low in most of your incubation depths (Table S1), the rates reported here should be attributed to 'potential rates.' While I acknowledge these potential rates are still very valuable, care should be taken in interpreting these results. For example, the authors measured some conspicuous high rates under substrate depleted samples, such as those high NO2- uptake rates (> 100nM/d) above the PNM where ambient NO2- is low. It tells the high potential for the phytoplankton to control the cycling and distribution of NO2-, but is it meaningful to use those potential rates to calculate the residence time?

We agree that the measurements presented are best characterized as *potential rates* and are likely stimulated by the addition of a large, but consistent, amount of 15N tracer. In the simple scatter plots below (rates vs ambient reactant DIN), many high rates occur at the lower end of the range of ambient substrate concentrations, where the addition of 200nM 15N tracer would be a significant alteration. It is ecologically relevant that some cells in low DIN locations are primed to respond robustly upon addition of DIN - perhaps during upwelling or eddy intrusion. However, not all low substrate samples resulted in high rates. This suggests that the potential rates were not fully driven by the addition of substrate, but also conditions of the source water at our sampling depths (with potentially variable abundances of active microbes) likely played a role in the observed rates.



While we cannot conclusively show whether low per volume rates were a result of low cell concentrations or lack of enhancement from tracer addition, the spatial distribution of rates plotted in Figure 4 shows how samples collected from a variety of ambient conditions – and therefore experiencing varying levels of rate enhancement due to 15N additions and varying cell concentrations – still reveal vertical zonation in maximum activity for each rate process. It remains unclear what controls this vertical distribution, although the MLR analysis suggests that nitrate and light are important environmental parameters relating to nitrite accumulation. Experiments that directly test environmental controls on microbial rates are needed, such as light and DIN manipulation experiments. While the rates presented here are per liter, not per cell, they are still useful in understanding bulk water column nitrite transformation.

We agree with the reviewer that our estimates of average residence time using these potential rates could be underestimated because of potential enhancement from tracer additions, and this caveat will be noted in the revised manuscript. We also note that steady state dynamics are an assumption too, and likely untrue. Residence times were on the order of days to weeks (albeit with a very large range). Perhaps summarizing these residence times into a mean was an oversimplification that hides the variance displayed in Fig S6, especially above the nitrite maxima. The median residence time (using total production input) was 7.8 days, while the mean was 30.4 days. We are also likely missing an input/output term from physical mixing, which could have a larger influence in dynamic coastal waters compared to offshore. Separating coastal stations and offshore stations shows that residence time estimates from rate data alone were slightly longer at offshore stations.

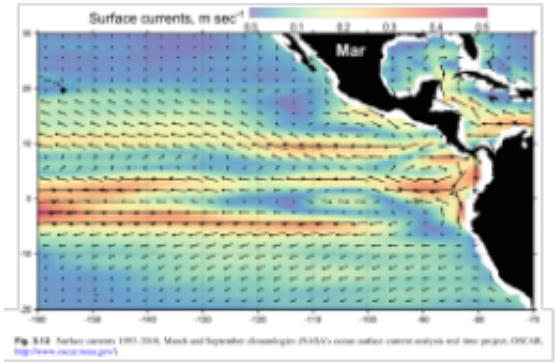
- Coastal - mean 17 days, median 5.8 days
- Offshore - mean 53 days, median 18.2 days

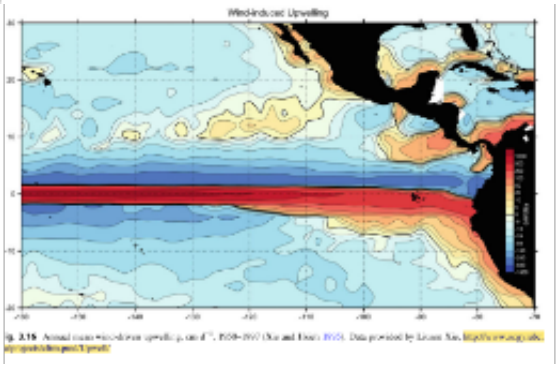
It is valuable to consider the differences in potential residence times between coastal and offshore conditions, even with the limitations and assumptions of making residence time estimates from nitrite production/consumption rates where the rates may be enhanced by tracer addition. It is likely that multiple methods will be needed to more accurately constrain the residence time of nitrite in the ETNP PNM.

3) The prominent accumulation of NO2- (i.e., >1μM) in the coastal stations is not surprising. It is interesting to see the absence of higher ammonia oxidation rates in these more eutrophic systems, as is frequently observed in other studies. On the other hand, the authors observed a mild increase of NO2- released by the phytoplankton, but I expect a long time is still required to get the high NO2- concentration observed here. The question is, can you find evidence of such a long residence time of the water mass here, as I expect a short residence time of these shallow, high dynamic coastal waters?

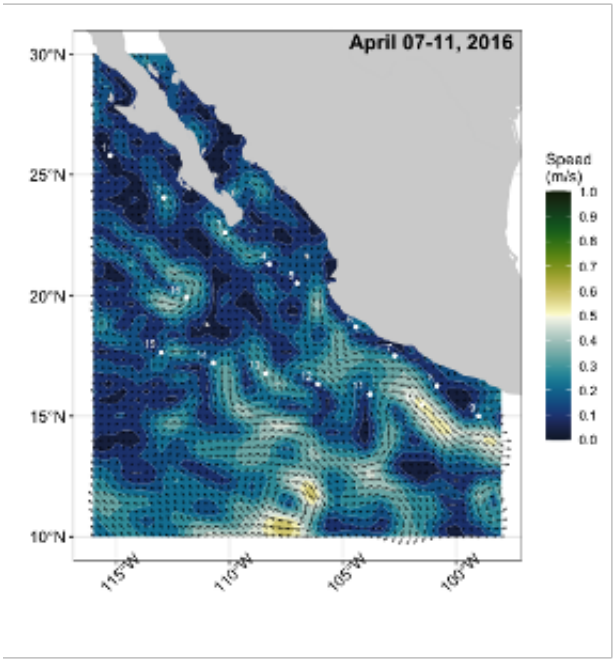
Coastal waters are, indeed, typically more dynamic than offshore, and the ETNP experiences upwelling along the coast. We can see increased wind-driven surface currents

occurring on the southern coastline of our study area in the spring months, and evidence of upwelling from historical data from the region (see cropped figures Fiedler and Lavín, 2017, below). A shorter residence time for coastal water is a good expectation as winds drive offshore transport.

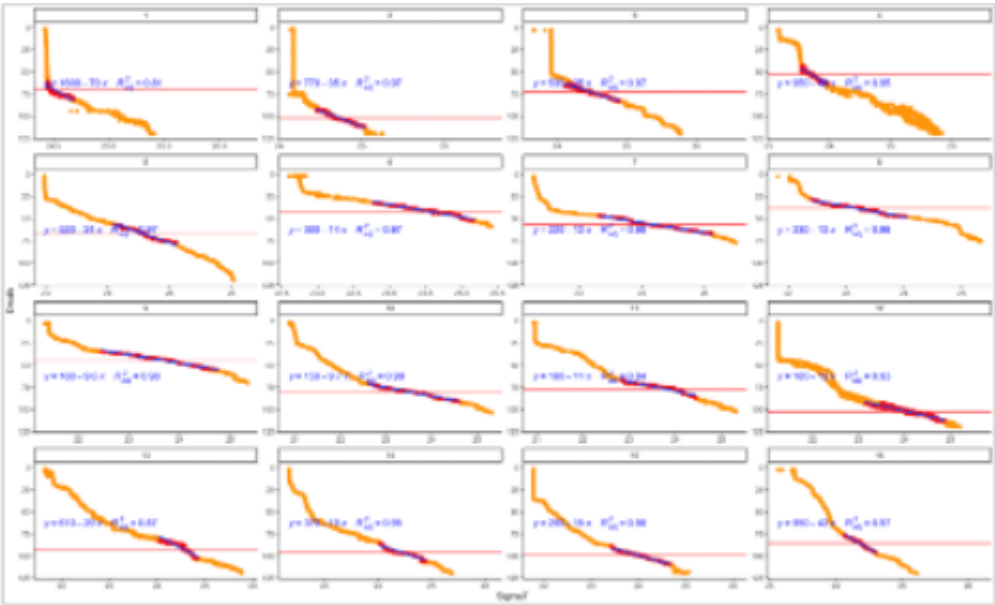




The surface current plot for April 07-11 2016 (5 day average from OSCAR; Earth & Space Research, below) showed fastest movement of surface waters near the southern coastal stations (6,7,8,9), which is similar to the averaged March surface currents by Fiedler and Lavín (2017). This subset of 4 southern coastal stations (used to inform the coastal MLR) have the largest observed nitrite maxima (800-1400 nM), larger chlorophyll maxima and shallower nitraclines. The nearby fast surface currents imply faster offshore transport of water, and a shorter residence time for near surface depths around the PNM feature during this study. In situ rates were only measured at stations 6 and 9 of this subset, where ammonia oxidation reached 73.8 and 46.8 nM/day, respectively. At the 40m depth at station 6, with low rates of nitrite uptake and nitrite oxidation, the net nitrite production rate was 73.5 nM/day (with total production at 86 nM/day). Considering this net production rate and the observed 824nM nitrite maxima, the nitrite accumulation would take a number of days to accumulate. However, even with the faster surface velocities at these stations, a median residence time of nitrite around 6 days seems feasible.



In addition, the density gradients at the coastal stations and the calculated Brunt-Vaisala frequencies gives some insight into physical mixing of water near the PNM, as high BV values indicate strong density gradients and reduced vertical mixing. The highest BV values (strongest density gradients) were found at the coastal stations, and corresponded with some of the largest nitrite maxima (Fig 2I, and station density profiles below). The full density profiles will be added to the supplement. As discussed in the manuscript, nitrite produced at coastal stations within compressed density gradients may not be diffusing/advecting vertically away from the production depth, thus leading to higher concentrations at those nitrite maxima even with similar net nitrite production rates to offshore stations. Our interpretation is that the higher rates together with the increased vertical stability allow the coastal stations to accumulate more PNM than offshore stations, and while the water moves through the coastal zone relatively quickly, its residence time in the coastal zone is long enough to support the required accumulation of nitrite over its 6 day residence time.



I am also very interested to know if the main findings (both the contributing processes

and time required for PNM formation) derived from the tracer experiment are consistent with dual NO2- isotope (i.e., 15N-18O natural abundance) in this region (if any). To my knowledge, the combined use of the natural abundance and isotope labeling approaches in exploring the PNM remains lacking, so any comparisons between these two independent methods would be very helpful.

We fully agree that using multiple approaches to understanding the PNM formation would be informative, and we plan to contribute that piece in a subsequent paper. However, the analysis and interpretation of the natural abundance nitrite isotopes is a substantial undertaking in its own right, and it is our assessment that adding it to the current paper would be unwieldy. Hopefully future analysis of the corresponding dual nitrite isotopes from this cruise will offer an opportunity to compare rates estimates (and residence time estimates) between these two methods.

2 Specific comments:

Line 41: Not sure how the authors derive the concentration of 300nM? Is it based on any specific statistics? As NO2- concentration at the PNM varied over space and time (as highlighted in the manuscript) and frequently fell below 300nM (e.g., in the subtropical gyres), justification is needed to better clarify the number.

The ~300 nM average PNM nitrite concentration was derived from the GLODAP Pacific Ocean nitrite measurements. Cast data from the Pacific Ocean was filtered to remove data with <3 μM oxygen or any data below 400m to exclude any secondary nitrite maximum measurements from oxygen deficient zones. PNM size was calculated from the maximum nitrite concentration at each station. This analysis yielded maxima ranging from 0 to 3.29 μM (excluding a single value >10μM), with an overall average primary nitrite maximum of 252 nM across 3485 stations. Because mean values can be skewed by outliers, we also calculated the median PNM size across the GLODAP Pacific dataset, median nitrite maximum of 237 nM. These values will be clarified and revised in the manuscript.

Line 56: I suggest revising the description of the three microbial groups to ammonia oxidizers, nitrite oxidizers, and phytoplankton.

Yes, microbial groups will be clarified as requested in the revised manuscript.

Line 57: Bacterial ammonia oxidizers should not be excluded as they might also play a role in some regions, such as the coastal zone.

We agree that bacterial ammonia oxidizers should not be excluded, and our rates represent the sum of all ammonia oxidizers in the community, since they were measured as bulk community rates. We did not attempt to identify what components of the ammonia oxidizing community were most active. Our intention was simply to note that archaeal ammonia oxidizers are typically dominant. This will be clarified in the revised manuscript.

Lines 138-154: Please clarify the detection limit and accuracy of the methods used for on-board and onshore nutrients measurement.

Detection limit for shipboard spectrophotometric nitrite measurements is typically 200nM (Strickland and Parsons, 1972) and in general agreement with what we directly derived from our standard curves. We will add detection limits and accuracy to the revised manuscript.

Line 151: The acronym of PPS has been defined in line 136.

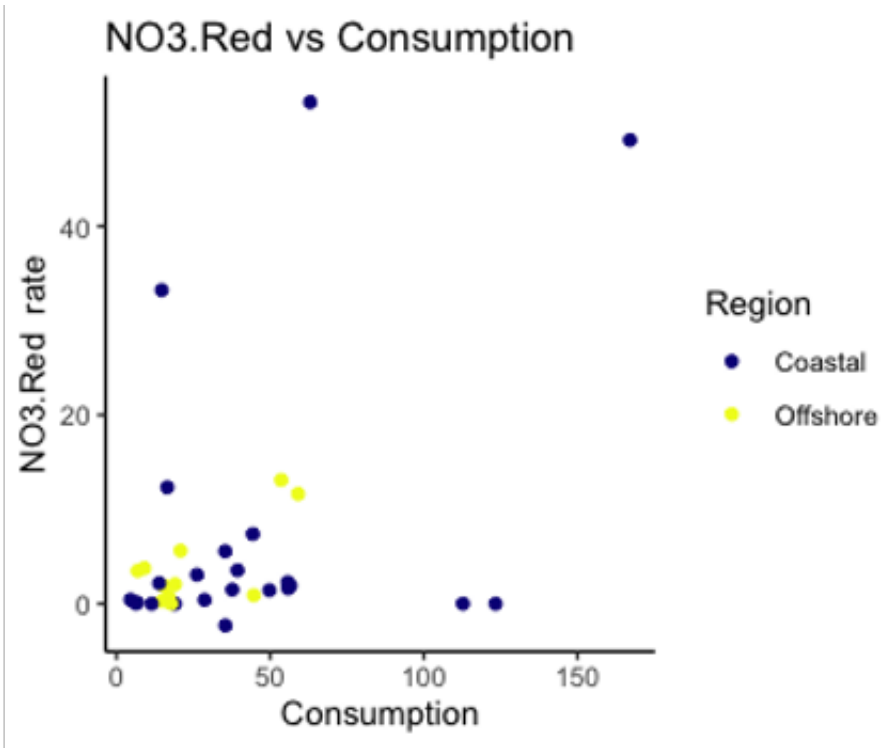
The PPS acronym will be removed from this line.

Lines 180-181: I have concerns about using the HDPE Nalgene bottles for light incubation due to the screen of light by the HDPE bottle. PC bottles are more widely used for simulated light incubation. Has the light intensity been measured inside the bottles? How accurate was the actual light intensity compared to the in-situ light intensity?

We apologize for the error during writing. The light incubations were indeed all conducted in clear polycarbonate bottles, as the reviewer states is typical for light incubations. The brown bottles used for dark incubations were HDPE, and we made a typographical error in describing the light incubations in the original manuscript. The light levels were not measured inside the PC incubations bottles, but each incubation tank was tested using a PAR meter to determine the incubation light levels. Percent surface PAR at each collection depth was also measured (see Table S1).

Line 213: For the samples with low in-situ NO2- concentration and high NO2- consumption rate (either by NO2- oxidation or assimilation), the newly produced 15NO2- via 15NO3- reduction might have been utilized by NOB or phytoplankton. The observed NO3- reduction rate thus might represent a conservative estimate.

Yes, it is possible that estimates of nitrate reduction were confounded by uptake of the 15N labeled product. In response to this comment, we explored our data for any patterns that might reveal the described issue. In reference to the figure below, we found that low nitrate reduction rates were found throughout the range of nitrite consumption rates. In addition, we found high nitrate reduction rates even at high rates of nitrite consumption. Two points showing low nitrate reduction rates at high rates of nitrite consumption were investigated further. These points derived from a coastal station (PS1) during the winter 2017 cruise at the shallowest depths measured (25m), where nitrite concentrations were near zero. It is possible that nitrate reduction measurements are underestimated for these samples.



Line 233: I have two questions/ concerns regarding the rate calculation: 1) the equation is used to derive the gross (i.e., in-situ NH4+ plus 15NH4+) oxidation rate (potential rate),

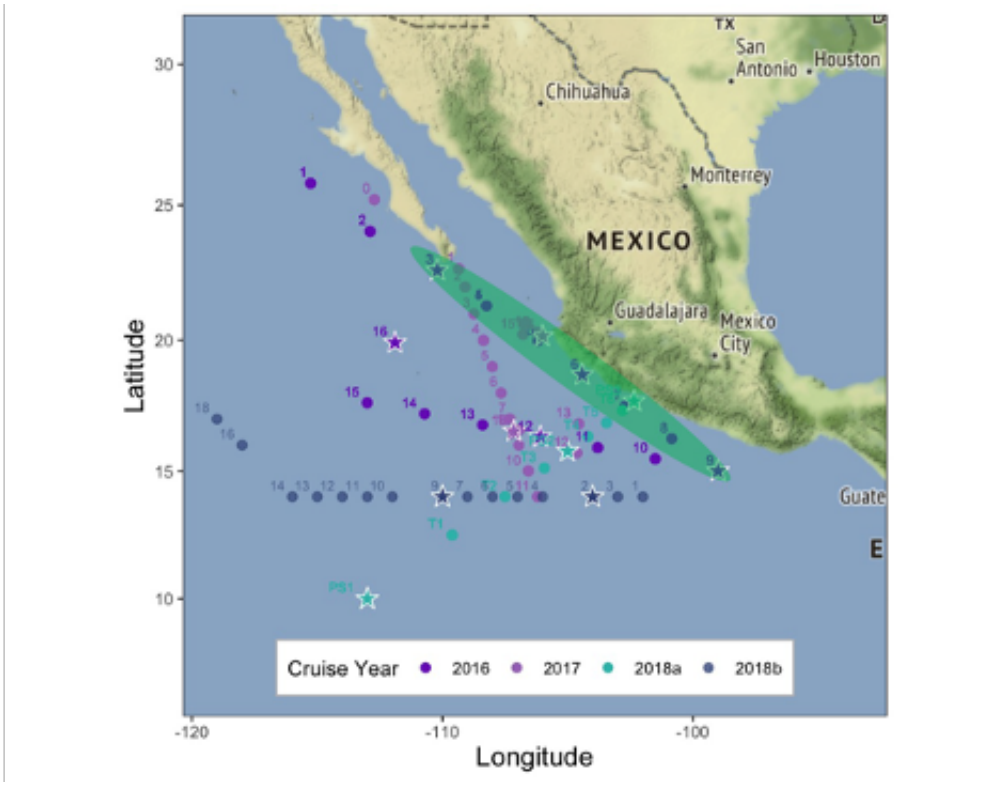
taking the different f15N for samples from various depths and stations, the enhancement of the rate due to tracer enrichment would also be varied. 2) it is unclear to me how the daily rate was calculated, have the rates from light and dark incubation been considered? These issues should be clarified.

- We did not correct for spike enhancement. This issue would affect NH4+ and NO2- because ambient DIN is highly variable near PNM depths. This is part of the reason why a single concentration was selected for 15N tracer addition, since it quickly became unfeasible to measure all ambient DIN pools and calculate a 10% tracer addition for each experimental sample. I believe this concern will have to be addressed as a caveat to the dataset, stating that these are most definitely *potential rates* with a high likelihood of being stimulated by the tracer additions in most incubations, and that the relative enhancement of the rates may vary across depths due to different ambient DIN concentrations. However, the initial 15N fraction in the experimental substrate pool was still calculated using the ambient concentrations and the tracer addition in order to determine final rates. Another limitation when comparing rate measurements across depth, is that these are bulk rates per volume, not per cell, and it is not known how cell concentrations varied with depth in these samples.
- Daily rates were originally calculated using only the light incubation rates, converted to an hourly rate and scaled to 24 hr. In response to the reviewer comment, these rates will be recalculated based on a 12 hr:12 hr daily cycle using light incubation rates for the 12 hr light and dark incubation rates for the 12 hr dark. We will try an analysis of the influence of light (percent inhibition/percent enhancement) by using the paired ambient light vs dark incubations. See also Major Comment 1.

Lines 311-312: The criteria for defining coastal and offshore stations should be clarified, e.g., by using the isobath or distance to the shore? Mark the boundary of the 'coastal zone' in Fig. 1a should also be helpful.

A subset of stations was selected based on water column traits such as their larger chl maximum, shallow nitracline, and shallow mixed layer depth specifically for training the "coastal" model in the multiple linear regression analysis using 2016 pump profiler cast data. The construction of the multiple linear regression models used subsets of example stations (four each) to train the "coastal" and "offshore" models, although there are other stations within the complete data set that could be characterized as coastal or offshore. Although they are not strictly defined spatially, we used the wording "coastal" because those water column traits are more common at coastal stations experiencing upwelling.

Marking the coastal stations on the map does seem helpful for visualizing where data was collected, so we will encircle all the spatially coastal stations in green (see updated map below) in the revised manuscript. We will also explain in the MLR section that only a subset of 4 spatially "coastal" stations from 2016 (Stations 6,7,8,9) were selected because they had water column features that were most representative of upwelling-like conditions (eg. shallow nitracline, larger chl maxima).



Line 349 and line 373: Fig. 2 and 3 contain a lot of statistical information that is very useful. But even though the regression of NO2- maxima /PNM depth against the parameters (listed in Table 1) was not fully shown in Fig. 2 and 3. Would you consider plotting a heatmap showing the result of Pearson correlation analysis (or other statistical information) between NO2- maxima /PNM depth and the collected parameters? That would provide more comprehensive information and save some space at the same time.

Yes, we agree that this would be helpful and will put the correlations in a table format.

In addition, as you have measured NO2- uptake rate, I assume you have also measured the PN concentration, which is a better indicator of organic matter stock and biological productivity than the Chl-a; thus should be included in the main results, and the statistical analysis.

Yes, we do have some PN concentration data, but they are limited to stations and depths where an uptake rate was measured, so there would be fewer data to use in the regression analyses if based on PN concentration data. Therefore, we have chosen to retain the correlations to Chl-a, as a proxy for PN concentration.

Lines 434-435: These results suggest either a quasi-homeostatic or physical dispersion plays a role in determining the magnitude of PNM.

Yes, we investigated this through the Brunt Vaisala frequencies, which correlated with PNM size. These correlations suggest that interaction with physical processes may indeed play an important role in formation and maintenance of the PNM feature. As we discuss, the concentration at the nitrite maxima cannot be explained with just the rate measurements. We investigated this further in response to Major Comment 3 (above).

Section 3.6 and 3.7: These sections are long and read dense; many results are similar between the sections. There are also a lot of similar descriptions in section 4.3. I suggest reducing these parts and focusing on the main findings.

Thank you for this suggestion. All MLR sections will be shortened and details moved to the supplement.

Lines 609-610: I concur that a net production rate is required for the formation and maintenance of NO2- accumulation at the PNM. On the other hand, negative feedback, either physical dispersion and/ or enhanced biological NO2- consumption, must be involved to restrict the magnitude of PNM to a certain degree. That saying, NO2- accumulation at PNM should be in the quasi-steady state that varies over the diel cycle, seasonal cycle, or some event disruption, making the concentration of NO2- at PNM less predictable.

Yes, we agree that the PNM is an observable manifestation of a lot of moving variables. And while the feature is ubiquitous, and seems constrained in terms of depth (always the base of the euphotic zone), its size (although usually in the 100-1500 nM range) is not easily predictable by snapshot measurements in the water column, such as environmental data or rate measurements because of periodically changing environmental conditions. Mackey et al. (2011) investigated the formation of a PNM at a single location over time, and the PNM feature went from low concentration (vertically homogenous) to a peak-shaped profile reaching near 1 uM at its peak in just 7 days with the onset of stratification and a chlorophyll bloom. It would be interesting to see how rate measurements might change along a sequence of 7 days during PNM formation.

Line 650: See my comment on sections 3.6 and 3.7 above. Please consider reducing these sections to avoid redundancy.

Thank you for this suggestion. All MLR sections will be highly reduced/summarized and details moved to the supplement in the revised manuscript

Lines 710-711: Check the format.

Thank you, we will remove the underlines

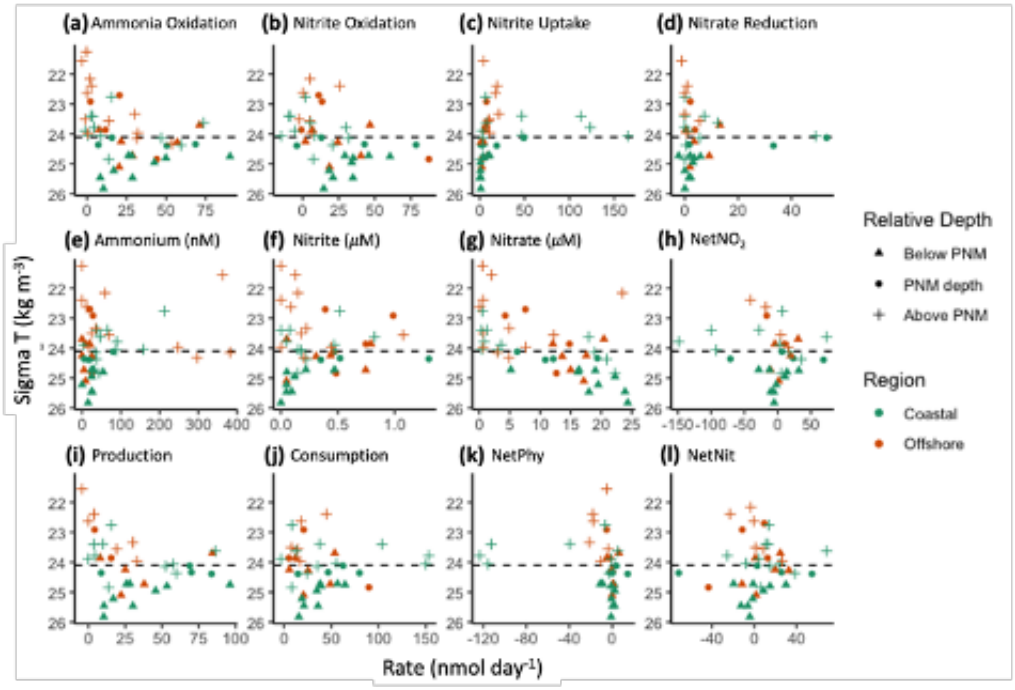
Lines 749-751: Again, these results indicate a dynamic feature of NO2- concentration at the PNM, which can't be fully explained by a snapshot of the measured rate.

We agree that while rate measurements are useful, more information may be needed in order to predict nitrite profiles accurately. Modeling efforts beyond simple MLR analysis may be a good way to combine information gained from direct rate measurements with natural abundance isotope constraints and physical processes.

Line 829: It looks to be the only difference between panels a and b is the role of phytoplankton in releasing NO2-, with a higher rate in the more eutrophic coastal upwelling zone. At the same time, the role of nitrifiers remains unchanged. The question is: what is the potential cause for the absence of stimulation on nitrification rate to the enhanced productivity (and thus organic regeneration and substrate supply)?

The schematic in Figure 9 was built using the observations from this dataset, namely that nitrification rates were not significantly different between the offshore stations and the coastal stations, while all of the highest nitrate reduction rates were coastal (see replotted Figure 4 below). The mean rates of ammonia oxidation were not significantly different between coastal stations (25.8 nM/day) and offshore stations (20.4 nM/day), $t(44)=0.78$, $p=0.44$. Rates of nitrite oxidation were also not statistically different between coastal (18.7 nM/day) and offshore stations (19.2 nM/day), $t(28)=-0.07$, $p=0.94$. However, there was high variance in the rate measurements, and all depths were included in the mean calculations.

We agree that it makes sense that enhanced productivity, regeneration, and nutrient cycling would lead to higher rates of nitrification. We could speculate on why this was not observed, such as the absence of an active nitrifying population, inhibition by light, or competition with phytoplankton for the released ammonium. To answer this question, we would likely need additional information, such as cell counts, or experimental data addressing light inhibition. Another question is whether the depth-integrated rates of ammonia oxidation are higher at coastal stations. We do have a separate dataset on experimental manipulations that will be analyzed for a subsequent manuscript. For this paper, we will determine the depth-integrated rates of ammonia oxidation, and report that if it shows an interesting pattern that might add insight to this question.



Lines 859-861: On the other hand, won't the upwelling reduce the residence time of the water mass at PNM due to the replacement of water by the upwelling? Also, can you see any clues of NO2- supply from SNM as I expect significantly higher NO2- can be seen in the oxygen-depleted subsurface water in at least part of your stations?

The SNM and PNM are separated spatially in the water column, so that SNM nitrite likely doesn't overlap with the PNM at these stations. All data with <3μM oxygen was removed from analysis. It's an interesting question about the effect of upwelling on the residence time of water in the PNM. Unfortunately, we don't have age tracers together with our data and would be speculating on the residence time of the water itself. The short residence time of the nitrite in coastal waters (around 6 days) does seem to be in keeping with a dynamic coastal environment.

Supplementary Line 70: What does this panel show?

Figure S7 shows a comparison of the observed nitrite concentrations and modeled nitrite concentrations from the full-variable multiple linear regression analyses. The bottom panel shows how the "coastal" MLR model (built using 4 example coastal stations) has a poor fit when applied across all 16 stations. We will update the caption to reflect the 4th panel.

*Higher resolution Figures attached as PDF

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2022-137/bg-2022-137-AC1-supplement.pdf