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Biogeosciences Discussions

Interactive comment on "Nitrogen budgets following a Lagrangian strategy in the Western Tropical South Pacific Ocean: the prominent role of N_2 fixation (OUTPACE cruise)" *by* Mathieu Caffin et al.

Anonymous Referee #2

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Summary and Overall Impressions

This is a well-written, interesting, enjoyable paper that quantifies the source of "new" nitrogen to the euphotic zone as well as the flux of nitrogen derived from N2 fixation with respect to diazotroph community and other factors. Data was collected from three stations in the western tropical south Pacific ocean. Two of these stations were oligotrophic while the third was "ultra" oligotrophic. Nitrogen fixation was overwhelmingly the largest source of N input due in part to very high rates of nitrogen fixation and lower than typical rates of atmospheric deposition. N flux from nitrogen fixation was

uncoupled from N inputs, and possible reasons for this are well discussed.

This paper is well presented and the "story" is clearly told. Everything is well organized and easy to follow. The conclusions are interesting and important, and I really liked the 5 day averaging and well resolved vertical profiles. Daily variability is high and important to measure! In addition, the comparison between in situ and on deck incubations is useful and important, as most researchers are unable to use in situ arrays. The introduction and discussion sections are very well done, but I do have some concerns about some of the methods and the presentation of the results.

My primary concerns are methodological and regard 1) the nitrogen fixation rate calculations including the absence of a reported detection limit and a failure to completely address the failings of the "bubble method" and 2) propogation of error throughout calculations and some additional statistical comparisons. These issues can all using data already in hand, and I think that these changes are unlikely to impact the conclusions of the manuscript. Nevertheless statistical rigor and proper calculations are important. I have also listed some line item concerns below.

General Comments

Throughout the paper, nitrate transport is refered to as a "diffusive flux", but "diffusion" brings to mind molecular diffusion, whereas this flux is really a result of vertical mixing. Please rename this term or, if this is standard terminology, indicate clearly that this is not molecular diffusion but is a physical mixing process.

Regarding atmospheric deposition, was atmospheric deposition of NH4 considered? What about DON? - These would be "new" N that look like regenerated N. Also, throughout the manuscript, "atmospheric deposition" reflects dry deposition only (as opposed to wet). Please modify the text throughout so that this distinction is clear.

Some of the notation was a bit confusing. was used both for integrated N2 fixation rates (i-N2) and density and nitracline density (NOÂň3). I know that these are established

conventions, but use of the same abbreviation is a bit confusing and these should be clarified somehow.

The methods section could use a bit more detail and/or additional references. I have indicated specific problems below.

In section 2.1, a more clear description of which parameters led to the designation of the three stations would be useful, i.e. LD A was oligotrophic and Tricho dominated, etc. A map of station locations would be helpful, to be referenced on p. 4 lines 11-14, which could be Figure 1 or similar.

Regarding the nitrogen fixation rate calculations, while I understand the reluctance to add equilibrated seawater to the incubations, especially given the oligotrophic nature of the samples, the compromise proposed here (to measure 15N2 at the conclusion of the incubation) does not adequately reconcile the rates calculated here with the problems associated with the bubble method. It has been shown that the fraction label of the dissolved N2 "source pool" changes during the course of the incubation (Mohr 2010 and others). However, the method used in this manuscript assumes a steady fraction label of that pool, based on the fraction label measured at the end of the incubation, inevitably resulting in an underestimation of nitrogen fixation rates. This is problematic because a) different organisms fix at different times of day and b) the rate of change likely varies based on physical and chemical parameters. Problem (a) is especially relevant to this study as the researchers have specifically set out to compare regions with different diazotroph assemblages. At this stage, this problem cannot be addressed directly, but some discussion of the implications of this in light of the conclusions is warranted. Problem (b) can and should be directly addressed by correcting the rate calculations for the rate of bubble dissolution. Examples of this correction can be found in Figure 1 of Mohr (2010) and the Supplemental Figure in Jayakumar (2017). Since these were incubated on an in situ array, differences in temperature with depth could have variably impacted rates of 15N2 dissolution. This is addressed to some degree in those two publications and should be discussed in this manuscript.

Atmospheric deposition is really a flux to the mixed layer. Depending on mixed layer depth relative to the nitracline, mixing speeds, and biological uptake rates the upward flux of NO3 may reflect a flux to the sub-mixed layer euphotic zone only. Nitrogen fixation rates also show vertical structure. I assume that diazotroph communities have vertical structure (might be nice to put this on Figure 2 if these data were collected, as well as density to show mixed layer depth); Tricho floats (if much of the Tricho is floating on the surface, was it even sampled?). Diatoms can adjust their bouyancy. The 200 m over which everything is integrated is a fairly large region. How does one account for these mini-environments within that 200 m in the budget? Could the vertical structure in N fixation reflect higher NO3 concentrations (even though they're undetectable - but the d.l. in this study was pretty high for oligotrophic waters) closer to the nitracline? Some discussion of the vertical structure within the euphotic zone may be informative.

The abstract could use more contextual information and conclusions. In its current form it reads as a bit of a data dump. The interesting conclusions of the paper could be better showcased in this section.

Technical and Line Item Comments

Abstract

Line 20: "...all major fluxes..." could be "...all major vertical fluxes..."

Line 21: Instead of "Thanks to a Lagrangian..." this would be more clear as "Using a Lagrangian..."

Line 22: "...allowing to consider..." Should this read "allowing us to consider"?

Line 23: Might it be easier to refer to stations as A, B, and C without the 'LD', as it does not seem to have any necessary significance.

Line 26: The N2 fixation measurements appear to have been depth integrated (units umol N/m2/d), but what depth region were these integrated over? Also, what is meant by "extremely high"? In comparison to what, are these high?

Line 30-31: "N2 fixation...at all stations." Does this refer only to the LD A, B, and C stations? If not, please state how many stations were sampled in each region for these data.

Line 31: PC and PP have not been defined.

Line 34: "there contribution" should be "their contribution"

Line 36-37: "This disequilibrium...summer season..." I don't understand this sentence. Does this mean that this disequilibrium is generally held to be true (confirmed by other studies) or that you found this here and that it was consistent across all stations?

Line 37: The mention of zookplankton seems strange. I would delete it.

Introduction

p 2 Line 5: "di-nitrogen" Is this journal format? I have generally seen it written as "dinitrogen".

p 2 Line 5: "ammonia" At seawater and physiological pH, it is primarily "ammonium" that is present.

p 2 Line 7-11: "In the oligotrophic...photic layer." Run-on sentence.

p 3 Line 8: "(see below)" This is not necessary.

p 3 Lines 9-13: What region are these N2 fixation rates integrated over?

Materials and Methods

p 3 Line 22: "strong thermal stratification" Please provide data or a reference for this statement or delete it. What qualifies as "strong" thermal stratification?

p 3 Line 23: "...along a west-east...French Polynesia." Please also indicate the location using lat/long, as the precise locations of New Caledonia and French Polynesia do not spring immediately to mind.

p 3 Line 25: "diazotrophs" should be "diazotroph"

p 3 Line 29: "firstly" should be "first"

p 4 Line 19: Doesn't PAR stand for "Photosynthetically Active Radiation", not "Photosynthetically Available Radiation", as written here?

p 4 Line 19: Does "fluorescence" refer to chlorophyll a fluorescence, or some other set of wavelengths? Please be specific.

p 5 Line 3: "In situ Chl a concentration was..." Should be "In situ Chl a concentrations were..."

p 5 Line 3: Was the AquaTraka III an in situ sensor attached to and deployed with the CTD package? So, this was used instead of the SeaBird chl sensor? Or is the AquaTraka for shipboard measurements? This is a little unclear because I expected to see the SeaBird chl sensor used with the SeaBird package. Please clarify.

p 5 Line 4-7: Please include more details on collection of the NO3 and PO4 samples- were they filtered? Were they stored or run immediately at sea?

p 5 Line 9: Were these incubations in glass bottles? please indicate. How many replicates were used?

p 5 Line 13-17: Were these integrated over the upper 200 m? It looks like that's so, but please state it specifically.

p 5 Line 19: "DIN" should include ammonium, which can account for $\sim$40% of total N deposition (Dentener et al. 2006). I suggest renaming the combined NO3/NO2 term to NOx or similar to avoid confusion.

p 5 Line 19-22: Since the reference is "submitted", these methods should be explained in greater detail or additional references given.

p 5 Line 26: "same depths as for NPP" Were these 15N2 incubations performed in the

same bottles as the 14C incubations?

p 6 Line 4: The danger of "trace metal contamination" is mentioned. Was the water for incubations collected using trace metal clean methods?

p 6 Line 13: "blue screening" I assume that the purpose of blue screening was to alter the quality of incident light on the bottles. Please provide additional information on the change in quality to the incident light achieved with the blue screening. Also, was the quantity of incident light altered (i.e. by using different sizes mesh screen or some other neutral density filter) in the deckboard incubators?

p 6 Line 16: What is meant by "gentle filtration"?

p 6 Line 21-22: Why was the initial PN only measured at two depths rather than for each rate measurement? Has it been determined that two depths are sufficient? If so, please be specific. How exactly were these two measurements used as 'initial' measurements for each rate measurement? Were they averaged and then used for all rates at that stations or for all stations or some other method? please specify. How many replicates were collected per depth for the initial measurements?

p 6 Line 25: How was the nitracline depth calculated? I am unclear on what NO3 is. Is this the density where the nitracline occurs?

p 6 Line 25- p 7 Line 3: Is there a reference for this flux calculation?

p 7 Line 6: Why were these depths chosen?

p 7 Line 8: "...buffered solution of formaldehyde..." Please include a reference for this statement.

p 7 Line 12: Please specify what a "swimmer" is. Were these living organisms found in the 'fresh' trap, or were they in the preserved samples too? If the latter, how were they separated from the rest of the material? Were these a certain size class of organisms? Why were they analyzed separately?

p 8 Line 6: What is meant by "gently filtered"?

p 8 Line 11-12: "The C contents...Luo et al. 2012)." This belongs in the results section.

p 8 Line 11-16: "As DDAs...asymbiotic)." It is unclear why this information is present; I thought the cell C content was only determined for Tricho and UCYN. Please add a topic sentence to this paragraph explaining for which organisms biovolume and cell C content were determined and which were determined directly v indirectly.

Results

p 9 Line 2: "...with the minimum concentration located at 60 m." It's best to refrain from calling this a minimum because the difference in concentration is slight and there are no replicates.

p 9 Line 5: "...while the DCM was deepening from 25 m to 70 m..." I suggest restating this as "...the depth of the DCM increased from 25 to 70 m during the five days that the station was occupied..." or something similar

p 9 Line 6: "...varying simultaneously between 115 and 155 m." This wording is confusing.

p 9 Line 11: "...below 0.2..." Is it below 0.2 or equal to 0.2, as Table 4 indicates? Where is the comparison of total N deposition and NO3+NO2 deposition, as suggested in the Methods?

p 9 Line 13: "...0.0-19.3..." What was the detection limit of this method? Instead of reporting "0.0" please report rates as below the limit of detection if they are. Two recent publications depict methods for calculating the detection limit of these rates (Gradoville et al. 2017; Jayakumar et al. 2017). Accounting for a DL will be especially important for LDC where the rates were very low and may be undetectable.

p 9 Line 17: "...below the quantification limit..." So there is a quantification limit! How was it calculated and what was it?

p 9 Line 17-19: Please compare the N2 fixation rates from days 1-3 using the Mann Whitney test, as was done comparing the in situ and shipboard incubations.

p 9 Line 23: "...the maximum at 40 mat LDC..." Was this small rate actually above the detection limit though?

p 9 Line 24-27: It is nice to see this comparison! It makes me feel better about all the shipboard measurements in the literature!

p 9 Line 31-p10 Line 1: "Strong time...(table 1)." This is repetitive with the previous sentence.

p 10 Line 22: "...maximum export...at LD C." PC export for LDB and LDC do not look significantly different, based on the overlap in standard deviation (Table 2).

p 10 Line 21-24: Why is there a discussion of trends in PC but not PN and PP? Line 24: "...averaged C:N ratios...LDC." Are these significantly different?

p 10 Line 31: "below detection limit" What was the detection limit? Elsewhere in the paper, (i.e. for NO3 and PO4 concentrations), a "quantification limit" is referenced. What is the difference between that quantification limit and a detection limit? Please define these terms in the Methods section and be consistent with their usage.

Discussion

p 11 Line 27: "...atmospheric deposition...lower end of fluxes..." I am curious what the mixed layer depth was at these stations, as atmospheric deposition is really a flux only to the mixed layer, not the entire euphotic zone. Since the flux is so small, it likely doesn't matter for this study, but it would be nice to see those depths in a Table for readers who study atmospheric deposition to the mixed layer.

p 12 Line 8-17: "In this study...to significant bias." This is a really good point, and it's nice to see a snapshot of this variability. It does make one wonder, however, if the snapshot had been even longer, how would the results have varied, given the large

differences seen between the use of an average Kz and the instantaneous Kz values and the seeming randomness of the spikes. Is 5 days long enough? A sentence or two addressing this concern would be useful.

p 12 Line 22: "N m-4" What is a m-4?

p 15 Line 11: "such as DON and/or zooplankton export" Please elaborate. Dissolved compounds are not exported like particles are; they do not sink. Or is this a reference to conversion of fixed N to the DON pool, followed up uptake and subsequent export? Both a direct DON flux and a delayed N flux following DON uptake are worth mentioning. Also, what about fixed N that is released as ammonium? Ammonium and many simple DON compounds (amino acids, urea...) cycle very quickly and likely would be taken up before they could be mixed downward. Also, it is unclear why DON and zooplankton export are lumped together like this... Is this referring to an active zooplankton flux (which is a completely different process than the DON pathway) or to the sinking out of dead zooplankton later?

p 16 Line 1: "zooplankton itself is" should be "zooplankton themselves are" Please make similar corrections throughout.

Conclusion

p 16 Line 16-17: "contributed to $\sim$15-21%" and "...and to 3%..." Delete "to".

Tables and Figures

The tables seem to be out of the order that they are mentioned in the text. Please check this.

Table 1. Please define Kz in the figure legend. For this and all figures and tables, please indicate what the $\pm$ error is. Standard deviation? Standard error? n = ?

Table 2. What is "DW matter"? Please indicate this in the table legend or with a footnote to the table. Why are the errors italicized in this table and Table 3 but not in Table 1?

Please standardize this across tables. If PC, PN, and PP should be PC flux, PN flux, and PP flux, please indicate that. Please propogate the error of PC, N, and P into the C:N:P ratio calculation.

Table 3. Please apply the comments from Tables 1 and 2 to this table. Also, please use a standard number of significant digits for all measurements (i.e. LD A 300 m is inconsistent).

Table 4: Please include the standard deviation or error of these values by propogating the error from the measurements. please define all non-obvious terms (i.e. d[NO3]/dp, NO3) and the calculation for the e-ratio in a footnote so that the reader does not have to dig through the text to find them. Is N2 fixation the in situ rate or the shipboard rate? Please use consistent significant digits (i.e. for NO3 diffusion and export N 150 m).

Fig 1: This figure seems needlessly complicated and the vertical component does not seem spaceworthy. The advantage of the vertical component is to show where the production arrays and traps were deployed, but it's difficult to tell the exact depths in this figure. A simple map may be preferable.

Fig. 2: Please indicate on the figure itself which units correspond to which parameter, as it is a bit confusing in current form (particularly for phosphate). It may be instructive to use a different scale for the N fixation rates in the third panel, since they can't be seen on this scale.

Fig 3: This is a cool figure! Nice dataset! I am a little confused on how 1% of surface PAR was calculated at night. Should there be breaks in the dotted line for nighttime?

References

Dentener, F., J. Drevet, J. F. Lamarque, I. Bey, B. Eickhout, A. M. Fiore, D. Hauglustaine, L. W. Horowitz, and others. 2006. Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. Global Biogeochem. Cycles. 20, GB4003, doi:10.1029/2005GB002672:

Gradoville, M. R., D. Bombar, B. C. Crump, R. M. Letelier, J. P. Zehr and A. E. White 2017. Diversity and activity of nitrogenâĂŘfixing communities across ocean basins. Limnol. Oceanogr. 62: 1895-1909

Jayakumar, A., B. X. Chang, B. Widner, P. Bernhardt, M. R. Mulholland and B. B. Ward 2017. Biological nitrogen fixation in the oxygen-minimum region of the eastern tropical North Pacific ocean. The ISME Journal. 11: 2356-2367

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