Response to Anonymous Referee #1

1. *Three different sets of data are used in this study (Time-series data from ALOHA and BATS - for specific locations; WOA 2013 - for basin scales in North Pacific and North Atlantic, and also for world ocean; and GLODAP v2 – for preformed nitrates vs CFC ages of waters) – Comments on comparisons/compatibility among these data sets and their products are worthy of inclusion in the manuscript.*

   We have added text at Lines 177-180 related to this comment: "The WOA and GLODAP data products provide our analysis with an annual time-mean picture of thermocline preNO_3 tracer distributions across the larger spatial scales of the North Atlantic, North Pacific, and the other subtropical gyre regions of the global ocean."

   And at Line 184-185: "All calculations of rNPN and rPPN anomaly formation rates at the time-series sites are performed from analysis of the monthly-resolved Station ALOHA and BATS datasets."

2. *Line 138: for the benefit of the reader it is important to state details of 'total dissolved nitrogen (TDN)' here though available elsewhere. Whether TDN includes PON component or not is unclear from the details provided.*

   We have added text at Lines 170-172 to clarify that samples for TDN are GF/F 0.7 μm filtered and that the time series computed DON concentrations include a negligible amount of ammonium.

3. *Line 183: "DON (measured)" is actually not measured but derived (obtained by difference between TDN and nitrate+nitrite, see lines 140-141).*

   The words "(computed) and (measured)" have been removed from the statement.

4a. Lines 186-191: Assumption of rDOM constancy over time in each density layer may compromise the examination of its temporal variability in the regions. More over near constancy of rDOM values shown in Table 1 for each region might oversimplify vertical variability. If such use is necessary why not consider the top 250 m as just one layer? Please see Comment 16 (a) and (b) below that seem to simplify or play down on actual temporal and spatial variabilities. I am afraid such assumptions may undermine understanding of natural variability and compromise the significance of this study.

Yes, this is an assumption, but necessitated due to the lack of statistically significant AOU vs. DON regressions in a subset of nearly half of the years of data. This is likely because each is a derived tracer subject to measurement and differencing errors. However, our goal of this study is to assess the main causative mechanism for the order of magnitude rPPN/rNPN formation rates we observe, not to report the detailed year-to-year variability of the rPPN/rNPN anomalies. The preformed NO3 anomalies represent a gap in our understanding of the system as the preNO3 tracer computation is meant to remove or account for the effects of biology on observed nitrate distributions. Thus, our goal is to first assess the biological mechanism giving rise to the anomaly before attempting to explain the nuances of its interannual variability. Furthermore, the significance of analyzing a detailed year to year record of rPPN/rNPN anomalies becomes muted when comparing these to the order of magnitude estimates of the contributions from TEP cycling, bacterial NO3 uptake and vertically migrating phytoplankton mechanisms. A detailed study of preNO3 anomaly interannual variability would also likely require a more highly resolved dataset than cruises every 1 – 1.5 months as with the time-series datasets. More detailed float observations as in Johnson et al (2010) will be very helpful in refining these calculations.

4b. Now that authors evaluated rDOM we know the values of rPOM (since DOM and POM remineralization should account for 100% of AOU, see also lines 190-191) specific for ALOHA and BATS. I believe this rPOM will be more realistic. Why use constants of 10.6 and 6.9 from literature? One can make a comparison with literature data but when one has an opportunity to use realistic values one should do so. In the present case the authors seem to prefer using literature values than their own results. Also I am not clear whether the values 10.6 and 6.9 are specific to PON or TON.

Decomposing the AOU into contributions from the remineralization of POM vs. DOM with differing stoichiometry requires 5 parameters: the AOU, fPOM, rPOM, fDOM, and rDOM. The only 2 that can be computed from observed quantities in the HOT and BATS datasets are AOU and rDOM. fDOM and fPOM can be estimated once rDOM is known, by choosing a value of rPOM, and from mass balance that fPOM+fDOM=1. Thus, with the mass balance equation and by prescribing a value of rPOM, one can solve the system of two equations with two unknowns and estimate fDOM and fPOM for the system. Under this constraint, that the value of rPOM must be prescribed, we chose to use the upper bound and lower bound values of rPOM from the literature (the 10.6 and 6.9 values) as a means to test the sensitivity (i.e. to assess the uncertainty) of the computed preNO3 anomalies to this undetermined parameter of our analysis. An observational estimation of rPOM would likely require an oxygen consumption incubation type measurement on field collected sinking POM. These data are unavailable.

5. *Lines 205 – 210: The periods of occurrence of seasonal rNPN and rPPN anomalies and their trends may be shown in x-y plots.*

   These x-y plots are now included in the Supplementary Materials as Figures S1-S5.

6. *Lines 223-225: 'DOM remineralization. . ...that utilizes the preNO3- tracer' is an appreciable observation and suggestion made in this study.*

   We agree. We now use this point and supporting text to begin the Discussion section of the revised manuscript.

7. *Lines 226-230: It appears that 40 to 67% of estimated AOU is explainable by POM oxidation and thus is equally important as that of DOM. Again authors chose to use climatological averages of fDOM than the actual values observed (Comments 2 and 3 are relevant here).*

   We have chosen to use climatological values of fDOM/fPOM in our calculation of preNO3 for the same reasons we discussed in our response to points 4a and 4b. Statistical significance is not present for each year's computation of rDOM leading us to take an approach of a climatological analysis of preNO3 anomalies. Also, we are aiming to assess the main contributing mechanism to rPPN/rNPN anomalies with only order of magnitude estimates to be made for TEP cycling, bacterial NO3 uptake, and vertically migrating phytoplankton with currently available datasets/studies.

8. *Lines 242-245: Are increases in rPPN in the euphotic zone and rNPN in the subsurface waters between May-June and Oct-Nov at ALOHA connected? It should be remembered that we have used near constant values of fDOM (50%), fPOM (50%), rDOM (18.1-18.9) and rPOM of 10.6 or 6.9 in the computations for the entire water column of 200 m (see Table 1). Then the results in Figure 1 are mainly reflective of changes and trends in TDN, Nitrate+Nitrite and Oxygen! I guess results in Figure 1a and b could be different if the authors used their evaluated values of fDOM, fPOM, rDOM and rPOM!!!*

rPPN anomalies in the surface and rNPN anomalies in the subsurface are connected temporally at both stations ALOHA and BATS. This is seen in Figures 1 & 2 but maybe more easily in the newly included Figures S1-S5. There is some degree of temporal offset between the initiation and termination of the two anomalies with rPPN in the surface generally beginning and ending earlier in the calendar year than does rNPN in the subsurface. In the text, we suggest there is a linkage consistent with vertical migrators acquiring nitrate at depth and then reducing it internally to biomass (with oxygen evolution) above the nutricline where nitrate values are vanishingly low (nM). Yes, the reviewer is correct in that because fDOM, fPOM, rDOM, and rPOM are all climatological values in our analysis, the seasonal trends observed in the figures exhibiting residual preNO3 concentrations are reflective of changes in TDN, nitrate+nitrite, and oxygen. As we point out on Line 232-233, the relative uncertainty in the literature values of rPOM is larger (~55%) than the empirically derived rDOM (~33%) variance. Thus, our reported ranges of rNPN and rPPN anomaly formation rates in Table 1 already include a larger estimate of the uncertainty than that which is introduced by using the climatological rDOM and fDOM values from Table 1 in our analysis.

9. *Line 369: "Having ruled out lateral mixing...." This seems to be a very tentative statement since lines 333-335 for BATS and 353-357 ALOHA clearly indicate lateral mixing influence is considerable in deeper layers. Ignoring mixing effects here is not justifiable.*

   Per our analysis, isopycnal mixing may influence the deep rPPN anomaly observed below the euphotic zone >200 m at ALOHA, but it is not a large contributor to the rPPN anomaly at the BATS station or the rNPN anomalies within the lower euphotic zone at either site. We've clarified these points with edited text at Lines 455-456: "This feature is noticeably absent at BATS, (Figure S6) consistent with a minor role for advective mixing there."

   Edits at Line 465-467: "Lateral mixing may explain the observed rPPN anomaly below ~200 m at Station ALOHA: however, the euphotic zone rPPN anomalies and upper mesopelagic zone rNPN anomalies at each site are generated by a local biological mechanism."

10. *Lines 402 and 427: A TEP gradient of 5-10 µg XG eq l-1 was used to assess its contribution to rPPN and rNPN anomalies. I wonder whether such small gradient is sufficient to assessing its role in view of the semi-quantitative nature of TEP measurements and results. The authors should clearly discuss the uncertainties associated with TEP measurements and justify that the gradients used between surface and deep layers are significantly above the analytical errors. Other constraints associated with TEP are identified by the authors in lines 460-465.*

    The reported analytical uncertainty in the studies we cite is 10-13% for µg XG eq L^{-1} which translates to a 3-4 µg XG eq L^{-1} uncertainty on the typically 30 µg XG eq L^{-1} TEP concentration in the upper ocean reported by these studies. Therefore, the TEP gradient we use in our analysis is 1.7 to 3.3 times greater than the analytical uncertainty. By using the published values and assuming they are statistically sound, we have maximized the contribution of TEP. Had we assumed that there was no gradient, the contribution of TEP to the rNPN anomaly would have been much smaller.

11. *Lines 413-414: "We assume TEP is comprised of pure carbohydrate with no N content..." – This is a simplified statement. The TEP has dominant polysaccharide composition but to assume that no other organic materials (nitrogen containing substances) are attached to TEP is not realistic.*

    We had addressed this comment with our text at Lines 466-474 (now Lines 504-514). In our budgeting analysis we used the assumption of no N content in TEP to provide the maximum upper

limit that TEP could contribute to the observed rPPN/rNPN anomalies given the observed upper ocean TEP vertical gradients.

12. *Lines 499-502: ". . .it is clear that neither remineralization of N-poor DOM and TEP or heterotrophic bacterial nitrate uptake can quantitatively explain both the. . ..." – How justifiable is this statement given several assumptions involved. The authors have to quantify uncertainties to give confidence to readers at some level!*

    The uncertainties to our assumptions are what lead to the ranges reported in Table 2. A major source of uncertainty is the value of rPOM (see response to point #8), for which we have chosen to report all of our results as ranges with respect to the lower bound and upper bounds for this value from the literature. In assessing TEP cycling, we have reported its maximum potential contribution based on a theoretical zero N content. Similarly, when assessing bacterial nitrate uptake, we reported its maximum potential contribution based on assigning all bacterial N demand to NO3 uptake when almost certainly a portion is satisfied from remineralization of organic matter. Even with these upper bound estimates of TEP cycling and bacterial NO3 uptake along with the uncertainties in rPOM, etc. we cannot close the residual preNO3 budget at either site, justifying our statement.

13. *Lines 547-550: The logic in the estimation of the contribution of vertically migrating phytoplankton to the rNPN and rPPN ignoring the contribution of physical N transports is not justified (see Comment 9).*

    From our analysis, we did not find large gradients in residual preNO3 necessary for lateral isopycnal mixing to be quantitatively responsible for the observed rPPN and rNPN anomalies above 150 m at BATS and 200 m at ALOHA. Similarly, vertical mixing cannot create the subsurface rNPN anomaly since waters both below and above this layer exhibit positive preNO3 values (from Fig. 1 & 2). The euphotic zone rPPN anomaly cannot be generated from vertical mixing as the waters below exhibit negative preNO3 values. Therefore, we ascribe the remainder of the residual preNO3 anomalies to vertically migrating phytoplankton, our last remaining hypothesized biological mechanism, and compare the anomaly formation rates to vertical nitrate transport rate estimates from the literature.

14. *Lines 557-558 and 564-565 are confusing! When vertically migrating phytoplankton can help explain the observed summertime DIC drawdown in the absence of measurable nitrate (557-558) why do they state 'the mixed layer DIC drawdown need not be entirely supported by migrator photosynthesis, instead their nitrate leakage could help explain. . .(564-565)'. Is not nitrate leaked through excretion used and included in migrator supported photosynthesis?*

    The statement has been edited by removing, "mixed layer DIC drawdown need not be entirely supported by migrator photosynthesis, instead their…". We have also added a new section to the Discussion at Lines 718-756, detailing the potential contribution of the euphotic zone rPPN anomaly formation to the mixed layer DIC drawdown at both sites. We find that the observed seasonal rPPN anomaly can potentially explain a significant fraction (>28%) of the summertime DIC drawdown at each site.

15. *Lines 591-592: "P-limited or P-stressed vertically migrating phytoplankton also take up phosphate at the nutricline. . ." is an important hypothesis.*

    Yes, and we state that this hypothesis can benefit from the collection of field data of vertically migrating phytoplankton intracellular phosphorus concentrations from the subtropical North Atlantic.

16a. SEVERAL ASSUMPTIONS: Lines 186-187: "our approach assumes rDOM is constant over time within each density horizon investigated at each station".

As we have acknowledged, not every year exhibits a statistically significant AOU vs. DON regression to estimate rDOM, diminishing the length and data density of the empirically derived rDOM dataset. We do not observe any trends with time in this dataset.

16b. Lines 202-205: "For the calculation of the preNO3 tracer within the euphotic zone, we made the assumption that the values of fDOM and rDOM were equivalent to those empirically derived for the upper mesopelagic density layer present immediately below the euphotic zone at each site".

This is valid because of our understanding of DOM production and consumption dynamics in the upper ocean. DOM accumulates in the euphotic zone from biological production processes and is subsequently transported vertically to depth, first entering the upper mesopelagic, from physical processes including advection, diffusion, and convection. There, it is remineralized by heterotrophic biological processes. In the absence of large horizontal DOM concentration and DOM stoichiometry gradients (as in the subtropical gyres near stations BATS and ALOHA) and for a system at steady-state on annual timescales, the stoichiometry and magnitude of DOM remineralized in the upper mesopelagic is balanced by that supplied from directly above in the euphotic zone.

16c. Lines 413-414: "We assume TEP is comprised of pure carbohydrate with no N content. . ." – This is probably highly simplified. We know that TEP has dominant polysaccharide composition but to assume that no other organic materials (say proteins etc.) attached to TEP is not realistic.

We had already acknowledged this caveat and how it impacts our results at Lines 466-474 (now Lines 502-514). We agree that it is highly likely there is some N component, either structurally or as attached particles. However, if we assume that TEP contains any % N, we are minimizing the contribution to rNPN. By assuming 0% N, the contribution of TEP is maximum and it makes our conclusion more defensible as maximum values. Any other assumption puts the values somewhere in a poorly defined center.

16d. Lines 453-457 related to assumptions on (i) TEP is pure carbohydrate and (ii) TEP sinks rapidly and account for annual carbon export flux.

We chose these assumptions in our analysis to provide an estimate of the maximum potential contribution for TEP to explain the residual preNO3 formation rates. Actual TEP contribution is likely lower and we stated this at Line 559-560 (now Line 714-715).

16e. Lines 533-535: "Nitrate transport calculations by vertical migration has a number of assumptions and caveats including considerable uncertainty in abundance estimates (Villareal et al., 2014)".

Our statement informs the reader that the largest source of uncertainty in nitrate transport calculations by vertically migrating phytoplankton arise from cell/mat abundance estimates (as detailed in Villareal et al., 2014).

17. *Figure 1 & 2: The captions need clarification. In (a) 'residual pre NO3- tracer' and in (b) 'monthly averaged pre NO3- climatology' have been shown as per the present caption. I wonder if (b) actually shows 'monthly averaged residual pre NO3- tracer climatology'!! If not I would expect different values of higher magnitude of pre NO3- in Figure (b) (according to Formula (1, line 178).*

Yes, we mistakenly left out the word "residual" in the caption to part (b) of Figures 1 & 2. It has been added.

18. *Figure 3: "the residual pre NO3- [µM] tracer (the amount remaining after accounting for DOM contributions to AOU, see lines 233-234)" its maximal value of zero in the world oceans (as shown in this figure) imply that hardly any PON oxidation is accountable for oxygen consumption at 150 m. This sounds unrealistic! (see Comment 7).*

    A value of zero in (now) Fig. 5 means there is no anomaly at 150 m, i.e. POM + DOM remineralization is accurately accounted for by the computation of the preNO3 tracer and there is no significant contribution from TEP, bacterial NO3 uptake, or vertically migration phytoplankton at that location.

19. *Figure S1 & S2: 'Residual pre NO3- is calculated using the values of fDOM and rDOM determined from the BATS station in Table 1 with a value of rPOM = 10.6' – Using fixed BATS values for the entire North Atlantic is not logical as it ignores spatial variability in these values. Figure S1 actually mimics that variability produced by changes in O2 and Nitrate+Nitrite listed in WOA2013!*

    Yes, this is a shortcoming of the mixing analysis arising from low data density in observations of DON across the larger North Atlantic basin, with better spatial coverage in DON data (not available) allowing for a more robust empirical determination of the values of rDON and rDON across the basin. We chose to apply the BATS station empirically derived values of fDOM and rDOM to the larger basin since our goal was to investigate for the presence of large spatial gradients in residual preNO3 distributions within the vicinity of the BATS station on the isopycnals of interest that are needed for lateral mixing to play a quantitatively important role in explaining the observed residual preNO3 distributions at BATS. In Figure S2 (now Fig. S7: plots residual preNO3 on the isopycnal bisecting the rNPN feature at BATS), we observe very little spatial gradient near BATS suggesting insignificant contributions of lateral mixing to the residual preNO3 observed at BATS. Figure S1 (now Fig. S6) plots a slightly deeper isopycnal for the North Atlantic and reveals there is very little vertical gradient comparing residual preNO3 near BATS on isopycnals 26.0 and 26.5, suggesting insignificant contribution from vertical mixing to the rNPN feature within density layer 25.8 – 26.3. Yes, there is likely to be spatial and probably temporal variability in the values of fDOM and rDOM on these isopycnals across the North Atlantic. For example, Carlson et al 2010 DSR II, report values of fDOC in the upper thermocline of the North Atlantic of 5-30%. However, this variability has a minor effect on plotted residual preNO3 concentrations with a change from prescribed fDOM from 0.4 to 0.5 changing the residual preNO3 concentration by < 20% and most typically by < 10% when applied to the WOA dataset for the North Atlantic. Therefore, the evidence suggests that spatial variability in fDOM and rDOM is not large enough to alter our conclusion: that lateral mixing plays an insignificant role in setting the residual preNO3 tracer distribution on the 25.8 – 26.3 isopycnal where seasonal rNPN anomaly formation is observed, with biological mechanisms dominating this process. It is clearly an area that requires more data and we welcome the opportunity to include it in the calculations when available.

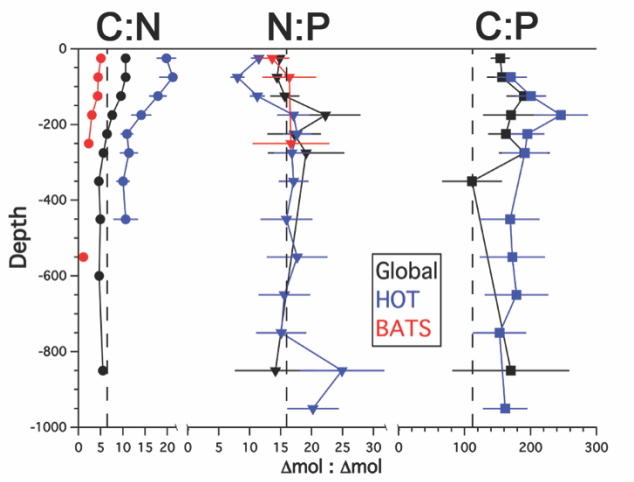
    Figures S3 & S4: See Comment 19 but for North Pacific Ocean.

    See our response to comment 19 which applies to the North Pacific as well. We do recognize that there are larger spatial gradients in residual preNO3 on the deeper 25.4 isopycnal across the North Pacific near station ALOHA which we state could be a significant contributor to the deeper rPPN anomaly observed at >200 m depth within the text at Line 454-455: "Mixing of these waters along γ^n

= 25.4 may explain the observed ~1 µM residual preNO$_3$ concentration present at 200 – 250 m depth in Figure 1."

20. *Figure S5 & S6: Why use Redfield ratio of 16 for N:P here? As the DON gets remineralized the associated phosphate is released in dissolved form. Then why not determine ΔDON/ΔPO4 with time-series data and use that ratio?*

Author Letscher has previously estimated the ΔDON:ΔDOP ratio in discrete depth intervals at stations ALOHA and BATS (figure adapted from Letscher & Moore 2015, GBC). At BATS (red line), remineralization of the non-refractory (semilabile) DON and DOP in the upper mesopelagic follows a ~16:1 ratio, justifying our use of that ratio here.



22a. Table 2: Lines 133-134 in text: 'Phytoplankton vertical migration. . .. . .at both stations ALOHA and BATS' is not convincing by the information provided in Table 2.

Table 2 has been updated and edited to clarify our approach, results, and conclusions.

22b. It is not clear whether the FOUR sources listed account for 100% of NPN or PPN features listed.

Please refer to our updated Table 2. New rows have been added to show the % contribution of TEP cycling, bacterial NO3 uptake, and vertically migrating phytoplankton to the total observed rNPN or euphotic zone rPPN feature at each site. As is stated in the discussion text, the % contribution from this last process is calculated from the total observed anomaly formation rate minus the contribution from TEP and bacterial NO3 uptake. Therefore, the sum of the three processes sum to 100%. The contribution of N-poor DOM remineralization is not included in the summing to 100% because the main thesis of our study is to quantify and then explain the *residual* preNO3 anomalies present after accounting for DOM remineralization and its stoichiometry to upper ocean AOU budgets.

22c. What are the total NPN and PPN values computed? What about contributions from lateral and vertical/diapycnal mixing, however small they are?

The total rNPN and rPPN values computed are reported in Table 1 as well as within the abstract. We do not include contributions from lateral and diapycnal mixing due to uncertainties in the spatial applicability

of the empirically determined values of fDOM and rDOM to the larger North Atlantic and North Pacific basins as detailed in our response to comments 19 and 20.

22d. Consider all factors/sources and show they account for 100% of NPN or PPN evaluated.

This is now included in our updated Table 2.

22e. Vertical migration appears more significant at BATS than at ALOHA? This will not be clear unless one shows the contributions of various sources in terms of percentage totaling to 100.

This conclusion is more easily gleaned from our updated Table 2.

"Probably a better approach to convince the readers on the significance of nutrient export by phytoplankton vertical migration is by conducting experiments to quantify intracellular accumulation of N and P and the extent of nutrient leakage through excretion by vertically migrating phytoplankton."

This work has been done and is cited in our manuscript. Singler & Villareal 2005 report nitrate, nitrite, and ammonium excretion rates from migrating *Rhizosolenia* mats across the subtropical North Pacific. Villareal & Lipschultz 1995, Villareal et al. 1996, and Woods & Villareal 2008 all document the high (millimolar) intracellular N content of *Rhizosolenia* cells that can only be acquired by direct uptake at μM concentrations, e.g. at the nitracline. Other workers have observed these phenomena in other taxa as well including dinoflagellates including Fraga 2001, Fraga et al 1992, Fraga et al 1999 and Cullen 1985.