## Response to Anonymous Referee #3

- 1. That is, the contribution of rNPN and rPPN anomaly formation which is said to be due to vertically migrating phytoplankton are computed by difference of other numbers only hence there seems to be no direct evidence of the role of migrating phytoplankton to contribute to the subject of this paper (rNPN and rPPN at BAST and HOT, respectively).
  - We have added a header to the last 3 columns of Table 2 entitled, "Proposed attributable process" to better identify these as putative following our analysis. The title of the manuscript has been edited, replacing "Vertically migrating phytoplankton drive" with "Evaluation of the". There is little direct evidence of TEP impact on the rNPN and rPPN anomaly, nor is there direct evidence of heterotrophic nitrate use. We have used the best available data to constrain the contributions of each.
- 2. the only evidence seems to be similarity of numbers and the possibility that a process observed and quantified elsewhere (eventually under different hydrographic and biogeochemical conditions?) may exist and dominate also at BATS and HOT. Actually, the two pages (23-24) that summarises very nicely the work of Villareal and others on vertically migrating phytoplankton appear to avoid to mention the locations and conditions of observations related to migrating phytoplankton explicitly.
  - Extensive addition of text has been added at Lines 622-687 to provide a review of the evidence for multiple taxa that are known to be vertical migrators and their meridional/zonal distribution across the global ocean. Multiple vertically migrating phytoplankton taxa have been observed within the vicinity of Station ALOHA and the BATS station. We have included 3 new figures in the Supplementary Materials detailing the distributions of *Pyrocystis* (Fig. S11), *Ethmodiscus* (Fig. S12), and *Rhizosolenia* (Fig. S13).
- 3. I am fine with papers to have a more speculative section. However, this needs to be clearly identified as being speculative, otherwise we leave the scientific ground that facts and clear evidence are the basis of our work.
  - We have updated our language throughout the manuscript when referring to the putative mechanisms with phrases like "potentially explain", "could explain", "proposed mechanism", etc.
- 4. Terminology: In the intro you introduce your terms 'residual negative preNO3' (rNPN) and rPPN, respectively. I found the usage of the term residual 'somewhat' misleading, in particularly since the description in M+M is somewhat unrelated to the usage of this term in the intro and in the rest of the paper. Perhaps 'apparent NPN' (etc) is better, particular since rNPN and rPPN rely on a variety of assumptions (fDOM etc.).
  - We have updated throughout the text to ensure that "residual" precedes "preNO<sub>3</sub>" wherever it is the tracer we refer to. There are some instances in the Intro and Methods in which we mean to refer to preNO<sub>3</sub>, as in the traditional formulation that is agnostic to differing POM vs. DOM remineralization  $-O_2$ :N stoichiometry. However, all data presented in the figures, tables, etc. of this study refer to the residual preNO<sub>3</sub> tracer which we now clearly define with our edits to the Intro/Methods and edit to Equation 1.
- 5. However, I was particularly surprise not to find these terms in M+M! Instead there you use 'traditional preNO3' (l 171) (of which data are never presented, I think) and 'updated calculation' (l

171), initially I wondered whether rNPN is something additional and did not find it well described in M+M. A more clear M+M is required.

We have edited this section for clarity such that we clearly define and refer to residual preNO<sub>3</sub> as the newly generated tracer data presented in this study including an important edit to Equation 1 which adds the word "residual".

6. Given the speculative nature of the quantitative role of migrating phytoplankton to the features discussed in this paper, I suggest to delete the last sentence of the Abstract.

This statement has been edited as: "These results based on geochemical distributions suggest that in the absence of additional mechanisms and rates, phytoplankton vertical migrators, although rare and easily overlooked, play a larger role in subtropical ocean nutrient cycling and the biological pump than generally recognized."

7. p 5, l 95 please give a reference for the O2:N ratio; generally, I wonder whether such a low ratio is consistent with the observations of variable elemental ratios in POM (e.g. the work of Martiny et al., but also older work, e.g. Anderson and Sarmiento, 1994, give higher values for –O2:N.)

We have added the reference, Anderson, 1995, which updates the POM –O2:N from Anderson and Sarmiento, 1994. The more recent work of Martiny et al. does not specifically address the –O2:C or –O2:N stoichiometry of marine POM, but does provide evidence for regionally variability in POC:PON ratios in the upper ocean. The global mean POC:PON is found to be 6.5 (Martiny et al 2013), i.e. very close to the Redfield value of 6.6. Low latitude oligotrophic regions exhibit elevated C:N closer to 7-7.5.

8. p5, 106ff: the quote to the Johnson et al 2010 work: do they really suggest what you cite them for? They rather observe a significant amount of Chla below the euphotic zone and argue that these phytoplankton may take advantage of deep nutrient pulses. Thereafter they cite actually earlier work of Villareal in support of upward transport of nitrate by migrating mats. In your intro it sounds as if Johnson provides independent evidence of the migration, which is not the case, I think.

We have edited this section for clarity at Lines 122-130: "Johnson et al. (2010) suggested that the subsurface NPN anomaly (calculated using a modified Redfield ratio) could be sustained by vertical separation between nitrate uptake in the nutricline and oxygen production/net community production near the surface. Their data supported the conclusion that the upper 250 m is in approximate balance between nutrient supply and demand, suggesting that there are processes that redistribute nitrate within this region. In the absence of other mechanisms, they suggested that the observed mixed layer net community production is mediated by 1) directly observed episodic vertical physical mixing events that deliver nitrate above 125 m and 2) inferred transport by vertically migrating phytoplankton transporting this nitrate upwards along a near-zero concentration gradient within the euphotic zone."

9. It might be helpful to mention the two papers of Fraga, which you discuss later, already in the intro

Our discussion of the work of Fraga is given within the concluding remarks section at Lines 802-811.

10. M+M, this section is partly confusing, and would benefit from careful reworking -p 8, l 171: 'calculation of the traditional preNO3 tracer', but this is never being used in the results, rephrase please.

-p7/8, l 173-192: shouldn't this procedure allow to provide error bars for fDOM and rDOM? how large are these? so far, if I understand correctly, the uncertainty in the Tabs mainly derive from literature assumptions about rPOM (10.6 vs. 6.9); how large is the range of rDOM values diagnosed from the data; I see that some of these numbers are provided on p 10 at the beginning of the results section

The Methods section has undergone extensive editing and reworking, most specifically at Lines 171-185; 198-202; 205-216; 225-236; 247-251; and 258-265.

11. p9/10, 1193-211: I found this part very confusing

This section has undergone significant editing at Lines 237-257.

12. I missed a data availability statement. The time series raw data are available from well known site, but what about the computed data from this study??

With the referee's suggestion we have made the residual preNO<sub>3</sub> and residual prePO<sub>4</sub> computed datasets available for the BATS and HOT sites via a supplemental data file to be published with the manuscript.

13. p 10, l 212: I suggest to add a suppl table with links to the original data used in this study, incl. dates of access, version numbers etc. (TS data, WOA, GLODAP, etc.)

We believe that the links listed at Lines 167-177 satisfy this request.

14. p 10, l 224-226: this is again confusing; do you present results based on the 'traditional preNO3' formulation in this paper?

We do not present any "traditional preNO<sub>3</sub>" tracer concentrations in this study. We have edited text for clarity.

15. p 11, l 242: I am not sure I really understand how the 'averaged climatology of residual preNO3' is computed; this goes back to the confusion of the M+M section and should be solved there

This statement here has been edited to reinforce and restate what is written in the caption for Fig. 1.

16. p 11, l251: from the text it seems that you sum up the /m3 data of two isopycnals, which can't be right, I think, please clarify

This statement has been removed and these values removed from Table 1.

17. p 12, l 274: really Fig. 1b, not 2b?

Yes, this was a mistake. Thank you for catching it.

18. p 13m 276ff: isn't the similarity of values of rPPN and rNPN an artefact of the way you select rDOM and fDOM for the upper layer (just by adopting it from the lower layer)?

No. Because from Equation 1, residual preNO<sub>3</sub> still varies as a function of in situ NO<sub>3meas</sub> and AOU within each depth/density layer investigated.

19. p 13, l 288/9: You compare /m3 rates (rPPN, rNPN) of different layers and argue that they are balanced. But does that make sence for /m3 values of layers of likely varying thickness?

This statement and a similar one at Line 340 have been edited to replace "in approximate balance" with "approximately equivalent".

20. I suggest to structure the discussion by subheadings

Subheadings to the discussion have been added.

21. p15, l 330: 'Advective mixing' you mean 'lateral mixing', right?

Yes. "Lateral" was added.

22. p15, l 333: reference for CFC data is missing; which kind of age is computed and used here, please provide details

Discussion of the methods for CFC diagnosed ventilation age have been added to the Methods at Lines 180-183 including three additional references: "Water mass ventilation ages on subsurface neutral density layers are computed from the GLODAP v2 CFC-11 and CFC-12 data using a method similar to that described in Doney and Bullister (1992) using the atmospheric time-history of the CFC gases provided by Walker et al. (2000) and the gas solubility constants of Warner and Weiss (1985)."

23. p16, l 354: plots of preNO3 vs. . . ., which preNO3 is given here, the traditional, the improved, the residual?

Residual. We have updated throughout the text to ensure "residual" precedes "preNO3" where appropriate.

24. p 18, l 403-404 (also p19, l 428): the units of the gradient are strange, shouldn't it be ug XGeq/L/m We have replaced "gradient" with "concentration excess".

25. p 18, 404-413: which physical process mechanism is assumed here, please specify; In general I miss details that feed into the computation in this part

We have added text at Line 518-522 to specify the physical mechanisms of sinking of negatively buoyant TEP and/or wintertime vertical mixing: "We take this upper 100 m excess to represent the fraction of the euphotic zone TEP pool that is exported annually to the upper mesopelagic 100-200 m depth layer by a combination of physical sinking of a fraction of the TEP pool that becomes negatively buoyant or is delivered to the subsurface with wintertime vertical mixing."

26. p 19, 435: the C:-O2 stoichiometry of TEP should be introduced earlier, p 18 or so; eventually in M+M

It has been moved up in the Discussion to Line 505.

27. p 20, l 454-459: this should be given earlier, before you do the computation, e.g. p 18 or so, or eventually in M+M\*

We have moved this section up in the Discussion to Line 500-514 before the discussion of the computation.

28. p 21, l 467 ff, bacterial N-uptake The underlying assumption is that bacterial biomass is to increase continuously, but aren't the bacteria grazed themselves and the respective N remineralised? I doubt that this process can support NPN anomalies. In particular, the simplistic calculation provided is not convincing and would f.e. require evidence of continuously increasing biomass of bacteria over the growth season that matches the time integrated TEP degradation rates.

We have added a statement at Line 594-598 addressing this point: "This proposed mechanism still requires a process that removes bacterial N from the  $\gamma^n = 25.8 - 26.3$  layer such that a rNPN anomaly is observed due to NO<sub>3</sub> uptake in the absence of stoichiometric O<sub>2</sub> accumulation. Diel vertically migrating grazers are a candidate mechanism whereby grazing on bacterial biomass and its eventual remineralization to NO<sub>3</sub> is spatially separated from respiration and O<sub>2</sub> consumption."

29. p 23-24: This is a nice summary of evidence for migrating phytoplankton 'somewhere' in the ocean. However, I miss a clear regional and at the same time quantitative link to the topic of this paper: rNPN, rPPN anomalies at BATS and HOT. Statements like 'buoyancy reversals, high internal nitrate pools and rapid ascent have been found in multiple taxa from the Atlantic and Pacific Oceans" (1 530f) or "the generalised rates are consistent with the required rNPN and rPPN rates at BATS and ALOHA" (1 544ff) is much too general.

Reiteration here of our response to point #2: "Extensive addition of text has been added at Lines 622-687 to provide a review of the evidence for multiple taxa that are known to be vertical migrators and their meridional/zonal distribution across the global ocean. Multiple vertically migrating phytoplankton taxa have been observed within the vicinity of Station ALOHA and the BATS station. We have included 3 new figures in the Supplementary Materials detailing the distributions of *Pyrocystis* (Fig. S11), *Ethmodiscus* (Fig. S12), and *Rhizosolenia* (Fig. S13)."

30. p 25: l551ff: The authors estimate the contribution of vertically migrating phytoplankton to rNPN and rPPN anomaly features . . . by difference, i.e. as the so far (mechanisms 1, 2, 3) unexplained. This is not sufficient to support a text entitled: "Vertically migrating phytoplankton drive seasonal formation of subsurface negative preformed nitrate anomalies . . ."

The title has been changed to remove reference to vertically migrating phytoplankton as the definitive causative mechanism.

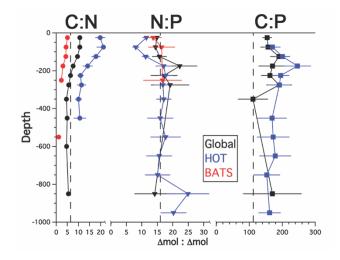
31. p 25, l 561ff (summertime DIC drawdown): again, this is highly speculative since you do not provide sound numbers for the role of vertically migrating phytoplankton for the two time series stations, based on data from the sites

Our language in this section had reflected this as speculative, using phrases including "can help explain", "can lead to", "could help explain".

32. p 26, l 575ff: Preformed PO4 is a very important aspect of the paper; I suggest to present the respective data already in the results section and with the same rigor as the rNPN etc data. You can

discuss / provide the interpretation her in the discussion, of course. Questions arise: You assume that fDOM and rDOM are the same for DOP and DON, due to lack of data, as you point out. However, this make the analysis a very weak one, I think. DOP and DON differ in their composition, shouldn't they also differ in their contribution to AOU, accordingly?

We have performed the suggestion and created new Figures 3 and 4, which replace Figures S5 and S6. Residual preformed PO4 is now defined in the Methods at Lines 258-265 and discussed in the Results at Lines 347-370 and the Discussion at Lines 757-784. We now cite our previous study justifying our prescribed rDOM value for the calculation of residual prePO4 which uses a semilabile DON:DOP remineralization stoichiometry of 16:1 (Letscher & Moore, 2015 GBC), adapted figure provided below.



33. p 27, 596ff: is there direct evidence for phosphate transport via migrating phytoplankton (at least from other sites); in which form is PO4 stored in the cells?

We have added text at lines 779-784: "Limited sampling in the waters between Hawai'i and California indicated N:P ratios were not significantly different between sinking and ascending *Rhizosolenia* mats (N:P  $\sim$ 26-30) while C:P ratios were significantly different (p=0.05, C:P sinkers = 388  $\pm$  66; C:P ascending = 221  $\pm$  43). This is consistent with simultaneous uptake of N and P at depth, but carbon consumption at depth relative to the surface. Further data on phosphate composition of vertically migrating phytoplankton are needed to confirm our hypothesis."

34. p27, l 600: I think your conclusions start here. Perhaps use a respective section title?

Yes, we now have added a section title beginning here, "Concluding Remarks".

35. p 28: l 622: "to confirm the conclusion" "confirm the hypothesis"

This change has been made.

36. p 28, l 622: "multiple authors": give at least some references

We have added four references to this statement: Cullen, 1985; Richardson et al 1998; Fraga, 2001; Villareal et al 2014.