

We thank the Reviewers for their comments. **Reviewer comments are in bold**, and our responses are in *non-bold italics*.

Comments from Reviewer #1

General: This manuscript is intended to be part of a special issue on mesocosm experiments undertaken to track the fate of nitrogen fixed by diazotrophs in a low nutrient low chlorophyll tropical environment (VAHINE project). Complimentary papers have been either published (Berthelot et al., BG, 2015) or are in review (Bonnet et al., BGD, 2015).

The major conclusion by the authors is that diazotrophically derived nitrogen (DDN; by UCYN-C) effectively contributes significantly to export of PON, but indirectly, after being recycled and incorporated into non diazotrophic phytoplankton (mainly diatoms). Aggregated UCYN-C cells are reported to contribute to export but only to a minor degree (<10%). This conclusion differs from the one in the Bonnet et al. paper (BGD, 2015) in which aggregation of UCYN-C cells into larger particles is highlighted. Such aggregates are reported by Bonnet et al. to contribute as much as 22.4% of the POC export. The other contributors to export effectively being non-diazotrophs who benefited N transfer from the diazotrophs. There is a need here to clarify and homogenise the conclusions formulated in these two papers.

We agree with the reviewer that it is essential for all manuscripts in the Special Issue to be consistent. After this manuscript was submitted, the evaluation of the contribution of UCYN-Cs to the sinking flux in the other manuscripts changed. We have communicated with K. Leblanc and S. Bonnet who have confirmed that our phrasing is consistent with the Leblanc manuscript, that UCYN-Cs contribute on average $\leq 10\%$ to export in the VAHINE experiments. For example, from the Bonnet et al., manuscript, "qPCR quantification of diazotrophs in the sediment traps revealed that $\sim 10\%$ of UCYN-C from the water column was exported to the traps daily, representing as much as $22.4 \pm 5.5\%$ of the total POC exported at the height of the UCYN-C bloom" Given these values, it is reasonable to conclude that over the course of P2 (days 15-23), UCYN-Cs contributed $< 10\%$ to the total export flux. In the current version of our manuscript, we have amended the text to be more explicitly consistent with the manuscript by Bonnet et al. For example, we include the following text in the abstract: "Despite comprising a small fraction of the total biomass, UCYN-C was largely responsible for driving export production during the last ~ 10 days of the experiments both directly (~ 5 to 22% of PN_{sink}) and through the rapid transfer of its newly fixed N to other phytoplankton" (p. 2, line 28-31), and in the discussion "UCYN-C are small cyanobacteria ($5.7 \pm 0.8 \mu\text{m}$; Bonnet et al. In prep.), but they were observed to aggregate into 100 to 500 μm particles that sank rapidly, constituting $22.4 \pm 5\%$ of the PC_{sink} flux at the height of the UCYN-C bloom (day 17) and $\sim 5\%$ as the bloom decayed (Bonnet et al., 2016a)" (p. 20, line 12-15). In any case, the exact fractional contribution of UCYN-Cs to the sinking flux does not change our interpretation that a significant fraction of DDN was transferred through the dissolved pool to be assimilated by non-diazotrophic phytoplankton that then rapidly sank.

In their introduction (and again at page 19920) the authors raise the point that while export of DDN would effectively transfer isotopically light N to the

thermocline region it cannot account for elevated NO₃/PO₄ ratios (i.e., regions with N* >0), since microorganisms who acquired DDN would export organic matter with Redfieldian stoichiometry. Would the fact that Bonnet et al. (BGD, 2015) indeed assign a significant part of the export to sinking UCYN-C cells (having N/P ratios 25:1 to 50:1) contribute to explain this condition?

The Reviewer asks a good question, and it is worth considering the consequences both inside and outside of the VAHINE mesocosms. Regarding the consequences for nutrient stoichiometry inside the mesocosm, the following calculation demonstrates that the export of the UCYN-Cs will not make a meaningful difference in subsurface N:P ratios because of the relatively small mass flux associated with export during the experiments:

Given that the total PN_{sink} flux during P2 ranges between 5.2 to 5.6 mmol N m⁻² d⁻¹, and if we assume that UCYN-Cs account for 10% of the sinking flux during P2, and if all of the UCYN-C-derived sinking PN is completely remineralized over a 100 m water column (which is not a good analogy to the VAHINE lagoon, but is a generously short estimate of the length scale over which sinking particulate matter may be remineralized in the open ocean), the remineralization of UCYN biomass would correspond to an increase in NO₃⁻ concentration of ~0.005 nM over that 100 m. If the water column was instead 10 m deep (i.e., the rest of the water column below the mesocosm-associated sediment traps in the New Caledonia lagoon) and UCYN-Cs contributed 22% of the sinking PN, the NO₃⁻ concentration would increase by ~0.10 nM upon their remineralization. This range in the possible increase in NO₃⁻ concentration due to the remineralization of diazotrophic biomass (0.005-0.10 nM N) is below the detection limit and precision of typical seawater nutrient measurements, and thus would be difficult to resolve. Given the relatively small addition of N from diazotrophs that would be unlikely to sink given “normal” conditions (i.e., non-large mesocosms where turbulence would prevent much of the UCYN-C biomass from sinking), it is hard to imagine how this small fraction of the sinking flux could influence ambient NO₃⁻:PO₄³⁻ concentration ratios regardless of whether they have a 25:1 or 50:1 N:P ratio when they sink. The calculation above further underscores that the UYCNs themselves represent a very small fraction of a relatively small sinking flux, even during the most productive P2 period.

To address the Reviewer’s question regarding the effects of UCYN-C export on subsurface N:P ratios in the open ocean requires similar calculations based on the rates at which UCYN-C sink in the open ocean. Given the increase in turbulence in “normal” environments, we expect that UCYN-C would be less likely to sink than in the VAHINE experiments, leading to an insignificant contribution to subsurface organic matter available for remineralization.

The section (pp 19920 to 19922) about the imbalance between DIP that was drawn down and the accumulation of P in different reservoirs is very long and it is unclear what exact purpose it serves.

We have removed this section of the text.

Specific: The mass balance considered to calculate the fraction of PN export supported by N₂ fixation sets isotopic signature of export = isotopic signatures of the

inputs (upward advection of thermocline NO₃ and N₂ fixation). This makes sense for a steady state system, but is this the case here? The approach is valid nevertheless because the NO₃ pool in surface waters is in a state of permanent depletion, and thus isotopic discrimination during uptake is probably muted. **Authors could clarify this in the ms.**

We appreciate the Reviewer's comment, and have revised the manuscript to emphasize that the $\delta^{15}\text{N}$ budgets in these experiments rely on the unique experimental design that provides a closed system (with the obvious exception of N supplied via N₂ fixation, the tracking of which is the goal of the experimental design). Moreover, as the Reviewer points out, the effectively complete NO₃⁻ consumption that occurred in these waters prior to the initiation of our experiments simplifies $\delta^{15}\text{N}$ calculations by removing the need to consider a potentially variable isotope effect for NO₃⁻ assimilation; because the NO₃⁻ is effectively entirely consumed prior to the initiation of the experiments, the isotope effect with which it was consumed need not be included in our calculations, and only the initial $\delta^{15}\text{N}$ of the NO₃⁻ is required. This has been clarified on p. 15, lines 10-16.

The issue about differences between DON results for P2 with those published by Berthelot et al. (2015) is a bit disturbing, and one wonders why methods have not been compared earlier.

We agree with the Reviewer that the differences are unsettling, but certainly not unusual given the relatively poor precision of DOM concentration measurements in general. Moreover, we emphasize that in all but three instances, our DON concentration measurements agree with those of Berthelot within the precision of the measurement. We were unaware that Berthelot et al. would also be making DON concentration measurements for the VAHINE experiments. Given that we report DON $\delta^{15}\text{N}$ for the same samples that we measured for DON concentration, we feel it is more appropriate to discuss our DON concentrations rather than those of Berthelot. However, we have included additional details regarding which measurements were made by which group, and we have added some discussion of the measurement discrepancy in the manuscript, including the lack of an impact on the $\delta^{15}\text{N}$ budget calculations if one were to assume the Berthelot DON concentration values (p. 18, line 14-21).

Page 19912, section 3.3: Decrease of the d15N-PN_{sink} during phases P1, P2. While this is clear for M1 and M2, M3 on the contrary shows an increase of d15N from P1 to P3. This should be discussed.

We have included a Supplementary Table containing these data in order to address the Reviewer's concerns. We have also revised the figures to include color symbols, which show that all three mesocosms have their lowest PN_{sink} $\delta^{15}\text{N}$ at the end of P2. Additionally, regarding the average $\delta^{15}\text{N}$ of PN_{sink} for M3 during P1 ($3.0 \pm 0.3\%$) relative to P2 ($3.3 \pm 1.9\%$), these PN_{sink} $\delta^{15}\text{N}$ values are not statistically different from each other. We also note that the integrated rate of N₂ fixation (measured and reported by Berthelot et al., 2015) is lower in M3 during P2, which is consistent with the PN_{sink} $\delta^{15}\text{N}$ data.

Page 19912, section 4.1: the wording 'complete' consumption of NO₃ and NH₄ does not make sense, since concentrations are never zero.

We have changed the text to read "effectively" complete consumption.

Page 19922, line 15: the sentence about silica matrices inhibiting recovery of the missing P is unclear.

We have removed this text.

Quality of graphs could be improved bu using coloured symbols.

We have included color symbols in Figures 1 and 2.

Rev #2

Overall quality of the manuscript:

Knapp et al. measured the concentration and isotopic composition of various N pools in response to an artificial addition of phosphate that deliberately induced nitrogen fixation in enclosed mesocosms. Based on the ^{15}N -depleted signature of diazotroph derived N (DDN), the authors attempted to track the fate of N supplied by nitrogen fixers into other N pools. The main finding of this study is that DDN was rapidly channeled into sinking particles and showed no accumulation in dissolved N or suspended particulate N pools. Given that various diazotroph had bloomed and were active throughout the experiments, the latter finding is puzzling. Because sinking particles were the only N pool that exhibited the ^{15}N -depleted signal, the authors conclude that the best geochemical estimates of N-fixation can be achieved by monitoring the $\delta^{15}\text{N}$ of sinking particles. However, as also pointed out by the authors, it is unclear how the findings of the ^{15}N budget determined for this mesocosm study apply to the open ocean.

*We agree with the Reviewer that the observations from the large-volume mesocosms are not necessarily an analog for what happens under “natural” conditions; indeed, we expect that many aspects of the VAHINE mesocosms are poor analogies for what happens in an open, oligotrophic water column. However, we emphasize that the value of the VAHINE large-volume mesocosm experiments is precisely the unusual experimental design: the closed system allows us to track the fate of newly fixed N in ways that are not possible in the open ocean, where we cannot distinguish whether the low- $\delta^{15}\text{N}$ of the sinking flux that is attributed to diazotrophy is due to the diazotrophs themselves directly sinking out, or whether diazotrophs growing in surface waters released their newly fixed N, which then supported the growth of other phytoplankton that ultimately sank out of the surface. In the VAHINE experiments, since the diatoms that bloomed during P2 do not have diazotrophic symbionts (a conclusion based both on phytoplankton taxonomy, see Leblanc et al., 2015, as well as the *nifH* study of Turk-Kubo et al., 2015), and because the $\delta^{15}\text{N}$ of the sinking flux has significantly decreased by this time, and decreased in proportion to the rate of N_2 fixation determined independently by Berthelot et al., 2015, the most reasonable interpretation of the data is that the primary fate of the newly fixed N was to be rapidly released by the UCYN-Cs and subsequently assimilated by the *Clyndrotheca* that bloomed during P2 immediately following the peak rates of N_2 fixation.*

*Having read through all of Reviewer #2’s comments, it seems that the majority of their concerns derive from the lack of observations of a low- $\delta^{15}\text{N}$ signal accumulating in the PN_{susp} pool. It is possible that the Reviewer has misunderstood this aspect of the paper, which we have endeavored to correct in the revised manuscript. Specifically, we seem to have communicated to the Reviewer that we do not expect the diazotroph-derived N (DDN) to ever accumulate in the PN_{susp} pool; this is not the message we mean to convey. We do very much expect that the *Clyndrotheca* that bloom during P2 were, for a short time, present in the PN_{susp} pool; however, the more-or-less invariant $\delta^{15}\text{N}$ of the PN_{susp} pool strongly suggests that low- $\delta^{15}\text{N}$ biomass (either diazotrophic, or produced through the consumption of DDN) did not accumulate in suspension above levels of 0.05-0.1 μM , as this would have been evident as a decline in the $\delta^{15}\text{N}$ of PN_{susp} , which was not*

observed; this is laid out in the manuscript (p. 12, line 27-p. 13, line 9). We see this as an analogous situation to the DDN passing briefly and undetectably through the dissolved pool – we expect that the same occurred for the PN_{susp} pool. However, the high concentrations of PN_{susp} and DON mask the small fluxes of DDN through these pools. However, these fluxes of DDN make up a significant and easily distinguished (by the $\delta^{15}N$) portion of the PN_{sink} flux – we go through the numerical argument regarding why it is easy to detect this shift in the PN_{sink} flux and not in the PN_{susp} and DON pools in the text. In response to the Reviewer’s concerns, we have amended the manuscript to clarify that DDN more than likely did pass through the PN_{susp} pool, albeit relatively quickly (p. 18, line 14; p. 20, lines 8-13; p. 21, lines 12, 19).

While we agree that the large-volume mesocosms are not necessarily a good analogy for the open ocean, our finding that the fate of newly fixed N is to be exported from surface waters via the sinking flux is consistent with most open ocean work, where there is little evidence for low $\delta^{15}N$ -N from diazotrophy accumulating in the PN_{susp} or DON pools (e.g., Altabet, 1988; Knapp et al, 2005; Knapp et al., 2008; Knapp et al., 2011, and other refs in text). So, to this end, we disagree with the Reviewer’s conclusion that our findings are “puzzling”, when they are consistent with prior observations from the open ocean. Again, due to a lack of clarity on our part, the Reviewer seems to have misinterpreted our manuscript as suggesting that the DDN never passes through the PN_{susp} pool – it most likely does pass through this pool in the same way that it passes through a dissolved pool undetected. Nonetheless, the evidence clearly indicates that it could not have accumulated there for long, given both the unchanging $\delta^{15}N$ of PN_{susp} , and that the turbulence typically associated with open-ocean conditions is removed in these large-volume mesocosms. This likely results in the rapid export from the PN_{susp} pool of both large, heavy phytoplankton like diatoms and smaller phytoplankton as well. This has been made clear in the amended manuscript text (p. 20, lines 8-13).

General Comments:

As pointed out by the first reviewer, it is disturbing that for the same experiments, there are two different data sets reported for the same parameter (i.e., DON concentration). Due to the uncertainty, and lack of an explanation for why the data may differ, the DON concentrations estimated by both studies should be considered in the authors’ interpretation of the ^{15}N budget, and perhaps also as a correspondence (i.e., errata) by Berthelot et al., as their main findings stem from these controversial data.

Please see our response above to Reviewer #1 who had a similar concern. In terms of the $\delta^{15}N$ budget, Berthelot et al. propose that the primary fate of the consumed DON is to accumulate in the PN_{susp} pool. This represents a redistribution of N between surface pools, such that it would not affect our $\delta^{15}N$ budget which relies on input and output fluxes. We have added text to our manuscript describing this (p. 18, lines 3-11).

How does N-fixed by diazotrophs immediately sink from the water column without existing as PN_{susp} ? Conceptually, particles that sink from the surface must first be suspended; this would apply to growing populations of plankton. Given the sampling resolution of ca. every other day, the PN_{susp} reservoir does not appear to

be large enough nor to turnover fast enough to obscure the passage of isotopically depleted DDN (up to 0.25 μM) through this reservoir before it sinks. As noted below, the insensitivity of $\delta^{15}\text{N}$ - PN_{susp} to input by N-fixation is in contrast to previous reports. The authors' final sentence raises a similar question regarding the bias of DDN toward sinking. Unfortunately, none of the companion studies of the VAHINE experiment have identified the composition (e.g., taxa, molecules, etc.) of sinking particles. This information seems to be well within the scope of the current study. Coincidentally with increased N-fixation in the late stages of the experiment, *Synechococcus* were shown to be most responsive to the addition of DDN, exhibiting the most substantial increases in non-diazotroph biomass (i.e., more biomass than diatoms; Leblanc et al. 2016; Biogeosciences Discuss., doi:10.5194/bg-2015-605, 2016). Given their small size and tendency to remain suspended in the water column, relatively to diatoms, it seems unlikely that DDN supporting the *Synechococcus* bloom would be channeled into PN_{sink} on the rapid timescales invoked by the authors. Moreover, the trend of increasing *Synechococcus* biomass begins on Day 7, just after the DIP spike and well before the onset of the high N-fixation period of the mesocosm experiment. In summary, DDN uptake is interpreted with respect to how the taxa composition of suspended particles changed in response to the DIP spike and N-fixation, but corresponding information for sinking particles, where the ^{15}N -depleted DDN accumulates, is largely unavailable.

*Regarding the Reviewer's concerns about the DDN consumed by *Cylindrotheca* during P2 not being observable in the PN_{susp} pool, we refer the Reviewer to responses above and below, where we state that we do expect that these diatoms were briefly, although largely undetectably, present in the PN_{susp} pool, much as the DDN was briefly and undetectably present in the TDN pool. We also reiterate that the 0.25 μM N added by N_2 fixation during P2 was not added on one day but over the course of 8 days, and so it would be difficult to resolve in measurements of PN_{susp} concentration and $\delta^{15}\text{N}$.*

Regarding the Reviewer's concerns regarding the identification of organisms in the sinking flux, we note that Bonnet et al. describe UCYN-Cs being observed in the sinking flux; these are small cyanobacteria that may not be expected to sink under "normal" open ocean conditions.

*Regarding the Reviewer's interest in *Synechococcus*, we agree that this genus blooms in P1 and P2. The low rates of N_2 fixation measured by Berthelot et al. during P1 may have supported the low biomass of *Synechococcus* observed during P1; even when the abundance of *Synechococcus* is high, the cells are so small that they likely account for a small fraction of the total phytoplankton, and thus an even smaller fraction of the PN_{susp} pool. It is possible that *Synechococcus* assimilated some DDN during P2 and, like UCYN-C, who are also typically considered too small to sink but managed to aggregate and sink in these low-turbulence mesocosms, also contributed to the sinking flux; our interpretation does not preclude this possibility. Similarly, our interpretation explicitly states that some of the DDN may have remained in the DON and/or PN_{susp} pools but was not resolvable given the precision of PN_{susp} and DON concentration and $\delta^{15}\text{N}$ measurements. However, we disagree with the Reviewer that "DDN uptake is interpreted with respect to how the taxa composition of suspended particles changed in response to*

*the DIP spike and N-fixation, but corresponding information for sinking particles, where the 15N-depleted DDN accumulates, is unavailable”; our interpretation of DDN uptake by *Cylindrotheca* is based on the mass and isotopic balance described throughout the manuscript. The phytoplankton taxonomy and diazotroph community composition data (from Turk-Kubo et al., 2015 and LeBlanc et al., in review) add detail to our interpretation, but do not change our fundamental interpretation, which is that the fate of newly fixed N is largely removal from surface waters via the sinking flux.*

The variability in d15N-PN_{sink} during P2 is largely disregarded by the authors (Page 19915, Line 25; Page 19916, Line 14), who rather focus on the overall trend as the principal finding of this study. But there appears to be a consistent trend among the replicate mesocosms in pulses of 15N-enriched particles sinking on days 15-18 and again on day 20.

The Discussion section should be reorganized, with a brief discussion of the changes observed in community composition (currently in Section 4.2), followed by a discussion of the components of the 15N budget. The current Section 4.1 is too long and builds confusion. It should be divided into smaller digestible sections with appropriate titles (e.g., one section for DON and PN_{susp} and another for PN_{sink}). The meaning of the current title of Section 4.1 is lost on me.

We respectfully disagree with the Reviewer; first, we note throughout the text that there is high variability in the $\delta^{15}\text{N}$ of PN_{sink} during P2 (e.g., p 15, lines 20-23). Second, we disagree that there is a consistent trend among the mesocosms for high- $\delta^{15}\text{N}$ PN_{sink} to be present on days 15 through 18, and again on day 20. We have now included the Supplementary Information Table 1 to illustrate this point and in response to the Reviewer’s concerns. It is also the case that the variability within and between mesocosms is too great to interpret day-to-day changes in the $\delta^{15}\text{N}$ of the PN_{sink} flux; we make this limitation clear in the text (p. 16, lines 10-16). In addition, our focus on large-scale patterns is consistent with how other manuscripts in the special issue have addressed biogeochemical changes, including the rate at which DIP is consumed in the three mesocosms, rates of C and N fixation in each mesocosm, and diazotrophic, phytoplankton, and heterotrophic microbial community shifts with time in the mesocosms. In spite of variations between the three mesocosms, there is a broad, statistically-significant trend towards lower PN_{sink} $\delta^{15}\text{N}$ with time, which is thus the focus of this manuscript.

The discussion of the “missing P” among the mesocosm experiments does not appear to fit within the scope of this manuscript and should be removed. However, the authors could instead comment on how the presumably diazotrophic biofilms, which were proposed to account for “missing P,” could have biased the 15N budget.

This text has been removed.

The Conclusions section is far too long. Some of the authors’ points are even redundant within this section (e.g., Page 19923, Line 2-3 vs. Page 19924, Lines 19-21).

We thank the reviewer for their input. While we feel that the conclusions section should include than just a summary of our findings, we have tried to reduce redundancy.

References to other studies throughout this section make it difficult to identify the key findings of the current study. Most text following the first paragraph could be removed.

We thank the reviewer for their input. Reference to other studies is required to underscore the potential implications of our work, but we have worked to separate the main points of the study from their implications, and reduce redundancy.

Specific Comments:

METHODS

Please provide more details for the method used to collect PN_{susp}. What was the pore size of the filter?

We have described the methods of Berthelot et al. who filtered their bulk water samples through a GF/F with a nominal 0.7 μm pore size (p.8, lines 30-31).

Was d15N-DON calculated by mass balance? Please state explicitly.

As we state on p. 9 in the Methods section, lines 13-15, DON concentration was determined by mass balance, as was the δ¹⁵N of DON (lines 18-20, p. 9)

RESULTS

Page 19911, Lines 3-4 – The logic is not clear. Are you implying that lagoon water has mixed with the mesocosm?

We are not implying that lagoon waters mixed with mesocosm waters; instead, we are indicating that Berthelot et al. observed a trend of decreasing DON outside the mesocosms, where it would not be expected due to the lack of DIP fertilization of N₂ fixation and where rates of C and N fixation did not increase to the same degree that they did inside the mesocosms. We have clarified this in the revised version of the manuscript.

Or are there inherent methodological differences, such that the data reported by Berthelot et al. (2015) over the last five days of the experiment are perhaps invalid? What are the methodological differences?

The French team collected all the samples, including the ones we analyzed. There is thus no reason to expect that sample collection contributed to the discrepancy between the measurements. The TN concentration samples were, however, measured separately. We have added details regarding methods to clarify which groups made which measurements (p. 9, lines 4-5; p. 10, lines 24-31).

As DON is calculated by mass balance in both studies, which of the other parameters (i.e., TN, PN, nitrate, ammonia) were similar or different between these two studies?

Berthelot et al. made the concentration measurements for PN_{susp}, NO₃+NO₂, and NH₄⁺; the only measurement that was duplicated by both groups was TN concentration. We used the same DIN and PN_{susp} data as Berthelot et al. to subtract from our TN measurements; if the discrepancy is real, its only possible source is the TN concentration measurement. We have added text to clarify that the DIN concentration measurements were made by others and reference the relevant studies.

It seems that TN data for both studies was determined after persulfate oxidation.

We note that while both Berthelot et al. and we used “wet chemical oxidation” to convert TN to NO_3^- , the specific methods used by each group are different. In particular, Berthelot et al. (2015) measured TN using the method of Pujo-Pay and Raimbault (1994, Marine Ecology Prog. Series), where the reagent used to chemically oxidize TN to NO_3^- includes boric acid and a relatively low concentration of sodium hydroxide, whereas we used the method of Knapp et al. (2005), where the persulfate oxidizing reagent does not include any boric acid and much higher concentrations of sodium hydroxide are used than outlined by Pujo-Pay and Raimbault (1994). We have included these details in the revised manuscript (p. 10, lines 24-31).

The values for PN_{susp} from the current study appear to be similar to those reported as PON by Berthelot et al. (2015).

Yes, the PN_{susp} data in our paper are from Berthelot et al., (2015). We have ensured that this is clear in the revised version of the manuscript (p. 10, lines 24-31).

Page 19911, Lines 4-7 – This argument is not so convincing, given the decoupling of DOC and DON reported previously for regions of N-fixation (Abell et al., 2000, 2005), which should be presented together with this statement.

We respectfully disagree with the Reviewer, and emphasize some mechanistic differences between what is observed by Berthelot and what is proposed by Abell et al. First, what Berthelot et al. observe is no change in DOC concentration, but a decrease in DON concentration that they primarily attribute to assimilation by phytoplankton. Since Berthelot et al. do not observe an increase in DON concentration over the 23-day experiment, the net DON that was consumed was some fraction of the DON that had been present throughout the experiment. DON, by definition, includes carbon, and marine DOM has a C:N ratio of 10-14. Given this stoichiometry, and the $0.9 \mu\text{M}$ DON decrease observed by Berthelot et al., this would apparently correspond to a ~ 9 to $13 \mu\text{M}$ decrease in DOC concentrations, which is not observed by Berthelot et al..

To us, the observations of Berthelot et al. are mechanistically different from the “decoupling” of TOC and TON proposed (but not directly observed) by Abell et al.. The Abell et al. paper describes a hypothetical situation where nitrogen fixation will produce both DOC and DON, but DON shows less accumulation because it will be rapidly consumed, without a parallel consumption of DOC. This should result in an accumulation of DOC without an accumulation of DON when N_2 fixation rates are high; this is in contrast to the Berthelot et al. data, which do not show an accumulation of DON or DOC during P2. Rather, they report a decline in DON concentration while DOC remains constant. We know of no mechanism that would result in such a large decrease in DON with no effect whatsoever on the concentration of DOC.

Page 19911, Lines 15-19 – The comparison of ^{15}N -DON to other studies should be moved to the discussion section.

We feel that the comparison of the $\delta^{15}\text{N}$ of DON measured in the VAHINE mesocosms with prior measurements from the Pacific does not play a role in the Discussion section. Additionally, it is often customary for the Results sections to include comparisons with prior results, and so we have chosen to keep this text in the Results section.

Page 19912, Lines 11-13 – Please point out here that there was much higher variability in $\delta^{15}\text{N}$ - PN_{sink} during P2 compared to the earlier phases of the experiment.

By definition, the higher standard deviation reported in these lines quantifies the higher variability associated with the mean $\text{PN}_{\text{sink}} \delta^{15}\text{N}$ during P2, and this variability is apparent in Figure 2. Additionally, we explicitly state that P2 has higher variability in the $\text{PN}_{\text{sink}} \delta^{15}\text{N}$ values (p. 15, lines 25-27, where the data are interpreted), and we also emphasize (p. 16, lines 10-19) that the overall trend in the $\text{PN}_{\text{sink}} \delta^{15}\text{N}$ of the mesocosms is more important than its daily variation, or the corresponding absolute value of the fractional contribution of N_2 fixation to export at any one time point. This is another way of stating that due to the high variability of the $\text{PN}_{\text{sink}} \delta^{15}\text{N}$ during P2, one should be wary of over-interpreting the data.

DISCUSSION

Page 19913, Line 6-8 – It remains unclear how PN_{susp} is decoupled from the N source that fuels export production, particularly when there are significant inputs of “new N.” This conclusion is in conflict with previous reports, which have identified PN_{susp} as a responsive reservoir to changes in N source, as suggested by the variability in $\delta^{15}\text{N}$ - PN_{susp} in regions of high N-fixation (Mino et al., 2002; Montoya et al., 2002; Mahaffey et al., 2003; Meador et al., 2007). The authors should include these findings and address this discrepancy, especially if the suggestion is that the changes in PN_{susp} documented by these previous studies are rather attributable to “recycled N.”

Here, we feel that a portion of the Reviewer’s concern results from a misunderstanding of the manuscript, which we have tried to rectify; we by no means expect that the diatoms that consumed low- $\delta^{15}\text{N}$ DDN during P2 were not part of the PN_{susp} pool at some point; however, the data indicate that they did not remain in the PN_{susp} pool for >1 day. This may be due to the low turbulence of the mesocosms, leading to the rapid sinking of these large, ballasted phytoplankton, compared to natural conditions, where diatoms can be more inhibited from sinking. In addition, the extremely shallow water column means that diatoms do not have to sink very far to no longer contribute to PN_{susp} . We underscore this latter point in the manuscript by calculating, using a version of Stokes’ law modified specifically for diatoms by Miklasz and Denny (2010), that diatoms with a diameter of 50 to 100 μm will sink at speeds >10 m/day (p. 16, line 29-31).

We do point out at the beginning of the first discussion section that the $\delta^{15}\text{N}$ of PN_{susp} has been used in the past to infer the sources of new N to oligotrophic surface waters. However, since PN_{susp} is a mass balance of N sources to and within the euphotic zone PN_{susp} should not be treated as the mass balance of N sources to the euphotic zone. Indeed, PN_{susp} can be significantly altered by N cycle processes within the euphotic zone, the fluxes of which are often far greater than those of new N sources to the euphotic zone, particularly in oligotrophic regions where regenerated N assimilation dominates over new N assimilation (as evidenced by f-ratios on the order of 0.1, indicating 10% new N assimilation, 90% regenerated N assimilation).

The $\delta^{15}\text{N}$ of PN_{susp} is often different from that of sinking PN, even on relatively short timescales. This is presumably due to regenerated N assimilation (see above; with the $\delta^{15}\text{N}$ of regenerated N typically being 3-6‰ lower than that of nitrate; Fawcett et al., 2011; 2014; Treibergs et al., 2014) as well as what is described in section 4.1, that PN_{susp} is a mixture of live phytoplankton, dead organic material, and heterotrophic microbes, all of which have distinct $\delta^{15}\text{N}$ signatures. Depending on the $\delta^{15}\text{N}$ of subsurface nitrate, the absolute value of these different components of the PN_{susp} pool will vary. For example, at BATS, Altabet (1988, 1989) measured the concentration and $\delta^{15}\text{N}$ of PN_{susp} every two months for 2.5 years and found both to be invariantly low (0.2-0.3 μM and $\sim 0\text{‰}$, respectively) throughout the euphotic zone (upper ~ 100 m), regardless of seasonal shifts in the relative and absolute importance of N_2 fixation vs. subsurface nitrate for fueling export production. Altabet also observed that the $\delta^{15}\text{N}$ of PN_{sink} collected at the base of the euphotic zone (100 m and 150 m) was significantly higher than PN_{susp} (PN_{sink} $\delta^{15}\text{N} = \sim 3\text{‰}$), despite overlapping in depth with PN_{susp} . Instead, PN_{sink} $\delta^{15}\text{N}$ was very similar to that of the nitrate supply to BATS surface waters ($\sim 2\text{-}3\text{‰}$; Knapp et al. 2005, 2008, Fawcett et al., 2015), even though this nitrate supports $<10\text{-}20\%$ of total phytoplankton production. While Altabet attributed the low $\delta^{15}\text{N}$ of PN_{susp} to recycled N dependence, he struggled to explain the $>3\text{‰}$ $\delta^{15}\text{N}$ difference between sinking and suspended PN; it has since been hypothesized to result from the disproportionately large contribution of high- $\delta^{15}\text{N}$ eukaryotic phytoplankton (deriving from their assimilation of the relatively high- $\delta^{15}\text{N}$ nitrate) to the sinking flux, with the smaller, numerically-dominant prokaryotic phytoplankton that depend mostly on recycled N remaining in surface waters as PN_{susp} (Fawcett et al. 2011, 2014). In this case, the eukaryotes also constituted PN_{susp} at some point, but their contribution was not large enough to significantly alter either the concentration or the $\delta^{15}\text{N}$ of the bulk PN_{susp} pool.

In the present manuscript, we emphasize that the total amount of DDN that was added to the mesocosms during P2 was 0.25 μM over the course of 8 days (Berthelot et al., 2015); even if all of this N were added on one day, it would still be difficult to resolve as a clear rise in the concentration of PN_{susp} . In the more likely scenario where this 0.25 μM N was added over several days, it would have represented too small a change in the PN_{susp} (and/or DON) concentration and $\delta^{15}\text{N}$ to be resolved; this is described in the manuscript on p.14-15. However, given the overarching question of the VAHINE experiment, which is “what is the fate of newly fixed N in the mesocosms”, the only pool or flux where low- $\delta^{15}\text{N}$ material is evident is in the sinking flux, which decreases in proportion to the rate of N_2 fixation measured independently.

Page 19914, Line 11-13 – In order to balance the There must be a fraction of PN_{susp} that is depleted in ^{15}N toward P2 balance the

We are not entirely sure what the Reviewer meant to say here. However, we refer the Reviewer to our replies immediately above, as well as to the mass balance calculations in section 4.1. Again, we emphasize that just because we do not see low- $\delta^{15}\text{N}$ DDN accumulating in the PN_{susp} pool does not mean that it does not pass through this pool – this is the same argument that we make for the DON pool. We have endeavored to clarify this misunderstanding throughout the revised manuscript (e.g., p. 13-14, lines 30-4; p. 19, lines 8-13).

Page 19914, Line 24 – It would be useful to also note that Trichodesmium is known to produce ^{15}N -depleted DON (Meador et al., 2007).

We have included the suggested reference.

Page 19914, Line 26-27 – The rapid uptake of DDN by N-limited non-diazotrophs suggests that DDN enters the PN_{susp} pool, which conflicts with the authors' conclusion that DDN did not accumulate as PN_{susp}. (see next comment)

Please see our replies above.

Page 19915, Line 11-15 – The addition of 0.25 μM DDN to the DON pool may not alter d^{15}N -DON, but is difficult to explain how DDN could enter the N budget via unicellular n-fixers without existing as PN_{susp}, which is a smaller reservoir of N (i.e., ca. 1.3 μM during P2). Furthermore, the 0.25 μM addition of DDN is similar to the increase in PN_{susp} concentration between P1 and P2 (Table 1), and would represent a significant fraction of PN_{susp} (ca. 20%).

Please see our replies above.

Page 19916, Line -14 – The logic is not clear. Is this a continuation of the caveats outlined in the previous paragraph?

We are uncertain what specifically the Reviewer is referring to here, although we think we have addressed this concern above.

Page 19917, Line 17 – The logic is not clear. Please clarify how the “more than half” estimate was derived.

Please see Equation 1 – this statement is based on the $\delta^{15}\text{N}$ budget equation, which we have indicated in the text (p. 17, line 17).

Page 19917, Line 20-23 – As above, if PN_{susp} increased with N-fixation rates from P1 to P2, why isn't the low d^{15}N value of the N supplied to the system imparted to the PN_{susp} pool? In other words, what is supporting the increase in PN_{susp} if not the supply of isotopically depleted N?

Please see our replies above. We also emphasize that we can only interpret the data that we have, which show that the $\text{PN}_{\text{sink}} \delta^{15}\text{N}$ decreases in proportion to the rate of N_2 fixation measured by Berthelot et al., while the $\delta^{15}\text{N}$ of PN_{susp} does not decrease. We also note that the PN_{susp} concentration in M3 increases more than in M1 and M2, where the PN_{susp} concentration increases in proportion to the N_2 fixation rates. Berthelot et al. attribute the increase in PN_{susp} concentration to the consumption of DON, the $\delta^{15}\text{N}$ of which could be anything, although if the consumption is of bulk DON, it is fairly high in $\delta^{15}\text{N}$ (~4.5‰).

Our conclusion that $\text{PN}_{\text{susp}} \delta^{15}\text{N}$ is not a good metric for the $\delta^{15}\text{N}$ of the sources of new N fueling export production is consistent with decades of work – starting with that of Altabet, 1988, DSR. As this original paper concluded, our data support the $\delta^{15}\text{N}$ of the sinking flux as being the best metric for the $\delta^{15}\text{N}$ of new N fueling export production, and indeed, the $\delta^{15}\text{N}$ of marine sediments confirms this (see Galbraith et al. 2013 and

references therein). Simply put, in the VAHINE mesocosms, the background concentration of PN_{susp} is too high to resolve the small addition of DDN as a change in either its concentration or $\delta^{15}N$ before this DDN leaves suspension via the sinking flux; the same argument holds for DDN passing quickly and undetectably through the dissolved pool. Moreover, the magnitude of the decrease in the $\delta^{15}N$ of PN_{sink} during P2 is directly proportionate to the increase in N_2 fixation during P2. These observations are consistent with this study being conducted in shallow mesocosms that are free from turbulence, such that there should be a very direct and rapid link between new production and the sinking flux. We make this point in the manuscript, and point out that this rapid sinking is thus not necessarily relevant to the open ocean (p. 15-16, lines 30-5).

Page 19918, Line 6-10 – It is difficult to envision either of these mechanisms of DDN export without DDN existing, at some point, as PN_{susp} .

Please see our replies above.

Page 19918, Line 15 – Given that Mino et al. (2002) and Meador et al. (2007) both observed that $15N$ - PN_{susp} appeared to be sensitive to N derived from N-fixation, it is difficult to know if this claim extends beyond the scope of the mesocosm study. For example, the “short timescales” referred to here represent a couple of weeks following an artificially induced diazotroph bloom, whereas the signals recorded by PN_{susp} in the open ocean integrate supply of N on seasonal timescales.

Please see replies above, as well as the work of Altabet, 1988, Fawcett et al., 2011, Knapp et al., 2005, and Knapp et al., 2011, which shows that it is quantitatively nearly impossible for the bulk PN_{susp} pool to respond to rates of N_2 fixation observed in the marine euphotic zone in the open oligotrophic ocean. Indeed, Mino et al. (2002) state in their abstract that the $\delta^{15}N$ of PN_{susp} is also correlated with rates of productivity, which is to say that low rates of productivity are associated with a low $\delta^{15}N$ for PN_{susp} ; this is consistent with the work of Fawcett et al. (2011) and Knapp et al. (2011), which shows that the $\delta^{15}N$ of PN_{susp} in oligotrophic gyres is significantly influenced by the low $\delta^{15}N$ of recycled N. Thus, N_2 fixation is not required to generate the low $\delta^{15}N$ of PN_{susp} in oligotrophic gyres. This is confirmed by the high $\delta^{15}N$ of shallow sinking PN in these regions, which is virtually indistinguishable from the $\delta^{15}N$ of the nitrate supply (with the exception of the stratified summer season near Hawaii, when N_2 fixation contributes ~25% to export production, Casciotti et al., 2008, DSRII), leaving very little room in the N budget for N_2 fixation. While Meador et al. (2007) show low $\delta^{15}N$ material incorporated into proteins and DNA, these pools represent a diminishingly small fraction of the bulk PN_{susp} pool, and it would not be expected for their isotopic composition to be setting the $\delta^{15}N$ of the bulk PN_{susp} pool.

Page 19920, Line > 15 – Most of the text summarizing the P imbalance of the mesocosm does not seem applicable to the current study. The “missing P” observed during this study is independent of the authors’ conclusion related to this topic, i.e., that DDN uptake by non-diazotrophs would yield sinking particles that carry an N:P similar to the Redfield ratio. As there is no attempt to balance an N^* budget, or a plot C:N:P stoichiometry of the different organic matter pools, I don’t understand the need to identify or explain the “missing P.”

Please see our replies above. We have removed this section from the manuscript in response to the suggestion of the Reviewers.

The conclusion derived from this discussion cannot be confirmed and has no application for the mesocosm study as a model for the open ocean. Save for the sentence beginning Page 19920, Line 29 (“Similarly, the N and C sinking fluxes...”), which could be appended to end of the previous paragraph, the discussion beginning here and continuing to the Conclusions section, as well as Fig. 3, could be removed without affecting the impact of this paper.

We have removed this text.

Page 19921, Line 13-20 – What is the basis for the assumptions of biofilm thickness or coverage of the mesocosm surface area? What are the implications of biofilms comprised by diazotrophs for the ^{15}N budget?

We have removed this text.

CONCLUSIONS

Page 19924, Line 14-16 – The phrase “strongly suggests” is not well supported by authors’ inference of diazotrophic DON production, which is largely a result of the lack of a depleted ^{15}N -signal in any organic matter pool other than PN_{sink} , and/or the observations reported in companion studies.

We respectfully disagree with the Reviewer – the interpretation we offer is the only one consistent with all the data: 1) The PN_{sink} flux decreases in proportion to N_2 fixation rates that were measured independently; 2) the low- $\delta^{15}\text{N}$ of N_2 fixation is not apparent in any other N pool measured in a closed system; 3) there is insufficient (by at least an order of magnitude) diazotrophic biomass in both PN_{susp} and PN_{sink} to attribute the low $\delta^{15}\text{N}$ of PN_{sink} solely to their direct contribution. Thus, the only plausible explanation is that DDN was channeled through the dissolved N pool before being consumed by non-diazotrophs and thus passing through the PN_{susp} pool (as the Reviewer has correctly pointed out to us), and then sinking into the sediment traps. Since we measured virtually every N pool in the mesocosms and carried out careful mass-balance calculations that show agreement between the timing of the $\delta^{15}\text{N}$ decrease in the sinking flux and the proportional increase in independently-measured N_2 fixation rates, we see no other plausible explanation for our observations and are inclined to retain our text as written. We have, however, ensured that we add mention of DDN passing through PN_{susp} .

Page 19924, Line 26-27 – I can’t think of a better way of answering this question than analyzing the molecular and taxonomic composition of the sinking particles, or repeating the mesocosm experiment.

We thank the Reviewer for their input.

Comments from Reviewer#3: Summary and Evaluation

This paper by Knapp et al. investigates the nitrogen budget of VAHINE mesocosms experiments by analyzing the nitrogen isotopic composition (d15N) and concentration of various nitrogen forms in the water and trap samples. They showed that the d15N values of the sinking particulate nitrogen (PNsink) at 15 m depth decreased during the 23 day experiments. In contrast, d15N values of the suspended PN (PNsusp) and dissolved organic nitrogen (DON) did not show significant changes. Based on these results, they suggested that the main fate of fixed nitrogen from increased N₂ fixation stimulated by DIP fertilization was the PNsink, not PNsusp nor DON. In addition, based on the results of community composition of phytoplankton and diazotrophs by concurrent studies, they discussed the possibility that nitrogen transfer occurred through dissolved phase from diazotrophs to non-diazotrophs, which could be an important pathway for the nitrogen transport from the surface ocean to the subsurface ocean. I think that, although this paper showed interesting and important datasets, several points listed below need to be amended or considered before publication in Biogeosciences.

General Comments

1. The authors should note that the term “PNsink” in this paper indicates a different thing from that usually used in field studies of oligotrophic oceans. The sampling water depth of PNsink of this paper is 15 m, which is much shallower than usual field sampling of PNsink in oligotrophic oceans (e.g., ~150 m at St. ALOHA). PNsink at 150 m is expected to reflect export flux out of nutrient-depleted euphotic zone via nitracline, but PNsink at 15 m may not represent it. The term “export production” would need some caution as well, because PNsink at 15 m would only reflect processes in the very upper part of euphotic zone but not in the lower part of euphotic zone. Such limitation of the experimental setup should be clearly stated in Abstract and Introduction. I also think that “export production” is not a suitable term for interpretation of the results of this paper. Readers may be confused by two different “export production” used in the paper: general term “export production” in the euphotic zone of the ocean (~150 m in the usual oligotrophic ocean) vs. special term “export production” in the upper 15 m of this VAHINE experiment.

We appreciate the Reviewer’s inclination towards open-ocean, oligotrophic ecosystems; we share the Reviewer’s partiality for these sorts of environments. However, we note that French PIs have been working in the New Caledonian lagoon for decades, and the goal of the VAHINE experiments was to examine the fate of newly fixed N in this shallow lagoon, where the water column is only 25 m deep. Given this relatively compressed water column, it is reasonable to collect the sinking particulate material (“export flux”) at 15 m depth. The sinking flux is operationally defined as material captured in a sediment trap, and not by the depth at which it is collected. Indeed, in the open ocean, the sinking flux is often collected at a range of depths that are not necessarily related to the depth of the euphotic zone or mixed layer (see Conte and Weber, 2014, Oceanography; Honjo et al., 1995, DSR II; Buessler and Boyd, 2009, L&O).

We have noted in the first sentence of the abstract that the entire water column is only 25 m deep.

2. I request the authors to show their individual data as tables (not only figures and averages) in Supplementary Materials (or anywhere else). Because the authors analyzed many samples and obtained interesting data sets, it would be beneficial for research community and future readers of this paper. In addition, because some symbols in the figures of this paper overlap each other and they are difficult to resolve, supplementary tables would help readers to understand the results.

We appreciate the Reviewer's suggestion and have included all the data generated for this manuscript in a Supplementary Table. We have also changed the symbols in Figures 1 and 2 to be in color, which should help with respect to overlapping data points.

3. The possibility of the assimilation of DIP by the biofilms (which is discussed in 4.2) is important, and it likely affects the interpretation of d15N budget of this study. If the assimilation of DIP by the biofilms is the primary sink for the "missing" DIP in the mesocosms, as concluded by the authors, it means that large amount of nitrogen was also assimilated by the biofilms (recycled N or N₂ fixation).

We have removed this section of the text.

A) Assimilation of recycled N by the biofilm: In the early part of 4.1, the authors discussed that DDN did not accumulate in the PN_{susp} pool in the mesocosms, based on the roughly constant d15N values of PN_{susp}. However, the d15N values of PN_{susp} (-3 permil) could be also explained by the addition of heterotrophic biomass which assimilated DDN and experienced trophic nitrogen isotopic fractionation by heterotrophic degradation of organic nitrogen and release of 15N-depleted ammonium (or anything else). Then, this 15N-depleted ammonium would be assimilated by the biofilms. Therefore, I think that, without closing the nitrogen budget of the mesocosms by analyzing d15N value and nitrogen quantity of the biofilm, accumulation of DDN in the PN_{susp} pool cannot be excluded.

While we have deleted this text from the manuscript, to address the Reviewer's comment, we refer them to our response immediately below, where we consider the timing of the DIP drawdown, which occurs early in P1, before the increase in water column N₂ fixation rates that peak during P2. Given the magnitude of N required to support the drawdown of the DIP, and the lack of change in any water column DON, PN_{susp} or DIN pool during the DIP drawdown, it is unlikely that the biofilms, which we believe were assimilating the DIP early in P1, could have been sustained by the low water-column rates of N₂ fixation during peak DIP drawdown. Consequently, we expect it is unlikely that the fate of the newly fixed N by the water column diazotrophs growing during P2 was to be assimilated by the biofilms, which were already well-established by the time the UCYN-Cs bloomed during P2.

We also refer the Reviewer to our other comments regarding the isotopic composition of the PN_{susp} pool, and what its constituents are (i.e., heterotrophic biomass, living phytoplankton and dead organic matter). Specifically, it is highly unlikely that the $\delta^{15}\text{N}$ of bacterial biomass will become significantly enriched – the work of Fawcett et al. (2011) demonstrates that the $\delta^{15}\text{N}$ of bacterial biomass is very similar to that of the substrate consumed. Additionally, while it is possible that bacteria may produce low- $\delta^{15}\text{N}$ NH₄⁺ the flux of this NH₄⁺ would be far too low to support the biofilm biomass.

Thus, while the biofilms likely introduced newly fixed N to the mesocosms, the timing of the increase in water column C and N₂ fixation rates, the shift in the water column diazotroph community composition, and the correspondence of both with the increased PN_{sink} flux and lower δ¹⁵N of the PN_{sink} flux when water column N₂ fixation rates increase all lead us to conclude that the primary fate of newly fixed N by water column diazotrophs was to be exported from surface waters via the sinking flux and not be incorporated into biofilm biomass.

B) N₂ fixation by the biofilm: If significant amount of N₂ fixation was conducted by the biofilm, it also does not support the conclusions of this study that, for example, “the primary fate of newly fixed N in the VAHINE mesocosms experiments was to be converted in to the PN_{sink} flux” (Page 19918 Lines 5–6). Therefore, the authors should estimate the amount of possible assimilation of N by the biofilm as well as P, and should discuss its effects on the discussion and conclusion in 4.1

Here we think the Reviewer might be conflating the fate of the DIP addition with the fate of newly fixed N by water column diazotrophs in the mesocosms. To distinguish between N₂ fixation in the water column and that in the biofilm, it is useful to consider: 1) the timing of the DIP drawdown (see Berthelot et al., 2015), and, 2) the timing of increases in water column N₂ fixation rates (also see Berthelot et al., 2015). We expect that given the rapid drawdown of DIP in the water column during P1, when there are no discernable changes to any other water column parameter (i.e., constant DIN, DON and PN_{susp} concentrations and C and N fixation rates relative to P0), that this DIP consumption largely occurred by organisms in the biofilms; since the mesocosm water column DIN concentrations were low (and PN_{susp} and DON concentrations did not change during P1), we assume that diazotrophs had to be part of the biofilm community in order to support the accumulation of the biofilm biomass. Importantly, we also expect that the biofilm biomass did not slough off the sides of the mesocosm and contribute to the sinking flux; this is confirmed by the work of Leblanc et al. and Bonnet et al. who establish phytoplankton taxonomy and verify the presence of diazotrophs growing in surface waters that were also present in the sediment traps.

Specific Comments

Page 19910 Lines 16 – Page 19911 Lines 12: Which parameter (TN, PN_{susp}, NH₄⁺, NO₃⁻, NO₂⁻, or else) is likely the main cause of the discrepancy of calculated DON concentration between the two studies? Specifying the main cause may be useful to understand the discrepancy. I’m also wondering whether the cutoff size of PN and DON filtering is same between the two studies.

Berthelot et al. made the concentration measurements for PN_{susp}, NO₃+NO₂, and NH₄⁺; the only measurement that was duplicated by both groups was TN concentration. We used the same DIN and PN_{susp} data as Berthelot et al. to subtract from our TN measurements; if the discrepancy is real, its only possible source is the TN concentration measurement. We have added text to clarify that the DIN concentration measurements were made by others and reference the relevant studies (p. 10, lines 24-31).

Page 19911 Line 21–23: What was the N source for the increased PN_{susp} concentration? While the authors concluded that DDN was not the N source for the increased PN_{susp} concentration, it seems that the authors did not suggest

alternative N sources. Nitrate is suggested as the origin of the elevated d15N values of PN_{susp}, but it would not explain the increased PN_{susp} concentration in the mesocosms, because the water in the mesocosms was depleted in nitrate.

Please see our replies above. The concentration of PN_{susp} increases in concert with the increase in carbon and N₂ fixation rates documented by Berthelot et al. (2015), in particular in M1 and M2. Thus, we expect that the PN_{susp} concentration would necessarily increase during P2 when C and N fixation rates increase; indeed, it would be hard to understand if they did not. We use the $\delta^{15}\text{N}$ budget to deduce the source of the N fueling the PN_{sink} during this time period, but we do not use the $\delta^{15}\text{N}$ budget to interpret the $\delta^{15}\text{N}$ of PN_{susp}. Indeed it would not be appropriate to do so for the reasons given above, including that PN_{susp} is a heterogenous mixture of different N pools that turnover at different rates and have different N sources. In section 4.1, we interpret the absolute value of the $\delta^{15}\text{N}$ of PN_{susp}, which is ~3‰ throughout the experiment and not significantly different from the PN_{susp} $\delta^{15}\text{N}$ measured outside the mesocosms in the lagoon (Figure 1d). A $\delta^{15}\text{N}$ for PN_{susp} of ~3‰ is high relative to other oligotrophic regions like Hawaii and Bermuda (see references in text), although we expect that discharge of anthropogenic waste from the island may contribute to the elevation of PN_{susp} $\delta^{15}\text{N}$ in this region (see discussion on p. 13 last paragraph through p. 14 first paragraph). We also expect that the consumption of regional subsurface NO₃⁻ with a $\delta^{15}\text{N}$ of 6.5‰, will produce PN_{susp} with a $\delta^{15}\text{N}$ that is relatively high (see first paragraph of p. 14). Thus, a 3‰ $\delta^{15}\text{N}$ for PN_{susp} reflects, to some degree, the incorporation of subsurface NO₃⁻ with a $\delta^{15}\text{N}$ of ~6.5‰.

We do not, however, mean to suggest that the growth of PN_{susp} can be attributed to NO₃⁻ consumption since, as the Reviewer points out, there was no detectable NO₃⁻ in the mesocosm waters at any point during the experiment. Finally, as noted above and in our revised text, Berthelot et al. attribute the increase in PN_{susp} during P2 to DON consumption. This is based on the observation of a decrease in the concentration of DON at the end of the experiments, which our DON measurements do not show. However, the consumption of DON would not alter our $\delta^{15}\text{N}$ budget calculations given that Berthelot et al. propose that the fate this DON is to accumulate as PN_{susp}, thus representing a redistribution of N between surface pools that does not impact the N fluxes in or out.

Page 19939: Figure 4 would be more suitable for Supplementary Materials. For me, the biofilms are not so obvious in the photos.

We have deleted this figure.