

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 important to be consistent with the other manuscripts in the Special Issue. After this manuscript was submitted, the evaluation of the contribution of UCYN-Cs to the sinking flux in the other manuscripts changed; while we will be sure that a revised version of this manuscript includes values of UCYN-C's contribution to the export flux that are consistent with the other manuscripts in the Special Issue, we emphasize that our estimate, that UCYN-Cs contribute <10% to export in the VAHINE experiments, is consistent with the overall contribution of the UCYN-Cs to time-integrated export. For example, from the Bonnet et al., manuscript, "qPCR quantification of diazotrophs in the sediment traps revealed that ~10% of UCYN-C from the water column was exported to the traps daily, representing as much as 22.4 +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 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 NO<sub>3</sub>/PO<sub>4</sub> 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, we think 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. For example, if the total PN sink flux during P2 ranges between 5.2 to 5.6 mmol N m<sup>-2</sup> d<sup>-1</sup>, and if we assume that UCYN-Cs account for 10% of the sinking flux during P2, and if all of that UCYN-C-derived sinking PN was 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) it would correspond to an increase of NO<sub>3</sub> of ~0.005 nM over that 100 m; if it was decreased to a 10 m water column (i.e., the rest of the water column below the traps in the New Caledonia lagoon) and a 22% contribution from UCYN-C-derived sinking PN, the NO<sub>3</sub> concentration would increase by ~0.10 nM. These increases in nutrient concentrations are below the detection limit and precision of typical seawater nutrient measurements, and thus would be difficult to resolve. Given this relatively small addition of N from diazotrophs that would be unlikely to sink given “normal” (i.e., non-large mesocosm) conditions, where turbidity would likely 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 NO<sub>3</sub>:PO<sub>4</sub> concentration ratios regardless of whether they have a 25:1 or 50:1 N:P ratio when they sink. This calculation further underscores how 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 to subsurface N:P ratios in the open ocean would require similar calculations based on the rates at which UCYN-C sink in the open ocean. Given the increase in turbidity in “normal” environments, we expect that UCYN-C would be less likely to sink than in the VAHINE experiments, and so we expect the relatively high rate of UCYN-C sinking observed in these large-volume mesocosms is one example of how these mesocosms are probably a poor analogy for the open ocean. Consequently, we are still faced with the question of how an elevated N:P ratio is exported from surface waters in “normal” circumstances.

**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 take this and other Reviewers’ concerns regarding this section of the text seriously, yet we feel that some discussion of the DIP imbalance is required, in particular to account for the lag in water column C and N fixation rates following DIP fertilization and drawdown. In a revised manuscript, we will remove discussion of the methodological concerns regarding particular P measurements of polyphosphate, and focus on the biofilms that were observed as the most likely fate for the “missing P”. We will also strengthen the link between this section and the rest of the manuscript by emphasizing the implications of the missing P for the mass and isotopic budgets.*

**Specific: The mass balance considered to calculate the fraction of PN export supported by N<sub>2</sub> fixation sets isotopic signature of export = isotopic signatures of the inputs (upward advection of thermocline NO<sub>3</sub> and N<sub>2</sub> fixation). This makes sense for a steady state system, but is this the case here? The approach is valid nevertheless because the NO<sub>3</sub> 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 will make sure that a revised manuscript emphasizes that the d15N budgets in these experiments rely on the unique experimental design that provides a closed system (with the obvious exception of N supplied via N<sub>2</sub> fixation, the tracking of which is the point of the experimental design). Moreover, as the Reviewer points out, the effectively complete NO<sub>3</sub> consumption that occurred in these waters prior to the initiation of our experiments simplifies d15N calculations by removing the need to worry about a potentially variable isotope effect for NO<sub>3</sub> assimilation; since the NO<sub>3</sub> is effectively entirely consumed, 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 NO<sub>3</sub><sup>-</sup> is required.*

**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 two instances, our DON concentration measurements agree with those of Berthelot within the precision of the measurement. However, yes, in future campaigns, we will work to reconcile differing measurements before publication; 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 in detail rather than those of Berthelot; indeed, we currently offer this caveat in the manuscript (p. 19911, line 7-12).*

**Page 19912, section 3.3: Decrease of the d15N-PNsink 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 feel that the inclusion of a Supplementary Table with the data will help address the Reviewer's concerns. Additionally, regarding the average d15N of PNsink for M3 during P1 ( $3.0 \pm 0.3\%$ ) relative to P2 ( $3.3 \pm 1.9\%$ ), these PNsink d15N are not statistically different from each other, and are driven by a couple of points. We also note that the integrated rate of N<sub>2</sub> fixation (measured and reported by Berthelot et al., 2015) is lower in M3 during P2, which is consistent with the PNsink d15N data.*

**Page 19912, section 4.1: the wording 'complete' consumption of NO<sub>3</sub> and NH<sub>4</sub> does not make sense, since concentrations are never zero.**

*We will change the text to read "effectively" complete consumption in a revised manuscript.*

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

*We have taken the Reviewer's suggestion and this text will be removed in a revised manuscript.*

**Quality of graphs could be improved by using coloured symbols.**

*Given that the focus of the manuscript is on the large-scale trends observed in the VAHINE experiments, we chose to keep all the figures in black and white. We found that using different colored symbols for the different mesocosms made the figures very difficult to read. However, a revised version of the manuscript will include modified symbols for clarity.*

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  $^{15}\text{N}$ -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  $^{15}\text{N}$ -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  $^{15}\text{N}$  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 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 supported the growth and sinking of other phytoplankton. 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 is significantly lower at this time, and is lower in proportion to the rate of  $\text{N}_2$  fixation determined independently by Berthelot et al., 2015, the only 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 *Clyindrotheca* that bloom during P2 immediately following the peak rates of  $\text{N}_2$  fixation.*

*Having read through all of Reviewer #2’s comments, we feel that the majority of their concerns are due to the lack of observations of a  $\delta^{15}\text{N}$  signal accumulating in the PN<sub>susp</sub> pool. It is possible that the Reviewer has misunderstood this aspect of the paper, which we will clarify in a revised manuscript. Specifically, the Reviewer’s concerns seem to be centered around an interpretation of our text that we do not expect the diazotroph-derived N (DDN) to ever accumulate in the PN<sub>susp</sub> pool; this is not the message we mean to convey and we will clarify this in a revised manuscript. We do very much expect that the *Clyindrotheca* that bloom during P2 were, for a short time, present in the PN<sub>susp</sub> pool.*

*While we agree that the large-volume mesocosms are not necessarily a good analogy to 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 all open ocean work, where there is little evidence for low  $d_{15}N$ -N from diazotrophy accumulating in the PNsusp or DON pool (e.g., Altabet, 1988; Knapp et al, 2005; Knapp et al., 2008; Knapp et al., 2011; Fawcett 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 paper to be saying that the DDN never passes through the PNsusp pool – it most likely does pass through that pool in the same way it passes through a dissolved pool undetected. Nonetheless, the evidence clearly indicates that it does not stay there for long, in particular in these large-volume mesocosms where the turbidity typically associated with open-ocean conditions is removed, leading not only large, heavy phytoplankton like diatoms to quickly leave the PNsusp pool and contribute to the PNsink flux, but smaller phytoplankton as well.

#### **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  $d_{15}N$  budget, the only way that the consumption of 1  $\mu M$  from the 5 to 6  $\mu M$  DON pool could change our interpretation is if the DON that was consumed had a very different  $d_{15}N$  from that of the bulk DON pool. Unfortunately, since Berthelot et al. did not measure the  $d_{15}N$  of DON, we cannot address this directly. However, our measurements of the bulk DON  $d_{15}N$  show no change across the time period of interest, which is the strongest direct evidence for no preferential consumption of low- $d_{15}N$  DON at the end of P2. Simple mass-balance calculations indicate that the  $d_{15}N$  of the 1  $\mu M$  DON that was hypothetically consumed would have to be much lower in  $\delta^{15}N$  than the bulk pool (i.e., >10 per mil lower) to make a difference to the  $d_{15}N$  budget; we are unaware of evidence for DON being consumed with a sufficiently large isotope effect that is required to drive a component of the bulk DON pool to such a low  $\delta^{15}N$ . In fact, the isotope effects that have been estimated for DON consumption are typically between 3 to 6 permil; see O'Leary and Kluetz, 1972; Macko et al., 1986; Bada et al., 1989; Silfer et al., 1992). Moreover, the consumption of 1  $\mu M$  DON with a large isotope effect would also act to raise the  $\delta^{15}N$  of the remaining 4-5  $\mu M$  DON; this is not observed. We thus feel that it is reasonable to exclude the possibility of the consumption of a low- $\delta^{15}N$  component of the DON pool. Moreover, we emphasize that Berthelot et al. (2015) observed a DON concentration decrease without a concurrent decrease in DOC concentrations; we are unaware of a process by which this could occur.*

**How does N-fixed by diazotrophs immediately sink from the water column without existing as PNsusp? 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<sub>susp</sub> 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  $\mu$ M) through this reservoir before it sinks. As noted below, the insensitivity of  $d_{15}N$ -PN<sub>susp</sub> 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<sub>sink</sub> 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<sub>susp</sub> pool, we refer the Reviewer to responses above and below, where we state that we do expect that these diatoms were briefly, although undetectably, present in the PN<sub>susp</sub> pool, much as the DDN was briefly and undetectably present in the TDN pool. We also reiterate that the 0.25  $\mu$ M N added by N<sub>2</sub> 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<sub>susp</sub> concentration and  $d_{15}N$ .*

*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.*

*Regarding the Reviewer's interest in *Synechococcus*, we agree that it blooms in P1 and P2. The low rates of N<sub>2</sub> fixation measured by Berthelot et al. during P1 may have supported the low biomass of Syn during P1; even when the abundance of Syn is high, Syn cells are so small that even a high abundance of Syn account for a small fraction of the total biomass, and thus an even smaller fraction of the PN<sub>susp</sub> 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-turbidity 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<sub>susp</sub> pools but was not resolvable given the precision of PN<sub>susp</sub> and DON concentration and  $d_{15}N$  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  $^{15}\text{N}$ -depleted DDN accumulates, is unavailable”; our interpretation of DDN uptake by *Cylindrotheca* is based on the mass and isotopic balance described throughout the manuscript, and the phytoplankton taxonomy and diazotroph community composition add detail to our interpretation, but do not change our fundamental interpretation, which is that the fate of newly fixed N leaves via the sinking flux.*

**The variability in  $d^{15}\text{N}$ -PNsink 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  $^{15}\text{N}$ -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  $^{15}\text{N}$  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 PNsusp and another for PNsink). The meaning of the current title of Section 4.1 is lost on me.**

*We disagree with the Reviewer; first, we note throughout the text that there is high variability in the  $d^{15}\text{N}$  of PNsink during P2. Second, we disagree that there is a consistent trend among the mesocosms for high- $d^{15}\text{N}$  PNsink to be present on days 15 through 18, and again on day 20; only mesocosm 3 shows higher  $d^{15}\text{N}$  on those days – M1 and M2 do not. This may become clearer in the Supplementary Information Table 1 included in a revised manuscript. We also believe there is too much variability between the mesocosms to interpret day-to-day changes in the  $d^{15}\text{N}$  of the PNsink flux; this is similarly evident in the rate at which DIP is consumed among the three mesocosms, rates of C and N fixation in each mesocosm, as well as how diazotrophic, phytoplankton, and heterotrophic microbial communities shift independently in all three mesocosm. In spite of variations between the three mesocosms, there are some general trends that we and the authors of other papers in the Special Issue feel are worth investigating. We also feel that if we were to examine the day-to-day variability in the PNsink  $d^{15}\text{N}$  of each mesocosm that would necessarily correspond to a longer, more complicated discussion section, which seems to be at odds with the Reviewer’s related comment that the Discussion section is currently too long.*

**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  $^{15}\text{N}$  budget.**

*Please see our response to Reviewer #1; we will remove the discussion of methodological challenges associated with polyphosphate recovery and instead focus on the biofilms discussion in a revised manuscript, and how they impact the  $d^{15}\text{N}$  budgets.*

**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 provide a summary of our findings in this section, as well as a discussion of the implications for the fate of newly fixed N, something that has interested the community for decades, and make suggestions for future work. We will work to reduce redundancy in a revised manuscript, but feel that a conclusion section should be more than a mere summary of the study findings such that discussion of implications and future work is appropriate.*

**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 will take the Reviewer's comments into consideration in a revised manuscript and ensure that the study's key findings are clearly stated.*

**Specific Comments:**

**METHODS**

**Please provide more details for the method used to collect PNsusp. What was the pore size of the filter?**

*Since we did not measure the PNsusp for this study, we had fewer details of these measurements. However, in a revised manuscript we will include the pore size of the filter; Berthelot et al. filtered their bulk water samples through a 0.7  $\mu\text{m}$  GF/F.*

**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 d15N 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 N<sub>2</sub> fixation. There is no reason to expect that a decrease in DON concentration in the mesocosms at the end of P2 would also be evident outside the mesocosms (i.e., in the lagoon waters), where rates of C and N fixation did not increase to the same degree that they did inside the mesocosms. It is possible that there were methodological complications/issues associated with the Berthelot et al. TN measurements, which led to the low values determined for P2 both inside and outside the mesocosms; however, since we were not privy to those measurements, we cannot comment on this in the manuscript. We can, however, point out that a decline in DON concentration outside the mesocosm (i.e., in the lagoon waters) is curious.*

**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.

**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 PNsusp, NO<sub>3</sub>+NO<sub>2</sub>, and NH<sub>4</sub>; the only measurement that was duplicated by both groups was TN concentration. We used the same DIN and PNsusp 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. In the current version of the manuscript, we state 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<sub>3</sub>, the specific methods used by each group are different. In particular, Berthelot et al. (2015) measured TN using the method of Pujol-Pay and Raimbault (1994, Marine Ecology Prog. Series), where the reagent used to chemically oxidize TN to NO<sub>3</sub> 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 than Pujol-Pay and Raimbault (1994). We have extensively tested this latter version of the method with other wet chemical oxidation methods to ensure: 1) that the method provides the highest recovery of the most diverse suite of organic N molecules, and, 2) that the N blank (which is typically large for the wet oxidation approach) is minimized, which is essential for N isotope work (Knapp et al., 2005, Fawcett et al., 2011). Indeed, in this particular study, the N blank was <<5% of the DON concentration, and it had a  $\delta^{15}\text{N}$  that was very similar to that of the bulk DON pool (~5‰). In a revised version of the manuscript, we will include these details.*

**The values for PNsusp from the current study appear to be similar to those reported as PON by Berthelot et al. (2015).**

*Yes, the PNsusp data in our paper are from Berthelot et al., (2015) – we did not measure PNsusp, but we use that data in our  $\delta^{15}\text{N}$  budget calculations. The only measurement duplicated between both papers is DON concentration, calculated by each group independently measuring TN concentration, and subtracting from that measurement the measurements of NO<sub>3</sub>+NO<sub>2</sub>, NH<sub>4</sub> and PNsusp made by the French group. We will ensure that this is clear in the revised manuscript.*

**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.**

*The “decoupling” of TOC and TON proposed (but not directly observed) by Abell et al. is not in the same sense as what is observed by Berthelot et al., and invoked by the Reviewer to explain the Berthelot et al. decrease in DON without a decrease in DOC. 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. Again, this hypothetical situation described by Abell et al. (that is to say, the authors did not observe this directly, but are speculating about what might happen in oligotrophic gyres) is in contrast to the Berthelot et al. data, which do not show an accumulation of DON or DOC. Instead, Berthelot et al. describe the consumption of DON without the consumption of DOC. This is fundamentally different from the Abell et al. paper – Abell et al. assume that both DOC and DON are produced by nitrogen fixation, which is not observed by Berthelot et al., who show constant DOC and DON concentrations throughout the experiment until the last few days of P2, when they observe a decrease in DON concentration without a decrease of DOC. Since by definition DON includes some carbon, it is difficult to imagine how DON could be consumed without a loss of carbon, too. Thus, the scenario proposed by Abell supports our interpretation of the data, not the Reviewer's. If the scenario of Abell et al. were to apply to the VAHINE mesocosms, then as N<sub>2</sub> fixation rates increase during P2, it should have resulted in an increase in DOC concentration, without a change in DON concentration relative to “background” values. Moreover, as calculated by Berthelot et al., the total amount of N<sub>2</sub> fixation during P2 only adds a net 0.25 μM N to the mesocosms; thus, the 1 μM DON drawdown described by Berthelot is greater than the amount added by N<sub>2</sub> fixation – it would require an additional 0.75 μM DON that did not originate from N<sub>2</sub> fixation. Moreover, this scenario would preclude any export of low-d<sup>15</sup>N material via the sinking flux, where it clearly accumulates. We feel the scenario proposed by the Reviewer is not only inconsistent with the Abell et al. paper, but also with the data from the VAHINE mesocosms.*

**Page 19911, Lines 15-19 – The comparison of <sup>15</sup>N-DON to other studies should be moved to the discussion section.**

*We feel that the comparison of the d<sup>15</sup>N of DON measured in the VAHINE mesocosms with prior measurements from the Pacific plays no role in the Discussion section. Additionally, it is customary for Results sections to include comparison with prior results, and so we chose to keep this text in the Results section.*

**Page 19912, Lines 11-13 – Please point out here that there was much higher variability in d<sup>15</sup>N-PNsink during P2 compared to the earlier phases of the experiment.**

*By definition, the higher standard deviation reported in these lines says precisely what the Reviewer requests. Moreover, the higher variability is readily apparent in Figure 2. We explicitly state that P2 has higher variability in the PNsink d<sup>15</sup>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 PNsink d<sup>15</sup>N of the mesocosms is more important than its daily variation, or the corresponding absolute value of the fractional contribution of N<sub>2</sub> fixation to export at any one time point. This is another way of stating that due to the high variability of the PNsink d<sup>15</sup>N during P2, one should be wary of over-interpreting the data.*

## **DISCUSSION**

**Page 19913, Line 6-8 – It remains unclear how PN<sub>susp</sub> 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<sub>susp</sub> as a responsive reservoir to changes in N source, as suggested by the variability in d<sup>15</sup>N<sub>PN<sub>susp</sub></sub> 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<sub>susp</sub> documented by these previous studies are rather attributable to “recycled N.”**

*Here, we feel the Reviewer has misunderstood the manuscript, which we recognize falls to us to rectify; we by no means expect that the diatoms that consumed low-d<sup>15</sup>N DDN during P2 were not part of the PN<sub>susp</sub> pool at some point; however, the data indicate that they did not remain in the PN<sub>susp</sub> pool for >1 day. This may be due to the low turbidity of the mesocosms, leading to the rapid sinking of these large, ballasted phytoplankton, compared to natural conditions, where diatoms would 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<sub>susp</sub>.*

*It is worth pointing out, however, that the  $\delta^{15}\text{N}$  of PN<sub>susp</sub> is often different from that of sinking PN, even on relatively short timescales. For example, at BATS, Altabet (1988, 1989) measured the concentration and  $\delta^{15}\text{N}$  of PN<sub>susp</sub> every two months for 2.5 years and found both to be invariantly low (0.2-0.3  $\mu\text{M}$  and  $\sim 0\%$ , respectively) throughout the euphotic zone (upper  $\sim 100$  m), regardless of month. Altabet also observed that the  $\delta^{15}\text{N}$  of PN<sub>sink</sub> collected at the base of the euphotic zone (100 m and 150 m) was significantly higher than PN<sub>susp</sub> (PN<sub>sink</sub>  $\delta^{15}\text{N} = \sim 3\%$ ), despite overlapping in depth with PN<sub>susp</sub>. Instead, PN<sub>sink</sub>- $\delta^{15}\text{N}$  was very similar to that of the nitrate supply to BATS surface waters ( $\sim 2$ - $3\%$ ; Knapp et al. 2005, 2008, Fawcett et al., 2015), even though this nitrate supports <10-20% of total phytoplankton production. While Altabet attributed the low  $\delta^{15}\text{N}$  of PN<sub>susp</sub> to recycled N dependence, he struggled to explain the  $>3\%$   $\delta^{15}\text{N}$  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 high- $\delta^{15}\text{N}$  nitrate source) to the sinking flux, with the smaller, numerically-dominant prokaryotic phytoplankton that depend mostly on recycled N remaining in surface waters as PN<sub>susp</sub> (Fawcett et al. 2011, 2014). In this case, the eukaryotes also constituted PN<sub>susp</sub> 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 suspended PN pool.*

*In the present manuscript, we emphasize that the total amount of DDN that was added to the mesocosms during P2 was 0.25  $\mu\text{M}$  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<sub>susp</sub>. In the more likely scenario where this 0.25  $\mu\text{M}$  N was added over several days, it would have represented too small a change in the PN<sub>susp</sub> concentration and d<sup>15</sup>N to be resolvable. 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-d<sup>15</sup>N material is evident is in the sinking flux.*

*We also reiterate what is stated in section 4.1, that PN<sub>susp</sub> is a mixture of live*

*phytoplankton, dead organic material, and heterotrophic microbes, all of which have distinct d15N signatures. Depending on the d15N of subsurface NO<sub>3</sub>, the absolute value of these different components of the PN<sub>susp</sub> pool will vary.*

**Page 19914, Line 11-13 – In order to balance the There must be a fraction of PN<sub>susp</sub> that is depleted in 15N 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 don't see low-d15N DDN accumulating in the PN<sub>susp</sub> pool does not mean it does not pass through it – this is the same argument we make for the DON pool. We will ensure that in a revised version of this manuscript it is made clear that DDN likely passes through PN<sub>susp</sub>, albeit rapidly.*

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

*The work of Meador et al. (2007) shows that low-d15N DON is produced in cultures of Trichodesmium where the only source of N to support Trichodesmium growth is atmospheric N<sub>2</sub> (with a d15N of 0 per mil); thus, it is unclear why one would expect anything other than DON with a d15N generally similar to that of the only source of N to the culture to be observed in this scenario.*

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

*Please see replies above. We will endeavor to clarify this misunderstanding in the revised manuscript.*

**Page 19915, Line 11-15 – The addition of 0.25  $\mu$ M DDN to the DON pool may not alter d15N-DON, but is difficult to explain how DDN could enter the N budget via unicellular n-fixers without existing as PN<sub>susp</sub>, which is a smaller reservoir of N (i.e., ca. 1.3  $\mu$ M during P2). Furthermore, the 0.25  $\mu$ M addition of DDN is similar to the increase in PN<sub>susp</sub> concentration between P1 and P2 (Table 1), and would represent a significant fraction of PN<sub>susp</sub> (ca. 20%).**

*Please see 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 may 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 d15N budget equation.*

**Page 19917, Line 20-23 – As above, if PN<sub>susp</sub> increased with N-fixation rates from P1 to P2, why isn't the low d15N value of the N supplied to the system imparted to**

**the PN<sub>susp</sub> pool? In other words, what is supporting the increase in PN<sub>susp</sub> if not the supply of isotopically depleted N?**

*Please see replies above. Again, we emphasize that the findings here, that PN<sub>susp</sub> d<sub>15</sub>N is not a good metric for the d<sub>15</sub>N of the sources of new N fueling export production, is consistent with decades of work – starting with that of Altabet, 1988, DSR. Instead, the d<sub>15</sub>N of the sinking flux is expected to be the best metric for the d<sub>15</sub>N of new N fueling export production, and indeed, the d<sub>15</sub>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<sub>susp</sub> is too high to resolve the small addition of DDN in its concentration or d<sub>15</sub>N before it leaves via the sinking flux; the same argument holds for the DDN passing quickly and undetectably through the dissolved pool. Moreover, the magnitude of the decrease in the d<sub>15</sub>N of PN<sub>sink</sub> during P2 is directly proportionate to the increase in N<sub>2</sub> fixation during P2. These observations are consistent with this study being conducting 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.*

**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<sub>susp</sub>.**

*Please see replies above.*

**Page 19918, Line 15 – Given that Mino et al. (2002) and Meador et al. (2007) both observed that <sup>15</sup>N-PN<sub>susp</sub> 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<sub>susp</sub> 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<sub>susp</sub> pool to respond to rates of N<sub>2</sub> fixation observed in the marine euphotic zone. Indeed, Mino et al. (2002) state in their abstract that the d<sub>15</sub>N of PN<sub>susp</sub> is also correlated with rates of productivity, which is to say that low rates of productivity are associated with low d<sub>15</sub>N of PN<sub>susp</sub>; this is consistent with the work of Fawcett et al. (2011) and Knapp et al. (2011), which shows that the d<sub>15</sub>N of PN<sub>susp</sub> in oligotrophic gyres is significantly influenced by the low d<sub>15</sub>N of recycled N. Thus, N<sub>2</sub> fixation is not required to generate the low d<sub>15</sub>N of PN<sub>susp</sub> in oligotrophic gyres; this is confirmed by the high δ<sup>15</sup>N of shallow sinking PN in these regions, which is virtually indistinguishable from the δ<sup>15</sup>N of the nitrate supply (with the exception of the stratified summer season near Hawaii, when N<sub>2</sub> fixation contributes ~25% to export production, Casciotti et al., 2008, DSR II), leaving very little room in the N budget for N<sub>2</sub> fixation. While Meador et al. (2007) show low d<sub>15</sub>N material incorporated into proteins and DNA, these pools represent a diminishingly small fraction of the bulk PN<sub>susp</sub> pool, and it would not be expected for their isotopic composition to be setting the d<sub>15</sub>N of the bulk PN<sub>susp</sub> 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 replies above.*

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 will consider the Reviewer's input in a revised version of the manuscript.*

**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 15N budget?**

*The estimates for biofilm thickness and coverage were intended to be conservative estimates based on the observations of those conducting the sampling in the mesocosms and based on the pictures of the biofilm growth on the mesocosms (see Fig. 4). We will more explicitly relate the biofilms to the d15N budget in a revised manuscript, but briefly, they are important because they provide an explanation for the fate of the DIP drawdown as well as the lag in water column C and N fixation rates, which are otherwise hard to understand. Please see response below to Rev. #3 for additional comments on how we interpret the biofilms in the context of the d15N budget.*

## **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 15N-signal in any organic matter pool other than PNsink, and/or the observations reported in companion studies.**

*We respectfully disagree with the Reviewer; the Reviewer does not provide an alternative interpretation of the data that we should consider, and 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  $\text{N}_2$  fixation rates, we are inclined to keep our text as it is written.*

**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 agree with the Reviewer that hindsight always provides a useful vantage point for how to best design future work. We note that this collaborative effort already assembled a wide array of expertise, and in particular, our manuscript benefits from the work of Turk-Kubo et al., which characterized the diazotrophic community during the*

*experiments, as well as the phytoplankton taxonomy of Leblanc et al.; without these contributions we would not be able to draw the conclusions we draw.*



### 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 N<sub>2</sub> 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 natural 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 25 m. 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).*

*While we state in the first sentence of our abstract that our study is, “In a shallow, coastal lagoon”, we will specify in a revised manuscript that by “shallow” we mean a 25 m water column.*

**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 would be happy to include all the data generated for this manuscript in a Supplementary Table in a revised version of the manuscript.*

**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 N<sub>2</sub> fixation).**

*We agree with the Reviewer and we expect that the biofilms included diazotrophs since NO<sub>3</sub> and NH<sub>4</sub> concentrations were very low throughout the experiment, and we see no change in DON or PNsusp concentration in the mesocosm water column during P1 when the DIP is drawn down from the water column. The only plausible source of N to support the biofilm biomass and its consumption of DIP is from diazotrophs growing within the biofilms. We will clarify this connection and its implications in a revised version of the manuscript.*

**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 PNsusp pool in the mesocosms, based on the roughly constant d15N values of PNsusp. However, the d15N values of PNsusp (−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 PNsusp pool cannot be excluded.**

*We refer the Reviewer 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 N<sub>2</sub> 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, PNsusp 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 N<sub>2</sub> 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 PNsusp pool, and what its constituents are (i.e., heterotrophic biomass, living phytoplankton and dead organic matter). Specifically, we expect it highly unlikely for the d15N of bacterial biomass to become significantly enriched – the work of Fawcett et al. (2011) demonstrates that the d15N of bacterial biomass is very similar to that which it consumed. Additionally, Fawcett et al. (2011) found it unlikely for heterotrophic microbial biomass to exude low d15N NH4, but even if it did, its flux would be too low to support the biofilm biomass.*

*Thus, while the biofilms likely introduced newly fixed N to the mesocosms, the timing of the water column C and N2 fixation rates, the shift with the water column diazotroph community composition, and their correspondence to the increased PNsink flux and lower d15N of the PNsink 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 leave 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 PNsink 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, and we will work to clarify this in a revised manuscript. 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 PNsusp 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 PNsusp and DON concentrations did not change during P1), we assume that diazotrophs had to be part of the biofilm community to support the 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., who establish phytoplankton taxonomy and verify the presence of diazotrophs growing in surface waters were also present in the sediment traps.*

*It is also worth considering the timing of the increase in water column N2 fixation rates as measured by Berthelot et al., who show rates increase at the end of P1/early P2, which is when all the other water column parameters also change, and when N2 fixation becomes evident in the PNsink flux. Given the high degree of correlation between the N added to the water column by water column N2 fixation rate measurements and the accumulation of low-d15N N in the sinking flux, we feel it is fair to conclude that the primary fate of newly fixed N by diazotrophs living in the water column (and not by the diazotrophs in the biofilm) was to leave via the sinking flux, and not to be assimilated by*

the biofilms.

### Specific Comments

**Page 19910 Lines 16 – Page 19911 Lines 12: Which parameter (TN, PN<sub>susp</sub>, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, 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.**

*All of the samples were collected at the same time and by the same methods (as described in Berthelot et al., 2015). We were given bulk water samples to measure TN concentration and TN d15N; from these measurements we subtracted the measurements of DIN and suspended PN (where our French colleagues measured PN<sub>susp</sub> concentration by filtering bulk water samples through a 0.7 μm GF/F). We expect that the measurement contributing the error in mass-balance calculations of DON concentrations was the TN measurement, which is notoriously difficult and associated with relatively poor precision compared to measurements of PN<sub>susp</sub>, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup>. We also emphasize here, as we did in the text, that our DON concentration measurements only disagree in two samples – the rest of the time they agree remarkably well within the precision of the measurement with those reported by Berthelot et al., 2015. We also note here as we did in the text that while Berthelot observed a decrease in DON concentration at the end of P2, they did not see a corresponding decrease in DOC concentration. Given that marine DOM has a C:N ratio of 10-14, it is unclear how a 1 μM decrease in DON could not be accompanied by a ≥10 μM drawdown of DOC in the same samples.*

**Page 19911 Line 21–23: What was the N source for the increased PN<sub>susp</sub> concentration? While the authors concluded that DDN was not the N source for the increased PN<sub>susp</sub> 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<sub>susp</sub>, but it would not explain the increased PN<sub>susp</sub> concentration in the mesocosms, because the water in the mesocosms was depleted in nitrate.**

*Please see our above replies to Rev. #2. As we describe in section 4.1, the concentration of PN<sub>susp</sub> increases in concert with the increase in carbon and N<sub>2</sub> fixation rates documented by Berthelot et al. (2015). Thus, we expect that the PN<sub>susp</sub> 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 d15N budget to deduce the source of the N fueling export during this time period; presumably the source of N fueling the sinking flux is closely related to the source of N fueling the C fixation at the same time. In section 4.1 (pages 15-17) we interpret the results of the d15N budget for each time period and describe how, even though NO<sub>3</sub> is effectively completely consumed in the water column throughout the experiment, the isotopic signature of the subsurface NO<sub>3</sub> is apparent in the d15N of the sinking flux during P0, when we expect that large phytoplankton that assimilated NO<sub>3</sub> prior to the initiation of the experiment rapidly sank out of the non-turbid surface waters; during P1, we expect that the majority of the sinking flux is comprised of accumulated PN<sub>susp</sub> material, which has a d15N identical to that of the PN<sub>sink</sub> flux during P1; and during P2, when the sinking flux is supported by N<sub>2</sub> fixation and PN<sub>susp</sub> material.*

*In section 4.1 we also interpret the absolute value of the  $d_{15}N$  of  $PN_{\text{susp}}$ , which is ~3 per mil throughout the course of the experiment, and does not vary significantly from the  $PN_{\text{susp}}$   $d_{15}N$  measured outside the mesocosms in the lagoon (Figure 1d). A  $d_{15}N$  of  $PN_{\text{susp}}$  of ~3 per mil 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  $PN_{\text{susp}}$   $d_{15}N$  in this region (see discussion on p. 13 last paragraph through p. 14 first paragraph). We also expect that the  $d_{15}N$  of regional subsurface  $NO_3$ , 6.5 per mil, will produce  $PN_{\text{susp}}$  with a  $d_{15}N$  that is relatively high (see first paragraph of p. 14). Thus, a 3 per mil  $d_{15}N$  for  $PN_{\text{susp}}$  reflects, to some degree, the incorporation of subsurface  $NO_3$  with a  $d_{15}N$  of ~6.5 per mil. We do not, however, mean to suggest that the growth of  $PN_{\text{susp}}$  can be attributed to  $NO_3^-$  consumption since, as the Reviewer points out, there was no detectable  $NO_3^-$  in the mesocosm waters at any point during the experiment. We will ensure that this is clear in the revised version of the manuscript.*

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

*We appreciate the Reviewer's feedback and would be happy to move this Figure to Supplementary materials.*