

Response to Anonymous Referee #2

We thank Anonymous Referee #2 for the time and effort devoted to the review of the manuscript. Below, we reproduce the reviewer's comments and address their concerns point by point. The reviewer's comments are copied below in regular font with our responses in red. Manuscript changes are shown with additions in bold, deletions in strikethrough.

In this manuscript Caffin et al. examine transfer of diazotroph-derived (DDN) through the foodweb using ^{15}N stable isotope probing, comparing sites dominated by *Trichodesmium* with a site dominated by UCYN-B as the dominant diazotroph. They find that over 48h in the UCYN-B dominated station, no DDN was detectable in the dissolved pool, whereas a significant fraction was detectable in the *Trichodesmium* stations. They further characterize DDN to different microbial and zooplankton groups, and find differences between the stations. These results have major ecological implications for our understanding of DDN fate. Overall, I thoroughly enjoyed the manuscript, and highly recommend it for publication. I do have a few general questions and suggestions regarding the interpretation of the results and the context those results are put in. I recognize that putting these results in context of the other research done on the same cruise is difficult to carve out one piece to focus on, but I think the manuscript could use some focusing.

- Regarding whether *Tricho* releases recalcitrant N and UCYN-B releases labile N, I'm not sure the data really tells us this. It might mostly be a matter of semantics, and how you define labile and recalcitrant. But for me those terms imply different molecules released by the diazotrophs. From the data I don't think we can rule out that *Tricho* and UCYN-B release the exact same molecules of N, but because of the difference in both the amount of N released and the composition and metabolic state of the resident community, you see different DDN transfer and efficiency. In fact, I think it's interesting, although maybe expected, that you see higher efficiency in the ultra-oligotrophic location, implying that that maybe that community have higher affinity responses and uptake relative to the resident community in the *Tricho* stations. *Prochlorococcus*, for example, is likely to be better at high affinity uptake than *Synechococcus* because of its smaller surface area to volume ratio and adaptation to oligotrophic environments. Maybe this knowledge could help us predict, by knowing community composition and amount of N fixed, how efficient DDN transfer will be?

We agree with the proposition of reviewer #2, that the differences that we observed in DDN release and transfer between the stations is probably the result of contrasted planktonic communities having different affinities due to the trophic state of the station. We have thus taken account to this proposition and discussed this point in the section '4.1 DDN release to the dissolved pool' which is now presented as one of the hypotheses explaining the discrepancy between the two diazotrophs:

*"The quantity and quality of N released by diazotrophs to the dissolved pool during N_2 fixation potentially plays a key role in shaping the planktonic and microbial food webs. In this study, *Trichodesmium* released 14 ± 4 % to 40 ± 57 % of the newly fixed N into the dissolved pool, which is in agreement with values reported in the literature for field studies (Mulholland, 2007; Bonnet et al., 2016a). DON accounted for ~95 % of the DDN released by *Trichodesmium* (Fig. 2), ~~which is in agreement~~ accordingly with contributions measured in culture (80 - 90 %; Berthelot et al., 2015) and in the field (Berthelot et al., 2016). The low contribution of NH_4^+ to the DDN release does not mean that it was not released, but is likely the results of immediate consumption by surrounding plankton, **which shows a great affinity for NH_4^+** . ~~as NH_4^+ is known to be the preferred N source for marine plankton. On the opposite~~ Similarly, part of the DON released by *Trichodesmium* was probably*

uptaken by heterotrophic and mixotrophic plankton (Bronk et al., 2007) **but a significant fraction was** ~~and part was~~ likely refractory (not easily available for organisms) **leading to,** ~~explaining~~ the observed accumulation in the dissolved pool. If not refractory, the DDN would likely have been immediately assimilated as the region where these experiments were performed are strongly limited by N availability (Van Wambeke et al., 2008; this issue; Bonnet et al., 2008).

In the E1 experiment, we noticed a large variability of N_2 fixation and DDN release rates among the three replicates, which explains the high standard deviations (Fig. 2): two replicates exhibited net N_2 fixation rates $\sim 25\text{--}30 \text{ nmol N L}^{-1} 48 \text{ h}^{-1}$ and DDN release rates $\sim 7\text{--}10 \text{ nmol N L}^{-1} 48 \text{ h}^{-1}$, whereas in the third replicate, the DDN release ($\sim 24 \text{ nmol N L}^{-1} 48 \text{ h}^{-1}$) exceeded net N_2 fixation rates ($5 \text{ nmol N L}^{-1} 48 \text{ h}^{-1}$). This can be attributed to the decline of *Trichodesmium* in this replicate as we counted much more degraded trichomes in the third replicate. This suggests that decaying *Trichodesmium* release DDN more efficiently than healthy *Trichodesmium*, which has already been observed by Bonnet et al. (2016a). This may also explain why the DDN transfer to non-diazotrophic plankton was slightly higher in E1 ($10 \pm 2 \%$) than in E2 ($7 \pm 1 \%$), despite both stations were dominated by *Trichodesmium*.

Conversely to E1 and E2, the DDN released by UCYN-B (E3), was not quantifiable in our study. However, significant DDN transfer into non-diazotrophic plankton was detected ($15 \pm 3 \%$ of the total fixed N, Fig. 4), suggesting that the DDN released to the dissolved pool is likely immediately transferred to surrounding communities. ~~Contrary to E1 and E2, DDN did not accumulate in the dissolved pool, suggesting either DDN is released by UCYN but is more labile than DDN released by Trichodesmium, or suggesting that UCYN only release NH_4^+ (which is immediately uptaken and thus does not accumulate as in Trichodesmium experiments).~~ To our knowledge, this is the first report of DDN release in the field in the presence of a diazotroph community dominated by UCYN-B. Bonnet et al., (2016b) report low release from UCYN-C in coastal waters of the WTSP ($16 \pm 6 \%$ of total N_2 fixation) compared to *Trichodesmium* ($13 \pm 2 \%$ to $48 \pm 5 \%$; Bonnet et al., 2016b). This seems to indicate that the DDN from UCYN is generally lower than the DDN from *Trichodesmium*. Several hypotheses may explain the differences observed between *Trichodesmium* and UCYN. ~~i) as stated above, the DDN compounds released by from UCYN may be more bio-available than the DDN from released by Trichodesmium, limiting its accumulation. therefore it does not accumulate in the dissolved pool~~ **The lack of accumulation in E3 could also be due to the more severe N limitation of planktonic communities in the ultra-oligotrophic waters as compared to MA waters (Van Wambeke, this issue), and to the nature of the resident community. *Prochlorococcus* was dominating the planktonic community at LD C (E3) and is known to have a high affinity to its small surface to volume ratio (Partensky et al., 1999). ii) the PCD causing *Trichodesmium* bloom demise can also be involved in the relatively high enhance the DDN release and accumulation during *Trichodesmium* dominated experiments (Bar-Zeev et al., 2013). iii) Exogenous factors, such as viral lyses (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992b; Vincent et al., 2007) are also suspected to enhance the DDN release. These factors were found to exert a higher pressure in the the E3 experiment (dominance of UCYN-B) was performed in the ultra-oligotrophic waters of the GY where exogenous factors such as viral lyses (Fuhrman, 1999) and sloppy feeding (O'Neil and Roman, 1992b; Vincent et al., 2007) (which usually enhance N release) are minimal compared to MA waters where the *Trichodesmium* dominated compared to ultra-oligotrophic waters experiments were performed (Bock et al., this issue), where UCYN-B dominated. Finally, part of the discrepancy might be due to a methodological artefact: different sampling procedures between E1 and E2 (pump) and E3 (Niskin bottles) as the pump is suspected to induce mechanical stress to the cells which may have potentially affected the DDN release. Lastly, the DDN release measured here**

~~for UCYN-B is close to the one measured in cultures ($1.0 \pm 0.3\%$ to $1.3 \pm 0.2\%$, Benavides et al., 2013; Berthelot et al., 2015), where the exogenous factors are reduced, which would plead for hypothesis ii)...~~

As stated by reviewer #2, this knowledge of the affinity responses of surrounding organisms, by knowing community composition and amount of N fixed, could help us to predict how efficient DDN transfer will be. We agree with this comment and we encourage the scientific community to perform further studies on the point to help understanding and prediction the DDN transfer efficiency.

- One of the points that the authors emphasize is novel is that this is the first open ocean study. But I am not getting the full context for moving to the open ocean-what do the authors expect will be different, other than diazotroph identity? If this is the focus, it would be nice to include an expectation in the introduction-do they expect the open ocean DDN transfer to be different from the other studies of coastal or mesocosms performed before by this group? Or the same? For example, P.4 line 15-what was expected, different or similar to what found for coastal? Also P.4 lines 25-27.

Our group performed similar studies to understand this DDN transfer in coastal water of the WTSP and measured specific transfer rates to the surrounding planktonic communities, but this study provides the first observation of these processes in the open ocean. Previous studies had shown differences of N release between culture and coastal field experiment suggesting a strong influence of the environment on the DDN release. We thus expected to see differences between coastal and open ocean waters in term of release and subsequently in term of transfer. We understand that reviewer #2 would like to see these expectations in the introduction section, thus we have modified the text in the following way: ***“The differences of DDN release and transfer rates observed between the different field experiments and the different diazotrophs suggest that these processes strongly depend on the physiological state of diazotrophs and the environment. Yet, To-date the transfer of DDN to different groups of plankton from different diazotroph (Trichodesmium vs. UCYN) in the open ocean, where most of global marine N_2 fixation takes place, has never been investigated.”***

Then, I think these experiments help give us a context to predict DDN transfer through the food web, so I would like some more discussion in that context at the end: i.e. Will we need to know both diazotroph identity and nutrient conditions to predict DDN transfer? Or other factors? In some ways focusing on "first time in the open ocean" might actually even sell the results a little bit short-is this maybe the first full food web study in this manner as well?

We agree with the fact that this study gives us a first estimate of the magnitude of the DDN transfer through the food web in the open ocean. Our long-term goal would be to be able to give solid parametrization for models to predict DDN transfer through the food web. This work has been initiated using a 1-D vertical biogeochemical mechanistic model (Gimenez et al., 2016, https://www.biogeosciences.net/special_issue193.html). However, we think that it might be an overkill to say that we could be able to predict the DDN transfer thanks to our measurements in coupled physical-biogeochemical models. More studies should be performed to have a wider understanding of the processes, in particular the effect of physical processes (not taken into account here), the effect of the physiological state of diazotrophs, the trophic status of the water mass, the plankton community composition, etc...

I also have some specific questions and suggestions:

P.8 lines 1-8-Flow sorting before analysis—I would like more information on this method included, when I looked up the referenced Bonnet et al, 2016b, it didn't include flow sorting-is there another paper with these details? If not, more information should be provided in this manuscript in order to verify that you had what was expected on the filter, and the NanoSIMS analysis was on the expected cells.

The good reference is Bonnet et al., 2016a. This is a mistake that has been corrected in the new version of the manuscript

Information and protocol about flow sorting are available in the supporting information file of Bonnet et al. (2016a) in the section 'Auto- and heterotrophic picoplankton analysis and sorting by flow cytometry' which is available on

<https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.1002/lno.10300> .

We understand that it was not clear in the text, thus we have changed the reference 'Bonnet et al. (2016b)' by 'Bonnet et al. (2016a, **Supp. Info.**)'.

For example, was there any correlated imaging of the filters (i.e. with fluorescence or SEM) to verify and map the cells other than the CCD camera on the NanoSIMS? It would be good to include some more raw data in supplemental with some examples of the NanoSIMS ion and secondary electron images for each group with examples of how ROIs were drawn. Particularly, it seems like the bacteria may have come through in the other sorts, was that a problem and were those identifiable in the NanoSIMS? *Prochlorococcus* and bacteria for example, might would look similar in the CCD camera?

We agree with the reviewer that *Prochlorococcus* and heterotrophic bacteria look very similar in the CCD camera of the nanoSIMS but also on a SEM. This is why we use cell sorting to discriminate the different groups, using in autofluorescence for photosynthetic cells and SYBR green staining for heterotrophic bacteria. In the new version of the manuscript, we added the figure below in the Supp. Info. showing representative cytograms where populations appeared clearly and were well clustered. This argues for a potentially low level of cross contamination in our samples, even though it cannot be excluded.

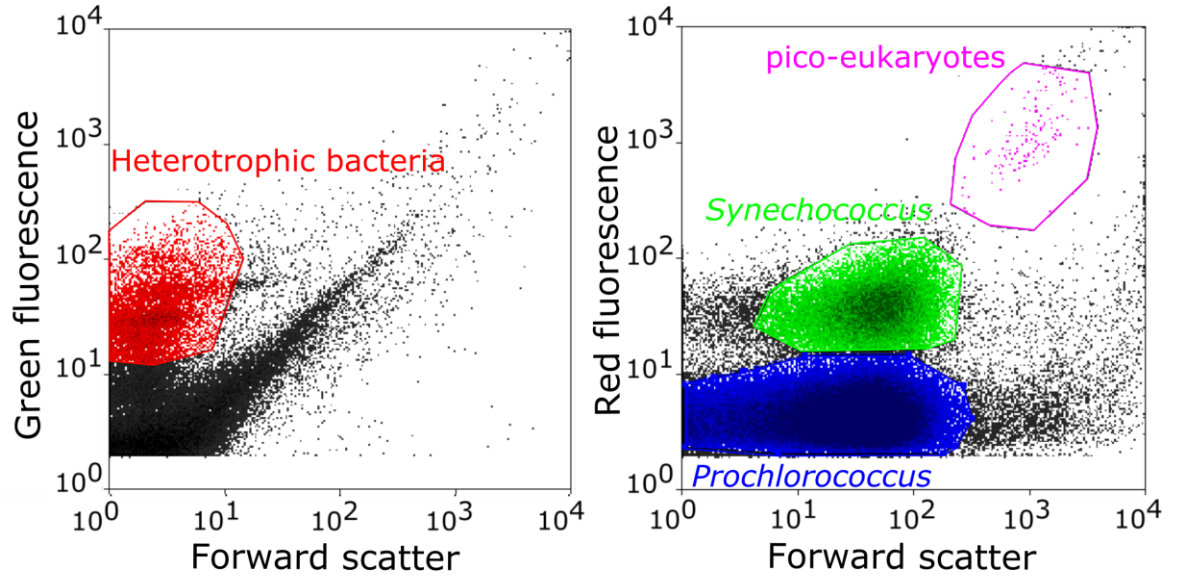


Figure 3: Clustering of planktonic communities by flow cytometry on green fluorescence vs. forward scatter cytograms (left) and red fluorescence vs. forward scatter (right): heterotrophic bacteria (red), *Prochlorococcus* (blue), *Synechococcus* (green), and the pico-eukaryotes (pink)

We agree that it is not clear that how the ROIs were drawn. To clarify this point we have modified the Figure 1 and have added some ROIs on the corresponding images, in the following way.

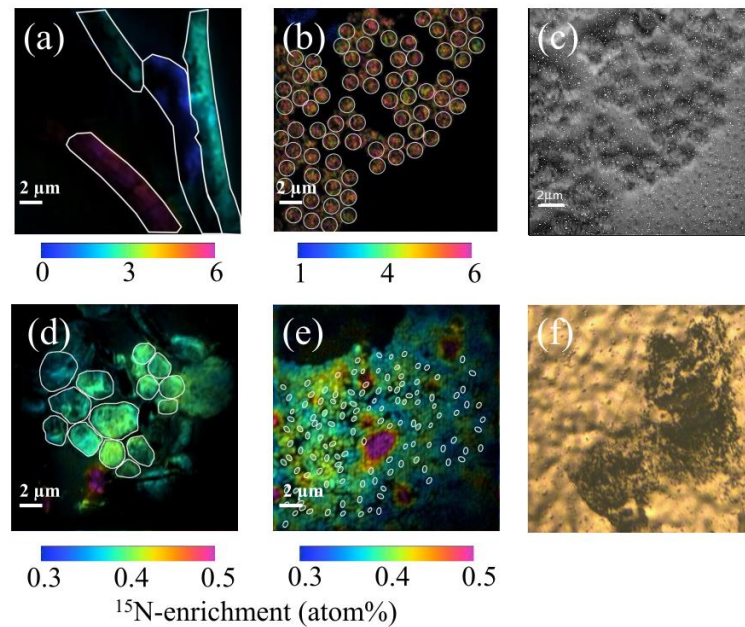


Figure 1: NanoSIMS images showing the ^{15}N -enrichment (a,b,d,e) after 48 h of incubation in the presence of $^{15}\text{N}_2$ for *Trichodesmium* (a), UCYN-B (b), Nano-Eukaryotes (d) and *Synechococcus* (e). The ROIs are represented in white line.

NanoSIMS images showing the secondary electrons channel of UCYN (e) (c) and optical camera image of *Prochlorococcus* spotted on the filter before NanoSIMS analyses (f).

In addition we have added complementary nanoSIMS images with ROIs in the Figure 2 of the Supp Info, in the following way:

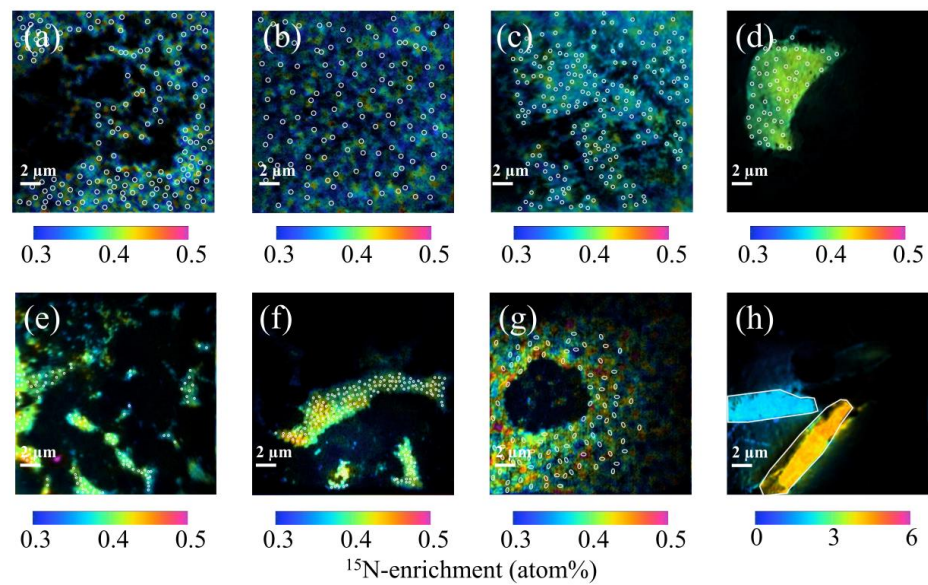


Figure 2: NanoSIMS images showing the ¹⁵N-enrichment after 48 h of incubation in the presence of ¹⁵N₂ for *Prochlorococcus* (a,b), pico-eukaryotes (c,d), heterotrophic bacteria (e,f), *Synechococcus* (g), and *Trichodesmium* (h). The ROIs are represented in white line.

P.8 line 24-25, a table of ROIs per sample in supp would help, i.e. n for each analysis

We have added the following table in the Supp. Info. file.

Table 1: Number of ROIs analyzed for diazotrophs (*Trichodesmium* in E1 and E2, UCYN-B in E3), *Synechococcus*, *Prochlorococcus*, bacteria, diatoms, pico-eukaryotes and nano-eukaryotes, for E1, E2 and E3.

Experiment	diazotrophs	<i>Synechococcus</i>	<i>Prochlorococcus</i>	bacteria	diatoms	pico-euk.	nano-euk.
E1	32	87	32	200	8	111	60
E2	25	156	213	85	33	200	29
E3	192	50	115	70	0	70	0

p.8 line 32-UCYN-B cell diameters from NS images-interesting and not typical-an example in supp would help, was it correlated with other imaging? (i.e. fluor or SEM?).

The UCYN-B cell diameters from nanoSIMS images were $2.3 \pm 0.3 \mu\text{m}$. Also, UCYN-B cell diameters, measured using a Zeiss Axio Observer epifluorescence microscope were $2.7 \pm 0.3 \mu\text{m}$. This has been specified in the ‘2.7 Cell-specific N content and DDN transfer calculations’ section in the following way: “For UCYN-B, cells diameters were directly measured on the nanoSIMS images and further confirmed on microscopic images.”

P.11 line 5-20 3.3-I couldn’t find the information on the T0 values, how many and how analyzed? Everything is relative to the T0 but unclear what the n is.

The T0 values were measured on 5 cells and analyzed on the nanoSIMS, with the same protocol as for all the measurements. T0 here are within the range of T0 values reported by Bonnet et al. (2016a; 2016b) et Berthelot et al. (2016). As the sentence is unclear, we have changed the text in the following way: “...compared to T0 samples (0.371 ± 0.005 atom%, $n=5$), ...”

P.11 Line 15-Sentence "For the three experiments.." -I don't get what this statement means and not sure how it relates to Figure 3

This statement relates to Figure 4 and not Figure 3. We apologize for this mistake which has been corrected in the new version of the manuscript.

P.11 line 26-Again, like the T0, how was the prelabelled plankton measured? NanoSIMS or IRMS? what is the n?

The T0 of the prelabelled plankton were measured by EA-IRMS for the zooplankton experiments. We performed triplicates for each experiment. We have added a new sentence in the Method section to clarify: “...stop to increase by fixing $^{15}\text{N}_2$. **The initial ^{15}N enrichment of the ^{15}N pre-labelled plankton was analyzed in triplicates by EA-IRMS. Meanwhile, zooplankton was collected...**”. In addition, we have added the number of measurements corresponding to the T0 in the Results section in the following way: “Before incubation with zooplankton, the isotopic enrichment of the ^{15}N pre-labelled plankton averaged 1.035 ± 0.091 atom% ($n=9$) in the experiments Zoo-1, Zoo-2 and Zoo-3 (dominated by *Trichodesmium*) and 0.385 ± 0.005 atom% ($n=3$) in the experiment Zoo-4 (dominated by UCYN-B).”

P.12 lines 3-4-when the error is bigger than the reported number, I worry this becomes meaningless to report-how else can the data be described?

We acknowledge that the reported error is high, potentially due to the *Trichodesmium* decay in one of the replicate bottle, as mentioned in the discussion section. However, we are confident that the results are still meaningful in regard of the transfer efficiency between diazotroph and non-diazotroph plankton.

P12-Because averaging to T0, lose some information about total N-fixation. Maybe Zoo4 is only different because lower total enrichment?

We averaged T0 of Zoo-1, Zoo-2 and Zoo-3 because there were not significantly different. T0 of Zoo-4 was not averaged with the others experiments because the ^{15}N enrichment was lower as stated by Reviewer #2. As it is unclear, we have modified the text in the following way: “Before incubation with zooplankton, the isotopic enrichment of the ^{15}N pre-labelled plankton **was not significantly different in the experiments Zoo-1, Zoo-2 and Zoo-3 (dominated by *Trichodesmium*) averaging 1.035 ± 0.091 atom% ($n=9$). ~~in the experiments Zoo-1, Zoo-2 and Zoo-3 (dominated by *Trichodesmium*) and~~ The isotopic enrichment was lower in the experiment Zoo-4 (dominated by UCYN-B) averaging 0.385 ± 0.005 atom% ($n=3$). ~~in the experiment Zoo-4 (dominated by UCYN-B).~~ After 24 h of incubation with zooplankton, the ^{15}N enrichment of the ^{15}N pre-labelled plankton **decreased down to 0.431 ± 0.014 atom% on average in Zoo-1, Zoo-2 and Zoo-3, and down to 0.372 ± 0.010 atom% in Zoo-4.**”**

P13 line 4-5: but the DDN in the dissolved pool doesn't show release by UCYN-B, the results do imply release because you see DDN transfer but then shouldn't this statement be in the next section?

The DDN measured in the dissolved pool can only come from UCYN-B, and thus show that DDN was released by UCYN-B. However, Reviewer #2 is true when mentioning that seeing DDN transfer implies that DDN was previously released. Thus, we agree that this sentence is suitable in both sections but we think that it makes more sense in this section.

P15 Line 29-Not clear what that 50-95

Here, we mean that diazotrophs contributed from 50 to 95 % to zooplankton biomass in the MA waters. As it is unclear, we have modified the text in the following way: *“This result is in agreement with the ones of Carlotti et al., (this issue) base on natural N isotopic measurements, who revealed Carlotti et al. (this issue) results based on ¹⁵N isotopic data showing that ~50-95 % and ~10-40 % of the zooplankton N content of the zooplankton originates from N₂ fixation in the MA waters and ~10-40 % in the GY waters, respectively.”*

P16 line 8 "The DDN transfer efficiency was more important..." not sure what is meant by "more important" more important how?

By ‘important’ we mean ‘higher’. The sentence has been clarified in the following way *“The DDN transfer efficiency was to non-diazotrophic plankton was higher more important to non-diazotrophic phytoplankton and bacteria ($\sim 15 \pm 3$ %) and zooplankton ($\sim 28 \pm 8$ %) when UCYN-B dominated the diazotroph community than when Trichodesmium dominated ($\sim 8 \pm 2$ % and 7 ± 6 % of transfer to phytoplankton and bacteria, and zooplankton, respectively).”*

P.16-last paragraph is a bit confusing and tangential to me. This is just a suggestion, but I would prefer more of a wrap-up on what this data presented means in the context of DDN transfer prediction, e.g. does this help to reconcile the differences between the culture and field studies, or coastal vs. open ocean? What are the implications from the results for predicting transfer through the food web in other areas?

To clarify the conclusion section, we have modified the text in the following way:

“5. Conclusion and ecological impact of N₂ fixation in the WTSP

N₂ fixation acts as a natural N fertilizer in the ocean, releasing DDN in the dissolved pool, which is available for surrounding marine organisms. To our knowledge, this study provides the first quantification of DDN transfer to phytoplankton, bacteria and zooplankton communities in open ocean waters. The main interest of this study was to compare DDN transfer and release under contrasting N₂ fixation activity and diversity.

*Here, we reveal that Trichodesmium released more DDN than UCYN-B, but a significant part of the DDN released by Trichodesmium **accumulated in the dissolved pool was refractory**, while the DDN released by UCYN-B was **more bio-available (NH₄⁺ and labile DON) and likely** immediately assimilated by the surrounding plankton communities. The DDN transfer efficiency **was to non-diazotrophic plankton was higher more important to non-diazotrophic phytoplankton and bacteria ($\sim 15 \pm 3$ %) and zooplankton ($\sim 28 \pm 8$ %) when UCYN-B dominated the diazotroph community than when Trichodesmium dominated ($\sim 8 \pm 2$ % and 7 ± 6 % of transfer to phytoplankton and bacteria, and zooplankton, respectively).** In the open ocean, most of the N₂ fixation is performed by Trichodesmium (Capone et al., 1997 Luo et al., 2012), thus on a global scale most of the DDN transfer can be attributed to Trichodesmium, moreover in the MA waters where Trichodesmium dominated diazotroph community. The regions where UCYN are the dominant diazotrophs **generally** present lower*

N_2 fixation rates than the ones **where** of *Trichodesmium* **dominates**, but **UCYN provide a continuous source of DDN to surrounding plankton communities** ~~it is not negligible and may provide a continuous background of DDN to surrounding plankton communities.~~ The DDN was ~~preferentially~~ transferred to pico-plankton, which **dominated** ~~is the most abundant plankton community in the WTSP,~~ suggesting that N_2 fixation fueled the growth of biomass in the N-depleted environment. This is consistent with Caffin et al., (2018), who revealed that N_2 fixation provides ~~> more than~~ 90 % of the new N to the photic layer of the **WTSP** ~~subsequently transformed into bio-available through DDN release, and indicated that N_2 fixation contributed to 15-21 % of the PP in the MA waters and ~4 % in the GY waters.~~ On a larger scale view, the simulation performed by Dutheil et al. (this issue) predicts that diazotrophs support a large part of PP (~15 %) in LNLC regions of the Pacific Ocean, comprising the WTSP.

Overall, this ~~study clearly~~ indicates that ~~in the WTSP the~~ N_2 fixation plays a key role on the marine biomass production, **the structure of** ~~subsequently on the planktonic food web associated,~~ and finally on the export of **organic matter** towards the deep ocean. The DDN can be exported to the deep ocean by different **pathways**: i) **the** direct export of diazotrophs, ii) **the** export of non-diazotrophs which benefited from the DDN ~~transfer,~~ and iii) **the** export of zooplankton which benefited from the DDN ~~transfer.~~ The direct export of **diazotrophs accounted for** ~~quantification in the WTSP, indicates a direct carbon export associated to diazotrophs of ~30 % of total C export at LD A (E1), 5 % at LD B (E2) and < 0.1 % at LD C (E3) (Caffin et al., 2018).~~ Using a $\delta^{15}N$ budget, Knapp et al., (This issue) found that 50-80 % of exported material was sustained by N_2 fixation (this includes both direct and indirect export of DDN). The low ^{15}N -enrichment of the particulate matter recovered in sediment trap deployed at LD A, LD B and LD C indicates that N_2 fixation significantly contributed to particulate export (Knapp et al., this issue), either by direct or indirect export, in the WTSP. Thus, N_2 fixation has ineluctably a key role on the biological carbon pump, as mentioned in Moutin et al. (this issue) who reveal a significant biological “soft tissue” carbon pump in the MA waters **almost exclusively** ~~almost exclusively~~ by N_2 fixation, and acting as a net sink ~~for~~ of atmospheric CO_2 **in the WTSP.”**

Figure 1: I think in the figure legend "secondary electrons channel of UCYN (e)" should be (c)? Also, does f correlate with anything? Is there a NanoSIMS image of *Prochlorococcus* cells?

In the figure legend "secondary electrons channel of UCYN (e)" has been corrected by “secondary electrons channel of UCYN (c)”. The image (f) does not correlate with any of the other images. NanoSIMS image of *Prochlorococcus* cells were added in the Supp Info as explained above.

Figure 4: The left pie charts numbers I think should correspond to P.11 lines 19-20 numbers-but they don't-how much N stays with the diazotrophs? Is it 50, 79 and 85

Reviewer #2 is right pie charts numbers did not corresponded to P.11 lines 19-20 numbers. This is a mistake that has been corrected in the new version of the manuscript. The right numbers are 50 ± 40 %, 79 ± 4 % and 85 ± 9 %.

Technical corrections:

P3 Line 15-16-this sentence is confusing to me, lower than what? In the field?

We agree that this sentence is confusing. The DDN release measured in culture studies is much lower than in field studies. We have clarified this sentence in the following way: “**The DDN release is generally much lower in (<5%) in monospecific cultures (Berthelot et al. 2015, Benavides et al. 2013) than in field experiment** ~~The DDN released to the dissolved pool measured by this direct approach is generally much lower in culture studies (<5 %) (Benavides et al., 2013a; Berthelot et al., 2016),~~ suggesting that external factors such as sloppy feeding and viral lysis have a strong influence on the DDN release by diazotrophs in field.”

P9 line 21-22 after "Plus an additional..." add "Zoo-2", if that is what that experiment is, confusing.

To avoid confusion we have had “Zoo-2” in the sentence, as recommended by Reviewer #2, in the following way: “...*plus an additional station (Zoo-2) located between LDA and LDB...*”