**Response to Anonymous Referee #1**

**We thank Anonymous Referee #1 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 blue.**

**We are responding to this review a long time after it was published on the *Biogeosciences Discussion* online forum because we had to submit the companion paper by Caffin et al. (this issue), cited in this article, before the closure of OUTPACE's special issue on December 31, 2017.**

## General comments:

In their study, Caffin *et al*. use a Lagrangian approach to determine N sources ($N_2$ fixation rates, $NO_3^-$ supply from vertical diffusion and N from atmospheric depositions) and sinks (particulate N export) at 3 stations in the Western South Tropical Ocean. They also measured primary production using $^{14}C$ tracer incubations. Their main findings are that 1) $N_2$ fixation is the major source of new N (>90%) at all stations, regardless of whether the diazotroph community was dominated by *Trichodesmium* or unicellular cyanobacteria, 2) carbon export relative to primary production is high in this region, and 3) the sum of N input in the photic zone exceeds particulate N export. Overall, this study is interesting and timely as this region has recently been identified as a $N_2$ fixation hotspot. However, I have some concerns. First, they report a low input from atmospheric depositions but did not consider atmospheric sources other than $NO_3^-$ and $NO_2^-$ (e.g., $NH_4^+$ DON, PON). They also measured relatively high $N_2$ fixation rates and I am wondering if they considered the possibility of a contamination of their $^{15}N$-$N_2$ stock with $^{15}N$-$NO_3^-$ and $NH_4^+$, which would artificially increase their $N_2$ fixation rates, as recently reported by Dabundo *et al*. (2014). Some of the references cited (this issue) were unavailable on the *Biogeosciences Discussions* online forum at the time of this review, making it impossible to evaluate these parts of the manuscript. I was also a bit confused regarding the novelty of their dataset: were the same $N_2$ fixation rates, qPCR or any other data collected at the same stations during the OUTPACE cruise already published in previous studies? The authors should make a clear distinction of the new data contributed by their study versus the data already published elsewhere in other manuscripts in the special issue.

**Reviewer #1 is right that we did not consider atmospheric sources other than $NO_3^-$ and $NO_2^-$. Our flux could be underestimated as it represents dry deposition and because gas and organic forms were not measured. At global scale, and depending on the location, organic nitrogen could represent up to 90 % of N atmospheric deposition (Kanakidou et al., 2012). Even if we double our estimated deposition flux, atmospheric deposition remained low (< 1.5 %) and consequently represented a minor contribution of the new N input. We are aware that Dabundo et al. (2014) report potential contamination of some commercial $^{15}N_2$ gas stocks with $^{15}N$-enriched $NH_4^+$, $NO_3^-$ and/or $NO_2^-$, and nitrous oxide ($N_2O$). To verify this, the Cambridge Isotopes batches that are routinely used by our team has been analyzed for potential contamination in Julie Granger and Richard Dabundo's lab, and this confirmed that the contamination of the $^{15}N_2$ gas stock was low: 1.4 x $10^{-8}$ mol of $^{15}NO_3^-$ per mol of $^{15}N_2$, and 1.1x$10^{-8}$ mol $NH_4^+$ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model described in Dabundo et al. (2014) indicates that our rates could only be overestimated by 0.01 to 0.12 %. We thus confirmed that the stock contamination issue did not affect the results reported here. We are aware that some of the references cited here (this issue) were unavailable on the *Biogeosciences Discussions* online forum at the time of this review, but most of them (except Bouruet-Aubertot et al., this issue) are available now. The objective**

**of the OUTPACE special issue is to provide a unique opportunity for a group of researchers to focus on the "Interactions between planktonic organisms and biogeochemical cycles across trophic and $N_2$ fixation gradients in the western tropical South Pacific Ocean". It is a multidisciplinary approach with a tight time schedule, and with the main aim of sharing the data in order to provide best study in a relatively short time of a new (exceptional) set of data. The data may be used several times in different papers of the special issue focusing on different scientific questions. In this case, the method is given with all details only in one paper that is clearly referenced as the main one for the method.**

**Kanakidou, M., Duce, R. A., Prospero, J. M., Baker, A. R., Benitez-Nelson, C., Dentener, F. J., ... & Sarin, M. Atmospheric fluxes of organic N and P to the global ocean. Global Biogeochemical Cycles, 26(3), 2017**

## Specific comments:

### Title

The title is a bit long and not focused on the main point of the study. I suggest changing for: "$N_2$ fixation as the dominant new N source in the Western Tropical South Pacific Ocean (OUTPACE cruise)"

**We have chosen another title containing the Lagrangian term, but because two reviewers suggested the same change, we have changed the title in accordance with this suggestion.**

### Introduction

Page 2, line 15: Knapp *et al.* (2008) and Bourbonnais *et al.* (2009) also observed a low $\delta_{15}N$ of $NO_3^-$ (relative to $\delta_{18}O$-$NO_3^-$) in surface waters in the western and eastern subtropical Atlantic Ocean, supporting the role of $N_2$-fixers in these regions.

**We have added these missing references in accordance with this suggestion**

Page 4, lines 6: What factors influence the distribution of *Trichodesmium* or UCYN? I believe temperature is an important factor (see Moisander *et al.*, 2010). This point should be discussed a bit more.

**We are aware that the activity and distribution of diazotrophs have been hypothesized to be controlled by several environmental variables in the open ocean, such as light (Fu and Bell, 2003; Breitbarth et *al.*, 2008; Levitan et *al.*, 2010), temperature (Capone et *al.*, 1997; Staal et *al.*, 2003; Breitbarth et *al.*, 2007; Moisander et *al.*, 2010) or nutrient availability (Van den Broeck et *al.*, 2004; Moutin et *al.*, 2005; Mills et *al.*, 2004; Ho, 2013). However, we did not focus on this in our study. In the context of the OUTPACE project, Bonnet et al. (this issue) have shown a correlation between diazotrophs abundance and temperature. A partition of niches between *Trichodesmium* and UCYN has been observed in this region (Moisander et al., 2010; Bonnet et al., 2015). Furthermore, this interesting scientific question has been studied in the WTSP by Stenegren et al. (this issue), who mentioned that the correlations between the different diazotroph communities and the environmental conditions observed in the WTSP were not always consistent with the meta-analysis of the external datasets.**

**Experimental procedures**

Page 5, line 19: They only considered $NO_3^-$ and $NO_2^-$ when quantifying N atmospheric depositions. They should also consider $NH_4^+$ or organic nitrogen (particulate or dissolved). For instance, Cornell *et al*. (1995) estimated that organic nitrogen was a significant component of atmospheric N depositions even in remove marine regions.

> **We understand and accept this suggestion that $NH_4^+$ and organic N (particulate and dissolved) should also be considered when quantifying atmospheric deposition. Our quantification of the atmospheric deposition could be underestimated as it only represents dry deposition, and gas and organic forms were not measured. At global scale, and depending on the location, organic nitrogen could represent up to 90 % of N atmospheric deposition (Kanakidou et al., 2012). Even if we double our estimated deposition flux, atmospheric deposition remained low (< 1.5 %) and consequently represented a minor contribution of the new N input.**

Page 5, line 26: Did they check their commercial $^{15}N$ Eurisotop gas for possible contamination with $^{15}N$-labeled dissolved inorganic nitrogen ($NO_3^-$, $NO_2^-$ and $NH_4^+$)? Microbial assimilation of contaminant $^{15}N$ labeled dissolved inorganic nitrogen would artificially increase $N_2$ fixation rates. Dabundo *et al*. (2014) recently reported significant concentrations of $^{15}N$ contaminants in $^{15}N$-labelled $N_2$ gas supplied by Sigma-Aldrich and Campro Scientific.

> **We are aware that Dabundo et al. (2014) reports potential contamination of some commercial $^{15}N_2$ gas stocks with $^{15}N$-enriched $NH_4^+$, $NO_3^-$ and/or $NO_2^-$, and nitrous oxide ($N_2O$). In their study, Dabundo et al. (2014) analysed various brands of $^{15}N_2$ (Sigma, Cambridge Isotopes, Campro Scientific) and found that the Cambridge Isotopes brand (i.e., the one used in these studies) contained low concentrations of $^{15}N$ contaminants, and the potential overestimated $N_2$ fixation rates modeled using this contamination level would range from undetectable to 0.02 nmol N $L^{-1}$ $d^{-1}$. The rates measured in this study were on average ~10 nmol N $L^{-1}$ $d^{-1}$, suggesting that stock contamination would be too low to affect the results reported here.**
>
> **To verify this, the Cambridge Isotopes batches that are routinely used by our team has been analyzed for potential contamination in Julie Granger and Richard Dabundo's lab, and this confirmed that the contamination of the $^{15}N_2$ gas stock was low: 1.4 x $10^{-8}$ mol of $^{15}NO_3^-$ per mol of $^{15}N_2$, and 1.1x$10^{-8}$ mol $NH_4^+$ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model described in Dabundo et al. (2014) indicates that our rates could only be overestimated by 0.01 to 0.12 %. We thus confirmed that the stock contamination issue did not affect the results reported here.**

Page 6, lines 5-7: A better way to assess whether equilibration was complete would be to try different treatments in triplicate, i.e., shake the bottles for different times and intensity before the *in-situ* incubations.

> **We agree that it would be a useful complementary study to try different treatments in triplicate in order to better assess whether the equilibration was complete. In the present study, we performed MIMS analyses to quantify the final $^{15}N$-enrichment in the $N_2$ pool and avoid any error due to an insufficient equilibration process.**

Page 6, line 6: Add "incomplete" before equilibration.

**This has been corrected in the new version**

Page 6, lines 7-10: I assume the 12 mL subsample was collected without contact with the atmosphere?

**The 12 mL subsample was collected rapidly from the 4.5 L to the Exetainers with contact with the atmosphere. We assume that the sampling was rapid enough to avoid sea-air exchanges that can affect the $^{15}N_2$ enrichment of the sample. In any case, this is more precise than using the theoretical $^{15}N_2$ enrichment, as revealed in Bonnet et al. (this issue).**

Page 6, lines 16-24: What is the detection limit for their $N_2$ fixation rates?

**The minimum quantifiable rate calculated using standard propagation of errors via the observed variability between replicate samples, measured according to Gradoville et al. (2017), was 0.035 nmol N $L^{-1}$ $d^{-1}$. This has been specified in the new version of the paper.**

**Gradoville, M. R., D. Bombar, B. C. Crump, R. M. Letelier, J. P. Zehr and A. E. White Diversity and activity of nitrogen fixing communities across ocean basins. Limnol. Oceanogr. 62: 1895-1909, 2017**

Page 7, line 21: Define UCYN-B, UCYN-A1, het-1 and het-2. Which bacteria are represented by these different groups?

**The sentence has been changed to "Diazotroph abundance for *Trichodesmium* spp., UCYN-B (*Crocosphaera watsonii*), UCYN-A1 (*Candidatus Atelocyanobacterium thalassa*), het-1 (*Richelia intracellularis* and *Rhizosolenia*), and het-2 (*Richelia intracellularis* and *Hemiaulus*) were quantified by qPCR analyses on the nifH gene using previously described oligonucleotides and assays (Foster et al. 2007; Church et al. 2005)."**

Page 8, line 1: Were samples with qPCR reaction efficiency below 95% reported? Why not repeat analysis for these samples?

**The samples with qPCR reaction efficiency below 95% were excluded. As the amount of water was restricted for each parameter measurement, we were not able to repeat the analysis for these samples.**

## Results

Page 9, lines 24 to 27: How are these rates different from the one measured in Bonnet *et al*., 2017 (This issue). Are they the same rates as reported in Bonnet *et al*. (2017)? The Bonnet *et al.* paper was not yet available at the time of this review, making it impossible to effectively evaluate this part of the manuscript.

**We understand that it was impossible for Reviewer #1 to evaluate this part of the manuscript as the Bonnet et al. paper was not available at the time of the review. The Bonnet el al. paper is now available in the *Biogeosciences Discussion* online forum. Bonnet et al. (this issue) reports rates from on-deck incubation at 15 short duration stations (that were not included in this study), and those of the 3 LD stations (presented here). Thus, the Bonnet et al. dataset is different and presented in a totally different perspective.**

Page 11, line 11: Were *Trichodesmium* data for LD A (150 m) not available or below detection limit (as stated on page 10, line 32)?

**Trichodesmium data for LD A (150 m) were not available as the qPCR efficiency was below 95 %. This has been changed on page 10 line 32 to: "… sediment material (< QL at LD C 330 m and not available at LD A 150 m) and …"**

## Discussion

Page 11, lines 27-29: Perhaps atmospheric deposition measured during OUTPACE are low because they neglected contributions from organic nitrogen and $NH_4^+$. This possibility should be discussed (see my previous comment, page 5, line 19).

**We agree with this comment of Reviewer #1, thus we have discussed the possibility of underestimation of the atmospheric input in the new version of the manuscript as follows: "Extrapolated NOx deposition from the atmosphere during OUTPACE (range: 0.34 – 1.05 µmol $m^{-2}$ $d^{-1}$) were one order of magnitude lower than predicted with major uncertainties by global models that include wet and gas deposition for that region (Kanakidou et al. (2012). Our flux could be an underestimation as it represents dry deposition and gas and organic forms were not measured. At global scale and depending on the location, organic nitrogen could represent up to 90 % of N atmospheric deposition (Kanakidou et al., 2012). Even if we double our estimated deposition flux, atmospheric deposition remained low (< 1.5 %) and consequently represented a minor contribution of the new N input (Table 4)"**

Page 12, lines 24-27: Again, it would be relevant to check for possible contamination of their $^{15}N_2$ Eurisotop stock by $^{15}N$-labelled dissolved inorganic nitrogen (see Dabundo *et al*., 2014).

**As previously explained, to verify this, the Cambridge Isotopes batches that are routinely used by our team has been analyzed for potential contamination in Julie Granger and Richard Dabundo's lab and this confirmed that the contamination of the $^{15}N_2$ gas stock was low: 1.4 x $10^{-8}$ mol of $^{15}NO_3^-$ per mol of $^{15}N_2$, and 1.1x$10^{-8}$ mol $NH_4^+$ per mol of $^{15}N_2$. The application of this contamination level to our samples using the model described in Dabundo et al. (2014) indicates that our rates could only be overestimated by 0.01 to 0.12 %. We thus confirmed that the stock contamination issue did not affect the results reported here**

Page 14, line 22-23: The Berman-Frank paper was not submitted at the time of this review. Also, define PCD.

**The Berman-Frank paper has been replaced by Spungin et al. (This issue), available in the *Biogeosciences Discussion* online forum at this time.**

**PCD (Programmed cell death) has been defined in the new version of the paper.**

**Spungin, D., Belkin, N., Foster, R., Stenegren, M., Caputo, A., Pujo-Pay, M., Leblond, N., Dupouy, C., Bonnet, S., and Berman-Frank, I.: Programmed cell death in diazotrophs and the fate of organic matter in the Western Tropical South Pacific Ocean during the OUTPACE cruise, Biogeosciences Discuss., in review, 2018.**

Page 16, lines 1-6: This paragraph is not clear. Do they mean the dead and live "swimmers" zooplankton were not distinguishable? Rewrite accordingly.

**This paragraph has been rewritten to explain more clearly that the dead and live "swimmers" zooplankton were not distinguishable.**

## References

The following cited references were not accessible on the *Biogeosciences Discussion* online forum at the time of this review:

Berman-Frank *et al.* (This issue)
Bonnet *et al.* (This issue)
Bouruet-Aubertot *et al.* (This issue)
Caffin *et al.* (This issue)
Moutin *et al.* (This issue) – there is a Moutin *et al.* submitted but with a different title
Van Wambeke *et al.* (This issue)

**To date, the Bouruet-Aubertot et al. (this issue) paper is not available yet and the Berman-Frank et al. (this issue) paper was submitted as Spungin et al. (this issue); all the other references are available in the *Biogeosciences Discussion* online forum.**

## Tables

Table 5: Include contributions from atmospheric depositions in this table.

**In most of the studies presented in Table 5, the N input associated to atmospheric deposition was not quantified. Only in the Mediterranean Sea, which is strongly affected by dust deposition, the atmospheric deposition was measured. Atmospheric models and global tropospheric budgets were performed (Duce et al., 2008; Kanakidou et al., 2012) that can give us a global picture of atmospheric deposition, however to be consistent with our study we have decided not to include those data in Table 5. In addition, the main objective of this table is to compare the contribution of $N_2$ fixation as a new N input in different regions of the world Ocean.**

## Figures

Figure 3: Why PAR and DCM are decoupled at station LD B?

**The decoupling between PAR and DCM observed at LD B remains in significant chlorophyll a concentration observed at the surface that was stirred, deformed and transported by the mesocscale circulation, as explained in de Verneil et al. (this issue) who focused their study on the significant surface chlorophyll a bloom sampled at LD B.**

**de Verneil, A., Rousselet, L., Doglioli, A. M., Petrenko, A. A., and Moutin, T.: The fate of a southwest Pacific bloom: gauging the impact of submesoscale vs. mesoscale circulation on biological gradients in the subtropics, Biogeosciences, 14, 3471-3486, https://doi.org/10.5194/bg-14-3471-2017, 2017.**

## Technical considerations:

Review the manuscript for grammatical errors and typos. Here are a few examples:

Page 1, line 21: replace "Thanks to a Lagrangian…" for "Using a Lagrangian…".

**This has been corrected in the new version**

Page 1, line 34: replace “while there contribution…” for “while their contribution…”

**This has been corrected in the new version**

Page 9, line 13: replace for “… in-situ incubation method ranged from 0.0 – 19.3 nmol N $L^{-1}$ $d^{-1}$ …”

**This has been corrected in the new version**

Page 10, line 11: replace by “was strongly influenced by the vertical diffusion coefficient”.

**This has been corrected in the new version**

Page 11, line11: replace “LDA” by “LD A”.

**This has been corrected in the new version**

Page 11, line 21: replace “whatever” for “regardless of”, i.e., “… $N_2$ fixation was the major external source of N to the WTSP regardless of the degree of oligotrophy, …”

**This has been corrected in the new version**

Page 12, lines 22-3: change for “… $NO_3^-$ input by turbulence always represented a minor contribution to the N budget.”

**This has been corrected in the new version**

Page 13, line 16: replace for “… and a clear dominance of …”

**This has been corrected in the new version**

Page 14, lines 9-10: Add a period after “in oligotrophic open ocean regions.” Start a new sentence with “To date, few qPCR *nifH* data from sediment traps are available…”.

**This has been corrected in the new version**

Perhaps the proportion of dead versus live zooplankton could be estimated from the flask not filled with formaldehyde collected on the fifth day of sampling and used for diazotroph quantification.

**Reviewer #1's suggestion is interesting as a way to estimate the proportion of dead versus live zooplankton. In fact, all the zooplankton (dead and alive) were recovered from the flask filled with formaldehyde, while only dead zooplankton could be recovered from the flask that was not filled with formaldehyde. However, zooplankton were not recovered from the poisoned and unpoisoned flask on the same day; that is a problem, as we observed daily variability in the amount of zooplankton recovered from the flask.**

Page 15, line 4: change for: “… in different oligotrophic regions of the ocean, for instance, the SPG …”

**This has been corrected in the new version**