Response to Carolin Löscher (Referee #3)

We thank Carolin Löscher for the time and effort devoted to the review of the manuscript. Below, we reproduce the reviewer's comments and address her concerns point by point. The reviewer's comments are copied in regular font, with our responses in green.

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. We recently obtained a 3 month extension.

The manuscript by Caffin et al. describes budgets of nitrogen at three stations in the oligotrophic western tropical South Pacific using a Lagrangian strategy thus being able to track the same water mass over time. The study reports exceptionally high N2 fixation rates and a corresponding high contribution of N2 fixation impacted material to export production. The study is very interesting to me particularly because of an approach that is more innovative than what is classically used when it comes to N budgets and N2 fixation. Overall, the paper doesn't need much changes to get into shape for publication, the study is clear and well presented. I personally think the title is not the best choice, it could make a statement on what the prominent role of N2 fixation is.

> **In accordance with the comments by Anonymous Referee #1, we have changed the title to "N_2 fixation as a dominant new N source in the Western Tropical South Pacific Ocean (OUTPACE cruise)"**

In order to make the study entirely convincing I have some main aspects, which should be and easily could be clarified:

1. The good old topic on using the bubble method: It is not convincing to just measure the dissolved fraction and not give any ranges. There are concerns with that method, everyone knows that, if you claim it is ok to use it you should have done a comparative measurement at least for some of your samples using both methods. In this context, I either need to see the data on the dissolved vs. particulate phase, or the rates have to be presented as potential rates.

> **The method mostly used here to measure N_2 fixation rates (addition of the $^{15}N_2$ tracer as a bubble in the incubation bottles -hereafter referred to as 'bubble addition method'- (Montoya et al., 1996)) has been seen to potentially underestimate rates (Großkopf et al., 2012; Mohr et al., 2010b; Wilson et al., 2012), compared to methods consisting in adding the $^{15}N_2$ as dissolved in a subset of seawater previously N_2 degased -hereafter referred to as '$^{15}N_2$-enriched seawater method'-, whereas other studies have not noted any difference between the two methods (Bonnet et al., 2016; Shiozaki et al., 2015).**
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> **In our lab, we have been using the bubble addition method for several years, e.g. (Bonnet et al., 2009; Bonnet et al., 2013; Bonnet et al., 2011; Dekaezemacker et al., 2013; Moutin et al., 2008) and the $^{15}N_2$-enriched seawater method in recent cruises and lab experiments (Benavides et al., 2015; Berthelot et al., 2015a; Berthelot et al., 2015b; Bonnet et al., 2016), and have compared the two methods. We have come to the conclusion that the associated drawbacks of degassing seawater and dissolving the $^{15}N_2$ are greater than the gain, considering the small differences observed between the two methods in Pacific waters (Bonnet et al. 2016, Shiozaki et al., 2015) and the high risk of sample**

contamination involved when manipulating sample seawater to prepare dissolved $^{15}N_2$ (Klawonn et al., 2015).

In addition to the contamination issues, preparing dissolved $^{15}N_2$ on board represents additional time with samples sitting on the bench or rosette before incubation, which is especially critical in tropical environments. This can be solved using dissolved $^{15}N_2$ prepared beforehand in the lab using, for example, artificial seawater or seawater sampled from another station. Such an approach is suitable when the sampling is always performed within the same water mass (e.g. sampling inside an eddy for example), but using in situ seawater (from the same sample) is strongly preferred in long transect cruises that encompass productivity gradients, as was the case in the present studies.

For these reasons, the bubble addition method was used here, but we paid careful attention to accurately measuring the $^{15}N/^{14}N$ ratio of the N_2 pool in the incubation bottles, whatever the method used. The potential -but not systematic- underestimation when using the $^{15}N_2$ bubble method has indeed been attributed to the incomplete equilibration of $^{15}N_2$ in the incubation bottles (Mohr et al., 2010a), which results in a lower $^{15}N/^{14}N$ ratio of the N_2 pool as compared to the theoretical value that can be calculated on the basis of gas constants. Here, we systematically performed MIMS measurements of the $^{15}N/^{14}N$ ratio of the N_2 pool in the incubation bottles and provided N_2 fixation rates that are not underestimated due to this issue. Our MIMS results reveal a ^{15}N enrichment of the N_2 pool of 6.145 ± 0.798 atom% when bottles were incubated in on-deck incubators, and 7.548 ± 0.557 atom% when bottles were incubated on the in situ mooring line, which is clearly lower than the theoretical value of ~8.2 atom% based on gas constants calculation (Weiss, 1970).

We are aware the dissolution kinetics of $^{15}N_2$ in the incubation bottles may have been progressive along the 24 h of incubation (Mohr et al., 2010), therefore, the N_2 fixation rates provided here represent conservative values.

2. In the same context, I don't know the gas quality of the company you bought from, but I assume you checked for purity as recommended in the Dabundo paper. Otherwise the high rates may as well come from an ammonia incorporation or similar. Please present your quality check, here.

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 1.1x10^{-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.

3. In addition, ammonia background measurements, fluxes and inputs are not mentioned- this would add enormous value to the stud, so please present if available. As you are making a suggestion on zooplankton moderated export, ammonia is a good part of this, too.

In our study, we focused on new N inputs (i.e. atmospheric deposition, N_2 fixation and vertical nitrate diffusion) thus associated with new production. Ammonium background was measured at the three LD stations (available on http://www.obs-vlfr.fr/proof/php/outpace/outpace.php) and was low (in the nM range) in the photic layer. We did not present ammonium data in our study, because ammonium fluxes were not measured during the cruise where the focus was essentially on new N budgets.

4. No sequencing was performed and no single cell rates were determined- how can you interpret on the key N2 fixers if you just look at 6 clusters via qPCR? What makes you conclude that Trichodesmium or UCYN clusters are important if you don't assess which diazotrophs are there?

We agree on this comment. The groups targeted by qPCR here are based on the companion paper of Stenegren et al. (this issue), who revealed that they were the major groups during the OUTPACE cruise.